RESEARCH ARTICLE

Synthesis, Spectral Characterization and Biological, DNA Binding, Molecular Docking and ADMET Studies of a New Benzyl (Z)-N'-((E)-3-Ethoxy-2-Hydroxybenzylidene) Carbamohydrazonothioate and Its Co (II), Ni (II), Cu (II) and Zn (II) Metal Complexes Derived From S-Benzyl Thiosemicabazide-Salicylaldehyde

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ABSTRACT

This study presents the synthesis, characterization, and biological evaluation of metal complexes derived from benzyl carbamohydrazonothioate. The ligand was synthesized through a condensation reaction and subsequently complexed with various metal ions, including Cu(II), Ni(II), Co(II), and Zn(II). The resulting metal complexes were characterized using a range of spectroscopic techniques, including FT-IR, UV-Vis, and NMR spectroscopy, confirming the successful coordination of the metal ions with the ligand. The structural elucidation was further supported by elemental analysis and molar conductance measurements. The biological activities of the synthesized complexes were evaluated for their antibacterial, antifungal, and antioxidant properties. The results indicated that the metal complexes exhibited enhanced biological activities compared to the free ligand, suggesting their potential as effective antimicrobial and antioxidant agents. This study highlights the significance of metal complexation in enhancing the biological efficacy of organic ligands.

Keywords: Spectroscopic techniques, Antioxidant, Thiosemicarbazide, Schiff base.

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INTRODUCTION

Thiosemicarbazide is a crucial one in the disciplines of organic part and inorganic parts of chemistry[1-5] as being applied in the industrial field, in biology and medicine. The coordination mode changes due to thioalkylation or arylation, which renders isothiosemicarbazides biologically active.[6-9] Although they are used in diverse applications, such as dyes, bio-analytical and others, thiosemicarbazides have not been thoroughly studied from the point of geometry, physicochemical, and properties.[10] In the chemical and pharmaceutical industries, thiosemicarbazide As a catalyst Schiff base is taken to make corrosion inhibitors and organic synthesis polymers.[11] The imine group's nitrogen participated in hydrogen formation bonds along with the cellular components' active site. This chemical structure is -CH=N-.[12-15] 'The nonlinear optical (NLO) characteristics of the thiosemicarbazide compounds have been observed, and they can be very important in optical communications.' Whereas, an organic Schiff base that coordinates with inorganic metal ions creates huge probabilities in advancements of NLO materials with excellent efficiency.[16] However, as the binding of metal to that of organic chromophore effectively increases the efficiency of quantum in singlet-triplet intersystem crossing of phosphorescence. To find excellent candidates for NLO materials, an increasing number of researchers are looking at how metals affect the characteristics of organic complexes.[17] The numerous examinations of the experimental results depict the metal complexes to bear biological activities that are remarkably more in comparison

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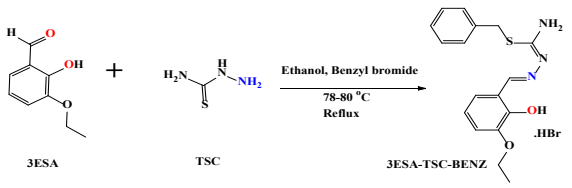


Scheme 1: Synthetic pathway of *S-benzyl thiosemicabazide-salicylaldehyde (3ESA-TSC-BENZ)*

with free organic – Schiff base compounds.[4,18,19] Due to their extensive use in medicinal inorganic chemistry, By inorganic chemists, Schiff bases are considered as raw compounds. Fields like bio-mimetic modeling, molecular-magnetic design, liquid crystal study, and the Schiff bases have grown in importance[20] In the improvement of pharmaceutically active compounds coordination chemistry and inorganic biochemistry, the azomethine group plays an important role.

A huge interest can be seen in synthesis and characterization along with the pharmacological importance of 'Co(II), Ni(II), Cu(II) and Zn(II) metal complexes' in this article. Schiff bases are used in this paper, and the Schiff bases are produced by the reaction between 3-Ethoxysalicylaldehyde, benzyl bromide and Thiosemicarbazide resulting in benzyl (Z)-N'-((E)-3-ethoxy-2-hydroxy benzylidene) carbamohydrazonohydrazonothioate) -(3ESA-TSC-BENZ). A docking simulation was used for in-depth exploration of the mechanism of interaction between compounds and their protein receptors.

MATERIALS AND METHODS

Materials and Measurements

All the required chemicals for the current experiment were of analytical grade and not purified. These were brought from companies like TCI and Sigma Aldrich. For the synthesized complexes, FTIR spectra (KBr pellets) were analyzed on a 'Bruker Alpha II FTIR Spectrometer in the range of (400–4000 cm^{-1})', whereas compounds were analyzed at ($2x10^{-5}$ M) concentration for determination of electronic spectrum, by measuring the 'UV-1800 Shimadzu Spectrophotometer in the range 200 to 800 nm'. Mass spectra of samples were recorded at room temperature using an ESI-MS Shimadzu instrument. With the aid of a Stuart melting point apparatus, capillaries determined all melting points. ^{1}H-NMR spectrum was recorded as a 'DMSO-d_6' solution on a 'Bruker 400 MHz Spectrometer' with the '^{1}H-NMR frequency of 400 MHz and ^{13}C-NMR frequency of 100 MHz' using (internal standard TMS). The JEOL Japan' JES FA 200 ESR Spectrometer' was used for analyzing the ESR spectrum with an X-band in normal temperature. Elemental analyses of CHNS samples were performed on the Elementar Unicube.

