*Available online at* www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

**Der Pharma Chemica, 2016, 8(10):198-204**
*(http://derpharmachemica.com/archive.html)*

# 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines: Synthesis, characterization, antimicrobial and antioxidant activity

**B. Chidananda[1], K. R. Venugopala Reddy[2]*, K. M. Pradeep[1], M. N. K. Harish[3], C. D. Mruthyunjayachari[1], S. D. Ganesh[4], N. S. Vijaykumar[1] and P. Malthesh[2]**

[1]*Department of Industrial Chemistry, Sahyadri Science College (Autonomous), Kuvempu University, Shivamogga - 577 203, Karnataka, INDIA*
[2]*Department of chemistry, Vijayanagar Srikrishna Devaraya University, Bellary, Karnataka, INDIA*
[3]*Department of Chemistry, Acharya Institute of technology, Soladevanahalli, Hesaraghatta Main Road, Bangalore, Karnataka, INDIA*
[4]*Department of Industrial Chemistry, Kuvempu University, Shankaraghatta - 577 451, Shivamogga, Karnataka, INDIA*

---

**ABSTRACT**

*The scheming and structural adornment of soluble metallophthalocyanines peripherally substituted with 4-bromo-2-methoxyaniline via amide bridged molecule is described. These molecules display admirable solubility in organic solvents such as methanol, ethanol, tetrahydrofuran, dimethylformamide and dimethyl sulfoxide and compound was characterized by a wide range of spectroscopic methods in addition to elemental analysis. Synthesized all of the new phthalocyanine compounds have been set to antimicrobial activity and antioxidant. The antioxidant activities of designed metallophthalocyanines were investigated by in vitro antioxidant assays such as free radical scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The title metallophthalocyanines (5-7) were screened against Gram-positive bacteria with comparing standard Streptomycin as reference. Antifungal activities against two different fungi have been evaluated and compared with Flucanazole as reference.*

**Keywords**: soluble metallophthalocyanines, amide bridged, spectroscopy, minimum inhibitory concentrations, antimicrobial, antioxidant.

---

## INTRODUCTION

We are involved in the synthesis of metallophthalocyanines (MPcs) and its derivatives are conventional pigments that were exposed almost a century ago. Investigation and design of phthalocyanines (Pcs) with novel structure and functional properties are becoming important precursors to obtained materials with extremely different functionalities by making changes in metal ions in their inner cores or in the substituent's on the periphery [1-4] and moreover, many reports describe the use of Pc materials in chemical sensors, liquid crystals, catalysts, conductive materials, electro chromic display devices, photovoltaic cells, xerography, optical disk, non-linear optics, photodynamic reagents for cancer therapy, and for other medical applications [5-10]. Selectivity applications, exceptional soluble and non-aggregated Pcs are desirable. The former property facilitates the purification and the fabrication processes, while the latter assists in characterization and allows these materials to function properly;

many properties of Pcs change upon aggregation [11-13]. In this consequence, a substantial number of Pcs with sterically demanding substituents have been reported [14]. Phenolic hydroxyl and amide substituted MPcs have been repeatedly tested for various wide of range biological activities because of enhanced solubility and their potent active groups[15-17]. Synthesized MPcs were demonstrating a prosperous array of biological activity and increasing administration of antimicrobial agents has led to the development of microbial resistance. Bibliographic survey reveals genetic mutations that result in resistance to clinically used drugs, especially Streptomycin for antibacterial and fluconazole for antifungal [18-19]. Studies in the literature involving very different classes of materials lead us to suggest that the discovery of new antimicrobial compounds could play a chief role as functional elements antimicrobial spectra and higher therapeutic indexes than Streptomycin and fluconazole. These versatile features have stimulated attempts on the synthesis of new Pc derivatives with objective of developing new materials containing improved or high functional characteristics and which are novel yet resemble known biologically active molecules by virtue of the presence of some critical structural features. In the interest of above mentioned features of MPc, we planned our synthesis by combining these two boilable components together to give a compact structure like title compounds.

