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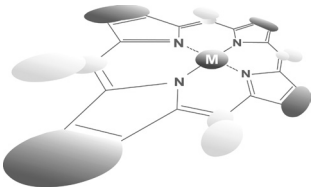

Efficient synthesis and *in vitro* photodynamic anticancer study of new purpurinimide-hydrazone conjugates

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ABSTRACT: A series of new purpurinimide-hydrazone conjugates were synthesized, and their *in vitro* anticancer efficacy against A549 lung cancer cell lines was evaluated. It was found that the incorporation of the hydrazone group to the purpurinimide could increase their anticancer activities *via* a combination of photodynamic therapy and chemotherapy.

KEYWORDS: chlorin, purpurinimide, hydrazone, photodynamic therapy, combination therapy.

INTRODUCTION

Photodynamic therapy (PDT) is a selective cancer treatment modality in which O_2, light, and a photosensitizing drug are used in combination to generate cytotoxic species, which consequently leads to cell death and tissue destruction [1–3]. Photofrin is the first photosensitizer to have received worldwide approval for the treatment of several cancers in humans [4, 5]. In recent years, several chlorin-based photosensitizers such as purpurinimide [6], HPPH [7], BPD-MA [8, 9], and NPe6 [10] have been developed and are at various stages of preclinical or advanced clinical trials. Generally, purpurinimide exhibits a long-wavelength absorption greater than 700 nm, which has attracted considerable attention in the development of new photosensitizers [5, 6, 11].

Combination therapies have increasingly received attention in the last few years because they offer dose-limited and selectivity-enhanced treatment options for cancer [12]. An important approach in the context of combination treatments involves the use of conjugates between the photosensitizer and chemotherapeutic agent. Porphyrin platinum conjugates (PPC), which consist of a porphyrin derivative and platinum fragment in the same molecule, are good examples of combination therapy agents for anticancer study [13, 14]. The hypothesis for the use of such systems is based not only on the combined effect of PDT and cytostatic activities but also on the porphyrin-mediated targeting of tumors [15, 16].

Hydrazones have been well-known to possess anticancer activity. Several studies have been published that show the antiproliferative activity of aroylhydrazone derivatives [17–19]. Hydrazones have been found to demonstrate their antiproliferative activity mainly through the inhibition of kinases [20], prevention of cell progression [21], inhibition of PI3K p110α enzymatic activity [22], generation of radicals and dissipation of the mitochondrial membrane potential [23], and inhibition of Complex III of the mitochondrial respiratory chain [24].

In this context, we decided to investigate whether the presence of a hydrazone moiety on the purpurinimide skeleton could also give rise to classes of anticancer active compounds. Therefore, in the present work, we proposed the efficient synthesis and evaluation for *in vitro* anticancer efficacy against A549 lung cancer cell lines of purpurinimide conjugates bearing a series of hydrazone moieties at the 13^2-position, with the expectation that the incorporation of these substituents would improve the anticancer activities of purpurinimide *via* the combination of photodynamic therapy and chemotherapy.

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EXPERIMENTAL

General

All of the reactions were monitored by thin layer chromatography (TLC) using 0.20-mm silica gel plates with or without a UV indicator (60F-254). Silica gel 60 (230-400 mesh, Merck) was used for flash column chromatography. The melting points (uncorrected) were measured using an Electrothermal IA9000 Series digital melting point apparatus. The electronic absorption spectra were measured using a SCINCO S-3100 UV-vis spectrophotometer. The absorption maxima (λ_{max}) values are given in nanometers and relative intensity. The ^{1}H NMR spectra were obtained using a Varian spectrometer (500 MHz). The chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane (TMS, 0 ppm), unless otherwise indicated. Elemental analyses were carried out using an automatic elemental analyzer (Flash 2000 series). Materials obtained from commercial suppliers were used without further purification. Methyl pheophorbide a (1) [25] and purpurin-18 methyl ester (2) [6] were prepared according to the procedures described in the literature.

Synthesis

Preparation of *N*-aminopurpurinimide 3. A mixture of purpurin-18 (2, 200 mg, 0.35 mmol) and hydrazine hydrate (0.1 mL) in 50 mL of dichloromethane was stirred for 5 h under a N_2 atmosphere at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with H_2O (3 × 100 mL). The organic layer was then dried over Na_2SO_4, concentrated, and purified by column chromatography using *n*-hexane and ethyl acetate (3:7) as the eluent to afford *N*-aminopurpurinimide **3** (139 mg, 67%): mp 120–123 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 421 (1.00), 515 (0.08), 555 (0.25), 661 (0.11), 714 (0.45). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.35 (s, 1H, 10-H), 9.20 (s, 1H, 5-H), 8.48 (s, 1H, 20-H), 7.83 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 6.25 (d, *J* = 17.8 Hz, 1H, 3^2-H), 6.13 (d, *J* = 11.5 Hz, 1H, 3^2-H), 5.70 (br s, 2H, 13^3-NH_2), 5.30–5.22 (m, 1H, 17-H), 4.32 (q, *J* = 7.3 Hz, 1H, 18-H), 3.67, 3.59, 3.3, 3.06 (each s, each 3H, OCH_3 + CH_3), 3.50 (q, *J* = 7.5 Hz, 2H, 8^1-CH_2), 2.82–2.69 (m, 1H, 17^{1a}-H), 2.52–2.38 (m, 2H, 17^{1b} + 17^{2a}-H), 2.01–1.88 (m, 1H, 17^{2b}-H), 1.72 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.59 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.16 (br s, 1H, NH), 0.11 (br s, 1H, NH). Anal. calcd. for $C_{34}H_{36}N_6O_4$: C 68.90; H 6.12; N 14.18. Found: C 68.93; H 6.13; N 14.20.

