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Synthesis and Photodynamic Activities of Pyrazolyl and Cyclopropyl Derivatives of Purpurin-18 Methyl Ester and Purpurin-18-*N*-butylimide

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The synthesis of new pyrazolyl and cyclopropyl derivatives of purpurin-18 methyl ester and purpurin-18-*N*-butylimide **1a**, **1b**, **2a**, **2b** and **8** is described. The new compounds were characterized by NMR, UV-vis spectroscopy and mass spectrometry. UV-vis spectra of the new compounds showed long wavelength absorption of ranges 692 - 708 nm (λ_{max}). Photodynamic effects of the chlorin derivatives **1a**, **1b**, **2a** and **2b** were investigated by WST-1 assay in A549 cells, and showed good photodynamic activities with high photocytotoxicity and low cytotoxicity in the dark. In comparison between pyrazolyl and cyclopropyl derivatives, purpurin-18 methyl ester compounds **1a** and **1b** showed comparable photocytotoxicity result of the cell viabilities, otherwise, pyrazolyl derivative of purpurin-18-*N*-butylimide **2a** showed better cell viabilities than those of cyclopropyl derivative **2b**. And cyclopropyl derivative of purpurin-18-*N*-butylimide **2b** showed higher dark cytotoxicity than that of others.

Key Words: Cyclopropyl and pyrazolyl units, *In vitro* study, Photodynamic therapy, Purpurin-18 methyl ester, Purpurin-18-*N*-butylimide

Introduction

Photodynamic therapy (PDT) is a promising cancer treatment because of its important advantages by a combination of visible light, photosensitizer (PS) and oxygen.[1-5] To date, many groups focused on the development of new PS to afford selective photodynamic effect through a photoirradiation results in a generation of singlet oxygen to destroy the tumour cells. Chlorins are second-generation PSs and good candidates for PDT on account of the long wavelength absorption within the photodynamic window (650 - 800 nm)[6] to allow sufficient tissue-penetration in cancer treatment.

Introduction of pyrazolyl[7] and cyclopropyl[8] units on the organic molecules has much attention because of their versatile applications that involves anticancer, antimicrobial, antiviral, antifungal and neurochemical agents. In addition, the pyrazole ring in a molecule is a hetero aromatic system which may afford biological significance to the molecules. Diazomethane[9] is a useful agent for the synthesis of pyrazolyl and cyclopropyl introduced chlorin derivatives through a well-known particular regioselective 1,3-dipolar cycloaddition reaction. Recently, Kozyrev group[10] reported the synthesis of pyrazolyl (before oxidation form) and cyclopropyl derivatives of methyl pyropheophorbide-a, purpurin-*N*-methylimide and protoporphyrin IX.

The PDT institute has many efforts to develop new PSs and to evaluate their photodynamic effects through *in vitro* and *in vivo* test. Previously,[11,12] we have reported the synthesis of pyrazolyl derivatives of methyl pheophorbide-a, methyl pyropheophorbide-a and purpurin-18 methyl ester by the reaction between 3-(2-acetyl-3-oxobutyl)-3-devinylpyropheophorbide-a methyl ester and hydrazine derivatives, and cyclopropyl derivative of methyl pyropheophorbide-a. And we have studied the photodynamic effect of the pyrazolyl derivatives of methyl pyropheophorbide-a.[12] Our continuous effort for developing potential PS afforded the synthesis of new chlorin derivatives incorporating the pyrazolyl (as both before and after oxdidation forms) and cyclopropyl units of purpurin-18 methyl ester and purpurin-18-*N*-butylimide. Especially, the purpurin-18-*N*-butylimide derivatives could allow deeper tissue-penetration than that of the purpurin-18 derivatives on account of longer wavelength absorption.[13]

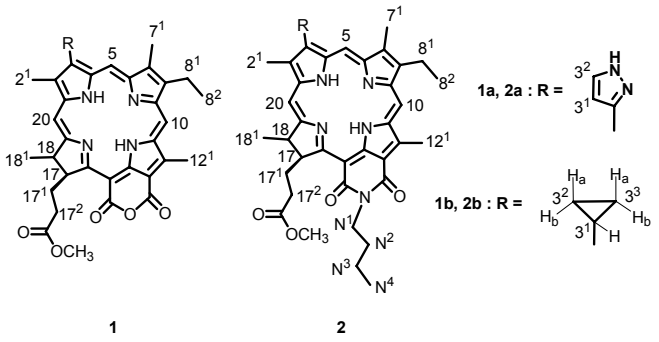

Figure 1. Structures of pyrazolyl and cyclopropyl derivatives of purpurin-18 methyl ester and purpurin-18-*N*-butylimide **1** and **2**.

