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ORIGINAL ARTICLE

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF Pd (II) COMPLEXES OF SOME NITROGEN- SULPHUR LIGANDS

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ABSTRACT

Pd (II) complexes of thiosemicarbazones (TSC) from *para* and *meta* substituted benzaldehyde were synthesized by ultrasonic irradiation method. These complexes were characterized by elemental analysis, molar conductance measurements, IR, electronic and ^{1}HNMR and ^{13}CNMR spectral studies. The molar conductance measurements of the complexes in DMSO determine the non-electrolytic nature of the complexes. The ligands and its metal complexes have been screened for antimicrobial activities. Square planar geometry may be assigned for Pd (II) complexes on the basis of spectral studies. Molecular docking studies were conducted to evaluate the inhibitory activities of ligand against DNA.

Keywords: Palladium (II) chloride. Ultrasonic irradiation and thiosemicarbazone antimicrobial activies, docking studies.

1.INDRODUCTION

Ultra sound assisted organic synthesis is used as a modern and eco-friendly technique and good yields that is being used to accelerate organic synthesis. Thiosemicarbazones are very versatile ligands. Thiosemicarbazones and their metal complexes have wide range of applications in analytical chemistry. They can coordinate to metal as neutral molecules or after deprotonation as anionic ligands and can adopt a variety of different coordination modes.

Thio-semicarbazones act as ligands because better co-ordination tendency, more stable complexes, better selectivity, macrocyclic ligands and they have the ability to produce some new and unique complexes with enhanced biological and analytical properties.

Thiosemicarbazones usually act as chelating ligands with transition metal ion bonding through the sulphur and hydrazine nitrogen atom. Thiosemicarbazones and their complexes have received considerable attention because of their pharmacological activities [1].

Thiosemicarbazones and their complexes have received considerable attention because of their antibacterial[2,3] antifungal[4], antitumor[5], antiamoebic[6,7], antimalarial[8], antiviral[9], radio protective[10] and anti-inflammatory activities[11]. Certain thiosemicarbazones are relatively specific inhibitors of ribonucleotide reductase, which is an important metabolic target for the development of chemotherapeutic agents against cancer[12,13]. The coordination chemistry of thiosemicarbazone appears to be very interesting from the point of view of both the number of metals forming complexes with them and the diversity of the ligand systems themselves which include macrocyclic systems[14,15].

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2. MATERIALS AND METHODS

All the chemicals were purchased from standard companies (Merck and Sigma- Aldrich). Analytical grade solvents ethanol, dimethylsulphoxide and acetonitrile were used. Synthesized compounds were purified and their physical constants were verified.

The complexes were found to be insoluble in methanol, carbon tetrachloride, nitrobenzene, chloroform, but sufficiently soluble in DMSO. The molar conductivity of these complexes was determined from their solutions in DMSO, which was found to be 130.10-160.15×10^{-3} Ohm^{-1} cm^{2} mol^{-1}. The extremely low values of molar conductivity indicate that these complexes are non – electrolytic in nature [16,17,18].

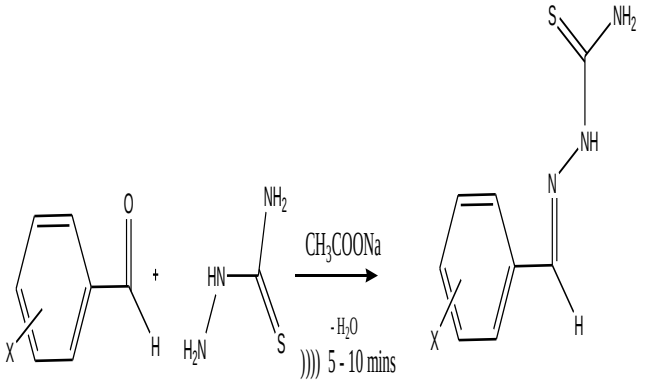
The elements Carbon, hydrogen, nitrogen analyzed by using 2400 series II CHNS/O analyzer. Molar conductance was measured by using the Elico (CM82T) conducting bridge. NMR spectra were recorded in Bruker AV 400 NMR spectrometer operating at 400 MHz has been utilized for recording ^{1}H NMR spectra and 100 MHz for ^{13}C spectra in DMSO solvent using TMS as internal standard. IR spectra (KBr) were recorded using FT IR spectrum BX-II

spectrophotometer. The electronic spectra were recorded in DMSO by using Shimadzu UV mini-1240 spectrophotometer.

