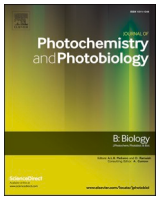
Journal of Photochemistry & Photobiology, B: Biology 268 (2025) 113187

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol



Quaternized phthalocyanines as a tool against melanoma and a broad spectrum of bacteria and fungi

Daniel Ziental [a,*], Eduardo Anaya-Plaza [b], Patrycja Talarska-Kulczyk [c], Agata Kubicka [d,e], Jakub Żurawski [c], Jolanta Dlugaszewska [f], Andrés de la Escosura [g], Tomas Torres [g,h], Lukasz Sobotta [a]

[a] Chair and Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland
[b] Department of Bioproducts and Biosystems, School of Chemical Engineering, Aalto University. Kemistintie 1, 00076 Aalto, Finland
[c] Department of Immunobiology, Poznan University of Medical Sciences, Rokietnicka 8, 60-806 Poznan, Poland
[d] Department of Cancer Pathology, Greater Poland Cancer Centre, Garbary 15, 61-866 Poznan, Poland
[e] Department of Tumour Pathology and Prophylaxis, University of Medical Sciences, Garbary 15, 61-866 Poznan, Poland
[f] Chair and Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Rokietnicka 3, 60-806 Poznan, Poland
[g] Departamento de Química Orgánica (C-I) and Institute for Advanced Research in Chemical Sciences (IAdChem), Universidad Autónoma de Madrid, 28049 Madrid, Spain
[h] Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanoscience), 28049 Madrid, Spain

ARTICLE INFO

Keywords:
Phthalocyanines
PACT
PDT
Melanoma
Antibiotic resistance
Doxycycline

ABSTRACT

Photodynamic therapy can be a powerful tool to combat two of the greatest challenges of 21st-century medicine – antibiotic-resistant infections and cancer. Each of these factors claims millions of human lives annually. In the presented study, a wide variety of cationic phthalocyanines was tested against selected pathogens: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* producing extended-spectrum β-lactamases, *Pseudomonas aeruginosa*, carbapenem resistant *Pseudomonas aeruginosa*, *Trichophyton mentagrophytes*, *Candida albicans*. The compounds used in this study, which are functionalized with pyridyloxy substituents and in some cases bear bulky substituents (*tert*-butylphenyl or pyrenyl moieties) at the 5th position of the pyridyloxy ring, proved to be remarkably effective against all pathogens, allowing for a reduction in the number of microorganisms between 1.4 and 5.6 logs. Additionally, coaction with the commonly used antibiotic – doxycycline – was achieved in in vitro studies. The effectiveness of these macrocycles was also evaluated against melanoma cells (MICH2), showing a significant reduction in melanoma cell viability up to 85 %. Therefore, the investigated compounds can be considered as promising photosensitizers in modern photodynamic therapy.

1. Introduction

Melanoma is one of the most common cancers worldwide. Only in United States of America, melanoma accounts for about 1 % of skin cancers but causes a large majority of skin cancer deaths. According to the American Cancer Society's projections for 2024 in the United States, it is estimated that approximately 100,640 new cases of melanoma will be diagnosed. Additionally, it is estimated that about 8290 individuals will die from melanoma, including approximately 5430 men and 2860 women [1]. This issue will continue to grow in the coming years with the advancing aging of the population in middle- and high-income countries, as age is one of the risk factors. New therapies improving prognosis and reducing mortality are still being developed. However, there is a lack of a universal and reliable treatment method. Radical surgical intervention, involving the removal of the lesion along with a wide margin of healthy tissue, remains the primary method of therapy [2]. However, such surgery has limited effectiveness when metastases occur, and it also leaves a necessary wound to heal. Among newer alternatives is immunotherapy, which stimulates the natural ability of the immune system to combat certain cancers. Unfortunately, some patients show resistance to this type of treatment or experience adverse effects resulting from excessive immune system activation. In recent years,

* Corresponding author.
E-mail address: dziental@ump.edu.pl (D. Ziental).

https://doi.org/10.1016/j.jphotobiol.2025.113187
Received 9 March 2025; Received in revised form 26 May 2025; Accepted 27 May 2025
Available online 28 May 2025

there has also been the development of therapies targeting genetic mutations, including patients with mutations in the BRAF gene. However, the effectiveness of such drugs is often short-lived.

Another major challenge for medicine in the 21st century is superficial infections, particularly including multi-drug-resistant infections. Many scientists are sounding the alarm, indicating that by 2050, most known antimicrobial drugs will cease to be effective. This is due to both reckless drug policies in many countries and irresponsible agricultural practices associated with industrial antibiotic overuse [3–6]. Among the greatest challenges are infections related to methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria producing extended-spectrum β-lactamases, which are often associated with post-operative wound infections, contributing to increased treatment costs and prolonged hospital stays [7–9]. Interestingly, MRSA infections are increasingly being observed not only in immunocompromised individuals but also in fully healthy athletes [10]. Infections caused by *Pseudomonas aeruginosa* are also particularly dangerous. They have exceptional ease in developing resistance, along with a tendency to form a rich, treatment-resistant biofilm [11–13]. In the presented work, *Enterococcus faecalis* bacteria characteristic of oral cavity infections, as well as *Candida albicans* and *Trichophyton mentagrophytes* fungi, were also utilized [14–16].

As a potential response to both presented medical challenges, photodynamic therapy (PDT) can be employed [17]. It is characterized by a broad spectrum of activity and a well-known safety profile. Already, it is increasingly being used in the treatment of psoriasis, rosacea, warts, and certain types of cancers such as head and neck, lung, bladder, gastrointestinal, and some types of prostate cancer. It has also found application in eye diseases, such as age-related macular degeneration (AMD). However, there is still room for improvement in the search for new photosensitizers (PSs). Desired characteristics include high efficacy, selectivity, and non-toxicity to healthy tissues [18]. On the other hand, the PS must be easily deliverable and remain active even in challenging conditions, such as environments rich in melanin in the case of melanoma or the specific structure of pathogen membranes and walls [19]. This also implies potential resistance both in microorganisms and cancers [20–23]. Among the wide range of PSs available, phthalocyanines (Pcs) hold a special position, given their outstanding optical properties like a sharp and intense absorption in the red region of spectra (c.a. 650–700 nm), and singlet oxygen ($^1O_2$) quantum yields [24–27]. Exceptional property of Pcs is their prone to the chemical modification by incorporation of metal ions or attaching specific moieties at their periphery. There are a large group of ions could be incorporated such as so-called closed-shell ions, which induce intensively spin coupling phenomenon being the crucial in efficient formation of singlet oxygen [24,28–30]. Taking account mentioned issues we decided to test zinc(II) phthalocyanines, where zinc(II) belongs to closed-shell ions group. As the peripheral substituents have been selected those with positive charges. The presence of positive charges within molecule is well-known to bring high activity against microbes. Moreover, introduction of positive charges to the structure of highly hydrophobic compounds such as Pcs enable to dissolve them in water environment. Solubility in water is one of the most important factor for achieving high rate of cancer cells killing [20,22]. Taking into account all mentioned issues Pcs bearing the quaternized pyridyloxy moieties varying in the size were chosen.

The presented study expands on the perspective of our previous article concerning the interaction between doxycycline and phthalocyanines, confirming our earlier assumption that this antibiotic sensitizes bacterial cells to ROS. Additionally, it is the first to suggest a certain universality of some quaternized phthalocyanines as both antimicrobial and anti-melanoma agents. At the same time, the study indicates that the universality of a PS's action depends on its chemical structure.

2. Experimental

Studied phthalocyanines **1–5** (Fig. 1) have been synthetized before [31–33].

2.1. Photochemistry

2.1.1. Photostability

Experiments were done at ambient temperature in DMSO according to the method described previously [34–36]. As irradiation source a high-pressure xenon lamp (model: ILLU XBO150CR, Optel, Opole, Poland) was chosen. The cutting filter (HCC-16) (transmittance >450 nm) separated visible light, used to sample irradiation. In the 1 min time intervals absorption spectra were recorded by OceanOptics system (Flame Spectrometer and source DT-MINI-2-GS) in total experiment

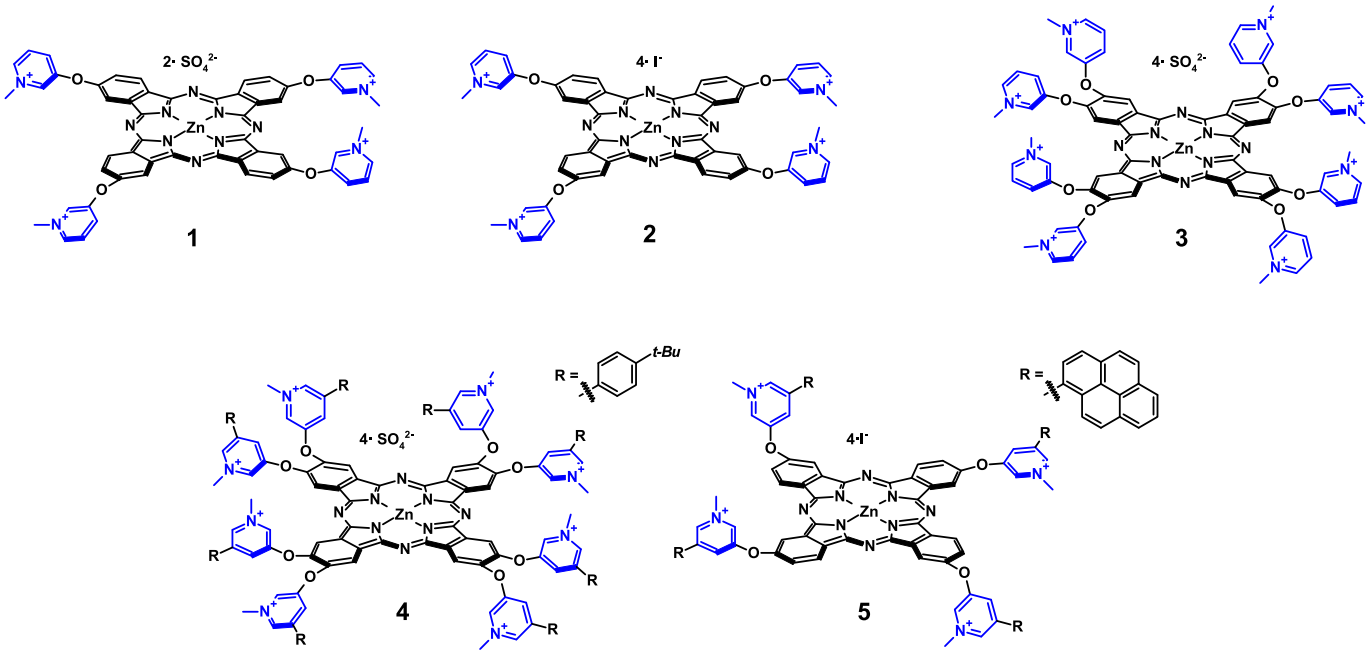


Fig. 1. Structures of macrocycles **1**–**5**.

time of 10 min.

2.1.2. Singlet oxygen formation

Experiments were made at ambient temperature in DMSO. Measurements were proceeded with a chemical quencher of singlet oxygen – 1,3-diphenylisobenzofuran (DPBF, Aldrich) and the reference compound – unsubstituted zinc(II) phthalocyanine (ZnPc, Aldrich). As the irradiation light source a high-pressure xenon lamp (150 W, Optel, Opole, Poland) was chosen. For separation of adjusted light wavelength to irradiate phthalocyanines at the absorption maximum in the Q band, a monochromator (M250/1200/U with 2 nm/mm (Dk = 4 nm) dispersion, Optel, Opole, Poland) was used. Delivered light to the sample was corrected to power of 0.5 mW/cm^2 and the light intensity with RD 0.2/2 with TD probe radiometer (Optel) was measured. In the irradiation intervals the absorption spectra with OceanOptics system (Flame Spectrometer and source DT-MINI-2-GS) were measured. As a final point, the obtained kinetic parameters of DPBF degradation were compared with those for reference, and quantum yield was determined [34,36–38].

