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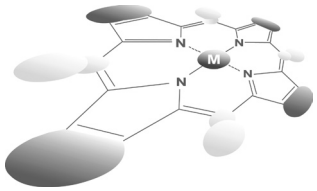

Synthesis of isoxazoline-linked chlorins and their *in vitro* cell viabilities

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ABSTRACT: A concise synthesis of isoxazoline-linked chlorins is described. This approach is carried out from methyl pyropheophorbide-a as the starting material *via* 1,3-dipolar cycloaddition of a vinyl group on the periphery with nitrile oxide to give regioselective products with excellent yields. This method represents an extensive and efficient entry into the functionalization of chlorins with a chlorophyll-α skeleton. Moreover, we have examined a preliminary *in vitro* effect of these new derivatives on mouse sarcoma S-180 cell line in photodynamic therapy.

KEYWORDS: photodynamic therapy, photosensitizer, chlorine, methyl pyropheophorbide-a, isoxazole, 1,3-dipolar cycloaddition, mouse sarcoma S-180 cell.

INTRODUCTION

Modification of the vinyl group at the 3-position in chlorins with a chlorophyll-α skeleton has been explored as an important way to develop new photosensitizers for photodynamic therapy (PDT) [1]. This double bond at the chlorin periphery has chemical properties similar to those of an ethylene moiety linked with an aromatic ring and undergoes most of the chemical reactions of a vinyl group. Several novel chlorins have been synthesized by modifying this vinyl group on the chromophore, such as its conversion to a formyl group by oxidization with osmium(III) tetroxide [2], to 1-bromoethyl by addition [3], to ethyl by reduction [4], and to a substituted cyclobutyl or condensed ring moiety by [2+2] or [4+2] cycloaddition [5]. An useful methodology for the functionalization of vinyl is the reaction with 1,3-dipolar compounds *via* a concerted *cis*-cycloaddition mechanism. An olefinic moiety can react with 1,3-dipoles, such as diazoalkanes, nitrile oxide, and azide to construct various five-membered heterocyclic structures.

In porphyrin chemistry, the 1,3-dipolar cycloaddition reaction has been used to convert porphyrin into chlorin and bacteriochlorin or to establish different chemical moieties containing heteroatoms on the periphery of an aromatic macrocycle [6]. Kozyrev *et al.* reported the application of 1,3-dipolar cycloaddition with diazomethane to the synthesis of pyrazolinyl- and cyclopropyl-substituted porphyrins and chlorins [7]. Recently, we demonstrated the reaction of methyl pheophorbide-α with diazomethane and diazoethane [8]. Until now, however, no study has reported on the same type of cycloaddition to the vinyl group of these chlorins using nitrile oxides as a 1,3-dipolar reagent to give isoxazoline derivatives. We postulated that a variety of functionalized chlorins might be synthesized from the reaction of chlorins with other 1,3-dipoles. To test this hypothesis, we synthesized isoxazoline-substituted chlorin by reacting methyl pyropheophorbide-a (MPPa) with nitrile oxides. In this study, novel isoxazoline-linked chlorins, a promising versatile starting material for further functionalization by chemical transformations, were synthesized *via* the 1,3-dipolar cycloaddition reaction of chlorins with nitrile oxide. We also carried out an *in vitro* assay of cell viability on

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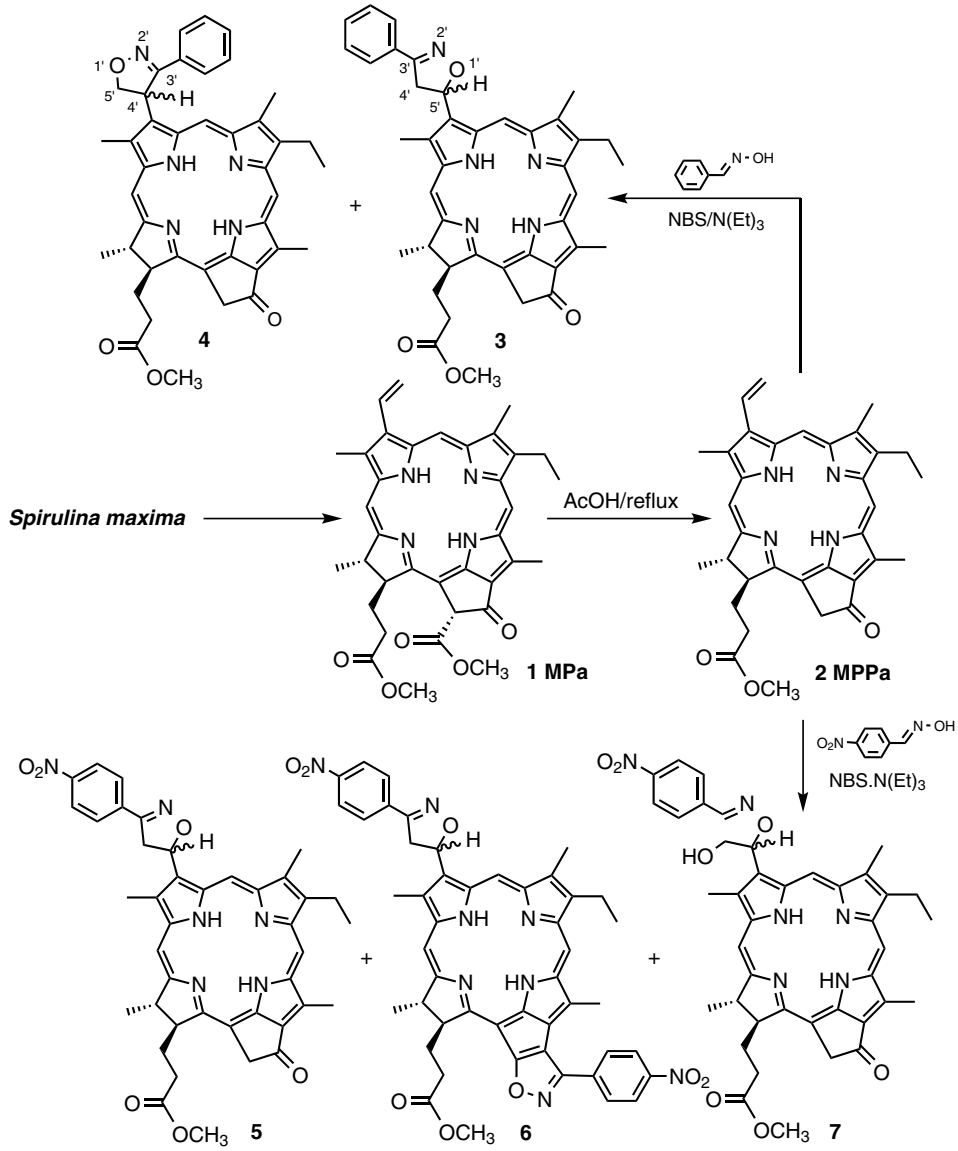