We describe herein a series of Pc complexes substituted with 4-bromo-2-methoxyaniline groups. These bulky and amide substituent not only enhance the solubility of Pcs in organic solvents and reduce their tendency to aggregate the substituted Pcs. 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines have been synthesized as the target compounds and characterized by FT-IR, $^{1}$H-NMR, UV-visible spectroscopy and elemental analysis. We described in order to examine the lead optimization in vitro microbiological activity against various Gram-positive, Gram-negative bacteria and the different fungi in comparison with control drugs. In addition, antioxidant properties of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines were investigated and it applied for antimicrobial activity and radical scavenging capacity was studied.

## MATERIALS AND METHODS

### MATERIALS

2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanine complexes (5-7) were prepared and characterized as previously described [31-32]. All reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. Reactions were performed under nitrogen. N,N-Dimethylformamide (DMF) was predried with barium oxide and distilled under reduced pressure. The chloroform used in the UV/Vis studies was ar grade, Commercial TLC plates (silica gel 60 F254, SDS) were used to monitor the progress of the reaction, with spots observed under UV light at 254 and 365 nm. N,N'-Dicyclohexylcarbodiimide (DCC) and 4-bromo-2-methoxyaniline were purchased from Sigma Aldrich , Bangalore, India and used without further purification. Ultrapure MilliQ water was used for all the experiments.

### METHODS

#### Spectral analyses

Elemental analyses (C, H and N) were recorded on a VarioMICROV 1.7.0. (Elementl Analysersysteme GmbH). The 1H NMR spectra were measured at 400 MHz Varian-AS NMR spectrometer in dimethylsulfoxide-d6 using tetramethylsilane as the internal standard. Elemental analysis was carried out using a Perkin Elmer 2400CHN instrument. UV-Vis spectra were measured on an ocean optics USB 4000.USA, using 1 cm path length cuvette at room temperature. Samples were prepared in dimethylsulphoxide at a concentration of $5.0 \times 10^{-5}$ mol $dm^{-3}$. Infrared spectra were recorded using FT-IR 8400s Shimadzu spectrometer with KBR pellets in the range of 400-4500$cm^{-1}$.

#### Chemistry

General procedure for the Synthesis of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines compounds (5-7). The template condensation reaction between a mixture of 4-bromo-2-methoxyaniline (1.9 g, 0.06 mmol), tetracarboxy metallo-phthalocyanines (1 g, 0.001 mol), $K_2CO_3$ (2.5 g, 0.0025 mmol) and DCC as catalyst in DMF (20 mL) and the mixture was stirred under nitrogen atmosphere for 24 h at room temperature. The progress of the reaction was observed by color change from blue to bluish green color and the precipitate was removed by filtration. The crude product so obtained was washed thoroughly with hot water and repeatedly with hexane. The product was dried in the oven for 1 h at 55ºC obtained bluish green solid.

**Synthesis of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted nickel(II)phthalocyanines (5)**

Yield: (80%) found: Anal. (%) Calc. for [$C_{64}H_{40}Br_4N_{12}O_8Ni$]: C, 51.82; H, 2.72; Br, 21.55; N, 11.33; O, 8.63; Ni, 3.96. Found: C, 51.55; H, 3.96; Br, 21.11; N, 11.02; O, 9.10; Ni, 3.81. IR (KBr, $cm^{-1}$): 3405.9 (-NH), 3020.56, 2983.33 (Ar-CH), 1637.0 (C=O), 1610.4 (C=N), 1531.4 (C=C), 1478.2 (C-C), 766.0 (C-Br), 1328.2, 1289.7, 1261.4, 1141.5, 1091.7, 1057.2, 926.1, 831.6, 738.1, 724.8, 702.2 are attributed to the various skeletal vibration of PC ring. $^1$H NMR (DMSO-d6, δ ppm): 9.0 to 9.3 (s, NH), 7.1 to 8.0 (m, Ar-H), 3.7 to 4.1 (s, $OCH_3$).

