Journal of Drug Delivery Science and Technology 33 (2016) 164–169

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Research paper

Evaluation of nuclear imaging potential and photodynamic therapy efficacy of symmetrical and asymmetrical zinc phthalocyanines

Kasim Ocakoglu [a, b, **], Ozge Er [c], Onur Alp Ersoz [c], Fatma Yurt Lambrecht [c, *], Mine Ince [b], Cagla Kayabasi [d], Cumhur Gunduz [d]

[a] Advanced Technology Research & Application Center, Mersin University, Ciftlikkoy Campus, TR-33343, Yenisehir, Mersin, Turkey
[b] Department of Energy Systems Engineering, Faculty of Technology, Mersin University, TR-33480, Tarsus, Mersin, Turkey
[c] Department of Nuclear Applications, Institute of Nuclear Science, Ege University, Bornova, 35100, Izmir, Turkey
[d] Department of Medical Biology, Faculty of Medicine, Ege University, Bornova, 35100, Izmir, Turkey

ARTICLE INFO

Article history:
Received 5 January 2016
Received in revised form
28 April 2016
Accepted 30 April 2016
Available online 30 April 2016

Keywords:
Photodynamic therapy
Cancer
Biodistribution
^{131}I
Radiolabeling
Phthalocyanine

ABSTRACT

Photodynamic therapy (PDT) is a medical treatment for the removal of target tissues involving the delivery of a photosensitizer agent followed by irradiation with visible light. In the present study, symmetric **Zn(II)*Pc* 1** and asymmetrically substituted **Zn(II)*Pc* 2** were synthesized and examined multifunctional agents for tumor nuclear imaging and PDT potential. The **Zn(II)*Pc* 1** and **Zn(II)*Pc* 2** were radiolabeled with ^{131}I with high efficiency (93.4 ± 1.6% and 91.4 ± 1.6%, respectively). The results of the biodistribution study showed that radiolabeled **Zn(II)*Pc* 1** had high uptake on lung, large intestine, ovary and pancreas. However, the uptake of radiolabeled **Zn(II)*Pc* 2** was statically significant in pancreas and intestine. In PDT studies, EMT6/P (mouse mammary cell) and HeLa (cervical adenocarcinoma cell) with **Zn(II)*Pc* 1** and **Zn(II)*Pc* 2** were exposed to red light at the doses of 10–30 J/cm^2. Although PDT activity of **Zn(II)*Pc* 2** in HeLa cell line was determined, **Zn(II)*Pc* 1** showed no phototoxic effect in both cell lines. In conclusion, radiolabeled **Zn(II)*Pc* 1** might be a promising imaging agent for the lung, the ovary pancreas, and the colon tumors. However, radiolabeled **Zn(II)*Pc* 2** might be a promising nuclear imaging agent for the colon and the pancreas tumors and promising PDT agent for cervical tumors.

1. Introduction

In the recent years, photodynamic therapy (PDT) gained speed in cancer treatment. PDT is a non-invasive, alternative method to chemotherapy, radiotherapy and surgery. This method is based on two steps. Firstly, a photosensitizer (PS) is accumulated in the cancer tissue and then flowed by irradiation with visible light. During the photoreaction Type II, energy transfer occurs between the PS and the molecular oxygen (O_2), the exposed photosensitizers produce highly toxic singlet oxygen ($^1O_2$). The singlet oxygen is responsible for the cell damage [5,12,21]. The life of $^1O_2$ is less than 0.05 μs because of its highly reactive feature and $^1O_2$could move only 0.02 μm from the released zone. The mutagenic effects of PDT are very low due to the localization area of the PS outside the nucleus. Thus, the cell death by the PDT consists of apoptosis, necrosis or a combination of them [6,7,10,11]. A good PS should follow some criteria: it should have strong absorption with the use of a high extinction coefficient in the red/near infrared region (600–850 nm) for allowing deeper tissue penetration, be an effective agent in the production of singlet oxygen, have suitable photophysical characteristics-high quantum yield of triplet formation, high singlet oxygen quantum yield, relatively long triplet state lifetime and high triplet-state energy, minimum dark toxicity, high target/non-target tissues rate, rapid clearance, stable drug formulation, be readily soluble in biological media, be easily transported to the target tissue with intravenous injection [10,11].