3. EXPERIMENTAL

(i) Synthesis of thiosemicarbazones

Aqueous solutions of thiosemicarbazide (0.01mol) and sodium acetate (0.01mol) were added to the dissolved subsitituted benzaldehyde (0.02mol) in ethanol solution. The mixture was irradiated with ultrasound for 5-10 minutes. The resulting reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was evaporated and recrystallized from ethanol **(Scheme 1)**.



(Scheme 1)

X = Substituents at para or meta position (Cl^- ,F^- and NO_2)

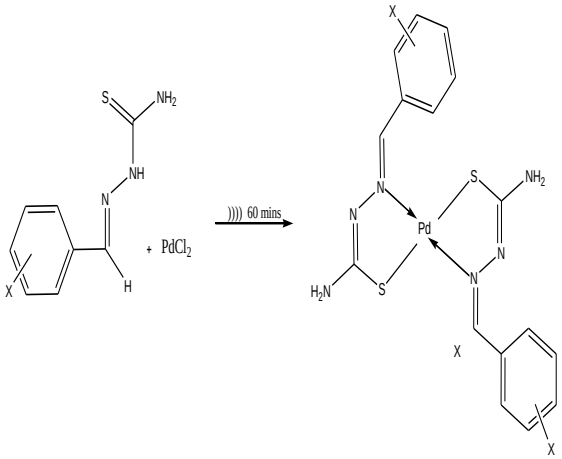
(E) -1-(4-chlorobenzylidene)thiosemicarbazide **(L^1)** Color : white , Yield: (65 %); m.p.: 202 °C. Anal. Calcd. for $C_8H_8N_3ClS$ (MW: 213): C, 45.03, H, 3.79; N, 19.72%. Found: C, 44.97; H, 3.77; N, 19.66 %. IR (KBr, cm^{-1}): 3238 (N-H), 3388 (NH_2), 1598 (C=N), 827 (–C=S–). ^{1}H-NMR (300 MHz, DMSO-*d*6, δ / ppm): 8.05 (CH=N), 7.46 (NH_2), 7.45–7.60 (Ar–H), 11.46 (N–H); UV–Vis (DMF) (λmax / nm): 234 (π→π*), 330 (n→π*). ^{13}C-NMR 178.08 (C=S), 140.81 (C=N), 128 - 134 (Ar–H).

(E) -1-(4-fluorobenzylidene)thiosemicarbazide **(L^2)** Color : white ; Yield: (70 %); m.p.: 191°C. Anal. Calcd. for $C_8H_8N_3FS$ (MW: 197): C, 45.03, H, 3.79; N, 21.36 %. Found: C, 48.72; H, 4.09; N, 21.3 %. IR (KBr, cm^{-1}): 3280 (N-H), 3433 (NH_2), 1598 (C=N), 819 (–C=S–). ^{1}H-NMR (300 MHz, DMSO-*d*6, δ / ppm): 8.18 (CH=N), 7.19 (NH_2), 7.83–8.03 (Ar–H), 11.41 (N–H); ^{13}C-NMR 177.99 (C=S), 148.35 (C=N), 115 - 130 (Ar–H) ; UV–Vis (DMF) (λmax / nm): 240 (π→π*), 333 (n→π*).

(E) -1-(3-nitrobenzylidene)thiosemicarbazide **(L^3)** Color : yellow; Yield: (68 %); m.p.: 234 °C. Anal. Calcd. for $C_8H_8N_4O_2S$ (MW: 224): C, 42.91, H, 3.63, N, 24.96 %. Found: C, 42.85; H, 3.60; N, 24.99 %. IR (KBr, cm^{-1}): 3165 (N-H), 3462 (NH_2), 1598 (C=N), 831 (–C=S–). ^{1}H-NMR (300 MHz, DMSO-*d*6, δ / ppm): 7.83 (CH=N), 7.24 (NH_2), 7.28–7.81 (Ar–H), 11.80 (N–H); ^{13}C-NMR 191.75 (C=S), 141.35 (C=N), 121–139 (Ar–H), UV–Vis (DMSO) (λmax / nm) 242 (π→π*), 336 (n→π*).

