Molecular Docking and Antimicrobial activity of heterocyclic thiosemicarbazone and their transition metal complexes

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Abstract: A Ligand (E) –N-Cyclohexyl-2-(1-(5-Bromo thiophen-2-yl) ethylidene) hydrazine carbothioamide was synthesised and characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, UV-Vis spectroscopy and elemental analysis. Cobalt(II),Nickel(II),Copper(II) and Zinc(II) complexes of thiosemicarbazones were characterized by elemental analysis, FT- IR,UV-Vis, EPR and ESI- Mass spectral studies. Ligand (HL) and its metal complexes have been screened *in vitro* antimicrobial activity against E.Coli,S.aureus,P.aeruginosa,E.facecalis and C.albicans . Further,the ligand and the complexes were docked with dihydrofolate reductase (DHFR).

Keywords: Thiosemicarbazone; metal complexes; antimicrobial screening; dihydrofolate reductase.

I. Introduction:

Transition metal complexes of thiosemicarbazones have a number of pharmacological applications [1-3] such as antibacterial, anti-fungal, antitumor, anti cancer and inflammatory [4-7]. Thiosemicarbazone have the coordination ability to form mononuclear (or) binuclear metal complexes [8-15].They can bind metal ions as bidentate NS- or tridentate NNS- donor ligands forming five membered chelate rings. Transition metal complexes of thiosemicarbazones are of considerable interest in chemistry because of their bioinorganic pertinence.

In the present work, we describe the synthesis and characterization of heterocyclic thiosemicarbazone and its Cobalt, Nickel, Copper and Zinc complexes along with their antimicrobial activity against E.Coli, S.aureus, P.aeruginosa, E.facecalis and C.albicans and further molecular docking studies were carried out with the dihydrofolate reductase (DHFR) receptor.

II. Experimental procedure

Materials

Cyclohexyl isothiocyanate (sigma Aldrich), 2-Acetyl-5-bromothiophene (Sigma-Aldrich), Hydrazine hydrate (Merck), Nickel (II) Perchlorate hexahydrate (Alfa Aesar), copper (II) per chlorate hexahydrate (Sigma- Aldrich), Cobalt (II) Chloride hexahydrate (Merk) and Zinc Chloride (Merk) were used as received. Analytical grade methanol and other solvents (Merck) were used without further purification as received. Antimicrobial strains were obtained from microbial type culture collection (MTCC), Indian Institute of Microbial Technology (IMTECH), Chandigarh.

Methods

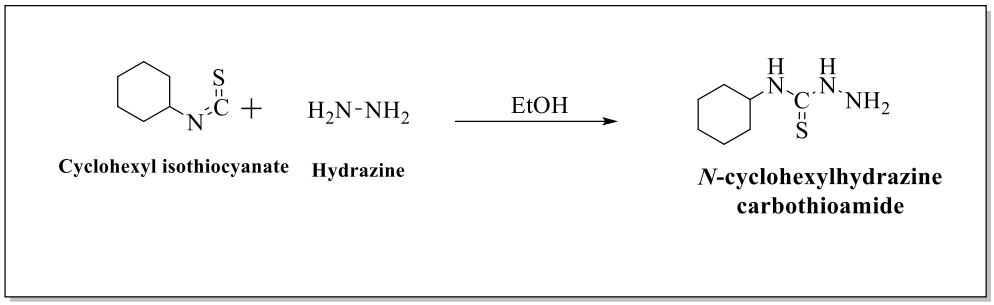
The FT-IR spectra were obtained in KBr disc by ABB Bomem (Model-MB 3000) spectrometer from 4000 cm^{-1} to 400 cm^{-1}. ^{1}H NMR and ^{13}C NMR were recorded on Bruker-Advance III 500 MHz NMR instrument using $CDCl_3$ as a solvent and TMS as internal reference (chemical shift in δ ppm). C, H, N, S analyses of the compounds were performed on Vario micro cubes elemental analyzer. The UV-visible spectra were recorded on UV-vis spectrophotometer (Elico SL159) in DMSO solvent. EPR spectra of the complexes were recorded in the solid state at room temperature using X band EPR spectroscopy. The ESI-mass spectral techniques were performed in Q-Tof-Mass Spectrophotometer.

Invitro antimicrobial screening of ligand and its complexes performed against human bacterial pathogens using well diffusion method. Molecular docking studies of metal (II) complexes were performed by using Schrodinger suite-2015 program.

