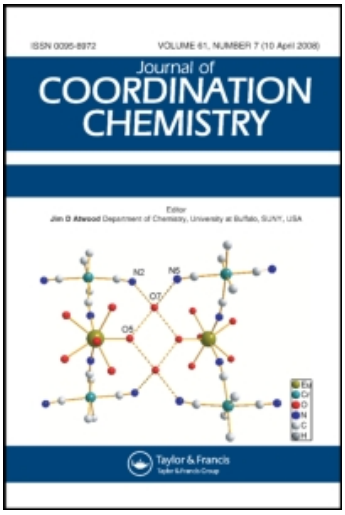


**Journal of Coordination Chemistry**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

# Radical scavenging and *in vitro* antifungal activities of Cu(II) and Co(II) complexes of the t-butylphenyl derivative of porphyrazine

Bahadir Keskin[a]; Aysegul Peksel[a]; Ulvi Avciata[a]; Ahmet Gül[b]
[a] Department of Chemistry, Yildiz Technical University, TR34210 Esenler, Istanbul, Turkey [b] Department of Chemistry, Istanbul Technical University, TR34469 Maslak, Istanbul, Turkey

First published on: 13 October 2010

**To cite this Article** Keskin, Bahadir , Peksel, Aysegul , Avciata, Ulvi and Gül, Ahmet(2010) 'Radical scavenging and *in vitro* antifungal activities of Cu(II) and Co(II) complexes of the t-butylphenyl derivative of porphyrazine', Journal of Coordination Chemistry, 63: 22, 3999 — 4006, First published on: 13 October 2010 (iFirst)
**To link to this Article: DOI:** 10.1080/00958972.2010.524930
**URL:** http://dx.doi.org/10.1080/00958972.2010.524930

*Journal of Coordination Chemistry*
Vol. 63, No. 22, 20 November 2010, 3999–4006

Taylor & Francis
Taylor & Francis Group

# Radical scavenging and *in vitro* antifungal activities of Cu(II) and Co(II) complexes of the t-butylphenyl derivative of porphyrazine

BAHADIR KESKIN*†, AYSEGUL PEKSEL†, ULVI AVCIATA† and AHMET GÜL‡

†Department of Chemistry, Yildiz Technical University, TR34210 Esenler, Istanbul, Turkey
‡Department of Chemistry, Istanbul Technical University, TR34469 Maslak, Istanbul, Turkey



(*Received 4 April 2010; in final form 30 August 2010*)

Co(II) and Cu(II) complexes and metal-free t-butylphenyl peripherally substituted porphyrazine (Pz) have been screened for *in vitro* antifungal (*Aspergillus niger*) and antioxidant (free radical scavenging, superoxide radical scavenging, and reducing power) activities. The results were compared with synthetic antioxidants, e.g., butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), trolox, or $\alpha$-tocopherol. The free radical scavenging activity of $H_2Pz$ was higher than the CuPz complex. However, CuPz complex showed higher superoxide radical scavenging activity than BHA, BHT, and trolox while $H_2Pz$ and CoPz showed weaker activity than BHA, BHT, and trolox. The reducing power of all complexes was similar to that of BHT and $\alpha$-tocopherol on a per molar basis. The ligand and complexes have antifungal activity against *A. niger*. The compounds have significant superoxide radical scavenging activity against various antioxidant systems *in vitro*.

*Keywords*: Porphyrazine; Antioxidant; Radical scavenging ability; Reducing power; 4-Tert-butylphenyl

## 1. Introduction

Reactive oxygen species (ROS) are chemical entities that include oxygen free radicals, such as superoxide anion radicals ($O_2^-$), hydroxyl radicals ($OH^{\cdot}$), nitric oxide (NO), peroxynitrite, and also non-radical species, such as hydrogen peroxide and singlet oxygen ($^1O_2$). Free radicals can be generated from metabolic pathways within body tissues and can also be introduced from external sources such as drugs, food, UV radiation, and environmental pollution [1]. *In vivo*, such species are securely coupled at their sites of generation or are detoxified by endogenous antioxidative defenses so as to preserve optimal cellular function. In pathological conditions, however, the detoxifying mechanisms are often inadequate as excessive quantities of ROS can be generated.

