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Microwave assisted synthesis of heterocyclic metal complexes and evaluation of their in vitro anticancer activity against oral cancer cells, antioxidant and molecular docking study

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Heterocyclic complexes of Cr(III) and Fe(III) have been synthesized through condensation of succinic dihydrazide with 5-chloroindoline-2,3-dione in alcoholic medium by template method in 1:1:1 ratio using microwave irradiation technique. On the basis of physicochemical and spectroscopic techniques the geometry of the complexes are identified as octahedral. The synthesized complexes are screened for their bioactivity. In vitro anticancer activity was evaluated against HNSC cell line and antioxidant activity is done by DPPH assay method. In-silico study is done by molecular docking with EGFR tyrosine kinase. The results show that synthesized compounds have significant anticancer as well as antioxidant property.

Keywords: Green Synthesis, Heterocyclic, Anticancer, Antioxidant, Molecular docking

The designing and synthesis of nitrogen containing heterocyclic bioactive compounds have been played an important role in the drug discovery[1-3]. Heterocyclic fragments constitute the core structure of large number of life saving medicine[4], such as anticancer[5-6] antitubercular[7] and antidiabetic[8] etc. Oral cancer is the most frequently found cancer among head and neck cancers. More than 90% oral cancer develops from the squamous epithelial lining[9].

As we know macrocycles with metal exists naturally in human being and plant kingdom. They take part in different stages of their vital life process[10-11]. A number of macrocyclic structure based broad spectrum antibiotics have been developed synthetically e.g. Erythromycin. For the treatment of cancer after the discovery of anticancer drug Cisplatin, a metal based drug discovery became interesting field for the researcher. Macrocyclic metal complexes possess antibacterial[12-13], anticancer[14], antioxidant[15] and antifungal[16] properties. According to Tweedy's chelation theory coordination reduces the polarity of metal compound, which results in easy penetration into lipid membranes of the cell. In some way metal ions may enhance the effect of therapeutic compounds[17].

Here, we have synthesized some heterocyclic metal complexes of Cr(III) and Fe(III) with succinic dihydrazide and 5-chloroindoline-2,3-dione by template method using microwave irradiation technique. A large number of indoline-2,3-dione (Isatin) derived compounds have been reported as antibacterial[18] and anticancer[19] agents. We have used microwave irradiation technique for the synthesis because of advantages i.e. shorter reaction times, higher yields and minimum solvent requirement[20-21]. All the complexes evaluated for in vitro anticancer as well as antioxidant activity because it is reported that sometimes dietary supplements which have antioxidant properties (e.g. vitamin C) are prescribed during antitumor treatment to protect healthy tissues against damage induced by oxidative stress[22]. Molecular docking study with epidermal growth factor receptor (EGFR) tyrosine kinase of the compounds was also carried out to check the anticancer property. EGFR tyrosine kinase is a significant target for drug development because EGFR inhibitors have been considered as anticancer agents[23-24]. The docking study results shows that synthesized compounds have good anticancer property.

Experimental Details

Materials and methods

AR grade chemical of Sigma-Aldrich, Himedia, CDH and Fisher Scientific brand were used. The

synthesized compounds were characterized by C H N analysis using elemental analyzer Perkin Elmer model-2400, Electronic spectroscopy using Perkin Elmer spectrophotometer model- LAMBDA 25, infrared spectroscopy using Thermo Scientific FTIR Spectrometer model-Nicolet iS50, Atomic absorption spectrophotometry using PG instruments model-AA500, Mass spectrometry using Agilent Mass Spectrometer model-G6530AA (LC-HRMS-Q-TOF). The Gouy balance was used to measure magnetic susceptibility and molar conductivity (10^{-3} M) was measured by Hach digital conductivity meter. Thermogravimetric analysis was done using Thermogravimetric Analyzers of TA Instruments.

