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Investigation of the Photophysical and Photochemical Properties of Amino Group Substituted Cationic Chlorin Derivatives for Photodynamic Therapy

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Abstract: Three of novel amino group substituted cationic chlorin derivatives were designed and synthesized using Methyl pheophorbide-a (MPa) as starting materials. The structures of new photosensitizers were characterized by elemental analysis, UV-vis and ^{1}H NMR spectra. They demonstrated a considerable hypsochromic shift of the Qy band in the visible region of the optical spectrum (658-661nm). And their log P values up to log 1. The obtained results proved that the novle cationic chlorin derivatives could be considered a potential photosensitizers for PDT application.

Keywords: photosensitizer, cationic, methyl pheophorbide-a, chlorin, photodynamic therapy

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1. INTRODUCTION

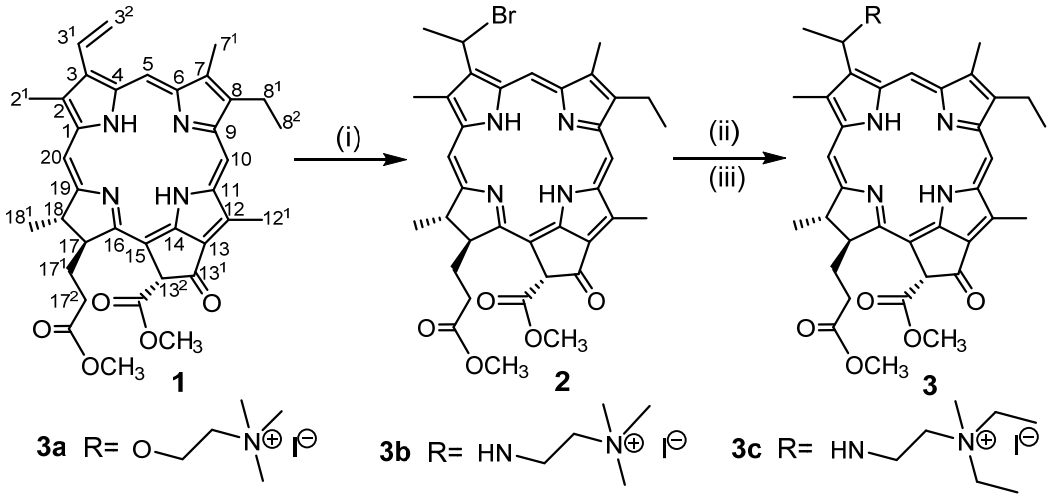
Over the past several decades, photodynamic therapy (PDT) has become a prospective and clinically approved minimally invasive or noninvasive modality for the treatment of a variety of malignant diseases, such as dermatological, respiratory and urinary tumors. PDT treatment involves the combination of photosensitizer, oxygen and light, forming highly reactive oxygen species (like ^{1}O2, OH) that lead to selectively destroying of tumor cells [1-3]. However, the limination of therapeutic efficacy of photosensitizers have been the critical factor for its clinical application.

The emphasis for development of new photosensitizers in PDT has been con- centrated on molecular design, chemical synthesis and biological action for porphyrin derivatives. In recent years a variety of photosensitizers related to chlorins, bacteriochlorins, porphycenes, phthalocyanines, naphthalocyanines, and expanded porphyrins have been synthesized and evaluated for PDT efficacy [4-7]. One of the limitation of photosensitizer is the lower water-solubility which due to aggregation in tumor. Therefore, numerous of photosensitizers have been synthesized and investigated as new generation photosensitizers in PDT applications over recent years [8-10].

Amino group was displayed excellent malignant tumor targeting ability, outstanding water-solubility and lower side effects in many drugs, and many amino group modified porphyrin, chlorin and phthalocyanine derivatives were prepared under this circumstances [11-12]. However, most of amino group modified photosensitizer has not reached the purpose of water-solubility, and its water-solubility was very poor. One of the method to solve this deficiency is to convert the nitrogen atom to ammonium salt using special reagents such as methyl iodide, dimethyl sulfate, etc. [13-15].

Thereby, in this paper, we have reported the synthesis, photophysical and photochemical properties of novel amino group substituted cationic chlorin derivatives (**3a**, **3b** and **3c**), and the water-solubility were also studied.

2. RESULTS AND DISCUSSION



Scheme 1. Synthesis route for cationic chlorin derivatives (**3a**, **3b** and **3c**). Reagents and conditions: (i) 33% HBr/AcOH, N_2, RT, 5h; (ii) Corresponding amines, DCM, N_2, RT, 4-5h; (iii) CH_3I, $CHCl_3$,N_2, RT, 20h.

