

## Synthesis, characterization and antibacterial activity of Co ( II ), Ni ( II ) , Mn ( II ), Cu( II ) and Zn( II ) complexes with 2-amino-7,7-dimethyl-5-oxo-4-chlorobenzen 5,6,7,8-tetra hydro-4H-chromene-3-carbonitrile

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### ABSTRACT

A novel series of complexes of the type  $[M (C_{18}H_{17}N_2O_2Cl)_2]$ , where M= Co( II ), Ni( II ), Cu( II ), Zn( II ) and Mn( II ) and  $C_{18}H_{17}N_2O_2Cl$  correspond to the bidentate ligands, were synthesized, and characterized by UV-Vis, FTIR, and Mass spectroscopies. The bidentate ligand was synthesized in a typical one-pot, three-component condensation of malononitrile, 4-chlorobenzaldehyde, and dimedone in the presence of alum as catalyst. The synthesized ligand and its metal complexes were screened for *in vitro* antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains. The most antibacterial activity of the synthesized compounds belongs to cobalt and copper complexes. The minimal inhibitory concentrations (MICs) against *E. Coli* and *S. aureus* were 16 and 32  $\mu\text{g/ml}$ , respectively. The results of these studies showed that the metal complexes have more antibacterial activities as compared with the non-complexed ligand.

**Keywords:** Bidentate ligand, antibacterial activity, metal complexes, escherichia coli, staphylococcus aureus.

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## INTRODUCTION

Pyran, as a modified prominent structure, is found in numerous active compounds [1]. Benzopyran derivatives have gained a unique position in drug discovery due to their broad range of biological activities such as anti-coagulants, anticancer agents, spasmolytic, anti-anaphylactic [2-5]. 4H-pyrans containing heterocyclic ring are increasingly used for their pharmaceutical activities [6]. These compounds can be used for the treatment of neurodegenerative diseases, including Alzheimer's disease, as well as for the treatment of schizophrenia and myoclonus [7]. Strategies for the synthesis of these compounds have varied from one-pot to multi-step approaches [8]. The common method involves one-pot, three-component condensation of malononitrile, aldehyde and dimedone in different conditions. Various catalysts such as  $\text{Na}_2\text{SeO}_4$ , acetic acid, tetramethylbenzyl ammonium bromide, KF-alumina and  $\text{K}_2\text{CO}_3$  have been used for these syntheses [9-13]. In this study, Potassium alum was used as catalyst for the synthesis of the compound. Moreover, the experimental procedure for this reaction is remarkably simple and without using of hazardous and expensive organic solvents. However, the discovery of

these complexes with metal ions is more effective routes for drug delivery [14]. Numerous studies showed these complexes can be used to treat diabetes mellitus, some bacterial infections or even cancers [15]. Due to their easy preparation and biological activity of pyran and its complexes, they have received considerable attention in medicinal chemistry [16-18].

In this study, we synthesized 2-Amino-4H-chromone-3-carbonitrile derivatives with various metal ions such as Cu (II), Co (II), Ni (II), Mn (II) and Zn (II). The antibacterial activity of the ligand and its metal complexes were reported.

## MATERIALS AND METHODS

Chemicals were purchased from Merck Company. The FTIR spectra (KBr), in the range of  $4000\text{-}450\text{ cm}^{-1}$ , were recorded on a standard 3577 Bruker IR spectrometer (USA). The  $^1\text{H}$ NMR spectra of the ligands were recorded on a Bruker 500 MHz spectrometer (USA),  $\text{CDCl}_3$  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 (China). The progress of the reaction was monitored using thin-layer

chromatography (TLC) using  $\text{CH}_2\text{Cl}_2/\text{EtOAc}$  (3:1) as an eluent.

### Synthesis of 2-Amino-7, 7-dimethyl-5-oxo-4-chlorobenzen5, 6, 7, 8-tetra hydro-4H chromene-3-carbonitrile

In a typical reaction for the preparation of tetrahydrobenzopyran [19] the mixture of an aromatic aldehyde, malononitrile, dimedone and Potassium Alum were dissolved in 10 ml of distilled water using mechanical stirring and refluxed for 6 hours. The progress of the reaction was monitored by TLC. The obtained mixture was cooled, and the product was refined by recrystallization process in hot ethanol.

