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Synthesis, Characterization, Biological Screening and Molecular Docking of New Schiff Base and Its Mononuclear Complexes with Pb2+, Cd2+, Zn2+ and Cu2+

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Abstract: This work deals with preparation of a Schiff base (L) by the condensation of 4-(dimethylamino)benzaldehyde with amino acid (Serine). The ligand (L) was reacted with metal ions Pb(II), Cd(II), Zn(II) and Cu(II) to form complexes with different properties. The metal complexes have been successfully synthesized in an alcoholic medium. The structures of all the synthesized compounds were characterized using TGA analysis, IR spectroscopy and UV–Vis spectroscopy. Moreover, the solvent effects for the maximum absorbance of the Schiff base using DMSO and DMF were recorded at different λmax (354 and 337 respectively). Furthermore, the ligand (L) and its metal complexes were tested for in-vitro antimicrobial activity and showed significant effects. The biological activity testing results showed that the complexes were more potent antibiotics than the free ligand. The Cu(II) and Cd(II) complexes displayed high antimicrobial potency while the Pb(II) and Zn(II) complexes did not. Finally, molecular docking simulation of the synthesized compounds were performed using AutoDock Vina to get insight into the binding interactions between the synthesized ligand and enzymes. The results revealed that Cu(II)/L and Cd(II)/L exhibited lower free energy of binding (FEB) compared to the free ligand. In addition, these complexes showed more interactions with the essential amino acids in the binding site. The in-vitro results were confirmed by docking analysis.
Keywords: Schiff base, Biological activities, Metal Complexes, Serine. Autodock Vina.

1 Introduction

There is currently considerable interest in the coordination chemistry of Schiff base ligands which are an important class of ligands in basic and applied science [1]–[3]. The synthesis of metallic complexes of Schiff bases are predominantly studied due to their biological and pharmaceutical applications which have been found to be effective and some have been made available as drugs.[4][5][6] These compounds have received attention due to their effective role in metallo enzymes and as biomimetic prototypical complexes owing to their closeness to regular enzymes and proteins.[7] However, Schiff bases derived from compounds containing NH_2-groups and a carbonyl compound that coordinate to metal ions *via* an azomethine N-atom have been studied. Herein, we present the synthesis and characterization of L by the condensation of 4-(dimethylamino)benzaldehyde and amino acid (Serine) followed by its complexes with some metal ions such as Pb(II), Cd(II), Zn(II) and Cu(II). Moreover, preliminary *in-vitro* antimicrobial screening activities of the complexes obtained are carried out and the results are reported. Finally, a molecular docking simulation using AutoDock Vina was performed to investigate the molecular interactions between the synthesised ligand and the amino acids in the target enzymes.

2 Experimental

2.1 Chemical, Reagents and Instruments

All chemicals and reagents were obtained from commercial sources and used as received without any further purification. Melting points were measured using Stuart SMP3 Apparatus at the central laboratory of the faculty of

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science, Omar Al-Mukhtar University Al-Bayda, Libya. Conductivity measurements were carried out using a HANNA conductivity meter. Infrared spectra were recorded on a Perkin Elmer FT-IR spectrophotometer in the region 4000–600 cm^{-1} using KBr disks. The electronic absorption spectra were recorded at the central laboratory of the faculty of science, Omar Al-Mukhtar University, Libya. Thermogravimetric analysis (TGA) was carried out using (shimadzu) at the Chemistry Department of El-Menia University, Egypt.

2.2 Preparation of Ligand (L)

The amino acid Schiff bases was prepared as follows: Methanolic solution (50 cm3) of NaOH (0.8 g, 20 mmol) was added to Serine. The mixture was stirred at room temperature and a solution of 4-dimethyl amino benzaldehyde (2.98 g, 20 mmol) in ethanol (50 cm^3) was added. The solution volume was reduced to 20% and 1 cm^3 of CH_3COOH was added. After 2 h, yellow crystals formed; the crystals were filtered, washed with ethanol, and recrystallized from hot ethanol to give yellow crystals.

