



NOVEL ANTIMICROBIAL AGENTS ON THE BASE OF NATURAL AND SYNTHETIC METALLOPORPHYRINS

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Abstract

Nowadays various infectious diseases are becoming more widespread in the world. The problem is becoming more and more complicated because of the “epidemic” of bacterial resistance, which makes broadly used agents almost not applicable. Patients infected with multi-resistant bacteria have more severe clinical courses and require prolonged hospital treatment. One of the ways to overcome this problem is the usage of fabrics with antimicrobial properties. At present, some antimicrobial-finished textile products have been accepted for use in clinical settings but they are insufficiently effective because of applied antimicrobial agents or dyes. In the present work, novel organo- and water-soluble argentum metalloporphyrins have been obtained and linked with known natural dye indigo carmine, which is widely used in the world to dye textile materials. Investigation of antimicrobial activities has shown that among evaluated metalloporphyrins pheophytins (**a** and **b**) Ag(II) metalloporphyrin has the highest antibacterial activity against methicillin-resistant *Staphylococcus aureus*. The highest antifungal activity has been demonstrated by pheophytins **a** Ag(II) and cationic AgTOEt4PyP (5,10,15,20-tetra[4-N-(2'-oxyethylpyridyl)]porphinato Ag (II) tetranitrate) metalloporphyrins on *Candida albicans*. The complex of indigo carmine with cationic AgTOEt4PyP metalloporphyrin has shown less antifungal activity than AgTOEt4PyP alone. However, the obtained complex with indigo dye possesses high antibacterial activity against *Staphylococcus aureus* similar with AgTOEt4PyP. Thus, obtained metallocomplexes can be recommended for treatment of cotton work clothes, wool textile materials, etc. These textile products can be used by people working in contaminated environment or insanitary conditions.

Keywords: porphyrins, metalloporphyrins, antimicrobial activity, indigo carmine, pheophytin.

Introduction

The “epidemic” of microbial resistance became a serious problem for the treatment of hospital infections [Neu H., 1992]. The problem is becoming more and more complicated because of ineffectiveness of commonly used antibiotic drugs. Despite the advance in our knowledge of nosocomial infections, those resulting from methicillin-resistant *Staphylococcus aureus* still cause clinically significant problems [Boyce J., 1990;

Panlilio A. et al., 1992; Harstein A. et al., 1995]. *Pseudomonas aeruginosa* is also frequently registered in hospital infections, especially in burn and intensive care units [Pruitt B. and McManus A., 1992; Kropec A. et al., 1993]. These microorganisms are shown to survive for extended period of time on materials worn by patients and healthcare workers and on various fabrics used in the hospital environment and are spread by the movement of contaminated articles such as bedclothes, bed curtains, towels, the protective clothing of nurses and by person-to-person contact [Babb J. et al., 1983; Nelly A. and Maley M., 2000]. This undesired

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and dangerous colonization could be prevented by the application of materials that contain antimicrobial agent.

Taking into account a great number of infections resulting from different bacterial species and fungi, the development of compounds with high antibacterial, antifungal activities and stability against environmental factors is an urgent need, as with the help of such compounds textile materials will be treated.

The ability for numerous chemical modifications and the large number of different mechanisms, by which porphyrins affect microbial and viral pathogens, place porphyrins into a group of compounds that present an outstanding source for discovery of novel procedures, materials, and agents active against a wide range of pathogenic microorganisms [Stojiljkovic I. et al., 2001]. A large number of natural and synthetic porphyrins of different chemical structure and properties can be isolated from natural sources or synthesized in the laboratory.

In the present work both natural and synthetic (metallo)porphyrins have been synthesized and their antimicrobial activities against methicillin-resistant *Staphylococcus aureus* and *Candida albicans* have been investigated. New type of complexes formed via interaction of synthetic porphyrins and known dye indigo carmine were also obtained. Indigo is among the oldest dyes to be used for dyeing and printing of cotton work clothes, wool textile materials. The dye was obtained by processing plant leaves (genus *Indigofera*). The obtained complex of indigo carmine and cationic metalloporphyrin has also been tested for antimicrobial activity. The testing was done in order to offer a dye with the help of which treated textile materials will possess antimicrobial activity.