**Synthesis of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted cobalt (II)phthalocyanines (6)**

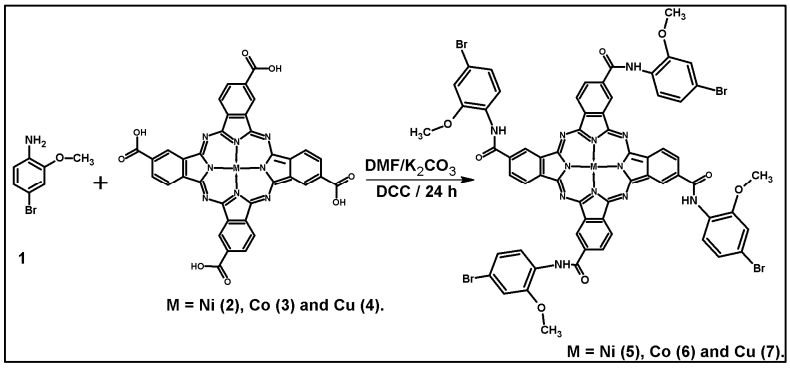
Yield: (81%) found: Anal. (%) Calc. for [$C_{64}H_{40}Br_4N_{12}O_8Co$]: C, 51.81; H, 2.72; N, 21.54; Br, 21.54; O, 8.63; Co, 3.97. Found: C, 51.78; H, 3.87; Br, 21.07; N, 10.95; O, 9.03; Co, 3.86. IR (KBr, $cm^{-1}$): 3398.3 (-NH), 3025.23, 2962.74 (Ar-CH), 1663.5 (C=O), 1609.6 (C=N), 1540.5 (C=C), 1518.7 (C-C), 765.8 (C-Br), 1328.4, 1289.3, 1261.4, 1141.4, 1096.7, 1054.5, 994.7, 831.6, 739.8, 725.5, 702.3, 682.0 are attributed to the various skeletal vibration of PC ring.

**Synthesis of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted cupper (II)phthalocyanines (7)**

Yield: (82%) found: Anal. (%) Calc. for [$C_{64}H_{40}Br_4N_{12}O_8Cu$]: C, 51.65; H, 2.71; Br, 21.48; N, 11.29; O, 8.60; Cu, 4.27. Found: C, 51.25; H, 3.89; Br, 21.13; N, 16.87; O, 9.08; Cu, 3.85. IR (KBr, $cm^{-1}$): 3406.2 (-NH), 3032.85, 2962.12 (Ar-CH), 1637.2 (C=O), 1610.8 (C=N), 1541.3 (C=C), 1505.0 (C-C), 763.8 (C-Br), 1399.8, 1316.1, 1289.4, 1140.5, 1093.8, 1051.8, 913.2, 832.6, 737.4, 724.0, 706.6, 679.0 are attributed to the various skeletal vibration of PC ring.

## RESULTS AND DISCUSSION

**Synthesis and Characterization**




**Scheme 1 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (5-7)**

2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (**5-7**) synthesized by tetracarboxy metallophthalocyanines (1 g, 0.001 mol), $K_2CO_3$ (2.5 g, 0.0025 mmol) and DCC as catalyst was dissolved in dimethylformamide (DMF, 20 mL) with constant stirred for 1 h. To this solution, 4-bromo-2-methoxyaniline (1.9 g, 0.006 mmol) was added followed by the above reaction mixture under stirring as shown in Scheme 1. After 24 h, green precipitate appeared and reaction mixture poured into water and repeatedly purified with hot water and followed by hexane. They were characterized by FT-IR, $^1$H-NMR spectroscopy and elemental analysis. The complexes were stable and readily soluble in MeOH, THF and DMSO. The proton NMR spectrum of the phthalocyanine compound (**5**) displayed characteristic signals as shown in figure 1. The singlets at 3.6 to 3.8 and 4.0 to 4.1 ppm in the spectrum revealed the presence of (O-$CH_3$) groups respectively. The -CO(NH) signals were observed at 9.0 to 9.2 ppm and the aromatic ring protons as unresolved multiplets (most likely due to the presence of isomers), integrating for a total of 24 protons in the range 7.1to 8.0 ppm respectively.

