ЖУРНАЛ СТРУКТУРНОЙ ХИМИИ

2014. Том 55, № 5 *Сентябрь – октябрь* *С. 956 – 961*

UDC 541.6:541.49:546.48

RELAXIVITY MEASUREMENT OF PORPHYRIN-BASED MAGNETIC RESONANCE IMAGING (MRI) CONTRAST AGENTS

Haroon-Ur-Rashid[1], M.N. Umar[2], K. Khan[3], M.N. Anjum[4], M. Yaseen[1]

[1]*Institute of Chemical Sciences, University of Peshawar, Peshawar, Pakistan*
E-mail: haroongold@gmail.com
[2]*Department of Chemistry, University of Malakand, Chakdara, Pakistan*
[3]*Department of Chemistry, Islamia College University, Peshawar, Pakistan*
[4]*Department of Applied Chemistry, Government College University, Faisalabad, Pakistan*

Received May, 7, 2013 *Revised September, 29, 2013*

Magnetic Resonance Imaging (MRI) has become a prominent imaging technique in medicine. MRI contrast agents are used to increase the sensitivity of this technique. Recently, a new method for cancer treatment with a less side effect called photodynamic therapy (PDT) has been introduced. In this method cancer tissues are selectively destroyed without injuring the surrounding healthy cells. However, for the successful use of this method, the selection of an appropriate photosensitizer is important. Hence, diagnosis-treat union of MRI and PDT will be significantly promoted if a new family of bi-functional agents is found, which would combine the effects of a contrast agent in MRI and of a photosensitizer in PDT. It will bring great improvement to the cancer diagnosis and treatment. Porphyrins have proved to be useful photosensitizing agents in PDT due to their promising photophysical efficiency and a less side effect. This work briefly describes the research development of porphyrins as a photosensitizer applied in PDT, and also highlights the recent progress in the research on bi-functional agents of MRI-PDT. Diethylenetriamine pentaacetic acid (DTPA) units are incorporated at a meso-position of the porphyrin ring to synthesize new bi-functional agents of MRI-PDT with sufficient water solubility and high relaxation potency. Gd^{3+} complexes are prepared by the reaction of the high molecular weight porphyrin-based ligand with $GdCl_3 \cdot 6H_2O$. The longitudinal relaxivity measurement indicates 154 % and 251 % enhancement compared to that of the widely used MRI contrast agent Gd—DTPA. These results indicate that the two complexes could possibly be used as bi-functional agents of MRI-PDT.

K e y w o r d s: contrast agent, porphyrin, relaxivity, gadolinium, ligand.

INTRODUCTION

Magnetic resonance imaging (MRI) is a noninvasive imaging modality used in modern clinical diagnosis to visualize a detailed internal structure and the limited function of the body. It is based on the detection of NMR signals emitted by protons of water and fat molecules in the body in a magnetic field. A magnetic field is applied to the region of interest which is then modulated by radio waves. The nuclear energy states of protons interact with the incident radio waves. The radio frequency emission by tissue that follows the absorption of photons can be exploited to produce images [1—3]. In this method, three-dimensional images of the body parts are obtained in thin slices [4]. Paul C. Lauterbur and Sir Peter Mansfield won Nobel Prize in 2003 for their work on MRI [5]. Compared to computed tomography (CT), MRI provides much greater contrast between the different soft tissues of the body. In modern medicine, MRI has emerged as one of the most powerful techniques for the diagnosis and treatment of human diseases [6, 7]. Porphyrin complexes are successfully used as MRI contrast