The band gap energy: E_g was calculated using following eq. (1) [39].

$$E_g[eV] = \frac{h \times c}{(\lambda_{onset} \times 10^{-9}) \times (1.6 \times 10^{-19})}$$

$$h = 6.626 \times 10^{-34} \left[\frac{J}{s}\right] c = 3.0 \times 10^{8} \left[\frac{m}{s}\right]$$

$$\lambda_{onset} = cut - off\ wavelength$$

2.2. Photodynamic antimicrobial activity

2.2.1. Microbial cultures

A modified procedure described in previous publications [14,40] was applied, in brief, as follows: the study used the Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), methicillin-resistant *S. aureus* (clinical isolate), *Enterococcus faecalis* (ATCC 29212), Gram-negative bacteria *Escherichia coli* (ATCC 25922), *E. coli* producing an extended spectrum of β-lactamases (ESBL+, clinical isolate), *Pseudomonas aeruginosa*, carbapenem resistant *Pseudomonas aeruginoa* (CRPA; clinical isolate) fungi *Trichophyton mentagrophytes* (ATCC 9533) and *Candida albicans* (ATCC 10231). All catalog bacteria were purchased from the American Type Culture Collection. Gram-positive bacteria and Gram-negative bacteria were cultivated in BHI broth (bioMerieux, France) at 36 ± 1 °C for approximately 24 h. *C. albicans* Sabouraud dextrose broth (Oxoid, UK) cultures were performed in Sabouraud dextrose broth (Oxoid, UK) at 35 ± 1 °C for 24 h. According to the procedure for dermatophytes, *T. mentagrophytes* was cultivated in Sabouraud dextrose agar (Oxoid, UK) at 35 ± 1 °C until adequate sporulation (ca. three weeks). All experiments were performed under aerobic conditions. After cultivation, the bacteria were collected by centrifugation for 15 min (3000 rpm). The bacteria were then re-suspended in physiological saline and diluted to a final amount of ca. 10^7 CFU/ml for bacteria, 10^6 CFU/ml for *C. albicans* and 10^5 CFU/ml for *T. mentagrophytes*.

2.2.2. Light and dark activity

A modified procedure described in previous publications [14,40] was applied, in brief, as follows: the bacterial or fungal suspension, prepared according to the procedure described above, was dispensed into the wells of a 96-well microtiter plate. Subsequently, an equal volume of a specific PS was added to each well and allowed to pre-incubate for 30 min. Following this, the plate was exposed to light emitted by an LED lamp (model: LS-P3R2030-G42T, Epistar, Hsinchu Science Park, Xinzhu, Tajwan), with a wavelength range of 620–680 with maximal emission corresponding to the maximum absorption of the compounds (ca. 660 nm). The fluence was administered at30 J/cm^2 (13.9 mW/cm^2; 36 min). Similarly, a dark control sample was prepared on a separate plate and was not exposed to light. Additionally, in parallel, a plate with a control sample was prepared on which a specific volume of bacteria or fungi was applied as in the tested samples, and then diluted with the same volume of physiological saline in order to achieve the same concentration as in the case of the tested sample and the dark control. A series of dilutions were executed for each sample, and the microorganisms from each dilution were plated onto solid media appropriate for the species, followed by incubation for 24 h at a constant temperature of 36 ± 1 °C. Following the incubation period, colony-forming units (CFU) were counted, and the decimal logarithm of reduction in the test sample relative to the control sample was calculated. Additionally, it was determined whether any cytotoxicity was evident in the unirradiated sample.

2.2.3. Determination of bacteria susceptibility to photodynamic antimicrobial chemotherapy (PACT) following habituation with sub-lethal antibiotic therapy

A modified procedure, as outlined in previous publication [41], was applied, briefly summarized as follows: a suspension containing methicillin-resistant *S. aureus* and *E. coli* (ESBL+), was prepared the day before the experiment. The suspension was exposed to doxycycline at concentrations corresponding to MIC, 1/2 MIC, 1/4 MIC, and 1/8 MIC. A control sample was left untreated with doxycycline. After 12 h of exposure, the optical density at 600 nm (OD600) of each culture was measured, and the bacterial suspension was diluted to achieve an OD600 of 0.3 for each microorganism, following the Lambert-Beer law. Subsequently, equal volumes of bacterial suspension and PS were dispensed into a 96-well microtiter plate and exposed to a sublethal light dose of 5 J/cm^2. Simultaneously, a dark control was conducted, where the mixture was not exposed to irradiation, and both dark and light control tests were carried out, replacing the PS with a physiological saline solution. A series of dilutions were prepared for each sample, and the microorganisms from each dilution were plated onto TSA plates, followed by incubation for 24 h at a constant temperature of 36 ± 1 °C. After the incubation period, colony-forming units (CFU) were counted. The decimal logarithm of reduction was calculated separately for the sample treated with the antibiotic alone (at concentrations corresponding to MIC, ½ MIC, ¼ MIC, and $^1/_8$ MIC), the PS alone, and in combination.

2.3. Photodynamic anticancer activity

2.3.1. Cell culture

The effects of the tested compounds on human cells were evaluated in vitro using the established human melanoma cell line MICH2 [42]. Cells were grown in DMEM (Dulbecco's Modification of Eagle's Medium, 1×, Corning) medium supplemented with 10 % FBS (Fetal Bovine Serum, Capricorn Scientific) and antibiotics (Penicilin, Streptomycin Solution, 100×, Corning) on 24-well plates (VWR Tissue Culture Plates, 24 wells, sterile). Cell culture were maintained at 37 °C in an atmosphere of 5 % CO_2 with elevated humidity. After 24 h of culture, the medium was replaced with the tested compounds (**1**–**5**) at two concentrations (10^{-5} i 10^{-6} M). FBS-free medium was used as the diluent. After another 24 h of incubation with the test compounds, the MICH2 cells on 24-well plates were exposed to light of LED lamp (model: LS-P3R2030-G42T, Epistar, Hsinchu Science Park, Xinzhu, Tajwan), with a wavelength range of 620–680 and maximal emission corresponding to the maximum absorption of the compounds (ca. 660 nm) at fluence rate of 5 J/cm^2 (13.9 mW/cm^2; 6 min) and then placed in the incubator.

After 30 and 60 min, respectively, the media were removed, the cells were washed with phosphate buffered saline (PBS, Dulbecco's PBS (1×) w/o Ca & Mg, w/o Phenol Red, Capricorn scientific) and detached from with EDTA buffered 0.25 % trypsin solution (Trypsin-EDTA 0.25 % in HBSS (1×) with Phenol Red, Capricorn Scientific), then washed and centrifuged. Into experiment cells were involved in following scheme: cells treated with studied compounds and not exposed for the radiation, cells without studied compounds and not exposed for the radiation, and

cells without studied compound and exposed for the radiation were used as controls. Negative controls were cells with culture media without FBS.

To observe the cells in a light microscope, a second set was cultured according to the above procedure. Hematoxylin (Chempur, catalog number: 124687401) and Eosin (Chempur, catalog number: 243767005) were used to stain melanoma cells. After removing the medium from 24-well plastic plates (VWR Tissue Culture Plates, 24 wells, sterile), all culture wells were filled with hematoxylin (exposition time – 1 min). The hematoxylin was removed and the culture plates were placed in a PBS solution (exposition time – 3 min). After removing the PBS, the culture wells were filled with eosin (exposition time – 1 min). The culture plates were again placed in the PBS solution and closed with the lid from the kit, securing the whole with Parafilm® M sealing foil (Bemis Company, Inc., catalog number LLG-9170003). The set prepared in this way was placed upside down under the stage of the light microscope. Microphotographic documentation was made using an Olympus BX43 microscope and an XC30 camera at a total magnification of 100×.

2.3.2. Cells viability

In order to determine the percentage of dead cells in the tested samples, staining with 7-Amino-Actinomycin D (7-AAD) was performed. 7-AAD is one of the viability dye which intercalates into double-stranded nucleic acids. 7-AAD can penetrate the cell membrane of dying or dead cells. Pre-collected, washed and centrifuged cells were used to perform the 7-AAD viability test. Each test trial was performed in duplicate. To each tube contains 100 µl (10^5 cells) cell pellets re-suspended in phosphate buffered saline (PBS) 5 µl of 7-AAD were added and incubated for 10 min, protected from light in room temperature. All stained samples were acquired with FACS Aria sorter (Becton Dickinson), results were analyzed with FACS Diva Software (Becton Dickinson). An unstained sample was used as a control for the test.

2.3.3. Cell cycle

Cell cycle assessment was performed using propidium iodide (PI) (1.0 mg/ml water solution; Merck-Sigma-Aldrich), a fluorescent dye that intercalates with DNA structure of replicating cells. Cell proliferative activity is assessed by the percentage of cells that are in S phase of the cell cycle and determined on the basis of mean fluorescent activity (MFI) emitted by propidium iodide. In addition, it is possible also to assess the percentage of cells entering mitosis (G2/M phase of cell cycle) and dead cells. Pre-collected, washed and centrifuged cells were used to perform the PI test. Each test trial was performed in duplicate. All tests samples were treated with PERM/WASH buffer (Becton Dickinson) for 30 min, 4 °C to permeabilize the membrane of the cells. Then propidium iodide were added, all test samples were incubated for 30 min, 4 °C, protected from light. Finally, the samples were added to acquisition with use of flow cytometer Navios (Beckman Coulter), and percentages of cells in each phases of cell cycle were calculated using Kaluza Analysis software (v. 2.1 Beckman Coulter). An unstained sample was used as a control for the test.

2.3.4. Apoptosis

To distinguish apoptotic cells from necrotic cells using flow cytometry commercially available FITC Annexin V Apoptosis Detection Kit I (BD Pharmingen) were used in accordance with the manufacturer's protocol. Pre-collected, washed and centrifuged cells were used to perform apoptosis analysis. Cells pellets were re-suspended in 1× Annexin V Binding Buffer and 100 µl were transferred to a 5 ml cytometer tubes. 5 µl of FITC Annexin V and 5 µl od PI were added to each tube and incubated for 15 min, room temperature, in the dark, then 1× Annexin V buffer was added. Each test trial was performed in duplicate. All stained samples were acquired within 1 h with FACS Aria sorter (Becton Dickinson), results were analyzed with FACS Diva Software (Becton Dickinson). With the staining used, depending on the proportion between FITC and/or PI fluorescence, cells can be defined as early apoptotic, late apoptotic or necrotic. An unstained sample, sample without PI and sample without FITC Annexin V were used as a control for the test.

