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New tridentate azo–azomethines and their copper(II) complexes: Synthesis, solvent effect on tautomerism, electrochemical and biological studies

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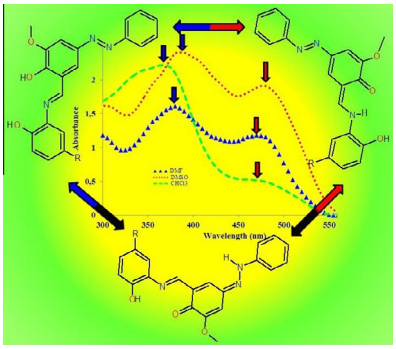
HIGHLIGHTS

- Three azo–azomethines and their copper(II) complexes are synthesized.
- The new compounds were characterized by IR, UV–Vis., ^{1}H and ^{13}C NMR, mass spectrometry and elemental analysis.
- The tautomeric behaviors of the azo–azomethines in solution were examined by UV–Vis. study.
- The redox behaviors of the compounds were investigated by cyclic voltammetry.
- The antibacterial activity of the compounds was also determined.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, three azo–azomethines and their copper(II) complexes were prepared and characterized by analytical and spectroscopic methods. The complexes prepared were found to be mononuclear and the chelation of the ligands to the copper(II) ions occurs through two phenolic oxygens and a nitrogen atom of the azomethine group of the ligand. The tautomeric behaviors of the azo–azomethines in solution were studied by UV–Vis. spectra in three organic solvents with different polarity ($CHCl_3$, DMSO and DMF) at room temperature. The redox behaviors of the azo–azomethines and their Cu(II) complexes were investigated by cyclic voltammetry (CV) in DMSO solution containing 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) as supporting electrolyte. Additionally, the antibacterial activity was also evaluated by the broth microdilution methods against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The compounds were found to be less effective against all bacteria tested than two reference antibiotics (ampicillin and gentamicin).

Introduction

Azo compounds are versatile molecules that find widespread applications as dyes and pigments in textile industry. They also have advanced applications in organic synthesis and high technology areas such as laser, liquid crystalline displays and ink-jet printers [1–3]. Azo dyes are an important class of the organic photoactive materials, due to their excellent optical switching properties [4–8]. In addition, the azo dyes and their metal complexes are involved in many biological reactions such as inhibition of DNA, RNA and biological activity against bacteria and fungi

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[9,10]. They are increasingly used in textile, leather and plastic industries [11]. These compounds have a great ability to coordinate with many metal ions and form stable complexes. The coordination compounds of azo–azomethine ligands are also widely used in medicine, for corrosion prevention, metal recovery as well as to treat nuclear wastes [12]. Furthermore, since the azo compounds have a good thermal stability, the azo–azomethine compounds find important applications in the optical data storage as recording layer on digital versatile disk-recordable [13]. Schiff bases derived from azo chromophore group containing aromatic aldehydes and their metal complexes have been extensively studied [14] because of their interesting physical and chemical properties [15] and potentially useful biological activities [16]. Azo–azomethine ligands contain both azo and azomethine groups. Azo groups possess excellent donor properties and play an important role in coordination chemistry [14,17,18].

Proton induced tautomerism has an importance in various fields of chemistry and particularly in biochemistry [19]. *o*-Hydroxy Schiff bases exhibit selfisomerisation *via* intramolecular proton transfer and they have potential applications in higher energy radiation detectors and memory storage devices [20]. They may exist in two tautomeric forms (enol and keto forms) in both solutions and the solid state and tautomerisms affects their photo-physical and photochemical properties [21].

Copper is an essential trace element are involved in a number of metabolic activities [22]. Copper complexes containing Schiff bases have attracted particular interest due to their potential antitumor activity and interaction with DNA [23,24]. Therefore, investigations on copper complexes are becoming more prominent in the research area of bioinorganic chemistry [25,26].

In our laboratory, we have been investigated the synthesis, spectral, structural and biological properties of new azo chromophore group containing ligands and their transition metal complexes [15,18,27–30]. In this study, novel azo–azomethine compounds, L^1H_2–L^3H_2, with *ONO* donor set were synthesized *via* condensation reaction of 2-hydroxy-3-methoxy-5-[(*E*)-phenyldiazenyl]benzaldehyde with 2-amino-4-(X) phenol (X = H, Me and Cl). The new azo–azomethines were characterized by analytical and spectroscopic methods such as elemental analysis, FT-IR, UV–Vis., mass, ^{1}H and ^{13}C NMR spectroscopy. Mononuclear copper(II) complexes of the azo–azomethine ligands were also prepared. To examine the tautomeric behavior of the azo–azomethine compounds L^1H_2–L^3H_2 in solution, electronic spectra in three organic solvents with different polarity ($CHCl_3$, DMSO and DMF) were performed at room temperature. In addition, the electrochemical properties and biological activities of the synthesized compounds were investigated.

Experimental

Reagents

Chemicals and solvents used were of analytical reagent grades and used without any further purification. Copper(II) acetate monohydrate was purchased from Merck and used without purification. Aniline, 2-aminophenol, 2-amino-4-methylphenol, 2-amino-4-chlorophenol, and 2-hydroxy-3-methoxybenzaldehyde were purchased from the Aldrich Chemical Company and 2-hydroxy-3-methoxy-5-[(*E*)-phenyldiazenyl]benzaldehyde was prepared according to a published procedure [31].

Apparatus

Carbon, hydrogen and nitrogen elemental analyses were performed with a model CHNS-932 (LECO) elemental analyzer. NMR spectra were performed using a Bruker Avance III HD 600 MHz. Spectrometer. Mass spectra were recorded on a Thermo Fisher Exactive + Triversa Nanomate mass spectrometer. The IR spectra were obtained (4000–400 cm^{-1}) using a Perkin Elmer spectrum 400 FTIR spectrophotometer (resolution; 0.5–4 cm^{-1}). Electronic absorption spectra were recorded on a T80-UV–Vis. Spectrometer PG Instruments Ltd. Melting points were obtained with a Electrothermal LDT 9200 Apparatus in open capillaries.

All the electrochemical experiments were performed using a Gamry Reference 600 workstation (Gamry, Pennsylvania, USA) electrochemical analyzer (Model 600C series) equipped with BAS C3 cell stand. The working electrode was a bare glassy carbon (GC) disk (BAS Model MF-2012) with a geometric area of 0.027 cm^2. The reference electrode was Ag/Ag^+ (0.01 M) in nonaqueous media, and the counter electrode was a Pt wire.

Electrochemical studies

GC electrodes were prepared by first polishing them with fine wet emery papers grain size 4000 (Buehler, Lake Bluff, IL, USA) followed by a 0.1 and 0.05 μm alumina slurry on a polishing pad (Buehler, Lake Bluff, IL, USA), to give them a mirror-like appearance. The electrodes were sonicated for 5 min in water and in 50:50 (v/v) isopropyl alcohol and acetonitrile (IPA + MeCN) solution purified over activated carbon. Before the electrochemical experiments, the electrodes were dried with an argon gas stream and the solutions were purged with pure argon gas (99.999%) at least for 10 min and an argon atmosphere was maintained over the solution during experiments. Electrochemical studies of the prepared azo–azomethines were performed in a solution of 1 mM of all the compounds in 0.1 M TBATFB in DMSO versus an Ag/Ag^+ (0.01 M) reference electrode using CV with a scan rate of 100 mV s^{-1} between 0 and −2.3 V.

