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Perchlorate mixed–ligand copper(II) complexes of β-diketone and ethylene diamine derivatives: Thermal, spectroscopic and biochemical studies

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Abstract

The present work carried out a study on perchlorate mixed–ligand copper(II) complexes which have been synthesized from ethylenediamine derivatives (**3a–c**) and β-diketones. These complexes, namely [Cu(DA-Cl)(acac)H_2O]ClO_4 **4**, [Cu(DA-Cl)(bzac)H_2O]$H_2O.ClO_4$ **5**, [Cu(DA-OMe)(acac)H_2O]ClO_4 **6**, [Cu(DA-OMe)(bzac)H_2O]ClO_4 **7**, [Cu(DA-H)(acac)H_2O]$2H_2O.ClO_4$ **8** and [Cu(DA-H)(bzac)H_2O]ClO_4 **9** (where acac, acetylacetonate and bzac, benzoylacetonate) were characterized by elemental analysis, spectral (IR and UV–vis) and magnetic moment measurements. Thermal properties and decomposition kinetics of all complexes are investigated. The interpretation, mathematical analysis and evaluation of kinetic parameters (E, A, ΔH, ΔS and ΔG) of all thermal decomposition stages have been evaluated using Coats–Redfern equation. The biochemical studies showed that, the diamines **3a–c** have powerful effects on degradation of DNA and protein. The antibacterial screening demonstrated that, the diamine (DA-Cl), **3b** has the maximum and broad activities against Gram +ve and Gram −ve bacterial strains.

Keywords: Ethylene diamine derivatives; Thermal degradation kinetics; Biochemical; DNA; Antibacterial

1. Introduction

The *vicinal* 1,2-diamine functional group represents the structural unit of a number of bioactive compounds such as peptide antibiotics, vitamin H, antitumor agents and opioid receptor agonists [1]. In addition, 1,2 diamines function as chiral building blocks [2] and bidentate ligands [3] for transition metals in asymmetric synthesis. Antitumor properties of cisplatin (*cis*-diamminedichloroplatinum) were serendipitously discovered by Rosenberg in the mid 1960s [4]. Its success in antitumor chemotherapy brought about the synthesis of many diamine-platinum complexes in a search for drugs having greater activity, less toxicity, and to circumvent drug resistance that may develop in certain tumors [5]. Among the 1,2-diaminoplatinum complexes described, several possess higher antitumor activity than cisplatin [6]. Some 1,2-diaminoplatinum compounds, either used clinically or at an advanced stage of testing, are depicted in Scheme 1 [7]. Moreover, Schiff bases such as **1**, which is a structural analogues of 1,2-diamines, were reported to posses antibacterial [8–13] antifungal [10–13] DNA binding [14] and antitumor activities [15,16]. In the presence of a co-oxidant or under aerobic conditions a cleavage of the DNA was observed in several cases. These studies could lead to the development of artificial restriction enzymes or antitumor drugs.

In present work, we study the synthesis, spectroscopic, biochemical and thermal behavior of six perchrolate mixed–ligand copper(II) complexes containing β-diketonates (dike) (where dike = acac, acetylacetonate or, bzac, benzoylacetonate) and diamines (diam) of general formula [Cu(dike)(diam)H_2O]ClO_4. Therefore, three novel diamine ligands namely 2-amino-1-benzylamino-1-phenylethane.2HCl, (DA-H) **3a**, 2-amino-1-benzylamino-1-(4-chlorophenyl)ethane.2HCl, (DA-Cl) **3b**, **2-**amino-1-benzylamino-1-(4-methoxyphenyl)ethane. 2HCl, (DA-OMe) **3c** were prepared in a search for new diamine-copper complexes, the structural analogues of **1**, of antitumor activity.

2. Experimental

2.1. Instrumentation and materials

All starting materials were purchased from Fluka, Riedel and Merck and used as received. Elemental analyses (C, H

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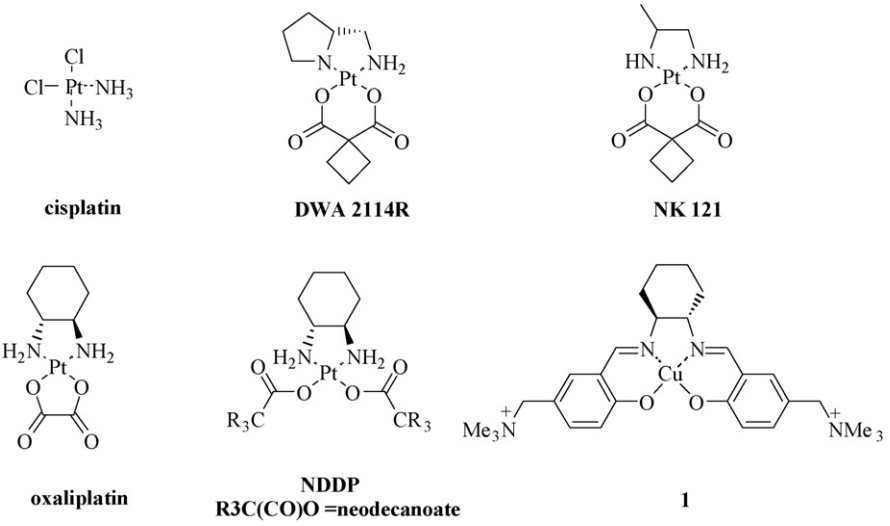


Scheme 1.

and N) were performed on a Perkin-Elmer 2400 Series II Analyzer. Electronic spectra were recorded on a UV-UNICAM 2001 spectrophotometer using 10 mm pass length quartz cells at room temperature. Magnetic susceptibility was measured with a Sherwood Scientific magnetic susceptibility balance at 297 K. Infrared spectra were recorded on a Perkin-Elmer FT-IR Spectrometer 2000 as KBr pellets and as Nujol mulls in the 4000–200 cm^{-1} spectral range. ^1H and ^{13}C NMR measurements at room temperature were obtained on a Jeol JNM LA 300 WB spectrometer at 250 MHz, using a 5 mm probe head in $CDCl_3$. HRMS spectra were recorded on a JEOL JMS-700 apparatus. Thermogravimetric (TG) and differential (DTG) thermogravimetric analysis were performed on a DTG-50 Shimazu instrument at heating rate of 10 °C/min.