(ii) Synthesis of palladium (II) complex

Palladium (II) chloride (0.02mol) was dissolved in acetonitrile (10ml) and semicarbazone ligands (0.04mol) were also dissolved in acetonitrile (10ml). Both the solutions were mixed and irradiated to ultrasound for 60 minutes. The resulting reaction was monitored by TLC. After completion of reaction, the reaction mixture was evaporated and recrystallized from acetonitrile **(Scheme 2).**



Scheme 2

X = Substituents at para or meta position (Cl^- ,F^- and NO_2)

[Pd(L^1)$_2$] Cl_2 Yield: (60 %); m.p.: 140 °C. Anal. Calcd. for $C_{18}H_{20}Cl_2N_6PdS_2$ (MW: 560): C, 38.52, H, 3.64; N, 14.89 %. Found: C, 38.48; H, 3.59; N, 14.96 %. IR (KBr, cm–1): 1593 (C=N), 3419 (NH $_2$), 507 (M-N), 437 (M-S). ^{1}H-NMR (300 MHz, DMSO-*d*6, δ / ppm): 8.27 (CH=N), 7.20 (NH_2), 7.21-8.26 (Ar–H), 11.46 (N–H); UV–Vis (DMF) (λmax / nm): 252 (π→π*), 369 (n→π*) LMCT (470). ^{13}C-NMR 192.65 (C=S), 140.81 (C=N), 128 - 134 (Ar–H).

[Pd(L^2)$_2$] Cl_2 Yield: (76 %); m.p.: 220 °C. Anal. Calcd. for $C_{18}H_{20}F_2N_6PdS_2$ (MW: 528): C, 45.03, H, 3.88; N, 15.89 %. Found: C, 40.87; H, 3.81; N, 15.86 %. IR (KBr, cm–1): 3417 (NH $_2$), 559 (M-N), 1596 (C=N) 511 (M-S), ^{1}H-NMR (300 MHz, DMSO-*d*6, δ / ppm): 8.35 (CH=N), 7.26 (NH_2), 7.31-8.33 (Ar–H), 11.41 (N–H); ^{13}C-NMR 162.65 (C=S), 151.66 (C=N), 128 - 142 (Ar–H) ; UV–Vis (DMF) (λmax / nm): 261 (π→π*), 378 (n→π*) LMCT (423).

[Pd(L^3)$_2$] Cl_2 Yield: (68 %); m.p.: 234 °C. Anal. Calcd. for $C_{18}H_{20}N_8O_4PdS_2$ (MW: 582): C, 40.91, H, 3.49, N, 19.28 %. Found: C, 37.09; H, 3.46; N, 19.22 %. IR (KBr, cm–1): 1589 (C=H), 3404 (NH $_2$), 509 (M=N), 457 (M-S). ^{1}H-NMR (300 MHz, DMSO-*d*6, δ / ppm): 8.21 (CH=N), 7.40 (NH_2), 7.44 – 8.06 (Ar–H); ^{13}C-NMR 161.11(C=S), 150.52 (C=N), 128-149 (Ar–H), UV–Vis (DMSO) (λmax / nm) 272(π→π*), 360 (n→π*) LMCT (434).

4. RESULT AND DISCUSSION

Infra red spectra

Infrared spectra of the thiosemicarbazone ligands showing bands in the region 1598 cm^{-1} may be assigned to the (C=N) vibrations. Strong band in the region 791–813 cm^{-1} in thiosemicarbazones are due to the (C=S)] groups, respectively. Strong band in the region ν 3165 – 3280 cm^{-1} in thiosemicarbazones are due to the [ν(N-H)] groups. Strong band in the region ν 3388 – 3462 cm^{-1} in thiosemicarbazones are due to the (NH_2) groups. On complexation the bands

corresponding to *ν*(–C=N) and bands of *ν*(–C=S) (in case of thiosemicarbazone) shifted towards lower side this suggests that the ligand acts as bidentate chelating agent coordinating through nitrogen of *ν*(–C=N) group and sulphur of *ν*(–C=S) group, respectively. Thus, it has been concluded that the thiosemicarbazones act as bidentate chelating agent [19]. The spectrum of the ligand shows a sharp and strong band at 800 cm^{-1}, which may be assigned [20] to ν(C=S). This band suffers downward shift at 780-770 cm^{-1} proposes linkage through thione sulphur atom of thiosemicarbazone moiety. The linkage through S atom of thione group is further confirmed by the appearance of a band in the far IR region at 470-450 cm^{-1} in the complexes which may be assigned [21] ν(M–S).