Synthesis of Schiff base S-benzyl thiosemicarbazide-salicylaldehyde (3ESA-TSC-BENZ)

A solution (0.166 g, 1 mmol) of 3-ethoxy salicylaldehyde (3ESA) ethanol (25 mL) at hot condition and added to (0.77 g, 1.1 mmol) benzyl bromide(BENZ) under stirring conditions for 15 minutes, and 0.48 g, 1 mmol of thiosemicarbazide (TSC) was added to this solution under stirring conditions for 15 minutes. The resulting mixtures refluxed for 6 hours at 90°C. Further, the reaction solution was made to chill in ambient temperature. The yellowish-colored compound obtained was filtered then ethanol (cold) washed and air dried (Scheme 1). Yield; 78%; color yellowish; m.p.: 242°C; Anal. Calcd. For ($C_{17}H_{19}N_3O_2S$) (%): C, 61.95; H, 5.78; N, 12.70, S, 9.68. Found. C, 61.98; H, 5.81; N, 12.76; S, 9.73. IR (KBr, cm^{-1}): νCH=N 1638, νC-OH 3456, νNH_2 3384, νC-S 778. UV-vis (DMSO, nm): 286, 330, 390. ESI-MS (m/z): 330 $[M+H]^+$ Figure 1. ^{1}H-NMR (DMSO, ppm): 8.76 (1H, CH=N), 9.40 (1H, OH), 9.77 (2H, NH_2), 4.66 (2H, $CH_{2\ benzyl}$), 4.06–4.11 (2H, $CH_{2\ alkyl}$), 1.34–1.38 (3H, $Ch_{3\ alkyl}$), 6.83–7.35 (5H, Aromatic $_{benzyl}$), 7.38–7.61 (3H, Aromatic $_{Sal}$), 13.03 (H, BrH), shown in Scheme 1.

Synthesis of metal complexes of $[M(3ESA\text{-}TSC\text{-}BENZ)_2]\ 2H_2O$

Using metal acetates equal moles (0.5 mmol) of 'Co(II), Ni(II), Cu(II), and Zn(II)' we attempted to form metal complexes with ligands in a 1:2 (M-L) molar ratio simply by dissolving them in hot ethanol periodically and mixing them with a (1.20 mmol) solution of (3ESA-TSC-BENZ). The mixture has to be refluxed for about 3 hours at 95°C. As the reaction progresses, TLC monitors it. Diethyl ether and water were used three times to filter solid products following reflux (Scheme 2). [Co*(3ESA-TSC-BENZ)]* $2H_2O$: Yield 75%; color: brown; m.p.: >275°C; Elem. (%). Ana. Calcd. for ($C_{34}H_{36}CoN_6O_4S_2$): C, 57.11; H, 5.71; N, 11.82; S, 8.92. Found: C, 57.06; H, 5.07; N, 11.74; S, 8.96. IR (KBr, cm^{-1}): νCH=N 1598, νC-O 1159, νNH_2 3389, νC-S 759. UV-vis (DMSO, nm): 281, 328, 425. ESI-MS *(m/z):* 715 $[M]^+$ (Figure 2).

[Ni(3ESA-TSC-BENZ)] $2H_2O$

Yield 75%; color: brown; m.p.: >275°C; Elem. (%). Ana. Calcd. for ($C_{34}H_{36}CoN_6O_4S_2$): C, 57.11; H, 5.71; N, 11.82; S, 8.92. Found: C, 57.06; H, 5.07; N, 11.74; S, 8.96. IR (KBr, cm^{-1}): νCH=N 1598, νC-O 1147, νNH_2 3404, νC-S 763. UV-vis (DMSO, nm): 310, 352, 412.

[Cu(3ESA-TSC-BENZ)] 2H2O

Yield 75%; color: brown; m.p.: >275°C; Elem. (%). Ana. Calcd. for ($C_{34}H_{36}CoN_6O_4S_2$): C, 57.11; H, 5.71; N, 11.82; S, 8.92. Found: C, 57.06; H, 5.07; N, 11.74; S, 8.96. IR (KBr, cm^{-1}): νCH=N 1600, νC-O 1155, νNH_2 3344, νC-S 786. UV-vis (DMSO, nm): 282, 350, 488.