Synthesis of Ligand (HL)

Synthesis of precursor

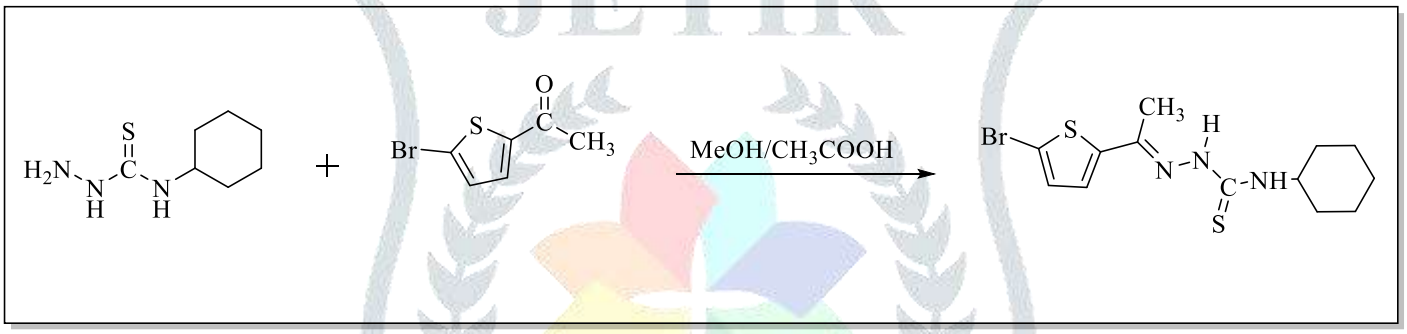
Ethanolic solutions of Cyclohexyl isothiocyanate (1.4123 g, 10 mmol) and hydrazine hydrate (0.5006 g, 10 mmol) were mixed with constant stirring for one hour and the final product was washed,dried and recrystallized from ethanol. Yield 93%.



Scheme 1: synthesis precursor

Synthesis of Ligand (HL)

N(4)-cyclo hexyl thiosemicarbazide (1.733g, 10 mmol) was dissolved in methanol (50 ml) and was added to the 2-Acetyl-5-bromo thiophene (2.051g, 10 mmol) dissolved in methanol (10 ml), reflux continuously for 4 hours after adding a few drops of acetic acid. The pale yellow crystalline product was kept aside for slow evaporation at room temperature, then filtered, washed with methanol and dried. The product was recrystallized from methanol.



Scheme 2: synthesis of the Ligand (HL)

III. Results and discussion

The physical characteristic and elemental analysis of the ligand and its complexes were represented in the **Table1.**

Table 1. Physical characteristic and elemental analysis of the ligand and its complexes.

S.No	Compound	Colour	Molecular Formula	Molecular Weight	Elemental Analysis % Calculated (Found)			
					C	H	N	S
1	Ligand (HL)	Pale Yellow	$C_{13}H_{18}$ Br N_3S_2	360.34	43.33 (43.30)	5.03 (5.27)	11.66 (12.01)	17.80 (17.65)
2	Co-HL	Light Blue	$C_{26}H_{36}Br_2Cl_2CoN_6S_4$	880.58	38.19 (38.23)	4.81 (4.32)	9.54 (9.75)	14.57 (14.48)
3	Ni- HL	Light Green	$C_{26}H_{36}Br_2NiN_6S_4$	779.37	40.07 (40.32)	4.66 (4.54)	10.78 (10.93)	16.46 (16.74)
4	Cu- HL	Dark Green	$C_{26}H_{36}Br_2CuN_6S_4$	784.22	39.82 (39.98)	4.63 (4.55)	10.72 (10.83)	16.36 (16.54)
5	Zn-HL	Pale Yellow	$C_{26}H_{36}Br_2Cl_2ZnN_6S_4$	887.03	37.91 (37.83)	4.77 (4.58)	9.47 (9.31)	14.46 (14.53)

IR Spectra

IR spectra of ligand and its metal complexes were given in the **Table 2.** The spectra of ligand and the metal complexes showed the broad band in the range of 3279 – 3333cm^{-1} due to NH Vibrations. The peaks occur in the range of 1528 – 1643cm^{-1} due to azomethine >C=N stretching frequency. The IR spectrum of the free ligand was compared with its metal complexes indentified that the ligand behaves as bidentate and coordinated towards metal ions through the azomethine nitrogen and the thione sulphur atom. The (C=S) stretching frequency was lowered in the spectra of the complexes, indicating the involvement of the thioketo sulphur coordination around metal ion [16]. Further, all the complexes exhibit band around 501 – 791 and 455 – 620 cm $^{-1}$ are assignable to ν(M-N) and ν(M-S) respectively.

