

Research Article

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Synthesis and physicochemical studies of a series of mixed-ligand transition metal complexes and their molecular docking investigations against Coronavirus main protease<https://doi.org/10.1515/chem-2020-0190>

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Abstract: A novel series of mixed-ligand complexes of the type, $[M(L_1)(L_2)Cl] \cdot 2H_2O$ [L_1 = 2-(α -methyl salicylidene hydrazine) benzimidazole (primary ligand), L_2 = 2,2'-bipyridine (bipy; secondary ligand), M = Co(II), Ni(II), Cu(II) and Zn(II)], were based on the physicoanalytical studies. The spectroscopic findings revealed tridentate nature of the Schiff base ligand (L_1) and its coordination to the metal ions via azomethine nitrogen, ring nitrogen and the deprotonated phenolic oxygen atoms. Furthermore, the synthesized compounds were evaluated for antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* microorganisms. In addition, molecular docking studies were carried out against Middle East respiratory syndrome coronavirus (PDB ID: 4ZS6) and severe acute respiratory syndrome coronavirus 2 main protease (PDB ID: 6W63).

Keywords: Schiff base, ternary complexes, spectral, antimicrobial, molecular docking studies

1 Introduction

Benzimidazole, an important precursor of the heterocyclic compounds, exhibits a wide range of biological

applications, i.e., antiviral, antifungal, antipyretic, antidepressant and inhibitory activities in various cancers [1–5]. The broad biological activity of these compounds is supposed to be due to the presence of sp^2 hybridized nitrogen donor atoms [6]. The ligational behavior of benzimidazole and its derivatives has been explored in coordination chemistry to form stable complexes through various modes of coordination [7]. The benzimidazole-based metal complexes exhibit broad spectrum of pharmaceutical activities, such as zinc complexes of benzimidazole find usage in anticancer activity, and reveal remarkable antimicrobial activity [8]. In addition, several benzimidazole complexes have been reported as anticancer agents [9,10]. It is also reported that the transition metal ions form stable complexes and have displayed several properties [11,12]. Over the years, bipy and its derivatives have received immense importance as binding blocks and ligands in the construction of various homo- and heteroleptic metal complexes with broad spectrum of applications in the area of both material and biological science [13–17].

Mixed-ligand complexes, which have two different ligands, find significant consideration in coordination chemistry because of their structural variation and diverse applications [18,19]. Besides, mixed-ligand complexes also exhibit various biological applications [20]. Recent outbreak due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has destroyed the economy and various socioeconomic sectors worldwide [21–23]. So far, not a single approved drug is available to treat the caused infection. Therefore, scientists are trying to find a drug to combat the COVID-19 pandemic. Several reports suggest that the progression of this pandemic can be controlled by targeting the main protease (M^{pro}) to develop the potential inhibitor. Molecular docking, a computational strategy to predict the binding site to assist drug repositioning for several diseases, plays a significant role in the pharmaceutical industry to bring new drugs to the market [24]. The HIV-1 protease inhibitors as the repurposed drugs for SARS-CoV-2 M^{pro} are discussed in literature [24–26]. Therefore, considering the versatile nature of mixed-ligand complexes, we are reporting four

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new mixed-ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) ions with benzimidazole-based Schiff base ligand (L_1) as the primary ligand, and bipy as the co-ligand, (L_2) in 1:1:1 molar ratio, and investigated them by various physicochemical studies. The synthesized metal complexes exhibited moderate antibacterial activity when screened against *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*. In addition, we analyzed the molecular docking of the complexes of protein obtained from Middle East respiratory syndrome coronavirus (MERS-CoV; PDB ID: 4ZS6) and SARS-CoV-2 M^{pro} (PDB ID: 6W63).

2 Experimental

2.1 Preparation of the benzimidazole derived Schiff base ligand, L_1

The precursor, 2-hydrazinobenzimidazole, was prepared following the previously reported protocol [27]. The primary ligand, L_1 , was prepared by the condensation reaction of 2-hydrazinobenzimidazole with *o*-hydroxyacetophenone in equimolar ratio in ethanol [27].

Yield 80%, color yellow; anal. calc. (%): C, 63.83; H, 4.96; N, 19.86, found (%): C, 63.76; H, 4.92; N, 19.82.

2.2 Preparation of mixed-ligand complexes

An ethanolic solution of the Schiff base ligand, L_1 (0.01 mol and 20 mL), metal(II) chloride (0.01 mol and 20 mL) and bipy, and L_2 (0.01 mol and 20 mL) were mixed in 1:1:1 molar ratio and refluxed for 2.5 h at pH = 7–8 by

adding catalytic amount of solid NaOH. A colored precipitate was obtained, which was separated by filtration. The precipitate was washed with methanol and dried in vacuo. The schematic representation of the synthesis of mixed-ligand complexes is given in Scheme 1.

