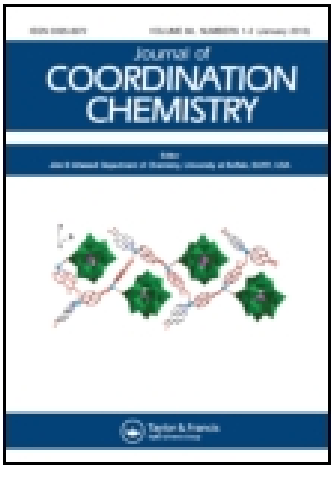

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Synthesis, characterization, equilibrium studies, and biological activity of complexes involving copper(II), 2-aminomethylthiophenyl-4-bromosalicylaldehyde Schiff base, and selected amino acids

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Synthesis, characterization, equilibrium studies, and biological activity of complexes involving copper(II), 2-aminomethylthiophenyl-4-bromosalicylaldehyde Schiff base, and selected amino acids

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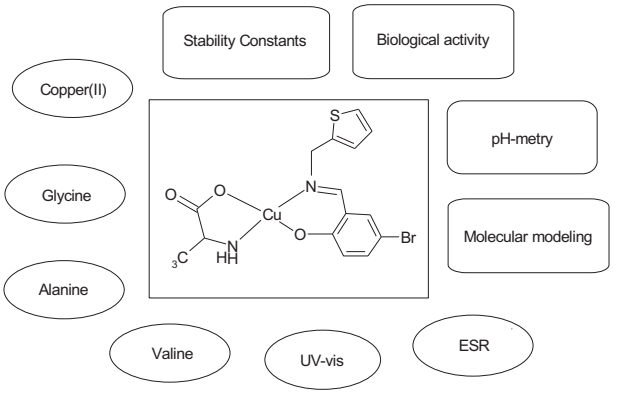

The present paper reports on the synthesis, characterization, and the electronic absorption spectra of Cu(II) ternary complexes involving ATS-Schiff base, and some selected amino acids. The antibacterial, antifungal, and antitumor activities were investigated. The geometry of the studied Cu(II) complexes has been fully optimized using parameterized PM3 semiempirical method. Protonation and complex formation equilibria were investigated.

Ternary complexes of copper(II) with 2-aminomethylthiophenyl-4-bromosalicylaldehyde (ATS) and some amino acids have been isolated and characterized by elemental analyses, IR, magnetic moment, molar conductance, UV–vis, mass spectra, and ESR. The proposed general formulas of the

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prepared complexes are [Cu(ATS)(AA)]·nH_2O (where AA = glycine, alanine, and valine). The low molar conductance values suggest the non-electrolytic nature of the complexes. IR spectra show that ATS is coordinated to copper in a bidentate manner through azomethine-N and phenolic-OH. The amino acids also are monobasic bidentate ligands via amino and ionized carboxylate groups. The magnetic and spectral data indicate the square-planar geometry of Cu(II) complexes. The geometry of the Cu(II) complexes has been fully optimized using parameterized PM3 semiempirical method. The Cu–N bond length is longer than that of Cu–O in the isolated complexes. Also, information is obtained from calculations of molecular parameters for all complexes including net dipole moment of the metal complexes, values of binding energy, and lipophilicity value (log *P*). The antimicrobial activity studies indicate significant inhibitory activity of complex **3** against the selected types of bacteria. The mixed ligand complexes have also been studied in solution state. Protonation constants of ATS and amino acids were determined by potentiometric titration in 50% (v/v) DMSO–water solution at ionic strength of 0.1 M NaCl. ATS has two protonation constants. The binary and ternary complexes of copper(II) involving ATS and some selected amino acids (glycine, alanine, and valine) were examined. Copper(II) forms [Cu(ATS)], [Cu$(ATS)_2$], [Cu(AA)], [Cu$(AA)_2$], and [Cu(ATS)(AA)] complexes. The ternary complexes are formed in a simultaneous mechanism.

Keywords: Schiff base; Copper(II); Amino acid; Electronic spectra; Stability constants; Antibacterial activity

1. Introduction

Copper is a bioessential element and its complexes have potential for treatment of cancers and many other diseases [1]. Schiff bases and their first-row transition metal complexes can exhibit fungicidal, bactericidal, antiviral, and antitumor activities in addition to their important roles in catalysis and organic synthesis [2–5]. Metal complexes of Schiff bases derived from substituted salicylaldehydes and various amines have been investigated because of their wide applicability [6–8], especially metal complexes of Schiff bases with heterocyclic compounds find applications as potential drugs [9]. Thiophene derivatives have antibacterial [10] and antitumor activities [11]. Therefore, synthesis of new Schiff bases and their complexes is a popular theme. Amino acids constitute the building blocks of proteins and are indispensable for performing a huge number of biological functions, as exemplified by the role of enzymes [12]. Interaction between metal ions and amino acids is of considerable interest as models for metal–protein reactions in a variety of biological systems. Reactions of peptides, proteins, and enzymes with metal cations are of biochemical importance, but they are yet to be fully elucidated. The explanation of these phenomena in the biological systems can be possible only by determination of protonation constants of the bioligands as well as their stability constants, which are the measure of their tendency to make possible complexes with metal ions. The elucidation of the various phenomena in the biological systems requires determination of the protonation constants of bioligands and their stability constants with various metal ions in a medium similar to those of biological systems. Most of these determinations have been carried out in aqueous media [13–17] due to the widespread belief that "*in vivo*" media are represented by aqueous media. However, it has been shown that water is not an ideal model for *in vivo* reactions. In enzymes, membranes and other biologically important media, the pK_a values are far different from those in water, because biological media show lipophilic character rather than hydrophilic [18, 19]. It has been suggested that non-aqueous media provide a better model for *in vivo* reactions [20, 21]. Hence, studies in media other than water should provide some understanding of the chemistry of bioligands in living systems. In addition, knowledge obtained from mixed solvents could complement the vast amount of knowledge collected from studies in aqueous media of the chemistry of bioligands like α-amino acids (A.A). There are various

techniques such as potentiometry, conductometry, and spectrophotometry that are used in the determination of protonation and formation constants. In this study, a potentiometric technique was employed because it has the widest area of applicability and reliability [22–24]. The ternary complexes of the Schiff bases and amino acids with metal ions have the importance of both Schiff bases and amino acids. In continuation of our earlier work [25–30], we report here the synthesis, characterization, and biological activity of the ternary system including Cu(II), Schiff base ligand (ATS), and selected amino acids (A.A = Glycine, Alanine, and Valine). The complexes were characterized based on elemental analyses, IR, UV–vis, ESR, magnetic moment, and molar conductance measurements. The isolated metal chelates were screened for their antibacterial, antifungal, and antitumor activities, and the results are reported and discussed.

2. Experimental

2.1. *Materials*

All chemicals used in this investigation were laboratory pure, including $CuCl_2{\cdot}2H_2O$, C_2H_5OH, DMSO, 5-bromosalicylaldehyde, glycine, valine, alanine, and 2-aminomethylthiophene, provided from Aldrich and Sigma chemical companies.

