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Original article

Amino acid derivatives of pyropheophorbide-*a* ethers as photosensitizer: Synthesis and photodynamic activity

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ABSTRACT

Ten new water-soluble amino acid conjugates of pyropheophorbide-*a* ethers **4a–4j** were synthesized and investigated for their in vitro photodynamic antitumor activity. The results showed that all compounds exhibited higher phototoxicity and lower dark toxicity against three kinds of tumor cell lines than BPD-MA. In particular, the most phototoxic compound **4d** and **4j** individually showed IC_{50} values of 41 nmol/L and 33 nmol/L against HCT116 cell, which represented 7.8- and 9.7-fold increase of antitumor potency compared to BPD-MA, respectively, suggesting that they were promising photosensitizers for PDT applications because of their strong absorption at long wavelength ($\lambda_{max} > 650$ nm), high phototoxicity, low dark cytotoxicity and good water-solubility.

1. Introduction

Photodynamic therapy (PDT) now is a well recognized approach to cancer therapy for the selective destruction of tumors by visible light in presence of a photosensitizer (PS) and cell oxygen [1]. It is based on the principle that the interaction between light and PS in tumor tissues generates cytotoxic reactive oxygen species (ROS) created through either electron transfer (type I) or energy transfer (type II) reactions to inactivate the tumor cells [2].

Porfimer sodium, the first clinically approved porphyrin-type PS for the treatment of bladder cancer in the world, has suffered some drawbacks such as its complex component, inefficient absorption ($\varepsilon = 1170\ L\ mol^{-1}\ cm^{-1}$) at long wavelength ($\lambda_{max} = 630$ nm), prolonged cutaneous phototoxicity up to 4–6 weeks due to its slow clearance in skin tissues [3].

In the recent decade, the so-called second generation chlorin-type PSs such as natural chlorophyll-*a* derivatives have generated interest due to its low skin phototoxicity, rapid clearance from tissues and strong absorption at long wavelengths ($\lambda_{max} > 650$ nm) to take full advantage of greater tissue penetration [4–6]. Among them, talaporfin [7] and verteporfin (BPD-MA) [8] were approved for PDT applications (Fig. 1).

Pyropheophorbid-*a* (**2**), the one of chlorophyll-*a* derivative as chlorin-type PS, has poor water solubility to hamper its clinical development. Introducing amino acid at 17^3-position and alkoxy at 3^1-position was reported to individually improve the water solubility and the biological activity of chlorin-based derivatives [9–12]. In this regard, a series of novel water-soluble amino acid conjugates of pyropheophorbide-*a* ethers **4a–4j** were synthesized (Scheme 1) and investigated their in vitro photodynamic antitumor activity against three kinds of tumor cell lines.

2. Experimental

2.1. Chemicals and instruments

Melting points were measured on a XRD micro melting point apparatus and uncorrected. ^{1}H NMR spectra were recorded on Bruker MSL-300 or MSL-600 using TMS as the internal reference. Mass spectra were collected on an API-3000 LC-MS spectrometer. UV absorption spectra were measured on an Agilent UV 8453 spectrophotometers. Elemental analysis was carried out using a PE2400 II instrument. Column chromatography was performed on silica gel (size 10–40 μm, Qingdao Haiyang

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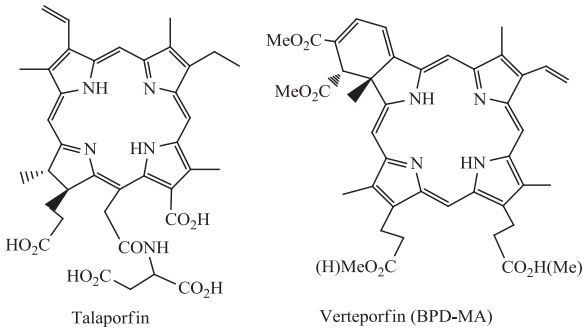


Fig. 1. Two clinical available semisynthetic chlorin-type photosensitizers.

