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SYNTHESIS, Cu(II) SCHIFF BASE COMPLEXATION AND STRUCTURAL ANNALYSIS OF THE LIGAND 4-{[2-(METHOXYCARBONYL)PYRROLIDIN-1-YL]METHYL}BENZOIC ACID

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ABSTRACT

A new proline-based ligand was synthesized by a convenient procedure. The ligand was characterized by ^{1}H NMR, ^{13}C NMR and IR spectroscopies. Copper (II) Schiff base complexes of 4-{[2-(methoxycarbonyl) pyrrolidin-1-yl] methyl} benzoic acid of this ligand were synthesized and characterized by the aforementioned spectroscopy techniques, i.e. ^{1}H NMR, ^{13}C NMR and IR. Moreover, its bactericidal activity was assessed through the determination of the minimum inhibitory concentration (MIC) values as well as the inhibition zone diameter for gram positive bacteria, such as *Staphylococcus aureus*, and gram negative bacteria like *Klebsiella pneumoniae*, *Citrobacter freundii*, *Salmonella typhi*, *Enterobacter cloacae* (the two bacteria *Citrobacter freundii* and *Salmonella typhi* are very sensitive against this molecule unlike Enterobacter cloacae bacteria which is resistant).

Keywords: Proline; complex; ligand; Schiff base; bactericidal activity; inhibitor.

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1. INTRODUCTION

Amino acids are of great biological importance and their metal complexes have attracted particular interest. A large number of researchers have studied complexes of various amino acids with different transition metals in solution.

It is worth recalling that metal complexes offer a versatile platform for the design of novel therapeutic compounds [1]. In addition, they play essential role in pharmaceutical industry and in agriculture [2]. Nowadays, the coordination chemistry of oxygen donor ligands is viewed as an active research area. Over the last few years, metal complexes have been widely investigated for their antimicrobial [3, 4], antitubercular [5,6], antiviral [7], anticonvulsant [8, 10], antitumor [11,12], and anti-inflammatory activities [13,14].

Today, it is widely admitted that metal complexes play an essential role in various biological systems. For this reason, the formation, stability and reactivity of these complexes have recently been active fields of research [15,16]. Proline or pyrolidine – 2 – carboxylic acid is an amino acid found in protein. Proline has several significant applications in biological systems [17-19]. Among twenty naturally occurring amino acids, proline possesses unique features as its side chain can bind to the main chain to form the pyrrolidine ring. It is well documented that proline plays unique roles in constructing three-dimensional protein structures such as an alpha-helix breaker or a reverse turn constituent to bend the main chain [20]. In addition, proline may be involved in diverse physiological phenomena [21,22]. Indeed, proline is a major organic osmolyte that accumulates in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation, or heavy metals [21]; it can also act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which is viewed as essential for plant recovery from stress [22]. Moreover, proline is an important molecule in the asymmetric catalysis regarding organic synthesis involving aldol condensation, as a prominent example [23].

The introduction of a copper atom into a molecule may have remarkable effects on its physical and chemical properties, as well as on its metabolic stability and biological activity.

2. METHODS AND MATERIALS

Reagents and analytical grade materials were obtained from commercial suppliers and used without further purification. Fourier transform infrared (FTIR) spectroscopy was performed on KBr pellets within the range of 4000 - 400 cm-1, with a Shimadzu FTIR-8300 instrument. In addition, the nuclear magnetic resonance (NMR) measurements were carried out on a Bruker AM300 MHZ Spectrometer (University of Oran, Es-Senia) relative to the internal standard tetramethylsilane (TMS) and the chemical shift values were expressed in parts per million (δ, ppm). Moreover, thin layer chromatography (TLC) was used. Indeed, silica gel TLC plates, coated with fluorescent indicator F254 supplied by Merck, were employed along with a mixture of different polar and non-polar solvents, in varying proportions. Using iodine as a visualizing agent, it was possible to observe points that had formed.

3. EXPERIMENTAL

3.1. Synthesis of L-proline ester: (G)

A quantity of 4 g of l-proline and an equimolar amount of phosphoryl trichloride were placed in a 200 ml flask, equipped with a magnetic stirrer. Next, a quantity of 10 mL of chloroforme was added as solvent. After that, excess chloride proline (G') was neutralized with sodium carbonate and then the solvent was evaporated and the resulting product was collected and washed with ether several times and dried under vacuum at room temperature; the final product was an orange gel. The yield of the reaction was around 43.5 %, with retention factor (Rf) = 0.85 ($CHCl_3$).

