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DRUG SYNTHESIS

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITIES OF MIXED LIGAND Zr(IV) COMPLEXES

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Abstract: Mixed ligand ternary Zr(IV) complexes of type $[M(Q)_2LNO_3 \times H_2O]$ have been synthesized using 8-hydroxyquinoline (HQ) as a primary ligand and N- and/O-donor amino acids (HL) such as L-serine, L-alanine and glycine as secondary ligands. These complexes were characterized on the basis of elemental analysis, conductance measurement, spectral and thermal studies. The molar conductance study of the complexes in DMF solvent signifies their non-electrolytic nature whereas the thermal analyses specify presence of a coordinated water molecule. The complexes were tested for antifungal and antibacterial activity by using agar well diffusion bioassay. The antibacterial activity was tested against the pathogenic bacteria *Staphylococcus aureus* and *Enterococcus faecium*. The results obtained were evaluated with antibacterial standard vancomycin. The antifungal activity was tested against *Candida albicans, Candida krusei, Aspergillus fumigatus* and the results obtained were compared with antifungal standard amphotericin B. The complexes were also screened for cytotoxicity studies against *Ehrlich ascites* cells and *Daltons lymphoma ascites* cells and show very low cytotoxicity.

Keywords: mixed ligand zirconium complexes; antibacterial activity; antifungal activity; thermal study; cytotoxicity study.

It is well known that mixed ligand complexes, particularly ternary complexes of some metals play an important role in the activation of enzymes and are used for storage as well as for transport of active materials (1). Mixed ligand complexes are established to be biologically active against pathogenic microorganisms (2, 3), further, metal complexes, which include 8-hydroxyquinoline as primary ligand exerts biological activity (4). Amino acids form complexes with metal atoms and exhibit significant biological and enzymatic activities (5, 6).

Previous reports (7, 8) on zirconium complexes revealed the applicability of the complexes in various fields. Complexes of Zr(IV) with bis-coumarin ligand exhibit cytotoxic activity (7). 8-Hydroxyquinoline-Zr(IV)-EDTA complex have potential to detect fluoride ions up to ppb levels (8). The literature on 8-hydroxyquinoline, amino acids and zirconium reveals that the complexes prepared from 8-hydroxyquinoline, amino acids and zirconium (IV) could present interesting metallo-organic compounds with significant biological activity. Further, no work has been carried out on mixed ligand zirconium (IV) complexes with 8-hydroxyquinoline and amino acids as ligands. It was, therefore, considered worthwhile to study the complexation and to determine the biological activity of these new complexes.

This paper reports synthesis and characterization of mixed ligand Zr(IV) complexes prepared with 8-hydroxyquinoline as a primary ligand and amino acids such as L-alanine/L-serine/glycine as a secondary ligand. Zirconium was used due to its high co-ordination number and ability to from stable complexes. These complexes were characterized on the basis of elemental analysis, conductance measurement, spectral and thermal studies and screened for their antibacterial, antifungal and cytotoxic properties.

EXPERIMENTAL

Materials

Metal salt zirconyl nitrate used for synthesis was procured from Thomas Baker, Mumbai, India. Primary ligand, 8-hydroxyquinoline, and amino acid, glycine, were acquired from E. Merck. Amino

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acids L-serine and L-alanine were obtained from S. D. Fine Chemicals, Mumbai, India and were used as received. The solvents: ethanol, DMSO and methanol, were of LR grade and purified by standard procedures (9).

Preparation of mixed ligand complexes

Mixed ligand Zr (IV) complexes were prepared from zirconyl nitrate using 8-hydroxyquinoline (HQ) as a primary ligand and amino acids (HL) like L-alanine/L-serine/glycine as a secondary ligand. 231 mg of zirconyl nitrate was dissolved in 10 mL of distilled water. To aqueous solution of zirconyl nitrate 10 mL ethanolic solution of 8-hydroxyquinoline (290 mg) was added. The mixture was stirred and kept in a boiling water bath for 10 min. To this hot solution 10 mL (1 mmol) of aqueous solution of amino acid (L-serine/L-alanine/glycine) was added with constant stirring. The complexes were obtained by raising the pH (from 2.8 to 6.5) of the reaction mixtures by adding dilute ammonia solution. The mixtures were cooled, filtered and washed with water, followed by ethanol. The solid complexes thus prepared were dried under vacuum.

