

## Photodynamic and Antioxidant Activities of Divalent Transition Metal Complexes of Methyl Pheophorbide-a<sup>†</sup>

Il Yoon,<sup>\*</sup> Ho Sung Park, Bing Cun Cui,<sup>a</sup> Jia Zhu Li, Jung Hwa Kim, Byambajav Lkhagvadulam, and Young Key Shim<sup>\*</sup>

PDT Research Institute, School of Nano System Engineering, Inje University, Gimhae 621-749, Korea

\*E-mail: yoonil71@inje.ac.kr (I. Y.); ykshim@inje.ac.kr (Y. K. S.)

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A comparative study of the photodynamic and antioxidant activities of methyl pheophorbide-a (MPa, **1**) and its transition metal(II) complexes (**2-5**) is described. Four transition metal complexes (palladium(II): **2**, zinc(II): **3**, cobalt(II): **4** and copper(II): **5**) of MPa were prepared by reaction between the corresponding transition metal and **1**, respectively, and were characterized by <sup>1</sup>H-NMR and UV-vis spectroscopic and mass spectrometric analyses. *In vitro* results show a photodynamic therapy (PDT) efficacy with A549 cells might be attributed to a heavy atom effect of the transition metal complexes of MPa. Among them, **4** and **5** showed higher photodynamic activity than that of **1** at the concentration of 5 μM at 24 h incubation after photoirradiation. The images of morphological change for **2-5** show evidence for the PDT effect with A549 cells. And the all transition metal complexes of MPa showed higher antioxidant activity than that of MPa, in which **4** showed the highest antioxidant activity.

**Key Words :** Antioxidant, *In vitro* study, Methyl pheophorbide-a, Photodynamic therapy, Transition metal(II) complex

### Introduction

Photodynamic therapy (PDT) has much attention in recent years due to noninvasive therapy in many kinds of human diseases through a combination of light, photosensitizer (PS) as a light-absorbing molecule and oxygen.<sup>1-3</sup> Methyl pheophorbide-a<sup>4</sup> (MPa) (**1** in Figure 1) is a widely used starting compound to synthesize new chlorin derivatives in PDT. Recently the chlorin derivatives with various functional groups have been synthesized by us<sup>5</sup> and by many other groups, and have been investigated for *in vitro* study to find out potential PSs.

It is of great interest that introduction of transition metal can afford a photoactivated biological activity, for example, highly effective anticancer drugs.<sup>6</sup> Some transition metal complexes were developed in PDT as good candidates of PS containing metalloporphyrin,<sup>7</sup> metallochlorin,<sup>8</sup> metallobacteriochlorin,<sup>9</sup> and metallophthalocyanine<sup>10</sup> (Pc) derivatives. Addition of a transition metal (heavy atom) generally increases the efficiency of the singlet-triplet intercrossing (the so-called heavy atom effect) due to the enhanced spin-orbital perturbations,<sup>11</sup> which results in an increased efficacy in PDT corresponding to an improvement of the singlet oxygen (<sup>1</sup>O<sub>2</sub>) quantum yield.<sup>8b,9c,12</sup>

Recently, Obata, Hirohara and Yano groups developed<sup>8b</sup> an *in vitro* heavy atom effect of the palladium(II) and platinum(II) complexes of pyrrolidine-fused chlorin in PDT.

And Fukuzumi, Pandey and Kadish groups have shown<sup>9c</sup> that the palladium(II) and zinc(II) bacteriochlorin complexes act as good PS candidates result in formation of <sup>1</sup>O<sub>2</sub> with a high quantum yield and in occurrence of the intermolecular photoinduced electron transfer to make both radical cation and radical anion due to the small HOMO-LUMO gap, respectively. In addition, the zinc(II) Pcs showed good <sup>1</sup>O<sub>2</sub> quantum yield<sup>10b</sup> and phototoxicity.<sup>10a</sup>

In addition, some of transition metal complexes have been shown interesting biological activities as model systems that artificial metallopeptidases, enzyme inhibitors, and free radical scavengers.<sup>13</sup> The free radical species may give oxidative DNA damage<sup>14</sup> leads to a wide variety of conditions, including cardiovascular disease, cancer, neurodegenerative diseases, and aging.<sup>15</sup> Therefore antioxidant supplementation is of interest to prevent or ameliorate those diseases.<sup>16</sup>

So far, several transition metal complexes of MPa, for example, palladium(II),<sup>17</sup> zinc(II),<sup>18</sup> copper(II),<sup>18a,18c,19</sup> nickel (II),<sup>18a,18c,20</sup> iron(II),<sup>21</sup> manganese(III),<sup>22</sup> and tin(IV)<sup>17a</sup> have been synthesized and characterized. Ol'shevskaya group reported<sup>17b</sup> the low dark cytotoxicity of the palladium(II) and tin(IV) complexes of MPa. However, to our knowledge, there is no more report for *in vitro* study of the transition metal complexes of MPa have been published. Furthermore, there is no report of comparative study of the photodynamic and antioxidant activities of MPa and its transition metal complexes.

In this report a comparative study of the photodynamic activity of MPa and its transition metal complexes was described by *in vitro* study with A549<sup>23</sup> (human lung carcinoma) cells. In addition, the antioxidant activity of **1-5** was shown by DPPH<sup>24</sup> (2,2-diphenyl-1-picryl hydrazyl) assay.

<sup>†</sup>This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.

<sup>a</sup>Current address: College of Medicine, Huangshi Institute of Technology, Huangshi, 435003, P. R. China

## Experimental Section

**General.** The UV-vis absorption spectra were recorded on Scinco S-3100 spectrophotometer using  $\text{CHCl}_3$  as a solvent. Thin layer chromatography (TLC) was performed on silica gel 60  $F_{254}$  (E. Merck). Preparative TLC was performed on silica gel 60  $F_{254}$  (Atlantic Scientific Co., Inc.). Melting points (uncorrected) were measured on an Electrothermal IA9000 Series digital melting point apparatus. Routine nuclear magnetic resonance (NMR) spectra were recorded at 25 °C on a Varian liquid chromatography-nuclear magnetic resonance spectrometer, with working frequencies of 500 MHz for  $^1\text{H}$  nucleus. Chemical shifts are quoted in ppm on the  $\delta$  scale and coupling constants ( $J$ ) are expressed in Hertz (Hz). Samples for NMR spectroscopic studies were prepared using solvents purchased from Aldrich. High resolution fast atom bombardment mass (HRFABMS) spectra were obtained with a Jeol JMS700 high resolution mass spectrometer at the Daegu center of KBSI, Kyungpook National University, Korea.

