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Synthesis, Characterization and Bacterial Activity Study of Some Mixed Ligand Cadmium(II) Complexes with Diclofenac and N-donor Ligands

Emad Nissan Abdullah , Ahmed A. Irzoqi , Mahmood Mahdi Salih
Department of Chemistry, College of Education for pure Science, University of Tikrit , Tikrit, Iraq

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Name: Ahmed A. Irzoqi
E-mail: ahmedirzoqi@tu.edu.iq
Tel:

ABSTRACT

New complexes of Cd(II) were prepared by reaction of one mole equivalent of cadmium chloride with two moles equivalent of sodium diclofenac and suitable moles of one of the following ligands (1,10-phenanthroline (Phen), bipyridine (bipy), 2-aminopyridine (2-apy), 3-aminopyridine (3-apy), 4-aminopyridine (4-apy) or cyanoguanidine (cg)). The obtained results indicated that the diclofenac behaved as a monodentate ligand coordinated to cadmium ion through oxygen atom of the carboxyl group, while (bipy and phen) behaved as bidentate ligands, the substituted aminopyridine and cyanoguanidine behaved as monodentate ligands. The prepared complexes have tetrahedral geometry.
The prepared complexes were characterized by conductivity measurements, element analysis CHN, also by infrared and ^{1}H-NMR spectroscopy.
Some of the prepared complexes showed biological activity against two bacterial types' *staphlycocas aureus*, *pseudomonas aeruginusa* in comparison with the antibiotic gentamycin.

Introduction

metals, which consist of bivalent metal clusters as in $[Ca(dic)_2.2H_2O]$, $[Mg(dic)_2.2H_2O]$, $[Zn(dic)_2.2H_2O]$, and a quaternary with a trivalent iron cluster $[Fe(dic)_3.3H_2O]$[12].
In the present work, a number of cadmium(II) complexes with diclofenac and mixed ligands such as (phen, bipy), 2-,3- or 4- aminopyridine and cyanoganidine were prepared and their bacterial activity were tested against two types of bacteria, *staphlycocas aureus* and *pseudomonas aeruginusa*.

Experimental:

The IR spectra were recorded on a Shimidzu FT-IR 8400S spectroscopy, using KBr disc. The ^{1}H-NMR spectra were recorded on Bruker (300 MHz) with TMS as internal reference and DMSO-d^{6} as a solvent in Gazi University in Turkey. Melting points were measured using SMP40 supplied by stuart company. The moler conductivity measureds were recorded on a Starter 3100C in DMSO as solvent at 1×10^{-3}M and 25C^{O}. Elemental analysis was measured using EuroEA3000 in the central service laboratory in college of education Ibn alhaytham, university of Baghdad in Iraq.

Synthesis of $[Cd(dic)_2(H_2O)_2]$ complex:

Sodium diclofenac is a non-steroidal anti-inflammatory drug (NSAID); it is used in treatment of minor and medium rheumatoid diseases, and pain analgesia after surgery. But It often may cause a gastrointestinal tract complications, especially in stomach and duodenum[1-4].There are a number of medicines and pharmaceutical preparations contains metals, this association is potentially to affect the biological activity of the drug and may also cause damage son their target biomolecules [5]. Metal complexes of diclofenac such as $[Cu(diclo)_2(H_2O)].2H_2O$, $[Pd(diclo)_2].2H_2O$, $[Fe(diclo)_2(H_2O)]$, $[Ni(diclo)_2(H_2O)].2H_2O$ and $[Co(diclo)_2(H_2O)].2H_2O$ exhibit promising anti-inflammatory activity and act as antioxidant compounds, a property that is absent in diclofenac itself [6-8].
Carboxylic complexes have emerged as an important species in the last few years, these complexes includes not only mono or dicarboxylates associated with transitional elements, rare earth, and the main group metals, but also a variety of mixed mono and dicarboxylates structures [9-11]. An ionic bond was formed between carbonyl diclofenac and central

Synthesis of $[Cd(dic)_2(amine)_2]$ complex (3-6)

A solution of Nadic (0.343g, 0.0018mol) in absolute ethanol 15ml was added to suspension of $CdCl_2$ (0.100g, 0.0005mol) in absolute ethanol 15ml, the mixture was stirred for 1 hour to give white precipitate, ethanolic solution of amine compounds (2-,3-,4-aminopyridine or cyanoganidine) in appropriate molar ratio was added, the final mixture was refluxed for (3) hour to give the corresponding complex as precipitate, which was filtered off, washed with cold absolute ethanol and dried under vacuum. As shown in equation 2.