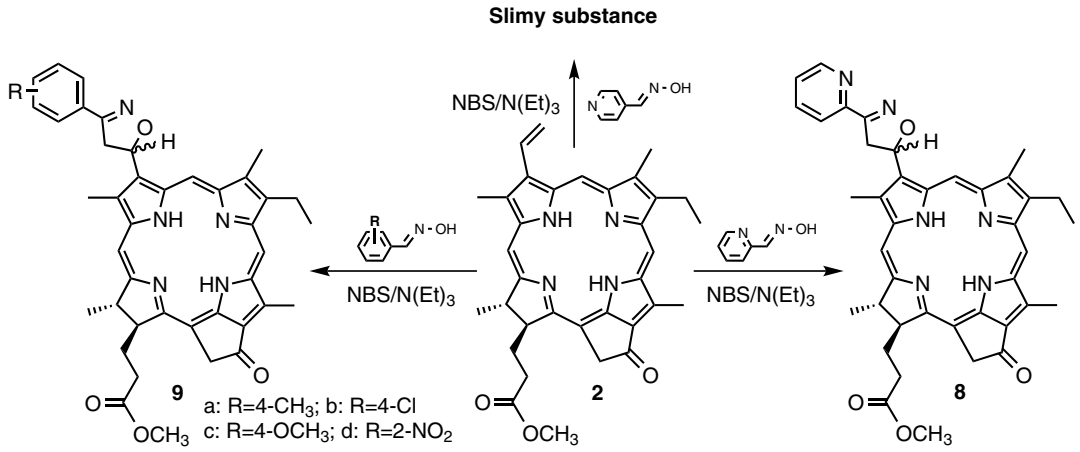
Scheme 1.

mouse sarcoma S-180 cell line with the aim of examining preliminary PDT activities of these new derivatives.

RESULTS AND DISCUSSION

Synthesis and characterization

Methyl pheophorbide-a (**1**, **MPa**) as dipolarphile was extracted from the alga Spirulina maxima [9] and converted into methyl pyropheophorbide-α (**2**, **MPPa**) by thermal demethoxycarbonylation in refluxing acetic acid. The corresponding hydroximoyl bromides were first obtained by treating aromatic aldoximes with *N*-bromosuccinimide (NBS) in an equimolar ratio in tetrahydrofuran at room temperature. Excess MPPa **2** was added to these precursors before generating the nitrile oxide by the slow addition of triethylamine over a period of 15 min. Completion of the reaction was assessed using thin layer chromatography (TLC). Under these conditions, 3-[5′(R/S)-isoxazolinyl]-substituted chlorins were obtained in excellent yields. The reaction results depended on the choice of different nitrile oxide as 1,3-dipoles in this cycloaddition. During the course of these investigations, we observed that treatment of MPPa **2** with phenyl aldoxime under the same reaction conditions generated chlorin **3** (68%) as the major adduct and chlorin **4** (3%) as a trace amount of the constitutional isomer. The formation and distribution of the product in these reactions indicated that the 1,3-dipolar cycloaddition reaction of chlorin derivative **2** with nitrile oxide at the 3-vinyl group is particularly regioselective. We also attempted to use *p*-nitrophenyl aldoxime as a precursor to nitrile oxide in this 1,3-dipolar cycloaddition of MPPa **2** to afford a mixture including standard adduct **5** (12%), the double cycloaddition product **6** (9%), and the cycloaddition-ring opening product **7** (58%). Compared to the 1,3-polar cycloaddition of **MPa 1** with *p*-nitrobenzonitrile oxide,



Scheme 2.

this result showed that the 4-nitro group of benzonitrile oxide has an important role in this reaction and prompted us to study the 1,3-polar cycloaddition of chlorin derivatives using another nitrile oxide linked with different groups.

