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Synthesis, Structural Elucidation and Antimicrobial Activity of Metal (II) Polypyridyl Complexes of 2-Amino-4-(Methylthio) Butanoic Acid

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Abstract

The interaction of selected biometals with dinitrogen donor ligand, 1,10-phennathroline (phen) and amino acid, 2-amino-4-(methylthio)butanoic acid (methionine; met) resulted in isolation of three mononuclear complexes. The prepared complexes were characterized by diverse techniques including elemental and spectroscopic analysis. From the analytical and spectral data, octahedral geometry was proposed to the synthesized metal (II) complexes. Bonding of the metal ion through N⁻ and O⁻ donor atoms of the ligands is revealed by infrared studies. The compounds showed moderate solubility in distilled water and in some common organic solvents. In vivo evaluation of the antimicrobial activities of the metal complexes showed improved inhibitory activity against some micro-organisms as compared to the ligands, with Co(II) and Cu(II) complexes showing the highest activities against the fungi.

Keywords: antimicrobial; mixed ligands; amino acid; polypyridyl

Introduction

Drug resistance, especially antibiotics, is one of the most important threats to public health. Antibiotic-resistant infections can sometimes lead to serious ill health or even mortality (de Sousa *et al*, 2020). Therefore, considering the swift spread of multi-drug resistance, the design and development of new antimicrobial agents which act on newly adapted microorganisms has become a priority. The use of metals in medicine is in fact an ancient practice stretching back thousands of years. Metals are found to play an astonishing number and variety of roles in modern medicine. Metal-based drugs play an important role in medicine for the treatment of various diseases. Modern medicinal chemistry seeks to intentionally investigate the structure and properties of metal complexes for medicinal function has developed immensely. The focus is on the design of safe metallotherapeutics, due to their ability to overcome the developed cell resistance, their biological activity, their side-

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effects, their low solubility and the advantages over the organic drugs (Thakkar & Thakkar, 2000; Shivankar & Thakkar, 2003; Theophanides *et al.*, 2003).

Design of complexes containing mixed ligands are established to be biologically active against pathogenic microorganisms (Thakkar & Thakkar, 2000; Shivankar & Thakkar, 2003), and polypyridyl as co-ligands in complexes, has shown promising biological activities (Howard-Lock, & Lock, 1987). The chemistry of amino acid coordination compounds has always been an intriguing challenge to the inorganic chemists. this class of molecules have been found throughout the life science and vary tremendously in their function and complexity. These compounds play an essential part of metabolism and cellular signaling and as a part of drugs (Ozturk et al., 2014). Amino acids form coordination compounds that show significant enzymatic and biological activities (Perrin & Agarwal, 1973; Sigel & Sigel, 2002; Bodkhe et al., 2012).

In this work, we synthesized three ternary metal (II) complexes containing an amino acid, methionine ligand, and the heterocyclic ligand, 1,10-phennathroline (phen). Further insight into the bonding and possible geometrical structure has been made by microanalytical, IR, UV–vis spectral studies., as well as magnetic susceptibility measurements. The antimicrobial activities of the complexes against some selected Gram positive and Gram-negative bacteria and fungi were also reported. The knowledge gained from this study should be useful for the development of potentially new therapeutic agents for combating antibiotic resistance.



Figure 1: Structure of ligands (a) methionine (b) 1,10-phenanthroline

Experimental

Materials

2-amino-4-(methylthio)butanoic acid was purchased from Loba Chemie Pvt Ltd, analytical grade purity, while Mn (II), Cu (II) and Co (II) chlorides, 1,10-phenanthroline, dimethylsulfoxide, ethanol, and methanol, Muller Hinton agar, Nutrient agar were purchased from Acros and Merck Laboratories, analytical grade purity. Elemental analyses were carried out using the CHNS-O EA1110 Analyzer. Electronic spectra were recorded on a Shimadzu UVPC-3001 spectrophotometer. Infrared spectra were recorded on a FTIR Spectrophotometer Spectrum 2000, Perkin Elmer; using KBr pellets.