In this report we describe the synthesis of new derivatives of pyrazolyl and cyclopropyl purpurin-18 methyl ester and purpurin-18-*N*-butylimide (Figure 1), which was characterized by NMR and UV-vis spectroscopic and high resolution fast atom bombardment mass (HRFABMS) spectrometric analyses. Moreover, we evaluated photodynamic activities of the new chlorin derivatives by *in vitro* study in A549[14] (human lung carcinoma) cells. Especially, cell viabilities for photocytotoxicity and cytotoxicity in the dark of the pyrazolyl and cyclopropyl derivatives

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of purpurin-18 methyl ester and purpurin-18-*N*-butylimide were compared each other.

Experimental Section

General. All reagents were purchased from Aldrich and used without further purification. Compounds **4**,[15] **5**,[15] **6**[11] and **7**[15] were prepared according to literature procedures. Thin layer chromatography (TLC) was performed on silica gel 60 F254 (E. Merck). Column chromatography was performed on silica gel 60F (Merck 9385, 0.040 - 0.063 mm). The UV-vis absorption spectra were recorded on Scinco S-3100 spectrophotometer using chloroform as a solvent. Melting points were recorded on an Electrothermal IA9000 Series digital instrument in open capillary tubes and are uncorrected. Routine nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a Varian liquid chromatography-nuclear magnetic resonance spectrometer, with working frequencies of 500 MHz for ^{1}H, and 125 MHz for ^{13}C nuclei. 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. High resolution fast atom bombardment mass (HRFABMS) spectra were obtained on a Jeol JMS700 high resolution mass spectrometer at the Daegu center of KBSI, Kyungpook national university, Korea.

Cell Culture and Photoirradiation. A549 cells (human lung carcinoma) were obtained from the cell line bank at Seoul national university's cancer research center (Korea). They were grown in a mixed medium of RPMI-1640 (Sigma-Aldrich) containing 10% fetal bovine serum (FBS), penicillin-streptomycin mixture and sodium pyruvate at 37 °C in a humidified atmosphere of 5% CO_2 in air. The PDT was carried out using a diode laser generator apparatus (BioSpec LED, Russia) equipped with a halogen lamp, a band-pass filter (640 - 710 nm), and a fiber optics bundle. The duration of light irradiation, under PDT treatment, is calculated taking into account the empirically found effective dose of light energy in J·cm^{-2}.

WST-1 Assay and Cell Viability. A549 cells (10×10^4 cells/well) in 100 μL of the mixed medium were placed in a 96-well plate and incubated for 24 h (37 °C, 5% CO_2). And the PS (0.5 - 10 μM) in 100 μL of the mixed medium was added in each well. After 24 h incubation, the mixed solution in each well was discarded. And the cells were washed with phosphate buffered saline (PBS, Sigma-Aldrich) (100 μL × 3), and 100 μL of the mixed medium was added in each well. The cells were irradiated with the LED (2 J·cm^{-2}) for 15 min. And absorbance of the cells was measured after 3 h, 24 h and 48 h incubation using WST-1 reagent (10 μL) by fluorescence multi-detection reader (BioTek, Synergy HT, USA) at 450 nm, respectively. Cell viability was calculated by normalization with respect to the value for no PS treatment. Standard deviation of the cell viability was calculated from the three replicate experiments.

Methylpheophorbide-a (MPa) 3. Chlorophyll-a paste (*excrementum bombycis*, 100 g) was dissolved in 500 mL of 5% sulfuric acid in methanol and stirred at room temperature for 50 h under nitrogen atmosphere in dark. Following the standard workup, methyl pheophorbide-a (MPa) was obtained in 5% yield. The analytical data were identical with those reported previously.[16]

Pyrazolyl Purpurin-18 Methyl Ester (1a). 6 (25 mg, 0.0403 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (10 mg, 0.0441 mmol) were dissolved in acetone (10 mL), and the reaction mixture was stirred for 24 h. The solvent was evaporated and the residue was purified by column chromatography (SiO_2: 2% acetone/dichloromethane) to afford **1a** as a dark purple solid (7.5 mg, 30%). **1a**: mp 243 - 244 °C; UV-vis ($CHCl_3$): λ ($10^{-3}\varepsilon$/M^{-1}·cm^{-1}) = 413 (130.7), 510 (6.67), 548 (27.67), 589 (2.55), 645 (9.84), 701 nm (55.40); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS) δ 10.01 (s, 1H, 10H), 9.60 (s, 1H, 5H), 8.60 (s, 1H, 20H), 7.98 (d, J = 3 Hz, 1H, 3^{2}H), 7.06 (d, J = 3 Hz, 1H, 3^{1}H), 5.20 (m, 1H, 17H), 4.40 (m, 1H, 18H), 3.80 (s, 3 H, 12^{1}H), 3.67 (q, J = 8 Hz, 2H, 8^{1}H), 3.60 (s, 3H, CO_2CH_3), 3.44 (s, 3H, 2^{1}H), 3.16 (s, 3H, 7^{1}H), 2.77-2.74 (m, 1H, 17^{2}H), 2.50-2.45 (m, 2H, 17^{1}H), 1.99 (m, 1H, 17^{2}H), 1.76 (d, J = 7 Hz, 3H, 18^{1}H), 1.67 (t, J = 8 Hz, 3H, 8^{2}H), 0.34 (br s, 1H, NH), 0.09 (br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS) δ 177.5, 176.5, 173.7, 164.3, 159.5, 156.8, 150.1, 145.9, 145.0, 144.5, 139.9, 139.2, 136.8, 136.7, 133.9, 132.1, 131.5, 128.0, 111.4, 107.6, 107.5, 106.5, 95.0, 92.7, 60.0, 55.0, 51.6, 49.2, 32.6, 31.2, 23.9, 19.4, 17.5, 12.4, 11.1; HRFABMS: calcd for $C_{35}H_{35}N_6O_5$ ([$M + H$]$^+$) 619.2669, found 619.2667.

