Current Enzyme Inhibition, 2018, *14*, 186-195

RESEARCH ARTICLE

Anti-Urease, Anti-Hyaluronidase, Antioxidant Properties of Some Zinc (II) Phthalocyanines

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ARTICLE HISTORY

Received: April 09, 2018
Revised: May 28, 2018
Accepted: June 21, 2018

DOI:
10.2174/1573408014666180627143643

Abstract: ***Background*:** Some of chemical syntheses are attractive because of their pharmacological application in the drug industry.

***Materials and Methods*:** In this study, water soluble peripherally and non-peripherally tetra substituted novel zinc (II) phthalocyanines, which were tagged as ZnPC, investigated against the inhibition of urease and hyaluronidase (HYA). In addition to enzyme inhibitory activities, antioxidant activity of synthesis molecules was measured by Ferric Reducing Antioxidant Power (FRAP) and DPPH radical scavenging activity assays.

***Results*:** The study also showed that four synthesis extracts exhibited a potent hyaluronidase and urease inhibitory effects with IC_{50} value ranges 7.095-115.878 µM and 4.227-35.678 µM respectively. Besides ZnPC-1 exhibited efficient urease and HYA inhibition, it showed good antioxidant activity with 0.341±0.001 µmol $FeSO_4.7H_2O$/mg sample for FRAP and 16.917±0.005 µM for DPPH. Also, the results obtained in the present study indicate that zinc (II) phthalocyanines compounds can be a potential source of bioactive agents.

Keywords: DPPH, FRAP, hyaluronidase, phthalocyanines, urease, zinc.

1. INTRODUCTION

Enzymes are biomolecules that are responsible for mediating the reactions involved in the metabolic pathway. These molecules are sometimes controlled by inhibitors that bind them and decrease their activities. Some synthesized compounds known as potential enzyme inhibitors lead a new way for potential drug strategy because these compounds are used for the treatment of various physiological disorders. Generally, the aim of the potential drug compounds is to kill a pathogen or correct a metabolic imbalance with inhibition activities. Although so many natural products are used enzyme inhibitors, synthesis materials are preferred due to their efficiencies in the medicine. Phthalocyanines have a large conjugation with many electrons, and also are able to consider as one of the most valuable groups because of bioactivities. They have been employed in several marvelous applications such as liquid crystals (LCs) [1], non-linear optics [2], solar cells [3], electronic devices [4], gas and chemical sensors [5], medical applications [6] and catalysis [7]. Due to the fact that diamagnetic central metal ions (such as Zn or Mg) phthalocyanines have been remarkably used to illustrate photodynamic reagents for cancer therapy [8-11]. In the literature, the phthalocyanine derivatives have been indicated as the potential leading compounds for the development of some enzyme inhibitors [12]. In many cases, applications of phthalocyanine depend on their solubility. But, it is vitally important to control the aggregation behaviors of phthalocyanines. Aggregation decreases their solubility and minimizes their applications in many organic solvents [13].

Inhibitions of urease and hyaluronidase (HAase) are the main subjects for the current research due to the previous results of the lack of investigations. The cause of numerous gastric disorders is an anaerobic microorganism known as *Helicobacter pylori*. The main survival way of this bacterium is being blocked in the gastric system with urease inhibitors [14]. Hyaluronidase is classified in the endoglycosidase and is tasked with hydrolyzing of the hyaluronic acid (HA) which is the only glycosaminoglycan, non-sulfated and not attached to a core protein and it repeats the unit of D-glucuronic acid and N-acetyl glucosamine. HA synthases, which are founded on the intracellular membrane surface, are the pioneer of HA polymers [15].

Besides some disorders of enzymes, another reason to the etiology of several chronic diseases, including cardiovascular disease, diabetes, cancer, and neurodegenerative disorders is oxidative stress that is the final and general process after the occurring of reactive oxygen species (ROS) in the human body [16]. Although ROS play a key role as a messenger in normal cell signal transduction and cell cycling at low levels,

