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Synthesis Characterization and Biological Influence of Some Newly Synthesized Mix ligand Chelates

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Abstract

Objective: A series of mixed ligand chelates of d10 were synthesized expending 3TC a potent nucleoside analogue and ACV, is a guanosine analogue antiviral drug.

Methods: The synthesized chelates were characterized by IR, Mass spectra, TGA analysis and Elemental analysis and were evaluated for their anti-fungal activity against a panel of two pathogenic fungal strains namely, *Aspergillus niger*, and *Candida albicans* by Broth-dilution method, antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. pyogenus*.

Results: All the compounds showed significant inhibitory activity against the microorganism. Anti-bacterial activity was determined using Ampicillin as a standard and antifungal activity was determined using standard Greseofulvin.

Conclusion: Out of all the chelates, the chelate of Cd^{2+} exhibited promising antimicrobial activity as a whole.

Keywords: ACV; 3TC; HIV/AIDS; Antibacterial; Antifungal

Introduction

Lamivudine (2′,3-dideoxy-3′-thiacytidine or 3TC) is a persuasive nucleoside analogue contrary transcriptase inhibitor (nRTI). It has been employed for exploit of chronic hepatitis B than for suppository of HIV at a lower dose. Lamivudine advances the seroconversion of e-antigen positive hepatitis B and histology enactment of the liver [1].

Both types (1 and 2) of HIV reverse transcriptase are introverted by a Cytidine analogue Lamivudine. It is phosphorylated to active metabolites that strive for integration into viral DNA. Lamivudine act as a chain terminator of DNA synthesis as well as impedes the HIV reverse transcriptase enzyme competitively. 5' to 3' phosphodiester, which formed, associate essential for DNA chain expansion is prevented due to lack of a 3'-OH group in the assimilated nucleoside analogue, which lay off the viral DNA growth. 3TC exhibited no evidence of carcinogenicity or mutagenicity in *in vivo* studies in mice and rats at doses from 10 to 58 times those used in humans and so it possess an advantage over other anti HIV agents [2].

Acyclovir (ACV), 9-[(2-hydroxyethoxy) methyl] guanine, an analogue of 29-deoxyguanosine. Several herpes viruses, especially HSV-1 and HSV-2 are introverted as a competent topically active acyclic nucleoside [3]. ACV is both the antecedent and the model for improvement of many of the purine nucleoside inhibitors. Acyclovir is well known antiviral as well as a broad spectrum antibiotic it is a chemical acyclic analogue of the natural nucleoside 2'-deoxyguanine. ACV has antiviral activity "*in vitro*", against herpes simplex viruses (HSV), varicella zoster virus (VZV), Epstein barr virus (EBV), cytomegalovirus (CMV) and human herpes virus 6 (HHV-6) [4], which is in use against gram positive and gram negative bacteria [5].

The study of mixed ligand chelates having two or more different ligands attached to metal ion has received a great importance in recent years because of their wide applications in various fields of chemicals activity and more particularly because of their presence in biological systems. As a matter of fact, many naturally, occurring metal complexes are mixed ligand chelates [6].

In the present work, isolation of mixed ligand chelates of Zn (II), Cd (II) and Hg (II) with Lamivudine and acyclovir has been carried out. Their physicochemical properties have been studied using analytical techniques. Structures and composition of the chelates have been established on the basis of their elemental analysis, magnetic measurements, infrared spectra, electronic spectra and thermo gravimetric analysis etc. This study is interesting because biochemical role of many metal ions have been well established [7].

Materials and Purification

Water

Conductivity water was used right through the work. Conductivity water was redistilled over alkaline potassium permanganate. The distilled conductivity water was boiled to expel carbon dioxide and stored in an air tight Pyrex aspirator. The pH of this water was found to be ~ 6.9. This water was used for preparing solutions of metal perchlorates and reagents.

Ligand and other chemicals

The primary ligand Lamivudine was obtained from Fluka and secondary ligand acyclovir (ACV) was obtained from different pharmaceuticals companies and their purities were checked by noting their melting point as well as TLC. All metal carbonates used were also

A.R. grade. Stock solutions of Zn (II), Cd (II), and Hg (II) perchlorates were prepared and analysed by complex metric methods. Sodium hydroxide (E Merk) was used.

