

A New Perspective of Ligands and their Metal Chelates for Anthelmintic Activity

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Abstract

Metal chelates of isomeric 1,2-Naphthoquinone mono oxime were prepared with Oxo metals like ammonium molybdate and sodium tungstate in a methanolic solution. The chemical composition was established as ML_2 . The structural investigations of the chelates were carried out by analytical tools such as TGA, XRD, SEM and elemental analysis. Further investigations were performed by spectral methods such as IR, Far IR, UV-VIS, 1H NMR and ^{13}C NMR. The ligands and their metal chelates of ammonium molybdate $[(NH_4)_6MoO_{24} \cdot H_2O]$ and sodium tungstate $[Na_2WO_4]$ exhibited significant biological activity. Therefore, the anthelmintic activity was performed. The results are indicative of the medicinal importance to the human health fare.

Key words: 1,2-Naphthoquinone(NQ)-1- oxime, 1,2-Naphthoquinone(NQ)-2- oxime, Ammonium molybdate, Sodium tungstate, Anthelmintic activity

1. Introduction

1,2-naphthoquinone mono oxime exists in two tautomeric forms known as the “quinone oximic form” and the “naphtholic form” [1]. Literature survey illustrates that, these ligands 1,2-Naphthoquinone(NQ)-1-oxime (L1) and 1,2-Naphthoquinone(NQ)-2-oxime (L2) are in oximic form and 1-Nitroso-2-Naphthol and 2-Nitroso-1-Naphthol are in naphtholic form. Oxime exhibits position isomerism resulting due to the exchange of ‘oxime’ and ‘carbonyl’ functional groups in their positions. 1,2-Naphthoquinone mono oximes possess powerful chelating ability [2], as well as useful analytical properties and antimicrobial activity [3]. In organic synthesis, naphthoquinones are the most widely utilized [4]. Oximes are commonly used as ligands. The metal complexes that oxime compounds create through their atoms are quite stable [5]. Oxime and oximate metal complexes have made significant contributions to coordination chemistry in a variety of ways [6].

Oxime complexes are also known to exhibit as sequestering agents for nanoparticle preparation [7] and an artificial sweetener [8]. They display biological activity [9]. Oximes and their derivatives are utilized as chemical inhibitors of enzyme activity, critical pharmaceutical, in clinical and synthetic chemistry applications [10]. Oxo- and dioxo-molybdenum complexes have been extensively investigated as catalysts for a wide range of chemical reactions, most notably sulfoxidation of organic compounds [11]. Smart windows, electrochromic devices, photothermal treatment, and NIR shielding have all been proven to benefit from WO₂ [12]. Anthelmintic is a category of antiparasitic medications that works by killing parasitic worms (helminths) and other internal parasites in the body without causing major harm to the host [13]. The prominent results make an appearance that chelates are potent. This fact depicts that chelates have medicinal potency.

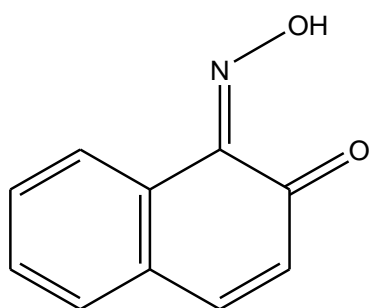


Figure.1 (L1)

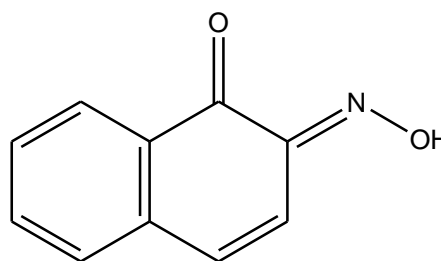


Figure. 2 (L2)

2. Experimental

All chemicals and solvents utilized in this study were of A.R. grade, so they were employed directly without further purification. 1,2-Naphthoquinone mono oxime and metal salt were bought from Loba. C, H, N elemental analysis was estimated by ELEMENTAR Vario EL III. Thermal analysis was performed using SDT Q600 V20.9Build 20. Shimadzu spectrometer was used to record UV-VIS spectra. Infra-red spectra were recorded from a 3000 Hyperion microscope with a vertex 80 FTIR system. ¹H and ¹³C NMR spectra were received from Advance III HD NMR 400 MHz, Bruker spectrometer using DMSO-d₆.

3. Synthesis of metal chelates:

The known methodology was used to make metal chelates as previously stated [14].