Materials and Methods

Chemicals. 4-N-pyridylcarboxaldehyde, pyrrole, 1-hydroxy-2-chloroethane, AgNO₃, solvents were purchased from Sigma-Aldrich Chemie GMBH, Germany. Analytical thin-layer chromatography was performed on silica-coated plastic plates ("Sorbfil"). Preparative separations were performed by flash column chromatography on alumina.

Measurement of spectral properties. The structure and purity of compounds synthesized were determined by NMR and electronic absorption spectroscopy. ¹H and ¹³C NMR spectra were obtained with a Mercury Varian 300 spectrometer. The absorbance of porphyrins and metalloporphyrins dissolved in water was measured in the wavelength range 350–800 nm using Perkin-Elmer Lambda 800 double-beam UV/Vis spectrometer. The absorption coefficients of the bands were determined in 10⁻⁴-10⁻⁶M porphyrin solutions by the Beer-Lambert law.

Antimicrobial testing. Antibacterial and antifungal activities of (metallo)porphyrins were determined *in vitro* by the systematic double and ten times dilution method in peptone broth on the methicillin-resistant *Staphylococcus aureus* 906 and *Candida albicans*. A known concentration of tested (metallo)porphyrin was added to 1 ml of microbial suspension with 10⁷ cells/ml concentration. After stepwise dilution, a 100 μl portion was spread onto an agar plate and incubated at 37°C for 24 hours. Colonies were then counted. Minimum inhibitory concentrations (MIC), – the minimal concentration of compound, at which microbial cell growth was not observed; were determined for each evaluated compound. All experiments were repeated at least three times for each evaluated compound.

Results

Obtaining natural porphyrins and metalloporphyrins. Chlorophylls (**a** and **b**) were separated from the leaves of nettle (*Urtica dioica*). Their extraction and de-metallization were performed in acetic acid medium with the help of ultrasound (20 kHz), which brought to appropriate mixture of pheophytins (**a** and **b**). Metallocomplexes of natural porphyrins (pheophytins **a** and **b**) were synthesized by interaction of argentum nitrate with the initial porphyrins (Figure 1). The structure of compounds was determined by ¹H and ¹³C NMR spectroscopy. The molar absorption coefficients along with absorption maxima of obtained natural compounds are summarized in Table 1.

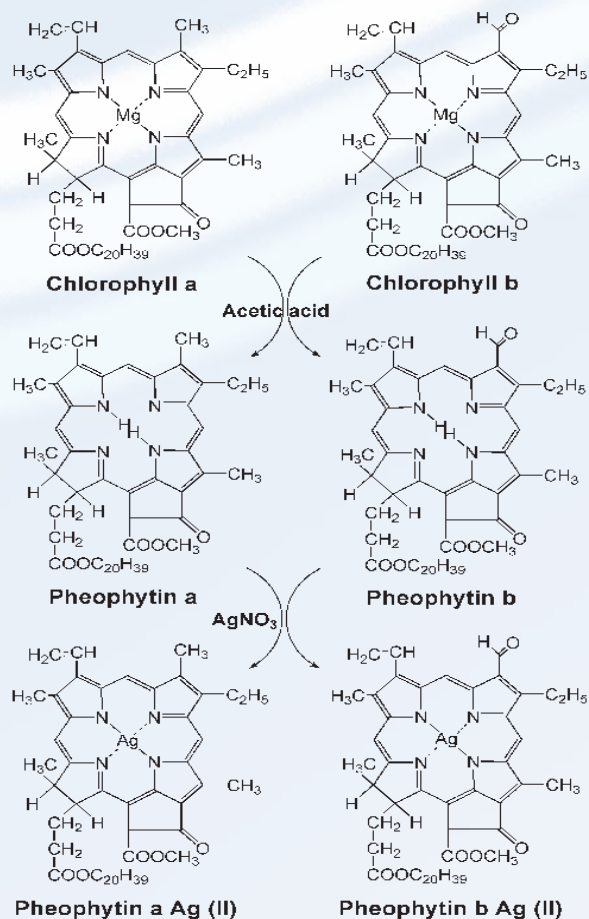


Figure 1. General procedure of obtaining pheophytins and their argentum metallocomplexes.