Experiments

Preparation of chelates

The synthesis of the chelates was carried out by mixing 25 ml of a 0.1 M aqueous solutions of Zn (II), Cd (II), and Hg (II) perchlorates with 25 ml 0.1 M solution of Lamivudine in DMSO. After stirring this mixture for 40-50 min, 25 ml of a 0.1 m ACV were taken in the form of DMSO solutions and was added slowly with constant mechanical stirring for 30 min. It was refluxed with water condenser for 2 h at 150°C temperature, until the mixture remains ¼ parts. There was no immediate precipitation. The pH of the above solutions was then raised to ~ 6.9 using 0.1 M sodium hydroxide solution which resulted in the precipitation of solids. The chelates thus obtained were washed well with a 1:1 mixture of absolute alcohol and water to remove unreacted metal salt and ligand. All chelates were dried in vacuum at room temperature.

Analyses and physical measurements

Elemental analyses were performed with a Perkin - Elmer model 2400 series - II C, H, N analyser. The IR spectra were recorded on a Perkin Elmer Impact model 400D FT-IR spectrophotometer. The samples were run as KBr pellets. The mass spectra of some selected compounds were recorded on a JEOL SX 102\DA-6000 Mass Spectrometer using Argon\xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. Thermal measurements were performed using a Meltler - Toledo model TG - 50 thermal analyser at a 15°C min - 1 heating rate. DSC measurements were also performed using a Meltler - Toledo model DSC-822e.

Results and Discussion

The analytical data of the chelates presented in Table 1A showed 1:1:1 stoichiometry. The general equation for the formation of the chelate with Lamivudine and acyclovir is as shown below.

Compound Molecular Formula	Colour	Molar conductance Mho cm-1	Rf value	Yield %	Formula Weight	Analysis (%) Calculated/ (Found)			M.P. °C
						C %	H %	N %	
$C_8H_{11}N_5O_3$ (L_1)	White	-	-	-	225.21	42.67 (42.77)	4.92 (4.99)	31.10 (31.89)	256.5°C
$C_8H_{11}N_3O_3S$ (L_2)	White	-	-	-	229.25624	41.91	4.84	18.33	76°C
[Zn (L_1) (L_2)]3H_2O	Lemon yellow	1.438	0.769	55.15	518	36.86 (37.38)	4.08 (4.26)	21.69 (20.98)	>300°C
[Cd (L_1) (L_2)]10H_2O	Lemon yellow	0.931	0.774	56.04	565	33.96 (33.63)	3.74 (4.06)	19.80 (19.75)	>300°C
[Hg (L_1) (L_2)] 10H_2O]	Green	5.776	0.745	29.64	654	29.38 (29.41)	3.24 (2.98)	17.13 (17.93)	>300°C
L1=Acyclovir and L2=Lamivudine									

Table 1A: Analytical data and some physical properties of the metal chelates.

$M^{2+} + HA + HL + H_2O \rightarrow [MAL].\ H + 2H^+$

$Zn^{2+} + HA + HL + H_2O \rightarrow [Zn\ AL].\ H_2O + 2H^+$

Where, HA = Lamivudine

HL = Conjugated base of acyclovir

$M^{2+} = Zn^{2+}, Cd^{2+}, Hg^{2+}$

The Zn (II), Cd (II), and Hg (II) chelates are coloured, all are crystalline or amorphous and stable in air. The chelates are soluble in DMSO and insoluble in water and other common organic solvents. The melting points of the chelates are higher, which suggest their greater thermal stability. To check the presence of single or more than one chelates, TLC of the solid was carried out using silica gel as the stationary phase and N-amyl alcohol, glacial acetic acid and water (6:1:2) as the solvent system. For all the four chelates only one clear spot was obtained indicating the formation of only a single product.

Conductance measurements

Chelates are found to be only slightly soluble in DMSO and insoluble in water and other organic solvents. The conductivities of all the chelates are measured in DMSO at 25°C at a concentration 10-3 M using systronics conductivity meter. All chelates were found to be non-conducting in nature. The molar conductance values of the chelates are listed in Table 1A.

