

Biologically active N/O/S donor ligands: Synthesis, characterization and biological activity with Metal chelates

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ABSTRACT

In the present study, we synthesized ternary metal complexes with few N/O/S donor Carbazole(CZ), phenothiazine(PT), anthranilic acid(AA), Proline(PR), acridone(AR) and phenazone(PN) ligands. The colored complexes were prepared by the addition of chloride salts of copper(II), cobalt(II) and manganese(II) to a solution of ligands. In conclusion, the structures of the obtained complexes were characterized by FT-IR, elemental analysis, UV-spectral studies conductmetric and magnetic susceptibility measurements. The synthesized metal complexes were investigated for biological activities. Enzymatic inhibition activity has been done by using acetylcholinesterase (AChE) and also antioxidant activity by DPPH assay. All tested metal complexes reveal effective biological activities against enzyme inhibition and free radical scavenging activity significantly.

Keywords: N/O/S donor ligands, Metal complexes, Enzymatic activity, acetylcholinesterase (AChE), Antioxidant activity.

1. INTRODUCTION

The enzyme acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine to acetate and choline at the cholinergic synapses, terminating nerve impulse transmission. It is known that AChE is effectively inhibited by organophosphate and carbamate pesticides, and also by metals [1]. Ternary metal complexes formed between metal ions and two different types of bio ligands, viz., heteroaromatic nitrogen base ligands. Chelating N/O/S donor play an important role in inorganic chemistry as they easily form stable complexes with most transition metal ions [2,3]. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species [4, 5]. Cu(II) amino acids complexes, in recent years have been receiving much attention due to their useful antibacterial agents. Copper-histidine treatment has been used for Menkes disease [6]. the synthesis and characterization of mixed ligand Cu(II), Ni(II) and Co(II) complexes of hippuric acid and nitrilotriacetic acid/ iminodiacetic/ histidine have been reported. Synthesis, structural, coordination and thermal stability studies of mixed ligand complexes of histidine with other ligands (adenine/guanine, ethylenediamine, diethylenetriamine or N,N,N',N'', N''-pentamethyldiethylenetriamine, glycine or L-

alanine, histamine and diethylglyoxime) [7] have also been carried out. During the 1970s–1980s, antipyrine complexes with certain metal ions, including Pt(II) and Co(II) ions, have been shown to act as antitumoral agents [8].

Mixed-ligand complexes containing, in addition to these Mannich bases, relevant biological molecules like imidazole [9], 2-aminobenzothiazole [10], or mercaptobenzothiazole [11] as coligands have been obtained and it has been demonstrated that their structure is designed by the big binucleating ligands BAMP and TAMEN. For some of the previously cited complexes cytotoxic/antiproliferative effects were proved [12]. Also, their toxicity was tested on marine Hydrozoon Hydractinia echinata [13]. As continuation of the work in this field, we report here the synthesis, characterization and biological activity with copper(II), cobalt(II) and manganese(II) cations. Evaluation of enzyme inhibition activity by using acetylcholine enzyme and also antioxidant activity by using *in vitro* assays such as 2, 2'-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity for the newly synthesized ligand and their metal complexes was undertaken.

2. EXPERIMENTAL

2.1. Materials and methods

All the reagents were of AR grade and used as received from Merck reagent, Germany, BDH, England and Sigma-Aldrich chemical company, USA. Melting point of the complexes were determined using Thomas Hoover apparatus and uncorrected. Molar conductance of the complexes were determined using Digisum Electronics DI-909/9009 direct reading digital conductivity meter, consisting of a cell with cell constant=0.9. IR spectra were recorded using Shimadzu 8201 PCFT-IR spectrometer by KBr technique. Magnetic susceptibility measurements were done using Gouy balance consisting of the type NP-53 electrometer with DC power supply type NP-1053 and Keroy semi microbalance supplied by Universal scientific company, Mumbai, India. Elemental analyses were done using Vario-EL III CHN and S elemental analyzer.

