Synthesis and Spectral Study of mono-ligand Complexes for Acriflavine with Some Metalions and Evaluation Their Antibacterial Activity

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Abstract

In this study, a new seven complexes have been prepared from acriflavine (LCl) with metal ions VO (II), Co (II), Ni (II), Cu (II), Cd (II), Zn (II) and Hg (II) with mole ratio (M:L) (2:2) all complexes have general composition [M₂(L₁)Cl₄(H₂O)₄]Cl₂, [(VO)₂(L₁)₂(SO₄)₂(H₂O)₂]Cl₂ in ethanol. The ligand and all complexes were characterized by modern spectroscopic (IR, UV-Vis, Mass, atomic absorption), along with elemental microanalysis, magnetic susceptibility measurements and molar conductance. In addition to evaluated their antibacterial activity against two type of bacterial. Based on date for all techniques we suggested that all the prepared complexes have octahedral geometry, the value of antibacterial activity of this prepared complexes showed higher inhibition activity compared to the free ligand

Keywods: Acriflavin, Acridine, Acriflavine metal complexes, Biological activity.

Introduction

Acridine derivatives have important role in different applications in medicine, industry, and used in sterilization as antiseptic agent due to a quaternary ammonium structure for treatment minor wounds and bacterial infections [1-3]. Acriflavine is one of these derivatives which obtained from coal tar with a 90% pigmentation ratio, it was used as antiseptic agent during the First World War and sleeping sickness due to his trypanocidal activity against trypanosomes [4-6].

Dilute solution of acriflavine used For treatment and prevention of external microbial infections in fish [7]. The scientific name of acriflavine is (3,6-di amino-10-methyl acridinium chloride), melting point about (258-260°C) [8]. Acriflavine is prepared first time by benda in 1912 by acetylation of proflavine and then reacted with methyl sulfate or methyl toluenesulfate [9,10] which dissolved in water with phosphorous color, dissolved slightly in alcohol and insoluble in ether, chloroform, and fixed oils [8,4] acriflavine has ability to formed complexes with DNA by intercalated with DNA nucleotide pairs which creating mutations [1,11,12] and used in urological treatment and gonorrhea [13]. In this paper, we report the formation of new acriflavine complexes obtained in the reaction of (LCl) with the metal ions VO (II), Co (II), Ni (II), Cu (II), Cd (II), Zn (II) and Hg(II) in ethanol as solvent. The newly complexes have been structurally characterize by elemental analysis, infrared (IR), electronic absorption spectroscopy and mass spectrum of some complexes, the antibacterial activity of prepared complexes also have been investigated against two various type of bacteria.

Fig 1. Structure of acriflavine
Materials and Methods of Characterization

Acriflavine (BDH), cobalt (II) chloride hexahydrate, nickel(II) chloride, copper(II) chloride dehydrate, cadmium(II) chloride, zinc(II) chloride, mercury(II) chloride, and vanadyl (II) Sulfate monohydrate, DMSO and ethanol were provided from Aldrich company. Melting points for prepared complexes were measured by electro thermal (Stuart melting point apparatus). Infrared spectra were performed using a Shimadzu (FT–IR)–8400S.

The electronic spectra of the compounds were recorded by using double-beam (U.V.-Vis) spectrophotometer type U.V 160A (Shimadzu), Elemental analysis recorder by using Euro Vector, model EA 3000 single V.3.Osingle, The Chloride contents were determined using (686-Titro processor-665). Dosimat Matron Swiss, Mass spectra were recorder by using the device (GLC-mass) QP SO A: shima (170 ev) (for complex [Co2(L1)2(0H2)4Cl]Cl2). Electrical conductivity measurements of the complexes were recorder at (25°C) for (10–3 mole.L–1) solution of the samples in DMSO by using (conductivity meter, model 4070), Magnetic measurements of the metal complexes were performed on a Magnetic Susceptibility Balance Mode (MSB _MKI).

Synthesis of Complexes

Synthesis of [M2(L1)2(H2O)4Cl4] Cl2

A hot EtOH solution (10 mL) of (0.476 g, 0.258, 0.340, 0.366, 0.272, 0.544) (2 m mole) of Co(II),Ni(II),Cu(II),Cd(II), Zn(II) and Hg(II), hot Et OH solution (60 mL) of acriflavine (0.518 g, 2mmole) were mixed in round flask with constant stirring, the mixture refluxed for an 1hr at (50°C), A colored complexes was precipitated, filtered and washed several times with cool ethanol.

Characterization of Ligand

IR spectrum of acriflavine (LCl): (3410) cm–1 νs(νy) NH2 (3317) cm–1 νs(νy) NH2 (1639) cm–1 δ N–H ,(1597) cm–1 νC=N, (1492) cm–1 ν(C=C) aromatic ring, (3178)cm–1 ν(CH3) aliphatic,(3039)cm–1 ν(C–H) aromatic ring [14,15]. The electronic spectrum date of (LCl) showed two high absorption peaks, the first at (267 nm) (37453) cm–1 resulted from electronic transition n→n*, the other peak appeared at (469 nm) (21322) cm–2 resulted from electronic transition n→n* [16].

