Synthesis, Characterization and Biological Studies of Metal Complexes Derived from 3–Hydroxyquinoxaline–2–Carboxaldehyde and 2–Aminobenzothiazole

P. Dharmapandian, **J. Joseph, J. Dharmaraja**

Abstract: ***Novel metal complexes were synthesized by the condensation of 3–hydroxyquinoxaline–2–carboxaldehyde and 2–aminobenzothiazole. Using elemental analysis, IR, 1H–NMR, UV–Vis., molar conductance, magnetic moment and electrochemical studies, the ligands and its metal complexes were characterized. The magnetic moment and electronic spectral data evidenced that all the complexes exhibit square planar geometry. The metal complexes showed better antimicrobial screening than that of its free ligands. Using the cyclic voltammetry technique, the DNA binding behavior of complexes with CT DNA has been studied. The DNA binding constants revealed that intercalation binding mode for all metal complexes with DNA. Antibacterial screening of the ligand and its complexes reveal that all the complexes show higher activities than the ligand.***

***Keywords**: 2–Aminobenzothiazole; Metal–Schiff base Complexes; Spectral; DNA binding; Antimicrobial; Antioxidant activities.*

Abbreviations:
2ABT (L) = *2–Aminobenzothiazole*;
AA = Ascorbic acid,
DPPH = 2, 2–Diphenyl–1–picrylhydrazyl,
CV = Cyclic voltammogram,
MIC = Minimum inhibitory concentration

I. INTRODUCTION

Quinoxalines and its derivatives have been synthesized by many synthetic procedures. The commonly used procedures are from aromatic 1,2–dicarbonyl compounds and 1,2–diamines. One of such familiar method used to prepare quinoxalines is the reaction of a 1,2 –dicarbonyl compound with o–phenylenediamine [1]. In the natural and biological systems, there are many quinoxaline derivatives available. So, it attracted more research interest and these metal complexes of quinoxaline derivatives have been widely reported in the field of photochemical, electrical, dyes, pharmaceuticals, diagnostic agents, as well as in genomic research [2–7]. Due to its better anti–microbial properties, several antibiotics *viz*., levomycin, actinoleutin and echinomycin consist of quinoxaline molecules have been used. Many quinoxaline derivatives have been found to be significant as biocidal agents, due to its high potential for DNA binding and DNA cleavage under physiological conditions. The synthetic value and pharmacological significance of these complexes have encouraged many scientists to design new complexes of quinoxaline derivatives [8,9]. The present study was focused on the synthesis and characterization of quinoxaline derivatives and their metal complexes from 3–hydroxyquinoxaline–2–carboxaldehyde and 2–aminobenzothiazole. They were subjected to DNA binding and SOD activity studies.

II. EXPERIMENTAL METHOD

2.1. Material

All chemicals and solvents were analaR grade and were purchased from Merck. All supporting electrolyte solutions were prepared using analytical grade reagents. Calf thymus DNA purchased from Genie Biolab, Bangalore, India.

2.2. Instrumentation

CNH analysis was done by using Elementar Vario ELIII Carlo Erba 1108 at CDRI Lucknow. Using ammonium oxalate of AnalaR grade, the quantity of metals present in the metal complexes was determined as its oxide gravimetrically [10,11]. Molar conductance of the complexes was measured using a coronation digital conductivity meter. The magnetic moment was determined using Gouy's magnetic balance. Infrared spectra of synthesized ligands and their metal complexes have been recorded using KBr pellets in a Shimadzu FTIR IR Affinity–1 spectrometer instrument in region from 4000 cm–1 to 350 cm–1. The NMR spectra of ligands and metal complexes were recorded using Bruker Avance II 400 MHz spectrometer from SAIF, Punjab University, Chandigarh. Electronic spectra were recorded in a Systronics 2201 Double beam UV–Vis., spectrophotometer within the range of 200–1100 nm regions. The Thermal Analyses (TGA/DTA) of metal complexes was measured by using a Shimadzu TG–50 Thermobalance, in the temperature range of 0ºC–1000ºC, under a dynamic atmosphere of nitrogen. The cyclic voltammogram of the metal complexes was recorded using CHI 604D electrochemical analyser.

2.3. Synthesis of Schiff bases

Hot ethanolic solutions of 2–aminobenzothiazole (2 M) was added drop wise to one mole of 3–hydroxyquinoxaline–2–carboxaldehyde in the presence of 40 mL ethanol. Then the mixture was refluxed using anhydrous potassium carbonate as a catalyst.