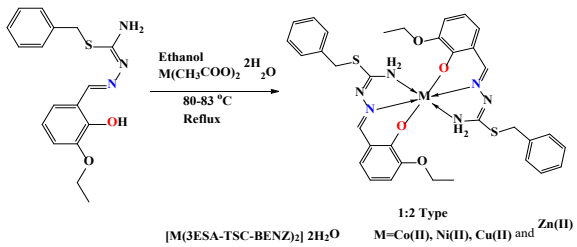
[Zn(3ESA-TSC-BENZ)] 2H2O

Yield 75%; color: brown; m.p.: >261**s**°C; Elem. (%). Ana. Calcd. for ($C_{34}H_{36}CoN_6O_4S_2$): C, 57.11; H, 5.71; N, 11.82; S, 8.92. Found: C, 57.06; H, 5.07; N, 11.74; S, 8.96. IR (KBr, cm^{-1}): νCH=N 1596, νC-O 1150, νNH_2 3386, νC-S 735. UV-vis (DMSO, nm): 318, 369, 465 as shown in Scheme 2.

Biological studies

- *Antimicrobial activity*

By using the "paper-disc diffusion method", the ligand 3ESA-TSC-BENZ and its metal complexes of 'Co(II), Ni(II), Cu(II), and Zn(II)' were selected for antibacterial activity over the bacterial species – *S. aureus, B. subtilis,* and *E. coli*,



Scheme 2: A synthetic pathway for metal complexes with M: L ratio of 1:2

as well as the fungal strains *S. cerevisiae* and *C. albicans*. The compounds used in the current study were dissolved in DMSO at varied concentrations (0.2 and 0.4 mg/mL). The chosen species were inoculated into the agar broth medium, & culture was grown for 24 hours. The 6 mm paper discs were placed on 'culture-impregnated agar plates' after being soaked into prepared test solutions. In an autoclave, the so prepared plates were kept under incubation for about 37°C for around 24 hours. Later, microorganisms were significantly affected; they were measured on a scale according to their zone of inhibition (ZoI),[21-24] while the experimental results were compared to that of amphotericin - B and streptomycin as standard drugs.

Quantum mechanical and docking study

A semi-empirical approach to calculate the structural parameters of the 3ESA-TSC-BENZ ligand with metal ions is implemented using the semi-empirical MINDOM3 method in ChemBioDraw 3D Ultra (version 12.0). 'Quantum mechanical parameters such as HOMO and LUMO energies, their energy gaps (ΔE), electronegativity (χ), and chemical hardness (η)' were also calculated.

Through molecular analysis of 2QLC proteins downloaded from the online protein data bank, in silico molecular docking has been completed. Using AutodockVina Ver. 4.2,[25] the binding modes of the compound structures were optimised for protein receptors, and ChemBioDrawPro (Ver. 12) was used to depict the compound structures. The Discovery Studio (Ver. 4) was used to visualise the results.[26]

RESULTS AND DISCUSSION

The synthetic process for the ligand 3ESA-TSC-BENZ and its complexes' Co(II), Ni(II), Cu(II), and Zn(II)' are shown in Schemes-I & II. '^{1}H-NMR, FTIR, LC-MS, and UV-vis spectroscopy are used to characterize the compounds. The 3ESA-TSC-BENZ is typically dissolve in all types of organic solvents while their complex dissolve in DMF & DMSO alone. The so produced compounds are meant to ne non-hygroscopic in nature stable at normal temperature. While the elemental testing of these compounds is in good association with calculated values reported in the experimental section and is in accordance with the stoichiometric ratios of 1:2 for metal-ligands of type ML_2. These were thoroughly characterized using spectroscopic, mass, and NMR techniques.

^{1}H-NMR spectrum: the ligand structure, 3ESA-TSC-BENZ, was established using a ^{1}H-NMR spectrum acquired with TMS reference dissolved in DMSO. A singlet peak at δ 9.40 ppm in the ^{1}H-NMR spectrum of 3ESA-TSC-BENZ was identified as phenol (OH) and was observed. A proton of azomethine that singlet appeared at δ 8.76 is responsible for the (CH=N) singlet peak. The broad singlet peak (HBr) observed at δ 13.03, the peak attributed to the alkyl benzyl ring (S-CH2-C6H5) at δ 4.66 ppm, and the benzene moiety observed from δ 6.83 to 7.35 ppm were all attributed to the aromatic moiety protons peaks. Additionally, salicylic aromatic protons peak in the δ 7.38 to 7.61 ppm range.

The molecular ion peak for ($C_{17}H_{19}N_3O_2S$), with a molecular weight of 299, was observed in the mass spectrum of 3ESA-TSC-BENZ at m/z = 330 $[M+1]^+$. The mass spectra of $[Co(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$ revealed the molecular io peak for ($C_{34}H_{36}CoN_6O_4S_2$) at m/z = 715 $[M]^+$, that is in line with the original molecular weight of 715 and supports the suggested complex depicted in Scheme 2.

