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Design and Anticancer, Cytotoxic, Nephrotoxic, DNA cleavage, DNA binding and Antimicrobial studies Co(II), Ni(II), Cu(II) and Zn(II) complexes derived from a Schiff bases of 2-substituted-3-formyl Quinoline and 2-amino-1H-purin-6(7H)-one.

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ABSTRACT

Novel transition metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Schiff base Ligands "(E)-2-((2-hydroxyquinolin-3-yl)methyleneamino)-1H-purin-6(7H)-one" abbreviated as GUOH and "(E)-2-((2-mercaptoquinolin-3-yl)methyleneamino)-1H-purin-6(7H)-one" abbreviated as GUSH derived by the condensation of 2-amino-1,9-dihydro-6H-purin-6-one (Guanine) with 3-formyl-2-hydroxy quinoline and with 3-formyl-2-mercapto quinoline respectively and characterized by elemental analysis, molar conductance, magnetic susceptibilities, UV, IR, ^{1}H-NMR, ESR and thermal studies. The elemental and spectral analysis of the complexes confirms $[M(GUOH)_2(H_2O)_2]$ and $[M(GUSH)_2(H_2O)_2]$ stoichiometry and exhibits octahedral geometry, where M= Co(II), Ni(II), Cu(II) and Zn(II) respectively. Both the ligands act as monobasic and didentate, coordinating through azomethine nitrogen, quinoline oxygen via deprotonation. The synthesized ligands and the metal complexes were screened for the antibacterial, antifungal, DNA cleavage, DNA binding, Cytotoxic, Nephrotoxic, and Anticancer studies. The results reveal that the metal complexes possess higher antimicrobial activity than their corresponding ligands and Cu(II) complexes are found to be more active than the other complexes.$[Ni(GUOH)_2(H_2O)_2]$, $[Co(GUSH)_2(H_2O)_2]$ and $[M(GUSH)_2(H_2O)_2]$have shown complete cleavage of CT-DNA where as other samples have displayed partial cleavage and DNA binding studies of selected compounds revels the Intercalative mode of bindings with CT-DNA.From the Anticancer analysis it is found that $[Cu(GUSH)_2.(H_2O)_2]$ is showing better activity against Cervical Canceramong other tested cell lines,the activity is in the order: Cervical Cancer(HeLa)> Breast Cancer(MCF-7)>Skeletal muscle Myoblast(L6)>Monkey kidney cancer cell lines(Vero)> HumanColon Cancer cell line(HT-29). Nephrotoxicity test against NRK 49F(KIDNEY) shows that the complex Cu(II) complex is showing Nephrotoxicity at CTC_{50}(µg/ml) =526.67±06.

Key words: Quinoline, Guanine, monobasic didentate, Antibacterial, DNA cleavage, Anticancer, Cytotoxic, nephrotoxic.

INTRODUCTION

Quinoline is a heterocyclic base whose potential as anti-inflammatory, analgesics, anti-convulsant, antibacterial, antipyretic, antihypertensive and interferon inducing activity has been reported recently [1-7].The Quinoline derivatives have also been used for many clinical purposes, such as antimuscarinic, noradrenergic receptor

antagonistic, antihypertensive, vasodilative, antithrombotic, antipyretic, anti-inflammatory and in the treatment of acute heart attack [8]. Quinoline containing drugs, particularly 4-aminoquinolines, have a long and successful history as antimalarials [9,10]. Metal complexes of Quinoline derivatives have proven their significance by entering into the field of diagnosis of wide variety of disease like heart disease, brain disorder, cancer, diabetics, tissue hypoxia etc and also to detect the multi-drug resistance [11, 12]. Many Schiff bases of Quinoline have been reported in the last decades. *M. R. Solanki et. al.* has synthesized 2-[(8-hydroxy-1-quinolin-5-yl) methyl]-1*H*-isoindole-1, 3 (2*H*)-dione and its complexes with Cu(II), Ni(II), Mn(II) and Zn(II) along with biological activities of these complexes[13]. *Nora H. Al-Sha'alan et. al*. described the synthesis and characterization of Cu(II), Ni(II), Co(II), Mn(II),Fe(III) and UO_2(VI) complexes of 7-chloro-4-(benzylidenehydrazo)Quinoline[14]. *Patel Sheetal Ashwinbhi et.al* have synthesized transition metal complexes with 2-(8-Hydroxy-quinolin-5-ylamino)-1-(5-methyl-4-methylene-1,4-dihydro-2H-quinazolin -3-yl)-ethanone (PEHQ) and characterized by elemental analyses, spectral studies, magnetic moment determination, molar conductivity and microbicidal activity. The antifungal activity of all the compounds measured for various plant pathogens. Inspection of the result indicates that all compounds are good toxic for fungi. Out of all the compounds copper chelates were more toxic than other[15]. *Antonino Mamo, et. al* have synthesized many substituted 2-Pyridyl-4-phenylquinolines, their transition metal complexes and studied their biological activities[16]. *Wolfgang S. et. al* have studied the fluorescent properties of fluorine substituted Quinoline and their transition metal complexes[17].

Guanine derivatives with various substitutions at N_7 and N_9 position have been synthesized and reported for their analgesic, anti-inflammatory and anti-pyretic activities.The metal complexes of guanine have considerable interest in the design of model complexes involving purines which could mimic three interactions of metal ions with DNA[18,19]. In addition, a few purine like guanine have shown significant anti-inflammatory activity, antitumor activity and different animal cancer[20]. *Shayma A. Shaker et. al*.have carried out extensive synthetic work on transition metal complexes of purine derivatives. They concluded that the chelating sites of copper (II) guanine complexes are probably formed due to the nitrogen atoms N(3) and N(9). [21]

However literature survey reveals that the Schiff bases derived from quinoline and guanine derivatives and their transition metal complexes have not been reported and studied so far. Hence the present study aims for the new transition metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) with the Schiff bases derived from the condensation of 2-amino-1H-purin-6(7H)-one (Guanine) with 3-formyl-2-hydroxy quinoline and with 3-formyl-2-mercapto quinoline.

RESULTS AND DISCUSSION

2.1. Chemistry

All the Co(II), Ni(II), Cu(II) and Zn(II) complexes are coloured, stable, non-hygroscopic and insoluble in common organic solvents like methanol, ethanol, acetone, benzene etc. but soluble in acetonitrile, DMF and DMSO. The elemental analysis and other spectroscopic analysis show that all the complexes possess octahedral geometry. The molar conductance values are too low to account for any dissociation of the complexes in DMF, indicating non electrolytic in nature. The analytical, magnetic and conductance data of the Schiff bases and their corresponding transition metal complexes are given in table-1.

2.2.1. Infrared spectral studies

The significant IR bands for the ligands GUOH and GUSH as well as for theirtransition metal complexes and their tentative assignments are compiled and represented in table-2. The broad band observed at 3403 cm^{-1} in the IR spectrum of the ligand (GUOH) was assigned to υ(OH), which disappeared in all their respective complexes, there by indicating the involvement of phenolic oxygen via deprotonation[22]. The band of υ(NH) observed at 3084cm^{-1} in ligand GUOH and at 3067cm^{-1} in GUSH remains unaltered in the,complexes. The broad band observed at 2685cm^{-1} in the IR spectra of the ligand (GUSH) assigned to υ(SH), which were found to have disappeared in all their respective complexes, there by indicating the involvement of thiolate sulphur in bonding with metal ions through deprotonation. This is further supported by the lower frequency band appeared in the region 634-662 cm^{-1} in the metal complexes due to ν(C-S)(fig1). The carbonyl ($\upsilon_{C=O}$) at 1702-1720cm^{-1} remains almost unaltered in all the complexes indicating its non involvement in complexation. The band at 1617-1618 cm^{-1} is assigned to the azomethine υ(C=N) group[23], lowering of υ(C=N) by 8-20 cm^{-1} in the complexes as compared to its ligands, is due to reduction of double bond character of carbon-nitrogen bond of the azomethine group due to coordination[24]. The

new bands in the region of 561-590cm^{-1}, 420-445cm^{-1} and 351-390cm^{-1} in the spectra of the metal complexes are assigned to stretching frequencies of (M-O), (M-N) and (M-S) bond formation respectively[25].