2.2. Synthesis of diamine ligands

2.2.1. General procedures of Strecker synthesis

Benzaldehydes (**1**) (0.1 mol) was added to a solution of sodium bisulfite (10.4 g, 0.1 mol) in water (100 mL) and the mixture was stirred for 20 min at room temperature till the benzaldehyde-bisulfite adduct is formed as a crystalline solid. An aqueous solution of benzyl amine was added dropwise to the above suspension over 20 min to give the corresponding imine. A solution of KCN (6.5 g, 0.1 mol) in water (30 mL) was added and the mixture was stirred for 2 h at room temperature. The product was extracted with ethyl acetate and the organic layer was washed with water, dried ($MgSO_4$) and concentrated to give the aminonitrile **2** in excellent yield (Fig. 1).

*2.2.1.1. 2-Benzylamino-2-phenylacetonitrile (**2a**).* According to the general procedures, benzaldehyde (10.61 g, 100 mmol) and benzylamine (10.72 g, 100 mmol) gave **2a** as oil (20.0 g, 90%) ν_{max} (CCl_4)/cm^{-1} 2240, 3346; ^1H NMR (250 MHz; $CDCl_3$) δ: 1.86 (br, NH), 3.95 (q, J 13.0, CH_2), 4.69 (s, CH), 7.27–7.52 (m, Ar-H); ^{13}C NMR (62.9 MHz; $CDCl_3$) 51.2, 53.4 (CH_2, CH), 118.7 (CN), 127.3, 127.6, 128.4, 128.6, 128.9, 129.0, 134.7 and 138.1.

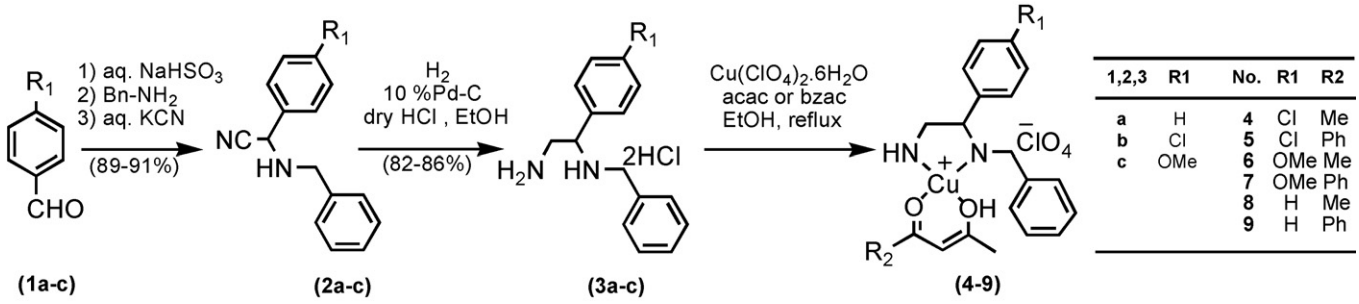
*2.2.1.2. 2-Benzylamino-2-(4-chlorophenyl)acetonitrile (**2b**).* According to the general procedures 4-chlorobenzaldehyde (14.06 g, 100 mmol) and benzylamine (10.72 g, 100 mmol) give **2b** as oil (23.40 g, 91%). ν_{max} (CCl_4)/cm^{-1} 2231, 3332; ^1H NMR (250 MHz; $CDCl_3$) 1.96 (br, NH), 3.89 (q, *J* 13.1, CH_2), 4.63 (s, CH), 7.23–7.42 (m, Ar-H); ^{13}C NMR (62.9 MHz; $CDCl_3$) 51.1, 52.7 (CH_2, CH), 118.3 (CN), 127.7, 128.3, 128.6, 128.7, 129.0, 133.3, 134.9 and 138.0.

*2.2.1.3. 2-Benzylamino-2-(4-methoxyphenyl)acetonitrile (**2c**).* According to the general procedures, 4-methoxybenzaldehyde (13.62 g, 100 mmol) and benzylamine (10.72 g, 100 mmol) gave **2c** was obtained as a yellow oil (22.46 g, 89%); ν_{max} (CCl_4)/cm^{-1} 2240, 3340; ^1H NMR (250 MHz; $CDCl_3$) 1.86 (br, NH), 3.70 (CH_3), 3.87 (q, *J* 13.1, CH_2), 4.58 (s, CH), 6.82–7.38 (m, Ar-H); ^{13}C NMR (62.9 MHz; $CDCl_3$) 51.0, 52.8, 55.2 (CH_3, CH_2, CH), 119.0 (CN), 114.2, 126.9, 127.5, 128.3, 128.5 (2C), 138.3, 159.9. Anal. Calcd for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 11.10. Found: C, 75.95; H, 6.38; N, 11.40.

2.2.2. General procedures of catalytic hydrogenation

Nitrile (**2**) (50 mmol) in absolute ethanol (250 mL) containing dry HCl (10 g, 0.27 mol) was hydrogenated over 10% Pd–C catalyst in an autoclave for 2–5 h at room temperature. After the completion of reaction the solution was filtered through celite pad and washed twice with absolute ethanol (50 mL). The filtrate was concentrated to give crude product of the diamine HCl salt **3** which was recrystallized from ethanol.

