

Note

Metallopharmaceuticals: Synthesis, characterization and bio-active studies

S Aruna Kumari¹, Bonige Kishore Babu^{1*}, Ch China Satyanarayana², M Padma¹ & B Swarna Latha³

¹Department of Engineering Chemistry, AU College of Engineering (A), Andhra University, Visakhapatnam- 530 003; & Department of H & BS, Aditya Engineering College, Surampalem- 533 437, Andhra Pradesh, India

²Sree Konaseema Bhanaji Ramars College, Amalapuram- 533 201, Andhra Pradesh, India

³Smt. Kandukuri Rajyalakshmi College for Women, Rajamundry- 533 103, Andhra Pradesh, India

Received 04 April 2019; revised 26 July 2019

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 pronounced¹⁻³⁵. 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 mononucleotide³⁶. 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.

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 $\text{C}_5\text{H}_{11}\text{O}_4$ (part) of Riboflavin ligand and at 245 (m/z) is $\text{C}_{12}\text{H}_9\text{N}_4\text{O}_2$. The peak at 484 (m/z) correspond to cobalt bound to three water molecules refer to $[\text{Co}(\text{Ribo})3\text{H}_2\text{O}]$. The peaks around 505 (m/z) refer to cobalt bound to Riboflavin and four water molecules $[\text{Co}(\text{Ribo})4\text{H}_2\text{O}]$.

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 $100\text{--}200^\circ\text{C}$ 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 $200\text{--}300^\circ\text{C}$ 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 $300\text{--}500^\circ\text{C}$ 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 $[\text{Co}(\text{Ribo})4(\text{H}_2\text{O})]$

In the title mono nuclear complex $[\text{Co}(\text{Ribo})4\text{H}_2\text{O}]$ 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 $[\text{Co}(\text{Ribo})4(\text{H}_2\text{O})]$

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).

