

Article

Synthesis, Singlet Oxygen Photogeneration and DNA Photocleavage of Porphyrins with Nitrogen Heterocycle Tails

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Abstract: Eight novel compounds were prepared by reaction of 5-(bromopropoxyphenyl)-10,15,20-triphenylporphyrin with oxazole thiols, 1,3,4-oxadiazole thiols and 1,3,4-thiadiazole thiols, and their structures confirmed by UV-vis, IR, ¹H-NMR, MS and elemental analysis. The assessment of indirectly measured ¹O₂ production rates against 5,10,15,20-tetraphenyl porphyrin (H₂TPP) were described and the relative singlet oxygen production yields were: porphyrin **5** > porphyrins **1**, **3**, **4**, **6-8**, H₂TPP > porphyrin **2**. Porphyrin **4** and porphyrin **7** showed substantial photocleavage activities toward DNA, with over 75% cleavage observed at 40 μM. It suggested that these those porphyrins with nitrogen heterocycle tails are potential photosensitive agents.

Keywords: porphyrin; singlet oxygen generation; DNA cleavage; heterocyclic thiol

1. Introduction

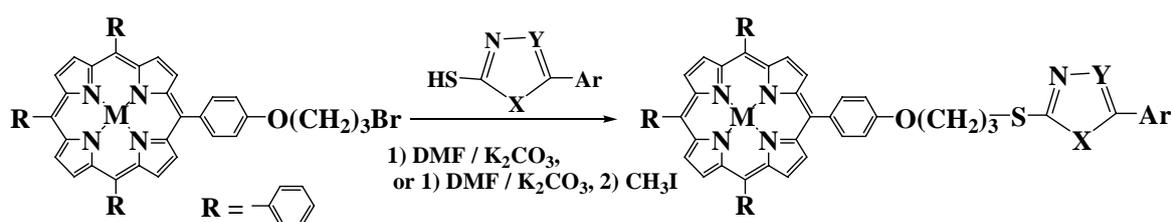
The human vascular endothelial growth factor (VEGF) gene locates at chromosome 6P^{21,3}, whose length is 28 Kb. The VEGF gene, of 14 Kb length, consists alternately of eight exons and seven

introns. The encoding product is a homogenous dimeric glycoprotein of 34–35 KD [1]. Against a target of VEGFR-2 tyrosine kinase VEGFR-2 plays a main receptor function in tumor angiogenesis mediated by VEGF, which prevents the activation of VEGFR-2 enzyme and limits VEGF signal transduction so as to inhibit tumor growth for cancer treatment. Compounds with oxazole, 1,3,4-oxadiazole and 1,3,4-thiadiazole structures display certain capability of acting in the VEGFR-2 tyrosine kinase domain and of binding VEGFR-2 in competition with ATP to prevent the growth of tumor vessels and cause tumor death [2-4]. So far, research on the use of angiogenesis inhibitors against the tumor metastasis has become a major topic.

Meanwhile, porphyrin-based compounds with unique structures have special affinity interactions with tumor cells and they can selectively remain in the tumor tissues [5,6]. Photodynamic therapy (PDT) is a promising therapy against cancer with intrinsic selectivity, which is used to eliminate deviant tissues, such as tumors. While gathering selectively around the tumors, the photosensitizers can absorb visible light under illumination and generate active oxygen (such as the singlet oxygen, $^1\text{O}_2$, generated when porphyrins are photoactivated by absorption at the Soret band [7]), which leads to tumor cell death [8,9]. The $^1\text{O}_2$ is produced by energy transfer from the photoexcited sensitizer (in its triplet state) to the ground-state (triplet) oxygen. This is known as the Type II photosensitization mechanism, which is considered to be more important in PDT [10,11]. The DNA photocleavage activity of a photosensitizer depends on its $^1\text{O}_2$ yield [12,13].