Characterization of [Co2 (L1)2(H2O)4Cl4] Cl2

Green yellowish, Yield:65 %. M.p. 206 °C. IR (KBr cm–1 ): 3421 (νs(νy) NH2 ), 3329 (νs(νy) NH2 ), 1631 (6 N – H ), 578 (v M – N ), 439 (v M – O ), 821 (δOH ). UV/visible (DMSO, nm): 295 (Intra-ligand) , 468 (Intra-ligand) , 614 (1T1g(F)→1T1g(P) ), 680 (1T1g (F)→4A1g (F) ), 984 (4T1g (F)→4T1g (F)).


Characterization of [Ni2 (L1)2(H2O)4Cl]Cl2

Reddish orange, Yield: 77 %. M.p. 242°C. IR (KBr cm–1): 3425 (νs(νy) NH2 ), 3329 (νs(νy) NH2 ), 1643 (6 N – H ) , 578 (v M – N ), 478 (v M – O ) , 337 (δOH ). UV/visible (DMSO, nm): 276 (Intra-ligand), 476 (Intra-ligand), 734 3A1g(F)→1T1g(F), 972 (4A1g(F)→4T1g(F)). (CH,N) found %, (calculated %): 40.21(39.56) C, 3.28 (4.73) H , 9.06 (9.88) N ,12.09 (13.76) Metal,14.67(16.70) Cl. 5eff (B.M) : 2.9.

Characterization of [Cu2(L1)2(H2O)4Cl]Cl2

Black, Yield: 84 %. M.p. 200 °C. IR (KBr cm–1): 3452 (νs(νy) NH2 ), 3332 (νs(νy) NH2 ), 1612 (6 N – H ), 528 (v M – N ), 451 (v M – O ) , 821 (δOH ). UV/visible (DMSO ,nm): 278 (Intra-ligand), 475 (Intra-ligand), 949 (5eg→5Tg) (C.H,N) found %, (calculated %): 38.43 (39.06) C, 4.33 (4.18) H , 8.27 (9.76) N ,14.00 (14.76) Metal,15.55(16.51) Cl. 5eff (B.M) : 1.79.

Characterization of [Cd2(L1)2(H2O)4Cl]Cl2

Characterization of [Zn₂ (L₁)₂(H₂O)₄Cl₄] Cl₂

Orange, Yield: 60 %. M.p. 320°C. IR (KBr cm⁻¹): 3433 (vasy NH₂ ) , 3325 (vasy NH₂ ), 1627 (6N – H ) , 528 (v M – N ) , 451 (v M – O ) , 937 (6OH ). UV/visible (DMSO ,nm): 279 (Intra-ligand) , 476 (Intra-ligand) . (C.H.N) found %,(cala.%): 38.07 (38.89) C , 6.01 (4.16) H , 8.77 (9.72) N ,16.64 (15.14) Metal ,17.43(16.43) Cl.

Characterization of [Hg₂ (L₁)₂(H₂O)₄Cl₄] Cl₂


Synthesis of [VO] 2(L₁)₂(SO₄)₂(H₂O)₂Cl₂

A hot EtOH solution (15 mL) of VOSO₄.H₂O (0.362g, 2 mmole) and hot EtOH solution (60 mL) of acriflavine (0.518 g, 2mmole) were mixed in round flask with constant stirring ,the mixture refluved for an two hour at (50°C), A brown complex was precipitated and washed several times with cool ethanol.

Characterization of [VO] 2(L₁)₂(SO₄)₂(H₂O)₂ Cl₂

Brown, Yield:73 %. M.p. 130°C IR (KBr cm -1): 3467 (vasy NH₂ ) , 3329 (vasy NH₂), 1635 (6 N – H ) , 578 (v M – N ) , 482 (v M – O ) , 879 (6OH ). UV/visible (DMSO, nm): 278 (Intra-ligand) , 478 (Intra-ligand), 673(²B₉g→³B₁g) , 841 (²B₉g→ ²Eg) . (C.H.N) found %,(cala.%) 36.89 (38.13) C , 3.02 (3.36) H , 10.14 (9.53) N ,10.82 (11.57) Metal ,8.90(8.05) Cl. ³eff (B.M): 1.79

Antibacterial Activity

Microorganisms and Media

The Bauer method was used to test the effect of all prepared complexes against the Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Pseudomonas aeruginosa) by agar-well diffusion method . The media were prepared by add 28 g of nutrient agar in 1000 mL of distilled water and disinfected in the autoclave to prevent contamination at 121°C for 30 min then spilled in petri dish ,after rigidity the media, the bacterial was spread, each of the prepared complexes was dissolved in (DMSO) to give concentration of (0.01) mg/ml, and incubated the media at 37 °C for 24 h and checked for the growth of inhibition zones. [17, 18]

