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Synthesis and properties of novel purpurinimide derivatives from methyl pheophorbide-a

Qiong Li, Lelin Li, Meiyan Zhu, Qian Zhang, Shuangqing Song and Bing Cun Cui*

College of Medicine, Hubei Polytechnic University, Huangshi, P. R. China

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ABSTRACT

The novel purpurinimide derivatives exhibiting long wavelength absorption and amphiphilicity were obtained from methyl pheophorbide-a (MPa) by modification of the peripheral functional groups. The vinyl group at 3-position was oxidized with OsO_4 and $NaIO_4$ to form the formyl group and the Grignard reaction of this aldehyde with the alkyl magnesium bromide was carried out to give the corresponding 3-(1-hydrroxylalkyl) pheophorbide-a. The E-ring of these chlorins was converted into imide ring to give purpurinimide derivatives by air oxidation and amidation reaction. These new compound have long wavelength absorption in the range of 726-727nm. In preliminary screening, the purpurinimides exhibit relatively high PDT effect than MPa.

Key words: Purpurinmide, Methyl pheophorbide-a, Photosensitizer, Photodynamic therapy

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INTRODUCTION

Photodynamic therapy (PDT) is a new procedure for the treatment of various types of malignant tumors. A very interesting novel approach for purging of neoplastic cell from autologous bone marrow has been presented by Jamieson and his co-workers [1-4]. PDT utilizes the ability of a selectively retained photosensitizer to elicit an efficient photodynamic reaction upon activation with tissue penetrating light Photofrin, an efficient hematoporphyrin derivative which is the only photosensitizer that has been approved worldwide for the treatment of various types of cancers by PDT, suffers with certain disadvantages: it is a chemically complex mixture, its longest absorption wavelength peak falls at 630 nm and it shows long-term skin phototoxicty. Now the emphasis on developing improved photosensitizers is molecular design, good amphiphilicity, reasonable stability and lack of toxicity of compounds with longer wavelength absorption at near or above 700 nm.

Recently, Zheng and his co-workers [5] reported in vivo photodynamic efficacy of a series of alkyl ether analogues of purpurinimide. Among the optimal photosensitizers, different lengths of alkyl ethers were regioselectively introduced at their 3-position. According to Quantitative Structure Activity Relationships (QSAR) for the analogues of purpurinimide series, we expanded this approach to photosensitizers with long alkyl chains, which improved their lipophilicity more efficiently comparing to alkoxy chain.

For the Qy bands of chlorophyll-a compound, the longest absorption band in visible spectra, was strongly affected by the substitutents on Qy axis (N^{21}-N^{23}). Constructing conjugated carbonyl group at 3-position and converting E-ring of chlorin into six-membered ring were found to greatly increase wavelength absorption and stability to meet the requirements of an improved photodynamic therapeutic agent [6-8]. Purpurinimides show a strong absorption band (Qy) in the near IR region, a key cytotoxic agent in PDT application [5, 9]. Therefore, the synthesis of novel purpurinimides has become the focus on PDT study. In continuation with our earlier study [7], it was thought that worthwhile to synthesize some new purpurinimide derivatives by incorporating the essential structural features of the above-mentioned potential cytotoxic drugs in order to obtain synergistic effects, which we report herein.

EXPERIMENTAL SECTION

General Methods

The UV-vis absorption spectra were recorded on Scinco S-3100 spectrophotometer using CH_2Cl_2 as a solvent. Thin layer chromatography (TLC) was performed on silica gel 60 F254 (E. Merck). Routine nuclear magnetic resonance (NMR) spectra were recorded on a Varian-500MHz spectrometer. Chemical shifts are given as δ values using TMS as the internal standard and J values in Hz. Chemical shifts are quoted in ppm on the δ scale and coupling constants (J) are expressed in Hertz (Hz). Samples for NMR spectroscopic studies were prepared using solvents purchased from Aldrich. Elemental analysis data were measured on Flash 2000 series (Thermo). All chemical reagents were commercially available and purified with standard methods before use. Solvents were dried in routine ways and redistilled. Methyl pheophorbide-a (MPa) 1 was obtained according to Smith's method 10.