The general procedure for the synthesis of purpurinimide-hydrazone conjugates **5**–**16** was as follows. A mixture of *N*-aminopurpurinimide (3, 100 mg, 0.17 mmol), corresponding arylaldehyde (1.1 equiv.), and *p*-toluenesulfonic acid (10 mg) in 10 mL of dichloromethane was stirred under nitrogen for 12 h at room temperature. After concentration, the residue was purified by column chromatography using *n*-hexane and ethyl acetate (1:1–2:1) as the eluent to afford purpurinimide-hydrazone conjugates **5**–**16**.

Purpurinimide-hydrazone conjugate 5. Yield: 93%, mp 135–137 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 514 (0.12), 553 (0.27), 651 (0.14), 710 (0.45). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.34 (s, 1H, 10-H), 9.15 (s, 1H, 5-H), 8.91 (s, 1H, 13^4-H), 8.52 (s, 1H, 20-H), 8.15 (dd, *J* = 8.0, 1.4 Hz, 2H, Ph-H), 7.80 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 7.63–7.51 (m, 3H, Ph-H), 6.23 (dd, *J* = 17.8, 1.2 Hz, 1H, 3^2-H), 6.11 (dd, *J* = 11.5, 1.2 Hz, 1H, 3^2-H), 5.31 (dd, *J* = 9.3, 2.4 Hz, 1H, 17-H), 4.35 (q, *J* = 7.3 Hz, 1H, 18-H), 3.69, 3.54, 3.30, 3.01 (each s, each 3H, OCH_3 + CH_3), 3.44 (q, *J* = 7.7 Hz, 2H, 8^1-CH_2), 2.79–2.72 (m, 1H, 17^{1a}-H), 2.53–2.38 (m, 2H, 17^{1b} + 17^{2a}-H), 2.04–1.96 (m, 1H, 17^{2b}-H), 1.72 (d, *J* = 7.4 Hz, 3H, 18-CH_3), 1.56 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.04 (br s, 1H, NH), -0.07 (br s, 1H, NH). Anal. calcd. for $C_{41}H_{40}N_6O_4$: C 72.33; H 5.92; N 12.34. Found: C 72.35; H 5.90; N 12.36.

Purpurinimide-hydrazone conjugate 6. Yield: 90%, mp 128–130 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 514 (0.16), 552 (0.32), 651 (0.17), 710 (0.47). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.13 (s, 1H, 10-H), 8.99 (s, 1H, 5-H), 8.86 (s, 1H, 13^4-H), 8.50 (s, 1H, 20-H), 8.03 (d, *J* = 8.0 Hz, 2H, Ph-H), 7.70 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 7.35 (d, *J* = 7.9 Hz, 2H, Ph-H), 6.16 (dd, *J* = 17.8, 1.0 Hz, 1H, 3^2-H), 6.04 (dd, *J* = 11.5, 1.0 Hz, 1H, 3^2-H), 5.32 (dd, *J* = 9.3, 2.3 Hz, 1H, 17-H), 4.35 (q, *J* = 7.3 Hz, 1H, 18-H), 3.60, 3.55, 3.27, 2.87 (each s, each 3H, OCH_3 + CH_3), 3.25 (q, *J* = 7.6 Hz, 2H, 8^1-CH_2), 2.80-2.74 (m, 1H, 17^{1a}-H), 2.56–2.38 (m, 2H, 17^{1b} + 17^{2a}-H), 2.47 (s, 3H, Ph-CH_3), 2.06–1.93 (m, 1H, 17^{2b}-H), 1.73 (d, *J* = 7.4 Hz, 3H, 18-CH_3), 1.47 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), -0.11 (br s, 1H, NH), -0.22 (br s, 1H, NH). Anal. calcd. for $C_{42}H_{42}N_6O_4$: C 72.60; H 6.09; N 12.10. Found: C 72.67; H 6.12; N 12.11.

Purpurinimide-hydrazone conjugate 7. Yield: 90%, mp 122–124 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 513 (0.06), 552 (0.22), 651 (0.10), 710 (0.43). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.29 (s, 1H, 10-H), 9.17 (s, 1H, 5-H), 8.73 (s, 1H, 13^4-H), 8.51 (s, 1H, 20-H), 7.84 (m, 3H, 3^1-H + Ph-H), 7.01 (d, *J* = 8.5 Hz, 2H, Ph-H), 6.26 (dd, *J* = 17.9, 0.9 Hz, 1H, 3^2-H), 6.14 (dd, *J* = 11.5, 1.0 Hz, 1H, 3^2-H), 5.32–5.27 (m, 1H, 17-H), 4.35 (q, *J* = 7.2 Hz, 1H, 18-H), 4.34 (br s, 1H, OH), 3.67, 3.56, 3.32, 3.01 (each s, each 3H, OCH_3 + CH_3), 3.42 (q, *J* = 7.9 Hz, 2H, 8^1-CH_2), 2.80–2.72 (m, 1H, 17^{1a}-H), 2.54–2.40 (m, 2H, 17^{1b} + 17^{2a}-H), 2.04-1.92 (m, 1H, 17^{2b}-H), 1.75 (d, *J* = 7.4 Hz, 3H, 18-CH_3), 1.56 (t, *J* = 7.6 Hz, 3H, 8^2-CH_3), 0.17 (br s, 1H, NH), 0.01 (br s, 1H, NH). Anal. calcd. for $C_{41}H_{40}N_6O_5$: C 70.67; H 5.79; N 12.06. Found: C 70.74; H 5.75; N 12.11.