*2.2.2.1. 2-Amino-1-benzylamino-1-phenylethane. 2HCl (**3a**).* According to the general procedures, the nitrile **2a** (2.22 g, 10 mmol) gave **3a** as 2HCl salt (2.53 g, 86%), mp 247–250 °C. ν_{max} ($CHCl_3$)/cm^{-1}; 3320, 3068; ^1H NMR (250 MHz; $CDCl_3$) δ: 1.48 (br, NH), 2.81 (dd, *J* = 7.3 and 12.5, 1H, H-2), 2.91 (dd, *J* = 5.5 and 12.5, 1H, H-2), 3.58 (d, *J* = 13.1, 1H, Ha-Bn), 3.71 (d, *J* = 13.1, 1H, Hb-Bn), 3.63 (dd, *J* = 5.5 and 7.3, H-1), 7.23–7.40 (m, Ar-H); ^{13}C NMR (75 MHz; $CDCl_3$) 48.9, 51.5, 64.9 (CH_2,



1,2,3	R1	No.	R1	R2
a	H	4	Cl	Me
b	Cl	5	Cl	Ph
c	OMe	6	OMe	Me
		7	OMe	Ph
		8	H	Me
		9	H	Ph

Fig. 1. Synthesis of diamine ligands.

CH), 126.9, 127.3, 127.4, 128.2, 128.4, 128.5, 140.6 and 142.3. HRMS *m/z* (M^+): calcd for $C_{15}H_{14}N_2{\cdot}2HCl$, 295.05694. Found: 295.05699.

*2.2.2.2. 2-Amino-1-benzylamino-1-(4-chlorophenyl)ethane. 2HCl (**3b**).* According to the general procedures, nitrile **2b** (2.56 g, 10 mmol) gave **3b** as 2HCl salt (2.7 g, 82%), mp 240–243 °C. ν_{max} ($CHCl_3$)/cm^{-1}: 3300 (br, NH_2) ^1H NMR ($CDCl_3$) δ: 1.53 (br, 3 H, NH&NH_2), 3.55 (d, $J = 13.1$, lH, Ha-Bn), 3.70 (d, $J = 13$ 1, 1H, Hb-Bn), 3.82–3.74 (m, 1H, H-1), 2.90 (m, 2H, Hz), 7.21–7.37 (m, 9H, Ar-H). HRMS *m/z* (M^+): calcd for $C_{15}H_{13}ClN_2{\cdot}2HCl$, 329.50200. Found 329.50207.

*2.2.2.3. 2-Amino-1-benzylamino-1-(4-methoxyphenyl)ethane. 2HCl (**3c**).* According to the general procedures, nitrile **2c** (2.52 g, 10 mmol) gave **3c** as 2HCl salt (2.76 g, 85%), mp 210–212 °C, ν_{max} ($CHCl_3$)/cm^{-1}; 1609, 1517; ^1H NMR (250 MHz; $CDCl_3$) 1.54 (br, 3 H, NH&NH_2), 2.80 (dd, $J = 7.2$ and 12.7, 1H, H-2), 2.86 (dd, $J = 5.5$ and 12.7, 1H, H-2), 3.56 (d, $J = 13.2$, Ha-Bn), 3.70 (d, $J = 13.2$, Hb-Bn), 3.53–3.58 (m, 1H, H-1), 3.81 (s, 3H, OCH_3), 6.89–7.31 (m, Ar-H); ^{13}C NMR (75 MHz; $CDCl_3$) 48.9, 51.4, 55.3, 64.3 (CH_3, CH_2, CH), 114.0, 126.8, 128.2, 128.4, 128.5, 134.3, 140.7 and 158.9. HRMS *m/z* (M^+): calcd for $C_{16}H_{16}N_2O{\cdot}2HCl$, 349.07432. Found 349.07428.

2.3. Synthesis of mixed-ligand complexes

To a solution of diamines **3a–c** (0.5 mmol) in EtOH (20 mL) was added acac or bzac (0.5 mmol). To this mixture was added a solution of $Cu(ClO_4)_2{\cdot}6H_2O$ (0.185 g, 0.5 mmol) in EtOH (10 mL) and the green solution was heated under reflux for 30 min. A dilute NH_4OH was added to form a green precipitate. The green solid was washed with EtOH and dried. Elemental analysis and magnetic moment values for all complexes are listed in Table 1.

2.4. Biological studies

2.4.1. DNA and protein electrophoreses

2.4.1.1. Agarose gels. Diamine (**3a–c**) or their metal complexes (**4–9**) (100 μM) were added individually to 1 μg of the DNA isolated from *E. coli*. These samples were incubated for 1 h at 37 °C. The DNA was analysed by using horizontal agarose gels electrophoresis. The electrophoresis was performed using 0.7% (w/v) agarose gels in TAE buffer (5 μM sodium acetate, 1 μM EDTA and 0.04 M Tris–HCl pH 7.9). The agarose gels were stained with ethidium bromide (0.5 μg/mL) and the DNA was visualised on a UV trans illuminator [17].

2.4.1.2. Polyacrylamide gel electrophoresis. Bovine serum albumin (BSA) (3 mg) was treated with diamine ligands (**3a–c**) or their metal complex (**4–9**) (100 μM) were added to Biovine serum albumin. The reaction mixtures were incubated for 1 h at 37 °C. The protein samples were analysed using vertical one-dimensional SDS-polyacrylamide gel electrophoresis according to the method of Laemmli [18].

The samples were prepared by adding 15 μL 2× SDS-gel loading buffer (100 μM Tris–HCl pH 6.8, 4% (w/v) SDS, 0.2% (w/v) bromophenol blue, 20% (v/v) glycerol, 200 μM DTT) and 15 μL protein samples and boiled for 3 min, 20 μL of denatured protein samples were loaded into the gel [18].

2.4.2. Determination of SOD-like activity

Diamine ligands or their complexes were assayed for activities on superoxide dismutase (SOD) enzyme according to the method of Bridges and Salin phenazine methosulphate (PMS) [19]. They were assayed to generate a superoxide anion radicals at pH 8.3.

