PDT potential of amphiphilic free-base and Zn(II) tripyridylporphyrins

Martina Mušković, Ivana Ratkaj, Nela Malatesti*

Department of biotechnology, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka

*e-mail: nela.malatesti@biotech.uniri.hr

međunarodni znanstveno-stručni skup
18. RUŽIČKINI DANI
DANAS ZNANOST – SUTRA INDUSTRIJA
16. – 18. rujna 2020. | Vukovar, Hrvatska

Introduction

Photodynamic therapy (PDT) is a relatively new treatment modality for malignant diseases, in which the main components are photosensitizer (PS), light of appropriate wavelength and oxygen. Their combination produces cytotoxic reactive oxygen species (ROS), which leads to tumour destruction [1].

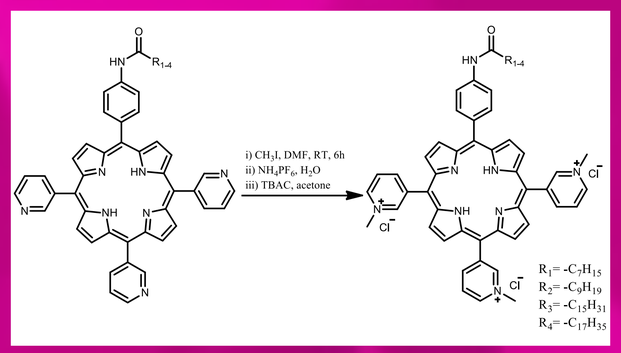
Some desirable properties of PS are good absorption in red region of the spectra, negligible dark toxicity, high production of singlet oxygen ($^1O_2$) and high stability. One way to increase production of singlet oxygen is chelation with paramagnetic metals since they increase the PS's lifetime of triplet state ($^3PS^*$)[2].

Amphiphilicity of a molecule also plays an important role in PDT effectiveness since its hydrophobic parts facilitate passage through membrane and hydrophilic parts increase solubility in water [3].

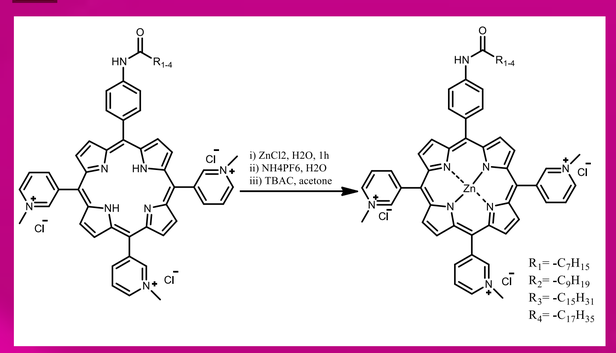
Here, in this work, we investigate two groups of tripyridylporphyrins conjugated with an alkyl chain consisting of 8C-atoms or more. Firstly, physiochemical properties, like stability, lipophylicity and singlet oxygen production were tested and then these properties were compared with cytotoxic activity on fibroblasts (HFF) and melanoma cell line (MEWO).

N-methylation and Zn(II) insertion

A



B



Scheme 1. *N*-methylation and Zn(II) chelation of tripyridylporphyrins conjugated with long alkyl chain of various lenght
All synthetised stuctures are confirmed with ^{1}H NMR spectra.

Absorbance and fluorescence properties

A

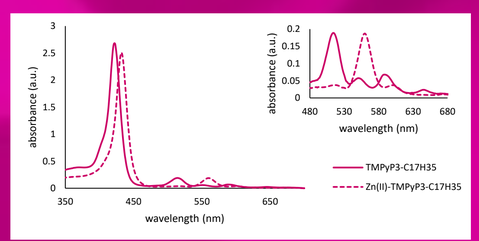
Compounds	λ_{abs}[nm] (ε[x10^{3}M^{-1}cm^{-1}])					λ_{em} [nm]	
	Soret (B)	*Qy (1-0)*	*Qy (0-0)*	*Qx (1-0)*	*Qx (0-0)*		
TMPyP3-C_7H_{15}	422 (258.4)	514 (16.9)	551 (4.4)	598 (5.4)	645 (1.5)	648	713
TMPyP3-C_9H_{19}	422 (249.1)	515 (16.5)	552 (5.9)	588 (5.7)	645 (2.8)	650	715
TMPyP3-$C_{15}H_{31}$	422 (189.3)	514 (12.5)	550(3.8)	588 (4.1)	644 (0.9)	649	715
TMPyP3-$C_{17}H_{35}$	422 (268.7)	514 (17.9)	551 (5.5)	589 (4.1)	645 (1.5)	650	714