Chloride proline (9 mmol) was added to a solution of methanol (0.3 ml, 9 mmol) with chloroform (20 ml). The mixture was stirred at reflux for 7 to 8 hours, at the temperature of 80°C, and then filtered; the solvent was evaporated afterwards and the resulting product was a white solid. The TLC technique was used to monitor the progress of the reaction. The excess acid was neutralized with sodium bicarbonate, then the solvent was evaporated and the resulting product was collected. For compound (G), the yield was 96.5 %, with Rf= 0.76 ($CHCl_3/CH_3OH$ = 8/2).

3.2. Synthesis of ligand HL: (1G)

The synthesis of proline-based ligand is illustrated in Scheme 1. Following the treatment with bromomethyl benzoic acid (3mmol), the mixture of proline ester (0.5g, 3 mmol) and acetone-water solution (60% acetone - 40% eau) in the presence of potassium carbonate (K_2CO_3) was gently refluxed for 4-5 hours using a water bath. After cooling down to room temperature, the solvent was evaporated and the residue were simply filtered and washed with cold diethyl ether. In this case, the yield was 40.54 %; the product obtained was in powder form with a melting point of 199 °C with Rf = 0.7 ($CHCl_3/CH_3OH$ = 8/2).

3.3. Synthesis of the metal complexes: ($1G_1$)

The HL ligand ($1G_1$) (1.0 mmol, 2 equivalents) was first placed in a flask, and then 1.0 equivalent of a solution of $Cu(NO_3)_2$ in 30 mL of methanol was added. It is noted that the color of the mixture turned into green color. After 6 h of reflux, the solution was further stirred at room temperature. It was then filtered and the solvent was evaporated; then, a green solid was obtained. In this case, the yield was 68.18 %; the resulting product was in powder form with a melting point of 148 °C with Rf = 0.62 ($CHCl_3/CH_3OH$ = 8/1).

3.4. Biology

The stock solutions of test compounds were prepared in dimethyl sulfoxide (DMSO) to attain a concentration of 75 mg/mL. From these stock solutions, serial dilutions of the test compounds (75, 50 and 25 mg/mL) were prepared. the inhibition zone was recorded in each case as the diameter of the compound that inhibited the visible growth of the tested microorganism. The DMSO solution was used as a negative control.

3.4.1. Antibacterial activity

In order to assess the antibacterial activity, four bacteria were used, namely *Klebsiella pneumoniae ATCC700603, Citrobacter freundii ATCC8090; Salmonella typhi ATCC13311, Enterobacter cloacae ATCC13047*, as gram negative bacteria and *Staphylococcus aureus ATCC25923*, as gram positive bacteria.

All microorganisms were obtained from the Microbiology Laboratory in the Institute of Biology at Dr. Moulay Tahar University in the City of Saïda (Algeria). The bacterial strains were maintained on Muller-Hinton agar and the tested compounds were dissolved in the

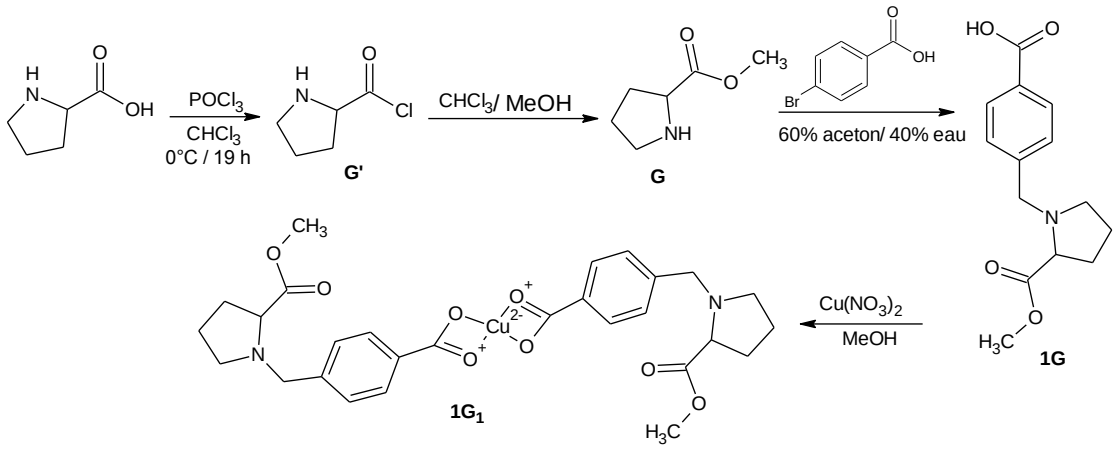
DMSO solution in order to make a 75 mg/mL stock solution; however, the other concentrations were prepared by dilution. A suspension of micro-organisms was spread over the surface of sterile agar plates which were then incubated at 37 °C for 24 hrs. After incubation, the diameter of inhibition zone was determined. it is to test the sensitivity of the bacterial strains by the diffusion of the complex on the solid medium in a Petri dish, the appearance and importance of the diameter of the inhibition zone reflects the impact on the bacterial strains. thus, the latter will be qualified as sensitive or very sensitive or resistant.