Results and Discussion:

Treatment of cadmium chloride with sodium diclofenac and N-donor ligands such as imines (phen, bipy), 2-,3-,4-aminopyridine or cyanoganidine in absolute ethanol gave new complexes **(1-6)** as shown in scheme(1) and (2). The new complexes were characterization by spectral data IR, ^{1}H-NMR, moler conductivity and elemental analysis measurements.

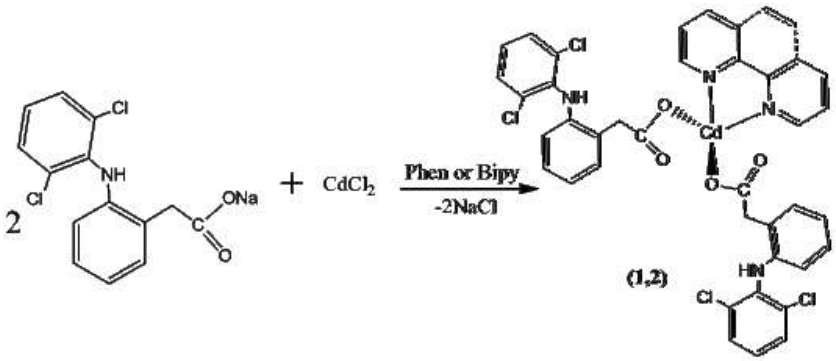
This complex was prepared by literature method [13].

Synthesis of $[Cd(dic)_2Phen]$ complex (1)

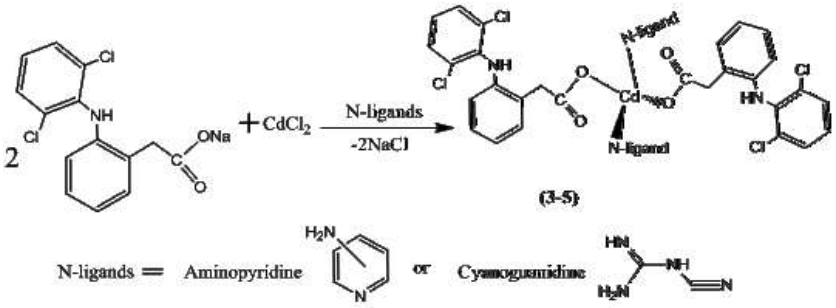
a) A solution of Phen (0.180g, 0.001mol) in absolute ethanol 10ml was added to solution of $[Cd(dic)_2(H_2O)_2]$ complex (0.738g,0.001mol) in absolute ethanol 10ml, the mixture was stirred and refluxed for 3 hour to give white precipitate, which was filtered off, washed with cold absolute ethanol and dried under vacuum.(0.732g, 83%).

b) A solution of Nadic (0.343g, 0.0018mol) in absolute ethanol 15ml was added to suspension of $CdCl_2$ (0.100g,0.0005mol) in absolute ethanol 15ml. The mixture was stirred for (1) hour to give white precipitate and added a solution of Phen (0.090g, 0.0005mol) in 10ml absolute ethanol, the final mixture was refluxed for (3) hour to give white precipitate, which was filtered off, washed with cold absolute ethanol and dried under vacuum (0.357g, 81%).

$[Cd(dic)_2bipy]$ **(2)** complex was prepared by two similar methods in used to prepare **(1)** complex.



Scheme (1) Reaction $CdCl_2$ with sodium diclofenac and Phen or Bipy



Scheme (2) Reaction $CdCl_2$ with sodium diclofenac and aminopyridine or cyanoguanidine

The results of the molar conductivity showed that the solutions of the prepared complexes (1×10^{-3}M) in DMSO were non-electrolytic. Elemental analysis results were in accord with the suggested formula for complexes **(1-6)** (Table 1).