Purpurinimide-hydrazone conjugate 8. Yield: 91%, mp 140–143 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 513 (0.07), 553 (0.23), 651 (0.09), 710

(0.42). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.22 (s, 1H, 10-H), 9.07 (s, 1H, 5-H), 8.81 (s, 1H, 13^4-H), 8.52 (s, 1H, 20-H), 8.09 (d, *J* = 8.6 Hz, 2H, Ph-H), 7.75 (dd, *J* = 17.9, 11.7 Hz, 1H, 3^1-H), 7.05 (d, *J* = 8.7 Hz, 2H, Ph-H), 6.19 (d, *J* = 17.9 Hz, 1H, 3^2-H), 6.08 (d, *J* = 11.5 Hz, 1H, 3^2-H), 5.31 (dd, *J* = 9.2, 2.2 Hz, 1H, 17-H), 4.35 (q, *J* = 7.3 Hz, 1H, 18-H), 3.9, 3.64, 3.54, 3.28 (each s, each 3H, OCH_3 + CH_3), 3.33 (q, *J* = 7.9 Hz, 2H, 8^1-CH_2), 2.93 (s, 3H, 4-Ph-OCH_3), 2.82–2.71 (m, 1H, 17^{1a}-H), 2.54–2.38 (m, 2H, 17^{1b} + 17^{2a}-H), 2.03–1.95 (m, 1H, 17^{2b}-H), 1.73 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.51 (t, *J* = 7.6 Hz, 3H, 8^2-CH_3), -0.06 (br s, 1H, NH), -0.16 (br s, 1H, NH). Anal. calcd. for $C_{42}H_{42}N_6O_5$: C 70.97; H 5.96; N 11.82. Found: C 70.99; H 5.95; N 11.80.

Purpurinimide-hydrazone conjugate 9. Yield: 88%, mp 127–129 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 514 (0.13), 553 (0.28), 651 (0.15), 710 (0.45). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.44 (s, 1H, 10-H), 9.24 (s, 1H, 5-H), 8.88 (s, 1H, 13^4-H), 8.53 (s, 1H, 20-H), 8.07 (m, 2H, Ph-H), 7.85 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 7.57-7.50 (m, 2H, Ph-H), 6.27 (dd, *J* = 17.8, 1.2 Hz, 1H, 3^2-H), 6.15 (dd, *J* = 11.5, 1.2 Hz, 1H, 3^2-H), 5.29 (dd, *J* = 9.2, 2.4 Hz, 1H, 17-H), 4.34 (q, *J* = 7.2 Hz, 1H, 18-H), 3.73, 3.54, 3.33, 3.08 (each s, each 3H, OCH_3 + CH_3), 3.52 (q, *J* = 8.0 Hz, 2H, 8^1-CH_2), 2.78–2.70 (m, 1H, 17^{1a}-H), 2.51–2.36 (m, 2H, 17^{1b} + 17^{2a}-H), 2.08-1.93 (m, 1H, 17^{2b}-H), 1.71 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.60 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.15 (br s, 1H, NH), 0.03 (br s, 1H, NH). Anal. calcd. for $C_{41}H_{39}ClN_6O_4$: C 68.85; H 5.50; N 11.75. Found: C 68.90; H 5.51; N 11.77.

Purpurinimide-hydrazone conjugate 10. Yield: 88%, mp 135–137 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 514 (0.13), 554 (0.27), 651 (0.14), 712 (0.43). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.55 (s, 1H, 10-H), 9.30 (s, 1H, 5-H), 8.67 (s, 1H, 13^4-H), 8.54 (s, 1H, 20-H), 8.01–7.96 (m, 2H, Ph-H), 7.87 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 6.82–6.77 (m, 2H, Ph-H), 6.27 (dd, *J* = 17.8, 1.3 Hz, 1H, 3^2-H), 6.14 (dd, *J* = 11.5, 1.3 Hz, 1H, 3^2-H), 5.32 (dd, *J* = 9.2, 2.6 Hz, 1H, 17-H), 4.34 (q, *J* = 7.3 Hz, 1H, 18-H), 3.80, 3.53, 3.33, 3.12 (each s, each 3H, OCH_3 + CH_3), 3.60 (q, *J* = 7.6 Hz, 2H, 8^1-CH_2), 3.11 (s, 6H, Ph-N$(CH_3)_2$), 2.76–2.70 (m, 1H, 17^{1a}-H), 2.52–2.35 (m, 2H, 17^{1b} + 17^{2a}-H), 2.04–1.93 (m, 1H, 17^{2b}-H), 1.69 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.64 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.11 (br s, 1H, NH), -0.01 (br s, 1H, NH). Anal. calcd. for $C_{43}H_{6.27}N_7O_4$: C 71.35; H 6.27; N 13.54. Found: C 71.37; H 6.28; N 13.52.

