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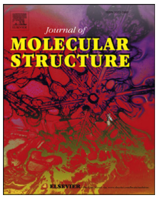

Synthesis, characterization and DNA interaction properties of the novel peripherally tetra 4-(3-methyl-4-(3-morpholinopropyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl) substituted water soluble Zn(II) and Cu(II) phthalocyanines

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The triazol compound 3-methyl-4-(3-morpholinopropyl)-1H-1,2,4-triazol-5(4H)-one **(3)**, phthalonitrile compound 4-(3-methyl-4-(3-morpholinopropyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)phthalonitrile **(5)**, peripherally tetra substituted zinc(II) **(6)** and copper(II) **(7)** phthalocyanines and their water soluble quaternized derivatives **(6a)** and **(7a)** were synthesized for the first time in this study. These novel compounds were characterized with FT-IR, ^{1}H NMR, ^{13}C NMR, UV–Vis and mass spectroscopy. The absorption titration, and thermal denaturation studies were preferred to investigate the interaction mode of the water soluble quaternized tetra substituted peripherally phthalocyanines zinc(II) **(6a)** and copper (II) **(7a)** with CT-DNA. **6a** and **7a** bound to CT-DNA with K_b values of $6.53 \pm (0.04) \times 10^{4}\,M^{-1}$ and $1.14 \pm (0.02) \times 10^{4}\,M^{-1}$, respectively. The observed ΔT_m of CT-DNA with the compounds were elucidated as 7.55 °C and 5.75 °C. In addition, supercoiled pBR322 plasmid DNA was used to determine cleavage properties of the compounds by agarose gel electrophoresis. The compounds had no cleavage activities in the absence of oxidizing agents in the dark whereas; the both compounds had excellent photo cleavage activities in the absence and presence of oxidizing agents such as H_2O_2, ME and AA. The results of electrophoresis indicated that singlet oxygen is responsible for the cleavage activities of the water soluble quaternized tetra substituted peripherally phthalocyanines zinc(II) **(6a)** and copper (II) **(7a)** with irradiation. These results showed that both compounds may be potential anticancer drug for photodynamic therapy.

1. Introduction

Photodynamic therapy (PDT), has emerged as a therapeutic approach for treatment of many cancer types such as esophageal, lung, skin, colorectal [1,2]. The principle of PDT is to destroy tumor cells by producing reactive oxygen species in the presence of light, oxygen and photosensitizer. There has been an increased interest in PDT in recent years due to its low side effect and ability to be applied in combination with other therapies (chemotherapy and surgery) [3,4]. Phthalocyanines are used as photosensitizer in PDT applications thanks to their high singlet oxygen yields, strong absorptions in the therapeutic windows, high stabilities and interaction properties with biomolecules [5,6].

Phthalocyanines are important macrocyclic compounds and they are used in biological applications as biomarkers, antineoplastic drugs, antiviral, antioxidant and antibacterial agents [7–12]. However, the solubility of phthalocyanines is low in organic solvents and this restricts their use in biological applications [13]. In order to increase solubility in apolar or polar solvents, various substituents such as alkyl, crown ether, alkoxy, sulfo or quaternary ammonium groups are attached to the phthalocyanine ring. The cationic phthalocyanines have superior water solubility, high cellular uptake efficiency and interaction with DNA due to their structures [1,14]. DNA is a crucial intracellular target to stop

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uncontrolled cell proliferation. For this reason, the interaction of anticancer drugs with DNA becames one of the most important targets for the anticancer treatments [15,16]. So, the water-soluble cationic phthalocyanines are valuable compounds for the design of new anticancer drugs [17–19].

1,2,4-Triazoles and morpholines demonstrate important biological and pharmacological such as anti-tubercular, antioxidant, anticonvulsant, antifungal, anticancer, anti-inflammatory, tyrosinase inhibitory and photodynamic therapy agents [20–24].

In this study, we firstly aimed to attach the triazole and morpholine units which have important biological and pharmacological properties to phthalocyanine ring and increase the biological properties of the molecule. So, we synthesized 4-(3-methyl-4-(3-morpholinopropyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl) phthalonitrile) and its peripherally tetra substituted zinc(II) and copper(II) phthalocyanines. Then, in order to increase solubility and the interaction with DNA we synthesized water soluble quaternized phthalocyanine derivatives. Finally, the DNA binding and DNA cleavage properties using absorption spectroscopy, thermal denaturation and agarose gel electrophoresis of water soluble quaternized phthalocyanines were investigated.

2. Experimental

Materials, equipments, synthesis, DNA binding experiments and DNA-cleavage experiments were given in supplementary material.

3. Results and discussion

3.1. Synthesis and characterizations

The synthetic route of novel compounds was shown Scheme 1. 3-Methyl-4-(3-morpholinopropyl)-1*H*-1,2,4-triazol-5(4H)-one **(3)** was synthesized by reaction of ethyl 2-(1-ethoxyethylidene) hydrazinecarboxylate **(1)** and 3-morpholinopropan-1-amine **(2)**. In the IR spectra of compound **(3),** the disappearance of the $-NH_2$ vibrations of compound (**2**) and the new vibrations appeared at 3140 (−NH) cm^{-1} verified the formation compound (**3**). The disappearance of the $-NH_2$ signal of compound (**2**) and presence of new −NH signals and new aliphatic protons in ^{1}H NMR spectra of compound **(3)** confirm the proposed structure of compound **(3)** according to ^{1}H NMR. The $[M+H]^+$ peak was monitored at 227.28 verified the formation of compound **(3)** according to mass spectra.