Various tetrapyrole derivatives such as chlorines, porphyrins, and pthalocyanines have been used for the PDT. Phthalocyanines (*Pc*) are the most commonly used due to the high tumor uptake efficiencies. Zinc (II) phthalocyanine, a second generation photosensitizer, has absorption Q bands at longer wavelengths (670–770 nm) that allows maximum penetration of the light into the tissues. Intercalary, the chemical purity and the high singlet

* Corresponding author.
** Corresponding author. Advanced Technology Research & Application Center, Mersin University, Ciftlikkoy Campus, TR-33343, Yenisehir, Mersin, Turkey.
E-mail addresses: kasim.ocakoglu@mersin.edu.tr (K. Ocakoglu), fatma.yurt.lambrecht@ege.edu.tr (F.Y. Lambrecht).

http://dx.doi.org/10.1016/j.jddst.2016.04.005
1773-2247/

oxygen quantum yield of zinc(II) phthalocyanine make it suitable for the PDT [1,10].

In this study, the synthesis of a symmetric and an asymmetric Zn(II) phthalocyanine was made for the PDT. In vitro PDT activities of the compounds were investigated in EMT-6 murine mammary carcinoma and HeLa human cervix carcinoma cell lines. However, the Zn(II)Pcs were radiolabeled with ^{131}I to determine their biological potential in Albino Wistar rats.

2. Material and methods

2.1. Materials

All chemicals used for the in vitro studies were obtained from the commercial supplier Biological Industries; all the other chemicals were supplied from Merck. The thin-layer chromatography-cellulose gel (ITLC-F plastic sheets 20 × 20) was purchased from Merck. Iodogen was supplied from Sigma-Aldrich. Radiolabeling experiments were analyzed using a Bioscan AR2000 TLC Scanner. Cell culture studies were performed in a Thermo MSC Advantage 1.2 laminar air flow cabinet. For counting cells, an Olympus Japan inverted light microscope was used. A thermo Multimode microplate reader was used for determining the IC_{50} values of cell cultures. The IR spectra were performed with Perkin-Elmer, FT-IR/MIR-FIR (ATR, Attenuated total reflectance) spectrophotometer. Mass spectrometry analysis was performed on an autoflex III MALDI TOF/TOF MS system (Bruker Daltonics, Bremen, Germany). ^1H NMR (400 MHz) spectra were recorded with the use of a Bruker AC-400 instrument. UV/Vis spectra were recorded with the use of an Analytic JENA S 600 UV–Vis spectrophotometer.

2.2. Synthesis of *Zn(II)Pc 1* and *Zn(II)Pc 2*

The symmetric **Zn(II)*Pc* 1** and asymmetrically substituted **Zn(II)*Pc* 2** are showed in Fig. 1 were synthesized according to a published procedure [19]. All compounds were fully characterized by ^1H NMR, UV–Vis, FT-IR and MALDI-TOF spectroscopies. The ^1H NMR spectra of tetra (*tert*-butyl) **Zn(II)*Pc* 1** and asymmetrically substituted **Zn(II)*Pc* 2** in $CDCl_3$ showed similar and several multiplets corresponding to the phthalocyanine protons in the aromatic region, between 9.6 and 7.8 ppm. MALDI-TOF mass spectra of **Zn(II)*Pc* 1** and **2** clearly showed intense signals for the molecular ion peaks at 803.793 and 873.146, respectively.

A mixture of 4-*tert*-butylphthalonitrile (950 mg, 5.16 mmol) and 4-iodophthalonitrile (262 mg, 1.03 mmol) was refluxed in dimethylaminoethanol (DMAE) (5 ml) under argon for 20 h in the presence of $Zn(OAc)_2$ (302 mg, 1.6 mmol). The solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel, (hexane/dioxane, 3:1). The symmetric **Zn(II)*Pc* 1** was eluted first, obtaining 290 mg (28% yield), followed by **Zn(II)*Pc* 2** 165 mg, 19% yield.

