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Research Article

Synthesis and Evaluation of Cytotoxicity and Molecular Docking of New Symmetrical Macroacyclic Schiff Base Ligands and Their Ni(II) and Zn(II) Complexes

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To synthesize new symmetrical macroacyclic Schiff base ligands (L_1 and L_2), polyamine (A) was condensed with 2-formylpyridine and salicylaldehyde. Furthermore, $Ni(ClO_4)_2.6H_2O$ and $Zn(ClO_4)_2.6H_2O$ in methanol were reacted with the ligands to prepare corresponding metal complexes. The cytotoxicity of the synthesized complexes against MCF-7 and A549 adenocarcinoma cells was evaluated, where Zn-complexes displayed greater cytotoxic effects than Ni-complexes. Conductivity measurements, ESI-MS, UV-Vis, molar conductivity, IR, ^1H, and ^{13}C NMR spectra were used to characterize the products. Furthermore, molecular docking was performed to assess the biological activity of the complexes, and it was observed that Zn-complexes exhibited the highest inhibition effect against cytotoxic receptors.

1. Introduction

Schiff base complexes are highly valuable in transition metal coordination chemistry due to their diverse structures and easy preparation. They have played a significant role in advancing coordination chemistry, leading to various research projects in physicochemical and biochemical investigations, as well as pure synthesis [1–3].

Extensive research has been conducted on metal complexes with chelate ligands to replicate the redox reactions of various metalloenzymes and the coordination and reactivity of dioxygen in select processes. Metalloenzymes possess distinct functions, such as redox [4–9] and structural and catalytic roles [10–14]. The study of complexes imitating metalloprotein active sites has been extensively pursued. To create similar conditions as found in proteins, model complexes can be derived from bulky polydentate ligands.

In this report, the synthesis of novel Schiff base ligands (L_1 and L_2) and their Ni(II) and Zn(II) complexes is presented along with their characterization using specific physicochemical techniques. The global rise in cancer cases leading to death, estimated to reach 13.1 million by 2030, has led to the development of various cancer treatment methods such as hormone and biological therapy, chemotherapy, surgery, and radiation. Chemotherapy, in particular, has been extensively used for destroying cancer cells in diverse types of cancers. However, the resistance of cancer cells to metal-based drugs used in chemotherapy has been a significant drawback, necessitating researchers to focus on the design and discovery of novel metal complexes and drugs [15–20].

One of the most important strategies is to develop innovative compounds that can effectively target infected cells and kill cancerous tumors. Therefore, the synthesis of these compounds can significantly impact the solution to this problem. In this study, we aimed to analyze the cytotoxicity properties of reported complexes and identify their pharmaceutical characteristics using a bioinformatics approach,

which proved to be effective. To predict the binding affinity and interactions between macromolecules and ligands, we employed AutoDock 4.2, AutoDock Vina, PyRx, and Molegro Virtual Docker (MVD) [21–23]. Moreover, we estimated the interactions between the complexes and Mutant (Y1248L) MET receptor tyrosine kinase (PDB ID: 3DKG) and ERK2 (PDB ID: 4ZXT) after determining their cytotoxic properties [24–28]. Our findings suggest that synthesized complexes can potentially inhibit the activities of these receptors. Importantly, we found good agreement between the cytotoxicity property analysis and molecular docking results.

2. Experimental

2.1. Materials. 2-Hydroxybenzaldehyde, 2-nitrobenzyl chloride, 1,2-diaminopropane, pyridine-2-carbaldehyde, 2-formylpyridine, Ni(II), and Zn(II) perchlorates were purchased from Merck, Aldrich, and Fluka and were used without further purification. Common techniques were used to purify the solvents which were of reagent grade. Significantly, only a small amount of perchlorate salts which are greatly explosive should be prepared.

2.2. Physical Measurements. It was on a Perkin Elmer Spectrum RXI FT-IR spectrophotometer that the infrared spectra were measured from 400 to 4000 cm^{-1} as KBr pellets. Besides, we applied a Bruker Avance DPX- 400 spectrometer in order to record ^{1}H and ^{13}C NMR spectra which were at 400 MHz. Furthermore, dimethyl sulfoxide (DMSO-d_6) was deuterated and $CDCl_3$ was applied as the solvent. Agilent Technologies (HP) 5973 mass spectrometer was used to record the mass spectra and an Elementar Analysen systeme GmbH was also applied to perform the elemental analysis.

2.3. Molecular Docking. Our primary objective was to evaluate the anticancer activity of the synthesized complexes through molecular docking with Mutant (Y1248L) MET receptor tyrosine kinase (PDB ID: 3DKG) and ERK2 (PDB ID: 4ZXT). We utilized the Molegro Virtual Docker (MVD) to calculate the binding affinity (kJ/mol) of the complexes. Initially, we used ChemDraw software to draw the structure of the synthesized complexes, and Gaussian 09 was employed to optimize their structure. The parameter settings for MVD were as follows: the MolDock Score was selected as the score function, ligand evaluation included internal ES, the algorithm used was MolDock SE, internal HBond was considered, the maximum population size was set at 50 sp2-sp2 torsions with all checked, 20 runs were performed, the neighbor distance factor was set to 1.00, maximum interactions were limited to 1500, and the maximum steps allowed were 300.