2.4. Statistical analysis

Statistical analyses were performed using Welch's *t*-test in GraphPad Prism (version 9.0.0) to compare group means while accounting for unequal variances. Data are presented as mean ± standard deviation. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

Studied phthalocyanines **1–5** (Fig. 1) have been synthetized before [31–33]. The crucial factor providing photodynamic treatment efficacy is singlet oxygen generated by the activated PS [17]. Therefore, singlet oxygen formation ability was assessed in DMSO with the relative method using DPBF as a chemical singlet oxygen quencher and ZnPc as a reference. Evaluated PSs **2–5** (Table 1) showed higher ability to form singlet oxygen than reference. Interestingly, comparing Φ_Δ values for **1** and **2**, it could be concluded that the anionic counterpart (SO_4^{2-} vs. I^-, respectively) has significant impact resulting in increased Φ_Δ from 0.53 to 0.88 [43]. The studied PSs reveal moderate-high Φ_Δ values, what is result of electron-deficient character of quaternized pyridoxyl ZnPcs as it was reported before [31]. The electron-withdraw phenomenon is known to reduce the singlet oxygen generation ability [44–46]. The presence of partial positive charges in the nitrogen atoms leads to a smaller HOMO-LUMO gap. This situation cause hampering photoinduced electron transfer within the molecule, which is associated with singlet oxygen generation [47–49]. Moreover, calculated energy band gap at the level of ca. 1.8 eV (Table 1) indicates that studied pcs are prone to losing electron and start the reactions cascade leading to form ROS. The showed values (Table 1) are much more lower that these reported before for zinc(II) pc bearing imidazole moieties at the periphery ($E_{g(ZnPc)} = 2.87$ eV) [50]. This comparison enable to assume that for the Eg value of pcs significant influence possess incorporated matal ion as well as nature of substituent (electron withdraw-donating).

Photodecomposition quantum yields (Table 1) indicate that compounds **1–5** present moderate stability in comparison to earlier reported phthalocyanine derivatives [7,38]. Noteworthy, **5**, with very high ability to generate $^1O_2$, decomposed under visible light exposure with a significantly lower with quantum yield of $6.5 \cdot 10^{-7}$ in contrast to the other evaluated PSs.

Numerous studies have confirmed that PDT can be a powerful tool in combating microorganisms [7,8,14,23,44,51–55]. It possesses several significant advantages, such as a broad spectrum of activity, selectivity towards pathogenic cells, and a low risk of developing resistance to this type of therapy. Unfortunately, commercially available PSs do not fully exploit the potential of PACT, and further exploration of structures with preferable properties is necessary [17,23,56,57]. In this study, we propose the utilization of pyrene-modified phthalocyanines as potential candidates for antimicrobial therapy.

The experiment utilized a very broad spectrum of potentially pathogenic microorganisms. Among the strains examined were

Table 1
Values of singlet oxygen and photodecomposition quantum yields in DMSO.

Compound	Φ_Δ	$\Phi_P \cdot 10^4$	λ_{onset} [nm]	E_g [eV]
1	0.53	1.06	704	1.76
2	0.88	1.06	709	1.75
3	0.77	0.47	712	1.74
4	0.63	1.45	696	1.78
5	0.73	0.0065	706	1.76

representatives of both Gram-positive bacteria (*S. aureus*, *E. faecalis*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*), as well as yeast-like fungi and dermatophytes (*C. albicans* and *T. mentagrophytes*). In the case of **1** and **2** at the concentration of 10 μM, very high activity against all bacteria was achieved, ranging between a minimum of 4 and a maximum of 6 log reduction in bacterial growth (Fig. 2). Interestingly, even a 10-fold decrease in concentration did not weaken the antimicrobial activity of the investigated PSs. Compound **1** exhibited slightly higher activity against fungi than compounds **2** and **3**, with approximately 4 log reduction observed for both *C. albicans* and *T. mentagrophytes*. Compounds **2** and **3** reduced *C. albicans* by 4.7 and 1.5 log, respectively, while the activity against dermatophytes remained around 2.7 for the concentration of 10 μM (Fig. 2). Such characteristics predispose compounds **1–3** to be promising candidates for further development as potential APIs (active pharmaceutical ingredients) in PACT, as activity exceeding 3 log reduction is considered borderline for compounds with potential antibiotic-like properties according to international guidelines [58].

The activity of the tested phthalocyanines was significantly stronger than in many previously published studies. For example, zinc(II) and metal-free phthalocyanines bearing nipagin-functionalized substituents studied before, even when used at a 10-fold higher concentration and with a more than 3-fold higher light dose, achieved a maximum activity against *E. coli* of approximately a 2 log reduction [51]. In the case of tetra- and octa substituted methimazole-phthalocyanine conjugates, at a concentration of 10 μM and a light fluence of 30 J/cm^2 (13.9 mW/cm^2; 36 min), a maximum reduction in the number of *S. aureus* bacteria by 1.8 log was observed [44]. Such promising results encouraged the authors to compare the sensitivity to PACT using pyrene-modified phthalocyanine between standard strains and those phenotypically manifesting resistance to selected types of antibiotics. For this purpose, *S. aureus* and methicillin-resistant *S. aureus* were selected as model strains, as well as *E. coli* and *E. coli* (ESBL+). In none of the mentioned cases were potentially clinically significant differences in the activity of the tested compounds observed. Results from other research groups on this topic are mixed and quite ambiguous.

Studies conducted by Grinholz et al. indicate that antibiotic-resistant bacteria are typically also more resistant to PDT [59]. On the other hand, phenomena called collateral sensitivity are described, where the cell's metabolic engagement to increase resistance to one factor (e.g., classical antibiotic) enhances sensitivity to another [60–62]. In the presented study, none of these phenomena were observed. It is also worth noting that for all tested compounds, very little impact of cell wall structure on the obtained results was observed. Gram-negative bacteria often exhibit

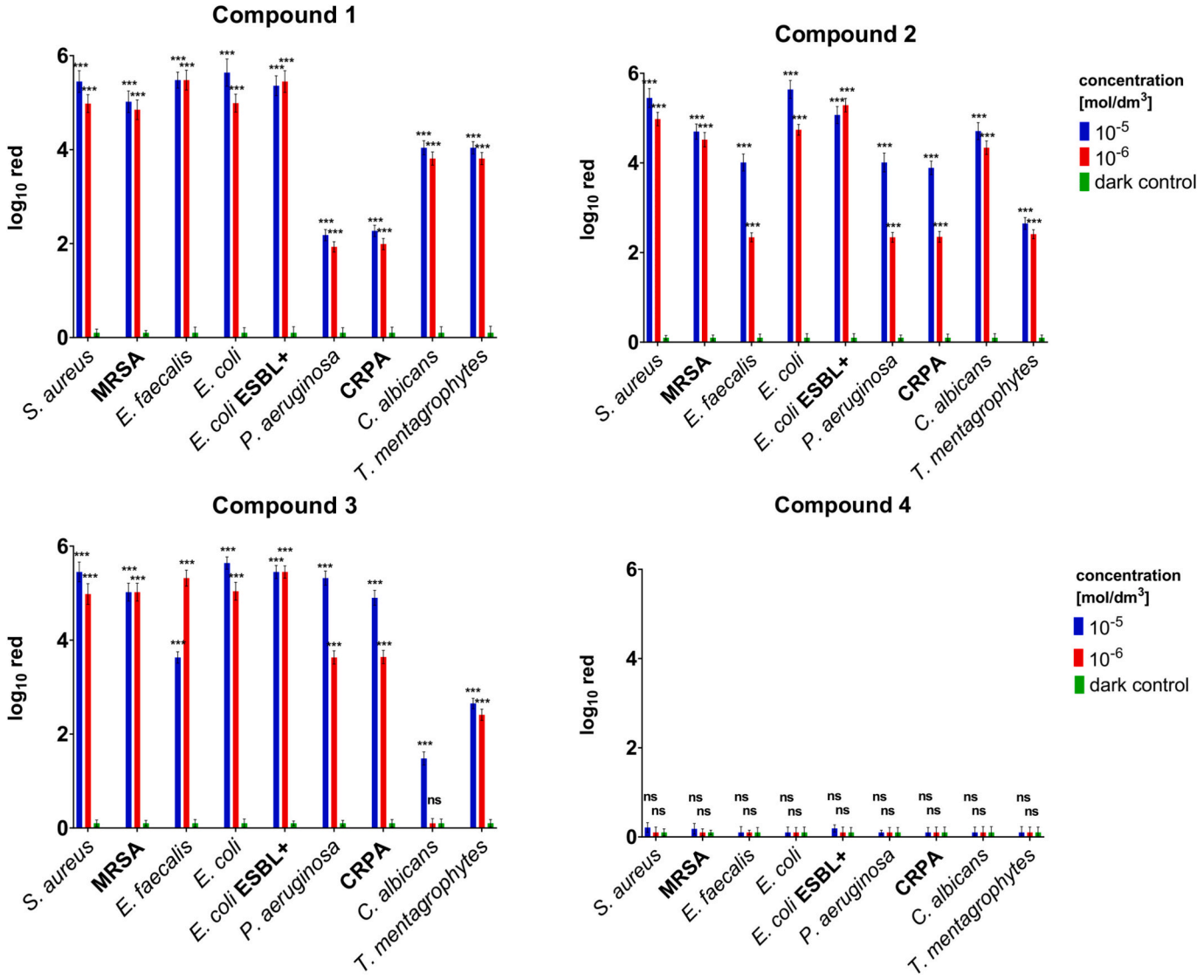


Fig. 2. Antimicrobial activity of the studied phthalocyanines (30 min pre-incubation; light dose 30 J/cm^2; 13.9 mW/cm^2; 36 min). * for $p > 0.05$, ** for $p > 0.01$, *** for $p > 0.001$.

lower sensitivity to PACT due to their outer membrane rich in phospholipids, proteins, and lipopolysaccharides. The presence of the outer membrane often hinders the activity of the PS by limiting its internalization [63,64]. The insensitivity of the applied phthalocyanines to this microbial structural feature constitutes an additional advantage as a potential means of infection treatment. Additionally, none of the analyzed macrocycles revealed dark toxicity. Compounds **4–5** exhibited marginal antimicrobial activity, which may be directly related to the presence of large, nonpolar substituents potentially limiting the molecule's interactions with the bacterial wall. Simultaneously, these substituents may pose steric hindrance, restricting the possibility of internalization. The obtained results indicate that the investigated phthalocyanines constitute a promising direction for further development as photosensitizers. The encouraging outcomes observed in this study provide a strong rationale for extending research into in vivo models in the future, particularly in light of previous investigations by Zheng et al., which demonstrated favorable in vivo efficacy of cationic phthalocyanines against *Staphylococcus aureus* infections [65]. These findings collectively suggest a strong potential for translational applications of this class of compounds in the treatment of bacterial infections.

In the photodynamic antimicrobial activity assessment we haven't noticed correlation between Φ_Δ values and the log reduction in bacterial growth. This phenomenon may be associated with the fact that crucial role in aPDT plays PS attraction to the bacterial membrane, what was provided by our structures with positive charges. The ability to form ROS is the second important factor and we assume that needed level of produced singlet oxygen (ROS) was achieved by our PSs. In the next step of evaluation of potential phthalocyanines for PACT we have studied their potential in synergistic activity with doxycycline. Doxycycline belongs to the group of tetracycline antibiotics, one of the most important antibiotic groups, and it has been in clinical use for over 50 years. At concentrations used in clinical practice, it has bacteriostatic properties, although at high doses it can also be bactericidal. Its main mechanism of action involves inhibiting the activity of bacterial ribosomes. Its activity includes both Gram-positive and Gram-negative bacteria as well as atypical strains. The clinical use of doxycycline is broad and includes infections of the upper respiratory tract, genitourinary system, some rickettsial diseases, bartonellosis, and malaria prophylaxis [66]. Of particular interest from the perspective of PDT are applications of doxycycline related to superficial bacterial diseases. It can be used in common acne caused by the overgrowth of pathological bacterial flora, primarily by the colonization of the skin by *Cutibacterium acnes*. It can also be used in rosacea, dermatoses, purulent skin inflammations, and in the treatment of infected wounds and ulcers [67,68]. It has been observed to prevent angiogenesis and apoptosis and may enhance fibroblast attachment and wound healing. It also has an indirect anti-inflammatory effect resulting from the inhibition of certain matrix metalloproteinases (MMPs) produced by inflammatory cells [66,67]. There is a very limited number of studies evaluating the additive or even synergistic effect of tetracyclines with PDT [69]. For instance Baccani et al. presented a typical combination of PDT and antibiotics, testing the sensitivity to excited PS of *Helicobacter pylori* bacteria exposed to sub-inhibitory concentrations of doxycycline. In their study, protoporphyrin IX (PPIX) was used as the PS along with light at a wavelength of 400 nm. In this experiment, an additive effect between PPIX and antibiotic therapy was also observed, which the authors described as a synergistic effect. Although the study did not include any calculations to confirm the actual synergistic effect, it can undoubtedly be confirmed that the combined effect of PDT and antibiotics is significantly stronger than each alone [70]. Comparing results from different experiments regarding the interaction between antibiotics and PDT will always be subject to methodological difficulties because the final effect may be highly dependent on the strength of PDT itself. The study by Baccani et al. [70] showed that even a relatively small increase in the reduction of bacterial counts in culture could greatly enhance the combined effect of antibiotics and PDT. Here, we adopted a different assumption, where we decided to use sublethal PDT, defining the cut-off point as a maximum of 1.5 log reduction resulting from the action of PDT alone. It is worth noting that unlike in the presented study and Baccani et al.'s experiment [70], bacteria were not cultured with a sublethal dose of antibiotic, but the antibiotic was added simultaneously with the PS.