Cyclopropyl Purpurin-18 Methyl Ester (1b). 6 (50 mg, 0.0806 mmol) was dissolved in *o*-dichlorobenzene (25 mL) and refluxed at 184 - 193 °C for 20 min. The reaction solution was cooled until room temperature and the solution was loaded into column chromatography (SiO_2: dichloromethane) to remove *o*-dichlorobenzene, and eluting with 4% acetone/dichloromethane afforded **1b** as a dark purple solid (34 mg, 71%). **1b**: mp 124 - 126 °C; UV-vis ($CHCl_3$): λ ($10^{-3}\varepsilon$/M^{-1}·cm^{-1}) = 410 (134.03), 506 (8.56), 543 (20.89), 662 (12.07), 692 nm (42.63); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS) δ 9.49 (s, 1H, 10H), 9.48 (s, 1H, 5H), 8.46 (s, 1H, 20H), 5.17 (m, 1H, 17H), 4.35 (m, 1H, 18H), 3.71 (s, 3H, 12^{1}H), 3.61 (q, J = 8 Hz, 2H, 8^{1}H), 3.59 (s, 3H, CO_2CH_3), 3.31 (s, 3H, 2^{1}H), 3.17 (s, 3H, 7^{1}H), 2.77 (m, 1H, 17^{2}H), 2.75 (m, 1H, 3^{1}H), 2.49-2.39 (m, 2H, 17^{1}H), 1.98 (m, 1H, 17^{2}H), 1.73 (d, J = 7 Hz, 3H, 18^{1}H), 1.64 (t, J = 8 Hz, 3H, 8^{2}H), 1.59-1.54 (m, 2H, 3^2_aH and 3^3_aH), 1.34-1.25 (m, 2H, 3^2_bH and 3^3_bH), 0.27 (br s, 1H, NH), −0.13 (br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS) δ 177.5, 177.1, 173.7, 164.3, 159.4, 156.5, 150.0, 146.1, 144.7, 142.2, 139.4, 138.9, 138.7, 136.4, 134.4, 131.2, 111.2, 107.6, 102.8, 94.5, 92.8, 54.9, 51.5, 49.3, 32.5, 31.2, 23.8, 19.4, 17.4, 12.3, 11.3, 11.0, 7.4, 6.6; HRFABMS: calcd for $C_{35}H_{37}N_4O_5$ ([$M + H$]$^+$) 593.2764, found 593.2767.

Pyrazolyl Purpurin-18-*N*-butylimide (2a). 8 (22 mg, 0.0326 mmol) and DDQ (10 mg, 0.0441 mmol) were dissolved in acetone (10 mL), and the reaction mixture was stirred for 24 h. The solvent was evaporated and the residue was purified by column chromatography (SiO_2: 2% acetone/dichloromethane) to afford **2a** as a dark purple solid (11 mg, 50%). **2a**: mp 85 - 86 °C; UV-vis ($CHCl_3$): λ ($10^{-3}\varepsilon$/M^{-1}·cm^{-1}) = 420 (202.19), 481 (7.18), 513 (9.74), 551 (35.25), 596 (3.49), 651 (11.79), 708 nm (71.44); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS) δ 9.98 (s, 1H, 10H), 9.61 (s, 1H, 5H), 8.61 (s, 1H, 20H), 7.96 (d, J = 3 Hz, 1H, 3^{2}H), 7.05 (d, J = 3 Hz, 1H, 3^{1}H), 5.40 (m, 1H, 17H), 4.47 (t, J = 7.5 Hz, 2H, N^{1}H), 4.37 (m, 1H, 18H), 3.82 (s, 3 H, 12^{1}H), 3.65 (q, J = 8 Hz, 2H, 8^{1}H), 3.56 (s, 3H, CO_2CH_3), 3.45 (s, 3H,