2.4.3. Antibacterial effect

The antibacterial investigation of the complexes was carried out using cup diffusion technique [20]. The test was done against the Gram-negative *Pesudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*) and the Gram-positive *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus* (*Staphylococcus* sp.). The tested complexes were dissolved in dimethylsulphoxide (DMSO) at concentration 1 mg/mL. The Luria–Bertani Agar (LBA) medium was used. An aliquot of the solution of the tested complexes equivalent to 100 μg was placed separately in each cup. The LBA plates were incubated for 24 h at 37 °C and the resulting inhibition zones were measured. DMSO, which exhibited no antibacterial activity, was used as a negative control.

Table 1
Infrared spectral bands (cm^{-1}), elemental analysis and magnetic moment of complexes **4–9**

Complex/empirical formula (formula weight)	ν(C=O) + ν(C=C) combination		$\nu(ClO_4^-)$		ν(Cu–O)	ν(Cu–N)	Found (calculated) (%)			μ_{eff} (BM)
							C	H	N	
[Cu(DA-Cl)(acac)H_2O]ClO_4, **4** $C_{20}H_{27}Cl_2CuN_2O_7$ (541.89)	1557	1533	1115	607	293	515	43.79 (44.33)	5.00 (5.02)	5.02 (5.17)	1.80
[Cu(DA-Cl)(bzac)H_2O]$H_2O.ClO_4$, **5** $C_{25}H_{31}Cl_2CuN_2O_8$ (621.97)	1557	1519	1117	603	291	513	48.00 (48.28)	4.96 (5.02)	4.39 (4.50)	1.69
[Cu(DA-OMe)(acac)H_2O]ClO_4, **6** $C_{21}H_{30}ClCuN_2O_8$ (537.47)	1555	1521	1111	609	289	519	46.13 (46.93)	5.47 (5.63)	5.12 (5.21)	1.90
[Cu(DA-OMe)(bzac)H_2O]ClO_4, **7** $C_{26}H_{32}ClCuN_2O_8$ (599.54)	1551	1523	1111	609	303	525	51.88 (52.09)	5.30 (5.38)	4.54 (4.67)	1.95
[Cu(DA-H)(acac)H_2O]$2H_2O.ClO_4$, **8** $C_{20}H_{32}ClCuN_2O_9$ (543.47)	1558	1515	1110	610	300	520	43.89 (44.20)	5.88 (5.93)	5.05 (5.15)	1.75
[Cu(DA-H)(bzac)H_2O]ClO_4, **9** $C_{25}H_{30}ClCuN_2O_7$ (569.51)	1557	1520	1110	610	297	517	52.66 (52.72)	5.19 (5.31)	4.88 (4.92)	1.80

3. Results and discussion

3.1. IR spectra of mixed-ligand complexes

The main IR absorption bands for the synthesized complexes are shown in Table 1. The observed bands may be classified into those originated from the ligands, and those emanating from the bonds formed between copper(II) and coordinating sites. The bands are assigned in comparison with similar Cu(II)-complexes. Infrared spectroscopic data suggest the coordination of both acac (in complexes **4**, **6** and **8**) and bzac (in complexes **5**, **7** and **9**) ligands to the copper(II) centre [21]. Strong band observed at around 1110 cm^{-1} (antisymmetric stretch) and the sharp band at around 610 cm^{-1} (antisymmetric bend), suggest uncoordinated perchlorate anions [22,23] in all studied complexes.

3.2. Electronic spectra and magnetic moment measurements of complexes

All complexes are insoluble in water and sparingly soluble in most organic solvents (only soluble in DMF and DMSO), thus the d–d transition bands of all complexes are recorded in Nujol mulls and in DMF solutions. These electronic spectra show only one broad band observed in the (720–740 nm) range. This position is in favor of the square pyramidal geometry [24,25] for all the isolated complexes in which two amine nitrogen atoms of the diamine ligand occupy the basal plane with the two oxygen atoms of the β-diketone ligand and one water molecule coordinated (confirmed by thermogravimetry) in the apical position [26].

Magnetic moment of complexes was observed at room temperature and the data are listed in Table 1. Complexes **4**, **5**, **8** and **9** show μ_{eff} value in the range 1.69–1.8 BM, consistent with the expected value for spin systems $S = 1/2$ with one unpaired electron (1.73 BM) in $3d^9$ systems. Complexes **6**, **7** with magnetic moment values of 1.90 and 1.95 BM, respectively, are typical of "magnetically dilute" [27,28] complexes where the individual copper(II) ions are separated from each other (no intermolecular magnetic interaction).

3.3. Thermal analysis

The stages of decomposition, temperature ranges, decomposition product loss as well as the found and calculated weight loss percentages of the complexes are given in Table 2. The TG, DTG curves of studied complexes (Fig. 2) show either three or four decomposition steps.

In all studied complexes the first decomposition step corresponding to the loss of coordinated water in the (135–280 °C) temperature range. Other decomposition steps involve the removal of some terminal and/or integral parts in both ligands. The final degradation step (centered at around 600 °C) involves the removal of ClO_4 ion. The high residue percentage left without degradation in all complexes reveals the stability of coordination sphere around the copper atom.

In order to access the influence of the structural properties of the ligand and the type of the metal on the thermal behavior of the complexes, the order, n, and the heat of activation E of the various decomposition stages were determined from the TG and DTG thermograms using the Coats–Redfern equations in the following forms:

$$\ln\left[\frac{1-(1-\alpha)^{1-n}}{(1-n)T^2}\right] = \frac{M}{T} + B \quad \text{for } n \neq 1 \tag{1}$$

$$\ln\left[\frac{-\ln(1-\alpha)}{T^2}\right] = \frac{M}{T} + B \quad \text{for } n = 1 \tag{2}$$

where $M = -E/R$ and $B = \ln AR/\Phi E$; E, R, A, and Φ are the heat of activation, the universal gas constant, pre-exponential factor and heating rate, respectively.