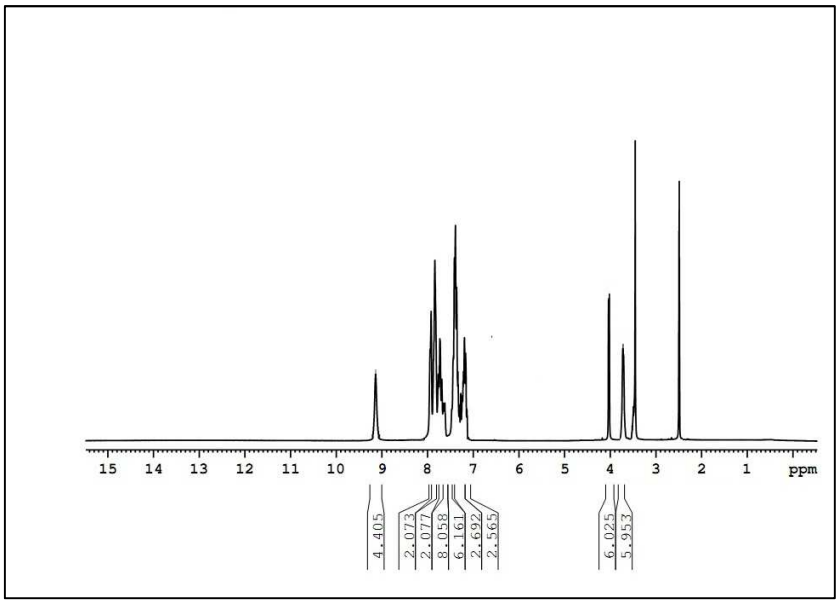



**Figure 1. 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted nickel(II)phthalocyanine (5)**

The IR spectra of the metallophthalocyanine complexes show characteristic peaks and were relatively well resolved as shown in figure 2. The presence of broad and intense absorption bands at region 3398.3-3406.2 $cm^{-1}$ for (Ar-NH) function, the presence of a band in the region 3033.85-2962.12 $cm^{-1}$ for (Ar-CH) group, the (C=O) group was observed as a sharp, intense band at 1663.5-16637.0 $cm^{-1}$, the stretching vibrations of (C=N) and (C=C) group were observed near 1610.0-1609.0 $cm^{-1}$ and 1540.5-1505.0 $cm^{-1}$, the sharp peak observed at 766.0-763.0 $cm^{-1}$,for (C-Br) group, 1313.19, 1227.67, 1086,18, 887.16, 837.40, 741.00, 636.82 $cm^{-1}$ are attributed to the various skeletal vibration of PC ring and IR spectral data supports the proposed target structures. The elemental analyses results are consistent with the expected one.

