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Original Article

Investigation into the Antibacterial Activity of Metal Complexes Derived from Substituted Chromone in Comparison with Tetracycline, and Cephradine as Standard Drugs against *Escherichia coli* and *Staphylococcus aureus*

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Introduction: The chemistry of metal complexes derived from heterocyclic compounds has attracted considerable interest due to the broad range of pharmacological activities of such compounds. The important pathogens such as *Escherichia coli*, and *Staphylococcus aureus* are wildly caused many diseases. So antibacterial activity of Zn (Ⅱ), Ni (Ⅱ), Co (Ⅱ) and Cu (Ⅱ) chromone complexes against two kinds of bacteria was established. **Methods:** In this study, antibacterial activity of metal complexes derived from 2-amino-7, 7-dimethyl-5-oxo-4-methylbenzen5, 6, 7, 8-tetra hydro-4H-chromone-3-carbonitrile were studied. The metal complexes were characterized by FTIR, UV-Vis and Mass spectroscopy. Antibacterial effect of these compounds was evaluated by disc diffusion and micro broth dilution methods. **Results**: The results obtained in this study demonstrate that all the complexes have square planner geometry with the stoichiometry 1:2 (Metal: Ligand). Among the tested compounds the most effective compound was the Cu complex with MIC value of 62.5 μg/mL against *E. coli* and 125 μg/mL against *S. aureus.* **Conclusion**: The results of these studies show that the metal complexes had higher antibacterial activity against species when compared to parent ligand. *J Med Microbiol Infec Dis, 2015, 3 (3-4): 75-79.*

Keywords: Heterocyclic Compounds, *Escherichia coli, Staphylococcus aureus.*

INTRODUCTION

Transition metal complexes have various roles in biochemistry [1, 2]. The multifarious role of these compounds in biochemistry has stimulated the development of new chemistry systems with metal ligands and enormous interest in the synthesis of transition metal complexes with nitrogen donor groups as such compounds have a broad range of pharmacological activity [3]. The chemistry of heterocyclic compounds has attracted considerable research interest and is considered necessary because some of these compounds are applied in anticancer, anti-inflammatory, anticonvulsant and antidiuretic treatments [4].

Isocyanides have been known for more than a hundred years. They were first synthesized by Lieke [5] and then followed by Meyer [6], but were erroneously assumed to be nitriles. The study of transition metal complexes of isocyanides developed slowly over the past century into a subarea of coordination chemistry [7].

Isocyanides are unique in their ability to coordinate with a variety of transition metals in states of low, medium and high oxidation to form stable complexes [8]. Isocyanides are good σ-donor and moderate π-acceptor ligands. Also, owing to their linear shape and small steric demand in the coordination sphere of metal centers, different well-defined coordination geometries such as linear, square planar and octahedral forms are quickly established [9]. The report on coordination polymers of transition metal complexes of isocyanides and studies of their properties such as electrical conductivity and liquid crystalline properties show that transition metal complexes of isocyanides can act as useful building blocks for solid-state molecular materials [10].

However, mixed cyano-nitrido complexes have been reported and studied for several different metal centers such as Cr, Mn, Tc, Re, OS, so it appears that mixed cyano-amino complexes should also be accessible [11,12].

In some cases, mixed cyano complexes are not formed. Instead, they have a strong tendency to be transformed into a complex with only cyanide ions as a ligand. Unfortunately, there is no satisfactory explanation for this phenomenon [13].

The present work describes syntheses of the 2-Amino-4H-chromone-3-carbonitrile derivative with various metal ions such as Cu (Ⅱ), Co (Ⅱ), Ni (Ⅱ) and Zn (Ⅱ). It is widely maintained that important pathogens such as *Escherichia coli* and *Staphylococcus aureus* cause many diseases, so the antibacterial activity of these complexes was established against these two kinds of bacteria.

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MATERIAL AND METHODS

All used chemicals were of analytical grade purchased from Merck. Melting points of compounds were measured by an electrothermal melting point apparatus and were not corrected. The FTIR spectra (KBr), in the range of 4000-450 cm^{-1}, were recorded on a standard stambd 3577 Brucker IR spectrometer. The ^{1}HNMR spectrum of the ligand was registered on a Brucker 500 MHz spectrometer in $CDCl_3$ with TMS as an internal standard. The UV-Vis spectra of the compounds were recorded on a Bio-TEK, kon TRON 922 spectrophotometer. The Mass spectra were run at 70 eV at 230°C with Agilent technologies. The progress of the reaction was monitored by Thin-layer chromatography (TLC) using n-hexane/EtOAc (1:2) as an eluent.