Synthesis of (metallo)porphyrins. The initial 5,10,15,20-tetra(4-N-pyridyl)porphine (H_2T4PyP) was synthesized according to the method of Adler [Adler A. et al., 1967] modified by R. Ghazaryan and co-workers [Ghazaryan R. et al., 2004; 2005; 2006]. Further quaternization of free porphyrin by 1-hydroxy-2-chloroethane led to the targeted water soluble cationic 5,10,15,20-tetra[4-N-(2'-oxyethylpyridyl)]porphine tetrachloride ($H_2TOEt4PyP$). By interaction of argentum nitrate with the $H_2TOEt4PyP$ porphyrin 5,10,15,20-tetra[4-N-(2'-oxyethylpyridyl)]porphinato Ag(II)tetranitrate ($AgTOEt4PyP$) metallocomplex was synthesized.

Spectral properties of (metallo)porphyrins. Absorption spectra of synthesized $H_2TOEt4PyP$ porphyrin are characterised by a strong Soret band in the near violet region and four Q-bands located in the 519–640 nm region. Only one Q-band is observed in the absorption spectra of the $AgTOEt4PyP$ metal derivative characteristic of metalloporphyrins. Both Soret and Q-bands arise from the $\pi-\pi^*$ transitions. The molar absorption coefficients along with absorption maxima of synthesized compounds are summarized in Table 1. The structure of compounds was determined by 1H and ^{13}C NMR spectroscopy.

Table 1.

The results of UV-vis spectra of porphyrins and their metallocomplexes in water; λ_{max} , nm ($\epsilon \times 10^{-3}$)

Compounds	Bands				
	1	2	3	4	5 (Soret)
Pheophytin a Ag (II)*				690(108)	430(240)
Pheophytins (a and b) Ag (II)*				648(240)	430(402)
$H_2TOEt4PyP$	640 (1.5)	585 (6.4)	556 (5.8)	519 (15)	424 (209)
$AgTOEt4PyP$				545 (12.3)	431 (125)
Complex of $H_2TOEt4PyP$ and indigo carmine				609 (45.6)	440 (91.5)
Complex of $AgTOEt4PyP$ and indigo carmine			609 (40.5)	570 (30)	445 (68)

Note: *Chloroform was used as a solvent

Table 2.

The values of observed bathochromic shifts of Soret band of $H_2TOEt4PyP$ porphyrin absorption spectra after titration with indigo dye solution

$H_2TOEt4PyP$: indigo carmine (molar coefficients)	1:0	1:1	1:2	1:3	1:4
Bathochromic shifts of Soret band (nm)	(424.14)	4.76 (424.14-428.90)	5.23 (428.90-434.13)	4.69 (434.13-438.82)	0.85 (438.82-439.67)

Synthesis of complexes of (metallo)porphyrin with indigo carmine. Synthesized $H_2TOEt4PyP$ porphyrin and its argentum metallocomplex $AgTOEt4PyP$ were reacting with indigo carmine dye. As a result, complexes of $H_2TOEt4PyP$ and $AgTOEt4PyP$ with indigo carmine were formed as green precipitates in methanol (Figure 2). It is of interest to state that though both (metallo)porphyrin and indigo carmine are soluble in methanol, formed complexes are non-soluble in methanol.