The broad bands of the –NH group observed at 3172–3277 cm^{-1} for the ligands disappear in the complexes of IR spectra, which indicates the deprotonation of the NH-CS group. In addition, the vibrational frequencies of the $–NH_2$ groups remain unchanged for both the ligands and the complexes. This indicates the non - coordination of the $–NH_2$ group to the Pd (II) center.

Electronic spectra

An adsorption bands observed in the range of 239 to 242 nm in the spectrum of the ligands may be due to π→π* transition. The electronic spectra of Pd (II) complexes consists of bands in the region 360 - 378 nm which are assigned to n→π* band of the azomethine group. The thiosemicarbazone and palladium (II) complexes have two bands; one centered at 239 and 242 nm and another at 360 and 378 nm. These bands are assigned to π→π* and n→π* transitions of phenyl rings and azomethine moiety respectively [22,23]. The charge transfer bands were observed around 423-470 nm and its broadness can be explained as due to the combination of ligand to metal charge transfer transitions [24, 25, 26].

The electronic spectra of the Pd(II) complexes show transitions from the lower lying *d* levels to the empty $dx^2–y^2$ orbital. These bands are $^1A_{1g} \rightarrow {}^1B_{1g}$ transitions respectively. The electronic spectra of these complexes indicate square planar geometry around the Pd(II) [27,28]

^{1}H NMR and ^{13}C NMR spectra

In the ^{1}H-NMR spectra of the ligands, the signals of the =N–NH protons were observed as singlets at *δ* 11.42–11.83. These signals disappeared in the ^{1}H-NMR spectra of the palladium (II) complexes indicating the deprotonation of the =N–NH group. The signals of the HC=N protons which appear as singlets at *δ* 8.03–8.17 in the ligands show a shift to downfield after complexation. This shift indicates the coordination of the imine nitrogen to the metal center. The signals of the aromatic protons of the ligands appeared in the range of *δ* 7.21–7.91ppm. These signals do not suffer relevant changes in the chemical shifts for the palladium (II) complexes. The NH_2 proton signal in the ligands (L^1, L^2 and L^3) appears at *δ* 7.98–8.45 due to the nonequivalence of the amine protons [29]. These signals are not changed in the NMR spectra of the complexes. The NMR spectrum of metal chelates confirms the non participation of NH_2 group in the coordination with metal ions. This evidence is attributed to the restricted rotation around C–N bond (thiocarbonyl carbon and terminal amine nitrogen) due to its partial double bond character [30].

The ^{13}C NMR spectrum provides direct information about the carbon skeleton of the synthesized compound. In the ^{13}C NMR spectra the signal due to (-CH=N-) carbon, in the range of 137.89, 137.80, 138.01 158.46 and136.88 ppm for L^1, L^2 and L^3 respectively. The- C=S carbon, appeared in the range of 156.59, 156.60, 161.22, 190.63 and 156.50 ppm for L^1, L^2 and L^3 respectively. All the (Ar- C) 121.99- 134.10 ppm was observed in the expected regions.

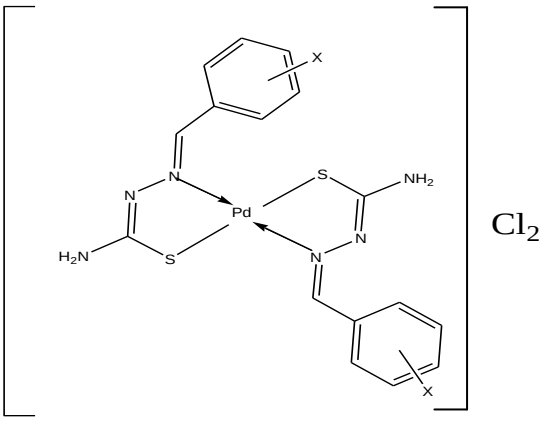


Figure 1: Suggested structure of Pd(II) complexes

Antibacterial activity

The antibacterial activity of the palladium (II) complexes was studied against Gram-positive bacteria *Bacillus subtilis, Streptococcus pyogenes, Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*. Each of the palladium complexes compounds dissolved in DMSO at a concentration of 2 mg/ cm3 was prepared. Paper discs of Whatmann filter paper No. 41 were cut and sterilized in an autoclave. The paper discs were saturated with 5µl of the palladium complexes compounds dissolved in DMSO as negative control and were placed aseptically in the Petri dishes containing Nutrient agar media inoculated with the above mentioned two bacteria separately. The petri dishes were incubated at 37^0C and the inhibition zones were recorded after 24 h of incubation.