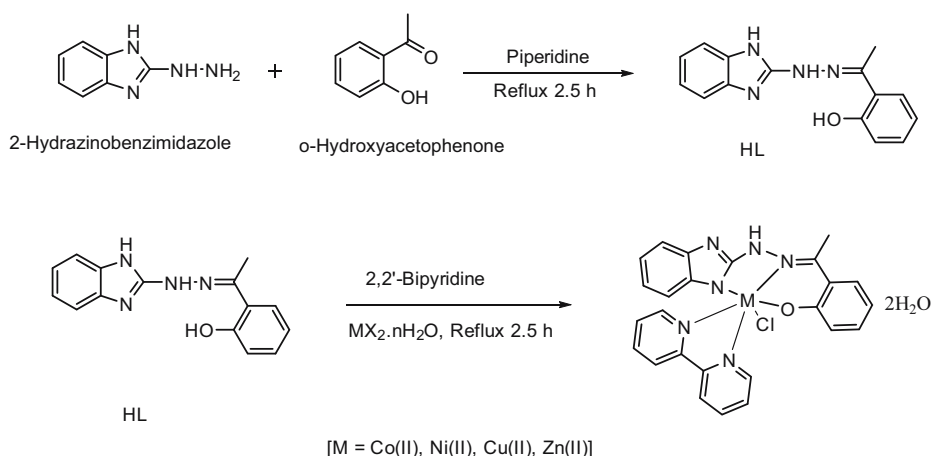
2.3 *In vitro* antibacterial activity

The *in vitro* antimicrobial activity of the studied mixed-ligand complexes was reported by Agar Well diffusion method [27] at $100\ \mu\text{g mL}^{-1}$ concentrations against *B. subtilis*, *E. coli* and *S. typhi* with ciprofloxacin as the standard antibacterial drug.

2.4 Molecular docking study

In order to evaluate the biological activity, molecular docking was analyzed on CLC Drug Discovery Workbench Software to obtain accurate predictions about the structure and interactions with a protein/enzyme receptor [28]. Some protein/enzyme receptors were imported from protein data bank (<http://www.rcsb.org/PDB>): MERS-CoV (PDB ID: 4ZS6 [29]) and SARS-CoV-2 M^{pro} (PDB ID: 6W63 [30]).

In the docking simulation, the compounds (Figure 1) were placed into a predictable binding site on the surface of a protein target. The CLC Drug Discovery Workbench utilized MMFF94 (Merck Molecular Force Field [MMFF]) force field to generate 3D structure on import. Rotation around bond generates several conformations. Thus, the ligand optimizer was realized by geometry minimization using MMFF94 force field [31], conforming the binding



Scheme 1: Schematic representation of preparation of mixed-ligand complexes.

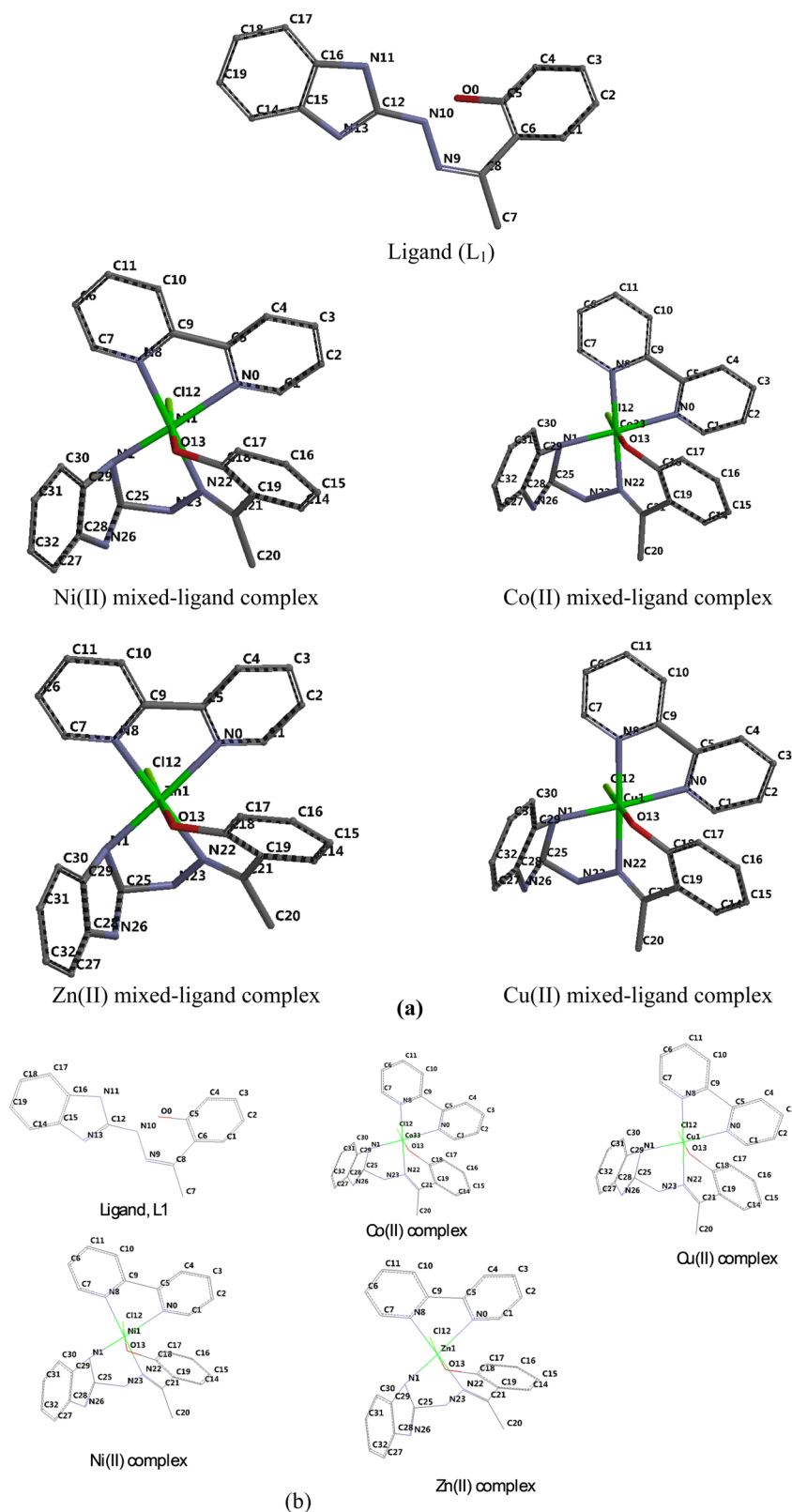


Figure 1: (a) Tube representation and (b) wire representation of the optimized molecular structure of ligand and mixed-ligand metal complexes (numbering of the atoms was done according to the software).

pocket geometry. The protein–ligand interaction was scored, and the best score-binding mode was returned for individual ligand and collected with the score. The ligand-binding mode search is effectuated inside the binding site (green sphere with a radius large enough to comprise the ligands docked to the receptor protein). After the import of the protein receptor from the PDB bank, the next step involved the setting up of the binding site and the binding pockets; binding pockets are necessary to guide the docking simulation. After the setup of the binding site and the binding pocket, the co-crystallized-natural ligand was extracted and redocked in the active binding site of the protein receptor to validate the method and the docking parameters obtained from the molecular docking studies.