Porphyrins linked with some anticancer drugs can effectively localize in tumor cells and increase the cell cytotoxicity through the synergistic effect of porphyrins and anticancer drugs [14]. To enhance targeted therapy role of porphyrins against the VEGFR-2 tyrosine kinase target some porphyrins with nitrogen heterocycle tails were prepared using as starting materials oxazole thiols, 1,3,4-oxadiazole thiols and 1,3,4-thiadiazole thiols linked by $-\text{O}(\text{CH}_2)_3\text{-S}-$ spacer group (shown in Scheme 1). Their $^1\text{O}_2$ productivity and DNA photocleavage activities were then investigated.

Scheme 1. Synthesis of eight porphyrins with nitrogen heterocycle tails.



Porphyrin	M	X	Y	Ar
1	2H	O	CH	-Ph
2	Cu	O	CH	-Ph
3	2H	O	N	

Scheme 1. Cont.

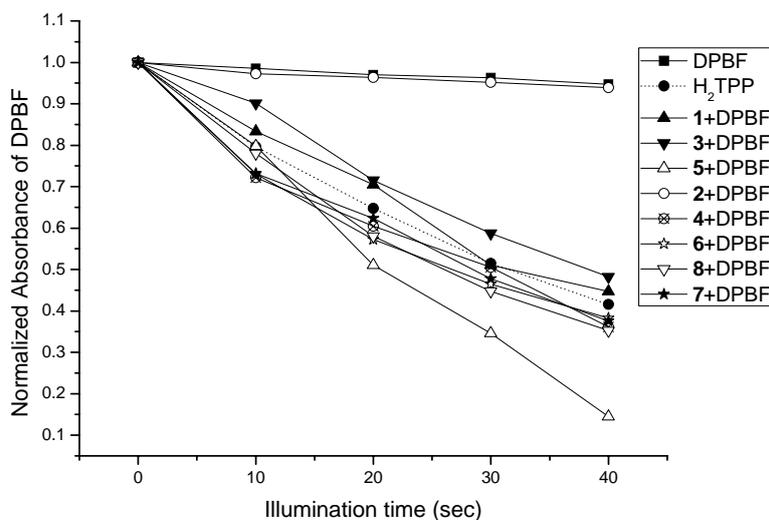
4	2H	O	N	
5	Zn	O	N	-Ph
6	2H	S	N	
7	2H	S	N	
8	2H	O	N	

2. Results and Discussion

2.1. Photogeneration of 1O_2

DNA photocleavage of the porphyrins was the result of generating the singlet oxygen. 1,3-diphenylisobenzofuran (DPBF) was able to capture the 1O_2 photogenerated by the porphyrins, which reduced its own light activity [15,16]. The relationship between A/A_0 absorbed by DPBF and illumination time indirectly reflected 1O_2 yield of those porphyrins compared with H_2TPP .

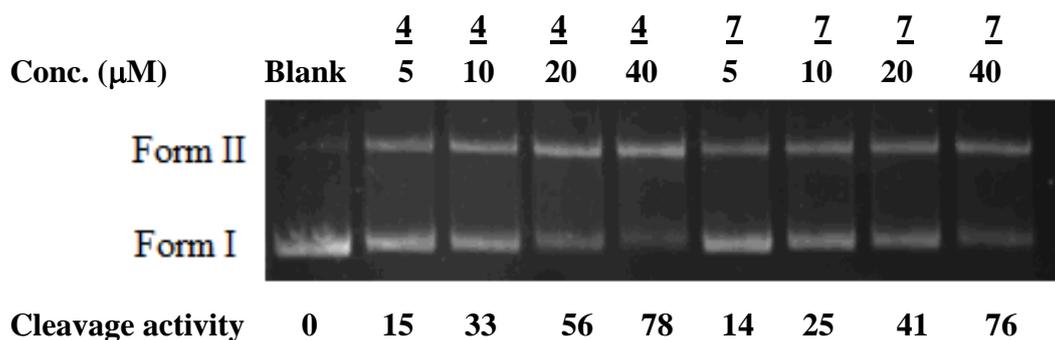
As shown in Figure 1 for H_2TPP and porphyrins **1-8**, the absorbance of DPBF at 418 nm decreased in the presence of each porphyrin with increasing illumination time. From the slope of the line, the relative rates of 1O_2 photogeneration of the porphyrins could be compared. With the increasing of the line slopes, 1O_2 yield was higher. It could be seen that the order was porphyrin **5** > porphyrins **1, 3, 4, 6-8, H_2TPP** > porphyrin **2**. The ability of photogenerating 1O_2 by the photosensitizers might be greatly affected through the interaction between the chromophoric groups [17]. The yield of 1O_2 photogeneration by porphyrins **1, 3, 4, 6-8** was similar to that of H_2TPP . The reason was that the conjugated system was not connected to the structure, but rather flexible chains were used, which made the porphyrins with oxazoles, oxadiazoles and thiadiazole rings show no significant change of 1O_2 photogeneration compared to H_2TPP . For copper (II) porphyrinate **2** no 1O_2 photogeneration was seen. This phenomenon has previously been observed in other paramagnetic metalloporphyrins with partially filled *d* orbitals, such as Ag^{II} -TPP [18] and Co^{II} -TCNPP [19], presumably due to a metal-facilitated relaxation of the porphyrin excited triplet state that precludes bimolecular quenching by oxygen. In contrast, Zn-porphyrin **5** showed a greater yield of 1O_2 , as other Zn (II)-porphyrin derivatives, and this is attributed to their correspondingly high triplet quantum yield, Φ_T [20].