*FTIR (KBr): max=3372, 3178, 2967, 2186, 1674, 1597  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (500 MHz,  $\text{DMSO-d}_6$ ):*

*$\delta = 1.08$  (s, 3H,  $\text{CH}_3$ ), 1.12 (s, 3H,  $\text{CH}_3$ ), 2.26 (s, 2H,  $\text{CH}_2$ ), 2.46-2.48 (m, 2H,  $\text{CH}_2$ ), 4.43 (s, 1H, CH), 6.58 (s, 2H,  $\text{NH}_2$ ), 7.18-7.28 (m, 4H, ArH) (ppm).*

### Synthesis of the transition metal complexes

The reaction of the ligands with Cobalt (II), Nickel (II), Copper (II), Manganese(II) and Zinc (II) ions with 2:1 molar ratio (ligand: metal) afforded the corresponding transition metal complexes.

Two different methods were used to prepare the metal complexes, as follows:

### Non-direct method

To a solution consisting of 2 mM of the ligand in 20 ml of DMSO, was slowly added a solution of 1 mM appropriate metal nitrate in 10 ml of DMSO with stirring. The mixture was refluxed for 3 hours and placed on ice-cold water. The resulting metal complex was filtered and washed with ether and dried over calcium chloride in a vacuum desiccator.

### Direct method

A mixture solution of 2 mM malononitrile, 2 mM dimedone, and 2 mM 4-chlorobenzaldehyde in distilled water was added to a solution of 1 mM metal nitrate in water containing Potassium alum as a catalyst. The solution was stirred for 30 min, and refluxed for 5 hours. The solid complex precipitation was filtered off and washed with hot ethanol. The complexes are air-stable and soluble in DMF and DMSO.

### Antibacterial activity

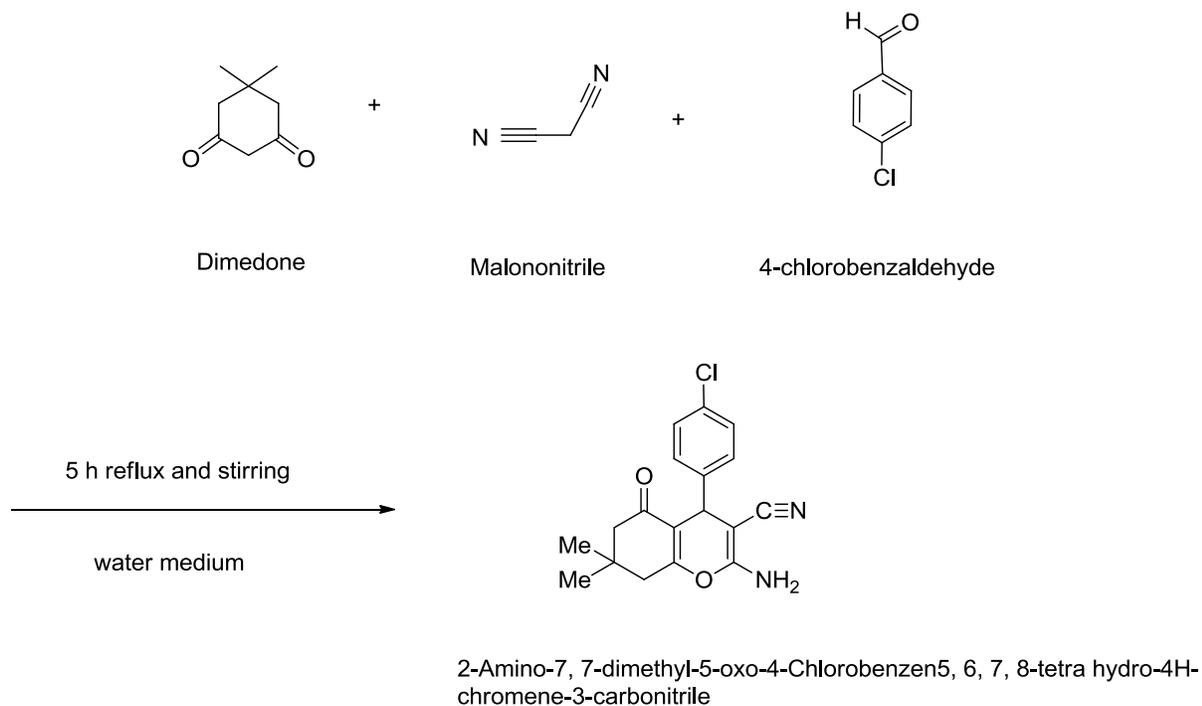
The antibacterial activities of the compounds in this study were determined by micro broth dilution method [20] against a Gram-positive (*Staphylococcus aureus* PTCC 1112), and a Gram-negative bacteria (*Escherichia Coli* PTCC 1330) using Muller-Hinton Agar (MHA) as the medium.

