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Novel cationic purpurinimides as potential photosensitizers: Design, synthesis and biological evaluation

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ABSTRACT

A series of novel cationic purpurinimides which had long wavelength absorption were designed and synthesized using purpurin-18-N-aminoimides as starting materials. They demonstrated a considerable bathochromic shift of the major absorption band in the red region of the optical spectrum (705.8-718.9nm). Newly synthesized purpurinimides were screened for their antitumor activities, and showed enhanced PDT efficiency in A549 cells as compared to purpurin-18-N-aminoimide. The results showed the novel cationic purpurinimides could be considered a promising photosensitizer in photodynamic therapy.

Key words: Purpurinimide, Chlorin, Photosensitizer, Photodynamic therapy

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INTRODUCTION

Photodynamic therapy (PDT) has become a feasible modality for the treatment of numerous cancers using a combination of a photosensitizer, light and molecular oxygen. PDT relies on several concepts: (a) preferential localization of the photosensitizer ; (b) activation of photosensitizer by light to sensitize the generation of singlet oxygen ($^1O_2$), which results in free radicals (•OH, HO•$_2$) and radicals ions (•O^{-2}) [1-3]. Because $^1O_2$ is so reactive, PDT induces oxidative damage to cell membranes, proteins (aromatic amino acids), lipids (double bonds), and DNA damage (bases and phosphate backbone); which are localized to areas no greater in diameter than the cell membrane thickness (bilayers 6 –10 nm, human tissue cell ~ 20 microns) [4,5].

The activating light is specifically chosen to be within 650-800 nm, because (a) The presence of endogenous chromophores such as hemoglobin results in very poor penetration of tissues by light at wavelengths below 650nm; (b) A compound which absorbs light above 800nm may not have a large enough energy gap between its triplet state and ground state to generate siglet oxygen [3,6].

Development of 2nd-generation photosensitizer candidates has focused on improving the photophysical and pharmacokinetic properties of potential photosensitizers to increase the efficacy and expand the utility of PDT, while simultaneously obviating negative side effects of the currently approved treatments. Chlorins represent a class of porphyrinoid with one pyrrole double bond missing, often times reduced. As the lowest energy Q-band of chlorins is normally red-shifted 20-30 nm and has a 10 times greater absorption intensity compared to that of porphyrins, which allows greater penetration into tissues in PDT applications [7]. Mono-L-aspartyl chlorin e6 (MACE, NPe6,Talaporfin, LS-11) and mono-carboxylic acid (BPD-MA, Visudyne™), which are obtained either from

chlorophyll or from protoporphytin, are important naturally derived second generation photosensitizers. These two, as PDT photosensitizers, are currently undergoing human clinical trials for the treatment of various cancers and age-related macular degeneration[8,9]. In the meanwhile, synthetically derived second generation photosensitizers, such as the symmetric meso-tetra(m-hydroxyphenyl)chlorin (m-THPC, Foscan®) and Sn(IV)-etiopurpin (SnET2, PuryltinTM), are in their early clinical trials for the treatment of various cancers[10,11].

The cationic photosensitizers play an important role in medicine, molecule identification, analytical chemistry, electrochemistry and photochemistry. The damage that occurs during PDT using cationic photosensitizers can take place both intra and extracellularly. The damage to the cell involves either the disruption of the plasma membrane or the mitochondrial membrane along with destruction of the cellular organelles. In recent years, several cationic dyes have been studied as sensitizers for PDT, such as cationic porphyrins, phthalocyanines, chlorins and bacteriochlorin [12-15]. These cationic dyes, however, still have shortcomings, most notably the shorter wavelength absorption or lower stability. Synthesis and photodynamic effects of functional chlorin molecules is one of our academic items in our group [16-18]. In the previous studies [19], data has been presented using purpurin-18-N-aminoimides as a PDT agent, and purpurinimide containing pyrazolyl and hydroxyl groups showed good PDT efficacy. [16]

With this in mind, and the knowledge that chlorins have increased absorption at longer wavelengths, we have designed, synthesized, and evaluated a series of novel long-wavelength cationic purpurinimides as potential photosensitzers for PDT.