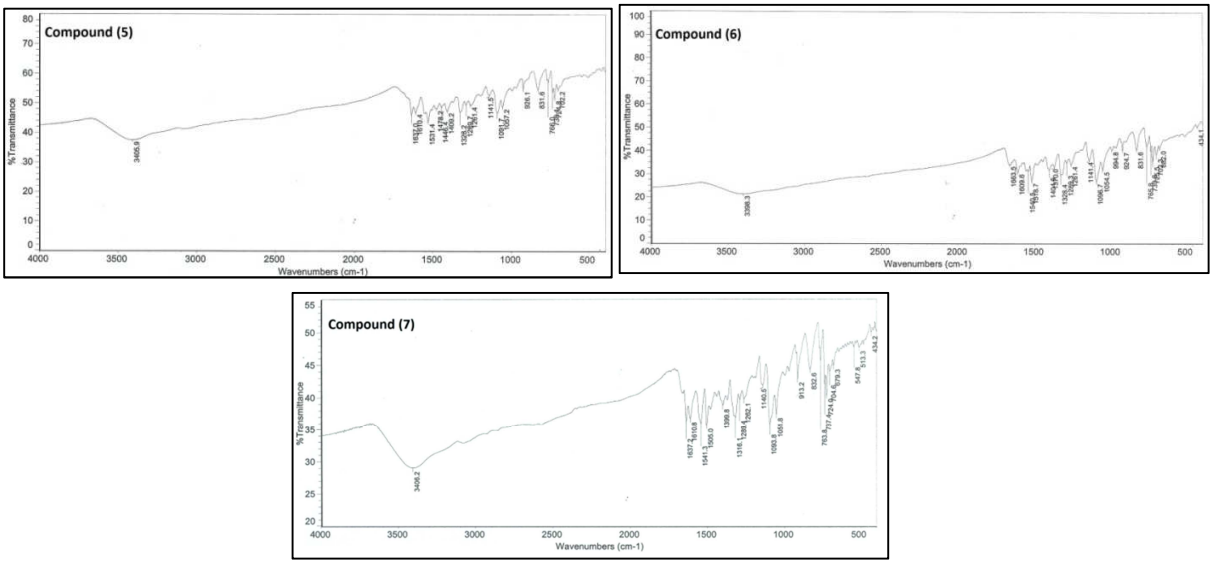



**Figure 2. 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (5-7)**

**In vitro antibacterial and antifungal assay**

2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (**5**-**7**) were scrneed for antimicrobial activity using six different laboratory control strains of bacteria, i.e., the Gram positive *Staphylococcus aureus*, Gram negative, *Klebsiella pneumonia, Pseudomonas aures, Escherichia coli*, two fungal strains were *aspergillus niger and candida albicans* with metallophthalocyanine complexes (**5**-**7**) was performed by the Agar well diffusion method [15-17]. The bacterial strains were collected from different infectious status of patients who had not administered any antibacterial drugs for at least two weeks with the suggestions of an authorized physician, in Kiran diagnostic health centre of Chitradurga, Karnataka state, India. Fungal strains were procured from the culture maintained at National College of Pharmacy Shimoga. The compounds were tested at 40 µg/mL concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Streptomycin (40 µg in100µl) and Fluconazole (40 µg in100µl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 $^{o}$C for antibacterial activity and 72 h at 25 $^{o}$C for antifungal activity. The results revealed that the tested compounds were considered to be modest since the values obtained were close to each other. The antibacterial and antifungal studies suggest that all synthesized metallophthalocyanine (5-7) were showed resistant to antibacterial and antifungal activity and microbial results are systematized in tables 1. In the case of bacteriological studies, the results were compared with the standard drug (Streptomycin). Antifungal activities revealed showed moderate to good fungal inhibition against the standard drug (Flucanazole). The complex showed a strong activity to Grampositive bacteria and the activity of the complex to Gram negative bacteria was reasonable on comparing little leesser to Streptomycin standard drug. Antifungal activity revealed moderate to good fungal inhibition against the standard drug (Flucanazole) but Among the compounds **5** and **6** exhibited higher antimicrobial activity than compound **7** as compared with standard drugs (Table 1). which make metallophthalocyanines (5-7) act as more powerful and potent bacteriostatic agents, thereby inhibiting the growth of the microorganisms.

**Table 1: Anti-microbial activity of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (5-7)**

| Zone of inhibition test (in cm) | | | | | | |
|---|---|---|---|---|---|---|
| bacterial strains | | | | fungal Strains | | |
| **Compound** | ***S.aureus*** | ***P.aeruginosa*** | ***K.pneumoniae*** | ***E.coli*** | ***A.niger*** | ***C.albicans*** |
| **5** | 3.6 | 2.6 | 2.8 | 2.9 | 2.0 | 2.0 |
| **6** | 3.7 | 2.8 | 2.9 | 2.7 | 2.1 | 2.2 |
| **7** | 3.8 | 2.8 | 2.7 | 2.8 | 2.1 | 2.1 |
| **DMSO** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **Standard** | 4.1 | 3.4 | 3.7 | 3.6 | 2.2 | 2.3 |

*Standard - Streptomycin (antibacterial)*
*Standard - Flucanazole (antifungal)*

**Minimum Inhibitory Concentrations (MIC)**

The Minimum Inhibitory Concentrations (MIC) of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (**5**-**7**) was determined by a micro dilution method [15-17]. The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The synthesized 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallo- phthalocyanines (**5**-**7**) at different concentrations viz. 10, 20, 30, 40 and 50 µg was dissolved respectively in 25, 50, 75, 100 and 125 µL of DMSO. And later loaded into corresponding wells. The standard drug Streptomycin (40 µg in100µl) and Fluconazole (40 µg in100µl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 $^{o}$C for antibacterial activity and 72 h at 25 $^{o}$C for antifungal activity. The results are recorded in mm in Table 2.