Spectral studies of complexes of (metallo)porphyrin with indigo carmine. Synthesized complexes of $H_2TOEt4PyP$ and its silver (II) metalloporphyrin with anionic dye (indigo carmine) have a wide absorption band in red area of spectra comparable with intensity of *a* Soret band. New complexes have been characterized

by the data of NMR and electronic absorption spectra (Table 1).

In order to determine the chemical composition of obtained complexes, the products of interaction of cationic $H_2TOEt4PyP$ porphyrin and indigo dye in molar ratio 1:1, 1:2, 1:3, 1:4 and above have been investigated by the method of electronic absorption spectroscopy in water (Figure 3). It has been shown that at their interaction in molar ratio 1:1, there is a bathochromic shift of all absorption bands of initial porphyrin. The further increase in amount of dye results in the gradual bathochromic shifts up to molar ratio 1:4, then no change of a spectrum from excess of the amount of dye occurs (Table 2). The received final spectrum is identical to an electronic absorption and NMR spectra of synthesized, individually allocated zwitterionic complexes.

Two-dimensional NMR methods have been applied for the elucidation of the structure of complex of $H_2TOEt4PyP$ porphyrin and indigo dye (Figure 4). The observation of both COSY and NOESY connectivities among signals of protons of cationic porphyrin and dye molecules allowed us to determine the geometry of binding as well as the stoichiometry of the complex (Figure 5).

Antimicrobial activity of natural and synthetic (metallo)porphyrins. Argentum metalloporphyrins of both natural and synthetic origin were tested for antimicrobial activity against Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus*) and fungi (*Candida albicans*). The minimal inhibitory concentrations determined for each evaluated compound are presented in Table 3. Data obtained suggest that among evaluated metalloporphyrins the pheophytins (*a* and *b*) $Ag(II)$

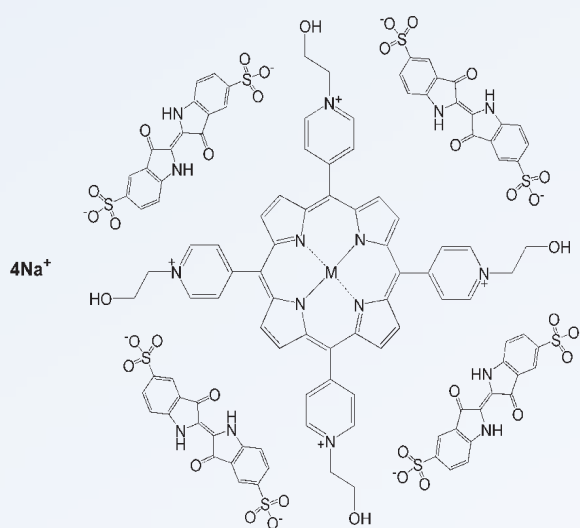


Figure 2. General formula of complexes of $H_2TOEt4PyP$ ($M=2H$) and $AgTOEt4PyP$ ($M=Ag$) with indigo carmine.

Table 3.

Antimicrobial testing data (MIC values) of natural and synthetic argentum metalloporphyrins

Tested microorganism	Minimal inhibitory concentrations ($\mu\text{g/ml}$)			
	Pheophytins <i>a</i> Ag(II)	Pheophytins (<i>a</i> and <i>b</i>) Ag(II)	AgTOEt4PyP	Complex of AgTOEt4PyP and indigo carmine
Methicillin-resistant <i>Staphylococcus aureus</i>	0.2	0.0625	0.125	0.125
<i>Candida albicans</i>	15.6	62.5	15.6	500

metalloporphyrin has the highest antibacterial activity against methicillin-resistant *Staphylococcus aureus* with a 0.0625 $\mu\text{g/ml}$ MIC value. However, the highest antifungal activity has been demonstrated by pheophytins *a* Ag(II) and cationic AgTOEt4PyP metalloporphyrins with MIC value equal to 15.6 $\mu\text{g/ml}$. The complex of indigo carmine with cationic AgTOEt4PyP metalloporphyrin has shown less antifungal activity than AgTOEt4PyP alone. Surprisingly, the obtained

complex with indigo dye possesses high antibacterial activity against *Staphylococcus aureus* similar with AgTOEt4PyP, although the molar content of metalloporphyrin is decreased about three times. Metal non-containing analogues of natural and synthetic argentum metalloporphyrins (pheophytins *a* and *b*, $\text{H}_2\text{TOEt4PyP}$ porphyrin and its complex with indigo dye) did not demonstrate antibacterial activity against tested microorganisms (data is not presented).