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).

General procedure for the preparation of cationic purpurinimides.
Purpurinimide (100 mg) and excess of methyliodine (5 ml) were dissolved in dry dichloromethane (20 ml), and the mixture was stirred under nitrogen atmosphere for 20 h at room temperature. Solvent and excess MeI were removed. The desired compound was obtained in nearly 100% yield.

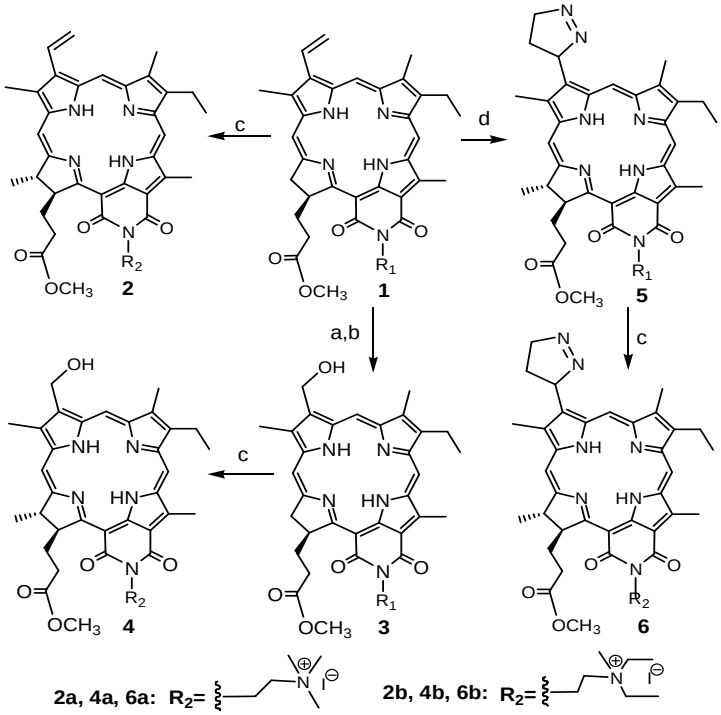
3^1-hydroxypurpurin-18-N-(N,N,N-trimethyl)ethylimide iodide 4a. UV-vis in CH_2Cl_2, λ max (nm, rel. intensity log ε), 417.8 (0.96), 479.9 (0.03), 508.9 (0.06), 547.8 (0.12), 650.4 (0.05), 705.8 (0.28). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.32 (s, 1H, for 10-H), 9.28 (s, 1H, for 5-H), 8.50 (s, 1H, for 20-H), 5.74 (s, 2H, 3-CH_2), 5.26 (m, 1H, 17-H), 4.65 (t, 2H, N-*CH_2*-CH_2-N-$(CH_3)_2$), 4.31 (m, 1H, 18H), 3.56 (s, 3H, 12-CH_3), 3.52 (q, 2H, 8^1CH_2), 3.49 (s, 3H, N^+-CH_3), 3.32 (s, 3H, 2-CH_3), 3.10 (s, 3H, 7-CH_3), 2.71 (s, 6H, N-$(CH_3)_2$), 2.70 -1.81 (m, 6H, NCH_2-*CH_2*-N-$(CH_3)_2$, 2 x 17^1H and 2 x 17^2H), 1.77 (d, *J* = 7.0 Hz, 3H, 18-CH_3), 1.58 (t, *J* = 7.5 Hz, 3H, 8^2CH_3), -0.37 and -0.50 (each br s, 1H, 2NH). Anal. calcd. For $C_{38}H_{47}N_6O_5I$: C, 57.43; H,5.96; N, 10.57. Found: C, 57.38; H, 5.92; N, 10.52.