**Materials.** DPPH (2,2-diphenyl-1-picryl hydrazyl) was purchased from Aldrich. Ascorbic acid was purchased from Samchun pure chemical. Compound **1** (MPa) was obtained from an extraction of chlorophyll-a paste (*excrementum bombycis*) in acidic methanol. Chlorophyll-a paste (100 g) was dissolved in 500 mL of 5% sulfuric acid in methanol and stirred at room temperature for 50 h under nitrogen atmosphere in dark. Following the standard workup, MPa was obtained in 5% yield. The analytical data are identical with those reported previously.<sup>4</sup>

**Preparation of Compound 2:** Compound **1** (50 mg, 0.082 mmol) and palladium acetate (92.5 mg, 0.412 mmol) were dissolved in dichloromethane (10 mL). The mixture was stirred for 2 h at room temperature, and then the mixed solution was evaporated. The product was purified by column chromatography on silica gel followed by preparative TLC in 2% acetone/dichloromethane as a greenish black powder **2** (23 mg, yield 40%). mp 183–185 °C; HRFABMS  $\text{C}_{36}\text{H}_{36}\text{N}_4\text{O}_5\text{Pd}^{[\text{M}]^+}$  calcd 710.1721, obsd 710.1719; UV-vis ( $\text{CHCl}_3$ ):  $\lambda$  ( $10^3 \text{ }\epsilon/\text{M}^{-1}\cdot\text{cm}^{-1}$ ) = 422 (156), 493 (14.4), 532 (14.7), 599 (32.9), 644 nm (168);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ , 25 °C, TMS):  $\delta$  9.71 (s, 1H, 10H), 9.49 (s, 1H, 5H), 8.63 (s, 1H, 20H), 7.96 (dd,  $J$  = 18 and 12 Hz, 1H, 3<sup>1</sup>H), 6.21 (d,  $J$  = 19 Hz, 1H, 3<sup>2</sup>H), 6.09 (d,  $J$  = 12 Hz, 1H, 3<sup>2</sup>H), 5.40 (s, 1H, 13<sup>2</sup>H), 4.57 (m, 1H, 17H), 4.21 (m, 1H, 18H), 3.76 (q,  $J$  = 8 Hz, 2H, 8<sup>1</sup>H), 3.71 (s, 3H, O13<sup>3</sup>CH<sub>3</sub>), 3.70 (s, 3H, 12<sup>1</sup>H), 3.63 (s, 3H, O17<sup>4</sup>CH<sub>3</sub>), 3.32 (s, 3H, 2<sup>1</sup>H), 3.27 (s, 3H, 7<sup>1</sup>H), 2.57–2.54 (m, 2H, 17<sup>1</sup>H), 2.28–2.22 (m, 1H, 17<sup>2</sup>H), 2.04 (d,  $J$  = 7 Hz, 3H, 18<sup>1</sup>H), 1.71 (t,  $J$  = 8 Hz, 3H, 8<sup>2</sup>H).

**Preparation of Compound 3–5:** Compound **1** (101 mg, 0.166 mmol) was dissolved in dichloromethane (5 mL) and the corresponding metal acetate (0.166 mmol) was dissolved in methanol (5 mL). The reaction mixture was refluxed for 30 min–1 h, and then the mixed solution was poured into water (200 mL), and extracted with dichloromethane (2 × 50 mL). The organic layer was dried over anhydrous sodium sulfate. After evaporating the product was purified by column

chromatography on silica gel with hexane/ethyl acetate (v/v 2:1) as an eluent to give the corresponding metal complex, respectively. **3** (95 mg, yield 85%): mp 144–145 °C; HRFABMS  $\text{C}_{36}\text{H}_{36}\text{N}_4\text{O}_5\text{Zn}^{[\text{M}]^+}$  calcd 668.1977, obsd 668.1981; UV-vis ( $\text{CHCl}_3$ ):  $\lambda$  ( $10^3 \text{ }\epsilon/\text{M}^{-1}\cdot\text{cm}^{-1}$ ) = 426 (77.1), 521 (3.92), 568 (5.94), 611 (10.8), 661 nm (51.5);  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ , 25 °C, TMS):  $\delta$  9.23 (s, 1H, 10H), 9.21 (s, 1H, 5H), 8.40 (s, 1H, 20H), 7.94 (dd,  $J$  = 18 and 12 Hz, 1H, 3<sup>1</sup>H), 6.16 (d,  $J$  = 19 Hz, 1H, 3<sup>2</sup>H), 6.05 (d,  $J$  = 12 Hz, 1H, 3<sup>2</sup>H), 4.74 (m, 1H, 17H), 4.49 (m, 1H, 18H), 4.17 (s, 1H, 13<sup>2</sup>H), 3.70 (s, 3H, O13<sup>3</sup>CH<sub>3</sub>), 3.52 (q,  $J$  = 8 Hz, 2H, 8<sup>1</sup>H), 3.47 (s, 3H, 12<sup>1</sup>H), 3.39 (s, 3H, O17<sup>4</sup>CH<sub>3</sub>), 3.32 (s, 3H, 2<sup>1</sup>H), 3.07 (s, 3H, 7<sup>1</sup>H), 2.49–2.42 (m, 2H, 17<sup>1</sup>H), 2.31–2.23 (m, 1H, 17<sup>2</sup>H), 1.83 (d,  $J$  = 7 Hz, 3H, 18<sup>1</sup>H), 1.58 (t,  $J$  = 8 Hz, 3H, 8<sup>2</sup>H). **4** (97 mg, yield 88%): mp 142–143 °C; HRFABMS  $\text{C}_{36}\text{H}_{37}\text{N}_4\text{O}_5\text{Co}^{[\text{M} + \text{H}]^+}$  calcd 664.2096, obsd 664.2093; UV-vis ( $\text{CHCl}_3$ ):  $\lambda$  ( $10^3 \text{ }\epsilon/\text{M}^{-1}\cdot\text{cm}^{-1}$ ) = 412 (51.6), 490 (4.53), 540 (4.48), 595 (9.33), 645 nm (23.9). **5** (106 mg, yield 96%): mp 107–108 °C; HRFABMS  $\text{C}_{36}\text{H}_{36}\text{N}_4\text{O}_5\text{Cu}^{[\text{M}]^+}$  calcd 667.1982, obsd 667.1987; UV-vis ( $\text{CHCl}_3$ ):  $\lambda$  ( $10^3 \text{ }\epsilon/\text{M}^{-1}\cdot\text{cm}^{-1}$ ) = 426 (147), 508 (6.23), 550 (7.24), 609 (16.2), 652 nm (71.7).

