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Synthesis of axially disubstituted quaternized silicon phthalocyanines as a promising photosensitizer for photodynamic treatment of HCT-116, A549 and SH-SY5Y cancer cell lines

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1. Experimental

1.1. Materials and equipments

The IR spectra were recorded on a Perkin Elmer 1600 FT-IR Spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometers in $CDCl_3$ and chemical shifts were reported (δ) relative to Me_4Si as internal standard. MALDI-MS of complexes were obtained in dihydroxybenzoic acid as MALDI matrix using nitrogen laser accumulating 50 laser shots using Bruker Microflex LT MALDI-TOF mass spectrometer Bremen, Germany). The UV-Vis absorption spectra were recorded on Perkin Elmer Lambda 25 UV-Vis spectrophotometer at room temperature. Acetic acid, agarose, bromophenol blue, ethidium bromide (EB), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ethylenediaminetetraacetate (EDTA), ethidium bromide (EB), dimethyl sulfoxide (DMSO), glycerol, hydrochloric acid (HCl), trypsin-EDTA, trizma-base (Tris), xylene cyanol, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and supercoiled pBR322 plasmid DNA were obtained from commercial sources.

The photocleavage and phototoxic studies were performed using a white lamp (100 W, Phillips). The DNA cleavage studies were photographed using BioRad Gel Doc XR system and the results were calculated by Image Lab Version 4.0.1 Software program. The power density was measured using power meter (Ophir sensor Nova II). The cytotoxicity and phototoxicity studies were measured using microplate reader (Thermo Fischer Scientific).

1.2. DNA cleavage experiments

The DNA cleavage effects of the compounds were investigated using agarose gel electrophoresis on supercoiled pBR322 plasmid DNA without / with irradiation. The DNA-photocleavage studies were performed under light irradiation using white lamp for 15, 30 and 60 min (17.5 mW/cm^2). In this study, supercoiled pBR322 plasmid DNA was treated with

increasing concentration of compounds **Si-3a** and **Si-4a** (5, 10 and 50 μM) in the buffer containing 50 mM Tris-HCl pH 7.0. All samples were incubated at 37 ºC for 1 h. After that, loading buffer (bromophenol blue, xylene cyanol, glycerol, EDTA, SDS) was added and the mixtures were loaded on 0.8% agarose gel with EB staining in TAE buffer (Tris-acetic acid-EDTA). Electrophoresis was carried out at 100 V for 90 min and the results were visualized using BioRad Gel Doc XR system and analyzed by Image Lab Version 4.0.1 Software programme [1].

1.3. Cell Culture

Human colorectal carcinoma (HCT-116), lung adenocarcinoma (A549) and bone marrow neuroblastoma (SH-SY5Y) cell lines were obtained from the American Type Culture Collection. Cells were cultured in high-glucose DMEM supplemented with 10% FBS and antibiotics (10.000 units/mL of penicillin and 10 mg/mL of streptomycin). Cultures were maintained in 25 cm^2 flasks and seeded as 10^4/well/100 μL in 96-plates for cytotoxicity tests. Cells were incubated in 37 ºC humidified incubator with 5% CO_2.

1.3.1. Exposure condition

The stock solutions of 10 mM compounds (**Si-3a** and **Si-4a**) dissolved in DMSO, after removing the culture medium, cells were treated with exposure medium containing compounds (final concentrations: 0.5, 1, 5, 10, 50, and 100 μM). SDS (50 ppm) and DMSO (0.1%) were used as positive and solvent controls, respectively. For the darkness groups plates were incubated directly in dark, 37 ºC, humidified incubator with 5% CO_2, while for the light ones, plates were incubated for 2 h; then plates were exposed to light (white, 17.5 mW/cm^2, 60 min). The distance between the light source and the surface of the irradiated solution was ~ 10 cm. Plates were re-incubated in the same incubation conditions with the darkness groups [2].

1.3.2. MTT assay

The cytotoxic/phototoxic effects of the compounds were investigated using MTT assay

on HCT-116, A549 and SH-SY5Y cell lines. In this test, after 24 hours exposure period, the exposure medium was removed and 100 μL MTT solution (0.5 mg/mL in serum-free cell culture medium) was added for each well. After incubation for 3 hours the MTT solution was thrown and 150 μL DMSO was added to solve the formazan crystals. Microplate spectrophotometer system at 570 nm to 690 nm was used for reading the optical densities (ODs). The inhibition of enzyme activity in exposed cells was calculated according to that of unexposed cells (solvent control, 0.1% DMSO). Then, the half maximal inhibitory concentration (IC_{50}) was expressed as the sample concentration that caused an inhibition of 50% in enzyme activities in cells [3].

1.4. Statistical analysis

In this study, all data were analyzed using GraphPad Prism 5.0 and expressed as mean ± standard deviation (n=3). The statistical analyses were performed by two-way ANOVA, followed by Bonferroni posttests.

References

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Table 1. Cell viabilities (%) of HCT-116 with compounds

μM	3a	3a + irradiation	4a	4a + irradiation
0.5	100 ± 3.60	100 ± 3.70	100 ± 3.90	96 ± 1.80
1	100 ± 3.40	100 ± 3.70	100 ± 6.80	94 ± 5.60
5	100 ± 0.50	100 ± 0.80	98 ± 7.80	92 ± 1.10
10	100 ± 4.80	100 ± 2.90	92 ± 5.20	87 ± 3.10
50	100 ± 2.80	89 ± 5.70	64 ± 7.70	59 ± 7.40
100	100 ± 0.10	87 ± 6.30	56 ± 4.30	55 ± 5.90

Table 2. Cell viabilities (%) of A549 with compounds

μM	3a	3a + irradiation	4a	4a + irradiation
0.5	80 ± 1.70	53 ± 3.70	100 ± 7.70	62 ± 3.60
1	74 ± 4.10	53 ± 3.40	100 ± 5.40	51 ± 4.40
5	69 ± 1.50	49 ± 3.40	100 ± 7.30	50 ± 3.30
10	63 ± 1.70	34 ± 2.40	83 ± 8.80	27 ± 3.70
50	63 ± 4.50	25 ± 1.40	53 ± 5.40	25 ± 4.10
100	63 ± 7.70	25 ± 4.50	50 ± 4.90	20 ± 4.10

Table 3. Cell viabilities (%) of SH-SY5Y with compounds

μM	3a	3a + irradiation	4a	4a + irradiation
0.5	70 ± 1.30	35 ± 2.10	74 ± 3.70	40 ± 1.60
1	67 ± 2.40	31 ± 2.30	73 ± 2.80	37 ± 3.70
5	67 ± 2.90	30 ± 1.60	71 ± 4.40	35 ± 1.70
10	63 ± 2.30	23 ± 2.00	65 ± 3.40	22 ± 0.20
50	63 ± 5.50	20 ± 1.10	64 ± 5.20	20 ± 0.80
100	62 ± 5.60	20 ± 1.10	63 ± 6.20	19 ± 0.90