

# Synthesis of Schiff Base (*E*)-2-(((3-Hydroxyphenyl)imino)methyl)-6-methoxyphenol Containing N and O Donors and its Metal Complexes: Spectral, Thermal, Redox Behaviour, Fluorescence Quenching, Antimicrobial and Anticancer Studies

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Convenient synthesis of new octahedral complexes from 3-aminophenol and 2-hydroxy-3-methoxybenzaldehyde with the molecular formula  $[M(L_2-2H)]$  (where  $M = Mn^{2+}$  and  $Co^{2+}$ ) have been described. The Schiff base behaves as a tridentate ligand with O and N donors and coordinates to metal ion *via* phenolic oxygens and azomethine nitrogen. The compositions of the ligand and its metal complexes have been established by elemental analysis and metal estimation. The ionic nature of the metal complexes has been deduced from conductance measurements. Structural features and bonding mode of Schiff base have been proposed by magnetic properties and spectral methods. Both the complexes exhibit *d-d* transitions, photo emissive and redox behaviour. The Schiff base ligand and its metal complexes have been screened for antimicrobial evaluation towards some clinically important microorganisms. The quenching studies were carried out for the [Co(L<sub>2</sub>-2H)] complex by alizarin red S dye in DMSO using fluorescence measurements. The evaluated highly biologically active [Co(L<sub>2</sub>-2H)] complex shows excellent cytotoxicity towards (MCF-7) cancer cell line.

Keywords: Schiff base ligand, 3-Amino phenol, 2-Hydroxy-3-methoxybenzaldehyde, Fluorescent quenching, Biological activities.

### **INTRODUCTION**

Schiff base ligands containing N and O donors are unique and multifaceted, known for their selectivity and sensitivity towards the metal ions. The biomedicinal properties of free organic molecules depend upon chelation with suitable metal ion led to the implementation of metal complexes for several biomedical applications as therapeutically active analgesic, antipyretic, antiinflammatory, cytotoxic, antiviral, antitumorous and antitubercular activity besides their applications as antimicrobial agents [1-5]. Now a days there is an increasing interest in the developement of flourescence sensors for the enantioselective recognition of chiral organic compounds and drug molecule [6]. In recent years, there have been numerous reports of their use in homogeneous and heterogenous catalyst. An interesting application of Schiff bases, is their use as an effective corrosion inhibitor, which is based on their ability to spontaneously forms a monolayer on the surface to be protected [7]. They have played a seminal role in the development of modern coordination chemistry, as well as they can also be found at key points in the development of inorganic biochemistry, catalysis and optical materials [8].

Previously a number of biologically important complexes have been reported by our group [9-13]. In order to broaden the scale of investigations on the Schiff bases, the present paper records the synthesis and characterization of Mn(II) and Co(II) complexes derived from 2-aminophenol and 2-hydroxy-3methoxybenzaldehyde. The structures of the ligand and its metal complexes were characterized by elemental analysis, IR, <sup>1</sup>H NMR, UV, molar conductance and magnetic susceptibility measurements. The biological activities are also studied against gram positive and gram negative bacterial and fungi organisms for Schiff base ligand and their complexes. The Co(II) complex shows excellent cytotoxicity towards human breast cancer cell (MCF-7). The fluorescence quenching studies were carried out for the [Co(L<sub>2</sub>-2H)] complex by the alizarin red S dye in DMSO using fluorescence measurements.