2.3 Preparation of complexes

This method was used for all metal salts. The L (2.1 g, 20 mmol) was dissolved in methanol (50 cm^3) containing NaOH (0.8 g, 20 mmol) and stirred at room temperature. A solution of 4-dimethyl amino benzaldehyde (2.98 g, 20 mmol) in ethanol (50 cm^3) was added into the mixture. The mixture was charged with the appropriate M-salt (10 mmol). The mixture was stirred for 3 h and the solution volume reduced to 75%. The product was filtered and recrystallized from methanol:ethanol (1:1).

2.4 Molecular docking

Molecular docking was performed for complexes of Cu(II), Cd(II) and free ligands using Autodock Vina.[8] The free ligand and complexes were built using Marvensketch, and energy minimization was performed Hyperchem 8 [9], then saved to pdb format. Crystal structures of Staphylococcus aureus (PDB: 1JIJ) and Escherichia coli (PDB: IFJ4) were obtained from Protein Data Bank with PDB ID: 1JIJ[10] and PDB ID: IFJ4[11], respectively. Proteins were edited using AutoDockTools (ADT) to remove unwanted water molecules and all hydrogen atoms were added. A grid box of 60 × 60 × 60 points, with a spacing of 0.375 A˚ was located at the center of the active site. The lowest FEB was selected. Visualizing the docking results was done using Discovery Studio visualizer 2016 (Accelrys, Inc., San Diego, CA, USA) and Ligplot.[12]

3 Results and Discussion

Schiff bases and their complexes have a variety of applications.[13] The coordinating possibility of 4-dimethyl amino benzaldehyde/Serine has been improved by condensing serine with carbonyl group. Physical characteristics (Table 1), IR data (Table 3), UV-spectroscopy (Table 2), TGA analysis (Table 4) of the ligand and the metal complexes are given below.

Table 1: Physical appearance and Melting points of L and complexes 1–4.

Compounds	Physical appearance	Melting points (°C)
L	Cinnamon	118
Zn(L)	Off white	72
Cd(L)	Brown	146
Cu(L)	Dark brown	103
Pb(L)	Sandy brown	79

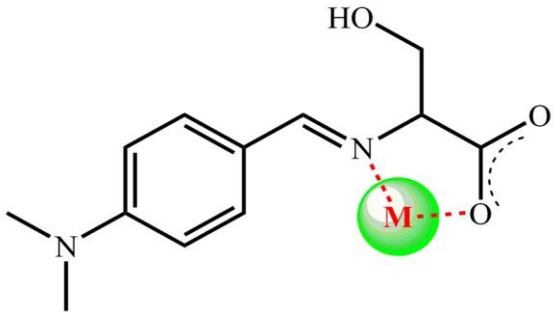


Fig.1: Proposed complexation of the ligand with metal ions

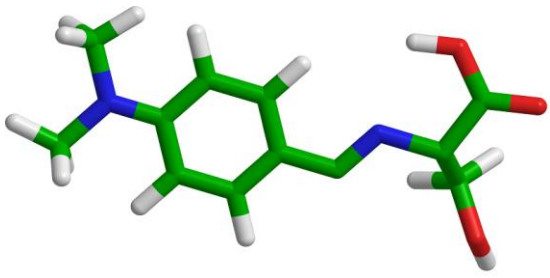


Fig.2: 3D view of the ligand L

The UV Spectrophotometric Studies

Electronic spectra of the Schiff base in the presence of different polar solvents are illustrated in table 2. Changing the solvent from DMF to DMSO leads to a red shift of the ʎmax band which occurs at 337 and 354 nm respectively, this band is due to n→π* electronic transition.[14]