3^1-hydroxypurpurin-18-N-(N,N,N-methyldimethyl)ethylimide iodide 4b. UV-vis in CH_2Cl_2, λ max (nm, rel. intensity log ε), 417.6 (0.96), 480.8 (0.03), 510.4 (0.05), 548.6 (0.13), 649.9 (0.05), 705.9 (0.28). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.74 (s, 1H, for 10H), 9.44 (s, 1H, for 5H), 8.60 (s, 1H, for 20H), 5.78 (s, 2H, 3-CH_2), 5.32 (m, 1H for 17H), 4.75 (m, 2H, N-*CH_2*-CH_2-N-(CH_2CH_3)), 4.33 (q, *J* = 7.5 Hz, 1H, 18H), 3.70 (s, 3H, 12-CH_3), 3.61 (m, 2H, 8^1CH_2), 3.55 (s, 3H, $17^2CO_2CH_3$), 3.51(s, 3H, N^+-CH_3), 3.12 (s, 3H, 7CH_3), 2.78 (m, 4H, N-(*CH_2*CH_3)$_2$), 2.76-1.85 (m, 6H, N-CH_2-*CH_2*-N-$(CH_2CH_3)_2$, 2 x 17^1H and 2 x 17^2H), 1.76 (d, *J* = 10.0 Hz, 3H, 18CH_3), 1.67 (t, *J* = 7.5 Hz, 3H, 8^2CH_3), 1.34(m, 6H, N-(CH_2-*CH_3*)$_2$), 0.19 and -0.48 (each br s, 1H, 2NH). Anal. calcd. For $C_{40}H_51N_6O_5I$: C, 58.39; H,6.25; N, 10.21. Found: C, 58.33; H, 6.29; N, 10.14.

3-Devinyl-3-[3'(R,S)-(1'-pyrazolinyl)]purpurin-18-N-(N,N,N-trimethyl)ethylimide iodide 6a. UV-vis in CH_2Cl_2, λ max (nm, rel. intensity log ε), 417.8 (0.98), 480.9 (0.04), 511.5 (0.05), 554.8 (0.09), 718.9 (0.29). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 9.66 (s, 1H, for 10H), 9.32 (s, 1H, for 5H), 8.58 (s, 1H, for 20H), 6.67 (dd, *J*=12.5, 7.5, Hz, 3'-H), 5.58 (dd, 1H, *J*=18.0, 9.5Hz, 5'$_a$-H), 5.32 (m, 1H for 17H), 4.77 (td 1H, *J*=18.0, 9.0 Hz, 5'$_b$-H), 4.70 (t, *J* = 7.0 Hz,

2H, N-CH_2-CH_2-N-$(CH_3)_2$), 4.38(q, *J* = 7.5 Hz, 1H, 18H), 3.78 (s, 3H, 12-CH_3), 3.66 (q, *J* = 8.0 Hz, 2H, 8^1CH_2), 3.52 (s, 3H, $17^2CO_2CH_3$), 3.42 (s, 3H, N^+-CH_3), 3.38(s, 3H, $2CH_3$), 3.12 (s, 3H, $7CH_3$), 2.79 (s, 6H, N-$(CH_3)_2$), 2.78 -1.96 (m, 8H, 4'$_a$-H, 4'$_b$-H, NCH_2-CH_2-N-$(CH_3)_2$, 2 x 17^1H and 2 x 17^2H), 1.79 (d, *J* = 7.5 Hz, 3H, $18CH_3$), 1.67 (t, *J* = 7.5 Hz, 3H, 8^2CH_3), 0.07 and -0.10 (each br s, 1H, 2NH). Anal. calcd. For $C_{40}H_{49}N_8O_4I$: C, 57.69; H, 5.93; N, 13.46. Found: C, 57.66; H, 5.96; N, 13.38.