*Corresponding author. Email: bahadirkeskin@gmail.com

*Journal of Coordination Chemistry*
ISSN 0095-8972 print/ISSN 1029-0389 online © 2010 Taylor & Francis
DOI: 10.1080/00958972.2010.524930

This resulting pro-oxidant shift, a process known as oxidative stress, can result in degradation of cellular components such as DNA, carbohydrates, polyunsaturated lipids, and proteins, or precipitate enzyme inactivation, irreversible cellular dysfunction, and ultimately cell death if the pro-oxidant-antioxidant balance is not restored [2]. ROS not only play an important role in numerous diseases, but also contribute to deterioration of foodstuffs, cosmetics, and pharmaceutical preparations. Thus, antioxidants are important inhibitors against oxidative damage [3–5]. Synthetic and natural compounds capable of reacting catalytically or stoichiometrically with one or more reactive species have been developed and proven effective in several *in vivo* models of oxidative stress.

The widespread use of antifungal agents has resulted in resistance to these drugs by pathogenic microorganisms [6]. Therefore, treatment of fungal infections is an area of increasing concern, because of the limited number of antifungal agents available for use and the emergence of clinically resistant pathogens.

Several classes of synthetic metal complexes are under investigation as catalytic antioxidants, including manganese complexes of porphyrins, salen, and cyclic polyamines [7, 8]. Metalloporphyrins have a broad range of antioxidant activities that include dismutation of superoxide and hydrogen peroxide and scavenging of peroxynitrite [9]. Symmetrical octakis substitution of planar tetrapyrrole derivatives (e.g., phthalocyanines, porphyrins, or porphyrazines) provide materials capable of interacting with metal ions, directly inside the inner core and through peripheral donor sites [10–14]. We reported earlier metal-free and metallo porphyrazines with symmetrical octakis(4-tert-butylbenzylthio), as shown in figure 1 [15]. Cobalt and copper complexes are biocompatible and offer new ways to induce, modify, and control molecular properties and high metal/ligand stability [16]. The diffuse $\pi$ electrons of porphyrazines may help understand long-range interactions. The presence of soft sulfur donors plays an important role in bringing physical and chemical properties of the porphyrazines comparable to those of phthalocyanines [17].



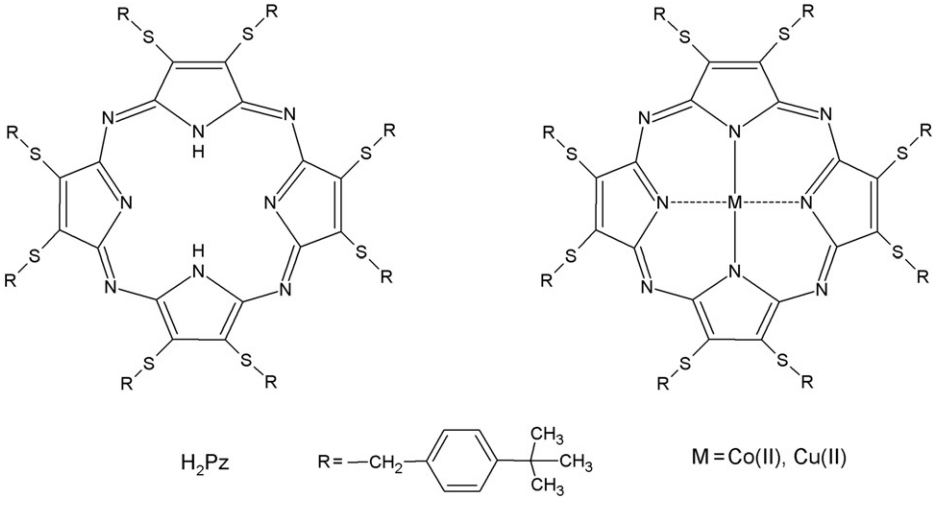


Figure 1. Octakis(4-tert-butylbenzylthio) porphyrazines (M = Cu; Co).

