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Synthesis of Palladium Phthalocyanine and Investigation of Sono-Photodynamic Therapy Properties

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Abstract

In this study, it is desired to apply the Sono-Photodynamic Therapy (SPDT) method to create a more effective therapeutic outcome. There are cell applications of SPDT in the literature, but the sonophotochemical studies are very limited. For this purpose, a new palladium phthalocyanine (PdPc) (**2**) was synthesized and characterized by standard spectroscopy methods. Then its photochemical and photophysical properties were determined in DMSO. Singlet oxygen quantum yield of the complex (**2**) was calculated by both photochemical and sono-photochemical methods. The SPDT method (Φ_Δ =0.90) was found to be more effective than the PDT method (Φ_Δ =0.74), to obtain a high singlet oxygen quantum yield. All the results show that, SPDT improves the therapeutic effects and the synthesized PdPc can be an effective sono/photosensitizer in PDT / or SPDT.

Keywords: Palladium Phthalocyanine, Sonodynamic Therapy, Photodynamic Therapy

1. Introduction

Photodynamic therapy (PDT) is one of the most noticeable applications for cancer treatment. PDT method is dependent on a light-sensitive agent (photosensitizer) injected into the patient. Then the photosensitizer accumulates in the tumor tissue by the addressing method to produce reactive singlet oxygen [1, 2]. PDT is a successful method of producing singlet oxygen. However, the penetration problem of PDT studies limited its applications. Because the depth of penetration of the laser light is more suitable for superficial lesions [3, 4]. To overcome this problem, the sonodynamic therapy (SDT) method has been developed by using ultrasound activated sensitizers inspired by PDT [5, 6]. The ultrasound is preferred in the SDT method because of the deeper penetrating and during the application, the free radicals are formed by the breakdown of water molecules as a result of acoustic cavitations. Afterward, these reactive radicals cause the death of the targeted cell [7]. With these properties, sonodynamic therapy method has developed the disadvantage of photodynamic therapy method, but the sonosensitizers in SDT have not been confirmed for clinical experiments [8, 9]. Because sonophotodynamic studies were required high concentration and this was increased the potential phototoxicity [10, 11]. All of the problems are required the Sono-photodynamic Therapy (SPDT), a combination of SDT and PDT [3, 12]. In the SPDT method, the sensitizer is activated by both light and ultrasound to obtain sono-photo chemical reactions [13, 14]. Previous studies about the sono-photochemical applications have shown that the sono-photochemical study has a more powerful therapeutic effect than the other therapy methods [14, 15]. SPDT bases on some parameters as the properties of used ultrasound and light, sono/photosensitizers, tumor response to the sensitizer, and light/sound irradiation time [3, 12]. Especially sensitizer selection is the most important issue in this method. The synthesized sono/photo sensitizers should have some properties like a stable chemical composition, high sono-photo sensitivity, high cleaning properties from healthy tissues, and should not be toxic [8, 16].

Singlet oxygen as a reactive oxygen species can kill a cancer cell due to its cytotoxicity if produced in sufficient quantity. The our previous studies show that the obtained singlet oxygen yield is quite affected by the used chemical method [17-19]. That's why, the purpose of this paper is to present that the heavy center metal atom and the sono-photochemical method increase singlet oxygen efficiency. In this way, the new palladium phthalocyanine was synthesized and investigated its photophysicochemical properties and also compared with the zinc and indium derivatives reported before [18, 20]. The singlet oxygen activity of the synthesized phthalocyanine was analyzed by both SDT, PDT and SPDT methods and presented by comparison.

2. Experimental

2.1. Materials and equipment

Details about the materials and equipment can be found in the supplementary material.

2.2. Synthesis

The synthesis of compound (**1**) was prepared according to the our previous study [20]. New palladium phthalocyanine (**2**) was synthesized in this study for SPDT studies. The synthesized complex was characterized by performing different techniques such as ^{1}H NMR, MALDI-TOF, FT-IR, UV-Vis spectroscopy, elemental analysis and then investigated photodynamic and sono-photodynamic therapy properties.