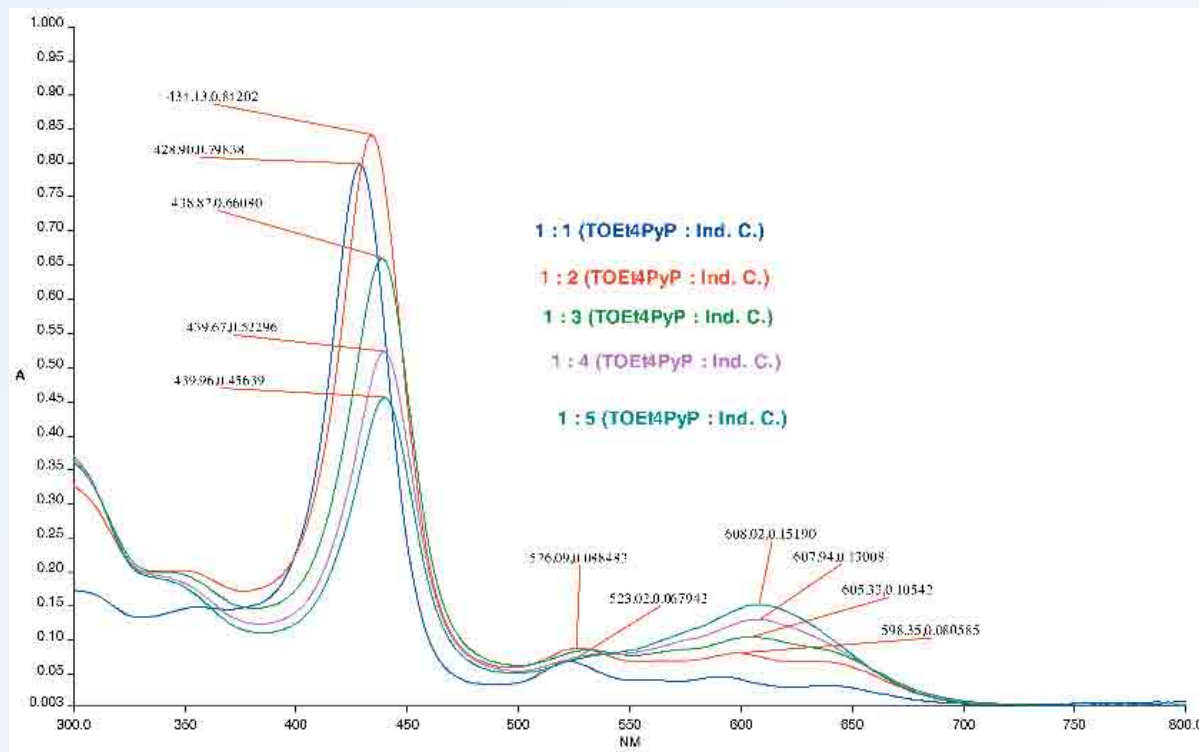


Figure 3. The observed changes of $\text{H}_2\text{TOEt4PyP}$ porphyrin spectra during titration with the equimolar solution of indigo carmine up to 1:5.

