



Copper(II) Complexes Bearing Unsymmetrical NNO-Tridentate Schiff Bases: Synthesis, Spectral Characterization, Electrochemical Behaviour and their Biological Activity

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In this work, three mono-condensation Schiff bases of *o*-phenyldiamine with 5-*t*-butylsalicylaldehyde (AIBP), 3,5-di-*t*-butylsalicylaldehyde (AIdBP) and 2-hydroxy-1-naphthaldehyde (AINA) were prepared and coordinated by CuCl₂·2H₂O without and with the presence of pyridine to form six tridentate Schiff base copper(II) complexes. The synthesized unsymmetrical Schiff base copper(II) complexes were characterized by diverse physico-chemical methods like mass, infrared, ultraviolet-visible spectroscopies and magnetic susceptibility measurements to indicate that the coordination of copper(II) with the synthetic Schiff bases at NNO atoms and the geometry around central ion is distorted square-planar or square-pyramidal probably. Then electrochemical properties of the obtained Schiff base copper(II) complexes were studied using cyclic voltammetry (CV). [Cu(II)(AINA)Cl] has exhibited that the peak current increases with the increase of the scan rates linearly. All the Schiff base copper(II) complexes have been screened for their *in vitro* antibacterial activity on Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus fermentum*. [Cu(II)(AIdBP)Cl] has expressed the effective activity for all three tested bacteria. The *in vitro* cytotoxicity of the synthetic Schiff base copper(II) complexes for human cancer cell lines, MCF-7 and HepG-2, were examined as well.

Keywords: Tridentate Schiff bases, Copper(II) complexes, Electrochemical properties, Antibacterial activity, Cytotoxicity.

INTRODUCTION

Schiff bases containing azomethine group (–HC=N–) in their structures are usually synthesized by condensation reaction of carbonyl compounds with primary amines. They are considered as one kind of the most distinguish organic compounds due to their ease synthesis, various structures and remarkable applications [1-6]. Most of the Schiff base ligands studied are bidentate and tetradentate ligands, tridentate Schiff base ligands are recently included [7-9]. In general, NNO tridentate Schiff base ligands are prepared from mono-condensation reactions of a carbonyl compound with one arm of a primary diamine. Such tridentate ligands can exist as tautomeric mixture of keto-amine and enol-imine forms [10-12]. In the presence of transition metal ions, the tridentate Schiff base ligands can form stable unsymmetrical metal complexes with their specific structures and new applicable properties such as catalytic features [13-16], advanced materials [17-19] and biomedical activities [20-22].

Copper, a biometal found in several natural enzymes, is important for the organ biotransformations. In metalloenzymes, copper can usually exist in organometallic and coordination compounds [23,24]. In published works on some useful copper (II) complexes were found out for biomedical applications such as antifungal, antimicrobial, antiviral and anticancer activities [25-31]. Although there are some outstanding researches, more works are needed to find the real bioactive copper(II) complexes which can be used clinically, especially in anticancer drugs. So in this work, the complexes of NNO tridentate Schiff base ligands with copper(II) ion are prepared and studied their spectral characterization, electrochemical behaviour and bioactive activities.

EXPERIMENTAL

All chemical reagents were received from several commercial companies and used without any more purification. All solvents were obtained from commercial resources and purified by usual laboratory procedures.

High resolution mass spectra (IDA-TOF-MS) were identified on Sciex X500 QTOF and Mass spectra (ESI-MS) were recorded on Agilent 6310 Ion Trap in electrospray ionization. Infrared spectra (FTIR) were determined on Perkin-Elmer Spectrum-2 using KBr pellets. ^1H NMR spectra were measured on Bruker Advance (500 Hz) using CDCl_3 as solvent and TMS as a standard reference. Electronic absorption spectra were recorded on Perkin-Elmer Lambda UV-35 spectrophotometer in methanol solution. Magnetic susceptibility measurements of the obtained copper(II) complexes were identified at room temperature using a magnetic susceptibility balance (Sherwood Scientific Mark 1 with serial No. 25179). The purity of the synthetic compounds was analyzed by TLC and HPLC-MS methods.

Synthesis of NNO-tridentate Schiff base ligands: The tridentate Schiff bases were synthesized following the reported condensation reaction between *o*-phenyldiamine and relative aldehydes [32,33]. *o*-Phenyldiamine (10.0 mmol, 1.11 g) and 15 mL ethanol were added into 100 mL flask containing a magnetic stirring bar. Then 10.0 mmol of relative aldehyde in 20 mL ethanol was slowly added. The condensation reaction was continuously stirred for 60 min in the presence of 10 mmol of anhydrous Na_2SO_4 (1.43g). The reaction was observed by TLC until the reaction was carried out completely. The precipitates were filtered and dried under low pressure and recrystallized in methanol (**Scheme-I**). The received products were analyzed by various spectroscopies to obtain their mass, IR, NMR and UV-Vis spectra.

(E)-2-(((2-Aminophenyl)imino)methyl)-4-(tert-butyl)phenol (AIBP): Yellow powder, yield: 91%; 2.44 g; ESI-MS (m/z): 269.0 $[\text{M}+\text{H}^+]$ (calcd. 269.3); FT-IR (KBr, cm^{-1}): 3484, 3382 $\nu(\text{N-H})$, 2953 $\nu(\text{C-H})$, 1608 $\nu(\text{C=N})$, 1489, 1265 $\nu(\text{C-O})$, 1173, 818, 754 $\delta(\text{C-H})$, 617, 488; ^1H NMR (500 MHz, CDCl_3 , δ ppm): 12.79 (s, 1H, OH), 8.62 (s, CH=N), 7.43 (dd, $J = 8.5$ Hz; 2.5, 1H-Sal), 7.38 (d, $J = 2.5$ Hz, 1H-Sal), 7.09 (dt, $J = 7.5$ Hz; 1.5, 1H-Ph), 7.04 (dd, $J = 8.5$ Hz; 1.0, 1H-Ph), 6.97 (d, $J = 8.5$ Hz, 1H-Sal), 6.79 (m, 2H-Ph), 3.99 (s, 2H- NH_2), 1.33 (s, 9H-*t*-Bu); UV-Vis (MeOH, 3.0×10^{-5} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{M}^{-1}$) 237 (13,000), 274 (8,333), 375 (6,000).

