Novel Aminoketooxime Ligand and Its Cu(II) and Mn(II) Complexes: Synthesis, Characterization and Molecular Docking Studies

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A novel ligand, \(N, N^2-(4\text{-methyl-1,2-phenylene})\text{bis}(2-(\text{biphenyl-4-yl})-N^1\text{-hydroxy-2-oxoacetimidamide})\) (\(H^2L\)) with its Cu(II) and Mn(II) complexes were synthesized in this study. All compounds synthesized were also characterized by \(^1\text{H-}\text{and}^\text{13}\text{C-NMR, the Fourier transform infrared, elemental analysis, inductively coupled plasma optical emission spectrometry, molar conductivity, magnetic susceptibility measurements and thermogravimetric analysis. Vascular endothelial growth factor-2 (VEGFR-2) and cyclooxygenase-2 (COX-2) inhibition is often used as a parameter for being a potent anticancer agent in docking studies. For this purpose, synthesized and characterized ligand was investigated by molecular docking study to test its inhibitory effect against angiogenic factors VEGFR-2 and COX-2.

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1. Introduction

Transition metals have the ability to coordinate ligands in a three dimensional conformation enabling them to act optimally on defined biological targets. This unique ability possessed by transition metals mostly depends on the higher electron affinity which can significantly polarize groups that are coordinated to them, leading to the generation of hydrolysis reactions [1]. Majority of pharmacologically active successful antineoplastic agents are either natural biological compounds or their synthetic analogs [2, 3]. Oximes are, one of these analogs, used extensively not only for their antitumor activity but also for their biological properties like fungicidal, bactericidal, insecticidal, analgesic, anti-inflammatory, antioxidant activity [4, 5]. These biologically active ligands, including oximes, give rise to more potent compounds when they coordinated with certain transition metals called metal complexes. Homo- and heteronuclear metal complexes derived from oxime-type ligands have been reported potential antioxidants due to their observed IC\_50 values [6]. The ability of transition metals to accept electrons from ligands (due to unfilled d-orbitals) play an important role in the interaction of ligands with other molecules [1]. Docking studies investigate the interaction of those molecules by simulating their shape considering their structures as well as their inter and intramolecular energies. It is still critically important to inhibit molecules increasing in pathological conditions like chronic inflammation, tumor growth, angiogenesis and metastasis. Angiogenesis plays a key role in tumor growth and metastasis with a cascade mechanism. Vascular endothelial growth factor (VEGF) is the essential factor for regulation of angiogenesis. It also promotes the angiogenic process by inducing cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS) [7–9].

Oxime compounds form an important part of the coordination chemistry. The oximes give stable coordination compounds with most of the transition metals and make coordination bonds in different forms via nitrogen or oxygen atoms to the metals. For this reason, it is aimed to have amine and oxime groups which have a good electron donor property in the structure of the synthesized compound. Thus, the ligand we synthesized has gained the ability to make stable metal complexes which are used for various medical and industrial purposes.

In the view of all progresses, we reported herein the synthesis and characterization of a new ligand \(N, N^2-(4\text{-methyl-1,2-phenylene})\text{bis}(2-(\text{biphenyl-4-yl})-N^1\text{-hydroxy-2-oxoacetimidamide})\) (\(H^2L\)) (biphenyl amino ketooxime derivative) with its homodinuclear Cu(II) and Mn(II) metal complexes having a general formula of \(M_2L^2(H_2O)_2\) (\(M: \text{Cu(II)}\) and \(\text{Mn(II)}\)). Molecular docking study involving the interaction of synthesized ligand with VEGFR-2 and COX-2 were carried out to illuminate the inhibition capacity of our ligand.