IR spectra of the Serine Schiff base complex

The FT-IR spectral data of the serine Schiff base (L) is characterized by the appearance of peaks at 3286 cm^{-1}, assigned to OH, the aliphatic CH peaks assigned at 2882-2913 cm^{-1} and the carbonyl group (C=O), assigned at 1568-1658 cm^{-1}. In addition, peaks at 1548, 1430, 1369, 1309, 1231 and 800 cm^{-1} peaks were assigned to the C-O, NH (bending), CH_2, C-N, CH_3 and C-C respectively. The spectra of the ligand showed a shift of the OH peak to low frequency in case of coordination with Cd(II) and Pb(II) whereas the OH-peak disappeared when the ligand was coordinated with Zn(II) and Cu(II). Notably, the change of the free ligand peak frequency indicated the coordination of the OH-group with the metal ions with a medium band at 3175–3200 cm^{-1} due to t(N-H).[15] The ligand shows one medium intensity band at ca. 1699 and 1444 cm^{-1}. The CH_2 band shifted to higher frequency in all L complexes except the cadmium complex, the band of CH (Aliphatic) shifted to lower frequency in case of Zn^{2+} and Pb^{2+} complexes and stayed in the same position in Cu^{2+} and disappeared in the case of the Cd^{2+} complex. The NH_2 band appeared at 3305 cm^{-1} in the Cu^{2+} complex although this band disappeared in their Schiff base with the NH serine Schiff base band located at 1548 cm^{-1} in all complexes. The C=O band in the serine Schiff base appeared at 1571 cm^{-1} and changed in the complexes of Cu, Cd, Zn and Pb to appear at 1547, 1548, 1591 and 1591 cm^{-1} respectively. This shifting suggests coordination with the metal through the oxygen atom of the hydroxyl group.[15] The C-O band which is located at 1231 cm^{-1} in serine Schiff base complexes held its position in all of the complexes. The C-C Schiff base band located at 800 cm^{-1} shifted to higher frequency in all serine Schiff base complexes. New bands in the range of 663–686 cm^{-1} which are not present in the free Schiff base are due to (M --- O).[15]

Thermogravimetric analysis of the prepared Schiff base and Schiff base complexes

The thermogravimetric analysis data for some serine Schiff base and serine Schiff base complexes are given in table 4. The mass lost from L Schiff base-Zn, Se Schiff base–Cd, Se Schiff base–Cu and Se Schiff base–Pb complexes correspond to the loss of water molecules in the temperature range of (25 to 80 ^oC), and the loss of CO_2 molecules which occurs in the temperature range (75 to 227 ^oC). In addition to decomposition, the residual of metal oxides (M-O) appeared in the temperature range of (115 to 376 ^oC).

Table 2: The maximum absorbance of the Schiff base in different solvents.

Solvent	L1 Schiff base (serine) $ʎ_{max}$(nm)
DMF	337
DMSO	354

Table 3: The IR spectra of the Serine Schiff base complexes.

	Se-L /Cu(II)	Se-L/ Cd(II)	Se-L /Zn(II)	Se-L/ Pb(II)
OH	-	3259	-	3281
C=O	1600-1654	1600-1656	1591-1659	1591-1659
C=C	-	-	-	-
NH bending	1547	1548	1547	1547
CH_3	1368	1367	1368	1336
CH_2	1485	1405	1445	1412
CH(Aromatic)	-	-	-	-
CH(Aliphatic)	2925	-	2903	2907
C-O	1232	1231	1231	1231
C-N	1834	1311	1311	1312
C-C	811	811	824	824

Table 4: Thermogravimetric analysis data for L and its complexes.

	H_2O (˚C)	CO_2(˚C)	MO(˚C)
L	50 – 75	75 – 125	125 – 310
Se-L/Zn	50 – 75	75 – 115	115 – 185
Se-L/ Cd	50 – 75	75 – 170	170 – 250
Se-L/ Cu	48 – 80	80 – 130	130 – 180
Se-L/ Pb	25 - 99	99 – 227	227 – 376

Antimicrobial studies

Antibacterial assay

The antibacterial tests were assayed according to the diffusion method. The strains of bacteria used were: Gram positive bacteria (*Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli*). All strains were isolated from laboratory of microbiology. The identity of all strains were confirmed. A bacterial suspension was prepared and added to the sterilized medium before solidification under aseptic conditions, different concentrations of complex were placed on the surface of the culture and incubated at 37 ^{o}C for 24 h. After incubation, the average of inhibition zones was recorded (mm).