### **EXPERIMENTAL**

All the chemicals used were of analytical reagent grade (AR) and of highest purity available. Solvents were purified and dried according to the standard procedures. All metal(II) compounds were used as chloride salts. IR spectra of the complexes

were recorded in KBr pellets with a Perkin Elmer RX1 FT-IR Spectrophotometer in the 4000-400 cm<sup>-1</sup> range. The electronic spectra were recorded in DMF on a Perkin Elmer Lambda 35 spectrophotometer in the 190-1100 nm range. The <sup>1</sup>H NMR spectra were recorded on a Bruker 400MHz FT-PMR spectrometer (DMSO-d<sub>6</sub>). Elemental analysis of the ligand and complexes were obtained using Elementar Vario EL CHN rapid analyzer. Cyclic Voltammetric measurements for the complexes were measured using Princeton applied Research-Multichannel VersaSTAT-II. Magnetic susceptibilities were measured on a Automagnetic Susceptibility meter (MSB-Auto) at room temperature. Melting points were determined using melting point apparatus (Elico) and are uncorrected. Conductivity measurements for the complexes were carried out on Elico Conductivity Bridge and a dip conductivity cell using dimethyl formamide as solvent. Fluorescence spectra were detected using Perkin Elmer LS 45 Spectrofluorometer.

**Synthesis of Schiff base ligand** (*E*)-2-(((3-hydroxyphenyl)imino)methyl)-6-methoxyphenol: The Schiff base was prepared by the condensation of equimolar amounts of 3-aminophenol (0.01 mol) and 2-hydroxy-3-methoxybenzaldehyde (0.01 mol) in minimum quantity of ethanol. The resulting mixture was then refluxed for 4-5 h. An orange coloured solid mass separated out on cooling is filtered, washed and dried over anhydrous CaCl<sub>2</sub> in desiccator. The purity of the ligand was checked by TLC and spectral data. The structure of Schiff base ligand is given in Fig. 1.



Fig. 1. Structure of ligand (*E*)-2-(((3-hydroxyphenyl)imino)methyl)-6methoxyphenol

**Synthesis of complexes:** Complexes of (*E*)-2-(((3-hydroxy-phenyl)imino)methyl)-6-methoxyphenol were synthesized by

mixing a hot ethanolic solution of Schiff base (L) (0.005 mol), ethanolic solution of corresponding M(II) chlorides (0.025 mol) according to the following equations:

$$MCl_2 \cdot nH_2O + 2L_2 \xrightarrow{\text{Ethanol/DMF}} [M(L)_2 - 2H] + nH_2O$$

where, M = Mn(II) and Co(II).

The mixture was refluxed for 7 h. The complexes obtained in each case were cooled, filtered and washed with ethanol several times to remove any excess ligand. Finally the complexes were washed with small amount of ether and dried in desiccator.

The micro analytical data, melting point, colour and other physical properties of the ligand and metal complexes are given in Table-1.

## **RESULTS AND DISCUSSION**

The physical characteristics of the Schiff base and its Mn(II) and Co(II) complexes are given in Table-1. The analytical data (Table-2) of the Schiff base and its complexes agree very well with the proposed molecular formulae and also indicates the formation of 1:2 (M:L) complexes of general formula of  $[M(L_2-2H)]$ .

**Molar conductance:** The molar conductance of metal complexes are measured using 10<sup>-3</sup> M DMF solutions and are found within the range 2.4 and 3.3 ohm<sup>-1</sup> cm<sup>-1</sup> mol<sup>-1</sup> suggesting the non-electrolytic nature [14] and indicate that no anions are present outside the coordination sphere (Table-2).

**IR spectra:** The Schiff base shows a strong absorption band at 1603 cm<sup>-1</sup> characteristic of v(>C=N-) [15] whereas the broad band at 3474 cm<sup>-1</sup> is characteristic of hydrogen bond v(O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm<sup>-1</sup> which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm<sup>-1</sup> in the ligand and 3360 cm<sup>-1</sup> the Co(II) complex corresponds to O-H stretching.