Determination of antibacterial activity

The minimal inhibitory concentration (MIC) were determined by broth microdilution method according to Clinical and Laboratory Standarts Institute (CLSI) guidelines (2011) in Mueller–Hinton broth (MHB) (Becton Dickinson, Sparks, MD) with an inoculum of approximately 5×10^5 colony-forming units (CFU)/mL [32]. The in vitro antibacterial activity of the substances was evaluated against standart strains; *Staphylococcus aureus* ATCC (American Type Culture Collection) 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 27853. All the synthesized complexes were weighted (10.24 mg) and dissolved in DMSO (10 mL) to prepare the stock solutions. The serial dilution from 256 to 0.25 μg/mL were made in a 96-wells microplate. To each well 100 μL of a bacterial suspension, obtained from a 24 h culture, containing $\sim 5 \times 10^5$ CFU/mL was added. The plate was incubated at 35 °C for 24 h. The data were reported as MICs, the lowest concentration of antibiotic and complexes inhibiting visible growth after 24 h of incubation at 35 °C. For quality control of the method ampicillin (IE Ulugay, Turkey) and gentamicin (IE Ulugay, Turkey) were tested as antimicrobial agents. These experiments were carried out in duplicate.

Preparation of azo–azomethines (L^1H_2, $L^2H_2{\cdot}1/2H_2O$ and L^3H_2)

Azo–azomethines (L^1H_2, $L^2H_2{\cdot}1/2H_2O$ and L^3H_2) were synthesized by addition of corresponding substituted 2-aminophenols (*2-aminophenol*, 0.109 g, 1 mmol; *2-amino-p-cresol*, 0.123 g, 1 mmol; *2-amino-4-chlorophenol*, 1 mmol, 0.143 g) in MeOH (10 mL) to a methanolic solution of 2-hydroxy-3-methoxy-5-[(*E*)-phenyldiazenyl]benzaldehyde (0.256 g, 1 mmol). The mixtures were stirred for about 3 h at room temperature and the

colored precipiates were collected, washed with cold methanol and dried in air.

L^1H_2, (***1***); yield: 0.21 g, 61%, color: orange. M.p.: 285–286 °C. Analysis Calc. for $C_{20}H_{17}N_3O_3$ (347.367): C, 69.15; H, 4.93 N, 12.10%. Found: C, 69.36; H, 5.02; N, 11.95. ESI-MS in MeOH (*m*/*z* (rel. intensity) assignment, positive ion): 347.36 (55%) $[M]^+$. NMR: ^1H (d_6-DMSO as solvent, δ in ppm,), 15.30 (b, 1H, —NH—), 10.46 (*b*, 1H, Ph-OH), 9.24 (*s*, 1H, =CH-N), 7.85 (s, 1H, aromatic-H), 7.83–7.82 (d, 2H, aromatic-H), 7.69–7.67 (d, 1H, aromatic-H), 7.58–7.55 (t, 2H, aromatic-H), 7.49–7.48 (d, 1H, aromatic-H), 7.46–7.45 (s, 1H, aromatic-H), 7.21–7.18 (t, 1H, aromatic-H), 7.05–7.03 (d, 1H, aromatic-H), 6.99–6.96 (t, 1H, aromatic-H), 3.85 (s, 3H, OCH_3). ^{13}C NMR (d_6-DMSO as solvent, δ in ppm): 167.87 (=CH—N), 158.33 and 152.63 (Ph—OH or Ph=O), 152.32, 149.89, 141.84, 130.47, 129.80, 129.07, 128.85, 128.76, 122.415, 120.35, 118.49, 116.86, 115.57, 102.92 (aromatic C atoms), 55.90 (OCH_3). IR (KBr, cm^{-1}): 3356–2925 (OH), 3061 (Ar—H), 2992, 2831 (aliphatic C—H), 1635 (C=O), 1613 (C=N), 1590 (C—C aromatic), 1541 (—N=N—), 1273 (C—O), 1127 (C—O—C).

$L^2H_2{\cdot}1/2H_2O$, (***2***); yield: 0.247 g, 68%, color: dark red. M.p.: 242–243 °C. Analysis Calc. for $C_{21}H_{20}N_3O_3{\cdot}1/2H_2O$ (370.40 g/mol): C, 68.09; H, 5.44; N, 11.34%. Found: C, 68.77; H, 5.518; N, 11.41%. ESI-MS in MeOH (*m*/*z* (rel. intensity) assignment, positive ion): 373.40 (20%) $[M+2]^+$. NMR: ^1H (d_6-DMSO as solvent, δ in ppm,), 15.31–15.29 (d, 1H, —NH—), 10.23 (*s*, 1H, Ph-OH), 9.23–9.22 (*d*, 1H, =CH—N), 7.84–7.83 (d, 2H, aromatic-H), 7.81 (s, 1H, aromatic-H), 7.57–7.54 (t, 2H, aromatic-H), 7.53 (s, 1H, aromatic-H), 7.48–7.46 (t, 1H, aromatic-H), 7.45 (s, 1H, aromatic-H), 7.01–7.00. (d, 1H, aromatic-H), 6.93–6.91 (d, 1H, aromatic-H), 3.84 (s, 3H, OCH_3), 2.30 (s, 3H, Ph—CH_3). ^{13}C NMR (d_6-DMSO as solvent, δ in ppm): 168.52 (=CH—N), 157.87 and 152.64 (Ph—OH or Ph=O), 152.46, 147.54, 141.675, 130.41, 129.80, 129.35, 129.27, 129.015, 128.35, 122.40, 118.57, 116.70, 115.40, 102.75 (aromatic C atoms), 55.88 (OCH_3), 20.79 (Ph—C). IR (KBr, cm^{-1}): 3338–2816 (OH), 3061 (Ar—H), 2967 (aliphatic C—H), 1633 (C=O), 1612 (C=N), 1590 (C—C aromatic), 1537 (—N=N—), 1263 (C—O), 1122 (C—O—C).

L^3H_2, (***3***); yield: 0.335 g, 88%, color: dark red. M.p.: 255–256 °C. Analysis Calc. for $C_{20}H_{16}ClN_3O_3$ (381.812 g/mol): C, 62.91; H, 4.22; N, 11.01%. Found: C, 63.18; H, 4.30; N, 10.98%. ESI-MS in MeOH (*m*/*z* (rel. intensity) assignment, positive ion): 383.81 (30%) $[M+2]^+$. NMR: ^1H (d_6-DMSO as solvent, δ in ppm,), 14.98–14.97 (*b*, 1H, —NH—), 10.60 (*s*, 1H, Ph-OH), 9.25 (*s*, 1H, =CH-N), 7.86–7.85 (d, 2H, aromatic-H), 7.84 (s, 1H, aromatic-H), 7.75 (s, 1H, aromatic-H), 7.76–7.75 (s, 1H, aromatic-H), 7.59–7.56 (t, 2H, aromatic-H), 7.51–7.49 (t, 2H, aromatic-H), 7.23–7.21 (dd, 1H, aromatic-H), 7.03–7.01 (d, 1H, aromatic-H), 3.87 (s, 3H, OCH_3). ^{13}C NMR (d_6-DMSO as solvent, δ in ppm): 164.85 (=CH—N), 160.06 and 152.54 (Ph-OH or Ph=O), 151.64, 149.31, 142.66, 131.73, 130.77, 129.85, 128.15, 127.33, 123.85, 122.52, 118.64, 118.18, 116.46, 109.18, 103.81 (aromatic C atoms), 56.05 (OCH_3). FT-IR (KBr, cm^{-1}): 3330–2843 (OH), 3060 (Ar—H), 2835 (aliphatic C—H), 1611 (C=N), 1587 (C—C aromatic), 1539 (—N=N—), 1257 (C—O), 1123 (C—O—C).

Preparation of the copper complexes

The general procedure for the preparation of the copper(II) complexes is as follows: To a solution of the 2-{(*E*)-[(2-hydroxyphenyl)imino]methyl}-6-methoxy-4-[(*E*)-phenyldiazenyl]phenol [L^1H_2], 2-{(*E*)-[(2-hydroxy-5-methylphenyl) imino]methyl}-6-methoxy-4-[(*E*)-phenyldiazenyl]phenol, [$L^2H_2{\cdot}1/2H_2O$], 2-{(*E*)-[(5-chloro-2-hydroxyphenyl) imino]methyl}-6-methoxy-4-[(*E*)-phenyldiazenyl]phenol [L^3H_2], (1 mmol) dissolved in $CHCl_3$ (15 mL) heating at 80 °C, methanolic solution of copper salt [$Cu(CH_3COO)_2{\cdot}H_2O$] (1 mmol), was added dropwise. The reaction mixture was refluxed for 3 h. After cooling, green colored precipitates from the mixtures were separated out and washed with cold MeOH and dried in air.