Thus the substituents placed a vital role in imparting enhanced antibacterial activity to the compounds. The screening results indicate that Complexes $[Pd(L^2)_2]$ Cl_2 and $[Pd(L^3)_2]$ Cl_2 were found to be moderate active against *B. Substilis*. Complex $[Pd(L^1)_2]$ Cl_2 and ligands, L^1, L^2 and L^3 were found to be less active against *B. Substilis*. Complexes $[Pd(L^1)_2]$ Cl_2 and $[Pd(L^2)_2]$ Cl_2 were found to be moderate active against *E. coli*. Complex $[Pd(L^1)_2]$ Cl_2 and ligands, L^1, L^2 and L^3 were found to be less active against *E.coli*. Complexes $[Pd(L^1)_2]$ Cl_2, $[Pd(L^2)_2]$ Cl_2 and $[Pd(L^3)_2]$ Cl_2 and ligand L^2 were found to be moderate active against *P. aruginosa* L^1 and L^2 were found to be less active against *P. aruginosa*.

Complexes $[Pd(L^1)_2]$ Cl_2 ,$[Pd(L^2)_2]$ Cl_2 and $[Pd(L^3)_2]$ Cl_2 were found to be moderate active against *St. aureus*. Complex (L^1), L^2 and L^3 were found to be less active against *St. aureus*. Complexes $[Pd(L^1)_2]$ Cl_2, $[Pd(L^2)_2]$ Cl_2 and $[Pd(L^3)_2]$ Cl_2 and ligand L^3 were found to be moderate active against *S.*

pyrogenes. Complexe (L^1), L^2 and L^3 were found to be less active against *S. pyrogenes*.

The antibacterial screening of palladium complexes exhibit more inhibitory effect than the parent ligand (Table 1). The increased activity of metal complexes is due to chelation and some properties like solubility, conductivity and bond length between the metal and the ligand. Thus the substituents placed a vital role in imparting enhanced antifungal activity to the compounds. Most of ligands and complexes were found to possess moderate antibacterial activity free ligands which have electron donating groups; this means that compounds with high electron density gave poor antibacterial activity which makes the diffusion of these compounds more difficult throw the body of the bacteria cell [29]. The increase in antibacterial activity is due to faster diffusion of the free ligands with electron withdrawing groups and metal complexes as a whole through the cell membrane or due to the combined activity effect of ligand and metal [30].

Table 1 Antibacterial activity by zone of inhibition of palladium complexes

Plate No	Bacteria	Standard	Zone of inhibition (mm)						
			1	2	3	4	5	6	Control
1	*B. subtilis*	28	06	12	12	02	10	9	-
2	*E. coli*	30	07	12	11	02	04	04	-
3	*P. aeruginosa*	30	13	11	11	06	10	06	-
4	*Sta. aureus*	32	13	10	11	01	03	03	-
5	*Str. Pyogenes*	30	11	09	11	02	03	12	-

Standard: ***ciprofloxacin 1** **1=[Pd(L^1)$_2$]Cl_2,2=[Pd(L^2)$_2$]Cl_2 3=[Pd(L^3)$_2$]Cl_2;* **4**=L^1 *(E) -1-(4-chlorobenzylidene)thiosemicarbazide;* **5**=L^2 *(E) -1-(4-fluorobenzylidene)thiosemicarbazide;*=L^3 *(E) -1-(3-*

Antifungal activity

The antifungal activity of the palladium (II) complexes was studied against *Aspergillus flavus*, *Aspergillus niger* and *Trichoderma viride*. Each of the palladium complex compounds dissolved in DMSO at a concentration of 2 mg/ cm3 was prepared. Paper discs of Whatmann filter paper No. 41 were cut and sterilized in an autoclave. The paper discs were saturated with 5µl of the palladium complex compounds dissolved in DMSO solution as negative control and were placed aseptically in the Petri dishes containing Nutrient agar media inoculated with the above mentioned fungi separately. The petri dishes were incubated at 37^0C and the inhibition zones were recorded after 24 h of incubation.