2.2.1. 4-[4-(Bromophenoxy) phenoxy] phthalonitrile (1)

Compound **1** was synthesized as given in the literature [20].

2.2.4. Palladium (II) chloride phthalocyanine (2)

A mixture of compound (**1**) (100 mg, 0.25 mmol), $PdCl_2$ (13.27 mg, 75.00 mmol) and DBU (0,2 mL, 0.13 mmol) in n-hexanol (2,0 mL) was refluxed and stirred under inert atmosphere for 12 h. The product was precipitated, centrifuged and then washed with hot solvents (hexane, methanol, ethanol). The final product was further purified by column chromatography over a silica gel using a mixture of $CHCl_3$: MeOH (100/2 v/v) as eluent. Yield: 0.065 g (15%). UV-Vis (DMSO): $\lambda_{max,}$ nm (log ε) 684 (5.22), 617 (4.36), 325 (4.81). FT-IR υ_{max} /cm^{-1} (KBr pellet): 2923 (Ar-CH), 1596 (C=C), 810 (C-Br) 1232, 1186 (C-O-C); ^{1}H NMR ($CDCl_3$): δ= 7.55-7.45 (12H, m, Pc-H), 7.15-6.95 (16 H, m, Ar-H), 6.85-6.70 (16 H, m, Ar-H). The results of elemental analysis, Calcd for $C_{80}H_{44}Br_4N_8O_8Pd$: C, 57.49; H, 2.65; N, 6.70: Found: C, 56.80; H, 2.34; N, 6.96; MS (MALDI-MS; Dithranol (DIT) as matrix) m/z: Calc. 1671; Found: 1684 [M+8 (^{81}Br- Ref. [18]+ 5H]$^+$ and 1797 [M+ 8+ 3K +H]$^+$.

2.3. Photophysical and photochemical studies

Single oxygen, photodegradation and fluorescence quantum yields were calculated performing the experimental setup described in the literature [21-23].

3. Results and discussion

3.1. Synthesis and characterization

Scheme 1 shows the synthetic route for the preparation of the phthalonitrile derivative (**1)** and the metallophthalocyanine (**2**). As shown, compound **1** was obtained by mixing of 4-nitrophthalonitrile and 4-4(bromophenoxy) phenol in DMSO at room tempareture for one day. This aromatic nucleophilic reaction was catalyzed by K_2CO_3 as the base. The pure compound **1** had a 50% yield after the recrystallization by methanol. To synthesis of palladium phthalocyanine (**2**), the cyclotetramerization process was performed using palladium chloride salt. After the reaction, on the FT-IR spectra, the distinctive C≡N peaks disappeared. Also, the typical vibrations corresponding to the C-O-C group occurred at 1232, 1186 cm^{-1}, and aromatic C-H peaks were observed at 2923 cm^{-1}, **Figure 1**.

In UV-Vis studies, the absorption bands of the complex were the typical spectrum of phthalocyanine. One of them was in the visible region at 684 nm (Q band) and the other band was in the UV region at 325 nm (B band) due to n → π * transitions [24].

In ^{1}H-NMR spectrum were showed signals with δ ranging from 7.74 to 6.92, integrating for a total of 11 protons for complex **1** and 7.55-6.70 integrating for a total of 44 protons (δ= 7.55-7.45 (12H, m, Pc-H), 7.15-6.95 (16 H, m, Ar-H), 6.85-6.70 (16 H, m, Ar-H)) for complex **2** as expected, **Figure 2**.

For mass spectrum, the presence of molecular ion peaks at m/z 392.0 [M+H]$^+$ for complex **1**, 1684 [M+8 (^{81}Br-Ref.[18]+5H]$^+$ and 1797 [M+8+3K+H]$^+$ for complex **2**, approved the recommended structures. All analytical results were consistent with the predicted structures.

