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Novel BODIPYs with dimethylamine substituents and their cationic and iodinated derivatives for anticancer and antibacterial photodynamic therapy

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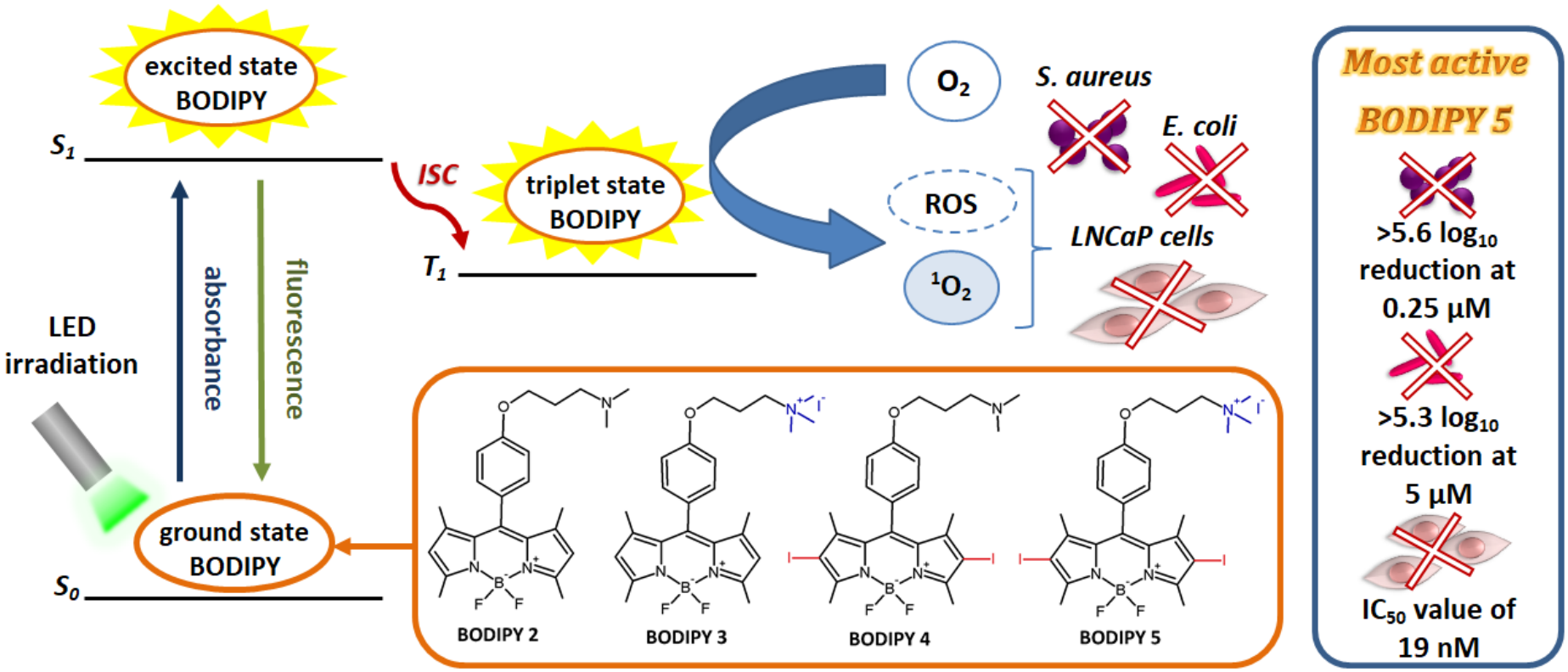
Graphical Abstract



Abstract:

Photodynamic therapy (PDT) is a minimally invasive modality, clinically approved for treating several localized cancer and non-cancer lesions and also perspective modality to treat microbial induced pathologies. It utilizes a photosensitizer activated with a light of a specific wavelength to produce reactive oxygen species responsible for the cytotoxic effect.

Boron-dipyrromethene (BODIPY) derivatives are organic chromophores possessing diverse applications, including fluorescent imaging probes, labeling reagents, chemosensors, and laser dyes. In addition, BODIPYs gain increasing attention as photosensitizers due to their distinctive photophysical properties attractive for PDT.

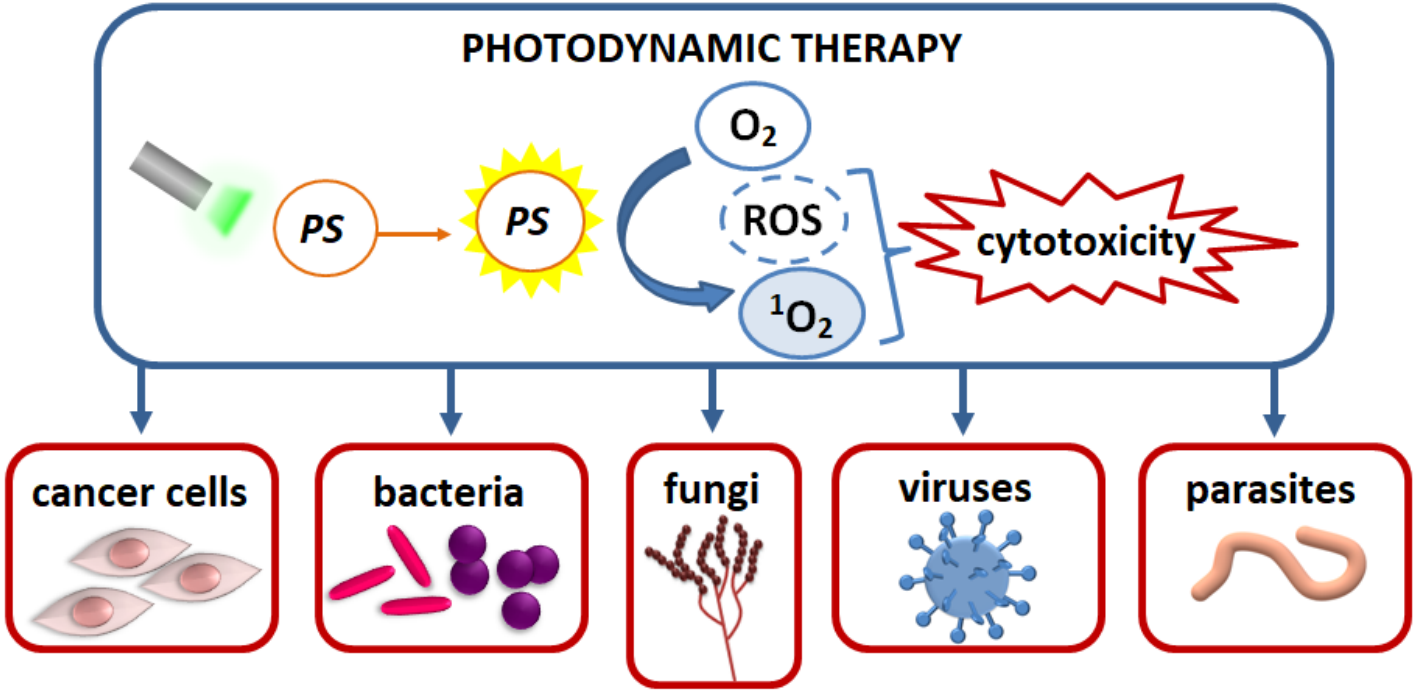
Novel BODIPYs with dimethylaminopropoxyphenyl substituents and their cationic and iodinated derivatives were synthesized and characterized using mass spectrometry, UV-Vis spectrophotometry, and various NMR techniques. Subsequent photochemical studies allowed evaluating their absorption and emission properties and the singlet oxygen generation ability. *In vitro* photodynamic activity studies were performed on human androgen-sensitive prostate adenocarcinoma cells (LNCaP) and two bacterial strains (Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*). Also, the impact of the presence of quaternary ammonium cation and iodine atoms, in the structure of BODIPYs, on physicochemical and photochemical properties as well as photodynamic activity was assessed. BODIPY derivative possessing both a positive charge and iodine atoms revealed the highest activity towards all studied cells.