Elemental analyses

Elemental analyses of the ternary chelates isolated in the present study are given in Table: 1B. They indicate 1:1:1 composition. This is also confirmed by observing their non-conducting nature. Both Zn^{2+} and Cd^{2+} have invariably +2 oxidation state Zn^{2+} is spherically symmetric with d^{10} configuration and hence no LFSE is present. As a result other factors decide the preference for coordination Zn^{2+} is typically tetrahedral or octahedral However trigonal bi pyramid and square pyramidal 5 coordinate complexes are also reported. Zn^{2+} compounds hydrolyse rapidly in water to increase their coordination number above4. For Cd^{2+} coordination numbers 3, 4, 5, 6 are known. Cd^{2+} and Hg^{2+} have a tendency to achieve 5 coordination by dimerizing Hg^{2+} has 2, 3, 4 and 6 coordination number Hg^{2+} has great affinity with sulphur. Hg^{2+} has various tendencies to form polymeric compounds, bridged compounds and also Hg^{2+} the mercurous state as a result isomeric compounds also may be present.

Electronic spectra study

Synthesis of chelates is analysed for UV-Visible absorption spectra and magnetic moment. These metals belong to II-B (12) group (d^{10}). This group has a usual characteristic of absence of d - d transition because no space for excited electron is present in the d orbital which is completely filled in these ions. The results indicate diamagnetic nature of the chelates with ligand to metal charge transfer bands [7]. The elemental analysis and other physical data of chelates are presented in Table 1A.

Infrared spectral study

Infrared spectra of metal chelate exhibit bands corresponding to lamivudine, acyclovir and metal chelates. Bands were observed in far IR region. It is known that in the IR spectra of lamivudine and acyclovir coordinate chelates; there is strong coupling between various vibrations modes of the spectra of metal chelates (MC) are obtained in the range of 4000-400 cm^{-1} using chelates in the form of KBr pellets. The IR spectra indicate the presence of lamivudine and acyclovir in the ternary chelates. The presence of water molecule in the chelates is indicated by broad band around 3400 cm^{-1} and some additional bands at 503 cm^{-1}, 517 cm^{-1} and 910 cm^{-1} assigned to M-OH_2 bands [8]. The lamivudine molecule shows the following characteristic bands; 3476 cm^{-1} - 3080 cm^{-1} (O-H and aromatic C-H stretching), 1219 cm^{-1} (C-O stretching), 1582 cm^{-1} and 1514 cm^{-1} (C=N stretching) [6]. The usual ring skeleton (C-C) and (C-N) bands are observed at 1600 cm^{-1}, 1450 cm^{-1}, 1400 cm^{-1} and 1370 cm^{-1} [9]. The acyclovir molecule shows the following characteristic bands; 1423 cm^{-1} and 1501 cm^{-1} (C-O) and (O-H) coupled, 1695 cm^{-1} (C=O), 1036 cm^{-1} (C-N) and 3406 cm^{-1} - 2916 cm^{-1} (O-H) and (N-H) [10]. In the chelates, the band around 498 cm^{-1} corresponds to (M-O) suggests that phenolic groups are involved in bond formation with metal ions. In the lamivudine molecule a band is observed at 3179 cm^{-1} -3050 cm^{-1} due to (O-H) band is absent in chelates due to M-O [11,12]. The (M-N) frequency in the lamivudine chelate is obtained at higher wave number because of the double bond character of M-N bond due to MN-interaction [13-15]. Nakamoto [16] has shown that (M-N) frequency undergoes coupling with other stretching bands. The bands around 788 cm^{-1} and 643 cm^{-1} may correspond to the coupled M-N vibrations. The Study confirmed the original assignments made by Nakamoto [16]. The complex Hg - ACV could be tentatively assigned as a Hg (ACV) polymer based on spectral data which show direct N (7)-Hg interaction, conductometric measurements (no electrolyte) and comparison with other similar structures [Hg (L_1) (L_2)] 10H_2O].

Mass spectra

In the mass spectra of individual ligand and chelates the following observations were made. The mass spectrum of lamivudine showed 230 m/z base peak due to lamivudine +1. All the three chelates of Zn^{2+}, Cd^{2+} and Hg^{2+} showed 178 m/z base peak due to ACV–1–C_2H_5OH. In the mass spectra of Zn^{2+} and Cd^{2+} chelates, peaks below 150 m/z were not observable whereas in Hg^{2+} chelate the molecular peaks were not observed largely because the molecule proposed seems too huge to be observable in mass spectra. However the 489 m/z molecular peak of Zn^{2+} chelate was observed a very tiny peak along with other [17,18].