Chemical, China). All reagents and solvents purchased from commercial vendors and were used without further purifications. The key intermediate pheophorbide-*a* (**1**) was obtained via cond. aqueous HCl degradation of chlorophyll-*a* in Et_2O (Scheme 1) by the methodology developed in our laboratory using crude chlorophyll extracts in silkworm excrements [13].

2.2. Chemical synthesis

Pyropheophorbide-*a* (**2**): A suspension of **1** (2.0 g, 3.38 mmol) in HOAc (100 mL) was refluxing under an atmosphere of N_2 for 4 h. The reaction mixture was poured into H_2O (1 L) and then extracted with CH_2Cl_2. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified on a silica gel column (CH_2Cl_2:CH_3COCH_3:CH_3OH:HCO_2H = 60:1:1:0.1 as eluent) to obtain **2** (1.5 g, 83.1%) as bright black solid. Physical and spectroscopic characterization data of compound **2** was given in Supporting information.

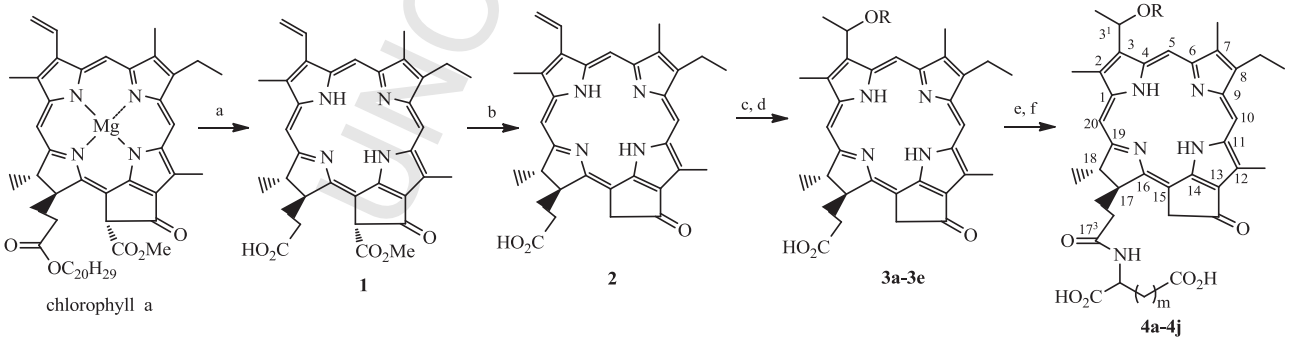
Pyropheophorbide-*a* ether derivatives (**3a**–**3e**): A suspension of **2** (0.5 g, 0.94 mmol) in 33% HBr–HOAc (50 mL) was stirred overnight at room temperature and then evaporated. The residue was added dissolved in CH_2Cl_2 (50 mL). K_2CO_3 (1.0 g) and the appropriate alcohol donors (10 mL) were then added and allowed to stir at room temperature for 2 h. The reaction mixture was added H_2O (300 mL) and then extracted with CH_2Cl_2. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The residue was purified on a silica gel column (CH_2Cl_2:CH_3COCH_3:CH_3OH:HCO_2H = 80:1:1:0.1 as eluent) to give compounds **3a–3e** as bright black solid in yield of 42.3–69.0%. Physical and spectroscopic characterization data of compounds **3a–3e** were given in Supporting Information.

Amino acid conjugates of pyropheophorbide-*a* ethers (**4a–4j**): To a solution of compounds **3a**–**3e** (0.40 mmol) in anhydrous CH_2Cl_2 (100 mL) was individually added 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochlorate (EDCI) (0.48 mmol, 1.2 equiv.) and 1-hydroxybenzotriazole (HOBt) (0.48 mmol l, 1.2 equiv.) to stir until completely dissolved under in ice-salt bath. After 30 min, L-Asp(OBut)$_2$·HCl or L-Glu(OBut)$_2$·HCl (0.48 mmol 1.2 equiv.) and *N*,*N*-diisopropylethylamine (DIPEA) (0.48 mmol l, 1.2 equiv.) were mixed in CH_2Cl_2 (30 mL) and poured into above reaction mixture. The mixture was allowed to stir at room temperature overnight under nitrogen. It was diluted with CH_2Cl_2 (150 mL) and then washed with 5% aqueous citric acid, brine and water, respectively. The organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was dissolved in dry CH_2Cl_2/TFA (1:1, 30 mL) and stirred at room temperature for 2 h. The resulting mixture was diluted with CH_2Cl_2 and adjusted to pH 5–6 with 10% $NaHCO_3$ and purified on a silica gel column (CH_2Cl_2:CH_3COCH_3:CH_3OH:HCO_2H = 80:1:1:0.1 as eluent) to give the target compounds **4a**–**4j**.