Keywords:

BODIPY; cationic photosensitizers ; heavy atom effect; photodynamic antimicrobial therapy; photodynamic therapy

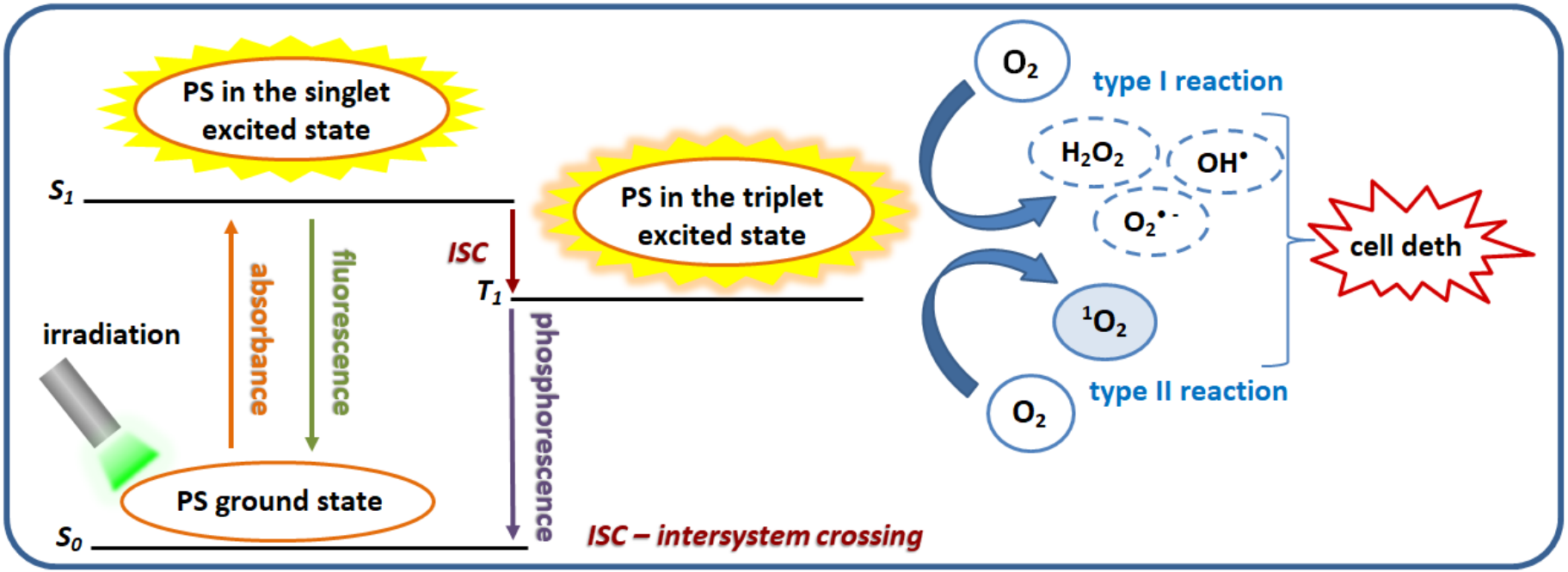
Introduction – Photodynamic therapy

Photodynamic therapy (PDT) is a medical treatment clinically approved to cure localized cancers such as esophageal cancer, lung adenocarcinoma, head and neck cancer, and also non-cancer diseases such as age-related macular degeneration, actinic keratosis, and some dermatological pathologies. PDT is also a perspective modality to treat microbial-induced pathologies, including those caused by antibiotic-resistant microorganisms [1,2].



