

An efficient synthesis of Zinc(II) complexes for possible viral applications

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Abstract : An efficient synthesis and design of three new zinc(II) complexes viz. $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4$ (**1**), $[\text{Zn}(\text{L})(\text{phen})]\text{ClO}_4$ (**2**) $[\text{Zn}(\text{L})_2]$ (**3**), (where, L= (Z)-N-phenyl-N'-((Z)-phenyl (pyridin-2-yl) methylene) carbamohydrazonic acid; en= ethylenediamine, bipy= 2,2'-bipyridyl, phen=1,10-phenanthroline) are described. These complexes were systematic characterized by various physicochemical and spectroscopic techniques. The Zn(II) complexes (**1**)-(**3**) were also evaluated through molecular docking assessment for their biological application theoreof and the results were used for various disease of the the viruses.

(Keywords : Zinc complexes. Efficient synthesis, viruses. Computational modelling)

Introduction

The identification of novel therapeutics against the pandemic Omicron variant of the SARS-CoV-2 virus is an indispensable new address of current scientific research^{1, 2}. The SARS-CoV-2 uses the spike protein receptor-binding domain (RBD) to bind it cognate receptor, ACE2, and initiate membrane fusion. Thus RBD is a key component in viral replication of the virus and an ideal target for vaccine developments³. Zinc deficiency is linked to poor prognosis in COVID-19 patients. Recently, the hypotheses have been explored that zinc ions or its salts or compounds, long used as medicinal supplement and known to support immune function, Zn^{2+} binds to active site and inhibits SARS CoV-2 main protease (M^{pro}) enzyme activity⁴. Structural data on the SARS-CoV-2

main protease (M^{pro}) in complex with a zinc containing inhibitor are already present in the literature and gave hints on the presence of a zinc binding site and its ability to inhibit the SARS-CoV-2 main protease⁵. Moreover, zinc plays an important role in strengthening the body's immune system, regulation of gene function and protein recognition⁶. Several application explorations on molecular targets and potential inhibition by Zn^{2+} ions for effective therapeutics against SARS-CoV-2 Omicron variants or other emerging viral disease have been explored⁷.

The Zn(II) coordination compounds possessing tridentate Schiff base and N-donor bidentate ligands (ethylenediamine, 2,2'-bipyridyl, and 1,10-phenanthroline) have been studied as anticancer and antiviral agents^[25-27]. The Schiff base derived from the condensation of 4-phenylsemicarbazide and 2-benzoylpyridine, may act as tridentate-NNO donor ligands. Herein, an efficient synthesis and design of three new zinc(II) complexes viz. $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4$ (**1**), $[\text{Zn}(\text{L})(\text{phen})]\text{ClO}_4$ (**2**) $[\text{Zn}(\text{L})_2]$ (**3**), (where, L= (Z)-N-phenyl-N'-((Z)-phenyl (pyridin-2-yl) methylene) carbamohydrazonic acid; en= ethylenediamine, bipy=2,2'-bipyridyl, phen=1,10-phenanthroline) are described. These complexes were systematic characterized by various physicochemical and spectroscopic techniques. The Zn(II) complexes (**1**)-(**3**) were also evaluated through molecular docking assessment for their biological application theoreof and the results were used for various disease of the the viruses.