**Cell Culture and Photirradiation:** A549 cells (human lung carcinoma) were obtained from the cell line bank at Seoul national university's cancer research center (Korea). They were grown in a mixed medium of RPMI-1640 (Sigma-Aldrich) containing 10% fetal bovine serum (FBS), penicillin-streptomycin and sodium pyruvate at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> in air. The PDT was carried out using a diode laser generator apparatus (BioSpec LED, Russia) equipped with a halogen lamp, a band-pass filter (640–710 nm), and a fiber optics bundle. The duration of light irradiation, under PDT treatment, is calculated taking into account the empirically found effective dose of light energy in  $\text{J}\cdot\text{cm}^2$ .

**Morphological Changes Induced by PDT:** A549 cells ( $10 \times 10^4$  cells/well) in 200  $\mu\text{L}$  of the mixed medium were placed in a 48-well plate and incubated for 24 h (37 °C, 5% CO<sub>2</sub>). And the PS (5  $\mu\text{M}$ ) in 200  $\mu\text{L}$  of the mixed medium was added in each well. After 24 h incubation, the mixed solution in each well was discarded. And the cells were washed with phosphate buffered saline (PBS) (Sigma-Aldrich) (200  $\mu\text{L} \times 3$ ), and 200  $\mu\text{L}$  of the mixed medium was added. The cells were irradiated with the LED ( $2 \text{ J}\cdot\text{cm}^{-2}$ ) for 15 min, and incubated for 24 h. The images of morphological changes were obtained by an optical microscopy (Olympus, CK40-32 PH, Japan), and compared with those in the cells with no irradiation.

**WST-1 Assay and Cell Viability:** A549 cells ( $10 \times 10^4$  cells/well) in 100  $\mu\text{L}$  of the mixed medium were placed in a 96-well plate (Falcon®, Becton Dickinson, USA) and incubated for 24 h (37 °C, 5% CO<sub>2</sub>). And the PS (5  $\mu\text{M}$ ) in 100  $\mu\text{L}$  of the mixed medium was added in each well. After 24 h incubation, the mixed solution in each well was discarded. And the cells were washed with PBS (100  $\mu\text{L} \times 3$ ), and 100  $\mu\text{L}$  of the mixed medium was added. The cells were irradiated with the LED ( $2 \text{ J}\cdot\text{cm}^{-2}$ ) for 15 min. And absorbance of the cells was measured after 3 h, 24 h and 48 h incubation using WST-1 reagent (10  $\mu\text{L}$ ) by fluorescence

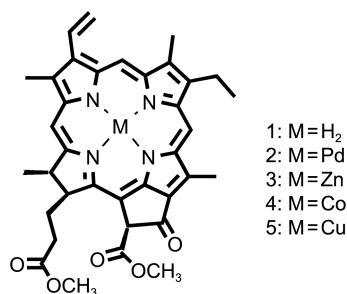
multi-detection reader (BioTek, Synergy HT, USA) at 450 nm, respectively. Cell viability was calculated by normalization with respect to the value for no PS treatment. Standard deviation of the cell viability was calculated from the three replicate experiments.

**DPPH Radical Scavenging Assay:** The antioxidant activity was measured using DPPH free radical absorption at a characteristic wavelength (517 nm), where the intensity of the absorbance decreases upon reduction by an antioxidant. 0.1 mM DPPH solution in methanol was prepared by dilution of 1 mM DPPH solution in methanol. 5 mM ascorbic acid (AA) and each PS solution were prepared by using dimethyl sulfoxide (DMSO). Each PS solution or AA solution in DMSO at 50  $\mu$ M is mixed with 0.1 mM DPPH solution in methanol in 96-well plate. After 1 h incubation at room temperature, the absorbance was measured by fluorescence multi-detection reader. The DPPH scavenging activity (%) was calculated by the following equation (We modified the method in ref. 24b): DPPH scavenging activity (%) =  $[1 - (A_s - A_{cb})/(A_c - A_{cb})] \times 100$ , where  $A_s$  is the absorbance of sample (DPPH with PS or AA) and  $A_{cs}$  is control sample (PS or AA only), the absorbance of the control (DPPH only) is  $A_c$  and control blank (solvent only) is  $A_{cb}$ .

## Results and Discussion

**Synthesis.** Figure 1 shows the structures of MPa (**1**) and its transition metal complexes (palladium(II): **2**, zinc(II): **3**, cobalt(II): **4** and copper(II): **5**) where the transition metal is inserted in the MPa macrocyclic ring system, respectively.

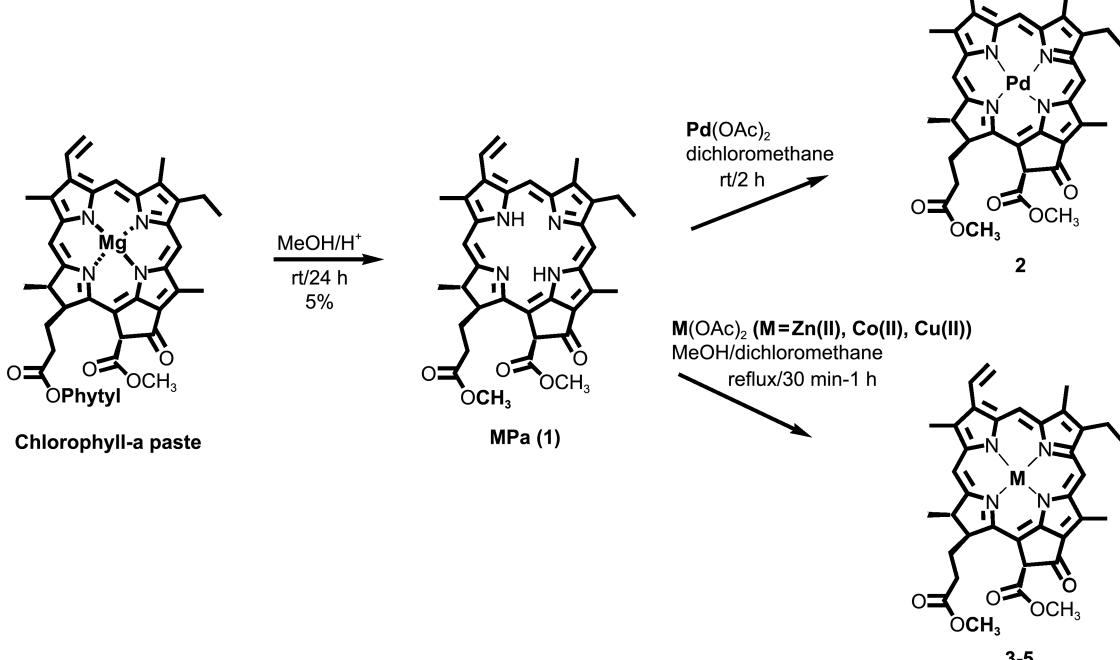
MPa was obtained from extraction of chlorophyll-a paste (*excrementum bombycis*) in an acidic methanol followed by column chromatography in 2% acetone/dichloromethane as