B

Compounds	λ_{abs}[nm] (ε[x10^{3}M^{-1}cm^{-1}])			λ_{em} [nm]	
	Soret (B)	*Qy (1-0)*	*Qx (1-0)*		
Zn(II)-TMPyP3-C_7H_{15}	432 (267.7)	560 (20.0)	602 (3.0)	613	660
Zn(II)-TMPyP3-C_9H_{19}	432 (227.0)	560 (17.0)	600 (1.7)	614	660
Zn(II)-TMPyP3-$C_{15}H_{31}$	432 (189.5)	560 (14.3)	602 (2.5)	614	660
Zn(II)-TMPyP3-$C_{17}H_{35}$	432 (211.1)	554 (18.2)	601 (3.0)	614	660

Table 1. Absorbance and fluorescence properties of free-base (A) and Zn(II) *N*-methylated tripyridylporphyrins (B)

A



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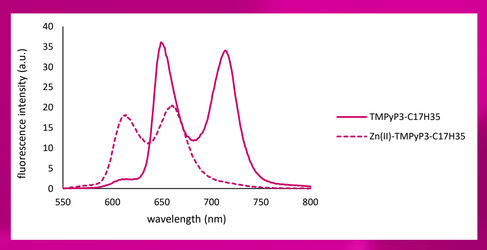
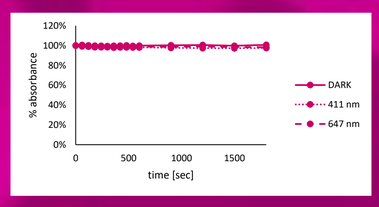
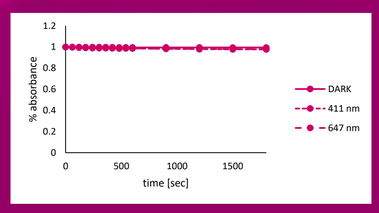


Figure 1. Comparison of an absorbance (A) and fluorescence spectra (B) of the free base (full line) and Zn(II) (dashed line) *N*-methylated tripyridylporphyrin with a 18C-chain.
All measurements were done in methanol, and excitation was at the Soret band wavelenght for the emission spectra.

Stability

A





B

	DARK	411 nm	647 nm
TMPyP3-C_7H_{15}	99.65%	97.21±0.02%	97.11±0.87%
TMPyP3-C_9H_{19}	98.69%	97.37±0.01%	98.60±0.53%
TMPyP3-$C_{15}H_{31}$	99.65%	96.99±0.05%	97.14±0.35%
TMPyP3-$C_{17}H_{35}$	99.47%	97.66±0.42%	97.96±0.05%

	DARK	411 nm	647 nm
Zn(II)-TMPyP3-C_7H_{15}	99.96%	97.62± 0.07%	98.2±0.33%
Zn(II)-TMPyP3-C_9H_{19}	99.58%	97.35±0.15%	98.63±0.05%
Zn(II)-TMPyP3-$C_{15}H_{31}$	99.91%	97.85±0.50%	98.42±0.03%
Zn(II)-TMPyP3-$C_{17}H_{35}$	99.91%	97.48±0.63%	98.29±0.16%

Figure 2. Photostability of **TMPyP3-$C_{17}H_{35}$** (top) and **Zn(II)-TMPyP3-$C_{17}H_{35}$** (bottom) detected by measuring decrease of *Soret band* after illumination with red or blue light (A) and percentage of initial absorbance after red or blue irradiation for 30 min (B)
All measurements were performed in methanol and compounds were irradiated for 30 min by LED based red light lamp (647 nm, 10.7 mW/cm^2, total light dose 19.3 J/cm^2) or LED based blue light lamp (411 nm, 11 mW/cm^2, total light dose 19.8 J/cm^2). Dark was used as a control. Data is presented as an average of two mwasurements ± SD.