[$CuL^1(CH_3OH)$], (***4***); yield: 0.083 g, 65%, color: green. M.p. > 250 °C. Analysis Calc. for $C_{21}H_{19}CuN_3O_4$ (440.939 g/mol): C, 57.20; H, 4.34 N, 9.53%. Found: C, 57.97 H, 3.877; N, 10.00%. ESI-MS in MeOH (*m*/*z* (rel. intensity) assignment, positive ion): 441.00 (40%) $[M+1]^+$. FT-IR (KBr, cm^{-1}): 3338–2816 (OH), 3056 (Ar—H), 2957, 2839 (aliphatic C—H), 1601 (C=N), 1583 (C—C aromatic), 1539 (—N=N—), 1270 (C—O), 1129 (C—O—C), 982 (C—O of MeOH), 868 (Cu—OHMe), 511 (Cu—O), 427 (Cu—N).

[$CuL^2(H_2O)$], (***5***); yield: 0.111 g, 91%, color: green. M.p. > 250 °C. Analysis Calc. for $C_{21}H_{19}CuN_3O_4$ (440.939 g/mol): C, 57.20; H, 4.34; N, 9.53%. Found: C, 56.73; H, 4.048; N, 9.636%. ESI-MS in MeOH (*m*/*z* (rel. intensity) assignment, positive ion): 440.94 (30%) $[M+H]^+$. FT-IR (KBr, cm^{-1}): 2915, 2835 (aliphatic C—H), 1595 (C=N), 1590 (C—C aromatic), 1538 (—N=N—), 1128 (C—O—C), 870 (Cu—OH_2), 514 (Cu—O), 421 (Cu—N).

[$CuL^3(H_2O)$], (***6***); yield: 0.119 g, 98%, color: green. M.p. > 250 °C. Analysis Calc. for $C_{20}H_{16}ClCuN_3O_4$ (461.358 g/mol): C, 52.07; H, 3.50; N, 9.11%. Found: C, 54.08; H, 3.39; N, 9.40%. ESI-MS in MeOH (*m*/*z* (rel. intensity) assignment, positive ion): 461.36 (5%) $[M-1]^+$. FT-IR (KBr, cm^{-1}): 3060 (Ar—H), 2994, 2835 (aliphatic C—H), 1602 (C=N), 1580 (C—C aromatic), 1538 (—N=N—), 1255 (C—O), 1129 (C—O—C), 871 (Cu—OH_2), 511 (Cu—O), 439 (Cu—N).

Results and discussion

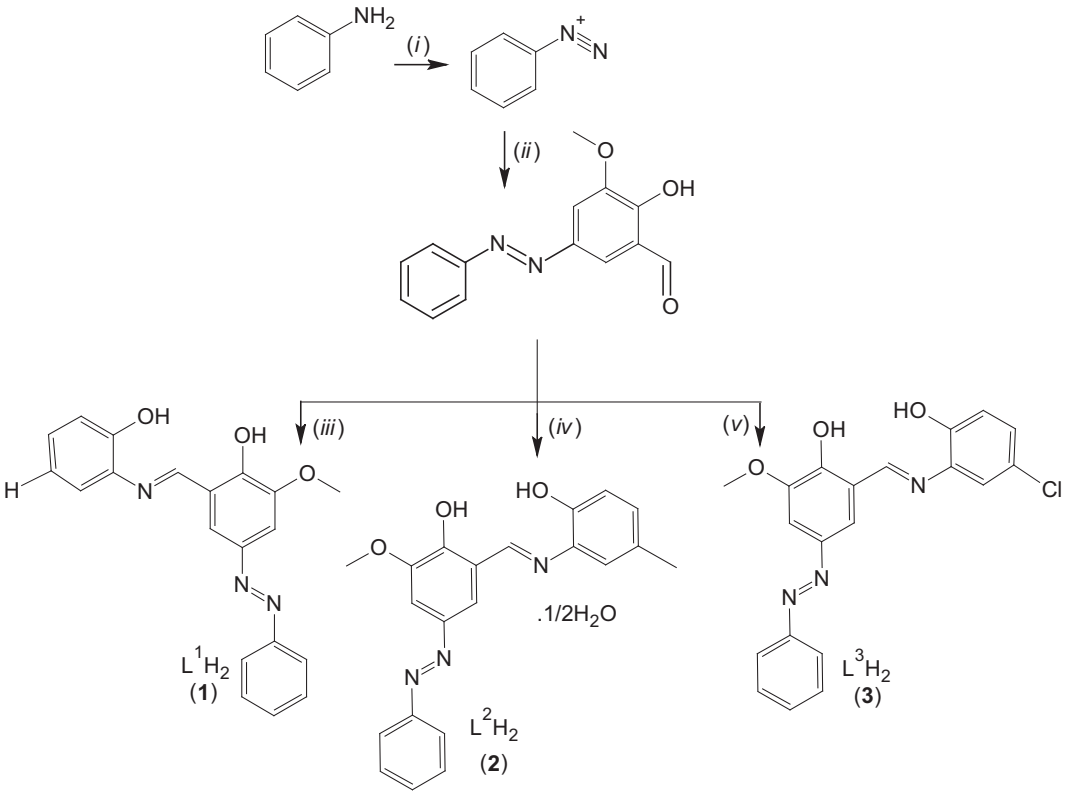
Synthesis

The azo–azomethine ligands L^1H_2–L^3H_2 were synthesized by the condensation reaction of equimolar quantities of 2-aminophenol, or its two derivatives with 2-hydroxy-3-methoxy-5-[(*E*)-phenyldiazenyl]benzaldehyde in MeOH. The compounds L^1H_2–L^3H_2 were obtained in yields 61, 68 and 88%, respectively. The purity of the ligands were confirmed by TLC technique and C, H, N elemental analyses. Mononuclear Cu(II) complexes [$CuL^1(CH_3OH)$] (***4***), [$CuL^2(H_2O)$] (***5***) and [$CuL^3(H_2O)$] (***6***) were prepared by the reaction of one equivalent of the ligands with one equivalent $Cu(OAc)_2{\cdot}H_2O$ according to the following equation:

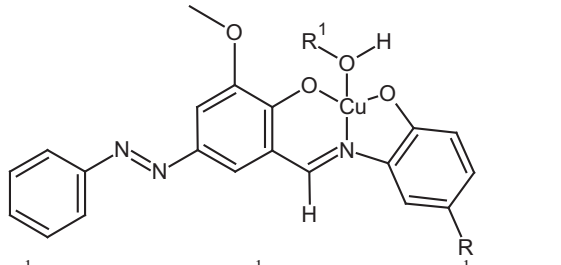
$$(CH_3COO)_2Cu.H_2O + L^xH_2 \xrightarrow[\text{Reflux}]{\text{MeOH}-CHCl_3} [CuL^x(ROH)]$$

$$(x = 1,\ R = H \text{ or } CH_3 \text{ and } x = 2,3\ R = H)$$

The copper(II) complexes derived from azo–azomethine ligands are green colored, stable toward air. The complexes are insoluble in water and some common organic solvents but soluble in dimethylformamide and dimethylsulfoxide. Single crystals of the new ligands synthesised and their copper(II) chelates could not be isolated from organic solvents, and so no definite structures are available. However, structures of the tridentate ligands and their Cu(II) complexes were proposed by the analytical and spectroscopic data as shown in Scheme 1, and Fig. 1, respectively. The possible tautomers of synthesized ligands are shown in Fig. 2. The physical characteristics and microanalytical data of the ligands and their copper(II) complexes are given in experimental section. The structures of the obtained ligands and their respective copper(II) complexes were elucidated by elemental analysis, and infrared, mass, and ultraviolet–visible spectroscopy. Microanalytical data for the ligands and their copper(II) complexes are in good agreement with theoretical values. The spectroscopic and analytical measurements showed that the complexes have general formulae of [CuL(X)], where L = dianionic azo–azomethines, X = MeOH ($L^2H_2{\cdot}1/2H_2O$) and H_2O (L^1H_2 or L^3H_2). The Cu(II):L ratio was found to be 1:1 for



Scheme 1. The general preparation route of azo–azomethines. (i) $NaNO_2$, HCl, 0 °C, (ii) 2-hydroxy-3-methoxybenzaldehyde, (iii) 2-aminophenol, (iv) 2-amino-*p*-cresol, and (v) 2-amino-4-chlorophenol.