2.4. Cytotoxicity In Vitro. We procured MCF-7 (IBRC C10082) and A549 (IBRC C10080) human breast and lung cell lines from the Iranian Biological Resource Center (IBRC) in Tehran. Ham's F12 medium supplemented with L-glutamine, 10% fetal bovine serum (FBS), 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin was used for culturing both cell lines in a humidified 5% CO2 atmosphere at 37°C. Compounds were dissolved in DMSO at 20 mM·L^{-1} concentration and then diluted in culture medium to a range of concentrations from 1.56 to 50 mM·L^{-1}. A maximum of 0.5% (v/v) concentration of DMSO was utilized to mitigate toxicity. Cells (5×104) were seeded in each well of a 96-well plate with 100 μL RPMI medium supplemented with 10% FBS and incubated for 24 hours before adding the compound dilutions (doubled) from 0 to 50 μM. After 12 hours, 10 μL MTT was added to each well followed by a 4-hour incubation period and subsequent addition of 100 μL DMSO to dissolve the formazan blue. OD at 490 nm was measured using an ELISA plate reader to estimate cell viability. The IC50 value represents the compound concentration that inhibited 50% of cell growth. The mean of three test repetitions was recorded to determine the cell survival curve for each cell line.

2.5. Synthesis

2.5.1. Synthesis of Polyamine (A). The octadentate Schiff base ligands were prepared based on the reported method in the literature [29]. Pyridine-2-carbaldehyde (2.14 g, 20 mmol) was dissolved in dry EtOH (30 mL), and 1,3-diamine propane (0.74 g, 10 mmol) was added to the warm solution. The mixture was refluxed for 24 h with stirring and allowed to cool to room temperature. The addition of solid sodium borohydride (3 g, 80 mmol) followed, and the reaction mixture was refluxed for 2 h. After filtration, rotary evaporation reduced the filtrate volume to 20 mL, excess water was added, and the product was extracted with chloroform ($3 * 25$ mL). The combined chloroform solutions were separated and dried over magnesium sulfate. Rotary evaporation removed the chloroform, resulting in yellow oil (89–91%). The product (9 mmol) was dissolved in acetonitrile (20 mL), followed by the addition of K2CO3 (3.7 g, 27 mmol), and the mixture was refluxed. A solution of 2-nitrobenzyl chloride (3.08 g, 18 mmol) in acetonitrile (40 mL) was added, and the mixture was refluxed for 48 h, filtered, and allowed to cool to room temperature. Water was added to the dried solvent, and the product was extracted with chloroform ($3 * 25$ mL) to yield an orange oil. yield: (95.8%); IR (Nujol mull, cm^{-1}) 1526, 1360 υ (NO_2). Furthermore, in order to prepare the desired solution, consisting of 20 mL water and 300 mL ethanol, our objective was to incorporate a suspension of the dinitro intermediate (8 mmol, 4.2 g). The resulting mixture was heated to boiling to dissolve the material. Next, we added ammonium chloride (6.95 g) and heated the mixture to dissolve the substance. Subsequently, we introduced zinc powder (13.07 g) slowly over a period of 1.5 hours into the heated solution. The solution was then refluxed for further 12 hours. To remove the catalyst, the reaction mixture was filtered with celite. Once the solvent was dried, we added 50 mL water and used chloroform (3×50 mL) to extract the product. Finally, we filtered the combined extracts using rotary evaporation and dried them with magnesium sulfate. As a result, a beige

powder diamine compound was obtained (Scheme 1). yield: (95%); IR (KBr, cm−1). 3423, 3327, 3022, 2924, 1606, 1590, 1495, 1458, 1370, 1312, 1281, 1155, 1048, 997 υ, 1H NMR (400 MHz, CDCl3); 1.63-4.2 (H Aliphatic), 4(4H, NH2), 7.16-8.65 (H Aromatic), ppm, 13C NMR (400 MHz, (CDCl3); 25.12, - 61.51(C Aliphatic), 120.64 - 158.5 (C aromatic) ppm; Anal. Calculated for C29H34N6 : C, 74.65%; H, 7.34%; N, 18.01%. Found C, 74.62%; H, 7.38%; N, 17.97% ; EI-MS: (m/ z): 466.54.

2.5.2. Synthesis of the Schiff Base Ligands (L_1 and L_2)

(1) Synthesis of L_1. We aimed at adding polyamine (A) (0.46 g, 1 mmol) in ethanol (20 ml) to a solution of 2-formylpyridine (0.214 g, 2 mmol) in ethanol (30 ml). Stirring and refluxing the mixture continued for 24 h, after which a brown precipitate was obtained, which was then filtered off and washed with cold methanol and dried in vacuo (Scheme 2). Yield: 0.46 g (71%). Anal. Calc. for $C_{41}H_{40}N_8$ (M. W: 644.81): C, 76.37; H, 6.25; N, 17.38. Found: C, 76.32; H, 6.21; N, 17.33%. ESI-MS (m/z): 644.78, IR (KBr, cm^{-1}): 1631 m(C=N), 1568, 1589 $[m(C=C)$ and $m(C=N)]_{Py}$. ^1H NMR ($CDCl_3$, ppm) dH = 1.71–3.95 (s, $H_{Aliphatic}$), 6.58–8.42 (m, H_{Aro}), 8.6 (s, H_{imin}). ^{13}C NMR ($CDCl_3$, ppm) dc = 29.67–60.24($C_{Aliphatic}$), 115.41–149.56 (C_{Aro}), 160.11 (C_{imin}), UV-Vis in DMSO (λ, nm) 266, 310,400.

