

Investigation of Biological and Photophysicochemical Properties of New Non-peripheral Fluorinated Phthalocyanines

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1 Apparatus

Electronic absorption spectra were obtained using a Scinco Lab Pro Plus UV/Vis spectrophotometer. ¹H NMR spectra were recorded on an Agilent VNMRS 500 MHz spectrometer. FT-IR spectra were obtained using a Perkin-Elmer Spectrum One spectrometer at room temperature. Mass spectra were obtained using a Perkin-Elmer Clarus 500 gas chromatography-mass spectrometry in positive electron impact mode and a Bruker Microflex matrix-assisted laser desorption-ionization-time of flight mass spectrometer, respectively. Elemental analyses were performed with a Thermo Finnigan Flash EA 1112 apparatus at 950 – 1000 °C. Fluorescence spectra were recorded on a Varian Eclipse spectrofluorometer at room temperature. Photo-irradiations for singlet oxygen determination were performed using a General Electric quartz line lamp (300 W). Light intensities were determined by utilizing a POWER MAX 5100 (Mol electron detector incorporated) power meter.

2 Biological studies

2.1 Antioxidant Activity

DPPH (2,2-difenil-1-pikrilhidrazil) [1] and chelating ability to ferrous ions [2] methods were used to determine the antioxidant activities of the molecules.

The radical scavenging ability of the tested compounds was monitored by measuring a decrease in UV absorption at 517 nm. Pc molecules were dissolved in DMSO and stock solutions were prepared at different concentrations.

Trolox was dissolved in methanol to obtain solutions of different concentrations and used as a positive control. The stock solutions of prepared Pc molecules and methyl laurate (ML) mixtures (1M ML+Pc molecules at different concentrations) were gained in DMSO. One mL of each molecule with prepared concentrations was taken into test tubes and 0.5 mL of 1 mM DPPH solution in methanol was added. These solutions were incubated for 1 h at room temperature and the absorbance was read at 517 nm using UV-Vis spectrophotometer (Optimizer). The DPPH radical scavenging activity percentage was calculated by using the following formula:

$$\text{DPPH radical scavenging activity(\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control reaction mixture, A_{sample} is the absorbance of the sample.

Ferrous ion chelating abilities of the Pc compounds and Pc+ML mixtures, competing with ferrozine were determined as in the method described by Dinis et al. [3]. Various concentrations ranging from 10 to 50 mg/mL of the Pc solutions and Pc compounds-methyl laurate mixtures were mixed with 2 mM FeCl_2 in deionized water. The solution was kept at room temperature for 30 min. and initiated by addition of 5 mM ferrozine. The mixture was incubated at room temperature for 10 min. and absorbance was monitored at 562 nm. FeCl_2 and a ferrozine mixture were used as the control and the inhibition percentage of the ferrozine- Fe^{2+} complex was calculated by the following equation:

$$\text{FIC effect (\%)} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

A_{control} is the control absorbance and A_{sample} is the absorbance of the Pc compounds, Pc-ML mixtures or standards. Ethylenediaminetetraacetic acid (EDTA) was used as a known standard.

2.2 Antimicrobial activity

Antimicrobial activities of Pc compounds and Pc-ML mixtures were determined by using disc diffusion method [4]. Antimicrobial activities of tested compounds were performed against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633 and *Bacillus subtilis* ATCC 6051. Bacteria cultures are incubated in Mueller Hinton Broth (MHB) at 37 °C overnight, and adjusted to 0.5 McFarland (1.5X 10⁸ CFU / mL) standard using UV visible spectrophotometer in tubes containing 5 mL of distilled water. The cultures taken from tubes by using sterile swab were inoculated on petri dish containing Mueller Hinton agar. Pc compounds, on the other hand, were dissolved in DMSO to a final concentration of 0.1, 0.2 and 0.5 M and methyl laurate were prepared at 1 M concentration. Then, Pc compounds at different concentrations (0.1, 0.2 and 0.5 M) were mixed with 1 M methyl laurate to investigate the synergistic effect (50:50 v/v). The discs were impregnated with 20 µL of prepared solutions and placed on the inoculated agar. Blank discs were impregnated with DMSO (20 µL for each blank disc) as negative control. Finally, inoculated petri dishes incubated at 37 °C for 24 h at the incubator and antimicrobial activities were evaluated by measuring the zone of inhibition against the test organisms.