2. Experimental methods

2.1. General materials and physical measurements

All air sensitive reactions were carried on a double manifold Schlenk vacuum line under N_2 with the resulting compounds stored under N_2 or Ar in a glovebox. All the solvents were freshly purified by an MBraun Manual solvent purification system packed with Alcoa F200 activated alumina desiccant. Reagents were purchased from commercial sources and used as received. All the solvents used for the synthesis, $Zn(ClO_4)_2 \cdot 6H_2O$, 4-phenylsemicarbazide, 2-benzoylpyridine, ethylenediamine, 2, 2'-bipyridyl, 1,10-phenanthroline were obtained from Sigma-Aldrich and were used without further purification. Various spectroscopic methods (NMR, FT-IR, FT-Raman, LC-MS, and UV-Vis and fluorescence spectroscopy) and electro chemical measurements were used to analyse the newly Zn(II) complexes (1)-(3). Elemental analytical data and quantum chemical calculations were also applied for these purposes. Microanalyses were carried out using an Elementar Vario EL III Carlo Erba 1108 elemental analyser. The LC-MS spectra were recorded with a water alliance E2695 / HPLC-TQD mass spectrometer (ESI/ LC-MS). The FT-IR spectra were recorded on a Perkin Elmer system with a FTIR/ATR spectrophotometer in the spectral range 4000-450 cm^{-1} using KBr pellets and the electronic spectra were taken on a Thermo scientific UV-Vis recording spectrophotometer Evolution-3000 in quartz cells. Fluorescence spectra were recorded at room temperature on a Horiba Scientific Fluoromax-4 spectrofluorometer in quartz cells. Melting point was measured on a Boetius micro melting point apparatus. NMR spectra were recorded on a Bruker Ultrashield 500 plus 500 MHz FT-NMR Spectrometer.

2.2. Synthesis procedure

2.3. Synthesis of Schiff base ligand

A Schiff base (L) ligand was synthesized as red coloured crystalline products by the

refluxing 4-phenylsemicarbazide (20.0 mmol, 3.02 g) with 2-benzoylpyridine (20.0 mmol, 3.66 g) in equimolar ratio 1:1 in EtOH for 8 hours. The red coloured crystalline product was obtained, which was filtered, washed with methanol and diethyl ether (2×5 mL), and stored in a desiccator over $CaCl_2$. Colour: Orange; Yield: 80%; M.p.: > 180°C. ^1H -NMR (600 MHz, $CDCl_3$): δ 11.97 (1H, s, -NH-), δ 10.04 (1H, s, Ar-NH-), δ 8.84 (1H, d, Ar-CH-N-), δ 7.70- 7.18 (10H, m, Ar-H), ^{13}C { ^1H } NMR (600 MHz, $CDCl_3$): 176.71, 166.89, 166.72, 145.45, 138.27, 133.68, 130.86, 128.66, 128.55, 128.38, 127.58, 126.49, and 125.69 ppm. Representative NMR spectra was given in Figure S1 and S2. FT-IR (KBr/cm^{-1}): 3280 (ν N-H), 2870 (ν O-H), 1638 ($>C=N$); 1375 (ν ArC=C). UV-Vis λ (nm): 311 (in EtOH). Fluorescence data (λ_{em}): 320, 350 (in EtOH). Elemental analyses: Anal. Calc. for $C_{19}H_{16}N_4O$, HL (%): C, 72.13, H, 5.10; N, 17.71. Found (%): C, 72.14; H, 5.11; N, 17.72.

2.4. Synthesis of $[Zn(L)(bipy)]ClO_4$ (1)

The synthetic procedure for Zn(II) complex $[Zn(L)(bipy)]ClO_4$ (2) was the same as mentioned above Zn(II) complex $[Zn(L)(en)]ClO_4$ (1) only with the difference of 2,2-bipyridyl (1.0 mmol, 0.156 g) in the place of ethylenediamine. In this case, we obtained a black and brown-colour precipitate, which was then filtered. The obtained products were washed with MeOH and then washed with diethyl ether (2×5 mL) and finally dried in vacuo. Yield: 75-80 %. M.p. 280-290 °C. FT-IR (KBr/cm^{-1}): 3387 (ν N-H), 2970 (ν O-H), 1655 ($>C=N$); 1411 (ν ArC=C), 881 (ν Zn-N), 660 (ν Zn-O). FT-Raman: 2839, 2802, 1656 and 1413 cm^{-1} . UV-Vis λ (nm): 308, 390 (in EtOH), 310, 389 (in MeOH), 309, 390 (in CH_3CN), 310, and 390 (in DMSO). Fluorescence data (λ_{em}): 319, 353, 402 (in EtOH). Elemental analyses: Anal. Calc. for $C_{29}H_{23}ClN_6O_5Zn$ (1) (%): C, 54.74; H, 3.64; N, 13.21. Found (%): C, 54.75; H, 3.65; N, 13.22. LC-MS: Obs. (Calcd.) 635.07 (634.37 g/mol.).