Table (1) Color, M.P., yield (%), moler conductivity and elemental analysis of the prepared complexes

NO.	Complexes	Color	M.P (°C)	Yield %	Λ ($\Omega^{-1}.cm^2.mol^{-1}$)	Elemental Analysis (%) Found(calc.)		
						C	H	N
1	$[Cd(dic)_2Phen]$	White	252d	83	22.0	54.69 (54.41)	3.76 (3.20)	6.62 (6.35)
2	$[Cd(dic)_2Bipy]$	White	175d	82	10.9	53.40 (53.14)	3.53 (3.29)	6.83 (6.52)
3	$[Cd(dic)_2(2apy)_2]$	White	177d	79	18.5	-	-	-
4	$[Cd(dic)_2(3apy)_2]$	White	188d	83	21.7	-	-	-
5	$[Cd(dic)_2(4apy)_2]$	White	190-192	85	24.0	51.68 (51.23)	3.91 (3.62)	9.59 (9.43)
6	$[Cd(dic)_2(cg)_2]$	White	265d	84	15.2	44.37 (44.13)	3.52 (3.24)	16.54 (16.28)

d = decomposition

IR Spectra:

The IR spectra of the prepared complexes were compared with the IR spectrum of free Nadic ligand in order to determine the coordination sites. For complexes that contains (COO^-) groups coordinated directly to metal, the major characteristic bands in IR spectra is the symmetric and asymmetric stretching vibration ν(COO^-) and the separation between them Δν(COO^-).

The prepared complexes shown bands within (1602-1639)cm^{-1} range attributed to asymmetric stretching vibration ($asCOO^-$) groups, while the stretching vibration of the symmetric stretching vibration ($sCOO^-$) groups appear within (1409-1450)cm^{-1} range, the separation between them Δν(COO^-) are (189-195)cm^{-1} which are greater than that of the free ion (sodium diclofenac, Δν(COO^-)=1575-1400=175cm^{-1}), this indicates a mondentate coordination mode[13-17].

A strong absorption bands with in (1548-1610) cm^{-1} range are attributed to ν(N=C) and (3353-3448) cm^{-1} range are assigned to ν(NH) and (3253-3346)cm^{-1} range are assigned to ν(NH_2) of the prepared complexes **3-6**, the ν(N≡C) group of cyanoguanidine of complex **6** showed bands at (2198,2156) cm^{-1} which was shift down compared with free ligand at (2163-2206)cm^{-1}, this is an indicating for coordination of this group[18,19]. Other IR data are given (Table 2).

Table (2) Selected IR stretching vibration bands (cm^{-1}) for complexes (1-6)

Complex	The IR vibration bands (cm^{-1})							
	$\nu_{as}COO^-$	**$\nu_{s}COO^-$**	**$\Delta\nu COO^-$**	**νNH**	**νNH_2**	**ν_{Ar}C-H**	**ν_{alph}C-H**	**νN=C**
1	1620	1425	195	3436	-	3049	2918	1587
2	1618	1427	191	3439	-	3048	2920	1576
3	1629	1436	193	3381	-	3064	2923	1589
4	1639	1448	191	3346	3448	3072	2921	1610
5	1602	1409	193	3253	3353	3036	2954	1573
6	1639	1450	189	3155 3222	3394 3375	3053	2920	1548

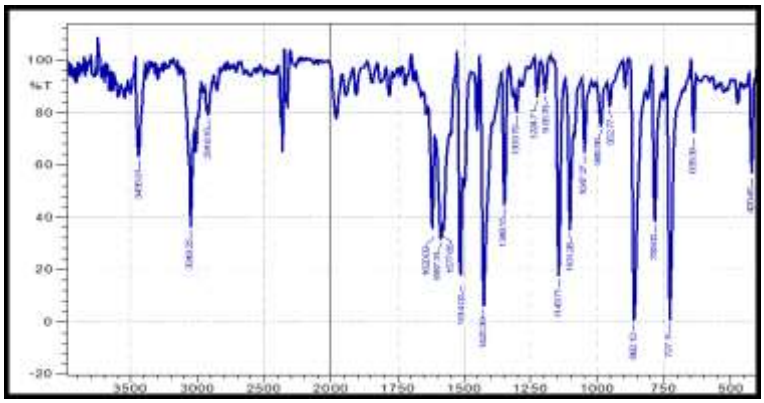


Figure (1) IR Spectrum of $[Cd(dic)_2Phen]$ Complex (1)