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1875-6662/18 $58.00+.00

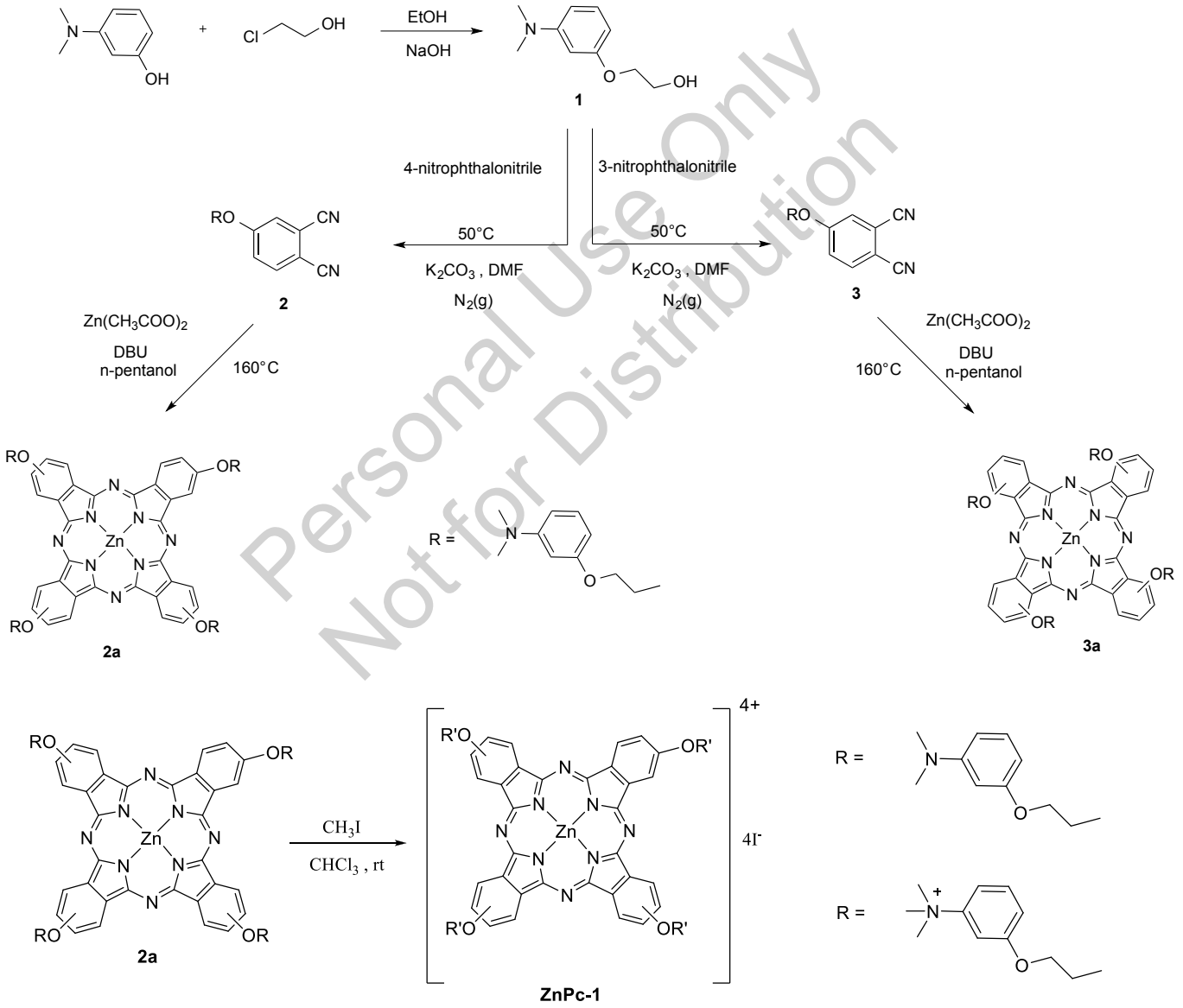
they may damage cellular macromolecules (DNA and RNA) and may act in apoptosis at high levels. For this reason, endogenous or exogenous antioxidants are responsible for these reactive molecules can sensitively regulate a part of oxidative stress damage. Many studies in recent years defined that some synthesis molecules except the well-known compounds such as phenolic acids and flavonoids might be evaluated as a potential antioxidant [16-19].

In the light of this reality, the study was prepared to determine the levels of anti-urease, anti-hyaluronidase, and antioxidant activity of some water-soluble zinc (II) phthalocyanines. To the best of our knowledge, this is the first study that combines directly some enzyme inhibition results and antioxidant levels in the current synthesis phthalocyanines.

2. MATERIALS AND METHODS

2.1. Chemical Synthesis

Peripherally and non-peripherally tetra-{2-[3-(dimethylamino)phenoxy]ethoxy} and tetra-{2-[3-(diethylamino) phenoxy]ethoxy} substituted zinc(II) phthalocyanines and their quaternized ionic derivatives (**ZnPC-1**, **ZnPC-2**, **ZnPC-3** and **ZnPC-4**) were prepared according to the literature [20, 21], respectively. All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. All solvents were dried and purified as described by Armarego and Perrin [22]. The synthesis route of peripherally and non-peripherally tetra substituted quaternerized ionic zinc phthalocyanines is shown in Fig. (**1**).