Figure 1. The relation between illumination time and DPBF's absorption value ratio (A/A_0).

2.2. DNA photocleavage ability

The DNA photocleavage of porphyrins **1-8** and H₂TPP was detected by monitoring the conversion of supercoiled form (form I) to the nicked circular form (form II). Only porphyrins **4, 7** with cationic groups showed certain photocleavage activities towards DNA, with over 75% cleavage activities observed at 40 μ M (see Figure 2). The reason might be that porphyrins **4, 7** with single cationic groups can bind the anionic area of DNA via electrostatic interactions. Although porphyrins **4, 7** have relatively weaker electrostatic interaction with DNA compared with other cationic porphyrins, such as *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin, H₂TMPyP, (98% cleavage activity at 2.5 μ M [19]), porphyrins **4, 7** also showed a good DNA photocleavage activity at higher concentrations. As for the remaining porphyrins and H₂TPP, their insignificant DNA photocleavage activities (data not shown) were likely the result of a lack of a binding interaction with DNA and a more effectively oxidative attack from close range despite the high ¹O₂ yields. This suggests the importance of close-range interactions with the target for the effect of the drugs and the positive charge of porphyrin structures to affect the interaction with DNA [21,22].

Figure 2. DNA photocleavage activity of porphyrin **4** (lanes 2-5) and porphyrin **7** (lanes 6-9) as a function of its concentration. Lane 1: DNA (Form I) control; lane 2, 6: 5 μ M; lane 3, 7: 10 μ M; lane 4, 8: 20 μ M; lane 5, 9: 40 μ M for porphyrin **4** and **7**. Photo-irradiation conditions: $\lambda_{\text{irrad}} = 455$ nm; duration, 60 min.



In PDT, a good cellular uptake effect depends more on lipophilicity of a photosensitizer than on its $^1\text{O}_2$ yield [23]. For example, H_2TMPyP showed strong DNA binding and photocleavage activity, but its PDT efficacy was poor due to its poor cellular uptake. Hence, more porphyrins with both hydrophilic and lipophilic groups, such as porphyrins **4** and **7**, can be an effective PDT agent.