(E)-2-(((2-Aminophenyl)imino)methyl)-4,6-di-tert-butylphenol (AIdBP): Yellow powder (Yield: 88%; 2.85 g); ESI-MS (m/z): 325.0 $[\text{M}+\text{H}^+]$ (calcd. 325.4); FT-IR (KBr, cm^{-1}): 3488, 3390 $\nu(\text{N-H})$, 2965 $\nu(\text{C-H})$, 1606 $\nu(\text{C=N})$, 1474, 1267 $\nu(\text{C-O})$, 1170, 813, 752 (δ , C-H), 644, 473; ^1H NMR (500 MHz, CDCl_3 , δ ppm): 13.71 (s, 1H, OH), 8.85 (s, CH=N), 7.48 (d, $J = 2.5$ Hz, 1H-Sal), 7.38 (d, $J = 2.5$ Hz, 1H-Sal), 7.15 (dd, $J = 8.0$ Hz; 1.5, 1H-Ph), 7.01 (dt, $J = 8.0$ Hz; 1.5, 1H-Ph), 6.80 (dd, $J = 8.0$ Hz; 1.0, 1H-Ph), 6.63 (dt, $J = 7.5$ Hz; 1.5, 1H-Ph), 4.98 (s, 2H- NH_2), 1.43 (s, 9H-*t*-Bu), 1.30 (s, 9H-*t*-Bu); UV-Vis (MeOH, 3.0×10^{-5} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{M}^{-1}$) 239 (10,667), 278 (7,000), 375 (5,000).

(E)-1-(((2-Aminophenyl)imino)methyl)naphthalen-2-ol (AINA): Yellow powder; yield: 85%; 2.23 g; ESI-MS (m/z): 262.9 $[\text{M}+\text{H}^+]$ (calcd. 263.3); FT-IR (KBr, cm^{-1}): 3472, 3375 $\nu(\text{N-H})$, 3048 $\nu(\text{C-H})$, 1610 $\nu(\text{C=N})$, 1558, 1493, 1306 $\nu(\text{C-O})$, 1162, 816, 752 (δ , C-H), 470, 418; ^1H NMR (500 MHz, CDCl_3 , δ ppm): 15.63 (s, 1H, OH), 9.60 (s, CH=N), 8.50 (d, $J = 9.0$ Hz, 1H-Naph), 7.93 (d, $J = 9.0$ Hz, 1H-Naph), 7.82 (d, $J = 8.0$ Hz, 1H-Naph), 7.54 (t, $J = 7.0$ Hz, 1H-Naph), 7.45 (d, $J = 7.5$ Hz, 1H-Naph), 7.36 (t, $J = 7.0$ Hz, 1H-Naph), 7.09 (d, $J = 9.0$ Hz, 1H-Ph), 7.04 (t, $J = 8.0$, 1H-Ph), 6.84 (d, $J = 8.0$, 1H-Ph), 6.71 (t, $J = 7.0$ Hz, 1H-Ph), 5.08 (s, 2H- NH_2); UV-Vis (MeOH, 3.0×10^{-5} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{M}^{-1}$) 262 (13,000), 320 (6,333), 440 (8,667).

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Synthesis of tridentate Schiff base copper(II) complexes:

The tridentate Schiff base copper(II) complexes were formed by the coordination of the synthetic tridentate Schiff bases with $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (1:1 molecular ratio) in ethanol using reflux. Schiff base ligand (2.0 mmol) dissolved in 15 mL ethanol was added slowly into a 100 mL flask containing 2.0 mmol (0.35 g) $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 20 mL ethanol. The reaction mixture was stirred continuously and reflux for 3 h. The reaction completion was checked by TLC. Then the reaction was cooled until room temperature. The blue precipitates were collected and dried (**Scheme-I**). The coordination products are soluble in polar solvents *e.g.* DMSO, CH_3OH , CH_3CN and CHCl_3 .

[Cu(II)(AIBP)Cl]: Green powder, yield: 90% (0.66 g); +IDA-TOF-MS (m/z): 330.0684 $[\text{M-Cl}]^+$ (calcd. 330.90); FT-IR (KBr, cm^{-1}): 3331 (N-H), 2955 (C-H), 1616 (C=N), 1521, 1494 (C=C), 1379, 1259 (C-O), 1154, 833, 742 (C-H), 511 (Cu-N), 465 (Cu-O). UV-Vis (MeOH, 1.0×10^{-4} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{M}^{-1}$): 247 (7,500), 301 (5,500), 425 (2,900). μ_{eff} (B.M.): 2.12.

[Cu(II)(AIdBP)Cl]: Green powder, yield: 87% (0.74 g); +IDA-TOF-MS (m/z): 386.1284 $[\text{M-Cl}]^+$ (calcd. 387.01); FT-IR (KBr, cm^{-1}): 3313, 3193 (N-H), 2951 (C-H), 1615 (C=N), 1524 (C=C), 1384, 1256 (C-O), 1165, 836, 761 (C-H), 535 (Cu-N), 442 (Cu-O). UV-Vis (MeOH, 1.0×10^{-4} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{M}^{-1}$): 242 (9,000), 303 (6,800), 429 (3,950). μ_{eff} (B.M.): 2.15.

[Cu(II)(AINA)Cl]: Green powder, yield: 85% (0.61 g); +IDA-TOF-MS (m/z): 324.0205 $[\text{M-Cl}]^+$ (calcd. 324.85); FT-IR (KBr, cm^{-1}): 3229 (N-H), 3058 (C-H), 1616 (C=N), 1581, 1534 (C=C), 1365 (C-O), 1182, 831, 738 (C-H), 570, 497 (Cu-N), 439 (Cu-O). UV-Vis (MeOH, 1.0×10^{-4} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{M}^{-1}$): 274 (4,800), 332 (2,700), 429 (2,900), 454 (2,800). μ_{eff} (BM): 2.14.

Synthesis of mixed-ligand Schiff base copper(II) complexes:

The mixed-ligand Schiff base copper(II) complexes were synthesized by the coordination of the synthesized tridentate Schiff bases and pyridine with $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (1:1:1 molecular ratio) in ethanol under reflux. The solution of 2.0 mmol (0.35 g) $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ dissolved in 20 mL ethanol was added slowly into a 100 mL flask containing 2.0 mmol of synthetic Schiff base ligand in 15 mL ethanol. Then, 2.0 mmol (0.16 g) of pyridine in 5.0 mL ethanol was added. The reaction mixture was stirred continuously and reflux for 3 h. The complete reaction was checked by TLC and cooled until room temperature. The blue precipitates were collected and dried (**Scheme-I**). The coordination products are soluble in polar solvents like DMSO, CH_3OH , CH_3CN and CHCl_3 .