(2) Synthesis of L_2. In the same manner, our objective was to combine a polyamine (A) (0.46 g, 1 mmol) dissolved in an ethanol solution (20 mL) with a solution of 2-Hydroxybenzaldehyde (0.24 g, 2 mmol) in ethanol (30 mL). After stirring, the mixture was heated to reflux for 4 h. A yellow solid was formed which was then filtered off, washed with cold methanol, and dried in vacuo (Scheme 2). Yield: 0.55 g (82%). Anal. Calc. for $C_{43}H_{42}N_6O_2$ (M. W:674.83): C, 76.53; H, 6.27; N, 12.45. Found: C, 76.50; H, 6.31; N, 12.40%. ESI-MS (m/z): 674.79. IR (KBr, cm^{-1}): 1614 m(C=N), 1565 m(C=C), 3419 m(OH). ^1H NMR ($CDCl_3$, ppm) dH = 1.68–4.55 (m, $H_{aliphatic}$), 6.49–8.35 (m, H_{Aro}), 8.45 (s, H_{imin}), 13.12 (s, 2H(OH)). ^{13}C NMR ($CDCl_3$–, ppm) dc = 28.36–58.64 ($C_{aliphatic}$), 116.01–148.91 (C_{Aro}), 161.06 (C-OH), 162.95 (C_{imin}), UV-Vis in DMSO (λ, nm) 269, 317,410.

2.5.3. Synthesis of Metal Complexes. To prepare the metal complexes, the ligand (either L_1 or L_2) was first dissolved in 25 ml of methanol. This solution was then added to a solution of $M(ClO_4)_2.6H_2O$ (where M is either Ni2+ or Zn2+) dissolved in 20 ml of methanol, at a molar ratio of 1 : 1. The resulting solution was refluxed for 24 hours and then cooled to room temperature. The complexes were then filtered to remove any unreacted metal salts and washed with excess ethanol before being dried under vacuum. It should be noted that the resulting products were pure compounds, as confirmed by their characterization (Scheme 3).

$[NiL_1](ClO_4)_2$. Green powder. Yield: 0.57 g (63%). Anal. Calc. for $C_{41}H_{40}Cl_2NiN_8O_8$ (M. W: 902.4): C, 54.57; H, 4.47; N, 12.42. Found: C, 54.53; H, 4.42; N, 12.38%. IR (KBr, cm^{-1}): 1623 m (C=N), 1597, 1445 [m (C=N) and $m(C=C)]_{Py}$, 1091, 623 $m(ClO_4^-)$. ESI-MS (m/z): 901.98, UV-Vis in DMSO (λ, nm) 271, 312,411,490. $\wedge$m:($SCm^2{\cdot}mol^{-1}$): 196.

$[ZnL_1](ClO_4)_2$. Light yellow powder. Yield: 0.54 g (59%). Anal. Calc. for $C_{41}H_{40}Cl_2ZnN_8O_8$ (M. W: 909.2): C, 54.17; H, 4.43; N, 12.33. Found: C, 54.23; H, 4.40; N, 12.37%. IR (KBr, cm^{-1}): 1619m(C=N), 1571, 1599 $[m(C=C)$ and $m(C=N)]_{Py}$, 1096, 623 $m(ClO_4^-)$. ^{1}HNMR (DMSO-d_6): 1.42–4.28 ($H_{aliphatic}$), 6.76-8.47($H_{aromatic}$), 8.63 (H_{imin}), ^{13}CNMR (DMSO-d_6): 19.48-65.81($C_{aliphatic}$), 119- 157.1($C_{aromatic}$),162.8 (C_{imin}) (ESI-MS (m/z): 908.97, UV-Vis in DMSO (λ, nm) 274, 314,421,493, $\wedge$m:($SCm^2{\cdot}mol^{-1}$): 180.

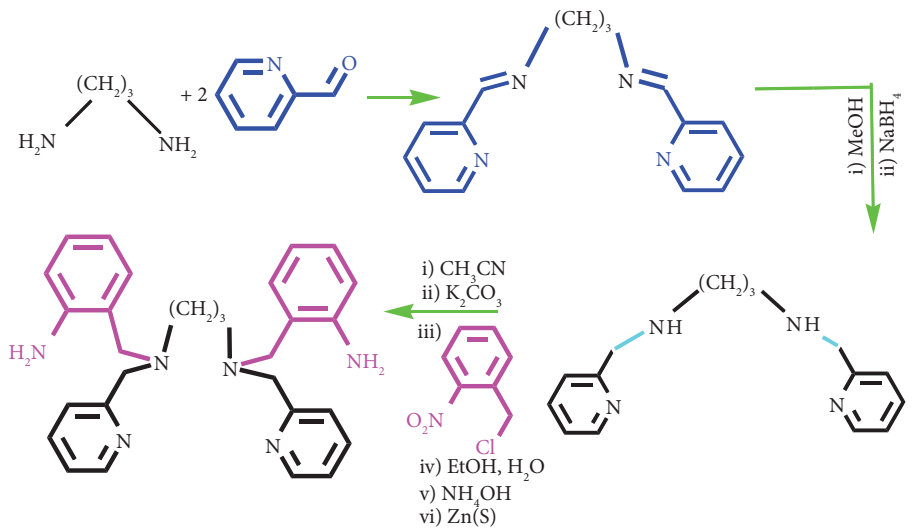
$[NiL_2](ClO_4)_2$. Green powder. Yield: 0.6 g (64%). Anal. Calc. for $C_{43}H_{40}Cl_2N_6NiO_{10}$ (F. W: 930.41): C, 55.51; H, 4.33; N, 9.03. Found: C, 55.48; H, 4.38; N, 8.97%. IR (KBr, cm^{-1}): 1620 m (C=N), 1594, 1445 $[m(C=N)$ and $m(C=C)]_{Py,}$ 1090, 623 (ClO_4^-). ESI-MS (m/z): 930.37, UV-Vis in DMSO (λ, nm) 275, 316,414,496, $\wedge$m: ($SCm^2{\cdot}mol^{-1}$): 183.