Table 2. IR (cm^{-1}) and UV-Vis (λ_{max} in nm) spectral data of the ligand and its metal complexes

Compound	ν(N-H)	ν(C=N)	ν(N-N)	ν(C=S)	ν(M-N)	ν(M-S)	λ_{max} (UV-Vis)
Ligand (HL)	3336	1643	1116	1294	-	-	267,358
Co-HL	3333	1605	1111	1257	501	455	265,338
Ni- HL	3333	1528	1041	1203	571	463	265,327
Cu- HL	3279	1628	1071	1211	791	620	269,300,366
Zn-HL	3456	1528	1111	1257	571	471	270,337

Electronic Spectra

The electronic spectra of the ligand (HL) and its metal complexes are given in the **Table 2.** In the UV – Visible spectra of the free ligand shows intense broad band at 358 nm with a small shoulder at 260 nm for n- π* and π – π* transitions respectively of the azomethine C=N bond [17]. The π – π* transition from the azomethine C=N is found at lower energy at 260 $\pm$ 10 nm.

NMR Spectral Data

^{1}H NMR spectral Data

The ^{1}H NMR spectrum of ligand showed two broad singlets at 8.43 ppm and 7.37 ppm assignable to the NH protons present in the molecule [18]. Ligand showed singlet signal at 2.2 ppm corresponding to the ketone methyl group [19].

^{13}C NMR spectral Data

The ^{13}C NMR spectrum of ligand revealed well defined peaks at 176.09 ppm assignable to thione carbon (C=S). Azomethine carbon (-C=N) peak appeared at 141.25 ppm [19].

EPR spectral studies

The solid state EPR spectra of the copper complex in the poly crystalline state at room temperature were recorded in the X-band region, using 100 KHz field modulation. The copper complex was found to be anisotropic with g-values g_x=2.047, g_y=2.012, g_z=1.988. The spectrum is slightly Rhombic with $g_x > g_y > g_z > 2.0023$.The g_x and g_y

values are in close proximity indicates that Cu complex is a square planar systems. The lack of copper hyperfine data shows that there is extensive delocalization on to the ligand or excess of g-/A strain in the system. This can be due to the deviations from strict square planar geometry [20]. The deviation of the g value from that of free electron (2.0023) is due to the covalent nature of the metal ligand bonding through NS (or) NNS [21-23].