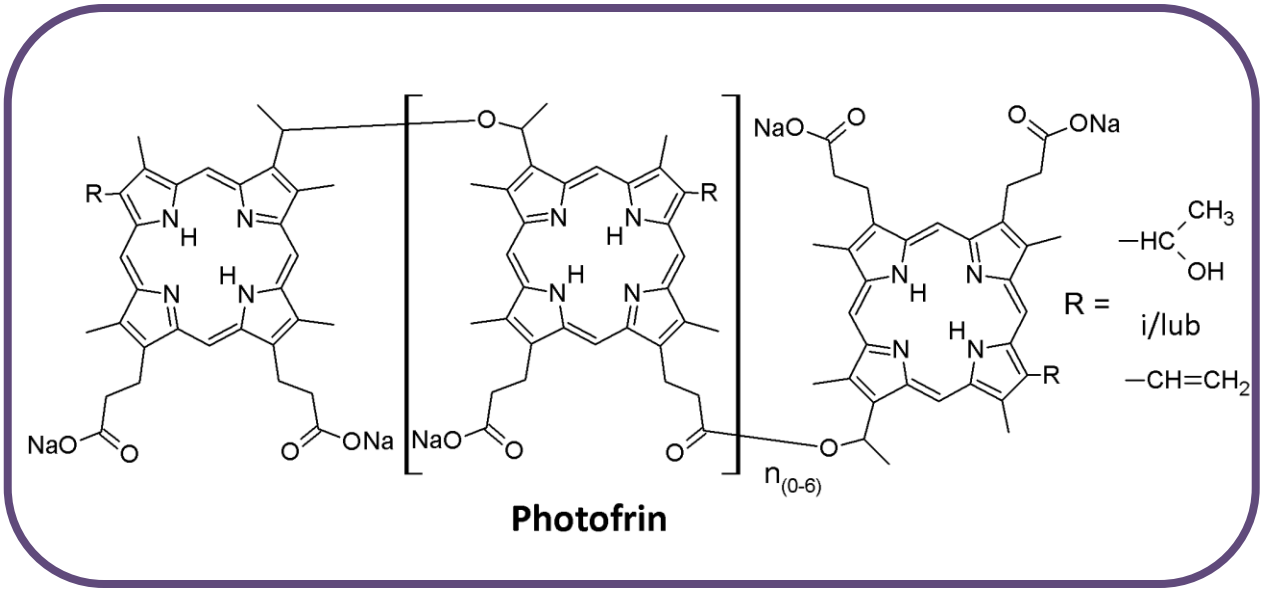
Introduction – Photodynamic therapy

PDT utilizes a light-sensitive drug called a photosensitizer (PS) and light of a wavelength corresponding to the maximum absorption peak of the selected PS. After irradiation, the PS molecule becomes excited to the singlet excited state, and then through intersystem crossing transforms into the triplet excited state. In this state, PS can react with molecular oxygen and other surrounding substrates to produce cytotoxic reactive oxygen species [1].

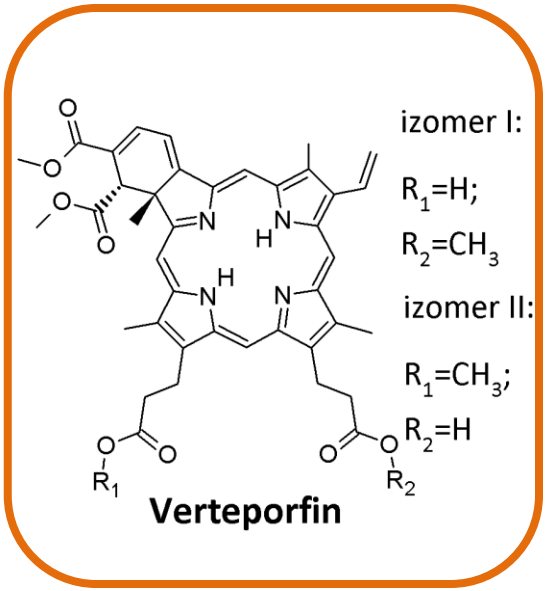


Introduction – Photosensitizers

The most commonly used compounds in photodynamic therapy belong to the group of porphyrinoids, which include e.g. clinically approved photosensitizers such as Photofrin, 5-aminolevulinic acid and verteporfin. However, currently used photosensitizers have significant disadvantages, such as poor water solubility, low absorption coefficients, or the need to use high doses, therefore there is a great interest in searching for new and more effective photosensitizers [3].



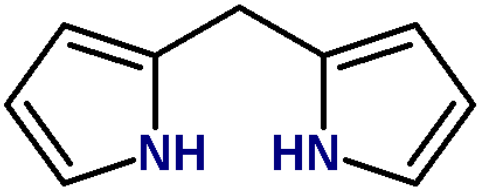
Photofrin



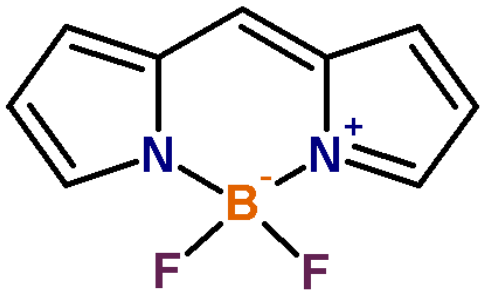
Verteporfin

Introduction – Boron-dipyrromethene derivatives (BODIPY)

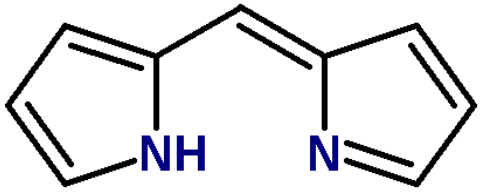
BODIPY dyes find a wide range of applications in various fields, such as DNA and protein labeling, diagnostic imaging, and photovoltaics. In recent years they have also been intensively researched for photodynamic therapy applications. These compounds are characterized by high molar absorption coefficients, good photostability, relatively simple synthesis, and the wide possibilities of functionalization. These features allow obtaining derivatives with the desired physicochemical properties and promising potential for application in photodynamic therapy. The basic structures of BODIPYs possess a few limitations, including high fluorescence quantum yields, low singlet oxygen generation, short maximum absorbance wavelengths, and high lipophilicity. However, the proper modifications can make them suitable as PSs [3,4].