Results and Discussion

Chemistry

A new seven complexes were synthesized by direct reaction between acriflavine (LCl) and metal ions ,this complexes were synthesis to confirm the suggested structures of complexes .In IR spectra of prepared complexes the band of vasy NH₂ and vṛ̃ NH₂ were shifting to higher frequencies compared to the spectrum of free ligand at range (3452_3367) cm⁻¹ and (3340_3317)cm⁻¹ respectively [19,20], this shifting refers to coordination of metal ions with two N atoms of NH₂ group of acriflavine, δ (N-H) band also has shifting to lower or higher frequencies at range (1645_1612) cm⁻¹ [21], for all complexes which also confirms the consistency between the metal ion and two N atom of the NH₂ group of acriflavine, New three bands has appeared, first one at range (937_821) cm⁻¹ assigned to ν(OH) stretching vibration of coordinated water molecules(aqua) [22], two weak bonds appeared at range (578_513) cm⁻¹ and (497_424) cm⁻¹ returned to (M-N) and(M-O) respectively [23],The IR spectrum of vanadyl ion complex showed a new bond at(983) cm⁻¹ refered to (V=O), and new four bonds at(1041,1064,651,659)cm⁻¹ due to the consistency of (SO⁴²⁻) ion which behaves as bidentate with vanadyl ion [24,25].

The electronic spectra

Of Zn (II), Cd (II), Hg (II) complexes showed that no (d,d) transition because it contains aftul d subshell [26]. (UV_Vis) transitions for all complexes have good agreement for octahedral geometry around the metal ion.

The Mass Spectra Data

Of [Co₂ (L₂)₂(H₂O)₄ Cl₄] Cl₂ has agreement with their molecular formula [27].

Elemental Analysis

Results also have a good agreement with the calculated values. At last the molar conductance showed that all complexes were electrolyte with ionic ratio (1:2)
Antibacterial Activity

The antimicrobial activities of prepared complexes were evaluated in vitro against Staphylococcus aureus and Pseudomonas aeruginosa which described in Table 1. All complexes give higher inhibition activity against Staphylococcus aureus ,While the complexes of Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) have no antimicrobial activity against Pseudomonas aeruginosa except VO(II), Co(II) complexes which inhibited this type of bacterial.

Conclusion

According to the characterization data for new prepared complexes we suggested that Co(II), Ni(II), Zn(II), Cd(II) and Hg(II) complexes are octahedral geometry, Cu (II) and VO(II) has distorted octahedral geometry, acriflavine behave as bidentate ligand through two (N) atoms of NH₂ groups. These prepared complexes have higher inhibition activity against bacterial that make it possible to use as drugs.

Table 1: The antimicrobial activities of prepared complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>St. aureus</th>
<th>p. aeruginosa</th>
</tr>
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<tbody>
<tr>
<td>Acriflavine (LCl)</td>
<td>25</td>
<td>—</td>
</tr>
<tr>
<td>[(VO)₂(L₁)(SO₄)₂(H₂O)₂]Cl₂</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>[Co₂(L₁)(H₂O)₄]Cl₄</td>
<td>21</td>
<td>10</td>
</tr>
<tr>
<td>[Ni₂(L₁)(H₂O)₄]Cl₈</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>[Cu₂(L₁)(H₂O)₄]Cl₈</td>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td>[Zn₂(L₁)(H₂O)₄]Cl₂</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td>[Cd₂(L₁)(H₂O)₄]Cl₂</td>
<td>26</td>
<td>—</td>
</tr>
<tr>
<td>[Hg₂(L₁)(H₂O)₄]Cl₂</td>
<td>25</td>
<td>—</td>
</tr>
</tbody>
</table>

St.aureus= Staphylococcus aureus , p.aeruginosa= Pseudomonas aeruginosa

![Fig. 2: suggested structure of complexes](image)

![Fig 3: IR spectrum of acriflavine](image)
Fig 4: UV-Vis spectrum of acriflavine

Fig 5: IR spectrum of \([\text{Co}_2(L_1)_2(\text{H}_2\text{O})_4\text{Cl}_4]\)\text{Cl}

Fig 6: UV-Vis spectrum of \([\text{Co}_2(L_1)_2(\text{H}_2\text{O})_4\text{Cl}_4]\)\text{Cl}_2 in (10^{-3}\text{M})

Fig 7: UV-Vis spectrum of \([\text{Co}_2(L_1)_2(\text{H}_2\text{O})_4\text{Cl}_4]\)\text{Cl}_2 in (6\times10^{-4}\text{M})
References

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