N-(3-Devinyl-3-(1-methoxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-aspartic acid (**4a**): Yield 54.2%, mp 168–169 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.10 (s, 2H, 2× CO_2H), 9.65 (s, 1H, 10-H), 9.38 (s, 1H, 5-H), 8.88 (s, 1H, 20-H), 8.20 (m, 1H, CONH), 6.02 (q, 1H, J = 6.8 Hz, 3^1-H), 5.22 (d, 1H, J = 21.0 Hz, 13^2-H$_b$), 5.10 (d, 1H, J = 21.0 Hz, 13^2-H$_a$), 4.56–4.59 (m, 1H, 17-H), 4.28–4.32 (m, 1H, 18-H), 3.82 (m, 1H, CONHCHCO), 3.69 (q, 2H, J = 7.2 Hz, 8^1-CH_2), 3.62 (s, 3H, 12-CH_3), 3.42 (s, 3H, 3^1-OCH_3), 3.35 (s, 3H, 7-CH_3), 3.17 (s, 3H, 2-CH_3), 2.22–2.03 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCHCH_2), 2.02 (d, 3H, J = 6.8 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 7.2 Hz, 18-CH_3), 1.59 (t, 3H, J = 7.2 Hz, 8^2-CH_3), −2.04 (s, 1H, NH); MS (ESI$^+$) m/z: 682.57 [M+H]$^+$ (100%); Anal. Calcd. for $C_{38}H_{43}N_5O_7$:C 66.94, H 6.36, N 10.27; Found: C 67.14, H 6.31, N 10.33.

N-(3-Devinyl-3-(1-propoxy)ethyl-pyropheophorbide-a-17^3-acyl)-L-aspartic acid (**4b**): Yield 55.8%, mp > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.80 (1H, splitted s, 10-H), 9.76 (s, 1H, 5-H), 8.83 (s, 1H, 20-H), 7.95 (m, 1H, CONH), 6.0 (q, 1H, J = 6.8 Hz, 3^1-H), 5.23 (d, 1H, J = 21.0 Hz, 13^2-H$_b$), 5.09 (d, 1H, J = 21.0 Hz, 13^2-H$_a$), 4.51–4.58 (m, 1H, 17-H), 4.28 (m, 1H, 18-H), 3.74 (m, 1H, CONHCHCO), 3.70 (q, 2H, J = 7.3 Hz, 8^1-CH_2), 3.63 (s, 3H, 12-CH_3), 3.49 (m, 2H, 3^1-OCH_2), 3.39 (s, 3H, 7-CH_3), 3.22 (s, 3H, 2-CH_3), 2.12–2.30 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCHCH_2), 2.02 (d, 3H, J = 6.8 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 7.6 Hz, 18-CH_3), 1.63 (t, 3H, J = 7.3 Hz, 8^2-CH_3), 0.82–0.93 (m, 5H, 3^1-$OCH_2CH_2CH_3$), −1.98 (s, 1H, NH); MS (ESI$^+$) m/z: 710.56 [M+H]$^+$ (100%); Anal. Calcd. for $C_{40}H_{47}N_5O_7$: C 67.68, H 6.67, N 9.87; Found: C 67.89, H 6.61, N 9.92.