The correlation coefficient, r, was computed using the least square method for different values of n, by plotting the left-hand side of Eqs. (1) or (2) versus $1000/T$ (Fig. 3). The n value which gave the best fit ($r \cong 1$) was chosen as the order parameter for the decomposition stage of interest. From the intercept and linear slope of such stage, the A and E values were determined. The other kinetic parameters, ΔH, ΔS and ΔG were computed using the relationships; $\Delta H = E - RT$, $\Delta S = R[\ln(Ah/kT) - 1]$ and $\Delta G = \Delta H - T\Delta S$, where k is the Boltzmann's constant and

Table 2
Thermal behavior of metal complexes

Compound (molecular weight)	DTG peak (°C)	Temperature range (°C)	Decomposition product lost (formula weight)	Weight (%) found (calculated)
$[Cu(DA\text{-}OMe)(bzac)H_2O]ClO_4$ (599.54)	223	187–280	H_2O (18.01)	3.43 (3.01)
	326	282–401	OCH_3 (31.03)	5.01 (5.10)
	442	402–502	C_2H_3 (27.05)	4.97 (4.50)
	694	635–748	$ClO_4 + CH$ (112.47)	20.47 (19.0)
		Residue	$C_{22}H_{23}O_2N_2Cu$ (410.98)	67.78 (68.39)
$[Cu(DA\text{-}OMe)(acac)H_2O]ClO_4$ (537.47)	249	182–338	H_2O (18.01)	3.90 (3.40)
	431	339–523	$OCH_3 + C_2H_3$ (58.08)	9.29 (10.8)
	677	614–728	ClO_4 (99.45)	19.04 (19.20)
		Residue	$C_{17}H_{21}O_2N_2Cu$ (348.91)	66.9 (66.6)
$[Cu(DA\text{-}H)(bzac)H_2O]ClO_4$ (569.51)	151	99–215	$H_2O + C_3H_4$ (58.08)	9.37 (10.2)
	375	215–501	C_6H_5 (77.10)	12.1 (13.5)
	711	647–711	$ClO_4 + C$ (111.46)	19.69 (18.1)
		Residue	$C_{16}H_{17}N_2O_2Cu$ (332.86)	59.5 (58.2)
$[Cu(DA\text{-}H)(acac)H_2O]2H_2O.ClO_4$ (543.47)	79	39–130	$2H_2O$ (36.02)	6.42 (6.90)
	196	130–282	$H_2O + C_4H_7$ (73.11)	12.34 (13.5)
	537	282–726	$C + ClO_4$ (111.46)	18.14 (20.5)
		Residue	$C_{17}H_{16}O_2N_2Cu$ (343.87)	55.5 (59.1)
$[Cu(DA\text{-}Cl)(bzac)H_2O]H_2O.ClO_4$ (621.97)	96	44–173	H_2O (18.01)	3.1 (2.9)
	278	175–355	$H_2O + C_6H_5$ (95.12)	15.5 (15.3)
	453	357–729	$ClO_4 + CH_3$ (114.49)	19.5 (18.4)
		Residue	$C_{18}H_{18}N_2O_2ClCu$ (393.35)	61.3 (63.4)
$[Cu(DA\text{-}Cl)(acac).H_2O]ClO_4$ (541.89)	181	135–219	H_2O (18.01)	3.58 (3.3)
	268	219–331	C_3H_4 (40.06)	7.62 (7.4)
	375	331–424	$Cl + C_2H_3$ (62.50)	10.4 (11.5)
	445	424–726	ClO_4 (99.45)	17.5 (19.0)
		Residue	$C_{15}H_{17}O_2N_2Cu$ (320.85)	61.02 (59.0)

Table 3
Temperature of decomposition, and the kinetic parameters of metal complexes

Complex	Step	T (K)	A (S^{-1})	E (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (kJ mol^{-1} K^{-1})	ΔG (kJ mol^{-1})
$[Cu(DA\text{-}OMe)(bzac)H_2O]ClO_4$	1st	496	16,654	218.33	214.2	−0.176	301.5
	2nd	599					
	3rd	705	15,807	239.9	233.99	−0.180	360.89
	4th	967	33,375	446.9	438.9	−0.176	609
$[Cu(DA\text{-}OMe)(acac)H_2O]ClO_4$							
	2nd	704	6,025	134	128.3	−0.188	260.66
	3rd	950	25,954	370.9	363	−0.178	532.45
$[Cu(DA\text{-}H)(bzac)H_2O]ClO_4$	1st	424	19,112	64.81	61.3	−0.174	135.16
	2nd	544	3,296	103.5	99.0	−0.190	202.83
	3rd	984	28,742	403.0	394.78	−0.178	569.75
$[Cu(DA\text{-}H)(acac)H_2O]2H_2O.ClO_4$	1st	353	11,402	153.65	150.7	−0.177	213.17
	2nd	469	4,704	95.18	91.28	−0.187	178.84
	3rd	662		50.7	45.2		
	4th	959	19,048	300.65	292.68	−0.181	466.27
$[Cu(DA\text{-}Cl)(bzac)H_2O]H_2O.ClO_4$	1st	369		19.1	16.0		
	2nd	551	877	67.84	63.26	−0.202	175
	3rd	725	31,364	113.0	106.96	−0.175	233.5
	4th	948	33,263	443.5	435.6	−0.176	435.78
$[Cu(DA\text{-}Cl)(acac).H_2O]ClO_4$	1st	454	4,707	93.65	89.88	−0.186	174.5
	2nd	541		59.0	54.57		
	3rd	648	18,551	260.46	255.69	−0.178	371
	4th	944	22,435	334.16	326.3	−0.179	496