The aim of this study is to investigate antifungal and antioxidant effects of CoPz, CuPz, and $H_2Pz$ and to elucidate their antioxidative action. Antifungal activity was determined against *Aspergillus niger* and compared with Fluconazole as standard antifungal drug. The complexes have been screened for free radical scavenging, superoxide radical scavenging, and reducing power. The results are compared with synthetic antioxidants, e.g., BHA, BHT, trolox, or $\alpha$-tocopherol.

## 2. Experimental

### 2.1. *Materials and methods*

IR spectra were recorded on a Mattson 1000 FT-IR spectrophotometer using KBr pellets and electronic spectra on an Agile 8453 UV-Visible spectrophotometer. Nicotinamide adenine dinucleotide (NADH), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\bullet}$), and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were provided from Fluka (Buchs). Unless specified otherwise, all other reagents and solvents used were of analytical grade obtained from commercial suppliers. The solvents were stored over molecular sieves.

The octakis(4-tert-butylbenzylthio)$^{21}$H,$^{23}$H porphyrazine, [octakis(4-tert-butylbenzylthio) porphyrazinato]Cu(II), and [octakis(4-tert-butylbenzylthio) porphyrazinato] Co(II) were prepared according to the previously reported procedures and characterized by comparing their spectral data to those reported earlier [15]. The Co(II) and Cu(II) complexes and metal-free porphyrazine were stable at room temperature, non-hygroscopic, insoluble in water, but soluble in many common organic solvents.



### 2.2. In vitro *antifungal assay*

The antifungal activities were determined on *A. niger* using agar well diffusion method as performed by Schillinger and Lücke [18]. The stock solution (1 mg mL$^{-1}$) of the test chemical was prepared by dissolving the test compound in *N*,*N*-dimethyl formamide (DMF). The stock solution was suitably diluted with sterilized distilled water to 25, 50, and 100 μg mL$^{-1}$. Control for each dilution was prepared by diluting the solvent instead of stock solution with sterilized distilled water. The fungus was subcultured in potato dextrose agar medium (PDA). PDA plates were inoculated with freshly grown culture by spreading. Wells (6 mm diameter) were punched in the agar and loaded with 150 μL samples of the complexes. Standard antifungal drug (Fluconazole) was used for comparison. The plates were incubated at 37°C for 48 h. Activity was determined by measuring the diameter of the zone showing complete inhibition (cm). In order to clarify any effect of DMF on the biological screening, separate studies were carried out with solutions of DMF alone, and they showed no activity against the fungal strain. Antifungal activity was calculated as a mean of three replicates.

### 2.3. *Antioxidant activity evaluation*

**2.3.1. Free radical-scavenging activity.** The free radical scavenging activities of the complexes were measured with DPPH$^{\bullet}$ using the slightly modified methods of Brand-Williams *et al*. [19]. Briefly, 20 mg $L^{-1}$ DPPH$^{\bullet}$ solution in methanol was prepared and 1.5 mL of this solution was added to 0.75 mL of the sample, BHA and BHT (25–100 μg $mL^{-1}$). The mixture was shaken vigorously and the decrease in absorbance at 517 nm was measured at 30 min. Water (0.75 mL) was used as control in place of the sample. The percent inhibition activity was calculated using the following equation:

$$\text{Inhibition activity (\%)} = [(A_0 - A_1)/A_0 \times 100],$$

where $A_0$ is the absorbance of the control reaction and $A_1$ is the absorbance in the presence of the sample solution. Sample concentration providing 50% inhibition ($IC_{50}$) was obtained plotting inhibition percentage *versus* sample solution concentration.