2.5 Synthesis of $[Zn(L)(phen)]ClO_4$ (2)

The synthetic procedure for Zn(II) complex $[\text{Zn}(\text{L})(\text{phen})]\text{ClO}_4$ (**2**) was the same as mentioned above Zn(II) complex $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4$ (**1**) only with the difference of 1,10-phenanthroline (1.0 mmol, 0.180 g) in the place of 2,2-bipyridyl (1.0 mmol, 0.156 g). In this case, we obtained a yellow-colored precipitate, which was then filtered. The obtained products were washed with MeOH and then washed with diethyl ether (2×5 mL) and finally dried in vacuo. Yield: 75-80 %. M.p. 280-290 °C. FT-IR (KBr/cm⁻¹): 3380 (νN-H), 2932 (5011PO-H), 1655 (>C=N); 1410 (νArC=C), 861 (νZn-N), 660 (νZn-O). FT-Raman: 2933, 2834, 1643 and 1434 cm⁻¹. UV-Vis λ(nm): 295, 307, 391 (in EtOH), 295, 308, 390 (in MeOH), 296, 307, 391 (in CH₃CN), 295, 308, and 391 (in DMSO). Fluorescence data (λ_{nm}): 318, 352 (in EtOH). Elemental analyses: Anal. Calc. for C₃₁H₂₃ClN₆O₅Zn (**2**) (%): C, 56.38; H, 3.51; N, 12.73. Found (%): C, 56.39; H, 3.52; N, 12.74. LC-MS: Obs. (Calcd.) 659.07 (658.07 g/mol.).

2.6. Synthesis of $[\text{Zn}(\text{L})_2]$ (**3**)

A MeOH solution (10 mL) of Schiff base (L) ligand (2.0 mmol, 0.632 g) was gradually added to another MeOH solution (10 mL) of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (1.0 mmol, 0.372 g). The solution was initially stirred at 80 °C and then subjected to reflux with constant stirring for 5 hours. After continuous stirring, a dark brown colour precipitate was obtained for the Zn(II) complex $[\text{Zn}(\text{L})_2]$ (**3**), and then filtered. The obtained product was washed with methanol, and store in a desiccator over CaCl₂. Yield: 80 %. M.p. 235 °C. FT-IR (KBr/cm⁻¹): 3410 (νN-H), 2932 (νO-H), 1651 (>C=N); 1438 (νArC=C), 880 (νZn-N), 660 (νZn-O). FT-Raman: 2940, 1658, 1410 and 1272 cm⁻¹. UV-Vis λ (nm): 295, 392 (in EtOH), 295, 393 (in MeOH), 295, 393 (in CH₃CN), 295, and 393 (in DMSO). Fluorescence data (λ_{nm}): 318, 352, 403, 429 (in EtOH). Elemental analyses: Anal. Calc. for C₃₈H₃₀N₆O₂Zn (**3**) (%): C, 65.57; H, 4.34; N, 16.10. Found (%): C, 65.58; H, 4.35; N, 16.11. LC-MS: Obs. (Calcd.) 695.18 (694.09 g/mol.).

3. Results and Discussion

3.1 An efficient synthesis and characterization

Scheme 1 represents proposed structures of the Schiff base Zn(II) complexes (**1**)-(**3**). The structural determination of the Zn(II) complexes (**1**)-(**3**) were ascertained by spectral studies such as FT-IR, FT-Raman, NMR, LC-MS, UV-Vis and fluorescence spectroscopy. The NMR spectra of free ligand (L) and the Zn(II) complexes (**1**)-(**3**) were documented using DMSO-d₆ as a solvent and spectral data are in accordance with their corresponding compositions. The ¹H-NMR spectra of free ligand (L) showed a singlet at 11.97 ppm, affirming the presence of -N-NH- group, which eventually confirms the formation of Schiff base ligand. The ¹³C-NMR results of the free ligand displayed a sharp characteristic peak due to azomethine carbon, which resonate at 166.89 ppm. Unlike free ligand, in the ¹H-NMR spectra of the Zn(II) complexes (**1**)-(**3**), the characteristic azomethine (-CH=N- group) peak at 8.56-8.59 ppm undergoes a negative shift in the Zn(II) complexes (**1**)-(**3**), demonstrating the coordination of Zn(II) towards azomethine nitrogen. Furthermore, multiple peaks for aromatic protons undergoes downfield shift, which further confirmed the coordination of ligands with Zn(II) centre.