**Table 2. (MIC) of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (5-7)**

| MIC (µg/µL) | | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | bacterial strains | | | | fungal Strains | |
| **10-50 (µg)** | ***S.aureus*** | ***P.aeruginosa*** | ***K.pneumoniae*** | ***E.coli*** | ***A.niger*** | ***C.albicans*** |
| **5** | 30 | 30 | 40 | 30 | 30 | 40 |
| **6** | 20 | 20 | 30 | 30 | 40 | 30 |
| **7** | 20 | 30 | 40 | 40 | 40 | 40 |
| Control DMSO | 0 | 0 | 0 | 0 | 0 | 0 |

**Antioxidant activity**

The DPPH radical scavenging activity of the compounds and the ascorbic acid (standard) was measured according to the method [15-17]. The DPPH radical is a stable free radical having $\lambda_{max}$ at 517 nm. Different concentration (5, 10, 25, 50, 100 and 200 µg/ml) of compounds and standard were prepared in methanol. In clean and labeled test tubes 2ml of DPPH solution (0.002% in methanol) was measured at 517 nm using UV-visible spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity was calculated using the formula: scavenging activity (%) = A-B/A x 100, where A is the absorbance of DPPH and B is the absorbance of DPPH in standard combination. The figure 3 reveals that antioxidant activity at different activity concentration of compounds (5-7) in methanol and ascorbic acid in terms of free radical scavenging ability which was evaluated using DPPH free radical assay. The compounds exhibited marked antioxidant activity by scavenging DPPH* found to be dose dependent. The compound **5** and **7** was shown to be more potent than compound **6** as compared with standard ascorbic acid and results were tabulated in table 3.

**Table 3. DPPH radical scavenging activity of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (5-7)**

| compounds (µg/ml) | Radical scavenging activity (%) | | | | | |
|---|---|---|---|---|---|---|
| | **5** | **10** | **25** | **50** | **100** | **200** |
| **5** | 59.23 | 70.52 | 75.67 | 83.68 | 82.69 | 88.98 |
| **6** | 60.27 | 71.12 | 76.42 | 84.15 | 85.24 | 87.78 |
| **7** | 60.82 | 72.35 | 75.98 | 83.36 | 84.67 | 88.16 |
| **Ascorbic acid** | 64.96 | 75.08 | 85.46 | 91.36 | 95.09 | 98.06 |

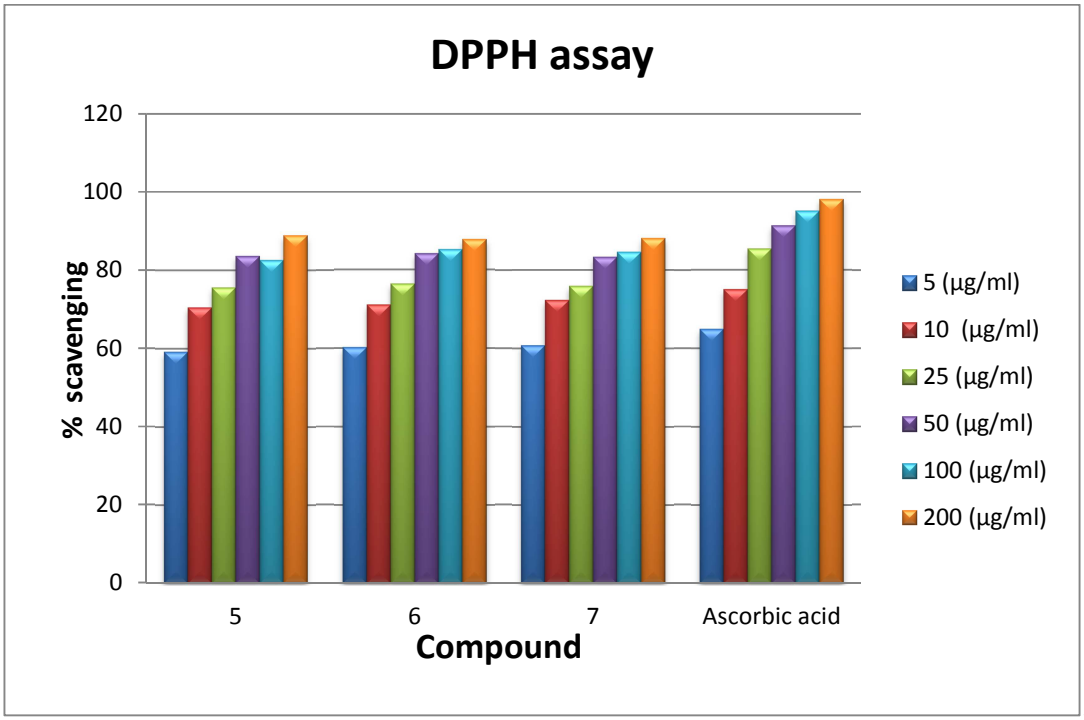



**Figure 3. Plots of the radical scavenging effects (%) of the 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (5-7) at various concentrations**