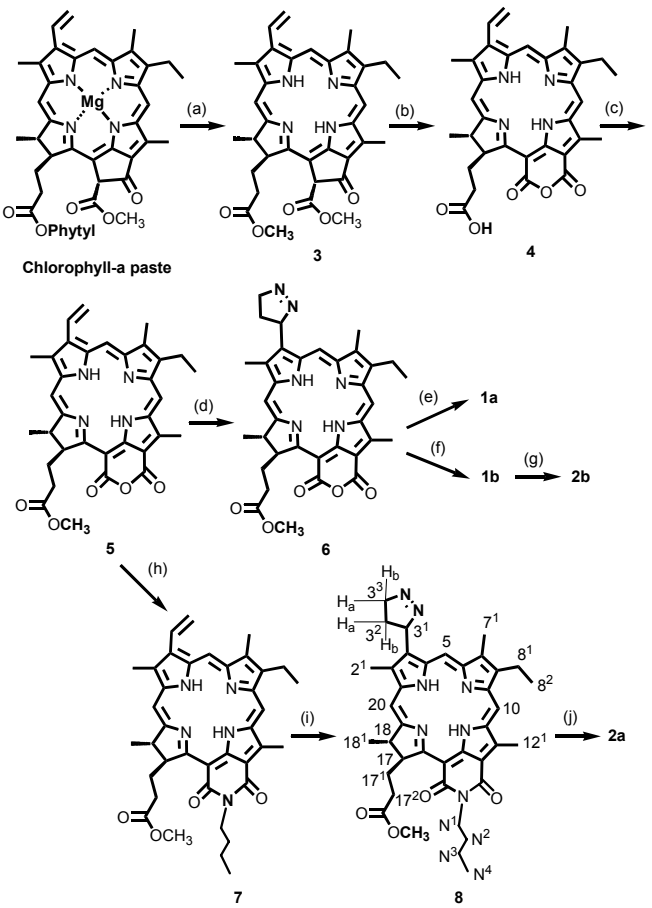
2^1H), 3.16 (s, 3H, 7^1H), 2.73-2.67 (m, 1H, 17^2H), 2.45-2.33 (m, 2H, 17^1H), 2.01 (m, 1H, 17^2H), 1.99 (m, 2H, N^2H), 1.77 (d, J = 7 Hz, 3H, 18^1H), 1.67 (t, J = 8 Hz, 3H, 8^2H), 1.65 (t, J = 7.5 Hz, 2H, N^3H), 1.10 (t, J = 7.5 Hz, 2H, N^4H), 0.02 (br s, 1H, NH), −0.03 (br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS) δ 176.5, 174.6, 173.9, 167.5, 163.5, 155.7, 149.8, 145.4, 145.3, 143.1, 139.5, 137.6, 136.4, 136.1, 133.1, 131.8, 131.4, 127.9, 115.9, 107.5, 106.8, 105.8, 97.5, 94.6, 60.2, 54.7, 51.5, 49.1, 40.1, 32.4, 31.1, 24.0, 20.8, 19.4, 17.5, 14.1, 12.5, 12.2, 11.1; HRFABMS: calcd for $C_{39}H_{44}N_7O_4$ ($[M + H]^+$) 674.3455, found 674.3451.

Cyclopropyl Purpurin-18-*N*-butylimide (2b). 1b (24 mg, 0.0405 mmol) and butylamine (100 μL, 1.008 mmol) were dissolved in dichloromethane (10 mL), and the reaction mixture was stirred at room temperature for 48 h. The reaction was monitored by TLC and the solvent was evaporated to afford dark green residue. The residue and CH_2N_2 (1 mL of 0.52 M solution in diethyl ether, 0.52 mmol) were dissolved in dichloromethane (7 mL) and the reaction mixture was stirred at room temperature for 5 min. The reaction was monitored by TLC and catalytic amount of methanolic KOH was added, and the reaction mixture was stirred at room temperature for 5 min. The solvent was evaporated and the residue was purified by column chromatography (SiO_2: 2% acetone/dichloromethane) to afford **2b** as a dark purple solid (13 mg, 50%). **2b**: mp 218 - 219 °C; UV-vis ($CHCl_3$): λ ($10^{-3}\varepsilon/M^{-1}\cdot cm^{-1}$) = 418 (304.43), 481 (9.04), 510 (14.99), 546 (37.09), 644 (13.87), 699 nm (79.62); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS) δ 9.52 (s, 1H, 10H), 9.46 (s, 1H, 5H), 8.47 (s, 1H, 20H), 5.38 (m, 1H, 17H), 4.46 (t, J = 7.5 Hz, 2H, N^1H), 4.33 (m, 1H, 18H), 3.78 (s, 3 H, 12^1H), 3.60 (q, J = 8 Hz, 2H, 8^1H), 3.56 (s, 3H, CO_2CH_3), 3.30 (s, 3H, 2^1H), 3.15 (s, 3H, 7^1H), 2.73 (m, 1H, 17^2H), 2.68 (m, 1H, 3^1H), 2.46-2.28 (m, 2H, 17^1H), 2.03-1.94 (m, 4H, N^2H and 17^2H), 1.75 (d, J = 7 Hz, 3H, 18^1H), 1.67 (t, J = 8 Hz, 3H, 8^2H), 1.66 (t, J = 7.5 Hz, 2H, N^3H), 1.57-1.53 (m, 2H, $3^2{}_a$H and $3^3{}_a$H), 1.29-1.25 (m, 2H, $3^2{}_b$H and $3^3{}_b$H), 1.10 (t, J = 7.5 Hz, 2H, N^4H), −0.05 (br s, 1H, NH), −0.26 (br s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS) δ 176.4, 175.3, 173.9, 167.5, 163.5, 155.3, 149.5, 145.4, 143.3, 141.2, 139.0, 137.9, 137.3, 136.1, 133.6, 131.4, 115.7, 106.8, 102.2, 97.6, 94.2, 54.6, 51.5, 49.1, 40.1, 32.3, 31.3, 31.1, 23.9, 20.8, 19.4, 17.5, 14.1, 12.4, 11.3, 11.0, 7.4, 6.5; HRFABMS: calcd for $C_{39}H_{46}N_5O_4$ ($[M + H]^+$) 648.3550, found 648.3548.