Synthesis of Complexes

Synthesis of [Cu(phen)₂(MET)] (1)

Complex 1 was prepared according to the method described previously (Mohamed et al., 2012). Phen (0.360 g, 2 mmol) was mixed with (0.170 g, 1 mmol) of CuCl₂· 2H₂O in 10 ml 80% (v/v) of ethanol-water solution for about 10 min at The aquamarine-colored temperature. room precipitate appeared after adding copper salt. The precipitate was dried under vacuum and assumed to be $[Cu(phen)_2Cl_2]$. Then, the precipitate (247 g, 0.5 mmol) was reacted with (0.075 g, 0.5 mmol) of methionine in 10 ml of 70% (v/v) ethanol-water solution for about 4 h at 353 K, until the volume of the reaction mixture was concentrated to ca. 2 ml. The concentrated deep green solution was allowed to stand at room temperature for slow evaporation. Green-blue prismatic crystals were obtained from the mother liquor.

FT-IR (KBr pellet, v cm⁻¹): 3256 (NH₂), 3078 (ArH), 2889, 2775 (CH₂), 1608 (C=O), 1577, 1454 (C=C, C=N), 594, 520 (Cu-N, Cu-O). Elem. Anal. Calc. (%) for C₂₉H₂₅N₅O₂SCu; C, 60.32; H, 4.52; N, 12.41. Found: C, 60.98; H, 4.41; N, 12.26. ESI-MS (m/z positive mode) Calc. 571.01, Found: 570.2.

[Mn(phen)₂(met)] (2). The complex was prepared using a procedure similar to that for 1 by using MnCl₂·4H₂O instead of CuCl₂·2H₂O. Yield: 0.254 g, 57%. FT-IR (KBr pellet, v cm⁻¹): 3059(ArH), 2823, 2752(CH₂), 1608(C=O), 1577, 1454(C=C, C=N), 513, 455(Mn-N, Mn-O). Elem. Anal. Calc. (%) for C₂₉H₂₅N₅O₂SMn; C, 61.40; H, 4.28; N, 13.11. Found: C, 61.92; H, 4.48; N, 12.45. ESI-MS (*m/z* positive mode) Calc. 562.24, Found: 562.11.

[Co(phen)₂(met)] (**3**). The complex was prepared using a procedure similar to that for **1** by using CoCl₂·6H₂O instead of CuCl₂·2H₂O. Yield: 0.254 g, 46%. FT-IR (KBr pellet, v cm⁻¹): 3375(N-H), 3059(ArH), 2823, 2752(CH₂), 1608(C=O), 1577, 1454(C=C, C=N), 513, 455(Co-N, Co-O). Elem. Anal. Calc. (%) for C₂₉H₂₅N₅O₂SCo; C, 61.24; H, 4.41; N, 13.31. Found: C, 61.48; H, 4.45; N, 12.36. ESI-MS (*m/z* positive mode) Calc. 564.21, Found: 566.11.

Results and Discussion

The percent composition of carbon, hydrogen, nitrogen and metal were determined to establish the composition of the coordination compounds. The results are listed in Table I. The mixed-ligand complexes were prepared according to the following general equations:



Figure 2: Synthetic scheme for the preparation of the complexes

Infrared Spectra

The IR spectra of the complexes shows the characteristic bands of the ligands involved (Table 2). The amino acid, methionine exhibit $v(NH_2)$ at 3365 cm⁻¹, v_s (COO-) and v_{as} (COO⁻) at 1410 cm⁻¹ and 1621 cm⁻¹, respectively. The peaks at 3365 cm⁻¹ and 3347 cm^{-1} which is due to asymmetric and symmetric stretching vibrations of N-H in a primary amine group which shows that the group of zwitterions of free ligand is deprotonated to -NH2 and participated in the coordination with metal ion. For the complexes, the stretching vibration of $v_s(COO^-)$ and $v_{as}(COO^-)$ appear in the range 1400– 1405 cm⁻¹ and 1570–1598 cm⁻¹, respectively. The – COO⁻ asymmetric and symmetric absorption bands are at relatively lower frequencies compared to those of free ligand. The reduction of absorption frequencies may be due to the formation of coordination bond through oxygen atom of -COO⁻ group with metal ion (Patil et al., 2011). No free carboxylic (COOH) groups could be detected from the IR spectral data, indicating coordination of all of the studied metal ions to the carboxylate anions. Since there is no significant absorption band at about $v > 3450 \text{ cm}^{-1}$ for v(O-H) absorption in any of the complexes, we can conclude that there is no water molecule in the complexes as coordinated water or as water of crystallization.