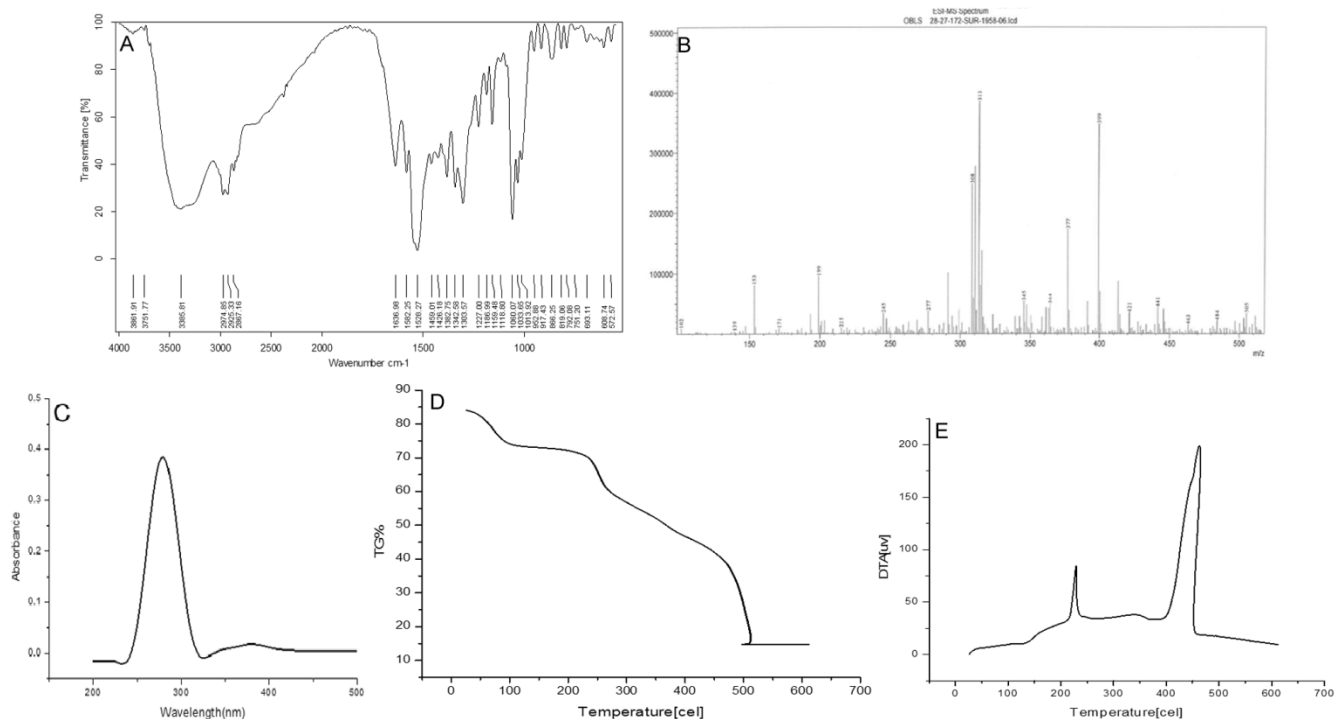


Fig. 1 — Cobalt Riboflavin Complex (A) IR Spectrum; (B) LC-MS Spectrum; (C) UV-Visible Spectrum; (D) TG Spectrum; and (E) DTA Gram

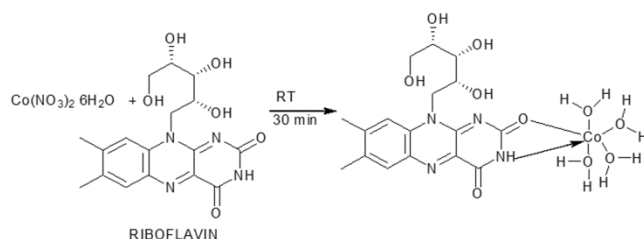


Fig. 2 — Synthetic route and proposed structure of complex 1

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

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

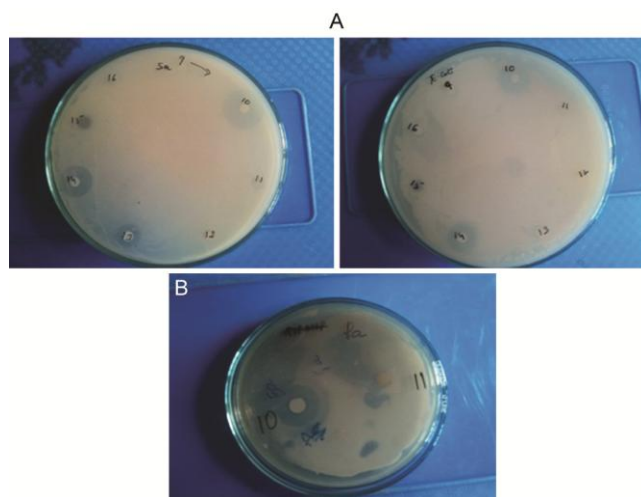


Fig. 3 — Inhibition zones for complex against (A) *S. aureus*, *E. coli*; and (B) *P. aeruginosa*

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 IC₅₀ (µg/mL) against MCF-7, A-431 and HepG-2 Cell lines. The IC₅₀ values of the all the complexes are listed in (Table 2).

Complex displayed low Cytotoxicity activities. Cytotoxicity results indicated that all tested complexes (IC₅₀ = 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
0.1	0.584	100	0
1	0.5605	95.97	4.03
10	0.538	92.12	7.88
100	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

0.1	0.8655	100	0
1	0.742	85.73	14.27
10	0.74	85.49	14.51
100	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

0.1	0.838	100	0
1	0.7775	92.78	7.22
10	0.6945	82.87	17.13
100	0.604	72.07	27.93
100	0.5285	63.06	36.94
500	0.4	47.73	52.27