dipyrromethane core

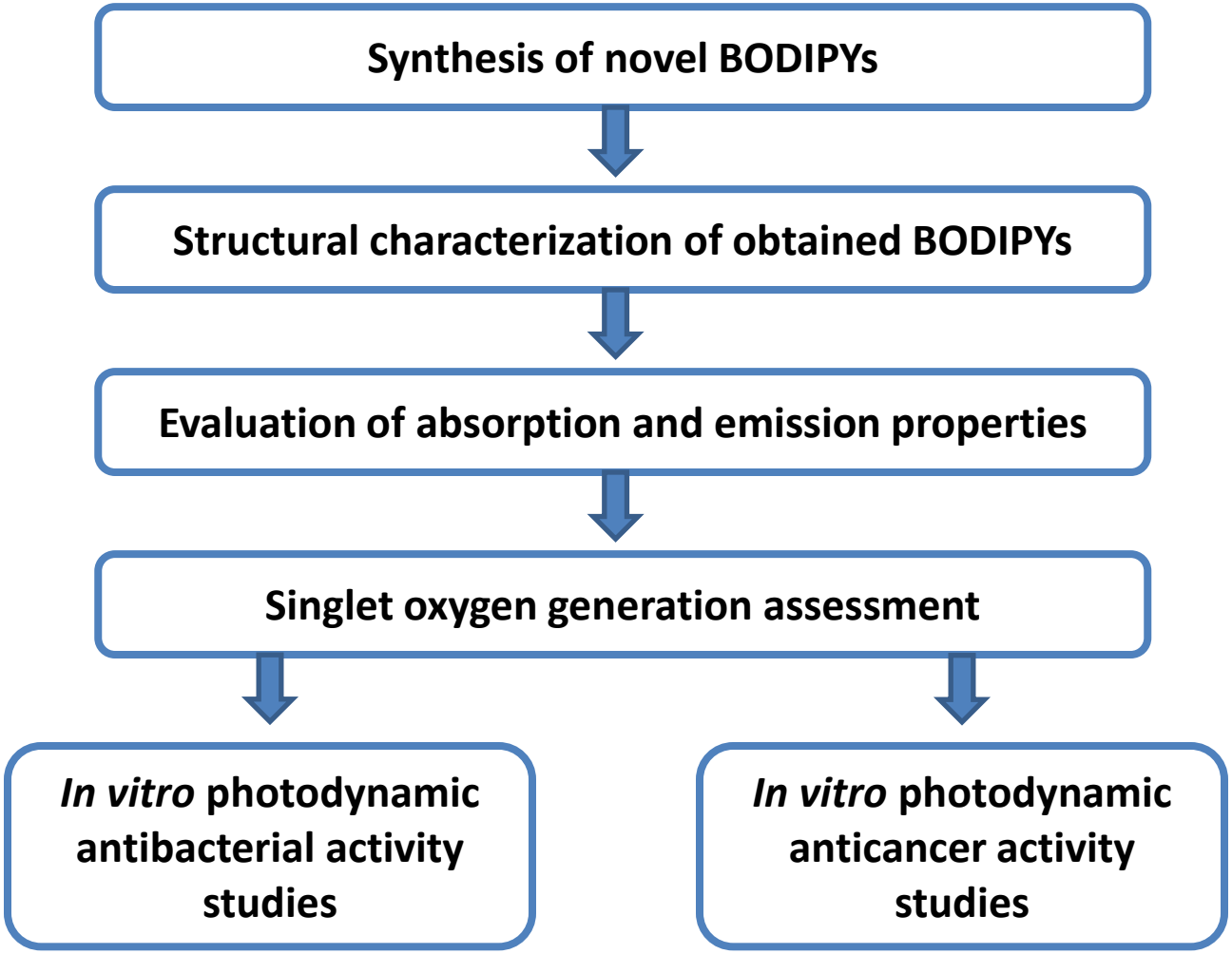


boron-dipyrromethene core (BODIPY)