Table 3. EPR Spectral parameter of the Cu (II) complex

Parameters/Complex	Cu-HL
g_x	2.047
g_y	2.012
g_z	1.988

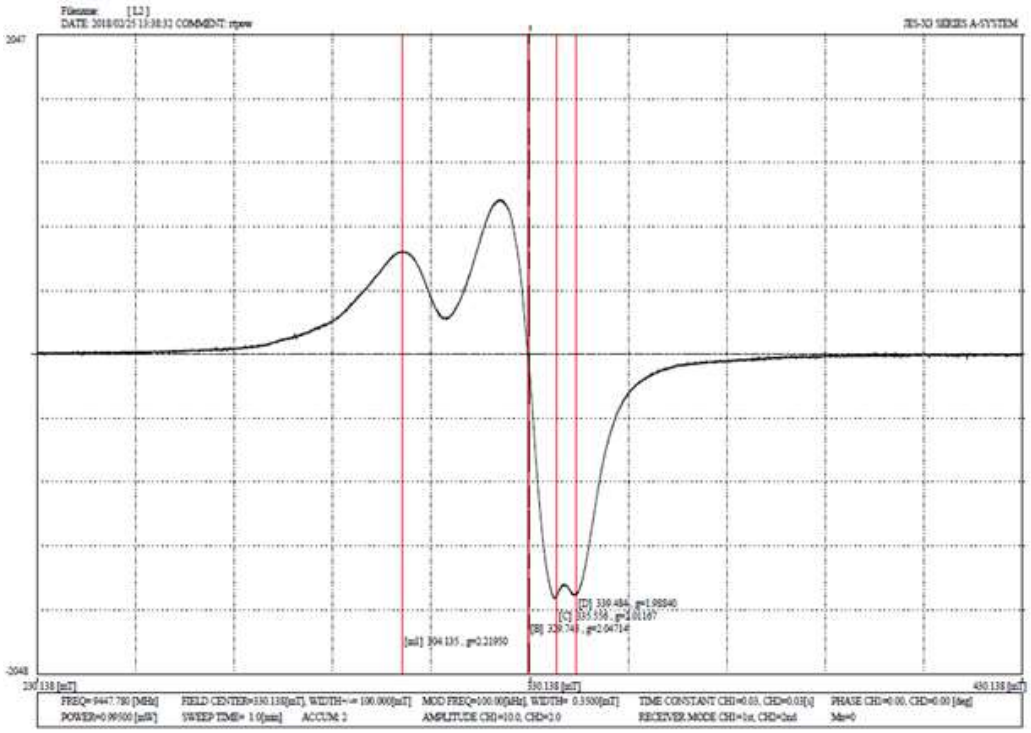


Figure 1. EPR Spectrum of Cu (II) complex

Electrospray mass spectral studies.

The ESI – mass spectral data for ligand and its metal complexes are given in the **Table 4**. The mass spectra of all the complexes invariably showed the base peak which corresponds to the molecular ion peak or stable carbocation, this is due to the formation of thermodynamically stable carbocation when compared to that of their corresponding complexes.

Table 4. Mass spectral data for Ligand and its metal complexes

Compound	Expected (m/z)	Observed (m/z)
Ligand(HL)	361.01	362.0$[M+H]^+$
Co-HL	880.94	881.94$[M+H]^+$
Ni- HL	777.96	778.96$[M+H]^+$
Cu- HL	782.95	783.95$[M+H]^+$
Zn-HL	885.93	886.93$[M+H]^+$

Anti microbial activity

All the compounds were screened for their antimicrobial activity against *S.aureus*, *Escherichia Coli*, *P.aeruginosa*, *E.facecalis* and *C.albicans* by well diffusion test. The zone of inhibition values were found out at the end of 24 hrs at 37 ^{0}C for the microbial stains. The antimicrobial results suggested that the schiff base derivative of thiosemicarbazone were found to be biologically active. It is observed that the Ni and Zn metal complexes higher activity against standard strains.

Standard microbial strains used:

S.aureus ATCC 25922

E. coli ATCC 25922

P. aeruginosa ATCC 700603

E. facecalis ATCC 29212

C. albicans ATCC 10231

Table 5. Zone of Inhibition (mm) of Ligand and its complexes

S.no.	Organism	Zone of inhibition at (100µl mm/ml)				
		S. aureus	*E. coli*	*P. aeruginosa*	*E. facecalis*	*C.albicans*
1	Ligand(HL)	16	18	17	20	22
2	Co-HL	20	21	16	24	18
3	Ni- HL	28	26	25	25	24
4	Cu- HL	25	21	22	20	22
5	Zn-HL	29	28	25	30	26

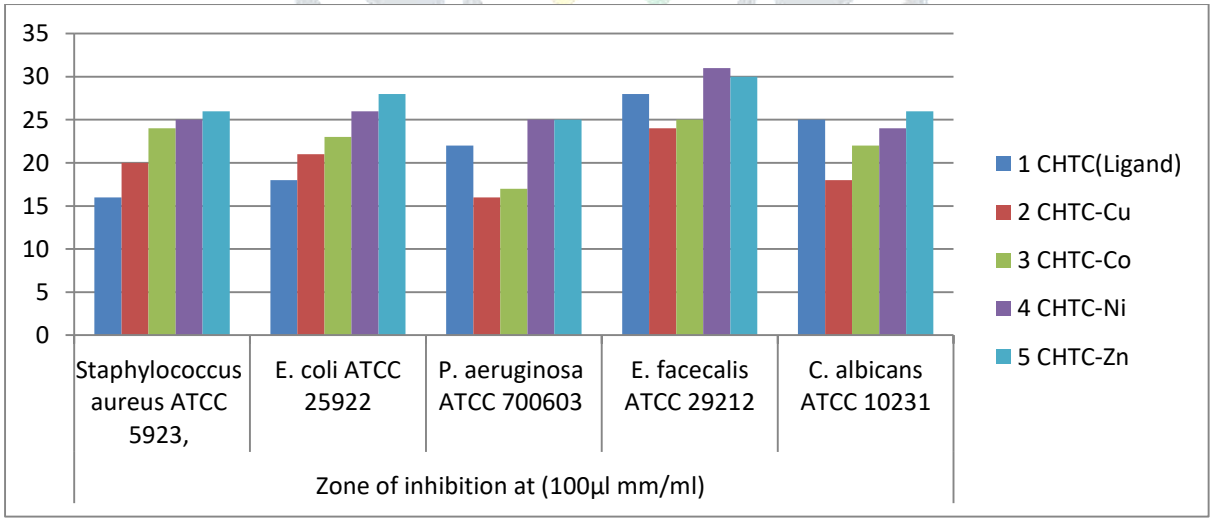


Figure 2. Graphical representation of Antimicrobial activity of Ligand and its complexes