Synthesis of complexes

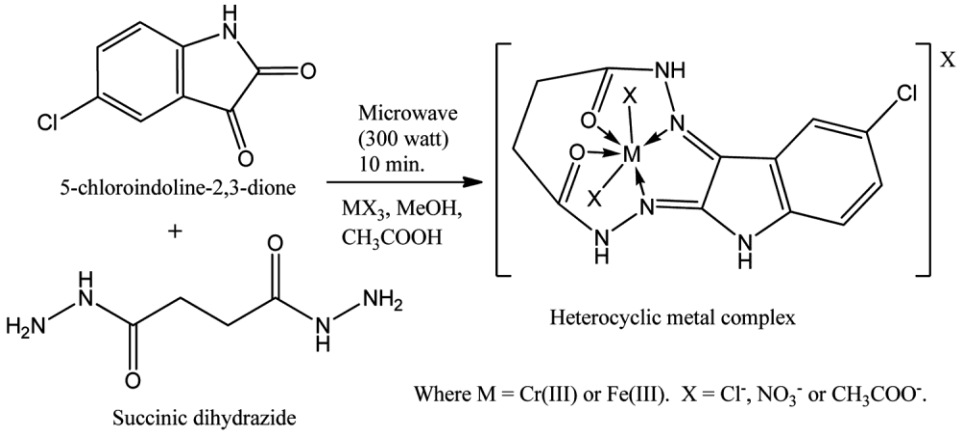
The mixture of succinic dihydrazide (10 mmol), 5-chloroindoline-2,3-dione (10 mmol) and metal salt (10 mmol) in 1:1:1 molar ratio was irradiated in microwave synthesizer by taking 10 mL of methanol as a solvent and few drops of glacial acetic acid for 10 min at 300 watt. The dark coloured metal complexes were obtained in the form of non crystalline solids. Products were filtered and washed with methanol and dried over anhydrous $CaCl_2$ in vacuum desiccators. The purity was checked by TLC. The product yield was about 78-84%. The syntheses of metal complexes may be represented as follows in Scheme 1**.**

Results and Discussion

Metal complexes were obtained in the form of dark colour non crystalline solids. All the synthesized metal complexes were soluble in DMSO and DMF. Molar conductance (10^{-3} M) in DMSO was found 30-80 ohm^{-1} cm^2 mol^{-1} indicates that electrolytic nature is 1:1, which predict the presence of single anion outside the coordination sphere of macrocyclic metal complex structure and support octahedral structure of complex[25]. Electronic spectroscopy and magnetic movement results conclude octahedral geometry[26] and supported with the elemental analysis results as listed in Table 1. The general formula of complexes is $[M(C_{12}H_{10}N_5O_2Cl)X_2]X$ as given in Scheme, 1.

Infrared spectroscopy

In FTIR spectra of synthesized complexes (Fig. 1) presence of a single medium band at 3181-3217 cm^{-1} may be assign to stretching vibrations of ν(NH).



Scheme 1 — Synthesis of heterocyclic metal complexes

Table 1 — Elemental analysis, melting point and molar mass

Complex	Elemental analysis, Found (Calculated)%				Colour	M.P. (°C)	Mol. mass
	M	C	H	N			
$[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$	11.46 (11.55)	31.90 (32.02)	2.19 (2.24)	15.49 (15.56)	Dark brown	284	450.04
$[Cr(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$	9.71 (9.82)	27.00 27.21)	1.82 (1.90)	21.05 (21.15)	Brown	288	529.70
$[Cr(C_{12}H_{10}N_5O_2Cl)(OAc)_2]OAc$	9.92 (9.98)	41.48 (41.51)	3.58 (3.68)	13.36 (13.45)	Brown	287	520.82
$[Fe(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$	12.20 (12.30)	31.66 (31.75)	2.13 (2.22)	15.40 (15.43)	Orange	282	453.89
$[Fe(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$	10.39 (10.47)	26.94 (27.01)	1.79 (1.89)	20.88 (21.00)	Dark red	290	533.55