Antifungal assay

The antifungal tests were assayed according to the diffusion method. The strains of fungi used were (*Rhizopus and penicillium*) All strains were isolated from laboratory of microbiology. The identity of all strains were confirmed. A fungal suspension was prepared and added to the sterilized medium before solidification under aseptic conditions, different concentrations of complex were placed on the surface of culture and incubated at 28 ^{o}C for 72 h. After incubation, the average of inhibition zones was recorded (mm).

Bacterial cultures

Plate cultures of agar medium nutrient were used for culture of bacteria. The medium was prepared by dissolving 14 g of powder in 500 mL of sterile distilled water, the medium was then sterilized by autoclaving at 121 ^{o}C for 15 min.

Fungal cultures

Plate cultures of subsaturated agar medium were used for culture of fungi. The medium was prepared by dissolving 32.5 g of powder in 500 mL of sterile distilled water, the medium was then sterilized by autoclaving at 121 ^{o}C for 15 min. [16]

Biological studies

Antimicrobial and Antifungal Activity

The antimicrobial and antifungal activity studies were carried out on two types of each organism. The Schiff bases and their complexes were tested on representatives of bacilli (*Mycobacterium phlei*), Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (Escherichia coli, and representative fungal species (*Penicillium and Rhizopus*). The agar diffusion method by cp plate technique (1tap) was applied, using trypticase soy agar for bacteria. The products were dissolved in sterile, distilled water at a concentration of 10 mg/mL, 100 mL was then aseptically transferred to preformed cups (100 mg/cup) in the dried, inoculated Triticale soy agar plates.[17] All culture plates were put on its surface from all concentrations of the solution complexes which were prepared from 10^{-1} to 10^{-5} mg/ml, thereafter the plates were incubated inverted at 37 ^{o}C for 24 h in the case of bacteria and at 25 ^{o}C for 48 h in the case of fungi. After incubation, the inhibition zones were recorded in mm. A diameter of less than 10 mm indicates no effect.[16] The minimum inhibitory concentration (MIC) was determined for the active compounds 100 mL of each dilution for the bacterial species were tested against a serial dilution which was transferred in cups preformed in nutrient agar inoculated with a suspension of 10 mL of microbial cells on the surface of agar plates and incubated at 37 ^{o}C for 24 h. After incubation, the lowest concentration producing inhibition was recorded as the minimum effective concentration. L Schiff base shows an antimicrobial effect against only Rhizopus fungi. In table 5, with respect to the minimum inhibitory concentration (MIC), the three tested compounds showed comparable antimicrobial activity. With respect to MIC, in general, the antimicrobial activity for the tested products was higher on Gram-negative than Gram- positive bacteria. In addition to the Schiff base, the Cu complex exhibited higher antimicrobial activity for all the tested organisms compared to the other complexes. The observed results revealed that the test compounds were very active as antimicrobial and antifungal agents.

Molecular docking studies

Validation of docking protocol

Before performing molecular docking of the synthesized ligands, control docking was performed to validate the docking procedure. The coordinated ligand of the 1JIJ and

1FJ4 for *Staphylococcus aureus* and *Escherichia coli* were extracted and re-docked into the same binding pocket. The docking results revealed that the binding conformation ligand pose was similar to the crystallographic pose (RMSD = 0.84 Å, and 0.78 and binding affinity –7.5 and 7.3 kcal/mol for 1JIJ 1FJ4, respectively Figure 3). Furthermore, the results indicated that the docking parameter used is reliable and reproduced the expected binding mode.

Molecular docking of the free ligand and its complexes with Cu(II) and Cd(II)

By conducting molecular docking simulations, reliable conformations were obtained of the synthesized ligands within the binding pocket of the selected enzymes. Furthermore, the docking results showed minimum FEB exhibited by the ligands with the metal ion using AutoDock Vina. Among the four synthesized compounds,

Table 5: Antimicrobial activities of the tested products (inhibition zone in mm).