3-(1-hydroxyl-1-amyl)-3-devinyl purpurin-18 methyl ester 4

Compound 3 (0.25 mmol) was dissolved in 5 mL of pyridine and was diluted by addition of ether (500 mL). The reaction mixture was stirred under air current, and then was added the solution of potassium hydroxide 2 g (0.05 mol) in poropanol (10 mL). Addition of ether, the reaction mixture was then poured into 100 mL of water after 30 min, separated the water layer and the organic layer was extracted with (2×100 mL) of water. From the extraction, the water layer was separated to be added to the forementioned water layer, adjusted with dilute sulphuric acid to pH = 4 and extracted with dichloromethane (3×300 mL). After the organic layer was dried (Na_2SO_4) and the solvent was evaporated under high vacuum, the residue was purified silica column chromatography, eluting with hexane/ ethyl acetate in 3:1 to afford 82 mg (0.13 mmol) of the title compound. Yeid: 52%. UV-vis (CH_2Cl_2) λmax : 693 (0.33), 542 (0.15), 505 (0.07), 477 (0.04), 410 (1.01); 1H NMR δ(500MHz, $CDCl_3$): 0.82 (m, 2H, NH), 1.28 (t, J = 6.8Hz, 3H, 36-Me), 1.36~1.41 (m, 8H, 32~35-H), 1.71 (t, J = 7.2Hz, 3H, 8-Me), 1.81 (d, J = 7.4Hz, 3H, 18-Me), 2.30~2.32, 2.60~2.65 (m, each 2H, 4H, 17a, 17b-H), 3.57 (m, 2H, 8-H), 3.22, 3.38, 3.65, 3.82 (s, each 3H, 15H, Me+OMe), 4.19~4.23, 4.42~4.44 (m, each 1H, 2H, 17, 18-H), 6.18 (m, 1H, 31-H), 8.52, 9.47, 9.70 (s, each 1H, 3H, meso-H).

3-(1-oxo-1-amyl)-3-devinyl purpurin-18 methyl ester 5

The compound 4 220 mg (0.35 mmol) was dissolved in the 15 ml of dichlorometnane, and then 4-methulmorpholine N-oxide 25 mg (0.02 mmol) was added. The mixture was stirred for 15 min under nitrogen. To this reactant mixture the tetrapropylamminium (2.5 mg) was added. When the starting material disappeared totally, the mixture was washed with water (2×20 mL) and extracted with dichloromethane, and then separated dichloromethane layer to be treated further by Drying (Na_2SO_4) and evaporating. The resulting crude residue was chromatographed on silica column with hexane / ethyl acetate in 3:1 to afford title compound (43 %). UV-vis (CH_2Cl_2) λmax (nm, rel. intensity logε): 717 (0.39), 549 (0.17), 510 (0.06), 484 (0.04), 411 (1.00); ^{1}H-NMR δ(500MHz, $CDCl_3$): -0.20 (m, 2H, NH), 1.36 (t, J = 7.4Hz, 3H, 3^6-Me), 1.65~1.66 (m, 2H, 3^3-H), 1.75 (t, J = 7.6Hz, 3H, 8-Me), 1.80 (d, J = 7.2Hz, 3H, 18-Me), 2.18 (t, J = 7.2Hz, 2H, 3^2-H), 2.55~2.57, 2.79~2.81 (m, 4H, each 2H, 17a, 17b-H), 3.55 (m, 2H, 8-H), 3.26, 3.60, 3.65, 3.81 (s, each 3H, 12H, Me+OMe), 4.40~4.41, 5.31~5.32 (m, each 1H, 2H, 17, 18-H), 8.72, 9.67, 9.80 (s, each 1H, 3H, meso-H). Anal. calcd. For $C_{38}H_{42}N_4O_6$: C, 70.13; H, 6.51; N, 8.61. Found: C, 70.17; H, 6.53; N, 8.60.