3.1 Synthesis of oxo molybdenum 1,2 -NQ-1-oximate (L1Mo) and oxo molybdenum 1,2-NQ-2-oximate (L2Mo) metal chelates:

In a three-necked flask, 0.01M ligand solutions of (L1) and (L2) were added to which an aqueous 0.01M solution of ammonium molybdate (Mo) metal salt solution was added drop by drop while the mixture was constantly stirred with a magnetic stirrer at room temperature. The ratio of metal salt to ligand solution was 1:2. After complete addition, pH was adjusted to around

7 with an aqueous ammonia to obtain the product. The entire mixture was stirred for three hours at room temperature. The mixture was kept in the refrigerator overnight. The brick red coloured amorphous solid (L1Mo) was filtered, washed with distilled water and dried. The yield was recorded as 81.22%. The (L2Mo) exhibited crystalline solids. It was grayish brown in colour. The yield obtained was 79.02%.

3.2 Synthesis of oxo tungsten 1,2-NQ-1-oximate (L1W) and oxo tungsten 1,2-NQ-2-oximate (L2W) metal chelates:

0.01M ligand solutions of (L1) and (L2) were added drop by drop to an aqueous 0.01M solution of sodium tungstate (W) metal salt solution in a three-necked flask, while the mixture was constantly agitated with a magnetic stirrer. Metal salts and ligand solutions were mixed in 1:2 ratios. To obtain the precipitation of the chelate, the pH was adjusted to about 7 using aqueous ammonia. The entire mixture was stirred for three hours at room temperature. The reaction mixture was then chilled overnight before being filtered under suction. It was washed with distilled water and dried. The brown coloured crystalline solid of (L1W) was obtained. The yield was 76.57%. The (L2W) appeared as a crystalline chocolate brown coloured solid. The yield was noted as 74.69%.

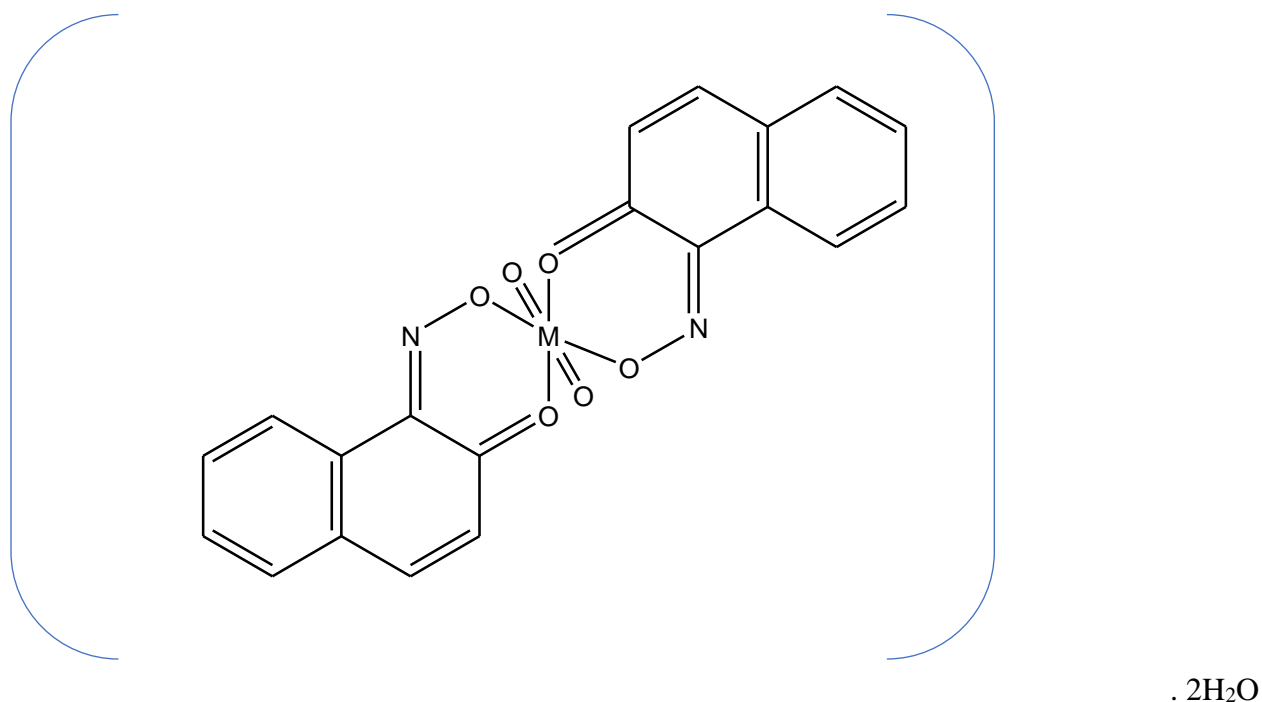


Figure. 3 (M=Mo, W)