2.3. Zinc (II) 2(3), 9(10),16(17),23(24)-tetra-tert-butyl-phthalocyaninato (2-)-N^{29}, N^{30}, N^{31}, N^{32} (1)

^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 8.7–8.2 (m, 8H), 8.0–7.8 (m, 4H), 1.7–1.6 (m, 36H, $C(CH_3)_3$). FTIR (ATR): ν (cm^{-1}) 2954, 2895, 1613, 1487, 1391, 1255, 1087, 921, 744 cm-1. UV/Vis (THF): λmax (log ε) = 672 (5.79), 607 (5.09), 346 (5.03). MS (MALDI-TOF): *m/z*: calcd. for $C_{48}H_{48}N_8Zn$ 802.338; found 803.798 $[M+H]^+$

2.4. Zinc (II) 9(10),16(17),23(24)-tri-tert-butyl-2-iodophthalocyaninato (2-)-N^{29}, N^{30}, N^{31}, N^{32} (2)

^1HNMR (400 MHz, d_8-THF): δ (ppm) = 9.6–9.0 (m, 7H), 8.9–8.8 (m, 1H), 8.4–8.2 (m, 4H), 1.9–1.8 (m, 27H, $C(CH_3)_3$). IR (ATR): υ = 2952, 2852, 1716, 1484, 1390, 1254, 1088, 918, 743 cm^{-1}. UV/Vis (THF): λ_{max} (log ε) = 674 (5.66), 607 (5.04), 350 (5.39). MS(MALDI-TOF): *m/z*: calcd. for $C_{44}H_{39}IN_8Zn$ 872.129; found 873.146 $[M+H]^+$

2.5. Radiolabeling and radiochemical purity analysis

Synthesized **Zn(II)*Pc* 1** and **Zn(II)*Pc* 2** were solved in DMSO and 50 μg of each one from DMSO stock were diluted with distilled water to 300 μl. Prepared Pcs were radiolabeled with 9.25 MBq ^{131}I via the iodogen method. The quality control of radiolabeling was determined using the TLRC method [3,14,22]. Cellulose-coated plastic (ITLC-cellulose) sheets (1 × 10 cm; Merck) and two different mobile phases [mobile phase 1: chloroform-acetic acid (9:1) and mobile phase 2: n-butanol-water-acetic acid (4:2:1)] were used to determine the radiolabeling efficiency. The cellulose sheets were scanned on a TLC-scanner.

2.6. Optimization conditions of radiolabeling *Zn(II)Pc 1* and *Zn(II) Pc 2*

Zn(II)Pcs were labeled with ^{131}I at various pH values (3, 5, 7 and 9) to investigate the effect of the reaction pH on the radiolabeling efficiency and the radiolabeling efficiencies were determined with the use of the TLRC method.

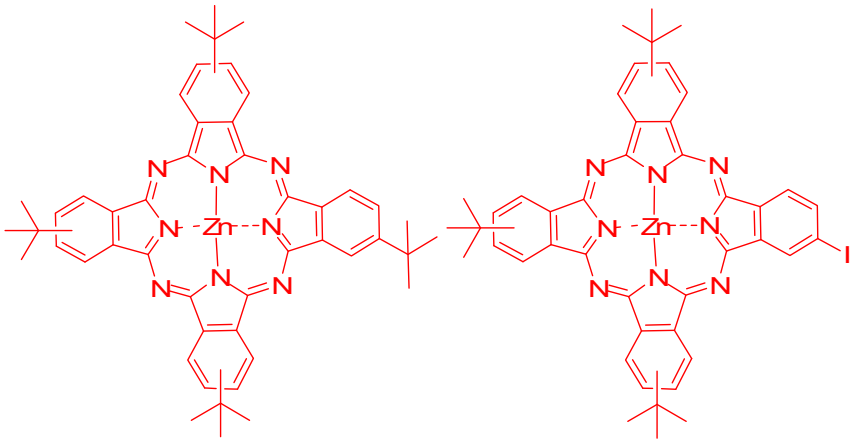

Fig. 1. Chemical structures of **Zn(II)*Pc* 1** and **Zn(II)*Pc* 2**.