R = H, R^1 = H (**4**); R = CH_3, R^1 = H (**5**); R = Cl, R^1 = H (**6**)

Fig. 1. The proposed structure of the mononuclear Cu(II) complexes.

all three complexes. The chelation of the ligands to the copper(II) ions occurs through the phenolic oxygen atoms and the nitrogen atom of the ligand. ESI mass spectra for the synthesized azo–azomethines and their Cu(II) complexes exhibit several fragmentation signals, however molecular ion peaks were observed with lower intensities.

FT-IR spectra

The characteristic FT-IR absorption bands of the azo–azomethine compounds and their mononuclear Cu(II) complexes were determined and the data are given in experimental section. FT-IR spectra of L^1H_2 and its Cu(II) complex [$CuL^1(CH_3OH)$] (***4***) are given in Figs. 3 and 4 and S1–S4, respectively. The solid state FT-IR spectra of azo–azomethines showed phenolic OH band in 3360–2900 cm^{-1} range. A strong sharp absorption band at 1613 cm^{-1} for L^1H_2, 1612 cm^{-1} for $L^2H_2{\cdot}1/2H_2O$, and 1611 cm^{-1} for L^3H_2 in the spectra of the azo–azomethines can be assigned to the ν(C=N) stretching. In addition to CH=N stretchings, compounds L^1H_2 and $L^2H_2{\cdot}1/2H_2O$ exhibits a band at 1635 and 1633 cm^{-1} which are assigned to ν(C=O) vibrations, respectively. These bands are indicative of keto-amine tautomer in solid state. However, the compound L^3H_2 do not exhibit a ν(C=O) vibration suggesting the enol-imine tautomer in the solid state. Previous X-ray crystallographic studies on *o*-hydroxy Schiff bases derived from salicylaldehyde and 2-hydroxy anilines indicated that both enol-imine and keto-amine forms may exist in solid state [33–36]. The azo group ν(N=N) stretching was observed at 1541, 1537 and 1539 cm^{-1} for the compounds L^1H_2–L^3H_2, respectively.

X-ray crystallographic study of *N*-(2-hydroxy-5-chlorophenyl) salicylaldimine derived from salicylaldeyhde and 5-chloro-2-hydroxyaniline showed that both tautomeric forms (keto and enol forms) are present in the solid state. This shows that there is a quick shift between two tautomeric forms suggesting the low energy difference between these two forms. Ledbetter proposed that the second *o*-hydroxy group in the aniline ring of *o*-hydroxy Schiff bases exhibits stronger keto-tautomerism due to this second hydroxy group [37].

Upon complexation with the Cu(II) ion, ν(C=O) vibrations in L^1H_2 and $L^2H_2{\cdot}1/2H_2O$ disappeared in the spectra suggesting the imine form in the complexes. In the FT-IR spectra of the complexes, azomethine ν(C=N) bands shifted to lower values 1601 cm^{-1} for [$CuL^1(CH_3OH)$], 1595 cm^{-1} for [$CuL^2(H_2O)$] and 1602 cm^{-1} for [$CuL^3(H_2O)$] suggesting coordination of the imine nitrogen to the metal center. The absence of the band due to phenolic OH group in the spectra of Cu(II) complexes confirm the coordination of ligands to the metal *via* phenolate oxygen atom. The vibrations of the Cu—O and C—N bonds are located at the low wavenumbers of 400–650 cm^{-1}.

NMR spectra

The ^1H and ^{13}C NMR spectra of azo–azomethines L^1H_2–L^3H_2 were recorded in d_6-dimethylsulfoxide (DMSO) solution using

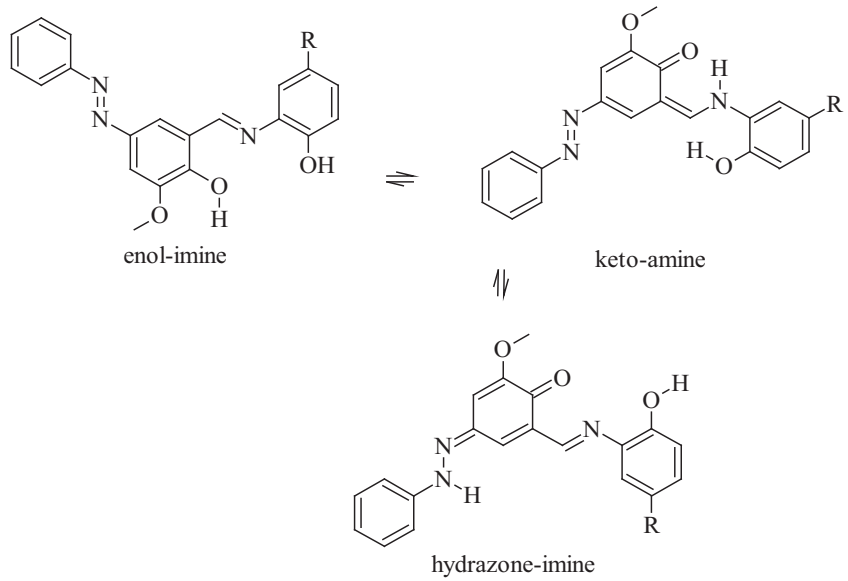


Fig. 2. The possible tautomers of synthesized azo–azomethines (where R = H for L^1H_2, R = CH_3 for L^3H_2 and R = Cl for L^3H_2.