In our approach, MPa 1 was was used as a starting material, it was reacted with 33% HBr in acetic acid to give bromine-substituted chlorin 2 at room temperature under N2 atomosphere. The preparation of cationic chlorin derivatives 3a, 3b and 3c were performed by mixing corresponding amines (N, N-dimethylethylenediamine, N, N-diethylethylenediamine, N, N-dimethylethanolamine) with chlorin 2 in dichloromethane and stirring under nitrogen. TLC was used to follow the progress of the reaction. The reaction was stopped when most of the starting material had been consumed. After separation on a silica gel column, the collection compounds were converted to the corresponding cationic chlorin derivatives (**3a**, **3b** and **3c**) by methylation and the product yields was nearly 100%.(**Scheme 1**)

All novel cationic chlorin derivatives were characterized by standard spectroscopic techniques such as UV-Vis, ^{1}H NMR, mass and elemental analysis as well. In the ^{1}H NMR spectra of studied cationic chlorin derivatives, the characteristic peaks of the N^+-CH3 protons

were observed as a singlet at 3.50ppm for **3a**, 3.48ppm for **3b** and 3.52ppm for **3c** , the other peaks of all other protons were very similar to peaks recorded for the MPa.

Table 1 Absorption properties of the purpurinimides (1, 3a–3c) in CH_2Cl_2

Compound	Absorption λmax (nm) (log ε) a			
	Soret	ΔSoret (Δε)	Qy	ΔQy (Δε)
1	412.0 (1.52)	0	666.0 (0.71)	0
3a	413.3 (1.69)	1.3 (0.17)	661.6 (0.80)	-4.4 (0.09)
3b	410.3 (1.66)	-1.7 (0.14)	660.1 (0.82)	-5.9 (−0.11)
3c	410.3 (1.69)	-1.7 (0.17)	658.9 (0.82)	-7.1 (−0.11)

[a] ΔSoret, ΔQ_y and Δε represent the change of the Soret band, Q_y band and absorbance intensity, respectively, between the cationic chlorin deratives and corresponding starting materials.

In the UV-Vis spectra, the Qy absorption maxima of cationic chlorin derivatives are observed in the vesible region of the spectrum at around 660nm occurred due to the large 20 carbon π-π* conjugation of chlorin stemnucleus. Compare with MPa **1**, these cationic chlorin derivatives show a hypsochromic shift (4.4-7.1nm) of the Qy band (**Table 1**), it's due to the 3-position double bond destruction which dropped the conjugate effect.

Table 2 Hydrophobicity parameters (log P) of the purpurinimides calculated by means of computer software ACD/Labs (version 12.01)

Compound	3a	3b	3c
Log P	1.51 ± 1.60	1.29± 1.64	1.35 ± 1.62

Research shows that the hydrophobicity parameter (log P) have affinities with the cellular uptake. We uesd a program module of the ACD/Labs software (version 12.01) to calculate the lipophilicity of the novel cationic chlorin derivatives (**Table 2**). All the cationic chlorin derivatives have log P values up to log 10, which theoretically should be expected to give good PDT tumor response.

3. EXPERIMENTAL SECTION

3.1 General methods

The ^1H NMR spectra were recorded on a Varian-500 MHz spectrometer. Chemical shifts are given as δ values using tetramethylsilane as the internal standard and J values are given in Hz. The UV-vis spectra were determined with a UV-1600PC spectrophotometer. Elemental analyses were performed on a Perkin-Elmer 240C Microanalyzer. The hydrophobicity

parameter (logarithm of the partition coefficient between n-octanol and water; log P) was calculated on the basis of the purpurinimide structure using ACD/Labs software (version 12.01). Thin-layer chromatography (TLC) was done on Merck silica gel 60 glass sheets.

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 the literature procedures [11].

3.2 General procedure for the preparation of cationic chlorins 3a, 3b and 3c

MPa (**1**) (100mg, 0.187mmol) was taken in a 50mL round-bottom flask, and 33% HBr/HOAc (5mL) was added after replacement of the atomosphere with nitrogen. The reaction mixture was stirred at room temperature for 5h, and the solvent was removed under high vacuum (bath temperature was maintained at 30-40^{o}C). The resulting concentrate (**2**) was redissolved in dry dichloromethane (10mL). Corresponding diamine (2.0mL) and potassium carbonate (200 mg) were added, and the reaction mixture was stirred at room temperature for 4-5 h under nitrogen atmosphere, poured into water (50mL), and extracted with dichloromethane (3×50mL). The organic layer was washed with water (2×100mL), dried over anhydrous sodium sulfate, concentrated and purified over Silica gel column unsing Hexane/EA (2:1) as eluant to furnish the product. The collection compound and excess of methyliodine (3 ml) were dissolved in dry dichloromethane (10 ml), and the mixture was stirred under nitrogen atmosphere for 20 h at room temperature. Solvent and excess MeI were removed. The desired compound **3** was obtained in nearly 100% yield.