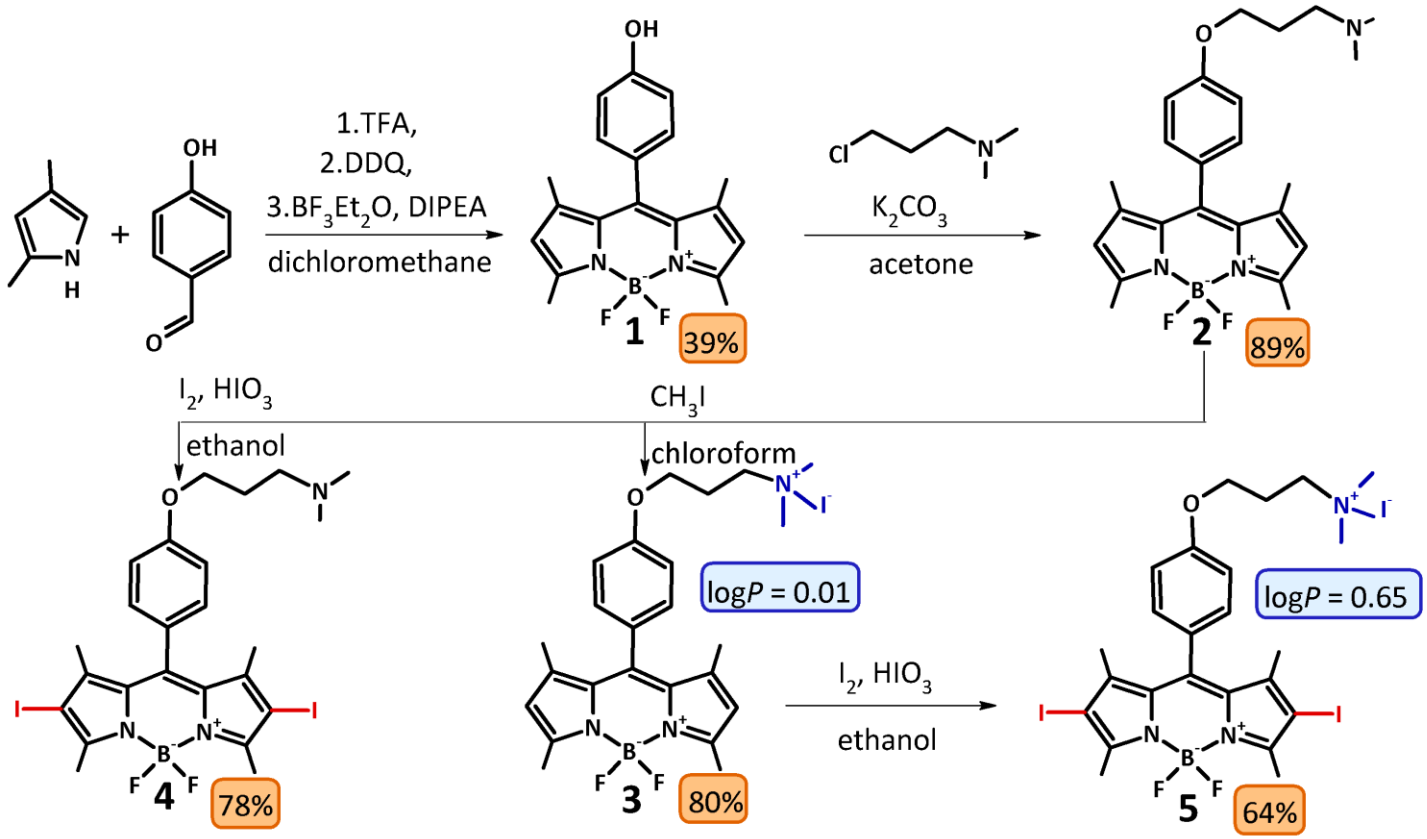
dipyrrin core

Results and discussion – general overview



Results and discussion – synthesis

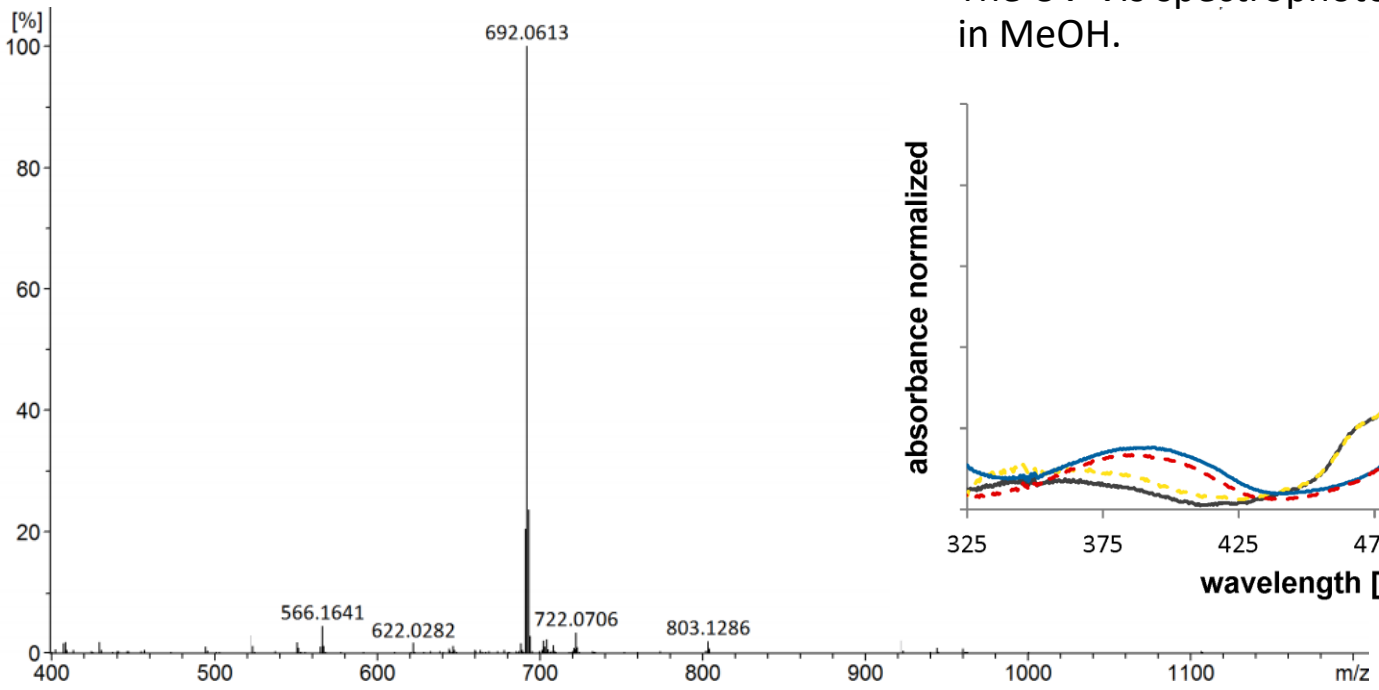
BODIPY **1** was synthesized using a three-step literature procedure [5], then the known derivative **2** was obtained by reaction with dimethylaminopropyl chloride in the presence of potassium carbonate [6]. BODIPY **2** was iodinated to obtain a new derivative **4** or quaternized to give amphiphilic BODIPY **3**. This derivative was then subjected to iodination, which increased the obtained BODIPY's **5** lipophilicity [7,8].



Results and discussion – structural characterization

The obtained compounds were characterized by mass spectrometry, UV-Vis spectrophotometry, and various NMR techniques. The two-dimensional spectra (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) were especially useful and allowed assigning signals to individual hydrogen and carbon atoms.

The mass spectrometry (ESI) of BODIPY **5**.

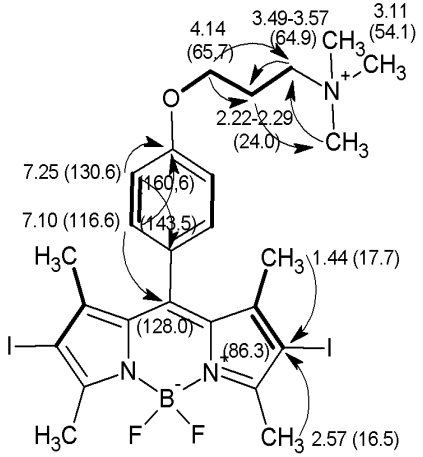


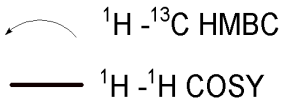
The UV-Vis spectrophotometry of BODIPYs **2-5** in MeOH.