3.2. Photophysical studies

3.2.1. Ground state electronic absorption spectra

The characteristic features of phthalocyanines can determined by examining of their absorption spectra and the aromatic 18π-electron structure of the phthalocyanine ring specifies the spectral properties of Pcs. The UV-Vis spectra of Pcs mainly include two strong bands denoted B- and Q-band. π-π* transition from the HOMO (highest occupied molecular orbital) to the LUMO (lowest unoccupied molecular orbital) of the Pc ring at visible region leads to the appearance of the Q-band giving the blue/green color of Pcs [25]. The absorption spectra of compound **2** was studied in DMSO and are presented in **Fig 3**. The Q-band of complex **2** appeared at 684 nm and its B-band was observed at 325 nm. The log ε values of the bands are 5.22 for Q-band and 4.81 for B-band. The heavier metal ions usually have more red-shift of the Q-band. Comparing the Q band of complex **2** with the ZnPc derivative (Q band= 680 nm) [20], the PdPc makes the complex 4 nm more red shift.

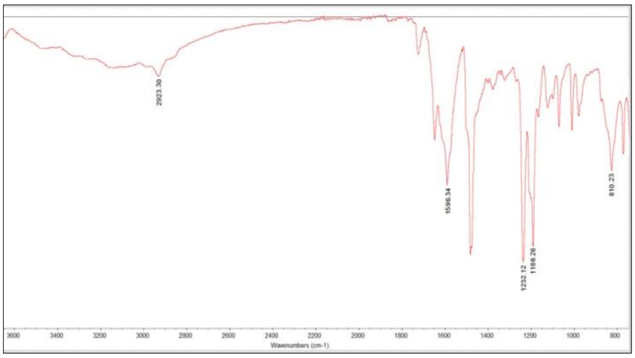


Figure 1. The FT-IR spectra of compound **2**

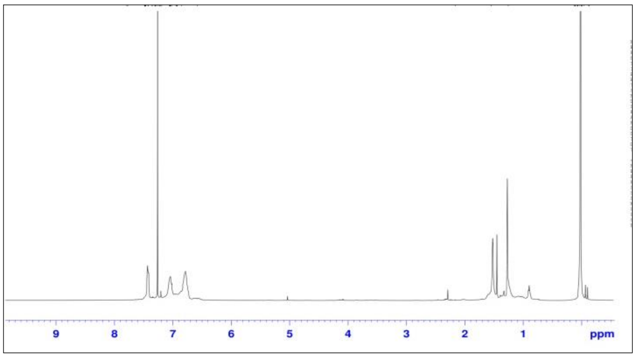
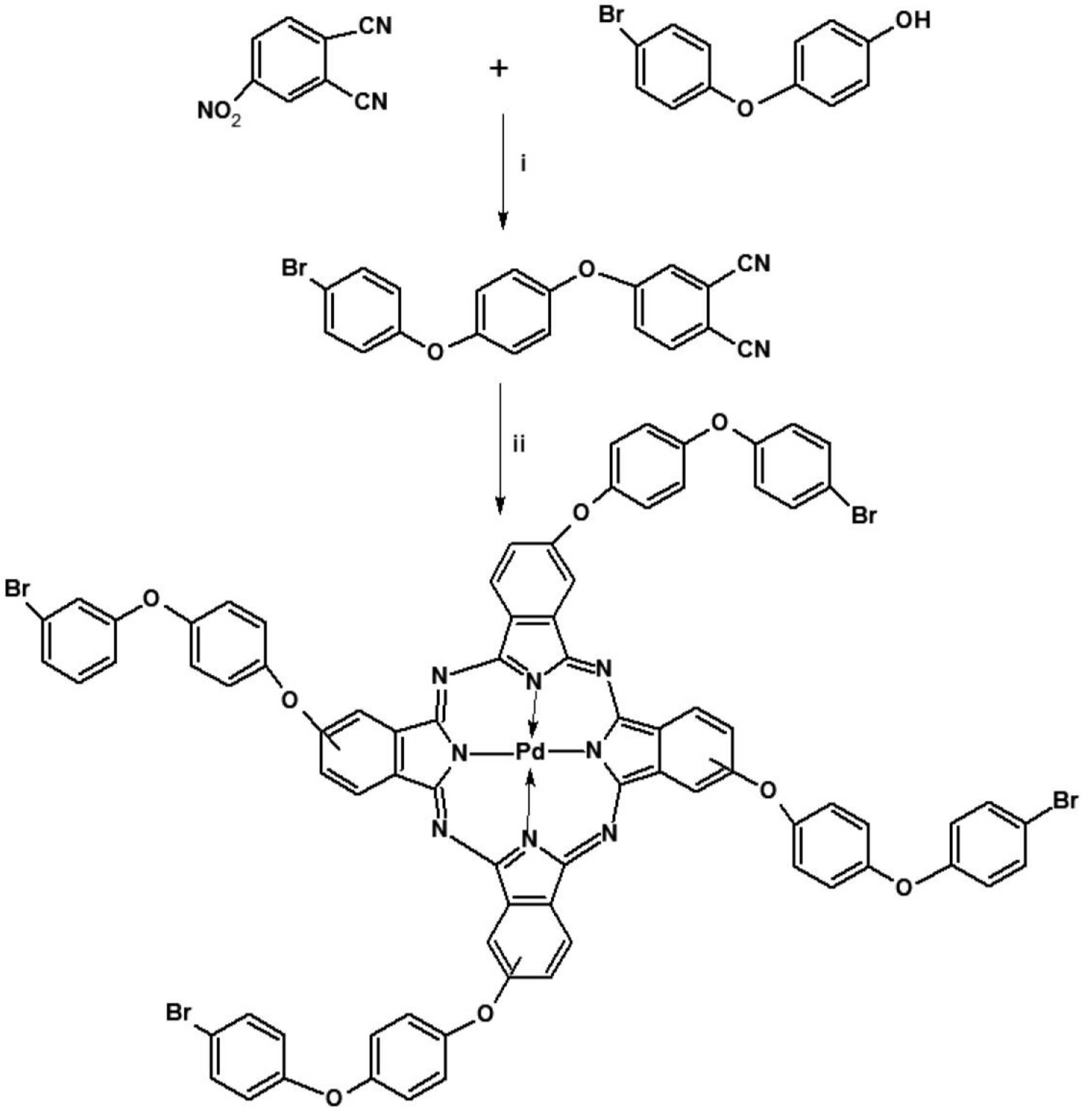


Figure 2. ^{1}H-NMR ($CDCl_3$) spectrum of compound **2**