This approach inherently favours bactericidal antibiotics. In our study, we used two strains – Gram-positive MRSA and Gram-negative *E. coli* (ESBL+) – to evaluate the interaction of doxycycline with PDT. In both cases, the log reduction of each species was greater than the sum of the individual effects of each therapy. The strongest effect in both cases was observed for **2** and the antibiotic at the minimal inhibitory concentration (MIC), where a combined effect with log reduction of 2.6 was achieved for MRSA (PDT alone 0.1 log and antibiotic alone 0.69 log) and 3.9 for *E. coli* (PDT alone 0.9 log and antibiotic alone 0.54 log). It is remarkable that reducing the antibiotic concentration to ½ MIC and ¼ MIC for *E. coli* (ESBL+) and ½ for MRSA still allowed for beneficial interaction of both pathways. Interestingly, compound **3** showed a significantly stronger effect for *E. coli* than for MRSA, although it was noticeable in both cases (Fig. 3). The mechanism of interaction between Pcs and doxycycline is still speculative, but based on previous publications, some scientifically supported hypotheses can be put forward [71–73]. Doxycycline binds to the 30S subunit of the bacterial ribosome and blocks the binding of aminoacyl-tRNA to the A site, thus preventing the synthesis of new proteins. This indirectly impairs the functioning of all cellular organelles, including the bacterial membrane and wall. Impairment of the function and structure of proteins, enzymes, and above all the wall and membrane of microorganisms sensitizes them to oxidative stress induced by PSs, the effect of which is stronger than in the case of traditional PACT [71].

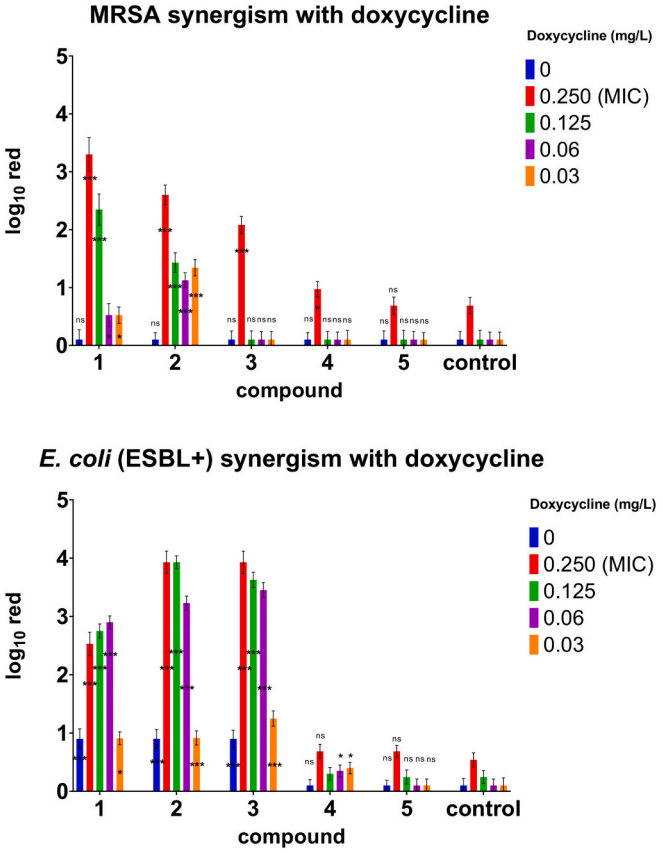


Fig. 3. Interaction of doxycycline with sublethal PDT. A. MRSA B. *E. coli* producing an extended spectrum of β-lactamases. * for $p > 0.05$, ** for $p > 0.01$, *** for $p > 0.001$.

The last part of the studies was evaluation of the photodynamic activity of studied PSs against melanoma. Melanoma remains one of the most challenging cancers to treat, attributable to several factors that contribute to its malignancy. It exhibits a high propensity for genetic variability, with possible occurrences of therapy-resistant alterations in genes such as BRAF, NRAS, or c-KIT. These alterations contribute, among other things, to increased tumor growth, resistance to chemotherapy or radiotherapy, and even disruptions in apoptotic pathways. Recent years have been seen rapid advancements in new therapies, including checkpoint inhibitor immunotherapy and targeted therapy

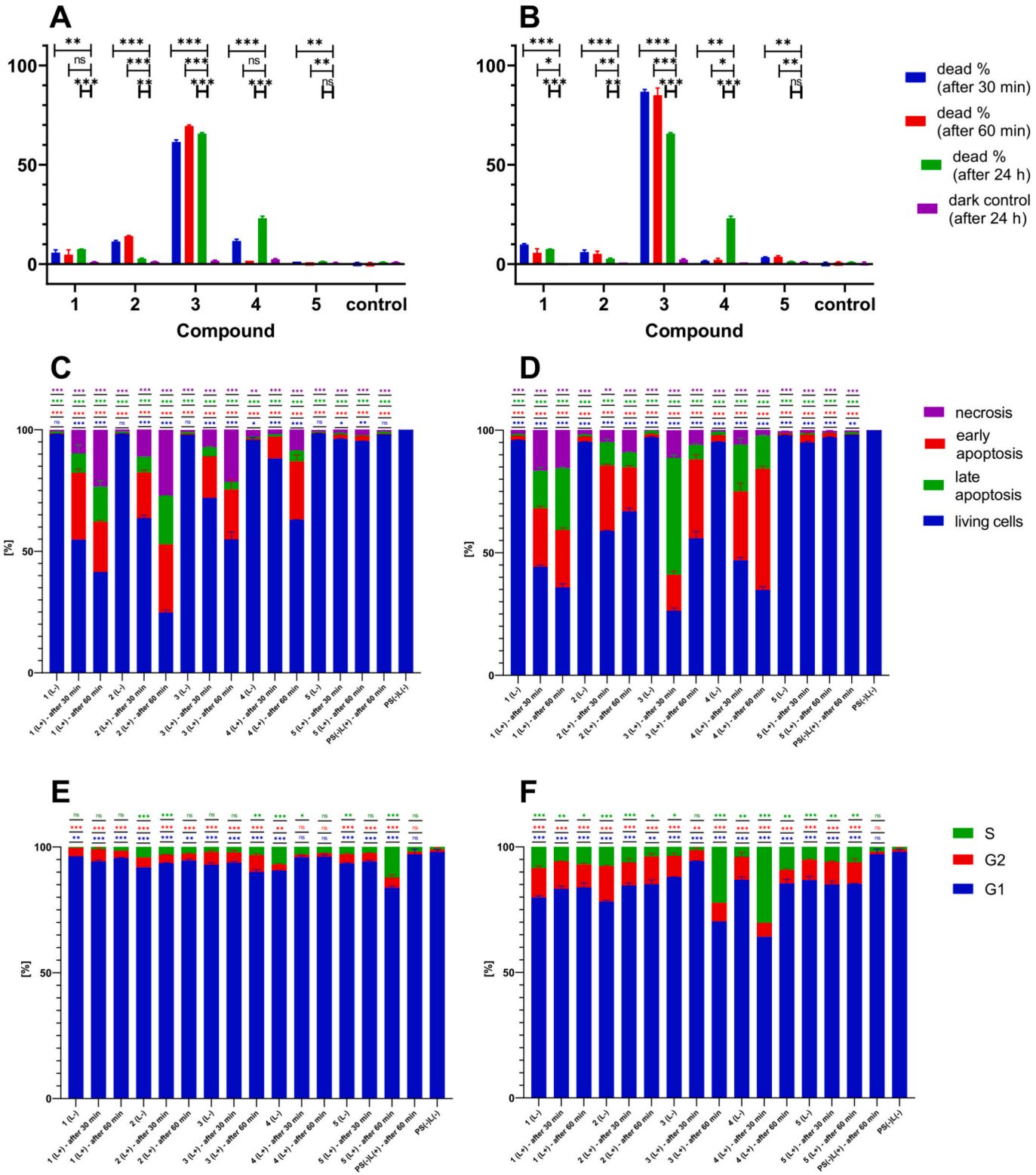


Fig. 4. Anti-melanoma activity of tested Pcs. A. Percentage of non-viable cells after using 1 μM dye. B. Percentage of non-viable cells after using 10 μM dye. C./E. Cell cycle assessment after using 1 μM dye D./F. Cell cycle assessment after using 10 μM dye; Readings taken after 30 min, 60 min and/or 24 h. * for $p > 0.05$, ** for $p > 0.01$, *** for $p > 0.001$.

focusing on genetic mutations in patients with an active MAPK pathway [2,74,75]. Despite this, in many cases, existing treatment methods remain ineffective. Therefore, the development of PDT remains intriguing [76]. The investigated compounds exhibited highly varied activity against melanoma cells. Compound **3** proved to be the most active. Even at a concentration of 1 μM, a reduction in the number of viable cells by approximately 59 % compared to the control group was achieved after just 30 min post-irradiation.

The delayed effect after 24 h did not differ significantly and amounted to 64 %. This indicates a rapid course of the process and limited ability of the cancer cells to regenerate. Increasing the PS concentration to 10 μM also led to increased activity in the reading after 30 min, reaching approximately 84 %, and 64 % after 24 h. This shows that increasing the concentration does not enhance the potential of the investigated compound in PDT. Characteristic of **3** is also its minimal dark toxicity. The compound without irradiation caused a reduction of only 2.3 % at a concentration of 10 μM and 1.7 % at a concentration of 1 μM. Interesting insights are also provided by studies on the mechanism of death in cancer cells subjected to therapy. In the case of **3** at a concentration of 1 μM, induction of early apoptosis is observed, which persists over time, potentially explaining the slight decrease in dead cell readings after 24 h from the start of the test, possibly associated with their partial recovery. At the concentration of 10 μM, was no longer observed such a relationship. Cells practically immediately converted into late apoptosis, which is typically an irreversible process. (Fig. 4).

Considering research indicating that both cells in early and late apoptosis stages are crucial in inducing an immune response compared to necrotic cells, the investigated compounds, especially **3**, appear to be potentially excellent candidate in adjuvant therapy for melanoma treatment. Their mechanism of action could therefore complement both radical surgical therapy and modern treatment based on checkpoint inhibitors [77,78]. Microscopic analysis of the examined cancer cells provides interesting data. The MICH2 melanoma cells not exposed to light were characterized by various morphologies. Mainly round and spindle-shaped cells were observed. Cells not exposed to light were larger and more numerous compared to cells exposed to radiation. However, the most noticeable differences were visible for **3** (at the concentration of 10^{-6} M) (Fig. 5).