The shift of frequency is due to the coordinate bonding of hydroxyl oxygen to the metal. The azomethine peak in ligand at 1603 cm<sup>-1</sup> is shifted to 1606 cm<sup>-1</sup> in Co(II) complex and 1614 cm<sup>-1</sup> Mn(II) complex suggesting the coordination through >C=N- group (Table-3). Further evidence for the coordination of the N and O atoms of the Schiff base with the metal atom was shown by the appearance of a new weak band in the

TABLE-1 PHYSICAL CHARACTERISTICS OF SCHIFF BASE AND ITS METAL COMPLEXES					8	
Schiff base and complexes	ff base and m.f. Colour m.p. (°C) Yield (%)					
L	$C_{14}H_{13}NO_{3}$	Orange	120	90	-	
$[Co(L_2-2H)]$	$C_{28}H_{24}N_2O_6Co$	Reddish brown	>300	65	2.35	
$[Mn(L_2-2H)]$	$C_{28}H_{24}N_2O_6Mn$	Brown	260	80	3.27	

TABLE-2 ANALYTICAL DATA OF SCHIFF BASE AND ITS METAL COMPLEXES							
Schiff base &	Elemental analysis (%): Calcd. (found)			Metal (%)			
complexes	С	Н	Ν	Calculated	Colorimetric method	Pyrolytic method	
L	69.15 (68.87)	5.36 (5.12)	5.82 (5.42)	_	-	-	
$[Co(L_2-2H)]$	61.85 (61.67)	4.42 (4.34)	5.15 (5.08)	10.84		11.05	
$[Mn(L_2-2H)]$	62.41 (62.65)	4.51 (4.32)	5.22 (4.98)	10.19	9.26	9.98	

TABLE-3           KEY IR BANDS (cm <sup>-1</sup> ) OF SCHIFF BASE AND ITS METAL COMPLEXES							
Schiff base & complexes	v(O-H) Phenolic v(C-O) v(>C=N-) v(M-O) v(M-N)						
L	3474	1460	1603	-	-		
[Co(L <sub>2</sub> -2H)]	3360	1471	1606	734	540		
$[Mn(L_2-2H)]$	[Mn(L <sub>2</sub> -2H)] 3421 1471 1614 740 558						

region 740-734 cm<sup>-1</sup> which may be assigned to v(M-O) and band at around 540-558 cm<sup>-1</sup> may be due to v(M-N) [18].

Electronic spectra and magnetic susceptibility measurements: The ligand is orange in colour and shows three absorption bands at 210 nm (47431 cm<sup>-1</sup>), 228 nm (43767 cm<sup>-1</sup>), 310 nm (32235 cm<sup>-1</sup>) accounts for  $\pi$ - $\pi^*$  and n- $\pi^*$  transitions [19]. The Co(II) complex is reddish brown in colour exhibits three absorption bands. The bands at 39384 and 36847 cm<sup>-1</sup> may be due to the intra ligand charge transfer and 30137 cm<sup>-1</sup> may be due to the transition from  ${}^{4}A_{2g} \rightarrow {}^{4}T_{1g}$  [20]. The Mn(II) complex is brown in colour exhibits two absorption bands (Fig. 2). The band at 36182 cm<sup>-1</sup> may be due to the intra ligand charge transfer and 30120 cm<sup>-1</sup> band is assigned to  ${}^{6}A_{1g} \rightarrow {}^{2}T_{2g}$ transition [21]. The magnetic moment value of Co(II) complex is 5.14 BM confirms the octahedral geometry whereas Mn(II) complex is 5.82 BM is confirms the distorted octahedral geometry [22] (Table-4).



<sup>1</sup>**H NMR:** <sup>1</sup>**H NMR** spectra of free ligand and its metal complexes were recorded in DMSO- $d_6$ . The multiplet which extends from  $\delta 6.7$  to  $\delta 7.3$  corresponds to the 7 protons of the aromatic rings [23]. The ligand NMR spectrum has a singlet peak at  $\delta 8.8$  ppm suggest the presence of CH=N- linkage (Fig. 3). The peak at  $\delta 13.2$  ppm indicates the phenolic hydroxyl



Fig. 3. <sup>1</sup>H NMR spectra of ligand (*E*)-2-(((3-hydroxyphenyl)imino)methyl)-6-methoxyphenol

proton signal at  $\delta$  3.81 ppm corresponds to  $-OCH_3$  proton [24]. The position of phenolic OH peak at  $\delta$  = 13.2 ppm in the ligand is unchanged in the complexes indicating the involvement of phenolic OH group in coordination with the metal without deprotonation [25]. In addition, the number of protons calculated from the integration curves and those obtained from the values of the CHN analysis, agree well with each other.

