Journal of Inorganic Biochemistry 189 (2018) 192–198

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Enhanced anti-microbial effect through cationization of a mono-triazatricyclodecane substituted asymmetric phthalocyanine

Huajian Lin[a], Jincan Chen[b], Yaxin Zhang[b], Azeem Ulla[b], Jianyong Liu[a], Fan Lin[c], Longguang Jiang[a], Mingdong Huang[a,b,*]

[a] College of Chemistry, Fuzhou University, Fujian 350118, China
[b] State Key Laboratory of Structural Chemistry, Fujian Institute of Research on the Structure of Matter, Fuzhou, Fujian 350002, China
[c] Qingdao Sundynamic Technology Co., Ltd, Qingdao, Shandong 266000, China

ARTICLE INFO

Keywords:
Cationic phthalocyanine
Photosensitizer
Antimicrobial photodynamic therapy (aPDT)
Bioluminescent bacteria
Synthesis

ABSTRACT

Antimicrobial photodynamic therapy (aPDT) is an effective way to combat infectious diseases and antibiotic resistance. Photosensitizer is a key factor of aPDT and has triggered extensive research interest. In this study, a new asymmetric Zn(II) phthalocyanine mono-substituted with a triazatricyclodecane moiety (compound **3**) and its cationic *N*-methylated derivative (compound **4**) were synthesized. Their photodynamic antimicrobial activities were evaluated using bioluminescent bacterial strains. Compound **3** showed phototoxicity only toward the Gram-positive bacteria, whereas the cationic derivative compound **4** exhibited strong anti-bacterial activity against both Gram-positive and Gram-negative strains. These bacterial species were eradicated (> 4.0 logs or 99.99% killing) at appropriate concentrations of compound **4** with 12.7 J/cm^2 of red light, demonstrating compound **4** as a potent aPDT agent.

1. Introduction

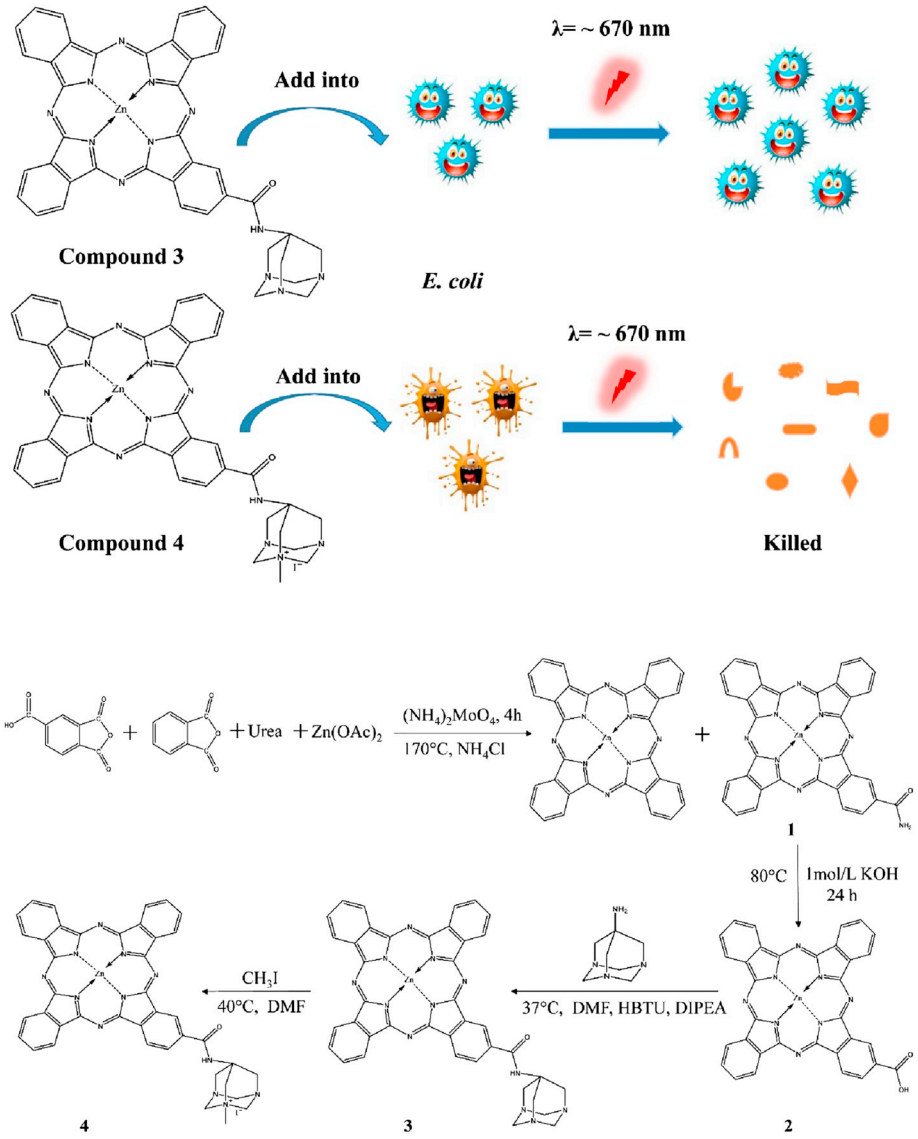
Phthalocyanines have many important industrial applications, including dyes and pigments in fabric, demonstrating that they are safe and environment-friendly materials. They also possess remarkable stability and unique photochemical and photophysical properties, broadening their uses in many high technology fields, including semiconductor materials [1,2], solar cells [3,4], optical data storage [5], chemical sensors [6,7], oxidation-reduction catalysts [8–11] and photocatalysts [12], as well as photodynamic therapy (PDT) for antimicrobial [13–15] or antitumor applications [16–18]. The emergence of multi-resistant bacteria due to the over-use of antibiotics has become a global challenge [19,20]. Phthalocyanines have emerged as a new class of photosensitizer possessing potent antimicrobial effect, even toward drug-resistant bacterial strains separated from hospitals [21–24].