The screening results indicate that Complexes [Pd(L^1)$_2$] Cl_2, [Pd(L^2)$_2$] Cl_2 and [Pd(L^3)$_2$] Cl_2 were found to be moderate active against *A. flavus* than corresponding ligands. Complexes [Pd(L^1)$_2$] Cl_2, [Pd(L^2)$_2$]Cl_2 and [Pd(L^3)$_2$] Cl_2 were found to be moderate active against *A. niger* than corresponding ligands. Complexes [Pd(L^1)$_2$] Cl_2 and [Pd(L^2)$_2$] Cl_2 were found to be more active against *Tri.veride* than corresponding ligands. Complexe [Pd(L^3)$_2$]Cl_2 were found to be less active against *Tri.veride* than corresponding ligand (L^3) due to complex show high electron density so its show lower antibacterial active (Table 2). A comparative study of the ligands and their complexes as antifungal active indicates that the metal complexes are more active than the free ligands.

Table 2 Antifungal activities by zone of inhibition of palladium complexes

Plate No	Fungal	Standard	Zone of inhibition (mm)						
			1	2	3	4	5	6	Control
1	*A. flavus*	22	17	16	15	16	12	13	-
2	*A. niger*	18	14	13	13	13	13	02	-
3	*Tri. Veride*	30	21	18	04	19	01	16	-

Standard: Amphotericin-B;1=[Pd(L^1)$_2$]Cl_2 ;2=[Pd(L^2)$_2$]Cl_2 3=[Pd(L^3)$_2$]Cl_2
4=L^1 *(E) -1-(4-chlorobenzylidene)thiosemicarbazide*
5=L^2 *(E) -1-(4-fluorobenzylidene)thiosemicarbazide*
6=L^3 *(E) -1-(3-nitrobenzylidene)thiosemicarbazide*

MOLECULAR DOCKING

Molecular docking is an important computational technique in structural biology and computer aided drug design. The major objective of molecular docking is to evaluate the feasible binding geometries of a putative ligand with a target protein of known three dimensional structures. To understand the interaction of all the synthesized molecules with DNA gyrase subunit B enzymes, the crystal structure of topoisomerase II was downloaded from the Protein Data Bank (PDB: 3U2D) and molecular docking studies were performed using the Glide program (version 6.3, Schrodinger, LLC, New York, 2014). To analyze the docking results and execute the protocol, the Maestro user interface (version 9.3, Schrodinger, LLC, NewYork, 2014) was employed.

Table. 3 Docking Data of thiosemicarbazones

Thiosemicarbazones	Glide Score	Binding Energy
L^1	-5.12	-35.18
L^2	-5.63	-33.62
L^3	-5.61	-33.21
Standard Ciprofloacin	-7.53	-30.06

L^1 = *(E) -1-(4-chlorobenzylidene)thiosemicarbazide*
L^2 = *(E) -1-(4-fluorobenzylidene)thiosemicarbazide*
$L^{3=}$ *(E) -1-(3-nitrobenzylidene)thiosemicarbazide*

The molecular docking studies were performed against crystal structure of DNA gyrase and the respective docking scoring functions were given in Table 3 and Figure 2 & 3 shows the X-ray crystal structure of protein DNA gyrase (PDB: 3U2D). Figures 4a, 4b and 4c show the docking images of thiosemicarbazone ligands of L^1, L^2 and L^3. The docking analysis was finished for the ligands with the target protein DNA gyrase by means of docking software GLIDE and the docked images are exposed. The structures docked by GLIDE are generally ranked according to the GLIDE Scoring Function (more negative). The scoring function of GLIDE docking agenda is accessible in the Gscore form. The majority straight forward technique of evaluating the accurateness of a docking procedure is to establish how

Docking images

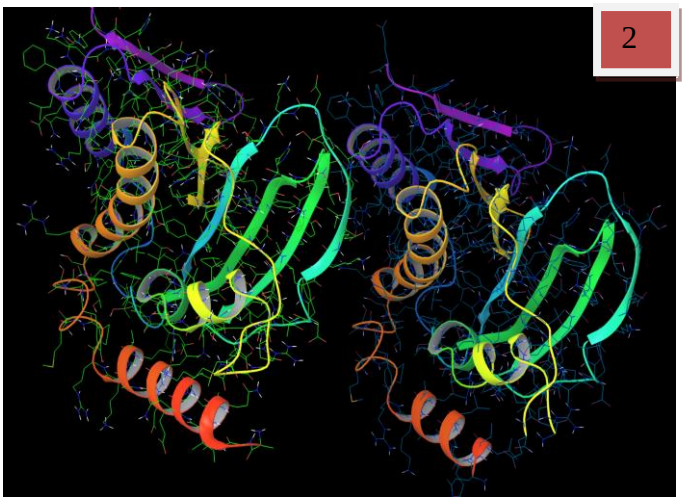


Figure 2: X-ray crystal structure of protein DNA gyrase (PDB: 3U2D)