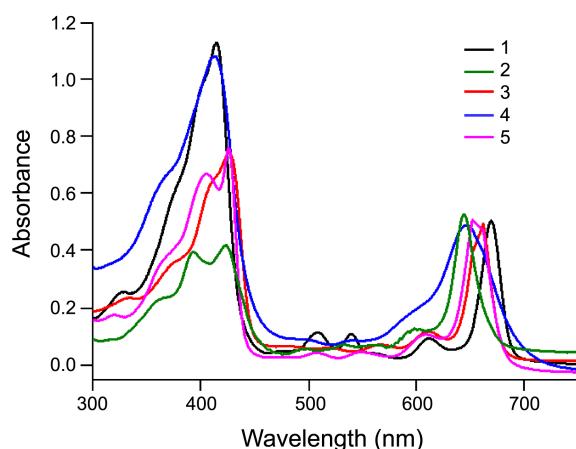
**Figure 1.** Representative structures of MPa (**1**) and its transition metal complexes (**2-5**).

an eluent in 5% yield. Characterization data of MPa were same with the previous report.<sup>4</sup>

In general, some transition metal complexes have been synthesized by using the direct and transmetalation approaches.<sup>25</sup> In this paper, we have used the direct method for preparation of the transition metal complexes of MPa, which has shown in Scheme 1. Palladium(II) complex (**2**) was prepared<sup>26</sup> by the reaction of **1** with palladium acetate in dichloromethane in 40% yield. And the other metal complexes (**3-5**) were prepared by reaction of **1** with the corresponding metal acetate in methanol and dichloromethane in reasonable (85-96%) yield, respectively. Compound **2** was purified by column chromatography on silica gel followed by preparative TLC (2% acetone/dichloromethane as eluent). Compounds **3-5** were purified by column chromatography on silica gel (hexane/ethyl acetate (v/v 2:1) as eluent). The structures of **2-5** were confirmed by <sup>1</sup>H-NMR (Figures S1-S3 in the Supporting Information) and UV-vis spectroscopies, and high resolution fast atom bombardment mass (HRFABMS) spectrometry (Figures S4-S7 in the Supporting Information), re-



**Scheme 1.** Preparation of the transition metal complexes (**2-5**) of MPa.



**Figure 2.** UV-vis absorption spectra of **1** and its transition metal complexes **2-5** in  $\text{CHCl}_3$  at  $25^\circ\text{C}$ .

**Table 1.** Spectral data of MPa (**1**) and its transition metal complexes (**2-5**) in  $\text{CHCl}_3$  at  $25^\circ\text{C}$ .

	$\lambda_{\text{max}}/\text{nm} (10^{-3} \epsilon/\text{M}^{-1}\cdot\text{cm}^{-1})$				
Soret band	Q bands				
<b>1</b>	413 (53.6)	508 (5.24)	539 (4.98)	611 (4.22)	669 (23.8)
<b>2</b>	422 (156)	493 (14.4)	532 (14.7)	599 (32.9)	644 (168)
<b>3</b>	426 (77.1)	521 (3.92)	568 (5.94)	611 (10.8)	661 (51.5)
<b>4</b>	412 (51.6)	490 (4.53)	540 (4.48)	595 (9.33)	645 (23.9)
<b>5</b>	426 (147)	508 (6.23)	550 (7.24)	609 (16.2)	652 (71.7)

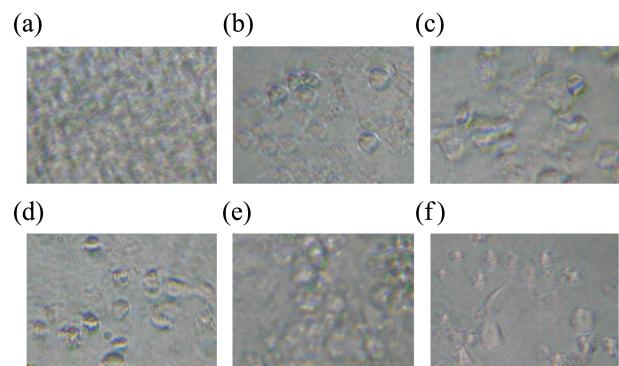
spectively.

**UV-vis Spectroscopic Investigation.** Figure 2 shows UV-vis absorption spectra of compounds **1-5** in chloroform at  $25^\circ\text{C}$  and Table 1 summarizes the spectral data. Addition of the transition metal into MPa, compounds **2-5**, allowed hypsochromic shifts of the Q bands and bathochromic shifts of the Soret band.<sup>27</sup>

