Synergism antibacterial activity for novel synthesized Schiff base ligands and semi-thiosemicarbazones with β-diketones and 4-aminoantipyrine

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Abstract. Schiff base ligands were synthesized in this work, the first is (Z)-2-((Z)-3-(((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)mimino)-1,3-diphenylpropyildene)hydrazine-1-carboxamide, the second is (Z)-2-((1E,5Z,6E)-5-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)mimino)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-dien-3-ylidene)hydrazine-1-carbothioamide. The Schiff base ligands both were synthesized using the same method (the single pot reaction), using EtOH as the reaction medium and GAA as the catalyst in fixed temperature at 70 °C and reflux for 6 hrs. The starting materials of HL1 were semicarbazide,4-aminoantipyrine and dibenzoylmethane, while the starting materials of HL2 were TSC, 4-AAF and Curcumin. The ligands proved to be bi-dentate ligands that coordinate from the azomethane groups. FT-IR, U.V-Visible, 1H and 13C-NMR, molar conductivity, and magnetic susceptibility were used to identify all the compounds produced. The metal ions used in preparing the complexes in this work were Co(II), Cu(II) and Cr(III). Schiff base and its complexes were evaluated for antibacterial activity against four bacterial strains of Gram-negative (Escherichia coli and Pseudomonas aeruginosa), Gram-positive (Staphylococcus aureus and Bacillus Subtilis) and two types of fungi (Candida albicans and Rhizopus Sporium), the results were positive for all compounds tested.

Keywords: Schiff base, Curcumin, 4-aminoantipyrine

Introduction

During the past few decades, the Schiff base metal complexes have been under attention due to their role in coordination chemistry, easy preparation, and the diversity of their structure. Schiff base is considered a bioactive compound; for example, these compounds behave as anti-cancer agents. The free ligands have a weaker biological effect when compared to their metal complexes. Antipyrines played an essential role in drug development fields; also, they helped in monitoring and observing patients with chronic liver illness (Hepatitis B virus (HBC), hepatitis C virus (HCV) and alcohol-related disease). 4-aminoantipyrine condensation with suitable compounds such as ketones, aldehydes, thiosemicarbazones, carbazones or compounds with a similar structure results in flexible ligands which can coordinate with various metal ions. β-diketones based Schiff base ligands have an important role in developing coordination chemistry; also, they play an important role in biochemical systems. In this study, the β-diketones used are Curcumin and dibenzoylmethane; both are known for their biological activity and behavior as an anti-inflammatory, anti-cancer, insecticidal, anti-Alzheimer’s, gastroprotective, antifungal, antibacterial agents. Also significant are semicarbazones and thiosemicarbazones; they are made up of the scaffolds NH2-CO-NH- and NH2-CS-NH- respectively. Thiosemicarbazones’ and semicarbazones’ capacity to coordinate with transition and main group metallic cations is due to the extended delocalization of electron density over their skeleton, which is increased by substitution at the N (4)-position. The goal of this study was to synthesize and characterize Schiff bases with two azomethine groups derived from diketones (Curcumin and dibenzoylmethane), 4-amino antipyrine, semicarbazide and thiosemicarbazide, in order to test the ligand and its complexes’ efficient biological activity against various types of bacteria and fungi.

Materials and methods

The device used to measure the melting point is (MPA160 Digi Melt); a device type (jew wary, model 4070) was used to measure the molar conductivity of the metal complexes. The FT-IR spectroscopy was evaluated using (Shimadzu FT-IR 8400S) spectrophotometer. The UV-Vis spectra of the ligands and their transition metal complexes were recorded in DMSO at 0.001M using the (UV 160A Shimadzu apparatus). Balance Johnson Mattey device was used to measure the magnetic susceptibility of the metal complexes, and NMR spectroscopy was recorded using device type (Inova 500). Finally, the Mass spectrum was recorded using (Sciex Esi) spectrometer.