3-(1-oxo-1-amyl)-3-devinyl purpurin-18-N-(N,N-dimethyl)ethylimide 6a

The compound 5 (200 mg) and excess of corresponding amine (0.15 ml) was dissolved in toluene (20 ml), and the mixture was refluxed under nitrogen atmosphere. When the starting material disappeared totally, the mixture was cooled to room temperature, solvent and excess amines were removed. The crude product was purified by silica column chromatography or preparative TLC plates with 10% methanol in dichloromethane to give purpurinimide 6a in 95% yield. UV-vis (CH_2Cl_2) λmax (nm, rel. intensity logε): 726 (0.38), 554 (0.16), 513 (0.05), 489 (0.04), 416(1.01); ^{1}H-NMR δ(500MHz, $CDCl_3$): -0.57 and -0.80 (each br s, 1H, 2NH), 1.39 (t, J = 7.2Hz, 3H, 3^6-Me), 1.68 (m, 2H, 3^3-H), 1.77 (t, J = 7.0Hz, 3H, 8-Me), 1.80 (d, J = 7.2Hz, 3H, 18-Me), 2.77 (s, 6H, N-$(CH_3)_2$), 2.81 -2.09 (m, 6H, NCH_2-*CH_2*-N-$(CH_3)_2$, 2 x17^1H and 2x 17^2H), 3.52 (q, 2H, 8-H), 3.25, 3.61, 3.67, 3.85 (s, each 3H, 12H, Me+OMe), 4.32 (m, 1H, 18-H), 4.72 (t, 2H, N-*CH_2*-CH_2-N-$(CH_3)_2$),5.33 (m, 1H, 17H), 8.74, 9.68, 9.86 (s, each 1H, 3H, meso-H). Anal. calcd. For $C_{42}H_{52}N_6O_5$: C, 69.97; H, 7.27; N, 11.66. Found: C, 70.11; H, 7.28; N, 11.65.

3-(1-oxo-1-amyl)-3-devinyl purpurin-18-N-(N, N-diethyl) ethylimide 6b

The title compound was synthesized by similar method for 6a. Yeid: 94%. UV-vis (CH_2Cl_2) λmax (nm, rel. intensity logε): 727 (0.40), 555 (0.18), 514 (0.07), 489 (0.05), 417 (1.06); ^{1}H-NMR δ(500MHz, $CDCl_3$): -0.22 and -0.49 (each br s, 1H, 2NH), 1.34 (t, J = 7.4Hz, 3H, 3^6-Me), 1.68 (m, 2H, 3^3-H), 1.76 (t, J = 10.0Hz, 3H, 8-Me), 1.85 (d, J = 7.2Hz, 3H, 18-Me), 2.18 (t, J = 7.2Hz, 2H, 3^2-H), 2.02~2.79 (m, 6H, each 2H, N-CH_2-*CH_2*-N-$(CH_2CH_3)_2$, 17a, 17b-H),2.78 (m, 4H, N-(*CH_2*$CH_3)_2$), 3.55 (m, 2H, 8-H), 3.12, 3.53, 3.62, 3.78 (s, each 3H, 12H, Me+OMe), 4.31~5.34 (m, each H, 4H, N-*CH_2*-CH_2-N-(CH_2CH_3),17, 18-H), 8.56, 9.27, 9.48 (s, each 1H, 3H, meso-H). Anal. calcd. For $C_{44}H_{56}N_6O_5$:C, 70.56; H, 7.54; N, 11.22. Found: C, 70.54; H, 7.53; N, 11.25.

In vitro photosensitizing efficacy

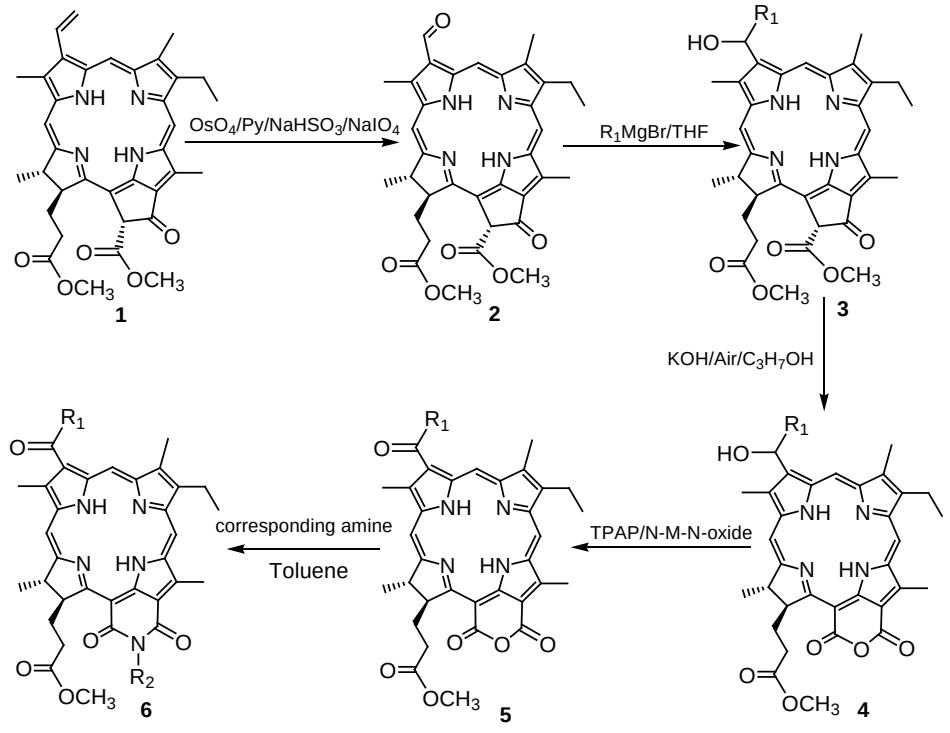
A549 cells were cultured at 37 ^{o}C in a humidified 5% CO_2 incubator using RFMI 1640 growth medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. For phototoxicity studies, A549 cells were plated in 96-well plates at a density of 10 x 10^4 cells well. After 24 h of incubation, 100 µL of 1 µM, 2 µM, 5 µM, 10 µM and 15 µM purpurin-18-N-aminoimides were added, respectively. Plates were returned to the incubator for 24 h. And then the cells were replaced with fresh media and exposed to light (2.0 J/cm^2) for 15 min. Following illumination, the plates were incubated at 37 ^{o}C in the dark. Every 3, 24, 48 hours later, WST-1 was put into each well and measure the absorbance on 450nm wavelength after photoirradiation or without light, respectively. Each experiment was done with three replicate wells. The percentage cell survival was calculated by normalization with respect to the value for no photosensitizer treatment.