2.2. *Preparation of Schiff base*

The Schiff base used was prepared by mixing an ethanolic solution (20 mL) of 2.01 g (0.01 M) of 5-bromosalicylaldehyde with 1.13 g (0.01 M) of 2-aminomethylthiophene in the same volume of ethanol. The mixture was refluxed with stirring for 1 h. The precipitate was collected by filtration through a Buchner funnel, recrystallized from ethanol, and dried at room temperature with 89% yield.

2.3. *Preparation of the solid complexes*

The 1 : 1 : 1 [Cu:ATS:AA] complexes were prepared from hot ethanolic solutions (90 °C) by the addition of 25 mL of $CuCl_2{\cdot}2H_2O$ (1 mM, 0.170 g) dropwise to 25 mL of ATS (1 mM, 0.295 g) and AA (amino acid) (1 mM, 0.075 g Gly, 0.089 g Ala, 0.117 g Val). An equivalent amount of $NaHCO_3$ was added to neutralize the released protons. The obtained mixture was refluxed with stirring for 3 h and then kept in the refrigerator overnight. Thus, the formed complexes were filtered, collected, and then washed several times with ethanol and then diethyl ether. The solid complexes were dried in a vacuum desiccator. The yield ranged from 70 to 74%. The dried complexes were subjected to elemental and spectroscopic analyses.

2.4. *Pharmacology*

2.4.1. *In vitro* antibacterial and antifungal activities. The antimicrobial bioassay was performed according to protocols described previously using a modified Kirby-Bauer disk diffusion method [31–33]. The antimicrobial activities of metal complexes were studied against Gram (+) bacteria as (*Staphylococcus epidermidis* and *Bacillus cereus*), Gram (−) bacteria as (*Pseudomonas aeruginosa*, *Escherichia coli*), and fungi as *Aspergillus fumigatus*

and *Aspergillus niger*. Standard disks of *Ciprofloxacin* (antibacterial agent) and *Ketoconazole* (antifungal agent) served as positive controls for antimicrobial activity, but filter disks impregnated with 10 µL of solvent (DMSO) were used as a negative control.

2.4.2. *In vitro* cytotoxicity. The synthesized complexes were screened for their cytotoxicity against colon carcinoma (HCT116) and larynx carcinoma (HEP2) cells by using the protocol of SRB assay [34]. Cells were plated in a 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cells to the wall of the plate. Different concentrations of the test chemical were added to the cell monolayer. Triplicate wells were prepared for each individual dose and IC_{50} is the mean of three values. Monolayer cells were incubated for 48 h at 37 °C in air with 5% CO_2. After 48 h, cells were fixed, washed, and stained with Sulfo-Rhodamine-B stain. Excess stain was washed away with acetic acid and attached stain was recovered with tris-EDTA buffer. Color intensity is measured in an ELISA reader. The average drug concentration (µg cm^{-3}) for 50% inhibition of tumor cell growth was determined by plotting the surviving fraction *versus* drug concentration for each tumor cell line.

2.5. *Molecular modeling*

To gain an insight on the molecular structure of the synthesized complexes, geometric optimization and conformation analysis have been performed using semiempirical parameterized PM3 method as implemented in HyperChem 7.5 [35]. A gradient of 1×10^{-2} cal $Å^{-1}$ M^{-1} was set as a convergence criterion in all the molecular mechanics and quantum calculations.

2.6. *Instruments*

Potentiometric measurements were made using a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland-Herisau). A thermostatted glass cell was used equipped with a magnetic stirring system, a Metrohm glass electrode, a thermometric probe, a microburet delivery tube, and a salt bridge connected with the reference cell filled with 0.1 M KCl solution in which saturated calomel electrode was dipped. Temperature was maintained constant inside the cell at 25.0 ± 0.02 °C by the circulating water of a thermostated bath (precision ± 0.02). All potentiometric measurements in this study were carried out in water–DMSO mixtures containing 50% DMSO because of low solubility of Schiff base and possible hydrolysis in aqueous solution. The microchemical analysis of the separated solid chelates for C, H and N were performed at the Microanalytical Center, Cairo University. The analyses were performed twice to check the accuracy of the analyses. Infrared spectra were recorded on an 8001-PC FTIR Shimadzu spectrophotometer using KBr pellets. Solid reflectance spectra were measured on a Shimadzu 3101 pc spectrophotometer. The molar conductance of the complexes was measured for 1.00×10^{-3} M DMSO solutions at 25 ± 1 °C using a Systronics conductivity bridge type 305. The room temperature magnetic susceptibility measurements for the complexes were determined by the Gouy balance using $Hg[Co(SCN)_4]$ as a calibrant. EPR signals were recorded at room temperature by using a Bruker EMX spectrometer (X-band) of Bruker, Germany. The operating conditions are: microwave power = 0.201 mW, modulation amplitude = 4.00 Gauss, modulation frequency = 100 kHz,

sweep width = 200 Gauss, microwave frequency = 9.775 GHz, time constant = 81.92 ms, and sweep time = 20.97 s. The detection limits of the EPR technique depend on the type of sample, sample size, detector sensitivity, and frequency of the incident microwave radiation.

2.7. *Potentiometric titrations*

The potentiometric cell was calibrated before each experiment to convert the pH-meter readings into hydrogen ion concentration as reported [36]. The pH-meter readings recorded in DMSO–water solutions were converted to hydrogen ion concentration [H^+] by using the widely used relation given by Van Uitert and Hass relations [37],

$$-\log_{10}[H^+] = B + \log_{10} U_H \tag{1}$$

where $\log_{10} U_H$ is the correction factor for the solvent composition and ionic strength for which B is read.

The ionic product ($K_w = [H^+][OH^-]$) was calculated at a constant ionic strength of 0.10 M with NaCl in 50% aqueous DMSO solutions based on measurements of [OH^-] and pH [38]. The pK_w value obtained is 15.50 in this medium. Potentiometric titrations were carried out at constant temperature and in an inert atmosphere of nitrogen with CO_2-free standardized 0.05 M NaOH as titrant in a 40.0 mL solution at constant ionic strength 0.10 M (adjusted with NaCl). The proton association constants of the ATS and amino acids were determined potentiometrically by titrating 1.25×10^{-3} M of the ATS or AA solution (40 cm^3). The stability constants of the binary complexes were determined using potentiometric data obtained from (40 cm^3) mixture containing $CuCl_2 \cdot 2H_2O$ (1.25×10^{-3} M) + (ATS or AA) (1.25×10^{-3}; 2.5×10^{-3} M). The hydrolysis constant of Cu^{II} was determined by titrating Cu^{II} (40 cm^3) solution (1.25×10^{-3} M) in 0.10 M NaCl. The stability constants of the ternary complexes were determined using potentiometric data obtained from mixtures (40 cm^3) of Cu^{II} (1.25×10^{-3} M), ATS, and the biorelevant AA solutions at concentration ratios of 1 : 1 : 1, 1 : 1 : 2, and 1 : 2 : 1 at constant ionic strength of 0.10 M NaCl.

Caution! For DMSO solutions only glass equipment and Hamilton Teflon valves can be used!