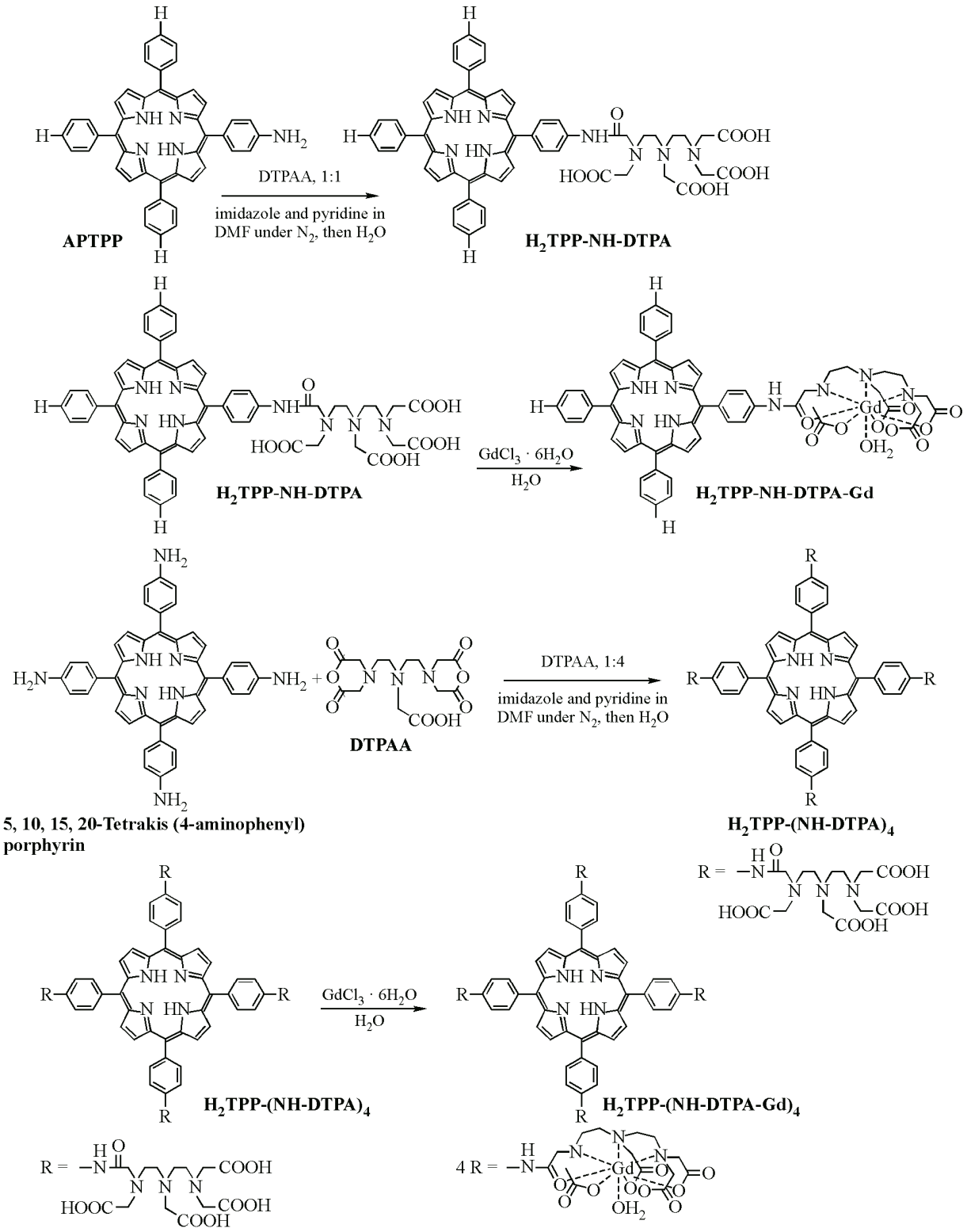
agents. They are one of the very important chemical units necessary for many processes on the earth. Many biological molecules work with prosthetic groups basically consisting of these units [8—10]. Porphyrins and some of their derivatives have been found to be malignant tumor localizers. However, free porphyrins do not sufficiently affect the relaxation time of water and therefore, are not effective MRI contrast agents. They have suitable chelation ability with paramagnetic metal ions. Therefore, complexes of porphyrins with manganese, iron, and gadolinium ions are being investigated for the detection of malignant tumors on MRI. Tetraphenylporphine sulfonate and hematoporphyrin have been found to possess sufficient affinity for cancerous tissues [11—13]. Porhyrins act as photosensitizers in photodynamic therapy. Photosensitizer is a drug or chemical compound used in photodynamic therapy (cancer treatment). They are absorbed by cancer cells. On exposure to light of specific wavelengths, photosensitizers are activated and thus kill the cancer cells. Photodynamic therapy (also called PDT, phototherapy, photo radiation therapy or photochemotherapy) is a useful method for the detection and treatment of cancer cells without surgery or chemotherapy. The basic principle of PDT is that photosensitizing agents (like porphyrins and pthalocyanins) can kill single-celled organisms on their exposure to a particular type of light. Thus, in PDT cancer cells are destroyed by the use of a fixed frequency (laser light) in combination with a photosensitizer. Porphyrins are considered to be ideal photosensitizers because they are water-soluble to a certain extent, non-toxic, selectively retained in tumor tissues in high concentrations, cleared from the body in a reasonable time and rapidly from the skin, thus preventing photosensitive reactions. Research shows that PDT can be as useful as radiation therapy or surgery in the treatment of specific types of cancers and precancerous conditions [14—18].