**2.3.2. Superoxide radical scavenging activity.** Measurements of superoxide anion scavenging were based on the method described by Liu *et al*. [20]. Superoxide anions were generated in a non-enzymatic PMS–NADH system by oxidation of NADH and assayed by reduction of NBT. In this experiment, the superoxide anion was generated in 3 mL of tris-HCl buffer (16 mmol $L^{-1}$, pH 8.0) containing 1 mL of NBT (50 μmol $L^{-1}$) solution, 1 mL of NADH (78 μmol $L^{-1}$) solution, and 100 μg $mL^{-1}$ concentration of sample solution. The reaction was started by adding 1 $mL^{-1}$ of PMS solution (10 μmol $L^{-1}$) to the mixture. The reaction mixture was incubated at 25°C for 5 min and absorbance at 560 nm was recorded against blank samples in a spectrophotometer. BHA, BHT, and trolox were used as standard samples (100 μg $mL^{-1}$). The inhibition of superoxide radical generation (%) was calculated by the following equation:

$$\text{Inhibition (\%)} = [(A_0 - A_1)/A_0 \times 100],$$

where $A_0$ is the absorbance of the control reaction and $A_1$ is the absorbance in the presence of samples or standards. Sample concentration providing 50% inhibition ($IC_{50}$) was obtained plotting inhibition percentage *versus* sample solution concentration.

**2.3.3. Reducing power.** The reducing power of the complexes were measured according to the method of Oyaizu [21]. Various concentrations of the samples (10–50 μg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 mol $L^{-1}$, pH 6.6) and 2.5 mL potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v), and the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 mL of TCA (10%, w/v) was added to the mixture and centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper-layer solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%, w/v), and the absorbance was measured at 700 nm. α-Tocopherol, BHA, and BHT were used as standard antioxidants. Higher absorbance of the reaction mixture indicated greater reducing power.

## 3. Results and discussion

### 3.1. In vitro *antifungal assay*

All the tested compounds showed remarkable antifungal activity against *A. niger*. The results are shown in figure 2. CoPz showed much enhanced activity at 100 μg mL$^{-1}$. CuPz and $H_2Pz$ exhibited similar activities at the same concentration. Activity significantly increased on coordination. Standard antifungal drug Fluconazole exhibited 2.5 cm of inhibition at 100 μg mL$^{-1}$. Coordination reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with donor groups within the chelate ring system formed during coordination, increasing the lipophilic nature of the metal, which favors its permeation through the lipid layer of the microorganism, thus destroying them more aggressively [22]. Redox potential is also important because living cells contain numerous redox couples. Intracellular concentrations of these couples may change selectively with changes in the cellular environment. Concentrations of specific redox couples play a role in the regulation of cellular functions such as transcription of DNA into mRNA, the translation of mRNA into proteins, and the activity of signal transduction enzymes. A change in the intracellular concentration of redox couples may cause vital changes in cellular functions [23].



### 3.2. *Antioxidant activity evaluation*

**3.2.1. Free radical-scavenging activity.** Proton radical scavenging action is an important mechanism of antioxidation; DPPH• is used as a free radical to evaluate the antioxidative activity of some natural sources [3]. The DPPH radical scavenging effects of the complexes are presented in figure 3. $H_2Pz$ and the Cu(II) complex show free radical scavenging activities at 50–100 μg mL$^{-1}$. Co(II) complex did not show activity at any concentrations tested. Free radical scavenging activity of $H_2Pz$ was

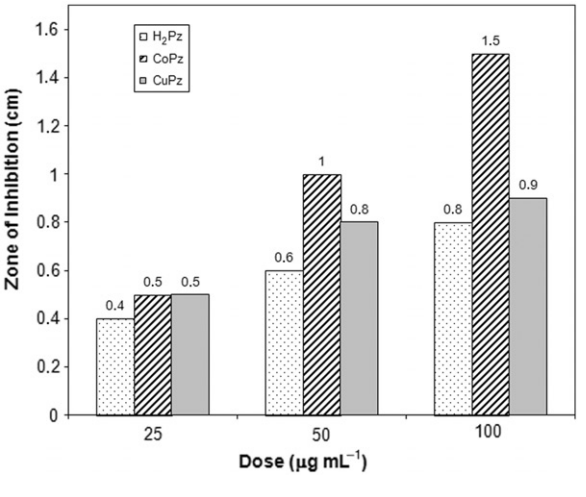


Figure 2. Antifungal activity of $H_2Pz$, CoPz, and CuPz against *A. niger* at different concentrations.