**Thermal analysis:** Thermogravimetric curves of the complexes were recorded heating rates of 10 °C/min under nitrogen atmosphere in the temperature range from room temperature to 800 °C. The complex is stable upto 350 °C as shown in Fig. 4. This excludes the possibility of lattice as well as coordinated water molecules in the complex. Above this temperature, decomposition begins slowly. A gradual weight loss starts from 300 °C which corresponds to the decomposition of the Schiff base ligand from the metal chelates. The horizontal thermal curve observed above 540 °C corresponds to the step by step removal of the ligand and the conversion of metal to a metal oxide residue. Thus the overall thermogravimetric results were consistent with the formulation of the complex along with the conclusion derived from infrared and mass spectral studies.

ELECTRONIC SPECTRAL AND MAGNETIC SUSCEPTIBILITY DATA OF SCHIFF BASE AND ITS METAL COMPLEXES						
Schiff base & complexes –	Electronic sp	pectral data	$\mu_{ m eff}$			
	Frequency (cm <sup>-1</sup> )	Transition	Calculated	Observed		
L	47431 43767, 32235	π-π* n-π*	-	-		
[Co(L <sub>2</sub> -2H)]	39384, 36847 30137	Intra ligand ${}^{4}A_{2g} \rightarrow {}^{4}T_{1g}$	5.23	5.14		
[Mn(L <sub>2</sub> -2H)]	36182 30120	Intra ligand ${}^{6}A_{1g} \rightarrow {}^{2}T_{2g}$	5.85	5.82		

TABLE-4



Based on the above interpretation the probable structure of complexes proposed in the present work is given in Fig. 5.



Fig. 5. Structure of metal complexes

Cyclic voltammetry measurements: Electrochemical cyclic voltammetry measurements were performed for all the complexes at room temperature in an air tight three electrode cell by using Glassy carbon electrode with 0.071 cm<sup>2</sup> surface area as a working electrode, a platinum wire served as the counter electrode and a Ag/AgCl in a saturated KCl solution as reference electrode. The electrochemical reaction cell was charged with DMF solution of each complex  $(1 \times 10^{-4} \text{ M})$  and tetra butyl ammonium perchlorate (0.1 M) as supporting electrolyte [26]. Cyclic voltammetric measurements were made over a potential range between -2.7 V to +2.1 V for all the complexes with a scan rate of 0.2 Vs<sup>-1</sup>. The important parameters are calculated from cyclic voltammogram and are shown in Table-5. The cyclic voltammogram obtained for Mn(II) complex (Fig. 6) inferred that the redox potential value is metal centered, one electron quasi reversible, since the  $\Delta Ep$  value greater than 100 mV (160 mV). The cyclic voltammogram obtained for Co(II) complex (Fig. 7), inferred that the redox potential value is metal centered, one electron irreversible.





Fig. 6. Cyclic voltammogram for [Mn(L<sub>2</sub>-2H)] complex



Fig. 7. Cyclic voltammogram for [Co(L2-2H] complex

Antimicrobial activities of the complexes: Antibacterial and antifungal activity of Schiff base and its metal complexes (Table-6) have been tested by disc diffusion technique [27,28]. Various gram positive and gram negative bacterial organisms such as gram negative bacteria (Klebsiella aurogenes) and fungi (Mucor) are used to find out the antimicrobial activity. The results were compared with standard drug ciprofloxacin 5 mg/disc and nystatin 100 mg/disc for fungi. It is evident that overall potency of the ligand is enhanced on coordination with metal ion. Both metal complexes showed a remarkable biological activity against bacteria and fungus. Hence it is evident that overall potency of uncoordinated ligand is enhanced on coordination with metal ion. Thus, both Mn(II) and Co(II) complexes were observed to be more active against *Mucor* and Klebsiella aerogenes. The enhancement in antimicrobial activity of the metal complexes as compared to Schiff base may be explained on the basis of Tweedy's chelation theory.