N-(3-Devinyl-3-(1-pentyloxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-aspartic acid (**4c**): Yield 60.1%, mp > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.80 (splitted s, 1H, 10-H), 9.75 (s, 1H, 5-H), 8.83 (s, 1H, 20-H), 7.95 (m, 1H, CONH), 5.98 (q, 1H, J = 6.8 Hz, 3^1-H), 5.23 (d, 1H, J = 18.0 Hz, 13^2-H$_b$), 5.09 (d, 1H, J = 18.0 Hz, 13^2-H$_a$),



Scheme 1. Synthetic route for the titled compounds **4a**–**4j**. Reagents and conditions: (a) *cond.* aqueous HCl–Et_2O, 0–5 °C, 30 min; (b) HOAc, reflux, 4 h; (c) 33% HBr–HOAc, 24 h; (d) alcohol, CH_2Cl_2, K_2CO_3, 2 h; Alcohol donors: R = CH_3 (**3a**), *n*-C_3H_7 (**3b**), *n*-C_5H_{11} (**3c**), *n*-C_6H_{13} (**3d**), *n*-C_8H_{17} (**3e**); (e) L-Asp(OBu^t)$_2$ or L-Glu(OBu^t)$_2$, EDCI, HOBt, DIPEA, CH_2Cl_2, r.t., 12 h; (f) CH_2Cl_2–TFA (1:1), r.t., 2 h. Alcohol donors and amino acid residues: m = 1 and R = CH_3 (**4a**), *n*-C_3H_7 (**4b**), *n*-C_5H_{11} (**4c**), n-C_6H_{13} (**4d**), *n*-C_8H_{17} (**4e**); m = 2 and R = CH_3 (**4f**), *n*-C_3H_7 (**4g**), *n*-C_5H_{11} (**4h**), *n*-C_6H_{13} (**4i**), *n*-C_8H_{17} (**4j**).

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4.59–4.56 (m, 1H, 17-H), 4.31–4.28 (m, 1H, 18-H), 3.74 (m, 1H, CONHCHCO), 3.71 (q, 2H, J = 7.7 Hz, 8^1-CH_2), 3.62 (s, 3H, 12-CH_3), 3.46–3.51 (m, 2H, 3^1-OCH_2), 3.38 (s, 3H, 7-CH_3), 3.22 (s, 3H, 2-CH_3), 2.33–2.12 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCHCH_2), 2.02 (d, 3H, J = 6.8 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 6.6 Hz, 18-CH_3), 1.63 (t, 3H, J = 7.7 Hz, 8^2-CH_3), 1.25 (m, 4H, 3^1-$OCH_2(CH_2)_2C_2H_5$), 0.72–0.86 (m, 5H, $O(CH_2)_3CH_2CH_3$), −1.98 (s, 1H, NH); MS (ESI^+) m/z: 738.51 $[M+H]^+$ (100%); Anal. Calcd. for $C_{42}H_{51}N_5O_7$:C 68.36, H 6.97, N 9.49; Found: C 68.58, H 6.92, N 9.56.

N-(3-Devinyl-3-(1-hexyloxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-aspartic acid (**4d**): Yield 51.6%, mp > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 9.80 (s, 1H, 10-H), 9.75 (s, 1H, 5-H), 8.81 (s, 1H, 20-H), 8.05 (m, 1H, CONH),6.21 (q, 1H, J = 6.8 Hz, 3^1-H), 5.20 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.05 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.55 (m, 1H, 17-H), 4.26 (m, 1H, 18-H), 4.12 (m, 1H, CONHCHCO), 3.71 (q, 2H, J = 7.5 Hz, 8^1-CH_2), 3.62 (s, 3H, 12-CH_3), 3.49 (m, 2H, 3^1-OCH_2), 3.39 (s, 3H, 7-CH_3), 3.22 (s, 3H, 2-CH_3), 2.12–2.33 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCHCH_2), 2.02 (d, 3H, J = 6.8 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 6.6 Hz 18-CH_3), 1.63 (t, 3H, J = 7.5 Hz, 8^2-CH_3), 1.27 (m, 6H, 3^1-$OCH_2(CH_2)_3C_2H_5$), 0.80–0.90 (m, 5H, 3^1-$O(CH_2)_4CH_2CH_3$); MS (ESI^+) m/z: 752.53 $[M+H]^+$ (100%). Anal. Calcd. for $C_{43}H_{53}N_5O_7$:C 68.69, H 7.10, N 9.31; Found: C 68.89, H 7.05, N 9.38.