2. Experimental

2.1. Physical measurements

Commercially obtained chemicals in their highest purity grade were used as they received. The instruments used in our experiments were as follows: \(^1\text{H-}\text{and}^\text{13}\text{C-NMR spectra of the ligand were recorded on a JEOL NMR-400 MHz spectrometer, using TMS as an internal standard and CDCl}_3\) as a solvent. Spectrophotometric
As a starting material, 2 mmol, 0.519 g of 2-(biphenyl-4-yl)-N-hydroxy-2-oxoacetimidoyl chloride was dissolved in 20 ml EtOH. The mixture was cooled and kept at -5 °C during dropwise addition of ethanol solution of 2 mmol 0.244 g 3,4-diamino toluene to the solution of chloroacetyl chloride and biphenyl in the presence of dry HCl gas [13]. With isopentyl nitride in the presence of dry HCl, then, 1-(biphenyl)-2-chloro-2-hydroxyimino-1-etanone was obtained by reacting 4-(chloroacetyl)biphenyl with isopentyl nitride in the presence of dry HCl. The final mixture was cooled to +4 °C and kept overnight. The solid product was filtered, washed with water and ethanol, respectively and dried on P₂O₅.

For Cu₂L₂(H₂O)₂, dark brown complex; yield: 61%; m.p.: 288°C. Anal. calc. for C₇₀H₅₀Cu₂N₈O₁₀: C, 64.86; H, 4.35; N, 8.64; Cu, 9.80; found: C, 64.59; H, 4.57; N, 8.46; Cu, 9.64%; ΛM (DMF solution, Ω cm² mol⁻¹): 10.1; μeff = 2.51 B.M.; FT-IR (KBr, cm⁻¹): 3445 s (O-H), 1602 w (C=N), 1452 w (C-N), 1419 m (N-O), 515 w (M-O), 445 w (M-N) (b, broad; s, strong; m, medium; w, weak); UV-Vis (DMF solution, nm): 274, 295, 305.

For Mn₂L₂(H₂O)₂, black complex; yield: 48%; m.p.: 220°C. Anal. calc. for C₇₀H₅₀Mn₂N₈O₁₀: C, 65.73; H, 4.41; N, 8.76; Mn, 8.59; found: C, 65.53; H, 4.62; N, 8.38; Mn, 8.74%; ΛM (DMF solution, Ω cm² mol⁻¹): 8.30; μeff = 7.16 B.M.; FT-IR (KBr, cm⁻¹): 3453 b (O-H), 1602 w (C=N), 1452 w (C-N), 1426 m (N-O), 526 m (M-O), 439 w (M-N) (b, broad; s, strong; m, medium; w, weak); UV-Vis (DMF solution, nm): 274, 295, 367.

2.2. Preparation of the ligand

As a starting material, 2 mmol, 0.519 g of 2-(biphenyl-4-yl)-N-hydroxy-2-oxoacetimidoyl chloride was dissolved in 20 ml EtOH. The mixture was cooled and kept at -5 °C during dropwise addition of ethanol solution of 2 mmol 0.244 g 3,4-diamino toluene to the solution of chloroacetyl chloride and biphenyl in the presence of dry HCl gas [13]. With isopentyl nitride in the presence of dry HCl, then, 1-(biphenyl)-2-chloro-2-hydroxyimino-1-etanone was obtained by reacting 4-(chloroacetyl)biphenyl with isopentyl nitride in the presence of dry HCl gas [13].

2.3. Preparation of the complexes

0.3 mmol ethanol solutions of acetate salts [Cu(CH₃COO)₂], H₂O. Mn(CH₃COO)₂, 4H₂O were used both for Cu(II) and Mn(II) complex preparation, respectively. Then, prepared solutions were added to a hot ethanol solution (0.6 mmol) of ligand added continuously stirring. A distinct change in colour and decrease in pH (pH ~ 3.0–3.5) was observed. Ethanol solution of KOH (0.1 M) was added dropwise to adjust a pH approximately between 5 and 6, until the solid product started to precipitate and kept on a water bath at 80 °C for one hour in order to complete the precipitation. The final mixture was cooled to +4 °C and kept overnight. The solid product was filtered, washed with water and ethanol, respectively and dried on P₂O₅.