The infrared spectral bands most useful for determining the mode of coordination of the Schiff base ligand (L) are U(C=N-), U(N-N) and U(C=O) vibrations. FT-IR spectra of the Zn(II) complexes (**1**)-(**3**) were recorded and presented in Figures S3-S5, respectively. The shift of the U(C=N-) bands of the free ligand to lower wavenumbers frequencies (1655-1658 cm⁻¹) in the IR spectra of the Zn(II) complexes support coordination of the ligand (L) to the metal atom via azomethine nitrogen atom. The ligand (L) showed U(C=O) mode at 1098 cm⁻¹. In the spectra of the complexes, the U(C=O) modes observed disappeared and consequently the U(C-O) mode is observed confirming that

complexation occurred. The presence of peaks at the region 2932-2934 cm^{-1} represents the vibrational modes of C-H groups in the IR spectrum of the Zn(II) complexes. The peaks at the region 3387-3410 cm^{-1} shows the presence of phenyl ring N-H stretching modes in the complexes. The broad band centred at the region 865-881 cm^{-1} for U(Zn-O) and 659-660 cm^{-1} for U(Zn-N) reveals the coordination of for U(C=O) and azomethine nitrogen for U(C=N) to the Zn(II) ions. The FT-Raman spectra of the Zn(II) complexes (1)-(3) show a broad band of azomethine group and $\delta(\text{C=O})$ at 1610-1656 and 1413-1454 cm^{-1} region, respectively. The phenyl ring C-H stretching modes are assigned at 2942 cm^{-1} in FT-Raman spectrum. The U(ClO_4) bands in Zn(II) complex (1), (2) and (3) are found at the range 1092-1096 cm^{-1} . Based on the above spectral evidences, it is confirmed that the free ligand (L) is tridentate-NNO, coordinating via pyridyl nitrogen, azomethine nitrogen and the carbonyl oxygen atoms. UV-Vis spectra of the Zn(II) complexes (1)-(3) were recorded in different solvents (EtOH, MeOH, CH_3CN and DMSO) in the concentration of 3×10^{-3} M at room temperature. Each complexes exhibits two bands in the region of 310-320 nm and 380-410 nm. The absorption band maxima exhibited by Zn(II) complexes in the range 310-320 nm are assigned to the $\pi-\pi^*$ transitions due to azomethine group (uC=N-). The other band maxima exhibited by the same complexes in the range 380-410 nm as a consequence of the $\pi-\pi^*$ transition of aromatic rings. These complexes do not show appreciable absorption in the region above 410 nm in different solvents used, in accord with the d^{10} electronic configuration of the Zn(II) ion, with the exception of MLCT maxima for complexes due to bipy and phen groups which are showing line broadening with a tail running into the visible part of the spectrum. The same behaviour have been observed in the electronic spectra of other similar five coordinated Zn(II) complexes reported in the literature⁶. The fluorescence spectra of the Zn(II) complexes were recorded in MeOH at room temperature. Metal mediated fluorescence

enhancement is observed on complexes (1) and (2) containing NNO donor Schiff base ligand (L) and N-donor bi-dentate co-ligands with Zn(II), whereas metal mediated fluorescence quenching occurs in a bis-Schiff base Zn(II) complex $[\text{Zn}(\text{L})_2]$ (3). The complexes were showed two maximum emission centred in the range of 350-410 nm and 420-430 nm upon excitation in the range of 430-435 nm. The aim of this study was to design and synthesis of some new Zn(II) complexes (1)-(3) as potential therapeutic drug candidates against the viruses. The synthesized Zn(II) complexes (1)-(3) shows good docking results with the viral protein; the binding energies (ΔG) and respective K_i (inhibition constants) correlation values are -9.1 (2.938), -9.5 (2.819), -9.9 (2.428) and -10.2 kcal/mol (1.296 μM), respectively.