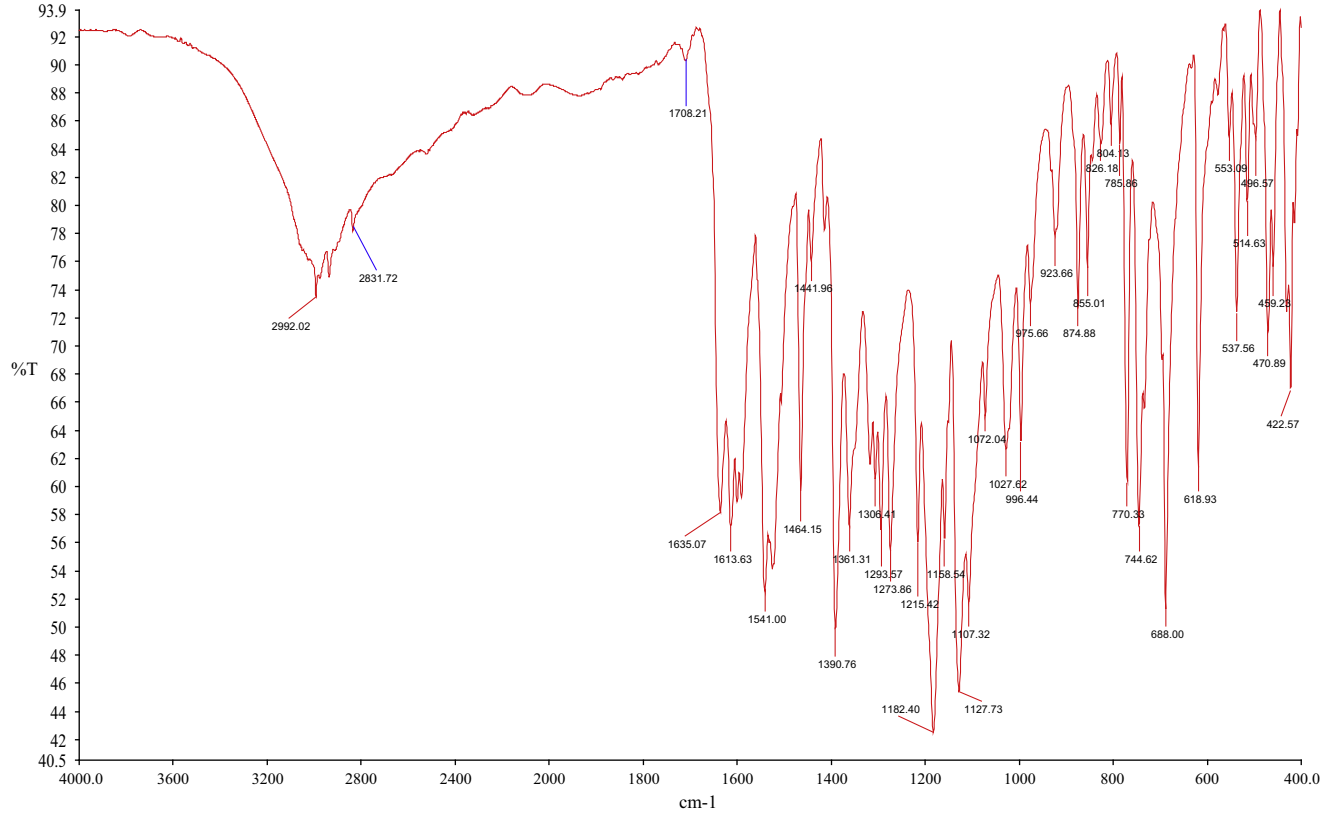


Fig. 3. FT-IR spectrum of L^1H_2.

tetramethylsilane (TMS) as an internal standard. The ^1H NMR and ^{13}C NMR data of the azo–azomethines are collected in experimental section. The ^1H NMR and ^{13}C NMR spectra of L^2H_2 are shown in Figs. 5 and 6 and rest of the NMR spectra are given in the supplementary file (Figs. S5–S8). The ^1H NMR spectra of L^1H_2–L^3H_2 display a singlet signal at δ 3.85, 3.80 and 3.87 ppm with an integration equivalent to three hydrogens corresponding to the methoxy (OCH_3) group. In the ^1H NMR spectrum of L^1H_2, a singlet at δ 2.30 ppm was assigned to the protons of methyl group (Ph—CH_3). The ^1H NMR spectra of L^1H_2–L^3H_2 exhibited broad signals for the phenolic proton in the aniline ring in the range of δ 10.23–10.60 ppm. The azo–azomethines also exhibit signals at δ 14.97–15.30 and 9.22–9.25 ppm ranges assigned to —NH— and =CH—N protons, respectively. In the spectrum of L^1H_2, doublet signals of —NH— and =CH—N protons were observed at δ 9.22–9.23 and 15.29–15.30 ppm as shown in Fig. 5. These chemical shifts suggests that compounds L^1H_2–L_3H_2 favor keto–amine tautomer in DMSO solution that are in agreement with the results of related

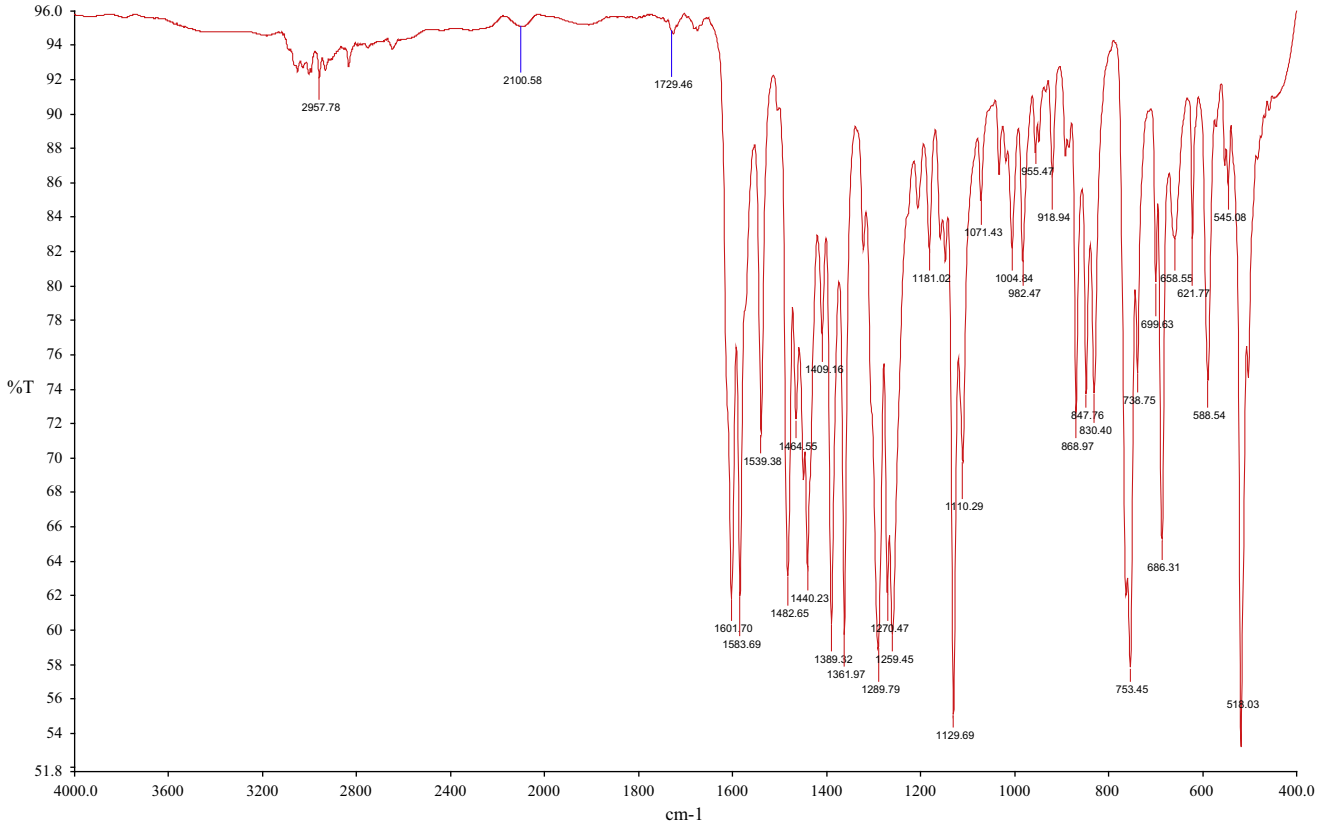


Fig. 4. FT-IR spectrum of [CuL^1(CH$_3$OH)] (***4***).