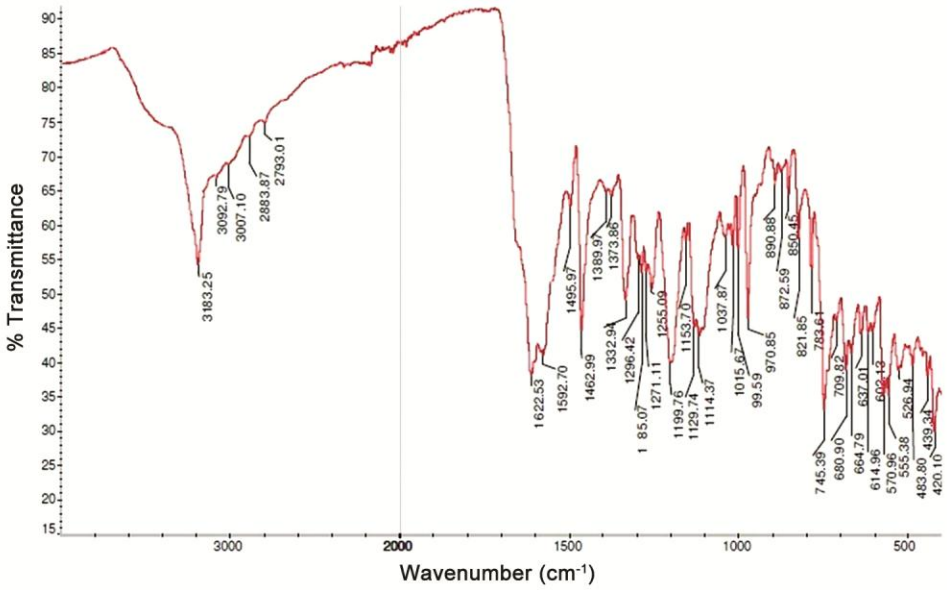


Fig. 1 — FTIR spectrum of $[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ complex

However, pair of bands from 3200-3400 cm^{-1} corresponding to vibrations of $\nu(NH_2)$ of succinic dihydrazide and strong bands of ν(C=O) vibrations from 1730-1745 cm^{-1} of 5-chloroindoline-2,3-dione are absent in all the metal complexes. A new strong bands observed at 1589-1595 cm^{-1} may be assigned to stretching vibrations of ν(C=N)[27]. The down values of ν(C=N) vibration suggests coordination of azomethine nitrogen with metal[28] and a strong band at 1620-1623 cm^{-1} corresponding to ν(C=O) of the CONH group suggesting coordination of carbonyl oxygen with the metal[29].

The bands were observed for ν(C-H) at 3008-3040 cm^{-1}, for ν(C=C) at 1409-1578 cm^{-1} and for ν(C-N) at 1018-1360 cm^{-1} may be of 5-chloroindoline-2,3-dione[28,30]. In nitrate containing complexes three bands at 1412-1455 cm^{-1} (ν_5), 1306-1320 cm^{-1} (ν_1) and 1015-1034 cm^{-1} (ν_2) were observed corresponding to unidentate coordination of nitrate groups with metal[31,32]. The complexes which have acetate showed bands at 1618-1640 cm^{-1} (ν_1) and 1369-1388 cm^{-1} (ν_2), support unidentate coordinates[33]. The bands for ν(M–O) vibrations were present at 526-534 cm^{-1} and for ν(M–N) at 479-491 cm^{-1}(Ref. 34,35).

UV-visible spectroscopy and magnetic studies

Electronic absorption spectra of complexes were recorded by UV-visible spectrophotometer and magnetic moment measurement by Gouy balance at room temperature. The magnetic moment measurement and absorption bands value agree well with the proposed octahedral geometry for all the complexes[36]. The ligand field parameters like B', β, and ν_2/ν_1 were also calculated to finalize the structure of complex. The electrolytic behaviour of the complexes supported the structure formula.

Chromium (III) complexes

In Cr(III) complexes absorption spectra, there observed three bands at 14806-15860(ν_1), 22172-22315(ν_2) and 28402-30211(ν_3) cm-^{1} corresponding to $^4A_{2g} \rightarrow {}^4T_{2g}$ (F), $^4A_{2g} \rightarrow {}^4T_{1g}$(F) and $^4A_{2g} \rightarrow {}^4T_{1g}$ (P) transitions, respectively, corresponding to octahedral geometry[36-38]. The complexes have Racah parameter value (B′ = 814-822) lower than free ion value (B′ = 918) and indicates a greater degree of covalence. The naphelauxetic parameter β, proposed covalent character in metal ligand bonding because of the values of the ν_2/ν_1 ratio found between 1.47-1.49 and support octahedral geometry[36]. The magnetic moment values were found from 3.80-4.50 B.M. corresponding to three unpaired electrons of Cr(III)[39].