$[ZnL_2](ClO_4)_2$. Yellow powder. Yield: 0.67 g (71%). Anal. Calc. for $C_{43}H_{40}Cl_2N_6O_{10}Zn$ (F.W: 937.11): C, 55.11; H, 4.30; N, 8.97. Found: C, 55.14; H, 4.33; N, 8.92%. IR (KBr, cm^{-1}): 1621 m(C=N), 1592, 1444 [m(C=N) and m(C=C)], 1096, 622 (ClO_4^-), ^{1}HNMR (DMSO-d_6): 1.43–4.3 ($H_{aliphatic}$), 6.68–8.42($H_{Aromatic}$), 8. 66 (H_{imin}), 12.9 (s, 2H(OH)). ^{13}CNMR(DMSO-d6): 20.68–64.70 ($C_{Aliphatic}$), 117–157.6 ($C_{Aromatic}$), 162.01 (C-OH), 163.8 (C_{imin}). ESI-MS (m/z): 937.07, UV-Vis in DMSO (λ, nm) 276, 318, 416, 499, $\wedge$m: ($SCm^2{\cdot}mol^{-1}$): 190.

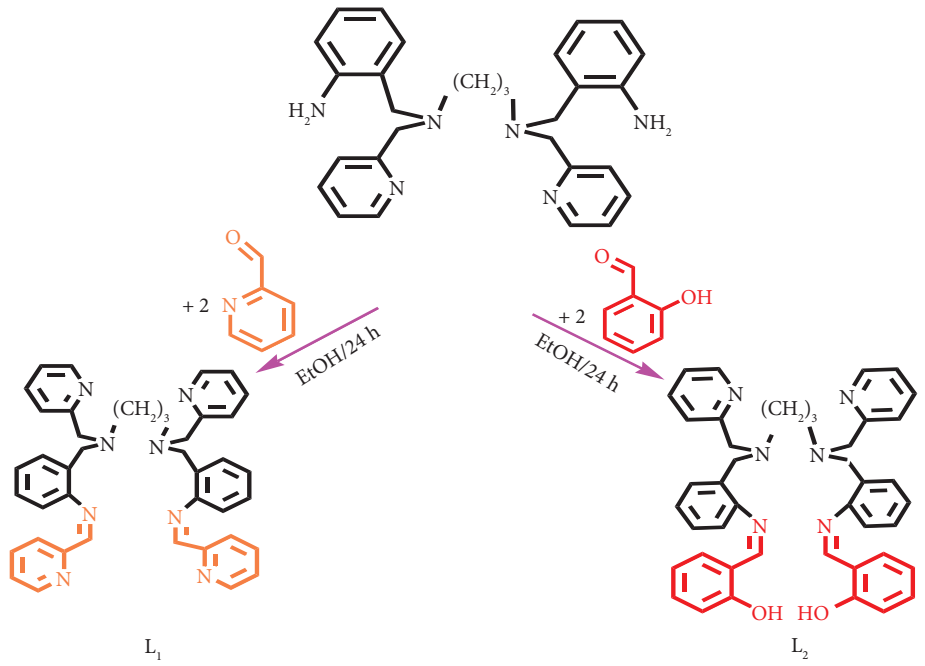
3. Results and Discussion

Our objective was to synthesize two Schiff base ligands through the condensation of polyamines with 2-formylpyridine and 2-hydroxybenzaldehyde in a 1 : 2 ratio. The structural formulas of L_1 and L_2 were confirmed by testing through elemental analysis, IR, 1H, and 13C NMR spectroscopy, all of which yielded results in agreement with the intended compounds. The infrared spectra of the ligands displayed a noticeable absorption band at around 1631–1633 cm^{-1}, indicating the formation of Schiff base ligands due to the C=N stretching vibration. Furthermore, the presence of C=N stretching vibration and the absence of C=O and NH_2 stretching vibrations suggest that the Schiff base condensation is consistent [30].

Octadentate N8 and N6O2 Schiff base ligands (L_1 and L_2, respectively) were synthesized through the condensation of 2-formylpyridine or 2-hydroxybenzaldehyde. Mass spectra were utilized to confirm the synthesis of ligands (L_1 and L_2), with peaks observed at 644.78 and 674.83 m/z. A mixed ethanolic and methanolic solution was used in an equimolar ratio (1 : 1) to directly react the ligands and metal salts, producing Ni(II) and Zn(II) perchlorate. The complexes were characterized through mass spectrometry, IR spectroscopy, and elemental analysis, with infrared spectra consistent with imine nitrogen coordination to the metal ions, attributed to m (C=N), and shifted towards lower



Scheme 1: The processes of synthesis of polyamine.