3. Experimental

3.1. Materials and methods

DMF was distilled from calcium hydride. 5-[4-(3-bromopropoxy)phenyl]-10,15,20-triphenylporphyrin and its Zn complex [24], 2-mercapto-5-phenyloxazole [3], 5-(3-pyridyl)-1,3,4-oxadiazole-2-thione [25], 5-(3-pyridinyl)-1,3,4-thiadiazole-2-thione [26] and 5-(2-hydroxyphenyl)-2-thioxo-1,3,4-oxadiazoline [27] were prepared according to the corresponding literature procedures. The other reagents were purchased from the China Sinopharm Company. NMR spectra were recorded with a Bruker ARX-300 (300 MHz) NMR spectrometer. Electronic absorption spectra in the UV/Vis region were recorded with a Shimadzu UV-PC 2401 spectrophotometer. The IR spectra (KBr pellets) were recorded with a Shimadzu FT-IR 3000 spectrometer. Melting points were measured with a Tianjing RY-1 melting point apparatus (the thermometer was not corrected). Elemental analyses were performed by using a Vario ELIII elemental analyzer. High-resolution mass spectra (+ve mode, CDCl_3) were recorded with a Bruker Autoflex MALDI-TOF mass spectrometer. All measurements were performed at ambient temperature (20 ± 2 °C) under atmospheric pressure.

3.2. Synthesis routes and procedures

3.2.1. Preparation of porphyrin **1**

5-[4-(3-Bromopropoxy)phenyl]-10,15,20-triphenylporphyrin (106.5 mg, 0.14 mmol) and 2-mercapto-5-phenyloxazole (25 mg, 0.14 mmol) were dissolved in dry DMF (15 mL) and anhydrous K_2CO_3 (1 g) was added to the solution. Under nitrogen protection, the mixture was heated to 65 °C for 5 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and filtered. The precipitate was purified on a silica gel column eluted with chloroform. The second fraction was the blue-violet title product. Yield: 90 mg, 76%. $^1\text{H-NMR}$ (CDCl_3) δ : -2.78 (2H, s, pyrrole, NH), 2.50~2.58 (2H, m, $-\text{CH}_2\text{CH}_2\text{CH}_2-$), 3.57~3.60 (2H, t, $J = 7.2$ Hz, $-\text{CH}_2\text{S}-$), 4.42 (2H, t, $J = 7.2$ Hz, $-\text{CH}_2\text{O}-$), 7.35~7.38 (6H, m, oxazole-PhH and oxazole-H), 7.62~7.64 (3H, m, PorPhH_p), 7.74~7.76 (8H, m, PorPhH_m), 8.10~8.12 (2H, m, PorPhH_o), 8.19~8.22 (6H, m, PorPhH_o), 8.83 (8H, s, H _{β}); UV-Vis (CHCl_3 , 20 °C), λ_{max} ($\log \epsilon$): 422 (5.58), 515 (4.18), 549 (3.85), 588 (3.70), 643 nm ($3.34 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$); IR (KBr), ν : 3316, 1600, 1242, 1173, 799 cm^{-1} ; HRMS: $m/z = 847.3006$ [$\text{M}]^+$ ($\text{C}_{56}\text{H}_{41}\text{N}_5\text{O}_2\text{S}$: calcd. 847.2981, $\Delta_m = 2.92$ ppm) Anal. Calcd for $\text{C}_{56}\text{H}_{41}\text{N}_5\text{O}_2\text{S}$: C 79.31, H 4.87, N 8.26; found C 79.42, H 4.97, N 8.33.

3.2.2. Preparation of porphyrin **2**

The complex was prepared by heating porphyrin **1** (50 mg, 0.059 mmol) at reflux with an excess amount of copper (II) acetate in $\text{CHCl}_3/\text{methanol}$ for 3 h. It was then purified by column

chromatography on silica gel with chloroform as eluent. Yield: 49 mg, 92%. UV-Vis (CHCl₃, 20 °C), λ_{\max} (log ϵ): 423 (5.86), 523 (4.83), 550 nm (4.52 dm³ mol⁻¹ cm⁻¹); IR (KBr), ν : 1601, 1508, 1242, 1175, 1000 (Cu^{II}, OSMB), 812 cm⁻¹; HRMS: m/z = 908.2150 [M]⁺ (C₅₆H₃₉N₅O₂SCu: calcd. 908.2121, Δ_m = 3.24 ppm). Anal. Calcd for C₅₆H₃₉N₅O₂SCu: C 73.95, H 4.32, N 7.70; found C 73.84, H 4.39, N 7.61.