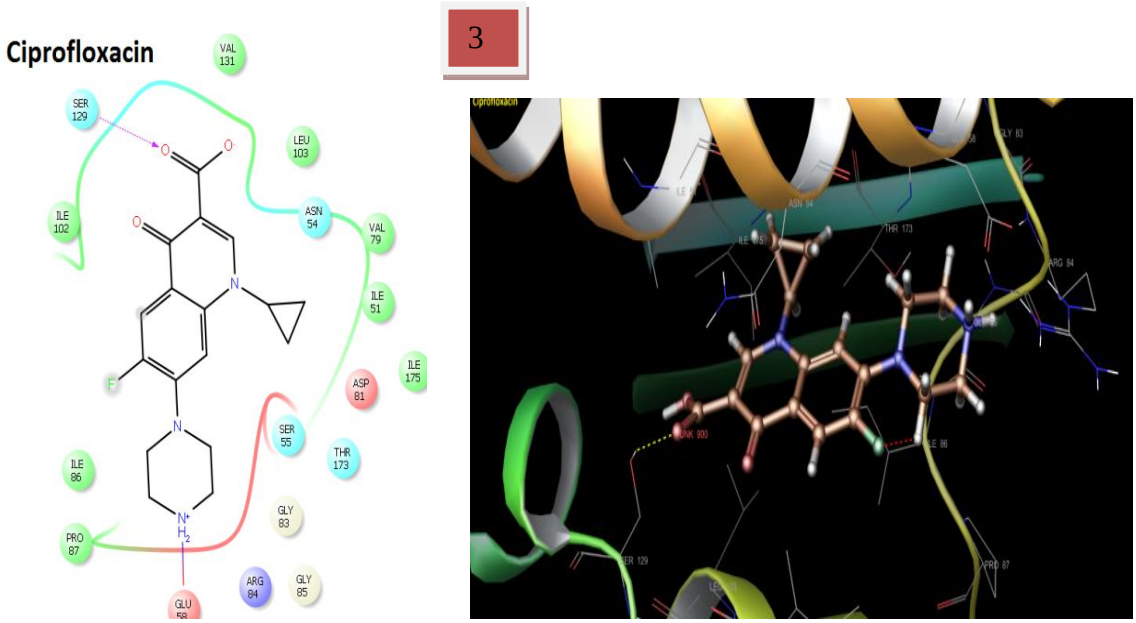


Figure 3: Docking structures of Ciprofloxacin (Standard)

closely the lowest energy pose (binding conformation) predicted by the object scoring function. The interaction energy includes vander Waals energy, electrostatic energy, as well as intermolecular hydrogen bonding were calculated for each minimized complex. The GLIDE Score for the standard ciprofloxacin, docked with DNA gyrase was -7.63. Generally for higher GLIDE score, good ligand affinity to the receptor may possibly be expected.

Thiosemicarbazone ligands (L^1, L^2 and L^3) were showed the noteworthy inhibition against DNA gyrase with -5.63 to –5.11 glide score. The synthesized thiosemicarbazone ligands are shown to be exhibit good binding its binding sites of DNA Gyrace. Hydrophobic, polar, glycine, charged positive and charged negative interactions present between the ligands (L^1, L^2 and L^3) and target DNA gyrace. Ligands L^1, L^2 and L^3 show more hydrophobic (ILE, LEU and PRO), more polar (ASN, SER and THR), charged positive (GLY), glycine (AEG) and charged negative (ASP and GLU) interaction.

In addition to –NH group in ligand L^1, L^2 and L^3 shows H-bond (side chain) with amino acid (ASP). Thiosemicarbazone ligand shows high glide score; good ligand affinity to the receptor may possibly be expected.