2.2. Synthesis of complexes

2.2.1. Synthesis of $[\text{Cu}(\text{PT})(\text{CZ})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 1a

One millimoles of metal(II) chloride (1mmol) dissolved in 10mL ethanol was treated with solid Carbazole (1mmol) and phenothiazine (1mmol) dissolved in ethanol (10 mL) at 80-90 °C under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 85%. M.P: 205-210 °C, Anal. Calcd for: $[\text{Cu}(\text{C}_{24}\text{H}_{18}\text{N}_2\text{S})(\text{H}_2\text{O})_2(\text{Cl})_2]$, C, 53.68; H, 4.13; Cl, 13.21; Cu, 11.83; N, 5.22; O, 5.96; S, 5.97. In the low-frequency region, one bands were observed for complexes at ~445-450 cm^{-1} which were attributed to $\nu(\text{M-N})$.

2.2.2. Synthesis of $[\text{Co}(\text{PT})(\text{CZ})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 1b

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid Carbazole (1mmol) and phenothiazine (1mmol) dissolved in ethanol (10 mL) at 80-90 °C under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 80%. M.P: 196-210 °C, Anal. Calcd for: $[\text{Co}(\text{C}_{24}\text{H}_{18}\text{N}_2\text{S})(\text{H}_2\text{O})_2(\text{Cl})_2]$, C, 54.15; H, 4.17; Cl, 13.32; Co, 11.07; N, 5.26; O, 6.01; S, 6.02. In the low-frequency region, two bands were observed for complexes at ~ 450-455 cm^{-1} which were attributed to $\nu(\text{M-N})$.

2.2.3. Synthesis of $[\text{Mn}(\text{PT})(\text{CZ})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 1c

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid Carbazole (1mmol) and phenothiazine (1mmol) dissolved in ethanol (10 mL) at 80-90 °C under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 75%. M.P: 180-190 °C, Anal.

Calcd for: $[\text{Mn}(\text{C}_{24}\text{H}_{18}\text{N}_2\text{S})(\text{H}_2\text{O})_2(\text{Cl})_2]$, C, 54.56; H, 4.20; Cl, 13.42; Mn, 10.40; N, 5.30; O, 6.06; S, 6.07. In the low-frequency region, two bands were observed for complexes at ~ 440-450 cm^{-1} which were attributed to $\nu(\text{M-N})$.

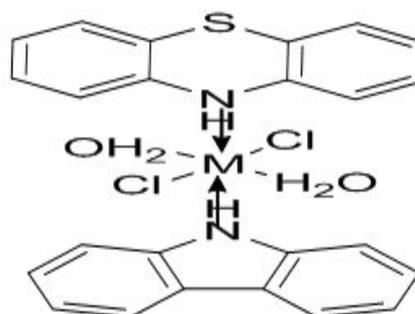


Figure 1: General structure of metal complexes 1(a-c)

2.2.4 Synthesis of $[\text{Cu}(\text{AA})(\text{PR})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 2a

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid anthranilic acid (1mmol) and Proline (1mmol) dissolved in ethanol/1:1 ethanol (10 mL) at 80-90 °C under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 79%. M.P: 203-208 °C, Anal. Calcd for: $[\text{Cu}(\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4)(\text{Cl})_2]$, C, 39.06; H, 4.29; Cl, 17.74; Cu, 15.90; N, 7.01; O, 16.01. In the low-frequency region, two bands were observed for complexes at ~ 450 and 465 cm^{-1} , which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

2.2.5. Synthesis of $[\text{Co}(\text{AA})(\text{PR})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 2b

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid anthranilic acid (1mmol) and Proline (1mmol) dissolved in ethanol/1:1 ethanol (10 mL) at 80-90 °C under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 82%. M.P: 190-192 °C, Anal. Calcd for: $[\text{Co}(\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4)(\text{Cl})_2]$, C, 39.52; H, 4.34; Cl, 17.95; Co, 14.92; N, 7.09; O, 16.20. In the low-frequency region, two bands were observed for complexes at ~440 and 450 cm^{-1} , which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