After exposure to light, the cells were round, spindle-shaped, and irregularly shaped, with a visible cell nucleus. Some of them formed clusters of cells with the presence of pseudopodia. Cells exposed to radiation were significantly smaller, and spherical with an irregular outline of cell membranes. Some of them had damaged cell membranes. In all tested samples, differences were found in terms of appearance, size, and number of cells compared to the control culture. The most noticeable differences, however, were visible for compounds **4** (at a concentration of 10^{-5} M), and **5** (at a concentration of 10^{-5} M) (Fig. 6).

The use of PDT in melanoma treatment is subject to certain controversies, especially due to the presence of pigment cells within the tumor, which can spontaneously absorb part of the delivered light [79]. It is worth noting that melanin, the pigment present in melanoma cells, exhibits maximum light absorption between 200 and 300 nm. However, for the compounds we investigated, the maximum absorption occurs around 650 nm, where melanin absorption is minimal [80]. Therefore, it is possible to apply light with limited loss due to light absorption by the cancer cells themselves. Moreover, with further development of the API, it may be possible to completely avoid unwanted interactions by using an optical clearing system [81]. Importantly, the pigments naturally present in melanosomes can potentially act as scavengers of free radicals. However, the observed cytotoxic effect induced by **3** contradicts the significance of this process. This could be related to the high efficiency in generating singlet oxygen for **3** ($\Phi_\Delta = 0.77$), while maintaining relatively good stability ($\Phi_P = 0.47 \cdot 10^{-4}$). These results correlate well with the findings from cell line studies. Phthalocyanine **3** induces an immediate cytotoxic effect without significant changes over time. Comparing the obtained results with those using other PSs, it can be concluded that the investigated phthalocyanines are a promising path for further development.

Based on the studies published to date, it can be concluded that phthalocyanine-induced cancer cell death is primarily associated with the mitochondrial apoptosis pathway. ROS lead to cytochrome *c* release, mitochondrial depolarization, and subsequent activation of caspases. Cell death involving death receptor signaling, such as through Fas (CD95) or TRAIL receptors, appears to be less well supported by the current literature in the context of phthalocyanine-based PSs [82–84]. Despite the relatively long history of using PDT in melanoma research, there are not numerous studies using pure phthalocyanines. Kolarova et al. utilized disulphonated chloroaluminum phthalocyanine ($ClAlPcS_2$) as a PS, which resulted in approximately 50 % reduction in cell viability at a significantly higher concentration of 6.4 μM and light dose of 10 J/cm^2 [85]. Interestingly, **3** in the concentration of 1 μM activated with 5 J/cm^2 gave similar killing rate. Comparable efficacy to **3** was achieved also by Valli et al. using cationic zinc(II) phthalocyanine 2,9(10),16(17),23(24)-tetrakis[(2-trimethylammonium)ethylsulfanyl] phthalocyaninatozinc(II) tetraiodide, where a decrease in cell viability of over 80 % was observed for B16F0 cells at a concentration of 1 μM and light dose of 0.68 J/cm^2 [86]. Compound **3** also showed a significantly stronger effect than tetrasulphonated chloroaluminum phthalocyanine ($AlPcS_4Cl$) at a concentration of 2.5 μM and under the influence of light 5 J/cm^2. In this case, Ndhundhuma and Abrahamse achieved a reduction of slightly over 40 % after 1 h [87]. However, the therapeutic efficiency of pyrene-modified phthalocyanines is very close to that of widely studied bacteriochlorins and porphyrins. In the study by Dabrowski et al., a reduction of 90 % melanoma cells (SK-MEL-188) was achieved for $TCPBSO_3H$ with a light dose of 5.4 J/cm^2, however, the concentration used in the study was twice as high as the concentration of compound **3**, which resulted in a reduction of approximately 85 % [88,89].

The main challenge for the presented active PSs (**1–3** against microorganisms or **3** against melanoma) is the future development of effective formulations. A potential approach to addressing this issue could involve the use of liposomes as carriers for the PSs, as also

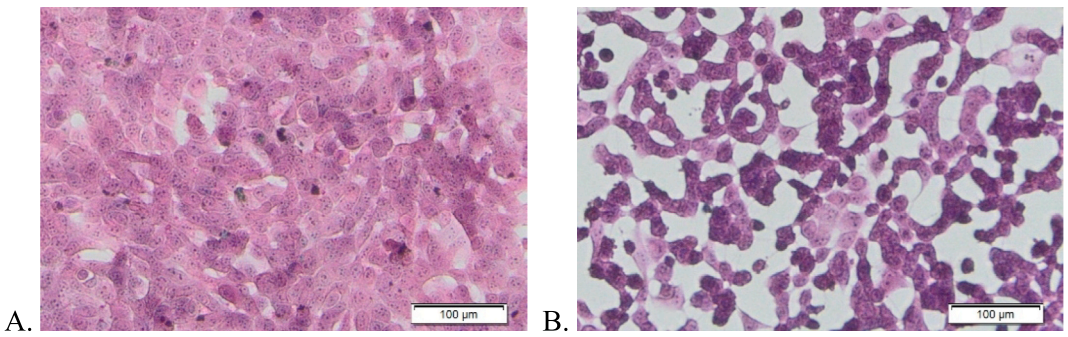


Fig. 5. The MICH2 cells not exposed to light A. Control, B. **3** in concentration 10^{-6} M.

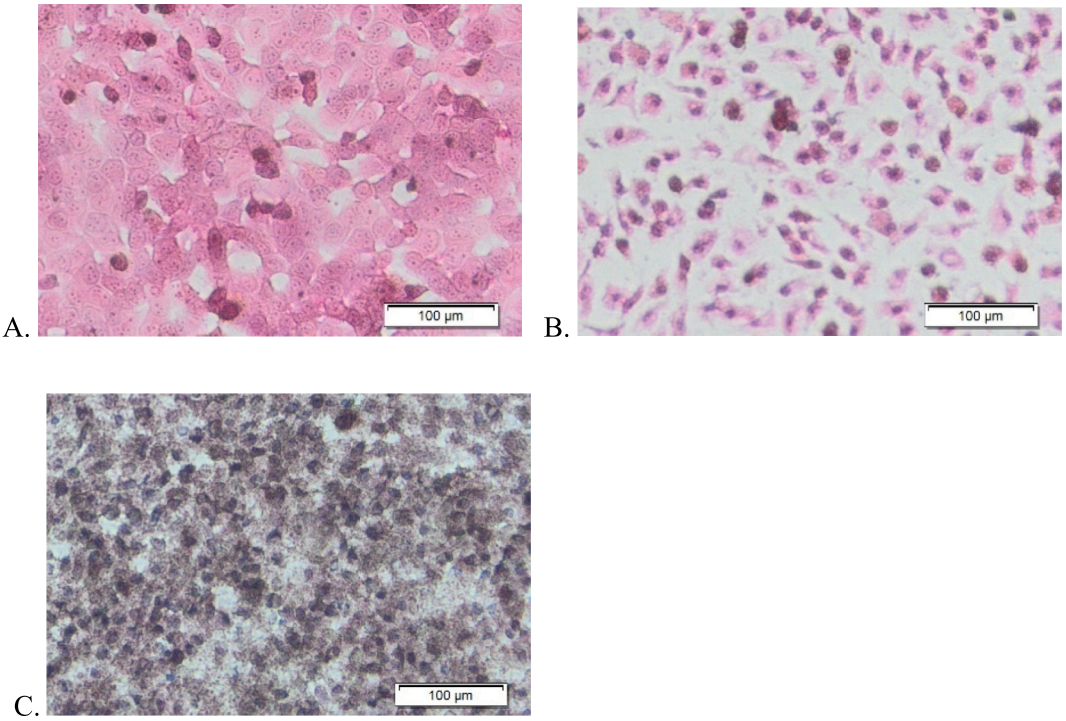


Fig. 6. The MICH2 cells exposed to light A. Control, B. MICH2 cells with **4** in concentration 10^{-5}, C. **5** in concentration 10^{-5} M.

supported by studies conducted on other macrocycles by our group before [14,35]. Another possible solution could be the combination with nanoparticles, such as gold or silver, which might be particularly attractive in the context of antimicrobial therapy [44,90–92]. Encapsulation in cyclodextrins could also be considered as an alternative strategy [93]. These are just a few of the many possible solutions that could improve the compounds' stability and solubility while also enabling selective molecular targeting. The main tasks in the further development of the product, enabling its introduction into clinical practice, will include continued preclinical studies focusing on safety, dosage, and the pharmaceutical form of the product [91]. We assume that the molecules may be particularly useful in surface applications. The presented studies, especially those conducted on microorganisms, indicate very low or even zero dark toxicity, which may facilitate the introduction of these molecules as potential clinical PSs [94,95]. At the same time, further in vivo studies will be necessary to optimize the treatment protocol and to assess the risk and severity of side effects typically associated with PSs, such as erythema, pain, burns, edema, itching, desquamation, pustular formation, urticaria, contact dermatitis at the site of PS application, and erosive pustular dermatosis [96].

The presented studies indicate a very good potential for combination with other therapies, such as antibiotic therapy; however, formulating generalized conclusions based on this study should be limited. Previous research by other authors shows that the synergy between antibiotics and PDT is highly dependent on the specific drug and PS chosen [71,97]. In our opinion, this often relates to poorly selected methodology for such studies. A typical synergy test for two antimicrobial agents is the FICI assay, but based on our experiences, this approach in the case of PACT may yield both false-positive and false-negative results (unpublished data). Additionally, our studies provide particularly good prospects for bacteriostatic antibiotics, such as doxycycline (where the bactericidal or bacteriostatic effect depends on concentration) [41]. In the case of using PDT in cancer treatment, particularly melanoma, a different perspective should be considered. First of all, after the surgical removal of the melanoma lesion, it is necessary to apply a large margin of healthy tissue to ensure a certain result. Enhancing surgical treatment with perioperative PDT may potentially contribute to reducing the required surgical margin, although this would require further in vivo studies. Moreover, an increasing number of papers point to additional mobilization of the immune system under the influence of PDT against cancer. This suggests the potential for combining PDT with both surgical intervention and immunotherapy, as well as with drugs such as checkpoint inhibitors [74,75,79].

4. Conclusions

Evaluated PSs showed high ability to form singlet oxygen. It could be concluded that anionic counterpart (SO_4^{2-} vs. I^-, respectively) has significant impact resulting in increased Φ_Δ. Photodecomposition quantum yields indicated that studied compounds present moderate stability. The photodynamic inactivation potential against pathogens was evaluated. Among the strains examined were of both Gram-positive bacteria (*S. aureus*, *E. faecalis*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*), as well as yeast-like fungi and dermatophytes (*C. albicans* and *T. mentagrophytes*). For two derivatives at the concentration of 10 µM, exceptionally high activity against all bacteria was achieved. Interestingly, even a 10-fold decrease in concentration did not weaken the antimicrobial activity of the investigated PSs. Moreover, it was noticed the strong synergistic effect of PDT mediated with studied phthalocyanines and antibiotic drug – doxycycline against MRSA and *E. coli* (ESBL+). The photodynamic anticancer activity against melanoma cells was evaluated as well. The most developed PS proved to be active against melanoma cells at both investigated concentrations, causing a reduction in viable cells by 85 % after 1 h. These results designate it as an excellent candidate for anticancer PDT. Interesting insights are also provided by studies on the mechanism of death in cancer cells subjected to therapy, for which the early apoptosis stage predominates. Moreover, presented results enable to conclude that to provide photodynamic activity, the amount of formed singlet oxygen by PS should achieved desired level above which the therapeutic effects doesn't increase. It appears to be potentially excellent candidate in adjuvant therapy for melanoma treatment.