2.1.2. ^{1}H-NMR spectral studies

^{1}H-NMR spectrum of the ligands and the Zn(II) complexes was scanned in the range 0-16 δ ppm in DMSO-d_6 solvent. The ligand GUOH shows a sharp peak at δ12.5 (S,1H) due to OH at 2-position of quinoline ring of 2-hydroxy quinoline, but in the case of Zn(II) complex which has been disappeared indicating the involvement of phenolic oxygen in the coordination via deprotonation. The Schiff bases exhibit the characteristic resonance at 8.7-8.9ppm due to the azomethine proton. The downfield shift of the azomethine proton from 8.7 ppm in the ligand to 8.2 ppm in the complexes indicate the participation of azomethine nitrogen in thecoordination[26]. A singlet corresponding to one proton observed at 10.92ppm is due to SH group in ligand GUSH which is found to have disappeared in the Zn(II) complex confirming the involvement of thiolate Sulphur in coordination with the metal via deprotonation. The sharp multiplet signals of the phenyl protons are found in the region 6.1-7.7ppm.

2.1.3. UV-visisble spectral studies

The electronic spectra of Co(II) complexes exhibit absorption bands in the region 8000-10,000cm^{-1} and 18,000-20,000cm^{-1}corresponding to ν_1 and ν_3 transitions, respectively which are attributed to the transitions $^{4}T_{1g}$ (F) → $^{4}A_{2g}$ (ν_1) and $^{4}T_{1g}$ (F) → $^{4}T_{1g}$ (P) (ν_3). In the present investigation, brownish Co(II) complexes show the absorption bands at 8954-8968 and 19,165-19,182cm^{-1} are corresponding to ν_1 and ν_3 transitions, respectively[27, 28]. The bands due to the $^{4}T_{1g}$ (F) → $^{4}A_{2g}$ (F) (ν_2) transition could not observed because of its very low intensity(table 3). However the position of the ν_2 band has been computed (16260 cm^{-1}) by the equation ($\nu_2 = \nu_1 + 10Dq$). The intense band around 30000 cm^{-1} may be a charge transfer band. The ligand field parameter such as Dq, B', β and β % have been calculated by using band-fitting equation given by Underhill and Billing[29], the crystal field splitting energy (Dq) value at 869 cm^{-1}. These values are well within the range reported for most of the octahedral Co(II) complexes. The Co(II) complex under present investigation process interelectronic repulsion parameter (B') 945 cm^{-1}. The Racha parameter (B) is less than free ion value (971) suggesting a considerable orbital overlap and delocalization of electrons on the metal ion. The nephelauxetic ratio (β) for the present Co(II) complex is 0.973. This is less than one, suggesting partial covalency in the metal ligand bond. The values Dq, β%, LFSE and ν_2/ν_1 suggest the octahedral geometry for Co(II) complex[30].

The electronic spectrum of Ni(II) complex(table 4) shows two bands at 10256 and 24691 cm^{-1} assignable to $^{3}A_{2g}$→ $^{3}A_{1g}$ (F) (ν_1) and $^{3}A^{2g}$ → $^{3}T^{1g}$(P) (ν_3) transitions respectively, in an octahedral environment[31]. The lowest band ν_2 (10 Dq) was not observed due to limited range of the instrument used. However, it is calculated by using equation suggested by Billing and Underhill. Racha parameter B^1 is less than the free ion value of 1040 cm^{-1} indicating the covalent character of the complex. The ratio ν_2 / ν_1 and β % are further support the octahedral geometry around the Ni(II) ion[32].

The electronic spectra of Cu(II) complexes (table 5)display two prominent bands. A low-intensity broad band of around 14,392 cm^{-1} is assignable to $^{2}T_g$← $^{2}E_g$ transition and another high intensity band at 25,548 cm^{-1} is due to symmetry forbidden ligand → metal charge transfer. On the basis of electronic spectra octahedral geometry around Cu(II) ion is suggested[33].

2.1.4. Magnetic properties

The magnetic measurement for Co(II) complexes exhibit magnetic moment values in the range of 4.60-4.80 B.M., which are well within the octahedral range of 4.3-5.2 BM. Ni(II) complexes showed the magnetic moment values of 3.20-3.28 BM within the range of 2.8-3.5 BM suggesting consistency with their octahedral environment(table-1). The Cu(II) complexes showed magnetic moment value of 1.77-1.79 BM, Which is slightly higher than the spin only value 1.73 BM expected for one unpaired electron, which offers possibility of an octahedral geometry [34].

2.1.5. ESR spectral studies

The powdwer state ESR spectrum of Copper complex was operated in the region 9000MHz with corresponding field intensity at ~3000 Gauss at room temperature. The spectrum exhibits isotropic intense broad signal with g_{iso} 2.072 and no hyperfine splitting was observed. ESR spectrums of this kind have been reported for complexes having large organic ligands [35]. The observed ESR spectrum is characteristic of octahedral geometry, g value averaged to overall directions and G which is measure of extent of exchange interaction between metal ion have been calculated. In present case the value of G was found to be 4.028 according to Hathway. If G value is greater than 4, the spin

exchange interaction is negligible where as G value less than 4 indicate considerable interaction between metal ions in solid complex clearly indicate that Cu(II) ion in the complex is mono-nuclear nature of the complex. The ESR spectrum of one of the representative Cu(II)complex of GUSH is shown in the figure1.

2.1.6. Molar conductivity measurements

The molar conductance value of complexes was obtained at room temperature in DMF solution with 10^{-3}mol/dm^3 concentration. The molar conductivity values of all the complexes fall in the range 16.76-25.10 ohmcm^{2}mol^{-1}, which is in agreement with non-electrolytic nature of the complexes [36].

2.1.6. Mass spectral studies

The mass spectrum of the ligands GUOH and GUSH shows molecular ion peaks M^+ at *m/z306*and *322*respectively. Apart from the molecular ion peaks, the spectrum shows some other peaks, which are due to molecular cations of various fragments of the ligands. A typical mass spectrum of the complex$[Cu(GUOH)_2(H_2O)_2]$is shown in figure 2 shows a molecular ion peak at m/z 710 which is equivalent to its molecular mass. This species on fragmentation gives a molecular ion $[Cu(GUOH)2]^+$ peak at m/z 674 by the loss of two water molecules. Further undergoes demetallation to form the species $[(GUOH) +H]^+$ with m/z 307. Other fragmentation corresponding to the dissociation of the ligands are all shown in the spectrum.

2.1.7. Thermal studies

In the present investigation TGA and DTG data's of Co(II), Ni(II), Cu(II) and Zn(II) complexes of GUOH and GUSH are given in the table-6. In all the complexes, the weight loss taken place in three steps. In the first step 4.85-4.89%, in the temperature range 220-250^{0}C attributed to the weight loss of coordinated water molecules and this process is endothermic in nature, which evident by the DTA signal at 250^{0}C. The weight losses in the second step is 46.36% observed in the temperature range 270-342^{0}C which is due to the loss of two quinoline moiety. In the third step, the weight loss observed is 40.16% in the temperature range 450-498^{0}C which indicate the loss of guanine moiety, and thereafter the curve became plateau due to the formation of stable metal oxides[37]. The TGA/DTA curve of one of the representative $[Cu(GUSH)_2(H_2O)_2]$ complex (9) has been reproduced in figure3.

2.2. Pharmacology

2.2.1. Anti-biogram analysis

The antibacterial and antifungal activities were done at 100, 50 and 25 mgL^{-1} concentrations in DMF solvent using two bacteria *Escherichia coli, Staphylococcus aureus*(table-6) and two fungi*Aspergillus niger and Candidaalbican*(table-7)strains by zone of inhibition method. These bacterial and fungi strains were incubated for 24h and 48h at 37^{0}C respectively. Standard antibacterial (*Gentamycin*) and antifungal drugs(*Fluconazole*) were used for comparison under similar conditions. Activity was determined by measuring the diameter of the zone of inhibition (mm). The results of antibacterial and antifungal activity are given in.