4. Anthelmintic activity:

The anthelmintic activity was assessed by the technique as reported previously [15]. It was performed on grown-up earthworms *Eudrilus Eugeniae*, because of its physical and physiological similarity with the gastrointestinal roundworm parasite of individuals [16]. Earthworms gathered from clammy soil and washed with normal saline to eliminate all faeces were utilized for the anthelmintic review. The worms were 8 to 12 cm long and 0.1 to 0.2 cm in width were utilized for the experimental protocol. The chelates were selected for anthelmintic examinations against earthworms at 5, 10 and 15 mg/ml by involving *Albendazole* as a standard. A small amount of DMSO and normal saline water were used as a control. The solution of metal chelate was freshly prepared by dissolving in a very small amount of DMSO and diluted up to 10ml with normal saline water [17]. The time was recorded for the paralyzing and death of earthworms and their mean was determined for three-fold sets. The time of death was determined by immersing the earthworms in warm water (50°C), which stimulated movement if the worm was alive.

5. RESULTS AND DISCUSSION

5.1 Molecular formula, colour, elemental analysis

Table-1 Molecular Formula, Colour, Elemental Analysis Data

Molecular formula	Colour	Elemental analysis - found (calculated)			
		%C	%H	%N	%M
C ₂₀ H ₁₆ O ₈ N ₂ Mo (L1Mo)	Bricked red	47.19 (47.24)	3.04 (3.15)	5.45 (5.51)	18.81 (18.89)
C ₂₀ H ₁₆ O ₈ N ₂ Mo (L2Mo)	Grayish brown	47.31 (47.24)	3.09 (3.15)	5.54 (5.51)	18.90 (18.89)
C ₂₀ H ₁₆ O ₈ N ₂ W (L1W)	Brown	40.01 (40.26)	2.55 (2.68)	4.62 (4.70)	30.84 (30.87)
C ₂₀ H ₁₆ O ₈ N ₂ W (L2W)	Chocolate brown	40.35 (40.26)	2.61 (2.68)	4.75 (4.70)	30.89 (30.87)

Newly synthesized metal chelates of oxo metals were the focus of recent research. Molecular formulas for all metal chelates based on the observations were shows in (Table-1).

5.2 UV- VIS Spectral analysis

Table-2 Uv-Vis Spectral Data of Metal Chelates

Compounds	Benzoid electron transfer		Quinonoid electron transfer		n → π*	
	(nm)	(cm ⁻¹)	(nm)	(cm ⁻¹)	(nm)	(cm ⁻¹)
L1	269	37174	349	28653	444	22522
L2	305	32786	341	29325	452	22123
(L1Mo)	243	41151	308	32467	351	28490

(L2Mo)	243	41151	300	33333	351	28490
(L1W)	225	44444	295	33898	351	28490
(L2W)	246	40650	287	34843	351	28490

UV- VIS SPECTRA supports benzenoid and quinonoid forms, in addition to this it has enone system which is in conjugation with benzene π bonds. These facts are confirmed by the UV spectrum, which shows three chromophores. Ligand L1 and L2 show peaks at 269nm (37174 cm^{-1}) and 305nm (32786 cm^{-1}) respectively due to $\pi \rightarrow \pi^*$ transitions. Quinonoid ring depicts more absorption at 349nm (28653 cm^{-1}) and 341nm (29325 cm^{-1}) due to more chromophoric length cause by benzene ring, C1 and C2 carbonyl group. Vibrational dissymmetry due to $n \rightarrow \pi^*$ transition of enone system in L1 and C1 carbonyl of L2 i.e. forbidden transition, which indicates low stability of excited state at higher absorption 444nm (22522 cm^{-1}), 452nm (22123 cm^{-1}) respectively.

In the formation of chelates, absorption due to $\pi \rightarrow \pi^*$ transition of benzenoid system have been reduced to 243nm (41151 cm^{-1}) in (L1Mo) and (L2Mo). Quinonoid system also exhibits same pattern as benzenoid system. The absorption at 308nm (32467 cm^{-1}) and 300nm (33333 cm^{-1}) noticed for (L1Mo) and (L2Mo). As expected, $n \rightarrow \pi^*$ transition have been reduced to 351nm (28490 cm^{-1}) in both chelates (L1Mo) and (L2Mo) due to chelation.

In case of oxo tungsten chelates of (L1W) and (L2W), reduction in $\pi \rightarrow \pi^*$ transition of benzenoid and quinonoid system is recorded. $n \rightarrow \pi^*$ transition also reduced in both chelates. The data is provided in above mentioned (Table-2).