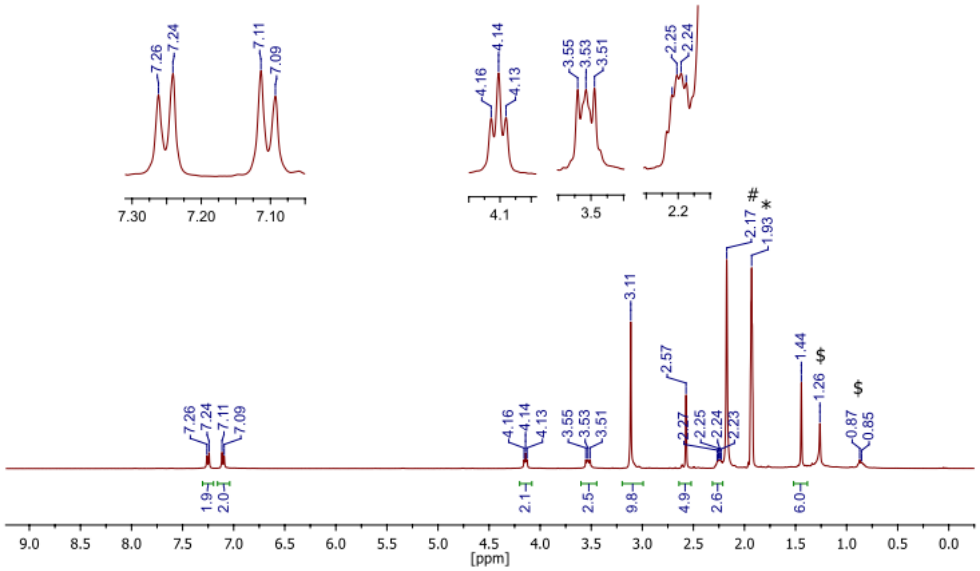
Results and discussion – structural characterization

NMR spectroscopy:

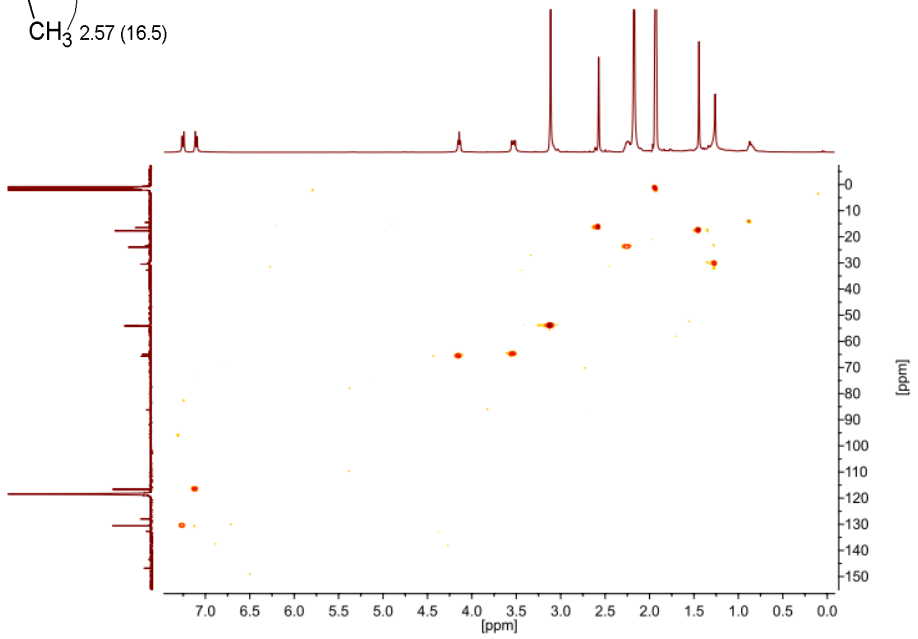
^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY,
^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC







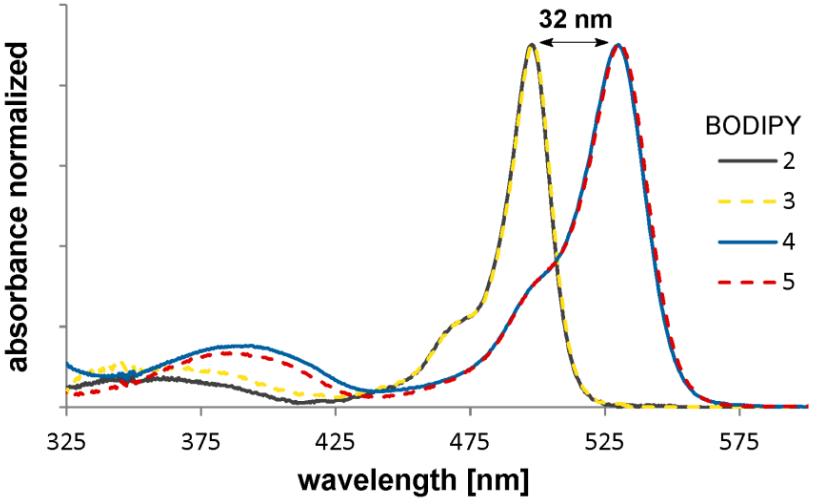
The ^{1}H NMR spectrum of **5** in CD_3CN.



The ^{1}H-^{13}C HSQC spectrum of **5** in CD_3CN.

Results and discussion – absorption properties

Obtained derivatives **2**-**5** were subjected to physicochemical studies, including the determination of the absorption and emission properties. It was observed that the derivatives **2** and **3** without iodine atoms showed the maxima of the absorption wavelength about 500 nm, while the introduction of iodine atoms into positions 2 and 6 of the BODIPYs **4** and **5** core caused a bathochromic shift of the absorption maxima by about 30 nm. All derivatives showed high molar absorption coefficients in the range of 4.54-4.80, favorable for the use in PDT.