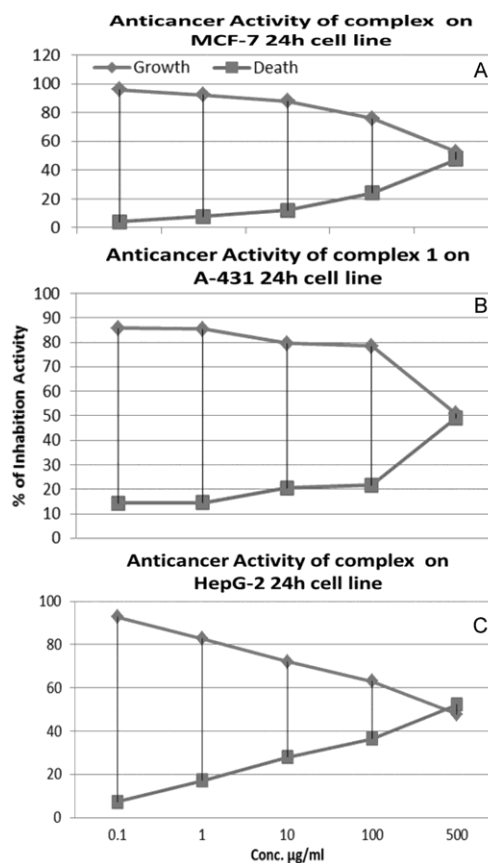


Fig. 4 — Effect of complex on (A) MCF-7; (B) A-431; and (C) HepG-2 cell viability for 24 h incubation time

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.

References

- Pattan SR, Pawar SB, Vetel SS, Gharate UD & Bhawar SB, The scope of metal complexes in drug design - a review. *Indian Drugs*, 49 (2012) 5.
- Katsarou ME, Efthimiadou EK, Psomas G, Karaliota A & Vourloumis D, Novel Copper(II) Complex of N-Propyl-norfloxacin and 1,10-phenanthroline with enhanced antileukemic and DNA nuclease activities. *J Med Chem*, 51 (2008) 470.
- Huang KB, Chen ZF, Liu YC, Wang M, Wei JH, Xie XL, Zhang JL, Hu K & Liang H, Copper (II/I) complexes of 5-pyridin-2-yl- [1,3]dioxolo[4,5-g] isoquinoline: Synthesis, crystal structure, antitumor activity and DNA interaction. *Eur J Med Chem*, 70 (2013) 640.
- Hindo SS, Frezza M, Tomco D, Heeg MJ, Hryhorczuk L, McGarvey BR, Dou QP & Verani CN, Metals in anticancer therapy: Copper(II) complexes as inhibitors of the 20S proteasome. *Eur J Med Chem*, 44 (2009) 4353.
- Gao EJ, Lin L, Zhang Y, Wang RS, Zhu MC, Liu SH, Sun TD, Jiao W & Andrey VZ, Synthesis, characterization, and study on HeLa cells activity of a dinuclear complex [Cu₄(phen)₄H₂O₂](pyri).3H₂O. *Eur J Med Chem*, 46 (2011) 546.
- Gligorijević N, Todorović T, Radulović S, Sladić D, Filipović N, Godevac D, Jeremić D & Anđelković K, Synthesis and characterization of new Pt(II) and Pd(II) complexes with 2-quinolinecarboxaldehyde selenosemicarbazone: Cytotoxic activity evaluation of Cd(II), Zn(II), Ni(II), Pt(II) and Pd(II) complexes with heteroaromatic selenosemicarbazones. *Eur J Med Chem*, 44 (2009) 1623.
- Kowalski K, Hikisz P, Szczupak Ł, Therrien B & Koceva-Chyła A, Ferrocenyl and dicobalt hexacarbonyl chromones e New organometallics inducing oxidative stress and arresting human cancer cells in G2/M phase. *Eur J Med Chem*, 81 (2014) 289.
- Liu YC, Wei JH, Chen ZF, Liu M, Gu YQ, Huang KB, Li ZQ & Liang H, Antitumor activity of zinc(II) and copper(II) complexes with 5,7-dihalo-substituted-8-quinolinoline. *Eur J Med Chem*, 69 (2013) 554.
- El-Morsy FA, Jean-Claude BJ, Butler IS, El-Sayed SA & Mostafa SI, Synthesis, characterization and anticancer activity of new zinc(II), molybdate(II), palladium(II), silver(I), rhodium(III), ruthenium(II) and platinum(II) complexes of 5,6- diamino-4-hydroxy- 2-mercaptopyrimidine. *Inorganica Chimica Acta*, 423 (2014) 144.
- Rosu T, Negoiu M, Pasculescu S, Pahontu E, Poirier D & Gulea A, Metal-based biologically active agents: Synthesis, characterization, antibacterial and antileukemia activity evaluation of Cu (II), V (IV) and Ni (II) complexes with antipyrine- derived compounds. *Eur J Med Chem*, 45 (2010) 774.