2.8. *Data processing*

The general four-component equilibrium can be written as follows (charges are omitted for simplicity):

$$l(\mathrm{Cu}) + p(\mathrm{ATS}) + q(\mathrm{AA}) + r(H) \rightleftharpoons (\mathrm{Cu})_l(\mathrm{ATS})_p(\mathrm{AA})_q(H)_r \tag{2}$$

$$\beta_{lpqr} = \frac{[\mathrm{Cu}_l(\mathrm{ATS})_p(\mathrm{AA})_q(H)_r]}{[\mathrm{Cu}]^l[\mathrm{ATS}]^p[\mathrm{AA}]^q[H]^r} \tag{3}$$

The calculations were obtained from ca. 100 data points in each titration using the computer program MINIQUAD-75 [39]. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere [39]. The fitted model was tested by comparing the experimental titration data

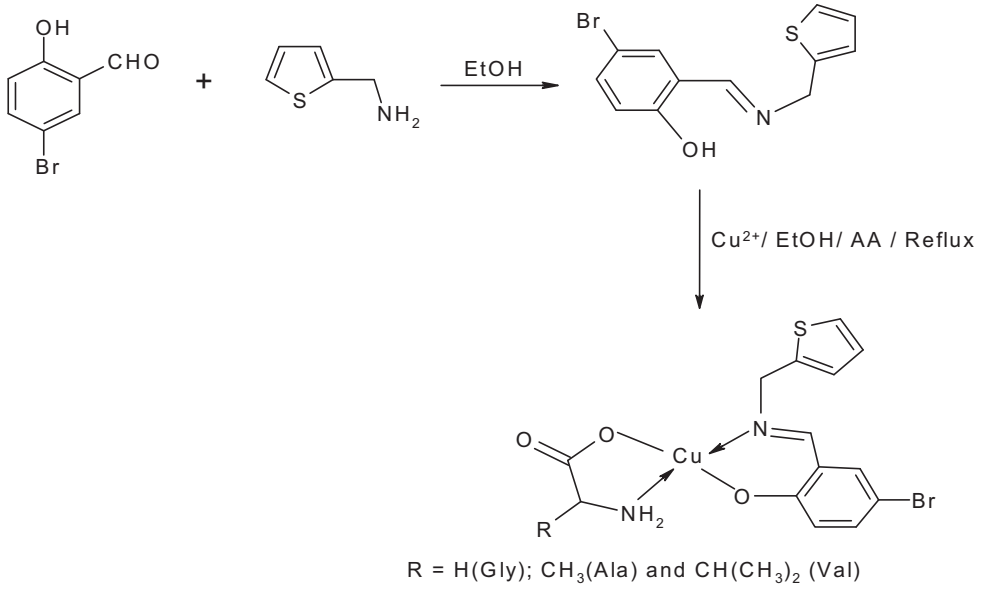
points and the theoretical curve calculated from the values of the acid dissociation constant of the ligand and the formation constants of the corresponding complexes. All equilibrium studies were carried out in our laboratory at Cairo University.

3. Results and discussion

Condensation of 5-bromosalicylaldehyde with 2-aminomethylthiophene in boiling ethanol yields a Schiff base (ATS) as reported [40–42]. Elemental analyses for the interaction of Schiff base and amino acids with copper(II) chloride in EtOH agree well with a 1 : 1 : 1 Cu (II) : ATS : AA stoichiometry for all the complexes. The results of elemental analysis (C, H, N, and S), along with molecular formulas, percent yield and color of the Cu(II) complexes are presented in table 1. The chemical equations concerning the formation of the Schiff base and the complexes are represented in scheme 1. The complexes are air-stable, insoluble in most organic solvents and water, but soluble in DMF and DMSO. The complexes have higher melting points than their corresponding ligands, indicating that they are thermally stable.

Table 1. Analytical and physical data of compounds.

Compound	Mwt	% Yield	Color	% Found (Calcd)			
				C	H	N	S
ATS	295.9	89	Yellow	48.49 (48.66)	3.39 (3.37)	4.80 (4.73)	10.89 (10.81)
[Cu(ATS)(Gly)]·H_2O (**1**)	450.9	70	Green	37.22 (37.27)	3.29 (3.33)	6.19 (6.21)	7.07 (7.09)
[Cu(ATS)(Ala)] (**2**)	446.9	74	Pale green	40.25 (40.32)	3.32 (3.38)	6.21 (6.27)	7.12 (7.15)
[Cu(ATS)(Val)] (**3**)	474.9	72	Green	42.98 (43.00)	4.01 (4.03)	5.87 (5.90)	6.69 (6.73)



Scheme 1. The general proposed structure for Cu-ATS-AA complexes.

3.1. *IR spectra and mode of bonding*

The results of IR measurements are listed in table 2 with assignments for most of the major peaks. The IR spectrum of the free ATS reveals a band at 3458 cm^{-1} due to υ(OH). This band is absent in the spectra of Cu(II) complexes, indicating coordination through the deprotonated phenolic OH [43, 44]. Also, it was evidenced from the shift in the position of υ(C–O) at 1319 cm^{-1} [45] to the lower frequency region in the spectra of complexes [46]. However, the strong band at 1675 cm^{-1} in free ATS due to azomethine group vibration is shifted to lower frequencies in the complexes (51–58 cm^{-1}), suggesting that azomethine is involved in coordination [47–49]. In free ATS, the sharp band at 825 cm^{-1} due to υ(C–S–C) stretch of the thiophene ring remained unchanged in all complexes confirming the non-involvement of the thiophene sulfur in complex formation [50]. The appearance of new bands at 506–510 and 431–434 cm^{-1} due to υ(Cu–O) and υ(Cu–N) vibrations [51], respectively, support the participation of nitrogen of the azomethine and oxygen of the OH in complexation with Cu(II) [51], as shown in scheme 1. The amino acid coordinates as bidentate, ligands and bound to copper through the carboxylate and amino groups. The δ^{+}_{NH3} band, which is the characteristic of a zwitter ion, disappears in the spectra of complexes, indicating that NH_2 must be involved in coordination. This is supported by the appearance of stretching vibrations of amino group as split bands at 3444–3464 and 3255–3215 cm^{-1} [52] in addition to δ_{NH2} (in plane deformation) at 1460–1463 cm^{-1}, assigned for the coordinated amino [52, 53]. One particularly interesting aspect to be analyzed in complexes of this type is the displacement of the characteristic carboxylic bands after coordination. This peculiar aspect has been summarized in table 2. The "free" amino acid exists as a zwitter ion in the crystalline state; thus, one expects two stretching vibrations for COO^{-} in these systems ($\upsilon_s(COO^{-})$ and $\upsilon_{as}(COO^{-})$). The symmetric stretch is usually of medium intensity in the IR spectrum, whereas the asymmetric one is strong and broad. Bands at 1594–1608 and 1410–1413 cm^{-1} due to $\upsilon_{asym}(COO^{-})$ and $\upsilon_{sym}(COO^{-})$ of the amino acids appear in the complexes at 1588–1582 and 1375–1381 cm^{-1}. Deacon showed that the magnitude of $\Delta\upsilon$ [$\Delta\upsilon = \upsilon_{asy}(COO^{-}) - \upsilon_{sym}(COO^{-})$] can be correlated with the coordination modes of carboxylate [54, 55]. This difference is >200 cm^{-1}, reflecting monodentate coordination of carboxylate of the amino acid in the complexes. The shift of these two bands suggests the involvement of carboxylate of the amino acids in complex formation. Hence, the amino acid moiety is chelated to copper forming a five-membered chelate ring. The OH stretch in the spectra of [Cu(ATS)(Gly)] at 3510 cm^{-1} is attributed to the presence of water of hydration. According to Stefov *et al.* [55], coordinated water should exhibit frequencies

Table 2. Tentative assignment of the important infrared bands of the synthesized complexes.