[Cu(II)(AIBP)(Py)Cl]: Brown powder, yield: 79% (0.71 g); +IDA-TOF-MS (m/z): 408.0721 $[\text{M-Cl}]^+$ (calcd. 410.12); FT-IR (KBr, cm^{-1}): 3348 (N-H), 2956 (C-H), 1616 (C=N), 1521,

1497 (C=C), 1380, 1258 (C–O), 1157, 833, 738 (C–H), 596, 511 (Cu–N), 464 (Cu–O). UV-Vis (MeOH, 1.0×10^{-4} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{ M}^{-1}$): 243 (19,500), 299 (14,700), 337 (10,300), 427 (6,700). μ_{eff} (BM): 1.81

[Cu(II)(AIdBPy)(Py)Cl]: Dark powder, yield: 77% (0.77 g); +IDA-TOF-MS (m/z): 466.1502 $[\text{M}-\text{Cl}]^+$ (calcd. 466.19); FT-IR (KBr, cm^{-1}): 3340 (N–H), 2952 (C–H), 1599 (C=N), 1519 (C=C), 1383, 1253 (C–O), 1163, 835, 745 (C–H), 538 (Cu–N), 441 (Cu–O). UV-Vis (MeOH, 1.0×10^{-4} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{ M}^{-1}$): 247 (16,700), 302 (12,500), 430 (7,900). μ_{eff} (BM): 1.83.

[Cu(II)(AInA)(Py)Cl]: Dark powder, yield: 75% (0.66 g); +IDA-TOF-MS (m/z): 402.0419 $[\text{M}-\text{Cl}]^+$ (Cal. 403.07); FT-IR (KBr, cm^{-1}): 3266 (N–H), 3060 (C–H), 1616 (C=N), 1579, 1534 (C=C), 1364 (C–O), 1183, 829, 739 (C–H), 551, 497 (Cu–N), 455 (Cu–O). UV-Vis (MeOH, 1.0×10^{-4} M, λ/nm , $\epsilon/\text{cm}^{-1} \text{ M}^{-1}$): 244 (8,700), 275 (5,500), 331 (3,000), 430 (3,500), 454 (3,400). μ_{eff} (BM): 2.11.

Electrochemical measurements: The electrochemical properties of Schiff base copper(II) complexes were studied on a Zahner Elektrik IM6 instrument using Ag/AgCl as the reference electrode, the counter electrode as a platinum wire and the working electrode was a glassy carbon electrode. The cyclic voltammograms were recorded using the complexes' concentration of 1.0×10^{-3} M in DMSO solution and LiClO₄ 0.1 M as supporting electrolyte. All experiments in standard cells at scan rate of 100 mV s^{-1} at room temperature were performed within the potential window -2.0 V to $+0.75 \text{ V}$ vs. the reference electrode.

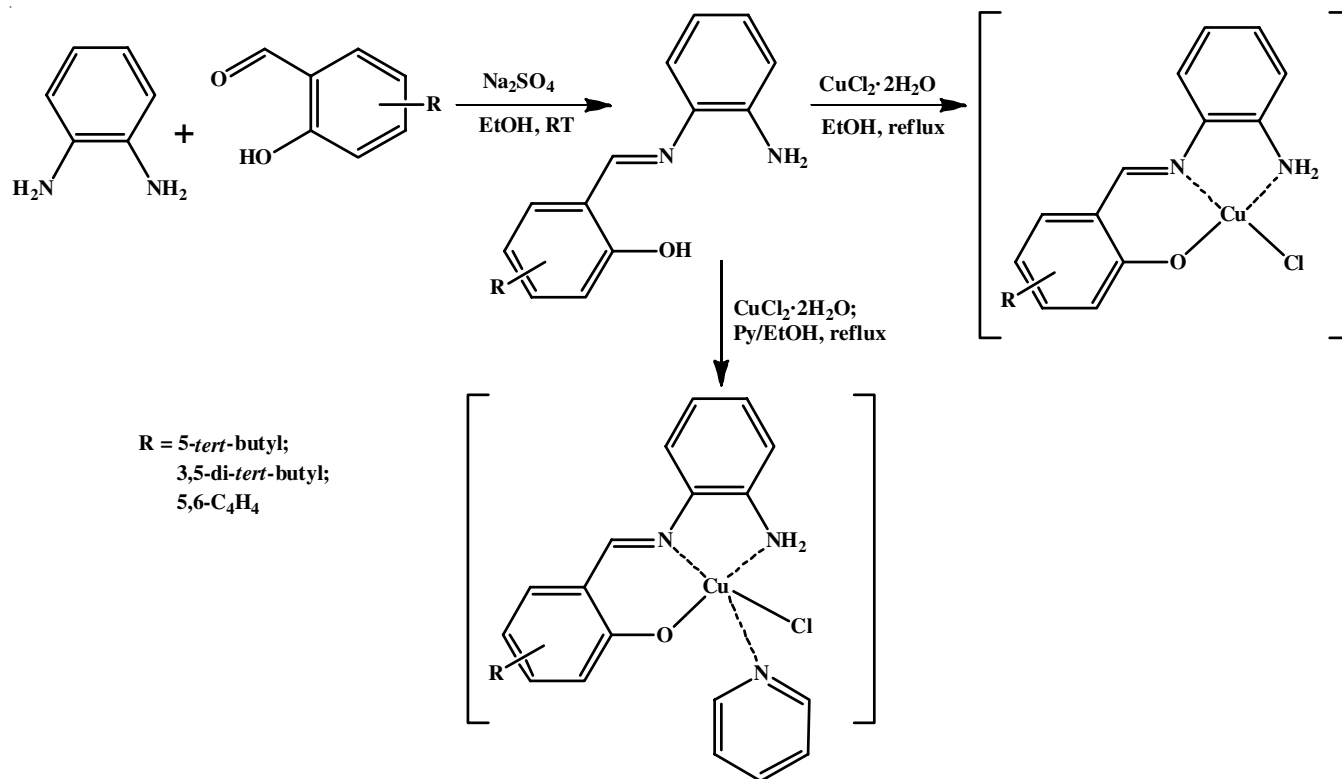
Antibacterial assay: All the obtained Schiff base copper(II) complexes were evaluated for their antibacterial activity against

the Gram-positive bacteria, *Staphylococcus aureus* (ATCC 13709), *Bacillus subtilis* (ATCC 6633), *Lactobacillus fermentum* (N4) using the microdilution method [34,35]. Ampicillin was used as the reference drug. Bacterial strains were suspended in nutrient broth at a concentration of approximately 5×10^5 CFU/mL. The nutrient broths containing microorganisms were transferred by 200 μL into 96 well plates and 10 μL of each solution with the different complexes' concentration of 128.0–4.0 $\mu\text{g/mL}$ were added. All the tests were grown at 37°C in an incubator for 24 h. The minimum inhibitor concentration (MIC, $\mu\text{g/mL}$), at which more than 90% bacteria inhibition was determined, was represented for at least three observations.