2.2.6 Synthesis of $[\text{Mn}(\text{AA})(\text{PR})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 2c

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid anthranilic acid (1mmol) and Proline (1mmol) dissolved in ethanol/1:1 ethanol (10 mL) at 80-90 °C under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 70%. M.P: 189-195

$^{\circ}\text{C}$, Anal. Calcd for: $[\text{Mn}(\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_4)\text{Cl}_2]$. C, 39.92; H, 4.38; Cl, 18.13; Mn, 14.05; N, 7.16; O, 16.36. In the low-frequency region, two bands were observed for complexes at ~ 450 and 460 cm^{-1} , which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

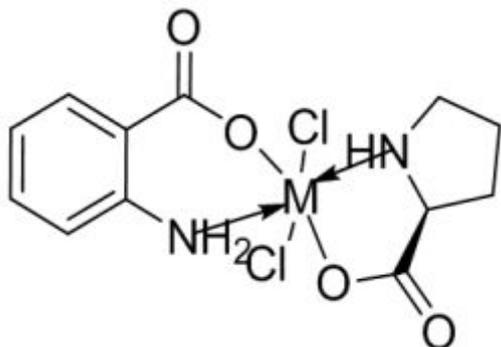


Figure II: General structure of metal complexes 2(a-c)

2.2.7. Synthesis of $[\text{Cu}(\text{AR})(\text{PN})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 3a

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid acridone(1mmol) and phenazone(1mmol) dissolved in ethanol/1:1 ethanol (10 mL) at $80\text{-}90\text{ }^{\circ}\text{C}$ under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 88%. M.P: $205\text{-}210\text{ }^{\circ}\text{C}$, Anal. Calcd for: $[\text{Cu}(\text{C}_{24}\text{H}_{21}\text{N}_3\text{O}_2)(\text{H}_2\text{O})_2\text{Cl}_2]$, Elemental Analysis: C, 52.04; H, 4.55; Cl, 12.80; Cu, 11.47; N, 7.59; O, 11.55. In the low-frequency region, two bands were observed for complexes at ~ 445 and 460 cm^{-1} , which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

2.2.8. Synthesis of $[\text{Co}(\text{AR})(\text{PN})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 3b

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid acridone(1mmol) and phenazone (1mmol) dissolved in ethanol/1:1 ethanol (10 mL) at $80\text{-}90\text{ }^{\circ}\text{C}$ under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 80%. M.P: $190\text{-}192\text{ }^{\circ}\text{C}$, Anal. Calcd for $[\text{Co}(\text{C}_{24}\text{H}_{21}\text{N}_3\text{O}_2)(\text{H}_2\text{O})_2\text{Cl}_2]$. C, 52.48; H, 4.59; Cl, 12.91; Co, 10.73; N, 7.65; O, 11.65. In the low-frequency region, two bands were observed for complexes at ~ 445 and 460 cm^{-1} , which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

2.2.9. Synthesis of $[\text{Mn}(\text{AR})(\text{PN})(\text{H}_2\text{O})_2(\text{Cl})_2]$ 3c

One millimoles of metal(II) chloride (1mmol) dissolved in 15mL ethanol was treated with solid acridone(1mmol) and

phenazone(1mmol) dissolved in ethanol/1:1 ethanol (10 mL) at $80\text{-}90\text{ }^{\circ}\text{C}$ under vigorous stirring. The reaction was finished after 2-3 h. The solid product was filtered off, washed with ethanol, and dried over calcium chloride in air. Yield: 76%. M.P: $190\text{-}192\text{ }^{\circ}\text{C}$, Anal. Calcd for: Yield: 80%. M.p: $188\text{-}196\text{ }^{\circ}\text{C}$, $[\text{Mn}(\text{C}_{24}\text{H}_{21}\text{N}_3\text{O}_2)(\text{H}_2\text{O})_2\text{Cl}_2]$. C, 52.86; H, 4.62; Cl, 13.00; Mn, 10.07; N, 7.71; O, 11.74. In the low-frequency region, two bands were observed for complexes at ~ 445 and 460 cm^{-1} , which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