3-(1-N,N,N -trimethylethyloxyethyl)-3-divinyl-pheophorbide-*a* methyl ester iodide 3a.
UV-vis (CH_2CI_2) λ_{max} = 661.6 nm (0.80), 604.5 (0.16), 536.1 (0.18), 504.8 (0.17), 413.3 (1.69).^{1}H NMR ($CDCl_3$, δ): 9.67, 9.43, 8.49 (s, 1H, *meso*-H), 5.95 (q, 1H, 3^1-H), 5.21 (dd, 2H, 13^2-H), 4.43 (q, 1H, 18H), 4.26 (d, 1H, 17H), 3.71(q, 2H, 8^1-H), 3.66, 3.50, 3.28, 3.22 (each s, 3H, N^+-CH_3, OCH_3+CH_3); 2.62 (s, 6H, $N(CH_3)_2$), 2.12 (s, 3H, 3^2-CH_3); 1.81 (d, 3H, 18-CH_3): 1.77 (t, 3H, 8^2-CH_3), 2.85-2.02 (m, 8H, 17^1+17^2-H, O-CH_2-CH_2-N), 0.23 and -1.68 (each brs, 1H, NH). Anal. calcd. For $C_{41}H_{52}N_5O_6I$: C, 58.78; H,6.26; N, 8.36. Found: C, 58.74; H, 6.17; N, 8.39.

3-(1-N,N,N -trimethylethylaminoethyl)-3-divinyl-pheophorbide-*a* methyl ester iodide 3b.
UV-vis (CH_2CI_2) λ_{max} = 660.1 nm (0.82), 603.5 (0.22), 536.7 (0.24), 505.8 (0.24), 410.3 (1.66). ^{1}H NMR ($CDCl_3$, δ): 9.87, 9.43, 8.35 (s, 1H, *meso*-H), 6.10 (1H, br, NH), 5.55 (q, 1H, 3^1-H), 5.11 (dd, 2H, 13^2-H), 4.35 (q, 1H, 18H), 4.16 (d, 1H, 17H), 3.69(q, 2H, 8^1-H), 3.65, 3.48, 3.42, 3.24 (each s, 3H, N^+-CH_3, OCH_3+CH_3); 2.60 (s, 6H, $N(CH_3)_2$), 2.02 (s, 3H, 3^2-CH_3); 1.80 (d, 3H, 18-CH_3): 1.67 (t, 3H, 8^2-CH_3), 2.85-2.02 (m, 8H, 17^1+17^2-H, N-CH_2-CH_2-N), 0.18 and -1.86 (each brs, 1H, NH). Anal. calcd. For $C_{41}H_{52}N_6O_5I$: C, 58.92; H,6.27; N, 10.06. Found: C, 58.86; H, 6.25; N, 10.05.

3-(1-N,N,N-methyldiethylethylaminoethyl)-3-divinyl-pheophorbide-*a* methyl ester iodide 3c.

UV-vis (CH_2CI_2) λ_{max} = 658.9 nm (0.82), 604.0 (0.15), 536.6 (0.16), 505.8 (0.17), 410.3 (1.69). ^{1}H NMR ($CDCl_3$, δ): 9.87, 9.44, 8.40 (s, 1H, *meso*-H), 6.12 (1H, br, NH), 5.65 (q, 1H, 3^1-H), 5.09 (dd, 2H, 13^2-H), 4.53 (q, 1H, 18H), 4.24 (d, 1H, 17H), 3.72(q, 2H, 8^1-H), 3.68, 3.52, 3.38, 3.21 (each s, 3H, N^+-CH_3, OCH_3+CH_3); 2.82 (q, 4H, N(CH_2CH_3)$_2$), 2.12 (s, 3H, 3^2-CH_3); 1.81 (d, 3H, 18-CH_3): 1.77 (t, 3H, 8^2-CH_3), 2.85-2.02 (m, 8H, 17^1+17^2-H, N-CH_2-CH_2-N), 1.21 (t, 6H, N(CH_2CH_3)$_2$), 0.23 and -1.68 (each brs, 1H, NH). Anal. calcd. For $C_{43}H_{56}N_6O_5I$: C, 59.79; H,6.53; N, 9.73. Found: C, 59.75; H, 6.55; N, 9.75.

4. CONCLUSION

In this study, the synthesis and characterization of novel amino group substituted cationic chlorin derivatives (**3a**, **3b**, **3c**) were described. In the electronic absorption spectra, the cationic chlorin derivatives displayed the Qy band in the region 658-661 nm. ^{1}H NMR spectroscopy confirmed the structure of target compounds, resulting in significant chemical shift of N^+-CH3 protons signals. And their log P values up to log 1, which revealed that they could be potential photosensitizers for PDT.

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