3-Devinyl-3-[3'(R,S)-(1'-pyrazolinyl)]purpurin-18-N-(N,N,N-methyldimethyl)ethylimide iodide 6b. UV-vis in CH_2Cl_2, λ max (nm, rel. intensity log ε), 418.6 (0.92), 482.3 (0.05), 512.8 (0.06), 555.1 (0.10), 669.8 (0.05), 718.5 (0.27). ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.48 (s, 1H, for 10-H), 9.21 (s, 1H, for 5-H), 8.57 (s, 1H, for 20-H), 6.68 (dd, *J*=12.5, 7.5, Hz, 3'-H), 5.56 (dd, 1H, *J*=18.0, 9.5 Hz, 5'$_a$-H), 5.40 (m, 1H for 17-H), 4.78 (td 1H, *J*=18.0, 9.0 Hz, 5'$_b$-H), 4.52 (t, 2H, *J* = 7.0 Hz, N-CH_2-CH_2-N-(CH_2CH_3)), 4.38 (q, *J* = 7.5 Hz, 1H, 18H), 3.71 (s, 3H, 12-CH_3), 3.62 (q, *J* = 8.0 Hz, 2H, 8^1-CH_2), 3.55 (s, 3H, N^+-CH_3), 3.32 (s, 3H, 2-CH_3), 3.02 (s, 3H, 7-CH_3), 2.91(q, 4H, N-($CH_2CH_3)_2$), 2.89 -1.98 (m, 8H, 4'$_a$-H, 4'$_b$-H, N-CH_2-CH_2-N-$(CH_2CH_3)_2$, 2 x 17^1-H and 2 x 17^2-H), 1.79 (d, *J* = 7.5 Hz, 3H, 18-CH_3), 1.68 (t, *J* = 7.5 Hz, 3H, 8^2-CH_3), 1.29 (t, *J* = 7.0 Hz, 6H, N-(CH_2-$CH_3)_2$), 0.11 and -0.09 (each br s, 1H, 2NH). Anal. calcd. For $C_{42}H_{53}N_8O_4I$: C, 58.60; H,6.21; N, 13.02. Found: C, 58.54; H, 6.15; N, 13.05.



Scheme 1. Synthesis of cationic purpurinimides. Reagents and conditions: (a)OsO_4, $NaIO_4$, 6h; (b) $NaBH_4$, 5h; (c)CH_3I, $CHCl_3$, 20h; (d)CH_2N_2, 20h.

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 wereadded, 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.

RESULTS AND DISCUSSION

The synthetic strategies adopted the target compounds are depicted in **Scheme 1**. In order to synthesize compound **3** with a hydroxyl group at 3-position, the vinyl group at 3-position of purpurin-18-*N*-aminoimides **1** was oxidized to

3-formyl analog by reacting with osmium tetraoxide/sodiumperiodate. The resulting 3-formyl derivative on reaction with sodium borohydride afforded 3-hydroxy-methyl-purpurin-18-*N*-aminoimides **3**. For synthesis of purpurinimides with a pyrazolinyl group at 3-position, The 1,3-dipolar [3+2] cycloaddition of purpurin-18-N-aminoimides **1** with ethereal diazomethane was also attempted. And the final desired compounds were obtained in good yield. Purpurinimides **1, 3, 5** were converted to the corresponding cationic purpurinimides by N-methylation and the product yield was nearly 100%. All the desired cationic purpurinimides were characterized by ^{1}H-NMR and UV-vis spectroscopies and elemental analysis.