3.2.3. Preparation of porphyrin 3

5-[4-(3-Bromopropoxy)phenyl]-10,15,20-triphenylporphyrin (106.5 mg, 0.14 mmol) and 5-(3-pyridyl)-1,3,4-oxadiazole-2-thione (27 mg, 0.14 mmol) were dissolved in dry DMF (15 mL) and anhydrous K₂CO₃ (1 g) was added to the solution. Under nitrogen protection, the mixture was heated to 65 °C for 5 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and filtered. The precipitate was purified on a silica gel column eluted with chloroform. The second fraction was the blue-violet title product. Yield: 84 mg, 70%. ¹H-NMR (CDCl₃) δ : -2.78 (2H, s, pyrrole NH), 2.55~2.59 (2H, m, -CH₂-), 3.71 (2H, t, J = 7.2 Hz, -CH₂S-), 4.43 (2H, t, J = 7.2 Hz, -CH₂O-), 7.43~7.47 (3H, m, PorPhH_p), 7.74~7.76 (8H, m, PorPhH_m), 8.10~8.13 (2H, m, -O-PorPhH_o), 8.19~8.22 (6H, m, PorPhH_o), 8.32~8.35 (1H, m, PyH₆), 8.75~8.76 (2H, m, PyH₄₋₅), 8.83 (8H, s, H _{β}), 9.26 (1H, s, PyH₂); UV-Vis (CHCl₃, 20 °C), λ_{\max} (log ϵ): 429 (5.66), 514 (5.19), 548 (4.76), 588 (4.23), 643 nm (3.68 dm³ mol⁻¹ cm⁻¹); IR (KBr) ν : 3313, 1600, 1242, 1177, 800 cm⁻¹; HRMS: m/z = 849.2923 [M]⁺ (C₅₄H₃₉N₇O₂S: calcd. 849.2886, Δ_m = 4.31 ppm). Anal. Calcd for C₅₄H₃₉N₇O₂S: C 76.30, H 4.62, N 11.53; found C 76.44, H 4.73, N 11.62.

3.2.4. Preparation of porphyrin 4

Porphyrin 3 (50 mg, 0.059 mmol) was added to CH₃I (10 mL). After heating at reflux for 4 h under nitrogen, the solution was concentrated to dryness in vacuum. The precipitate was purified on a silica gel column and eluted with chloroform and methanol (v/v 20:1). The second fraction was the blue-violet title product. Yield: 53 mg, 90%. ¹H-NMR (CDCl₃) δ : -2.85 (2H, s, Pyrrole NH), 2.53~2.56 (2H, m, -CH₂-), 3.73 (2H, t, J = 7.5 Hz, -CH₂S-), 4.42 (2H, t, J = 7.5 Hz, -CH₂O-), 4.46 (3H, s, -N-CH₃), 7.39~7.43 (3H, m, PorPhH_p), 7.72~7.75 (8H, m, PorPhH_m), 8.11~8.12 (2H, m, -O-PorPhH_o), 8.18~8.21 (6H, m, PorPhH_o), 8.34~8.36 (1H, m, PyH₆), 8.73~8.75 (2H, m, PyH₄₋₅), 8.85 (8H, s, H _{β}), 9.65 (1H, s, PyH₂); UV-Vis (CHCl₃, 20 °C), λ_{\max} (log ϵ): 426 (5.47), 514 (5.16), 548 (4.80), 586 (4.27), 645 nm (3.60 dm³ mol⁻¹ cm⁻¹); IR (KBr) ν : 3315, 1602, 1504, 1242, 1175, 805 cm⁻¹; HRMS: m/z = 991.2181 [M]⁺ (C₅₅H₄₂IN₇O₂S: calcd. 991.2165, Δ_m = 1.65 ppm). Anal. Calcd for C₅₅H₄₂IN₇O₂S: C 66.60, H 4.27, N 9.88; found C 66.54, H 4.33, N 9.91.