Measurement of singlet oxygen production rate

1, 3-Diphenylisobenzofuran (DPBF) was used as a selective singlet oxygen ($^1O_2$) acceptor, which was bleached upon reaction with $^1O_2$. Two sample solutions of DPBF in DMSO (50 µM) containing, respectively no purpurinimide (control sample), 6a (1 µM) and 6b (1 µM) were prepared in the dark. Each sample container was covered with aluminum foil. All samples were then exposed to light (2 J·cm^{-2}) for 15 min. After irradiation, visible spectra of the sample solutions were measured spectrophotometrically. The normalized absorbances of DPBF at 418 nm in these samples were reported. From this plot, the rates of $^1O_2$ production of purpurinimides6a-6b relative to the DPBF were determined.

RESULTS AND DISCUSSION

As shown in Scheme 1, methyl pyropheophorbide-a **1**, used as starting material, was oxidized with osmium(VIII) oxide in tetrahydrofuran containing a catalytic amount of pyridine at 0 ^{o}C, which was followed by glycol cleavage with sodium periodate in aqueous THF to form methyl pyropheophorbide-d (MPPd) in 88 % yield. The formyl group of **2** at 3-position reacted with straight chain alkyl or cyclic alkyl mabnesium bromide in tetrahydrofuran at 0 ^{o}C to give corresponding sec-alcohol 3in 58% yield. Subsequently, thechlorin**3**were converted into derivatives of purpurin-18 methyl ester **4**by air oxidation in propanol containing KOH. The hydroxyl group of chlorin4 was converted into carbonyl group by oxidation with tetrapropylammoniumperruthenate (TPAP) and N-methylmorphloine N-oxide to give 3-alkylacyl-substituted purpyrin-18 methyl ester **5** which exhibited longer wavelength absorption above 717 nm in visible spectra. The intermediate adduct **5** was reacted with N,N-dimethylethylenediamine and N,N-diethylethylenediamine in refluxing toluene under nitrogen atmosphere produces the corresponding purpurinimides**6a-6b** in excellent yield, respectively.



6a. R_1=n-amyl,R_2=$(CH_2)_2N(CH_3)_2$ **6b.**R_1=n-amyl,R2=$(CH_2)_2N(CH_2CH_3)_2$

Scheme 1. Synthetic route to purpurinimides

Table1. Absorption properties of the purpurinimides (1, 4, 5, 6a–6b) in CH_2Cl_2

Compound	Absorption λ_{max} (nm) (log ε)[a]			
	Soret	ΔSoret (Δε)	Q_y	ΔQ_y (Δε)
1	406 (1.00)	0	666 (0.38)	0
4	410 (1.01)	4 (0.01)	693 (0.33)	27 (-0.05)
5	411(1.00)	5(0.00)	717 (0.39)	51 (0.01)
6a	416 (1.01)	10 (0.01)	726 (0.38)	60 (0.00)
6b	417 (1.06)	11 (0.06)	727 (0.40)	61 (0.02)

[a] *ΔSoret, ΔQy and Δε represent the change of the Soret band, Qy band and absorbance intensity, respectively, between the substituted purpurinimides and corresponding starting materials.*

All the novel compounds were successfully characterized by a combination analysis of 1H-NMR and UV-vis spectroscopies, and elemental analysis. The spectroscopic properties of the novel purpurinimides in CH2Cl2 are summarized in Table 1. The purpurinimides 6a–6b containing a 3-formyl group (electron-withdrawing group) showed the maximum red shift and exhibited a strong absorption in the range of 726–727 nm, compared with MPa 1 (666 nm), these purpurinimides shows a great bathochromic shift of the Qy band, and the shifts were significantly found to depend on the nature of the substituents present at the peripheral positions of the chromophore. These purpurinimides had the "ideal" photochemical properties required for an effective PDT agent.