Thermal gravimetric study

It has been observed that Zn (II) and Cd (II) chelates show loss in weight corresponding to two water molecules in range 100°C-150°C and Hg (II) chelate show loss in weight corresponding to one water molecule in 100°C-150°C. This shows that there is water molecules present in each metal chelate. Decomposition of Zn (II) chelate starts above 350°C, Cd (II) chelate starts above 350°C, and Hg (II) chelate starts above 200°C. In general, the water of hydration may be considered as either the crystallization water or coordinated water. According to the study water eliminating below 150°C can be considered as the water of crystallization and water eliminated above 150°C may be due to its coordination in the metal.

Compound	0	>NH	-OH	>C=O	C–O–C	C–O	C–O	0	-C–N	M–N
Molecular Formula						Ether	Alcohol			
$C_8H_{11}N_5O_3$ (L_2)	3471, 3440, 1573	3302, 1541	3522	1715	1106	1183	1048	3183, 2927-2854	1346, 1308	-
$C_8H_{11}N_3O_3S$ (L_1)	3325, 3201	-	3179	1760	1281, 1160	1202	1040	-	1381	-
[Zn (L_1) (L_2)]$_3H_2O$	-	3344	-	1695	1109	1175	1036	2916	1371, 1328,	410
[Cd (L_1) (L_2)]$_{10}H_2O$	3406	-	-	1691	1107	-	1018	2293	1390, 1364, 1323	498
[Hg (L_1) (L_2)] $_{10}H_2O$]	-	3336	-	1673	1107	1180	1088	2922	1382, 1318	493

Table 1B: Infrared spectra of the metal chelates (cm^{-1}).

Antibacterial activity

All synthesized chelates were screened for their antimicrobial activity *in vitro* against both Gram-positive and Gram-negative bacteria. *Staphylococus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* were the microorganism employed. All the synthesized chelates gave extreme activity against different antimicrobial genus in comparison with the ligand as well as the standard drug which indicates that due to chelation the antimicrobial activity of organic counterpart intensifies.

In comparison with the standard drug Ampicillin, chelates of Cd^{2+} and Hg^{2+} offers enhanced activity against *Escherichia coli*, while the rest of chelates as well as both the ligands did not give even equal activity abutting *Escherichia coli*. Similarly in comparison with the same standard drug Ampicillin, the chelate of Cd^{2+} reveals excellent activity and chelate of Hg^{2+} demonstrate equal activity against *Staphylococcus aureus*. Moreover 3TC also proves better activity towards *Staphylococcus aureus* in comparison with the standard drug Ampicillin. In this development, when the synthesized chelates were screened in conjunction with *Pseudomonas aeruginosa*, only the chelate of Hg^{2+} shows parallel activity as the standard drug while the rest of chelates as well as both the ligands did not give even equal activity against *Pseudomonas aeruginosa*.

Finally when the synthesized chelates were screened for *Streptococcus pyogenes*, not only the chelates but also both the ligands did not give even equal activity abutting *Streptococcus pyogenes*. But the study revealed interesting changes in antimicrobial activity on chelation including significant activity by some chelates as they compared with the ligand's activity. The ligand was not sowing even equal activity in comparison with the standard drug, while on chelation it not only gave better activity than the standard drug Ampicillin but also exhibits equal activity as another standard drug such as Chloramphenicol as well as ciprofloxacin (e.g. chelates of Cd^{2+}, Hg^{2+}… etc.) (Tables 2 and 3).

Sr. No	Code No.	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenus*
	Compound Molecular Formula	**MTCC 443**	**MTCC 1688**	**MTCC 96**	**MTCC 442**
1	$C_8 H_{11} N_5 O_3$ (L_1)	250	250	500	500
2	$C_8H_{11}N_3O_3S$ (L_2)	200	500	150	200
3	[Zn($_{L1)}$ (L_2)]3H_2O	250	250	500	500
4	[Cd (L_1) (L_2)]10H_2O	50	125	125	250
5	[Hg (L_1) (L_2)] 10H_2O]	62.5	100	250	250

Table 2: Antibacterial activity table minimal inhibition concentration.

Drug	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenus*
	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
(Microgramme/ml)				
Gentamycin	0.05	1	0.25	0.5
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50
Ciprofloxacin	25	25	50	50
Norfloxacin	10	10	10	10

Table 3: The standard drugs: Minimal bactericidal concentration.