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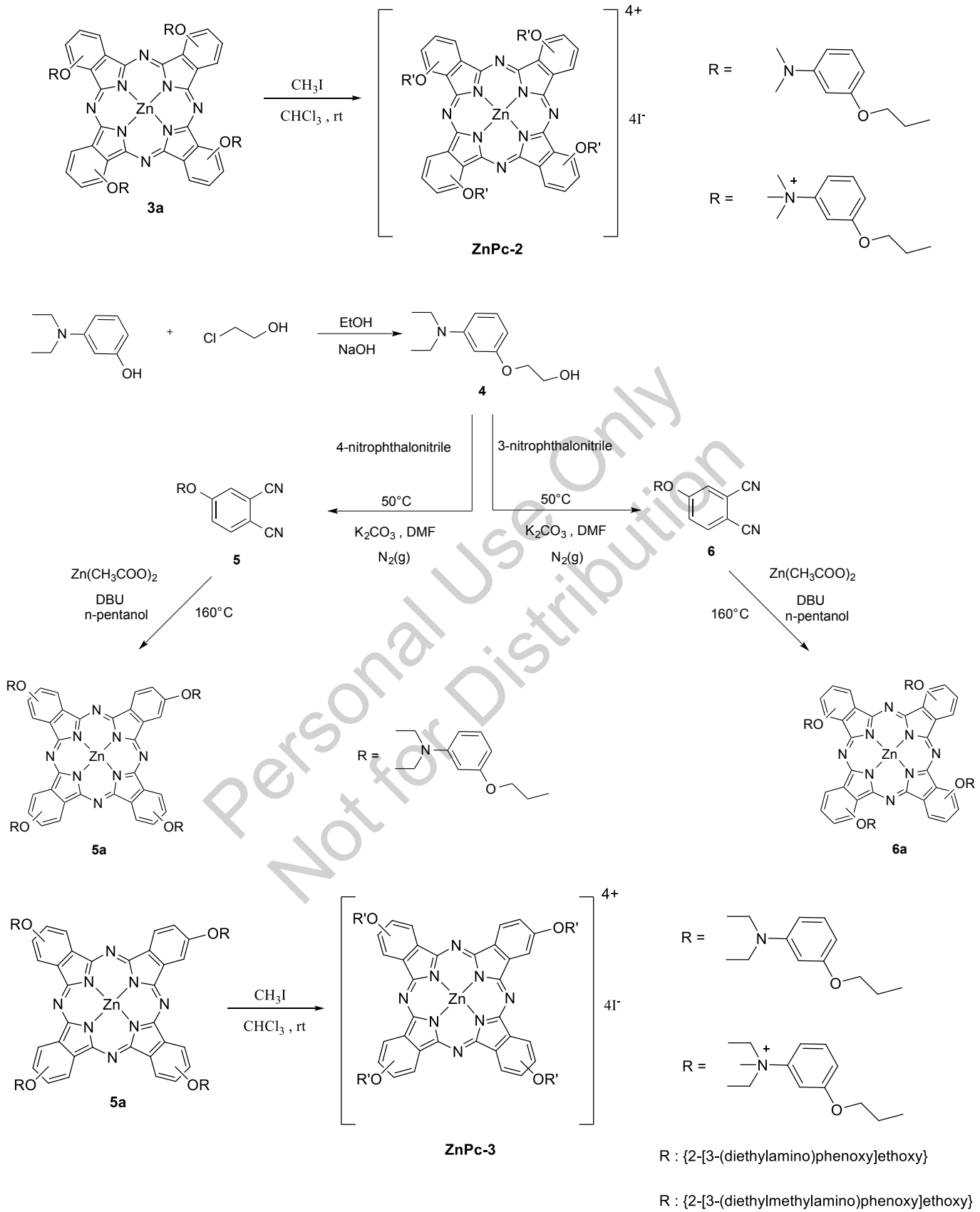

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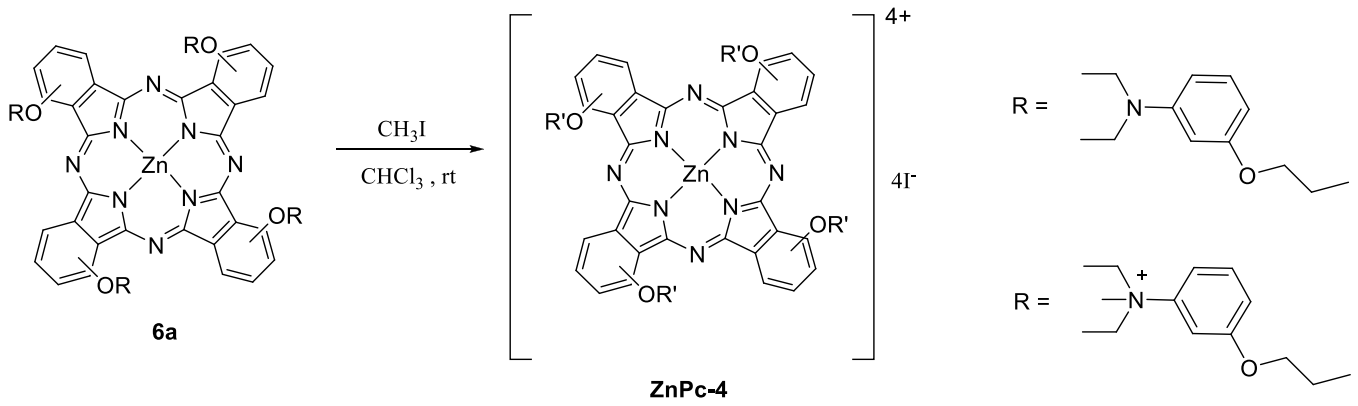


Fig. (1). The synthesis of the quaternerized ionic zinc phthalocyanine complexes (ZnPc-1, 2, 3 and ZnPc-4). ZnPc-1 and ZnPc-3 are peripherally tetra substituted; ZnPc-2 and ZnPc-4 are non- peripherally position.