Ligand/ complex	υ (cm^{-1})										
	υ_{OH}	$\upsilon_{(C=N)}$	δ_{NH3+} (Free AA)	υ_{asym} (COO)	υ_{sym} (COO)	δ_{NH2} (Coord. AA)	υ_{NH2} (Coord. AA)	$\upsilon_{(C-O)}$ phenolic	$\upsilon_{(C-S)}$	υ_{Cu-N}	υ_{Cu-O}
ATS	3361–3543	1675	–	–	–			1319	825	–	–
Gly	–	–	1585	1608	1412	–	–	–	–	–	–
Ala	–	–	1623	1605	1413	–	–	–	–	–	–
Val	–	–	1622	1594	1410	–	–	–	–	–	–
[Cu(ATS)(Gly)].H_2O	3510	1617	–	1588	1377	1463	3464; 3255	1182	826	432	506
[Cu(ATS)(Ala)]	–	1617	–	1585	1381	1462	3454; 3215	1181	827	431	510
[Cu(ATS)(Val)]	–	1618	–	1582	1375	1460	3444; 3205	1185	826	434	508

Note: ATS = 2-aminomethylthiophenyl-4-bromosalicylaldehyde; Gly = glycine; Ala = alanine; and Val = valine.

Table 3. Molar conductance, magnetic moment, electronic spectra, and electron spin resonance data of [Cu-ATS-AA] complexes.

Complex	Λ_M[a]	$M_{eff.}$ (B.M.)	λ_{max} (cm^{-1})			Electron spin resonance data			
			$n\rightarrow\pi^*$	$\pi\rightarrow\pi^*$	d–d transition	$g_{\parallel}$	$g_{\perp}$	g_{avg}	G
ATS	–	–	28409	40816	–				
[Cu(ATS)(Gly)]·H_2O	11.2	1.75	29499	41240	16233	2.252	2.059	2.123	4.410
[Cu(ATS)(Ala)]	10.5	1.78	35816	43651	15873	2.285	2.071	2.142	4.114
[Cu(ATS)(Val)]	8.7	1.81	37125	45523	15625	2.277	2.067	2.137	4.245

[a]Molar conductance measured for 10^{-3} M DMSO solution, $\Omega^{-1}\,cm^2\,M^{-1}$; AA = amino acid, and ATS = 2-aminomethylthiophenyl-4-bromosalicylaldehyde.

at 825, 575, and 500 cm^{-1}. The absence of these spectral bands in the spectra of [Cu(ATS)(Gly)] indicates that water is not coordinated but present as lattice water.

As a general conclusion, the Schiff base participated in bonding to copper as a monobasic bidentate (N, O donor) ligand and the amino acids also as monobasic bidentate ligands with a total of four coordination sites around Cu(II) whose charge is neutralized by the deprotonation of the Schiff base phenolic OH and the amino acid COOH (scheme 1). The non-electrolytic nature of the complexes was evidenced from the low values of the molar conductance of the complexes measured in DMSO [56] (table 3).

3.2. *Electronic spectra and magnetic properties*

Unable to obtain a single crystal for X-ray analyses to confirm the structure for these complexes, solid reflectance spectra and magnetic moment measurements are used. The complexes show bands at 41,240–45,523 and 29,499–37,125 cm^{-1}, which are assigned to the intraligand transitions [57]. In general, due to Jahn–Teller distortion, square-planar Cu(II) complexes give a broad absorption between 16,666 and 14,286 cm^{-1} (600–700 nm). Electronic spectra of the [Cu(ATS)(AA)] complexes show d–d absorptions centered at 16,233–15,625 cm^{-1} (616–640 nm), due to $^2B_{1g}\rightarrow{}^2A_{1g}$, as reported for square-planar Cu(II) complexes [58]. The magnetic moments of the complexes were measured at room temperature, and are listed in table 3. The room temperature magnetic moments for Cu(II) complexes (μ_{eff} = 1.75–1.81 BM) fall in the normal range for copper(II) species with $S = 1/2$, and this confirms that the copper(II) complex has square-planar geometry [59, 60] with $d_{x^2-y^2}$ ground state [61].

3.3. *Conductivity measurements*

The chelates were dissolved in DMSO and the molar conductivities of 10^{-3} M of their solutions at 25 ± 1 °C were measured. As seen from table 3, the molar conductivity values for M(II) chelates are 8.7–11.2 $\Omega^{-1}\,cm^2\,M^{-1}$, indicating the non-electrolytic nature of the complexes.

3.4. *Mass spectra*

Appearance of final peak at *m/z* 295 ($C_{12}H_{10}NOSBr$, calculated atomic mass 295 amu) confirms the proposed formula of ATS-Schiff base and other peaks at 200, 184, 171, 156, 143, and 63 amu may be due to fragmentation of ATS as a result of the rupture of different bonds inside the molecule [62].

The mass spectra of [Cu(ATS)(Gly)]·H_2O, [Cu(ATS)(Ala)] and [Cu(ATS)(Val)] are reported and their molecular ion peaks are in agreement with their assigned formulas. The mass spectrum of [Cu(ATS)(Gly)]·H_2O showed a molecular ion peak (M^+) at *m/z* 451 that is equivalent to its molecular weight $CuC_{14}H_{13}N_2SO_3Br·H_2O$, suggesting a monomer. Thus, the mass spectral data along with elemental analyses agree with the formation of [Cu(ATS)(Gly)]·H_2O of 1 : 1 : 1 stoichiometry. Additionally, the mass spectrum of [Cu(ATS)(Gly)]·H_2O also showed fragmentation patterns corresponding to the successive degradation of the complex. The peaks at 433 (Calcd 432.7) and 359 (Calcd 358.7) correspond to $[Cu(ATS)(Gly)]^+$ and $[Cu(ATS)]^+$, respectively. The mass spectra of [Cu(ATS)(Ala)] and [Cu(ATS)(Val)] showed signals at *m/z* 447 (Calcd 446.9) and 475 (Calcd 474.9). The fragmentation pattern is similar to that of [Cu(ATS)(Gly)] in stepwise ligand loss.