We explored the 1,3-dipolar cycloaddition of MPPa **2** with other nitrile oxide. The results of several attempted cyclizations with various substituted benzonitrile oxides to optimize the conditions are listed in Table 1. The reactions with various aromatic aldoximes were monitored until **2** had disappeared on TLC. With bases such as K_2CO_3, NaOH, and NaOMe, the yield of adduct was lower than that when Et_3N was used. With 4- or 2-substituted phenyl aldoxime or 2-pyridinyl aldoxime, chlorins **8** and **9a–d** were formed with yields of 48% to 72% at different times without any identifiable side products [10]. The reaction results of chlorin with these 1,3-polar cycloadditions were definitely related to the polarity of the dipolar compounds. Surprisingly, the 1,3-dipolar cycloaddition of MPPa **2** with active hydroximoyl bromides, prepared from *p*-nitrophenyl aldoxime, can occur in two regions. Chlorin **5** was formed by formal cycloaddition with an ethylenic linkage at the 3-position in the yields of 38%, and chlorin **6** was generated by cycloaddition with an enolic double bond in the exocyclic ring with relatively low yields. The cycloaddition-ring opening product **7** derived from chlorin **5** was obtained in the high yield. However, when the reaction was carried out using 4-pyridinyl aldoxime as a more active dipolar precursor, an inseparable slimy mixture was obtained. The higher yield and short reaction time demonstrated that the electron withdrawal of the substituted group had a positive effect on the cycloaddition. As expected, the reactions involving the peripheral functional groups of chlorin depended on the character of the substituted group linked with the aromatic aldoximes: the stronger the electron withdrawal by the aryl group linked to the nitrile oxide, the greater the reactivity. The position of the substituted groups on the aromatic aldoxime, in addition to their character, also influenced the cycloaddition (*e.g.* entry 7).

The possible formation process for **6** and **7** is outlined in Fig. 1. The 1,3-dipolar cycloaddition at 3-vinyl in the congeneric reaction of chlorin **5a** to the enolic double bond in the exocyclic ring afforded chlorin **6** *via* the dehydration of intermediate A under basic conditions. As a reasonable explanation for the appearance of chlorin **7**, we postulated that the S_N2 substitution attack at the 4-position of the isoxazolinyl group on chlorin **5** by a hydroxide ion resulted in ring-opening to form anionic intermediate B, which has an extensive delocalized region. Lastly, hydroxyliminoyl-substituted chlorin **7** was generated from this anionic intermediate *via* the seizure of a proton.

Table 1. 1,3-cycloaddition between 1 and aromatic aldoxime as the precursor for aromatic nitrile oxide

Entry	Chlorin/aromatic aldoxime	Compounds	Time, h	Yield, %
1	MPPa **2**/PhCH=NOH	**3, 4**	12	71
2	MPPa **2**/4-NO_2PhCH=NOH	**5, 6, 7**	12	79
3	MPPa **2**/2-PyrCH=NOH	**8**	18	70
4	MPPa **2**/4-CH_3PhCH=NOH	**9a**	24	48
5	MPPa **2**/4-ClPhCH=NOH	**9b**	24	72
6	MPPa **2**/4-OCH_3PhCH=NOH	**9c**	24	57
7	MPPa **2**/2-NO_2PhCH=NOH	**9d**	48	44