Purpurinimide-hydrazone conjugate 11. Yield: 86%, mp 145-147 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 513 (0.11), 552 (0.26), 651 (0.12), 708 (0.40). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.20 (s, 1H, 10-H), 9.10 (s, 1H, 5-H), 8.90 (s, 1H, 13^4-H), 8.52 (s, 1H, 20-H), 8.26–8.21 (m, 2H, Ph-H), 8.11–8.07 (m, 2H, Ph-H), 7.77 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 6.22 (dd, *J* = 17.8, 1.0 Hz, 1H, 3^2-H), 6.11 (dd, *J* = 11.5, 1.0 Hz, 1H, 3^2-H), 5.29 (dd, *J* = 9.3, 2.2 Hz, 1H, 17-H), 4.36 (q, *J* = 7.4 Hz, 1H, 18-H), 3.59, 3.55, 3.30, 2.96 (each s, each 3H, OCH_3 + CH_3), 3.35 (q, *J* = 7.6 Hz, 2H, 8^1-CH_2), 2.81–2.72 (m, 1H, 17^{1a}-H), 2.52–2.41 (m, 2H, 17^{1b} + 17^{2a}-H), 2.00 (m, 2H, 17^{2b}-H), 1.77 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.52 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.03 (br s, 1H, NH), -0.08 (br s, 1H, NH). Anal. calcd. for $C_{41}H_{39}N_7O_6$: C 67.85; H 5.42; N 13.51. Found: C 67.88; H 5.45; N 13.49.

Purpurinimide-hydrazone conjugate 12. Yield: 89%, mp 175–177 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 513 (0.09), 552 (0.24), 651 (0.10), 710 (0.40). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.56 (s, 1H, 10-H), 9.49 (s, 1H, 5-H), 9.23 (s, 1H, 13^4-H), 8.64 (dd, *J* = 7.7, 1.5 Hz, 1H, 6′-Ph-H), 8.53 (s, 1H, 20-H), 8.24 (dd, *J* = 8.4, 1.2 Hz, 1H, 3′-Ph-H), 7.83 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 7.89–7.84 (m, 1H, 5'-Ph-H), 7.78–7.71 (m, 1H, 4′-Ph-H), 6.26 (dd, *J* = 17.8, 1.2 Hz, 1H, 3^2-H), 6.14 (dd, *J* = 11.5, 1.2 Hz, 1H, 3^2-H), 5.27 (dd, *J* = 9.2, 2.2 Hz, 1H, 17-H), 4.35 (q, *J* = 7.3 Hz, 1H, 18-H), 3.76, 3.54, 3.32, 3.08 (each s, each 3H, OCH_3 + CH_3), 3.55 (q, *J* = 7.6 Hz, 2H, 8^1-CH_2), 2.82–2.71 (m, 1H, 17^{1a}-H), 2.53–2.40 (m, 2H, 17^{1b} + 17^{2a}-H), 2.06-1.94 (m, 1H, 17^{2b}-H), 1.71 (d, *J* = 7.4 Hz, 3H, 18-CH_3), 1.62 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.21 (br s, 1H, NH), 0.06 (br s, 1H, NH). Anal. calcd. for $C_{41}H_{39}N_7O_6$: C 67.85; H 5.42; N 13.51. Found: C 67.84; H 5.44; N 13.55.

Purpurinimide-hydrazone conjugate 13. Yield: 82%, mp 141–142 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 514 (0.14), 552 (0.28), 651 (0.15), 710 (0.46). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.49 (s, 1H, 10-H), 9.24 (s, 1H, 5-H), 9.22 (s, 1H, 13^4-H), 8.53 (s, 1H, 20-H), 7.82 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 7.50 (d, *J* = 8.3 Hz, 2H, 4,6-Ph-H), 7.36 (dd, *J* = 8.6, 7.7 Hz, 1H, 5-Ph-H), 6.25 (dd, *J* = 17.8, 1.1 Hz, 1H, 3^2-H), 6.12 (dd, *J* = 11.5, 1.2 Hz, 1H, 3^2-H), 5.31 (dd, *J* = 9.2, 2.2 Hz, 1H, 17-H), 4.36 (q, *J* = 7.5 Hz, 1H, 18-H), 3.79, 3.55, 3.32, 3.07 (each s, each 3H, OCH_3 + CH_3), 3.54 (q, *J* = 8.0 Hz, 2H, 8^1-CH_2), 2.83–2.72 (m, 1H, 171a-H), 2.56–2.40 (m, 2H, 17^{1b} + 17^{2a}-H), 2.08–1.94 (m, 1H, 17^{2b}-H), 1.72 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.62 (t, *J* = 7.7 Hz, 3H, 8^2-CH_3), 0.13 (br s, 1H, NH), -0.02 (br s, 1H, NH). Anal. calcd. for $C_{41}H_{38}Cl_2N_6O_4$: C 65.69; H 5.11; N 11.21. Found: C 65.75; H 5.14; N 11.23.