2.3 Decolorization studies

Decolorization abilities of the bacterial strains on Pc compounds were investigated against four different bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC 6633 and *Bacillus subtilis* ATCC 6051). One night incubated bacterial cultures were adjusted to the 0.5 McFarland standard (5x10⁸ CFU/mL) in MHB by using spectrophotometer. Compound 4 alone at 0.5 M and compound 4-ML mixture were prepared at 1M ML+0.5 M Pc 50:50 (v/v) concentration in DMSO. Dye solutions were diluted with MHB (25:75 dye solution to MHB) and 1 mL bacterial culture adjusted to the 0.5 McFarland standard was added to each mixture while the control mixture was prepared without bacterial culture. Dye solutions were incubated at 37 °C by using an incubator. Biodegradation experiments were performed in the form of static samples culturing for 2, 5 and 10 days at 37 °C. After 2 days of biodegradation, samples were filtered, centrifuged for 15 min at 4400 rpm and UV/vis absorption spectra were measured [5].

3. Photophysical and Photochemical Studies

3.1 Fluorescence quantum yields

Fluorescence quantum yields (Φ_F) were determined by the comparative method (Eq. 1) [6],

$$\Phi_F = \Phi_{F(\text{Std})} \frac{F \cdot A_{\text{Std}} \cdot n^2}{F_{\text{Std}} \cdot A \cdot n_{\text{Std}}^2} \quad (1)$$

where F and F_{Std} are the areas under the fluorescence emission curves of the samples and the standard, respectively. A and A_{Std} are the respective absorbances of the samples and standard (Unsubstituted ZnPc) at the excitation wavelengths, respectively. n^2 and n_{Std}^2 are the refractive indices of solvents used for the sample and standard, respectively. Unsubstituted ZnPc in DMSO ($\Phi_F = 0.20$) [6] was used as the standard. Both the samples and standard were excited at the same wavelength.

3.2 Singlet oxygen quantum yields

Quantum yields of singlet oxygen photogeneration were determined in air (no oxygen bubbled) using the relative method with ZnPc as reference and DPBF as chemical quencher for singlet oxygen, using formula 2

$$\Phi_{\Delta} = \Phi_{\Delta}^{\text{Std}} \frac{R \cdot I_{\text{abs}}^{\text{Std}}}{R_{\text{Std}} \cdot I_{\text{abs}}} \quad (2)$$

where $\Phi_{\Delta}^{\text{Std}}$ is the singlet oxygen quantum yields for the standard ZnPc ($\Phi_{\Delta}^{\text{Std}} = 0.67$ in DMSO). R and R_{Std} are the DPBF photobleaching rates in the presence of the respective samples and standard, respectively. I_{abs} and $I_{\text{abs}}^{\text{Std}}$ are the rates of light absorption by the samples and standard, respectively. Solutions, that contain DPBF, were prepared in the dark and irradiated in the Q band region. The degradation of DPBF at 417 nm was monitored after each 5 s irradiation [7]. The light intensity of 7.05 x 10¹⁵ photons s⁻¹ cm⁻² was used for Φ_{Δ} determinations. The absorption band of DPBF reduced by light irradiation [6, 7].

3.3 Photodegradation quantum yields

Photodegradation quantum yields were determined using formula 3,

$$\Phi_d = \frac{(C_0 - C_t) \cdot V \cdot N_A}{I_{\text{abs}} \cdot S \cdot t} \quad (3)$$

where " C_0 " and " C_t " are the sample concentrations before and after irradiation respectively, " V " is the reaction volume, " N_A " is the Avogadro's constant, " S " is the irradiated cell area, " t " is the irradiation time, " I_{abs} " is the overlap integral of the radiation source light intensity and the absorption of the samples. A light intensity of 7.05 x 10¹⁵ photons s⁻¹ cm⁻² was employed for Φ_d determinations [8].

4. Characterization

4.1 Mass Spectra

Mass spectrometry is an analytical technique in which samples are ionized into charged molecules and the ratio of their mass-to-charge (m/z) can be measured. In MALDI-TOF mass spectrometry, the ion source is matrix-assisted laser desorption/ionization (MALDI), and the mass

analyzer is a time-of-flight (TOF) analyzer. Albeit mass spectra of all the studied compounds assigned to the ionized intact phthalocyanines, some differences could arise from the medium (matrix nature) in which the measurement is performed. Some of these differences have been reported in the literature [9-14].

4.2 ^1H NMR Spectra

^1H NMR spectra of all the newly synthesized phthalocyanine complexes were observed broader than that of the phthalonitrile derivative since the isomeric mixture show up slightly different chemical shifts that can result in broadening the bands. Additionally, aggregation in the concentrated solutions prepared for NMR spectroscopy can broaden the bands [15-20].