Iron (III) complexes

In Fe(III) complexes absorption spectra have three bands at 13490-14046, 18998-19432 and 31548-31608 cm^{-1} may be assigned to $^6A_{1g} \rightarrow {}^4T_1$ (^{4}D), $^6A_{1g} \rightarrow {}^4T_{1g}$ and $^6A_{1g} \rightarrow {}^4T_{2g}$ transitions, respectively, observed corresponding to octahedral geometry[40]. The magnetic moment value were found from 5.85-5.90 B.M. corresponding to five unpaired electrons of Fe(III) and predicted high spin values[39].

Mass spectrometry

The molecular ion peaks observed in mass spectra of the complexes are mentioned in the Table 1. The metal complex showed molecular ion peak (M^+) for $[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ m/z at *449.90*, $[Cr(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$ m/z at *528.96*, $[Cr(C_{12}H_{10}N_5O_2Cl)(OAc)_2]OAc$ m/z at 520.03, $[Fe(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ *m/z* at *453.89* as shown in Fig. 2 and $[Fe(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$ m/z at *532.95* agree well with the proposed molecular formula of the complexes on the bases of elemental analysis.

Thermo-gravimetric analysis

Thermo-gravimetric analysis of $[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ complexes (Fig. 3) do not show any weight loss upto 200 °C. It shows the nonexistence of any lattice/coordinated water. The decomposition starts from 200 °C corresponding to loss of chloride ions upto 300 °C and continue to 650 °C may be due to continuous decomposition of organic moieties. The remaining air stable residue about 12% may be due to the formation of respective metal oxides. These results support the stoichiometry of formula of the complex.

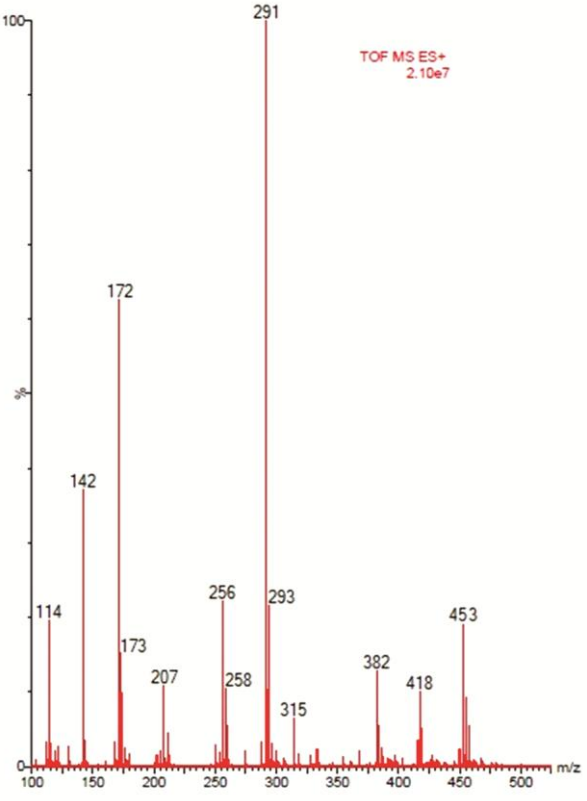


Fig. 2 — Mass spectrum of $[Fe(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ complex

Biological activity

In- vitro anticancer activity

In vitro MTT assay was performed to analyze the anticancer effect of synthesized compounds against SCC-4 (Squamous Cell Carcinoma) cell line of head and neck cancer at different times and compared with IC_{50} value of the Cisplatin. A 100 µL cell suspension in growth medium ($5x10^3$ cells/well) was seeded in each wells of 96 well ELISA plate and incubate at 37 °C in a highly humidified atmosphere with 5% CO_2 for about 24 h. Then growth medium was replaced with 200 µL of appropriate concentration (From 10 µM to 100 µM in 5% DMSO) of test compounds. The plates were incubated for 24 h, 48 h and 72 h in CO_2 incubator at 37 °C. A blank sample with same volume of DMSO was used as control. After the incubation, 20 µL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5 mg/mL phosphate buffer saline) was added to the each well. The plates were further incubated for 4 h at 37°C. Then medium and MTT were discarded without disturbing monolayer and 150 µL DMSO to each well was added to dissolve formazan crystals at room temperature. Absorbance was taken on ELISA plate reader at 570 nm. The IC_{50} value for each compound was calculated and compared with a standard anticancer drug i.e.Cisplatin. Triplicate samples were analyzed.

$$\text{Cell inhibition (\%)} = \left(\frac{\text{Mean OD}_{\text{control}} \text{ of untreated cells} - \text{Mean OD of treated cells}}{\text{Mean OD}_{\text{control}} \text{ of untreated cells}}\right) \times 100$$