Ethics approval: The conducted research is not related to either human or animal use.

3 Results and discussion

All the synthesized mixed-ligand complexes are stable at room temperature, non-hygroscopic and soluble in dimethyl sulfoxide and *N,N*-dimethylformamide. The physicoanalytical data are shown in Table 1.

3.1 Fourier-transform-infrared (FT-IR) spectra

The FT-IR spectra revealed the coordination of the metal ion through the deprotonated phenolic oxygen atom by the disappearance of the band at $\sim 3,300\text{ cm}^{-1}$ due to the phenolic $-\text{OH}$ stretching vibration [32,33]. This was further confirmed by the hypsochromic shift of phenolic $\nu_{(\text{C}-\text{O})}$ band at $\sim 1,280\text{ cm}^{-1}$ in the free ligand and L_1 to $\sim 1,500\text{ cm}^{-1}$ in the spectra of the mixed-ligand complexes [33,34]. However, the position of the band due to $\nu_{\text{N}-\text{H}}$

(exocyclic) remains practically unaltered in the spectra, suggesting its noninvolvement in coordination [33] (Figure S1). Furthermore, noncoordination of ring nitrogen atom $\nu_{(-\text{C}=\text{N})}$ of benzimidazole moiety in the spectra of the complexes is also ascertained by finding no change in the positions of the characteristic IR bands at $\sim 1,540$ and $\sim 1,320\text{ cm}^{-1}$ due to $\nu_{(\text{C}=\text{N})}$ (cyclic) and $\nu_{(\text{C}-\text{N})}$ (cyclic) modes of vibration, respectively [33]. On the other hand, the position due to benzimidazole $\nu_{(\text{N}-\text{H})}$ at $\sim 3,150\text{ cm}^{-1}$ reduced to lower frequency of ca. $20\text{--}25\text{ cm}^{-1}$, suggesting participation of $-\text{NH}$ group of benzimidazole in coordination [33,35]. However, the bands due to $\nu_{(\text{C}=\text{N})}$ and $\nu_{(\text{N}-\text{N})}$ vibrations also show negative shift of ca. $10\text{--}20\text{ cm}^{-1}$ in the spectra of the complexes, suggesting their role in coordination [33,36]. Moreover, the vibration at $\sim 3,450\text{ cm}^{-1}$ is assigned to $\nu_{(\text{O}-\text{H})}$ of the lattice water [33,34]. In addition, a sharp band at $655\text{--}680\text{ cm}^{-1}$ due to $\nu_{(\text{C}=\text{N})}$ of pyridine is observed in the spectra of the complexes [33,37].

3.2 Electronic spectra

The electronic spectral data and the magnetic moment values of the mixed-ligand complexes are listed in Table S1. The electronic spectrum of Co(II) complex showed two main bands at $\sim 10,745\text{ cm}^{-1}$ (broad) and $\sim 22,136\text{ cm}^{-1}$ (strong) due to ${}^4\text{T}_{1\text{g}}(\text{F}) \rightarrow {}^4\text{T}_{2\text{g}}(\text{F})$ (ν_1) and ${}^4\text{T}_{1\text{g}}(\text{F}) \rightarrow {}^4\text{T}_{1\text{g}}(\text{P})$ (ν_3) transitions, respectively [38,39]. However, ${}^4\text{T}_{1\text{g}}(\text{F}) \rightarrow {}^4\text{A}_{2\text{g}}(\text{F})$ (ν_2) transition was not observed because of its association with a large amount of energy cause by two electron transitions ($\text{t}_{2\text{g}}^5 \text{e}_\text{g}^2 \rightarrow \text{t}_{2\text{g}}^3 \text{e}_\text{g}^4$) [39,40]. Furthermore, the observed magnetic moment, μ_{eff} (4.72 BM), supported an octahedral geometry around Co(II) ion [39,41]. The studied mixed-ligand complex of Ni(II) complex showed split bands at $\sim 8,850$ and $\sim 10,420\text{ cm}^{-1}$ assigned to ${}^3\text{B}_{1\text{g}} \rightarrow {}^3\text{E}_\text{g}$ and ${}^3\text{B}_{1\text{g}} \rightarrow {}^3\text{B}_{2\text{g}}$ transitions, respectively. In addition, two characteristic bands at $\sim 15,350$ and $\sim 24,575\text{ cm}^{-1}$ were assigned to ${}^3\text{A}_{2\text{g}}(\text{F}) \rightarrow {}^3\text{T}_{1\text{g}}(\text{F})$ (ν_2) and ${}^3\text{A}_{2\text{g}}(\text{F}) \rightarrow {}^3\text{T}_{1\text{g}}(\text{P})$ (ν_3) transitions, respectively, and suggested an octahedral