EXPERIMENTAL

All reagents were of the best grade, commercially available and were distilled, crystallized or used without further purification, as appropriate. Chloroform was dried over $CaCl_2$ whereas N,N-dimethylformamide (DMF) was dried over molecular sieves. Flash chromatography was performed on silica gel (particle size 200—300 mesh). IR, ^{1}H NMR and UV/Vis spectra were recorded at 25 °C. The IR spectra were recorded on a Bruker FTEQUINOX55 IR spectrophotometer as KBr discs. ^{1}H NMR were recorded on Bruker AV 400 (400 MHz) using TMS as the internal standard. Gd—DTPA was purchased from Shandong Hongfuda Pharm Chem Company Limited Shandong. $GdCl_3 \cdot 6H_2O$ was purchased from Hangzhou Ocean Chemical Co., Ltd. Zhejiang.

Synthesis of the H_2TPP—NH—DTPA ligand (5,10,15,20-tetraphenylporphyrin-NH-diethylenetriamine pentaacetic acid). Diethylenetriamine-N,N′,N″-triacetic N,N″-dianhydride (DTPAA) (71.4 mg, 0.2 mmol) was dissolved in 10 ml of dry pyridine. Imidazole (118 mg, 1.4 mmol) was then added to it and the mixture was heated to 50 °C. A mixture consisting of dry pyridine (111 mg, 1.4 mmol) and 5-(4-aminophenyl)-10,15,20-triphenylporphyrin (APTPP) (126 mg, 0.2 mmol) in 10 ml of refined DMF was added dropwise to the reaction flask via a pressure-equalizing dropping funnel in 30 min. Then the reaction mixture was stirred at 50 °C under nitrogen protection. When the reaction was carried on for 5 h, 12 μl of H_2O was added into the reaction solution. Then the reaction mixture was stirred for about 30 min. It was filtered and the filtrate was reserved. The filtrate was then concentrated to its saturation point. A mixture solution of acetone/diethyl ether (V:V = 1:10) was added into the filtrate and stirred for 30 min. The solid precipitate was collected and washed with a mixture solution of acetone/diethyl ether three times. Furthermore, acetone was used to wash it three times. Finally, diethyl ether was used to wash it two times. After drying some purple solid compound (120 mg, 0.12 mmol) was obtained. The yield is 72 %. ^{1}H NMR (in DMSO-d_6, 400 mHz): δ 10.55 (br, 1H, C(O)NH), 8.91—8.83 (m, 8H, β-H), 8.24—8.14 (m, 6H, Ar—H), 7.85—7.61 (m, 9H, Ar—H), 7.48 (d, 2H, J = 8.0 Hz, Ar—H), 7.11 (d, 2H, J = 8.0 Hz, Ar—H), 3.72—2.89 (a set of very broad and multiple peaks with an integration corresponding to 18H, CH_2 of DTPA), –2.91 (s, 2H, pyrrole N—H) [19, 20].

Synthesis of the H_2TPP—NH—DTPA—Gd complex. $GdCl_3 \cdot 6H_2O$ (45 mg, 0.12 mmol) was dissolved in 5 ml of water. The H_2TPP—NH—DTPA ligand (101 mg, 0.1 mmol) was added to the above solution under severe stirring. NaOH solution (0.1 M) was used to adjust the solution pH



Scheme. Synthetic route of the H_2TPP—NH—DTPA—Gd and H_2TPP—(NH—DTPA—Gd)$_4$ complexes

value to 6—7 during the reaction. The reaction was carried out for 48 h at 50 °C. It was then ceased and the reaction solution was added to the mixture solution of ethanol/diethyl ether (V:V = 1:2). A solid matter precipitated which was then collected. The precipitate was washed three times each

with a mixture solution of ethanol/diethyl ether and ethanol. Finally, it was washed with diethyl ether. After drying a purple solid material (70 mg, 0.06 mmol) was obtained. The yield is 60 %.