Pyrazolyl Purpurin-18-*N*-butylimide (8). 7 (52 mg, 0.0820 mmol) and CH_2N_2 (2.5 mL of 0.52 M solution in diethyl ether, 1.30 mmol) were dissolved in dichloromethane (15 mL) and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the residue purified by column chromatography (SiO_2: 2% acetone/dichloromethane) to afford **8** as a dark purple solid (22 mg, 40%). **8**: mp 101 - 103 °C; UV-vis ($CHCl_3$): λ ($10^{-3}\varepsilon/M^{-1}\cdot cm^{-1}$) = 418 (171.80), 481 (5.68), 508 (8.79), 546 (23.79), 598 (3.63), 666 (12.0), 706 nm (55.76); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS) δ 9.63 (s, 1H, 10H), 9.02 and 9.01 (two s, each 0.5H, 5H), 8.62 (s, 1H, 20H), 6.58 (t, J = 9 Hz, 1H, 3^1H), 5.50-5.44 (m, 1H, $3^3{}_a$H), 5.42 (m, 1H, 17H), 4.79-4.73 (m, 1H, $3^3{}_b$H), 4.47 (t, J = 7.5 Hz, 2H, N^1H), 4.37 (m, 1H, 18H), 3.83 (s, 3 H, 12^1H), 3.64 (q, J = 8 Hz, 2H, 8^1H), 3.57 (s, 3H, CO_2CH_3), 3.23 and 3.22 (two s, each 1.5H, 2^1H), 3.12 (s, 3H, 7^1H), 2.79 (m, 1H, $3^2{}_a$H), 2.70 (m, 1H, 17^2H), 2.46-2.34 (m, 2H, 17^1H), 2.06 (m, 1H, $3^2{}_b$H), 2.01-1.94 (m, 3H, 17^2H and N^2H), 1.77 (d, J = 7 Hz, 3H, 18^1H), 1.67 (t, J = 8 Hz, 3H, 8^2H), 1.64 (t, J = 7.5 Hz, 2H, N^3H), 1.10 (t, J = 7.5 Hz, 2H, N^4H), −0.23 (br s, 2H, NH); ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C, TMS) δ 176.6, 174.7, 173.9, 167.4, 163.4, 154.8, 150.1, 145.4, 142.3, 140.0, 137.6, 136.4, 135.9, 135.1, 132.4, 132.0, 116.3, 106.8, 102.1, 97.8, 94.6, 85.0, 78.1, 54.7, 51.5, 49.1, 40.2, 32.4, 31.3, 31.1, 27.5, 24.0, 20.8, 19.4, 17.5, 14.1, 12.5, 11.4, 11.1; HRFABMS: calcd for $C_{39}H_{46}N_7O_4$ ($[M + H]^+$) 676.3611, found 676.3607.

Results and Discussion

Synthesis. The route for the synthesis of pyrazolyl and cyclopropyl derivatives of purpurin-18 methyl ester and purpurin-18-*N*-butylimide is shown in Scheme 1. The synthesis was carried out by a combination of modification of the vinyl, the ester and the exo cycle groups, and demetallation from chlorophyll-a. Methyl pheophorbide-a **3** as a starting material was obtained