Table 1: Physicoanalytical data of the compounds

Sl. no.	Compounds	Molecular mass	Yield (%)	$\Lambda_{\text{m}}^{\text{a}}$	C found (calcd)	H found (calcd)	N found (calcd)	M found (calcd)
1	$[\text{CoL}_1\text{L}_2\text{Cl}]\cdot 2\text{H}_2\text{O}$	533.5	66	14.5	53.92 (53.98)	4.08 (4.12)	15.69 (15.74)	11.01 (11.06)
2	$[\text{NiL}_1\text{L}_2\text{Cl}]\cdot 2\text{H}_2\text{O}$	538	71	11.3	53.69 (53.53)	4.02 (4.09)	15.55 (15.61)	11.75 (11.80)
3	$[\text{CuL}_1\text{L}_2\text{Cl}]\cdot 2\text{H}_2\text{O}$	533	68	12.7	53.96 (54.03)	4.07 (4.12)	15.71 (15.76)	10.93 (10.97)
4	$[\text{ZnL}_1\text{L}_2\text{Cl}]\cdot 2\text{H}_2\text{O}$	539.5	65	9.2	53.32 (53.38)	4.03 (4.07)	15.50 (15.57)	11.98 (12.04)

^a $\text{Ohm}^{-1}\text{ cm}^2\text{ mole}^{-1}$.

Table 2: TGA data for the ternary compounds

Compounds	Temp. range of water loss (°C)	% of water loss		Decomposition temperature (°C)	% of residue		Composition of the residue
		Found	Calc.		Found	Calc.	
[CoL ₁ L ₂ Cl]2H ₂ O	60–110	6.69	6.74	270	14.01	14.05	CoO
[NiL ₁ L ₂ Cl] 2H ₂ O	55–110	6.70	6.75	260	13.92	13.98	NiO
[CuL ₁ L ₂ Cl]2H ₂ O	45–95	6.65	6.69	255	14.72	14.77	CuO
[ZnL ₁ L ₂ Cl]2H ₂ O	50–100	6.62	6.67	240	14.93	15.01	ZnO

geometry around Ni(II) ion, which was confirmed by the observation of magnetic moment at 2.98 BM [39,42]. The mixed-ligand complex of Cu(II) displayed two bands at $\sim 14,320$ and $\sim 16,775 \text{ cm}^{-1}$ attributed to ${}^2B_{1g} \rightarrow {}^2B_{2g}$ (ν_2) and ${}^2B_{1g} \rightarrow {}^2E_g$ (ν_3) transitions, respectively, thus suggesting a distorted octahedral geometry [39,43]. However, the band due to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ was not observed in the studied copper complex [39,44]. The μ_{eff} value for copper complex at 1.88 BM also suggested an octahedral geometry [39,45].

3.3 Thermal analysis

The thermogravimetric analysis (TGA) data for the studied mixed-ligand complexes displayed similar pattern of thermal decomposition. The lattice water degraded at temperature below 100°C in all the studied complexes. This is followed by the degradation of the anhydrous complexes in two distinct stages at temperatures 250–280°C and 360–390°C. However, the degradation of organic constituents continued until the formation of stable metal oxide as the end product. The temperature ranges of decomposition, peak temperature and the possible fragments removed are presented in (Table 2).

3.3.1 Proton nuclear magnetic resonance (${}^1\text{H-NMR}$) spectra

The ${}^1\text{H-NMR}$ spectra of Schiff base ligand, L₁, and its mixed-ligand complex of Zn(II) displayed a multiplet due to the aromatic protons at δ 7.3–8.1 ppm. The signals at δ 6.7 and δ 9.0 ppm were attributed to the ring NH and exocyclic NH protons, respectively (Figure S2). However, the observed de-shielding in the ring –NH proton at δ 7.0 ppm indicated its participation in coordination. Furthermore, disappearance of phenolic –OH proton in the metal complexes confirmed its role in coordination. Moreover, $-\text{CH}_3$ signal was also observed at δ 2.4 ppm.

3.4 Antibacterial activity

The *in vitro* antibacterial activity was reported by Agar well diffusion method [27] against *B. subtilis*, *E. coli* and *S. typhi* at $100 \mu\text{g mL}^{-1}$ concentrations. The zone of inhibition data was studied using ciprofloxacin as the standard antibacterial drug as shown in Figure 2. The studied complexes exhibited better activity than the free ligand, which is likely due to the presence of $-\text{C}=\text{N}$ linkage and its involvement in coordination. Furthermore, coordination through the metal ions reduces the electron density due to the partial sharing of its positive charge with the donor groups and possible π -electron delocalization [46]. In addition, solubility, dipole moment, the nature of the ligand and geometry are supposed to be the possible factor for the higher antibacterial activity displayed by the mixed-ligand complexes [47]. However, the mixed-ligand Zn(II) complex remained inactive against *E. coli* and *S. typhi*.

3.4.1 Docking evaluation against MERS-CoV

Docking studies were performed to obtain accurate predictions on the optimized conformations for both the ligands and protein target to form a stable complex. All

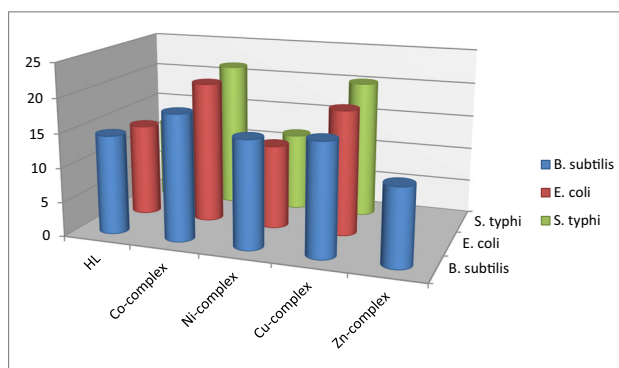


Figure 2: *In vitro* antibacterial activity of the ligand and its mixed-ligand complexes.