The results reveal that the metal complexes show higher activity than their corresponding ligands. The copper complex show highest activity i.e. 73.75 and 71.37% zone of inhibition against the bacterial stains at 100µg concentration, which is more than the ligand activity. In antifungal studies, copper complexes exhibits extremely high activity, 100% zone of inhibition against A. Niger which is as good as the internal standard at all the concentrations.

This enhancement in the activity may be rationalized on the basis that their structures mainly possess an additional C=N bond. It has been suggested that the ligands with nitrogen and oxygen donor systems inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. Moreover, coordination reduces the polarity [38] of the metal ion mainly because of the partial sharing of its positive charge with the donor groups [39] within the chelate ring system formed during coordination. This process, in turn, increases the lipophilic nature of the central metal atom, which favors its permeation more efficiently through the lipid layer of the microorganism [40], thus destroying them more aggressively.

2.2.2. DNA cleavage studies by gel-electrophoresis method

The Schiff bases GUOH, GUSH, Co(II), Ni(II)and Cu(II)complexes (figure 4) were studied for their DNA cleavage activity by agarose gel electrophoresis method. Lanes M= Standard DNA molecular weight marker (λ DNA HindIII digest, Merck, Bangalore),C=Control DNA,1=GUOH, 2=$[Cu(GUOH)_2,(H_2O)_2]$, 3=$[Ni(GUOH)_2,(H_2O)_2]$,

4=GUSH,5=[Co(GUSH)$_2$,(H$_2$O)$_2$], 6=[Cu(GUSH)$_2$,(H$_2$O)$_2$] complexes respectively on the isolated DNA of E. coli. Control experiment using DNA alone does not show any significant cleavage of DNA even after a longer exposure time.[Ni(GUOH)$_2$,(H$_2$O)$_2$], [Co(GUSH)$_2$,(H$_2$O)$_2$] and [Co(GUSH)$_2$,(H$_2$O)$_2$] have shown complete cleavage of DNA where as other samples have displayed partial cleavage.

2.2.3. DNA binding analysis using viscosity measurement

The Hydrodynamic method(viscometric measurement) is a crucial tool to find the nature of binding of metal complexes to the DNA, in which the solution viscosity of DNA is sensitive to the changes in the effective length of DNA molecules is one of the most critical tests for inferring the binding mode(intercalation or other binding modes) of DNA. This study was regarded as the least ambiguous and the most critical tests of binding mode in solution state in absence of crystallographic structural data[41-43]. Under the appropriate conditions intercalation causes noteworthy increase in the viscosity of DNA solution due to the disjointing of base pairs at intercalation spots. The results of the viscosity measurement for all the complexes that are bound to DNA show increase in relative viscosities with an increase in the [complex]/[DNA] ratio (where [complex]is 50,100,150 and 200 µl) as shown in Figure 5. Thus, the increase in the viscosity has been attributed to the enlargement of the separation between base pairs, which are pushed apart to accommodate the intercalating molecule[44-46]. These results suggested an intercalative binding mode of the complexes with DNA.

2.2.4. DNA melting temperature (T_m) studies

The T_m of *E. coli* DNA is the temperature at which 50% of the nucleotide and its perfect complement are in duplex. Typically, annealing or hybridizations are performed at 5-10^{0}C below the T_m of a duplex. Stability of the DNA double helix influences the melting temperature (T_m) of DNA, while the binding of compounds to DNA alters the T_m depending on the strength of interactions. The intercalation of the complexes into the DNA base pairs causes stabilization of base stacking and hence raises the melting temperature of the double stranded DNA. The DNA melting experiment is useful in establishing the extent of intercalation [47]. As shown in figure 6, the T_m of DNA in the absence of any added complex was found to be 58$\pm$ 1^{0}C, under our experimental conditions [48]. Under the same set of conditions, the presence of [Cu(GUSH)$_2$(H$_2$O)$_2$] complex increased the T_m of about 5^{0}C, which is characteristics of an intercalating behavior of the complexes of the DNA[49].

2.2.5. DNA binding studies by Spectroscopic method:

Electronic absorption spectroscopy is one of the most powerful experimental techniques for probing metal ion–DNA interactions. Binding of the macromolecule leads to changes in the electronic absorption spectrum of the metal complex. Base binding is expected to perturb the ligand field transition of the metal complex. Intercalative mode of binding usually results in hypochromism and bathochromism due to the strong stacking interaction between an aromatic chromophore and the base pairs of DNA. The extent of hypochromism parallels the strength of intercalative binding. On the other hand, metal complexes, which bind non-intercalatively or electrostatically with DNA, may result in hyperchromism or hypochromism. The electronic absorption titration of complex [M(GUSH)$_2$(H$_2$O)$_2$], M= Co(II), Ni(II), Cu(II) and Zn(II) has been carried out at a fixed concentration of complexes (100 µM) in aqueous media at 25^{0}C, while varying the concentration of DNA (0-150 µM). The absorption spectra of the complex [M(GUSH)$_2$(H$_2$O)$_2$] in the absence and presence of DNA is depicted in the Figure 7. Addition of increasing amount of DNA results in an appreciable decrease in absorption intensity of LMCT band at 392 nm with insignificant shift in wavelength. The complex [M(GUSH)$_2$(H$_2$O)$_2$] showed hypochromism (24%) and the K_b value is 2.1 x 10^4 M^{-1}. Isosbestic points are observed near 292 nm for [M(GUSH)$_2$(H$_2$O)$_2$], while binding to DNA, suggesting that the complex has a single mode of binding to DNA. Determinations of intrinsic binding constant, K_b, based upon these absorption titrations may be made with the following equation.

$$[DNA]/ (\varepsilon_A-\varepsilon_F) = [DNA]/ (\varepsilon_B-\varepsilon_F) + 1/K_b (\varepsilon_B-\varepsilon_F)$$

Arrow shows the absorbance change upon the increase of DNA concentration where ε_A, ε_F, and ε_B correspond to A_{obsd}/[complex], the extinction coefficient for the free complex and the extinction coefficient for the complex in the fully bound form, respectively. The slope and y intercept of the linear fit of [DNA]/(εA-εF) *versus* [DNA] give 1/(εB-εF) and 1/Kb(εB-εF) respectively. The intrinsic binding constant, K_b can be obtained from the ratio of slope to the intercept. The K_b values observed here are lower than those observed for typical classical intercalators (ethidium-DNA, 7.0 x10^7 M^{-1} in 40 mM Tris-HCl buffer, pH 7.9, and 1.4 x 10^6 M^{-1} in 40 mM NaCl-25 mM Tris-HCl; proflavin with *Escherichia coli* DNA, 50% GC content, 4.1 x 105 M-1 in 0.1 M Tris-HCl) with a proven DNA-binding mode involving the complete insertion of the planar molecules between the base pairs.

2.2.6. Anticancer studies:

HT-29 (Human Colon adenocarcinoma), MCF-7 (Breast carcinoma), HeLa (Cervix carcinoma), L6 (Rat muscle) and Vero (African green monkey kidney) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). (figure-8)

Preparation of Test Solutions

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

Principle: The ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.(table-8)

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

2.2.7. Cytotoxic studies :

The E.coli AB 1157, a wild-type strain, proficient to repair damage in the DNA is considered for this study.Initially, the stock culture of bacteria was revived by inoculating in broth medium and grown at 37ºC for 18 hrs. The LB Agar plates were prepared and wells were made in the solidified LB agar plate. Each plate was inoculated with 18 h old cultures (100 µl, 10-4 cfu) and spread evenly on the plate. After 20 min, the wells were filled with compound at different concentrations. Standard compound plate was also prepared in the same manner. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone were noted. The results are presented in Table-9as diameter of inhibition zones in mm and minimum inhibitory concentration (MIC).None of the compounds showed significant cytotoxicity. Compounds $[Co(GUSH)_2(H_2O)_2]$ and $[Co(GUSH)_2(H_2O)_2]$ showed a MIC value of 2.0 whereas other compounds did not show any cytotoxicity effect which indicates that these compounds do not exhibit any deleterious effect and non-toxic to the bacterial cell in this study. Stannous chloride, a toxic chemical which induces free radicals, showed an MIC of 0.25µg.