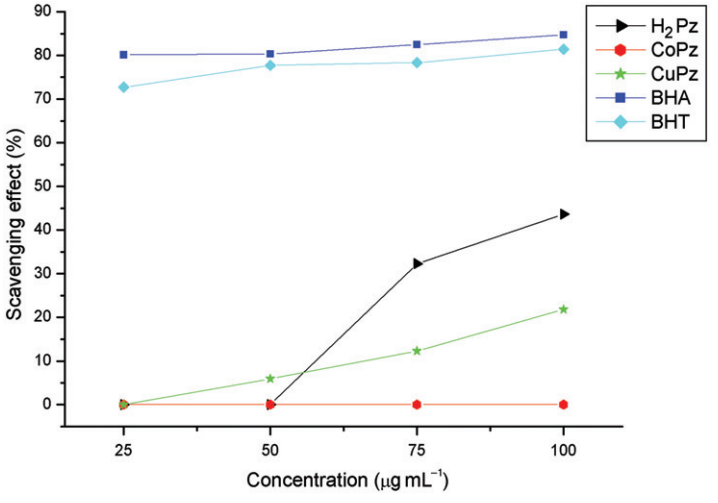


Figure 3. Scavenging activities of $H_2Pz$, CoPz, and CuPz against the DPPH radical.



higher than CuPz. Scavenging activity of BHA and BHT, known antioxidants, were higher than that of samples. $IC_{50}$ value (the effective concentration at which the DPPH radicals were scavenged by 50%) of $H_2Pz$ ($11.5 \pm 0.4\,\mu g\,mL^{-1}$) was higher than that of CuPz ($23 \pm 1\,\mu g\,mL^{-1}$), which were comparable. $IC_{50}$ value was not determined for CoPz. $IC_{50}$ values were $5.90 \pm 0.22$ and $6.12 \pm 0.29\,\mu g\,mL^{-1}$ for BHA and BHT, respectively. From these results, the samples have moderate ability to scavenge free radicals and could serve as free radical inhibitors or scavengers.

**3.2.2. Superoxide radical scavenging activity.** Superoxide radical is very harmful to cellular components as a precursor of more ROS, contributing to tissue damage and various diseases. In a biological system, its toxicity can be eliminated by superoxide dismutase [3]. The radicals may also play an important role during peroxidation of unsaturated fatty acids and other potential susceptible substances. In the PMS–NADH–NBT system, superoxide anion derived from dissolved oxygen by PMS–NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates consumption of the superoxide anion in the reaction mixture. Figure 4 shows the superoxide radical scavenging activity by $100\,\mu g\,mL^{-1}$ of $H_2Pz$, CoPz, or CuPz in comparison to the same amount of BHA, BHT, and trolox. CuPz shows higher superoxide radical scavenging activity than BHA, BHT, and trolox while $H_2Pz$ and CoPz show weaker activity than BHA, BHT, and trolox. $IC_{50}$ values for scavenging abilities on superoxide radicals were $16 \pm 1$, $13.3 \pm 0.6$, $6.2 \pm 0.4$, $6.40 \pm 0.01$, $8.4 \pm 0.2$, and $6.2 \pm 0.4\,\mu g\,mL^{-1}$ for $H_2Pz$, CoPz, CuPz, BHA, BHT, and trolox, respectively. Many metal complexes that can undergo such redox cycling can function as superoxide radical scavengers; many copper complexes have been synthesized and tested especially for SOD-like activity [24–26]. Some cobalt [21] SOD mimics have also been reported showing marked SOD activity [27].