5.3 FT-IR Spectral analysis

Table-3 FT-IR Data of Metal Chelates (Cm^{-1})

Compounds	-OH	>C=O	>N-O-	>C=C<	>C=N-	O=M=O (sym)	O=M=O (asym)	M-O
L1	3425 (br)	1622 (s)	1073 (s)	1559 (w)	1588 (w)	--	--	--
L2	3202 (br,s)	1668 (s)	1068 (m)	1628 (m)	1574 (m)	--	--	--
(L1Mo)	3129 (br)	1591 (m)	1253 (s)	1533 (m)	1554 (w)	876 (m)	907 (m)	436 (m)
(L2Mo)	3147 (br,s)	1629 (w)	1302 (w)	1538 (w)	1550 (w)	877 (s)	929 (m)	451 (s)
(L1W)	3434 (br)	1621 (s)	1216 (s)	1527 (s)	1559 (w)	854 (s)	960 (m)	431 (m)
(L2W)	3201 (br,w)	1627 (s)	1338 (m)	1600 (m)	1573 (s)	849 (w)	974 (s)	424 (br,w)

(s=strong, m=medium, w=weak, br=broad)

>N-OH stretching frequency in ligand L1 and L2 shows at 3425 and 3202 cm^{-1} . Stretching vibration of -OH is attributed to the broad band at 3434-3129 cm^{-1} for all metal chelates, indicating the presence of water molecules in the region. This was confirmed by a thermal analysis(TGA), ^1H NMR and D_2O exchange study. The observed band $>\text{C}=\text{C}<$ in chelates transfer to lower frequency due to conjugation of double bond. In all chelates, $>\text{C}=\text{N}<$ and $>\text{C}=\text{O}<$ bands shifted to lower absorption due to formation of chelate. This was further authenticated by the absorption band between 424-451 cm^{-1} of M-O band, which is absent in free ligand. It displays the formation of metal chelates between ligand and metal ion. $>\text{N}-\text{O}<$ absorption band authorizes metal chelate formation through N-O bond. $\text{O}=\text{M}=\text{O}$ absorption band also confirms the formation of chelation. The data is provided in above mentioned (Table-3).

5.4 ^1H NMR AND ^{13}C NMR Spectral analysis

Table-4 ^1H And ^{13}C NMR Data of Metal Chelates

Compound	^1H NMR	^{13}C NMR
L1	8.90 (Intramolecular $\underline{\text{H}}$, 1H); 14.40 (br, s, OH); 6.39 (d, 1H, J=9.60 Hz, $\text{C}_3\text{-H}$); 6.42 (d, 1H, J=9.60 Hz, $\text{C}_4\text{-H}$); 7.52 (dd, 1H, J=8.00 & 2.0 Hz, $\text{C}_6\text{-H}$); 7.51 (dd, 1H, J=8.40 & 1.6 Hz, $\text{C}_7\text{-H}$); 7.58 (dd, 1H, J=8.80 & 2.0 Hz, $\text{C}_5\text{-H}$); 7.70 (dd, 1H, J=8.80 & 2.4 Hz, $\text{C}_8\text{-H}$)	145 (C=N); 183 (C=O); 109 (C_3); 155 (C_4); 123 (C_6); 123 (C_7); 127 (C_5); 128 (C_8); 131 (C_{4a}); 131 (C_{8a})
L2	9.10 (Intramolecular $\underline{\text{H}}$, 1H); 13.14 (s, OH); 6.57 (d, 1H, J=10.40 Hz, $\text{C}_3\text{-H}$); 6.60 (d, 1H, J=10.40 Hz, $\text{C}_4\text{-H}$); 7.61 (dd, 1H, J=7.60 & Hz $\text{C}_6\text{-H}$); 7.58 (dd, 1H, J=7.60 & Hz $\text{C}_7\text{-H}$); 7.67 (dd, 1H, J=8.00 & Hz $\text{C}_5\text{-H}$); 7.97 (dd, 1H, J=7.60, $\text{C}_8\text{-H}$)	185 (C=O); 148 (C=N); 116 (C_3); 148 (C_4); 129 (C_6); 126 (C_7); 130 (C_5); 133 (C_8); 135 (C_{4a}); 136 (C_{8a})
L1Mo	6.42 (d, 1H, J=9.60 Hz, $\text{C}_3\text{-H}$); 6.50 (d, 1H, 9.60 Hz, $\text{C}_4\text{-H}$); 7.51 (br, 1H, $\text{C}_6\text{-H}$); 7.39 (br, 1H, $\text{C}_7\text{-H}$); 7.70 (br, s, $\text{C}_5\text{-H}$); 7.80 (br,s, 1H, $\text{C}_8\text{-H}$); 3.40 (br, s, H_2O)	150 (C=N); 183 (C=O); 105 (C_3); 141 (C_4); 121 (C_6); 120 (C_7); 129 (C_5); 129 (C_8); 130 (C_{4a}); 131 (C_{8a})
L2Mo	6.64 (d, 1H, J=10.4 Hz, $\text{C}_3\text{-H}$); 6.67 (d, 1H, J=10.4 Hz, $\text{C}_4\text{-H}$); 7.72 (d, 1H, J=8.80 Hz, $\text{C}_6\text{-H}$); 7.70 (d, 1H, J=8.80, $\text{C}_7\text{-H}$); 7.74 (d, 1H, J=6.80 Hz, $\text{C}_5\text{-H}$); 7.81 (d, 1H, J=9.20 Hz, $\text{C}_8\text{-H}$); 3.42 (br, s, H_2O)	185 (C=O); 146 (C=N); 116 (C_3); 134 (C_4); 126 (C_6); 123 (C_7); 129 (C_5); 130 (C_8); 131 (C_{4a}); 133 (C_{8a})