2.2.8. Nephrotoxicity studies:

The Nephrotoxicity analysis of the most active compound $[Cu(GUSH)_2(H_2O)_2]$ was carried out against NRK 49F(Rat kidney cell line) and found that itis showing nephrotoxicity at CTC_{50}(µg/ml) =526.67±0.6(table-10, figure-8)

3. Experimental protocol

All the chemicals were of reagent grade and the solvents were dried and distilled before use according to the standard procedures. The metal chlorides used were in the hydrated form. Elemental analysis (C, H and N) were performed on a Parkin-Elmer 2400 CHN elemental Analyzer Model 1106, Carloerba Strumentazione. Molar conductivity measurements were recorded on an ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51. The electronic spectra of the complexes were recorded in DMF on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1100nm. The IR spectra of the ligands and their Cu (II), Ni (II), Cu (II) and Zn (II) complexes were recorded on a HITACHI-270 IR Spectrophotometer in the 4000-250 cm^{-1} region in KBr discs. The ^{1}H-NMR spectra of ligands were recorded in $CDCl_3$ and Zn(II) complexes in DMSO-d_6 on BRUKER 300 MHz spectrometer at room temperature using TMS as an internal reference. The mass spectra of the ligands were taken in a Thermo Finnigan MAT 1020 ion trap, Type: ESI operating at 70eV.The EPR spectra of the Cu(II) complexes were recorded on a variant E-4´, X-band ESR spectrometer using cylindrical quartz sample tube at room temperature and at LNT using Polycrystalline diphenylpicrylhydrazyl(DPPH) as "g" marker. The fluorescence spectra of the ligands and the complexes were recorded in a VARIAN CARY 50-BIO fluorescence - spectrophotometer in the region of 200-700nm.Thermogravimetric data were measured from room temperature to 1000^0C at a heating rate of 10^0C/min using PERKIN-ELMER DIAMOND TG/DTA instrument.

3.1. Synthesis

3.1.1. Synthesis of 2-chloro-3-formyl Quinoline.

This compound was synthesized by Vilsmier reaction using acetanilide, $POCl_3$ and DMF at 80^0C as per the procedure given in the literature[50, 51]. Yellow crystals (ethyl acetate), yield= 92.24%, m.p. = 172-173^{0}C.

3.1.2. Synthesis of 2-hydroxy-3-formyl Quinoline.

2-Chloro-3-formyl Quinoline(0.1mol) was refluxed for 10h in HCl(4M) and allowed to cool to room temperature. The reaction mixture was poured into crushed ice to get yellow product[52]. Recrystallized from aqueous acetic acid. Yield=89%, m.p.= 295-297^{0}C.

3.1.3. Synthesis of 2-Marcapto-3-formyl Quinoline.

A mixture of 2-Chloro-3-formyl Quinoline (5.73g, 29.98mmol) and sodium sulphide (8.4g, 9.2mmol) was refluxed for 10min on a water bath in ethanol (50ml). DMF (15ml) was added drop wise to the reaction mixture. The marcapto compound precipitates as a yellow crystalline solid which was further filtered, washed with ethanol, dried and crystallized from ethyl acetate and benzene (8:2) [53]. Yield= 84%, m.p. = 193^{0}C.

3.1.4. Preparation of the ligands (GUOH and GUSH):

The Schiff base ligands were prepared by condensation of 3-formyl-2-hydroxy quinoline (0.1M) with 2-amino-1,9-dihydro-6H-purin-6-one (Guanine) (0.1M) in ethanol and refluxed on water bath for 5-6 hours in presence of few drops of acetic acid. The reaction mixture was cooled to room temperature and the separated Schiff base was filtered, washed with hot alcohol and recrystallized from alcohol to get a pure sample (GUOH). Similar methods were used for the preparation of the ligand (GUSH) by the condensation of 3-formyl-2-mercapto quinoline(0.1M) with 2-amino-1,9-dihydro-6*H*-purin-6-one (Guanine). The synthesis of ligands and the structure is given in Scheme 1.

3.1.5. Preparation of complexes

For the Synthesis of transition metal complexes, hot ethanolic solution of the respective metal (II) chloride (0.01mol) and the Schiff base(0.02mol) were refluxed for 4-5h on a water bath at the pH 7.0-7.7 and the precipitate obtained was filtered, washed successively with ethanol and ether and finally dried over fused $CaCl_2$ in vacuum. Yield of all the complexes lie in the range of 67-73%.

Table 1. Analytical, magnetic and conductance data of the ligands and their transition metal complexes

SL. No.	Compound code (Emp. Formula)	Molar Mass	C% found (calc)	H% found (calc)	N% found (calc)	S% found (calc)	M% found (calc)	Molar conductance $Ohm^{-1} cm^{-2} mole^{-1}$	μ_{eff} BM
1	GUOH ($C_{15}H_{10}N_6O_2$)	306	57.98 (58.82)	3.12 (3.29)	27.58 (27.44)	-	-	-	-
2	$[Co(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}CoN_{12}O_6$)	705	50.29 (51.07)	3.04 3.14)	23.12 (23.82)	-	7.95 (8.35)	18.09	4.80 (4.87)
3	$[Ni(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}N_{12}NaiO_6$)	706	50.38 (51.09)	3.07 (3.14)	23.45 (23.83)	-	8.12 (8.32)	17.59	3.20 (2.82)
4	$[Cu(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}CuN_{12}O_6$)	710	51.20 (50.74)	3.29 (3.12)	24.20 (23.67)	-	8.30 (8.95)	16.76	1.77 (1.73)
5	$[Zn(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}ZnN_{12}O_6$)	712	50.61 (53.20)	3.11 (3.20)	23.61 (24.50)	-	9.18 (7.30)	19.76	Dia
6	GUSH ($C_{15}H_{10}N_6OS$)	322	59.30 (58.81)	2.93 (3.29)	27.30 (27.43)	10.38 (10.47)	-	-	-
7	$[Co(GUSH)_2(H_2O)_2]$ ($C_{30}H_{22}CoN_{12}O_4S_2$)	737	50.85 (51.06)	2.97 (3.14)	23.34 (23.82)	9.34 (9.09)	7.24 (7.42)	25.1	4.60 (4.67)
8	$[Ni(GUOH)_2(H_2O)_2](C_{30}H_{22}N_{12}NiO_4S_2)$	738	51.78 (51.08)	2.98 (3.14)	24.08 (23.83)	8.78 (9.09)	5.78 (6.33)	19.23	3.28 (2.82)
9	$[Cu(GUSH)_2(H_2O)_2]$ ($C_{30}H_{22}CuN_{12}O_4S_2$)	742	50.08 (48.54)	3.35 (2.99)	22.46 (22.64)	8.94 (8.64)	7.87 (8.56)	17.28	1.79 (1.73)
10	$[Zn(GUSH)_2(H_2O)_2]$ ($C_{30}H_{22}N_{12}ZnO_4S_2$)	744	50.12 (49.42)	2.98 (2.98)	22.59 (22.59)	8.62 (8.62)	8.79 (8.79)	18.09	Dia

Note: GUOH and GUSH = deprotonated ligands

Table-2: Infrared spectral data of Ligands and their metal complexes:

Sl. No.	Compound code (Empirical Formula)	$\nu_{(OH)H_2O}$	$\nu_{(O-H)Quinoline}$	$\nu_{(N-H)}$	$\nu_{(S-H)}$	$\nu_{(C-S)}$	$\nu_{(C=O)}$	$\nu_{(C=N)}$	$\nu_{(H2O)}$	$\nu_{(M-O)}$	$\nu_{(M-N)}$	$\nu_{(M-S)}$
1	GUOH ($C_{15}H_{10}N_6O_2$)	-	3403b	3084	-	-	1702s	1617s	-	-	-	-
2	$[Co(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}CoN_{12}O_6$)	3448b	-		-	-	1705s	1593s	827s	574w	438m	-
3	$[Ni(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}N_{12}NaiO_6$)	3352b	-		-	-	1715s	1595s	827s	585w	421m	-
4	$[Cu(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}CuN_{12}O_6$)	3386b	-		-	-	1710s	1600s	829s	590w	433w	-
5	$[Zn(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}ZnN_{12}O_6$)	3373b	-		-	-	1716s	1597s	831s	561w	438m	-
6	GUSH ($C_{15}H_{10}N_6OS$)	-	-	3067	2685	634s	1710s	1618s	-	-	-	-
7	$[Co(GUSH)_2(H_2O)_2]$ ($C_{30}H_{22}CoN_{12}O_4S_2$)	3431b	-		-	649s	1707s	1596s	830s	-	445w	354w
8	$[Ni(GUOH)_2(H_2O)_2]$ ($C_{30}H_{22}N_{12}NiO_4S_2$)	3273b	-		-	651m	1719s	1587s	827s	-	441m	375w
9	$[Cu(GUSH)_2(H_2O)_2]$ ($C_{30}H_{22}CuN_{12}O_4S_2$)	3431b	-		-	662s	1704s	1597s	824s	-	437w	390w
10	$[Zn(GUSH)_2(H_2O)_2]$ ($C_{30}H_{22}N_{12}ZnO_4S_2$)	3298b	-		-	647s	1720s	1611s	829s	-	438w	351w

Table 3. Electronic spectral data of octahedral Co(II) complexes (in DMF solution)

Code	Complex	ν_1	ν_2	ν_3	Dq	B'	B	ν_2/ν_1	LFSE Kcal/mol
1	$[Co(GUOH)_2(H_2O)_2]$	10152	16260	20618	869	945	0.973	1.601	14.89
5	$[Co(GUSH)_2(H_2O)_2]$	10146	16250	20605	868	944	0.972	1.602	14.88

Free ion value for Co(II) = 971cm^{-1}; LFSE = 12Dq

Table 4. Electronic spectral data of Ni(II) complexes in DMF solution.

Code	Complex	ν_1	ν_2	ν_3	Dq	B'	B	ν_2/ν_1	LFSE Kcal/mol
2	$[Ni(GUOH)_2(H_2O)_2]$	11049	15302	26115	933	895	0.860	1.385	31.98
6	$[Ni(GUSH)_2(H_2O)_2]$	10256	15455	24691	866	830	0.798	1.506	29.68

Free ion value for Ni(II)=104cm^{-1}; LFSE=12Dq; 350 cm^{-1} Kcal

Table 5. Electronic spectral data of Cu(II) complexes in DMF solution.

Complex Code	Complex	λ_{max} nm	λ_{max} cm^{-1}	Assignment
		584	17123	$^2T_{2g}<\text{-----}^2E_g$
		342	29240	
3	$[Cu(GUOH)_2(H_2O)_2]$	297	33670	Ligand
		258	38760	
		658	15198	$^2T_{2g}<\text{-----}^2E_g$
7	$[Cu(GUSH)_2(H_2O)_2]$	385	25974	
		332	30121	Ligand
		264	37879	

Table-6: Antibacterial activities

Bacteria	Conc. (mg/ml)	Std. drug (Gentamycin)	GUOH	Ni-OH	Co-OH	Cu-OH	Zn-OH	GUSH	Ni-SH	Co-SH	Cu-SH	Zn-SH
E.coli	100	16	14	13	14	16	6	15	12	12	18	4
	50	10	10	10	9	13	1	11	10	9	11	1
	25	7	3	4	5	7	0	3	3	4	4	0
S aureus	100	18	13	13	13	17	7	14	13	14	17	2
	50	12	9	11	11	13	2	10	9	8	10	0
	25	6	5	3	4	8	0	6	3	0	2	0

The antibacterial activity of the ligands and their Co(II), Ni(II), Cu(II), and Zn(II) metal complexes are assayed against two bacteria viz., Escherichia coli and Staphylococcus aureus by cup-plate method. (Zone of inhibition in mm)

Table-7: Antifungal Studies:

The compounds were tested for their actvities against *A.Niger*. and *C. Albican*. The MIC results are given below.(Zone of inhibition in mm)

Fungi	Conc. (mg/ml)	Std. drug (Fluconazole)	GUOH	Ni-OH	Co-OH	Cu-OH	Zn-OH	GUSH	Ni-SH	Co-SH	Cu-SH	Zn-SH
A. Niger	100	7	2	4	4	6	0	3	4	2	8	0
	50	2	0	1	1	3	0	0	1	1	3	0
	25	0	0	0	0	0	0	0	0	0	0	0
C. Albian	100	8	3	3	3	7	0	4	5	4	7	0
	50	2	0	1	1	3	0	0	1	2	4	0
	25	0	0	0	0	0	0	0	0	0	0	0

Table-8: Anticancer Analysis against the following cell lines:

Cancer cell lines.	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
HT-29 (Human Colon Cancer cell line)	RR 1318 (GUSH)	1000 500 250 125 62.5	21.00±0.2 13.52±0.7 9.40±0.5 5.02±0.2 2.91±0.3	>1000±0.00
HT-29 (Human Colon Cancer cell line)	RR 1319 $[Cu(GUSH)_2(H_2O)_2]$	1000 500 250 125 62.5	53.47±0.5 26.64±3.2 15.94±0.4 10.18±0.5 4.72±0.3	936.67±1.0
MCF-7 (Human Breast Cancer cell line)	RR 1428 $[Cu(GUSH)_2(H_2O)_2]$	1000 500 250 125 62.5	85.50±1.1 40.97±1.0 30.49±1.2 17.24±0.6 12.26±0.8	610.00±0.9
HeLa (Human Cervical Cancer cell line)	RR 1428 $[Cu(GUSH)_2(H_2O)_2]$	1000 500 250 125 62.5	75.46±0.8 73.38±1.2 52.55±0.5 26.41±1.1 10.85±0.3	236.67±0.8
Vero (Monkey kidney cell line)	RR 1428 $[Cu(GUSH)_2(H_2O)_2]$	1000 500 250 125 62.5	58.67±0.4 31.18±0.8 21.09±0.6 11.39±0.7 4.95±0.4	846.60±0.6
L6 (Skeletal muscle cell line, myoblast.)	RR 1428 $[Cu(GUSH)_2(H_2O)_2]$	1000 500 250 125 62.5	65.61±0.4 39.02±0.5 31.37±1.1 18.28±0.6 13.37±0.8	706.67±0.7

Table- 9: Cytotoxic Activity of E. coli AB 1157-Zone of Inhibition in µg (mm):

Compounds	0.0625 µg	0.125 µg	0.25 µg	0.5 µg	1.0 µg	2.0 µg	MIC
GUOH	0	0	0	0	0	0	>2.0
$[Ni(GUSH)_2(H_2O)_2]$	0	0	0	0	0	0	>2.0
$[Co(GUSH)_2(H_2O)_2]$	0	0	0	0	0	4	2.0
$[Cu(GUSH)_2(H_2O)_2]$	0	0	0	0	0	7	2.0
GUSH	0	0	0	0	0	0	>2.0
Stannous chloride	0	0	4	6	8	15	0.25

Table-10: Nephrotoxicity test against NRK 49F (RAT KIDNEY) cell line:

Sl. No	Name of Test sample	Test Conc. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	RR 1428 $[Cu(GUSH)_2(H_2O)_2]$	1000 500 250 125 62.5	69.20±0.5 48.77±0.7 38.96±0.4 29.57±0.6 21.34±0.8	526.67±0.6