3.5. *Electron spin resonance*

Electronic paramagnetic resonance spectra of Cu complexes were recorded in the solid state at room temperature to obtain further information about their stereochemistry. The "g" tensor values of the copper(II) complexes can be used to derive the ground state [63, 64]. In axially elongated octahedral and square-planar complexes, the unpaired electron occupies the d_{x2-y2} orbital with $^2B_{1g}$ ground state resulting in $g_{\|} > g_{\perp} > 2$, while the unpaired electron lies in the d_{z2} orbital giving $^2A_{1g}$ as the ground state with $g_{\perp} > g_{\|} > 2$. The $g_{\|}$ and $g_{\perp}$ values computed from the ESR spectrum using DPPH free radical are listed in table 3. The observed "*g*" values of [Cu(ATS)(Gly)] ($g_{\|} = 2.252$, $g_{\perp} = 2.059$, $g_{avg} = 2.123$), [Cu(ATS)(Ala)] ($g_{\|} = 2.285$, $g_{\perp} = 2.071$, $g_{avg} = 2.142$) and [Cu(ATS)(Val)] ($g_{\|} = 2.272$, $g_{\perp} = 2.067$, $g_{avg} = 2.137$) suggest that the unpaired electron lies predominantly in the d_{x2-y2} orbital, indicating square-planar geometry around copper(II) [65]. Kivelson and Neiman [66] have shown that $g_{\|}$ is a moderately sensitive function for indicating covalency. Relatively speaking $g_{\|} > 2.3$ is characteristic of ionic bonding and $g_{\|} < 2.3$ of a covalent environment in M−L bonding. By applying this criterion, the complexes under study have mainly covalent Cu–ligand bonding. In addition the exchange coupling interaction between two copper centers in the solid state given by Hathaway expression, $G = g_{\|} - 2.0023/g_{\perp} - 2.0023$ [67, 68], led to the value of 4.114–4.403. According to Hathaway, if the value of *G* is greater than four, the exchange interaction is negligible, whereas when the value of *G* is less than four a considerable interaction is indicated in solid complexes. Accordingly, the exchange interactions between Cu(II) ions in the solid state are negligible. No signal at half field was observed in the spectrum, ruling out the possibility of a dimer [69]. Based on elemental analysis, IR, electronic spectral data and ESR data, the copper(II) complexes have square-planar geometry.

3.6. *Structure of the complexes*

It is concluded from elemental analysis, IR, conductance, electronic spectra, magnetic measurements and ESR, the ATS-Schiff base behaves as a monobasic bidentate ligand coordinated to Cu(II) through hydroxo and azomethine-N while the amino acid is a neutral bidentate ligand coordinated through the amino and carboxylate groups. On the basis of the elemental analysis and spectral data square-planar geometry is suggested for the complexes.

Table 4. Complex formation equilibria of [Cu(II)-ATS-AA] in 50% (v/v) DMSO–water mixture and 0.10 M ionic strength using NaCl.

System	log β [a]	S[b]	log K_1	log K_2	log K_1 − log K_2
Schiff base (ATS)					
(1) $ATS^- + H^+ \rightleftharpoons HATS$	9.99 ± (0.02)	2.1E−7			
(2) $ATS^- + 2H^+ \rightleftharpoons H_2ATS$	17.50 ± (0.04)				
Cu-ATS					
(3) $Cu^{2+} + ATS^- \rightleftharpoons [Cu(ATS)]^+$	9.32 ± (0.05)	4.3E−8	9.32	7.79	1.53
(4) $Cu^{2+} + 2ATS^- \rightleftharpoons [Cu(ATS)_2]$	17.11 ± (0.06)				
Cu-ATS-Gly					
(1) $Gly^- + H^+ \rightleftharpoons HGly$	9.91 ± (0.01)	5.7E−8			
(2) $Gly^- + 2H^+ \rightleftharpoons H_2Gly^+$	14.13 ± (0.02)				
(3) $Cu^{2+} + Gly^- \rightleftharpoons [CuGly]^+$	8.12 ± (0.01)	4.9E−8	8.12	7.02	1.20
(4) $Cu^{2+} + 2\ Gly^- \rightleftharpoons [Cu(Gly)_2]$	15.24 ± (0.03)				
(5) $Cu^{2+} + ATS^- + Gly^- \rightleftharpoons [Cu(ATS)(Gly)]$	17.49 ± (0.09)	3.1E−7			
Cu-ATS-Ala					
(1) $Ala^- + H^+ \rightleftharpoons HAla$	10.01 ± (0.01)	2.2E−8			
(2) $Ala^- + 2H^+ \rightleftharpoons H_2Ala^+$	14.21 ± (0.02)				
(3) $Cu^{2+} + Ala^- \rightleftharpoons [CuAla]^+$	8.27 ± (0.02)	5.7E−8	8.27	7.03	1.24
(4) $Cu^{2+} + 2Ala^- \rightleftharpoons [Cu(Ala)_2]$	15.36 ± (0.05)				
(5) $Cu^{2+} + ATS^- + Ala^- \rightleftharpoons [Cu(ATS)(Ala)]$	17.69 ± (0.07)	3.8E−7			
Cu-ATS-Val					
(1) $Val^- + H^+ \rightleftharpoons HVal$	9.89 ± (0.02)	1.9E−8			
(2) $Val^- + 2H^+ \rightleftharpoons H_2Val^+$	13.86 ± (0.03)				
(3) $Cu^{2+} + Val^- \rightleftharpoons [CuVal]^+$	8.34 ± (0.02)	6.4E−8	8.34	7.09	1.25
(4) $Cu^{2+} + 2Val^- \rightleftharpoons [Cu(Val)_2]$	15.41 ± (0.05)				
(5) $Cu^{2+} + ATS^- + Val^- \rightleftharpoons [Cu(ATS)(Val)]$	17.86 ± (0.06)	4.5E−7			

[a]Standard deviations are given in parentheses.
[b]Sum of square of residuals.

3.7. *Equilibrium studies*

Complex formation equilibria of Cu(II)-ATS-AA complexes cannot be carried out in aqueous solution because the complexes as well as the ligands are insoluble in water. The DMSO–water mixture of 50% : 50% ratio was chosen for our study. In such a medium, the Schiff base and its metal complexes give stable solutions. The use of this mixed solvent has some advantages over pure DMSO. Pure DMSO is very hygroscopic and controlling its water content is difficult [70], affecting reproducibility. However, DMSO–water 50% : 50% has only small hygroscopic character. A further advantage is compatibility with the standard glass electrode, so that pH measurements may be carried out in a similar way to that employed in a purely aqueous solution; use of pure DMSO is not recommended for potentiometry. Another advantage of the DMSO–water mixture is its large acidity range ($pK_w = 15.50$) which allows the investigation of deprotonation equilibria of weak acids, which are difficult to study in water [71, 72]. The stoichiometric protonation constants of ATS and amino acids were determined in 50% DMSO–water mixture at 25 °C, and are tabulated in table 4.

3.7.1. Protonation constants of ligands. Protonation constants of the amino acids investigated have been determined mostly in aqueous media and rarely in organic solvent–water mixtures [73–75]. The protonation constants of ATS and amino acids were re-determined under the same experimental conditions of ionic strength and temperature used to study the binary and the ternary complexes, and the results and comparisons are given in table 4. Analysis of the potentiometric titration curve using Miniquad-75 gave best fit for two

protonation constants of the investigated ligands (ATS, glycine, alanine, and valine). The $\log_{10} K_1$ value is related to the attachment of H^+ to the phenolic oxygen in ATS, and $\log_{10} K_2$ corresponds to the attachment of a proton to the imino group in ATS. For amino acids, the $\log_{10} K_1$ values are related to protonation of the α-amino group in amino acids and $\log_{10} K_2$ corresponds to protonation of α-carboxylate.

3.7.2. Metal–ligand binary systems. Analysis of potentiometric titration curves in the presence of metal ions indicates that addition of metal ion shifted the buffer region of the ligand to lower pH. The observed decrease in the binary ML curve in comparison to the free ligand curve indicates the formation of binary complexes in solution. The formation constants of all binary complexes with ATS and amino acids were computed, taking into account possible species (H_2L, HL, L, M(II), ML, and ML_2). Table 4 presents the logarithms of the stability constants for all of the complex species detected by potentiometric titrations.