	L	Cu^{2+}	Zn^{2+}	Cd^{2+}	Pb^{2+}
Escherichia coli	-	33	-	36 23	-
Staphylococcus aureus	-	21	-	-	-
Penicillium	-	12	-	41	-
Rhizopus	-	21	-	-	12

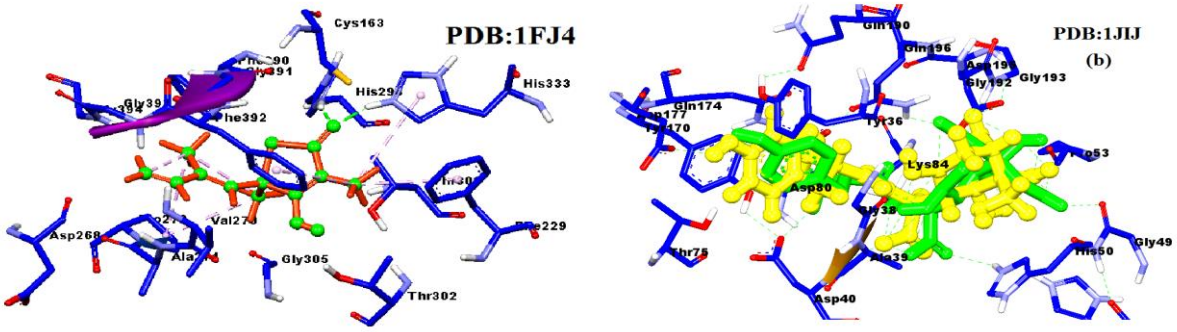


Fig. 3: Superimposition of the docked and crystallographic poses green and yellow, respectively for 1JIJ) and (green and orange respectively for 1FJ4).

Table 6: Details of binding interactions of the Free and synthesized ligand docked into active site of the enzymes 1JIJ and 1FJ4.

No	**Ligands**	**AutoDock Vina (kcal/mol)**	**Residue**	**Type of interactions**
1	FL-JIJ	-7.5	Tyr170	H-Bond
			Asp177, Gln174, Gly38, Gly193, Gly193, His50, Asp195, Ala93	van der Waals
			Asp40	Attractive Charge

2	FL-1FJ4	-7.2	Gly305	H-Bond
			Phe390, Pro272, Phe229, Thr203,	van der Waals
			Phe392	Pi-Alkyl
			Val270	Conventional H-Bond
3	L-Cu(II) 1JIJ	-9.0	Gln196, Asp195, Asp177, Gly38	H-Bond
			Thr36, Gln190, Gly38, Ala39, Asp40, Lys84, Thr75, His50, Gln174	van der Waals
			Asp80	Pi-Anion
	L-Cu(II) 1FJ4	-8.9	Gln391, His298,	H-Bond
			Ala39, Gly394, His74, Pro272, Ala271, Phe392, Val270, Phe229, Gln190, Tyr36, Asp177, Gln305, Thr300, Cys163, Phe390	van der Waals
			Thr302	Conventional H-Bond
	L-Cd(II) 1JIJ	-8.2	Leu70	H-Bond
			Tyr36, Gln174, Gln190, Cys37, Gly38, Ala39, Asp40, Gln196, Lys84, Asp195, Thr75, His50	van der Waals
			Tyr170,	Conventional H-Bond
			Asp80	Pi-Anion
	L-Cd(II) 1FJ4	-8.4	His333	H-Bond
			Pro272, Met204, Gly391, Ala271, Phe392, Val270, Gly305, Thr300, Phe390	van der Waals
			Thr302, Ala206, Gly205	Conventional H-Bond

The L-Cu(II) and L-Cd(II) exhibited the best interactions with 1JIJ and 1FJ4. The L-Cu(II) showed FEB of –9.0, –8.9 kcal/mol toward 1JIJ and 1FJ4, while L-Cd(II) showed FEB –8.2 and –8.4 kcal/mol toward 1JIJ and 1FJ4, respectively.

The FL with the 1JIJ was found to show one H-bond with the amino acid Tyr170 and van der Waals interactions with Asp177, Gln174, Gly38, Gly192, Gly193, His50, Asp195 and Ala39. Attractive charge interaction was shown with Asp40. The FL with 1FJ4 exhibited one hydrogen bond

with the amino acid Gly305, van der Waals interactions were displayed with amino acid residues Phe390, Pro272, Phe229 and Thr302. A conventional H-bond is shown with the amino acid Val270 and a Pi-alkyl interaction with Phe392 (Figure 4 and Table 6).