N-(3-Devinyl-3-(1-octyloxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-aspartic acid (**4e**): Yield 53.0%, mp > 300 °C; ^1H NMR (300 MHz, $(CD_3)_2CO$): δ 9.87 (s, 1H, 10-H), 9.71 (s, 1H, 5-H), 8.78 (s, 1H, 20-H), 7.45 (m, 1H, CONH), 6.02 (q, 1H, J = 6.8 Hz, 3^1-H), 5.21 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.04 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.77 (m, 1H, 17-H), 4.60 (m, 1H, 18-H), 4.38 (m, 1H, CONHCHCO), 3.69 (q, 2H, J = 7.5 Hz 8^1-CH_2), 3.58 (s, 3H, 12-CH_3), 3.55 (m, 2H, 3^1-OCH_2), 3.39 (s, 3H, 7-CH_3), 3.22 (s, 3H, 2-CH_3), 2.31–2.20 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCHCH_2CO_2H), 2.06 (d, 3H, J = 6.8 Hz, 3^2-CH_3), 1.78 (d, 3H, J = 6.6 Hz, 18-CH_3), 1.64 (t, 3H, J = 7.5 Hz, 8^2-CH_3), 1.20 (m, 4H, 3^1-$OCH_2(CH_2)_2C_5H_{11}$), 0.81–0.82 (m, 8H, 3^1-$O(CH_2)_3(CH_2)_4CH_3$), 0.63 (t, 3H, J = 7.5 Hz, 3^1-$O(CH_2)_7CH_3$), −1.84 (s, 2H, NH × 2); MS (ESI^+) m/z: 780.62 $[M+H]^+$ (100%). Anal. Calcd. for $C_{45}H_{57}N_5O_7$:C 69.30, H 7.37, N 8.98; Found: C 69.49, H 7.29, N 9.05.

N-(3-Devinyl-3-(1-methoxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-glutamic acid (**4f**): Yield 73.3%, mp > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 9.70 (s, 2H, 10- and 5-H), 8.85 (s, 1H, 20-H), 8.15 (m, 1H, CONH), 5.95 (q, 1H, J = 6.6 Hz, 3^1-H), 5.20 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.07 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.56 (m, 1H, 17-H), 4.30 (m, 1H, 18-H), 4.10 (m, 1H, CONHCHCO), 3.68 (q, 2H, J = 7.4 Hz, 8^1-CH_2), 3.58 (s, 3H, 12-CH_3), 3.47 (s, 3H, 3^1-OCH_3), 3.40 (s, 3H, 7-CH_3), 3.21 (s, 3H, 2-CH_3), 2.25–2.03 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCH(CO_2H)$CH_2CH_2CO_2H$), 2.02 (d, 3H, J = 6.6 Hz, 3^2-CH_3), 1.77 (d, 3H, J = 6.9 Hz, 18-CH_3), 1.61 (t, 3H, J = 7.4 Hz, 8^2-CH_3), 1.20–1.40 (m, 2H, CONHCH(CO_2H)$CH_2CH_2CO_2H$), −1.99 (s, 1H, NH); MS (ESI^+) m/z: 696.55 $[M+H]^+$ (100%). Anal. Calcd. for $C_{39}H_{45}N_5O_7$:C 67.32, H 6.52, N 10.07; Found: C 67.51, H 6.48, N 10.12.