Preparation of Ligand. Preparation of chromone derivative was reported by Foroughifar *et al.* previously [14]. A mixture of 4-methyl benzaldehyde (1 mmol), malononitrile (1 mmol), dimedone (1 mmol) and catalyst (Potassium Alum) (0.03 g) in distilled water was refluxed for 6 h. The progress of the reaction was monitored by TLC. After the mixture had been cooled down to room temperature, the crude product was purified by recrystallization in hot ethanol.

IR (KBr): ν_{max} = 3426, 3330, 3139, 2191, 1675, 1509 cm^{-1}. ^{1}HNMR (250 MHz, $CDCl_3$): δ=1.02 (s, 3H, CH_3), (s, 2H, NH_2, 7.06 (m, 4H, Ar-H (ppm)).

Syntheses of the transition metal complexes. The coordination compounds were prepared according to a modification of previously reported method [15]. The reaction of the ligand with Cobalt (Ⅱ), Nickel (Ⅱ), Copper (Ⅱ) and Zinc (Ⅱ) ions in the molar ratio 2:1 (2 ligand: 1 metal) afforded the corresponding stoichiometry transition metal complexes.

Two different methods are used in preparing the metal complexes, as follow:

Non-direct method: A solution of 1 mmol of a metal (II) salt in DMSO (10 ml) was added dropwise to the solution of 2 mmol of ligand in DMSO (15 ml) with constant stirring. The mixture was then refluxed for 2-3 h and poured on the ice-cold water. The resulting metal compound was filtered and washed with petroleum ether and dried over calcium chloride in a vacuum desiccator.

Direct method: A mixture of a solution of malononitrile (2 mmol), dimedone (2 mmol) 4-methylbenzaldehyde (2 mmol) and potassium alum (0.03 g) in distilled water was added gradually to a solution of metal ions (1 mmol) in constant stirring. The reaction mixture was refluxed for 6 h. The solid complex was precipitated, filtered off and washed with hot ethanol. The complexes were air stable, soluble in DMF and DMSO.

Antibacterial activity. *E. coli* (ATCC: 25922) as gram negative bacteria and *S. aureus* (ATCC: 6838) as gram positive bacteria were used to test the antibacterial activity of the ligand and its metal complexes. The antibacterial activity of the synthesized compounds was determined by disc diffusion and micro broth dilution methods.

Disc diffusion method: Müller-Hinton agar medium (38 g Müller-Hinton agar in 1000 ml of distilled water) was prepared and sterilized. A bit of each bacteria was dissolved in a sterile distilled water tube similar to 0.5 McFarland turbidity standard (the suspension had a final inoculum of 5×10^8 CFU/ml). The Muller-Hinton Agar plates were inoculated with bacteria by two sterile cotton swabs. The substance (0.02 g) was dissolved in 1 ml of DMSO. The sterile blank discs (Whitman no. 1 filter paper, 5 mm diameter) were dipped in 0.1 ml of each sample. The discs were placed on plates at specified intervals by sterile forceps. After an incubation period of 24 h at 35°C, the diameter of each zone of inhibition was measured with a ruler (mm). Cephradine and Tetracycline (30 µg per disc) were chosen as standards for the antibacterial activity measurements. To clarify any participating role of DMSO in the biological screening, a separate study was carried out with the solution of DMSO alone, and it showed no activity against any bacterial strains. The test results are presented in table 1. The results were confirmed by performing the procedure conditions for three times.

Micro-broth dilution method: The Minimal Inhibitory Concentrations (MICs) of the ligand and complexes were also determined for the bacterial strains [16, 17]. MIC is the lowest concentration of the antimicrobial compound, which inhibits the visible growth of a microorganism after overnight incubation. 13 sterile tubes containing one ml of Mueller nutrient broth medium were prepared and sterilized for each substance. Each substance (2 mg) was dissolved in 1 ml of DMSO. Then the samples were prepared by two-fold serial dilution.

As a result, the concentration in each tube was half of the previous one. The extra solution (1 ml) from the 12th tube was discarded. Thus, the 13th tube acted as control bacteria. In the next step to each tube, 0.1 ml of the standard microorganism (1.5×10^8 CFU/ml) was added except the tube 12. Turbidity was observed after incubating the inoculated tubes at 35°C for 24 h. The MIC values of free ligand and complexes are presented in table 2.