The IC_{50} values for 24 h, 48 h and 72 h were calculated by dose survival curves for MTT assay and are listed in Table 2. All the compounds show time dependent effect with decrease in IC_{50} value. Fe(III) complexes found more cytotoxic against cancer cells than Cr(III) complex but higher than Cisplatin IC_{50}

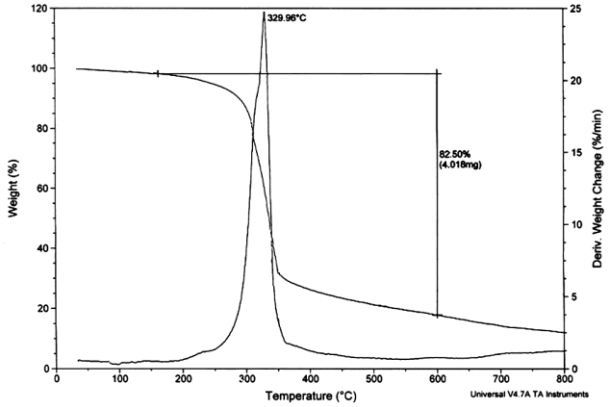


Fig. 3 — TGA analysis of $[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ complex

Table 2 — MTT Assay results against SCC-4 cell line

S.N.	Compound	IC_{50} value (μM)		
		24 h	48 h	72 h
1	$[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$	>100	>100	98.3
2	$[Cr(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$	>100	99.0	96.8
3	$[Cr(C_{12}H_{10}N_5O_2Cl)(OAc)_2]OAc$	>100	>100	>100
4	$[Fe(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$	80.2	78.5	75.8
5	$[Fe(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$	88.7	85.2	83.6
6	Cisplatin	8	6	5

value. Among these complexes, Fe(III) complex $[Fe(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ with IC_{50} 75.8 μM at 72 h exhibited most anticancer effect against SCC-4 cell line.

Antioxidant activity

Antioxidant assay of compounds was done by DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay method[41]. Butylated hydroxyanisole (BHA) was used as standard antioxidant. Three test tubes (different concetrations) for each test compound were prepared using 10 μg, 50 μg and 100 μg/100μL in DMF and 5 mL of 0.1mM methanolic DPPH were added to each. DPPH solution was used as control without any test compound. All solutions were allowed to stand for 30 min at room temperature and absorbance was measured 517 nm by spectrophotometer.

Free radical scavenging activity calculated by the given formula:

% Radical scavenging activity = $[(OD_{Control} - OD_{Sample}) / OD_{Control}] \times 100$

All complexes exhibited free radical scavenging activity (Fig. 4). $[Cr(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$ complex showed highest free radical scavenging activity ($IC_{50} \geq 50$μg) among all the complexes in comparison to BHA ($IC_{50} \leq 50$μg).

Molecular docking study

Discovery Studio 4.0, Accelrys Software was used for preparation of ligand and molecular docking studies. The EGFR-erlotinib co-crystal structure (PDB ID: 1M17) (resolution, 2.6A°) was downloaded from Data Bank (www.rcsb.org). The erlotinib and water molecules were removed from the protein and hydrogen molecules were added. 3D structures were prepared and minimized using Conjugate Gradient Algorithm. RMS gradient was obtained up to 0.009. CDOCKER protocol was used to dock ligand. It is a grid based method using CDOCKER algorithm[42,43]. The minimized structure of ligand (complex) was docked at rigid active site of 10.5A° radius using CHARMM force field[44].The binding free energy was obtained for the best docked pose of the ligand[45].