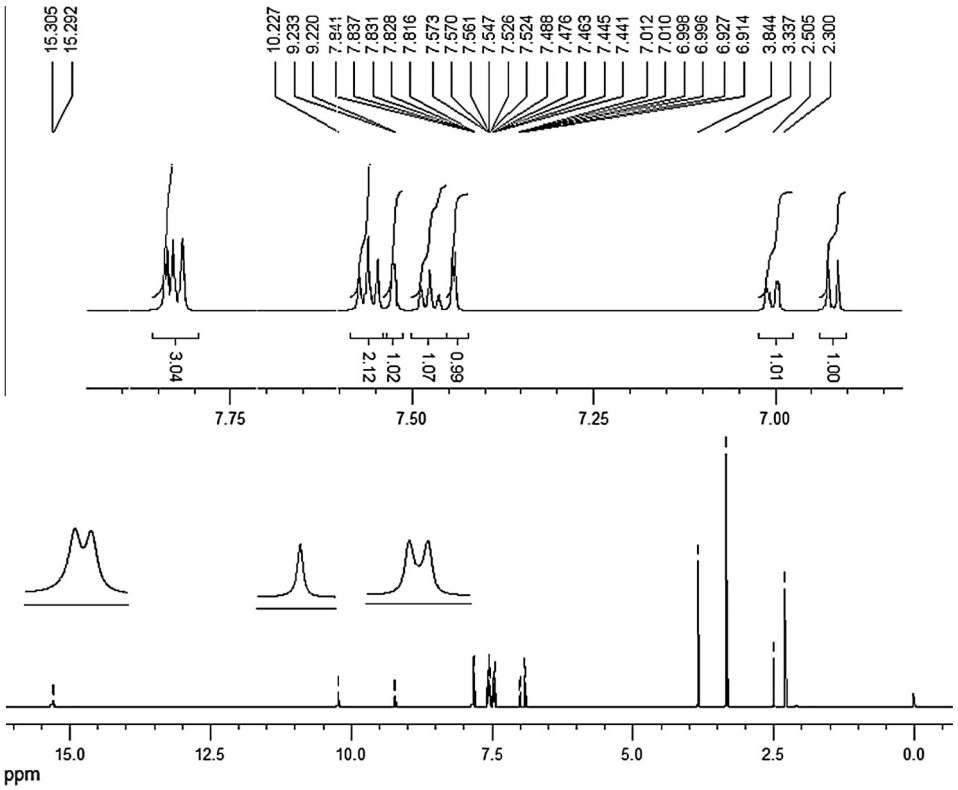


Fig. 5. The ^{1}H NMR spectrum of L^{2}H$_2$ azo–azomethine.

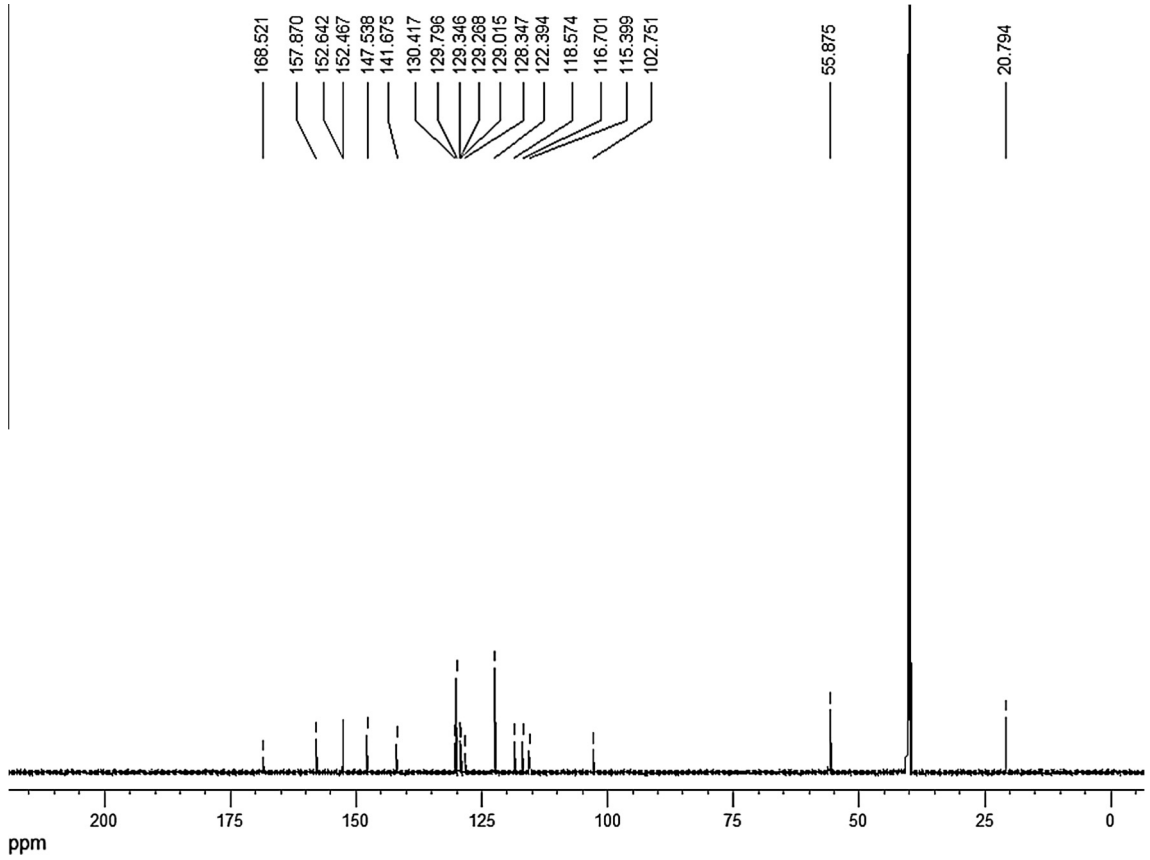


Fig. 6. The ^{13}C NMR spectrum of L^2H_2 azo–azomethine.

compounds [38,39]. These chemical shifts were also supported by UV–Vis. spectra in DMSO solution.

The ^{13}C NMR spectra of azo–azomethine compounds L^1H_2–L^3H_2 exhibit consistent carbon atom signals with their proposed structures. In the ^{13}C NMR spectra of compounds L^1H_2–L^3H_2 display signals at δ 55.90, 55.875 and 56.05 ppm assigned to the carbon atoms of the methoxy groups (OCH_3), respectively. The methyl carbon (Ph—CH_3) signal in L^2H_2 was observed at δ 20.79 ppm. In ^{13}C NMR spectra of L^1H_2–L^3H_2, the signals at δ 167.865 and 168.521 and 164.848 ppm was assigned to the carbon atom of the C=O group, respectively. The signal belong to carbon atom phenolic group (C—OH) were seen at δ 158.33, 157.87, 160.055 ppm for L^1H_2–L^3H_2, respectively. Rest of the carbon atom signals were observed in the range of δ 152–102 ppm for all three compounds.

UV–Vis. spectra

There are several different spectroscopic techniques including FT-IR, UV–Vis., NMR and X-ray crystallography techniques to evaluate tautomeric species of *o*-hydroxy Schiff bases both in solutions and solid state [40–42]. UV–Vis. spectroscopy is very useful tool for studying tautomerism in Schiff bases involving a hydroxyl group in ortho position to the azomethine group. The previous reports indicated that *o*-hydroxy Schiff bases with azo group (—N=N—) may exist in keto and/or enol forms in solid state [43] and solvent media [10]. There are several factors influencing the tautomeric equilibrium including substitute groups in the molecule, solvent polarity and temperature [39].

To examine the tautomeric behavior of the azo–azomethine compounds L^1H_2–L^3H_2 in solution, electronic spectra were performed in three organic solvents with different polarity ($CHCl_3$, DMSO and DMF) at room temperature. UV–Vis. spectra of $L^2H_2{\cdot}1/2H_2O$ and L^3H_2 are shown in Figs. 7 and 8 and electronic data are illustrated in Table 1. The absorption spectra of azo–azomethines are similar and exhibit two absorpiton maximums in the solvents studied $CHCl_3$, DMSO and DMF. Maximum absorption bands observed in the range of 320–420 nm were assigned to the π–π* transitions of the aromatic rings. The π–π* transitions shifted to higher wavelength values (batochromic effect) with the increase in polarity. The second band was observed in the range of 450–550 nm. The previous tautomeric studies on *o*-hydroxy Schiff bases suggested that a band above 400 nm is indicative of existence of keto-amine tautomer in solution [39].