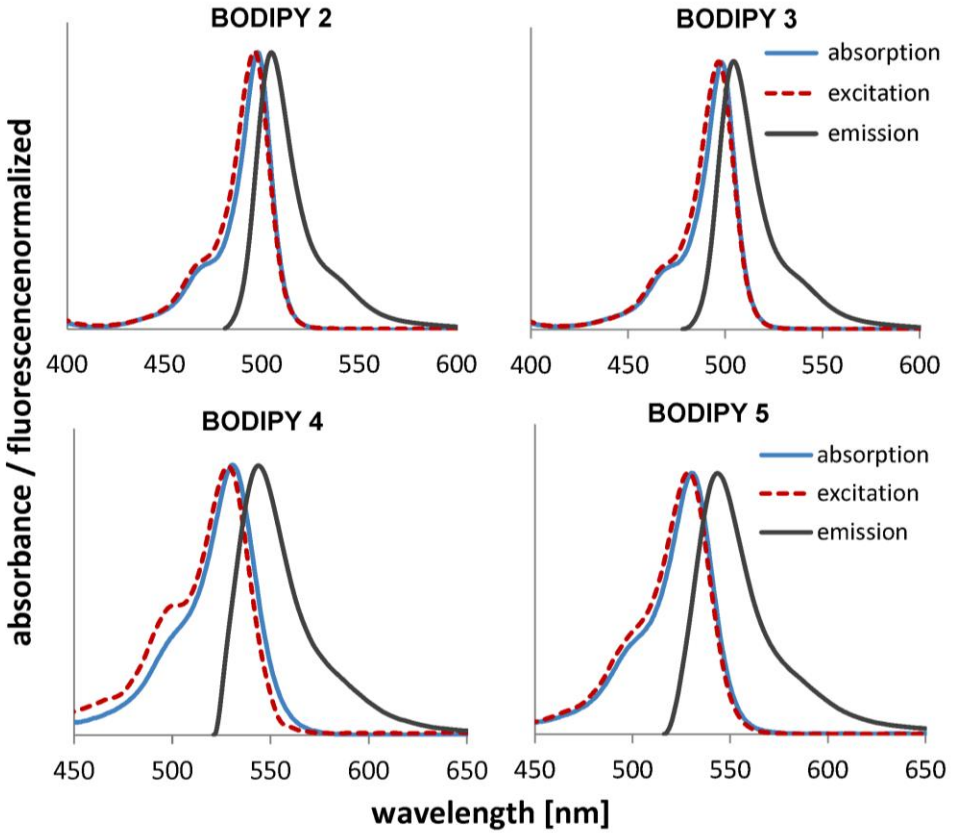
Normalized absorption spectra of BODIPYs **2-5** in methanol.

Maximum absorption (λ_{max}) and molar absorption coefficients (logε) for derivatives 2-5 in methanol				
	BODIPY			
	2	**3**	**4**	**5**
λ_{max}	498	498	530	530
(logε)	(4.80)	(4.54)	(4.57)	(4.75)

Results and discussion – emission properties

The non-iodinated derivatives **2** and **3** showed high fluorescence efficiency. The introduction of iodine atoms to the structure of derivatives **4** and **5** caused a 30-35-fold reduction in fluorescence efficiency [7]. This phenomenon, called the heavy atom effect, is caused by an increase in the intersystem crossing from the first singlet excited state to the triplet excited state and resulting reduction in fluorescence intensity.

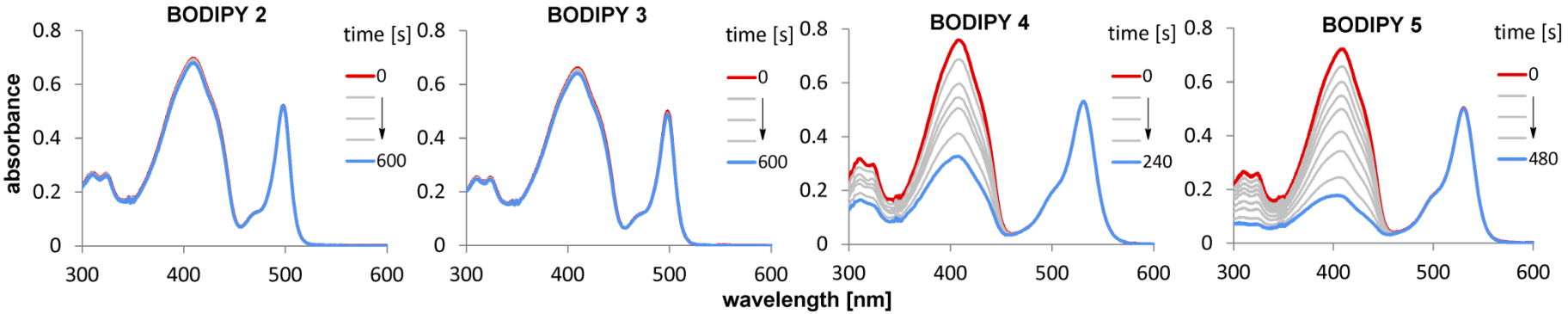
Fluorescence quantum yields (Φ_F) for BODIPYs 2-5				
solvent	**2**	**3**	**4**	**5**
methanol	**0.69**	**0.70**	0.02	0.02
ethanol	**0.61**	**0.60**	0.02	0.02



The long-wavelength absorption, excitation and emission bands for BODIPYs **2-5** in methanol.

Results and discussion – singlet oxygen generation

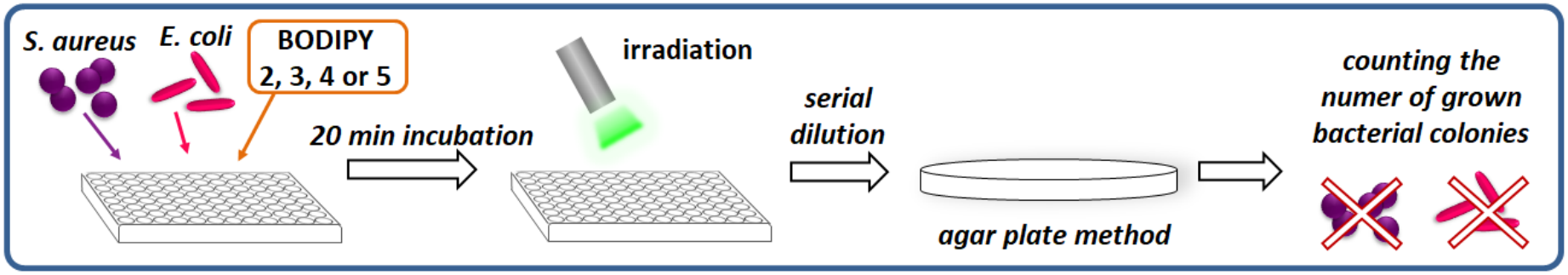
The non-iodinated BODIPY derivatives **2** and **3** showed low singlet oxygen generation capacity, in contrast to iodinated derivatives **4** and **5**, which revealed exceptionally high singlet oxygen generation quantum yields (0.69-0.97) [7]. It is caused by the aforementioned increase in the formation of the triplet state, in which the compound can react with molecular oxygen to form highly reactive singlet oxygen.