FTIR Spectra: The 3ESA-TSC-BENZ synthesized ligands and its complexes' FTIR spectra were captured in 4000 to 400 cm^{-1}. In order to identify any potential changes that might take place during their formation, they were compared. The azomethine group was observed to have participated in complexation through the nitrogen atom to metal ion as evidenced by ligand IR spectrum exhibiting a strong band at 1638 cm^{-1} due to CH=N corresponding to the azo-methine group', which was moved onto lower frequency i.e., 1600-596 cm^{-1}.[27,28] The shift of the 'C-O band' at 1147 to 1159 cm^{-1} in metal complexes towards a higher or lower frequency region[29,30] is evidence that the hydroxyl group is coordinated. Additional bands seen between 738 and 786 cm^{-1} are attributed to C-S. Furthermore, all complexes revealed 2 new bands at around the 536 to 566 cm^{-1} and 451 to 482 cm^{-1} range, which proves that 'N' atom of azo-methine and 'O' atom of phenolics, also known as M-O and M-N, had taken part in coordination. Table 1 lists some of the significant compounds' FTIR absorption frequencies.

Table 1: FTIR spectral data of the metal complexes of 3ESA-TSC-BENZ in (cm^{-1})

Compound	ν_{OH}	$\nu_{HC=N}$	ν_{NH}	ν_{C-O}	ν_{C-S}	ν_{M-O}	ν_{M-N}
3ESA-TSC-BENZ	3456	1638	3384	-(31)	778	-	-
$[Co(3ESA\text{-}TSC\text{-}BENZ)_2]$ $2H_2O$	-	1598	3389	1159	759	564	463
$[Ni(3ESA\text{-}TSC\text{-}BENZ)_2]$ $2H_2O$	-	1598	3404	1147	763	566	470
$[Cu(3ESA\text{-}TSC\text{-}BENZ)_2]$ $2H_2O$	-	1600	3344	1155	786	539	452
$[Zn(3ESA\text{-}TSC\text{-}BENZ)_2]$ $2H_2O$	-	1596	3386	1150	735	534	471

Table 2: Electronic spectral data of 3ESA-TSC-BENZ and its complexes

Compounds	*Bands λ_{max} (nm)*	*Assignments*
3ESA-TSC-BENZ	282, 311 and 365	π-π*, π-π* and n-π*
[Co(3ESA-TSC-BENZ)$_2$] 2H$_2$O	281, 328 and 425	π-π*, n-π* and CT
[Ni(3ESA-TSC-BENZ)$_2$] 2H$_2$O	310, 352 and 412	π-π*, n-π* and CT
[Cu(3ESA-TSC-BENZ)$_2$] 2H$_2$O	282, 350 and 488	π-π*, n-π* and CT
[Zn(3ESA-TSC-BENZ)$_2$] 2H$_2$O	318, 369 and 465	π-π*, n-π* and CT

Electronic spectra

The ligand 3ESA-TSC-BENZ, UV-Vis absorption spectra and those of its metal complexes' Co(II), Ni(II), Cu(II), and Zn(II)', were captured in DMSO at room temperature. The results are shown in Table 2. The ligand's and its metal complexes' absorption was measured between 280 and 600 nm. The 3ESA-TSC-BENZ revealed 3 bands at 286, 311 & 365 nm, which are attributed to the 'n-π* transitions of the -C=N- and OH groups' and the 'π - π* transition of aromatic rings', respectively.[16,31] These bands were moved to higher wavelength ranges of approximately 280 to 330 and 330 to 470 after complexation at the metal center, which denoted metal complex formation. When comparing to the free ligand, 3ESA-TSC-BENZ, a hipsochromic shift was seen in each complex. The 'C=N and C=O' groups in metal complexes cause the absorption band transitions that have been associated with L-M charger transfer transitions.

EPR spectral studies of [Cu(3ESA-TSC-BENZ)$_2$] 2H$_2$O

The observed g values for the spectrum were 2.27 > 2.15 > 2.0023, following the trend' $g_{\|} > g_{\perp} > 2.0023$'. Depicts that in 'dx2-y2 orbital' unpaired electrons are present, which is in accordance with the square planer geometry of the Cu(II) center and conforms to the 2B1g ground state.[16] A covalent nature of the metal-ligand bond is indicated by the $g_{\|}$ value, which is found to be 2.[3.30] The calculated expression below has to calculate geometric parameter G that portrays in between Cu (II) centers, the magnitude of interaction exchange is seen.

$$G = \frac{g_{\|} - 2.0023}{g_{\perp} - 2.0023}$$

When G is greater than 4, exchange interactions are minimal, while G < 4 assuming that interaction exchange among metal centers can be possible.[32] The complex [Cu(3ESA-TSC-BENZ)$_2$] 2H$_2$O G value is found to be 1.81, indicating that there are significant metal-metal exchange interactions between the Cu(II) metal ion.

Quantum chemical calculations

For the ligand 3ESA-TSC-BENZ and its complexes, [M(3ESA-TSC-BENZ)2]2H2O, 'the highest occupied (HOMO) and lowest unoccupied (LUMO)' orbitals, respectively, explains susceptibility of molecules by attacking nucleophiles/electrophiles.[33] Table 3 contains a list of the quantum chemical properties of 3ESA-TSC-BENZ and its [M(3ESA-TSC-BENZ)$_2$]2H$_2$O complexes, including 'HOMO, LUMO, energy gap (ΔE), electronegativity (χ), and chemical hardness (η)'. According to calculations, the HOMO-LUMO energy difference (ΔE) values for 3ESA-TSC-BENZ and its complexes, [M(3ESA-TSC-BENZ)$_2$]2H$_2$O, are 2.242, 2.737, 0.842, 1.795, and 3.005 eV, respectively.