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Synthesis of Schiff base ligand HL¹

For the preparation of HL¹ a mixture of 4-aminoantipyrine, semicarbazide and dibenzoylmethane was refluxed for six hrs. with stirring at a fixed temperature of 70 °C. after that, the solution was left to evaporate for a day the resulted ligand was a yellow-colored, needle-shaped crystal and odorless.

The structure was confirmed by the data collected from elemental analysis, FT-IR, ¹H-NMR and ¹³C-NMR; the synthesis of the ligand and it complexes is illustrated in Figure 1.

HL¹: (Z)-2-((Z)-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro1H-pyrazol-4-yl)imino)-1,3-diphenylpropylidene) hydrazine-1-carboxamide. Yield: 75 %, elemental analysis found%: 69.49 C, 5.621 H, 18.11 N, 6.78 O, Calculated%: 69.51 C, 18.01 N, 6.66 H, 6.86 O.

IR (KBr): 1624 (C=N), 1670 (C=O), 3398(NH), 3210 and 3175 (NH₂). ¹H-NMR (δ/ppm): 2.44 (s, 3H, C-CH₃); 3.21 (s, 3H, N-CH₃); 10.74 (s, N-H), 7.33-7.71(m,15H aromatic); 7.21 (s, NH₂). ¹³C-NMR δ/ppm: 150.69 and 161.85 (C=N), 124.86-137.60 (Caromatic), 157.67 and 159.69, 12.76 (C-CH₃), 37.32 (N-CH₃), 41.15 (CH₂).

Synthesis of Schiff base ligand HL²

Curcumin (0.368 gm, 1 mmol) was dissolved in 20 ml ethanol, then adding four drops of GAA as a reaction catalyst, 0.204 grams (1mmol) 4-aminoantipyrine dissolved in 20 mL ethanol and 0.09 gm (1mmol) thiosemicarbazide dissolved in 20 mL ethanol were all progressively added at the same time. This mixture was refluxed by stirring at 70 °C for 6 hours; TLC was used to monitor the reaction. A dark brown precipitate was isolated and recrystallized from hot ethanol. The structure was confirmed by the data collected from elemental analysis, FT-IR, ¹H NMR and ¹³C NMR. The synthesis of the ligand is illustrated in Figure 2.

HL²: (Z)-2-((1E,5Z,6E)-5-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)imino)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-dien-3-ylidene)hydrazine-1-carbothioamide. Yield: 77%. Elemental analysis, calculated for C₃₁H₃₀N₆O₃S: C 65.71, H 5.34, N 14.83, S 5.66%. The analysis found: C 65.64, H 5.39, N 14.92, O 8.51, S 5.54 %. IR (KBr, cm⁻¹): v (C = O ) 1670, v (C = N ) 1627, v (N-H) 3441, v (C=S) 817. ¹H NMR δ/ppm: 2.43 (s, 3H, –CH₃); 3.17 (s, 3H, –CH₃); 10.69 (s, N-H), 7.33-7.51(m, 15H aromatic); 9.23 (s, NH₂), 9.35 (O-H), 4.26 (s, 2H, NC-CH₂-CN). ¹³C NMR δ/ppm: 150.69 and 161.85 (C=N), 124.86-137.60 (Caromatic), 157.67 and 159.69 (HC=CH), 12.76 (C-CH₃), 37.32 (N-CH₃), 41.15 (CH₂).

Synthesis of metal complexes

a) [HL¹] complexes: complexes were prepared by dissolving 0.466 gm (1mmol) of HL¹ ligand in 10 ml ethanol, followed by adding an equivalent of KOH (0.5 mmol) of the metal salt (CoCl₂.6H₂O, CuCl₂.2H₂O, CrCl₃.2H₂O) dissolved in 3 ml ethanol which was added as dropwise on the mixture of the ligand with KOH, the mixture was refluxed for 6hrs with continuous stirring at fixed temperature 70-80 0C. The mixture was filtered, and the participants washed several times with cold ethanol and then recrystallized from hot ethanol. Figure 1 depicts the suggested geometry of the recrystallized structures.