Methionine (ν , cm⁻¹): 3414 , ν_{str} (NH₃⁺) 2914 ν_{str} (C–H), 1630 ν_{as} (NH₃⁺) , 1580 ν_{as} (COO⁻), 1516 ν_{sb} (NH₃⁺), 1413 ν_{s} (COO⁻), 1220 ν_{b} (S–CH).

Complex	Empirical formula	Molecular weight	Element Found (tal analy Calcula	ysis ted)	Melting point	Colour
			%C	%Н	%N	(°C)	
[Cu(phen)2(met)]	C ₂₉ H ₂₅ N ₅ O ₂ SCu	570.20	60.98 (60.32)	4.41 (4.52)	12.26 (12.41)	266-268	Green
[Mn(phen)2(met)]	C ₂₉ H ₂₅ N ₅ O ₂ SMn	562.11	61.92 (61.50)	4.48 (4.28)	12.45 (13.11)	248-250	Cream
[Co(phen)2(met)]	C ₂₉ H ₂₅ N ₅ O ₂ SCo	566.11	61.48 (61.24)	4.45 (4.41)	13.31 (12.45)	254-256	Blue

 Table 1: Empirical formula, molecular weight, elemental analysis data and molar conductance of

	Table 2: I	Table 2: Infrared Spectral data of the complexes (cm ⁻¹)					
	υs(COO ⁻)	vas(COO ⁻)	v(N-H) str	υ(C=N)	υ(M-O)	υ(M-N)	
[Cu(phen)2(met)]	1400 m	1610 s,b	3341 m	1498	466 m	414 w	
[Zn(phen)2(met)]	1465 m	1620 s	3232 m	1520	481 m	410 w	
[Co(phen)2(met)]	1411 m	1615 s,b	3420 m	1615	476 m	416 w	

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KEY: s (strong), m (medium), w (weak)

From the foregoing band positions of $v(NH_2)$, $v(COO^{-})$, v(M-O) and v(M-N) and comparison with similar compounds (Amolegbe et al., 2014; de Sousa et al, 2020), it may be concluded that the involved amino acid in the complexes are bidentate coordinating through the -NH₂ and COO⁻ groups.



Figure 3: Infrared spectra of compounds

Electronic Spectra

The electronic absorption spectra of the mixed metal complexes and their characteristics absorption bands with tentative assignments were recorded in dimethylformamide at various concentrations and are presented in Table 3. The assignments have been done on the basis of some standard references (Singh et al., 1995; Amolegbe et al., 2014; Vusak et al., 2017). The absorption bands between 200 and 400 nm were observed for the organic part of the complexes and that of >400 nm was due to the metal ion. The bonding pattern of the complexes were similar in most of the cases. The bands due to $\pi \rightarrow \pi^*$ transition in all the metal

complexes at ~236–253 nm were broad. Whereas in the ligand, it was found at about 224 nm.

Table 3: Electronic Spectra Data of the complexes

	λmax (nm)	Assignment
Methionine	224	$\pi \rightarrow \pi^*$
	262	$n \mathop{\rightarrow} \sigma^*$
	300, 331	$n \rightarrow \pi^*$
[Cu(phen) ₂ (met)]	253,	$\pi \rightarrow n^*$
	298,	$n \rightarrow \sigma^*$
	338, 365	$n \rightarrow \pi^*$
	413	$d \rightarrow d$
[Mn(phen) ₂ (met)]	238	$\pi \rightarrow n^*$
	296	$n \rightarrow \sigma^*$
	341, 349	$n \rightarrow \pi^*$
	604	$d \rightarrow d$
[Co(phen) ₂ (met)]	236	$\pi \rightarrow n^*$
	297	$n \rightarrow \sigma^*$
	346. 337	$n \rightarrow \pi^*$
	2.0,007	