Synthesis of Ligand H_2TPP—(NH—DTPA)$_4$. DTPAA (280 mg, 0.8 mmol), imidazole (472 mg, 5.6 mmol), and dry pyridine (443 mg, 5.6 mmol) were dissolved in 10 ml of distilled DMF. The temperature of the reaction mixture was then raised to 50 °C. Then 5, 10, 15, 20-tetrakis (4-aminophenyl) porphyrin (135 mg, 0.2 mmol) dissolved in 10 ml of DMF, was added dropwise to the reaction mixture via a pressure equalizing funnel. The reaction was carried on for 25 h. Then 16 μl of distilled water was added into the reaction mixture. The reaction was then ceased after 2 h. The reaction mixture was filtered. The filter liquor was concentrated to its saturation point. A mixture solution of acetone/diethyl ether (V:V = 1:10) was then added to the filter liquor. The mixture was stirred for 30 min. The solid precipitate was collected. It was washed with the mixture solution of acetone/diethyl ether three times. Acetone was used three times to wash the ligand. The product was washed two times with diethyl ether. It was purified via column chromatography using dichloromethane as eluent. The first fraction from the column was collected. The solvent was removed via rotary evaporator and thus a solid purple material H_2TPP—(NH—DTPA)$_4$ (315 mg, 0.144 mmol) was obtained. Yield is 72 %. ^{1}H NMR (in D_2O, 400 mHz): δ 8.60 (s, 8H, β-H), 7.37 (m, 16H, Ar—H), 3.79—2.90 (a set of very broad and multiple peaks with an integration corresponding to 72H, CH_2 of DTPA) [19, 20].

Synthesis of Complex H_2TPP—(NH—DTPA—Gd)$_4$. $GdCl_3 \cdot 6H_2O$ (180 mg, 0.48 mmol) was dissolved in 5 ml of water. Then the H_2TPP—(NH—DTPA)$_4$ ligand (218 mg, 0.1 mmol) was added under severe stirring conditions. Sodium hydroxide solution (0.1 M) was used to adjust the solution pH value to 6—7 during the reaction. The reaction was carried out for 24 h at room temperature. Then a mixture solution of ethanol/diethyl ether (V:V = 1:2) was added to the reaction mixture to precipitate the product. The solid material was collected. It was washed three times each with a mixture solution of ethanol/diethyl ether and ethanol. Finally, the product was washed two times with diethyl ether. After drying a solid purple compound (155 mg, 0.056 mmol) was obtained. Yield is 56 %.

Determination of the longitudinal relaxation time T_1. For the measurement of the longitudinal relaxation time T_1, solutions of certain concentration of Gd—DTPA, H_2TPP—NH—DTPA—Gd, and H_2TPP—(NH—DTPA—Gd)$_4$ complexes in distilled water were separately prepared. A 2 mmol solution of Gd—DTPA (5.63 mg in 5 ml of solution) was prepared. Similarly, 2 mmol solutions of both H_2TPP—NH—DTPA—Gd (11.76 mg in 5 ml of solution) and H_2TPP—(NH—DTPA—Gd)$_4$ (28.62 mg in 5 ml of solution) complexes were prepared in separate flasks. The pH of all the solutions was adjusted to 7. The longitudinal relaxation time $(T_1)_d$ of pure water and the longitudinal relaxation time $(T_1)_{obs}$ of water in the presence of the paramagnetic complex were measured with the inversion recovery (IR) method on a Bruker BIOSPEC 47/30 magnetic resonance imaging (4.7 T) instrument [20].

RESULTS AND DISCUSSION

The relaxation phenomenon of water protons in aqueous solutions of gadolinium complexes affects the *in vivo* properties of such complexes during the MRI investigation. The relaxation ability is denoted as the relaxation rate (R_1); the greater the value of R_1, the stronger would be the relaxation ability and consequently the better would be *in vivo* imaging results. The pure water spin-lattice relaxation rate $(1/T_1)_d$ and the observed relaxation rate $(1/T_1)_{obs}$ of water in the presence of paramagnetic complexes were measured under the same conditions using the following equation. This equation is used to calculate the relaxation rate R_1 of various paramagnetic complexes [21, 22].

$$R_1[M] = (1/T_1)_{obs} — (1/T_1)_d,$$

where [M] indicates the Gd^{3+} concentration.