MOLECULAR DOCKING STUDIES

The molecular docking studies of ligand and its metal complexes were carried out by maestro program inbuilt in the Schrödinger suite. Initially, the ligand structures are sketched by maestro building tools and then the ligand was

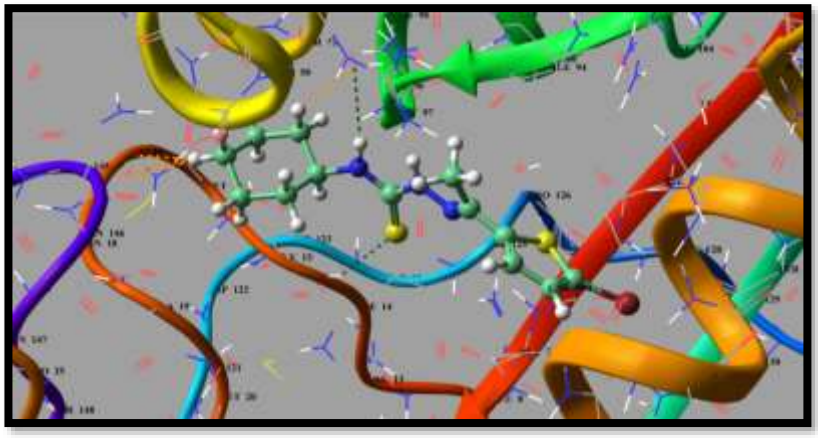
pre-processed in the ligand preparation step by using force field OPLS-2005 and gave low energy structure conformers which is suitable for molecular docking studies. The three-dimensional structure of dihydrofolate reductase DHFR (PDBID: 4DFR) was downloaded from protein data bank (http://www.pdb.org). In the protein preparation step used to assign bond orders, add hydrogen atoms and removed water beyond 5Å. Finally, the protein structure was fully optimized by using force field (OPLS-2005). The prepared ligand structure was docked into binding site of the DHFR using the glide with standard precision mode. The energy calculations were made using genetic algorithms. The outputs were exported to PyMol for visual inspection of the binding modes and for possible π-π stacking, hydrogen bond and hydrophobic exchanges of the compounds with DHFR.

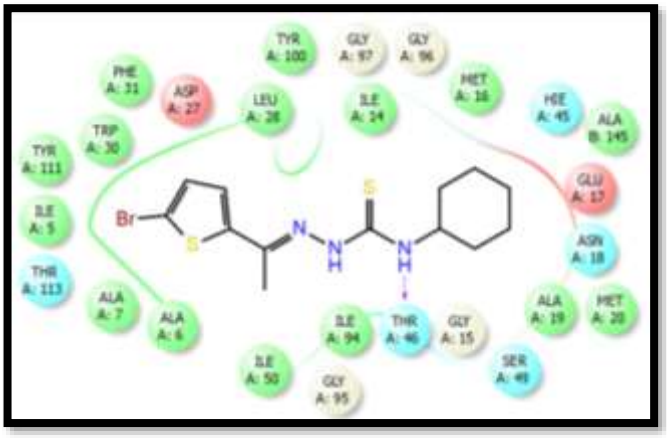
Molecular docking of complexes against target DHFR

Molecular docking program is easiest way to interpret interaction between ligand and protein, and also find various mode of interaction between them. The 3D and 2D interaction site of the complexes with DHFR shown in Figure 3. The docking studies results revealed that the ligand and the metal complexes located within the hydrophobic site of DHFR receptor. The observed docking score values found to be, -6.266, -2.942, -4.876, -4.896, -6.558 kcal mol^{-1} for ligand and complexes HL, Co-HL, Ni-HL, Cu-HL and Zn-HL respectively, which showed effective interaction with receptor DHFR. Moreover, the binding results showed various mode of interaction via H-bond, π-π stacking, and hydrophobic contacts. From the results Zn-HL complex showing highest docking score with DHFR. The complex also exhibit hydrophobic interactions with many amino acid residues: TYR 100, ILE 5, TRP 30, PHE 31, ALA 7, ALA 6, MET 20, TRP 22, ILE 50, PRO 25, ALA 19, LEU 28, LEU 54, ALA 145, ILE 14, MET 16, and ILE 94. Therefore the results obtained from molecular docking analysis concluded that the ligand and their metal complexes could effectively bind with DHFR receptor and the interaction

