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Note

Metallopharmaceuticals: Synthesis, characterization and bio-active studies

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Riboflavin can be described as a biological chelating ligand due to the existence of nitrogen and oxygen atoms on its structure that can act as coordinating sites for metal ion on chelation. Co (II) with Riboflavin have been synthesized and characterized by IR, LC-MS, UV, TG-DTA confirms the coordination of ligand. Complex is screened for Anti-microbial activity and Cytotoxicity.

Keywords: Cytotoxicity, Differential thermal analysis (DTA), Riboflavin

Many of the biologically active agents are complexes and even the simpler types of chelating compounds have served as model compounds in bodily process and the living system is partially supported by coordination complexes. In literature, the use of transition metal complexes as therapeutic compounds has become more and more pronounced[1-35]. Metals like Copper, Nickel, Cobalt and Zinc are bio essential elements and responsible for numerous bio-activities in living organism. Riboflavin (7, 8-dimethyl-10-ribityl-isoalloxazine) is a water soluble vitamin present in a wide variety of foods. Its metabolism is controlled by different hormones which regulate its conversion in flavin adenine dinucleotide and flavin mononucleotide[36]. These two coenzymes catalyse many oxidation-reduction reactions and are essential for production of energy[37,38]. The risk of cancer at certain site increase due to Riboflavin deficiency, in some cases Riboflavin reduces the effect of carcinogen this is due to metabolism by flavin-dependent enzymes. Literature survey shows that the synthesis and characterization of Riboflavin complexes have not been fully exploited by researchers. In this paper we present the synthesis and bio-chemistry of Riboflavin complexes with cobalt.

Materials and Methods

Chemicals

All chemical reagents and solvents used were of analytical grade and used without further purification and used as received.

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Instruments

IR spectra are obtained with a Shimadzu IR Prestige 21 FT-IR spectrophotometer. Electronic spectra are recorded on Labindia UV3000$^+$ UV- VIS spectrophotometer. LC-MS Spectra is recorded on Agilent QQQ (ESI-MS) mass spectrometer. TG-DTA spectra are obtained using SDT Q600 V20.9 BUILD 20.

Synthesis of metal complex

A methanolic (10 mL) solution of Cobalt nitrate (0.219 g, 1.0 mM) is added to a sodium hydroxide solution (10 mL) of Riboflavin (0.376 g, 1.0 mM) resulted into brown precipitate under stirring conditions. After constant stirring at room temperature for 30 min, the solution turned to yellowish brown and is filtered off, later green precipitate is formed. It is washed with methanol. Yield is 0.428 g (72%).

Results and Discussion

Characterization of metal complexes

IR spectrum of complex

The strong vibration bands at 1733 cm^{-1} and 1666 cm^{-1} in Riboflavin were assigned as ν (C=O) stretching vibration. In Riboflavin, the band shifted to the range 1647 cm^{-1} in the Cobalt (II) complexes, respectively. This confirmed the coordination of Riboflavin *via* carbonyl oxygen atom. The azomethine, ν (C=N) stretching vibration at 1649-1546 cm^{-1} in Riboflavin shifted in the Cobalt (II) complexes to 1647-1544 cm^{-1} indicative of coordination of the imine nitrogen to the Cobalt (II)

ions. The broad band at 3384 cm^{-1} is indicative of coordination of the ν (OH) of coordinated water molecules.

LC-MS spectrum

The peak at 139 (*m/z*) correspond to $C_5H_{11}O_4$ (part) of Riboflavin ligand and at 245 (*m/z*) is $C_{12}H_9N_4O_2$. The peak at 484 (*m/z*) correspond to cobalt bound to three water molecules refer to [Co(Ribo)3H_2O]. The peaks around 505 (*m/z*) refer to cobalt bound to Riboflavin and four water molecules [Co(Ribo)4H_2O].

Electronic spectrum of complex

The UV–VIS spectrum of the cobalt complexes are recorded in DMF solution in the wavelength range 200–800 nm. The electronic spectrum of Co (II) complex showed bands at 280 nm and 380 nm assignable to d-d transitions, respectively, which are characteristic to the octahedral configuration.

Thermogravimetric analysis

TG-DTA spectrum of complex: Thermal decomposition of the complex takes place in three stages. In the first stage evaporation of water takes in between 100-200°C with a mass loss of 14% (obs 16.7%). The maximum rate of mass loss is indicated by DTA peak at 150°C. After the evaporation of water thermal degradation of ligand starts which is the second stage takes on place at 200-300°C with mass loss of 52.5% (obs 59.5%) which is indicated by DTA peak at 250°C. The third stage is complete decomposition of Riboflavin takes place between 300-500°C with mass loss of 86.4% (obs 89.3%). The maximum rate of mass loss is indicated by DTA peak at 450°C. The end product estimated is oxide of cobalt (Fig. 1).