The results of the relaxation performance test show that compared to the current widely used clinical MRI contrast agent Gd—DTPA, the relaxation performance of the complexes synthesized in this work has been significantly improved. Since the H_2TPP—NH—DTPA—Gd and H_2TPP—(NH—DTPA—Gd)$_4$ complexes have a larger molecular size than Gd—DTPA, therefore, their molecules rotate slowly in an aqueous solution causing their longer rotational correlation time compared to

Table 1

Relaxivity measurement of gadolinium complexes

Gd^{3+} complex	Molecular weight of ligands, $(g \cdot mol)^{-1}$	[M], $(mmol \cdot l)^{-1}$	$T_{1(obs)}$, s	R_1/Gd^{3+}, $(L^{-1} \cdot mmol \cdot s)^{-1}$	Enhancement, %
H_2TPP—NH—DTPA—Gd	1005	1.532	0.081	7.82	154
H_2TPP—(NH—DTPA—Gd)$_4$	2190	1.361	0.0564	12.76	251
Gd—DTPA	393	1.241	0.1498	5.09	100

$T_{1(d)}$ = 2.798 s; Temperature: 25 °C, Frequency: 200 MHz, $T_{1(d)}$ represents the longitudinal relaxation time of pure water, $T_{1(obs)}$ represents the observed longitudinal relaxation time of water in the presence of a paramagnetic complex, [M] is the Gd^{3+} ion concentration, R_1/Gd^{3+} indicates the ratio of the relaxation rate to the Gd^{3+} ion concentration.

the relatively smaller molecules of Gd—DTPA. This effect results in a decreased T_1 relaxation time and an increased relaxation rate of the H_2TPP—NH—DTPA—Gd complex.

UV-Visible absorption spectra. Metal-free porphyrins show characteristic UV-Vis absorptions, consisting of two sets of band. First is called the B band or soret band (appears in 400—450 nm range). The second set of bands called the Q bands appears at lower energy (550—700 nm range). UV-Visible absorption spectra of the H_2TPP—(NH—DTPA)$_4$ ligand is shown in Fig. 1, *a*. In this spectrum both bands (soret and Q bands) can be seen. An intense soret band can be seen around 450 nm. Molar absorptivities of four Q bands (in the 525—675 nm range) have increased due to a high number of DTPA introduced on the porphyrin ring. The UV-Visible spectrum of the H_2TPP—(NH—DTPA—Gd)$_4$ complex is shown in Fig. 1, *b*. This complex is more symmetrical, resulting in its simple spectrum containing only one Q band. In this spectrum the highest molar absorptivity (about 50,000 $M^{-1} \cdot cm^{-1}$) for a single Q band has appeared at 670 nm indicating that this complex could possibly be used as a photosensitizer in photodynamic therapy.

CONCLUSIONS

The use of contrast agents have proved to be very useful in the imaging of various pathologies via the use of the MRI technique. The successful use of porphyrins in PDT has motivated chemists to carry out the advanced research in this field. The synthesis of two porphyrin complexes is reported. The (H2TPP—NH—DTPA—Gd) agent was sufficiently soluble in water. The longitudinal relaxivity measurement indicated 154 % enhancement compared to that of the widely used Gd—DTPA MRI contrast. Due to the large size of the H_2TPP—(NH—DTPA—Gd)$_4$ complex, it showed significantly high relaxivity. The UV-Visible spectrum of the complex indicates that this agent could possibly be used as a photosensitizer in PDT.