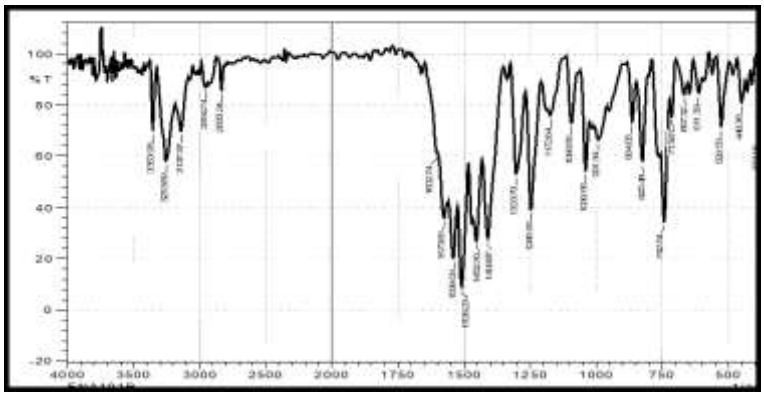


Figure (2) IR Spectrum of $[Cd(dic)_2(4\text{-apy})_2]$ complex (5)

^{1}H-NMR Spectra:

The ^{1}H-NMR spectrum of complex **(1)** showed a signal as a singlet at δH=3.59ppm attributed to CH_2 protons of diclofenac ligand, the H_C proton of diclofenac ligand appeared as a doublet at δH=6.24ppm with coupling constant (^3JH_C-H_D =7.96Hz), while H_E proton appeared as a triplet at δH=6.82ppm with coupling constant (^3JH_E-H_F =7.9Hz), the spectrum of complex **(1)** showed a triplet at δH=7.02ppm with coupling constant (^3JH_D-H_C =7.96Hz) attributed to H_D proton, while H_A and H_F protons appeared as a pseudo triplet at δH=7.13ppm, the H_B protons appeared as a doublet at δH=7.42ppm with coupling constant (^3JH_B-H_A =7.3Hz), the NH proton appeared as a singlet at δH=7.57ppm[20,21], the spectrum of complex **(1)** showed a multiplet within δH=8.02-8.07ppm range attributed to H_2 protons of Phen ligand, the spectrum showed a singlet at δH=8.22ppm attributed to H_4 protons, the H_3 protons appeared as a doublet at δH=8.81ppm, while the H_1 protons appeared as a doublet at δH=9.17ppm.

The ^{1}H-NMR spectrum of complex **(4)** showed a singlet at δH=3.59ppm attributed to CH_2 protons of diclofenac ligand, the H_C proton of diclofenac ligand appeared as a doublet at δH=6.31ppm with coupling constant (^3JH_C-H_D =7.7Hz), while H_E proton appeared as a triplet at δH=6.83ppm with coupling constant (^3JH_E-H_F =7.0Hz), the spectrum of complex **(4)** showed a triplet at δH=7.02ppm with coupling constant (^3JH_D-H_C =7.7Hz) attributed to H_D proton, while H_A and H_F protons appeared as a pseudo triplet at δH=7.17ppm, the H_B protons appeared as a doublet at δH=7.48ppm with coupling constant (^3JH_B-H_A =8.0Hz), the NH proton appeared as a singlet at δH=8.66ppm[20,21], the spectrum of complex **(4)** showed a doublet at δH=6.44ppm with coupling constant ($^3JH_2$-H_3 =8.0Hz) attributed to H_2 protons of 3-apy ligand, the spectrum showed a doublet at δH=6.48ppm with coupling constant ($^3JH_4$-H_3 =7.7Hz) attributed to H_4 protons, the H_3 protons appeared as a triplet at δH=7.36ppm with coupling constant ($^3JH_3$-H_4=7.7Hz), while the H_1 protons appeared as a doublet at δH=7.88ppm with coupling constant ($^4JH_1$-H_4 =7.2Hz), the NH_2 protons appeared as a singlet at δH=5.92ppm.

The ^{1}H-NMR spectrum of complex **(5)** showed a signal as a singlet at δH=3.56ppm attributed to CH_2 protons of diclofenac ligand, the H_C proton of diclofenac ligand appeared as a doublet at δH=6.30ppm with coupling constant (^3JH_C-H_D =7.8Hz), while H_E proton appeared as a triplet at δH=6.82ppm with coupling constant (^3JH_E-H_F =7.3Hz), the spectrum of complex **(5)** showed a triplet of doublets at δH=7.01ppm with coupling constant (^3JH_D-H_C =7.5Hz) and (^4JH_D-H_F =1.65Hz) attributed to H_D proton, while H_A proton appeared as a triplet at δH=7.09ppm with coupling constant (^3JH_A-H_B=8.0Hz), the H_F protons appeared as a doublet of doublets at δH=7.16ppm with coupling constant (^3JH_F-H_E =7.3Hz) and (^4JH_F-H_D =1.58Hz), the H_B protons appeared as a doublet at δH=7.45ppm with coupling constant (^3JH_B-H_A =8.0Hz), the NH proton appeared as a singlet at δH=8.71ppm[20,21], the spectrum of complex **(5)** showed a doublet at δH=6.50ppm with coupling constant ($^3JH_2$-H_1 =8.85Hz) attributed to H_2 protons of 4-apy ligand, the spectrum showed a doublet at δH=6.63ppm with coupling constant ($^3JH_1$-H_2 =8.89Hz) attributed to H_1 protons, the NH_2 protons appeared as a singlet at δH=4.58ppm.