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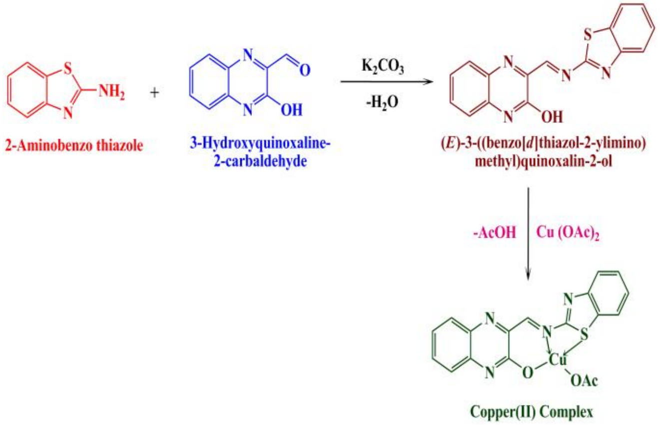
The obtained product was set aside in a refrigerator for 12 hrs. The progress of reaction was monitored by TLC. After completion of reaction the solid material was removed by filtered and recrystallized from ethanol.

2.4. Synthesis of Metal Complexes

Ethanolic solutions of ligand(s) (2 M) and metal acetate(s) (1M) were refluxed for about 6 hrs at room temperature (Scheme 1). The course of reaction was observed by thin layer chromatography up to the formation of product. Then the solution was poured on crushed ice. Thus, obtained solid compound was filtered and recrystallized from ethanol.

2.5. DNA Binding Studies

The DNA binding efficiency of metal complexes were carried out in accordance with the procedure reported by Joseph et al. [12]. All the analyses were made in three replicate and the results were compared with the control.



Scheme I: General Synthetic Route of Copper (II) Complex

III. RESULTS AND DISCUSSION

All the metal complexes possess poor solubility in commonly used non–polar solvents, but have high solubility in DMSO, DMF and insoluble in water. Using ammonium oxalate of AnalaR grade, the quantity of metals present in the metal complexes was determined as its oxide gravimetrically [10,11]. The metal complexes were dissolved in DMSO and their molar conductance was measured. The conductance values were observed in the range, 1.5 to 19.8 mho cm2 mol–1 suggested that the non–electrolytic behavior of metal complexes [13]. The chemical analysis of acetate ion (by the addition of ferric chloride solution) was observed that no precipitate formed. This observation also confirmed the presence of acetate ion inside the coordination sphere. The magnetic moments of copper(II) complex is observed around 1.91 B.M. which is very close to spin–only value i.e. 1.73 B.M. corresponds to mononuclear copper(II) compounds having d9–electronic configuration.

3.1. FT– IR and ^{1}H NMR studies

Schiff base ligand, the characteristic stretching frequencies of C=O and NH2 groups at 1680 cm–1 and 3500 cm–1 were absent respectively. This proves the Schiff base formation. The free ligand exhibits a strong intense band around 1640 cm–1 which is assigned to the azomethine group ν(C=N), confirms the formation of the preferred Schiff base ligand. On complexation, this band is shifted to a lower value of about ~30 cm–1 indicating that imine nitrogen present in the azomethine group undergoes chelation with the central metal ions in the complexes [14]. A new band at 435–455 cm–1 region is seen in the metal complex, which further supported the imine nitrogen coordination to metal ion, ν(M—N).

^{1}H NMR of free Schiff base ligand, exhibited resonance signals at 8.65 ppm assigned to azomethine (–HC=N) proton of the preferred Schiff base moiety. The azomethine proton in the complex shows a peak at 8.9 ppm, which is slightly downfield shift of 0.25 ppm. The shielding detected confirms the azomethine nitrogen atom coordination to the copper (II) ion.

3.2. Electronic Spectral Features Studies

UV–Visible absorption spectra of Schiff base ligands and their metal complexes were measured using DMSO solvent. In the electronic absorption spectrum of all the Schiff base ligands two absorption bands have been seen, 36,000 – 43,000 cm^{-1} and at 22,000 – 35,000 cm^{-1} regions, designated to π–π* and n–π* transitions which can be assigned to aromatic ring present in the quinoxaline moiety and the hetero atom (N, O and S) present respectively. Metal complexes also showed these transitions; however, they are shifted towards lower and higher frequencies, confirming the coordination of the ligand to the metallic ions. The Schiff base ligand, exhibited absorption maximum at 43,859 cm^{-1} (228 nm) and 31,250 cm^{-1} (320 nm) which is assigned to π–π* and n–π* transitions.