L1W	6.39 (d, 1H, J=9.20 Hz, C ₃ -H); 6.42 (d, 1H, J=9.20, C ₄ -H); 7.60 (merged dd, 1H, J=8.00 Hz, C ₆ -H); 7.50 (merged dd, 1H, J=8.00 Hz, C ₇ -H); 7.71 (br, d, 1H, J=8.00 Hz, C ₅ -H); 7.71 (br, d, 1H, J=8.00 Hz, C ₈ -H); 3.18 (s, H ₂ O)	145 (C=N); 183 (C=O); 107 (C ₃); 144 (C ₄); 128 (C ₆); 128 (C ₇); 131 (C ₅); 131 (C ₈); 130 (C _{4a}); 130 (C _{8a})
L2W	6.62 (d, 1H, J=10.4 Hz, C ₃ -H), 6.64 (d, 1H, J=10.4 Hz, C ₄ -H); 7.67 (d, 1H, J=7.60 Hz, C ₆ -H); 7.66 (d, 1H, J=7.60, C ₇ -H); 8.03 (d, 1H, J=8.00 Hz, C ₅ -H); 8.17 (d, 1H, J=8.00 Hz, C ₈ -H); 3.41(s, H ₂ O)	185 (C=O); 148 (C=N); 116 (C ₃); 137 (C ₄); 126 (C ₆); 123 (C ₇); 128 (C ₅); 129 (C ₈); 130 (C _{4a}); 133 (C _{8a})

¹H NMR spectra of L1 and L2 demonstrates intramolecularly bonded proton at δ 8.90 and 9.10 ppm. Similarly, hydroxy proton of L1 and L2 appeared at δ 14.40 and 13.14 ppm as a broad singlet, which were absent in chelates, showing the deprotonation of hydroxy and intramolecularly bonded proton. In chelates, olefinic C₃ and C₄ protons show slight downfield shift as those are in conjugation with carbonyl group, which is bonded to the metal. Aromatic de shielded proton in L1 and their metal chelates vary from δ 7.39 to 7.80 ppm. For L2 and their metal chelates, aromatic protons show in the range of δ 7.58- 8.17 ppm. The presence of a signal about δ 3.18-3.42 ppm verified the existence of water molecules in chelates. >N-OH gives the signals at δ 14.40 and 13.14 ppm for ligands L1 and L2.

In ¹³C NMR spectra of ligands and their metal chelates, singlet of carbonyl carbon (>C=O) atoms were observed from δ 183 to 185 ppm. Another singlet of carbon (>C=N-) atoms were recorded from δ 145 to 150 ppm. Aromatic carbon atoms doublet noticed minute change. Fused aromatic carbon (C_{4a} and C_{8a}) atoms appeared as a singlet in the range from δ 130 to 133 ppm. Olefinic C₃ and C₄ carbon atoms displayed doublet at δ 105 to 155 ppm.

¹H and ¹³C NMR of ligands and their chelates are present in (Table-4).

5.5 Anthelmintic activity

The significances of anthelmintic investigations are summarized in the (Table-5).

Table- 5 Anthelmintic Activity of Ligands, Metal Salts and Metal Chelates

Compounds	Time (min.)			Time (min.)		
	For paralysis concentration (mg/ml)			For death concentration		
	5	10	15	5	10	15
L1	5	8	11	24	37	47
L2	13	10	7	99	84	41
L1Mo	22	20	17	47	33	28

L2Mo	10	7	5	39	32	22
L1W	10	8	12	21	16	25
L2W	7	6	8	47	34	36
Mo	145	124	32	312	245	88
W	124	85	115	341	205	258
Standard (Albendazole)	120	27	21	348	284	118
Control (DMSO+ Normal saline water)	-	-	-	-	-	-

Table 6- Data of Albendazole.

Concentration(mg/ml)	Time (min.) paralyzed	Time (min.) death
5	120	348
10	27	284
15	21	118

Standard *albendazole* is potent at higher concentration (15mg/ml) for paralysis and death of earthworms.