4. RESULTS AND DISCUSSION

4.1. Chemistry

The target compounds ($1G_1$) were synthesized by a multiple-step procedure, as shown in Scheme 1. The synthetic route started from the esterification of the pyrrolidine-2-carbonyl chloride with methanol.

The ester used reacted with the 4-bromobenzoic acid in a mixture of 60% acetone and 40% water to give compound (1G). The products ($1G_1$) were finally obtained by refluxing compound (1G) in methanol with the appropriate metal.



Scheme.1. Synthetic route to title compound $1G_1$

The formation of the ligand and its metal complex were detected by thin layer chromatography (TLC) via their retention factors (Rf) which were different from those of the starting materials. The metal complex color (green) was different from the color of the ligand,

indicating that the resulting color depends on the metal ions. The melting point of the ligand was found different (higher) from that of the metal complex, which is an evidence for complexation. The physical and analytical data of all compounds studied are summarized in Table 1.

Table 1. Physical properties and analytical data of compounds, ligand and it metal complexe

Compound	Color	%Yield	Melting Point °C	Rf Value	Solvent system	Suggest formula
G’	Orange	43.5	-	0.85	$CHCl_3$	C_5H_8ClNO
G	white	96.5	-	0.76	$CHCl_3/CH_3OH$ = 8/2	$C_6H_{11}NO_2$
1G	white	40.54	199	0.7	$CHCl_3/CH_3OH$ = 8/2	$C_{14}H_{17}NO_4$
$1G_1$	green	68.18	148	0.62	$CHCl_3/CH_3OH$ = 8/1	$C_{28}H_{32}CuN_2O_8$

4.2. Infrared spectra studies of compounds, the ligand and it metal complexe

The IR spectra in the (4000–400 cm^{-1}) region provide information regarding the coordination mode in the complexe are analyzed by comparison with the data for the free ligand. The IR data of the ligand and complexes are shown in Table 2.

Table 2. The characteristic infrared absorptions of compounds, the ligand and it metal complexe

Compound	υ(NH)	υ(OH)	υ(C=O)	υ (C=C)	υ (C-O-C)	υ (M-O)	υ(Cl)
G’	3425.3	-	1658.7	-	1255.6		634.5
G	3436.9	-	1745.5	-	1244.0		
1G	-	2856.4-3400.3	1703.0	1575.7	1097.4	-	-
$1G_1$	-	-	1620.1			1384.8	-

The IR spectra of ligand HL showed a wide band corresponding to the OH function within the interval extending from 2856.4 to 3400.3 Cm^{-1} which was absent in the spectra of the complex, with lowering of the intensity of the absorption function C = O in the spectra of the complex, which indicated the deprotonation and complexation of carboxylate anions.

4.3. NMR Analysis studies of compounds, the ligand and it metal complexe

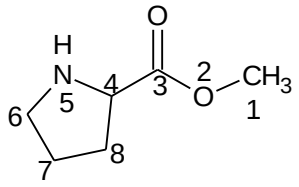


Fig.1. Structure of L-proline ester

^{1}H NMR (300MHz, D_2O), δ (ppm):3.388 (1H, NH), 4.698 (1H, C1), 4.452 (1H, C4), 2.399 (2H, C8), 3.778 (2H, C6), 2.013 (2H, C7).

^{13}C NMR chemical shifts are given in the following signals ^{13}C-NMR (300MHz, D_2O), δ (ppm): 59.743 (C4), 53.848 (C1), 46.147 (C6), 23.556 (C7), 170.382 (C3), 28.095(C8).

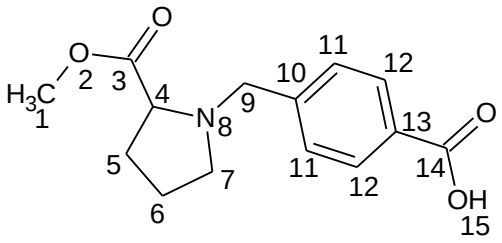


Fig.2. Structure of the ligands

^{1}H NMR (300MHz, DMSO), δ (ppm): 3.488 (2H, C9), 10.134 (H, OH), 3.668 (1H, C1), 3.336 (1H, C4), 7.568 (1H, C11), 7.968 (1H, C12), 1.919 (1Ha, C6), 1.973 (1Hb, C6), 2.501 (1Ha, C7), 2.375 (1Hb, C7), 2.099 (Ha, C5), 1.855 (Hb, C5)

^{13}C-NMR (300MHz, DMSO), δ (ppm): 65.771 (C4), 63.565 (C1), 39.964 (C6), 45.502 (C7), 167.679 (C3), 40.242 (C5), 166.997 (C14), 63.927 (C9), 142.791 (C10), 131.117 (C12), 129.213(C11, C13).