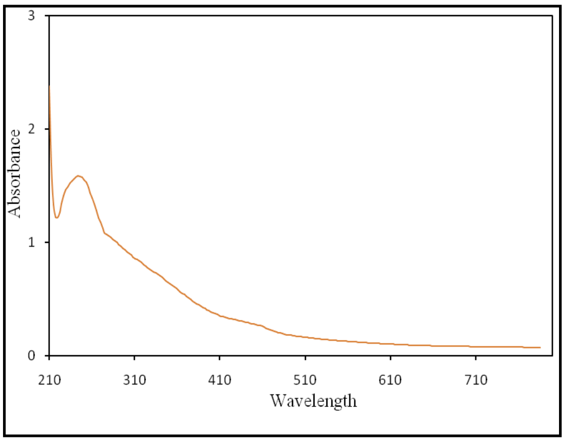


Fig. 1. UV – Visible Spectrum of ligand

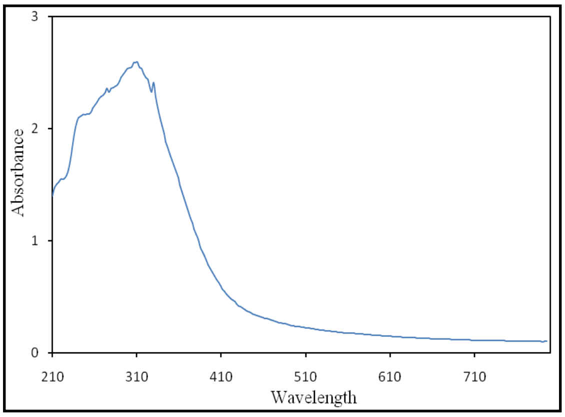


Fig. 2. UV – Visible Spectrum of Cu (II) complex

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On coordination with copper (II) ion, these transitions were shifted to lower or higher wavelength. Broad absorption peaks at 41,841 cm^{-1} (239 nm), 29,850 cm^{-1} (335 nm) and 23,310 cm^{-1} (429 nm) have been observed for the copper complex derived from quinoxaline derivative. The first two bands ν_1 and ν_2 are designated to be $^2B_{1g}\rightarrow{}^2A_{1g}$ transition, $(dx^2{-}y^2\rightarrow dz^2)$, $^2B_{1g}\rightarrow{}^2B_{2g}$ $(dx^2{-}y^2\rightarrow dxy)$ transition and the third band perhaps caused by charge transfer. These transitions are consistent with a square planar geometry around copper(II) ion which is further confirmed by its magnetic moment value of 1.82 B.M [15–18]. It is revealed that in the electronic spectra of copper complex there is more domination of intense intra ligand and charge transfer bands. Hence, it causes the appearance of weak shoulders for low energy bands.

3.3. Thermo gravimetric Analysis

Copper complex involves three decomposition steps. Till 195 °C the complex showed thermal stability. This evidences the absence of water molecules in the complex. Initially, both the acetate molecules present in the complex were lost at about 205 – 220 °C. Then, the complex undergoes decomposition at about 240 – 405 °C, related to the removal of part of the organic moiety present in the ligand (quinoxaline and benzothiazole). Finally, in the last step, the complex undergoes complete decomposition and resulted in the copper oxide formation as end products. In DTA curve, three peaks of strong exothermic decomposition were seen for the copper complex at 315 °C, 605 °C, 765 °C and it is shown in the figure. Similar curves were obtained for all the other complexes. The amount of copper present in the complex was determined from the obtained copper oxide weight and compared. The amount of copper present in all the complexes agrees well with those of the results obtained earlier.