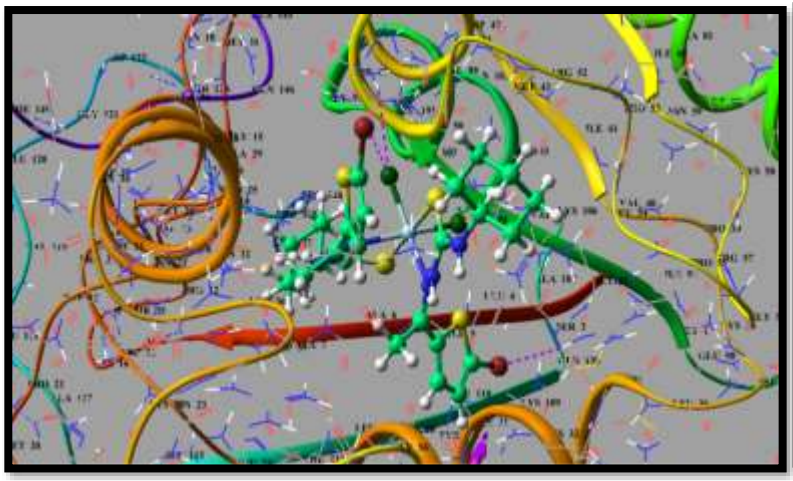
between them was governed by hydrogen bond, π-π stacking and hydrophobic forces.