Ethics approval and consent to participate

The study was conducted on cell lines and bacterial strains, and the scope of the research did not require approval from the bioethics committee.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Daniel Zietal: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Eduardo Anaya-Plaza:** Writing – review & editing, Resources. **Patrycja Talarska-Kulczyk:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Agata Kubicka:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation. **Jakub Żurawski:** Methodology, Investigation. **Jolanta Dlugaszewska:** Resources, Methodology, Investigation. **Andrés de la Escosura:** Writing – review & editing, Resources. **Tomas Torres:** Writing – review & editing, Resources. **Lukasz Sobotta:** Writing – review & editing, Writing – original draft, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The research was funded by the FENG.02.02-IP.05-0257/23 grant from the Foundation for Polish Science, co-financed by the European Union funds under the European Funds for Smart Economy 2021-2027 (FENG).

EAP acknowledges funding from the Research Council of Finland, project 341057.

T.T. acknowledges financial support from the Spanish MCIN/AEI/10.13039/501100011033 and European Union NextGenerationEU/ PRTR (PID2020-116490GB-I00, MCIU /AEI /10.13039/501100011033 / FEDER, UE (PID2023-151167NB-I00), the Comunidad de Madrid and the Spanish State through the Recovery, Transformation and Resilience Plan [“Materiales Disruptivos Bidimensionales (2D)” (MAD2D-CM) (UAM1)-MRR Materiales Avanzados], and the European Union through the Next Generation EU funds. IMDEA Nanociencia is appreciative of support from the “Severo Ochoa” Programme for Centers of Excellence in R&D (CEX2020-001039-S).

T. T. also acknowledges the Alexander von Humboldt Foundation (Germany) for the A. v. Humboldt - J. C. Mutis Research Award 2023 (Ref 3.3 - 1231125 - ESP-GSA).

The authors would like to thank Dr. Paulina Skupin-Mrugalska (Chair and Department of Inorganic and Analytical Chemistry Poznan University of Medical Sciences Rokietnicka 3, 60-806 Poznan, Poland) for help in preparing the graphical abstract (created using the BioRender application).

Data availability

Data will be made available on request.

References

[1] Melanoma Skin Cancer Statistics, American Cancer Society. https://www.cancer.org/cancer/types/melanoma-skin-cancer/about/key-statistics.html, 2024 (accessed December 4, 2024).
[2] L.E. Davis, S.C. Shalin, A.J. Tackett, Current state of melanoma diagnosis and treatment, Cancer Biol. Ther. 20 (2019) 1366–1379, https://doi.org/10.1080/15384047.2019.1640032.
[3] J.M.A. Blair, M.A. Webber, A.J. Baylay, D.O. Ogbolu, L.J.V. Piddock, Molecular mechanisms of antibiotic resistance, Nat. Rev. Microbiol. 13 (2015) 42–51, https://doi.org/10.1038/nrmicro3380.
[4] A. Cassini, L.D. Högberg, D. Plachouras, A. Quattrocchi, A. Hoxha, G.S. Simonsen, M. Colomb-Cotinat, M.E. Kretzschmar, B. Devleesschauwer, M. Cecchini, D. A. Ouakrim, T.C. Oliveira, M.J. Struelens, C. Suetens, D.L. Monnet, Burden of AMR Collaborative Group, Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: A population-level modelling analysis, Lancet Infect. Dis. 19 (2019) 56–66, https://doi.org/10.1016/S1473-3099(18)30605-4.
[5] P. Dadgostar, Antimicrobial resistance: implications and costs, IDR Volume 12 (2019) 3903–3910, https://doi.org/10.2147/IDR.S234610.
[6] A.O. El-Gendy, Y. Obaid, E. Ahmed, C.S. Enwemeka, M. Hassan, T. Mohamed, The antimicrobial effect of gold quantum dots and femtosecond laser irradiation on the growth kinetics of common infectious eye pathogens: an in vitro study, Nanomaterials 12 (2022) 3757, https://doi.org/10.3390/nano12213757.
[7] D. Zietal, D.T. Mlynarczyk, E. Kolasinski, E. Güzel, J. Dlugaszewska, Ł. Popenda, S. Jurga, T. Goslinski, L. Sobotta, Zinc(II), palladium(II), and metal-free Phthalocyanines bearing Nipagin-functionalized substituents against Candida auris and selected multidrug-resistant microbes, Pharmaceutics 14 (2022) 1686, https://doi.org/10.3390/pharmaceutics14081686.
[8] D. Zietal, J. Zajac, K. Lewandowski, J. Dlugaszewska, M.J. Potrzebowski, L. Sobotta, Oxospirochlorins as new promising photosensitizers against priority pathogens, Dyes Pigments 201 (2022) 110240, https://doi.org/10.1016/j.dyepig.2022.110240.
[9] E. Ahmed, A.O. El-Gendy, M.R. Hamblin, T. Mohamed, The effect of femtosecond laser irradiation on the growth kinetics of Staphylococcus aureus: an in vitro study, J. Photochem. Photobiol. B Biol. 221 (2021) 112240, https://doi.org/10.1016/j.jphotobiol.2021.112240.
[10] S. Karanika, T. Kinamon, C. Grigoras, E. Mylonakis, Colonization with methicillin-resistant Staphylococcus aureus and risk for infection among asymptomatic athletes: a systematic review and Metaanalysis, Clin. Infect. Dis. 63 (2016) 195–204, https://doi.org/10.1093/cid/ciw240.
[11] E. Drenkard, F.M. Ausubel, Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation, Nature 416 (2002) 740–743, https://doi.org/10.1038/416740a.
[12] C. Jonkergouw, N.K. Beyeh, E. Osmekhina, K. Leskinen, S.M. Taimoory, D. Fedorov, E. Anaya-Plaza, M.A. Kostiainen, J.F. Trant, R.H.A. Ras, P. Saavalainen, M.B. Linder, Repurposing host-guest chemistry to sequester virulence and eradicate biofilms in multidrug resistant Pseudomonas aeruginosa and Acinetobacter baumannii, Nat. Commun. 14 (2023) 2141, https://doi.org/10.1038/s41467-023-37749-6.
[13] E. Ahmed, A.O. El-Gendy, N.A. Moniem Radi, T. Mohamed, The bactericidal efficacy of femtosecond laser-based therapy on the most common infectious bacterial pathogens in chronic wounds: an in vitro study, Lasers Med. Sci. 36 (2021) 641–647, https://doi.org/10.1007/s10103-020-03104-0.
[14] L. Sobotta, D. Zietal, J. Sniechowska, J. Dlugaszewska, M.J. Potrzebowski, Lipid vesicle-loaded meso -substituted chlorins of high in vitro antimicrobial photodynamic activity, Photochem. Photobiol. Sci. 18 (2019) 213–223, https://doi.org/10.1039/C8PP00258D.
[15] A.O. El-Gendy, S. Ezzat, F.A. Samad, O.A. Dabbous, J. Dahm, M.R. Hamblin, T. Mohamed, Studying the viability and growth kinetics of vancomycin-resistant enterococcus faecalis V583 following femtosecond laser irradiation (420–465 nm), Lasers Med. Sci. 39 (2024) 144, https://doi.org/10.1007/s10103-024-04080-5.
[16] L. Sobotta, J. Dlugaszewska, P. Kasprzycki, S. Lijewski, A. Teubert, J. Mielcarek, M. Gdaniec, T. Goslinski, P. Fita, E. Tykarska, In vitro photodynamic activity of lipid vesicles with zinc phthalocyanine derivative against enterococcus faecalis, J. Photochem. Photobiol. B Biol. 183 (2018) 111–118, https://doi.org/10.1016/j.jphotobiol.2018.04.025.
[17] M. Wysocki, B. Czarczynska-Goslinska, D. Zietal, M. Michalak, E. Güzel, L. Sobotta, Excited state and reactive oxygen species against Cancer and pathogens: a review on Sonodynamic and Sono-photodynamic therapy, ChemMedChem 17 (2022), https://doi.org/10.1002/cmdc.202200185.
[18] M. Michalak, S. Mazurkiewicz, J. Szymczyk, D. Zietal, Ł. Sobotta, Photodynamic therapy applications – review, journal of, Med. Sci. 92 (2023) e865, https://doi.org/10.20883/medical.e865.
[19] D. Langerreiter, K. Solin, M. Jordà-Redondo, R. Bresolí-Obach, L. Fliri, S. Nonell, M.A. Kostiainen, E. Anaya-Plaza, Antimicrobial efficacy of solar disinfection in cellulose fiber supported photoactive materials, Mater. Today Commun. 38 (2024) 107858, https://doi.org/10.1016/j.mtcomm.2023.107858.
[20] L. Sobotta, P. Skupin-Mrugalska, J. Piskorz, J. Mielcarek, Porphyrinoid photosensitizers mediated photodynamic inactivation against bacteria, Eur. J. Med. Chem. 175 (2019) 72–106, https://doi.org/10.1016/j.ejmech.2019.04.057.
[21] L. Sobotta, P. Skupin-Mrugalska, J. Piskorz, J. Mielcarek, Non-porphyrinoid photosensitizers mediated photodynamic inactivation against bacteria, Dyes Pigments 163 (2019) 337–355, https://doi.org/10.1016/j.dyepig.2018.12.014.
[22] A.A. Glowacka-Sobotta, B.D. Zietal, C.L. Sobotta, Chapter 12 Porphyrinoids Used for Photodynamic Inactivation against Bacteria, in: Applications of Porphyrinoids