2.2. Aggregation Study of Phthalocyanines

Optical spectra in the UV-vis region were recorded with a Perkin Elmer Lambda 25 Spectrophotometer (PERKIN ELMER CA, USA). Ground state electronic absorption spectra of all Pcs were recorded at room temperature. Aggregation behavior of water-soluble ZnPc-1, ZnPc-2, ZnPc-3 and ZnPc-4 complexes was investigated in different solvents (DMSO, DMF, Methanol, PBS and Water). Aggregation experiments were performed by $2.00x10^{-5}$ M of quaternized zinc (II) phthalocyanines in DMSO, DMF, Methanol, PBS and Water solutions (3 mL) and the changes in the UV-Vis absorption spectra of quaternized zinc (II) phthalocyanines were recorded (Fig. **2**).

2.2.1. In vitro Urease Inhibition Assay

Indophenol method was used to get some data about the inhibition of the urease [23]. Reaction mixtures including 200 µL of Jack Bean Urease from Sigma-Aldrich Co. (St. Louis, Missouri, USA), 500 µL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA and 0.01 M LiCl, pH 8.2) and 100 µL of the sample extract were incubated at room temperature for 20 min. The phenol reagent (550 µL, 1% w/v phenol and 0.005% w/v sodium nitroprusside) and alkali reagent (650 µL, 0.5% w/v sodium hydroxide and 0.1% v/v NaOCl) were added to each tube and an increasing absorbance at 625 nm was measured after 50 min, using a UV/vis spectrophotometer.

2.2.2. In vitro Hyaluronidase Inhibition Assay

The inhibition of hyaluronidase was conducted by using UV spectroscopy technique with a slight modification of Sigma protocol and an actual reference method [24]. Briefly, the reaction mixture consisted of 100 µL of bovine testes hyaluronidase from Sigma-Aldrich Co. (St. Louis, Missouri, USA) (1.67 U/mg), 100 µL of phosphate buffer (200 mM, pH 7, 37°C) with 77 mM sodium chloride and 0.01% BSA were mixed with 25 µL sample extract solution. After preincubation at 37°C for 10 min, the reaction was initiated by the addition 100 µL of substrate solution in the form of hyaluronoic acid (0.03% in 300mM sodium phosphate, pH 5.35). The assay mixture was incubated at 37°C for 45 min. The undigested hyaluronoic acid was precipitated with 1 mL acid albumin solution made up of 0.1% bovine serum albumin in 24 mM sodium acetate and 79 mM acetic acid, pH 3.75. After leaving the mixture at room temperature for 10 min, the absorbance was seen at wavelength 600 nm.

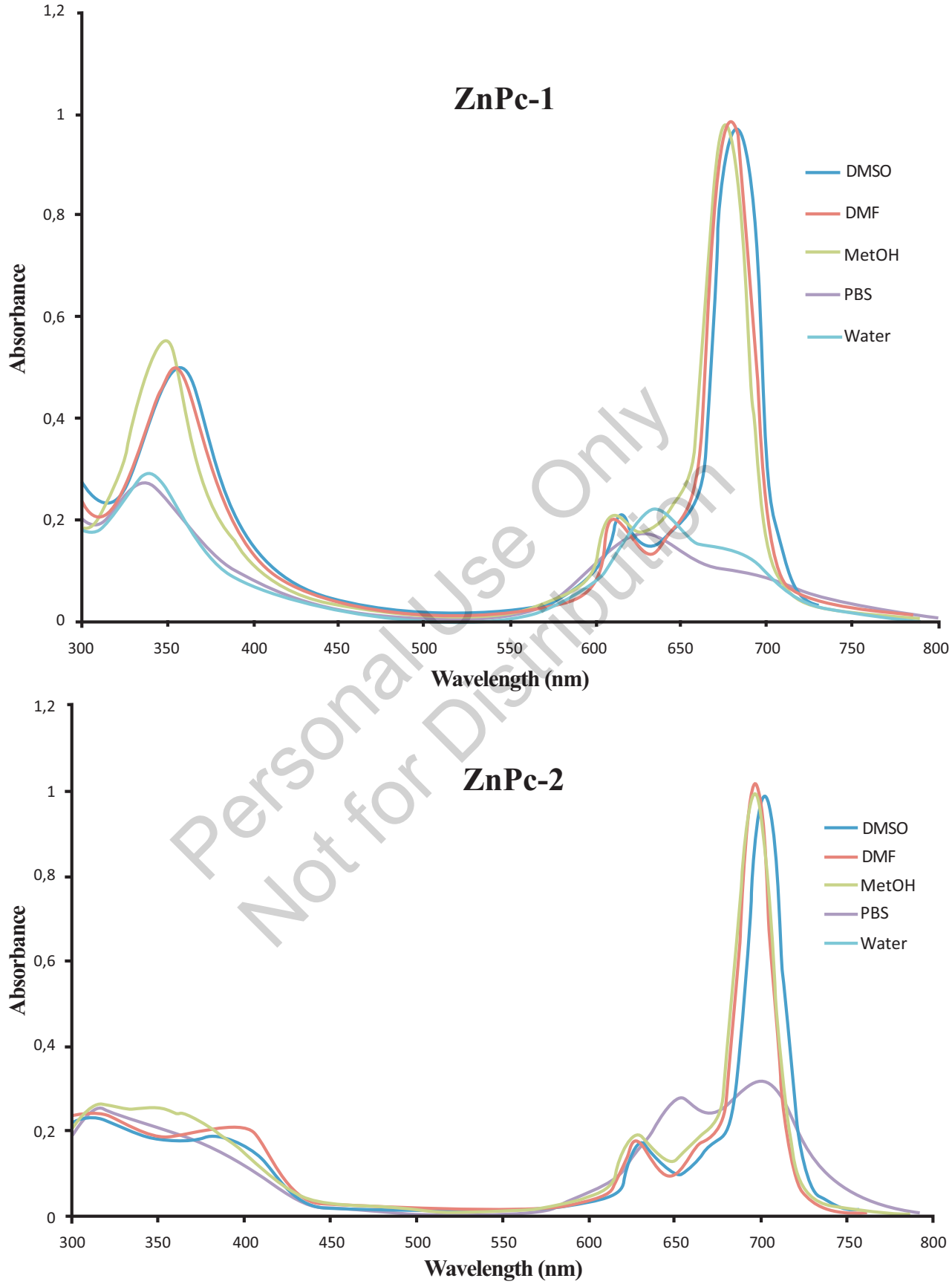
2.2.3. Calculation of Inhibition Concentration 50 (IC_{50})