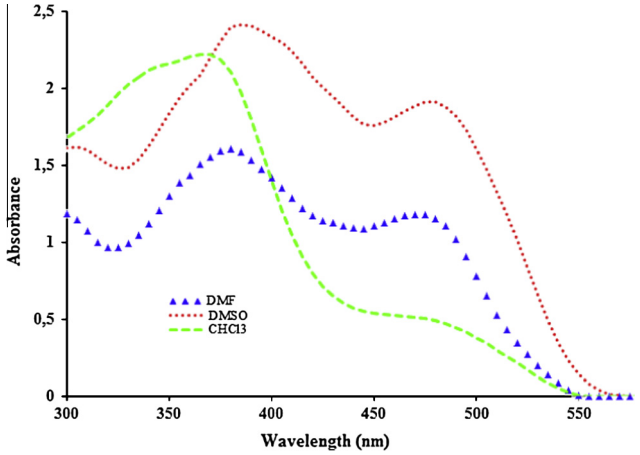


Fig. 7. UV–Vis. spectra of $L^2H_2{\cdot}1/2H_2O$ ligand in solvents DMSO, DMF and $CHCl_3$.

The keto-amine band intensity increases with the increase in the solvent polarity. In $CHCl_3$ solution, the intensity of the keto-amine band is lower than in DMSO and DMF for all three compounds. The electronic absorption spectra of the azo–azomethine compounds at different volume ratios of the applied pair solvents DMSO/H_2O were also measured. The absorption curves of $L^2H_2{\cdot}1/2H_2O$ in DMSO/H_2O mixtures are shown in Fig. 9. In DMSO/H_2O mixture, the absorption bands shifted to lower wavelengths (hypsochromic effect) and absorption values (hypochromic effect).

Absorption spectra for Cu(II) complexes were also recorded in DMF solution. The complexes show only one absorption band in the range of 330–500 nm assigned to the π–π^* and M → L charge transitions. The keto-amine band in the range of 450–550 nm observed for ligands disappeared in the spectra of the complexes. In the spectra, no metal induced d-d transitions were observed in the solvents and concentrations studies. UV–Vis. spectrum of the complex $[CuL^1(CH_3OH)]$ (***4***) is shown in Fig. 10.

Electrochemistry

Electrochemical behaviors of the new azo–azomethines (L^1H_2–L^3H_2) and their copper(II) metal complexes were investigated using CV technique in DMSO solution containing 0.1 M TBATFB in the range from 0 to −2.3 V.

For all azo–azomethine ligands studied, cyclic voltammograms at 100 mV s^{-1} consist of two cathodic peaks in the range −1.45–(−1.58) V and −2.05–(−2.12); no anodic wave occurs in the reverse scan. This behavior was observed for a wide range of scan rates from 25 to 1000 mV s^{-1}. Hence, such a reduction process should correspond to a totally irreversible electron transfer. The CV curves for L^1H_2 and $[CuL^1(MeOH)]$ are shown in Fig. 11 and electrochemical data of all these compounds are given in Table 2. It is believed that the reduction peaks correspond to the —N=N— (azo) and —C=N— (imino) groups of the organic ligands in solution [44]. The electrochemical properties of Cu(II) metal complexes, have been studied in order to consider spectral and structural changes accompanying electron transfer. The cyclic voltammogram of the complex $[CuL^1(MeOH)]$, displays three reduction waves in potentials −0.83, −1.19 and −1.59 V. The peak at E_{pc} = −0.83 V and E_{pc} = −1.19 V is characteristic for $Cu^{II} \rightarrow Cu^{I}$ and $Cu^{I} \rightarrow Cu^{0}$ reductions, respectively [45]. The irreversible reduction wave at −1.59 V is related to reduction of L^1H_2 ligand [46,47]. In other complexes, the cyclic voltammetric $Cu^{II} \rightarrow Cu^{I}$ reduction process was observed at −0.72 and −0.80 V, for $[CuL^2(H_2O)]$ and $[CuL^3(H_2O)]$, respectively. For Cu(II) complexes, the absence of the reduction peak of the imine group indicates that, owing to the lack of transferable hydroxylic protons, the reduction potentials of the dianionic ligands have been shifted beyond the lower limit of the potential interval considered in the experimental measurements [48].

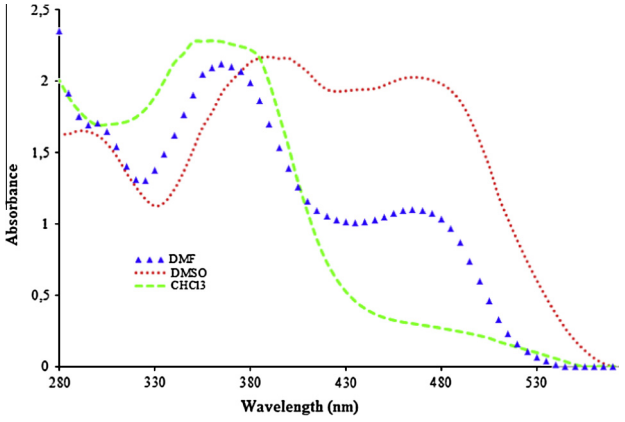


Fig. 8. UV–Vis. spectra of L^3H_2 ligand in various solvents.

Table 1
UV–Vis. absorption bands for the synthesized azo–azomethines in various solvents [λ_{max} (nm), absorbance].

Compounds	$CHCl_3$	DMSO	DMF
L^1H_2 (***1***)	365(2.389), 482(0.257)	382(2.389), 482(0.257)	365(2.063), 475(1.307)
$L^2H_2{\cdot}1/2H_2O$ (***2***)	360(2.181), 475(0.518)	385(2.411), 473(1.956)	380(1.607), 475(1.178)
L^3H_2 (***3***)	365(2.278), 480(0.269)	390(2.17), 475(2.007)	365(2.12), 470(1.091)
$[CuL^1(MeOH)]$ (***4***)	–	–	420(1.167)
$[CuL^2(H_2O)]$ (***5***)	–	–	415(2.654)
$[CuL^3(H_2O)]$ (***6***)	–	–	435(2.868)

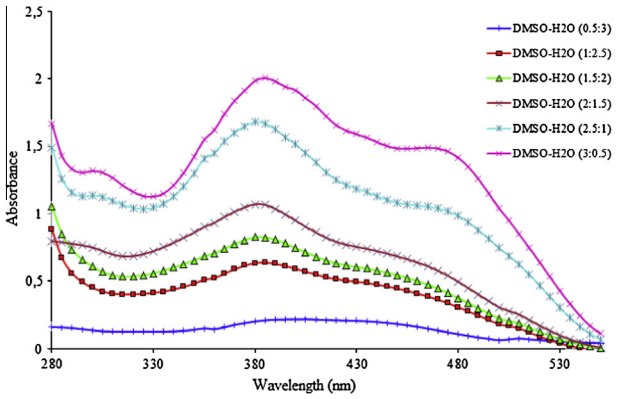


Fig. 9. UV–Vis. spectra of $L^2H_2{\cdot}1/2H_2O$ ligand in DMSO-H_2O mixtures.

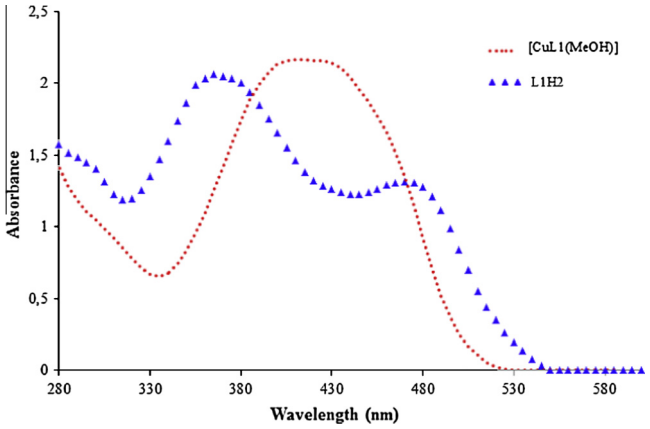


Fig. 10. UV–Vis. spectra of the ligand L^1H_2 and its Cu(II) complex in DMF.