Cytotoxicity assay: MTT assay was used to determine cytotoxic activity of the Schiff base Cu(II) complexes against human cancer cell lines, MCF-7 and HepG-2, according to Mosmann's modified method [36]. The % cell inhibition was calculated from the obtained optical densities following the reported formulae [37]. The IC₅₀ values, at which the tested complex concentration has caused 50% cell inhibition, were estimated from the plots performed % cell inhibitions based on tested complex concentrations.

RESULTS AND DISCUSSION

Mono-condensation was carried out at room temperature with 1:1 molecular ratio of *o*-phenyldiamine and relative aldehyde to synthesized one-arm Schiff base ligands (**Scheme-I**) in high yields (85–91%). From these Schiff bases, their Cu(II) complexes were prepared following two ways with and without pyridine to form the green or dark Schiff base Cu(II) complexes (**Scheme-I**) in high yields up to 90% and high purity (> 99% examined by HPLC). The obtained Schiff base copper(II) com-



Scheme-I: Synthesis of Schiff base compounds

plexes are soluble in polar organic solvents such as chloroform, methanol, acetonitrile, DMF, *etc.*

Spectral features: From the recorded mass spectra, pseudo-molecular peaks are found as $[M+H]^+$ for the Schiff base ligands or $[M-Cl]^+$ for the synthesized copper(II) complexes. These mass peaks are well suitable to the molecular mass of the proposed formulae.

On 1H NMR, the synthetic Schiff base ligands, there are typical signals at 15.63-12.79 ppm as single peaks for OH protons, at 9.60-8.62 ppm as single peaks for protons of $CH=N$ bonds which are formed by the mono-condensation, at 8.50-6.63 ppm for aromatic protons and 5.08-3.99 ppm as single peaks for NH_2 protons. The protons of *t*-butyl are appeared at 1.43-1.30 ppm as single peaks reasonably.

From FT-IR spectra of the synthesized Schiff base ligands, the typical signals are observed at $3488-3375\text{ cm}^{-1}$, which can present for stretching vibrations of two N-H bonds. Special signals at $1610-1606\text{ cm}^{-1}$ must be stretching vibrations of $C=N$ bonds, at $1306-1265\text{ cm}^{-1}$ for stretching vibrations of $C-O$ bonds and at $754-752\text{ cm}^{-1}$ for bending vibrations of $C-H$. In IR spectra of the Schiff base copper(II) complexes, the typical bands at $3331-3193$ and $3340-3266\text{ cm}^{-1}$ can exhibit for stretching vibrations of two N-H bonds, which show that the NH_2 of the Schiff base ligands are existed in the synthesized Cu(II) complexes [38,39]. There are typical signals at $1616-1615\text{ cm}^{-1}$ and $1616-1599\text{ cm}^{-1}$ for stretching vibrations of $C=N$ bonds, at $1365-1256$ and $1364-1253\text{ cm}^{-1}$ for stretching vibrations of $C-O$ bonds, at $761-738$ and $745-738\text{ cm}^{-1}$ for bending vibrations of $C-H$. There are new signals at $535-497$ and $465-439\text{ cm}^{-1}$; at $538-497$ and $464-441\text{ cm}^{-1}$ for vibrations of $Cu-N$ and $Cu-O$ bonds, which have proved the coordination of ion Cu^{2+} with atoms N, O in the studied Schiff base ligands of both two kinds of copper(II) complexes, respectively.

In UV-visible spectra of the obtained Schiff bases, there are absorption maxima at 237-262 nm, which can be assigned to $\pi \rightarrow \pi^*$ electron transitions of aromatic rings; at 274-320 nm and 375-440 nm which can be attributed to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electron transitions of azomethine, hydroxyl and amine groups [40] (Fig. 1). In the coordinated complexes, the absorption bands found in Schiff base ligands were moved to the longer wavelength as usual. Electron transitions of $\pi \rightarrow \pi^*$ of aromatic rings were found at 242-275 nm. Electron transitions of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ of azomethine, hydroxy and amine groups were found at 299-337 nm and 425-554 nm, respectively. There is no significant difference on the electronic spectra of tridentate Schiff base copper(II) complexes and mixed-tridentate Schiff base copper(II) complexes (Figs. 2 and 3). The studied Schiff base copper(II) complexes have the effective magnetic moments (μ_{eff}) in the range 1.81-2.15 B.M. consistent with one unpaired electron d^9 system and distorted square-planar or square-pyramidal geometry around Cu(II) centers [41,42].

Electrochemical studies: Electrochemical behaviour of all Schiff base copper(II) complexes was investigated in DMSO using 0.1 M $LiAlO_4$ as supporting electrolyte. The cyclic voltammetry (CV) of 1 mM copper(II) complexes show that the reduction peaks at 1.44-1.94 V due to $Cu^{I/0}$ redox processes and at 1.05-1.47 V for $Cu^{II/I}$ redox processes probably. From the

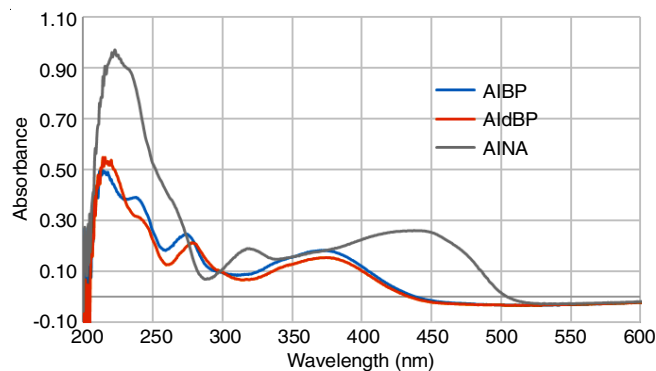


Fig. 1. The electronic spectra of studied unsymmetrical tridentate Schiff base ligands

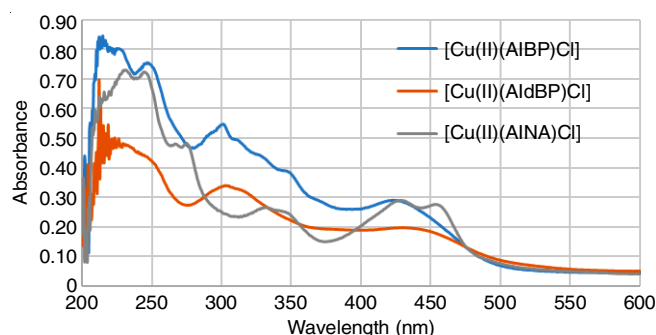


Fig. 2. The electronic spectra of the obtained unsymmetrical tridentate Schiff base copper(II) complexes

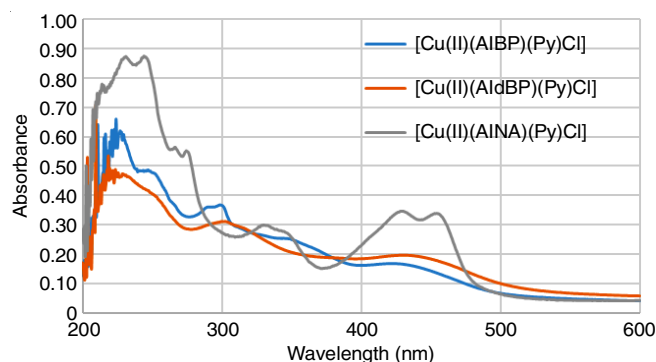


Fig. 3. The electronic spectra of the mixed-tridentate Schiff base copper(II) complexes

CVs of the copper(II) complexes, the reduction potentials and relative currents (mA) are identified and presented in Table-1.