Urease and hyaluronidase inhibition assays were done in triplicate for calculating the standard deviation. Degrees of urease and hyaluronidase inhibition activity were performed by calculating IC_{50}. In order to calculate these values, different concentrations of each sample extracts were assayed at the same reaction conditions. Although only one initial concentration of phthalocyanine samples was prepared, different diluted ranges of them were used for each assay. While in the range of ZnPc-1, ZnPc-2, ZnPc-3, and ZnPc-4 was applied as 33.417-1.044 µM, 36.997-2.312 µM, 30.395-0.950 µM, 32.647-1.020 µM for urease inhibition graphs, the concentration ranges of ZnPc-1, ZnPc-2, ZnPc-3, and ZnPc-4 were chosen as 7.347-0.918 µM 14.186-1.773 µM 12.904-1.613 µM 13.859-1.732 µM for hyaluronidase inhibition graphs. A reagent blank without enzyme was subtracted from the apparent absorbance to give the net absorbance. The inhibition concentration of the sample extracts (IC_{50}) was calculated from the dose-response curve using exponential function formula, which reduced absorbance by 50%. IC_{50} graphs of urease and hyaluronidase are described in Fig. (**3**).

2.3. Antioxidant Activity

2.3.1. Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant activities of the samples were determined by FRAP assay [25]. The method is based on the measurement of the iron-reducing capacities of the synthesized compounds. Working FRAP reagent was prepared as required by mixing 25 mL of 0.3 M acetate buffer at pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$. 100 µL of the sample were mixed with 3 mL of freshly prepared FRAP reagent. Then, the reaction mixture was incubated at 37°C for 4 min. After that, the absorbance was determined at 593 nm against a blank that was prepared using distilled water and incubated for 1 h instead of 4 min. A calibration curve was used, using an aqueous solution of ferrous sulphate $FeSO_4.7H_2O$ concentrations in the range of 100-1000 µM,