as Functional Materials, The Royal Society of Chemistry, 2021, pp. 352–404, https://doi.org/10.1039/9781839164149-00352.
[23] D. Zietal, D.T. Mlynarczyk, B. Czarczynska-Goslinska, K. Lewandowski, L. Sobotta, Photosensitizers mediated photodynamic inactivation against Fungi, Nanomaterials 11 (2021) 2883, https://doi.org/10.3390/nano11112883.
[24] X. Li, B.-D. Zheng, X.-H. Peng, S.-Z. Li, J.-W. Ying, Y. Zhao, J.-D. Huang, J. Yoon, Phthalocyanines as medicinal photosensitizers: developments in the last five years, Coord. Chem. Rev. 379 (2019) 147–160, https://doi.org/10.1016/j.ccr.2017.08.003.
[25] P.-C. Lo, M. Salomé Rodríguez-Morgade, R.K. Pandey, D.K.P. Ng, T. Torres, F. Dumoulin, The unique features and promises of phthalocyanines as advanced photosensitisers for photodynamic therapy of cancer, Chem. Soc. Rev. 49 (2020) 1041–1056, https://doi.org/10.1039/C9CS00129H.
[26] S. Singh, A. Aggarwal, N.V.S.D.K. Bhupathiraju, G. Arianna, K. Tiwari, C.M. Drain, Glycosylated porphyrins, Phthalocyanines, and other Porphyrinoids for diagnostics and therapeutics, Chem. Rev. 115 (2015) 10261–10306, https://doi.org/10.1021/acs.chemrev.5b00244.
[27] D. Langerreiter, M.A. Kostiainen, S. Kaabel, E. Anaya-Plaza, A greener route to blue: solid-state synthesis of Phthalocyanines, Angew. Chem. Int. Ed. 61 (2022) e202209033, https://doi.org/10.1002/anie.202209033.
[28] İ. Ömeroğlu, M. Göksel, V. Kussovski, V. Mantareva, M. Durmuş, Novel water-soluble silicon(IV) Phthalocyanine for photodynamic therapy and antimicrobial Inactivations, MHC 12 (2019) 255–263, https://doi.org/10.6060/mhc190763m.
[29] K. Harmandar, M.F. Saglam, I.F. Sengul, G. Ekineker, P. Balcik-Ercin, M. Göksel, D. Atilla, Novel triazole containing zinc(II)phthalocyanine Schiff bases: determination of photophysical and photochemical properties for photodynamic cancer therapy, Inorg. Chim. Acta 519 (2021) 120286, https://doi.org/10.1016/j.ica.2021.120286.
[30] K. Ishii, Functional singlet oxygen generators based on phthalocyanines, Coord. Chem. Rev. 256 (2012) 1556–1568, https://doi.org/10.1016/j.ccr.2012.03.022.
[31] E. Anaya-Plaza, M.M. Oliva, A. Kunzmann, C. Romero-Nieto, R.D. Costa, A. De La Escosura, D.M. Guldi, T. Torres, Quaternized Pyridyloxy Phthalocyanines render aqueous Electron-donor carbon nanotubes as unprecedented supramolecular materials for energy conversion, Adv. Funct. Mater. 25 (2015) 7418–7427, https://doi.org/10.1002/adfm.201503002.
[32] E. Anaya-Plaza, J. Joseph, S. Bauroth, M. Wagner, C. Dolle, M. Sekita, F. Gröhn, E. Spiecker, T. Clark, A. De La Escosura, D.M. Guldi, T. Torres, Synergy of electrostatic and π–π interactions in the realization of nanoscale artificial photosynthetic model systems, Angew. Chem. Int. Ed. 59 (2020) 18786–18794, https://doi.org/10.1002/anie.202006014.
[33] A. Shaukat, E. Anaya-Plaza, S. Julin, V. Linko, T. Torres, A. De La Escosura, M. A. Kostiainen, Phthalocyanine–DNA origami complexes with enhanced stability and optical properties, Chem. Commun. 56 (2020) 7341–7344, https://doi.org/10.1039/D0CC01916J.
[34] L. Sobotta, P. Fita, W. Szczolko, M. Wrotynski, M. Wierzchowski, T. Goslinski, J. Mielcarek, Functional singlet oxygen generators based on porphyrazines with peripheral 2,5-dimethylpyrrol-1-yl and dimethylamino groups, J. Photochem. Photobiol. A Chem. 269 (2013) 9–16, https://doi.org/10.1016/j.jphotochem.2013.06.018.
[35] L. Sobotta, J. Dlugaszewska, M. Gierszewski, A. Tillo, M. Sikorski, E. Tykarska, J. Mielcarek, T. Goslinski, Photodynamic inactivation of enterococcus faecalis by non-peripherally substituted magnesium phthalocyanines entrapped in lipid vesicles, J. Photochem. Photobiol. B Biol. 188 (2018) 100–106, https://doi.org/10.1016/j.jphotobiol.2018.09.003.
[36] I. Seotsanyana-Mokhosi, N. Kuznetsova, T. Nyokong, Photochemical studies of tetra-2, 3-pyridinoporphyrazines, J. Photochem. Photobiol. A Chem. 140 (2001) 215–222, https://doi.org/10.1016/S1010-6030(01)00427-0.
[37] A. Ogunsipe, D. Maree, T. Nyokong, Solvent effects on the photochemical and fluorescence properties of zinc phthalocyanine derivatives, J. Mol. Struct. 650 (2003) 131–140, https://doi.org/10.1016/S0022-2860(03)00155-8.
[38] M. Stolarska, A. Glowacka-Sobotta, D.T. Mlynarczyk, J. Dlugaszewska, T. Goslinski, J. Mielcarek, L. Sobotta, Photodynamic activity of Tribenzoporphyrazines with bulky periphery against wound Bacteria, IJMS 21 (2020) 6145, https://doi.org/10.3390/ijms21176145.
[39] M.S. Kang, K.-J. Hwang, Synthesis and band gap analysis of Meso-Arylporphyrins containing exclusively Electron donating or withdrawing groups, J. Korean Chem. Soc. 67 (2023) 175–180, https://doi.org/10.5012/JKCS.2023.67.3.175.
[40] J. Dlugaszewska, W. Szczolko, T. Koczorowski, P. Skupin-Mrugalska, A. Teubert, K. Konopka, M. Kucinska, M. Murias, N. Düzgüneş, J. Mielcarek, T. Goslinski, Antimicrobial and anticancer photodynamic activity of a phthalocyanine photosensitizer with *N*-methyl morpholiniumethoxy substituents in non-peripheral positions, J. Inorg. Biochem. 172 (2017) 67–79, https://doi.org/10.1016/j.jinorgbio.2017.04.009.
[41] A.B. Domínguez, D. Zietal, J. Dlugaszewska, L. Sobotta, T. Torres, M.S. Rodríguez-Morgade, Multicationic ruthenium phthalocyanines as photosensitizers for photodynamic inactivation of multiresistant microbes, Eur. J. Med. Chem. 285 (2025) 117214, https://doi.org/10.1016/j.ejmech.2024.117214.
[42] A. Przybyła, K. Lamperska, A. Mackiewicz, Analysis of sequence variants in the 3'UTR of CDKN2A gene in melanoma patients, Wo 4 (2015) 275–279, https://doi.org/10.5114/wo.2015.54227.
[43] M.C. DeRosa, R.J. Crutchley, Photosensitized singlet oxygen and its applications, Coord. Chem. Rev. 233 (2002) 351–371.
[44] M. Wierzchowski, D. Zietal, D. Łażewski, A. Korzanski, A. Gielara-Korzanska, E. Tykarska, J. Dlugaszewska, L. Sobotta, New Metallophthalocyanines bearing 2-Methylimidazole moieties—potential photosensitizers against Staphylococcus aureus, IJMS 23 (2022) 5910, https://doi.org/10.3390/ijms23115910.
[45] J. Szymczak, T. Rebis, M. Kotkowiak, B. Wicher, L. Sobotta, E. Tykarska, J. Mielcarek, M. Kryjewski, Regioisomers of magnesium(II) phthalocyanine bearing menthol substituents - synthesis, spectral, electrochemical and computational studies, Dyes Pigments 191 (2021) 109357, https://doi.org/10.1016/j.dyepig.2021.109357.
[46] J. Szymczak, L. Sobotta, J. Dlugaszewska, M. Kryjewski, J. Mielcarek, Menthol modified zinc(II) phthalocyanine regioisomers and their photoinduced antimicrobial activity against Staphylococcus aureus, Dyes Pigments 193 (2021) 109410, https://doi.org/10.1016/j.dyepig.2021.109410.
[47] V. Çakır, D. Çakır, M. Pişkin, M. Durmuş, Z. Bıyıklıoğlu, Water soluble peripheral and non-peripheral tetrasubstituted zinc phthalocyanines: synthesis, photochemistry and bovine serum albumin binding behavior, JOL 154 (2014) 274–284, https://doi.org/10.1016/j.jlumin.2014.04.030.
[48] H. Yanık, S.Y. Al-Raqa, A. Aljuhani, M. Durmuş, The synthesis of novel directly conjugated zinc(II) phthalocyanine via palladium-catalyzed Suzuki–Miyaura cross-coupling reaction and its quaternized water-soluble derivative: Investigation of photophysical and photochemical properties, Dyes Pigments 134 (2016) 531–540, https://doi.org/10.1016/j.dyepig.2016.08.009.
[49] P. Skupin-Mrugalska, T. Koczorowski, W. Szczolko, J. Dlugaszewska, A. Teubert, H. Piotrowska-Kempisty, T. Goslinski, L. Sobotta, Cationic porphyrazines with morpholinoethyl substituents – syntheses, optical properties, and photocytotoxicities, Dyes Pigments 197 (2022) 109937, https://doi.org/10.1016/j.dyepig.2021.109937.
[50] B. Jamoussi, R. Chakroun, A. Timoumi, K. Essalah, Synthesis and characterization of new imidazole Phthalocyanine for Photodegradation of Micro-organic pollutants from sea water, Catalysts 10 (2020) 906, https://doi.org/10.3390/catal10080906.
[51] D. Zietal, D.T. Mlynarczyk, E. Kolasinski, E. Güzel, J. Dlugaszewska, Ł. Popenda, S. Jurga, T. Goslinski, L. Sobotta, Zinc(II), Palladium(II), and Metal-Free Phthalocyanines Bearing Nipagin-Functionalized Substituents against Candida auris and Selected Multidrug-Resistant Microbes 19, 2022.
[52] B. Czarczynska-Goslinska, M. Stolarska, D. Zietal, M. Falkowski, A. Glowacka-Sobotta, J. Dlugaszewska, T. Goslinski, L. Sobotta, Photodynamic antimicrobial activity of magnesium(II) porphyrazine with bulky peripheral sulfanyl substituents, Phosphorus Sulfur Silicon Relat. Elem. 197 (2022) 705–710, https://doi.org/10.1080/10426507.2021.2012780.
[53] M. Wysocki, D. Zietal, M. Jozkowiak, J. Dlugaszewska, H. Piotrowska-Kempisty, E. Güzel, L. Sobotta, Porphyrazine/phthalocyanine hybrid complexes – antibacterial and anticancer photodynamic and sonodynamic activity, Synth. Met. 299 (2023) 117474, https://doi.org/10.1016/j.synthmet.2023.117474.
[54] A. Glowacka-Sobotta, D. Zietal, B. Czarczynska-Goslinska, M. Michalak, M. Wysocki, E. Güzel, L. Sobotta, Nanotechnology for dentistry: prospects and applications, Nanomaterials 13 (2023) 2130, https://doi.org/10.3390/nano13142130.
[55] A. Glowacka-Sobotta, M. Wrotynski, M. Kryjewski, L. Sobotta, J. Mielcarek, Porphyrinoids in photodynamic diagnosis and therapy of oral diseases, J. Porphyrins Phthalocyanines 23 (2019) 1–10, https://doi.org/10.1142/S108842461850116X.
[56] M.R. Hamblin, Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes, Curr. Opin. Microbiol. 33 (2016) 67–73, https://doi.org/10.1016/j.mib.2016.06.008.
[57] M.R. Hamblin, H. Abrahamse, Oxygen-independent antimicrobial Photoinactivation: type III photochemical mechanism? Antibiotics (Basel) 9 (2020) 53, https://doi.org/10.3390/antibiotics9020053.
[58] Anti-Infective Drugs Advisory Committee, Notice of Meeting, Fed. Regist. (2013). https://www.federalregister.gov/documents/2013/08/07/2013-19036/anti-infective-drugs-advisory-committee-notice-of-meeting (accessed May 4, 2024).
[59] M. Grinholc, B. Szramka, J. Kurlenda, A. Graczyk, K.P. Bielawski, Bactericidal effect of photodynamic inactivation against methicillin-resistant and methicillin-susceptible Staphylococcus aureus is strain-dependent, J. Photochem. Photobiol. B Biol. 90 (2008) 57–63, https://doi.org/10.1016/j.jphotobiol.2007.11.002.
[60] D. Nichol, J. Rutter, C. Bryant, A.M. Hujer, S. Lek, M.D. Adams, P. Jeavons, A.R. A. Anderson, R.A. Bonomo, J.G. Scott, Antibiotic collateral sensitivity is contingent on the repeatability of evolution, Nat. Commun. 10 (2019) 334, https://doi.org/10.1038/s41467-018-08098-6.
[61] V. Lázár, A. Martins, R. Spohn, L. Daruka, G. Grézal, G. Fekete, M. Számel, P. K. Jangir, B. Kintses, B. Csörgő, Á. Nyerges, Á. Györkei, A. Kincses, A. Dér, F. R. Walter, M.A. Deli, E. Urbán, Z. Hegedűs, G. Olajos, O. Méhi, B. Bálint, I. Nagy, T. A. Martinek, B. Papp, C. Pál, Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides, Nat. Microbiol. 3 (2018) 718–731, https://doi.org/10.1038/s41564-018-0164-0.
[62] C. Pál, B. Papp, V. Lázár, Collateral sensitivity of antibiotic-resistant microbes, Trends Microbiol. 23 (2015) 401–407, https://doi.org/10.1016/j.tim.2015.02.009.
[63] L. Huang, Y. Xuan, Y. Koide, T. Zhiyentayev, M. Tanaka, M.R. Hamblin, Type I and type II mechanisms of antimicrobial photodynamic therapy: an in vitro study on gram-negative and gram-positive bacteria, Lasers Surg. Med. 44 (2012) 490–499, https://doi.org/10.1002/lsm.22045.
[64] F.F. Sperandio, Y.-Y. Huang, M.R. Hamblin, Antimicrobial photodynamic therapy to kill gram-negative Bacteria, Recent Pat. Antiinfect. Drug Discov. 8 (2013) 108–120.
[65] B.-D. Zheng, S.-L. Li, Z.-L. Huang, L. Zhang, H. Liu, B.-Y. Zheng, M.-R. Ke, J.-D. Huang, A non-aggregated zinc(II) phthalocyanine with hexadeca cations for antitumor and antibacterial photodynamic therapies, J. Photochem. Photobiol. B Biol. 213 (2020) 112086, https://doi.org/10.1016/j.jphotobiol.2020.112086.