For Cu₂L₂(H₂O)₂, dark brown complex; yield: 61%; m.p.: 288°C. Anal. calc. for C₇₀H₅₀Cu₂N₈O₁₀: C, 64.86; H, 4.35; N, 8.64; Cu, 9.80; found: C, 64.59; H, 4.57; N, 8.46; Cu, 9.64%; ΛM (DMF solution, Ω cm² mol⁻¹): 10.1; μeff = 2.51 B.M.; FT-IR (KBr, cm⁻¹): 3445 s (O-H), 1602 w (C=N), 1452 w (C-N), 1419 m (N-O), 515 w (M-O), 445 w (M-N) (b, broad; s, strong; m, medium; w, weak); UV-Vis (DMF solution, nm): 274, 295, 305.

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3. Results and discussion

Synthetic pathway for the synthesis of aminoketoamide ligand can be seen from Fig. 1. Consecutively, 4-(chloroacetyl)biphenyl was obtained from chloroacetyl chloride and biphenyl in the presence of aluminum chloride according to Friedel-Crafts acylation. Then, 1-(biphenyl)-2-chloro-2-hydroxyimino-1-etanone was obtained by reacting 4-(chloroacetyl)biphenyl with isopentyl nitride in the presence of dry HCl gas [13]. N, N′-(4-methyl-1,2-phenylene)bis(2-(biphenyl-4-yl)-N-hydroxy-2-oxoacetimidamide) was synthesized.
by 1-(biphenyl)-2-chloro-2-hydroxyimino-1-etanone reacting with 3,4-diaminotoluene.

Synthesized ligand reacted with Cu(II) and Mn(II) metal salts in a hot medium resulting in the formation of metal complexes. They were further purified by washing ethanol and water.

3.1. $^1$H- and $^{13}$C-NMR spectra

CDCl$_3$ is used for $^1$H- and $^{13}$C-NMR spectra as solvent. From ligand’s $^1$H-NMR spectrum, a singlet peak at 8.15 ppm corresponding to oxime group and multiple peaks corresponding to aromatic C–H protons between 6.74 and 7.68 ppm were observed. Signals belong to N–H and aliphatic C–H protons were observed at 4.73 ppm and 2.34 ppm as singlet peaks, respectively. From ligand’s $^{13}$C-NMR spectrum, a signal obtained from the chemical shift corresponding to the carbonyl carbon was observed at 153.59 ppm. Chemical shifts attributed to aromatic carbons were seen between 113.48 and 142.97 ppm while aliphatic carbon at 20.85 ppm. Results obtained were in accordance with the previous NMR and other analytical data carried out for characterization [14–19].

3.2. FT-IR spectra

FT-IR spectra of the ligand and its metal complexes are shown in Figs. 2–4. Characteristic bands originated from the ligand and its Cu(II) and Mn(II) complexes were investigated and yielded such results: the broad band observed at 3234 cm$^{-1}$ which corresponds to the oxime group of the ligand disappearing in the spectra of the complexes indicates oxime protons which were separated upon complex formation and bonded to the metals. The medium band 3386 cm$^{-1}$ attributable to $\nu$(N–H) of the ligand shifted to lower frequencies to 3334 cm$^{-1}$ as a strong band for Cu$_2$L$_2$(H$_2$O)$_2$ and to 3361 cm$^{-1}$ as a medium band for Mn$_2$L$_2$(H$_2$O)$_2$, respectively. This shift is the indication of nitrogen atom of aromatic amine moiety which was involved in the complex formation [20].