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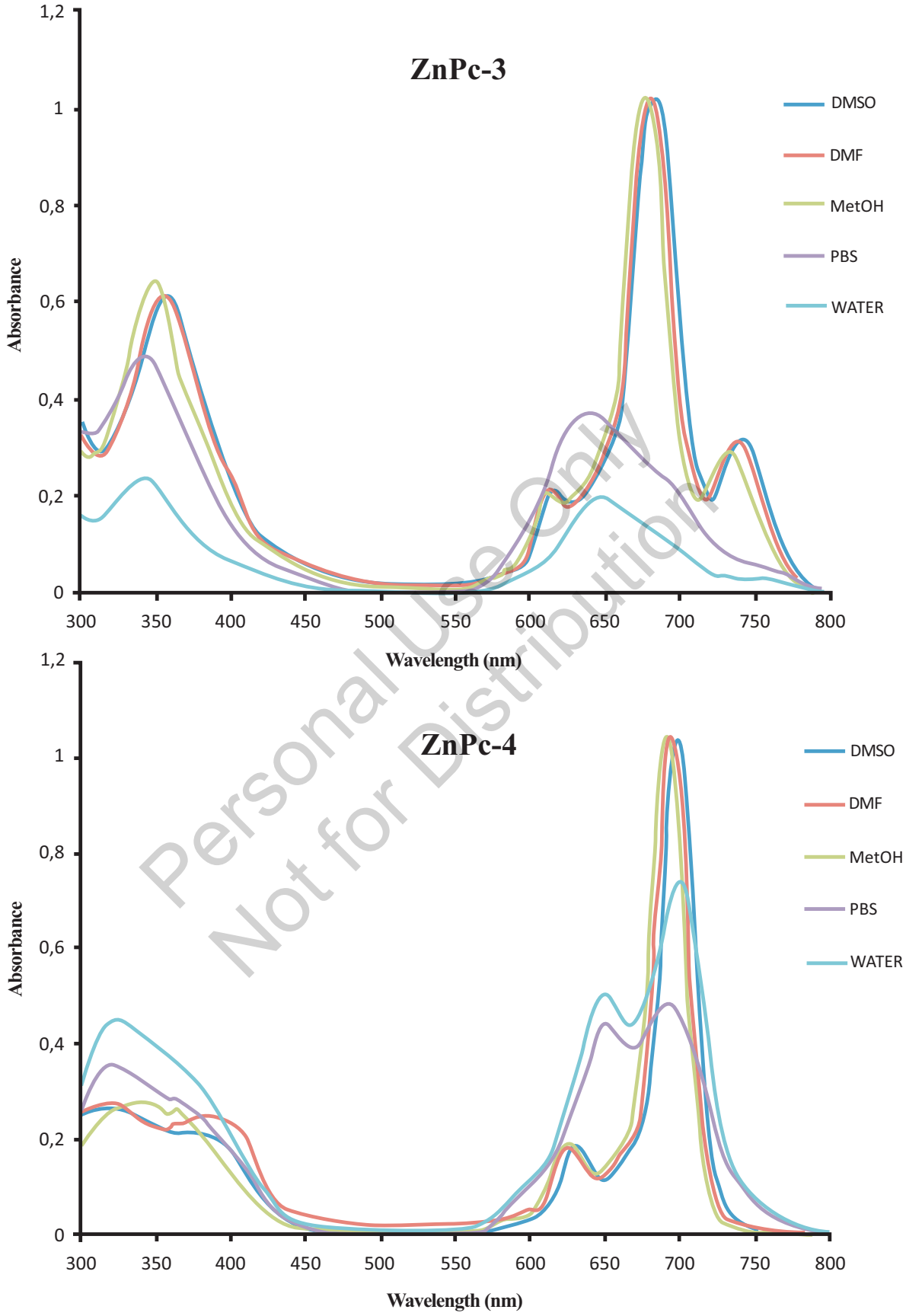
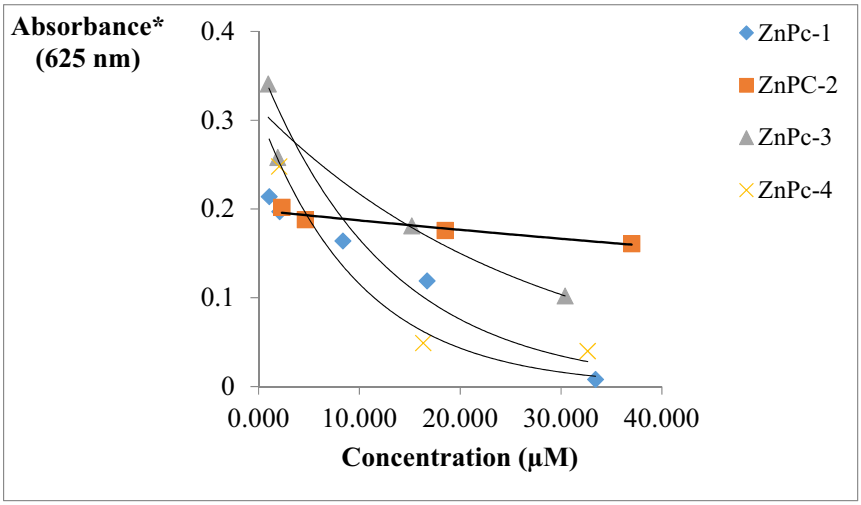
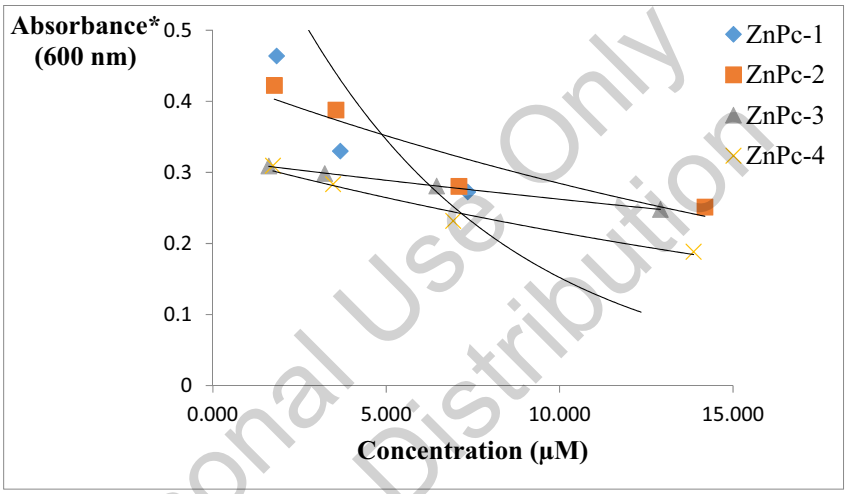


Fig. (2). UV-vis spectral changes of ZnPc-1, ZnPc-2, ZnPc-3, ZnPc-4 in Dimethyl sulfoxide (DMSO), Dimethylformamide (DMF), Methanol (MetOH), Phosphate-buffered saline (PBS), and water, respectively. (Concentration = $2x10^{-6}$ M).