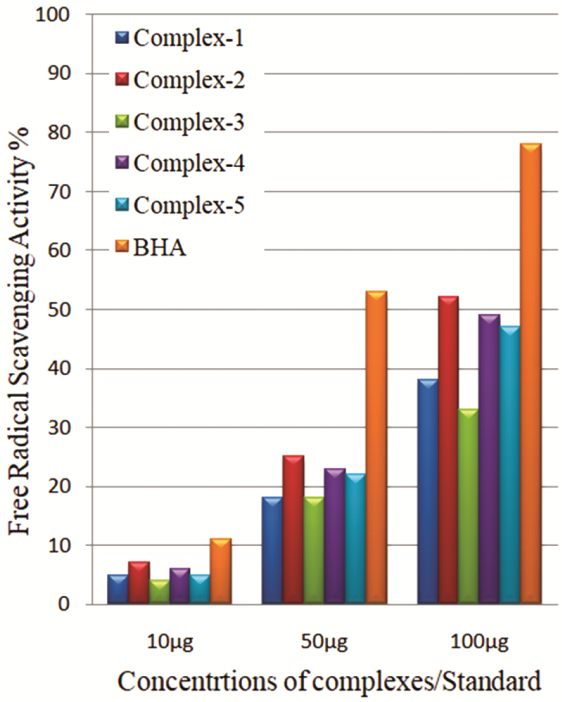


Fig. 4 — Antioxidant activity results

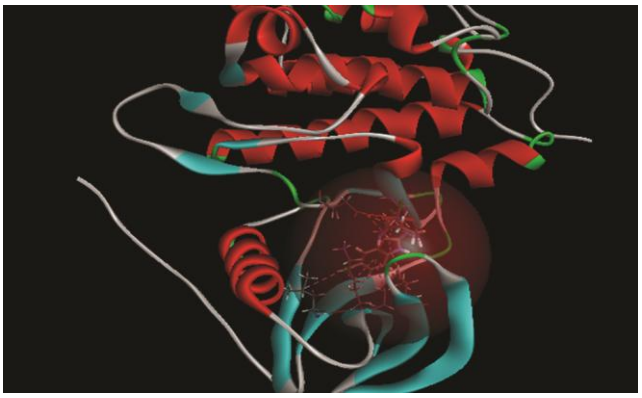


Fig. 5 — Molecular docking of $[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ with EGFR tyrosine kinase

The synthesized compounds were successfully docked with EGFR and studied for type of interactions. The best docked pose of $[Cr(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ (SDH_ligand1) complex with EGFR is shown in Fig. 5. The results showed that compounds have good affinity with the EGFR protein, as indicated by the hydrophobic interactions in Fig. 6. The complex exhibited hydrophobic and electrostatic interactions with amino acids. The binding energy of compound found -267.99 kcal/mol. The binding energies of other complexes were -203.46-kcal/mol to -218.08 kcal/mol. These results indicate that the compound may have good anticancer potential.

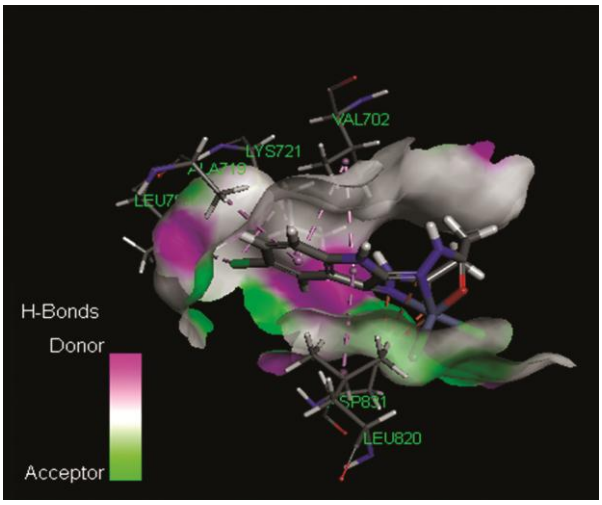


Fig. 6 — Interaction of metal complex with amino acid of EGFR at active site

Conclusions

In this work we have synthesized five new heterocyclic metal complexes. The metal ion exists in coordination with two carbonyl oxygen and two azomethine nitrogen atoms of heterocyclic structure. Physicochemical and spectroscopic studies confirmed octahedral geometries of the complexes. In vitro anticancer and antioxidant activities were investigated. All complexes existed anticancer as well as antioxidant effect. It was observed that $[Fe(C_{12}H_{10}N_5O_2Cl)Cl_2]Cl$ compound exhibited most anticancer effect against SCC-4 cells and $[Cr(C_{12}H_{10}N_5O_2Cl)(NO_3)_2]NO_3$ had highest antioxidant effect. Also the Molecular docking study with EGFR protein results support the anticancer potential of the synthesized compounds. On the basis of these findings it may conclude that this study may be helpful for the development of novel metal containing heterocyclic anticancer and antioxidant compounds.

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