Table 3: The list of intermolecular interactions between the compounds docked with 4Z6S

Ligand	Score*	RMSD (Å)	Group interaction	Hydrogen bond**	Bond length (Å)
Co-crystallized	-21.63	0.04	LEU 417, LEU 389, LYS 413, LEU 414, LEU 411, ASN 410, TYR 409, LYS 587, ASN 408, GLU 382, CYS 383, ASP 384, SER 386, PHE 385	N sp ² (N2)–O sp ² -GLU 382	3.078
				O sp ² (O1)–N sp ² -PHE 385	3.016
				O sp ³ (O1)–N sp ² -SER 386	3.043
				O sp ³ (O3)–O sp ² -GLU 382	2.866
				O sp ³ (O3)–N sp ³ -LYS 413	3.333
Schiff base ligand	-41.40	0.05	LEU 417, LEU 389, LEU 414, LYS 413, PHE 385, LEU 411, ASN 410, TYR 409, CYS 383, GLU 382, ASN 408, LYS 587	O sp ³ (O4)–N sp ³ -LYS 413	3.082
				O sp ³ (O0)–O sp ² -GLU 382	3.077
				N sp ² (N10)–O sp ² -GLU 382	3.018
				N sp ³ (N10)–N sp ³ -LYS 413	3.155
				N sp ² (N13)–O sp ² -GLU 382	2.696
[NiL ₁ L ₂ Cl]2H ₂ O	-26.64	0.04	LEU 417, SER 416, LYS 413, LEU 414, LEU 389, SER 386, ASN 410, LYS 587, LEU 411, TYR 409, GLU 382, PHE 385, ASP 384, CYS 383	N sp ² (N11)–N sp ³ -LYS 413	3.084
				N sp ² (N11)–N sp ² -ASN 410	2.849
				O sp ³ (O13)–N sp ³ -LYS 413	3.140
				O sp ³ (O13)–N sp ³ -LYS 413	3.156
				O sp ³ (O13)–N sp ³ -ASN 410	3.044
[ZnL ₁ L ₂ Cl]2H ₂ O	-26.10	0.02	LEU 417, SER 416, LYS 413, LEU 414, LEU 389, SER 386, ASN 410, LYS 587, LEU 411, TYR 409, GLU 382, PHE 385, ASP 384, CYS 383	O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
[CoL ₁ L ₂ Cl]2H ₂ O	-25.85	0.04	SER 416, LYS 413, THR 412, LEU 411, ASN 410, ALA 434, SER 435, TYR 409, LYS 587, PRO 586	O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
[CuL ₁ L ₂ Cl]2H ₂ O	23.86	0.08	LEU 417, SER 416, LYS 413, LEU 414, LEU 389, SER 386, ASN 410, LYS 587, LEU 411, TYR 409, GLU 382, PHE 385, ASP 384, CYS 383	O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014
				O sp ³ (O13)–N sp ³ -LYS 413	3.014

*The docking score (PLANTS_{SP,IP} score) is a function described in Korb et al. [31]. **The numbering of the atoms was done according to the software (Figures 1 and 2).