The ^{1}H-NMR spectrum of complex **(6)** showed a signal as a singlet at δH=3.49ppm attributed to CH_2 protons of diclofenac ligand, the H_C proton of diclofenac ligand appeared as a doublet at δH=6.27ppm with coupling constant (^3JH_C-H_D =7.9Hz), while H_E proton appeared as a triplet at δH=6.79ppm with coupling constant (^3JH_E-H_F =7.2Hz), the spectrum of complex **(6)** showed a triplet at δH=6.98ppm with coupling constant (^3JH_D-H_C =7.4Hz) attributed to H_D proton, while H_A and H_F protons appeared as a multiple whithin δH=7.06-7.13ppm range, the H_B protons appeared as a doublet at δH=7.46ppm with coupling constant (^3JH_B-H_A =8.0Hz), the NH proton appeared as a singlet at δH=7.98ppm[20,21], the spectrum of complex **(6)** showed a singlet at δH=5.52ppm attribute to NH proton of secondary amine of cyanoguanidine ligand and a singlet at δH=6.60ppm attribute to NH_2 proton

of cyanoguanidine ligand, while the proton of C=NH group of cyanoguanidine ligand and H_A, H_F protons assigned to diclofenac which was as unresolved multiplet within the δH=7.06-7.13ppm range, the integration value confirms the presence of 6 protons. H-NMR spectral data of complexes **1,4,5** and **6** are listed in Table (3).

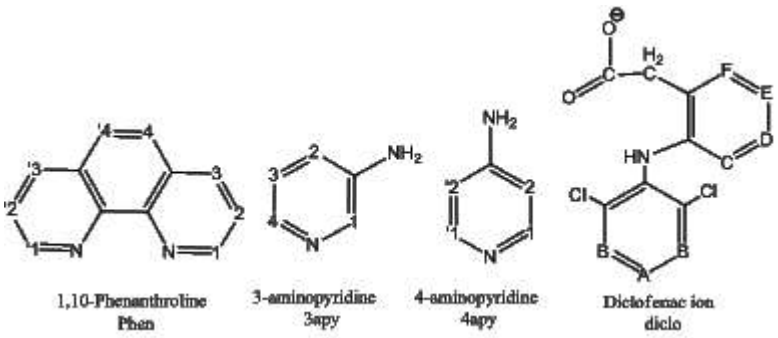


Table (3) ^{1}H NMR Chemical Shifts (ppm) for the Prepared Complexes (1,4,5 and 6)

Ligands	Complexes			
diclofenac	1	4	5	6
CH_2	3.59(s,4H)	3.59(s,4H)	3.56(s,4H)	3.49(s,4H)
Hc	6.24(d,2H)	6.31(d,2H)	6.30(d,2H)	6.27(d,2H)
H_E	6.82(t,2H)	6.83(t,2H)	6.82(t,2H)	6.79(t,2H)
H_D	7.02(t,2H)	7.02(t,2H)	7.01(td,2H)	6.98(t,2H)
H_A,H_F	7.13(t*,4H)	7.17(t*,4H)	7.09(t,2HA) 7.16(dd,2HF)	7.06-7.13(m,6H)
H_B	7.42(d,4H)	7.48(d,4H)	7.45(dd,4H)	7.46(d,4H)
NH	7.57(s,2H)	8.66(s,2H)	8.71(s,2H)	7.98(s,2H)
Other ligands	Phen	3-apy	4-apy	Cg
H_1	9.17(d,2H)	7.88(d,2H)	6.63(d,4H)	-
H_4	8.22(s,2H)	6.48(d,2H)	-	-
H_3	8.81(d,2H)	7.36(t,2H)	-	-
H_2	8.02-8.07(m,2H)	6.44(d,2H)	6.50(d,4H)	-
NH_2	-	5.92(s,4H)	4.58(s,4H)	6.60(s,4H)
NH	-	-	-	5.52(s,2H)