Singlet oxygen ($^1O_2$) production

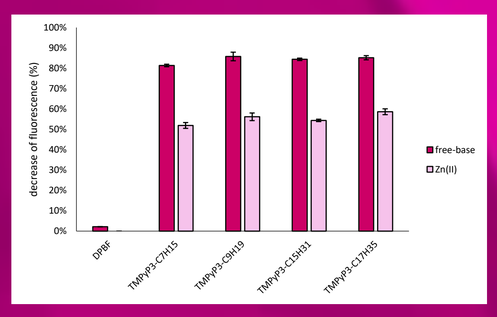


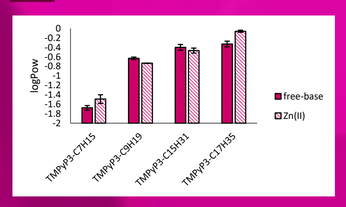
Figure 3. Singlet oxygen production ($^1O_2$) measured by photodegradation of 1,3-diphenylisobenzofurane (DPBF)
Methanol was used as solvent, irradiation time was 15 min, and as a light source LED based red lamp was used (647 nm, 38 mW/cm^2, overall light dose 34.2 J/cm^2). Data is presented as an average of duplicate measurements ± SD.

Lipophylicity

A

	ALOGPS 2.1	OSIRIS Property Explorer
TMPyP3-C_7H_{15}	-0.52	-6.52
TMPyP3-C_9H_{19}	-0.11	-5.61
TMPyP3-$C_{15}H_{31}$	1.46	-2.89
TMPyP3-$C_{17}H_{35}$	1.95	-1.98
Zn(II)-TMPyP3-C_7H_{15}	0.39	-6.81
Zn(II)-TMPyP3-C_9H_{19}	0.49	-5.9
Zn(II)-TMPyP3-$C_{15}H_{31}$	0.71	-3.18
Zn(II)-TMPyP3-$C_{17}H_{35}$	0.78	-2.27

B



C

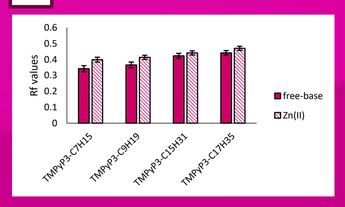
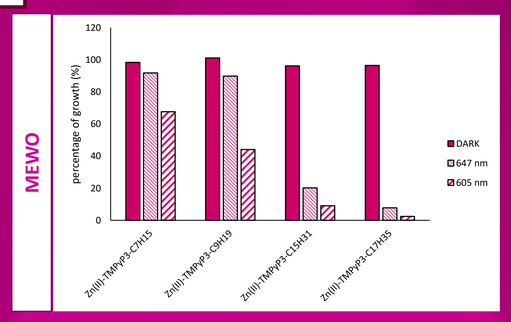


Figure 4. Lipophylicity of free-based and Zn(II) tripyridylporphyrins determined using computational programmes ALOGPS 2.1 and OSIRIS Property Explorer (A), using modified „shake-flask" method (B) and R_f values
Shake-flask method was done as in literature, with logPow calculated using equation: $logP_{ow} = 1.55logP_{bw} - 0.54$ [4] and R_f values determined on TLC silica plates using 8(CH_3CN): 1(H_2O):1 ($KNO_{3(sat.)}$) as a solvent.

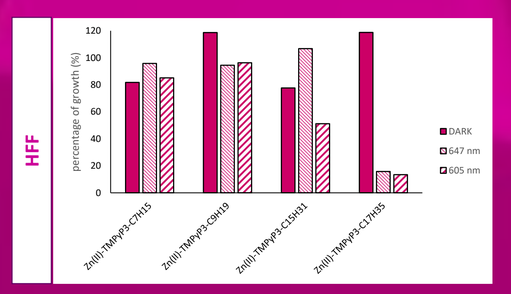
Citotoxicity on HFF and melanoma cells

A

MEWO



HFF



B

	IC$_{50}$ [μM]		
	DARK	645 nm	605 nm
TMPyP3-C7H15	> 50	5.28	
Zn(II)-TMPyP3-C7H15	> 50	33.31	7.33
TMPyP3-C9H19	> 50	14.85	
Zn(II)-TMPyP3-C9H19	> 50	29.25	4.53
TMPyP3-C15H31	> 50	0.67	
Zn(II)-TMPyP3-C15H31	> 50	3.42	0.87
TMPyP3-C17H35	> 50	0.45	
Zn(II)-TMPyP3-C17H35	> 50	2.18	0.57