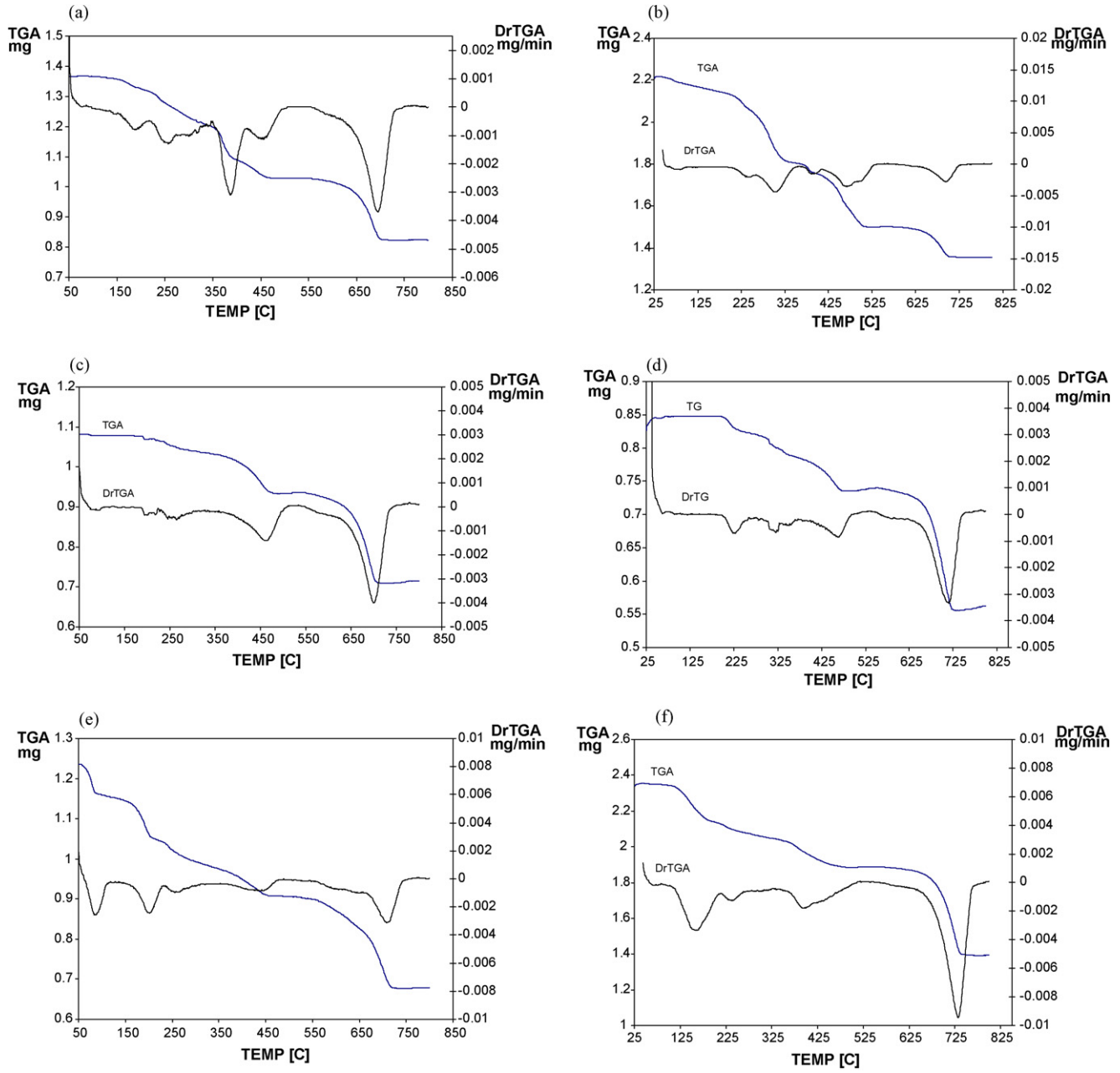


Fig. 2. TG and DTG of (a) [Cu(DA-Cl)(acac).H_2O]ClO_4; (b) [Cu(DA-Cl)(bzac)H_2O]$H_2O.ClO_4$; (c) [Cu(DA-OMe)(acac)H_2O]ClO_4; (d) [Cu(DA-OMe)(bzac)H_2O]ClO_4; (e) [Cu(DA-H)(acac)H_2O]$2H_2O.ClO_4$; (f) [Cu(DA-H)(bzac)H_2O]ClO_4 complexes.

h is the Planck's constant. The kinetic parameters are listed in Table 3. The following remarks can be pointed out: (i) all complexes decomposition stages show a best fit for ($n = 1$) indicating a first order decomposition in all cases. Other n values (e.g. 0, 0.33 and 0.66) did not lead to better correlations; (ii) the value of ΔG increases significantly for the subsequently decomposition stages of a given complex. This is due to increasing the values of $T\Delta S$ significantly from one stage to another which overrides the values of ΔH. Increasing the values of ΔG of a given complex as going from one decomposition step subsequently to another reflects that the rate of removal of the subsequent ligand will be lower than that of the precedent ligand [29,30]. This may be attributed to the structural rigidity of the remaining complex after the expulsion of one and more ligands, as compared with the precedent complex, which require more energy, $T\Delta S$, for its rearrangement before undergoing any compositional change; (iii) the negative values of activation entropies ΔS indicate a more ordered activated complex than the reactants and/or the reactions are slow [31]; (iv) the positive values of ΔH mean that the decomposition processes are endothermic.

3.4. Biochemical effect of ligand and its metal-complexes on the DNA in vitro

The degradation effect of 100 μM diamines (**3a**, **3b** and **3c**) and its copper(II) complexes (**4**, **5**, **6**, **7**, **8** and **9**) on the DNA