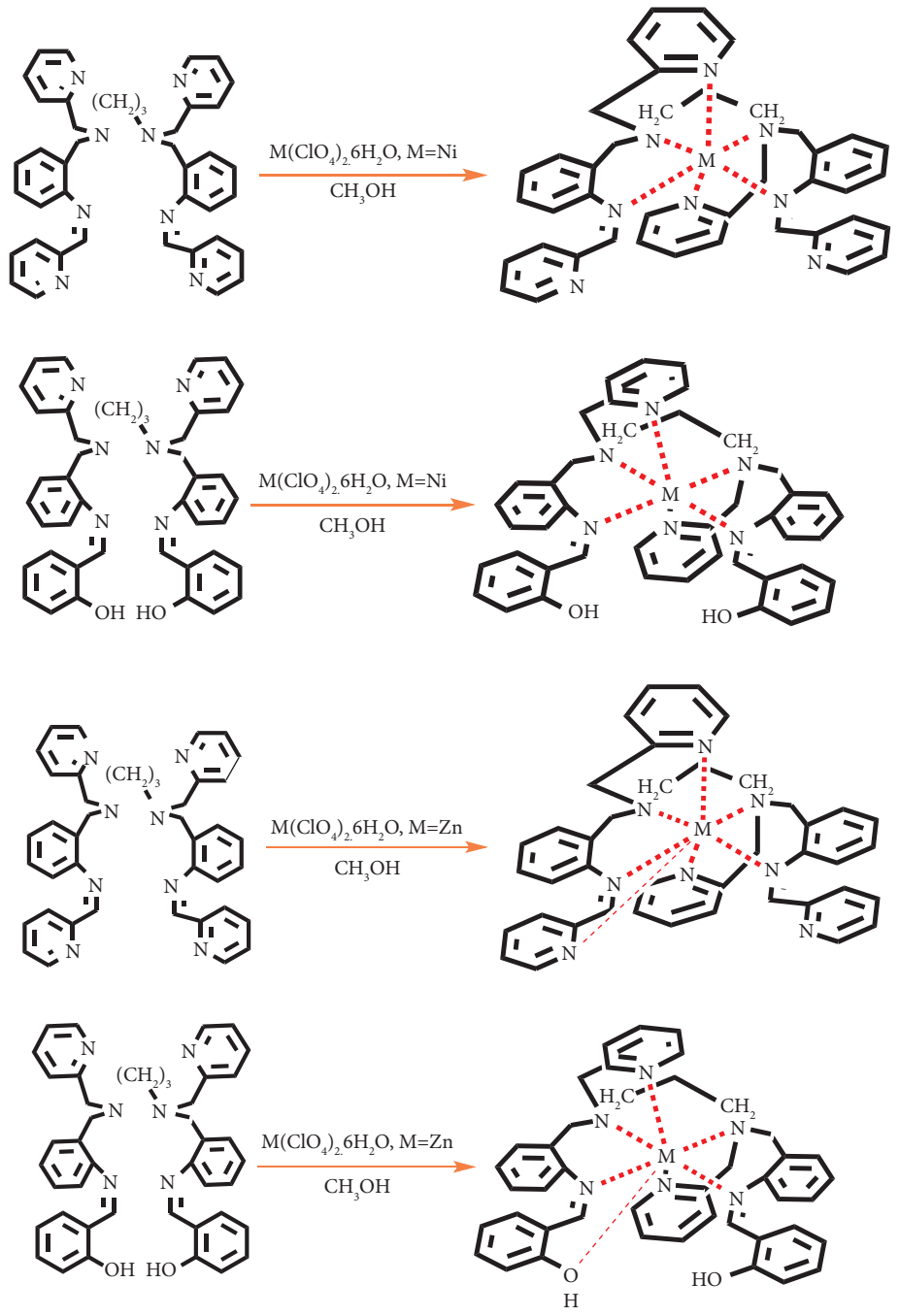


Scheme 2: The processes of synthesis of L_1 and L_2.

frequencies. The absence of NH_2 stretching vibrations and C=O indicated the occurrence of Schiff base condensation. O-H stretching bands were seen in the IR region of 3400–3300 cm^{-1} for [L_2] complexes, confirming that deprotonation did not occur [31, 32].

Additionally, the presence of perchlorate anions at 1100 and 623 cm^{-1} and the non-split perchlorate bands indicate that the perchlorate ions were not coordinated to the metal ion [33]. The ligand and complexes exhibit solubility in most organic solvents. Based on previous studies and elemental analysis, Ni-complexes exhibit a distorted octahedral geometry, while Zn-complexes exhibit a pentagonal bipyramidal geometry as depicted in Table 1. Furthermore, it is noteworthy that these compounds remain stable in air.

3.1. IR Spectral Data. IR spectrum of the Schiff base ligand (L_2) illustrates a band at 3419 cm^{-1} which is present in the complexes. Besides, it shows that coordination did not occur through the deprotonated phenolic OH groups. IR spectrum of the Schiff base ligands (L_1 and L_2) reveals an absorption band at 1631 and 1633 cm^{-1} which can be related to the C=N stretching mode. Shifting toward lower frequencies by 8–13 cm^{-1}, it would reveal the coordination of the imine nitrogen atom to the metal. Accordingly, weak bands at 523–568 cm^{-1} can be assigned to the ν(M-N) vibrations, supporting the participation of the nitrogen atom of the azomethine group of the ligand. Besides, the absence of C=O and NH_2 stretching vibrations would be connected to the aldehyde and diamine, which can be the result of Schiff base



Scheme 3: The processes of synthesis of metal complexes: L_1 and L_2.

Table 1: The elemental analysis data of complexes and the ligands.