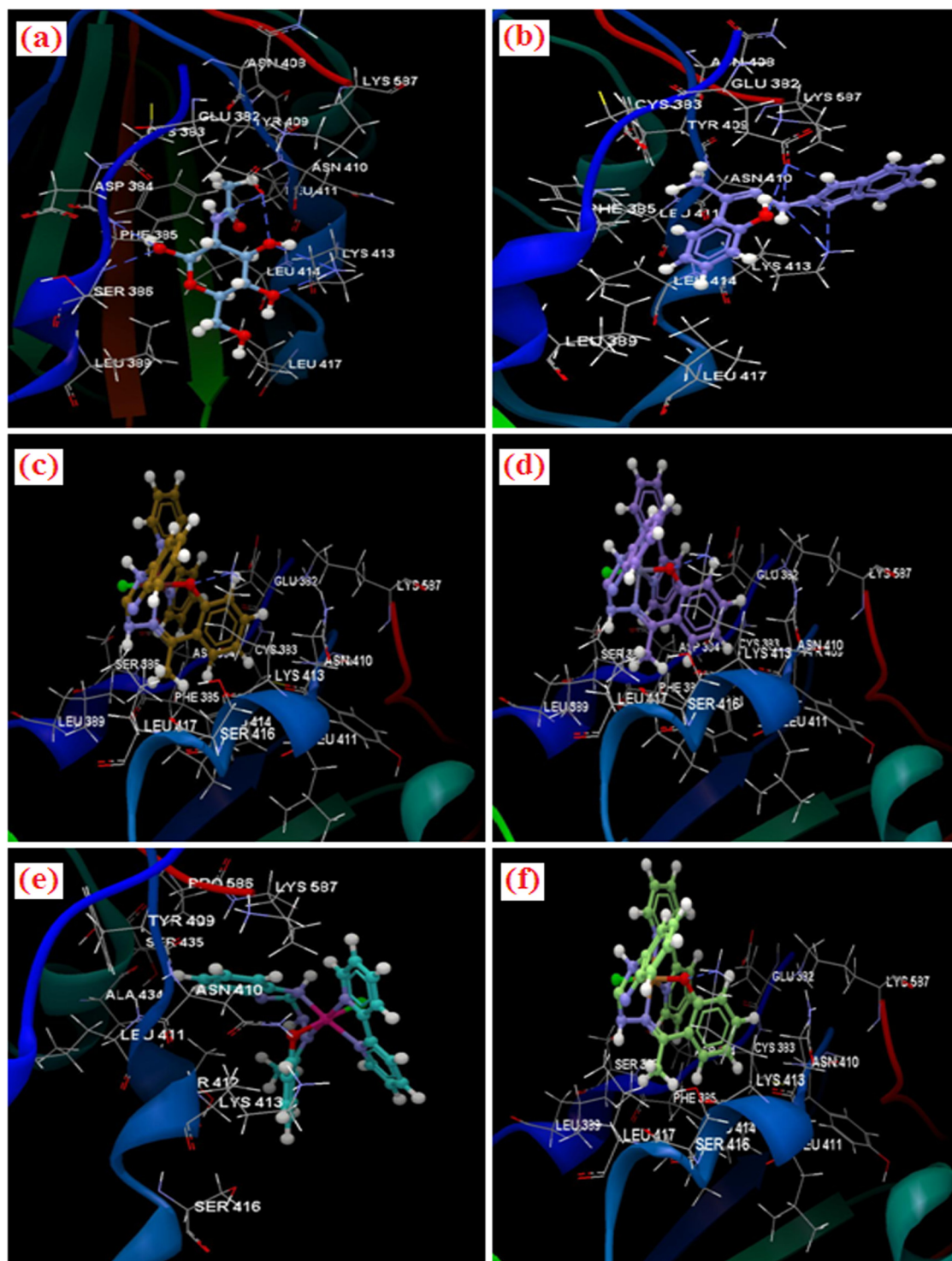


Figure 3: Docking pose of the compounds interacting with the residues of amino acid of binding site of 4Z6S; (a) co-crystallized NAG, (b) Schiff base ligand, (c) Ni(II) complex, (d) Zn(II) complex, (e) Co(II) complex and (f) Cu(II) complex.

the compounds were docked on the crystal structure of MERS CoV (PDB ID: 4Z6S). The docking pose of the co-crystallized *N*-acetyl-*D*-glucosamine (NAG) interacting with the residues of amino acid of active site are listed in

Figure 3. The co-crystallized NAG displayed the occurrence of six hydrogen bonds: two with GLU 382 (3.078 and 2.866 Å), two with LYS 413 (3.333 and 3.082 Å), PHE 385 (3.016 Å) and SER 386 (3.043 Å). The co-crystallized

Table 4: The list of intermolecular interactions between the compounds docked with 6W63

Ligand	Score*	RMSD (Å)	Group interaction	Hydrogen bond**	Bond length (Å)
Co-crystallized	-56.57	1.53	THR 190, GLN189, VAL 186, ARG 188, MET 49, TYR 54, ASP 187, PRO 168, LEU 167, GLU 166, MET 165, HIS 164, HIS 172, HIS 163, CYS 145, ASN 142, GLY 143, SER 144, LEU 141, PHE 140, GLY 143, LEU 127, THR 26, THR 25, VAL 42, HIS 41, CYS 44	O sp ² (O01)–N sp ² -GLY 143	3.202
				O sp ² (O13)–N sp ² -GLU 166	2.721
Schiif base ligand	-49.91	0.87	ALA 191, VAL 186, GLN 192, THR 190, GLN 189, ARG 188, ASP 187, HIS 41, LEU 50, PRO 52, ASP 48, CYS 44, TYR 54, MET 49, PRO 168, LEU 167, GLU 166, MET 165	O sp ³ (O0)–N sp ² -GLN 192	3.028
				O sp ³ (O0)–O sp ² -THR 190	2.558
				O sp ³ (O0)–N sp ² -THR 190	2.924
				O sp ³ (O0)–O sp ² -ARG 188	3.025
				N sp ³ (N23)–N sp ² -GLN 189	3.143
[CoL ₁ L ₂ Cl]2H ₂ O	-52.52	0.002	ALA 191, GLN 192, THR 190, GLN 189, ARG 188, ASP 187, MET 49, THR 169, PRO 168, LEU 167, GLY 170, GLU 166, MET 165, HIS 164, HIS 163, CYS 145, ASN 142	N sp ³ (N23)–N sp ² -GLN 189	3.145
[NiL ₁ L ₂ Cl]2H ₂ O	-52.35	0.006	ALA 191, GLN 192, THR 190, GLN 189, ARG 188, ASP 187, MET 49, THR 169, PRO 168, LEU 167, GLY 170, GLU 166, MET 165, HIS 164, HIS 163, CYS 145, ASN 142	N sp ³ (N23)–N sp ² -GLN 189	3.136
[ZnL ₁ L ₂ Cl]2H ₂ O	-52.23	0.01	ALA 191, GLN 192, THR 190, GLN 189, ARG 188, ASP 187, MET 49, THR 169, PRO 168, LEU 167, GLY 170, GLU 166, MET 165, HIS 164, HIS 163, CYS 145, ASN 142	N sp ³ (N23)–N sp ² -GLN 189	3.134
[CuL ₁ L ₂ Cl]2H ₂ O	-51.97	0.01	ALA 191, GLN 192, THR 190, GLN 189, ARG 188, ASP 187, MET 49, HIS 41, VAL 186, THR 169, PRO 168, LEU 167, GLY 170, GLU 166, MET 165, HIS 164, HIS 163, CYS 145, ASN 142	N sp ³ (N23)–N sp ² -GLN 189	3.134

*The docking score (PLANTS_{PLIP} score) is a function described in Korb *et al.* [31]. **The numbering of the atoms was done according to the software (Figures 1 and 2).