The synthesized compounds were dissolved in DMSO. The plates were incubated for 24 hours at 35 °C.

### RESULTS

The 2-amino-7,7-dimethyl-5-oxo-4-chlorobenzen-5,6,7,8-tetra hydro-4H-chromene-

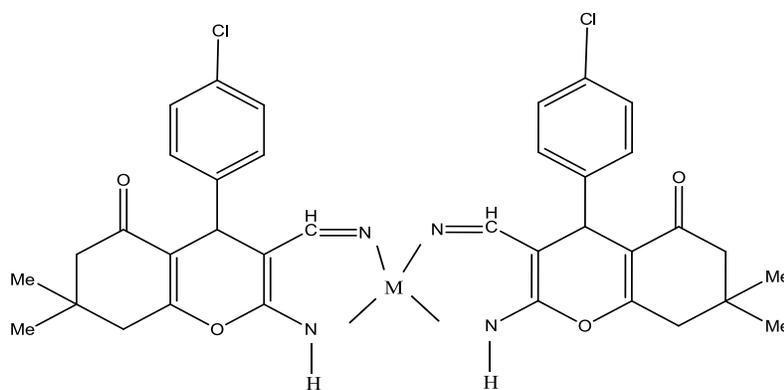
3-carbonitrile was synthesized by the reaction of dimedone, malononitrile and 4-chlorobenzaldehyde in the presence of potassium Alum and water (Scheme 1).



**Scheme. 1.** Synthesis of ligands

The complexes were prepared by the reaction of ligand with metal salts in the molar ratio 2:1 in refluxing conditions. The complexes of Co (II), Ni (II), Cu (II), Mn (II) and Zn (II) are stable at room temperature. These complexes are insoluble in water and many common organic solvents but are readily soluble in strong solvents

such as DMSO and DMF. The analytical data showed that the complexes had 1:2 stoichiometry of the type  $ML_2$ , where  $M = Co(II), Ni(II), Cu(II), Mn(II), Zn(II)$  and  $L =$  pyran ligand. Fig.1 shows the proposed structure of metal complexes.



M= Co( II ), Ni( II ), Cu( II ), Mn( II ) and Zn( II )

**Figure. 1.** Proposed structure of Metal complexes

## DISCUSSION

### FTIR spectra

The FTIR spectra of the prepared ligand, and its metal complexes exhibited characteristic bands of the functional groups and are presented in Table 1.

The FTIR spectrum of the ligand shows bands at 3372 and 3178  $\text{cm}^{-1}$  assigned to  $\nu(\text{N-H})$ . The other bands at 1674 and 1361  $\text{cm}^{-1}$  are assigned to  $\nu(\text{C=O})$  (carbonyl) and  $\nu(\text{C-O})$  stretching vibrations, respectively [19]. The spectrum of the free ligand had shown a band in 2156  $\text{cm}^{-1}$  assigned to  $\nu(\text{C}\equiv\text{N})$  indicating the formation of the cyano ligand. No band was observed in the FTIR spectra of the metal complexes in 2100  $\text{cm}^{-1}$ . The absence of  $\nu(\text{C}\equiv\text{N})$  (cyanide) suggests coordination of cyanide group to the metal ions. The presence of a new band in 1660  $\text{cm}^{-1}$  can be assigned to  $\nu(\text{C=N})$ .

### Electronic absorption spectra

The electronic absorption spectra of the ligand, and its metal complexes were obtained at room temperature using DMSO as the solvent. UV-vis spectrum of ligand shows essential bands at 274 and 321 nm, which were attributed to  $\pi\rightarrow\pi^*$  and  $n\rightarrow\pi^*$ , respectively [21]. The electronic absorption spectrum of Ni (II) complex was characterized by a broad band in the region 350 and 420 nm which may be assigned to two allowed transitions  ${}^1\text{A}_{1g}\rightarrow{}^1\text{E}_g$  and  ${}^1\text{A}_{1g}\rightarrow{}^1\text{B}_{2g}$ , respectively [21]. The Co (II) complex shows absorption in the region 350 nm [22]. The UV-Vis spectrum of Cu (II) complex shows bands at 243 and 399 nm which may be due to  ${}^2\text{B}_{1g}\rightarrow{}^2\text{E}_g$  and  ${}^2\text{B}_{1g}\rightarrow{}^2\text{A}_{2g}$  respectively [23]. The electronic spectrum of Mn (II) complex showed weak

absorption at 310 nm, which may be assigned to  ${}^6A_{1g} \rightarrow {}^4E_g$  transition [24].