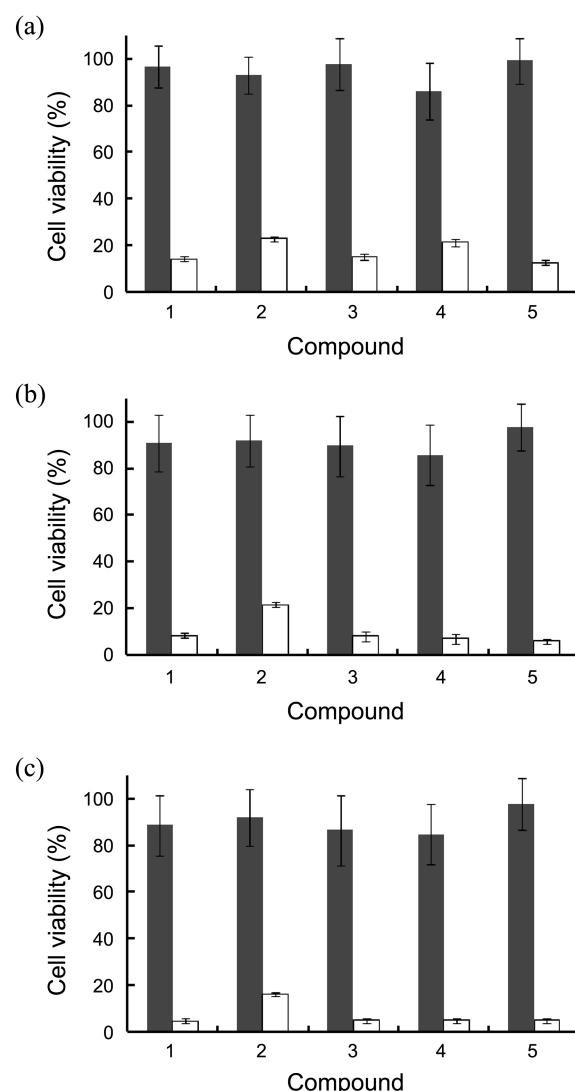
**Photodynamic Activity and Morphological Change Investigations.** Photocytotoxicity and cytotoxicity in the dark of MPa (**1**) and its transition metal complexes (**2-5**) were investigated in A549 cells using a halogen lamp equipped with band-pass filter (640-710 nm) as a light source. A549 cells ( $10 \times 10^4$  cells/well) were incubated with PSs for 24 h and photoirradiated for photocytotoxicity test. At 3 h, 24 h and 48 h incubation after photoirradiation, the cell viability (%) was estimated based on the mitochondrial activity of NADH dehydrogenase using WST-1<sup>28</sup> (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assay.

Figure 3 shows images of morphological change of A549 cells from an optical microscope for **1-5** at the concentration of  $5 \mu\text{M}$  at 24 h incubation after photoirradiation. A549 cells without PS treatment as a control (Figure 3a) showed no morphological changes. But A549 cells with PSs (Figures 3b-f) showed cell shrinkage, indicating cell death processes commenced, resulted in loss of cell viability (Figure 4). This result suggests that **1-5** induced apoptosis.<sup>29</sup>

Figure 4 shows cytotoxicity in the dark and photocytotoxicity-



**Figure 3.** Images of morphological change in A549 cells without PS treatment as a control (a) and treatment with **1** (b), **2** (c), **3** (d), **4** (e) and **5** (f) at 24 h incubation after photoirradiation. The concentration of each PS was  $5 \mu\text{M}$ .



**Figure 4.** Cytotoxicity in the dark (left bar) and photocytotoxicity (right bar) of MPa (**1**) and its transition metal complexes (**2-5**) in A549 cells. The concentration of PS was  $5 \mu\text{M}$ . The percentage of cell viability was determined by WST-1 assay at 3 h (a), 24 h (b) and 48 h (c) incubation after photoirradiation. Error bars represent the standard deviation of three replicate experiments.

city of **1** and its transition metal complexes **2–5** at the concentration of 5  $\mu\text{M}$  at different incubation time (3 h, 24 h and 48 h) after PDT (Table S1 in the Supporting Information).

In all the compounds, upon photoirradiation, the cell viability was decreased corresponding to the increased incubation time after PDT, for example, 13% at 3 h, 6% at 24 h and 5% at 48 h incubation for compound **5** (Table S1 in the Supporting Information). At 3 h and 24 h incubations, in the cobalt(II) (**4**) and copper(II) (**5**) complexes the cell viability for photocytotoxicity was decreased than that of MPa (**1**).

And in the zinc(II) (**3**) complex the cell viability for photocytotoxicity was almost the same with that in **1**. Otherwise, in the palladium(II) (**2**) complex the cell viability for photocytotoxicity was increased than that of **1** at those incubation times. The increased photocytotoxicity in **4** and **5** might be attributed to the heavy atom effect of the transition metal, respectively. And at 48 h incubation after PDT, the cell viability for photocytotoxicity was almost the same in **1** and **3–5**.

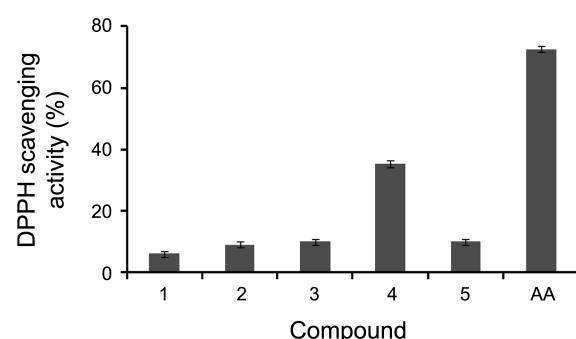
All compounds **1–5** showed low dark cytotoxicity, and the cytotoxicity also increased corresponding to the increased incubation time after PDT. In the zinc(II) (**3**) and cobalt(II) (**4**) complexes the cytotoxicity was higher than that in **1**. But in the copper(II) (**5**) complex the cytotoxicity was lower than that in **1**. And in the palladium(II) (**2**) complex the cytotoxicity was almost the same with that in **1**.

Zinc(II) complexes of Pcs showed comparable PDT activities with that of **3** against the employed cells.<sup>10</sup> Photocytotoxicity of **2** was higher than those of the metalloporphyrins, but lower than those of the pyrrolidine-fused metallochlorins<sup>8b</sup> (palladium(II) and platinum(II) complexes) which showed appreciable photocytotoxicity even at the low concentration (0.5  $\mu\text{M}$ ) in HeLa cells. Copper(II) complex of the porphyrin derivative<sup>7c</sup> exhibits inferior PDT effect corresponding to its lower singlet oxygen yield. But in this work, copper(II) complex of the chlorin, **5** showed good PDT effect. In addition, a theoretical study of the transition metal bacteriochlorin complexes<sup>9a</sup> (cobalt(II), copper(II) and zinc(II)) has been carried out by density functional theory (DFT), where the cobalt(II) and copper(II) complexes showed stronger interaction with the bacteriochlorin than that of the zinc(II) complex.

**Antioxidant Activity Investigation.** For antioxidant activity test we have used DPPH free radical scavenging assay which is one of the short methods for investigation of the hydrogen donating potency.<sup>24</sup> DPPH is a purple-colored stable free radical that becomes reduced to the yellow-colored diphenyl picryl hydrazine.

Ascorbic acid (AA) has been used as control standard. AA is a common reference of antioxidant activity test due to its high antioxidant activity.<sup>30</sup> It was expected that AA showed excellent antioxidant activity (72.6%) in DPPH radical scavenging assay.