b) [HL²] complexes: In 20 mL of ethanol, 0.627 gm (1mmol) of the Schiff base (ligand) was dissolved, and the equivalent of KOH was added; the solution of the
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Figure 2. Synthesis route for HL\(^2\) and its metal complexes

Metal chloride (0.5 mmol) was added dropwise addition with continual stirring and reflux for 6 hours at fixed temperature 70-80 °C. (3 mmol) of CoCl\(_2\)·6H\(_2\)O, CuCl\(_2\)·2H\(_2\)O, CrCl\(_3\)·2H\(_2\)O (dissolved in 3 mL ethanol) was added progressively to the mixture. The residues that formed were separated, rinsed with cold ethanol multiple times, and then recrystallized from hot ethanol. The proposed geometry of the recrystallized structure is shown in Figure 2.

Results

All complexes were generally synthesized by reacting the metal salts with the Schiff base in a 1:2 mole ratio (metal salts: Schiff base). All of the complexes were colored solid compounds. Table 1 shows that the analytical findings corresponded with the proposed formula of a 1:2 metal: Schiff base molar ratio. The complexes synthesized using HL\(^1\) were soluble in dimethylformamide and dimethyl sulfoxide, and it has partial solubility in chloroform, ethanol, methanol, and water. In contrast, the complexes of HL\(^2\) were soluble in water, DMSO, and DMF and had a partial solubility in ethanol and methanol. Some of the physical and chemical characteristics are shown in Table 1.

**FT-IR spectra**

The spectrum of Schiff base ligands showed a band at 1624 cm\(^{-1}\) that referred to the imine group in HL\(^1\) and 1627 cm\(^{-1}\) for HL\(^2\) ligand, also a band appeared at 1670 cm\(^{-1}\) that assigned to the carbonyl group (C=O), the thione band was assigned at 817 cm\(^{-1}\) for HL\(^2\). The NH indicating band is set for HL\(^1\) at 3441 cm\(^{-1}\) and 3417 cm\(^{-1}\) for HL\(^2\), and the OH group band of [HL\(^1\)] appears at 3525 cm\(^{-1}\). Otherwise, the spectrum of the complexes showed shifting in the imine band, and the shifting range was 1600-1624 cm\(^{-1}\); the carbonyl group and the thione group did not show any noticeable shifting, which means the two groups did not participate in the coordination with the metal ions. Also, new bands for stretching frequencies of M-N have been allocated at 460-420 cm\(^{-1}\). Another band was observed at a range of 243-260 cm\(^{-1}\), indicating the formation of the M-Cl coordination bond. The data are listed in Table 2, and the FT-IR spectrum is shown in Figures 3 and Figure 4.

| Compounds | M.Wt | M.P | Color | Yield % |
|-----------|------|-----|-------|--------|
| HL\(^1\) (Schiff base) C\(_3\)H\(_6\)NeO\(_2\) | 456  | 228-230 | Yellow | 80     |
| [Ce(HL\(^1\)\(_2\)) Cl\(_2\)] | 1062 | 284-286 | Green  | 73     |
| [Cr(HL\(^1\))\(_2\) Cl] | 1055 | 280-282 | Green  | 77     |
| [Cu(HL\(^1\)\(_2\)) Cl\(_2\)] | 1067 | 190-192 | Brown  | 65     |
| HL\(^2\) (Schiff base) C\(_{13}\)H\(_{34}\)N\(_4\)O\(_5\)S | 627  | 241-243 | Brown  | 88     |
| [CO(HL\(^2\)\(_2\)) Cl] | 1383 | 170-172 | Dark Green | 68 |
| [Cr(HL\(^2\))\(_2\) Cl] | 1376 | 190-192 | Green  | 88     |
| [Cu(HL\(^2\))] Cl] | 1388 | 215-217 | Brown  | 60     |

Table 1. Some of the physical and chemical characteristics of the synthesized compounds
Figure 3. FT-IR spectrum of HL1