*= pseudo triplet

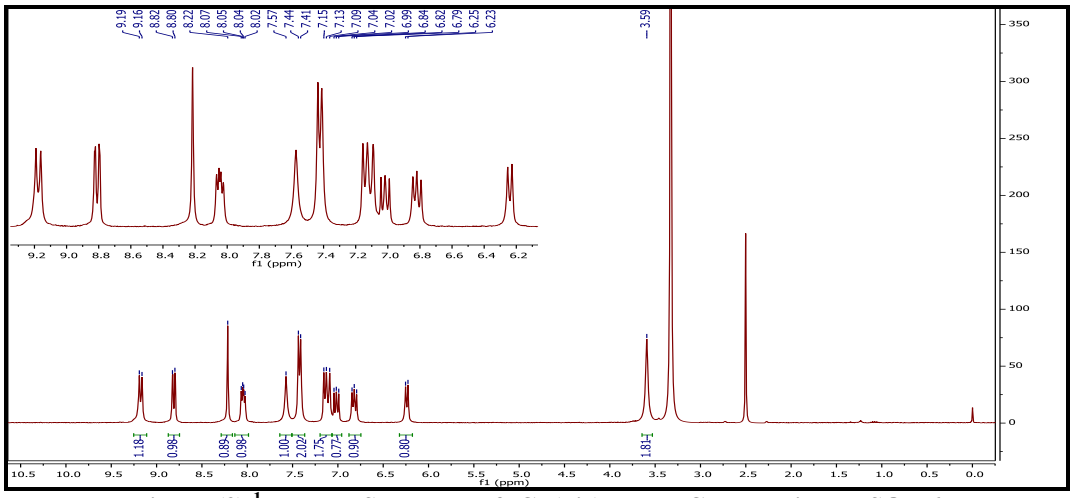


Figure (3) ^{1}H-NMR Spectrum of [Cd(dic)$_2$Phen] Complex in DMSO-d6

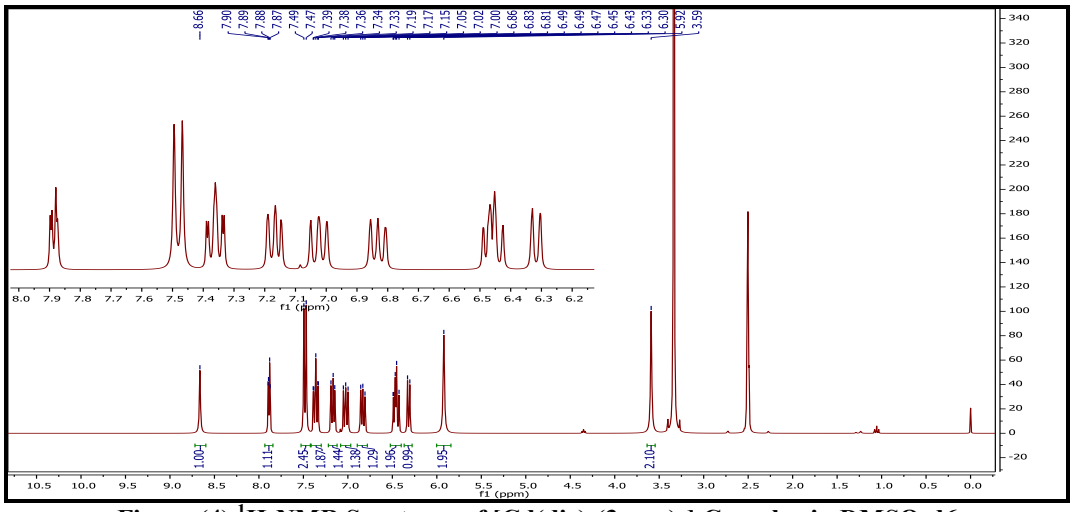


Figure (4) ^{1}H-NMR Spectrum of [Cd(dic)$_2$(3-apy)$_2$] Complex in DMSO-d6

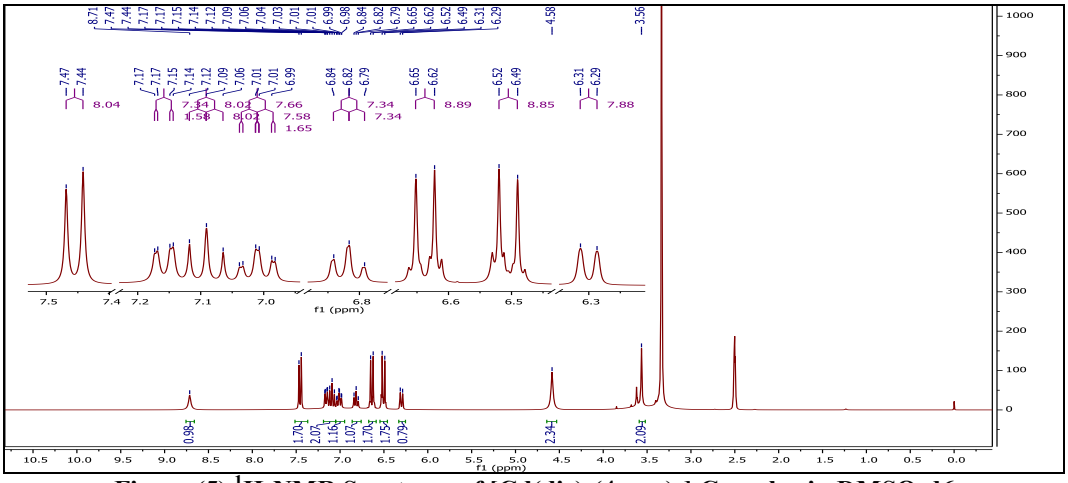


Figure (5) ^{1}H-NMR Spectrum of [Cd(dic)$_2$(4-apy)$_2$] Complex in DMSO-d6

Antibacterial activity:

The results of antibacterial activity of the prepared complexes **1**, **5**, **6** were tested against two bacterial types *staphlycocas aureus* (gram positive), *pseudomonas aeruginusa* (gram negative) and compared with the antibiotic gentamycin exclusive the complex **6**. The hole methods used to tested of the prepared complexes and three concentration from the prepared complexes ($1x10^{-1}$, $10^{-3}$and 10^{-5} M) while the DMSO used as solvent and control [5], the anti-bacterial activity was determined by measuring inhibition zone diameter (IZD) in millimeter (mm) to gentamycin and complexes **1**, **5** and **6**. The results are summarized in (Table 4).