Purpurinimide-hydrazone conjugate 14. Yield: 91%, mp 155–157 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 514 (0.12), 553 (0.27), 651 (0.12), 711 (0.43). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.31 (s, 1H, 10-H), 9.10 (s, 1H, 5-H), 8.75 (s, 1H, 13^4-H), 8.52 (s, 1H, 20-H), 7.96 (s, 2H, 3',5'-Ph-H), 7.76 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 6.20 (dd, *J* = 17.8, 1.2 Hz, 1H, 3^2-H), 6.08 (dd, *J* = 11.5, 1.2 Hz, 1H, 3^2-H), 5.70 (br s, 1H, OH), 5.33 (dd, *J* = 9.2, 2.7 Hz, 1H, 17-H), 4.35 (q, *J* = 7.3 Hz, 1H, 18-H), 3.70, 3.54, 3.29, 2.96 (each s, each 3H, OCH_3 + CH_3), 3.39 (q, *J* = 7.6 Hz, 2H, 8^1-CH_2), 2.79-2.70 (m, 1H, 17^{1a}-H), 2.55-2.36 (m, 2H, 17^{1b} + 17^{2a}-H), 2.05–1.96 (m, 1H, 17^{2b}-H), 1.72 (d, *J* = 7.4 Hz, 3H, 18-CH_3), 1.54 (t, *J* = 7.6 Hz, 3H, 8^2-CH_3), 1.53 (s, 18H, 2′,6′-$C(CH_3)_3$), -0.04 (br s, 1H, NH), -0.15 (br s,

1H, NH). Anal. calcd. for $C_{49}H_{56}N_6O_5$: C 72.75; H 6.98; N 10.39. Found: C 72.79; H 7.04; N 10.43.

Purpurinimide-hydrazone conjugate 15. Yield: 82%, mp 117–119 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 513 (0.11), 553 (0.26), 651 (0.13), 710 (0.43). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 9.51 (s, 1H, 10-H), 9.27 (s, 1H, 5-H), 8.78 (s, 1H, 13^4-H), 8.53 (s, 1H, 20-H), 7.86 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 7.75 (d, *J* = 1.4 Hz, 1H, 5′-H), 7.22 (d, *J* = 3.5 Hz, 1H, 3′-H), 6.65 (dd, *J* = 3.5, 1.4 Hz, 1H, 4′-H), 6.26 (dd, *J* = 17.8, 1.0 Hz, 1H, 3^2-H), 6.15 (dd, *J* = 11.5, 1.0 Hz, 1H, 3^2-H), 5.28 (dd, *J* = 9.2, 1.9 Hz, 1H, 17-H), 4.34 (q, *J* = 7.3 Hz, 1H, 18-H), 3.77, 3.55, 3.33, 3.11 (each s, each 3H, OCH_3 + CH_3), 3.57 (q, *J* = 8.0 Hz, 2H, 8^1-CH_2), 2.81–2.74 (m, 1H, 17^{1a}-H), 2.53–2.40 (m, 2H, 17^{1b} + 17^{2a}-H), 2.08–1.91 (m, 1H, 17^{2b}-H), 1.70 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.63 (t, *J* = 7.6 Hz, 3H, 8^2-CH_3), 0.17 (br s, 1H, NH), 0.04 (br s, 1H, NH). Anal. calcd. for $C_{39}H_{38}N_6O_5$: C 69.83; H 5.71; N 12.53. Found: C 69.87; H 5.74; N 12.55.

Purpurinimide-hydrazone conjugate 16. Yield: 94%, mp 139–141 °C. UV-vis ($CHCl_3$): λ_{max}, nm (rel. intensity log ε) 422 (1.00), 512 (0.06), 552 (0.19), 650 (0.06), 708 (0.33). ^{1}H NMR (500 MHz, $CDCl_3$): δ, ppm 10.14 (s, 1H, CHO), 9.44 (s, 1H, 10-H), 9.24 (s, 1H, 5-H), 9.03 (s, 1H, N=C-H), 8.53 (s, 1H, 20-H), 8.29 (d, *J* = 8.1 Hz, 2H, Ph-H), 8.06 (d, *J* = 8.2 Hz, 2H, Ph-H), 7.85 (dd, *J* = 17.8, 11.5 Hz, 1H, 3^1-H), 6.27 (dd, *J* = 17.8, 1.2 Hz, 1H, 3^2-H), 6.15 (dd, *J* = 11.5, 1.2 Hz, 1H, 3^2-H), 5.29 (dd, *J* = 9.2, 2.3 Hz, 1H, 17-H), 4.35 (q, *J* = 7.3 Hz, 1H, 18-H), 3.53 (m, 2H, 8^1-CH_2), 3.73, 3.54, 3.32, 3.08 (each s, each 3H, OCH_3 + CH_3), 2.78–2.70, 2.51–2.38, 2.02–1.94 (each m, total 4H, 17^1 + 17^2-CH_2), 1.72 (d, *J* = 7.3 Hz, 3H, 18-CH_3), 1.61 (t, *J* = 7.6 Hz, 3H, 8^2-CH_3), 0.19, 0.07 (each br s, each 1H, NH). Anal. calcd. for $C_{42}H_{40}N_6O_5$: C 71.17; H 5.69; N 11.86. Found: C 71.20; H 5.72; N 11.90.