TABLE-6 in vitro CYTOTOXICITY ASSAY OF [Co(L2-2H)] AGAINST MCF-7 CELL LINE				
Concentration (µm) Cell inhibition (%)				
0.25	0.200535			
2.5	2.339572			
25	12.36631			
50	66.24332			
100 85.36096				
IC 42 (7 / I. D? 0.0041				

$IC_{50} = 42.67$	µg/mL; R	$^{2} = 0.9841$
20		

TABLE-5							
ELECTROCHEMICAL DATA OF SCHIFF BASE METAL COMPLEXES							
Complex	$E_{pa}\left(mv ight)$	$E_{pc}(mv)$	$\Delta E_{p} (mv)$	ip <sub>a</sub> (× 10 <sup>-5</sup> A)	ip <sub>c</sub> (× 10 <sup>-5</sup> A)	$ip_{a}/ip_{c} (\times 10^{-5} A)$	
[Co(L <sub>2</sub> -2H)]	-300	-	-	5.0	-	-	
$[Mn(L_2-2H)]$	-300	-1000	700	2.5	-0.35	-7.1	

According to Tweedy's chelation theory, chelation reduces the polarity of the metal ion mainly because of the partial sharing of the positive charge with the donor groups present in the ligand and possibly the electron delocalization within the whole chelate ring system thus formed during coordination.

The metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The variation in the activity of different complexes against different organisms depend either on the impermeability of the cells of the microbes or difference in ribosomes of microbial cells. Apart from this, other factors such as solubility, conductivity may also be the possible reasons for increasing this activity.

**Cytotoxic activity of Co(II) complex:** The metal complexes may also be a vehicle for activation of the ligand as the cytotoxic agent. The *in vitro* cytotoxicity of the newly-synthesized complexes with remarkable antimicrobial activity were carried out in three different human tumor cell lines. Cell line namely human breast cancer cell line (MCF-7) were assayed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay [29]. The complexes were initially dissolved in neat dimethyl sulfoxide to make a stock solution and additional five series of dilutions (6.25, 12.5, 25, 50 and 100  $\mu$ M) were made to provide a total of five sample concentrations. The concentration of the compounds at % cell ihibition growth (IC<sub>50</sub>) was calculated. The percentage cell inhibition was determined using the following formula:

Cell inhibition (%) = 
$$\left(100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}}\right) \times 100$$

Non-linear regression graph was plotted between % cell inhibition and log concentration and IC<sub>50</sub> was determined using GraphPad Prism software [30].

The Co(II) complex derived from (*E*)-2-(((3-hydroxyphenyl)imino)methyl)-6-methoxyphenol shows an excellent cytotoxicity towards human breast cancer cell (MCF-7). The complex exhibit considerable IC<sub>50</sub> value 42.67 µg/mL. The concentration of the complex at 50 % cell growth was inhibited and IC<sub>50</sub> was calculated (Table-6). The better cytotoxicity activity of Co(II) complex against (MCF-7) may play a significant role in metallodrug formulation.

