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The substituted amino group type dependent sensitivity enhancing of cationic phthalocyanine derivatives for photodynamic activity

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ABSTRACT

Many reports indicated that the cellular uptaken and photodynamic anti-cancer activity of amino group modified phthalocyanine (Pc) could be greatly improved in the acid environment around cancer cells because such Pc could transfer to their hydrochloride protonated form and inhibit the intramolecular photoinduced electron transfer (PET) effect. However, no more researches are carried out to indicate whether this law has the extensive applicability in all kinds of amino modified Phthalocyanines (Pcs). So, here, four amino groups modified Pcs and their hydrochloride or quaternized cationic derivatives, with two kinds of amino groups (phenylamine and benzylamine) substituted were synthesized. Their solubility in aqueous system, cancer cell uptaken ability and photodynamic activities were also systematically compared. Our research results indicated that above law had the extensive applicability in benzylamine amino groups modified Pcs. But for phenylamine amino groups modified ones, the cancer cell uptaken ability and anti-cancer activity of hydrochloride derivatives were obviously decreased comparing with the unmodified Pc because its nitrogen atom was closer to the Pc ring, which would induce its nitrogen atom and the ring to form p–π conjugation system, furthermore, the relatively free state of lone pair electrons of surrounding nitrogen atom leaded to PET strengthening.

1. Introduction

Photodynamic therapy (PDT) is a promising and clinically approved noninvasive modality for treatment of a variety of malignant diseases [1,2], including lung, esophageal, bladder and skin tumors [3,4]. In the PDT process, the highly reactive oxygen species (ROSs), including superoxide anion, singlet oxygen ($^1O_2$) and hydroxyl radical, was formed after energy transfer from an excited photosensitizer by light [5,6], which was lethal to tumor cells and caused irreversible tumor damage [7,8]. Phthalocyanines are the synthetic analogues of naturally occurring porphyrin family [9,10] and considered as the promising photosensitizers for PDT owning to strong absorption in the phototherapeutic window (600–900 nm), high ROSs generation efficiency [11], low dark toxicity and high light stability [12]. Amino group modified drugs always showed high potential tumor targeting ability and low intrinsic toxicity because the rapid proliferation of tumor cells determined that they required a large amount of amino to sustain. Based on this concept, many amino groups modified Pc was reported [13,14]. However, researches indicated that the photodynamic activity of amino modified Pcs was not ideal. This may be due to the enhanced reductive quenching of amino in its structure, which disfavor the intersystem crossing because of the intramolecular photoinduced electron transfer (PET) effect. Furthermore, the water solubility of many amino modified Pcs was very poor. The commonly method to solve these problems is quaternizing the nitrogen atoms to obtain cationic derivatives. The amino modified Pcs bearing cationic substituents always have good solubility in aqueous system and cancer cell uptaken ability because of their electropositive property. Besides, their photodynamic activities were also greatly improved because of the inhibition of PET effect [15,16]. On the contrary, many researches indicated that these problems would be easily solved because of the acid environment around cancer cells, which would induce the amino modified Pcs to transfer to their hydrochloride protonated form [17]. The formation of hydrochloride can greatly improve its water solubility and prevent the PET process by protonation, resulting in the increase of photodynamic activity [18–20]. Such researches implied that preparing hydrochloride of amino modified Pcs can greatly improve its water solubility and photodynamic activity. However, no more researches were carried out to indicate whether this law and result had the extensive applicability in all kinds of amino modified Pcs.

Thereby, in this manuscript, we synthesized four series of amino group modified zinc phthalocyanine (ZnPc) and their

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hydrochloride or quaternized derivatives and also systematically compared their water solubility, ROSs generation ability, cellular uptake in HeLa cells and the photosensitive anti-tumor activity. Our researches indicated that above law and result did not have the extensive applicability in all kinds of amino modified Pcs. Phthalocyanine modified with benzylamine derivative (pc-benzylamine), which hydrochloride protonation or quaternizing derivatives cannot only greatly improve their solubility in aqueous system but also effectively improve cellular uptake in HeLa cells, ROSs generation ability, and the photosensitive anti-tumor activity. However, the phthalocyanine modified with phenylamine derivative (pc-phenylamine) after hydrochloride protonating also can effectively improve Pcs low water solubility. But due to nitrogen atoms on substituents are closer to the phthalocyanine ring, nitrogen atom and the ring forms p–π conjugation system and the relatively free state of lone pair electrons of surrounding nitrogen atom leads to PET strengthening, which result the ROSs generation ability, cellular uptake in HeLa cells and the photosensitive anti-tumor activity are not nearly as superior as quaternized derivative.