3.2.5. Preparation of porphyrin 5

Zn^{II}(II), 5-[4-(3-bromopropoxy)phenyl]-10,15,20-triphenylporphyrinate (50 mg, 0.061 mmol) and 5-phenyl-2-sulfydryl-1,3,4-oxadiazole (11 mg, 0.061 mmol) were dissolved in dry DMF (20 mL) and anhydrous potassium carbonate (1 g) was added to the solution. Under nitrogen protection, the mixture was heated to 65 °C for 4 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform. The second fraction was the blue-violet title product. Yield: 40 mg,

71%. $^1\text{H-NMR}$ (CDCl_3), δ : 2.53~2.59 (2H, m, - $\text{CH}_2\text{CH}_2\text{CH}_2$ -), 3.68 (2H, t, $J = 7.5$ Hz, $-\text{CH}_2\text{S}$ -), 4.43 (2H, t, $J = 5.1$ Hz, $-\text{CH}_2\text{O}$ -), 7.28~7.29 (3H, m, oxadiazole- $\text{PhH}_{m,p}$), 7.51~7.52 (3H, m, PorPhH_p), 7.73~7.76 (8H, m, PorPhH_m), 8.03~8.06 (2H, m, oxadiazole- PhH_o), 8.10~8.13 (2H, m, $-\text{O-PorPhH}_o$), 8.20~8.22 (6H, m, PorPhH_o), 8.92 (8H, s, H_β); UV-Vis (CHCl_3 , 20 °C), λ_{max} ($\log \epsilon$): 427 (5.80), 521 (4.45), 554 nm ($3.76 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$); IR (KBr) ν : 3316, 1598, 1240, 1174, 996 (Zn^{II} , OSMB), 810 cm^{-1} ; HRMS: $m/z = 910.2103 [\text{M}]^+$ ($\text{C}_{55}\text{H}_{38}\text{N}_6\text{O}_2\text{SZn}$: calcd. 910.2068, $\Delta_m = 3.87$ ppm). Anal. Calcd for $\text{C}_{55}\text{H}_{38}\text{N}_6\text{O}_2\text{SZn}$: C 72.40, H 4.20, N 9.21; found C 72.53, H 4.31, N 9.25.

3.2.6. Preparation of porphyrin 6

5-[4-(3-Bromopropoxy)phenyl]-10,15,20-triphenylporphyrin (106 mg, 0.14 mmol) and 5-(3-pyridinyl)-1,3,4-thiadiazole-2-thione (27 mg, 0.14 mmol) were dissolved in dry DMF (15 mL) and anhydrous potassium carbonate (1 g) was added to the solution. Under nitrogen protection, the mixture was heated to 65 °C for 6 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform. The second fraction was the blue-violet title product. Yield: 92 mg, 76%. $^1\text{H-NMR}$ (CDCl_3) δ : -2.78 (2H, s, pyrrole NH), 2.55~2.59 (2H, m, $-\text{CH}_2$ -), 3.76~3.81 (2H, t, $J = 6.0$ Hz, $-\text{CH}_2\text{S}$ -), 4.42~4.45 (2H, t, $J = 6.0$ Hz, $-\text{CH}_2\text{O}$ -), 7.28~7.31 (5H, m, PorPhH_p and $\text{PyH}_{4,5}$), 7.35~7.40 (1H, m, PyH_6), 7.75~7.77 (8H, m, PorPhH_m), 8.11~8.14 (2H, m, $-\text{O-PorPhH}_o$), 8.20~8.23 (6H, m, PorPhH_o), 8.53 (1H, s, PyH_2), 8.84 (8H, s, H_β); UV-Vis (CHCl_3 , 20 °C), λ_{max} ($\log \epsilon$): 424 (5.66), 513 (4.32), 550 (3.85), 586 (3.77), 633 nm ($3.56 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$); IR (KBr) ν : 3315, 1601, 1503, 1243, 1176, 803 cm^{-1} ; HRMS: $m/z = 865.2693 [\text{M}]^+$ ($\text{C}_{54}\text{H}_{39}\text{N}_7\text{OS}_2$: calcd. 865.2658, $\Delta_m = 4.05$ ppm). Anal. Calcd for $\text{C}_{54}\text{H}_{39}\text{N}_7\text{OS}_2$: C 74.89, H 4.54, N 11.32; found C 75.01, H 4.65, N 11.38.