The ligand with the Cu(II) metal ion at the binding site of 1JIJ was found to show three hydrogen bond interactions with Gly38, Asp177 and Gln196. van der Waals interactions were displayed between the ligand with amino acids Thr38, Gln190, Ala39, Asp40, Lys84, His50, and Thr57. A conventional hydrogen bond formed with Thr170. Likewise, Asp80 exhibited Pi-Anion interactions at the binding pocket (Figure 5 and Table 6). Meanwhile, the ligand with the Cu(II) metal ion at the binding site of 1FJ4

displayed two hydrogen bonds with amino acids Gln391 and His298. Amino acid Thr302 formed a conventional H-bond at the binding pocket. Likewise, van der Waals interactions were formed between the ligand, and the amino acid residues Ala39, Gly394, His74, Pro272, Ala271, Phe392, Val270, Phe229, Gln190, Tyr36, Asp177, Gln305, Thr300, Cys163 and Phe390.

The L-Cd(II) with 1JIJ displayed one hydrogen bond with Leu70, Amino acids Tyr36, Gln174, Gln190, Cys37, Gly38, Ala39, Asp40, Gln196, Lys84, Asp195, Thr75, His50 showed van der Waals interactions; another interaction was a conventional H-bond formed with Tyr170. Likewise, a Pi-Anion interaction is formed with the amino acid Asp80. Finally, L-Cd (II) with 1FJ4 exhibited different interactions at the binding pocket of the enzyme. These compounds displayed one hydrogen bond with the amino acid His333, van der Waals interactions were also formed between the amino acids Pro272, Met204, Gly391, Ala271, Phe392, Val270, Gly305, Thr300 and Phe390. In addition, the compound had three conventional H-bonds with amino acids Thr302, Ala206 and Gly205 (Figure 6 and Table 6).

As can be seen from the above findings, the addition of metal ions to the free ligands enhanced the potency of the ligand. The ligand with metal ions exhibited lower FEB, high affinity, and higher binding interactions than the free ligand, which showed lower affinity and higher FEB. Therefore, these compounds were inhibitor targets for the target enzymes due to the interaction with the essential amino acids in the binding pocket in a way similar to the coordinated ligand in the crystal structure; these affinities offer additional benefits of inhibiting the microbial activity. Moreover, compounds with the metal ions show an advantage over the free ligand in terms of amount and types of interactions and FEB, making them potential inhibitors for Gram + and Gram – bacteria.