Table (4) The anti-bacterial activity of complexes 1, 5 and 6

No.	Complexes	Concentration	*staphlycocas aureus*	*pseudomonas aeruginusa*
1	[Cd(diclo)$_2$phen]	10^{-1}	S	S
		10^{-3}	S	S
		10^{-5}	S	R
5	[Cd(diclo)$_2$(4-apy)$_2$]	10^{-1}	S	S
		10^{-3}	R	R
		10^{-5}	S	R
6	[Cd(diclo)$_2$(cy)$_2$]	10^{-1}	S	S
		10^{-3}	S	S
		10^{-5}	S	S

(R: 12mm, M: 13-14mm, S: 15mm)

Conclusion

From the results obtained we can conclude that the diclofenac was behaved as a monodentate ligand coordinated to cadmium ion through oxygen atom of the carboxyl group, while the bipy and phen were behaved as bidentate ligands. The substituted aminopyridine behaved as monodentate ligands bonded through nitrogen atom of the heterocyclic ring. The cyanoguanidine ligand behaved as a

monodentate ligand bonded through nitrogen of the (N≡C) group. The proposed geometrical structures of the prepared complexes **(1-6)** were tetrahedral around cadmium ion. Bacterial activity revealed that the metal complexes showed good activity on both type of gram positive and gram negative of tested bacteria.