3.2.7. Preparation of porphyrin 7

Porphyrin 6 (50 mg, 0.058 mmol) was added to CH_3I (10 mL). After heating at reflux for 4 h under nitrogen, the solution was concentrated to dryness in vacuum. The precipitate was purified on a silica gel column and eluted with chloroform and methanol (v/v 20:1). The second fraction was the blue-violet title product. Yield: 54 mg, 92%. $^1\text{H-NMR}$ (CDCl_3) δ : -2.82 (2H, s, pyrrole NH), 2.50~2.55 (2H, m, $-\text{CH}_2$ -), 3.77 (2H, t, $J = 7.5$ Hz, $-\text{CH}_2\text{S}$ -), 4.40 (2H, t, $J = 7.5$ Hz, $-\text{CH}_2\text{O}$ -), 4.48 (3H, s, $-\text{N-CH}_3$), 7.74~7.76 (11H, m, PorPhH_m and PorPhH_p), 8.10~8.12 (2H, m, $-\text{O-PorPhH}_o$), 8.19~8.22 (6H, m, PorPhH_o), 8.83~8.85 (11H, m, H_β and $\text{PyH}_{4,6}$), 9.70 (1H, s, PyH_2); UV-Vis (CHCl_3 , 20 °C), λ_{max} ($\log \epsilon$): 423 (5.70), 514 (4.40), 548 (3.80), 588 (3.69), 638 nm ($3.40 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$); IR (KBr) ν : 3313, 1600, 1242, 1177, 805 cm^{-1} ; HRMS: $m/z = 1007.1962 [\text{M}]^+$ ($\text{C}_{55}\text{H}_{42}\text{IN}_7\text{OS}_2$: calcd. 1007.1937, $\Delta_m = 2.53$ ppm). Anal. Calcd for $\text{C}_{55}\text{H}_{42}\text{IN}_7\text{OS}_2$: C 65.53, H 4.20, N 9.73; found C 65.45, H 4.29, N 9.69.

3.2.8. Preparation of porphyrin 8

5-[4-(3-Bromopropoxy)phenyl]-10,15,20-triphenylporphyrin (106.5 mg, 0.14 mmol) and 5-(2-hydroxyphenyl)-2-thioxo-1,3,4-oxadiazoline (13 mg, 0.14 mmol) were dissolved in dry DMF (25 mL) and anhydrous potassium carbonate (1 g) was added to the solution. Under nitrogen protection,

the mixture was heated to 65 °C for 5 h. After cooling to room temperature, the reaction mixture was poured into water saturated with sodium chloride (30 mL) and filtered. The precipitate was purified on a silica gel column and eluted with chloroform. The second fraction was the blue-violet title product. Yield: 130 mg, 61%. $^1\text{H-NMR}$ (CDCl_3) δ : -2.77 (4H, s, pyrrole NH), 2.44~2.48 (2H, m, -OCH₂-CH₂-CH₂-S-), 2.54~2.57 (2H, m, -OCH₂-CH₂-CH₂-O-), 3.63 (2H, t, $J = 6.6$ Hz, -OCH₂-CH₂-CH₂-S-), 4.18 (2H, t, $J = 5.1$ Hz, -OCH₂CH₂CH₂-O-Por), 4.49 (2H, t, $J = 6.0$ Hz, Por-O-CH₂CH₂CH₂S-), 4.59 (2H, t, $J = 6.0$ Hz, Por-O-CH₂CH₂CH₂O-), 7.33~7.35 (4H, m, PhH₃₋₆), 7.54~7.61 (6H, m, PorPhH_p), 7.65~7.76 (16H, m, PorPhH_m), 7.98~8.00 (4H, m, -O-PorPhH_o), 8.05~8.07 (4H, m, -O-PorPhH_o), 8.14~8.22 (12H, m, PorPhH_o), 8.75~8.89 (16H, m, H _{β}); UV-Vis (CHCl_3 , 20 °C), λ_{max} (log ϵ): 421 (5.50), 520 (4.18), 552 (3.90), 589 (3.60), 651 nm ($3.59 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$); IR (KBr) ν : 3312, 1607, 1240, 1173, 798 cm^{-1} ; HRMS: $m/z = 1535.5677$ [M]⁺ ($\text{C}_{102}\text{H}_{74}\text{N}_{10}\text{O}_4\text{S}$: calcd. 1535.5649, $\Delta_m = 1.83$ ppm). Anal. Calcd for $\text{C}_{102}\text{H}_{74}\text{N}_{10}\text{O}_4\text{S}$: C 79.77, H 4.86, N 9.12; found C 79.68, H 4.91, N 9.20.