Scheme 1. Synthesis of new palladium phthalocyanine. Reagents and conditions; **(i)** K_2CO_3, DMF, 24 hours **(ii)** Palladium chloride, hexanol, DBU, argon atm, reflux 12 hours.

3.2.2. Fluorescence Spectra and Quantum Yield (Φ_F)

The fluorescence properties of photosensitizing molecules are important measures to determine the suitability of these molecules as biological imaging materials [26]. The fluorescence property of complex **2** was investigated in DMSO and fluorescence quantum yield is listed in **Table 1**. The absorption, excitation, and emission spectra of the molecule are shown in **Fig 3**. Fluorescence emission maxima was observed at 695 nm and the absorbance spectra was a mirror image of the excitation spectra. The effect of substituent nature and metal ion on the value of Φ_F were examined and the fluorescence quantum yield was found as 0.17. The value was determined using the comparative method. Unsubstituted zinc phthalocyanine (ZnPc) in DMSO ($\Phi_F = 0.20$) was used as the standard [16].

Generally, the heavy metal effect leads to lower values of Φ_F due to the heavy atom which promotes intersystem crossing [27] and the new palladium phthalocyanine have lower fluorescence quantum yields than standard ZnPc (Φ_F = 0.20 in DMSO) [16] and its previously reported ZnPc pattern (Φ_F = 0.23) [20].