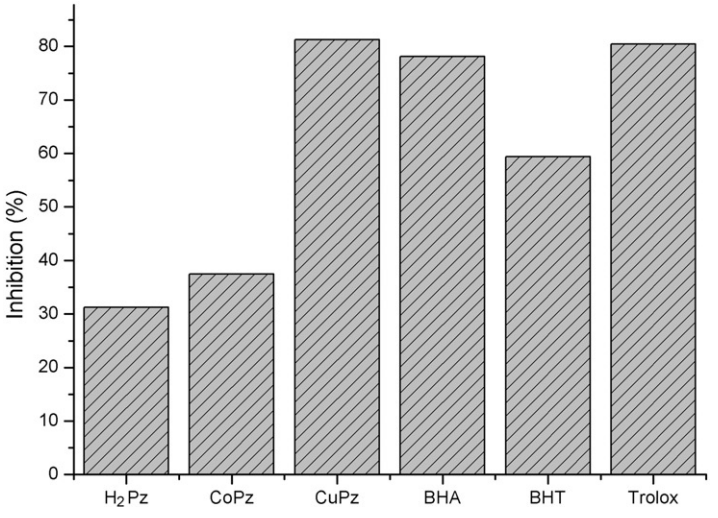


Figure 4. Superoxide radical scavenging activity of $H_2Pz$, CoPz, CuPz, BHA, BHT, and trolox at $100\,\mu g\,mL^{-1}$ concentration.



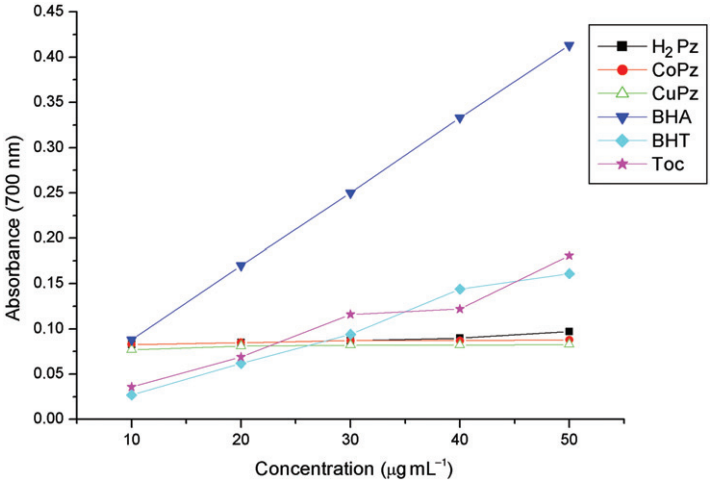


Figure 5. Reducing power of $H_2Pz$, CoPz, and CuPz at different concentrations.

**3.2.3. Reducing power.** Figure 5 shows the reducing power of $H_2Pz$, CoPz, and CuPz; reducing power of the samples was not concentration dependent. Based on a comparison of the absorbance at 700 nm, the reducing power of all complexes was similar to that of BHT and $\alpha$-tocopherol per molar basis. These results revealed that the ligand and their complexes were good electron and hydrogen donors and could terminate the radical chain reaction, converting free radicals to more stable products.

## 4. Conclusions

This study shows that porphyrazines with bulky (tert-butylphenyl) groups on the periphery and Co(II) and Cu(II) complexes exhibited different levels of antioxidant activity. The complexes have significant free radical scavenging, superoxide anion radical scavenging activity, and reducing power against various antioxidant systems *in vitro*. Complexes showed excellent activity for scavenging the superoxide radical. Therefore, these complexes may be a new kind of effective scavengers of ROS. The ligand and complexes also have antifungal activity against *A. niger*.

## Acknowledgments

This study was supported by the Research Fund of the Yildiz Technical University, Project no: 23-01-02-01.