3.3. Measurement of singlet oxygen production rate

1,3-Diphenylisobenzofuran (DPBF) was used as a selective singlet oxygen ($^1\text{O}_2$) acceptor, which was bleached upon reaction with $^1\text{O}_2$. Eight sample solutions of DPBF in DMSO (50 μM) containing, respectively no porphyrin (control sample), H₂TPP (1 μM) and porphyrins **1-8** (1 μM) were prepared in the dark. Each sample container was covered with aluminum foil with a yellow filter (with cutoff wavelength <500 nm) on one side. The samples were then exposed to light (50 watt) through the filter. After irradiation, visible spectra of the sample solutions were measured spectrophotometrically. The normalized absorbances of DPBF at 418 nm in these samples were reported as a function of the photo-irradiation time. From this plot, the rates of $^1\text{O}_2$ production of porphyrins **1-8** relative to those of H₂TPP were determined.

3.4. DNA photocleavage assay

The DNA photocleavage activities of porphyrins **1-8** and H₂TPP were measured using the plasmid DNA relaxation assay. Briefly, the plasmid DNA (pBluescript, 0.5 μg), enriched with the covalently-closed circular or supercoiled conformer (Form I), and the one-phor-all plus buffer (10 mM Tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, pH 7.5) was vortexed. Aliquots of the DNA were pipetted into different Eppendorf tubes. Various amounts of autoclaved water (control sample) or porphyrins (test sample) were added into the Eppendorf tubes to give a final volume of 20 μL in each sample tube. The sample mixtures were then photo-irradiated at 400–450 nm for 60 min using a transilluminator (Vilber Lourmat) containing 4×15 W light tubes (Aqua Lux) with maximum emission at 435 nm. After photo-irradiation, 2 μL of the 6x sample dye solution (which contained 20% glycerol, 0.25% bromophenol blue and 0.25% xylene cyanol FF) was added to each Eppendorf tube and mixed well by centrifugation. The sample mixtures were loaded onto a 0.8% (v/v) agarose gel (Gel dimension: 13 cm \times 10 cm), with 1 \times TBE buffer (89 mM Tris-borate, 1 mM EDTA, pH 8) used as supporting electrolyte, and electrophoresized at 1.3 Vcm^{-1} for 3 h using a mini gel set (CBS Scientific Co., Model No. MGU-502T). After electrophoresis, the gel was stained with 0.5 $\mu\text{g/mL}$ ethidium

bromide solution for 30 min and then destained using deionized water for 10 min. The resulting gel image was viewed under 365 nm and captured digitally using a gel documentation system (BioRad).

4. Conclusions

Eight porphyrins with nitrogen heterocycle tails were synthesized and characterized. Compared with H₂TPP, the ¹O₂ yields of porphyrins **1-8** were measured indirectly and their DNA photocleavage activities were tested. Two porphyrins with cationic groups, **4** and **7**, which showed certain singlet oxygen yields and DNA photocleavage activities could be potential photosensitizers. The *in vitro* PDT activities against VEGFR-2 receptors and tumor angiogenesis are currently under investigation.

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Sample Availability: Samples of the porphyrin **1-8** and some intermediates are available from the authors.

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