Scheme 1. Proposed structures of the Zn(II) complexes (1)-(3).

3.2 Zinc(II) complexes for possible viral applications

As an effective viral inhibitor, we have performed the molecular docking of the Zn(II) complexes (1)-(3) with viral proteins. We show that zinc(II) complexes not only inhibits the viral proteins with micromolar to nonomolar affinity, but also viral replication. The prediction of the active binding site have been identified. Along with this, we cross-verified predictions of the active binding site using the COACH meta-server. Predicted residues and residues identified from the viral proteins were used to perform molecular docking. The important interacting residues (δ -interacting residues, H-bond forming residues and hydrophobic interactions) have been identified between docked Zn(II) complexes (1)-(3) with viral proteins. The synthesized Zn(II) complexes (1)-(3) shows good docking results

with the viral protein; the binding energies (ΔG) and respective K_i (inhibition constants) correlation values are -9.5 (2.819), -9.9 (2.428) and -10.2 kcal/mol (1.296 μ M), respectively. Good inhibitor drugs have their K_i /binding constant in the range of nanomolar to picomolar. Higher the value, the more impractical is the use of the molecule as a drug. The expected trend is that the value of K_i/K_d should decrease when ΔG also decreases. The lower the energy (ΔG) value, the better is the ligand binding and the lower will be the K_i/K_d value. Figure 1 shows the graphical view of the docked Zn(II) complex $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4(\mathbf{1})$ inside the active site of the viral protein with its focussed view for interacting amino acid residues.

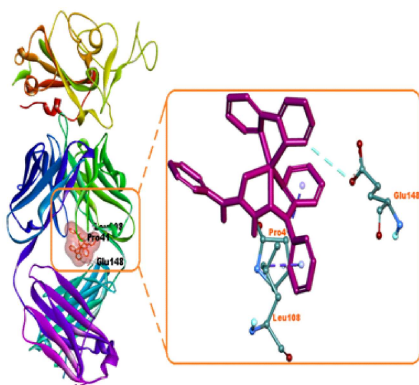


Figure 1. Graphical view of the docked Zn(II) complex $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4(\mathbf{1})$ inside the active site of the viral protein with its focussed view for interacting amino acid residues.

The interaction between the $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4(\mathbf{1})$ with viral protein

predicted that there is observed one δ -hydrogen bond with a distance of 5.475 Å between ARG-408 residue and δ -electrons of docked $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4(\mathbf{1})$. Besides the δ -H bond, there are also seen two δ -anionic electrostatic interaction with a distance of 4.24 and 4.93 Å between (COO^-) group of anionic LYS-378 and VAL-231 residue of the viral protein and docked complex $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4(\mathbf{1})$. The third kind of interactions is seen as hydrophobic, which are between the δ -electrons of PRO-412 and ASP-230 residue of the viral protein and docked complex $[\text{Zn}(\text{L})(\text{bipy})]\text{ClO}_4(\mathbf{1})$ with a distance of 4.22 and 3.85 Å was identified. It was observed that the similar intermolecular interactions seen for remaining Zn(II) complex $[\text{Zn}(\text{L})(\text{phen})]\text{ClO}_4(\mathbf{2})$ and $[\text{Zn}(\text{L})_2](\mathbf{3})$. Overall, the molecular docking study reveals that the Zn(II) complexes ($\mathbf{1}$)-(3) may be considered as potential drug candidate against the viral proteins.

4. Conclusion

The aim of this study was to in-silico design and synthesis of a series of the Zn(II) complexes ($\mathbf{1}$)-(3). The newly synthesized complexes were fully characterized by using various physicochemical and spectroscopic methods. The docking results of the Zn(II) complexes ($\mathbf{1}$)-(3) showed that the highest binding affinities of the complexes predicted that they might serve as better inhibitors against the viral proteins.

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