Fluorescence quenching studies of  $[Co(L_2-2H]]$ : The excited state interaction of  $[Co(L_2-2H]]$  with alizarin red S dye was carried out through fluorescence measurements. Fig. 8 shows the emission spectrum of  $[Co(L_2-2H]]$  complex measured in DMSO and the complex was effectively quenched by increasing the concentration of alizarin red S dye  $(0-5 \times 10^{-5} M)$ . The observed quenching is entirely due to interaction of complex with alizarin red S and not due to inner filter effect or re absorption. Hence the binding constant for this type of interaction was calculated using fluorescence quenching data using the formula [31]:

$$\frac{1}{(F^0 - F)} = \frac{1}{(F^0 - F')} + \frac{1}{K(F^0 - F)[Q]}$$

where K is the binding constant,  $F^0$  is the initial fluorescence intensity of the complex, F' is the fluorescence intensity of alizarin red S adsorbed Co(II) complex and F is the observed

fluorescence intensity at its maximum. The plot of  $1/(F^0-F)$ versus 1/[Q] gives a straight line (inset Fig. 8) and from the slope the calculated binding constant  $(K_b)$  are found to be 7.6  $\times 10^3$  for the complex. Thus there is a good linear relationship [R = 0.997] between  $1/(F^0-F)$  and the reciprocal concentration of alizarin red S. The fluorescence quenching can be enlightened by a number of possible mechanisms such as electron transfer, energy transfer, proton transfer or hydrogen atom transfer. For energy transfer, there should be an overlap between emission spectrum of donor complex and absorption spectrum of the acceptor (alizarin red S). Since there is no wavelength overlap between absorption of acceptor (alizarin red S) and emission of donor (complex), energy transfer between donor and acceptor is negligible. The free energy change can be calculated for the above mechanisms which can be used to predict the feasibility of the reaction [32].



Fig. 8. Fluorescence quenching of [Co(L<sub>2</sub>-2H)] complex by alizarin red S dye Inset: plot of 1/(F<sup>0</sup>-F) vs. 1/[Q]

#### Conclusion

Synthesis, spectral characterization, electrochemical, photochemical behaviour and in vitro biological evaluation of transition metal complexes containing Schiff base of 2-aminophenol and 2-hydroxy-3-methoxybenzaldehyde has been described. The low molar conductance values indicate the absence of anion outside the sphere. Schiff base behaves as a tridentate N and O donors. The presence of v(M-O) and v(M-N) bonds in the complexes have been confirmed by IR spectra. The absence of water molecules has been confirmed by thermal analysis. Based on spectral studies, octahedral geometry has been proposed for the metal complexes. The comparison of antimicrobial activities of Schiff base and its metal complexes indicates that the Schiff bases have been found to be biologically active and most of their metal complexes exhibit significantly enhanced antimicrobial activities against the microorganisms. It is observed that the potency to act against the cancerous cell is highly influenced by the nature of metal ion present in the chelate. The feasibility of the reaction has been established from fluorescence quenching studies.

### REFERENCES

- 1. L. Mishra and S. Ragini, Indian J. Chem., 29A, 1131 (2000).
- 2. J. Mannad and J.C. Crabbe, Bacterial and Antibacterial Agents, Spectrum Academic Publishers, Oxford (1996).
- A.M. Abu-Dief, I.M.A. Mohamed, Beni-Suef Univ. J. Basic Appl. Sci., 4, 119 (2015); https://doi.org/10.1016/j.bjbas.2015.05.004.