Changes in the UV-Vis spectra for DPBF and BODIPYs **2-5** in methanol.

Singlet oxygen generation quantum yields (Φ_Δ) for derivatives 2-5				
solvent	**2**	**3**	**4**	**5**
methanol	0.02	0.02	**0.90**	**0.94**
ethanol	0.02	0.02	**0.69**	**0.97**

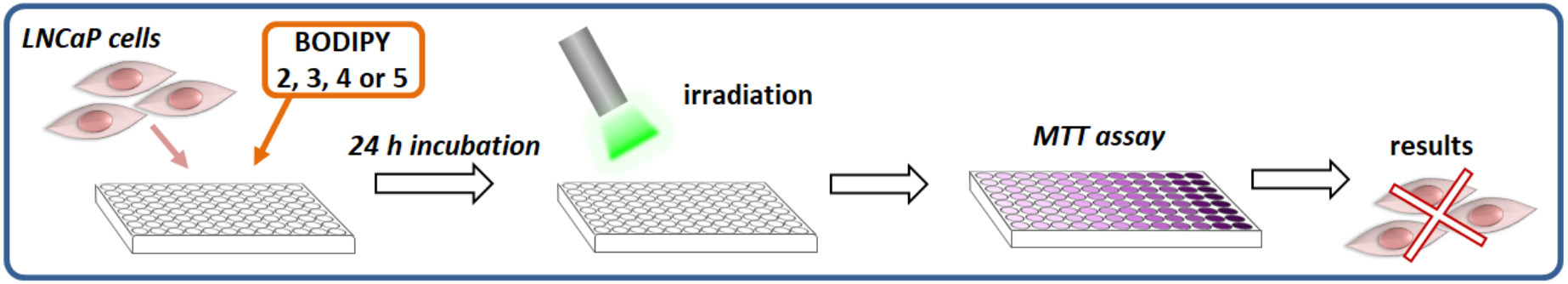
Results and discussion – *In vitro* photodynamic antibacterial activity



Log_{10} reductions of S. aureus and E. coli treated with BODIPYs 2-5				
BODIPY	**2**	**3**	**4**	**5**
S. aureus	**2.5 µM**		**0.25 µM**	
	4.1 log_{10}	>5.8 log_{10}	3.9 log_{10}	>5.6 log_{10}
E. coli	**500 µM**			**5 µM**
	1.7 log_{10}	>5.3 log_{10}	>5.4 log_{10}	>5.3 log_{10}

In vitro photodynamic activity studies showed high activity of all tested compounds against Gram-positive *Staphylococcus aureus*. Iodinated derivatives **4** and **5** showed high activity at a low concentration of 0.25 µM, while non-iodinated BODIPYs **2** and **3** required a 10-fold higher concentration. However, for Gram-negative *Escherichia coli*, high inactivation was obtained only for cationic, iodinated derivative **5** at a concentration of at least 5 µM [7].

Results and discussion – *In vitro* photodynamic anticancer activity



The IC_{50} values of BODIPYs 2-5 against prostate adenocarcinoma cell line (LNCaP)		
BODIPY	non-irradiated	irradiated
2	9.7±1.4	3.3±1.5
3	>10	>10
4	>2	**0.048±0.012**
5	>0.06	**0.019±0.004**
5 (hypoxia)	>10	>10

Both iodinated derivatives **4** and **5** showed high photodynamic anticancer activity with IC_{50} values of 48.3 nmol and 19.3 nmol, respectively. Due to the best photodynamic activity, derivative **5** was also assessed under hypoxic conditions with an oxygen content reduced to 1%. It was shown that lowering the oxygen concentration inhibited the activity of derivative **5**. These results indicate that oxygen is essential for the activity of this compound and the mechanism of action is most likely related to the generation of singlet oxygen, the formation of which depends on the presence of molecular oxygen [7].

Conclusions

- The introduction of iodine atoms to the amphiphilic derivative **3** resulted in an increase in lipophilicity, confirmed by a change in the log*P* value from 0.01 to 0.65.

- The non-iodinated BODIPYs **2** and **3** showed strong fluorescent properties, proving that they can be used in diagnostics, e.g. as fluorescent markers.

- The iodinated derivatives **4** and **5** are characterized by very high values of the quantum yields of singlet oxygen generation (Φ_{Δ}) in the range of 0.69-0.97.

- BODIPYs **4** and **5** showed 10-fold greater activity against gram-positive *S. aureus* than their iodine-free analogs **2** and **3**. The presence of iodine atoms in the structure of BODIPY showed a greater impact on photodynamic activity than the presence of a positive charge.

Conclusions

- In the case of *in vitro* photodynamic activity against Gram-negative *E. coli*, the influence of both the presence of iodine atoms and the positive charge in the structure of compounds on increasing their activity was observed.

- *E. coli* showed less susceptibility to photodynamic therapy than *S. aureus*. The most active BODIPY **5** eradicated these bacterial strains at 0.25 µM and 5 µM concentrations, respectively.

- The study of anticancer activity against prostate cancer cells showed a greater effect of the presence of iodine atoms in the BODIPY structure on their activity, than the presence of a positively charged substituent.

- Both iodinated derivatives **4** and **5** showed very high photodynamic anticancer activity (IC_{50} = 48.3 nmol and 19.3 nmol). BODIPY **5** showed the highest photodynamic activity related to all tested cells, which confirms its promising properties and the possibility of application in both antimicrobial and anticancer photodynamic therapy.

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Acknowledgments

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