Figure 4. FT-IR spectrum of HL2.
The ligands’ absorption spectra revealed peaks at 270-290 nm due to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ peaks around 340 nm; Figure 3 shows the ranges of the ligands. The complexes revealed intra-ligand fields at 267-289 and 340-347 nm; this slight shift is considered to approve the ligand→metal coordination, charge transfer peaks at 363 nm $^{13-15}$ and d-d transition peaks as follows; The electronic spectra of ligand’s HL₁ metal complexes confirmed an octahedral geometry of the synthesized complexes where the peaks exhibited was like the following: Co(II) complex exhibited a peak at 782nm that is related to the electronic transition $^4T_{1g} \rightarrow ^4T_{2g}$. Cr(III) complex showed a peak at 779 nm which refers to the electronic changes $^{4}A_{2g}(F) \rightarrow ^{4}T_{2g}(F)$. The Cu(II) complex d-d transitions display two peaks at the d-d region at 778 nm and 760 nm which are related to the following transitions: $^{2}B_{1g} \rightarrow ^{2}A_{2g}$. The HL₂ complexes spectral data were as follows; Co(II) complex displayed peaks at the d-d region at 700 nm related to the electronic transition $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$.
the complex’s magnetic moment equals 3.88 BM, the data suggested an octahedral geometry. The Cr(III) complex spectra exhibited a peak at 803 nm related to $^4A_{2g} \rightarrow ^4T_{2g}$; the electronic spectrum and the magnetic moment of the complex, which equals 2.91 BM confirm an octahedral geometry structure. According to the electronic spectrum data collected, an octahedral geometry was suggested for the Cu(II) complex, where peaks were detected at 626 nm and 682 nm that are referring to $^2E_g \rightarrow ^2T_{2g}$ and $^2B_{1g} \rightarrow ^2A_{1g}$, and the magnetic moment of the complex is 2.15 BM. Also, the magnetic susceptibility were measured and the results were: [Co (HL)$^\cdot$_1]Cl$_2$ (3.962), [Cr (HL)$^\cdot$_1]Cl (4.05), [Cu (HL)$^\cdot$_1]Cl$_2$ (2.23), [Co (HL)$^\cdot$_2]Cl$_2$ (3.88), [Cr (HL)$^\cdot$_2]Cl$_2$Cl (3.91) and for [Cu (HL)$^\cdot$_2]Cl$_2$ (2.15).

**Mass spectroscopy**

In the Schiff base ligands, mass spectrum Figure 4 the HL1 spectrum has a well-defined molecular ion peak at m/z = 467 amu, which correlates (M + 1) with the Schiff base ligand’s molecular formula (C$_{27}$H$_{26}$N$_6$O$_2$). The spectra of HL1 ligand reveal a succession of peaks at m/z 409, 332, 290, 199, 184, 105, 70 and 56, amu, which correspond to the fragments. The strength of these peaks indicates the pieces’ stabilities. At the same time, the Mass of HL2 exhibited peaks at m/z+=627 amu attributed to

![Figure 4: Mass spectrum of HL1 and HL2](image)

**Figure 5.** This is a Figureure.: (a) mass spectrum of HL$^\cdot$_1 (b) mass spectrum of HL$^\cdot$_2
[M+1] of the ligands molecular formula (C33H34N6O5S). Also, it revealed peaks at 594.1, 490.1, 449.1, 390.1, 367, 339, 227, 164, 136, 77, and 54 amu that are attributed to the fragments 18.

The ¹H and ¹³C -NMR spectra of the ligands

According to a literature review, NMR spectroscopy is required to determine the structure of various compounds; the NMR spectra were recorded in DMSO-d₆ (dimethyl sulfoxide) using TMS (tetramethyl silane) as standard.

For HL¹ the signals were at 10.74ppm (NH), 7.21 ppm (NH₂), 5.16 ppm (N=CH₂-C=N), 7.33-7.71 ppm (H aromatic) and the signals referring to the CH₂ is overlapped by the DMSO signal. While for HL² the signs were 9.23 ppm (NH₂), 9.35 ppm (OH), 10.69 ppm (NH), 7.33-7.51 ppm (H aromatic), 4.26 ppm (N=CH₂-C=N). A slight downfield shift was observed in the signals of significant complexes. The spectra are shown in Figure 6 and Figure 7.