Table 1. Characteristic IR frequencies (cm^{-1}) of the ligand and its metal complexes

Compounds	ν (NH)	ν (C≡N)	ν (C=N)	ν (C=O) (carbonyl)	ν (C-O) (phenolic)	ν (M-N)
Ligand, $C_{19}H_{20}O_2N_2$	3426	2191	-	1675	1290	-
[$C_{38}H_{38}O_4N_4Zn$]	3433	-	1660	1680	1211	550
[$C_{38}H_{38}O_4N_4Co$]	3432	-	1662	1675	1290	535
[$C_{38}H_{38}O_4N_4Ni$]	3433	-	1660	1670	1295	510
[$C_{38}H_{38}O_4N_4Cu$]	345	-	1660	1670	1215	540

Table 2. Inhibition zone of ligand and metal complexes against bacterial strains

Compounds	Bacteria	
	Escherichia coli	*Staphylococcus aureus*
	Inhibition zone (mm)	
Free Ligand, $C_{19}H_{20}O_2N_2$	12	8
$[C_{38}H_{38}O_4N_4Zn]$	22	18
$[C_{38}H_{38}O_4N_4Co]$	20	14
$[C_{38}H_{38}O_4N_4Ni]$	14	12
$[C_{38}H_{38}O_4N_4Cu]$	24	20
Tetracycline	12	14
Cephradine	16	22
DMSO	0	0

Table 3. Minimal Inhibitory Concentration of ligand and complexes against bacterial strains

Compounds	Bacteria	
	Escherichia coli	*Staphylococcus aureus*
Free Ligand, $C_{19}H_{20}O_2N_2$	500 µg/ml	1000 µg/ml
$[C_{38}H_{38}O_4N_4Zn]$	250 µg/ml	500 µg/ml
$[C_{38}H_{38}O_4N_4Co]$	125 µg/ml	250 µg/ml
$[C_{38}H_{38}O_4N_4Ni]$	500 µg/ml	500 µg/ml
$[C_{38}H_{38}O_4N_4Cu]$	62.5 µg/ml	125 µg/ml

RESULTS

The synthesis of tetrahydro chromone derivative was achieved by three-component condensation of 4-methylbenzaldehyde (1 mmol), malononitrile (1 mmol) and dimedone (1 mmol) in the presence of potassium alum (0.03 g) and distilled water to give a product with the highest yield (Fig. 1).

Two different methods were used to prepare metal complexes; direct and non-direct methods. The non-direct method consists of two steps: Synthesis of ligand and preparation of metal complexes. In the direct method, the reagents for the preparation of ligand and metal salt are directly added to a round bottom flask and then refluxed for 6 h. Both methods were checked by TLC, and the preparation of new product were confirmed. In the non-direct approach, the yields of the compounds were higher, so the products of the non-direct method were characterized using various spectroscopy methods. The structure of metal complexes is shown in figure 2. These complexes are insoluble in water and many common organic solvents, but are readily soluble in DMSO and DMF. The chelation of the metal ions to the ligand occurs through the cyano and amino groups of the ligand. Based on various spectral data, it is concluded that the complexes may tentatively be suggested to have square planner geometry.