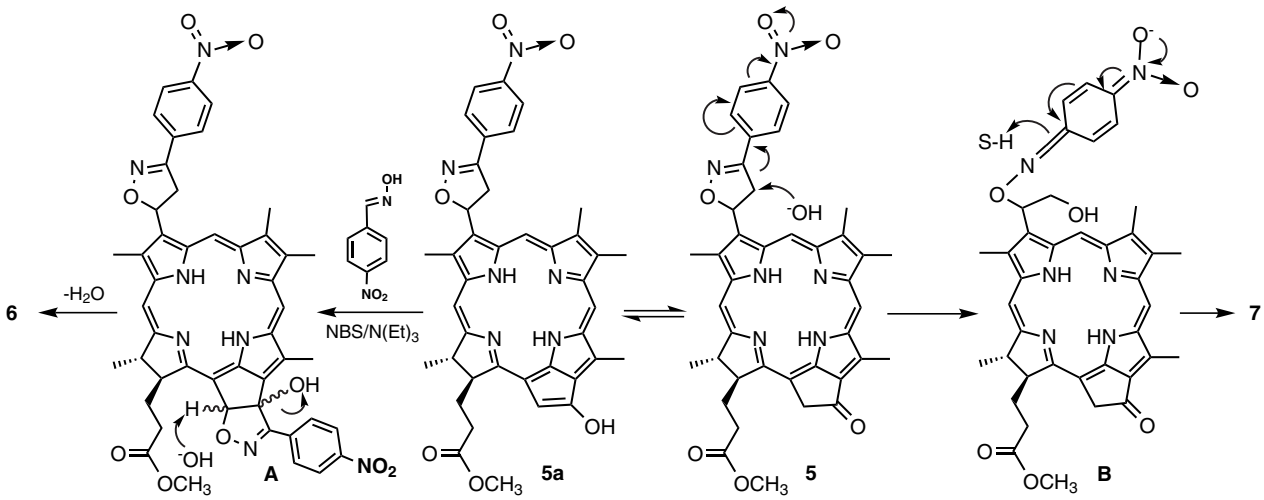


Fig. 1. Possible mechanism of the production of compounds **6** and **7**

All standard adducts comprised a pair of epimers and their certain chemical shifts appeared in pairs in equal intensities. The ^{1}H NMR analyses of **3** and **4** clearly indicate they were a pair of regioisomers. The chemical shifts of the protons, linked in C3-position, were found at δ = 7.00 and 6.85(6.86) ppm, while the two *gem*-carbon protons of C3-heterocyclic ring appeared at δ = 3.95, 4.15 and 5.02, 5.34 ppm, respectively. These oppositely shifted absorption peaks of isoxazsline rings showed their attaching positions to the periphery of chlorins. In the ^{1}H NMR spectrum of chlorin **6** the chemical shifts of the *gem*-protons at 13^2-position were not discovered and the eight *AB*-type phenyl group signals appeared at δ = 8.25, 8.13, 8.06, 7.13 ppm, each of which was ascribable to two protons on symmetrical position of *p*-substituted phenyl group. These ^{1}H NMR data demonstrated that the 1,3-dipolar cycloaddition of chlorin **6** with nitrile oxide formed isoxazol ring fused with the exocyclic *E*-ring. Compared with the Qy band (664 nm) of normal adduct **5**, the Qy absorption of **6** shifted to 678 nm due to the expansion of π-system by combining with the conjugated heterocyclic ring. The ^{1}H NMR spectra of chlorin **7** as a pair of regioisomers showed two $C13^2$-methylene signals with large coupling constant (δ = 5.26 and 5.13 ppm, J = 19.9 Hz) to indicate that the original exocyclic ring was still intact, but newly linked *p*-nitrobenzaldehyde oxime moiety showed two sets of *AB*-type phenyl peaks at δ = 8.06, 7.64 ppm and a 1H singlet [8.38(8.46) ppm] corresponding to the proton attached to C=N of the aldehyde oxime, which reflected C3-vinyl group reacted with nitrile oxide. The C3a-H triplet appeared at δ = 7.00, and mutual coupled two *gem*-protons of hydroxymethyl group linked in 3a-position showed signals with large coupling constant (J = 10.5 Hz) at δ = 4.59 and 4.35 ppm, respectively.

In vitro photosensitizing efficacy

In the present study, viability of the cell using the methodology reported by Ahn *et al*. [11] was determined by a comparison with that of mTHPC and MPPa for new photosensitizers on mouse sarcoma S-180 cell line at 0.01, 0.1, 1 and 10 μM after PDT (Fig. 2). For PDT treatment, compounds **7** and **8** among the tested photosensitizers showed 20.3% and 21.7% cell viability at 0.1 μM after PDT, respectively. With an increase in the concentration of

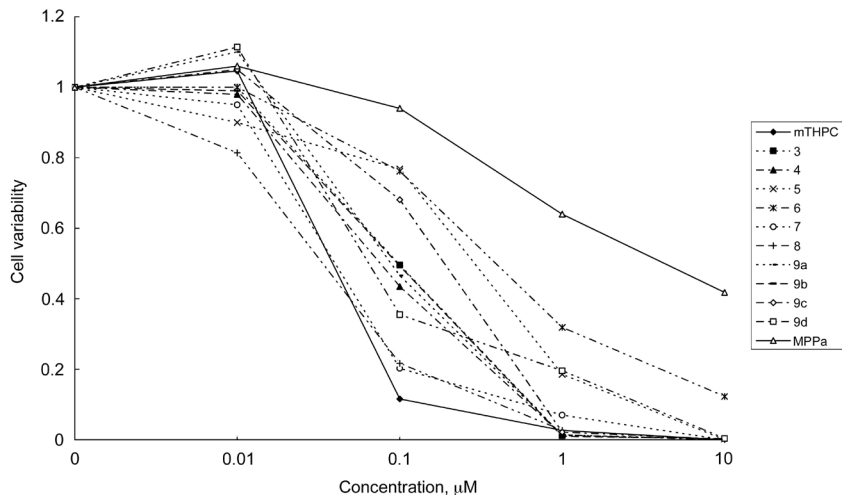


Fig. 2. Cell viability results of photosensitizers on mouse sarcoma S-180 cell line. Compounds were tested at 0.01, 0.1, 1 and 10 μM concentrations in triplicate. mTHPC was used as a reference compound. Mouse sarcoma S-180 cell line at 80% confluency in 96-well plates was used for *in vitro* PDT tests. Cells were illuminated with a lamp in a wavelength range of 640–700 nm, with a peak at 660 nm, for 20 min. Total light dose was 8.4 J. Statistical analyses were performed using unpaired Student's *t* test

Table 2. IC_{50} results of photosensitizers on mouse sarcoma S-180 cell line

Compound	mTHPC	**3**	**4**	**5**	**6**	**7**
IC_{50} (μM)	0.064	0.098	0.091	0.495	0.601	0.067
Compound	**8**	**9a**	**9b**	**9c**	**9d**	**MPPa**
IC_{50} (μM)	0.056	0.096	0.099	0.345	0.085	6.812

the photosensitizer, the cell viability revealed decreasing results, for example, the cell viability for compounds **7** and **8** was 20.3% and 21.7% at 0.1 μM and 7.0% and 2.7% at 1 μM after PDT, respectively. As for reference compound mTHPC, it showed a promising effect after PDT. From the experimental results, we observed that all compounds showed an improved effect for cell death or cell viability as the concentration of the photosensitizer increased. And all compounds obtained showed better effect than that of MPPa. Among all the compounds we obtained, compounds **7** and **8** showed high effects when compared with the others.

Table 2 shows the IC_{50} values of these new photosensitizers on mouse sarcoma S-180 cell line after PDT. The reference compound mTHPC showed a promising effect after PDT (IC_{50} = 0.064 μM), another reference compound MPPa showed a relative low effect after PDT (IC_{50} = 6.812 μM), compounds **7** and **8** among the tested photosensitizers showed relatively high PDT effect (IC_{50} = 0.067 and 0.056 μM, respectively). compounds **8** was even better than the reference compound mTHPC. While the double cycloaddition product **6** showed relatively low PDT effect (IC_{50} = 0.601 μM) among the tested photosensitizers, which probably due to its relatively higher lipophilic value than other 3-[5′(R/S)-isoxazolinyl]-substituted products. Furthermore, we are aiming to explore, in greater depth, the other biological effects of these compounds for PDT.