5.CONCLUSION

The thiosemicarbazone ligand and its metal complexes were characterized by elemental analysis, molar conductance and

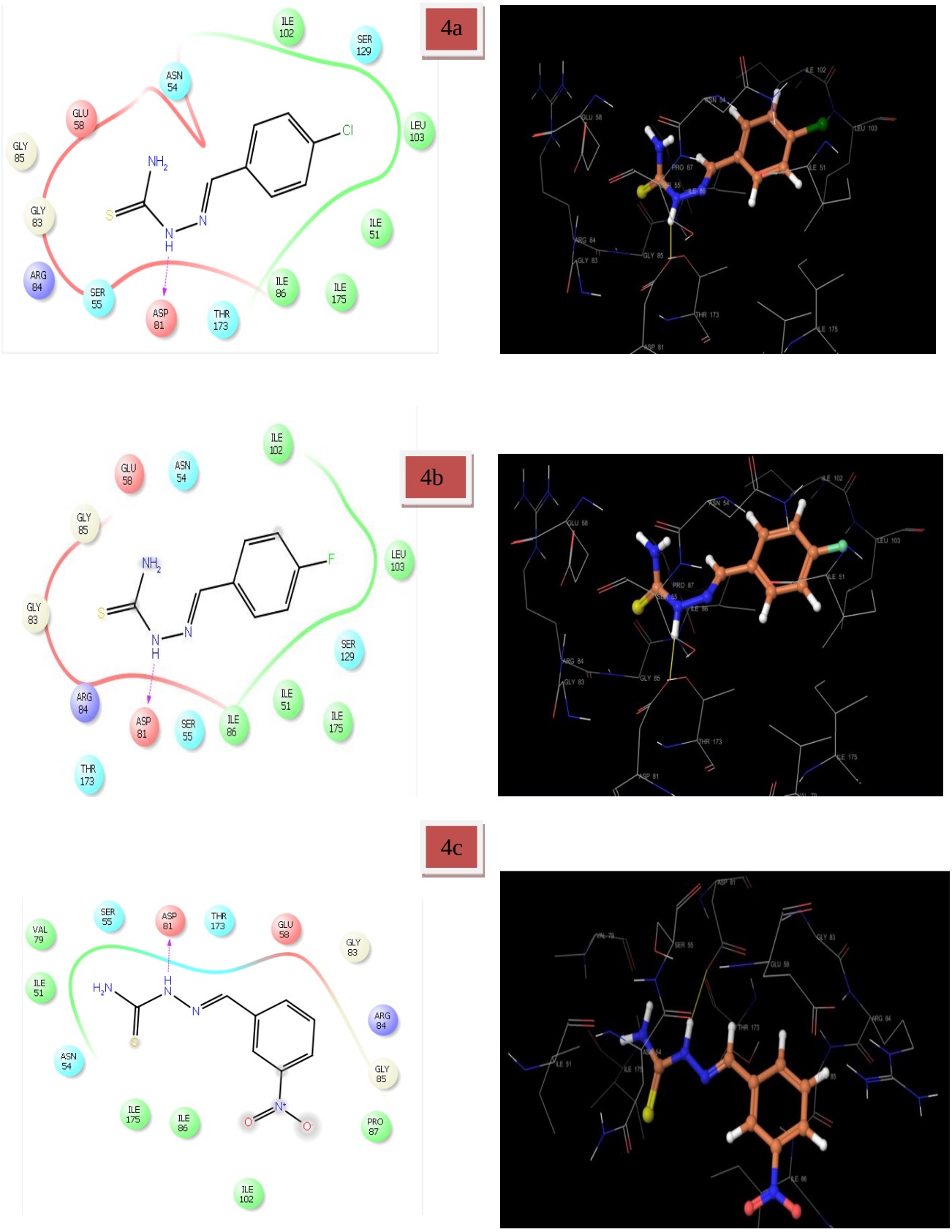


Figure 4a, 4b and 4c shows docking structures of thiosemicarbazone (L^1) , (L^2) and (L^3)

spectral analysis. On the basis of above data the thiosemicarbazone appear to behave as bidentate ligand coordinating though the azomethine nitrogen and the thione sulphur atom. The results of the above studies suggest that palladium (II) complexes probably posses square planar geometry respectively. The antimicrobial properties of ligand and its complexes were studied. The obtained results of the complexes show enhanced activity compared to the ligand, which indicate that the coordinated metal have an influence on the antimicrobial effects. Ligands were showed the noteworthy inhibition against DNA gyrase with (-5.63 to –5.42) high glide score, good ligand affinity to the receptor may possibly be expected. So, Ultra sound irradiation provides several advantages such as enhanced reaction rates, selectivity, shorter time, higher yield and eco-friendly technique.

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