Instrumentation

The complexes were analyzed for C, H and N contents using Thermo Finnigan Elemental Analyzer (Model–FLASH EA–1112). Metal content was estimated gravimetrically by standard procedure (10). Simultaneous TG/DTA curves of the complexes were recorded using Mettler-Toledo TG instrument (Model-851) in nitrogen atmosphere, at the heating rate of 10°C per min. The FT-IR spectra were recorded in KBr discs on a Perkin-Elmer FT-IR spectrophotometer (Model 160). The conductivities of the complexes were measured in DMF solvent using Equiptronics digital conductivity meter (Model No. EQ-DCM-P)

Antibacterial and antifungal screening

The antibacterial and antifungal activity of the complexes were assayed against the bacterial strains S. *aureus* 209P and *E. faecium* VRE323 and fungal strains C. *albicans*, *C. krusei* GO3 and *A. fumigatus* using agar well diffusion method. The complexes dissolved in methanol were used for study.

Agar well diffusion method

Agar well diffusion using sabourauds agar (Hi-Media) medium was employed to study the antibacterial and antifungal activity of the complexes. Each complex (25 mg) was dissolved in 50 mL of methanol (500 µg/mL), which was used as sample solution. Sample size for all the compounds was fixed at 0.1 mL. The wells were made by scooping out agar medium with sterilized cork borer in a Petri dish, which was previously inoculated with the microorganisms. The solution of each test compound (0.1 mL) was added in the wells and Petri dishes were subsequently incubated at 37°C for 24 h in dark. Vancomycin and amphotericin B were used as reference drugs for antibacterial and antifungal study, respectively. Zone of inhibition produced by each complex was measured in mm.

Cytotoxicity study

In vitro cytotoxicity study on the representative complexes, were carried out on established tumor cell lines *Ehrlich ascites* cells and *Dalton's lymphoma ascites* cells. The *Ehrlich ascites* cells were originally procured from Cancer Research Institute, Mumbai, India and *Dalton's lymphoma ascites* cells from Cancer Research Institute, Chennai, India. For the experiment, these cells were aspirated, washed with phosphate buffered saline and made up to a concentration of 10 million/mL. Different concentrations of the complexes (10 to 100 µg) in DMSO (20 µL) were incubated with 1 million cells in a total volume of 1 mL with phosphate buffered saline (PBS) used as a control in the experiment. Cells were incubated at 37°C for 3 h. After incubation, 0.1 mL of 1% trypan blue was added and cytotoxicity was determined by counting live and dead cells using hemocytometer.

RESULTS AND DISCUSSION

All three mixed ligand Zr(IV) complexes are colored, non-hygroscopic, and thermally stable solids, signifying a possibility of presence of strong metal ligand bond. These complexes are found to be soluble in organic solvents like ethanol, methanol, DMSO but insoluble in water.

The reaction for the synthesis of Zr mixed ligand complexes may be represented as:

$$ZrO(NO_3)_2.H_2O + 2\ HQ + HL \rightarrow [Zr(Q)_2(L)\times NO_3\times H_2O] + HNO_3 + H_2O$$

(where HQ is 8-hydroxyquinoline and HL is an amino acid)

The elemental analysis data obtained for Zr mixed ligand complexes are presented in Table 1. These data suggest that the mixed ligand complexes are formed in the ratio 1: 2: 1 and they are of the type $[M\ (Q)_2\ (L) \times NO_3 \times H_2O]$. The molar conductance values of the complexes in DMF solvent at 10^{-3} M concentration are very low (< 1) signifying their non-electrolytic nature (11).

Table 1. Elemental analysis data of the complexes

Complex	Empirical formula	Color	Elemental analysis Found (Calculated)			
			%M	%C	%N	%H
$[Zr(Q)_2(Ala)NO_3\times H_2O]$	$ZrC_{21}H_{20}O_8N_4$	Light yellow	16.20 (16.65)	45.64 (46.06)	10.83 (10.23)	3.3 (3.68)
$[Zr(Q)_2(Ser)NO_3\times H_2O]$	$ZrC_{21}H_{20}O_9N_4$	Light yellow	15.92 (16.18)	45.68 (44.75)	10.79 (9.94)	3.42 (3.57)
$[Zr(Q)_2(Gly)NO_3\times H_2O]$	$ZrC_{20}H_{18}O_8N_4$	Light yellow	16.95 (17.11)	45.82 (45.06)	11.26 (10.51)	3.26 (3.40)

Table 2. Thermal data of the complexes

Complex	Temp. range (^oC)	% Weight loss		Decomposition product
		Observed	Expected	
$[Zr(Q)_2(Ala)NO_3\times H_2O]$	120-200 220-350 360-780	2.95 15.62 57.05	3.29 16.086 58.12	$[Zr(Q)_2(Ala)NO_3]$ $[Zr(Q)_2NO_3]$ $[ZrO_2]$
$[Zr(Q)_2(Ser)NO_3\times H_2O]$	120-200 220-350 360-800	3.01 18.99 57.06	3.196 18.466 56.47	$[Zr(Q)_2(Se)NO_3]$ $[Zr(Q)_2NO_3]$ $[ZrO_2]$
$[Zr(Q)_2(Gly)NO_3\times H_2O]$	120-200 210-360 380-810	3.87 14.61 60.10	3.37 14.08 59.62	$[Zr(Q)_2(Gly)NO_3]$ $[Zr(Q)_2NO_3]$ $[ZrO_2]$