The ¹³C-NMR For HL¹ the signals of the aromatic carbons are located at 124.86-137.6 ppm, the carbon of the imine groups

| Functional groups | ¹H-NMR (ppm) | ¹³C-NMR (ppm) | ¹H-NMR (ppm) | ¹³C-NMR (ppm) |
|-------------------|--------------|---------------|--------------|---------------|
| C=H (aromatic)    | 7.33 - 7.72  | 124.86 - 137.6| 7.33 - 7.51  | 124 - 137     |
| C=O               | 7.21         | 150.69 & 161.85| 10.69        | 155.18 & 156.71|
| OH                | 5.16         | 41.15         | 4.26         | 12.76 & 37.32 |
| N=CH₂             | 2.39 & 3.18  | 12.73         | 2.37 & 3.18  | 39.70         |
| OH                |              | 9.35          |              | 115.3         |
| C=O               |              | 159.69 & 157.67| 7.33 - 7.51  | 179.61        |

Table 4. NMR spectral data of the ligands

Figure 6. ¹HNMR spectrum of the first Schiff base HL¹
Figure 7. $^1$HNMR spectrum of the first Schiff base HL$^2$.

Figure 8. $^{13}$CNMR spectrum of the first Schiff base HL$^1$. 
(C=N) group signal at 150.69 and 161.85 ppm, the signal of the carbonyl group was located at 157.67 and 159.69 ppm, finally, for HL\textsuperscript{1} the signals of the aliphatic carbons were located at 12.76 (C-CH\textsubscript{3}) and 37.32 ppm (N-CH\textsubscript{3}) and 41.15 ppm (CH\textsubscript{2}) [19-21]. When speaking of HL\textsuperscript{2} signals, they were explained as the following 179.61 ppm indicates the thione (C=S) group, the aromatic carbons are located at 124-137 ppm, while the aliphatic carbons signals are located at 12.76 (CH\textsubscript{2}) and 41.5 ppm indicating the CH\textsubscript{2} group that is located between the imine groups, and finally, the carbons of the imine group have two signals 155.18, and 156.71 ppm and the deference in signals results from the deference in the electronic environment surrounding the group [19-21]. The spectra are shown in Figure 8 and Figure 9.

**Figure 9.**\textsuperscript{13}CNMR spectrum of the first Schiff base HL\textsuperscript{2}

**Table 5.** Biological activity data of the ligands and their metal complexes

**Biological activity studies**

The effect of the synthesized compounds was evaluated using the agar diffusion technique on four types of bacteria (E. Coli, P. aeruginosa, S aureus and B. Subtilis) and two types of fungi (C. albicans and Rhizopus sporium). The inhibition zone (IZ) of 1x10\textsuperscript{-3} M of the compounds was compared to the IZ of Ceftriaxone antibiotic as standard [22]. The bacteria and fungi were chosen for their recognized impact on various illnesses; they exhibit varying resistance to medicines and medicinal compounds. The ligand and its complexes demonstrated a reasonable zone of inhibition.
Discussion

Schiff compounds were synthesized using various techniques, and were approved to have better biological activity than the antibiotic used as a standard and the physical activity.

Conclusions

The Schiff base ligand was synthesized by reacting dibenzoxy methane 1mmol with 4-aminantipyrine 1mmol and semicarbazide 1mmol (HL). The imine group atoms (N) performed it as bidentate coordinates. The Schiff base ligand (HL) was synthesized by reacting curcumin 1mmol with 4-aminantipyrine 1mmol and thiosemicarbazide 1mmol. It turned out to be a bidentate ligand coordinate from the imine group. All complexes were produced based on available data such as FT-IR, UV-Visible, 13C,1H-NMR, molar conductance, and magnetic susceptibility to adopt an octahedral geometric form. When tested for biological effectiveness against four types of bacteria and two types of fungi, all compounds gave a positive result; the synthesized compounds showed adequate inhibition in various ranges against the bacterium species and the effect on the fungi species.

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