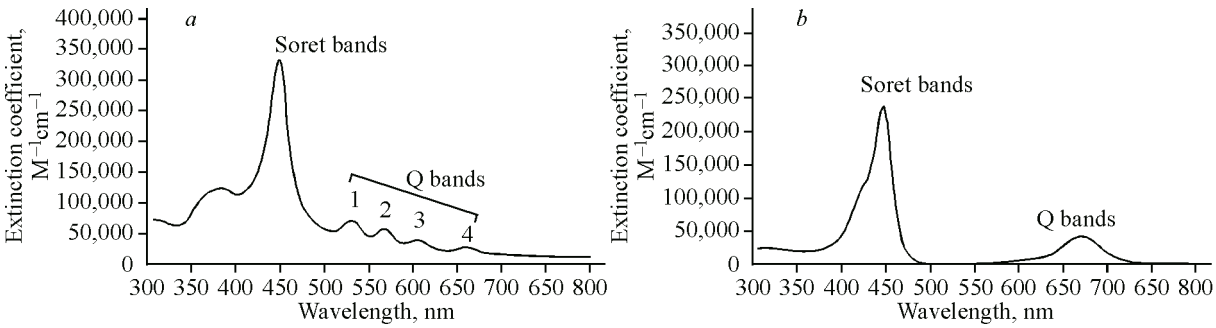


Fig. 1. UV-Visible spectrum of the complex H_2TPP—(NH—DTPA)$_4$ (*a*) and H_2TPP—(NH—DTPA—Gd)$_4$ (*b*)

REFERENCES

1. *Lauffer R.B.* // Chem. Rev. – 1987. – **87**. – P. 901 – 927.
2. *Squire L.F., Novelline R.A.* Squire′s fundamentals of radiology. (6th ed.) – Harvard University Press, 2004. – P. 36.
3. *Rajan S.S.* MRI: a conceptual overview. – New York: Springer-Verlag Inc., 1997. – P. 1 – 5.
4. *Wong W.S., Tsukuda J.S., Kortman K.E., Bradley W.G.* Practical Magnetic Resonance Imaging, A case study approach. – USA: Aspen publishers Inc., 1987. – P. 1.
5. *Ratnakar S.J., Alexander V.* // Eur. J. Inog. Chem. – 2005. – **19**. – P. 3918 – 3927.
6. *Mettler F.A., Muroff L.R., Kulkarni M.V.* Magnetic Resonance Imaging and Spectroscopy. – New York: Churchill Livingstone, 1986. – P. 231.
7. *Gallez B., Swartz H.* // NMR in Biomedicine. – 2004. – **17**. – P. 223 – 225.
8. *Buchler J.W.* ″The Porphyrins″. (Ed.) D. Dolphin. – New York: Academic, Part A, 1978, Vol. 1. – P. 427 – 428.
9. *Ostfeld D., Tsutsui M.* // Acc. Chem. Res. – 1974. – **7**. – P. 52 – 58.
10. *Golubchikov O.A., Berezin B.D.* // Russ. Chem. Rev. – 1986. – **55** (8). – P. 768 – 785.
11. *Kim T.K., Choi B.I., Park S.W., Lee W., Han J.K., Han M.C., Weinmann H.J.* // Amer. J. Roentgenology. – 2000. – **175**. – P. 227 – 234.
12. *Cohen J.S., Chen C.W., Myers C.E., Sohn M.* US Patent Number, 1991. – 4. – P. 256, 986.
13. *Axtell D.D., Tsutsui M.* Synthetic porphyrins and Metalloporphyrins. Department of Chemistry Texas, A & M University, Office of Naval Research, Contract N00014-75-C-0417, Task No. NR 053-559, Technical report No. 15, 1976.
14. *Lukyanets E.A.* // J. Porphyrins and Phthalocyanines. – 1999. – **3**. – P. 424 – 432.
15. *Macdonald I., Dougherty J.* // J. Porphyrins and Phthalocyanines. – 2001. – **5**. – P. 105 – 129.
16. http: // www.thenakedscientists.com/HTML/articles/article/davinacolumn1.htm/
17. http: // www.cancer.org/docroot/ETO/content/ETO_1_3X_Photodynamic_Therapy.asp
18. *Platzek J., Niedballa U.* A1, WO 2002059076 Germany, 2002.
19. *Li M., Selvin P.R.* // Bioconjugate Chem. – 1997. – **8**. – P. 127 – 132.
20. *Hines J.V., Ammar G.M., Buss J., Schmalbrock P.* // Bioconjugate Chem. – 1999. – **10**. – P. 155 – 158.
21. *Saab-Ismail N.H., Simor T., Gaszner B., Lorand T., Szollosy M., Elqavish G.A.* // J. Med. Chem. – 1999. – **42**. – P. 2852 – 2861.
22. *Mikawa M., Kato H., Okumura M., Narazaki M., Kanazawa Y., Miwa N., Shinohara H.* // Bioconjugate Chem. – 2001. – **12**. – P. 510 – 514.