NAG was considered as the reference ligand to compare the docking results of the studied compounds. The docking studies revealed that the docking score of all the metal complexes are greater than the co-crystallized NAG (docking score: -21.63; root-mean-square deviation [RMSD]: 0.04 Å) but smaller than L₁ (docking score: -41.40; RMSD: 0.05 Å; Table 4). The L₁ showed the presence of six hydrogen bonds: three with GLU 382 (3.077, 3.018 and 2.696 Å), two with LYS 413 (3.155 and 3.084 Å) and one with ASN 410 (2.849 Å). The Ni(II) complex with the best docking score (-26.64; RMSD: 0.04) displayed one hydrogen bond with LYS 413 (3.140 Å). With LYS 413 amino acid, Zn(II) complex (3.156 Å) and Cu(II) complex (3.014 Å) realized one more hydrogen bond. However, Co(II) complex realized one hydrogen bond with ASN 410 (3.044 Å). The docking pose of the compounds interacting with amino acid residues is presented in Figure 3. The amino acid residues that formed the interacting group of each compound are listed in Table 3. After analyzing the data, it was noticed that all the studied compounds were placed in the same binding site (represented by a green sphere) of 4ZS6 as the co-crystallized one and have the same orientation as the co-crystallized NAG (Figure 4).

3.4.2 Docking evaluation against SARS-CoV-2 M^{pro}

All the compounds were docked on the crystal structure of SARS-CoV-2 M^{pro} (PDB ID: 6W63). The docking pose of the co-crystallized X77 interacting with amino acid residues of the active site and the hydrogen bonds created with GLU 166 (2.721 Å) and GLY 143 (3.202 Å) is shown in Figure 5. The co-crystallized X77 (*N*-(4-*tert*-butylphenyl)-*N*-[(1*R*)-2-(cyclohexylamino)-2-oxo-1-(pyridin-3-yl)ethyl]-1*H*-imidazole-4-carboxamide) was considered as a standard ligand to compare the docking results of the studied compounds. The docking studies revealed that the docking

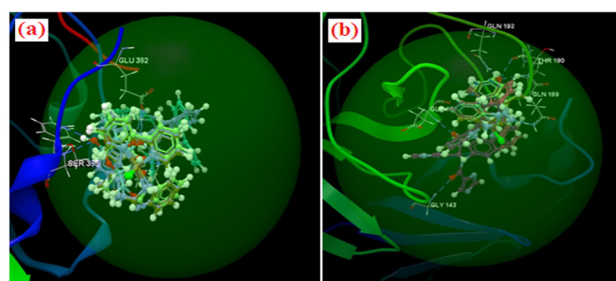


Figure 4: (a) Docking pose of the co-crystallized NAG and all compounds in the binding site of 4Z6S and (b) docking pose of the co-crystallized X77 and all compounds in the binding site of 6W63.

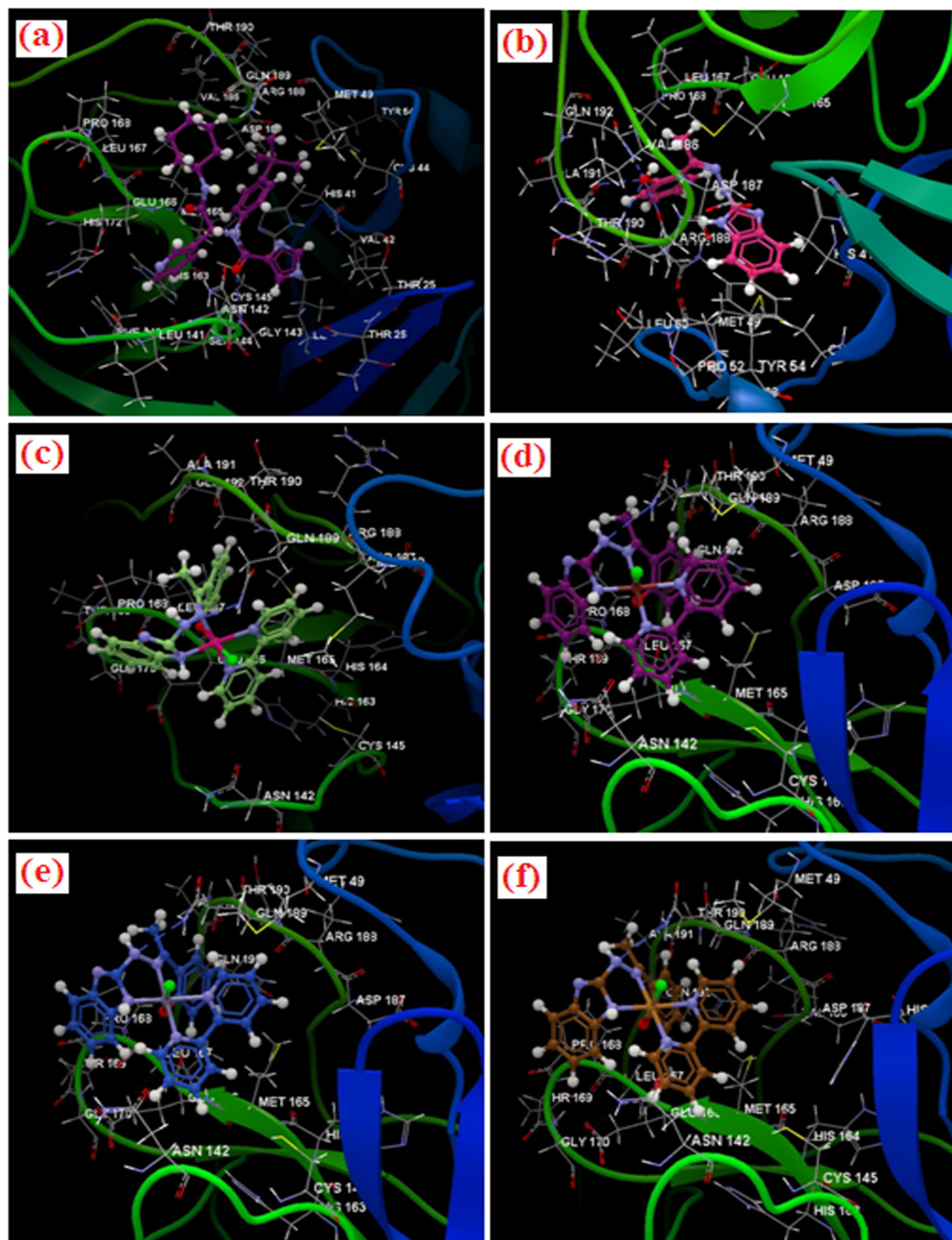


Figure 5: Docking pose of the compounds interacting with the amino acid residues of binding site of 4Z6S; (a) co-crystallized X77, (b) Schiff base ligand, HL, (c) Co(II) complex, (d) Ni(II) complex, (e) Zn(II) complex and (f) Cu(II) complex.