- Li MX, Zhang LZ, Zhang D, Ji BS & Zhao JW, Synthesis, crystal structures, and biological evaluation of manganese (II) and nickel (II) complexes of 4- cyclohexyl-1-(1-(pyrazin-2-yl) ethylidene) thiosemicarbazide. *Eur J Med Chem*, 46 (2011) 4383.
- Machado I, Marino LB, Demoro B, Echeverría GA, Piro OE, Leite CQ, Pavan FR & Gambino D, Bioactivity of pyridine-2-thiolato-1-oxide metal complexes: Bi (III), Fe (III) and Ga (III) complexes as potent anti-Mycobacterium tuberculosis prospective agents. *Eur J Med Chem*, 87 (2014) 267.
- Kalinowska-Lis U, Szewczyk EM, Chęcińska L, Wojciechowski JM, Wolf WM & Ochocki J, Synthesis, characterization, and antimicrobial activity of Silver(I) and copper(II) complexes of phosphate derivatives of pyridine and benzimidazole. *Chem Med Chem*, 9 (2014) 169.
- Thompson KH & Orvig C, Metal complexes in medicinal chemistry: new vistas and challenges indrug design. *Dalton Trans*, 14 (2006) 761.
- Oehninger L, Rubbiani R & Ott I, N-Heterocyclic carbene metal complexes in medicinal chemistry. *Dalton Trans*, 42 (2013) 3269.
- Sobiesiak M, Lorenz IP, Mayer P, Woźniczka M, Kufelnicki A, Krajewska U, Rozalski M & Budzisz E, Synthesis, X-ray structure and cytotoxic effect of nickel(II) complexes with pyrazole ligands. *Eur J Med Chem*, 46 (2011) 5917.
- Desbois N1, Pertuit D, Moretto J, Cachia C, Chauffert B & Bouyer F, cis-Dichloroplatinum(II) complexes tethered to dibenzo[c,h][1,6] naphthyridin-6- ones: synthesis and cytotoxicity in human cancer cell lines *in vitro*. *Eur J Med Chem*, 69 (2013) 719.
- Chen Y, Qin MY, Wu JH, Wang L, Chao H, Ji LN & Xu AL, Synthesis, characterization, and anticancer activity of ruthenium(II)-b-carboline complex. *Eur J Med Chem*, 70 (2013) 120.
- Kumar SV, Lo WKC, Brooks HLJ & Crowley JD, Synthesis, structure, stability and antimicrobial activity of a ruthenium(II) helicate derived from a bis- bidentate “click” pyridyl-1,2,3-triazole ligand. *Inorganica Chimica Acta*, 425 (2015) 1.
- Zhao B, Shang X, Xu L, Zhang W & Xiang G, Novel mixed ligand di-n-butyltin(IV) complexes derived from acylpyrazolones and fluorinated benzoic acids: Synthesis, characterization, cytotoxicity and the induction of apoptosis in Hela cancer cells. *Eur J Med Chem*, 76 (2014) 87.
- Balas VI, Verginadis II, Geromichalos GD, Kourkoumelis N, Male L, Hursthouse MB, Repana KH, Yiannaki E, Charalabopoulos K, Bakas T & Hadjikakou SK, Synthesis, structural characterization and biological studies of the triphenyltin(IV) complex with 2- thiobarbituric acid. *Eur J Med Chem*, 46 (2011) 2835.