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- G. Valarmathy and R. Subbalakshmi, Asian J. Chem., 25, 2077 (2013); https://doi.org/10.14233/ajchem.2013.13322.
- 5. R.K. Dubey, J. Indian Chem. Soc., 83, 1087 (2006).
- P.S. Reddyb B. Satyanarayana and Jayatyagaraju, J. Indian Chem. Soc., 83, 1204 (2006).
- B.T. Thaker and R.S. Barvalia, J. Coord. Chem., 63, 1597 (2000); https://doi.org/10.1080/00958972.2010.483767.
- V. Govindaraj and S. Ramanathan, *Turk. J. Chem.*, 38, 521 (2014); <u>https://doi.org/10.3906/kim-1301-83</u>.
- V. Gomathi, R. Selvameena, R. Subbalakshmi and G. Valarmathy, *Orient. J. Chem.*, 29, 533 (2013); https://doi.org/10.13005/ojc/290220.
- 10. G. Valarmathy and R. Subbalakshmi, *Indian J. Appl. Res.*, **3**, 43 (2013); https://doi.org/10.15373/22495555X/APR2013/14.
- 11. P.C.S. Rani, L. R.K and G.S. Thara, *Orient. J. Chem.*, **29**, 315 (2013); https://doi.org/10.13005/ojc/290151.
- 12. G. Valarmathy and R. Subbalakshmi, *Int. J. Pharm. Pharm. Sci.*, **5**, 368 (2013).
- W.J. Geary, Coord. Chem. Rev., 7, 81 (1971); <u>https://doi.org/10.1016/S0010-8545(00)80009-0</u>.
- 14. L.N. Sharda and M.C. Ganokar, Indian J. Chem., 27A, 617 (1988).
- 15. P. Chattopadhyay and C. Sinha, Indian J. Chem., 34A, 76 (1995).
- T.R. Rao, S. Shrestha, A. Prasad and K.K. Narang, *Synth. React. Inorg. Met.-Org. Chem.*, **32**, 419 (2002); <u>https://doi.org/10.1081/SIM-120003216</u>.
- 17. R.C. Maurya and P. Patel, *Spec. Lett.*, **32**, 213 (1999); https://doi.org/10.1080/00387019909349979.
- P.P. Bhargava, R. Bembi and M. Tyagi, J. Indian Chem. Soc., 60, 214 (1983).

- R.C. Maurya, D.D. Mishra, S. Mukherjee and J. Dubey, *Polyhedron*, 14, 1351 (1995);
  - https://doi.org/10.1016/0277-5387(94)00394-T.
- J.D. Lee, Concise Inorganic Chemistry, Blackwell Science Publishers, edn 5, p. 967, (1999).
- 21. H.R. Singh and B.V. Agarwala, J. Indian Chem. Soc., 65, 591 (1988).
- B.V. Agarwala, S. Hingorani, V. Puri, C.L. Khetrapal and G.A. Nagangowda, *Transition Met. Chem.*, **19**, 25 (1994); <u>https://doi.org/10.1007/BF00166260</u>.
- 23. D.J. Pasto, Organic Structure Determination, Prentice Hall, International, London (1969).
- Z.H. Chohan and M.M. Naseer, *Appl. Organomet. Chem.*, 21, 728 (2007); https://doi.org/10.1002/aoc.1279.
- 25. G.A. Mabbot, J. Chem. Educ., **60**, 697 (1983); https://doi.org/10.1021/ed060p697.
- 26. Indian Pharmacopoeia, IIA, 105 (1996).
- R. Cruickshank, J.O. Dugid, B.P. Marnion and R.H. Swain, Medical Microbiology, Churchill Livingston Publication, New York, edn 12, vol. II, p. 98 (1975).
- T. Mosmann, J. Immunol. Methods, 65, 55 (1983); <u>https://doi.org/10.1016/0022-1759(83)90303-4</u>.
- M. Navarra, M. Celano, J. Maiuolo, S. Schenone, M. Botta, A. Angelucci, P. Bramanti and D. Russo, *BMC Cancer*, **10**, 602 (2010); <u>https://doi.org/10.1186/1471-2407-10-602</u>.
- H.R. Park, C.H. Oh, H.C. Lee, J.G. Choi, B.I. Jung and K.M. Bark, *Bull. Korean Chem. Soc.*, 27, 2002 (2006); https://doi.org/10.5012/bkcs.2006.27.12.2002.
- S. Jagadeeswari, M.A. Jhonsi, A. Kathiravan and R. Renganathan, J. Lumin., 131, 597 (2011); https://doi.org/10.1016/j.jlumin.2010.10.037.