Table 7 – Potency of Ligand (L1)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	24 times	14.5 times
10	3.37 times	8.35 times
15	1.90 times	2.51 times

Ligand (L1) is potent at 5mg/ml concentration for paralysis and death of earthworms.

Table 8– Potency of Ligand (L2)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
50	9.23 times	3.51 times
10	2.7 times	3.3 times
15	3 times	2.87 times

Ligand (L2) is potent at 5mg/ml concentration for paralysis and death of earthworms.

Table 9 – Potency of Metal Salt (Mo)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	0.82 times	1.12 times
10	0.28 times	1.16 times
15	0.66 times	1.34times

Metal salt (Mo) is potent at 5mg/ml concentration for paralysis and 15mg/ml concentration for death of earthworms.

Table 10 – Potency of Metal Salt (W)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	0.97 times	1.02 times
10	0.31 times	1.39 times
15	0.18 times	0.45times

Metal salt (W) is potent at 5mg/ml concentration for paralysis and 10mg/ml concentration for death of earthworms.

Table 11 – Potency of Metal Chelates (L1Mo)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	5.45 times	7.40 times
10	1.35 times	8.60 times
15	1.24 times	4.21 times

Metal chelate (L1Mo) is potent at 5mg/ml concentration for paralysis and 10mg/ml concentration for death of earthworms.

Table 12– Potency of Metal Chelates (L2Mo)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	12 times	8.92 times
10	3.86 times	8.87 times
15	4.2 times	5.36 times

Metal chelate (L2Mo) is potent at 5mg/ml concentration for paralysis as well as for death of earthworms.

Table 13 – Potency of Metal Chelate (L1W)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	12 times	16.57 times
10	3.37 times	17.75 times
15	1.75 times	4.72 times

Metal chelate (L1W) is potent at 5mg/ml concentration for paralysis and 10mg/ml concentration for death of earthworms.

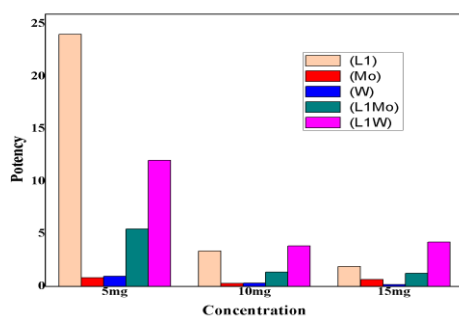
Table 14 – Potency of Metal Chelate (L2W)

Concentration(mg/ml)	Efficacy (paralyzed)	Efficacy (death)
5	17.14 times	7.40 times
10	4.5 times	8.35 times

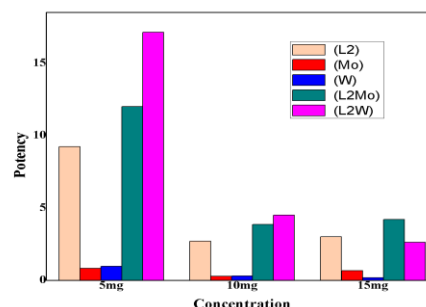
15	2.63 times	3.28 times
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Metal chelate (L2W) is potent at 5mg/ml concentration for paralysis and 10mg/ml concentration for death.

A literature survey reveals that electronegative atoms play an important role in biological activities. This is seen in the oxime group results. The orientation of the groups indicates that stereochemistry also affects the activity against helminths. It may be due to the planarity of the aromatic ring, insertion of electrons and/or hydrogen bond formation with the substrate moiety. The relevant results indicate that, the efficacy of the ligands and their molybdate and tungstate metal chelates. The results indicate that ligand (L1) is more powerful than ligand (L2).



Graph-1



Graph-2

Figure 4. Potency of Ligand, Metal Salts and their Chelates for Paralysis of Earthworms

(From Table 7 To 14)

5.5.1 At various concentrations for paralysis of earthworms: (From Graph 1 and 2)

5.5.1.1 Ligands at 5, 10 and 15mg/ml

Ligand (L1) is nearly 3 times more potent than ligand (L2). It may be due to, more electronegative nature of “O” atom (3.5) than “N” atom (3). Ketonic oxygen of ligand (L1) is in conjugation with double bond. Therefore, π electrons are more efficiently moved on “O” atom when it is compared with “N” atom of ligand (L2). In this ligand, the effective stabilization of electron cloud on “N” atom is less due to lower electronegativity. Such a type of electron density may cause the binding of cell matrix of a substrate (earthworm). This may be the reason for greater potency of ligand (L1) than ligand (L2). By increasing the concentration in double amounts, potency is significantly decreased in both ligands. The efficacy of both ligands is reduced, when the concentration is increased by three times.