## CONCLUSION

In conclusion, new series of 2,9,16,23-tetra-N-(4-bromo-2-methoxyphenyl)benzamide substituted metallophthalocyanines (**5-7**) were synthesized and the formation title compounds were characterized by elemental analysis, FT-IR, $^1$H NMR spectra and elemental analysis. These complexes has stable and good solubility in methanol, tetrahydrofuran, N,N-dimethylformamide and dimethylsulfoxide. Investigated all the compounds acknowledged for biological evaluation and antioxidant activities. Title compound were found to be highly active and results suggested the very diffusion of the complexes into the bacterial cells and were able to kill the bacterium

as indicated by the zones of inhibition of bacterial growth. The minimum inhibition concentration results showed w 50% of the bacterial inhibition at w1.20 mM MPc concentration in DMSO. Moreover, complexes have modest antioxidant activity when compared with standard.

**Acknowledgement**
One of the author (Dr. Chidananda B) thankful to Indian institute of science Bangalore for spectral analysis.

## REFERENCES

[1] O. Bekaroglu, *J Porphyr Phthalocyanines*, **2000**, 4, 465-473.
[2] C.C. Leznoff, and A.B.P. Lever, Phthalocyanines Properties and Applications VCH Publishers, New-York, **1989-1993**, 1-3.
[3] K.H. Schweikart, and M. Hanack, *Eur. J. Org.Chem*, **2000**, 2551-2556.
[4] M. Hanack, and M. Lang, *Adv. Mater*, **1994**, 6, 819-833.
[5] J.J.R. Fraústo da Silva, R.J.P. Williams, (Eds.) The Biological Chemistry of the Elements-The Inorganic Chemistry of Life Clarendon Press, Oxford, **1991**, 343.
[6] P. Erk, H. Hengelsberg, K.M. Kadish, K.M. Smith, R. Guilard, (Eds.) The Porphyrin Handbook Academic Press, **2003**, 19, 105.
[7] H. Mustroph, M. Stollenwerk, V. Bressau, *Angew. Chem. Int. Ed*, **2006**, 45, 2106.
[8] B. Meunier, A. Sorokin, *Acc. Chem. Res*, **1997**, 30, 470.
[9] B. Hasenknopf, J. M.Lehn, N. Boumediene, A. Dupont-Dervais, A. Van Dorsselaer, B. Kneisel, D. Fenske, J. *Am. Chem. Soc*, **1997**, 119, 10956.
[10] B. Simic-Glavaski, C.C. Leznoff, A.B.P. Lever, (Eds.) Phthalocyanines-Properties; Applications. VCH, New York, **1993**, vol. 3, 119.
[11] A. Graul, J. Castaner, *Drugs Future*, **1997**, 22, 956-968.
[12] A.A. Patchett, *J. Med. Chem*, **1993**, 36, 2051-2058.
[13] M. de Gasparo, S. Whitebread, *Regul. Pept*, **1995**, 59, 303-311.
[14] V. S. Ananthanarayanan, S. Tetreault, A. Saint-Jean, *J. Med. Chem*, **1993**, 36, 1324-1332.
[15] B. Chidananda, K.R. VenugopalaReddy, M.N.K. Harish, K.M. Pradeep, C.D. Mruthyunjayachari, S. D. Ganesh and T. R. prashith kekuda, *Der Pharma Chemica*, **2013**, 5(4), 293-300.
[16] B. Chidananda, K.R. VenugopalaReddy, M.N.K. Harish, K.M. Pradeep, C.D. Mruthyunjayachari, S. D. Ganesh and T. R. prashith kekuda, *Res. J. Chem. Sci*, **2013**, 3(9), 1-4.
[17] B. Chidananda, K.R. Venugopala Reddy, M.N.K. Harish, K.M. Pradeep, C.D. Mruthyunjayachari, S.D. Ganesh, *J. Heterocyclic Chem*, **2014**, 52, 1782-17913.
[18] P.G. Lou, F.J. Stutzenberger, *Adv. Appl. Microbial*, **2008**, 63, 145-181.
[19] G. Shahriar, A. Mina, and S. Sajjad, *Sci. Res. Essays*, **2012**, **7**, 3751-3757.