3.7.3. Metal–ligand ternary systems. Depending on the chelating ability of ATS and the amino acids, ternary complex formation proceeds through either a stepwise or simultaneous mechanism. The formation constants of the binary Cu(II)-AA and Cu(II)-ATS complexes are given in table 4. The formation constants of the binary Cu(II) complexes with ATS and the selected amino acids were of the same order. Consequently, ligation of ATS and the mentioned amino acids occur simultaneously according to equilibrium (4), charges are omitted for simplicity. The coincidence between the experimental potentiometric data and the computer simulation support complex formation.

$$\mathrm{Cu} + \mathrm{ATS} + \mathrm{AA} \rightleftharpoons [\mathrm{Cu(ATS)(AA)}] \tag{4}$$

The stability constants for equation (4) of mixed-ligand complexes giving the best fit of the pH-metric titration curves are listed in table 4. The overall formation constants expressed as $\log \beta_{1110}$ were calculated considering the acid dissociation constants of the ligands and formation constants of the binary complexes as known quantities. In general, stability constants of the Cu(II) complexes obey the order ($\log K_{[\mathrm{Cu(ATS)(Val)}]} = 17.86 > \log K_{[\mathrm{Cu(ATS)(Ala)}]} = 17.69 > \log K_{[\mathrm{Cu(ATS)(Gly)}]} = 17.49$).

3.7.4. Comparison of the stability constant of the ternary complexes with those of the binary complexes. The parameters generally used for indicating stabilization of the mixed complexes with respect to the binary ones are discussed in the following sections.

3.7.4.1. *Δ log K parameter.* $\Delta \log K$ represents the difference between the stabilities of the binary and mixed ligand complexes. One expects to obtain negative values for $\Delta \log_{10} K$ (table 5), since more coordination positions are available for the bonding of ligand (L) in

Table 5. Evaluated values of $\log \beta$; $\Delta \log K$; $\Delta \log \beta$, and $\log X$ for the formation of the ternary complexes [Cu(ATS)(AA)] at 25 °C and 0.1 M NaCl ionic strength.

Complex	$\log \beta_{1110}$	$\log \beta_{\mathrm{Stat.}}$	$\Delta \log \beta$	$\Delta \log K$	$\log X$
$[\mathrm{Cu(ATS)(Gly)}]\cdot H_2O$	17.49	16.48	1.01	0.05	2.63
[Cu(ATS)(Ala)]	17.69	16.54	1.18	0.10	2.91
[Cu(ATS)(Val)]	17.86	16.56	1.30	0.20	3.20

the binary than in the ternary complexes. According to Sigel [76], the relative stability of a ternary complex [Cu(ATS)AA] (1110) compared to its binary complexes Cu(ATS) (1100) and Cu(AA) (1010) can be expressed quantitatively by following equations.

$$\mathrm{Cu(ATS)} + \mathrm{Cu(AA)} \rightleftharpoons [\mathrm{Cu(ATS)(AA)}] + \mathrm{Cu} \tag{5}$$

$$\Delta \log K_{[\mathrm{Cu(ATS)(AA)}]} = \log \beta_{[\mathrm{Cu(ATS)(AA)}]} - (\log \beta_{\mathrm{Cu(ATS)}} - \log \beta_{\mathrm{Cu(AA)}}) \tag{6}$$

The theoretical Δ log K value should be negative and have a value between −0.5 and −2.0 [77], depending on the geometry of the complex. The $\Delta \log_{10} K$ values for ternary complex of valine ($\Delta \log_{10} K = 0.2$) is more positive than that of alanine ($\Delta \log_{10} K = 0.1$), because hydrophobic intramolecular interactions between the hydrophobic moieties of ATS and the valine amino acid are more than alanine. Positive values of $\Delta \log_{10} K$ may be considered as evidence for enhanced stabilities involving π back-donation from the negatively charged amino acid to the π-system of ATS. A similar behavior was also exhibited by complexes of 1,2,4-triazoles and some α-amino acids [78].

3.7.4.2. *Disproportionation constant (log X)*. The "disproportionation" constant, log X (equations (7) and (8)) [77], can be used besides $\Delta \log_{10} K$ to quantify the stability of ternary complexes. The values of log X for [Cu(ATS)AA] complexes are defined by equations (7) and (8)] (table 5).

$$\mathrm{Cu(ATS)_2} + \mathrm{Cu(AA)_2} \rightleftharpoons 2\mathrm{Cu(ATS)(AA)}; X_{[\mathrm{Cu(ATS)(AA)}]} = \frac{[\mathrm{Cu(ATS)(AA)}]^2}{[\mathrm{Cu(ATS)_2}][\mathrm{Cu(AA)_2}]} \tag{7}$$

$$\log X_{[\mathrm{Cu(ATS)(AA)}]} = 2 \log \beta_{[\mathrm{Cu(ATS)(AA)}]} - (\log \beta_{\mathrm{Cu(ATS)_2}} + \log \beta_{\mathrm{Cu(AA)_2}}) \tag{8}$$

The expected value for log X is +0.6 [79] for all geometries. The values of log X in table 5 are >>0.6, indicating marked stabilities of the ternary complexes. This was attributed to π back-donation from the metal ion to the aromatic moiety [80] in addition to the hydrophobic interaction between the moieties of ATS and amino acids. The same finding was obtained for the ternary complexes formed by Cu(pyrocatecholate) with 2-picolylamine, bipyridine, and 2-aminomethylbenzimidazole [81].

3.7.4.3. *Δ log β parameter.* The stability of the ternary complexes can also be calculated using a statistical method [82] according to the following equation:

$$\log_{10} \beta_{\mathrm{stat}} = \log_{10} 2 + 1/2 \log_{10} \beta_{1020} + 1/2 \log_{10} \beta_{1200} \tag{9}$$

The values of $\log_{10} \beta_{\mathrm{stat}}$ for the mixed ligand complexes are shown in table 5. The large differences of $\log_{10} \beta$ values ($\log_{10} \beta_{\mathrm{exp.}} - \log_{10} \beta_{\mathrm{stat}}$) indicate that the [Cu(ATS)AA] system is more stable than both $\mathrm{Cu(ATS)_2}$ and $\mathrm{Cu(AA)_2}$ complexes.

3.8. *Biological activity*

3.8.1. Antibacterial activity. To assess the biological potential of the synthesized compounds, the complexes were tested against different species of bacteria. The organisms used in the present investigations included two Gram-positive (*S. epidermidis and B. cereus*) and two-Gram negative (*E. coli, P. aeruginosa*) bacteria. The disk diffusion method was used to evaluate the antibacterial activity of the complexes [39]. The medium used for growing the culture was nutrient agar. The results of the antimicrobial activities of the synthesized compounds are recorded in table 6. The biological activity may arise from hydroxyl groups, which may play an important role in the antibacterial activity [83]. The mode of action of the compounds may involve the formation of a hydrogen bond through azomethine (>C=N–) with the active centers of various cellular constituents, resulting in interference with normal cellular processes [84]. It has been suggested that ligands with nitrogen and oxygen donors inhibit enzyme activity, since the enzymes appear to be more susceptible to deactivation by metal ions on coordination [85, 86]. The antibacterial activity can be ordered as [Cu(ATS)(Val)] > [Cu(ATS)(Ala)] > [Cu(ATS)(Gly)] (figure 1), suggesting that lipophilic behavior increases in the same order. This result is in accord with the stabilities of these complexes. The lipophilicity of complexes increases due to delocalization of π-electrons in the chelate ring with an increase in the stability constants $\log K_{[Cu(ATS)(Val)]} = 17.86 > \log K_{[Cu(ATS)(Ala)]} = 17.69 > \log K_{[Cu(ATS)(Gly)]} = 17.49$ [87, 88]. Similar increased antibacterial activity in accordance with stability constant values was also exhibited by copper(II) complexes of isoxazole Schiff base [89]. Cu(II) complexes of Gly and Ala are inactive towards *E. coli* while Cu(II) complex of Val is active with inhibition zone of 11.2 mm.