Scheme 1. Synthesis of pyrazolyl and cyclopropyl derivatives of purpurin-18 methyl ester and purpurin-18-*N*-butylimide: (a) 5% H_2SO_4/MeOH, room temp. (rt), 50 h, 5%; (b) KOH, 1-propanol, pyridine, Et_2O, rt, 1 h, 57%; (c) CH_2N_2, dichloromethane (DCM), rt, 5 min, 95%; (d) CH_2N_2, DCM, rt, 24 h, 51%; (e) DDQ, acetone, rt, 24 h, 30%; (f) *o*-dichlorobenzene, reflux, 20 min, 71%; (g) i: butylamine, DCM, rt, 48 h; ii: CH_2N_2, DCM, rt, 5 min; iii: KOH, MeOH, 5 min, 50%; (h) i: butylamine, DCM, rt, 48 h; ii: CH_2N_2, DCM, rt, 5 min; iii: KOH, MeOH, 5 min, 38%; (i) CH_2N_2, DCM, rt, 24 h, 40%; (j) DDQ, acetone, rt, 24 h, 50%.

from extraction of chlorophyll-a paste by 5% sulfuric acid in methanol. Purpurin-18 carboxylic acid **4** and purpurin-18 methyl ester **5** were prepared according to the literature[15] procedure. Exo cycle transformation of **3** followed by esterification with diazomethane afforded **5**.

Pyrazolyl purpurin-18 methyl ester **1a** was prepared by reaction between **5** and diazomethane followed by oxidation of **6**[11] with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 30% yield. Purpurin-18-*N*-butylimide **7** was prepared according to the literature[15] procedure. Reaction of **5** with butylamine followed by addition of diazomethane and methanolic KOH afforded purpurin-18-*N*-butylimide **7**. Pyrazolyl purpurin-18-*N*-butylimide **8** was prepared by reaction between **7** and diazomethane as the 'anti-Markovnikov'-type product in 40% yield. Oxidation of **8** by DDQ afforded pyrazolyl purpurin-18-*N*-butylimide **2a** in 50% yield. Cyclopropyl purpurin-18 methyl ester **1b** was prepared by reaction between **6** and *o*-dichlorobenzene in 71% yield. Cyclopropyl purpurin-18-*N*-butylimide **2b** was prepared by reaction between **1b** and butylamine followed by addition of diazomethane and methanolic KOH in 50% yield.

Mass Spectrometric Investigation. Pyrazolyl and cyclopropyl derivatives of purpurin-18 methyl ester and purpurin-18-*N*-butylimide were characterized by HRFABMS which revealed (see Figures S1 - S5 in the Supporting Information) the corresponding $[M + H]^+$ peak, respectively.

NMR Spectroscopic Investigation. ^{1}H and ^{13}C NMR spectra of the compounds **1a**, **1b**, **2a**, **2b** and **8** have shown in Figures S6-S15 in the Supporting Information. In compounds **1a** and **2a** the signals of 3^1H and 3^2H on oxidized pyrazolyl unit were found at aromatic region (δ = 7.98 and 7.06, 7.96 and 7.05 ppm, respectively) and the signals of 3^1H, 3^2H and 3^3H on pyrazolyl unit in compounds **6** and **8** were disappeared, respectively. In compound **8** the signals of 3^1H, 3^2H and 3^3H on pyrazolyl unit clearly showed at δ = 6.58, 2.79 and 2.06, 5.50-5.44 and 4.79-4.73 ppm, respectively, with disappearance of the signals of ethylene unit in compound **7**. The signals of cyclopropyl unit in compound **1b** were found at δ = 2.75, 1.59-1.54, 1.34-1.25 ppm with disappearance of signals of pyrazolyl unit in compound **6**. The proton signals of the pyrazolyl (before oxidation) and cyclopropyl units in the chlorins were comparable with those in the previous reports.[10,11] *N*-Alkylation in compound **2b** was confirmed with signals of N^1-N^4 clearly. The signal of 17H in purpurin-18-*N*-butylimides **2a** and **2b** showed downfield shift (*ca*. 0.2 ppm) as compared with that in purpurin-18 methyl esters **1a** and **1b**, respectively, presumably due to an electronic effect induced by the *N*-butyl group.

UV-vis Spectroscopic Investigation. The UV-vis spectra of the new compounds **1a**, **1b**, **2a**, **2b** and **8** showed long wavelength absorptions at λ_{max} 701, 692, 708, 699 and 706 nm, respectively (Figure 2 in $CHCl_3$ at 25 °C). Converting as purpurin-*N*-butylimides **2a** and **2b** from purpurin-18 methyl ester **8** and **1b** afforded a bathochromic shift of λ_{max} with 7 nm, respectively. And the oxidation of pyrazolyl compounds **6** and **8** to form compounds **1a** and **2a** allowed slight bathochromic shift of λ_{max} with 1 - 2 nm, respectively.