N-(3-Devinyl-3-(1-propoxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-glutamic acid (**4g**): Yield 59.5%, mp > 300 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 12.38 (s, 2H, 2× CO_2H), 9.80 (splitted s, 1H, 10-H), 9.74 (s, 1H, 5-H), 8.84 (s, 1H, 20-H), 8.16 (m, 1H, CONH), 6.0 (q, 1H, J = 6.3 Hz, 3^1-H), 5.21 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.09 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.55 (m, 1H, 17-CH), 4.31 (m, 1H, 18-CH), 4.20 (m, 1H, CONHCHCO), 3.71 (q, 2H, J = 7.7 Hz, 8^1-CH_2), 3.62 (s, 3H, 12-CH_3), 3. 49 (m, 2H, 3^1-OCH_2), 3.43 (s, 3H, 7-CH_3), 3.21 (s, 3H, 2-CH_3), 2.25–2.07 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CONHCH(CO_2H)$CH_2CH_2CO_2H$), 2.02 (d, 3H, J = 6.3 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 6.8 Hz, 18-CH_3), 1.63 (t, 3H, J = 7.7 Hz, 8^2-CH_3), 1.13–1.38 (m, 4H, CONHCH(CO_2H)$CH_2CH_2CO_2H$ + 3^1-$OCH_2CH_2CH_3$), 0.90 (t, 3H, J = 7.5 Hz, 3^1-$O(CH_2)_2CH_3$), −1.98 (s, 1H, NH); MS (ESI^+) m/z: 724.54 $[M+H]^+$ (40%); MS (ESI^-) m/z: 722.55 $[M-H]^+$ (70%), 1445.46 $[2M-H]^+$ (100%). Anal. Calcd. for $C_{41}H_{49}N_5O_7$:C 68.03, H 6.82, N 9.68; Found: C 68.23, H 6.77, N 9.73.

N-(3-Devinyl-3-(1-pentyloxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-glutamic acid (**4h**): Yield 53.5%, mp 180–182 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 12.53 (s, 2H, 2× CO_2H), 9.81 (s, 1H, 10-H), 9.75 (s, 1H, 5-H), 8.84 (s, 1H, 20-H), 8.10 (m, 1H, CONH), 6.00 (q, 1H, J = 6.0 Hz, 3^1-H), 5.22 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.09 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.56 (m, 1H, 17-H), 4.32 (m, 1H, 18-H), 4.18–4.20 (m, 1H, CONHCHCO), 3.71 (q, 2H, J = 8.3 Hz, 8^1-CH_2), 3.62 (s, 3H, 12-CH_3), 3.48–3.50 (m, 2H, 3^1-OCH_2), 3.39 (s, 3H, 7-CH_3), 3.21 (s, 3H, 2-CH_3), 2.33–2.10 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CH_2CO_2H), 2.01 (d, 3H, J = 6.0 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 6.8 Hz, 18-CH_3), 1.63 (t, 3H, J = 8.3 Hz, 8^2-CH_3), 1.20 (m, 8H, CONHCH(CO_2H)CH_2CH_2-CO_2H + 3^1-$OCH_2(CH_2)_3CH_3$), 0.90 (t, 3H, J = 6.8 Hz, $O(CH_2)_4CH_3$), −1.98 (s, 1H, NH); MS (ESI^+) m/z: 752.99 $[M+H]^+$ (100%). Anal. Calcd. for $C_{43}H_{53}N_5O_7$:C 68.69, H 7.10, N 9.31; Found: C 69.81, H 7.05, N 9.37.

N-(3-Devinyl-3-(1-hexyloxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-glutamic acid (**4i**): Yield 69.3%, mp 184–185 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 12.29 (s, 2H, 2× CO_2H), 9.79 (s, 1H, 10-H), 9.71 (s, 1H, 5-H), 8.83 (splitted s, 1H, 20-H), 8.14 (m, 1H, CONH), 5.95 (q, 1H, J = 5.7 Hz, 3^1-H), 5.21 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.09 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.56 (m, 1H, 17-H), 4.32 (m, 1H, 18-H), 4.22 (m, 1H, CONHCHCO), 3.69 (q, 2H, J = 7.3 Hz, 8^1-CH_2), 3.60 (s, 3H, 12-CH_3), 3.42 (m, 2H, 3^1-OCH_2), 3.35 (s, 3H, 7-CH_3), 3.20 (s, 3H, 2-CH_3), 2.23–2.11 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CH_2CO_2H), 2.02 (d, 3H, J = 5.7 Hz, 3^2-CH_3), 1.76 (d, 3H, J = 6.5 Hz, 18-CH_3), 1.61 (t, 3H, J = 7.3 Hz, 8^2-CH_3), 1.11–1.28 (m, 10H, CONHCH(CO_2H)CH_2CH_2-CO_2H + 3^1-$OCH_2(CH_2)_4CH_3$), 0.81 (t, 3H, J = 5.4 Hz, 3^1-$O(CH_2)_5CH_3$), −1.97 (1H, s, NH); MS (ESI^+) m/z: 766.26 $[M+H]^+$ (100%). Anal. Calcd. for $C_{44}H_{55}N_5O_7$:C 69.00, H 7.24, N 9.14; Found: C 69.23, H 7.17, N 9.20.