2. Experimental

2.1. Materials and methods

2.1.1. Materials

4-nitrophthalonitrile was used after being recrystallized from methanol. Other necessary chemicals grade is analytically pure and obtained from commercial suppliers, which are used without further purification unless otherwise stated. 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) and the 9,10-antracenediyl-bis (methylene) dimalonic acid (ADMA) were all purchased from established suppliers (Sigma–Aldrich) and used as received. Fetal calf serum (FCS) and dulbecco's minimum essential medium (DMEM) were from Gibico. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) and Hoechst 33342 were from Amosco. TLC was performed on silica gel GF254 plates. 300–400 mesh silica gel was used for preparative column chromatography.

2.1.2. Methods

IR spectra were recorded on IR Spectrometer Nicolet Nexus 670 by KBr tableting. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Advance 400 MHz NMR spectrometer. Mass spectra were obtained on 1290 Infinity LC/6460 QQQ MS spectrometer or UltraflexXreme MALDI-TOF-MS spectrometer. Elemental analyses were taken with Vario MICRO Elementar. Fluorescence spectra were carried out using PerkinElmer LS 50B fluorescence spectrophotometer. UV–vis spectra were recorded on spectrophotometer Cary 5000, Varian. Cell morphology changes were observed under a Zeiss Observer fluorescence microscope. A 665 nm LED (7W) was used as light source.

2.2. Synthesis

2.2.1. 4-(4-Aminophenoxy) phthalonitrile (A1)

All the compounds was designed and synthesized according to literature methods [21].

The synthesis of A1 was similar to the literature had reported [22,23] and certificated by checking the melting point (M.P.), infrared spectra (IR), and spectral imaging hydrogen (^{1}H NMR) and was the same as the literature. M.P. 130 °C; IR (KBr, cm^{-1}): 3463 (—NH_2), 3372 (—NH_2), 2233 (—CN), 1590, 1490, 1250 (C—O—C), 1070, 837; ^{1}H NMR (400 Hz, DMSO-d6): δ (ppm) 8.02 (d, 8.0 Hz, 1H, Ar—H), 7.606 (d, 2.4 Hz, 1H, Ar–H), 7.25–7.22 (m, 1H, Ar–H), 6.84 (d, 8.0 Hz, 2H, Ar–H), 6.64–6.61 (t, 6 Hz, 2H, Ar–H), 5.17 (S, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d6): δ (ppm) 163.1, 147.4, 143.4, 136.6, 121.9, 121.8, 121.1, 116.9, 116.5, 116.0, 115.4, 107.4. MS (ESI–MS) *m*/*z*: Calcd. for $C_{14}H_9N_3O$: 235.24. Found: $[M+Na^+]^+$ 258.1. Anal. Calcd. for $C_{14}H_9N_3O$: C, 71.48; H, 3.86; N, 17.86. Found: C, 71.40; H, 3.92; N, 17.78.

2.2.2. 4-(4-(Tritylamino) phenoxy) phthalonitrile (A2)