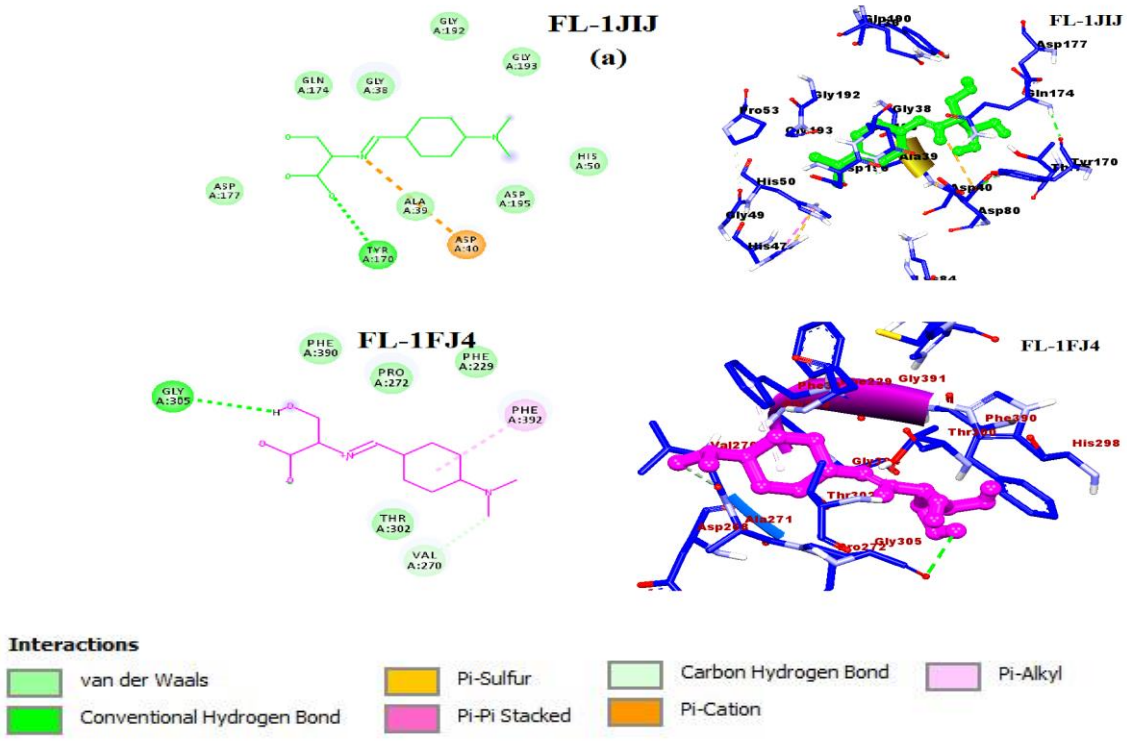


Fig.4: 2D and 3D interactions of the free ligand FL in the binding pocket of 1JIJ and 1FJ4.

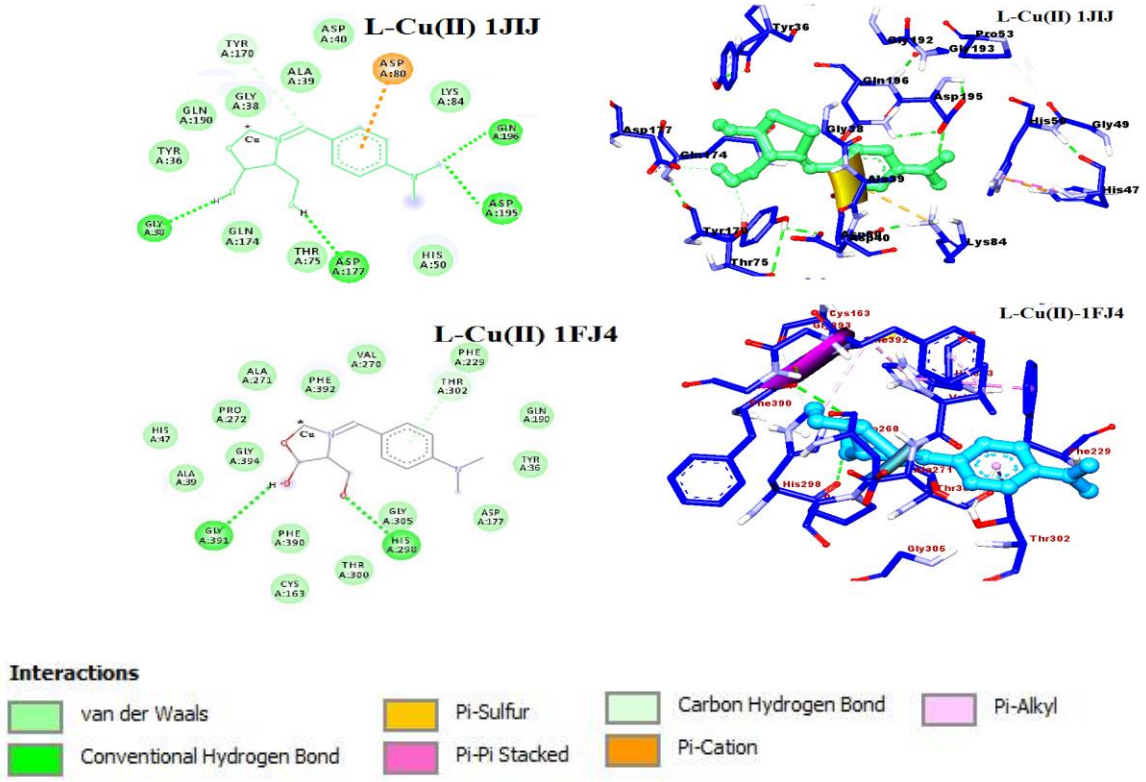
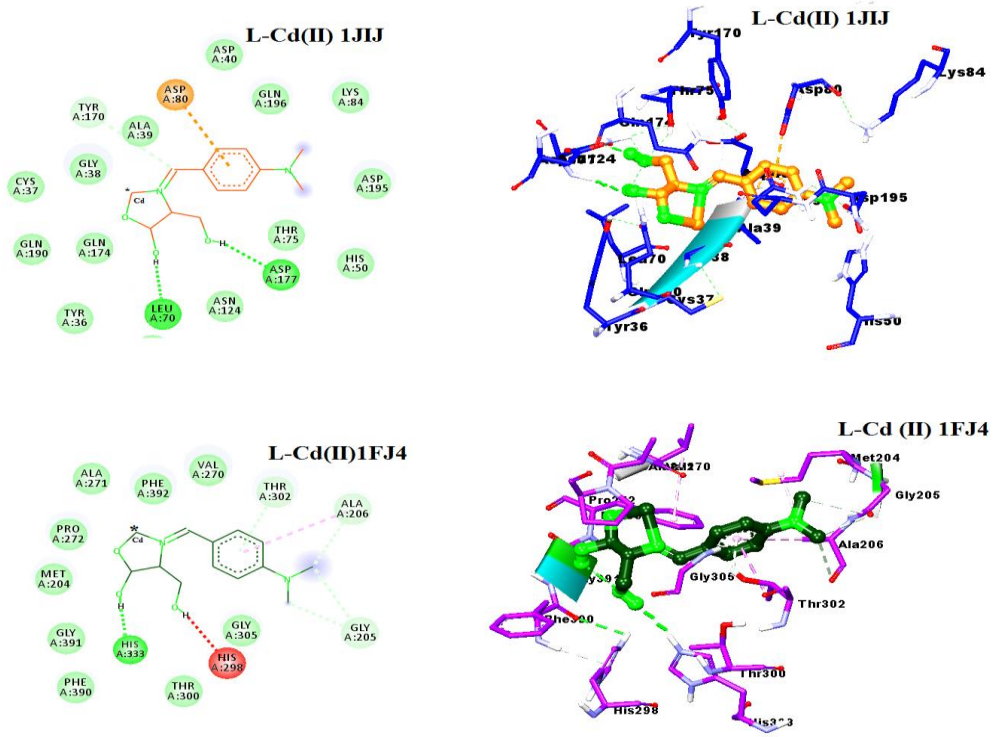