Among the synthesized Schiff base copper(II) complexes, $[Cu(II)(AINA)Cl]$ has expressed favourable reduction potentials at -1.44 and -1.05 V for $Cu^{I/0}$ and $Cu^{II/I}$ redox processes, respectively. Especially, the observed current assigned to $Cu^{II/I}$ redox process is remarkable, -2.05×10^{-2} A. Then the electrochemical property of $[Cu(II)(AINA)Cl]$ was continuously carried out at different scan rates of 100, 200, 300 and 400 $mV\ s^{-1}$ (Fig. 4). The results (Table-1) revealed the relationship between the square root of the scan rates and the relative currents of $[Cu(II)(AINA)Cl]$ is the similar linear dependence. This result shows that $[Cu(II)(AINA)Cl]$ can electrocatalyze hydrogen evolution [43,44].

Antimicrobial activity: The antibacterial activity of tridentate Schiff base copper(II) complexes was evaluated by

TABLE-1 REDUCTION POTENTIAL (V) AND CURRENT (mA)			
Sample	Scan rate (mV s ⁻¹)	Reduction potential (V)	Current (mA)
Blank	100	-0.725	-1.98 × 10 ⁻²
[Cu(II)(AIBP)Cl]	100	-1.47 (Cu ^{II/0})	-1.58 × 10 ⁻²
		-1.12 (Cu ^{III/I})	-1.39 × 10 ⁻²
		-0.713	-1.67 × 10 ⁻²
[Cu(II)(AIdBP)Cl]	100	-1.79 (Cu ^{II/0})	-1.45 × 10 ⁻²
		-1.25 (Cu ^{III/I})	-1.18 × 10 ⁻²
		-0.742	-1.18 × 10 ⁻²
[Cu(II)(AINA)Cl]	100	-1.44 (Cu ^{II/0})	-1.84 × 10 ⁻²
		-1.05 (Cu ^{III/I})	-2.05 × 10 ⁻²
		-0.739	-1.69 × 10 ⁻²
[Cu(II)(AINA)Cl]	200	-1.47 (Cu ^{II/0})	-2.27 × 10 ⁻²
		-1.07 (Cu ^{III/I})	-2.49 × 10 ⁻²
		-0.758	-2.08 × 10 ⁻²
[Cu(II)(AINA)Cl]	300	-1.52 (Cu ^{II/0})	-2.70 × 10 ⁻²
		-1.11 (Cu ^{III/I})	-2.83 × 10 ⁻²
		-0.784	-2.36 × 10 ⁻²
[Cu(II)(AINA)Cl]	400	-1.51 (Cu ^{II/0})	-3.16 × 10 ⁻²
		-1.08 (Cu ^{III/I})	-3.32 × 10 ⁻²
		0.769	-2.65 × 10 ⁻²
[Cu(II)(AIBP)(Py)Cl]	100	-1.45 (Cu ^{II/0})	-1.51 × 10 ⁻²
		-1.13 (Cu ^{III/I})	-1.39 × 10 ⁻²
		-0.733	-1.63 × 10 ⁻²
[Cu(II)(AIdBP)(Py)Cl]	100	-1.94 (Cu ^{II/0})	-2.36 × 10 ⁻²
		-1.47 (Cu ^{III/I})	-1.60 × 10 ⁻²
		-0.753	-1.74 × 10 ⁻²
[Cu(II)(AINA)(Py)Cl]	100	-1.76 (Cu ^{II/0})	-1.42 × 10 ⁻²
		-1.32 (Cu ^{III/I})	-1.21 × 10 ⁻²
		-0.745	-1.18 × 10 ⁻²

minimum inhibitory concentration (MIC) against some Gram-positive strains (*Staphylococcus aureus*, *Bacillus subtilis* and *Lactobacillus fermentum*). Some of the synthesized Schiff base copper(II) complexes like [Cu(II)(AIBP)Cl], [Cu(II)(AIdBP)Cl], [Cu(II)(AINA)Cl] and [Cu(II)(AIBP)(Py)Cl] showed the effective antimicrobial activity to *S. aureus* with MIC 64.0, 4.0; 32.0 and 64.0 µg/mL, respectively (Table-2). [Cu(II)(AIdBP)Cl] has exhibited the good efficiency for all the studied Gram-positive strains, *S. aureus*, *B. subtilis* and *L. fermentum* with MIC = 4.0, 8.0 and 16.0 µg/mL, which can compared to the standard drug ampicillin, with MIC = 0.125, 32.0 and 32.0 µg/mL, respectively. The presence of pyridine as a co-ligand adversely impacts the antibacterial activity, as it tends to enhance the solubility of copper(II) complexes [45,46].

Cytotoxic activity: *In vitro* cytotoxic activity of the synthesized Schiff base copper(II) complexes were evaluated against

TABLE-2 <i>In vitro</i> ANTIMICROBIAL ACTIVITY OF THE SCHIFF BASE COPPER(II) COMPLEXES			
Compound	MIC (µg/mL)		
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>L. fermentum</i>
[Cu(II)(AIBP)Cl]	64.0	> 128.0	> 128.0
[Cu(II)(AIdBP)Cl]	4.0	8.0	16.0
[Cu(II)(AINA)Cl]	32.0	> 128.0	> 128.0
[Cu(II)(AIBP)(Py)Cl]	64.0	> 128.0	> 128.0
[Cu(II)(AIdBP)(Py)Cl]	> 128.0	> 128.0	> 128.0
[Cu(II)(AINA)(Py)Cl]	> 128.0	> 128.0	> 128.0
Ampicillin	0.125 ± 0.0	32.0 ± 0.0	32.0 ± 0.0