In the ^{1}H-NMR spectra, the structure of the intermediates and title photosensitizers were clearly indicated. The proton signals of the 3-amyl groups of the chlorin 5 were observed at δ 2.18 (3^2-H), 1.65 (3^3-H) and 1.36(3^6-H) ppm. Chlorin**6a** showed each triplet at δ 4.77ppm for the protons of CO-N-CH_2-. In compound 6b, the protons of N-(CH_2CH_3)$_2$ show a multiplet at δ 2.78 ppm. And the N-(CH_3)$_2$ protons of 6a appears as a singlet at δ 2.77 ppm. Elemental analysis data for the purpurinimides reveal good match between calculated and experimental values to the compounds, respectively.

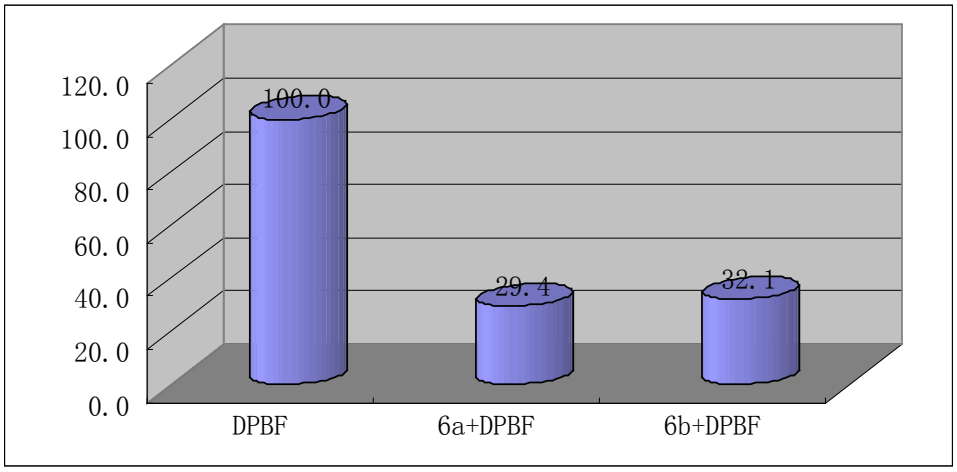


Fig. 1 Comparative absorbance decay (%) of DPBF (50 μM in DMSO) at 418 nm after photoirradiation (total light dose 2 J.cm^{-2}, irradiation time 15 min) in the absence (control) and presence of 6a–6b

1,3-diphenylisobenzofuran (DPBF) was able to capture the $^1O_2$photogenerated by the purpurinimides, which reduced its own light activity [11].As shown in **Figure 1** for purpurinimides**6a-6b**, the absorbance of DPBF at 418 nm decreased in the presence of each purpurinimde. The yield of $^1O_2$photogeneration by purpurinimides **6a-6b** was similar. And purpurinimide**6a** showed relatively higher $^1O_2$photogeneration. The ability of photogenerating$^1O_2$ by the purpurinimides might be greatly affected through the interaction between the chromophoric groups.

Table. 2 IC_{50} values of the purpurinimides against A549 cell lines.

Compound	**1**	**6a**	**6b**
IC_{50} (μM)	1.02	0.27	0.31

The in vitro activity of purprinmides 6a-6b was determined in A549 cells. **Table 2** shows the IC50 values of these novel purpurinimides on A549 cell line after PDT. Compared to MPa, the purpurinimide have higher PDT effect after PDT. And other biological effects of these purpurinimides are currently under investigation.

CONCLUSION

Novel purpurinimides 6a and 6b were prepared and their structures were confirmed by ^{1}H-NMR, UV-vis and elemental analysis. This method by reconstruction for these chemical groups performed the synthesis of purpurinimide derivatives conveniently. The electronic spectrum of these compounds showed a bathochromic shift of the Qy band in comparison with starting materials. The two purpurinimides exhibited certain singlet oxygen yields and photosensitizing efficacies could be potential photosensitizers.

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