In conclusion, we developed a new synthesis of isoxazoline-linked chlorins from methyl pheophorbide-a *via* cyclization with various aromatic nitrile oxides. The synthetic methodology utilizing the 1,3-cycloaddition of vinyl-substituted chlorins lays the groundwork for important peripheral functionalization of the tetrapyrrolic ring system. This reaction conveniently constructed five-membered heterocycle moieties on the macrocycle, and these modifications to the parent ring of chlorin derivatives may be useful for generating novel new photosensitizers for PDT. Among all the compounds we obtained, compounds **7** and **8** showed relatively high PDT effects (IC_{50} = 0.067 and 0.056 μM, respectively). Compound **8** (IC_{50} = 0.056 μM) was even better than the reference compound mTHPC (IC_{50} = 0.064 μM).

EXPERIMENTAL

General

The ^{1}H NMR spectra were recorded on a Varian-400 MHz spectro-meter in solvent $CDCl_3$ at 400 MHz. Chemical shifts are in δ values *versus* the internal standard tetramethylsilane and J values in Hz. The IR spectra were measured with a Shimadzu FTIR 8300 instrument. Elemental analysis were performed on a Perkin Elmer 240-C microanalyzer. All chemical reagents were commercially available and purified with standard methods before use. Methyl pyropheophorbide-a **1** was obtained according to the method used by Smith [9]. All reactions were monitored by thinlayer chromatography (TLC) using Merck Silica gel 60 F254 precoated (0.2 mm thickness) glass-backed sheets. Silica gel 60 (70–230 or 230–400 mesh, Merck) and neutral alumina (~150 mesh, 58 A^0) were used for column chromatography. The alumina was deactivated with 10% water (Brockmann Grade IV) before use.

General procedure for the synthesis of isoxazoline-linked chlorins

For each reaction, we prepared 3-[5′(*R*/*S*)-(3′-aryl-isoxazolinyl]-substituted chlorin. The *N*-bromosuccinimide (NBS) (1.3 mmol) and the corresponding aromatic aldoxime (1.5 mmol) were dissolved in dry dichloromethane (2 mL) in a 50-mL two-necked round-bottom flask equipped with a magnetic stirrer and stirred for 20 min to finish the bromination. MPPa 2 (0.4 mmol) was added to this mixture, and then triethylamine (1.5 mmol) in 2 mL of dry dichloromethane was added slowly dropwise under nitrogen at room temperature over 15 min. The resulting mixture was stirred under nitrogen at the same temperature and monitored using TLC. The reaction mixture was partitioned with dichloromethane and water. The obtained organic phase was washed with 1 N HCl solution and brine, and dried over Na_2SO_4. The crude products were purified by short column chromatography over silica gel using *n*-hexane and ethyl acetate (6:1-2:1) as the eluent.

3. Yield: 68%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 410 (1.31), 505 (0.14), 536 (0.13), 608 (0.12), 666 (0.68). ^{1}H NMR (400 MHz, $CDCl_3$): δ, ppm -1.95, 0.30 (each br s, 2H, NH), 1.57 (t, *J* = 7.4 Hz, 8^b-CH_3), 1.79 (1.78) (3H, d, *J* = 7.0 Hz, 18-CH_3), 2.14~2.29, 2.48~2.67 (each m, 4H, 17^a + 17^b-H), 3.44 (q, *J* = 7.4 Hz, 8^a-CH_2), 2.91 (2.90), 3.35, 3.46 (3.44), 3.59 (each s, 12H, CH_3 + OCH_3), 3.95 (dd, *J* = 9.8, 11.7 Hz, 1H, 4′-H), 4.15 (td, *J* = 4.6, 11.7 Hz, 1H, 4′-H), 4.22 (d, *J* = 8.7 Hz, 1H, 18-H), 4.43 (q, *J* = 7.3 Hz, 1H, 17-H), 5.03 (d, *J* = 19.8 Hz, 1H, 13^2-H), 5.17 (dd, *J* = 2.1, 19.8 Hz, 1H, 13^2-H), 7.00 (t, *J* = 10.4 Hz, 1H, 5′-H), 7.42~7.48 (m, 3H, Ph-H), 7.81~7.87 (m, 2H, Ph-H), 8.54, 9.19 (9.17), 9.25 (9.21) (each s, each 1H, *meso*-H). IR (KBr): ν, cm^{-1} 2958~2856 (C-H), 1735 (C=O), 1691 (C=N), 1620 (C=C). Anal. calcd. for $C_{41}H_{41}N_5O_4$: C 73.74; H 6.19; N 10.49. Found: C 73.70; H 6.25; N 10.55.