Each PS solution or AA solution in DMSO at 50  $\mu\text{M}$  is mixed with 0.1 mM DPPH solution in methanol. After 1 h incubation at room temperature, the reduction of the DPPH free radical is measured by reading the absorbance at 517 nm. The DPPH results are shown at Figure 5 and compared



**Figure 5.** DPPH scavenging activity(%) of **1–5** and ascorbic acid (AA) as control standard.

with the control standard AA (Table S2 in the Supporting Information). All the transition metal complexes **2–5** (9.0–35.3%) showed better DPPH scavenging activity than that of **1** (6.1%), free base MPa. Among them, cobalt(II) complex of MPa, **4** showed the highest antioxidant activity.

Copper(II) complex has many interest<sup>13a,13d</sup> and its antioxidant activity contains experimental and theoretical results, in which the antioxidant activity is related to stable coordination ability of copper(II). Petrović and Hadjipavlou-Litina have shown<sup>13e</sup> palladium(II) complexes afforded high antioxidant activity is related to Lewis acidic site of palladium(II) ion, as an electrophile, reacts as free radical scavengers<sup>31</sup> by trapping radical intermediate, which is supported by DFT calculation results. Asayama and Kawakami have reported<sup>13b</sup> manganese (Mn) porphyrin derivatives conjugated with a peptide showed good antioxidant activity.

For the highest antioxidant activity of the cobalt(II) complex of MPa, **4**, it is related to its significant biological properties. For example, cobalt was shown to have a strong antioxidant effect on iron-induced lipid peroxidation and subsequent biochemical changes in tissues that comparable with vitamin E.<sup>32</sup> Also it was shown, as it is expected, that the cobalt(II) complex of quercetin is much more effective antioxidant than the free quercetin.<sup>31d</sup> Consequently, it is noted that the biological activity of transition metal complexes of free base is higher than that of the free base because of its ability to act as free radical acceptor.<sup>31</sup> This results of stronger photocytotoxicity of the higher antioxidant metal complexes may support Type III mechanism<sup>33</sup> (modified Type I) which suggests combined activities of radical scavengers (acceptors) and PDT through the interactions between the long-lived triplet PS and the native free radicals in the tumor cells.

## Conclusions

The transition metal complexes (palladium(II): **2**, zinc(II): **3**, cobalt(II): **4** and copper(II): **5**) of MPa (**1**) were prepared by reaction between MPa and the corresponding transition metal, respectively, and were characterized by <sup>1</sup>H-NMR and UV-vis spectroscopies and HRFABMS spectrometry. UV-vis absorption spectra for **2–5** showed hypsochromic shifts of the Q bands and bathochromic shifts of the Soret band

relative to those for **1**. *In vitro* results showed increased PDT effect for the cobalt(II) (**4**) and copper(II) (**5**) complexes resulted in decreased cell viability than that for **1**, which might be attributed to the heavy atom effect. The copper(II) (**5**) complex showed the lowest dark cytotoxicity. The images of morphological change of A549 cells for **1-5** confirmed the loss of cell viability. All the transition metal complexes **2-5** showed higher antioxidant activity than that of free base MPa (**1**) due to their ability to act as free radical acceptor. The cobalt(II) (**4**) complex showed the highest antioxidant activity. These results could be useful for understanding of the transition metal complexes of chlorin and its heavy atom effect both in PDT and in antioxidant activity as well as for developing design of new transition metal complexes as good candidates of PS and antioxidants for reactive oxygen species (ROS).