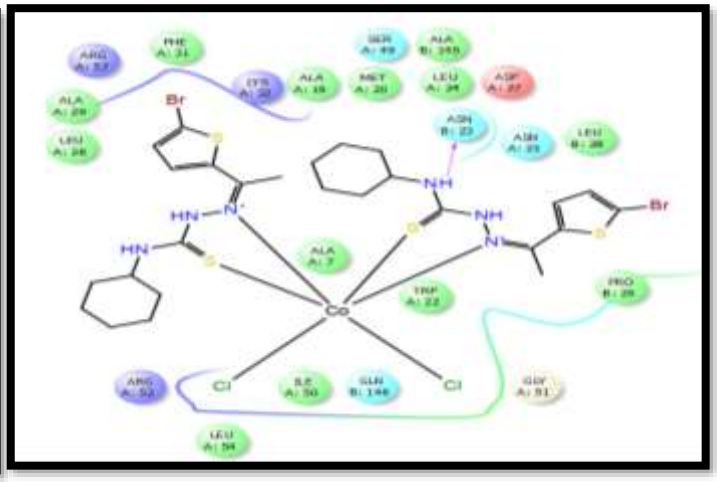
HL



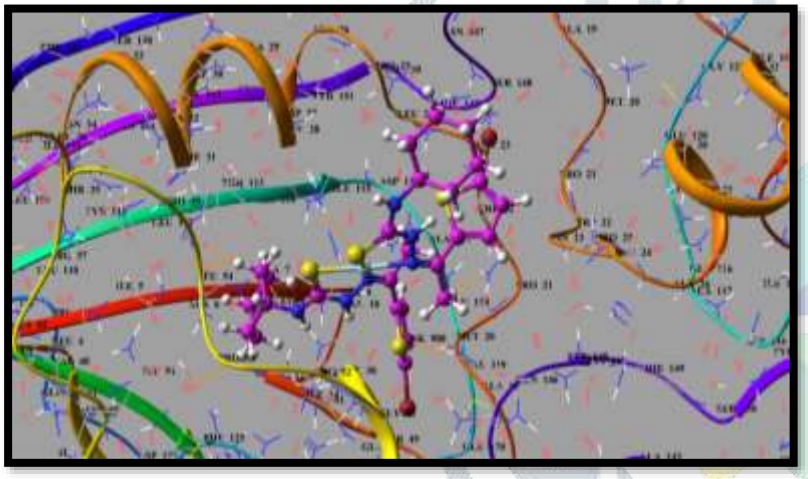


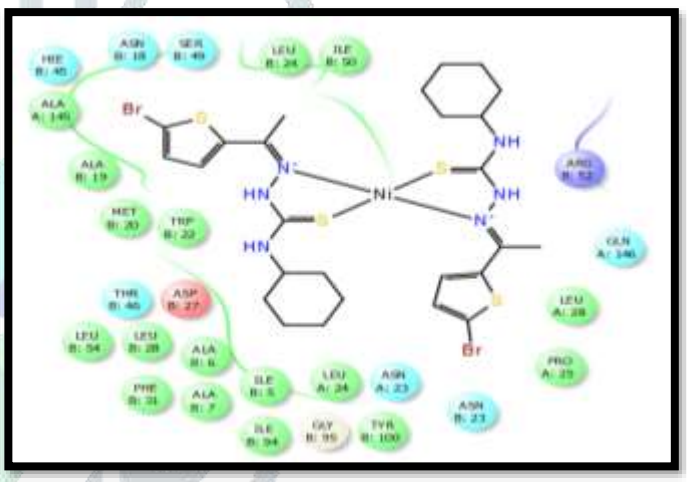
Co-HL



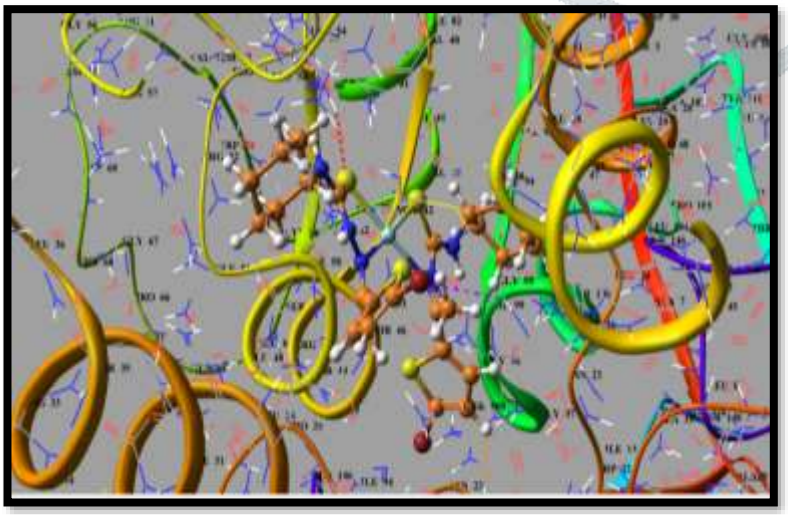


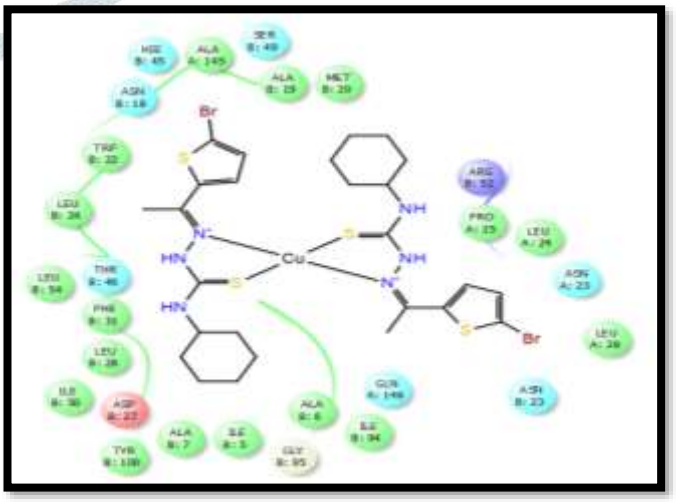
Ni-HL





Cu-HL





Zn-HL

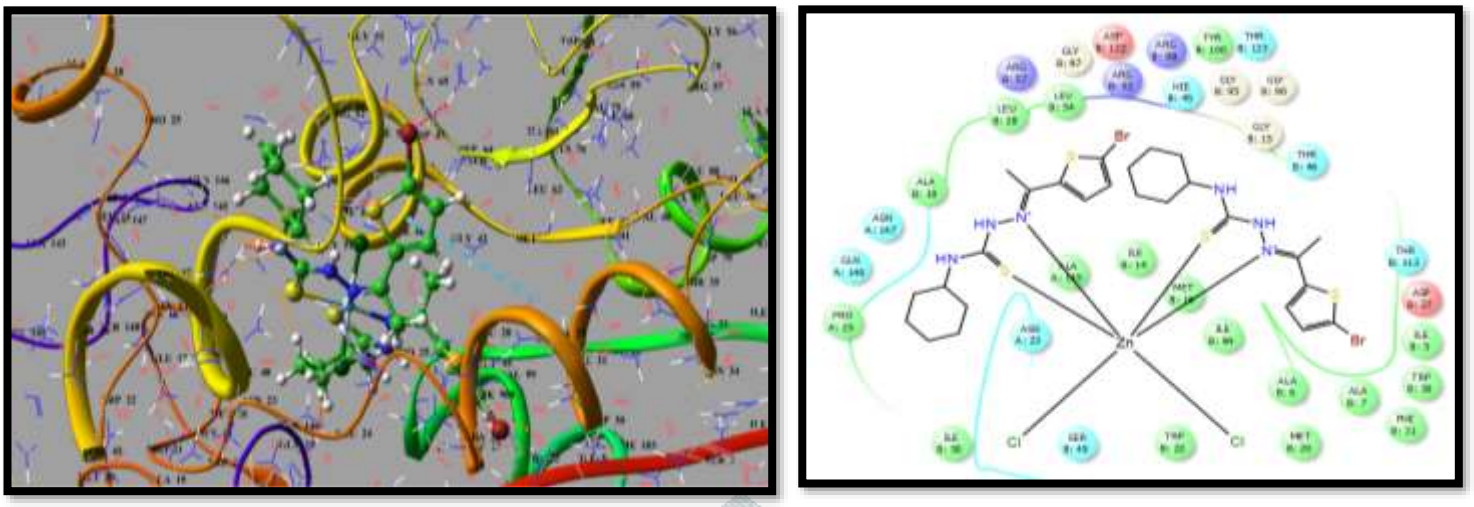


Figure 3. 3D and 2D interaction of Ligand(HL), Co(II)-HL, Ni(II)-HL, Cu(II)-HL and Zn(II) HL complexes located in the hydrophobic site of the DHFR receptor.

Table 6. Docking parameters of the Ligand and its metal (II) complexes with DHFR receptor

Complexes	Docking score kcal.mol^{-1}	Active sites with a mode of interaction		
		H-bond	π-π stacking	Hydrophobic contacts (Cut off at 5Å)
HL	-6.266	THR 46	--	ILE 14, MET 16, ALA 145, MET 20, ALA 19, ILE 94, ILE 50, ALA 6, ALA 7 ILE 5, TYR 111, TRP 30, PHE 31, LEU 28, TYR 100
Co-HL	-2.942	ASN 23	--	ALA 19, MET 20, ALA 145, LEU 24, LEU 28, PRO 25, ILE 50, ALA 29, PHE 31, ALA 7, TRP 22, ILE 50
Ni-HL	-4.876	--	--	LEU 28, PRO 25, TYR 100, ILE 94, ALA 7, PHE 31, LEU 54, LEU 24, ILE 5, ALA 6, LEU 28, TRP 22, MET 20, ALA 19, ALA 145, ILE 50
Cu-HL	-4.896	--	--	ALA 145, ALA 19, MET 20, PRO 25, LEU 24, LEU 28, ILE 94, ALA 6, ILE 5, ALA 7, TYR 100, ILE 50, PHE 31, LEU 54, TRP 22,
Zn-HL	-6.558	--	--	TYR 100, ILE 5, TRP 30, PHE 31, ALA 7, ALA 6, MET 20, TRP 22, ILE 50, PRO 25, ALA 19, LEU 28, LEU 54, ALA 145, ILE 14, MET 16, ILE 94.

Conclusion

The thiosemicarbazone ligand (HL) and its metal complexes were characterized by elemental analysis, NMR, IR, UV-vis, EPR and ESI mass spectral studies. On the basis of above data the thiosemicarbazone ligand (HL) behave as a bidentate ligand coordinating through the azomethine nitrogen and the thione

sulphur atom. The results of the above studies suggest that the metal complexes of Cu and Ni have been assigned square planar geometry whereas the metal complexes of Co and Zn confirm the octahedral geometry. The results of the H^1 NMR and ^{13}C NMR spectral data revealed that the number of hydrogen atoms and carbon atoms present in the ligand were exact when compared to the expected compounds. Antimicrobial screening of metal complexes showed excellent activity than compare to that of the ligand. All the complexes were strongly interact with DHFR inhibitor and the best docking score value observed for Complex Zn was consistent with experimental results obtained from an antimicrobial activity.

Conflict of Interest

All the authors declare that they have no conflict of interests.

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