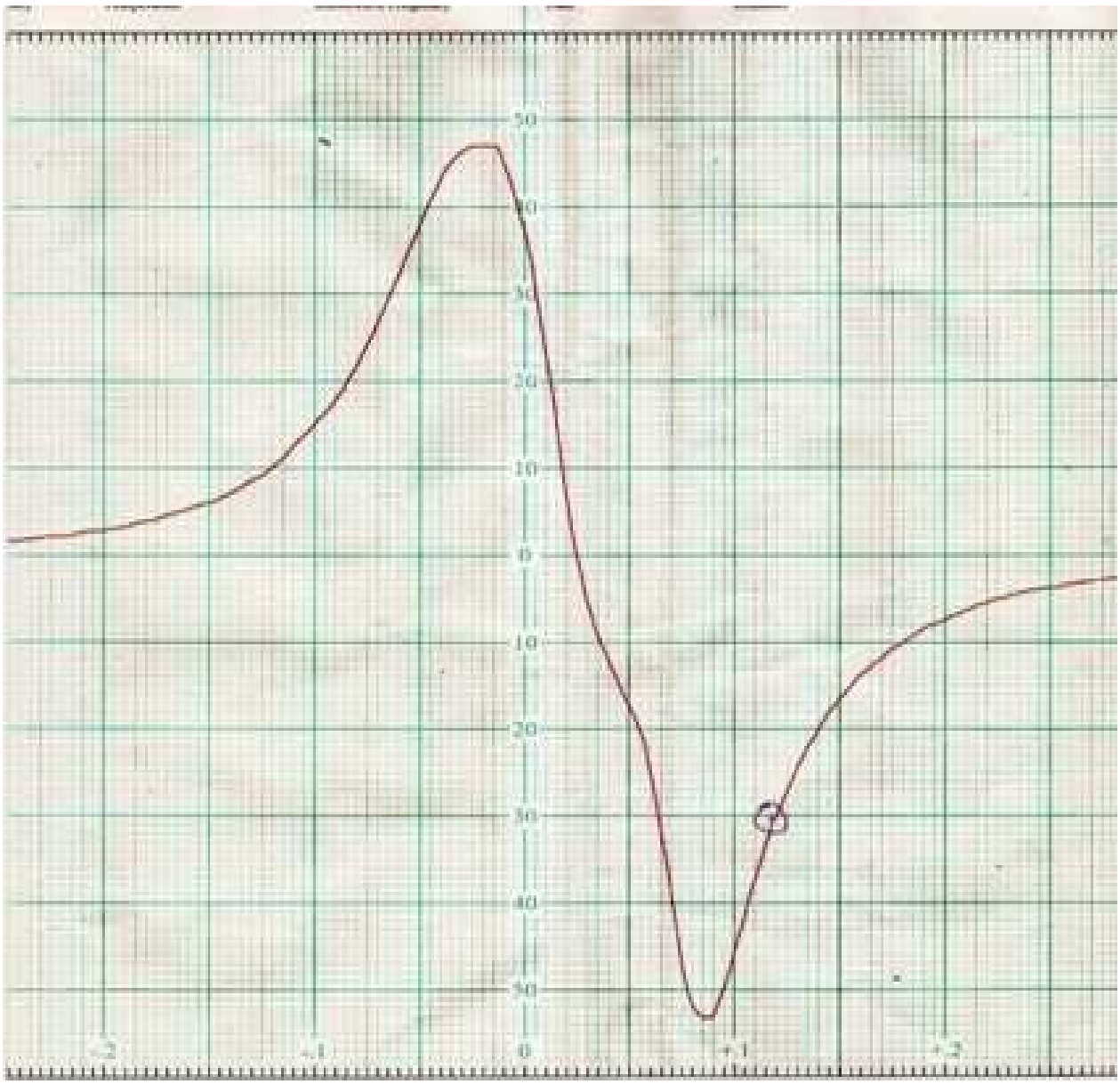


Figure-1: ESR spectrum of one representative $[Cu(GUSH)(H_2O)]_2$ complex:

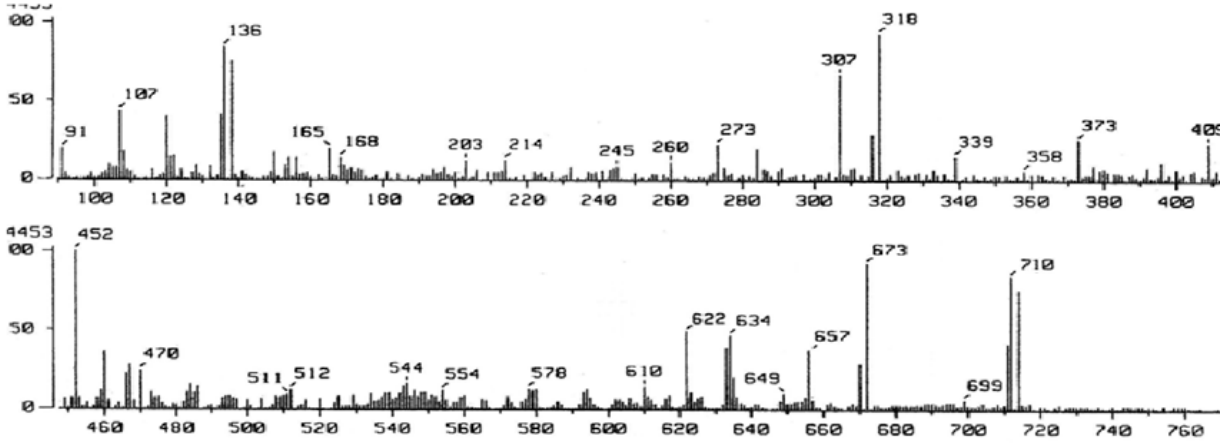


Figure-2: Mass Spectrum of [Cu(GUOH)$_2$(H$_2$O)$_2$]

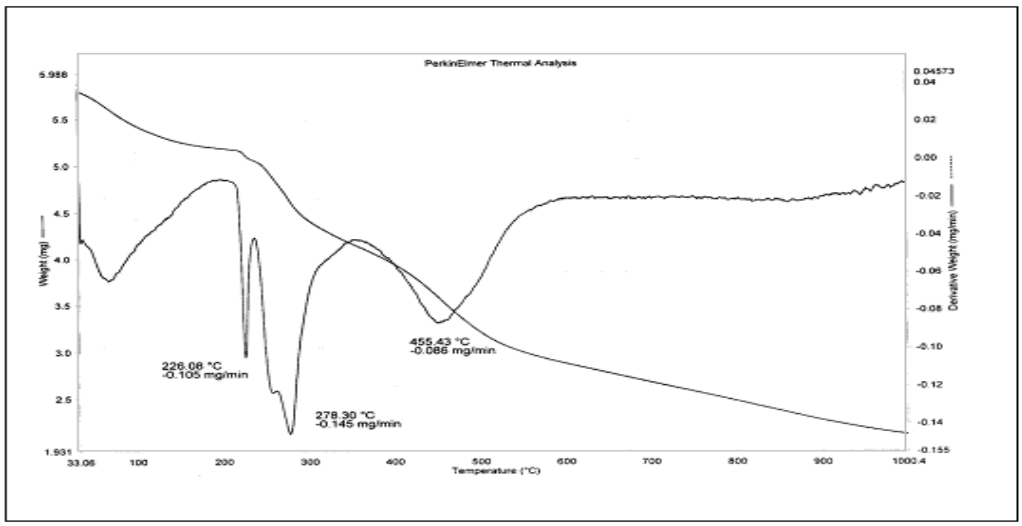
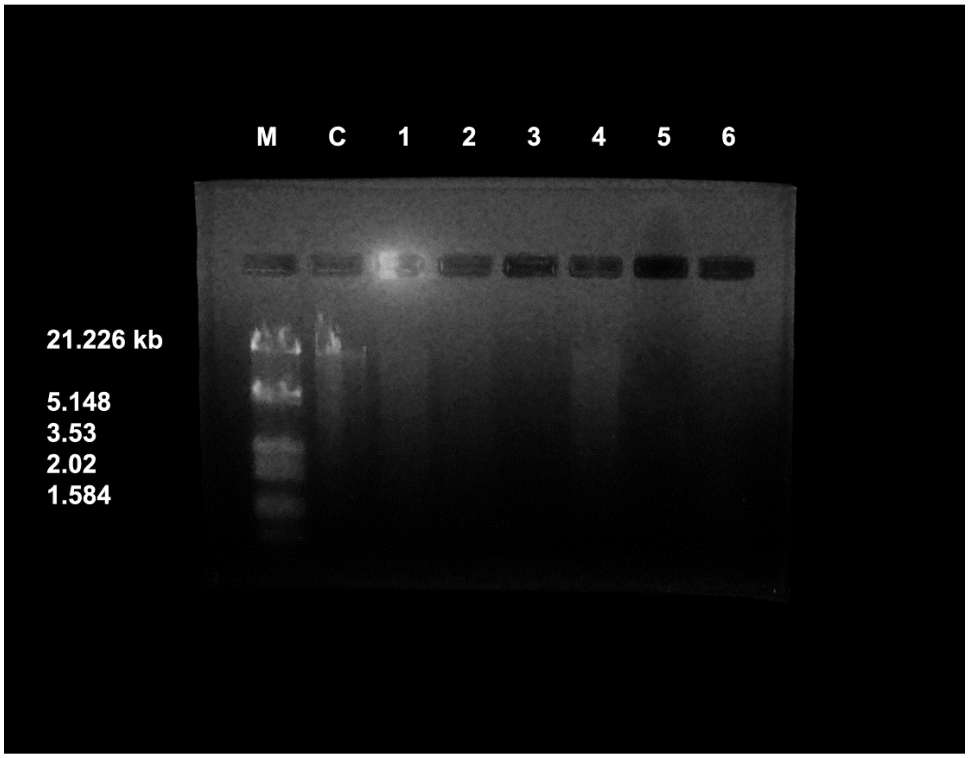