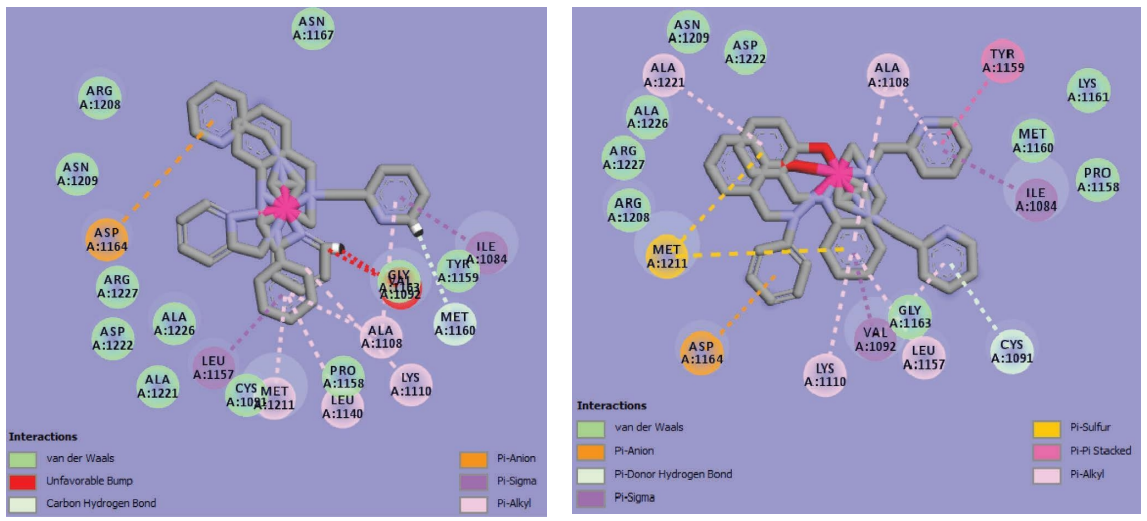
Compound	Yield (%)	% found (calc)		
		C	H	N
L_1	71	76.32 (76.37)	6.21 (6.25)	17.33 (17.38)
$[NiL_1](ClO_4)_2$	63	54.53 (54.57)	4.42 (4.47)	12.38 (12.42)
$[ZnL_1](ClO_4)_2$	59	54.23 (54.17)	4.40 (4.43)	12.37 (12.33)
L_2	82	76.50 (76.53)	6.31 (6.27)	12.40 (12.45)
$[NiL_2](ClO_4)_2$	64	55.48 (55.51)	4.38 (4.33)	8.97 (9.03)
$[ZnL_2](ClO_4)_2$	71	55.14 (55.11)	4.33 (4.30)	8.92 (8.97)

Table 2: Tentative assignment of the significant infrared bands of the synthesized compounds.

	V (cm^{-1})			
	V (O-H)	V (C=N)	V (M-N)	V (C=N)py
L_1	—	1631	—	1589
$[NiL_1](ClO_4)_2$	—	1623	523	1597
$[ZnL_1](ClO_4)_2$	—	1619	547	1599
L_2	3419	1633	—	—
$[NiL_2](ClO_4)_2$	3410	1620	535	—
$[ZnL_2](ClO_4)_2$	3412	1621	568	—

Table 3: Docking results (kJ/mol) of the synthesized complexes.

Synthesized complexes	Receptors	
	3DKG	4ZXT
Zn-L_1	−120.494	−139.6
Ni-L_1	−95.74	−93.46
Zn-L_2	−100.62	−99.02
Ni-L_2	−82.33	−85.25



(a) (b)

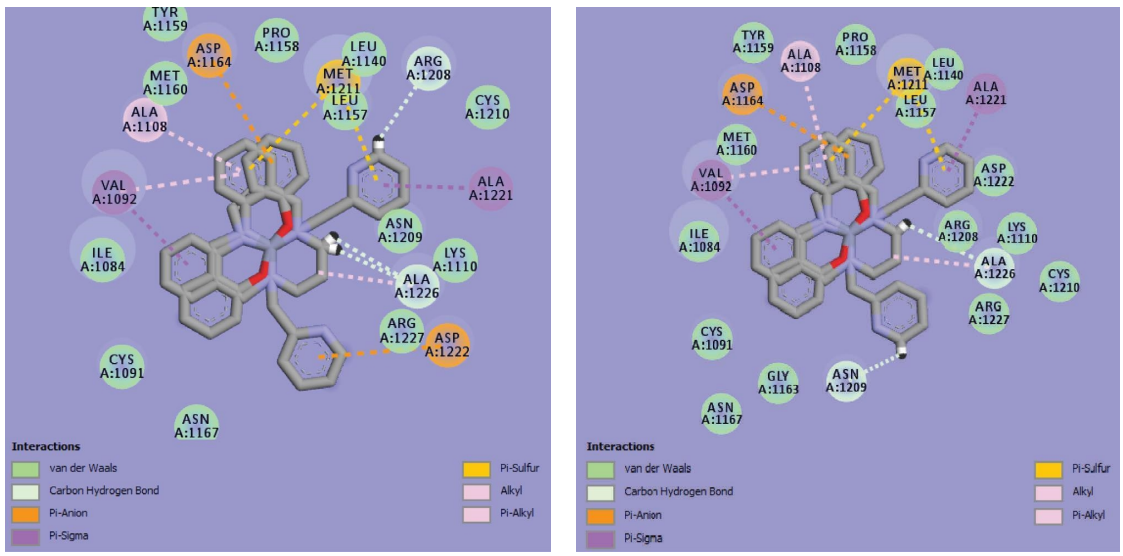
Figure 1: Interactions and binding residues of MET receptor tyrosine kinase (PDB ID: 3DKG) with (a) Ni-L_1 and (b) Ni-L_2.

condensation regarding the IR region of 3400–3300 cm^{-1} for $[L_2]$ complexes, and the presence of O-H stretching bands did not result in deprotonation of phenolic functions. Besides, some special adsorptions were found at 1100 and 620 cm^{-1} and also perchlorate bands did not split, showing that the coordination of perchlorate ions to metal ion did not occur (Table 2) [34, 35].

3.2. NMR Spectra. The 1H NMR spectra of both Schiff base ligands, L_1 and L_2, show a single 1H imine resonance at 8.45 and 8.52 ppm, indicating the equivalence of the imine environments. For ligand L_2, the OH group peak appears at 13.12 ppm, but in the Zn-L_2 complex, the OH group peak at 12.9 ppm confirms that deprotonation of phenolic functions did not occur. The displacement of the phenolic group peak in the zinc complex is attributed to the attachment of one oxygen atom of the phenolic group to the metal. According to the proposed structure, only one phenolic group can attach to the metal. In the 13C NMR spectra, the imine carbon atoms (~160.0 ppm) are equivalent and appear in the region matching the signals of the aromatic ring carbons (115–149 ppm).