4-(4-Aminophenoxy) phthalonitrile (A1) (1.5 g, 6.38 mmol) was reacted with Et_3N (5 mL) in CH_2Cl_2 (40 mL) at room temperature. Then, triphenylchloromethane (2.13 g, 7.65 mmol) in CH_2Cl_2 (40 mL) was added dropwise to the solution over a period of 4 h, and stirring was continued overnight after the titration was completed. Overed, the solvent was evaporated in vacuum. The residue was purified by column chromatography with silica gel as column material and petroleum ether/ethyl acetate (1:1) solvent system as elution. Yield: 2.44 g (80.0%). M.P. > 200 °C; IR (KBr, cm^{-1}) 3406 (—NH), 3089 (Ar–H), 2231 (—CN), 1591, 1512, 1506, 1485, 1247 (C—O—C), 702; ^{1}H NMR (400 Hz, DMSO-d6): δ (ppm) 8.02 (d, 8.8 Hz, 1H, Ar–H), 7.46 (s, 1H, Ar–H), 7.46–7.16 (m, 15H, Ar–H), 7.13 (d, 2.4 Hz, 1H, Ar–H), 6.68(d, 9.2 Hz, 2H, Ar–H), 6.58(d, 8.8 Hz, 2H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d6): δ (ppm) 162.7, 145.8, 145.6, 144.2, 136.6, 129.4, 128.3, 128.0, 127.0, 122.0, 121.2, 120.4, 118.1, 116.9, 116.5, 115.9, 107.5, 71.4. MS (ESI–MS) *m*/*z*: Calcd. for $C_{33}H_{23}N_3O$: 477.56. Found: $[M+H]^+$ 478. Anal. Calcd. for $C_{33}H_{23}N_3O$: C, 83.00; H, 4.85; N, 8.80. Found: C, 83.02; H, 4.88; N, 8.75.

2.2.3. 2(3), 9(10), 16(17), 23(24)-Tetra-(aminophenoxy) phthalocyaninato-zinc(II) (ZnPc1)

A mixture of anhydrous zinc acetate (0.12 g, 0.654 mmol), 4-(4-(tritylamino) phenoxy) phthalonitrile (A2) (0.5 g, 1.05 mmol) and DBU (0.4 g, 2.63 mmol) was stirred and heated at 140 °C in dry n-pentanol (10 mL) for 24 h under nitrogen atmosphere. After cooling to room temperature, the reaction solution was poured into CH_3OH (60 mL). The deep blue solid product was precipitated and collected by filtration, then washed with H_2O and methanol till the filtrate was colorless. The green crude product was purified by passing through a silica gel column with methanol/CH_2Cl_2 (1:5) as elution. The blue fraction was collected, evaporated under vacuum, and afford the product as a dark green solid. Yield: 0.83 g (40.05%). Then, under the condition of ice-water bath, A3 (0.6 g, 0.303 mmol) and excess trifluoroacetic acid (TFA) (0.5 mL) were dissolved in CH_2Cl_2 (20 mL) and stirred for 3 h. After the mixture recovered the room temperature, the crude product was collected by filtration and washed successively with CH_2Cl_2. Thereafter, the green solid was dissolved in water and precipitated by adjusted pH to 9–10. The residue product collected by filtration was washed successively with water and methanol. Finally, a dark green solid was gained. Yield: 232.4 mg (76.04%) M.P. > 200 °C; IR (KBr, cm^{-1}): 3436 (—NH_2), 3058 (Ar–H), 1610, 1506, 1228 (C—O—C), 1043, 945, 835; ^{1}H NMR (400 Hz, DMSO-d6): δ (ppm) 9.08 (br, 20.4 Hz, 4H, Pc–H), 8.63 (s, 4H, Pc–H), 7.84–7.78 (t, 12 Hz, 8H, Pc–H, Ar–H), 7.63–7.30 (m, 12H, Ar–H), 5.70 (s, 8H, NH_2). MS MALDI-TOF (matrix DHB) *m*/*z*: Calcd. for $C_{56}H_{36}N_{12}O_4Zn$: 1006.34. Found: $[M+H]^+$ 1007.09. Anal. Calcd. for $C_{56}H_{36}N_{12}O_4Zn$: C, 66.84; H, 3.61; N, 16.70. Found: C, 66.80; H, 3.64; N, 16.75.

2.2.4. Hydrochloride derivative of 2(3), 9(10), 16(17), 23(24)-tetra-(aminophenoxy) phthalocyaninato-zinc(II) (ZnPc2)

ZnPc1 (0.2 g, 0.198 mmol) was suspended in 6 mL redistilled water in a reactor and heated to reflux. Excess 5% HCl aqueous was added into the solution drop-wise until **ZnPc1** was totally dissolved. Concentrate the reaction liquid and 20 mL acetone was added into solution. The solid blue product was separated out and collected by filtration then thoroughly washed by dichloromethane and dried in vacuum. The title product **ZnPc2** was