References

1. Davies N.M., Saleh J.Y., Skjodt N.M.: **J. Pharm. Pharm. Sci.**, 3 (2000)137.
2. Wallace J.L., Del Soldado P.: **Fundam. Clin. Pharmacol.**, 17 (2003)11.
3. Martindale: **The Complete Drug Reference.** 37th edn., The Pharmaceutical Press, London (2011).
4. Peterson, S. P.,Killurman, I., **Gastrointestinal effects of NSAIDs:Difficulties in detection and management. Postgrad. Med.**, (1996).
5. Moamen S. Refat, Mohamed G. G., Mohamed Y. S. Ibrahim, Killa H. M. A., and H. Fetooh, **Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry**, 44 (2014)161–170.
6. Kovala-Demertzi, D., **J. of Inorg. Biochem.**, 79(2000) 153-157.
7. Konstandinidou, M.; Kourounakis, A.; Yiangou, M.; Hadjipetrou, L.; Kovala-Demertzi, D.; Hadjikakou, S.; Demertzis, M., **J. of Inorg. Biochem.**, 70 (1998) 63-69.
8. Khan, F. M. F., Marwat, I. K., Khan, G. M., Khan, H., **J. Chem. Soc. Pak.**, 32 (2010)462-466.
9. Batten, S. R., Hoskins, B. F., Moubaraki, B., Murray, K. S., Robson R., **Chem. Commun.** (2000) 1095.
10. Mao, J. G., Wang, Z., Clearfield, A., **J. Inorg. Chem.**, 41 (2002) 2334.
11. Eddaoudi M., Kim J., Vodak D., Sudik A., Wachter J., O'Keeffe M., Yaghi O. M. Proc., **Natl. Acad. Sci. USA**, 99 (2002) 4900.
12. Kenawi I. M., Barsoum B. N., Youssef M. A., **Eur. J. Pharma. Sci.** (2005) 26, 341–348.
13. Tarushi A., Totta X., Papadopoulos A. N., Kljun J., Turel I., Kessissoglou D. P., Psomas G., **Eur. J. Med. Chem.,** *74* (2014) 187–198..
14. Doyle A., Feleman J., Do Prado Gambardella M. T., Verani C. N., Braganca Tristao, M. L., **Polyedron**; 19, 2621–2627 (2000).
15. Nakamoto K., "**Infrared Spectra of Inorganic and Coordination Compounds**", 2nd edn. Wiley Interscience, New York (1970).
16. Imad I. Hamdan, Dina El-Sabawi, and Mariam Abdel Jalil; **Drug Dev Ind Pharm, Early Online**: 1–11(2015).
17. Ahmed S. M. Al-Janabi, Ahmed A. Irzoqi, Shihab A. O. Ahmed; **Tikrit Journal of Pure Science;** 21 (3) (2016).
18. Marianthi Z., Vassilis T., Catherine P. R., Vassilis P., Athanasios N. P. and George P., **Eur. J. Inorg. Chem.,** (2015), 2285–2294.
19. Mahmood M. Salih, Ahmed A. Irzoqi; **Tikrit Journal of Pure Science**; 23 (1) (2018).
20. Mohammed B., A. Sallam, I. Hamdan, M. Al-Hussaini, A. Bani-Jaber, **Drug Development and Industrial Pharmacy**, 34:434–444, (2008).
21. Akitt, J. W., Mann B. M., **"NMR and Chemistry"**, 4th edn, Chapman and Hall, London, (2000).

تحضير وتشخيص ودراسة الفعالية البكتيرية لبعض من معقدات الكادميوم مع مزيج ليكاندات دايكلوفيناك وليكاندات واهبة للنتروجين

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قسم الكيمياء ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق

الملخص

حضرت معقدات جديدة للكادميوم (II) من تفاعل مول مكافئ واحد من كلوريد الكاميوم مع مولين مكافئة من دايكلوفيناك الصوديوم واضافة مولات مناسبة من ليكاندات التالية (الفينولفثالين (bipy) اوالبابيريدين (phen) او 2–امينوبريدين (2-apy) او 3–امينوبريدين (3-apy) او 4– امينوبريدين (4-apy) اوسيانوكواندين (Cg)). دلت النتائج سلوك دايكلوفيناك كليكاند احادي السن مع ايون الكادميوم يرتبط من خلال ذرة اوكسجين مجموعة الكاربوكسيل، بينما سلكت ليكاندات (bipy, phen) كليكاندات ثنائية السن. اما معوضات امينوبريدين والسانوكواندين (Cg) فقد سلكت سلوك ليكاندات احادية السن. تمتلك المعقدات المحضرة شكل رباعي السطوح.

شخصت المعقدات المحضرة بواسطة وقياسات التوصيلية الكهربائية والتحليل الدقيق للعناصر CHN،كذلك شخصت طيفيا بالاشعة تحت الحمراء و طيف الرنين النووي المغناطيسي للبروتون.

اظهرت نتائج الفعالية المضادة للبكتيريا للمعقدات المحضرة 1،5،6 فعالية ضد نوعين من البكتيريا هي *staphlycocas aureus*, *pseudomonas aeruginusa* بالمقارنة مع المضاد الحيوي gentamycin.