4. Yield: 3%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 411 (1.31), 506 (0.14), 537 (0.13), 608 (0.10), 665 (0.68). ^{1}H NMR (400 MHz, $CDCl_3$): δ, ppm -1.75, 0.40 (each br s, 2H, NH), 1.66 (t, *J* = 7.7 Hz, 8^b-CH_3), 1.81 (1.80)

(d, J = 7.2 Hz, 18-CH_3), 2.22~2.38, 2.51~2.74 (each m, 4H, 17^a + 17^b-H), 3.63 (q, J = 7.7 Hz, 2H, 8^a-CH_2), 2.96 (2.95), 3.39, 3.61 (3.60), 3.66 (each s, 12H, OCH_3 + CH_3), 4.45-4.53 (m, 17-H + 18-H), 5.02 (td, J = 9.9, 3.1 Hz, 1H, 4′-H), 5.11 (d, J = 19.8 Hz, 1H, 13^2-H), 5.27 (d, J = 19.8 Hz, 1H, 13^2-H), 5.34 (t, J = 9.9 Hz, 1H, 4′-H), 6.86 (6.85) (t, J = 10.7 Hz, 5′-H), 7.51~7.68 (m, 3H, Ph-H), 8.32 (dd, J = 8.4, 1.3 Hz, 2H, Ph-H), 8.55, 9.37, 9.49 (9.48) (each s, each 1H, *meso*-H). IR (KBr): ν, cm^{-1} 2962~2875 (C-H), 1730 (C=C), 1689 (C=N), 1627 (C=C). Anal. calcd. for $C_{41}H_{41}N_5O_4$: C 73.74; H 6.19; N 10.49. Found: C 73.53; H 6.31; N 10.66.

5. Yield: 12%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 410 (1.30), 506 (0.13), 536 (0.12), 608 (0.09), 664 (0.66). ^{1}H NMR ($CDCl_3$): δ, ppm -1.86, 0.15 (each br s, 2H, NH), 1.65 (t, J = 7.5 Hz, 8^b-CH_3), 1.82 (d, J = 7.3 Hz, 3H, 18-CH_3), 2.24~2.36, 2.54~2.64, 2.66~2.77 (each m, 4H, 17a + 17b-H), 3.03, 3.44, 3.61, 3.66 (each s, each 3H, OCH_3 + CH_3), 3.63 (q, 2H, J = 7.7 Hz, 8^a-CH_2), 4.09 (dd, 1H, J = 17.1, 10.5 Hz, 4′-H), 4.32 (d, 17.1, 4′-H), 4.27~4.35 (m, 1H, 17-H), 4.51 (q, J = 7.3 Hz, 1H, 18-H), 5.13, 5.27 (each d, 2H, J = 19.9 Hz, 13^2-H), 7.24 (t, 1H, J = 10.5 Hz, 5′-H), 8.05 (dd, 1H, J = 8.8, 2.9 2H, Ph-H), 8.35 (dd, 1H, J = 8.8, 3.2 Hz, 2H, Ph-H), 8.61, 9.31, 9.51 (each s, each 1H, *meso*-H). IR (KBr): ν, cm^{-1} 2945~2880 (C-H), 1738 (C=C), 1702 (C=N), 1620 (C=C). Anal. calcd. for $C_{41}H_{40}N_6O_6$: C 69.09; H 5.66; N 11.79. Found: C 68.98; H 5.73; N 11.90.

6. Yield: 9%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 414 (1.31), 518 (0.10), 550 (0.17), 618 (0.05), 678 (0.67). ^{1}H NMR ($CDCl_3$): δ, ppm -1.96, -1.44 (each br s, 2H, NH), 1.73 (t, J = 7.8 Hz, 8^b-CH_3), 1.80 (d, J = 7.2 Hz, 18-CH_3), 2.12~2.24, 2.46~2.62 (each m, 4H, 17a + 17b-H), 3.73 (q, J = 7.5 Hz, 2H, 8^a-CH_2), 3.35, 3.58 (3.57), 3.68, 3.74 (each s, each 3H, OCH_3 + CH_3), 4.22~4.38 (m, 2H, 18H + 4′-H), 4.58~4.67 (m, 4′-H), 4.87~4.93 (m, 1H, 17-H), 7.09 (t, J = 6.8 Hz, 5′-H), 7.70 (d, J = 8.5 Hz, 2H, Ph-H), 8.06 (d, J = 8.5 Hz, 2H, Ph-H), 8.13 (d, J = 8.5 Hz, 2H, Ph-H), 8.25 (d, J = 8.5 Hz, 2H, Ph-H), 8.53, 9.62, 9.90 (9.89) (*meso*-H). IR (KBr): ν, cm^{-1} 2985~2878 (C-H), 1740 (C=O), 1693 (C=N), 1615 (C=C). Anal. calcd. for $C_{48}H_{42}N_8O_8$: C 67.12; H 4.93; N 13.05. Found: C 67.29; H 4.98; N 13.10.

7. Yield: 58%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 410 (1.32), 506 (0.13), 536 (0.12), 608 (0.09), 666 (0.63). ^{1}H NMR ($CDCl_3$): δ, ppm -1.89, 0.18 (each br s, 2H, NH), 1.70 (t, J = 7.6 Hz, 8^b-CH_3), 1.81 (d, J = 7.0 Hz, 3H, 18-CH_3), 2.21~2.34, 2.48~-2.59, 2.62~2.74 (each m, 4H, 17a + 17b-H), 3.31, 3.52, 3.60, 3.65 (each s, each 3H, OCH_3 + CH_3), 3.70 (q, 2H, J = 7.6 Hz, 8^a-CH_2), 4.31 (d, J = 10.4 Hz, 3b-H), 4.35 (td, J = 10.5 Hz, 5.4 Hz, 1H, 3b-H), 4.50 (q, J = 7.3 Hz, 1H, 18-H), 4.59 (dd, J = 10.5 Hz, 2.1 Hz, 3b-H), 5.13, 5.26 (each d, 2H, J = 19.9 Hz, 13^2-H), 7.00 (t, 1H, J = 6.8 Hz, 3a-H), 7.64 (d, 1H, J = 8.8 Hz, 2.1 Hz, 2H, Ph-H), 8.06 (d = d, 1H, J = 8.9 Hz, 1.6 Hz, 2H, Ph-H), 8.48 (8.46) (s, 1H, H-C=CN-OH), 8.63, 9.52 (9.51), 9.57 (each s, each 1H, *meso*-H). IR (KBr): ν, cm^{-1} 2968~2866 (C-H), 1728 (C=O), 1689 (C=N), 1624 (C=C). Anal. calcd. for $C_{41}H_{42}N_6O_7$: C, 67.38; H, 5.79; N, 11.50. Found: C, 67.45; H, 5.89; N, 11.54.