Table 3. Antifungal and antibacterial activity (ZOI in mm)

Compound	Antifungal test models			Antibacterial test models	
	C. albicans	*C. krusei* GO3	*A. fumigatus*	*S. aureus* 209P	*E. faecium* VRE 323
	Zone of inhibition in mm				
$[Zr(Q)_2(Ala)\ NO_3\cdot H_2O]$	10	20	15	28	15
$[Zr(Q)_2(Gly)\ NO_3\cdot H_2O]$	10	21	16	26	17
$[Zr(Q)_2(Ser)NO_3\cdot H_2O]$	10	22	16	26	16
Amphotericin B	19	15	16	-	-
Vancomycin	-	-	-	15	12

Infra-red spectra

The FT-IR spectra of the metal complexes were obtained in KBr discs over the range 4000-400 cm^{-1}. These spectra were assesed with the infra-red spectra reported for amino acid, 8-hydroxyquinoline and their metal complexes (12, 13) and important bands identified are:

1) A broad band present in the region 3600-3200 cm^{-1} which may possibly be due to asymmetric and symmetric O-H stretching vibrations. Another band present at ~1605 cm^{-1} is due to H-O-H bending vibrations, representing the presence of a coordinating water molecule which was further confirmed by thermal studies.

2) A presence of a strong C-O band at ~1108 cm^{-1} which is due to oxine moiety which is coordinated through oxygen and nitrogen atom acting as non-negative bidentate ligand (14). Charles et al. (15) reported that several metals which forms complexes with HQ show a C-O band at ~1120 cm^{-1}. The position of the observed band may vary for different metal ligand complexes. In the spectra of free HQ ligand, C=N band is observed near 1580 cm^{-1} which is found to shift to lower frequency i. e. ~1498 –1497 cm^{-1} in the spectra of complexes. A negative shift in this vibrational mode on complexation specifies the coordination through tertiary nitrogen donor of HQ. In plane and out of plane deformation

Table 4. Cytotoxicity study

Sample	Conc. µg/mL	Cells	No. of EA cells		% death	No. of DLA cells		% death
			Live	Dead		Live	Dead	
Control	-	1×10^6	100	0	0	100	0	0
DMSO	20 µL	1×10^6	100	0	0	100	0	0
$[Zr(Q)_2(Ser)NO_3\cdot H_2O]$	10 µg	1×10^6	99	1	1	100	0	0
	20 µg	1×10^6	98	2	2	99	1	1
	50 µg	1×10^6	97	2	2	94	0	0
	100 µg	1×10^6	98	3	3	99	1	1
	200 µg	1×10^6	92	8	8	99	1	1
$[Zr(Q)_2(Gly)\ NO_3\cdot H_2O]$	10 µg	1×10^6	99	1	1	100	0	0
	20 µg	1×10^6	97	3	3	100	0	0
	50 µg	1×10^6	96	4	4	99	1	1
	100 µg	1×10^6	94	6	6	99	1	1
	200 µg	1×10^6	90	10	10	99	1	1

modes observed at ~515 and ~786 cm^{-1}, confirm coordination though the HQ nitrogen atom.

3) A band observed at ~3046 cm^{-1} due to N-H symmetric vibration, is at higher wave number compared with spectra of free amino acid moiety. This reveals that the amino group is coordinated through nitrogen atom. The n_{assym} (COO^-) band of free amino acid observed at 1610-1590 cm^{-1} shifted to lower wave number in the spectra of metal complexes i.e. ~1574 cm^{-1}. The n_{sym} (COO^-) band of free amino acid observed at 1400 cm^{-1} is shifted to lower wave number in the spectra of metal complexes i.e. ~1378 cm^{-1}, representing coordination of carboxylic acid group with metal ion through the oxygen atom (16). The (C-N) symmetric stretching band observed at 950 cm^{-1} in the spectra of amino acids is found to be shifted to lower wave number ~909 cm^{-1} in the spectra of complexes, which corroborate coordination through amino group of amino acid.

4) The spectra show no absorption peak near 1352 cm^{-1} where ionic nitrate is known to absorb (17) what indicates the absence of ionic nitrate. Other bands present at ~1465, ~1275, ~1031, ~739 cm^{-1} correspond to $\nu_1, \nu_4, \nu_2, \nu_3$ vibrations, respectively and comply with frequencies reported for bidentate nitrate group (18, 19). The separation between ν_1 and ν_4 bands indicates bidentate character of nitrate group (20).

5) Some new bands of weak intensity observed in the regions 650-600 cm^{-1} and 520-480 cm^{-1} may be ascribed to M-N and M-O vibrations, respectively (21, 22), which are absent in the spectra obtained for the ligands.