Fig. 5: 2D and 3D interactions of the synthesized L-Cu(II) in the binding pocket of 1JIJ and 1FJ4.



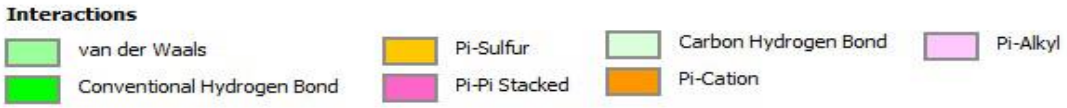


Fig. 6: 2D and 3D interactions of the synthesized L-Cd(II) in the binding pocket of 1JIJ and 1FJ4.

4 Conclusions

This study was carried out on some Schiff bases (L) of the Amino acid (Serine) with some transition metals to prepare Schiff base complexes by direct reaction and using some properties and spectral studies to identify the complexes. The data showed that most of the metals which were selected can form complexes with the Schiff base. The results also showed that some of the prepared complexes gave significant antimicrobial and antifungal effects. Molecular docking simulations revealed that good hydrogen bonding, and other interactions with several essential amino acids residues in both enzymes' binding pockets, clarifies the potency of the synthesized ligands with the metal ions. The information obtained from molecular docking of the synthesized ligands was in good agreement with the *in-vitro* results which stated that the ligands with Cd(II) and Cu(II) exhibited a significant antimicrobial effect.

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Conflicts of interest

The authors claim that the researchers in this study have no conflict of interest.

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