- 22 Darawsheh M, Abu Ali H, Abuhijleh AL, Rappocciolo E, Akkawi M, Jaber S, Maloul S & Hussein Y, New mixed ligand zinc(II) complexes based on the antiepileptic drug sodium valproate and bioactive nitrogen-donor ligands. Synthesis, structure and biological properties. *Eur J Med Chem*, 82 (2014) 152.
- 23 Ruan BF, Zhu YZ, Liu WD, Song BA & Tian YP, Synthesis, characterization, cytotoxicity and antibacterial activity of an anthracenyl-linked bis(pyrazolyl)methane ligand and its zinc(II) complexes. *Eur J Med Chem*, 72 (2014) 46.
- 24 Salem ML, Salama A, El-Gowily AH, Mansour MA & Ali El-Said MM, Cisplatin augments the anti-schistosomal effect of praziquantel in a schistosoma-infected cancer model. *Indian J Biochem Biophys*, 56 (2019) 57.
- 25 Raja DS, Ramachandran E, Bhuvanesh NSP & Natarajan K, Synthesis, structure and *in vitro* pharmacological evaluation of a novel 2-oxo-1,2- dihydroquinoline-3-carbaldehyde (20-methylbenzoyl) hydrazone bridged copper(II) coordination polymer. *Eur J Med Chem*, 64 (2013) 148.
- 26 Raja DS, Bhuvanesh NSP & Natarajan K, Synthesis, crystal structure and pharmacological evaluation of two new Cu(II) complexes of 2-oxo-1,2- dihydroquinoline-3-carbaldehyde (benzoyl) hydrazone: A comparative investigation. *Eur J Med Chem*, 47 (2012) 73.
- 27 Rajarajeswari C, Loganathan R, Palaniandavar M, Suresh E, Riyasdeen A & Akbarsha MA, Copper(II) complexes with 2NO and 3N donor ligands: synthesis, structures and chemical nuclease and anticancer activities. *Dalton Trans*, 42 (2013) 8347.
- 28 Raman N, Jeyamurugan R, Senthilkumar R, Rajkapoor B & Franzblau SG, *In vivo* and *in vitro* evaluation of highly specific thiolate carrier group copper(II) and zinc(II) complexes on Ehrlich ascites carcinoma tumor model. *Eur J Med Chem*, 45 (2010) 5438.
- 29 Tabassum S, Asim A, Arjmand F, Afzal M & Bagchi V, Synthesis and characterization of copper(II) and zinc(II)-based potential chemotherapeutic compounds: Their biological evaluation *viz.* DNA binding profile, cleavage and antimicrobial activity. *Eur J Med Chem*, 58 (2012) 308.
- 30 Ramachandran E, Raja DS, Bhuvanesh NSP, Natarajan K, Synthesis, characterization and *in vitro* pharmacological evaluation of new water soluble Ni(II) complexes of 4N-substituted thiosemicarbazones of 2-oxo-1,2-dihydroquinoline-3-carbaldehyde. *Eur J Med Chem*, 64 (2013) 179.
- 31 Jopp M, Becker J, Becker S, Miska A, Gandin V, Marzano C & Schindler S, Anticancer activity of a series of copper(II) complexes with tripodal ligands. *Eur J Med Chem*, 132 (2017) 274.
- 32 Lazarević T, Rilak A & Bugarčić ŽD, Platinum, palladium, gold and ruthenium complexes as anticancer agents: Current clinical uses, cytotoxicity studies and future perspectives. *Eur J Med Chem*, 142 (2017) 8.
- 33 Low ML, Maigre L, Tahir MI, Tiekink ER, Dorlet P, Guillot R, Ravoo TB, Rosli R, Pagès JM, Policar C, Delsuc N & Crouse KA, New insight into the structural, electrochemical and biological aspects of macrocyclic Cu(II) complexes derived from S-substituted dithiocarbamate schiff bases. *Eur J Med Chem*, 120 (2016) 1.
- 34 Gouda AM, El-Ghamry HA, Bawazeer TM, Farghaly TA, Abdalla AN & Aslam A, Antitumor activity of pyrrolizines and their Cu(II) complexes: Design, synthesis and cytotoxic screening with potential apoptosis-inducing activity. *Eur J Med Chem*, 145 (2018) 350.
- 35 Qin QP, Wang SL, Tan MX, Liu YC, Meng T, Zou BQ & Liang H, Synthesis of two platinum(II) complexes with 2-methyl-8-quinolinol derivatives as ligands and study of their antitumor activities. *Eur J Med Chem*, 161 (2019) 334.
- 36 Barker BM & Bender DA (Eds.) *Vitamins in medicine*. (William Heinemann Medical Books, London) 1995.
- 37 Chadar SN & Khan F, *Electrode Kinetics and Ternary Complexes of [MnII-antibiotics-vitamine-B2] vis-à-vis Kinetics of Electrode Reaction*. *J Indian Chem Soc*, 83 (2006) 1242.
- 38 Escott-Stump S, (Ed.) *Nutrition and Diagnosis-Related Care*. 6th Ed. (Philadelphia, Pa: Lippincott Williams and Wilkins) 2008.