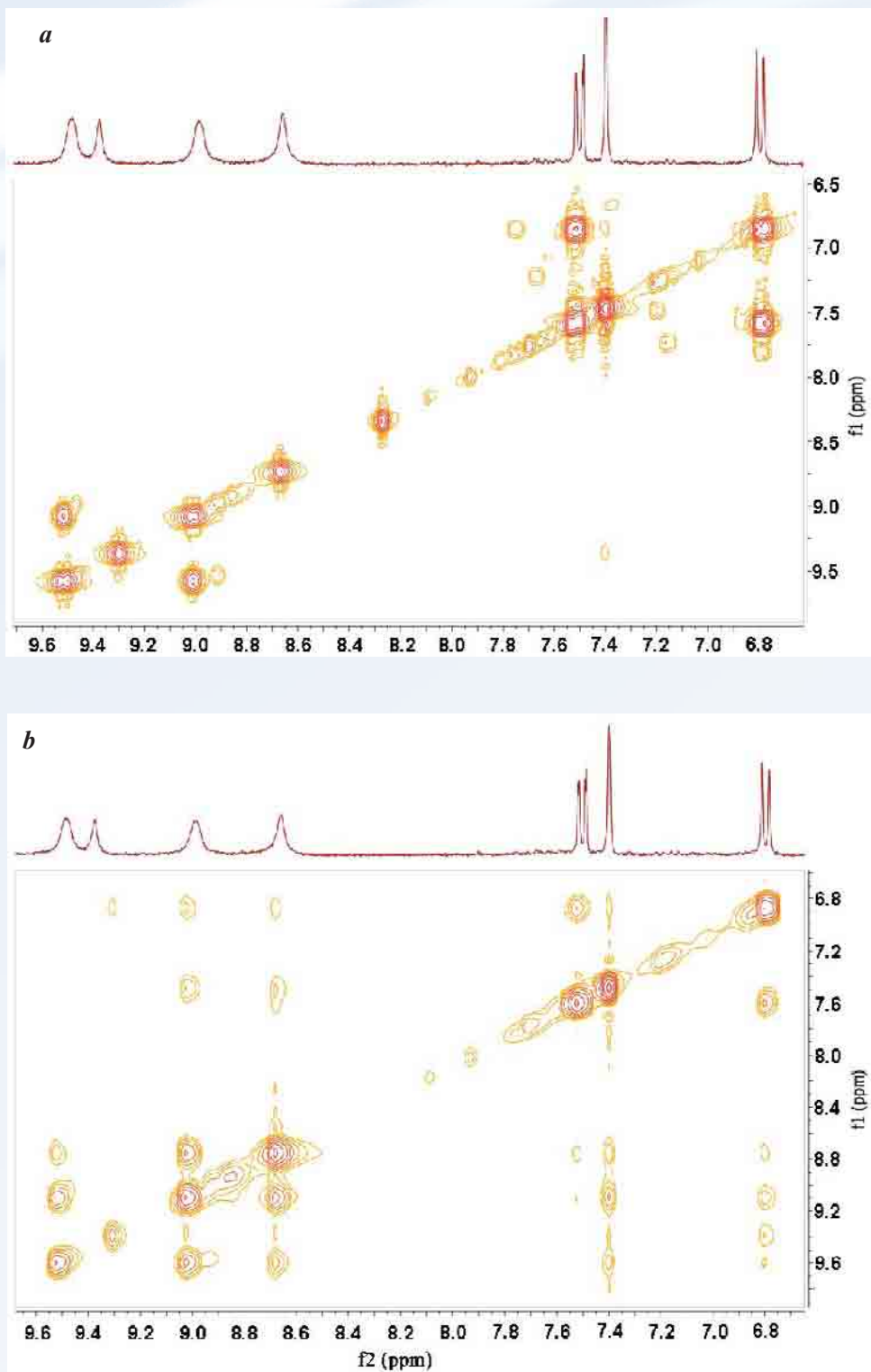


Figure 4. a) COSY and b) NOESY spectra of complex of H_2TOEt_4PyP and indigo carmine