The presence of the absorption band at ~ 265 -295 nm in the complexes was due to $n \rightarrow \sigma^*$ transitions that was observed at 262 nm in ligand. The $n \rightarrow \pi^*$ transition bands were observed at 325– 344 nm (at 300 and 331 nm in ligand) in all the metal complexes. The presence of $\pi \to \pi^*$, $n \to \pi^*$ and $n \rightarrow \sigma^*$ bands in all the complexes indicate the presence of the functional groups of the parent ligands (e.g. -C=O, -NH₂ and C-S) intact in the complexes. A large shifting of the absorption bands in the complexes and appearing of a new band for d-d transitions also indicate the probability of forming $M^+ \leftarrow L$ coordination bonds in the complexes.

Transition metal complexes are generally colored and this color arises due to the absorption of light in visible region. Therefore, the broad bands centering around 450, 515 and 514 nm in Cu (II), Mn (II) and Co (II) complexes respectively are clearly due to the d–d electronic transitions, which causes color of the complexes.



Figure 4: Electronic spectra of compounds

From the foregoing data the structure of the complexes can be postulated as follows (Fig. 5)



Figure 5: Optimized Structure of metal complexes (1-3)

Antimicrobial Activity

The prepared metal complexes were evaluated for their antimicrobial potential against B. *subtilis*, S. *aureus* (gram-positive), E. *coli* and P. *aeruginosa* (gram-negative) and A. *niger* and C. *albicans* (strains of fungi) using the disc diffusion method. The results of the screening of the prepared complexes are recorded in Table 4. The solvent and metals ions used showed no growth inhibition, suggesting that they do not interfere in the antimicrobial activity of the complexes.

The methionine has moderate activity with S. *aureus*, E. *coli*, P. *aeruginosa* and B. *subtilis*. The complexes exhibited significantly improved antibacterial activity against the tested microbial strains in comparison to the free ligand.

The Cu(II) and Co(II) complexes displays higher inhibitory activity with Gram positive bacteria than negative ones. It is clearly observed that the complexes are also very active against the fungi C. *albicans* and A. *niger*. However, Mn(II) complexes show a higher antimicrobial activity than the Co(II) against the fungi. The mixed complexes of Co(II) and Cu(II) of methionine shows the highest antimicrobial inhibitory activities overall. Thus, the antimicrobial activity increases on going from ligand to complexation.



Figure 6: Chart showing the inhibition zones of ligand and complexes

Inhibition zone diameter (mm)						
Methionine	S. aureus 14	B. subtilis 12	E. coli 14	P. aeruginosa 14	C. albicans 10	A. niger 11
[Cu(MET)(phen)2]	26	28	26	30	28	28
[Mn(MET)(phen)2]	21	22	24	22	26	24
[Co(MET)(phen)2]	24	28	26	26	22	21

Table 4: Results of antimicrobial screening of ligand and metal complexes

In complexes, the part sharing of the positive charge on the central metal atom with the ligands reduces the polarity of the metal atom and there is an electron delocalization over the whole chelated ring (Singh et al., 1995; Thangadurai & Natarajan, 2001). Indeed, typical chelation tends to improve the lipophilic character of the central atom, which subsequently favours its permeation through the lipid layers of the cell membrane and obstructing the metal binding sites on enzymes of microorganism (Al-Amiery et al., 2012). Thus, we infer that complexation increases the antimicrobial activity.

Conclusion

Summarily, the synthesis and the characterization of three mononuclear M(II) complexes with the amino acid methionine, as ligands in the presence of the nitrogen-donor ligand, phen has been discussed. Based on the physico-chemical results of and spectral techniques, the prepared complexes possess an octahedral geometry. The in vitro antimicrobial screening of the compounds were investigated. The results revealed that the mixed complexes showed improved inhibitory activity than the parent ligand. This is attributed to the chelation, which reduces the polarity of metal ion due to partial sharing of its positive charge with donor ligands and also due to the delocalization of π electrons over while, chelate ring.

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