Photodynamic activity investigation. Photocytotoxicity and cytotoxicity in the dark of the compounds **1a**, **2a**, **1b** and **2b** were

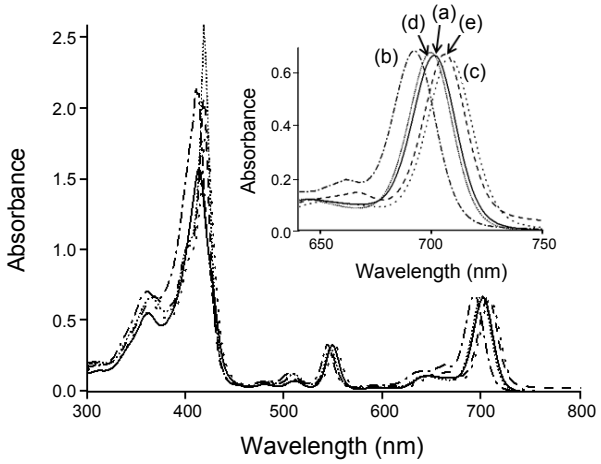


Figure 2. UV-vis absorption spectra of **1a** (a), **1b** (b), **2a** (c), **2b** (d) and **8** (e) in $CHCl_3$ at 25 °C. Insert: Expansion of the region 640 - 750 nm.

investigated in A549 cells (Figures 3-6). A549 cells (10×10^4 cells/well) were incubated with the compounds **1a**, **2a**, **1b** and **2b** as PSs for 24 h and photoirradiated for photocytotoxicity test. At 3 h, 24 h and 48 h incubation after photoirradiation or with no photoirradiation (cytotoxicity in the dark), the cell viability (%) was estimated based on the mitochondrial activity of NADH dehydrogenase using WST-1[17] (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) assay.

In all the compounds, upon photoirradiation, the cell viability was decreased corresponding to the increased incubation time after PDT, for example, 37.8% at 3 h, 9.8% at 24 h and 5.2% at 48 h incubation for compound **1a** at 2.5 μM (Table S1 in the

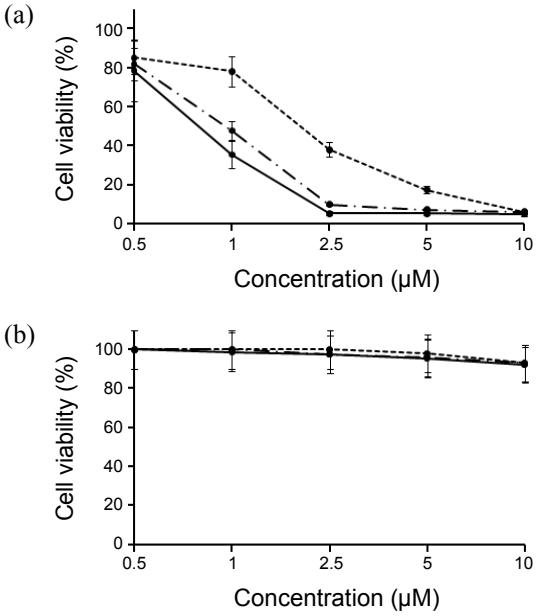


Figure 3. Photocytotoxicity (a) and cytotoxicity in the dark (b) of **1a** in A549 cells. The percentage of cell viability was determined by WST-1 assay at 3 h (-----), 24 h (–·–·–) and 48 h (——) incubation after photoirradiation. Error bars represent the standard deviation of three replicate experiments.

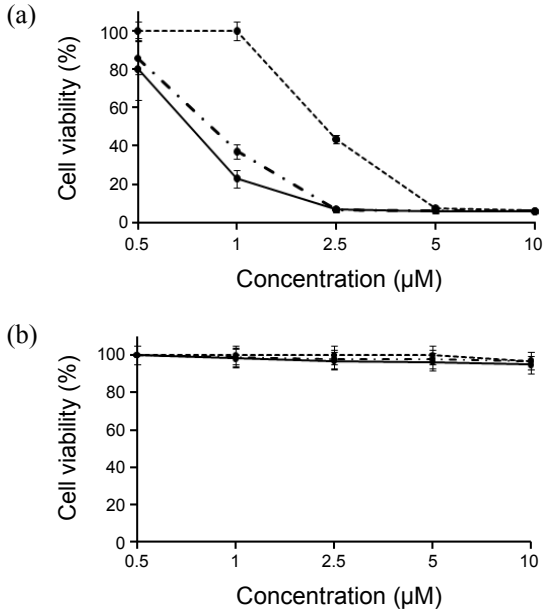


Figure 4. Photocytotoxicity (a) and cytotoxicity in the dark (b) of **1b** in A549 cells. The percentage of cell viability was determined by WST-1 assay at 3 h (-----), 24 h (–·–·–) and 48 h (——) incubation after photoirradiation. Error bars represent the standard deviation of three replicate experiments.