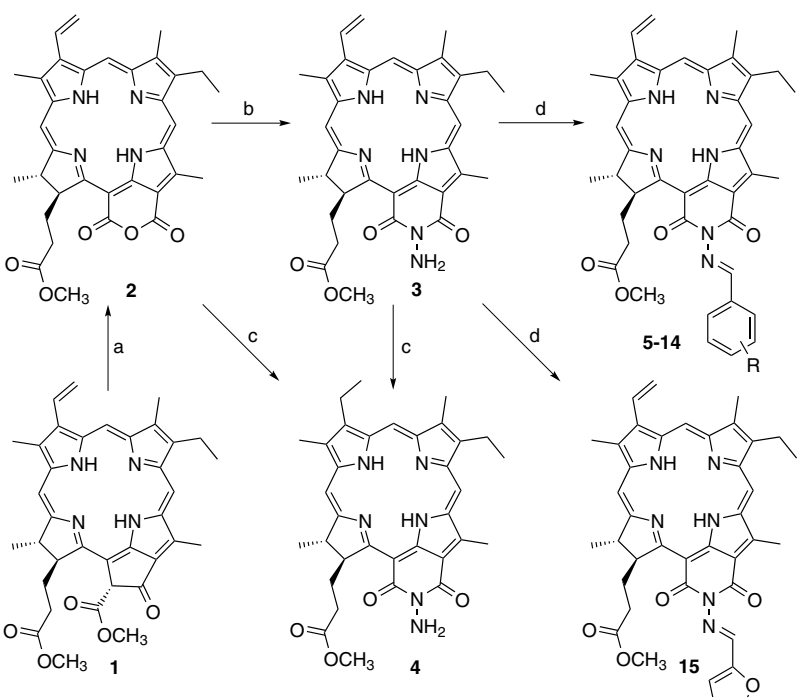
In vitro photosensitizing efficacy

General method. A549 cells were cultured at 37 °C in a humidified 5% CO_2 incubator using the RFMI 1640 growth medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. For phototoxicity studies, A549 cells were plated in 96-well plates at a density of 10×10^4 cells per well. After 24 h of incubation, one hundred μL of the 0.01 μM, 0.05 μM, 0.1 μM, 0.5 μM, 1 μM, and 10 μM purpurinimide-hydrazone conjugates was added to different plates. The plates were returned to the incubator for 24 h, after which the cells were replaced with fresh media and exposed to light (Bio-Spec LED, 670–700 nm, 2.0 J/cm^2) for 15 min. Following the illumination, the plates were incubated at 37 °C in the dark. After 12 h, WST-1 was put into each well, and the absorbance at the 450-nm wavelength after photoirradiation or without light was measured. Each experiment was performed with three replicate wells. The cell survival percentage was calculated using normalization with respect to the value obtained for the treatment in which no photosensitizer was used.

RESULTS AND DISCUSSION

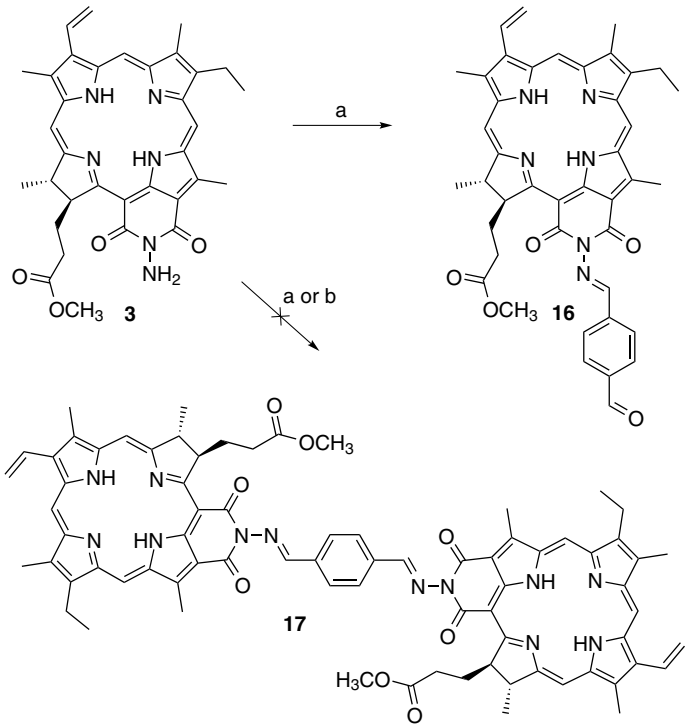
It was found that N_2 protection was essential for the successful preparation of *N*-aminopurpurinimide **3**, which possesses a primary vinyl group on its 3-position. Otherwise, the 3-vinyl group will be hydrogenated to give 3-devinyl-3-ethyl-*N*-aminopurpurinimide **4**, as has been reported by Mironov *et al*. [26]. The different results between N_2-protected and unprotected reactions suggest that the hydrogenation of the 3-vinyl group may be the result of the formation of diimide from hydrazine in the presence of O_2.

The condensation of *N*-aminopurpurinimide **3** with different arylaldehydes was accomplished by dissolving compound **3** and the corresponding arylaldehyde in dichloromethane and stirring for 12 h with the catalysis of *p*-toluenesulfonic acid and the protection of N_2. The reaction sequences are illustrated in Scheme 1.



5: R = 4-H; **6**: R = 4-CH_3; **7**: R = OH; **8**: R = 4-OCH_3; **9**: R = 4-Cl; **10**: R = 4-$N(CH_3)_2$; **11**: R = 4-NO_2; **12**: R = 2-NO_2; **13**: R = 2,6-Cl; **14**: R = 4-OH, 3,5-^{t}Bu.