DNA binding studies

- *UV-visible absorption titration studies*

To investigate CT-DNA stock solution pure CT–DNA along with complex interaction. CT-DNA purity and stock preparation were briefly described in previous literature.[34] With the aid of UV-vis studies Kb Constant can be calculated and the DNA binding graph. The study of DNA binding with newly synthesized metal complexes (a-d) was conducted with the most prevalent technique, electronic absorption spectroscopy. In all the comlpexes in Fig 4, the DNA linkages in them permits direct interactions with a central metal ion. The studies revealed a substantial bathochromic shift with hypochromism, indicating that the complexes used have a strong binding interaction with the DNA intercalating mode. The intercalative binding mode includes interaction between the 'complexes' π*-orbital and the π-orbital of DNA base pairs', resulting in a red shift in the electronic spectrum and a decrease in the π-π* orbital energy levels. In the present study, the complexes exhibit distinct isobestic points throughout the entire [complex/DNA] spectrum investigated. The presence of isosbestic points at various binding ratios suggests that complexes bound to DNA are homogeneous, i.e., the system is composed of CT-DNA-free and CT-DNA-bound complexes. One isosbestic point, approximately at 300 nm absorption band is formed by binding adducts generated in the reaction of CT-DNA with all complexes concentrated. As to estimate

Table 3: Computed E_{HOMO}, $E_{LUMO,}$ energy gap (ΔE), electronegativity (χ) and chemical hardness (η) of 3ESA-TSC-BENZ and its complexes (eV unit)

Compound	*E_{HOMO}*	*E_{LUMO}*	*ΔE*	*χ*	η
3ESA-TSC-BENZ	-3.163	-0.921	2.242	2.042	1.121
[Co(3ESA-TSC-BENZ)$_2$]2H$_2$O	-4.193	-1.4559	2.737	2.891	2.826
[Ni(3ESA-TSC-BENZ)$_2$]2H$_2$O	-6.381	-5.539	0.842	5.960	0.421
[Cu(3ESA-TSC-BENZ)$_2$]2H$_2$O	-3.162	-1.367	1.795	2.264	0.897
[Zn(3ESA-TSC-BENZ)$_2$]2H$_2$O	-4.095	-1.090	3.005	2.590	1.502

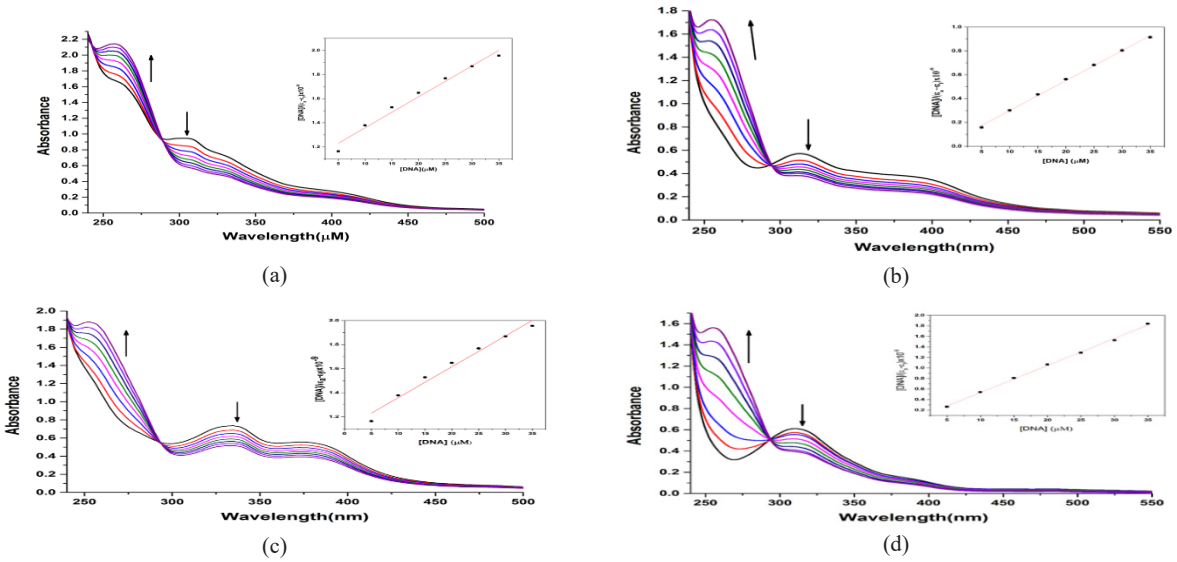


Figure 1: Electronic spectra of complexes (a-d) absence and presence of concentration increment of CT-DNA= 5-80 μM and [complex] =10 μM in Tris-HCl buffer. The arrow depicts addition of CT-DNA concentration alters the absorbance. A linear plot calculation for calculation of $K_{b,}$ for [DNA]/(e_a-e_f) vs [DNA] binding with complex

intrinsic binding of metal complexes (Kb), the following 'Wolfe-Shimer equation (1)' was used for:

$$= [DNA]/(e_a\text{-}e_{f)\,=\,[DNA]}/\,(e_b\text{-}e_f) + 1/K_b(e_b\text{-}e_f) \_\_\_\_\_ \quad (1)$$