6) An important feature of infra-red spectra of metal complexes with 8-HQ is the absence of the band at ~3440 cm^{-1} due to the O-H stretching vibration of the OH group of HQ (23). This observation specifies that the complexes are formed by deprotonation of the hydroxyl group of HQ moiety.

Thermal studies

The simultaneous TG/DTA curves of metal complexes were recorded in the nitrogen atmosphere at a heating rate 10°C/min. The TG curves of the complexes elucidate that as temperature increases the decomposition of complexes takes place due to fragmentation and show considerable loss in weight. The TG curves also designate that the stability of these complexes vary from complex to complex. In the first step, weight loss observed in the temperature range of 120-200°C may be due to loss of water molecule. The TG illustrate further loss in weight in the temperature range of 220-350°C, which may be due to decomposition of amino acid moiety. The final step confirms weight loss in the temperature range 380-600°C, which is due to decomposition of nitrate and HQ molecule present in the complexes. Above 600°C a constant plateau is observed which explicate complete decomposition of the complex. Thus, the TG curve indicates that the final decomposed product is ZrO_2.

The DTA of all the complexes illustrate a broad endothermic peak ~ 200°C, which confirms

that there is water molecule present in the complexes. As the temperature increases, the DTA curve shows small exothermic peaks in the range 200-380°C and a broad exothermic peak in the temperature range of 380-650°C, which is owed to decomposition of amino acid moiety, nitrate and 8-hydroxyquinoline groups present in the complexes. The broad band is formed due to simultaneous decomposition of ligand groups and their gaseous products like CO_2, H_2O and NO_2 formed due to oxidation reaction. The data obtained from TG and DTA curves are summarized and presented in Table 2.

On the basis of above physicochemical studies the bonding and structure for the metal complexes can be assigned as indicated in Figure 1.

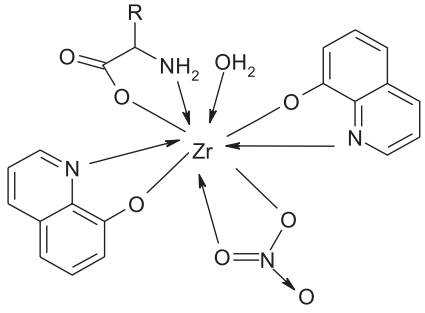
Similar bonding pattern and structures have been reported (6) for thorium mixed ligand complexes.

Antifungal and antibacterial studies

The results obtained for antifungal and antibacterial test models studied by agar well diffusion bioassay revealed biological activity of the complexes (Table 3). The zone of inhibition (ZOI) for *C. krusei* GO3 was elevated as compared to the standard amphotericin, whereas *C. albicans* showed low inhibition with hazy margins. *A. fumigatus* demonstrated the same ZOI as that of standard amphotericin for all the complexes. *S. aureus* 209P and *E. faecium* VRE 323 both showed higher zone of inhibition as compared with standard antibacterial agent, vancomycin. *E. faecium* VRE 323 showed very hazy margins against all the three complexes. All the complexes are almost similar in their potency against all the studied bacterial and fungal strains.

Cytotoxicity studies

The results of the *in vitro* cytotoxicity studies on the two representative complexes:



R = CH3 (Ala), R = CH2OH (Ser), R = H (Gly)

Figure 1. Proposed structures and bonding for the complexes

$[Zr(Q)_2(Ser)NO_3 \times H_2O]$ and $[Zr(Q)_2(Gly)NO_3 \times H_2O]$ are presented in Table 4. The Table clearly indicates that both the complexes have very low cytotoxicity even at the highest concentration i.e. 200 µg/mL. These complexes show more cytotoxic activity against *Ehrlich ascites* cells compared to *Dalton's lymphoma ascites* cells. IC_{50} value is the concentration at which cell number is reduced by 50%. The standard cytotoxic compound, curcumin have been reported (24) to have IC_{50} values of 4 µg/mL and 22 µg/mL against *Ehrlich ascites* and *Dalton's lymphoma ascites* cells, respectively. Comparisons of these cytotoxic activity values (of standards) with those of complexes (presented in Table 4) prove that the complexes prepared in the present work have very low cytotoxic activity.

CONCLUSION

A relatively high decomposition temperature of the mixed ligand complexes indicates that there is a presence of strong metal-ligand bond. The spectral study of the complexes shows that metal ion is bonded through the N- and O- donor atoms of the ligands. Thermal analysis confirms the presence of a coordinated water molecule. On the basis of the above results a nine coordinated structure is proposed for zirconium complexes. All the complexes demonstrate affirmative antifungal and antibacterial activity against selected strains. The cytotoxicity studies of the complexes show very low cytotoxic activity.

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