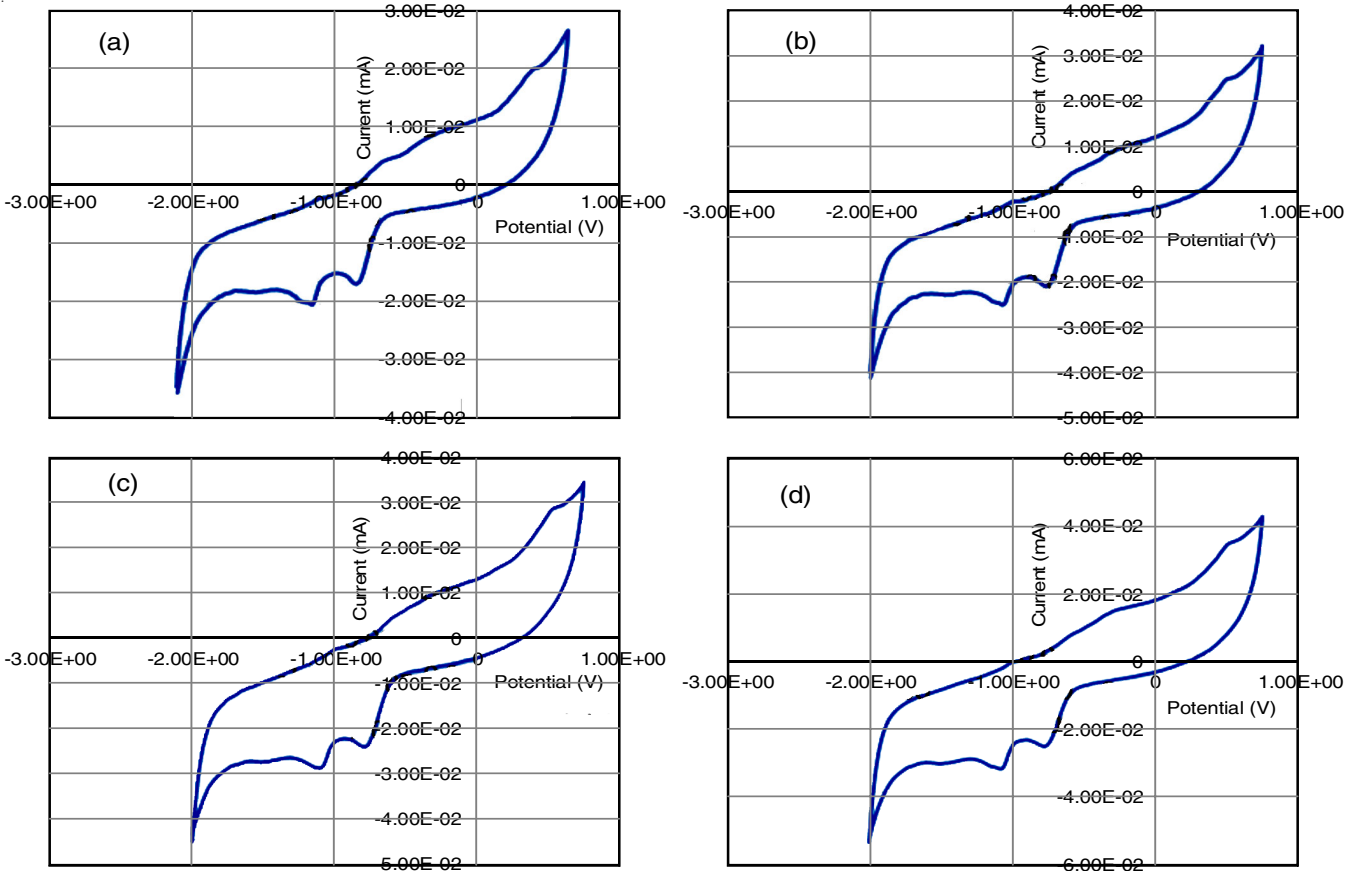


Fig. 4. The cyclic voltammograms of [Cu(II)(AINA)Cl] in DMSO at various scan rates of 100 mV s⁻¹ (a), 200 mV s⁻¹ (b), 300 mV s⁻¹ (c), 400 mV s⁻¹ (d)

human cancer cell lines, breast cancer cells (MCF-7) and hepatocellular carcinoma cell line (HepG-2). The experimental data of IC_{50} at which 50% cells inhibited are presented in Table-3. The obtained results have showed that all copper(II) complexes are good cytotoxic to cancer cells with $IC_{50} < 30.0 \mu\text{g/mL}$ except $[\text{Cu}(\text{II})(\text{AIdBP})(\text{Py})\text{Cl}]$. $[\text{Cu}(\text{II})(\text{AIBP})(\text{Py})\text{Cl}]$, a copper(II) complex containing pyridine as a co-ligand, has performed the good effect to MCF-7 with $IC_{50} = 15.25 \mu\text{g/mL}$, which is better than cisplatin with $IC_{50} = 18.47 \mu\text{g/mL}$ while $[\text{Cu}(\text{II})(\text{AInA})\text{Cl}]$ without pyridine possess the best $IC_{50} = 7.47 \mu\text{g/mL}$ for HepG-2, which are quite close to the cytotoxicity of the reference cisplatin with $IC_{50} = 5.65 \mu\text{g/mL}$. The steric structure and electronic nature of ligands and co-ligands can affect the lipophilicity and the geometry around Cu^{2+} are usually key reasons, which induce to the effective interactions of synthetic copper(II) complexes with the studied human cancer cell lines [47].

TABLE-3
In vitro CYTOTOXICITY OF THE STUDIED
SCHIFF BASE COPPER(II) COMPLEXES

Compound	IC_{50} ($\mu\text{g/mL}$)	
	MCF-7	HepG-2
$[\text{Cu}(\text{II})(\text{AIBP})\text{Cl}]$	19.14 ± 1.06	8.51 ± 0.61
$[\text{Cu}(\text{II})(\text{AIdBP})\text{Cl}]$	17.41 ± 0.98	11.01 ± 0.65
$[\text{Cu}(\text{II})(\text{AInA})\text{Cl}]$	23.27 ± 1.29	7.47 ± 0.42
$[\text{Cu}(\text{II})(\text{AIBP})(\text{Py})\text{Cl}]$	15.25 ± 0.9	15.75 ± 1.19
$[\text{Cu}(\text{II})(\text{AIdBP})(\text{Py})\text{Cl}]$	99.76 ± 8.41	> 128.0
$[\text{Cu}(\text{II})(\text{AInA})(\text{Py})\text{Cl}]$	22.30 ± 1.23	27.42 ± 1.62
Cisplatin	18.47 ± 0.35	5.65 ± 0.06