Where, '[DNA] is the concentration of CT-DNA base pairs, and e_a, e_f, and e_b are the extinction coefficients of test compound at a given concentration (Aobs/[complex]), in its free and fully DNA-bound forms, respectively. Kb is the binding constant, gets from the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA]'.

'The observed K_b values for the explored complexes are 2.10 x 10^4 M^{-1} Co(II) (a); 2.33 x 10^4 M^{-1} Ni(II) (b); 5.27 x 10^4 M^{-1} Cu(II) (c); 2.37 x 10^4 M^{-1} Zn(II) (d)'. The binding constant (K_b) order calculated from equation (1) complexes affinities; 'Cu(II) > Zn(II) > Ni(II) > Co(II)'.

Biological activity studies

- *Antibacterial and fungal activity*

The screened ligand, 3ESA-TSC-BENZ, and its complexes, [M(3ESA-TSC-BENZ)$_2$]2H_2O biological activity was performed 'using two gram-positive and two gram-negative bacterial strains, viz., *S. aureus, B. subtilis,* and *P. aeruginosa, E. coli;* and two fungal strains, viz., *S. cerevisiae* and *C. albican,* respectively. Data regarding the results of the 'zone of inhibition (ZoI)' is depicted in Table 4. The streptomycin standard is chosen in order to compare the resultant values of ligands & complexes. Figure 2 shows that the metal complexes exhibited more activity than that of free ligand 3ESA-TSC-BENZ, while the activity was lower than the standard drug. The larger potential of bio-cidal for complexes can be explained depending on 'Overtone's concept'[35] and 'Tweed's chelation theory'.[36] All the tested compounds possess different antimicrobial activities for both gram-positive & negative bacterial species also fungal species.

On the basis of the zones of inhibition, the compounds that have been tested against the test organisms are listed in the resulting order - "Ni(II) > Zn(II) > Co(II) > Cu(II) > Ligand". The inhibition potential of compounds, viz., [Ni(3ESA-TSC-BENZ)$_2$]2H_2O is more active against S. aureus bearing 18 mm of ZoI while compounds of [Zn(3ESA-TSC-BENZ)$_2$]2H_2O, [Co(3ESA-TSC-BENZ)$_2$]2H_2O and [Cu(3ESA-TSC-BENZ)$_2$]2H_2O is efficient over B. *subtilis* species having ZoI ranging from 7–15 mm. Compound [Zn(3ESA-TSC-BENZ)$_2$]2H_2O has the most effective 17-mm zone of inhibition against gram-negative bacteria compared to amphotericin-B as a standard drug. [Cu(3ESA-TSC-BENZ)$_2$]2H_2O shows effective antifungal inhibition values ranging between 3-8% against C. albicans and S. cerevisiae as observed in Fig 2.

- *Molecular docking studies*

'Molecular docking is an established technique for predicting the interactions between ligands and proteins'. In addition, it can be used to find the ligand with the lowest overall energy, based on its specific site of binding, affinity character,& optimal orientation.[37] According to the literature,[37-40] 'hydrogen bonds between 2.4 and 3.1 Å are strong interactions, and those between 3.1 and 3.55 Å are weak interactions'. The 2D and 3D plots and docking energies of 3ESA-TSC-BENZ & their metal complexes with the RadC receptor are depicted in Table 5 and Figure 3 Compounds can be compared based on their affinity for the target receptor (PDB ID: 2QLC) and their interactions. Tests were conducted on all compounds with 2QLC amino acid residues to determine binding interaction, binding energy, and bond length.

The 3ESA-TSC-BENZ with target protein 2QLC binding energy (-5.0 kcal/mol) with various interaction types involved, such as two H bondings with bond distances of 2.57 and 2.66 Å, other amino acid residue interactions are LYS 97, LYS 97, HIS

Table 4: *In-vitro* antibacterial and antifungal activity data of 3ESA-TSC-BENZ and its $[M(3ESA\text{-}TSC\text{-}BENZ)_2]\ 2H_2O$

Zone of Inhibition (ZoI) of bacterial growth (mm)						
	Gram-positive bacteria		*Gram-negative bacteria*		*Fungal strains*	
Compounds	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*S. cerevisiae*	*C.albicans*
3ESA-TSC-BENZ	9	7	5	8	3	5
$[Co(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$	14	10	8	13	4	5
$[Ni(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$	18	15	13	17	3	4
$[Cu(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$	9	7	9	6	5	8
$[Zn(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$	17	18	14	15	0	2
Streptomycin	20	22	19	18	-	-
Amphotericin-B	-	-	-	-	9.0	12.2