Proposed structure of [Co(Ribo)4(H_2o)]

In the title mono nuclear complex [Co (Ribo)4H_2O] Co (II) is coordinated by one oxygen and one nitrogen from Riboflavin and oxygen of four aqua ligands. Co (II) in the complex adopts perfect distorted octahedral based structure (Fig. 2).

Antimicrobial screening of [Co(Ribo)4(H_2o)]

The complex is screened *in vitro* for Antibacterial activity against *E. coli*, *S. aureus*, by Disc diffusion method. The Antimicrobial activities of complex are listed in (Table 1).

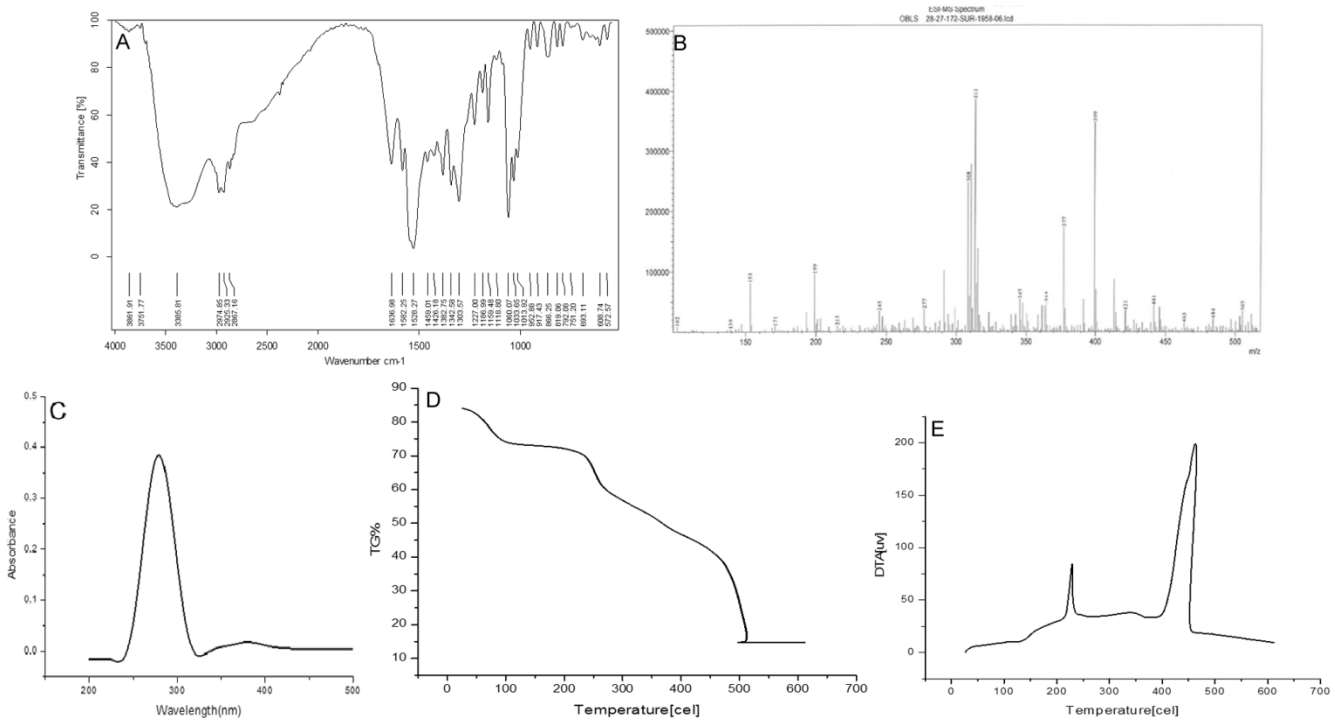

Fig. 1 — Cobalt Riboflavin Complex (A) IR Spectrum; (B) LC-MS Spectrum; (C) UV-Visible Spectrum; (D) TG Spectrum; and (E) DTA Gram

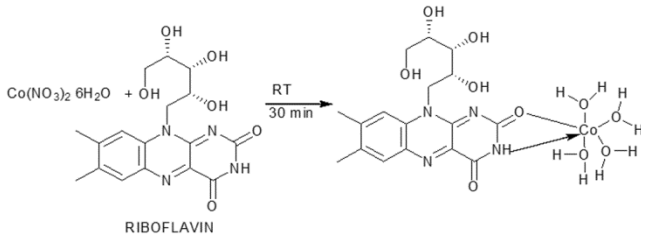

Fig. 2 — Synthetic route and proposed structure of complex 1

Table 1 — Inhibition zones for complex in comparison with standard drug

Bacteria	Inhibition zone (mM)	Streptomycin
E. Coli	Nil	1.8
S. aureus	3.5	1.7
P. aeruginosa	2	1.9