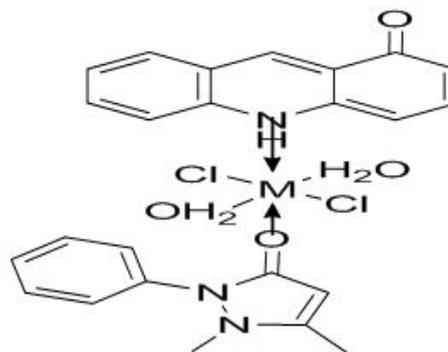


Figure III: General structure of complexes 3(a-c)

2.3. Biological activity

2.3.1. Enzymatic Activity (Acetylcholine esterase activity (AChE))

Procedure

Acetylcholine esterase activity was assessed as described by and modified by Briefly, claw muscles were homogenised on ice in 20 mM phosphate buffer, pH 7.0, 0.1% Triton X-100 (1/3 volume/weight) using an Ultra-Turax homogenizer and centrifuged for 20 min at 10000g. The acetylcholine esterase activity of 100 μL supernatant in presence of 0.01 M dithionitrobenzoic acid (DTNB) as reagent and 2.8 mM acetylthiocholine iodide (AcSCh) as substrate was then measured at 412 nm using a microplate reader at a controlled temperature of $20\text{ }^{\circ}\text{C}$. No attempt was made to distinguish between AChE and pseudo-cholinesterase. The activity of the enzyme was expressed in nmol of substrate hydrolysed per minute per milligram of protein.

2.3.2. Antioxidant evaluation

Procedure

The evaluation of antioxidant activity of newly synthesized compounds was done by DPPH radical scavenging activity assay ^[14]. Internal standard BHA and the synthesized compounds of different concentrations were prepared in distilled ethanol, 1 mL of each compound solutions having different concentrations (10 μM , 25 μM , 50 μM , 100 μM , 200 μM and 500 μM) were

taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decrease in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

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

Where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound.

3. Results and discussion

3.1. Synthesis

The Cu(II), Co(II) and Mn(II) complexes containing metal salts with Carbazole(CZ), phenothiazine(PT), anthranilic acid(AA), Proline(PR), acridone(AR) and phenazone(PN) the corresponding ligands in ethanol. The nature of the products seem to depend on the molar ratio of the reactants but, the best yields have been obtained for the molar ratio M:L(1:2) as described in the experimental part [15]. The complexes are stable in dry air, insoluble in most common solvents like water, acetone, dichloromethane, and ethyl ether, but they are soluble in DMSO and DMF. The complexes have been formulated based on elemental analyses, IR spectroscopy, conductometric and magnetic susceptibility measurements.

3.2. General characterization

3.2.1. Infrared spectroscopy

IR spectra of the new metal complex as reveal the following points.

- The spectra in the region 3350-3370 cm^{-1} of all the complexes show the characteristic vibrations of water molecule of the complexes.
- IR data supports the involvement of the antipyrine oxygen in coordination of metal chelates [16]. The spectra shows a band at 445-460 cm^{-1} and also IR data supports the involvement of nitrogen of Anthranillic acid and also nitrogen

containing moieties [17,18] which shows the spectra at 430-450 cm^{-1} .

- The metal-chloride vibrations were identified in the range 305-360 cm^{-1} [19].

3.2.2. Molar conductance studies

The molar conductivity values in the range 15.25-22.00 $\text{ohm}^{-1} \text{cm}^2$ in DMSO denote that all the complexes behave as 1 : 2 electrolytes in these solvents [20] and all metal complexes shows non-electrolytic characteristic property.

3.2.3. Electronic spectra

Electronic spectra in the metal complexes, bands below ~340 nm are attributed to intraligand transition [21]. A small shift should be observed for the second band in all complexes, these $\pi \rightarrow \pi^*$ transitions probably involving metal and ligand orbitals. Bands above ~340 nm are ascribed to charge transfer processes, probably from ligand to metal and mainly associated with the amine and acidic groups. Absorption bands at higher wavelengths are due to d-d transitions [22].