(A)



(B)

Fig. (3). The dose response curve against reducing absorbance of urease (**A**) and hyaluronidase (**B**) inhibition.

r=0.9966. In order to make a comparison, $FeSO4.7H_2O$ was also tested under the same conditions as a standard antioxidant compound FRAP values were expressed as 1000 µM of $FeSO_4.7H_2O$ equivalent of g sample.

2.3.2. Scavenging of Free Radical (DPPH) Assay

Radical scavenging activity of samples against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was spectrophotometrically at 517 nm [26]. The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants. Briefly, various concentrations of 0.75 mL of compounds extracts were mixed with 0.75 mL of a 0.1 mM of DPPH in methanol. Radical scavenging activity was expressed as SC_{50}, the concentration of the samples that cause 50% scavenging of DPPH radical.

3. RESULTS AND DISCUSSION

In the pharmacological area, drug manufacturing focuses on the right process of industrial-scale synthesis. In addition, effective dose (ED), therapeutic response and desired effect are irreplaceable factors in the manufacturing of pharmaceutical drugs. Hence, large numbers of new compounds, that phthalocyanines are one of them, are tried or modified in the scientific area. They are ordinarily arranged with centrally coordinated metal atoms to avoid aggregation and improve photochemical properties. The photochemical properties provide different powerful advantages such as ambient initiation, temporal control, and they have the ability to control the desired reaction spatially [27]. So, these advantages form the basis of the strong bioactivities that can be mentioned as some enzyme inhibition, antiradical, antibacterial, anticancer activity and antioxidant activity [28, 29]. Here, this article focused on the inhibition degrees of four quaternerized ionic zinc (II) phthalocyanines against urease and hyaluronidase.

Urease which catalyzes the hydrolysis of urea and increases the local pH as it produces ammonia. This enzyme can be found in a wide variety of plants and a broad range of bacterial species. *Helicobacter pylori* is a gram-negative bacterium that also neutralizes the acid in its environment by producing large amounts of urease [30]. It has been known that this bacterium is acid sensitive and only replicates at pH of 7-8, but it survives in the stomach under highly acidic conditions [31]. As a result, *H. pylori* has a tendency to attack the stomach lining and causes gastric ulcer, duodenal

Table 1. Anti-urease, Anti-HYA, and antioxidant values of studied Zn-PC extracts.

Sample Code	Enzyme Inhibition		Antioxidant Activity	
	Urease[a]	Hyaluronidase[a]	FRAP[b]	DPPH[c]
Zn-Pc1	**7.095±0.062**	**4.227±0.055**	**0.341±0.001**	**16.917±0.058**
Zn-Pc2	115.878±0.200	16.380±0.818	0.190±0.002	22.178±0.150
Zn-Pc3	18.791±0.086	35.678±1.294	0.177±0.002	21.426±0.140
Zn-Pc4	7.725±0.032	17.083±0.059	0.195±0.000	22.985±0.161
Thiourea	21.023±0.012			

[a]Enzyme inhibition data are expressed as IC_{50}-µM.
[b]FRAP values are expressed as µmol $FeSO_4.7H_2O$/mg sample.
[c]DPPH scavenging data are expressed as SC_{50}-µM.

ulcers, noncardia gastric cancer and gastric MALT lymphoma. Besides this current information, some investigations have shown that *H. pylori* is also related to esophageal cancer, functional dyspepsia, gastroesophageal reflux disease, asthma, cardiovascular diseases, iron deficiency anemia and idiopathic thrombotic purpura [32].

In the developmental processing of epithelial branching morphogenesis, skeletal development, and stem cell maintenance, Extracellular matrix (ECM) remodeling is tightly regulated. Namely, ECM and its degradation products function normally for the development of organs and tissue in homeostasis. Also, they prefer some important ways, which are controlling of cell behaviors or initiation of disease progression, under various diseases conditions such as tumor metastasis, atherosclerosis, and angiogenesis [33]. Especially, in abnormal conditions, production ways of ECM have to cut with enzyme inhibition, one of which is hyaluronidase (HAases). HAases can degrade hyaluronic acid (HA) which is found exclusively in the ECM [34].

Many natural products and chemical synthesis are used to inhibit urease and hyaluronidase. Besides existing inhibitors, it was also reported that some designs of new enzymes inhibitors had been documented in the literature. While some oxadiazole, benzimidazole, benzohydrazones derivatives and schiff base hydrazide ligands were used for urease inhibition; dihydropyrimidine, some triazole- and tetrazole-xyloside analogues were examined [10, 35-39]. Also, some triterpenoids, high molecular mass poly (styrene-4-sulfonate) (PSS), gossypol, sodium aurothiomalate, fenoprofen, glycerrhizic acid, heparin, and O-sulfated HA could be given as HAase inhibitors [33, 40].