Conclusion

Three one arm Schiff bases were synthesized from mono-condensation of *o*-phenyldiamine with the relative salicylaldehydes. From the synthetic unsymmetrical Schiff bases, six Schiff base copper(II) complexes were synthesized and characterized by various physico-chemical properties such as mass, FT-IR, UV-visible spectroscopies and magnetic susceptibility measurements. Data indicated that the coordination of copper(II) with the synthesized tridentate Schiff base ligands at the NNO sites occurs in a distorted square-planar or square-pyramidal geometry around the copper(II) centre. Electrochemical studies was carried out using cyclic voltammetry. The $[\text{Cu}(\text{II})(\text{AInA})\text{Cl}]$ has indicated the observed current assigned to $\text{Cu}^{\text{II/I}}$ redox process is remarkable and the increase of the scan rates has caused the linear increase of the relative currents. The synthesized Schiff base copper(II) complexes were also screened for their *in vitro* antibacterial activity on Gram-positive bacteria, *S. aureus*, *B. subtilis*, *L. fermentum*. $[\text{Cu}(\text{II})(\text{AIdBP})\text{Cl}]$ has possessed the effective activity for all three tested bacteria. The *in vitro* cytotoxicity of the synthetic Schiff base Cu(II) complexes for human cancer cell lines, MCF-7 and HepG-2, were examined as well. The $[\text{Cu}(\text{II})(\text{AInA})\text{Cl}]$ has performed the best activity for HepG-2 with $IC_{50} = 7.47 \mu\text{g/mL}$ while $[\text{Cu}(\text{II})(\text{AIBP})(\text{Py})\text{Cl}]$ has expressed the good effect to MCF-7 with $IC_{50} = 15.25 \mu\text{g/mL}$, which is closely similar to the activity of cisplatin with $IC_{50} = 5.65$ and $18.47 \mu\text{g/mL}$.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

- Nidhi, Siddharam, D.P. Rao, A.K. Gautam, A. Verma and Y. Gautam, *Results Chem.*, **13**, 101941 (2025); <https://doi.org/10.1016/j.rechem.2024.101941>
- K. Sztanke, A. Maziarka, A. Osinka and M. Sztanke, *Bioorg. Med. Chem.*, **21**, 3648 (2013); <https://doi.org/10.1016/j.bmc.2013.04.037>
- A.L. Berhanu, Gaurav, I. Mohiuddin, A.K. Malik, J.S. Aulakh, V. Kumar and K.-H. Kim, *Trends Anal. Chem.*, **116**, 74 (2019); <https://doi.org/10.1016/j.trac.2019.04.025>
- C.M. da Silva, D.L. da Silva, L.V. Modolo, R.B. Alves, M.A. de Resende, C.V.B. Martins and Á. de Fátima, *J. Adv. Res.*, **2**, 1 (2011); <https://doi.org/10.1016/j.jare.2010.05.004>
- P. Mahadevi and S. Sumathi, *Synth. Commun.*, **50**, 2237 (2020); <https://doi.org/10.1080/00397911.2020.1748200>
- A.T. Odularu, *BioMed Res. Int.*, 2023, 1626488 (2023); <https://doi.org/10.1155/2023/1626488>
- J. Lakshmipraba, C. Ebenezer, R.V. Solomon and M. Venkatesan, *Chem. Phys. Impact*, **7**, 100286 (2023); <https://doi.org/10.1016/j.chphi.2023.100286>
- M. Jayendran, P.M.S. Begum, M.R.P. Kurup, *J. Mol. Struct.*, **1206**, 127682 (2020); <https://doi.org/10.1016/j.molstruc.2020.127682>
- M. Kumar, A.K. Singh, A.K. Singh, R.K. Yadav, S. Singh, A.P. Singh and A. Chauhan, *Coord. Chem. Rev.*, **488**, 215176 (2023); <https://doi.org/10.1016/j.ccr.2023.215176>
- H. Louis, G.J. Ogunwale, E.C. Agwamba, T.O. Unimuke, E.F. Ahuekwe and A.S. Adeyinka, *J. Mol. Struct.*, **1279**, 134978 (2023); <https://doi.org/10.1016/j.molstruc.2023.134978>
- W. Schilf, B. Kamieński and K. U•arevic, *J. Mol. Struct.*, **1031**, 211 (2013); <https://doi.org/10.1016/j.molstruc.2012.10.004>
- V.Z. Mota, G.S.G. de Carvalho, F.R.G. Bergamini, A.L.B. Formiga, P.P. Corbi, R. Diniz, M.C.R. Freitas, A.D. da Silva and A. Cuin, *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, **99**, 110 (2012); <https://doi.org/10.1016/j.saa.2012.09.002>
- Z. Hao, J. Zhang, X. Zhang, Z. Ma, Z. Han, J. Lin and G.-L. Lu, *Polyhedron*, **221**, 115869 (2022); <https://doi.org/10.1016/j.poly.2022.115869>
- X. Liu, C. Manzur, N. Novoa, S. Celedón, D. Carrillo and J.-R. Hamon, *Coord. Chem. Rev.*, **357**, 144 (2018); <https://doi.org/10.1016/j.ccr.2017.11.030>
- W.-Y. Lu, H.-W. Ou, C.-N. Lee, J.K. Vandavasi, H.-Y. Chen and C.-C. Lin, *Polymer*, **139**, 1 (2018); <https://doi.org/10.1016/j.polymer.2018.02.002>
- K. Kim, S. Nayab, Y. Cho, H. Jung, H. Yeo, H. Lee and S.-H. Lee, *RSC Adv.*, **12**, 35896 (2022); <https://doi.org/10.1039/D2RA07241F>
- K. Mohanan, R. Aswathy, L.P. Nitha, N.E. Mathews and B.S. Kumari, *J. Rare Earths*, **32**, 379 (2014); [https://doi.org/10.1016/S1002-0721\(14\)60081-8](https://doi.org/10.1016/S1002-0721(14)60081-8)
- S. Kagitkar and D. Sunil, *J. Electron. Mater.*, **50**, 6708 (2021); <https://doi.org/10.1007/s11664-021-09197-9>
- M. Jayendran, S. Ponnann, P.M.S. Begum and M.R.P. Kurup, *Inorg. Chim. Acta*, **495**, 118968 (2019); <https://doi.org/10.1016/j.ica.2019.118968>