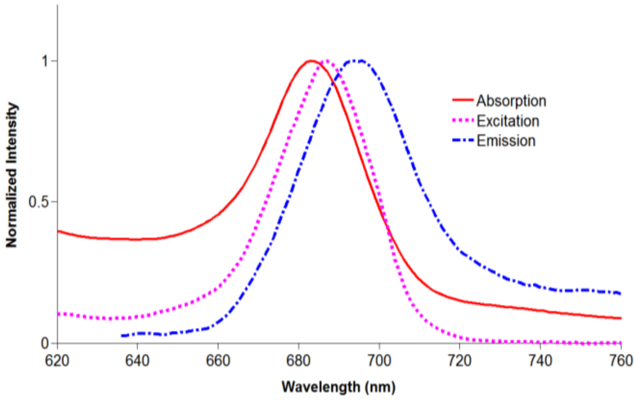


Figure 3. Absorption (684), excitation (687) and emission (695) spectra of compound **2** in DMSO.

Table 1. Spectral parameters of photophysical and photochemical properties of compound

Solvent	Q band λ_{max}, nm	log ε	Excitation λ_{Ex}, nm	Emission λ_{Em}, nm	Φ_F	$\Phi_{\Delta\ (PDT)}$	$\Phi_{\Delta\ (SPDT)}$	$\Phi_d(10^{-4})$
DMSO	684	5.22	687	695	0.17	0.74	0.90	4

3.3. Photochemical studies

3.3.1. Singlet oxygen quantum yields (Φ_Δ) for PDT and SPDT

A sono/or photosensitizer should have effective singlet oxygen yield to be used in photodynamic or sonodynamic therapy. In this part, the singlet oxygen quantum yield was calculated by both sono-photochemical and photochemical studies to achieve higher yields. The yields were determined by using DPBF as a chemical quencher. The spectrum of the intensity of DPBF absorbance at about 417 nm reduced by ultrasound activation and light irradiation and the spectra are present in **Figs**. **3-6** and **Table 1**.

In the literature, ultrasound at a frequency of approximately 1MHz is used in cell applications of SPDT [3, 9]. However, in this study, ultrasound at a frequency of 35 kHz was preferred because it was desired to show the singlet oxygen formation in the same UV spectrum. In both PDT and SPDT studies, there were no changes in the Q band intensities, approving that the compound is not degraded. According to the obtained yields, compound **2** have significantly higher Φ_Δ values than its ZnPc derivative (Φ_Δ= 0.63) [20] and unsubstituted ZnPc (Φ_Δ= 0.67) in DMSO and also have lower Φ_Δ values than the indium derivative (Φ_Δ= 0.79 for PDT method and 0.95 for SPDT method) [18] because of the heavy atom effect that may support intersystem crossing (ISC) [28].

When PDT, SDT, and SPDT were compared, it was seen that the highest decrease in the intensity of DPBF was obtained by the SPDT method, and the lowest decrease was obtained by the SDT method, **Fig. 2**. While the efficiency of the singlet oxygen in the PDT method was Φ_Δ= 0.74, it was found Φ_Δ= 0.90 in the SPDT method. This result shows that sensitizer **2** would generate a more therapeutic effect by a combination of sonochemical and photochemical studies.