5.5.1.2 Metal salts at 5, 10 and 15mg/ml-

All experiments illustrate negligible potency of metal salts even by increasing the concentration.

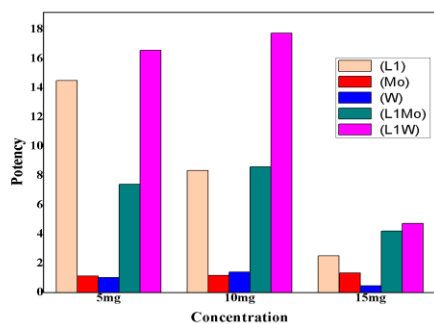
5.5.1.3 Metal chelate 5, 10 and 15mg/ml-

5.5.1.3.1 For (L1Mo) and (L2Mo) chelates

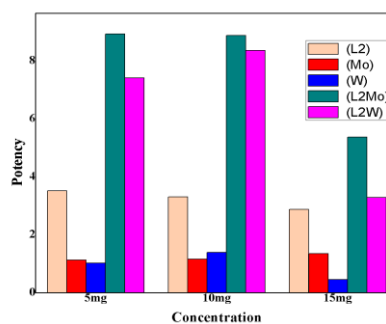
In case of (L1Mo) chelate, it is noticed that by increasing the concentration of chelate, potency decreases rapidly. In case of (L2Mo), the potency marked is nearly 1.5 times more than (L2) at 5mg/ml. Increasing the concentration by 10 and 15mg/ml of (L2Mo) is shown to be 3 times less potent than at a lower concentration of 5mg/ml.

5.5.1.3.2 For (L1W) and (L2W) chelates

In the case of (L1W) chelate, it is observed that when the concentration of chelate increases, potency rapidly declines. At 5mg/ml, the efficacy of (L2W) is almost 2 times higher than that of (L2). It is proven that increasing the concentration of (L2W) by 10 and 15mg/ml, makes it 3 times less powerful than at a lower concentration of 5mg/ml. In the case of (L1W), it shows nearly 2.5 times more potency than (L1Mo) at 5mg/ml concentration, while (L2W) shows nearly 1.5 times more potency than (L2Mo) at 5mg/ml concentration. Increasing the concentration by 10 and 15mg/ml, a potency of (L1W) and (L2W) is dramatically decreased and observed to be nearly the same.



Graph-3



Graph-4

Figure 5. Potency of Ligand, Metal Salts and their Chelates for Death of Earthworms (From Table 7 To 14)

5.6 At various concentrations for death of earthworms: (From Graph 3 and 4)

5.6.1 Ligands at 5, 10, and 15mg/ml-

The ligand (L1) is almost three times as powerful as the ligand (L2). The increased potency of ligand (L1) is for the same reason as 1.1. The ligand (L1) has about 2.5 times the strength of the ligand (L2). The potency of both ligands is dramatically reduced by increasing the concentration by two fold. At 15mg/ml concentration, the potency of ligand (L1) is almost the same as that of ligand (L2). The graphs indicate higher potency at lower concentrations of L1 and L2, while L1 is three times more potent than L2.

5.6.2 Metal salts at 5, 10, 15mg/ml-

Even when metal salt concentrations are raised, all investigations demonstrate that it is ineffective. The nature of metal salts shows that, ligands and their metal chelates are responsible for effectiveness.

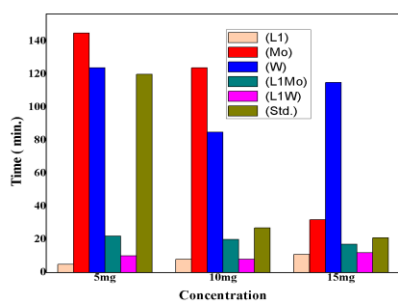
5.6.3 Metal chelate at 5, 10, 15mg/ml-

5.6.3.1 (L1Mo) and (L2Mo) chelates

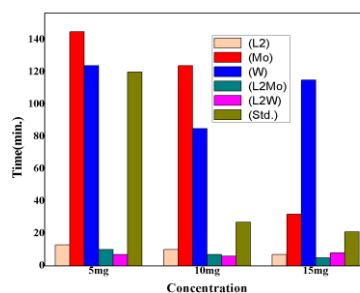
The potency of (L2Mo) is greater, to some extent, at all concentrations than (L1Mo).

5.6.3.2 (L1W) and (L2W) chelates

The efficacy of (L1W) is higher at all concentrations than (L2W).