References

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Figures

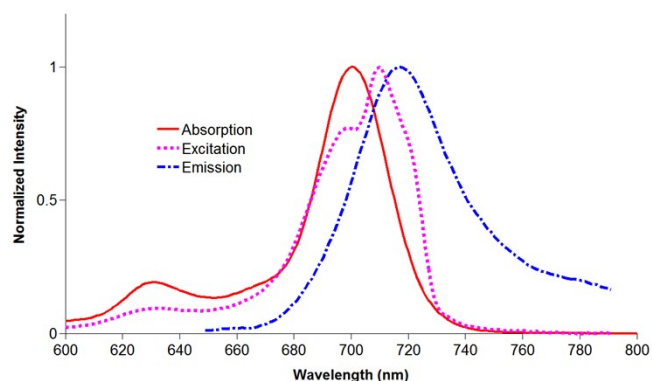


Figure SI-1 Absorption (700), excitation (710) and emission (717) spectra of complex **3** in DMSO.

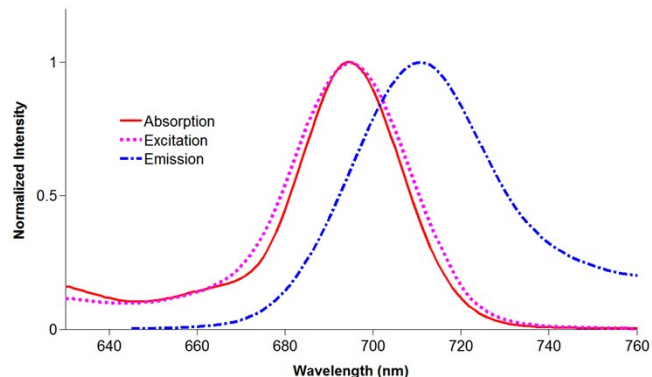


Figure SI-2 Absorption (695), excitation (695) and emission (711) spectra of complex **7** in DMSO.

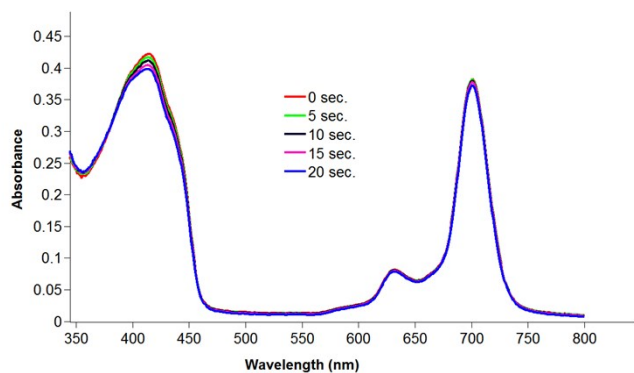


Figure SI-3 A typical spectrum for the determination of singlet oxygen quantum yield of complex **3** in DMSO.

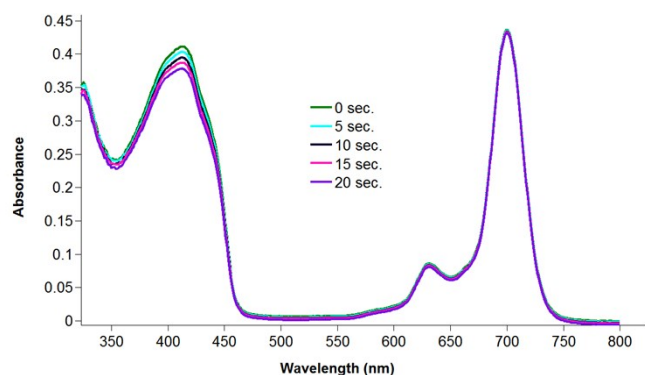


Figure SI-4 A typical spectrum for the determination of singlet oxygen quantum yield of complex **6** in DMSO.

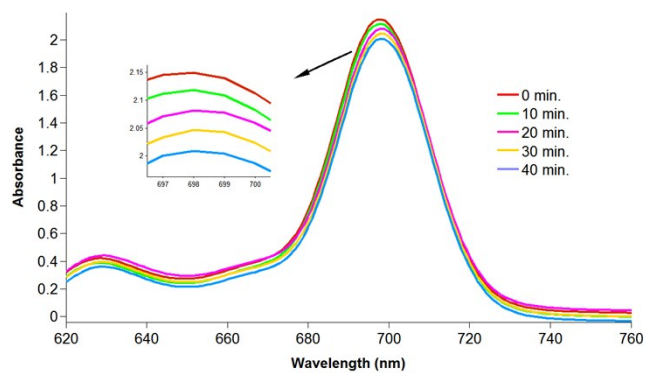
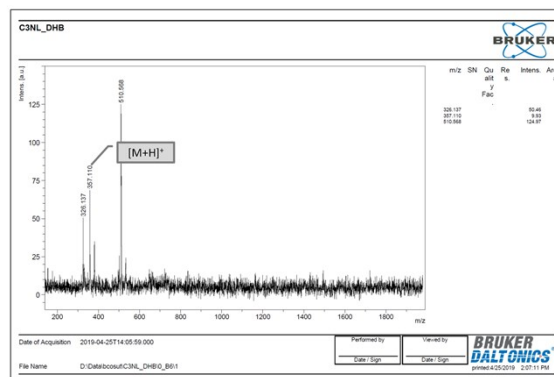