[66] N.E. Holmes, P.G.P. Charles, Safety and efficacy review of doxycycline, clinical medicine, Therapeutics 1 (2009) CMT.S2035, https://doi.org/10.4137/CMT.S2035.
[67] M. Henehan, M. Montuno, A. De Benedetto, Doxycycline as an anti-inflammatory agent: updates in dermatology, J. Eur. Acad. Dermatol. Venereol. 31 (2017) 1800–1808, https://doi.org/10.1111/jdv.14345.
[68] S. Valentín, A. Morales, J.L. Sánchez, A. Rivera, Safety and efficacy of doxycycline in the treatment of rosacea, Clin. Cosmet. Investig. Dermatol. 2 (2009) 129–140, https://doi.org/10.2147/ccid.s4296.
[69] Y. Feng, C. Coradi Tonon, S. Ashraf, T. Hasan, Photodynamic and antibiotic therapy in combination against bacterial infections: efficacy, determinants, mechanisms, and future perspectives, Adv. Drug Deliv. Rev. 177 (2021) 113941, https://doi.org/10.1016/j.addr.2021.113941.
[70] I. Baccani, P. Faraoni, M. Marini, A. Gnerucci, B. Orsini, P. Pecile, G. Romano, F. Fusi, G.M. Rossolini, A. Antonelli, Synergistic effect of photodynamic therapy at 400 nm and doxycycline against *helicobacter pylori*, Future Microbiol. 14 (2019) 1199–1205, https://doi.org/10.2217/fmb-2019-0129.
[71] X. Hu, H. Zhang, Y. Wang, B.-C. Shiu, J.-H. Lin, S. Zhang, C.-W. Lou, T.-T. Li, Synergistic antibacterial strategy based on photodynamic therapy: Progress and perspectives, Chem. Eng. J. 450 (2022) 138129, https://doi.org/10.1016/j.cej.2022.138129.
[72] S. Afrasiabi, A. Partoazar, N. Chiniforush, In vitro study of nanoliposomes containing curcumin and doxycycline for enhanced antimicrobial photodynamic therapy against Aggregatibacter actinomycetemcomitans, Sci. Rep. 13 (2023) 11552, https://doi.org/10.1038/s41598-023-38812-4.
[73] S.D. Astuti, P.S. Puspita, A.P. Putra, A.H. Zaidan, M.Z. Fahmi, A. Syahrom, Null Suhariningsih, The antifungal agent of silver nanoparticles activated by diode laser as light source to reduce C. Albicans biofilms: an in vitro study, Lasers Med. Sci. 34 (2019) 929–937, https://doi.org/10.1007/s10103-018-2677-4.
[74] B. Domingues, J.M. Lopes, P. Soares, H. Pópulo, Melanoma treatment in review, ImmunoTargets Ther. 7 (2018) 35–49, https://doi.org/10.2147/ITT.S134842.
[75] C. Lee, F. Collichio, D. Ollila, S. Moschos, Historical review of melanoma treatment and outcomes, Clin. Dermatol. 31 (2013) 141–147, https://doi.org/10.1016/j.clindermatol.2012.08.015.
[76] J.M. Dąbrowski, Chapter Nine, Reactive oxygen species in photodynamic therapy: Mechanisms of their generation and potentiation, in: R. van Eldik, C.D. Hubbard (Eds.), Advances in Inorganic Chemistry, Academic Press, 2017, pp. 343–394, https://doi.org/10.1016/bs.adioch.2017.03.002.
[77] G.M. Cramer, E.K. Moon, K.A. Cengel, T.M. Busch, Photodynamic therapy and immune checkpoint blockade†, Photochem. Photobiol. 96 (2020) 954–961, https://doi.org/10.1111/php.13300.
[78] V.A. Patel, A. Longacre, K. Hsiao, H. Fan, F. Meng, J.E. Mitchell, J. Rauch, D. S. Ucker, J.S. Levine, Apoptotic cells, at all stages of the death process, trigger characteristic signaling events that are divergent from and dominant over those triggered by necrotic cells: implications for the delayed clearance model of autoimmunity, J. Biol. Chem. 281 (2006) 4663–4670, https://doi.org/10.1074/jbc.M508342200.
[79] I. Baldea, L. Giurgiu, I.D. Teacoe, D.E. Olteanu, F.C. Olteanu, S. Clichici, G.A. Filip, Photodynamic therapy in melanoma - where do we stand? CMC 25 (2019) 5540–5563, https://doi.org/10.2174/0929867325666171226115626.
[80] N. Ahmad, H. Alqahtani, S. Al-Terary, A. Laref, A. Haseeb, Control of optical absorption and fluorescence spectroscopies of natural melanin at different solution concentrations, Opt. Quant. Electron. 51 (2019), https://doi.org/10.1007/s11082-019-1936-3.
[81] L. Pires, V. Demidov, B.C. Wilson, A.G. Salvio, L. Moriyama, V.S. Bagnato, I. A. Vitkin, C. Kurachi, Dual-agent photodynamic therapy with optical clearing eradicates pigmented melanoma in preclinical tumor models, Cancers 12 (2020) 1956, https://doi.org/10.3390/cancers12071956.
[82] D. Kessel, N.L. Oleinick, Cell death pathways associated with photodynamic therapy: an update, Photochem. Photobiol. 94 (2018) 213–218, https://doi.org/10.1111/php.12857.
[83] P. Mroz, A. Yaroslavsky, G.B. Kharkwal, M.R. Hamblin, Cell death pathways in photodynamic therapy of Cancer, Cancers 3 (2011) 2516–2539, https://doi.org/10.3390/cancers3022516.
[84] I. Mfouo-Tynga, H. Abrahamse, Cell death pathways and Phthalocyanine as an efficient agent for photodynamic Cancer therapy, Int. J. Mol. Sci. 16 (2015) 10228–10241, https://doi.org/10.3390/ijms160510228.
[85] H. Kolarova, P. Nevrelova, R. Bajgar, D. Jirova, K. Kejlova, M. Strnad, In vitro photodynamic therapy on melanoma cell lines with phthalocyanine, Toxicol. in Vitro 21 (2007) 249–253, https://doi.org/10.1016/j.tiv.2006.09.020.
[86] F. Valli, M.C. García Vior, L.P. Roguin, J. Marino, Oxidative stress generated by irradiation of a zinc(II) phthalocyanine induces a dual apoptotic and necrotic response in melanoma cells, Apoptosis 24 (2019) 119–134, https://doi.org/10.1007/s10495-018-01512-w.
[87] I.M. Ndhundhuma, H. Abrahamse, Susceptibility of *in vitro* melanoma skin Cancer to Photoactivated Hypericin versus Aluminium(III) Phthalocyanine chloride Tetrasulphonate, Biomed. Res. Int. 2017 (2017) e5407012, https://doi.org/10.1155/2017/5407012.
[88] J.M. Dąbrowski, M. Krzykawska, L.G. Arnaut, M.M. Pereira, C.J.P. Monteiro, S. Simões, K. Urbańska, G. Stochel, Tissue uptake study and photodynamic therapy of melanoma-bearing mice with a nontoxic, effective Chlorin, ChemMedChem 6 (2011) 1715–1726, https://doi.org/10.1002/cmdc.201100186.
[89] J.M. Dąbrowski, M.M. Pereira, L.G. Arnaut, C.J.P. Monteiro, A.F. Peixoto, A. Karocki, K. Urbańska, G. Stochel, Synthesis, Photophysical studies and anticancer activity of a new halogenated water-soluble porphyrin, Photochem. Photobiol. 83 (2007) 897–903, https://doi.org/10.1111/j.1751-1097.2007.00073.x.
[90] S. Ghosh, K.A. Carter, J.F. Lovell, Liposomal formulations of photosensitizers, Biomaterials 218 (2019) 119341, https://doi.org/10.1016/j.biomaterials.2019.119341.
[91] D. Ziental, B. Czarczynska-Goslinska, M. Wysocki, M. Ptaszek, Ł. Sobotta, Advances and perspectives in use of semisolid formulations for photodynamic methods, Eur. J. Pharm. Biopharm. 204 (2024) 114485, https://doi.org/10.1016/j.ejpb.2024.114485.
[92] D. Ziental, P. Błaszkiewicz, J. Długaszewska, E. Güzel, A. Dudkowiak, L. Sobotta, Modified gold nanoparticles modulated fluorescence and singlet oxygen generation of Pheophorbide a as an effective platform for photodynamic therapy against Staphylococcus aureus, Eur. J. Inorg. Chem. 27 (2024) e202300668, https://doi.org/10.1002/ejic.202300668.
[93] A.M. Ayoub, B. Gutberlet, E. Preis, A.M. Abdelsalam, A. Abu Dayyih, A. Abdelkader, A. Balash, J. Schäfer, U. Bakowsky, Parietin Cyclodextrin-inclusion complex as an effective formulation for bacterial Photoinactivation, Pharmaceutics 14 (2022) 357, https://doi.org/10.3390/pharmaceutics14020357.
[94] M. Wainwright, Photodynamic therapy: the development of new Photosensitisers, Anti Cancer Agents Med. Chem. 8 (2008) 280–291, https://doi.org/10.2174/187152008783961888.
[95] M. Wainwright, A. McLean, Rational design of phenothiazinium derivatives and photoantimicrobial drug discovery, Dyes Pigments 136 (2017) 590–600, https://doi.org/10.1016/j.dyepig.2016.09.015.
[96] F. Borgia, R. Giuffrida, E. Caradonna, M. Vaccaro, F. Guarneri, S.P. Cannavò, Early and late onset side effects of photodynamic therapy, Biomedicines 6 (2018) 12, https://doi.org/10.3390/biomedicines6010012.
[97] V. Pérez-Laguna, Y. Gilaberte, M.I. Millán-Lou, M. Agut, S. Nonell, A. Rezusta, M. R. Hamblin, A combination of photodynamic therapy and antimicrobial compounds to treat skin and mucosal infections: a systematic review, Photochem. Photobiol. Sci. 18 (2019) 1020–1029, https://doi.org/10.1039/c8pp00534f.