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## References

- (a) Pandey, R. K. *J. Porphyrins Phthalocyanines* **2000**, *4*, 368-373. (b) Bonnet, R. In *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science Publishers: Netherlands, 2000. (c) MacDonald, I. J.; Dougherty, T. J. *Porphyrins Phthalocyanines* **2001**, *5*, 105-129. (d) Detty, M. R.; Gibson, S. L.; Wagner, S. J. *J. Med. Chem.* **2004**, *47*, 3897-3915.
- (a) Pandey, R. K.; Zheng, G. In *Porphyrins as Photosensitizers in Photodynamic Therapy*, In *The Porphyrin Handbook*; Kadish, Smith, Guillard, Eds.; Academic Press: New York, 2000; Vol. 6, pp 157-230. (b) Dolmans, D. E. J. G. J.; Fukumura, D.; Jain, R. K. *Nat. Rev. Cancer* **2003**, *3*, 380-387. (c) Luguya, R.; Jensen, T. J.; Smith, K. M.; Vicente, M. G. H. *Bioorg. Med. Chem.* **2006**, *14*, 5890-5897.
- (a) Derycke, A. S. L.; de Witte, P. A. M. *Adv. Drug Delivery Rev.* **2004**, *56*, 17-30 and references therein. (b) Ikeda, A.; Nagano, M.; Akiyama, M.; Matsumoto, M.; Ito, S.; Mukai, M.; Hashizume, M.; Kikuchi, J.-I.; Katagiri, K.; Ogawa, T.; Takeya, T. *Chem. Asian J.* **2009**, *4*, 199-205.
- Smith, K. M.; Goff, D. A.; Simpson, D. J. *J. Am. Chem. Soc.* **1985**, *107*, 4946-4954.
- See the recent papers: (a) Bayarmaa, B.; Bayarmaa, B.; Lee, W.-K.; Shim, Y. K. *Bull. Korean Chem. Soc.* **2008**, *29*, 237-240. (b) Sengee, G.-I.; Badraa, N.; Shim, Y. K. *Int. J. Mol. Sci.* **2008**, *286*, 131-145. (c) Sengee, G.-I.; Badraa, N.; Lee, W.-K.; Shim, Y. K. *Bull. Korean Chem. Soc.* **2008**, *29*, 2505-2508. (d) Galindev, O.; Badraa, N.; Dalantai, M.; Sengee, G.-I.; Dorjnamjin, D.; Shim, Y. K. *Photochem. Photobiol. Sci.* **2008**, *7*, 1273-1281. (e) Sengee, G.-I.; Badraa, N.; Shim, Y. K. *J. Porphyrins Phthalocyanines* **2009**, *13*, 819-822. (f) Galindev, O.; Dalantai, M.; Ahn, W. S.; Shim, Y. K. *J. Porphyrins Phthalocyanines* **2009**, *13*, 823-831. (g) Bayarmaa, B.; Bayarmaa, B.; Shim, Y. K. *J. Porphyrins Phthalocyanines* **2009**, *13*, 832-841. (h) Cui, B. C.; Cha, M. U.; Li, J. Z.; Park, H. S.; Yoon, I.; Shim, Y. K. *Bull. Korean Chem. Soc.* **2010**, *31*, 3313-3317. (i) Yoon, I.; Park, H. S.; Cui, B. C.; Kim, J. H.; Shim, Y. K. *Bull. Korean Chem. Soc.* **2011**, *32*, 169-174.
- (a) Farrer, N. J.; Sadler, P. J. *Aust. J. Chem.* **2008**, *61*, 669-674. (b) Schatzschneider, U. *Eur. J. Inorg. Chem.* **2010**, 1451-1467.
- (a) Strauss, S. H.; Silver, M. E.; Long, K. M.; Thompson, R. G.; Hudgens, R. A.; Spartalian, K.; Ibers, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 4207-4215. (b) Harvey, J. D.; Ziegler, C. J. *Coord. Chem. Rev.* **2003**, *247*, 1-19 and references therein. (c) Huang, Q.; Pan, Z.; Wang, P.; Chen, Z.; Zhang, X.; Xu, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3030-3033.
- (a) Singh, A.; Huang, W.-Y.; Wgbujor, R.; Johnson, L. W. *J. Phys. Chem. A* **2001**, *105*, 5778-5784. (b) Obata, M.; Hirohara, S.; Tanaka, R.; Kinoshita, I.; Ohkubo, K.; Fukuzumi, S.; Tanihara, M.; Yano, S. *J. Med. Chem.* **2009**, *52*, 2747-2753.
- (a) Petit, L.; Adamo, C.; Russo, N. *J. Phys. Chem. B* **2005**, *109*, 12214-12221. (b) Chen, Y.; Potter, W. R.; Missert, J. R.; Morgan, J.; Pandey, R. K. *Bioconjugate Chem.* **2007**, *18*, 1460-1473. (c) Fukuzumi, S.; Ohkubo, K.; Zheng, X.; Chen, Y.; Pandey, R. K.; Zhan, R.; Kadish, K. M. *J. Phys. Chem. B* **2008**, *112*, 2738-2746.
- (a) Dreshsler, U.; Pfaff, M.; Hanack, M. *Eur. J. Org. Chem.* **1999**, 3441-3453. (b) Das, B.; Tokunaga, E.; Tanaka, M.; Sasaki, T.; Shibata, N. *Eur. J. Org. Chem.* **2010**, 2878-2884.
- Lanzo, I.; Russo, N.; Sicilia, E. *J. Phys. Chem. B* **2008**, *112*, 4123-4130.
- (a) Azenha, E. G.; Serra, A. C.; Pineiro, M.; Pereira, M. M.; de Melo, J. S.; Arnaut, L. G.; Formosinho, S. J.; Rocha Gonsalves, A. M. d. *Chem. Phys.* **2002**, *280*, 177-190. (b) Scalise, I.; Durantini, E. N. *J. Photochem. Photobiol. A* **2004**, *162*, 105-113.
- (a) Ji, H.-F.; Zhang, H.-Y. *Chem. Res. Toxicol.* **2004**, *17*, 471-475. (b) Asayama, S.; Kawamura, E.; Nagaoka, S.; Kawakami, H. *Mol. Pharm.* **2006**, *3*, 468-470. (c) Liu, J.; Lagger, G.; Tacchini, P.; Girault, H. H. *J. Electroanal. Chem.* **2008**, *619-620*, 131-136. (d) Alagona, G.; Ghio, C. *J. Phys. Chem. A* **2009**, *113*, 15206-15216. (e) Petrović, Z. D.; Hadjipavlou-Litina, D.; Pontiki, E.; Simijonović, D. *Bioorg. Chem.* **2009**, *37*, 162-166. (f) Bhuyan, B. J.; Mugesh, G. *Org. Biomol. Chem.* **2011**, *9*, 1356-1365.
- (a) Milne, L.; Nicotera, P.; Orrenius, S.; Burkitt, M. J. *Arch. Biochem. Biophys.* **1991**, *304*, 102-109. (b) Hermes-Lima, M.; Goncalves, M. S.; Andrade, R. G., Jr. *Mol. Cell. Biochem.* **2001**, *228*, 73-82. (c) Warner, D. S.; Sheng, H.; Batinic-Haberle, I. *J. Exp. Biol.* **2004**, *207*, 3221-3231. (d) Battin, E. E.; Perron, N. R.; Brumaghim, J. L. *Inorg. Chem.* **2006**, *45*, 499-501.
- (a) Singal, P. K.; Khaper, N.; Palace, V.; Kumar, D. *Cardiovasc. Res.* **1998**, *40*, 426-432. (b) Halliwell, B. *Drugs Aging* **2001**, *18*, 685-716. (c) Valko, M.; Izakovic, M.; Mazur, M.; Rhodes, C. J.; Telser, J. *Mol. Cell. Biochem.* **2004**, *266*, 37-56. (d) Karanjawala, Z. E.; Lieber, M. R. *Mech. Aging Dev.* **2004**, *125*, 405-415.
- (a) Malvy, D. J. M.; Favier, A.; Faure, H.; Preziosi, P.; Galan, P.; Arnaud, J.; Roussel, A.-M.; Briancon, S.; Hercberg, S. *Cancer Detect. Prev.* **2001**, *25*, 479-485. (b) El-Bayoumy, K. *Mutat. Res.* **2001**, *475*, 123-139. (c) Clarke, R.; Armitage, J. *Cardiovasc. Drugs Ther.* **2003**, *16*, 411-415. (d) Soriano-Garcia, M. *Curr. Med. Chem.* **2004**, *11*, 1657-1659.
- (a) Ol'shevskaya, V. A.; Nikitina, R. G.; Savchenko, A. N.; Malshakova, M. V.; Vinogradov, A. M.; Golovina, G. V.; Belykh, D. V.; Kutchin, A. V.; Kaplan, M. A.; Kalinin, V. N.; Kuzmin, V. A.; Shtil, A. A.; *Bioor. Med. Chem.* **2009**, *17*, 1297-1306. (b) Ol'shevskaya, V. A.; Savchenko, A. N.; Zaitsev, A. V.; Kononova, E. G.; Petrovskii, P. V.; Ramonova, A. A.; Tatarskiy, V. V., Jr.; Uvarov, O. V.; Moisenovich, M. M.; Kalinin, V. N.; Shtil, A. A. *J. Organometal. Chem.* **2009**, *694*, 1632-1637.
- (a) Boucher, L. J.; Katz, J. *J. Am. Chem. Soc.* **1967**, *89*, 4703-4708. (b) Pandey, R. K.; Shiao, F. Y.; Smith, N. W.; Dougherty, T. J.; Smith, K. M. *Tetrahedron* **1992**, *48*, 7591-7600. (c) Helfrich, M.; Rüdiger, W. Z. *Naturforsch. C* **1992**, *47*, 231-238.
- (a) Pesch, R.; Budzikiewicz, H. *Heterocycles* **1976**, *5*, 749-770. (b) Nonomura, Y.; Yoshioka, N.; Inoue, H. *Inorg. Chim. Acta* **1994**, *224*, 181-184. (c) Berezin, M. B. *Russ. J. Coord. Chem.* **1998**, *24*, 40-42.
- Belykh, D. V.; Tarabukina, I. S.; Matveev, Y. S.; Kuchin, A. V. *Russ. J. Gen. Chem.* **2007**, *77*, 1300-1307.
- Strell, M.; Zuther, F. *Liebigs Ann. Chem.* **1958**, *612*, 264-271.
- (a) Loach, P. A.; Calvin, M. *Nature* **1964**, *202*, 343-345. (b) Boucher, L. J.; Garber, H. K. *Inorg. Chem.* **1970**, *9*, 2644-2649.
- Nishizaki, M.; Meyn, R. E.; Levy, L. B.; Atkinson, E. N.; White,