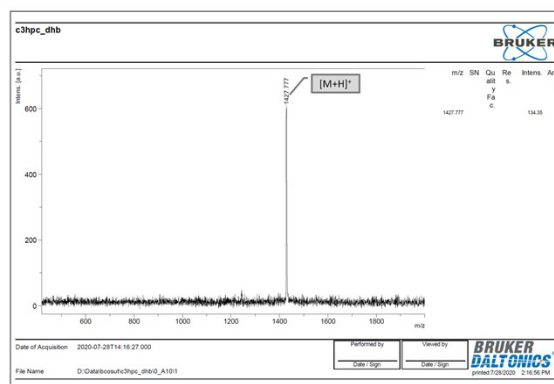
Figure SI-5 A typical spectrum for the determination of photodegradation of complex **7** in DMSO.

Mass spectrum of compound **1**

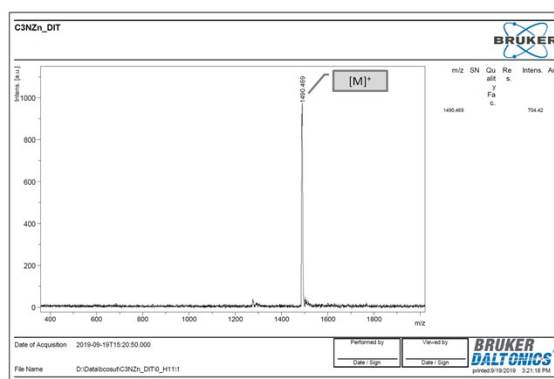


$[M+DHB]^+ = 510.568 \text{ m/z}$

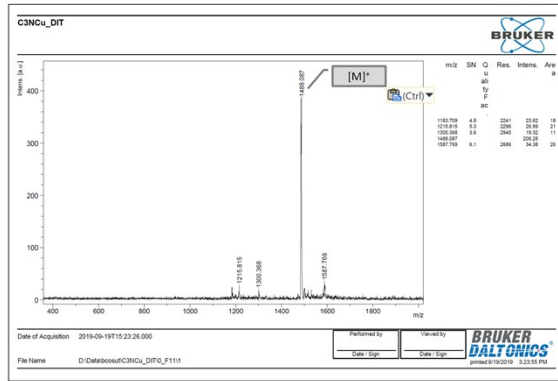
Mass spectrum of compound **2**



Mass spectrum of compound **3**

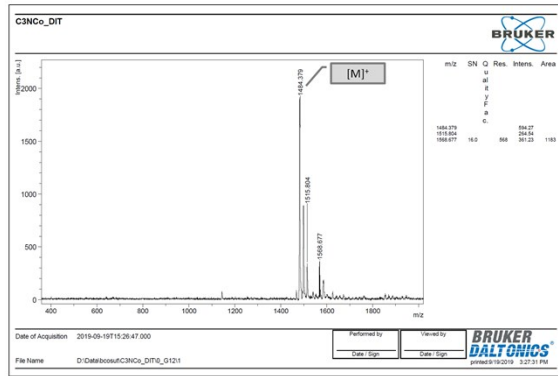


Mass spectrum of compound 4



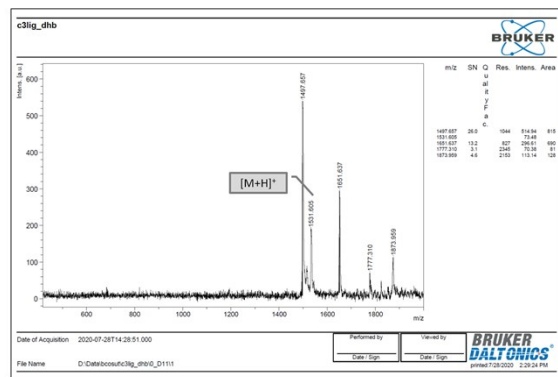
$[M-4CF_3-Cu+DIT+Li]^+ = 1587.769m/z$; $[M-2CF_3-Cu+2Li]^+ = 1300.368m/z$; $[M-4CF_3+3H]^+ = 1215.815m/z$