Antifungal activity

Three fungii entitled viz. *C. albicans*, *A. niger* and *A. clavatus*. The synthesized chelates showed antifungal activity against only one organism C.albicans compared to the standard drug Nystatin and Greseofulvin. The chelates exerted less activity against *A. niger* and *A. clavatus*. The chelates of Cd^{2+} exhibited better while Hg^{2+} furnishes identical activity than Greseofulvin against *C. albicans*, while ligand 3TC showed moderate effect against *C. albicans*. Out of all the chelates, the chelate of Cd^{2+} exhibited promising antimicrobial activity as a whole.

Conclusion

The confirmation and characterization of the compounds prepared in the present studies are interesting. The formation of metal chelates from various considerations and the structure is tentatively assigned on the basis of above studies. It is suggested that all metal chelates are diamagnetic, four coordinate, two of them crystalline, having ternary; metal: primary ligand: secondary ligand (1:1:1) composition.

References

1. J Chinen, WT Shearer (2008) Secondary immunodeficiencies, including HIV infection. J Allergy Clin Immunol 121: S388-S392.
2. C Stoddart, R Reyes (2006) Models of HIV-1 disease: A review of current status. Drug Discovery Today: Disease Models 3: 113-119.

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3. Schaeffer HJ, Beauchamp L, de Miranda P, Elion GB, Bauer DJ, et al. (1978) 9-(2-hydroxyethoxymethyl) guanine activity against viruses of the herpes group. Nature 272: 583-585.
4. Wagstaff AJ, Faulds D, Goa KL (1994) Aciclovir. Drugs 47: 153-2015.
5. Mayne PD (1999) Clinical Chemistry in Diagnosis and Treatment (6th edn), Oxford University Press, New York, USA. 57 - 64.
6. Perrin DD, Agrawal RP (1973) Metal Ion in Biological Systems. New York, USA. 2: 167.
7. Perrin DD, Agarwal RP, Sigel HC, Dekker M (1973) Metal ions in biological systems, New York, USA.
8. Lever ABP (1968) Inorganic Electronic Spectroscopy. Elsevier Publishing Company Inc 224: 246-247.
9. Patel GP, Sharma SS, Vora JJ, Joshi JD, Synth (2002) Synthesis and Characterization of Nickel (II), Cadmium (II) and Zinc (II) Mixed - Ligand Complexes with 2,2'-Bi pyridyl amine and Phenol. React Inorg Met-Org Chem, 32: 1792-1742.
10. Pretsch E, Buhlmann P, Affolter C, Springer VB (2000) Heidelberg Structure Determination of Organic Compounds. New York 258: 263-264.
11. Karitzky AR, Jones RA. The Infrared Spectra of Polycyclic Hetero aromatic Compounds. J Chem Soc 2942.
12. Williams D, Fleming I (1988) Spectroscopic Methods in Organic Chemistry (4thedn). Tata McGraw Hill, New Delhi, India, pp: 42-58.
13. Ferraro JR (1971) Low Frequency Vibration of Inorganic and Coordination Compounds, Springer, USA.
14. Patel NH, Patel KN, Patel MN (2002) Synthesis and Characterization of Coordination Chain Polymers of Some Transition Metals with Schiff Base. Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry 10: 1879-1887.
15. Schilt AA, Taylor RC (1959) Infra-red Spectra of 1:10 Phenanthroline Metal Complexes in the Rock Salt Region Below 2000 Cm– 1. Inorganic and Nuclear Chemistry 9: 211-221.
16. Nakamoto K (1993) Infrared and Raman Spectra of Inorganic and Coordination Compounds (4thedn), John Wiley and Sons Inc, New Yark, USA.
17. Patel AK, Patel VM, Rasmikanth AP, Joshi JD, (1999) Synthesis Characterization and Antimicrobial Activities of Nickel (II), Copper (II), Zinc (II) and Cadmium (II) Ternary Complexes Involving 8 - Quinolinol as Primary Ligand and Bidentate Glycine or Tridentate Aspartic Acid as Secondary Ligand. Synthesis and Reactivity in Inorganic and Metal - Organic Chemistry 29: 193-203.
18. Nakamoto K, Morimoto T, Fujita J (1962) Proc Inst Conf Coord Chem (7thedn), Stocholm 15.