Scheme 1. Synthesis of purpurinimide-hydrazone conjugates. Reagents and conditions: (a) (1) KOH/pyridine/air, (2) CH_2N_2; (b) hydrazine hydrate, N_2, rt; (c) hydrazine hydrate, air, rt; (d) corresponding arylaldehyde, *p*-TsOH, rt



Scheme 2. Attempts at synthesizing purpurinimide-hydrazone dimer. Reagents and conditions: (a) terephthalaldehyde, *p*-TsOH, rt; (b) terephthalaldehyde, *p*-TsOH, benzene, reflux

Purpurinimide-hydrazone conjugates **5**–**15** were obtained in good yields (82–94%). However, when we attempted to synthesize purpurinimide dimer **17** by the condensation of *N*-aminopurpurinimide **3** with terephthalaldehyde, we found that only compound **16** was obtained, even under refluxing in benzene, which was probably because of steric hindrance (Scheme 2).

The structures of the new compounds, **3** and **5**–**16**, were confirmed by the ^{1}H NMR spectra. Consider the ^{1}H NMR spectrum of compound **5** as an example. As shown in Fig. 1, the disappearance of the two-proton broadened singlet signal at $\delta = 5.70$ (13^2-NH_2, **3**) and the appearance of the one-proton singlet signal at $\delta = 8.91$ (13^4-H, **5**) and the monosubstituted phenyl group signal at δ = 8.15, 7.63–7.51 indicated the formation of the hydrazone group.

Further evidence for the transformation came from the electronic absorption spectra, where the peak at 710 nm of purpurinimide-hydrazone **5** was slightly blue-shifted compared to the corresponding peak (714 nm) of *N*-aminopurpurinimide **3** because of the steric effect of the hydrazone group (Fig. 2). Compounds **6**–**16** exhibited long-wavelength absorptions in the range of 708–712 nm, which were not distinctly different from those of compound **5**.

In the present study, the *in vitro* activities of purpurinimide-hydrazone conjugates **5**–**16** were determined by comparison with that of *N*-aminopurpurinimide **3** on A549 cells at 0.01, 0.05, 0.1, 0.5, 1, and 10 μM in the dark or after PDT (Fig. 3). As shown in Fig. 3a, generally none of the compounds showed a significant dark toxicity at low concentrations (≤0.1 μM), whereas at higher concentrations, some of the compounds showed relatively high cytotoxicities. Among them, compounds **10** and **13** showed relatively high dark toxicities compared to the other purpurinimide-hydrazone conjugates. At 1 μM, the dark toxicities were 20.7% and 17.1% for compounds **10** and **13**, respectively, whereas at 10 μM, their dark toxicities reached 40.4% and 31.2%, respectively.

In the PDT treatment, all of the purpurinimide-hydrazone conjugates except compound **14** showed higher cytotoxicities than that of the reference compound, purpurin-18 **2**. As we assumed, compounds **10** and **13** among the tested photosensitizers showed relatively high cytotoxicities. As shown in Fig. 3b, compounds **10** and **13** showed cell viabilities of 56.5% and 57.1% at 0.05 μM