In this study, we designed a new asymmetric Zn(II) phthalocyanine (ZnPc): triazatricyclo-substituted Zn(II) phthalocyanine (compound **3**) by conjugating with a Nitrogen-rich compound (1,3,5-triazatricyclo [3.3.1.1(3, 7)] decane-7-amine, Scheme 1). The substituent is quite bulky in size and can reduce the aggregation of phthalocyanine. Aggregation of Pc typically leads to the quench of photodynamic effect. Moreover, the triazatricyclodecane is water soluble, and will be protonated at aqueous solution, potentially rendering the conjugate aqueous solubility and positive charges in a weakly acidic environment. The positive charge is a common property of antimicrobial agents, which allows the adsorption and binding to bacterial surface that carry large amount of negative charges [25,26]. We also wanted to avoid the positional isomer on ZnPc during conjugation because single compound is a key for approval by regulatory agent, should the compound proceeds to clinical trial stage.

It turned out that compound **3** was not water soluble, and did not show desirable antimicrobial effect toward bacterial strains. We suspect that the compound **3** was not protonated at aqueous solution. Quaternization of aliphatic or aromatic nitrogen atom at the end of the synthetic pathway of phthalocyanine is a common way to prepare cationic phthalocyanine [27]. Most of the published cationic phthalocyanines with quaternary amine groups exhibit excellent photodynamic antimicrobial effect [28–32]. Moreover, it was discovered recently by Hamblin group that aPDT can be greatly enhanced by the addition of simple inorganic salts especially iodine ion at micromolar concentration [33]. Thus, we carried out methylation on the tertiary amine group of compound **3** in a hope to form an cationic phthalocyanine compound **4**. Photophysical and photochemical properties including UV–Vis spectra,

* Corresponding author at: College of Chemistry, Fuzhou University, Fujian 350118, China.
E-mail address: HMD_lab@fzu.edu.cn (M. Huang).

https://doi.org/10.1016/j.jinorgbio.2018.10.001
Received 14 June 2018; Received in revised form 29 September 2018; Accepted 1 October 2018
Available online 06 October 2018
0162-0134/



Scheme 1. Synthesis of compound **3** and **4**.

photodynamic antibacterial and hemolysis activities of these compounds were characterized and evaluated. Encouragingly, the compound **4** exhibited very potent antimicrobial effect.

2. Results and discussion

2.1. Synthesis of compound 3

The target compound (**3**) was synthesized using the scheme shown in Scheme 1. The intermediate compound (**1**) was prepared by statistic condensation method. Condensation of trimellitic anhydride or phthalic anhydride in the presence of urea and catalyst at high temperature can produce tetra-formamido-phthalocyanine or phthalocyanine, respectively. Using a mixture of trimellitic anhydride and phthalic anhydride at a ratio of 1:7 for condensation, we made a mixture of 2-formamidophthalocyanine zinc (**1**) and phthalocyanine zinc with only trace amount presumably polycarboxy substituted phthalocyanine zinc. The amide group of compound **1** was hydrolyzed to carboxy group, leading to 2-carboxyphthalocyanine (compound **2**), which was separated out of the mixture to a high purity (> 90%, Fig. S1). It is important to carry out the hydrolysis before the purification, because the separation of the compound **1** out of the condensation mixture was quite difficult. The purified carboxy compound **2** was then conjugated to compound 1,3,5-triazatricyclo [3.3.1.1(3, 7)] decan-7-amine by amine coupling chemistry. The product was precipitated out with water and collected by centrifugation, followed by washing with deionized water, acetonitrile and CH_2Cl_2, and dried to give blue solid. The compound **3** was further purified by semi-preparative HPLC system. The structure of compound **3** was fully confirmed by ^{1}H, ^{13}C NMR spectrum (Figs. S2, S3) and mass spectrometry measurement (Electrospray Ionization, ESI, Fig. S4, *m/z* calculated for compound **3** $[M + H]^+$ 757.1879, found 757.1846).

2.2. Antimicrobial effects of compound 3

Phthalocyanine and its derivatives are highly potent photosensitizers in photodynamic antimicrobial therapy. They sensitize oxygen under red light (~670 nm) to generate reactive oxygen species (ROS) and damage effectively nearby bacteria or cells [34]. In this study we evaluated the photodynamic antibacterial activity of compound **3** against two luminescent bacterial strains, Gram-positive (*S. aureus*) and Gram-negative (*E. coli*). These are genetically engineered bacterial strains expressing both luciferase and luciferase substrate, and are luminescent while alive with bioluminescence intensity (relative