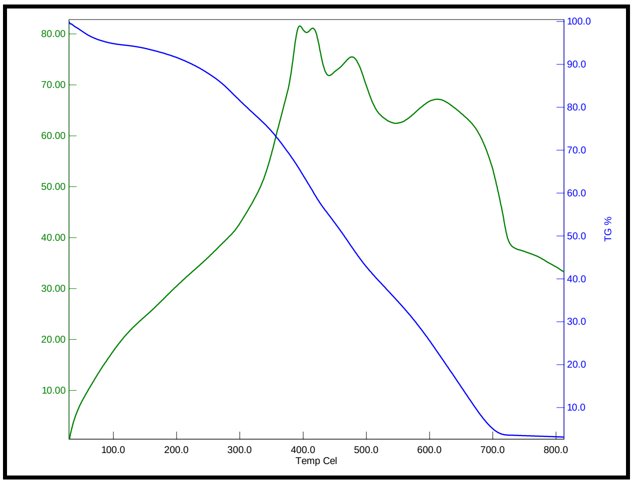

Fig. 3. Thermogravimetric analysis of Cu(II) complex

3.4. DNA Binding Experiments

3.4.1. Electrochemical Studies

Cyclic voltammogram displayed a non–nernstian behaviour for the control experiment i.e., without DNA, although quasi–reversible one electron redox process involving the copper(II)/copper(I) couple have been observed. In the first segment, the reduction and oxidation peaks were seen at –0.7 V and –0.15 V, respectively. This demonstrates the reduction from copper(II) to copper(I) form. Similarly, in the second segment the reduction and oxidation peaks were seen at +0.26 V and +0.5 V which relates to oxidation and reduction behaviour of the ligand, respectively. Upon the addition of CT–DNA, the peak currents were decreased. The decrease in current is due to gradual diffusion of the solution to the surface of the electrode. Moreover, copper(II) ion form strong coordination bond with guanine bases. This leads to the strong DNA binding affinity with the complex. This indicates that the copper complex binds to the DNA via intercalation mode [19, 20].

3.4.2. Absorption Spectral Titrations

UV region, the copper complex showed absorption bands at ca. 415, 430 nm. As the DNA concentration increases, hypochromism was observed with a shift in greater wavelength. This indicates that the copper complex interacts with DNA via N^7 atom of guanine base pair. The measured bathochromism and hypochromism for complexes are less as compared to that of potential intercalators [21]. The intrinsic binding constant (K_b) was determined by observing the shift in absorbance with respect to DNA concentration. The determined K_b values for the copper complexes are less than that of the classical intercalators *viz.*, EthBr K_b, 1 × 10^6 M^{-1} in 25 mM Tris–HCl/40 mM. NaCl

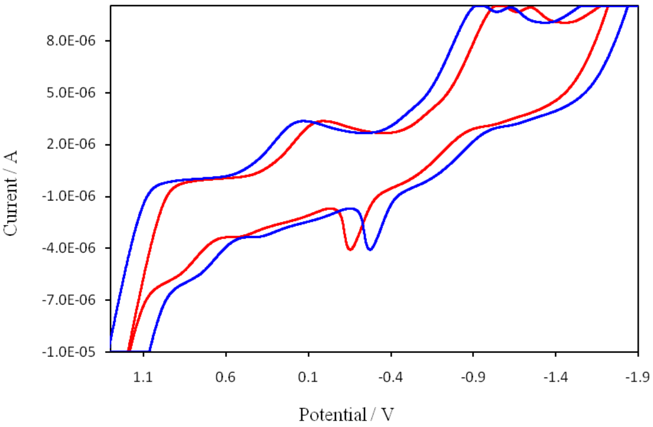

Fig. 4. Cyclic Voltammogram of Copper Complex in the Presence and Absence of DNA

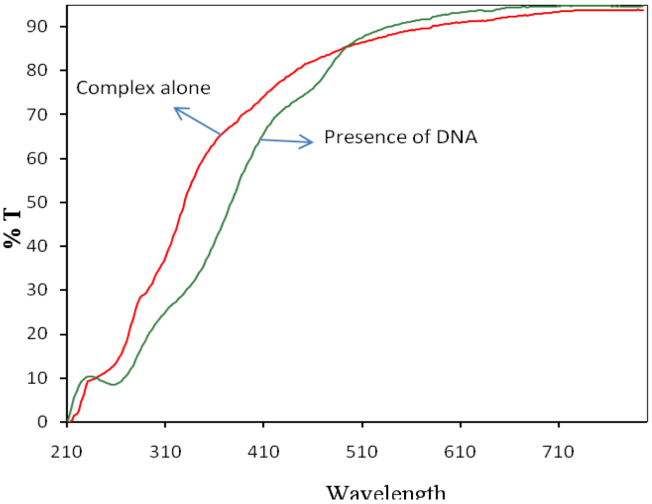

Fig. 5. UV Absorption Spectrum for Copper Complex in the Presence and Absence of DNA

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Table 1. Antimicrobial Inhibition Zone (in mm) for Ligand, its Metal Complexes and Standard Drugs

Strains	L	Cu (L)	Co (L)	Ni (L)	Zn (L)	Ampicillin	Chloramphenicol	Norflaxacin
S.aureus	10	23	16	18	15	35	36	35
P.vulgaris	09	22	15	17	14	30	31	30
K.pneumoniae	11	24	17	19	16	30	31	31
E.coli	10	22	17	18	16	31	32	31

Buffer, pH 7.9 [22]. This value evidences that the imine complexes involved in DNA binding via the DNA base pair. The high binding affinity observed by the metal complex is usual on the basis of extra aromatic ring in condensation which improves the degree of stacking of the imine with the base pairs of DNA. We can conclude that the free ligand and the copper(II) complex can interact with CT–DNA through the intercalation mode of binding.