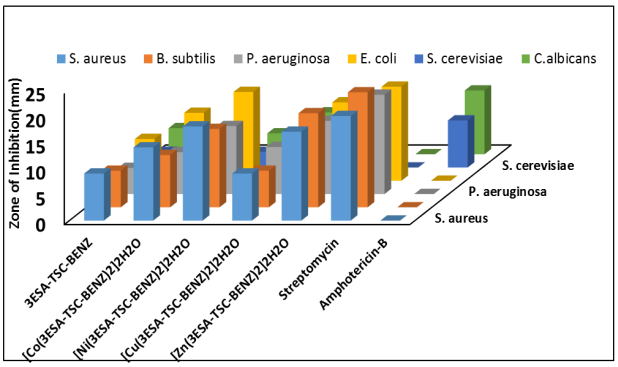


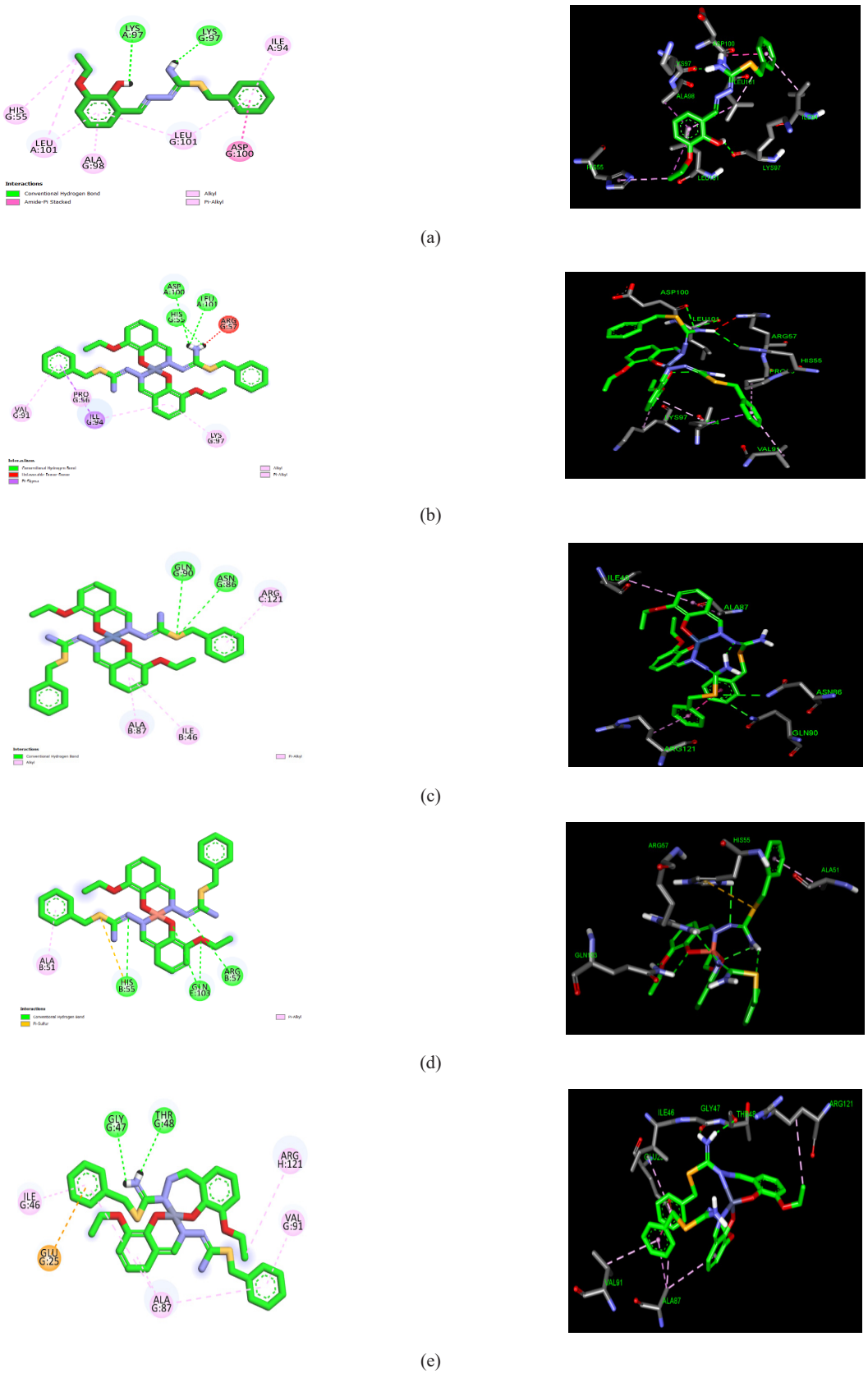
Figure 2: Statistical representation zone of inhibition of 3ESA-TSC-BENZ and its $[M(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$ compared with standard drugs