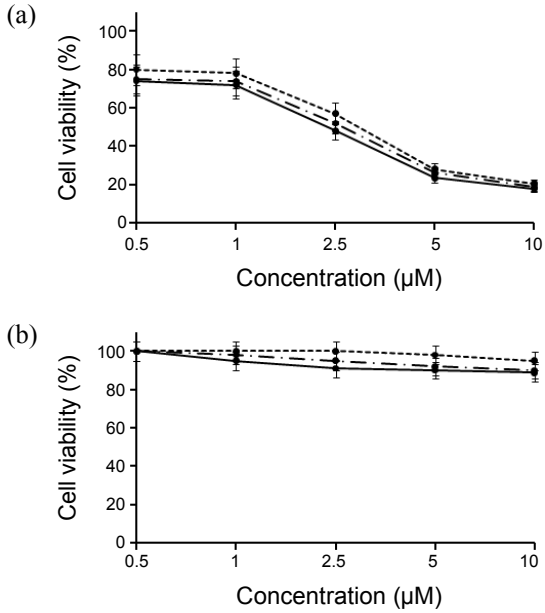


Figure 5. Photocytotoxicity (a) and cytotoxicity in the dark (b) of **2a** in A549 cells. The percentage of cell viability was determined by WST-1 assay at 3 h (-----), 24 h (–·–·–) and 48 h (——) incubation after photoirradiation. Error bars represent the standard deviation of three replicate experiments.

Supporting Information). In compounds **1a** and **1b** the cell viability showed good photocytotoxicity results in *ca*. 5 - 6% at 5 - 10 μM and 48 h incubation time. And in compounds **2a** and **2b** the cell viability showed lower photocytotoxicity than that in **1a** and **1b** results in *ca*. 18 - 32% at 5 - 10 μM and 48 h incubation time. The pyrazolyl derivatives of purpurin-18 methyl

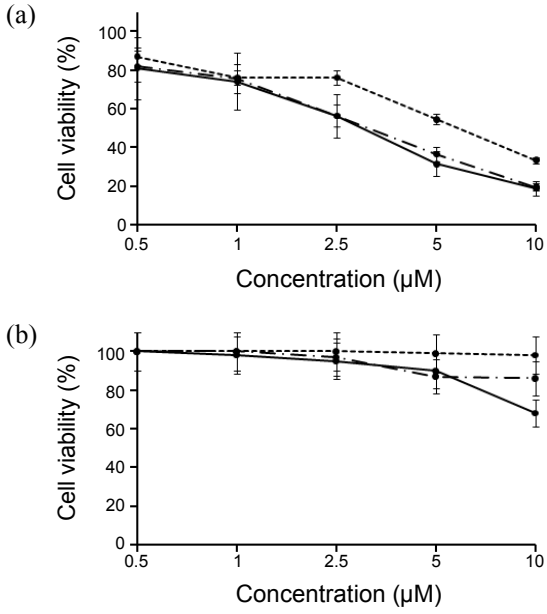


Figure 6. Photocytotoxicity (a) and cytotoxicity in the dark (b) of **2b** in A549 cells. The percentage of cell viability was determined by WST-1 assay at 3 h (-----), 24 h (–·–·–) and 48 h (——) incubation after photoirradiation. Error bars represent the standard deviation of three replicate experiments.

ester **1a** and purpurin-18-*N*-butylimide **2a** showed better cell viabilities than those of the pyrazolyl derivatives of methyl pyropheophorbide-a have been reported[12] previously. In purpurin-18 methyl ester derivatives the pyrazolyl and the cyclopropyl compounds **1a** and **1b** showed comparable result of cell viabilities. Otherwise, in purpurin-18-*N*-butylimide derivatives the pyrazolyl compounds **2a** showed better cell viabilities than those of the cyclopropyl compound **2b**. This different result between the pyrazolyl and the cyclopropyl derivatives might be attributed to the hetero aromatic system of the pyrazolyl ring.

Compounds **1a**, **2a** and **1b** showed low dark cytotoxicity, and the cytotoxicity also increased corresponding to the increased incubation time (Table S2 in the Supporting Information). Compound **2b** showed higher cytotoxicity than that of others.

Conclusions

Novel purpurin-18 methyl ester and purpurin-18-*N*-butylimide derivatives incorporating pyrazolyl or cyclopropyl unit were synthesized through the 1,3-dipolar cycloaddition reaction using diazomethane, and were characterized by a combination of NMR, UV-vis spectroscopy and HRFABMS spectrometry. UV-vis spectra of the new chlorin derivatives showed long wavelength absorption of λ_{max} ranges of 692 - 708 nm, which could allow good tissue-penetration in PDT. *In vitro* investigations of the chlorins showed good photodynamic activities upon photoirradiation and low cytotoxicity in the dark. Cell viabilities of purpurin-18 derivatives **1a** and **1b** were comparable each other, otherwise, the pyrazolyl derivative of purpurin-18-*N*-butylimide **2a** afforded better cell viabilities than those of the cyclopropyl derivative **2b**. These results could be useful

for the growth in the understanding of pyrazolyl and cyclopropyl containing purpurin-18 methyl ester and purpurin-18-*N*-alkylimide derivatives, as well as for developing new PSs in PDT.

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