The band observed at about 1608 cm$^{-1}$ corresponded to the C–N bond of the oxime group of the ligand. This group has taken place in complex formation not directly, but neighboring oxygen atom has been in coordination with the metal ion. The insignificant shifts observed at the related band for both complexes may be explained by the involvement of neighboring oxygen atom in coordination. The band at 1402 cm$^{-1}$ which is characteristic for the N–O bond of the oxime group of the ligand was shifted to higher frequency values to 1419 and 1426 cm$^{-1}$ for Cu$_2$L$_2$(H$_2$O)$_2$ and Mn$_2$L$_2$(H$_2$O)$_2$, respectively, revealing that oxygen atom of the oxime group has taken part in coordination. Also the C–N group gave a signal at 1482 cm$^{-1}$ has shifted to lower values indicating that the moiety has taken place in the coordination process.

The new bands appeared at 515, 445 cm$^{-1}$ for Cu(II) and 426, 439 cm$^{-1}$ for Mn(II) complexes are corresponding to $\nu$(M–O) and $\nu$(M–N), respectively. The appearance of these bands is a further support to the involvement of oxime oxygen and amine nitrogen being in coordination with the metal ions.

3.3. UV-Vis

From the ligand UV-Vis spectrum there has been yielded three bands: $\pi \rightarrow \pi^*$ transitions originating from benzene group, the $\pi \rightarrow \pi^*$ transitions from C–N group of oxime, the $n \rightarrow \pi^*$ transitions corresponding to the same C–N group are observed at 295, 265, and 420 nm, respectively.

The signal for $\pi \rightarrow \pi^*$ in the spectra of Cu(II) and Mn(II) complexes have transitions at about 295 nm which correspond to benzene group. The $\pi \rightarrow \pi^*$ transitions observed at 265 nm for ligand were shifted to 285 nm for complexes which may be due to taking place of oxygen atom in coordination adjacent to the imine nitrogen. The 9 transitions observed at 420 nm for ligand were not observed for Cu$_2$L$_2$(H$_2$O)$_2$ and shifted to 367 nm for Mn$_2$L$_2$(H$_2$O)$_2$. The red and blue shifts revealed that the oxime group has been involved in the coordination (Fig. 5).
3.4. Thermogravimetric (TG-DTG) analysis

$\text{H}_2\text{L}$ ligand has a general formula of $[\text{C}_{35}\text{H}_{28}\text{N}_4\text{O}_4]$ decomposed in three steps. The first step of decomposition occurred between 160 and 220°C leaving two moles of $-$OH and one mole of CH$_3$ with a mass reduction of 8.37% (8.62% theoretical) during this interval. Second decomposition was seen between 220 and 530°C suggesting the loss of 2 mol of each biphenyl, carbonyl and $-$CN groups. The mass reduction in the second step was estimated as 72.6% (72.8% theoretical). The third decomposition step started at 530°C. Although the system temperature was raised up to 1000°C, the ongoing decomposition was still observed (Fig. 6).

$\text{Mn}_2\text{L}_2(\text{H}_2\text{O})_2$ complex with a general formula of $[\text{C}_{70}\text{H}_{56}\text{Mn}_2\text{N}_8\text{O}_{10}]$ has been decomposed thermally in five steps. The first decomposition was between 0 and 150°C with a 2.70% estimated mass loss (calculated mass loss 2.81%) leaving two moles of coordination water. At the second step between 150 and 340°C total mass reduced 2.30% (calculated mass loss 2.34%) indicating the separation of two moles of $-$CH$_3$ groups. The third step occurred between 340 and 720°C with an estimated mass reduction of 22.88% leaving two biphenyl groups (calculated mass loss 23.92%). The forth step with a 4.25% estimated mass loss was observed between 720 and 920°C in which two carbonyl groups were removed (calculated mass loss 4.38%). The last step started at 950°C was still decomposing within the temperature limits of the system (Fig. 7).

3.5. Molecular docking study

In order to understand the interaction between the synthesized ligand-VEGFR-2 and ligand-COX-2 at the molecular level we performed double molecular docking study one with SwissDock and 1-Click Docking web servers. Figures 9 and 10 show the interaction between optimized structures of the ligand with crystal structure of the target molecules. Results obtained were highly...
Fig. 9. Visualization of interaction between (a) H2L-2XIR and (b) H2L-1CX2 driven by SwissDock®.