3.2.4. Magnetic Susceptibility

The magnetic susceptibility values of the complexes show paramagnetic $d9$ configuration of Cu^{2+} complexes, while the Co^{2+} complexes are also paramagnetic shows $d7$ configuration and Mn^{2+} enrich the $d5$ configuration with paramagnetic behavior. The measured values for the Cu^{2+} , Co^{2+} and Mn^{2+} complexes are 1.39, 3.75 and 5.81 B.M., respectively. These values show that the Cu^{2+} , Co^{2+} and Mn^{2+} complexes are octahedral in nature. All data agree with Arslan *et al.* [23].

3.2.5. Enzymatic activity

In present investigation, we studied the Ache enzyme inhibition activity percentage of our synthetic compounds for the discovery of new acetyl cholinesterase inhibitors. Various structural studies reveals that the specific positioning of the nitrogen atom and the planar structure of the metal complexes in relation to the aromatic ring will shows better inhibition activity and it is essential for the strong affinity between the antagonists and the receptor. Examined the overlap factor (HOMO) through an indirect approach receptor mapping [24]. According to his calculations, the metal complexes of 1a, 1b, 3a, 3b and 3c are very active compounds and the metal complexes of 1c and 2a shows moderate enzymatic inhibition activity the data shown in Table 3 and Figure IV.

Table - 1: Physical, spectral and analytical data of the metal chelate

Compounds	Mol. Formula	Mol.wt	M.p. °C	Calc. (found) (%)				Yield (%)	μ_{eff} (B.M.)
				C	H	N	O		
1a	[Cu(C ₂₄ H ₁₈ N ₂ S)(H ₂ O) ₂ Cl ₂]	536.96	205-210	52.00 (52.85)	4.45 (3.92)	5.32 (4.23)	5.81 (4.12)	85	1.39
1b	[Co(C ₁₂ H ₁₆ N ₂ O ₄)Cl ₂]	532.34	196-210	54.40 (53.64)	4.28 (3.75)	5.74 (4.65)	6.35 (5.84)	80	3.75
1c	[Mn(C ₁₂ H ₁₆ N ₂ O ₄)Cl ₂]	528.35	180-190	54.15 (53.81)	4.51 (3.91)	5.71 (5.01)	6.63 (5.56)	75	5.81
2a	[Cu(C ₁₂ H ₁₆ N ₂ O ₄)Cl ₂]	399.73	203-208	39.13 (38.57)	4.71 (3.51)	7.10 (6.74)	16.44 (15.23)	79	1.29
2b	[Co(C ₁₂ H ₁₆ N ₂ O ₄)Cl ₂]	395.12	195-205	39.74 (39.04)	4.76 (3.46)	7.83 (6.23)	16.50 (15.14)	82	3.80
2c	[Mn(C ₁₂ H ₁₆ N ₂ O ₄)Cl ₂]	391.12	189-195	39.02 (39.11)	4.68 (3.62)	7.76 (6.48)	16.27 (15.74)	70	5.74
3a	[Cu(C ₂₄ H ₂₁ N ₃ O ₂)(H ₂ O) ₂ Cl ₂]	553.92	205-210	52.74 (51.20)	4.15 (3.14)	7.89 (6.14)	11.85 (10.86)	88	1.30
3b	[Co(C ₂₄ H ₂₁ N ₃ O ₂)(H ₂ O) ₂ Cl ₂]	549.31	190-192	52.18 (50.98)	4.39 (4.03)	7.70 (6.23)	11.85 (10.12)	80	3.75
3c	[Mn(C ₂₄ H ₂₁ N ₃ O ₂)(H ₂ O) ₂ Cl ₂]	545.31	188-196	52.86 (52.00)	4.12 (3.51)	7.55 (6.82)	11.62 (10.27)	76	5.68