Figure- 3: TGA/DTG spectrum of [Cu(GUSH)$_2$(H$_2$O)$_2$]



M- Standard DNA molecular weight marker (λ DNA HindIII digest, Merck, Bangalore), C- ControlDNA, 1-GUOH, 2-[$Cu(GUOH)_2(H_2O)_2$], 3-[$Ni(GUOH)_2(H_2O)_2$], 4-GUSH, 5-[$Co(GUSH)_2(H_2O)_2$], 6-[$Cu(GUSH)_2(H_2O)_2$]

Figure-4: DNA cleavage Analysis of Calf-thymus DNA (Bangalore Genei, Bengaluru, Cat.No 105850).

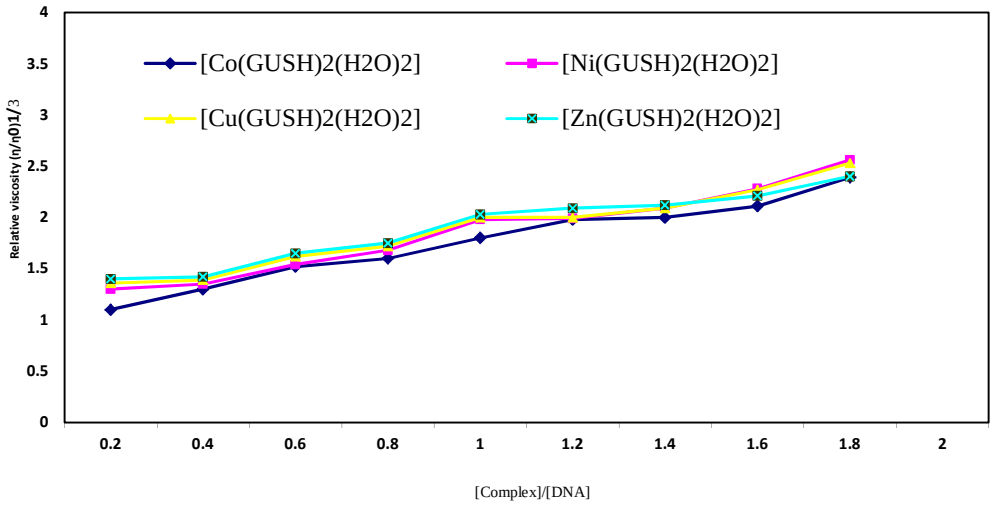
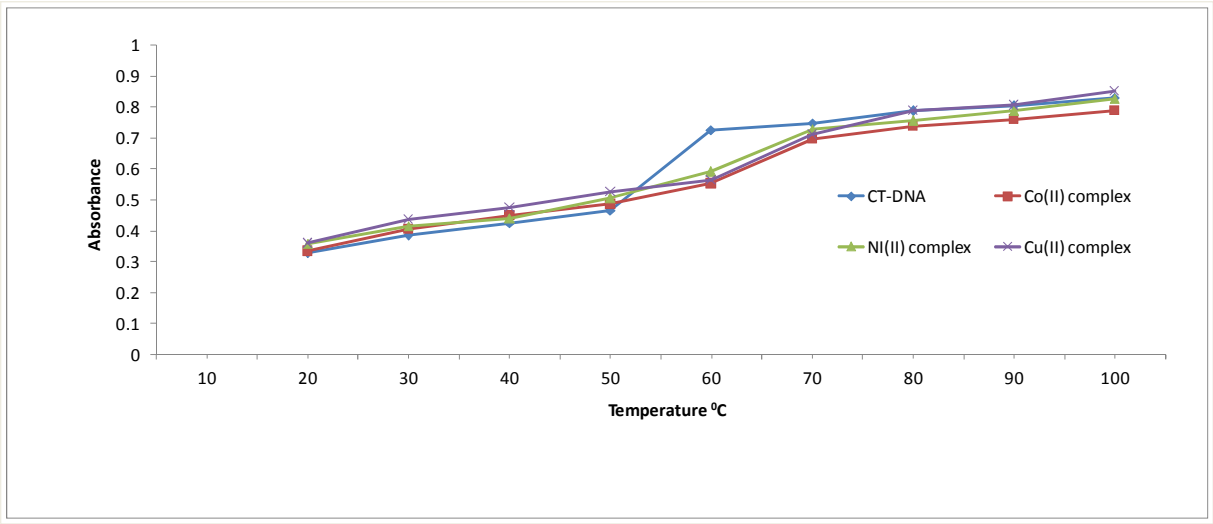


Figure-5: DNA binding analysis using viscosity measurement



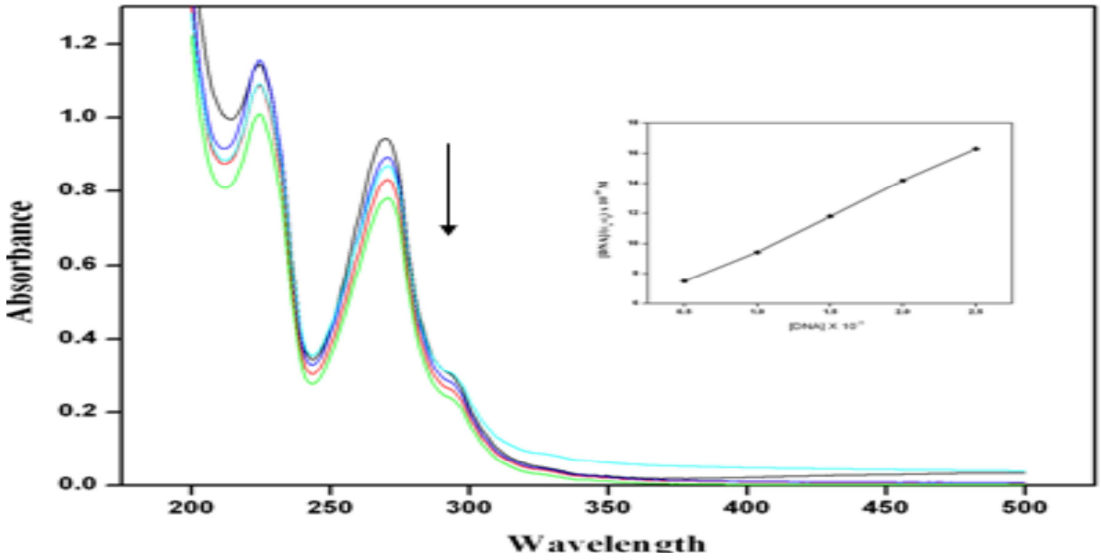


Figure-7: DNA binding studies by Spectroscopic method:

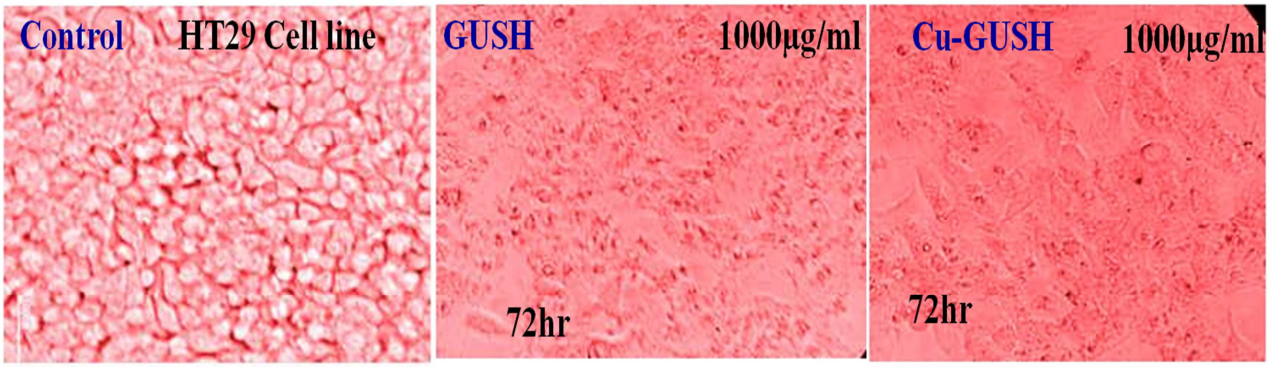
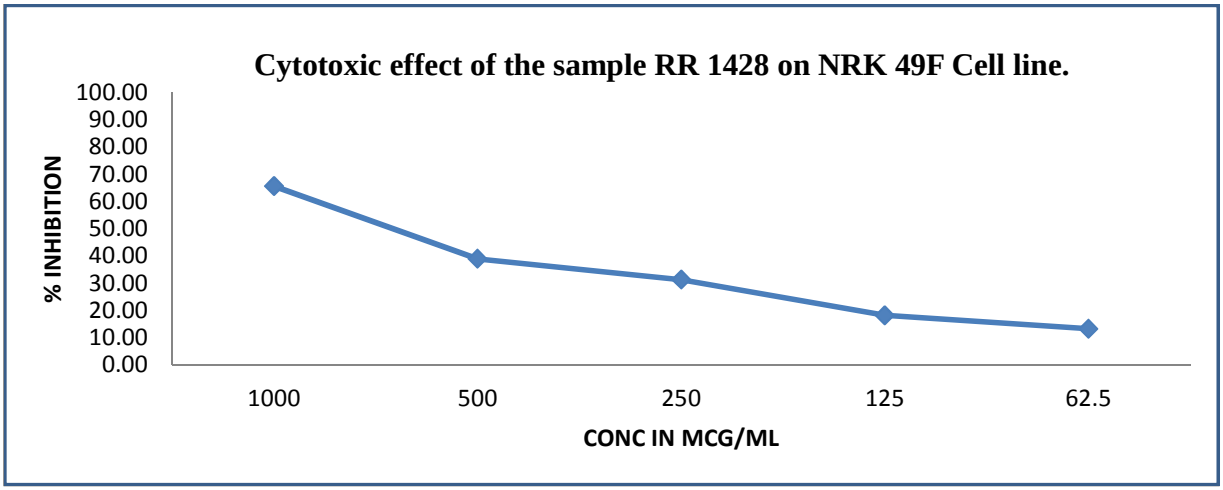
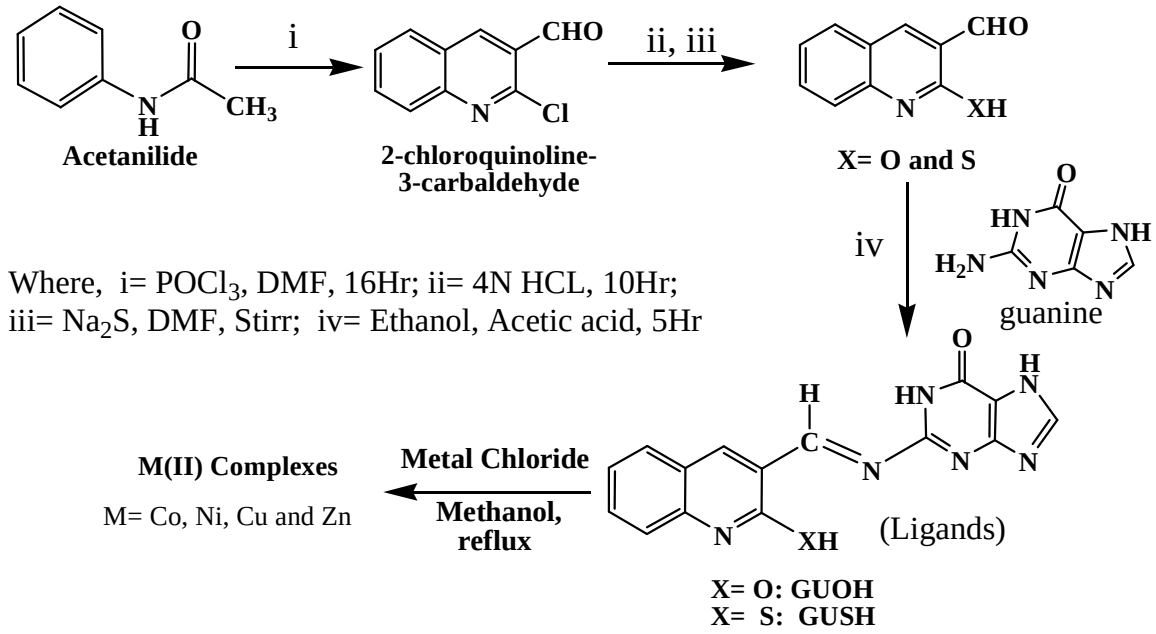


Figure-8: Anticancer properties of test drugs against HT-29 cell line:



The graph showing nephrotoxicity at CTC_{50}(µg/ml) =526.67±0.6

Figure-9:Nephrotoxicity test of $[Cu(GUSH)_2(H_2O)_2]$against NRK 49F(KIDNEY) cell line:



Scheme-1: Synthesis of Ligands and Metal complexes:

CONCLUSION

We have synthesized novel transition metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Schiff base Ligands "(E)-2-((2-hydroxyquinolin-3-yl)methyleneamino)-1H-purin-6(7H)-one" abbreviated as GUOH and "(E)-2-((2-mercaptoquinolin-3-yl)methyleneamino)-1H-purin-6(7H)-one" abbreviated as GUSH derived by the condensation of 2-amino-1,9-dihydro-6*H*-purin-6-one (Guanine) with 3-formyl-2-hydroxy quinoline and with 3-formyl-2-mercapto quinoline respectively and characterized by elemental analysis, molar conductance, magnetic susceptibilities, UV, IR, ^{1}H-NMR, ESR and thermal studies. The elemental and spectral analysis of the complexes confirms $[M(GUOH)_2(H_2O)_2]$ and $[M(GUSH)_2(H_2O)_2]$ stoichiometry and exhibits octahedral geometry, where M= Co(II), Ni(II), Cu(II) and Zn(II) respectively. Both the ligands act as monobasic and didentate, coordinating through azomethine nitrogen, quinoline oxygen via deprotonation. The synthesized ligands and the metal complexes were screened for the antibacterial, antifungal, DNA cleavage, DNA binding, Cytotoxic, Nephrotoxic, and Anticancer

studies. The results reveal that the metal complexes possess higher antimicrobial activity than their corresponding ligands and Cu(II) complexes are found to be more active than the other complexes. $[Ni(GUOH)_2(H_2O)_2]$, $[Co(GUSH)_2(H_2O)_2]$ and $[M(GUSH)_2(H_2O)_2]$have shown complete cleavage of CT-DNA where as other samples have displayed partial cleavage and DNA binding studies of selected compounds revels the Intercalative mode of bindings with CT-DNA. From the Anticancer analysis it is found that $[Cu(GUSH)_2.(H_2O)_2]$ is showing better activity against Cervical Cancer among other tested cell lines, the activity is in the order: Cervical Cancer(HeLa) > Breast Cancer(MCF-7) >Skeletal muscle Myoblast(L6) >Monkey kidney cancer cell lines(Vero) > Human Colon Cancer cell line(HT-29). Nephrotoxicity test against NRK 49F(Rat KIDNEY) shows that the complex Cu(II) complex is showing Nephrotoxicity at CTC_{50}(µg/ml) =526.67±06.

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