3.8.2. Antifungal activity. Antifungal activities of the synthesized complexes were tested against *A. niger* ATCC 9029 and *A. fumigatus* ATCC 46645 *in vitro* using the disk diffusion method. Ketoconazole was used as a standard drug. The susceptibility of the selected strains of fungi towards the complexes was judged by measuring the diameter of the formed zone of inhibition. The results are given in table 6. Metal ions are essential for growth inhibitory effects [90]. [Cu(ATS)(Gly)] was less effective against the selected fungal strains and [Cu(ATS)(Val)] is the most active one among the synthesized Cu(II) complexes. Thus, the antifungal activity can be ordered as [Cu(ATS)(Val)] > [Cu(ATS)(Ala)] > [Cu(ATS)(Gly)] (figure 2). The order of antifungal activity is in accordance with the stability order of metal ions with chloro and bromo amino acid salicylaldehyde Schiff bases [91].

3.8.3. Cytotoxic activities. *In vitro* potential cytotoxicity of Cu(II) complexes was tested against colon carcinoma (HCT116) and larynx carcinoma (HEP2) cells. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line (figure 3); IC_{50} values are given in table 7. Cytotoxicity of the compounds against HCT116 and HEP2 indicates that [Cu(ATS)(Val)] shows significant activity against colon and larynx cancer cells with IC_{50} values of 0.70 and 0.49 $\mu g\,mL^{-1}$, respectively (figure 3), classifying this compound as chemotherapeutically significant. IC_{50} is the concentration which can reduce the growth of cancer cells by 50%. The rank order of potency as a function of chelated amino acid follows the order Gly<Ala<Val against HEP2 and HCT116 cancer cells. [Cu(ATS)(Val)] exhibits significant decrease in the surviving fraction of HCT116 and HEP2 cancer cells, and induced apoptosis of these cell lines.

Table 6. Antibacterial and antifungal activities of [Cu(ATS)(AA)][a] complexes.

		Diameter of inhibition zone (in mm)[b]					
		Bacteria				Fungi	
		(G^-)		(G^+)			
Compounds	log *K*	*Pseudomonas aeruginosa* ATCC 2853	*Escherichia coli* ATCC 25922	*Bacillus cereus* ATCC 11778	*Staphylococcus epidermidis* ATCC 155	*Aspergillus niger* ATCC 9029	*Aspergillus fumigatus* ATCC 46645
Conc. ($\mu g\ mL^{-1}$)		100	100	100	100	100	100
[Cu(ATS)(Gly)]·H_2O	17.49	13.7 ± 0.42	NA[c]	20.3 ± 0.14	21.1 ± 0.35	15.2 ± 0.33	14.7 ± 0.12
[Cu(ATS)(Ala)]	17.69	14.8 ± 0.21	NA	22.4 ± 0.37	22.3 ± 0.34	18.4 ± 0.45	17.2 ± 0.52
[Cu(ATS)(Val)]	17.86	15.4 ± 0.54	11.2 ± 0.24	23.1 ± 0.62	27.3 ± 0.44	23.7 ± 0.64	21.3 ± 0.34
Standard[d]	–	22 ± 0.15	27 ± 0.18	24 ± 0.28	28 ± 0.14	28 ± 0.12	30 ± 0.38

[a]ATS = 2-aminomethylthiophenyl-4-bromosalicylaldehyde, AA = glycine, alanine and valine.
[b]Mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms.
[c]NA: not detected.
[d]Ciprofloxacin is used as standard for antibacterial activity and Ketoconazole is used as standard for antifungal activity.

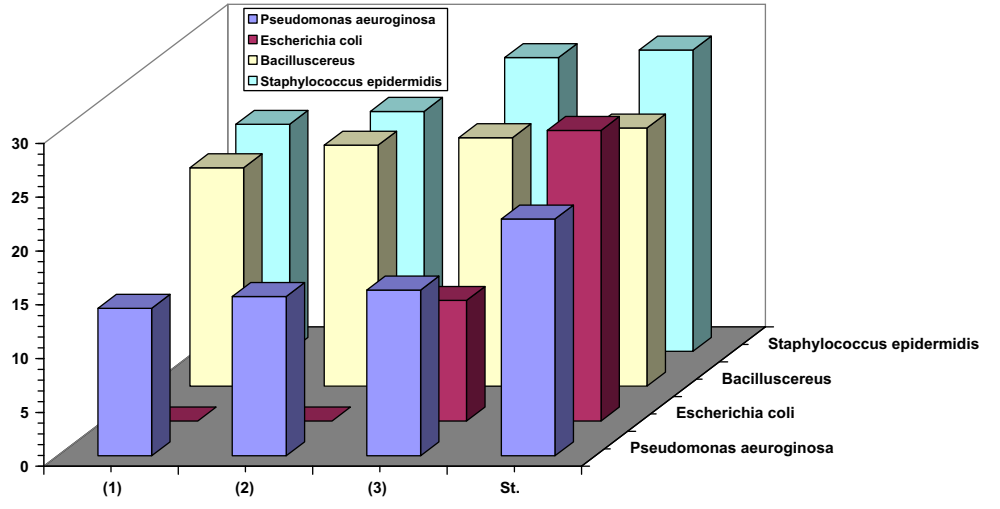


Figure 1. Antibacterial activity of Cu(II) complexes towards different types of bacterial strains.

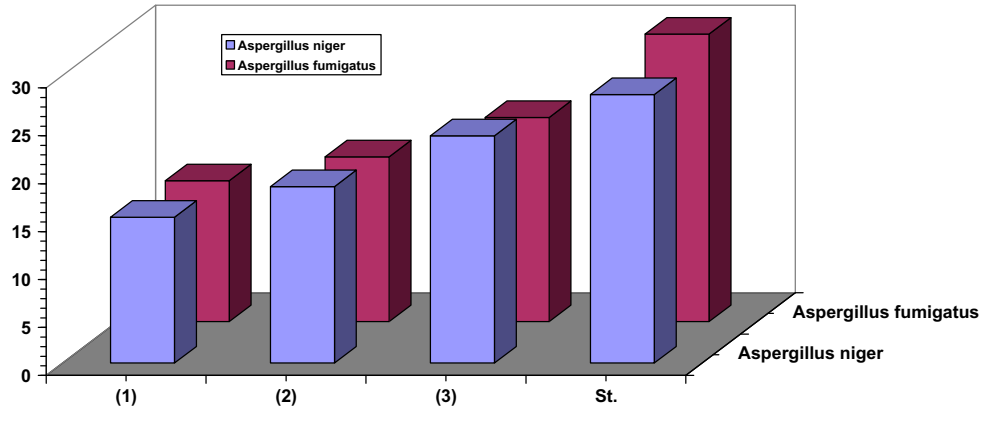


Figure 2. Antifungal activity of Cu(II) complexes towards different types of fungal strains.