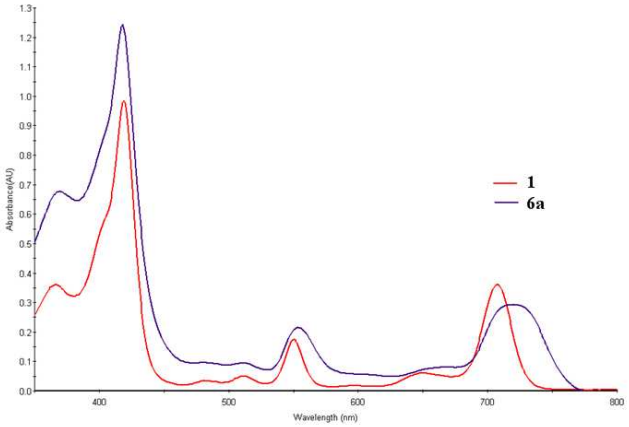


Figure 1. Electronic absorption spectra of 1 and 6a in dichloromethane

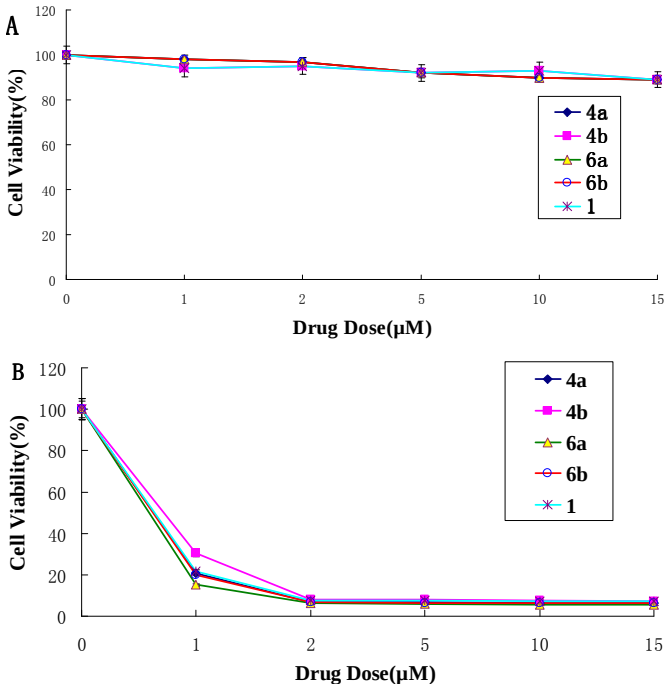


Figure 2. Comparative in vitro photosensitizing efficacy for 4a, 4b, 6a, 6b at various drug dose in A549 cells at 24 h post incubation: A) Dark toxicity; B) Photocytotoxicity

The spectroscopic properties of the novel cationic purpurinimides are shown in **Figure 1**. In the UV-vis spectra, the Qy absorption maxima of cationic purpurinimides **2a-2b**, **4a-4b** and **6a-6b** in dichloromethane are in the range of 705-719 nm, compared with methyl pheophorbide a (MPa), these cationic purpurinimides show a large bathochromic shift (39-53 nm) of the Qy band, which makes them as potential photosensitizers for PDT(**Table 1**). In the ^{1}H-NMR spectra of cationic purpurinimide **4a**, **4b**, **6a**, **6b** the N^+-CH_3 protons appears as a singlet at δ 3.49, 3.51, 3.42, 3.55ppm, respectively. For compounds **4a**, **4b**, **6a, 6b**, the resonances of all the protons of the 3-position also appear at characteristic chemical shifts.

Table 1. Absorption maxima of 2a-2b, 4a-4b and 6a-6b in dichloromethane and their red-shift values of the redmost Q_y band

Compound	Absorption maxima, nm		ΔQ_y (nm)
	Soret	Redmost Q_y	
MPa	412.0	666.0	0
2a	419.7	710.6	44.6
2b	418.8	710.9	44.9
4a	417.8	705.8	39.8
4b	417.6	705.9	39.9
6a	417.8	718.9	52.9
6b	418.6	718.5	52.5

To investigate their potential in PDT, preliminary in vitro photodynamic effects of these novel long wavelength cationic purpurinimides were evaluated against A549 cell exposed to increasing concentrations of each compound up to 15 μM. As shown in **Figure 2**, none of the cationic purpurinimides up to 1 μM concentration have any significant dark cytotoxicity. Most of the newly synthesized long wavelength cationic purpurinimides have exhibited enhanced PDT efficacy over compound **1**, which itself is a quite effective photosensitizer. Among the cationic purpurinimides investigated, the compound **6a** produced the best efficacy, followed by **6b**, **4a** and **4b** under similar experimental conditions.

CONCLUSION

We described the synthesis, photophysical properties, and preliminary in vitro photosensitizing efficacy of the novel long wavelength cationic purpurinimides. It is clearly indicated that cationic purinimides does improve efficacy, which revealed that they could be used as potential candidate of PDT. Further PDT efficacy tests of these cationic purpurinimides are presently in progress in our laboratory.

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