**Table 1.** FTIR frequencies ( $\text{cm}^{-1}$ ) of the ligand and its metal complexes

mpounds	$\nu(\text{NH})$ $\text{cm}^{-1}$	$\nu(\text{C}\equiv\text{N})$ $\text{cm}^{-1}$	$\nu(\text{C}=\text{N})$ $\text{cm}^{-1}$	$\nu(\text{C}=\text{O})$ $\text{cm}^{-1}$	$\nu(\text{C}-\text{O})$ (phenolic $\text{cm}^{-1}$ )	$\nu(\text{M}-\text{N})$
Ligand, $\text{C}_{16}\text{H}_{16}\text{O}_2\text{N}_2\text{Cl}$	3372	2191	-	1674	1361	-
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Co}]$	3446	-	1669	1687	1314	512
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Cu}]$	3327	-	1650	1710	1352	514
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Zn}]$	3497	-	1660	1790	1310	520
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Ni}]$	3425	-	1660	1685	1314	515
$[\text{C}_{36}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Mn}]$	3358	-	1647	1693	1358	510

### Mass spectral studies

Mass spectroscopy has been used as a powerful structural characterization technique in coordination chemistry. The mass spectrum of the ligand showed a molecular ion peak at  $m/z$  328 which is equivalent to its molecular weight whereas the molecular ion peak for the Co(II), Ni(II), Cu(II) Mn(II) and Zn complexes were observed at  $m/z = 731, 731, 736, 727$  and  $738$  respectively, which are equal to their molecular mass.

### Antibacterial activity

The antibacterial activities of the synthesized compounds were investigated against *E.coli* and *S.aureus* bacteria strains by minimal inhibitory

concentration (MIC) method which is presented in Table 2.

It has been suggested that the ligands with the nitrogen and oxygen donor atoms inhibit enzyme activity, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by metal ions. Moreover, coordination reduces the polarity of the metal ion essentially because of the partial sharing of its positive charge with the donor groups within the chelate ring system formed during coordination. Increasing the lipophilic nature of the central metal atom leads to effective permeation through the lipid layer of microorganism to destroy it aggressively [25]. As depicted in Table 2, the cobalt and copper

complexes showed the highest antibacterial activity against bacterial strains while manganese and zinc complexes showed the lowest activity

against *S.aureus* and *E.Coli*, respectively. The other compounds have moderate activity against all bacterial strains.

**Table. 2.** Antibacterial activities of the synthesized compounds

Compounds	MIC( $\mu\text{g/ml}$ ) bacteria	
	<i>E.Coli</i>	<i>S.aureus</i>
Ligand, $\text{C}_{18}\text{H}_{17}\text{O}_2\text{N}_2\text{Cl}$	64	128
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Co}]$	16	32
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Cu}]$	16	32
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Zn}]$	64	64
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Ni}]$	32	64
$[\text{C}_{37}\text{H}_{37}\text{O}_4\text{N}_4\text{Cl}_2\text{Mn}]$	32	128

## CONCLUSION

The pyran ligand containing cyano and amino groups, 2-Amino-7, 7-dimethyl-5-oxo-4-Chlorobenzen 5, 6, 7, 8-tetra hydro-4H-chromene-3-carbonitrile was synthesized. The ligand and its metal complexes with Copper (II), Cobalt (II), Nickel (II) Zinc (II) and Manganese (II) ions were characterized by FTIR, UV-Vis and mass spectroscopies. The complexes coordinated by N carbonitrile and N amine atoms of the ligand. In addition, the ligand and its metal complexes were evaluated for *in vitro* antibacterial activity against Gram-negative bacteria; *Escherichia coli*, and Gram-positive bacteria; *Staphylococcus aureus* using the micro broth dilution method. The MIC values indicate

that complexes have more antibacterial activity compared to free ligand. The pyran ligand had more effect on *E.Coli* compared to *S.aureus*. The most antibacterial activity of synthesized compounds belongs to Co and Cu complexes. Their MICs against *E.Coli*, *S.aureus*, were 16 and 32  $\mu\text{g/ml}$ , respectively.

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