Mass spectrum of compound 5



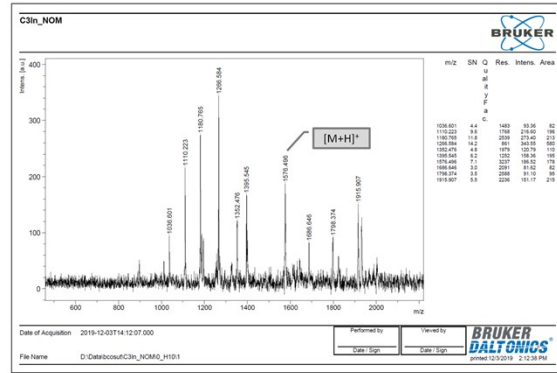
$[M-6H+5H_2O]^+ = 1568.677m/z$; $[M-4H^++2H_2O]^+ = 1515.804m/z$

Mass spectrum of compound 6



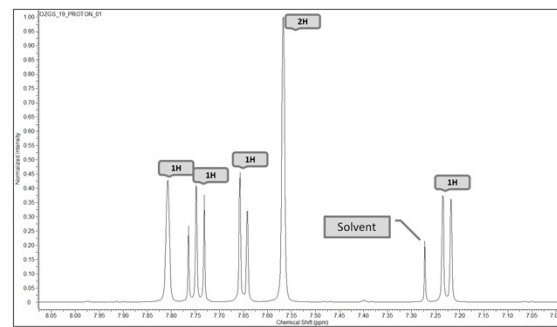
$[M+6H+8Na+DHB]^+ = 1873.959m/z$; $[M+H+4Na+DHB]^+ = 1773.310m/z$; $[M-Cl+2H+DHB]^+ = 1651.637m/z$; $[M-Cl+2H]^+ = 1497.657m/z$

Mass spectrum of compound 7

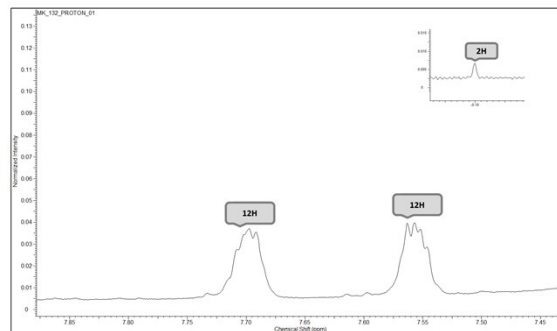


$[M+19H_2O-H]^+ = 1915.907m/z$; $[M+12H_2O+7H]^+ = 1798.374m/z$; $[M+6H_2O+3H]^+ = 1686.646m/z$; $[M-3CF_3+4H+Na]^+ = 1395.545m/z$; $[M-InCl-CF_3-4H]^+ = 1352.476m/z$; $[M-Cl-4CF_3+2H]^+ = 1266.584m/z$; $[M-6CF_3-4H+Na]^+ = 1180.765m/z$; $[M-7CF_3-5H+Na]^+ = 1110.223m/z$; $[M-8CF_3-10H+Na]^+ = 1036.601m/z$

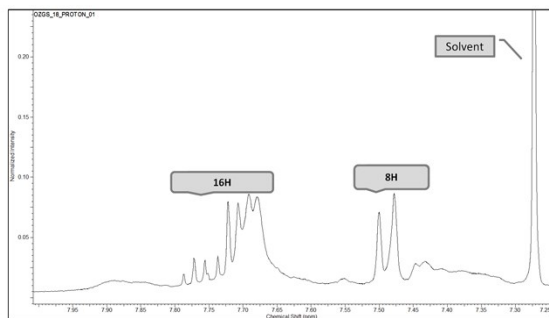
¹H NMR spectrum of compound 1



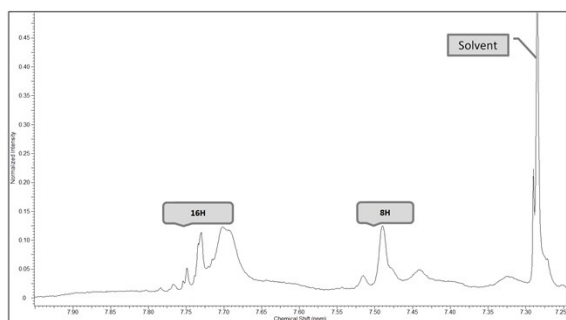
¹H NMR spectrum of compound 2



¹H NMR spectrum of compound 3



¹H NMR spectrum of compound 6



¹H NMR spectrum of compound 7