8. Yield: 70%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 410 (1.31), 506 (0.13), 536 (0.12), 608 (0.09), 666 (0.65). ^{1}H NMR ($CDCl_3$): δ, ppm -1.80, 0.32, (each br s, 2H, NH), 1.65(t, J = 7.7 Hz, 8^b-CH_3), 1.81 (d, J = 6.9 Hz, 3H, 18-CH_3), 2.00~2.12, 2.24~2.38, 2.52~2.74 (each m, 4H, 17a + 17b-H), 3.04, 3.43, 3.61, 3.65 (each s, each 3H, OCH_3 + CH_3), 3.62 (q, 2H, J = 7.7 Hz, 8^a-CH_2), 4.14~4.26 (m, 1H, 18-H), 4.30 (d, J = 9.1 Hz, 1H, 17-H), 4.40, 4.51 (each d, 2H, J = 12.0 Hz, 4′-H), 5.12, 5.27 (each d, 2H, J = 19.9 Hz, 13^2-H), 7.20 (t, 1H, J = 12.0 Hz, 5′-H), 7.36~7.44 (m, 1H, Pyr-H), 7.90 (t, 1H, J = 7.9 Hz, Pyr-H), 8.39 (d, 1H, J = 7.9 Hz, Pyr-H), 8.64~8.67 (m, 1H, Pyr-H), 8.59, 9.38, 9.47 (each s, each 1H, *meso*-H). IR (KBr): ν, cm^{-1} 2980~2893 (C-H), 1740 (C=O), 1699 (C=N), 1621 (C=C). Anal. calcd. for $C_{40}H_{40}N_6O_4$: C 71.84; H 6.03; N 12.57. Found: C 71.90; H 6.08; N 12.71.

9a. Yield: 48%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 411 (1.32), 506 (0.12), 536 (0.12), 607 (0.11), 665 (0.70). ^{1}H NMR ($CDCl_3$): δ, ppm -1.83, 0.28 (each br s, 2H, NH), 1.61 (t, J = 7.5 Hz, 8^b-CH_3), 1.81 (d, J = 7.2 Hz, 18-CH_3), 1.98~2.12, 2.19~2.34, 2.47~2.72 (each m, 4H, 17a + 17b-H), 2.43 (s, 3H, Ph-CH_3), 3.58 (q, J = 7.5 Hz, 2H, 8^a-CH_2), 3.01, 3.40, 3.59, 3.60 (each s, each 3H, OCH_3 + CH_3), 4.02 (dd, J = 10.0, 7.0 Hz, 1H, 4′-H), 4.21~4.32 (m, 2H, 18-H + 4′-H), 5.09 (d, J = 19.8 Hz, 1H, 13^2-H), 5.25 (d, J = 19.8 Hz, 1H, 13^2-H), 7.08 (t, J = 11.8 Hz, 5′-H), 7.29 (d, J = 7.4 Hz, 2H, Ph-H), 7.78 (d, J = 7.4 Hz, 2H, Ph-H), 8.56, 9.35 (9.34), 9.39 (9.38) (*meso*-H). IR (KBr): ν, cm^{-1} 2980~2890 (C-H), 1741 (C=O), 1699 (C=N), 1620 (C=C). Anal. calcd. for $C_{42}H_{43}N_5O_4$: C 73.99; H 6.36; N 10.27. Found: C 74.05; H 6.41; N 10.30.

9b. Yield: 72%. UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 411 (1.31), 507 (0.12), 536 (0.12), 607 (0.11), 665 (0.72). ^{1}H NMR ($CDCl_3$): δ, ppm -1.85, 0.28 (each br s, 2H, NH), 1.64 (t, J = 7.5 Hz, 8^b-CH_3), 1.81 (d, J = 7.2 Hz, 18-CH_3), 2.21~2.35, 2.50~2.74 (each m, 4H, 17a + 17b-H), 3.59 (q, J = 7.5 Hz, 2H, 8^a-CH_2), 3.02, 3.41, 3.61, 3.63 (each s, each 3H, OCH_3 + CH_3), 4.02 (dd, J = 17.1, 10.0 Hz, 1H, 4′-H), 4.22 (dd, J = 17.1, 12.0 Hz, 1H, 4′-H), 4.30 (t, J = 6.6 Hz, 18-H), 4.49 (q, J = 7.2 Hz, 17-H), 5.11 (d, J = 19.8 Hz, 1H, 13^2-H), 5.26 (d, J = 19.8 Hz, 1H, 13^2-H), 7.13 (t, J = 10.3 Hz, 5′-H), 7.47 (d, J = 8.4 Hz, 2H, Ph-H), 7.82 (d, J = 8.4 Hz, 2H, Ph-H), 8.58, 9.33, 9.45 (9.46) (*meso*-H). IR (KBr): ν, cm^{-1} 2981~2890 (C-H), 1744 (C=O), 1695 (C=N), 1621 (C=C). Anal. calcd. for $C_{41}H_{40}ClN_5O_4$: C 70.12; H 5.74; N 9.97. Found: C 70.19; H 5.78; N 10.06.