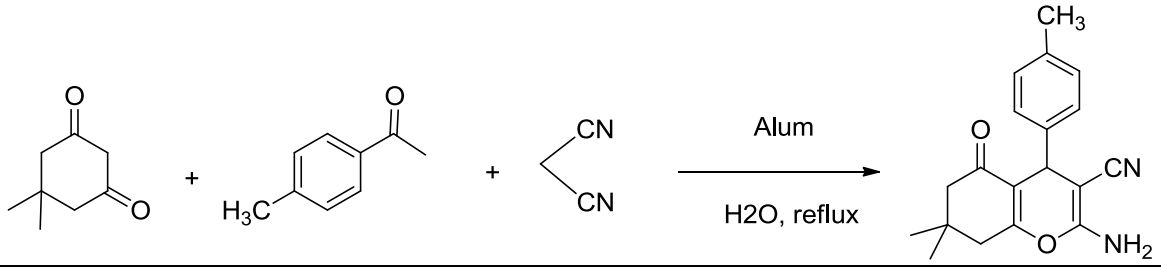

Fig. 1. Preparation of ligand

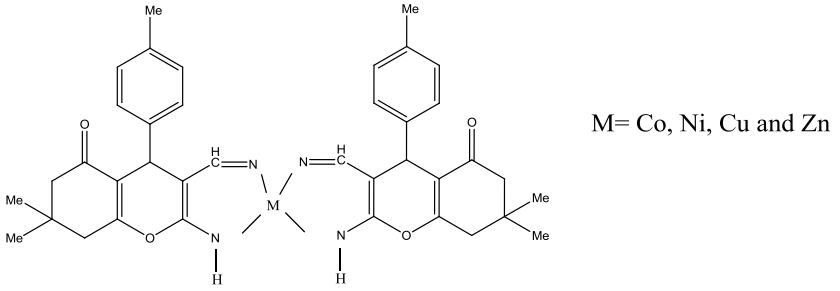

Fig. 2. The proposed structures for the complexes

FTIR spectra. Important spectral bands for the ligand and its metal complexes are presented in table 1. The IR spectrum of the ligand shows bands at 3426 and 3220 cm^{-1} assignable to ν (N-H). The other bands at 1675 and 1290 cm^{-1} are assignable to ν (C=O) (carbonyl) and ν (C-O) (phenolic) stretching modes, respectively [18]. The spectrum of the free ligand has shown a band in 2191 cm^{-1} region characteristics of the ν (C ≡ N) indicating the formation of the cyano ligand. No band was observed in the IR spectra of the metal complexes in the region 2100 cm^{-1}. The absence of ν (C≡N) (cyanide) suggests coordination of cyanide group to the metal ions. The presence of a new band in the region 1660 cm^{-1} can be assignable to ν (C=N). According to the above data, the ligand behaved as bidentate, and the coordination occurred via the Amin and Cyanide moiety.

The *in vitro* antibacterial studies of the complexes showed the higher activity of the complexed compounds compared to the free ligand. In view of the biological activity, the ligand and its metal complexes have shown a moderate activity. The results are listed in table 2 and 3.

DISCUSSION

Electronic absorption spectra. The electronic absorption spectra of the ligand and its metal complexes were recorded at room temperature using DMSO as the solvent. Electronic absorption of the free ligand shows tow bands at 265 and 320 nm which is assigned to $\pi \rightarrow \pi^2$ and $n \rightarrow \pi^*$ transitions, respectively. The Cu (Ⅱ) complex shows bands at 243 and 399 nm due to the $^2B_1g \rightarrow {}^2Eg$ and $^2B_1g \rightarrow {}^2A_1g$ transitions which support square planner geometry [19]. The Co (Ⅱ) complex shows absorption in the region 310 nm corresponding to $^4A_1g \rightarrow {}^4B_1g$ transition which also supports square planner geometry [20]. The Ni (Ⅱ) complex shows absorptions in the regions 280 and 490 nm corresponding to the $^3A_2g \rightarrow {}^3T_2g$ and $^3A_2g \rightarrow {}^3T_1g$ d-d transitions respectively. These electronic transitions account for the square planner geometry [21].

Mass. The mass spectra of the complexes were in good agreement with the proposed structures. The ligand shows a molecular ion peak at *m/z* =308 whereas the molecular ion peaks for the Co (Ⅱ), Ni (Ⅱ), Cu (Ⅱ) and Zn complexes were observed at *m/z*= 675, 674, 679 and 677 respectively, which are equal to their molecular mass. The other peaks in the mass spectrum were attributed to the fragmentation of complex obtain from the rupture of different bonds inside the molecule.

Antibacterial study. The ligand and its metal complexes were evaluated for their antibacterial activity against *S. aureus* and *E. coli*. Tetracycline and cephradine were used as standard antibiotics and compared with synthesized compounds in disc diffusion method. The results are listed in table 2 and 3. Inhibition zone of compounds shows that they have moderate to high antibacterial activity. The *in vitro* antibacterial activities demonstrated that the complexes have higher antibacterial activity in comparison with the ligand. According to the tweedy's theory [22] chelation reduces the polarity of the metal atom because of partial sharing of its positive charge with a donor group and the possible π-electron delocalization over the whole ring. Such a chelation could enhance the lipophilic character of the central metal atom, with subsequently favors its permeation through the lipid layers of the cell membrane and blocking the metal binding sites on enzymes of microorganism. The chromone derivative had more effect on *E. coli* compared to *S. aureus*. All the complexes showed moderate to high antibacterial activity against the species. The difference in antibacterial activity of the complexes for *E. coli* was in the following order: Cu> Co> Zn> Ni while for the *S. aureus* was Cu> Co> Zn=Ni. The most antibacterial activity of synthesized compounds belongs to Cu complex. The minimal inhibitory concentrations (MICs) against *E. coli* and *S. aureus*, were 62.5 and 125 μg/ml, respectively. In our previous work about chromone complexes, also the highest antibacterial activity of synthesized compounds belonged to copper and cobalt complexes [23].

In this contribution, we reported on the synthesis of Cu (II), Ni (II), Co (II) and Zn (II) complexes with 2-Amino-7, 7-dimethyl-5-oxo-4-methylbenzen5, 6, 7, 8-tetrahydro-4H-chromene-3-carbonitrile ligand. The complexes coordinated by N carbonitrile and N amin atoms of the ligand. They were characterized by spectral data. Square planner geometry was assigned for all the complexes. The antibacterial activities were screened for all the compounds by disc diffusion and micro broth dilution methods. The Cu complex demonstrated higher antibacterial activity compared to other compounds.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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