Antibacterial activity

MIC values of the synthesised azo–azomethines and their mononuclear copper complexes against bacteria were between 8 and 128 μg/mL. The ligand L^3H_2 showed the lowest MIC value (8 μg/mL) against *E. faecalis* strain but this value is higher than the reference antibiotics (ampicillin and gentamicin). $[CuL^1(MeOH)]$ and L^1H_2 substances had the highest MIC values (128 μg/mL)

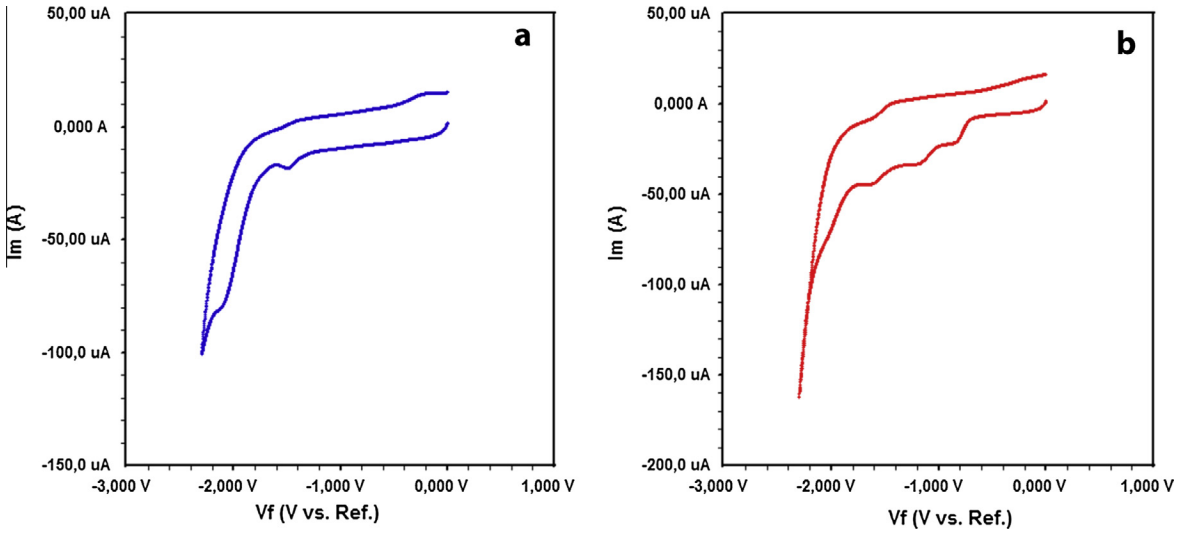


Fig. 11. Cyclic voltammograms recorded for the reduction of 1.0 mM L^1H_2 (a) and $[CuL^1(MeOH)]$ (b) at a GC electrode in DMSO containing 0.1 M TBATFB in the range from 0 to −2.3 V. Scan rate is 100 mV s^{-1}.

Table 2
Cyclic voltammetric parameters for the azo–azomethines and their copper(II) complexes.

Compounds	E_{pc} (V); ligand reductions	E_{pc} (V); $Cu^{II} \rightarrow Cu^{I}$	E_{pc} (V); $Cu^{I} \rightarrow Cu^{0}$
L^1H_2(***1***)	−1.48, −2.08	–	–
$L^2H_2{\cdot}1/2H_2O$ (***2***)	−1.45, −2.05 V	–	–
L^3H_2 (***3***)	−1.58, −2.12	–	–
$[CuL^1(MeOH)]$ (***4***)	−1.59	−0.83	−1.19
$[CuL^2(H_2O)]$ (***5***)	−1.63	−0.72	−1.28
$[CuL^3(H_2O)]$ (***6***)	−1.62	−0.80	−1.14

E_{pc} indicates the cathodic peak potential for irreversible reduction processes.

against *K. pneumoniae and P. aeruginosa* strains and this value is higher than gentamicin but is equal to that of ampicillin. These substances had the same MIC values (128 μg/mL) against *E. coli* and higher than gentamicin and ampicillin. Also *S. aureus* and E. *faecalis* which are Gram positive bacteria were more sensitive to these substances $[CuL^1(MeOH)]$ (64 μg/mL) and L^1H_2 (32 μg/mL)] but higher than reference antibiotics. The results are given in Table 3. All substances had the higher MIC values against Gram negative bacteria than Gram positive bacteria. L^1H_2 and $[CuL^1(MeOH)]$ substances had the highest MIC values against all bacteria but the MIC values for Gram negative bacteria (128 μg/mL) higher than Gram positive bacteria. L^1H_2 substance showed a lower MIC value (32 μg/mL) than $[CuL^1(MeOH)]$ (64 μg/mL) against *S. aureus* and *E. faecalis*. $L_2H_2{\cdot}1/2H_2O$ and L_3H_2 had the same MIC value (64 μg/mL) against all Gram negative bacteria but while $L_2H_2{\cdot}1/2H_2O$ had the same MIC value (16 μg/mL) against all Gram positive bacteria. $[CuL^2(H_2O)]$ and $[CuL^3(H_2O)]$ substances had the lower MIC value (32 μg/mL) against all Gram negative bacteria than the other substances. The complexes $[CuL^2(H_2O)]$ (***5***) $[CuL^3(H_2O)]$ (***6***) exhibit more biological activity than their corresponding free ligands against *E. coli*, *P. aeruginosa* and *K. pneumoniae* under identical experimental conditions. The possible cause of the increased biological activity of the metal chelates compared to that of the free ligand may be explained in terms of Tweedy's chelation theory [49]. According to this theory, the polarity of the central metal ion is reduced by chelation *via* partial sharing of positive charge of metal ion with the donor atoms and possible p-electron delocalization within the whole chelate ring. In addition, the lipophilic character of the central metal atom is increased by chelation which subsequently favors its permeation through the lipid layer of the cell membrane [49–51].

Conclusion

The azo–azomethines L^1H_2–L^3H_2 and their copper(II) complexes were synthesized and characterized by spectroscopic and analytical methods. The analytical data show that the metal ligand stoichiometry in all these copper(II) complexes is 1:1. The spectral data show that the ligands act as tridentate coordinating through nitrogen atom of the azomethine and two oxygen atoms of hydroxyl groups of the ligands. Proton induced tautomerism for L^1H_2–L^3H_2 was investigated by ^1H, ^{13}C NMR, FT-IR and UV–Vis. spectral methods. Existence of keto-amine tautomer for ligands L^1H_2–L^3H_2 were confirmed by an absorption band in the range of 450–550 nm in solutions ($CHCl_3$, DMSO and DMF). It is evident from the cyclic voltammogram of the tridentate ligands and the copper(II) complexes that all the compounds are electroactive over

Table 3
Antibacterial activity of the prepared azo–azomethines and their copper(II) complexes as MICs values (μg/mL).

Compounds	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*K. pneumoniae* ATCC 700603	*S. aureus* ATCC 29213	*E. faecalis* ATCC 29212
L^1H_2 (***1***)	128	128	128	32	32
$L^2H_2{\cdot}1/2H_2O$ (***2***)	64	64	64	16	16
L^3H_2 (***3***)	64	64	64	16	8
$[CuL^1(MeOH)]$ (***4***)	128	128	128	64	64
$[CuL^2(H_2O)]$ (***5***)	32	32	32	16	16
$[CuL^3(H_2O)]$ (***6***)	32	32	32	16	16
Ampicillin	8	128	128	0.5	2
Gentamicin	1	0.5	1	1	4

a range from 0.0 to −2.3 V in DMSO solvent. MIC values of azo–azomethines against bacteria were between at 8 and 128 μg/mL. The synthesized compounds were found to exhibit lower MIC values against Gram positive bacteria than Gram negative bacteria.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molstruc.2015.04.043.

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