20. C.S. Novoa-Ramírez, A. Silva-Becerril, M.M. González-Ballesteros, V. Gomez-Vidal, M. Flores-Álamo, L. Ortiz-Frade, J. Gracia-Mora and L. Ruiz-Azuara, *J. Inorg. Biochem.*, **242**, 112097 (2023); <https://doi.org/10.1016/j.jinorgbio.2022.112097>
21. A. Soroceanu and A. Bargan, *Crystals*, **12**, 1436 (2022); <https://doi.org/10.3390/cryst12101436>
22. L.A. Alfonso-Herrera, S. Rosete-Luna, D. Hernández-Romero, J.M. Rivera-Villanueva, J.L. Olivares-Romero, J.A. Cruz-Navarro, A. Soto-Contreras, A. Arenaza-Corona, D. Morales-Morales and R. Colorado-Peralta, *ChemMedChem*, **17**, e202200367 (2022); <https://doi.org/10.1002/cmdc.202200367>
23. P.K. Mudia, N. Bandopadhyaya, M. Joshib, M. Shitc, S. Pauld, A.R. Choudhuryb and B. Biswas, *Inorg. Chim. Acta*, **505**, 119468 (2020); <https://doi.org/10.1016/j.ica.2020.119468>
24. A.K. Patel, N. Patel, R.N. Patel and R.N. Jadeja, *Heliyon*, **8**, e09373 (2022); <https://doi.org/10.1016/j.heliyon.2022.e09373>
25. M. Pradeep Kumar, D. Ayodhya and Shivraj, *Results Chem.*, **5**, 100845 (2023); <https://doi.org/10.1016/j.rechem.2023.100845>
26. D. Fabra, J. Melones-Herrero, J. Velazquez-Gutierrez, A.I. Matesanz, P.D. Aliseda, S. Figueiras, F. Aguilar-Rico, C. Calés, I. Sánchez-Pérez and A.G. Quiroga, *Eur. J. Med. Chem.*, **280**, 116994 (2024); <https://doi.org/10.1016/j.ejmech.2024.116994>
27. T.J.P. McGivern, S. Afsharpour and C.J. Marmion, *Inorg. Chim. Acta*, **472**, 12 (2018); <https://doi.org/10.1016/j.ica.2017.08.043>
28. M. Naqi Ahamad, K. Iman, M.K. Raza, M. Kumar, A. Ansari, M. Ahmad and M. Shahid, *Bioorg. Chem.*, **95**, 103561 (2020); <https://doi.org/10.1016/j.bioorg.2019.103561>
29. S. Kumar, A. Arora, V.K. Maikhuri, A. Chaudhary, R. Kumar, V.S. Parmar, B.K. Singh and D. Mathur, *RSC Adv.*, **14**, 17102 (2024); <https://doi.org/10.1039/D4RA00590B>
30. P.M. Utsu, T.E. Gber, D.O. Nwosa, A.D. Nwagu, I. Benjamin, I.J. Ikot, E.A. Eno, O.E. Offiong, A.S. Adeyinka and H. Louis, *Polycycl. Aromat. Compd.*, **44**, 1109 (2024); <https://doi.org/10.1080/10406638.2023.2186444>
31. A. Das, T.P. Mohammed and M. Sankaralingam, *Coord. Chem. Rev.*, **506**, 215661 (2024); <https://doi.org/10.1016/j.ccr.2024.215661>
32. M. Kuate, M.A. Conde, E. Ngandung Mainsah, A.G. Paboudam, F.M.M. Tchieno, K.I.Y. Ketchemen, I. Tonle Kenfack and P.T. Ndifon, *J. Chem.*, **2020**, 5238501 (2020); <https://doi.org/10.1155/2020/5238501>
33. S. Meghdadi, M. Amirasr, M. Majedi, M. Bagheri, A. Amiri, S. Abbasi and K. Merciter, *Inorg. Chim. Acta*, **437**, 64 (2015); <https://doi.org/10.1016/j.ica.2015.08.006>
34. A.S. Stella Shalini, M. Amaladasan, N. Prasannabalaji, J. Revathi and G. Muralitharan, *Arab. J. Chem.*, **12**, 1176 (2019); <https://doi.org/10.1016/j.arabjc.2014.11.033>
35. I. Elaaraaj, N. Moukrad, A. Bouymajane, S.E. Raouan, A. Nakkabi, O. Oulidi, F.R. Filai, I.K. Saad, F. Cacciola, N. El Moualij and M. Fahim, *Results Chem.*, **5**, 100787 (2023); <https://doi.org/10.1016/j.rechem.2023.100787>
36. L.M. Balsa, M.C. Ruiz, L. Santa Maria de la Parra, E.J. Baran and I.E. León, *J. Inorg. Biochem.*, **204**, 110975 (2020); <https://doi.org/10.1016/j.jinorgbio.2019.110975>
37. Q.T. Nguyen, P.N. Pham Thi and N. Van Tuyen, *J. Chem.*, **2021**, 8028064 (2021); <https://doi.org/10.1155/2021/8028064>
38. M. Das, B.N. Ghosh, A. Valkonen, K. Rissanen and S. Chattopadhyay, *Polyhedron*, **60**, 68 (2013); <https://doi.org/10.1016/j.poly.2013.04.045>
39. L. Ghasemi, M.H. Esfahani, A. Abbasi and M. Behzad, *Polyhedron*, **220**, 115825 (2022); <https://doi.org/10.1016/j.poly.2022.115825>
40. S.I. Sheikhshoae, Y. Ebrahimipour, N. Lot, J.T. Mague and M. Khaleghi, *Inorg. Chim. Acta*, **442**, 151 (2016); <https://doi.org/10.1016/j.ica.2015.11.026>
41. C. Adhikary, S. Banerjee, J. Chakraborty and S. Ianelli, *Polyhedron*, **65**, 48 (2013); <https://doi.org/10.1016/j.poly.2013.08.019>
42. L. Rigamonti, A. Forni, R. Pievo, J. Reedijk and A. Pasini, *Inorg. Chim. Acta*, **387**, 373 (2012); <https://doi.org/10.1016/j.ica.2012.02.030>
43. T.M. Alshammari, A.K.D. Alsukaibi, V. Jevtovic and K.M. Alenezi, *Int. J. Electrochem. Sci.*, **17**, 221112 (2022); <https://doi.org/10.20964/2022.11.33>
44. J.-P. Cao, T. Fang, L.-Z. Fu, L.-L. Zhou and S.-Z. Zhan, *Int. J. Hydrogen Energy*, **39**, 13972 (2014); <https://doi.org/10.1016/j.ijhydene.2014.07.030>
45. Z. Albobaledi, M. Hasanazadeh Esfahani, M. Behzad and A. Abbasi, *Inorg. Chim. Acta*, **499**, 119185 (2020); <https://doi.org/10.1016/j.ica.2019.119185>
46. S. Alimirzaei, M. Behzad, S. Abolmaali and Z. Abbasi, *J. Mol. Struct.*, **1200**, 127148 (2020); <https://doi.org/10.1016/j.molstruc.2019.127148>
47. O. Krasnovskaya, A. Naumov, D. Guk, P. Gorelkin, A. Erofeev, E. Beloglazkina and A. Majouga, *Int. J. Mol. Sci.*, **21**, 3965 (2020); <https://doi.org/10.3390/ijms21113965>