N-(3-Devinyl-3-(1-octyloxy)ethyl-pyropheophorbide-*a*-17^3-acyl)-L-glutamic acid (**4j**): Yield 79.3%, mp 180–182 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 12.58 (s, 2H, 2× CO_2H), 9.79 (s, 1H, 10-H), 9.72 (s, 1H, 5-H), 8.82 (s, 1H, 20-H), 7.90 (m, 1H, CONH), 5.95 (q, 1H, J = 6.8 Hz, 3^1-H), 5.21 (d, 1H, J = 18.0 Hz, 13^2-H_b), 5.08 (d, 1H, J = 18.0 Hz, 13^2-H_a), 4.56 (m, 1H, 17-H), 4.30 (m, 1H, 18-H), 4.16 (m, 1H, CONHCHCO), 3.69 (q, 2H, J = 7.8 Hz, 8^1-CH_2), 3.60 (s, 3H, 12-CH_3), 3.45 (m, 2H, 3^1-OCH_2), 3.35 (s, 3H, 7-CH_3), 3.20 (s, 3H, 2-CH_3), 2.20–2.13 (m, 6H, 17^2-CH_2 + 17^1-CH_2 + CH_2CO_2H), 2.02 (d, 3H, J = 6.8 Hz, 3^2-CH_3), 1.75 (d, 3H, J = 6.8 Hz, 18-CH_3), 1.62 (t, 3H, J = 7.8 Hz, 8^2-CH_3), 0.82–1.37 (m, 17H, CONHCH(CO_2H)CH_2CH_2-CO_2H + 3^1-$OCH_2(CH_2)_6CH_3$), −1.98 (s, 1H, NH); MS (ESI^+) m/z: 792.63 $[M+H]^+$ (100%). Anal. Calcd. for $C_{46}H_{59}N_5O_7$:C 69.58, H 7.49, N 8.82; Found: C 69.76, H 7.41, N 8.90.

The determination method of in vitro dark toxicity and phototoxicity for target compounds **4a**–**4j** was given in Supporting Information.

3. Results and discussion

3.1. Synthesis

It was designed that pyropheophorbide-*a* ethers (**3a**–**3e**), which was generated via the addition of pyropheophorbide-*a* (**2**) with hydrobromic acid followed by substitution with alcohol donors (ROH), coupled with carboxyl-protected amino acids in the presence of condensation agent and catalyst followed by removal of protective group with trifluoroacetic acid (TFA) to yield the target compounds (**4a**–**4j**).

Compound **2** was prepared by acid degradation of pheophorbide-*a* (**1**) in refluxing HOAc. Compound **1** was synthesized according to our reported procedure [13]. All of the target amino acid conjugates of pyropheophorbide-*a* ethers **4a**–**4j** were new compounds and their structures were confirmed by ^1H NMR, MS and elemental analysis. The UV–vis spectral data showed that they possessed more efficient absorption ($\varepsilon = 1.7 \times 10^4$ to

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Table 1
UV–vis data of the titled compounds **4a**–**4j**.