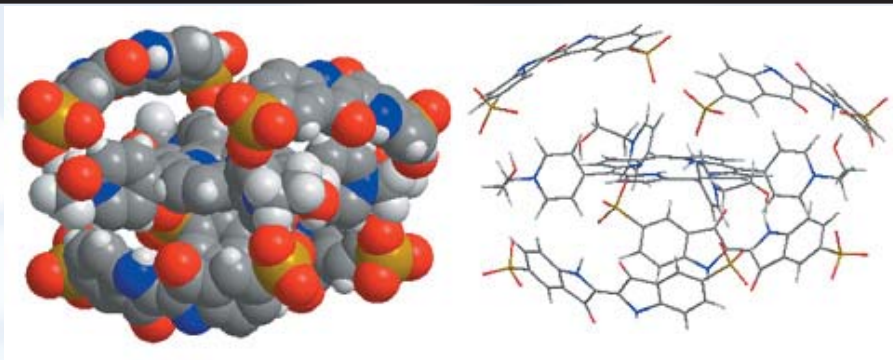


Figure 5. The proposed structure of complex of $H_2TOEt4PyP$ with indigo carmine.

Discussion

Porphyric compounds of both natural and synthetic origin are well described in the scientific literature as a promising group of potential photosensitizers for photodynamic cancer therapy and diagnosis [Vicente M., 2001; Berg K. et al., 2005; Juzeniene A. et al., 2007]. Some of them successfully passed preclinical and clinical testing and were accepted for use in hospitals [Dougherty T., 2002; Detty M. et al., 2004]. Photosensitizing activity of these compounds is also used to destroy Gram-positive, Gram-negative bacteria (including drug-resistant strains), fungi and viruses [Wainwright M., 1998; Vzorov A. et al., 2002; Hamblin M., Hasan T. et al., 2004; Jori G., 2006]. Porphyric structures are also demonstrated to possess a marked anticancer activity both in vitro and in vivo at dark conditions [Grand C. et al., 2002; Gasparyan G. et al., 2007; Tovmasyan A. et al. 2007a,b]. Dark antimicrobial activity of porphyrins is also widely investigated and reviewed [Stojiljkovic I. et al., 2001].

The purpose of this investigation was to synthesize natural and synthetic biologically active (metallo)porphyrins, link them with textile dye and evaluate antibacterial, antifungal properties *in vitro* for obtaining highly effective non-toxic antimicrobial compounds-dyes for textile products.

Some antimicrobial-finished textile products have been accepted for use in clinical settings to block the transmission of pathogens [Infante M. et al., 1996; Okuda K. and Kashiwagi S., 1996; Tsai G. and Su W., 1999]. However, there are differences in antibacterial properties among

commercially available antimicrobial-finished textile products concerning their water stability, non-effectiveness against some bacterial species and strains, incubation time [Takai K. et al., 2002].

Chlorophylls are the most available source of natural porphyrins, it is possible to use them for industrial production of various porphyrins and their metallocomplexes. Thus, chlorophylls (*a* and *b*) were separated as a form of pheophytins (*a* and *b*) from the leaves of nettle and argemum metallocomplexes were synthesized.

New type complexes were obtained via interaction of indigo textile dye with meso-substituted cationic $H_2TOEt4PyP$ porphyrin and its $AgTOEt4PyP$ metallocomplex.

Data on antimicrobial testing suggest that the bioactivity of porphyrins against microbial infections (methicillin-resistant *Staphylococcus aureus* and *Candida albicans*) is due to presence of a central metal atom in porphine ring. All argemum metallocomplexes of both natural and synthetic porphyrins demonstrated high antibacterial and antifungal activity.

Thus, organo- and water-soluble non-toxic dyes have been obtained on the basis of natural and synthetic silver metalloporphyrins. Synthesized compounds demonstrate high bactericide and fungicide activities and could be used for treatment of various materials. Treated materials can be very useful in the development of various medical fabrics and household products (bed linen, curtains, towels, protective means, dressings, etc.) in order to prevent the spread of the infections.

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