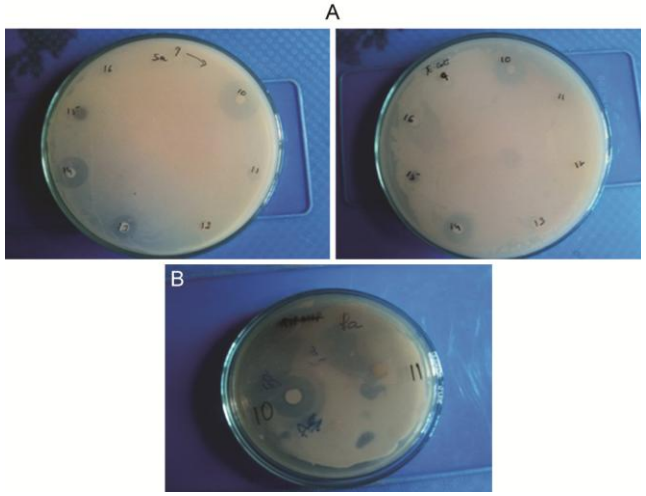

Fig. 3 — Inhibition zones for complex against (A) *S. aureus*, *E. coli*; and (B) *P. aeruginosa*

The *in vitro* antimicrobial properties cobalt mixed ligand complex are tested against these gram–positive and gram–negative bacteria- *S. aureus*, *P. aeruginosa*, *E. coli* the diameters of the inhibition zone equal 3.5 and 2 mM, respectively, and no inhibition zone found for *E. coli* (Fig. 3).

Cytotoxic studies

The complex is screened for its Cytotoxicity MCF-7, A-431 and HepG-2 Cell lines. From the data, it is observed that the complex displayed their Cytotoxicity activities as IC_{50} (µg/mL) against MCF-7, A-431 and HepG-2 Cell lines. The IC_{50} values of the all the complexes are listed in (Table 2).

Complex displayed low Cytotoxicity activities. Cytotoxicity results indicated that all tested complexes (IC_{50} = 440–500 µg/mL) (Fig. 4 & Table 3).

Table 2 — Dose response of complex on MCF-7 cell line incubation time 24 h

Conc (µg/mL)	OD of extract	% Cell Survival	% Cell inhibition
	0.584	100	0
0.1	0.5605	95.97	4.03
1	0.538	92.12	7.88
10	0.513	87.84	12.16
100	0.443	75.85	24.15
500	0.3075	52.65	47.35
Dose response of complex on A-431 cell line incubation time 24 h			
	0.8655	100	0
0.1	0.742	85.73	14.27
1	0.74	85.49	14.51
10	0.688	79.49	20.51
100	0.6785	78.39	21.61
500	0.44	50.83	49.17
Dose response of complex on HEPG-2 cell line incubation time 24 h			
	0.838	100	0
0.1	0.7775	92.78	7.22
1	0.6945	82.87	17.13
10	0.604	72.07	27.93
100	0.5285	63.06	36.94
500	0.4	47.73	52.27

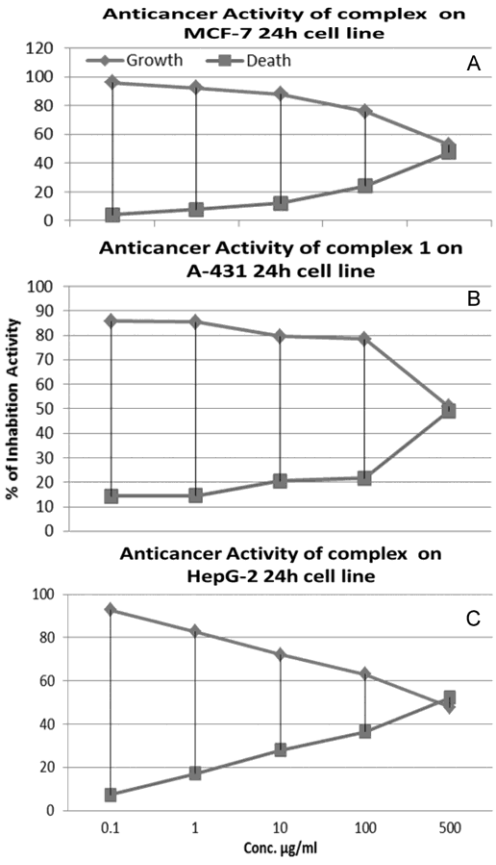

Fig. 4 — Effect of complex on (A) MCF-7; (B) A-431; and (C) HepG-2 cell viability for 24 h incubation time

Table 3 — Cytotoxic activity of complex

Cell line	Incubation period	IC_{50} µg/mL
MCF-7	24 h	>500
A-431	24 h	>500
HepG-2	24 h	440.01

Conclusion

This complex which had been studied for Antimicrobial activity proved to have better activity than the standard drug like streptomycin. This complex is also studied for cytotoxicity and is found to exhibit activity. From the current research Riboflavin deficiency has been suggested as a risk factor for cancer, hence coordination of Riboflavin with cobalt are good candidates exhibiting both microbial activity and cytotoxicity on further preclinical studies may lead to development as effective therapeutic strategies for treating cancer.

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