## References

[1] G. Miliauskas, P.R. Venskutonis, T.A. Van Beek. *Food Chem.*, **85**, 231 (2004).
[2] B. Halliwell, J.M.C. Gutteridge. In *Free Radicals in Biology and Medicine*, E. Haslam (Ed.), 2nd Edn, pp. 617–623, Clarendon Press, Oxford (1989).
[3] Y.C. Chung, S.J. Chen, C.K. Hsu, C.T. Chang, S.T. Chou. *Food Chem.*, **91**, 419 (2005).
[4] T. Juntachote, E. Berghofer. *Food Chem.*, **92**, 193 (2005).
[5] C.S. Ku, S.P. Mun. *Bioresour. Technol.*, **99**, 4503 (2008).
[6] A.M. Farag, A.S. Mayhoub, S.E. Barakat, A.H. Bayomi. *Bioorg. Med. Chem.*, **16**, 4569 (2008).
[7] D.P. Riley. *Chem. Rev.*, **99**, 2573 (1999).
[8] R. Kachadourian, M.M. Flaherty, A.L. Crumbliss, M. Patel, B.J. Day. *J. Inorg. Biochem.*, **95**, 240 (2003).
[9] M.J. Perez, A.I. Cederbaum. *Free Radical Biol. Med.*, **33**, 111 (2002).
[10] G. Gümüş, A. Gül, V. Ahsen. *New J. Chem.*, **21**, 589 (1997).
[11] N. Kobayashi, R. Higashi, K. Ishii, H. Kazuaki, K. Ohta. *Bull. Chem. Soc. Jpn.*, **72**, 1263 (1999).
[12] N.B. McKeown. *Phthalocyanine Materials: Synthesis, Structure and Function*, Cambridge University Press, Cambridge (1998).
[13] T.F. Baumann, M.S. Nasir, J.W. Sibert, A.J.P. White, M.M. Olmstead, D.J. Williams, A.G.M. Barrett, B.M. Hoffman. *J. Am. Chem. Soc.*, **118**, 10479 (1996).
[14] S.L.J. Goldberg, A.J.P. Michel, D.J. White, A.G.M. Williams, A.G.M. Barrett, B.M. Hoffman. *Inorg. Chem.*, **37**, 2100 (1998).
[15] B. Keskin, Y. Köseoğlu, U. Avcıata, A. Gül. *Polyhedron*, **27**, 1155 (2008).
[16] C.F. Van Nostrum, R.J.M. Nolte. *Chem. Commun.*, 2385 (1996).
[17] A. Rosa, E. Baerends. *J. Inorg. Chem.*, **33**, 584 (1994).
[18] U. Schillinger, F.K. Lücke. *Appl. Environ. Microbiol.*, **55**, 1901 (1989).
[19] W. Brand-Williams, M.E. Cuvelier, C. Berset. *LWT Food Sci. Technol.*, **28**, 25 (1995).
[20] F. Liu, V.F.C. Ooi, S.T. Chang. *Life Sci.*, **60**, 763 (1997).
[21] M. Oyaizu. *Jpn. J. Nutr.*, **44**, 307 (1986).
[22] G.B. Bagihalli, P.G. Avaji, S.A. Patil, P.S. Badami. *Eur. J. Med. Chem.*, **43**, 2639 (2008).
[23] J.D. Rabinowitz, J.F. Vacchino, C. Beeson, H.M. McConnell. *J. Am. Chem. Soc.*, **120**, 2464 (1998).
[24] R.N. Patel, K.K. Shukla, A. Singh, M. Choudhary, D.K. Patel. *J. Coord. Chem.*, **63**, 586 (2010).
[25] A.M. Diaz, R. Villalonga, R. Cao. *J. Coord. Chem.*, **62**, 100 (2009).
[26] J.R. Anacona, D. Lorono, M. Azocar, R. Atencio. *J. Coord. Chem.*, **62**, 951 (2009).
[27] R.N. Patel. *J. Coord. Chem.*, **63**, 1207 (2010).