55, LEU 101, ALA 98, LEU 101, ASP 101, and ILE 94. Co(II) metal ions are held together by three hydrogen bonds with lengths of 2.74, 2.68, and 2.71 kcal/mol. Amino acid residues ASP 100, LEU 101, LYS 97, ILE 94, VAL 91, ARG 57, and PRO 56 are also involved. Ni(II) metal ions are surrounded by two hydrogen bonds operating at bond distances of 3.77 kcal/mol, and ASN 86, GLN 90, ARG 121, ALA 87, and ILE 46 interact with the hydrogen bonds. Also, the Cu(II) complex interacts with four hydrogen bonds at distances of 2.58, 2.60, 2.79, and 2.98, as well as the active site amino acids GLN 103, ARG 57, HIS 55, and ALA 51, along with -5.3 Kcal / mol. Binding energy. While, the Zn(II) complex's observed binding energy (-6.6 kcal/mol) that includes 2 H bonds formation with bond distances of 2.14 and 2.34. The active site binds differently with amino acid residues GLY 47, THR 48, ILE 46, GLU 25, VAL 91, ALA 87, and ARG 12, respectively. In general, the docking energy ranking order is Ni(II) = -7.2 > Zn(II) = -6.6 > Co(II) = -6.3 > Cu(II) = -5.7 > ligand (kcal/mol). As a result, in vitro antimicrobial studies validate *in-silico* docking analyses.

Drug-likeness property: **'**Lipinski's rule of five' assesses the oral activity is tested by the active chemicals' drug likeliness. Orally non-bioavailable compounds bearing 2 or more than 2 volatile properties, according to the 'Lipinski rule of five' c[37,41,42] 'The log P, TPSA, MW, HBA, and HBD values' reveal excellent membrane permeability and hydrophobicity for therapeutic molecules. 'The acceptable molecules of NROTB and MR reflect effective intestinal absorption and oral bioavailability of drugs'.

Table 5: Docking energy and binding interactions of docked ligands into the active pocket amino acids of RadC protein receptor (PDB ID: 2QLC)

Ligands	*Interacting amino acid residues*	*Interacting atoms*	*H-bond distance (Å)*	*No. of H-bonds*	*Docking energy kcal/mol*
3ESA-TSC-BENZ	LYS 97, LYS 97, HIS 55, LEU 101, ALA 98, LEU 101, ASP 101, ILE 94	LYS G: 97…HN LYS A: 97…HO	2.57 2.66	2	-5.0
$[Co(3ESA\text{-}TSC\text{-}BENZ)_2]\ 2H_2O$	ASP 100, LEU 101, LYS 97, ILE 94, VAL 91, ARG 57, PRO 56	ASP 100 CO…HN LEU 101 CO…HN HIS N…HN	2.74 2.68 2.71	3	-6.3
$[Ni(3ESA\text{-}TSC\text{-}BENZ)_2]\ 2H_2O$	ASN 86, GLN 90, ARG 121, ALA 87, ILE 46	ASN G: 86 CN…SC GLN G: 90… CN…SC	3.77 3.5	2	-7.2
$[Cu(3ESA\text{-}TSC\text{-}BENZ)_2]\ 2H_2O$	GLN 103, ARG 57, HIS 55, ALA 51	GLN E: 103 NH…OC, GLN 103 NH…OC, ARG B: 57 NH…NC, HIS B: 55 NH…NC	2.58 2.60 2.79 2.98	4	-5.7
$[Zn(3ESA\text{-}TSC\text{-}BENZ)_2]\ 2H_2O$	GLY 47, THR 48, ILE 46, GLU 25, VAL 91, ALA 87, ARG 121	GLY G: 47 CO…HN THR G: 48 CO…HN	2.14 2.34	2	-6.6



(a)

(b)

(c)

(d)

(e)

Figure 3: 2D and 3D plots of molecular interactions of - (a) ligand, (b) Co(II) complex, (c) Ni(II) complex, (d) Cu(II) complex, (e) Zn(II) complex with the RadC protein receptor (PDBID: 2QLC)

CONCLUSION

In this article, a novel ligand, 3ESA-TSC-BENZ and its complexes, $[M(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$ were made and studied using different spectroscopic and analytical methods. The mass spectral, FTIR, and elemental data support 'the metal-ligand stoichiometric ratio of 1:2 of ML_2 type'. In accordance with all spectral findings, an octahedral geometry was proposed over the central metal. The ability of Complexes to bind to CT-DNA was confirmed by the UV spectroscopic studies. Compound (C) exhibited a prominent binding constant value of Kb = 5.27 $x10^4$ M^{-1}. Further, for all compounds using quantum mechanical approaches, the 'HOMO and LUMO energies' were analyzed. On the target bacterial and the fungal species the so ligands and their complexes were examined. The complexes $[Zn(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$ and $[Ni(3ESA\text{-}TSC\text{-}BENZ)_2]2H_2O$ exhibited powerful effects over selected fungal and bacterial species. Further, the results were studied for the molecular docking method. The study revealed that Ni(II), Zn(II), & Co(II) complexes had more efficient docking energies with the target protein *2QLC* than the corresponding ligand. Molecular docking simulations further support the *in-vitro* evidence.

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