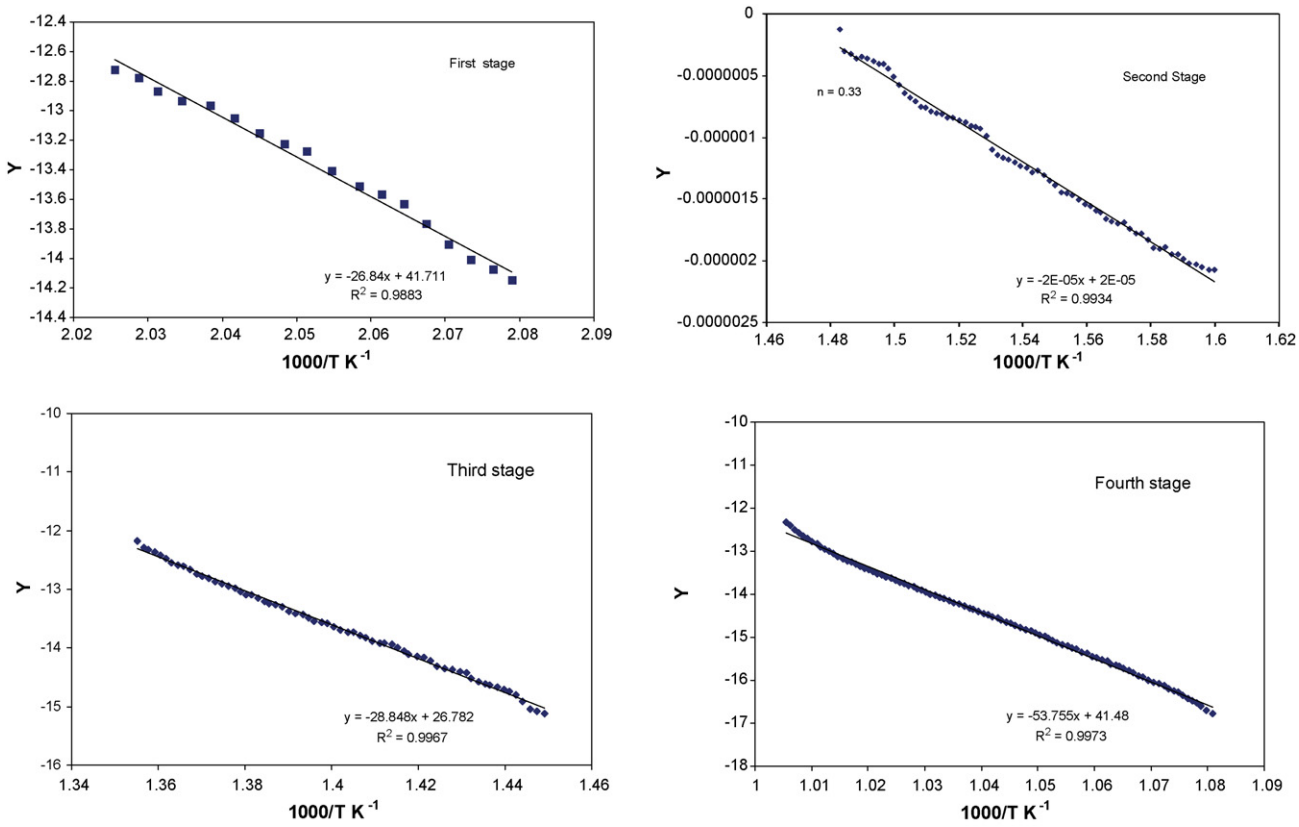


Fig. 3. Coats–Redfern plots for $[Cu(DA\text{-}OMe)(bzac)H_2O]ClO_4$, where $Y = [-\ln(1-\alpha)/T^2]$.

is illustrated in Fig. 4. The ligands **3a** and **3c** have a complete degradation on the tested DNA (Fig. 4), lanes 4 and 7, respectively. Ligand **3b** has less degradation effect on the DNA (Fig. 4), lane 10. On the other hand, complexes **4–9** have weak degradation effect on the DNA compared to the control (Fig. 4), lanes 2, 3, 5, 6, 8 and 9, respectively. It is clear that the diamine ligands (**3a**, **3b** and **3c**) have more degradation effect on the DNA than that recorded for **4–9** complexes. Therefore, the diamines, particularly **3a** and **3c** can be evaluated, after more detailed study, as degradation factor for DNA.

Further biochemical studies to illustrate the exact role of the promising degredative effect on DNA displayed by diamines **3a–c** and its Cu-complexes **4–9** were carried out. Therefore, we decided to study the effect of these diamines and its Cu-complexes on the protein as another important biomacromolecule. The effect of the parent diamine ligands (**3a–c**) and their complexes (**4–9**) on the BSA was carried out and the results illustrated in Fig. 5.

The diamine (DA-H), **3a** and its complexes **8** and **9** have a strong degradation effect on the BSA (Fig. 5), lanes 3–5, respectively. The diamine (DA-OMe), **3c** cleaves completely the BSA;

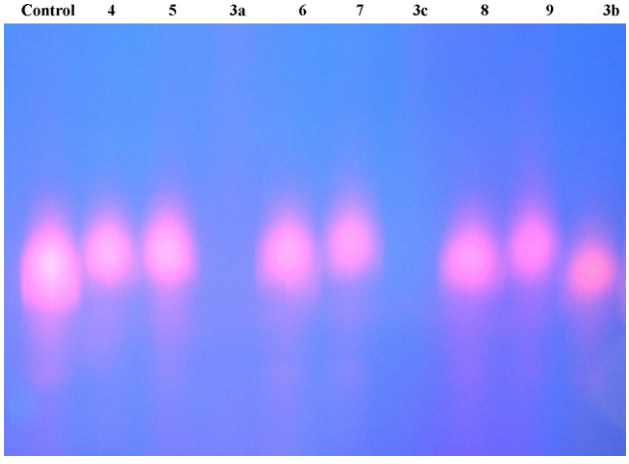


Fig. 4. Effect of 100 μM of the diamine ligands and their metal complexes on the DNA *in vitro*.

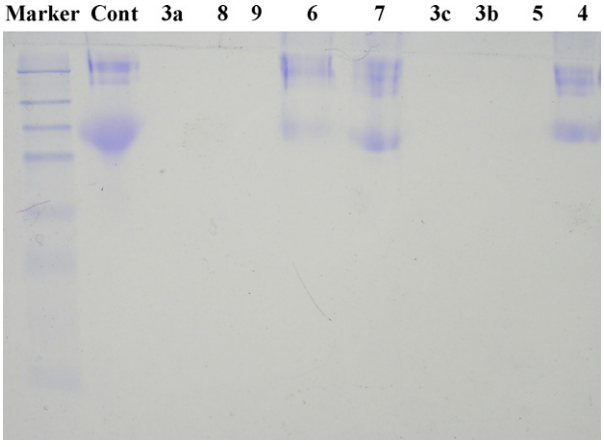


Fig. 5. Effect of 100 μM of the diamine ligands and their metal complexes on the BSA protein *in vitro*.