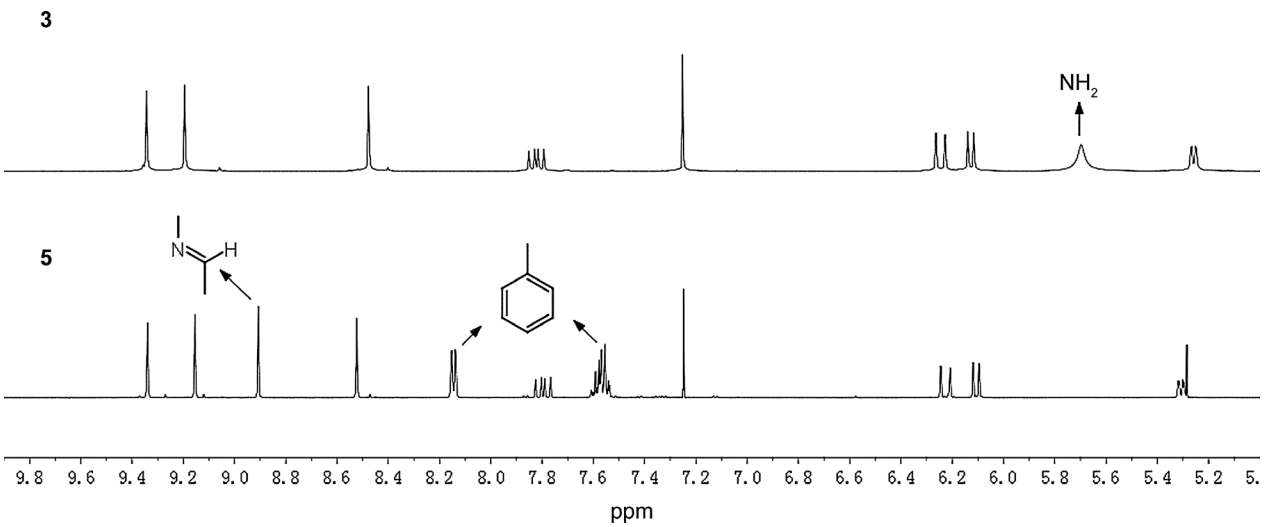


Fig. 1. Fragments of ^{1}H NMR spectra of compounds **3** and **5**

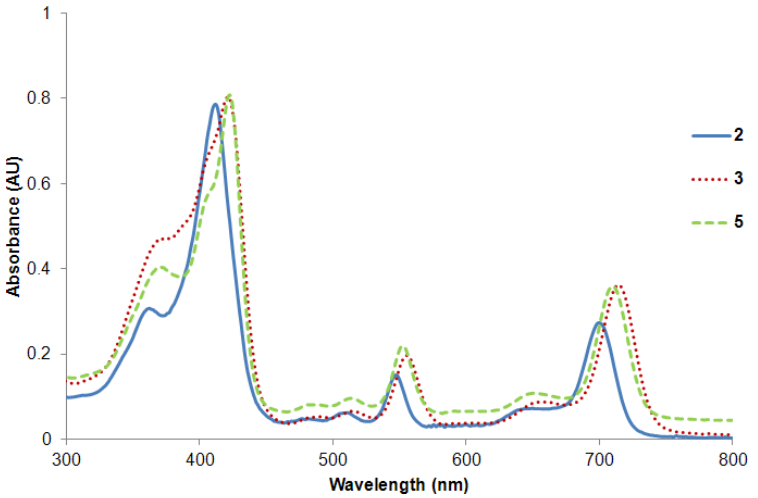
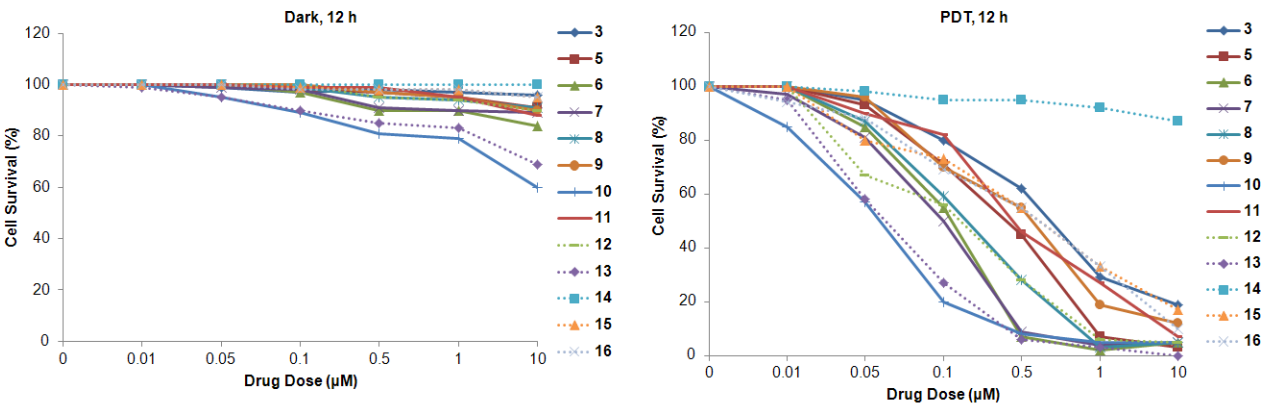


Fig. 2. UV-vis spectra of compounds **2** (blue solid line), **3** (red dotted line), and **5** (green dashed line)



(a) (b)

Fig. 3. Cell viability results for photosensitizers in dark (a) and after PDT (b) on A549 cells. The cell viabilities of **5-16** were assigned for PDT in the concentration ranges of 0.01 μM, 0.05 μM, 0.1 μM, 0.5 μM, 1 μM, and 10 μM after 12 h by comparing with that of purpurinimide **3**

Table 1. IC_{50} results of photosensitizers on A549 cell line

Compound	**3**	**5**	**6**	**7**	**8**	**9**	**10**
IC_{50} (μM)	0.687	0.424	0.142	0.100	0.218	0.571	0.059
Compound	**11**	**12**	**13**	**14**	**15**	**16**	
IC_{50} (μM)	0.456	0.186	0.063	>10	0.619	0.618	

after PDT, respectively. With an increase in the concentration of the photosensitizer, the cell viability revealed decreasing results; for example, the cell viabilities for compounds **10** and **13** were 20.0% and 26.8% at 0.1 μM and 4.9% and 3.3% at 1 μM after PDT, respectively. As for compound **14**, it showed little effect before and after PDT, which should be explained by its high antioxidant activity.

From the experimental results, we observed that all of the compounds showed an improved effect for cell death or cell viability as the concentration of the photosensitizer increased. In addition, all of the compounds obtained except compound **14** showed better effects than that shown by *N*-aminopurpurinimide **3**. Table 1 shows the IC_{50} values of these new photosensitizers on mouse sarcoma S-180 cell line after PDT. The reference compound *N*-aminopurpurinimide **3** showed a relative low effect after PDT (IC_{50} = 0.687 μM), compounds **10** and **13** among the tested photosensitizers showed relatively high PDT effect (IC_{50} = 0.059 and 0.063 μM, respectively). While compounds **14** showed relatively low PDT effect (IC_{50} > 10 μM) among the tested photosensitizers. It was also observed that a compound with a high PDT efficacy also showed a relative high dark toxicity. Thus, the overall cytotoxicities of these compounds may be the result of a combination of photodynamic therapy and

chemotherapy. To prove the combining result, further *in vitro* and *in vivo* studies about the cell uptake, subcellular localization and other biological test of the new purpurinimide-hydrazones are currently underway.

CONCLUSION

A series of new purpurinimide-hydrazone conjugates were synthesized and characterized. The incorporation of the hydrazone group into purpurinimide was found to increase its anticancer activity *via* a combination of photodynamic therapy and chemotherapy.

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