The ^{1}H and ^{13}C NMR chemical shifts of free complexe in DMSO at room temperature show the following signals.

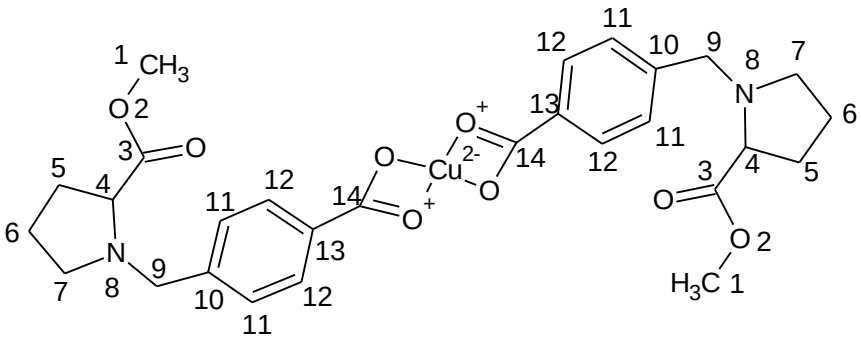


Fig.3. Proposed structure metal complexes of CuL_2

^{1}H NMR (300MHz, DMSO), δ (ppm):3.628 (2H, C9), 3.669 (1H, C1), 3.186 (1H, C4), 7.169 (1H, C11), 7.337 (1H, C12), 1.641 (1Ha, C6), 1.543 (1Hb, C6), 2.402 (1Ha, C7), 2.305 (1Hb, C7), 2.069 (Ha, C5), 1.851 (Hb, C5).

^{13}C-NMR (300MHz, DMSO), δ (ppm): 71.277 (C4), 51.955 (C1), 22.096 (C6), 57.504 (C7), 173.319 (C3), 26.622 (C5), 63.927 (C9), 138.627 (C10), 126.184 (C12), 128.425 (C11, C13).

In ^{13}C NMR spectra of free ligand C9, C10, C11, C12, C13 and C14 of the methyl benzoic acid were observed in 63.927, 142.791, 129.213, 131.117, 129.213 and 166.997 ppm.

Respectively, indicated the formation of the ligand. The spectra ^{1}H NMR of ligand, HL showed a sharp peak, δ(OH) at 10.134 ppm which was absent in the spectra of the complexe and indicated the deprotonation and complexation of carboxylate anions to metal ions. On the basis of the preceding discussion, the structure of the ligand and it metal complexe may be suggested as follows, the proposed structures are presented in Figures 2-3.

4.4. Antibactrial activties tests

The chemical substances were synthesized in our laboratory; they were then tested for the purpose of enhancing its antibacterial capacities against gram negative bacteria (Klebsiella pneumoniae ATCC700603, Citrobacter freundii ATCC8090, Salmonella typhi ATCC13311, Enterobacter cloacae ATCC13047) and gram positive bacteria (Staphylococcus aureus ATCC25923). The sensitive detection of these pathogenic bacteria against the test molecules was achieved by means of an antibiogram. The results obtained are summarized in Table 3.

Table 3. Antibacterial activity of the synthesized compounds

Compound	Relative inhibition rate (mm)														
	Klebsiella pneumoniae			*Citrobacter freindii*			*Salmonella typhii*			*Enterobacter coloacae*			*Staphylococcus aureus*		
	Concentration %														
	75	*50*	*25*	*75*	*50*	*25*	*75*	*50*	*25*	*75*	*50*	*25*	*75*	*50*	*25*
1G₁	*14*	*11*	*11*	*19*	*11*	*10*	*17*	*13*	*08*	*09*	*09*	*08*	*15*	*14*	*10*

The evaluation of antibactrial activity revealed that all the synthesized compounds have a significant biological activity.

The results also showed that *Citrobacter freindii* and *Salmonella typhii* bacteria are very sensitive to compound $1G_1$ "complexes of 4-{[2-(methoxycarbonyl) pyrrolidin-1-yl] methyl} benzoic acid". Unlike *Enterobacter coloacae* bacteria, it is resistant. For the remaining bacteria (*Klebsiella pneumonia* and *Staphylococcus aureus)*, they are also sensitive.

5. CONCLUSION

In conclusion, new compounds containing multiple active moieties based on amino acid methyl esters with attached bromomethyl benzoic acid were successfully synthesized and characterized; in addition, their physical and antibacterial properties were studied.

All the synthesized compounds were structurally characterized using IR and NMR spectroscopic techniques. The newly synthesized compound is evaluated for his antibacterial activity. The results of biological tests indicated of the synthesized compound exhibited promising results. Thus, the mixed compounds containing both amino acid methyl esters can serve as interesting lead molecules for further synthetic and biological evaluation.

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