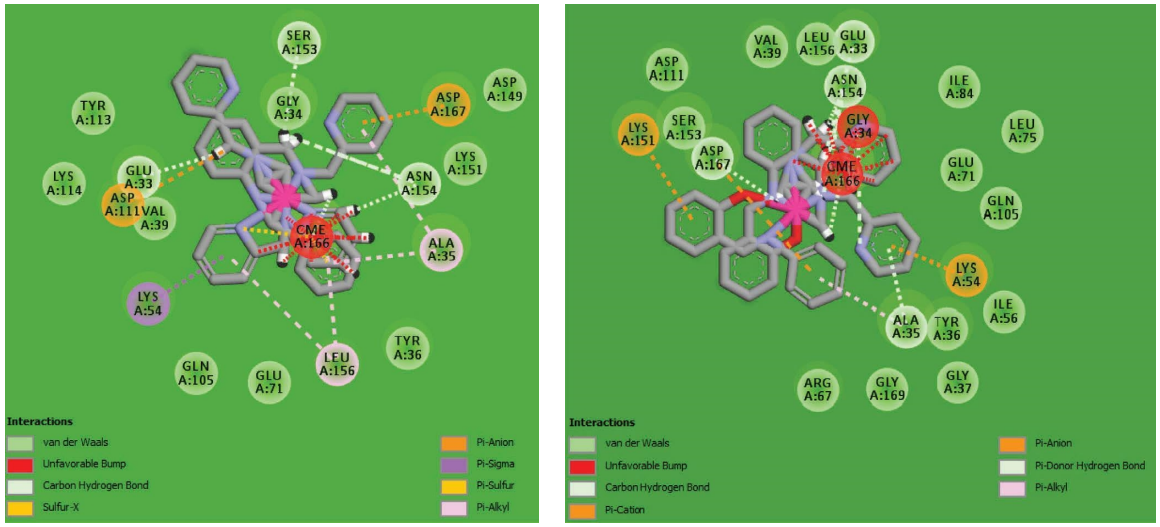
3.3. Molar Conductivity. The molar conductivity (ʌM) data were determined at 25°C using 10^{-3} M solutions of the complexes in CH3CN solvent. The value of molar conductivity (ʌM) of complexes (180–196 SCm^2·mol^{-1}) indicates that these compounds are electrolytes because of the presence of at least two counter ions in their structures [36].

3.4. UV-Visible. The UV-visible absorption spectra of the ligands and its complexes were measured in DMSO solutions at room temperature. The ligands displayed intense



(a) (b)

Figure 2: Interactions and binding residues of MET receptor tyrosine kinase (PDB ID: 3DKG) with (a) Zn-L_1 and (b) Zn-L_2.



(a) (b)

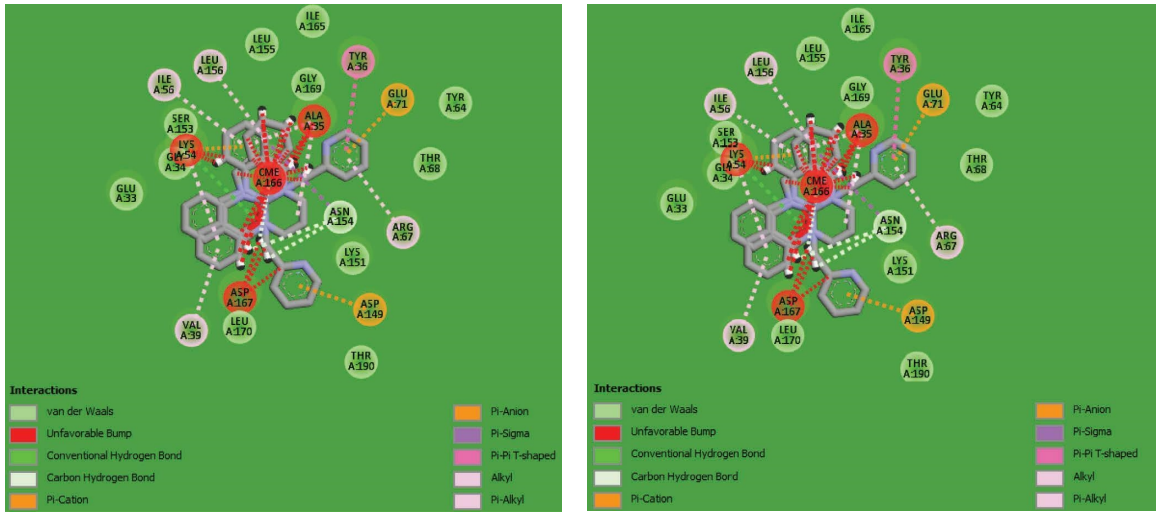
Figure 3: Interactions and binding residues of MET receptor ERK2 (PDB ID: 4ZXT) with (a) Ni-L_1 and (b) Ni-L_2.

bands at 266 and 269 nm, corresponding to $\pi \longrightarrow \pi^*$ transitions of the azomethine, and a peak at 310 and 317 nm, attributed to $n \longrightarrow \pi^*$ transition. These transitions were still prominent in the spectra of the complexes, albeit with a slight shift in position. Additionally, a charge transfer band at 400–454 nm was observed in both the ligands and complexes, while the complexes exhibited a band at 490–499 nm, indicative of MLCT transitions.

3.5. Molecular Docking Studies. The MVD program was utilized to carry out molecular modeling for biological assessment. Our objective was to remove the individual ligands and water molecules from the receptor structure. The anticancer property of the complexes was determined using the MolDock Score. According to Table 3, the Zn-L_1 complex exhibits the most significant inhibitory impact against 3DKG (–120.494 kJ/mol) and 4ZXT (–139.6 kJ/mol) receptors.