Table 4
Superoxide (SOD) like activity of the metal complex as antioxidative enzyme

Compound	Δ through 5 min	% inhibition
(DA-Cl), **3b**	0.110	81.57
[Cu(DA-Cl)(acac).H_2O]ClO_4, **4**	0.169	71.7
[Cu(DA-Cl)(bzac)H_2O]$H_2O.ClO_4$, **5**	0.365	38.86
(DA-OMe), **3c**	0.153	74.37
[Cu(DA-OMe)(acac)H_2O]ClO_4, **6**	0.281	52.93
[Cu(DA-OMe)(bzac)H_2O]ClO_4, **7**	0.383	58.63
(DA-H), **3a**	0.086	85.59
[Cu(DA-H)(acac)H_2O]$2H_2O.ClO_4$, **8**	0.226	62.14
[Cu(DA-H)(bzac)H_2O]ClO_4, **9**	0.167	72.03

% inhibition = ((ΔControl − ΔTest)/ΔControl) × 100.

Table 5
Effect of metal complex on some microorganisms the results expressed as zone inhibition in millimetre diameter

Compound	*E. coli*	*P. arg.*	*B. st.*	*S. st.*
(DA-Cl), **3b**	22	3	3	2.8
[Cu(DA-Cl)(acac).H_2O]ClO_4, **4**	12	1.1	1.1	1.5
[Cu(DA-Cl)(bzac)H_2O]$H_2O.ClO_4$, **5**	9	1.1	−ve	1.5
(DA-OMe), **3c**	12	1.5	1.4	1.8
[Cu(DA-OMe)(acac)H_2O]ClO_4, **6**	11	1.5	1.2	1.7
[Cu(DA-OMe)(bzac)H_2O]ClO_4, **7**	11	1.8	1.2	1.2
(DA-H), **3a**	18	1.4	1.7	2.1
[Cu(DA-H)(acac)H_2O]$2H_2O.ClO_4$, **8**	16	1.7	1.6	2.1
[Cu(DA-H)(bzac)H_2O]ClO_4, **9**	11	1.7	1.3	2.3

lane 8 (Fig. 5) while, their complexes **6** and **7** have little cleavage effect on the BSA (Fig. 5, lanes 6 and 7, respectively). It is clear that ligands **3a** and **3c** have strong degradation effect on both the DNA and the BSA. The diamine (DA-Cl), **3b** and its complex **5** have a strong degradation effect on the BSA (Fig. 5, lanes 9 and 10, respectively) while, complex **4** has less degradation effect on BSA (Fig. 5, lane 11). It is clear that the parent ligands have a powerful degradation effect on both DNA and BSA more that the effect of their complexes.

Parent compounds (**3c**, **3a** and **3b**) demonstrate the presence of a strong SOD like activity (Table 4) and this is represented in the as inhibition percent 85.59%, 81.57% and 74.37%, respectively. Also, complexes **9**, **4**, **8** and **6** demonstrated a considerable SOD like activity 72.03%, 71.7%, 62.14 and 52.93%, respectively. On the other hand, complexes **5** and **7** represented low SOD like activity of 38.86% and 38.86%, respectively, as illustrated in Table 4. The SOD like activity of the parent ligands indicates that these compounds can be used to prevent the formation of superoxide-radical.

The diamine (DA-Cl), **3b** displayed the maximum antibacterial activity regarding with inhibition zone diameter (30 mm against both *P. aeruginosa* and *B. subtilis*, 28 mm against *Staphylococcus* sp. and 22 mm against *E. coli*). On the other hand, complexes **4** and **5** present low antibacterial activity compared to their free diamine (DA-Cl). Complexes **4** and **5** have the same antibacterial activity regarding with inhibition zone diameter (15 mm) against *Staphylococcus* sp. and (11 mm) against *P. arg*. Complex **4** has remarkable antibacterial against *E. coli* (12 mm) compared to complex **5** (9 mm) against the same microorganism. Also complex **4** has higher antibacterial effect against *B. subtilis* (11 mm) in contrast; complex **5** has no antibacterial effect against *B. subtilis* as illustrated in Table 5.

The free ligand **3c** (DA-OMe), and its complexes **6** and **7** express a remarkable antibacterial activity against the tested microorganisms Table 5. It shows high effect regarding *Staphylococcus* sp. (18 mm) while, complexes **6** and **7** show antibacterial effect of 17 and 12 mm against the same microorganism. The inhibition zone diameter of (DA-OMe) against *B. subtilis* and *E. coli* were 14 and 12 mm, respectively, while complexes **6** and **7** exhibited inhibition zone diameter of 12 and 11 mm against *B. subtilis* and *E. coli*, respectively.

The free ligand **3a** (DA-H), display considerable antibacterial activity against *E. coli* (18 mm) compared to complex **8** (16 mm) and complex **9** (11 mm) for the same microorganism. On the other hand, complexes **8** and **9** exhibit the same antibacterial activity against *P. arg*. (17 mm) more than that recorded for **3a** (14 mm) against *P. arg*.

4. Conclusion

A new series of copper(II) complexes were prepared and characterized by the elemental analysis and spectral studies, based on these studies they have been assigned square-pyramidal geometry. TGA data support the presence of coordinated water. Thermal analysis are investigated and showed either three or four decomposition steps. The stability of complexes was explained and kinetic parameters (E, A, ΔH, ΔS and ΔG) of all thermal decomposition stages have been evaluated using Coats–Redfern equation.

Three novel diamine ligands are synthesized and characterized. These diamines show a noticeable effect as antibacterial and DNA-degradative action "which maybe a sign for anti-tumor activity" even much higher than the recorded with their copper complexes.

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