For a new contribution, four water-soluble zinc (II) phthalocyanines were tried to inhibit the urease and hyaluronidase. Non-aggregated and water-soluble phthalocyanines are very important and potentially useful materials for many applications. The main limitation in the applications of MPc complexes is their low solubility and aggregation in common organic solvents. Aggregation also affects seriously their spectroscopic, photophysical, photochemical and electrochemical properties. Generally, aggregation highly depends on concentration, temperature, nature of the substituents, nature of solvents and complexed metal ions. The aggregation behavior of the studied peripherally and non-peripherally ionic tetra-substituted zinc phthalocyanines (**ZnPC-1**, **ZnPC-2**, **ZnPC-3** and **ZnPC-4**) were investigated in different solvents (DMSO, DMF, MeOH, PBS and water) [20, 21]. These phthalocyanines did not show any aggregation in DMSO, DMF and MeOH, but they formed H-type aggregates in aqueous solutions (Fig. **2**) [20, 21]. For this reason, **ZnPC-1**, **ZnPC-2**, **ZnPC-3** and **ZnPC-4** were dissolved with DMSO to remove some aggregation effect and to create a concentrated stock solution that could be further diluted to the desired final concentration. Hence, the efficient correlation was done without aggregation effects about bioactivity.

The inhibitory degrees of the zinc (II) phthalocyanines derivatives were expressed by the half maximal inhibitory concentration (IC_{50}). All results were summarized in Table **1**. According to results, it was clear from both enzyme inhibition degrees that all molecules were found to act as low or moderate µM concentration. Comparing the ability of inhibition of urease, three phthalocyanine derivatives were better than Thiourea known as a standard inhibitor. Also, Thiourea was showed low activity with an IC_{50} value of 21.023 µM. The relationship between structures of the complexes and IC_{50} values indicated that the linking trimethylamino ligands with the substituents at the periphery position (**ZnPC-1**) had stronger activities than others. But also, a non-periphery position with diethylmethylamino ligands (**ZnPC-4**) had 7.725±0.032µM was nearly same value as the strongest compound.

As can be observed in Table **1**, all chemicals showed that they possessed strong to moderate hyaluronidase inhibition activity with varying degrees of dose-dependent. As well as urease inhibition results, the periphery position (**ZnPC-1**) was suitable for inhibiting the current enzyme but other results were not far away from the most effective compounds. The range 16.380-35.678 µM was the evidence that generally water-soluble zinc (II) phthalocyanines provided moderate conditions to inhibit the HAase.

The balance of metabolic system has to set up between the production of reactive species and the strength of the antioxidant defenses. Otherwise, oxidative degeneration known as impairments of cell function and cell death is inevitable [16]. Different sources to support the antioxidative balance with exogenous compounds such as *via* synthetic antioxidants, diets rich in fruits and vegetables and taking supplements are an actual investigation topic. From this per-

spective, antioxidant activity in zinc (II) phthalocyanines samples was determined using FRAP and DPPH radical scavenging activity method. One advantage of current study design was the limit of information about the anti-oxidative effect of water-soluble zinc (II) phthalocyanines because the outcome of literature database said to us that the current study was one of the precursors. Hence, it was chosen a direct way which was given the results of antioxidant effects of zinc (II) phthalocyanine compounds to show the superiority. The maximum antioxidant capacity in the FRAP method was observed for the compound **ZnPC-1** (Table **1**). The non-peripherally compounds **ZnPC-2** and **ZnPC-4** showed nearly same activity. The synthesized zinc phthalocyanines (especially peripherally tetra substituted **ZnPC-1**) showed relatively higher antioxidant capacity in DMSO.

For DPPH activity, the results were expressed as SC_{50} (μM), which was calculated from the curves by plotting absorbance values, the SC_{50} values representing the concentration of the compound (μM) required to inhibit 50% of the radicals [41]. The compound **ZnPC-1** had the lowest SC_{50} (16.917±0.058μM) value than other synthesized compounds. There was an efficient correlation between DPPH scavenging activity and FRAP assay because the compound **ZnPC-1** also had the best antioxidant activity. On the other hand, all compounds recorded moderate activity and the general preference of DPPH activity was periphery position side for each quaternerized amino ligands. The investigated samples in this study particularly with their different levels of the inhibition of the enzymes urease and hyaluronidase may be considered as a good source of antioxidant.

CONCLUSION

In consequence, there are many ways to show bioactive efficiencies of phthalocyanines on the basic of *in vitro* investigations. *In vitro* urease and hyaluronidase inhibition and antioxidant activity of four synthesized phthalocyanines were evaluated in the current study. It was known that a high percentage of the aggregation in solution was a common problem limiting the comparison and evaluation of phthalocyanines in a wide range of biochemical applications. Therefore, DMSO was used as a solvent and positive data was obtained. Thanks to types of substitute groups and linking positions, inhibition degrees of urease and hyaluronidase showed various effects. But especially, the **ZnPC-1** presents interesting properties as a good source with potential applications in bioactivity assays. We believe that the current results will lead the way to the future studies concerning the phthalocyanines.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Authors would like to thank Ebru Turk of the Giresun University for her input and critical review of the manuscript about linguistic and grammatical perspective.

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