9c. Yield: 57%, UV-vis ($CHCl_3$): λ_{max}, nm (ε × 10^5) 411 (1.31), 506 (0.14), 537 (0.13), 607 (0.11), 665 (0.67). ^{1}H NMR ($CDCl_3$): δ, ppm -1.82, 0.27 (each br s, 2H, NH), 1.61 (t, J = 7.6 Hz, 8^b-CH_3), 1.81 (3H, d, J = 6.9 Hz, 18- CH_3), 2.22~2.36, 2.51~2.62, 2.63~2.72 (m, 4H, 17a + 17b-H), 3.59 (q, J = 7.6 Hz, 8^a-CH_2), 3.02, 3.41, 3.58,

3.62 (each 3H, CH_3 + OCH_3), 3.87 (s, 3H, $PhOCH_3$), 4.02 (1H, dd, J = 12.0, 17.0 Hz, 4′-H), 4.25 (1H, dd, J = 9.9, 17.0 Hz, 4′-H), 4.28 (d, 1H, J = 8.9 Hz, 18-H), 4.48 (q, 1H, J = 6.5 Hz, 17-H), 5.10, 5.26 (each d, each 1H, J = 19.8 Hz, 13^2-H), 6.84~6.92 (m, 1H, 5′-H), 7.01 (d, 2H, J = 8.4 Hz, Ph-H), 7.83 (d, 2H, J = 8.4 Hz, Ph-H), 8.57, 9.36 (9.35), 9.43 (9.42) (*meso*-H). IR (KBr): ν, cm^{-1} 2977~2889 (C-H), 1740 (C=O), 1700 (C=N), 1616 (C=C). Anal. calcd. for $C_{42}H_{43}N_5O_5$: C 72.29; H 6.21; N 10.04. Found: C 72.35; H 6.28; N 10.11.

9d. Yield: 44%. UV-vis ($CHCl_3$): λ_{max}, nm ($\varepsilon \times 10^5$) 411 (1.31), 506 (0.12), 537 (0.11), 609 (0.09), 666 (0.69). ^{1}H NMR ($CDCl_3$): δ, ppm -1.85, 0.23 (br s, 2H, NH), 1.71 (t, J = 7.5 Hz, 8^b-CH_3), 1.81 (3H, d, J = 7.3 Hz, 18-CH_3), 2.21~2.34, 2.51~2.61, 2.64~2.75 (each m, 4H, 17a + 17b-H), 3.74 (q, J = 7.5 Hz, 8^a-CH_2), 3.31, 3.51, 359, 3.68 (each s, each 3H, CH_3 + OCH_3), 4.22 (d, J = 7.5 Hz, 1H, 18-H), 4.34 (dt, J = 10.6, 3.2 Hz, 1H, 4′-H), 4.50 (q, J = 7.3 Hz, 1H, 17-H), 4.56 (dd, J = 10.6, 7.4 Hz, 4′-H), 5.13 (d, J = 19.8 Hz, 1H, 13^2-H), 5.28 (dd, J = 2.1, 19.8 Hz, 1H, 13^2-H), 7.12 (m, J = 10.4 Hz, 1H, 5′-H), 7.41~7.47 (m, 2H, Ph-H), 7.69 (d, 1H, J = 6.9 Hz, Ph-H), 7.98 (d, 1H, J = 7.4 Hz, Ph-H), 8.62, 9.08 (9.07), 9.56 (*meso*-H). IR (KBr): ν, cm^{-1} 2950~2882 (C-H), 1742 (C=O), 1696 (C=N), 1618 (C=C). Anal. calcd. for $C_{41}H_{40}N_6O_6$: C 69.09; H 5.66; N 11.79. Found: C 69.14; H 5.73; N 11.82.

MTT assay for *in vitro* photosensitizing activity

The photosensitizing activity of compounds (**3**–**9d**) and mTHPC was determined in the mouse sarcoma S-180 cell line. The cells were grown in α-MEM with 10% fetal calf serum, L-glutamine, penicillin, and streptomycin. Cells were maintained in 5% CO_2, 95% air, and 100% humidity. Cells were plated in 96-well plates at a density of 5×10^3 cells per well in complete medium. After an overnight incubation at 37 °C, the photosensitizers were added at varying concentrations and incubated at 37 °C for 3 h in the dark. Prior to light treatment the cells were replaced with drug-free complete medium. Cells were then illuminated with a lamp in a wavelength range of 640–700 nm, with a peak at 660 nm, for 20 min. Total light dose was 8.4 J. After PDT, the cells were incubated for 48 h at 37 °C in the dark. Following the 48 h incubation, 10 μL of 4.0 mg/mL solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) (Sigma) dissolved in PBS was added to each well. After a 4 h incubation at 37 °C, unreacted MTT and medium were removed and 100 μL DMSO was added to solubilize the formazan crystals. The 96-well plate was read on a microtiter plate reader (ELISA-reader, BioTek, Synergy HT, USA) at an absorbance of 570 nm. The results were plotted as percentage survival of the corresponding dark (drug no light) control for each compound tested after subtracting medium only control absorbance. Each data point represents the mean from three separate experiments with six replicates at each dose, and the standard errors were less than 10%.

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