score of all the metal complexes are close to that of the co-crystallized X77 (docking score: -56.57 ; RMSD: $1.53 \text{ \AA}$) and greater than the Schiff base ligand, L_1 (docking score: -49.91 ; RMSD: $0.87 \text{ \AA}$; Table 5). The Schiff base ligand, L_1 ,

showed the occurrence of four hydrogen bonds: one with GLN 192 ($3.028 \text{ \AA}$), two with THR 190 (2.558 and $2.924 \text{ \AA}$) and one with ARG 188 ($3.025 \text{ \AA}$). The Co(II) complex with the best docking score (-52.52 ; RMSD: 0.002) displayed

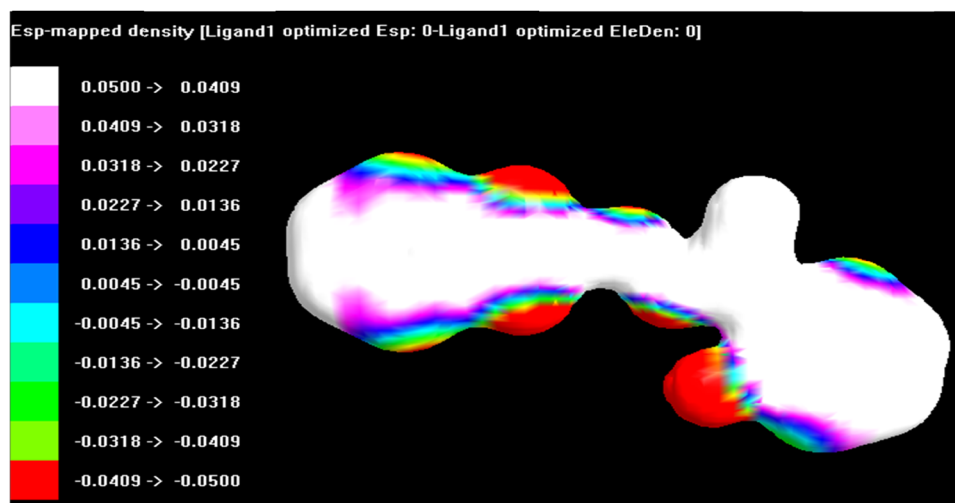


Figure 6: The ESP surface of the Schiff base ligand, L_1 .

one hydrogen bond with GLN 189 (3.143 Å). With the GLN189 amino acid, Ni(II) complex (3.145 Å), Zn(II) complex (3.145 Å) and Cu(II) complex (3.134 Å) realized one more hydrogen bond. The docking pose of the compounds interacting with amino acid residues is presented in Figure 5. The amino acid residues that formed the interacting group of each compound are listed in Table 4. After analyzing the data, it was noticed that the all studied compounds were placed in the same binding site (represented by a green sphere) of 6W63 as the co-crystallized one and have the same orientation as the co-crystallized X77 (Figure 5).

According to Lipinski's rule of five, the calculated parameters (Table S2) may predict the property of a molecule to turn into an active drug [48] on the basis of the number of violations made. It is observed that all metal complexes have two violations of Lipinski's rule of five (Lipinski violation is 2), namely, molecular weight >500 Da and octanol–water partition coefficient ($\log P$) > 5.

3.5 Electrostatic potential (ESP) surface analysis

The ESP surface of Schiff base ligand, L_1 , was analyzed by using Argus Lab 4.0.1 software as shown in Figure 6. The surface contains a number of possible sites for electrophilic attack. The ESP surface displayed a specific data about the charge distribution. The negative regions were mainly over phenolic oxygen, azomethine nitrogen and benzimidazole ring nitrogen atoms as indicated in red and were involved in coordination. However, the other

nitrogen atom was not involved in coordination likely due to the steric effect.

4 Conclusion

A series of mixed-ligand complexes with Cu(II), Co(II), Ni(II) and Zn(II) ions were investigated. The antibacterial activity suggested the moderate antibacterial activity for all the complexes in comparison to the Schiff base ligand. However, the Zn(II) complex was inactive against *E. coli* and *S. typhi*. Moreover, molecular docking studies recorded against MERS-CoV (PDB ID: 4ZS6) and SARS-CoV-2 M^{Pro} (PDB ID: 6W63) revealed that all the studied metal complexes demonstrated two violations of all the parameters involved in the Lipinski's rule of five. The Ni(II) complex displayed the best docking score (−26.64; RMSD: 0.04) against MERS-CoV whereas the Co(II) complex displayed the best docking score (−52.52; RMSD: 0.002) against SARS-CoV-2 M^{Pro}. This study may be helpful for the researchers in designing new potent drugs. In addition, the ESP surface of the Schiff base ligand, L_1 , was analyzed to understand the possible sites for electrophilic attack.

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Conflict of interest: There are no conflicts to declare.

Data availability statement: All data presented in the study are included in the article and supplementary material.

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