3.4.3. Antimicrobial Activity

Using disc diffusion process, a comparative study of *in–vitro* antimicrobial activities for the ligand (L) and its metal complexes have been done [23]. About 30 µg of chloramphenicol was used as positive control for bacterial study. From the experiments, the MIC (minimum inhibitory concentration) values were determined for all the metal complexes. The studies confirm that all the metal [cobalt(II), nickel(II), copper(II) and zinc(II)] complexes exhibit better antibacterial activity as compared to free ligands. For the bacterial study we have used two gram–negative bacteria, *K.pneumoniae* and *E.coli,* and two gram–positive bacteria, *S.aureus* and *P.vulgaris*. The copper complex showed higher antimicrobial activity owing to its extensive conjugation. The considerable activity of the ligand may be due to the presence of azomethinne (imine group). Among the metal complexes the copper complexes showed greater activity than that of the nickel, cobalt and zinc complexes. The antimicrobial observations evidently display that the activity of the Schiff base ligands turns out to be more prominent while coordinated to copper(II) ion. However, all compounds exhibit good activity on the yeast cultures with appreciable MIC value.

IV. CONCLUSION

In this study, we have synthesised a novel metal Schiff base complexes by the condensation of 3–hydroxyquinoxaline–2–carboxaldehyde and 2–aminobenzothiazole. Using various physico–chemical and spectral studies confirms all the complexes exhibit square planar environment around the central metal ions. Moreover, all the metal complexes showed significant antimicrobial screening as well as DNA binding activities than that of its free Schiff base ligand. Such increased activity of the metal chelates can be explained as polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with electron releasing groups.