The phthalonitrile compound **(5)** was synthesized by the reaction of 3-methyl-4-(3-morpholinopropyl)-1H-1,2,4-triazol-5(4H)-one **(3)** and 4-nitrophthalonitrile **(4)** in the presence of K_2CO_3. In the IR spectra of **(5),** the disappearance of the NH vibrations of compound (**3**) and the new vibrations appeared at 2229 (C≡N) cm^{-1} verified the formation phthalonitrile compound (**5**). The disappearance of the NH signal of compound (**3**) and presence of new aromatic protons in ^{1}H NMR spectra of compound **(5)** confirm the proposed structure according to ^{1}H NMR. In the ^{13}C NMR spectra the peaks seen at 116.063 (C≡N) and 109.723 (C≡N) ppm belonging to nitrile carbons show that substitution was occurred. The $[M+H]^+$ peak was monitored at 353.42 verified the formation of phthalonitrile (**5**) according to mass spectra.

The peripherally tetra substituted zinc(II) **(6)** and copper(II) **(7)** phthalocyanines were synthesized by cyclotetramerisation reaction of phthalonitrile compound **(5)**. The structural characterization of novel phthalocyanines **(6,7)** was performed by FT-IR, ^{1}H NMR, UV−Vis and mass spectroscopy. In the IR spectra of phthalocyanines **(6, 7)**, the disappearance of −C≡N vibration of phthalonitrile **(5)** confirm the formation of phthalocyanine compounds via cyclotetramerisation reaction. The ^{1}H NMR spectra of zinc(II) phthalocyanine **(6)** was very similar with stating phthalonitrile **(5)** except peaks were weak and broad. The ^{1}H NMR spectra of compound **(7)** could not be determined because of the presence of paramagnetic copper (II) ion [29]. The peaks at 1473.84 ($[M+H]^+$ for **6**) and 1472.38 ($[M+H]^+$ for **7**), confirm the proposed structures according to mass spectra. The absorption spectra of phthalocyanines **(6, 7)** were recorded in DMF (Fig. 1). The Q band of phthalocyanines causing from transitions from π-HOMO to π*-LUMO energy levels were observed at 692, 689 nm, respectively. The B band of the phthalocyanines, causing from electronic transitions from deeper level of HOMO to LUMO were observed at 330 and 340 nm, respectively.

The novel quaternized tetra substituted peripherally phthalocyanines zinc(II) **(6a)** and copper (II) **(7a)** were synthesized by mixing related phthalocyanine with iodomethane at room temperature. In the ^{1}H NMR spectra of zinc(II) phthalocyanine **(6a),** the new signals caused from new aliphatic protons confirm the structure of the target compound. The ^{1}H NMR spectra of compound **(7a)** could not be determined because of the presence of paramagnetic copper (II) ion [29]. The $[(M-4I)/4]^+$ peaks were observed in mass spectra, at 384.92 (for **6a**) and 382.88 (for **7a**) confirm the proposed structures of. The Q bands of phthalocyanines (**6a, 7a**) were observed at 695 and 691 nm, respectively. The B bands of the phthalocyanines (**6a, 7a**) were observed at 336 and 334 nm, respectively (Fig. 2).

3.2. DNA binding experiments

Absorption spectroscopy is one of the efficient methods of examining the binding mode of the compounds with CT-DNA. It is well known that changes in absorption intensity relate to binding mode of CT-DNA due to stacking of aromatic ring with bases of DNA or damage of the CT-DNA double helix structure [30]. The binding data of the water soluble quaternized tetra substituted peripherally zinc(II) **(6a)** and copper (II) **(7a)** phthalocyanines in the absence and presence of CT-DNA were presented in Table 1. The absorption spectra of the water soluble quaternized tetra substituted peripherally zinc(II) **(6a)** and copper (II) **(7a)** phthalocyanines in the absence and presence of CT-DNA at different concentrations were shown in Figs. 3 and 4. The both compounds showed maxima absorption intensity at 637 and 623 nm, respectively in the buffer. Figs. 3 and 4 claimed that both compounds showed hypochromicities (23.20%, 24.00%) and red shifts (3 nm and 2 nm) with the increasing concentrations of CT-DNA in the absorption spectra. The observed hyprochromicities with red shifts suggest that both compounds interact to CT-DNA via intercalation or minor groove binding mode. Therefore, we examined K_b values of both compounds to decide binding mode. K_b values of the water soluble quaternized tetra substituted peripherally zinc(II) **(6a)** and copper (II) **(7a)** phthalocyanines were calculated as $6.53 \pm (0.04) \times 10^4\ M^{-1}$ and $1.14 \pm (0.02) \times 10^4\ M^{-1}$, respectively using formula 2. The calculated K_b values are lower than classical intercalator ethidium bromide ($1.23\ (\pm 0.07) \times 10^5$), whereas both compounds were the similar level as well-known intercalation agents (10^4-10^6) [31–33]. Gup reported that triazole substituted Ni(II) complex exhibited hyperchromism on addition of CT-DNA at different concentrations and indicated partial intercalative binding with K_b value of $3.0 \times 10^3\ M^{-1}$ while K_b value of water soluble morpholine substituted Zn(II) phthalocyanine was elucidated as $4.76 \pm (0.06) \times 10^4\ M^{-1}$ according to our previous studies [5,34]. These results showed that **6a** bound to stronger effect that of **7a** to CT-DNA and the interaction mode of both compounds with CT-DNA was a moderate intercalation.

To confirm the interaction of the water soluble quaternized tetra substituted peripherally zinc(II) **(6a)** and copper (II) **(7a)** phthalocyanines with CT-DNA, thermal denaturation studies were