Table - 2: IR spectra and Molar conductance data of the metal complexes

Compounds	(M-N)(Cm ⁻¹)	(M-O)(Cm ⁻¹)	(M-Cl)(Cm ⁻¹)	(H ₂ O)(Cm ⁻¹)	Molar conductance (ohm's cm ² mol ⁻¹)
1a	445-450	--	350-360	3365-3360	18.7
1b	450-455	--	345-355	3355-3340	16.9
1c	440-450	--	342-350	3358-3362	21.2
2a	450-460	465-470	323-330	3364-3355	17.65
2b	440-455	445-460	341-360	3362-3352	15.23
2c	450-455	455-465	320-335	3363-3360	18.45
3a	445-455	450-460	345-3500	3360-3350	19.24
3b	445-460	465-470	348-355	3367-3355	14.31
3c	445-450	455-465	320-335	3361-3355	16.25

3.2.6. Antioxidant Activity

In the present program, we synthesized new metal complexes with different ligands and these complexes undergone Antioxidant activity by using DPPH assay. We tested all metal complexes with DPPH assay method and the

complexes of 2a, 2b, 2c and 1a shows effective free radical scavenging property and the remaining complexes shows moderate free radical scavenging activity. The reason would be the presence of electron releasing hydroxyl and electron donating Nitrogen groups in ligand moiety endowed notable improvement in radical scavenging activity. After the complexation with

metal ions reveals that the antioxidant activity increase due to the presence of positively charged metal ions as well as different electron withdrawing and electron donating groups present in the moiety as shown in Table 4 and Figure V.

Table - 3: Enzymatic Inhibition percentage inhibition of metal complexes

Compounds	% Inhibition
1a	51.89
1b	44.91
1c	48.61
2a	40.54
2b	38.07
2c	27.56
3a	29.45
3b	27.42
3c	32.61

Table - 4: IC₅₀ values of metal complexes

Compounds	IC ₅₀ values
1a	5.01
1b	3.98
1c	4.80
2a	6.01
2b	6.65
2c	7.05
3a	4.07
3b	5.28
3c	4.65
Ascorbic acid	8.25

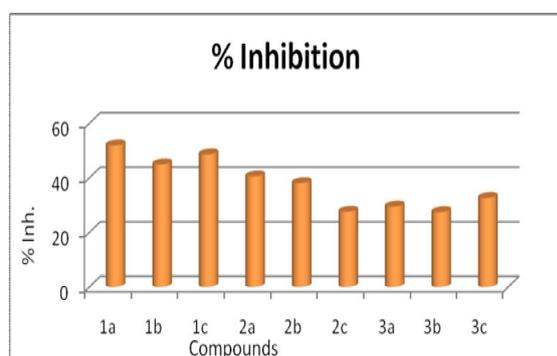


Figure IV: Enzymatic inhibition percentage of metal complexes

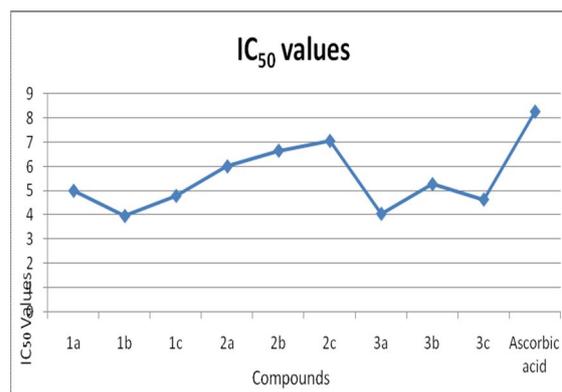


Figure V: Antioxidant activity of metal complexes

4. CONCLUSIONS

The N/S/O chelating metal complexes [M(PT)(CZ)(H₂O)₂(Cl)₂] 1(a-c), [M(AA)(PR)(H₂O)₂(Cl)₂] 2(a-c), [M(AR)(PN)(H₂O)₂(Cl)₂] 3(a-c) have been obtained by the reaction of metal salts with the corresponding chelating ligands. Their formulae have been proposed on the basis of elemental analyses and agree well with the Spectral properties. Based on physico-chemical data, as well as our biological investigation of metal complexes shows that the enzymatic inhibition efficacy of these complexes and also the free radical scavenging activity shows effective results.

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