No.	λ_{max} (CH_2Cl_2, nm) ($\varepsilon \times 10^4$ L mol^{-1} cm^{-1})				
	Soret band	Visible bands			
4a	413 (4.8)	507 (0.49)	537 (0.43)	608(0.71)	666 (1.7)
4b	415 (6.3)	–	536 (0.41)	602 (0.44)	651 (1.7)
4c	414 (7.1)	507 (0.63)	538 (0.65)	605(0.69)	660 (2.4)
4d	411 (4.1)	507 (0.35)	539 (0.36)	608 (0.44)	662 (1.7)
4e	415 (11.0)	–	537 (0.71)	603(0.81)	651 (3.1)
4f	412 (8.6)	507 (0.60)	–	614 (0.53)	665 (1.9)
4g	411 (16.0)	507 (1.6)	541 (1.7)	605(1.7)	661 (7.5)
4h	411 (34.0)	–	538 (2.1)	602 (2.4)	651 (9.8)
4i	410 (11.0)	507 (1.2)	541 (1.2)	602(1.2)	662(5.2)
4j	411 (3.8)	507 (0.42)	540 (0.43)	605 (0.44)	661 (1.7)

Table 2
Cytotoxicity (IC_{50}, μmol/L) for the titled compounds against tumor cells.

Compd.	HCT116		MDA-MB-231		MKN45	
	DT[a]	PT[a,b]	DT[a]	PT[a,b]	DT[a]	PT[a,b]
4a	10.08	0.183	36.11	0.297	32.70	0.298
4b	11.82	0.076	33.06	0.108	35.35	0.182
4c	35.19	0.061	61.48	0.11	>100	0.16
4d	15.06	0.041	38.23	0.09	24.56	0.12
4e	18.01	0.050	42.03	0.12	>100	0.13
4f	62.30	0.126	100	0.21	>100	0.15
4g	11.41	0.093	23.69	0.17	37.51	0.15
4h	11.99	0.092	26.74	0.13	16.24	0.14
4i	10.48	0.063	21.84	0.13	66.20	0.13
4j	11.80	0.033	39.73	0.10	81.40	0.10
BPD-MA	1.95	0.32	9.5	0.56	10.75	0.92

[a] *Abbreviation*: DT, dark toxicity; PT, phototoxicity.
[b] Cells irradiated with the diode laser at 660 nm for a light dose of 10.8 J/cm^2.

9.8×10^4 L mol^{-1} cm^{-1}) at longer maximum absorption wavelength ranged from 651 nm to 666 nm than porfimer sodium (Table 1), indicating their greater tissue penetration [4–6].

3.2. In vitro dark cytotoxicity and photodynamic efficacy

The dark toxicity and phototoxicity of the titled compounds were screened with the MTT assay against HCT116, MDA-MB-231 and MKN45 cells using BPD-MA as control (Table 2). In order to realize parallel control to eliminate the experimental error caused by the solvent, the tested compounds **4a**–**4j** and BPD-MA were both prepared into the DMSO-mediated 10% PBS solution. The results displayed that all compounds exhibited better phototoxicity and less dark toxicity against three kinds of tumor cell lines than BPD-MA. A structure-activity relationship analysis revealed that their PDT antitumor activity, particularly against HCT116 cells, enhanced with ether carbon chain growth, the one with 6–8 carbon atoms as the best. In addition, the type of water-soluble amino acid such as L-aspartic acid or L-glutamic acid had little effect on the activity. Among them, compound **4d** and **4j** exhibited the best in vitro PDT antitumor efficacy and their IC_{50} values for HCT116 cell were individually 41 nmol/L and 33 nmol/L, which represented 7.8- and 9.7-fold increase of antitumor potency compared to BPD-MA, respectively. In the next in vivo animal antitumor trials, they could be made into water-soluble sodium salt for intravenous administration.

4. Conclusion

In summary, 10 new water-soluble amino acid conjugates of pyropheophorbide-*a* ethers (**4a**–**4j**) were synthesized and evaluated for their in vitro photodynamic antitumor activity against HCT116, MDA-MB-231 and MKN45 cells. All compounds exhibited more potent phototoxicity and lower dark toxicity against all tested tumor cell lines than BPD-MA. In particular, compounds **4d** and **4j** were the two most effective PSs, which showed 7.8- and 9.7-fold more potent antitumor activity against HCT116 cells compared to BPD-MA. These results demonstrated that compounds **4d** and **4j** could be promising PSs for PDT applications because of their strong absorption at long wavelength, high phototoxicity, low dark cytotoxicity and good water-solubility, and worthy of further study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2016.03.019.

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