- R. A.; Roth, J. A.; Ji, L. *Clinical Cancer Research* **2001**, *7*, 2887-2897.
24. (a) Ruiz-Roca, B.; Navarro, M. P.; Seiquer, I. *J. Agric. Food Chem.* **2008**, *56*, 9056-9063. (b) Kwon, Y.; Kim, H.; Park, S.; Jung, S. *Bull. Korean Chem. Soc.* **2010**, *31*, 3035-3037. (c) Kim, H. J.; Noh, J. S.; Kwon, M. J.; Song, S.; Suh, H.; Kim, M. J.; Song, Y. O. *Bull. Korean Chem. Soc.* **2010**, *31*, 3327-3332.
25. (a) Hartwich, G.; Fiedor, L.; Simonin, I.; Cmiel, E.; Schafer, W.; Noy, D.; Scherz, A.; Scheer, H. *J. Am. Chem. Soc.* **1998**, *120*, 3675-3683. (b) Kozyrev, A. N.; Chen, Y.; Goswami, L. N.; Tabaczynski, W. A.; Pandey, R. K. *J. Org. Chem.* **2006**, *71*, 1949-1960.
26. Also we prepared the palladium(II) complex of MPa by same method in ref. 17a, and we confirmed that in both methods by us the  $\lambda_{\text{max}}$  (644 nm in Table 1) of the complex was different with that (625 nm) in ref. 17a.
27. See the following examples: The palladium(II) complexes showed hypsochromic shift in refs. 8b and 25b. Otherwise, the zinc(II) complex exhibited bathochromic shift in ref. 25b.
28. WST-1 assay is more useful for rapid analysis of cell viability than MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay. In MTT assay water-insoluble crystallized purple formazan is formed via the cleavage of the tetrazolium ring by the succinate dehydrogenase within the mitochondria following with addition of solubilizing buffer. Otherwise, WST-1 is one-step test and has no solubilisation step due to formation of water-soluble yellow formazan. See the following papers: (a) Kau, J. H.; Lin, C. G; Huang, H. H.; Hsu, H. L.; Chen, K. C.; Wu, Y. P.; Lin, H. C. *Curr. Microbiol.* **2002**, *44*, 106-111. (b) Moravek, M.; Dietrich, R.; Buerk, C.; Broussolle, V.; Guinebretière, M.-H.; Granum, P. E.; Nguyen-the, C.; Märtblauer, E. *FEMS Microbiol. Lett.* **2006**, *257*, 293-298. (c) Ngamwongsatit, P.; Banada, P. P.; Panbangred, W.; Bhunia, A. K. *J. Microbiol. Methods* **2008**, *73*, 211-215.
29. (a) Engeland, M. V.; Nieland, L. J.; Ramaekers, F. C.; Schutte, B.; Reutelingsperger, C. P. *Cytometry* **1998**, *31*, 1-9. (b) Wan, Q.; Liu, L.; Xing, D.; Chen, Q. *Photochem. Photobiol.* **2008**, *84*, 250-257.
30. (a) Lu, X.; Nan, M.; Zhang, H.; Liu, X.; Yuan, H.; Yang, J. *J. Phys. Chem. C* **2007**, *111*, 14998-15002. (b) Oh, C.; Li, M.; Kim, E.-H.; Park, J. S.; Lee, J.-C.; Ham, S. W. *Bull. Korean Chem. Soc.* **2010**, *31*, 3513-3514.
31. (a) Bors, W.; Heller, W.; Michel, C.; Saran, M. *Methods Enzymol.* **1990**, *186*, 343-355. (b) Afanas'ev, I. B.; Ostrakhovitch, E. A.; Mikhal'chik, E. V.; Ibragimova, G. A.; Korkina, L. G. *Biochem. Pharmacol.* **2001**, *61*, 677-684. (c) Mordini, M. Y.; Pourahmad, J.; Bui, H.; Siraki, A.; O'Brien, P. J. *Free Radic. Biol. Med.* **2003**, *34*, 243-253. (d) Bukhari, S. B.; Memon, S.; Tahir, M. M.; Bhanger, M. I. *J. Mol. Structure* **2008**, *892*, 39-46.
32. İnan, Ç.; Kilinç, K.; Kotiloglu, E.; Akman, H. O.; Kılıç, I.; Michl, J. *J. Lab. Clin. Med.* **1998**, *132*, 157-165.
33. (a) Gál, D. *Biochem. Biophys. Res. Commun.* **1992**, *186*, 1032-1036. (b) Gál, D. *Biochem. Biophys. Res. Commun.* **1994**, *202*, 10-16. (c) Kriska, T.; Maltseva, E.; Gál, D. *Biochem. Biophys. Res. Commun.* **1996**, *223*, 136-140. (d) Gál, D.; Kriska, T.; Maltseva, E. *Biochem. Biophys. Res. Commun.* **1997**, *233*, 173-176. (e) Németh, A.; Jakus, J.; Kriska, T.; Keszler, Á.; Vanyur, R.; Gál, D. *Biochem. Biophys. Res. Commun.* **1999**, *255*, 360-366. (f) Shutova, T.; Kriska, T.; Németh, A.; Agabekov, V.; Gál, D. *Biochem. Biophys. Res. Commun.* **2000**, *270*, 125-130. (g) Gál, D.; Shutova, T.; Kriska, T.; Németh, A. *J. Biochem. Biophys. Methods* **2003**, *55*, 11-21.