Graph-5



Graph-6

Figure 6. Activity of Ligand, Metal Salts and their Chelates for Paralysis of Earthworms (From Table 5)

5.7 At various concentrations for paralysis of earthworms: (From graph 5 and 6)

5.7.1 At 5 mg/ml-

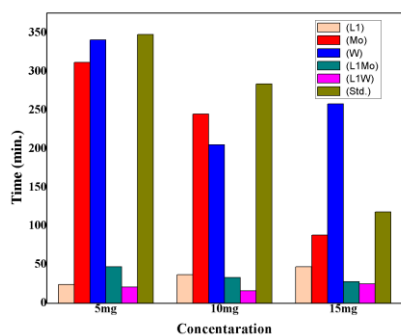
Ligand (L1) shows a minimum time as compared to ligand (L2) for the paralysis of earthworms. Metal salts, i.e., (Mo) and (W), show slightly higher times than the standard to demonstrate a paralyzed earthworm. This indicates the lower activity of metal salts. In comparison with ligands, (L1Mo) and (L2Mo) chelates require more time and less time to paralyze earthworms, respectively. (L2W) requires less time for paralysis than (L2), while (L1W) requires more time for paralysis.

5.7.2 At 10 mg/ml-

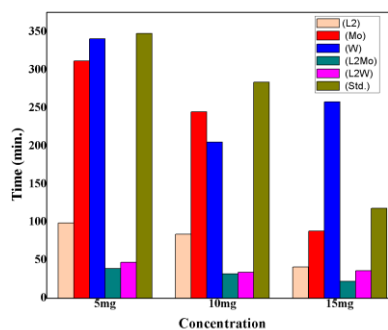
Ligands (L1) and (L2) react in the same manner as at 5mg/ml concentration. The activities of metal salts (Mo) and (W) appear to differ significantly from standard. Ligands with their metal chelates function similarly to a 5mg/ml concentration.

5.7.3 At 15 mg/ml-

At this concentration, (L2) shows a minimum time than (L1). Metal chelates react similarly to ligands at 5 and 10 mg/ml concentrations. Paralyzed earthworms with (L1), (L1Mo) and (L1W) take more time, while (L2), (L2Mo) and (L2W) require less time.



Graph-7



Graph-8

Figure 7. Activity of Ligand, Metal Salts and their Chelates for Death of Earthworms (From Table 5)

5.8 At various concentrations for death of earthworms: (From Graph 7 and 8)

5.8.1 At 5mg/ml-

At this concentration, (L1) is more active than (L2) considering the time. Metal salts are comparable to the standard for the death of earthworms. (L1Mo) requires more time than (L1), while (L2Mo) requires less time than (L2). (L2Mo) is more active than (L1Mo) by considering the time for the death of earthworms. Looking at the time of death of earthworms, (L1W) and (L2W) need less time than their respective ligands.

5.8.2 At 10mg/ml-

Ligands (L1) and (L2) functions as 5 mg/ml concentration. Metal salts (Mo) and (W) takes less time for the death of earthworm. (L1Mo) and (L2Mo) kill earthworms in a short time, when compared to their ligands. (L1W) takes less time for earthworms to die, so (L1W) is more active than (L2W).

5.8.3 At 15mg/ml-

At this concentration, (L2) is more active than (L1) as it takes a shorter time for earthworms to die. (Mo) metal salt shows less time than the standard and vice versa for (W). (L2Mo) takes a lower time to kill earthworms as compared with (L1Mo). (L1W) and (L2W) behave the same as 10mg/ml of concentration.

6 Conclusions

The spectral data, UV-VIS, FT-IR revealed the stated structures. The structures of ligand and their metal chelates were also confirmed by up-field and down-field shift of protons from ^1H NMR. Similarly, PND ^{13}C NMR have also confirmed corresponding up- field and down-field shift of carbon atoms. Thus, above mentioned structures were established. Metal salts are inactive than (L1), (L2) and their metal chelates, whereas standard *albendazole* takes much longer time to paralyzed and death of earthworms (from graph 5, 6, 7 and 8). Ligands are much more potent than

standard *albendazole* for paralysis and the death of helminths. Metal chelates are also potent than standard *albendazole*. The minimum time necessary for paralysis and death of earthworms using ligand (L1) is 5 and 24 min. respectively at 5mg/ml concentration. A 15 mg/ml concentration of ligand (L2) causes earthworms to become paralyzed and death in 7 and 41 min., respectively. At 10 mg/ml, L1W shows prominent results for the paralysis and death of earthworms in 8 and 16 min., respectively. The (L2W) metal chelate shows more activity than the ligand (L2). Chelates of (W) are more active than (Mo) chelates.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal, Bharati Vidyapeeth (Deemed to be University), Yashwantrao Mohite College of Arts, Science, and Commerce, Pune, for their encouragement and assistance, as well as to Department of chemistry, Savitribai Phule Pune University, Pune; SAIF, IIT Mumbai; CFC, Kolhapur; and SAIF, Cochi for providing facilities.

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