Fig. 10. Visualization of interaction between (a) H2L-2XIR and (b) H2L-1CX2 driven by 1-Click Docking®.

TABLE I
Docking energy and H bond location and length within the ligand-target molecule couples.

| Ligand–target | ΔG [kcal/mol] | H bond location (length) |
|---------------|---------------|--------------------------|
|               |               | SwissDock web server     |
| H2L-2XIR      | -8.76         | N of C=NOH & ODI of Asp 156 (3.175 Å) |
| H2L-1CX2      | -9.30         | N of C=NOH & O of Lys 468 (3.079 Å) |
|               |               | 1-Click Docking web server |
| H2L-2XIR      | -10.90        | H of –NH & O of Lys 105 (1.891 Å) |
|               |               | O of C=NOH & H of Tyr 324 (1.737 Å) |
| H2L-1CX2      | -9.50         | H of –NH & O of Tyr 324 (2.293 Å) |
|               |               | H of –NH & O of Tyr 324 (2.421 Å) |

Docking energy and H bond location and length within the ligand-target molecule couples.

Exothermic which reveals good orientation and proximity (Table I). The docking energy of ligand-VEGFR-2 couple was −8.76 and −10.90 kcal/mol while ligand-COX-2 was −9.30 and −9.50 kcal/mol in SwissDock and 1-Click Docking, respectively. Energy derived from ligand-COX-2 interaction was lower compared to the energy derived from ligand-VEGFR-2 interaction suggesting that the ligand-COX-2 couple is more stable according to SwissDock. Docked pose in Fig. 9b involves H-bonding which indicates a stronger interaction between ligand and COX-2 enzyme. The H-bonding occurred between nitrogen atom of oxime group of the ligand and oxygen atom of Lys 468 which is 3.079 Å in length. Similarly, ligand and VEGFR-2 molecule also have a hydrogen bond between nitrogen atom of oxime group of the ligand and OD1 atom of Asp 156 amino acid having a length of 3.175 Å. Lower docking energy and shorter hydrogen bond length confirm a stronger interaction between the synthesized ligand and COX-2 enzyme.

On the other hand, when 1-Click Docking was used, ligand-VEGFR-2 interactions exhibited the lowest free energy at −10.90 kcal/mol. This interplay was involved a hydrogen bond (1.891 Å) between proton of amine group and oxygen of Lys 105 in the active site of 2XIR (Fig. 10a). The ligand-COX-2 interaction was found to contain three hydrogen bonds despite it had a higher binding energy. The amino acid Tyr 324 has formed hydrogen bonding with oxygen of oxime group (1.737 Å) and proton of amino groups (2.293 and 2.421 Å).

The ligand has the potential to inhibit VEGFR-2 and COX-2 molecule according to both docking results. Synthesized ligand interact with both of VEGFR-2 and COX-2 molecules which means it may inhibit both at the same time enabling the two-way inhibition of angiogenesis and eventually means the inhibition of tumor growth. This makes the synthesized molecule a good candidate for anticancer use which has to be confirmed by the biological study.

4. Conclusion

The present study reports the synthesis and characterization of a new ligand containing dioxime group and its dinuclear Cu(II) and Mn(II) complexes, moreover the docking study of ligand molecule. Experimental results showed that the ligand acted as a tetradentate which coordinates via both oxime oxygen and both amine nitrogen atoms. Metal:ligand ratio of the synthesized complexes was found as 2:2. Results showed that each metal ion in the complex has pentacoordinated with a molecule of coordination water for both Cu(II) and Mn(II) complexes. Docking studies revealed that the synthesized ligand is in a good interaction with COX-2 and VEGFR-2. Synthesized ligand is a good candidate for the inhibition of angiogenic agents VEGFR-2 and COX-2 synchronously.

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