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REFERENCES

1. D. J. Brown, *Quinoxalines, Supplement II*, The Chemistry of Heterocyclic Compounds, Volume 61, John Wiley & Sons, Inc., Hoboken, New Jersey (2004).
2. A. Dell, D. H. Williams, H. R. Morris, G. A. Smith, J. Feeney, G. C. K. Roberts, Structure revision of the antibiotic echinomycin, *Journal of the American Chemical Society* 97 (1975) 2497.
3. S. M. Kher, S. X. Cai, E. Weber, J. F. W. Keana, Regiospecific Oxidative Nitration of 3,4–Dihydro–6,7–disubstituted Quinoxaline–2(1H)–ones Gives 1,4–Dihydro–5–nitro–6,7–disubstituted Quinoxaline–2,3–diones, Potent Antagonists at the NMDA/Glycine Site, *Journal of Organic Chemistry* 60 (1995) 5838.
4. K. Toshima, T. Kimura, R. Takano, T. Ozawa, A. Ariga, Y. Shima, K. Umezawa, S. Matsumura, Molecular design, chemical synthesis and biological evaluation of quinoxaline–carbohydrate hybrids as novel and selective photo–induced DNA cleaving and cytotoxic agents, *Tetrahedron*, 59 (2003) 7057.
5. A. Azqueta, L. Arbillaga, G. Pachón, M. Cascante, E. E. Creppy, A. L. de Cerain, A quinoxaline 1,4–di–N–oxide derivative induces DNA oxidative damage not attenuated by vitamin C and E treatment, Chemico–Biological Interactions 168 (2007) 95.
6. S. Budagumpi, N. V. Kulkarni, G. S. Kurdekar, M. P. Sathisha, V. K. Revankar, Synthesis and spectroscopy of CoII, NiII, CuII and ZnII complexes derived from 3, 5–disubstituted–1H–pyrazole derivative: a special emphasis on DNA binding and cleavage studies, European Journal of Medicinal Chemistry 45 (2010) 455.
7. K. Toshima, R. Takano, T. Ozawa, S. Matsumura, Molecular design and evaluation of quinoxaline–carbohydrate hybrids as novel and efficient photo–induced GG–selective DNA cleaving agents, Chemical Communications (2002) 212.
8. P. Corona, G. Vitale, M. Loriga, G. Paglietti, Quinoxaline chemistry. Part 11. 3–Phenyl–2[phenoxy and phenoxymethyl]–6(7)or 6,8–substituted quinoxalines and N–[4–(6(7)–substituted or 6,8–disubstituted–3–phenylquinoxalin–2–yl) hydroxy or hydroxymethyl] benzoylglutamates. Synthesis and evaluation of in vitro anticancer activity and enzymatic inhibitory activity against dihydrofolate reductase and thymidylate synthase. Il Farmaco 55 (2000) 77.
9. S. Banerjee, A. K. Mukherjee, R. Mukhopadhyay, N. G. Kundu, A. J. Welch, A series of three (E)–2–alkyldene–1,4–di–p–tosyl–1,2,3,4–tetra¬hydro¬quinoxaline compounds, Acta Crystallographica C 57 (2001) 861.
10. A.I. Vogel, Practical Organic Chemistry, 4th Edn., Longmann, 1978.
11. Angellici R.J, Synthesis and Techniques in Inorganic Chemistry, W.B. Saunders Company, 1969.
12. J. Joseph, K Nagashri, G. B Janaki, Novel metal based anti–tuberculosis agent: synthesis, characterization, catalytic and pharmacological activities of copper complexes, European Journal of medicinal chemistry 49 (2012) 151.
13. Geary W. J, The use of conductivity measurements in organic solvents for the characterisation of coordination compounds, Coordination Chemistry Reviews 7 (1971) 81.
14. M. A. Ali, Magnetic and spectroscopic studies on nickel (II) and copper (II) complexes of some neutral tridentate ONS ligands, Canadian Journal of Chemistry 58 (1980) 727.
15. Maria D. M. C, Da silva R, Jorge M. G, Ana L. R. S, Paula C. F. C, Schroder B and Manual A.V, Journal of Molecular Catalysis A: Chemical 224 (2004) 207.
16. Spinu , Kriza A, I, Ni(II) and Cu(II) complexes of bidentate Schiff bases, Acta Chimica Slovenica, 47 (2000) 179.
17. Sathyadevi P, Krishnamoorthy P, Butorac R. R, Cowley A.H and Dharmaraj N, Synthesis of novel heterobimetallic copper(I) hydrazone Schiff base complexes: a comparative study on the effect of heterocyclic hydrazides towards interaction with DNA/protein, free radical scavenging and cytotoxicity, Metallomics 4 (2012) 498.

18. Chaviara A. T, Christidis P. C, Papageorgiou A, Chrysogelou E, Hadjipavlou–Litina D. J, Bolos C. A, In vivo anticancer, anti–inflammatory, and toxicity studies of mixed–ligand Cu(II) complexes of dien and its Schiff dibases with heterocyclic aldehydes and 2–amino–2–thiazoline. Crystal structure of [Cu(dien)(Br)(2a–2tzn)](Br)(H2O), Journal of Inorganic Biochemistry 99 (2005) 2102.
19. Coggan D. Z. M, Haworth I. S, Bates P. J, Robinson A, Rodger A, DNA binding of ruthenium tris(1,10–phenanthroline): Evidence for the dependence of binding mode on metal complex concentration, Inorganic Chemistry 38 (1999) 4486.
20. Tan C, Liu J. L, Chen S, and Synthesis structural characteristics, DNA binding properties and cytotoxicity studies of a series of Ru(III) complexes, Journal of Inorganic Biochemistry 102 (2008) 1644.
21. Dimiza F, Perdih F, Tangoulis V, Turel I, Kessissoglou D. P, Psomas G, Interaction of copper(II) with the non–steroidal anti–inflammatory drugs naproxen and diclofenac: Synthesis, structure, DNA– and albumin–binding, Journal of Inorganic Biochemistry 105 (2011) 47.
22. T. Nogrady, J. Keshmirian, Rotifer neuropharmacology–II. Synergistic effect of acetylcholine on local anesthetic activity in Brachionus calyciflorus (Rotifera, Aschelminthes), Comparative biochemistry and physiology. Part C, Pharmacology, toxicology and endocrinology 83 (1986) 339.
23. Butterfield D. A, Howard B, Yatin S, Koppal T, Drake J, Hensley K, Aksenov M, Aksenova M, Subramaniam R, Varadarajan S, Harris–White ME, Pedigo NW Jr, Carney J. M. Elevated oxidative stress in models of normal brain aging and Alzheimer's disease, Life Sciences 65 (1999) 1883.