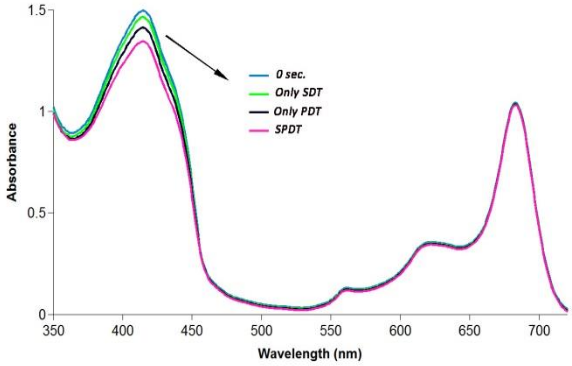


Figure 4. Comparison of SDT, PDT and SPDT spectrum of compound **2** in DMSO

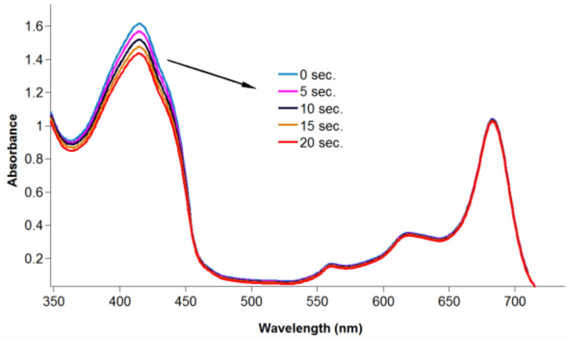


Figure 5. A typical spectrum for the determination of singlet oxygen quantum yield (for PDT method) of compound **2** in DMSO.

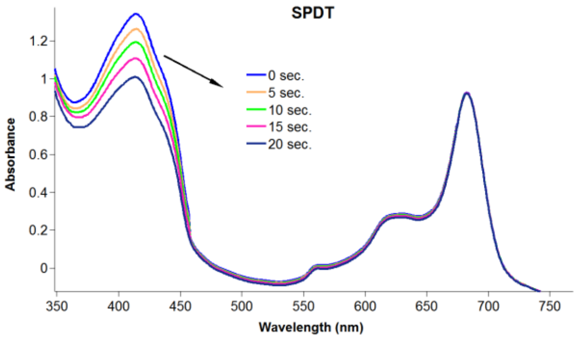


Figure 6. A typical spectrum for the determination of singlet oxygen quantum yield (for SPDT method) of compound **2** in DMSO.

3.3.2. Photodegradation quantum yield (Φ_d)

In photodynamic /or sonodynamic therapy, photosensitizers should be as stable as possible to light in the process of producing singlet oxygen. To investigate the stability, the molecule is irradiated by the light every ten minutes and the maxima Q band is examined using UV-vis spectrometry. The Q band of the molecule was decreased during the irradiation and the observed spectral changes are shown in **Fig. 7** and the quantum yield listed in **Table 1**. The palladium complex showed average stability with Φ_d in the order of 10^{-4}. But PdPc is less stable than its ZnPc derivative ($\Phi_d = 0.8\ .10^{-4}$) because of the heavy metal effect.

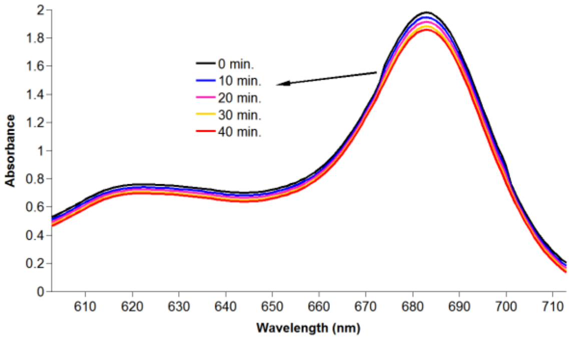


Figure 7. A typical spectrum for the determination of photodegradation of the compound **2** in DMSO.

4. Conclusion

The target of this paper was to enhance the photophysicochemical effect of phthalocyanines by the SPDT method and heavy central atom. In this way, a new palladium phthalocyanine was synthesized and investigated its photochemical and sono-photochemical properties for the first time. A very high result (Φ_Δ =0.90) was obtained in singlet oxygen quantum yield with the SPDT method and palladium center atom. Compared to its previously reported ZnPc derivative, the new PdPc is a more powerful performing intersystem crossing and could be a better sensitizer. Besides, while the efficiency in the PDT method was Φ_Δ =0.74, the efficiency reached Φ_Δ =0.90 with the SPDT method. The yields indicated that only the photochemical study is not sufficient and the sono-photochemical study can be a more effective method for anticancer treatment.

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Author's Contributions

Göknur Yaşa Atmaca: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ethics

There are no ethical issues after the publication of this manuscript.

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