	IC$_{50}$ [μM]		
	DARK	645 nm	605 nm
TMPyP3-C7H15	> 50	9.52	
Zn(II)-TMPyP3-C7H15	> 50	> 50	> 10
TMPyP3-C9H19	> 50	26.38	
Zn(II)-TMPyP3-C9H19	> 50	> 50	> 10
TMPyP3-C15H31	> 50	1.26	
Zn(II)-TMPyP3-C15H31	> 50	31.66	5.19
TMPyP3-C17H35	> 50	2.35	
Zn(II)-TMPyP3-C17H35	> 50	3.60	3.37

Figure 5. Citotoxicity of Zn(II) porphyrins in 5μM concentration after irradiation with red (645 nm) and orange light (605 nm) (A) and calculated IC_{50} values of both free-base and Zn(II) chelated tripyridylporphyrins (B)
MTT assay was used to determine cytotoxicity of tripyridylporphyrins in dark conditions and after illumination for 30 min with red light lamp (645 nm, 2mW/cm^2, total light dose 3.6 J/cm^2) or orange light lamp (605 nm, 1.5 mW/cm^2, total light dose 3.7 J/cm^2).

Conclusion

Zn(II) chelation of free-base *N*-methylated porphyrins was succesful using $ZnCl_2$, within 1h, in yields above 70%. Confirmation of Zn(II) insertion was done using UV-Vis spectroscopy, where chelated porphyrins, as expected, show slightly red-shifted Soret (B) band (from ~420 nm to ~430 nm) and decrease of Q-band number from four to two. Singlet oxygen production was determined using photodegradation of DPBF, where free-base porphyrins showed significantly higher results compared to Zn(II) chelated analogues with same alkyl chain lenght, when they were illuminated with red light (647 nm).

Lipophilicity was determined using modified „shake-flask" method, R_f values and using two computational programmes. All of the methods showed slightly higher lipophilicity of Zn(II) tripyridylporphyrins compared to free-base analogues. As expected, lipophilicity was higher proportional to number of C-atoms in alkyl chain, both in free-base and Zn(II) porphyrins. Stability of porphyrins was determined after illumination for 30 min with red light (647 nm) and blue light (411 nm). Both, free based and Zn(II) chelated porphyrins showed high stability after 30-min irradiation.

Higher activity of porphyrins with longer alkyl chain was confirmed using MTT cytotoxicity assay, where free-based porphyrins conjugated with 16C-atom and 18C-atom chain showed the lowest IC_{50} value. It was shown also that Zn(II) porphyrins could not be as efficient as free-base analogues when they are irradiated with red light wavelength (645 nm). Their activity was significantly increased when they were irradiated with orange light (605 nm). These results are in agreement with previously mentioned singlet oxygen results.

References

[1] L. B. Josefsen and R. W. Boyle, *Metal-Based Drugs* 2008 (2008) 276109.

[2] L. Benov, J. Craik, I. Batinic-Haberle, *Anti-Cancer Agents in Medicinal Chemistry* 2011 (11) 233-241.

[3] S. Pisarek, K. Maximova, D. Gryko, *Tetrahedron* 2014 (70) 6685-6715.

[4] I. Kos, J. S. Reboucas, G. DeFreitas-Silva, D. Salvemini, Z. Vujaskovic, M.W. Dewhrist, I. Spasojević, I. Batinić-Haberle, *Free Radical Biology & Medicine* 2009 (47) 72-78.

This work was supported by the University of Rijeka grants No. 17.12.2.1.01. and uniri-prirod-18-173. The equipment used in this work is part of the project "Research Infrastructure for Campus-based Laboratories at the University of Rijeka", financed by European Regional Development Fund (ERDF) and the Ministry of Science, Education and Sport of the Republic of Croatia (RC.2.2.06-0001). We are grateful to dr. Martin Lončarić and Anton Radman at the Laboratory for Photonics and Quantum Optics at Ruđer Bošković Institute in Zagreb for the light source used for singlet-oxygen measurements.