Also, binding residues and interactions of MET receptor tyrosine kinase (PDB ID: 3DKG) and ERK2 (PDB ID: 4ZXT) with complexes has been shown in (Figures 1–4). Different interactions, i.e., pi-sigma, pi-alkyl, pi-donor hydrogen bond, van der Waals, alkyl, pi-sulfur, carbon hydrogen bond, and pi-anion, can be identified from the docking results [37–40].

3.6. Cytotoxicity In Vitro. Synthesized compounds were evaluated for cytotoxicity profiling using the MTT assay on lung (A549) and breast (MCF-7) cell lines (Table 4). Cell



(a) (b)

Figure 4: Interactions and binding residues of MET receptor ERK2 (PDB ID: 4ZXT) with (a) Zn-L_1 and (b) Zn-L_2.

Table 4: Comparison of cytotoxicity (IC_{50} values based on μM).

Structure	MCF-7	A549
Zn-L_1	7.48 ± 0.24	8.15 ± 0.6
Ni-L_1	12.16 ± 0.79	13.63 ± 0.91
Zn-L_2	8.37 ± 1.09	9.07 ± 1.02
Ni-L_2	14.49 ± 1.4	15.33 ± 1.85

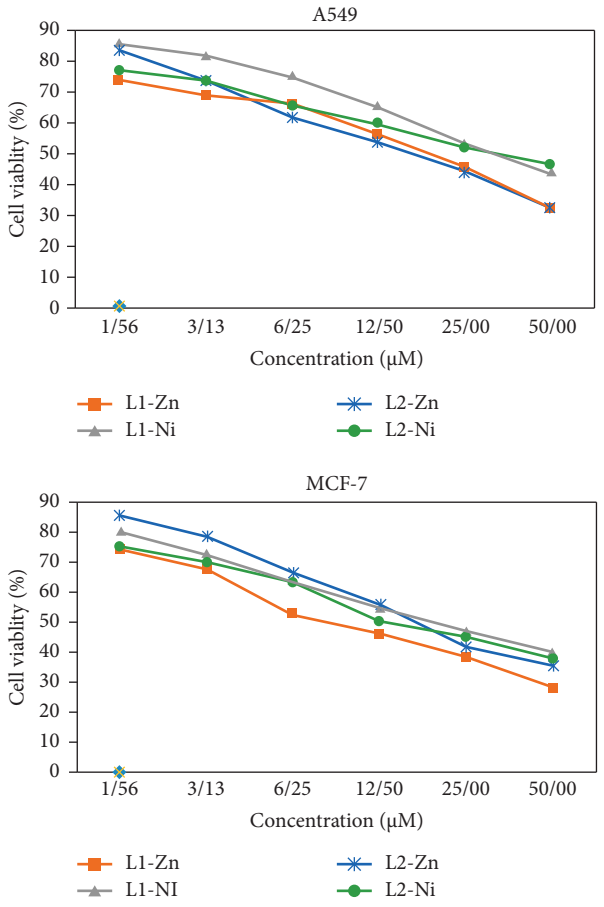


Figure 5: Graphical representation of the anticancer activity of the synthesized complexes.

viability was analyzed by measuring the reduction of yellow MTT through mitochondrial dehydrogenase enzymatic activity. Figure 5 displays cell viability percentages and IC50 values, indicating the dose needed to destroy 50% of the cells. The L_1-Zn and L_2-Zn complexes exhibited high efficiency in inhibiting cell growth. Comparing IC50 values revealed that MCF-7 cells were more chemosensitive than A549 cells. The L_1-Zn complex displayed extreme cytotoxicity, with an average IC50 value of $<8\,\mu$M, exceeding that of other complexes. Our study focused on the compounds' cytotoxic potency on MCF-7 cells due to the high prevalence of breast cancer as a leading cause of death. The L_1-Zn complex had greater toxicity properties than other complexes, with an IC_{50} value of 7.48. Lung cancer is a significant public health problem, causing millions of deaths and new cases annually. The L_1-Zn complex exhibited more toxicity with an IC50 value of $8.15\,\mu$M. [41, 42].

4. Conclusions

In this study, we propose the synthesis of octadentate N8 and N6O2 Schiff base ligands, named L_1 and L_2, respectively. These ligands are derived from the condensation of 2-formylpyridine or 2-hydroxybenzaldehyde. Our objective was to produce Ni(II) and Zn(II) perchlorate by directly reacting metal salts with equimolar ratios of ligands in a mixed methanolic and ethanolic solution. To characterize these complexes and ligands, we employed IR spectroscopy, mass spectrometry, and elemental analysis. In addition, we investigated the cytotoxic properties of the synthesized complexes using in vitro studies and molecular docking. Remarkably, we obtained similar results using both methods, with the Zn-complexes showing greater effectiveness against cell lines than the Ni-complexes. Employing Molegro Virtual Docker software, we calculated and predicted the interactions and binding affinity of the synthesized compounds. Based on the results, Zn-L_1 had the highest MolDock Score compared to other complexes. Overall, the order of the cytotoxic properties of the compounds in both methods was as follows: Zn-L_1 > Zn-L_2 > Ni-L_1 > Ni-L_2.

Data Availability

The data used to support the findings of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Hajari. Saadat, Keypour. Hassan, and Rezaei. Mohammadtaher conducted the main manuscript text writing, material preparation, data collection, and analysis. Jamshid. mahdi carried out the molecular docking studies, while Moazzami Farida. Seyed Hamed prepared the in vitro cytotoxicity activity. The manuscript was reviewed by all authors.

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