A similar behavior of [Cu(AMBI)(Gly)] (where AMBI = 2-aminomethylbenzimidazole and Gly = glycine) showed significant activity against HEP2 cells with IC_{50} values of 0.83 μg mL^{-1} [92].

3.9. *Molecular modeling and biological activity*

Molecular mechanics attempts to reproduce molecular geometries, energies, and other features by adjusting bond length, bond angles, and torsion angles to equilibrium values that are dependent on the hybridization of an atom and its bonding. To obtain estimates of structural details of these complexes, we have optimized the molecular structures of the complexes. Energy minimization studies were carried out by the semiempirical PM3 level provided by HyperChem 7.5 software. Energy minimization was repeated several times to find the global minimum. The calculated dipole moment, total energy, binding energy,

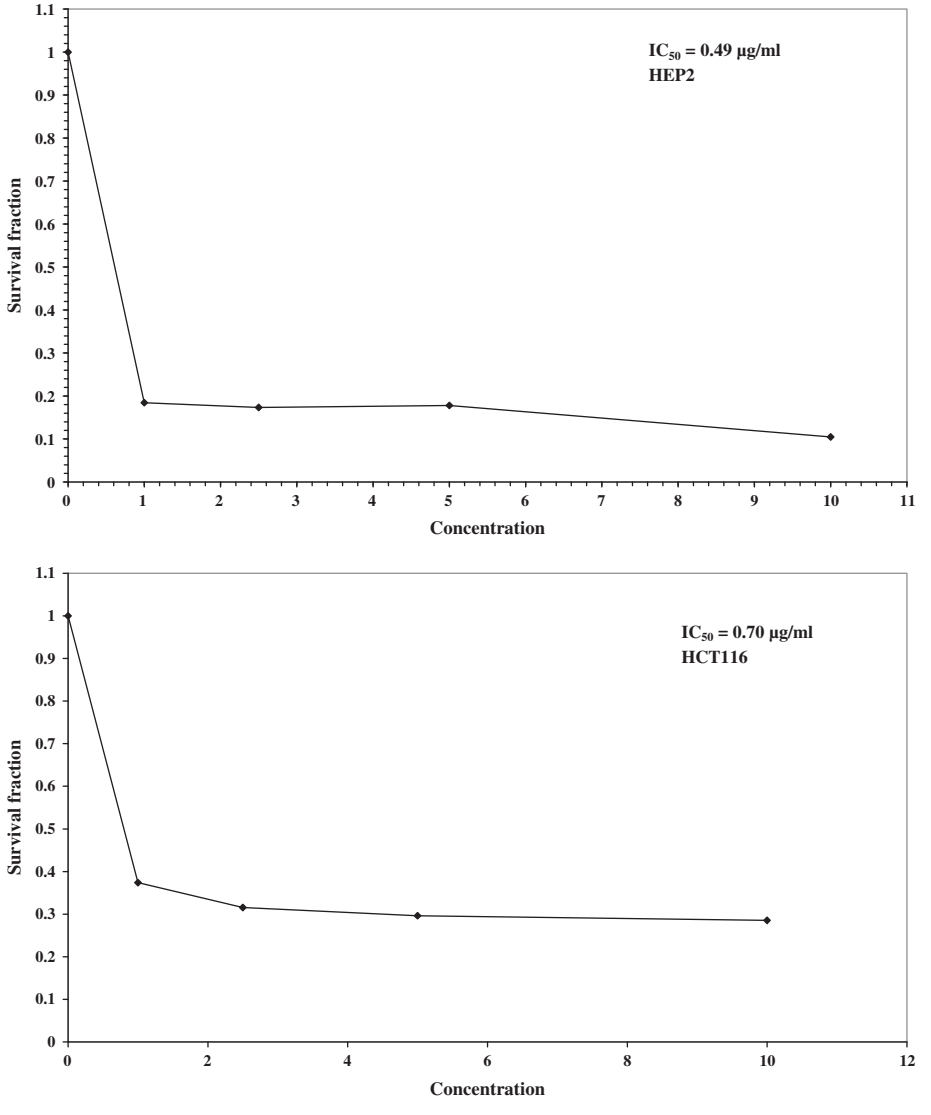


Figure 3. Effect of [Cu(ATS)(Val)] on surviving fraction of HEP2 and HCT116 tumor cells.

Table 7. Effect of synthesized [Cu(ATS)(AA)][a] complexes on tumor cell growth *in vitro* (IC_{50} values in $\mu g\,mL^{-1}$).

	IC_{50}[b]	
Compounds	HCT116	HEP2
[Cu(ATS)(Gly)]·H_2O	0.85	0.74
[Cu(ATS)(Ala)]	0.76	0.70
[Cu(ATS)(Val)]	0.70	0.49
Doxorubicin (standard)	0.69	0.40

[a]ATS = 2-aminomethylthiophenyl-4-bromosalicylaldehyde; AA = glycine, alanine, and valine.
[b]IC_{50} = cytotoxic dose at 50%, i.e. the drug concentration to inhibit the growth of the cancer cells by 50%.

lipophilicity (log *P*), HOMO, and LUMO energies after geometrical optimization of the structures of Cu(II) complexes are given in table 8.

The molecular structure of [Cu(ATS)(Val)], as a representative example of Cu(II) complexes along with the atom numbering scheme, are given in figure 4 while selected bond lengths and angles are given in Supplementary material. The Cu–N bond length is longer than that of Cu–O for the Cu(II) complexes. The bond angles around Cu(II) (~90) prove that the geometry is square planar as proposed by the analysis mentioned previously. From elemental analyses, spectral data, magnetic susceptibility measurements at room temperature, conductivity measurements, and QM calculations, it is possible to draw tentative square-planar structures of the copper(II) complexes.

Quantum chemical parameters of organic compounds are obtained from calculations, such as the energy of the highest occupied molecular orbital, E_{HOMO}, and energy of the lowest unoccupied molecular orbital, E_{LUMO}. Additional parameters, such as separation energies (ΔE), absolute electronegativitiy (χ), chemical potentials (Pi), absolute hardness (η), absolute

Table 8. Some properties of [Cu(ATS)(AA)][a] calculated by the PM3 method.

Complex	Total energy kcal M^{-1}	Binding energy kcal M^{-1}	Electronic energy kcal M^{-1}	HOMO	LUMO	log P[b]
[Cu(ATS)(Gly)]·H_2O	−110463.18	−3777.39	−692337.28	−3.70	−1.06	1.11
[Cu(ATS)(Ala)]	−113917.08	−4063.26	−754186.56	−3.72	−0.49	3.09
[Cu(ATS)(Val)]	−120465.18	−4576.78	−833991.08	−9.26	−1.55	4.57

[a]ATS = 2-aminomethylthiophenyl-4-bromosalicylaldehyde, AA = glycine, alanine, and valine.
[b]Lipophilicity (log *P*) values calculated using QSAR.

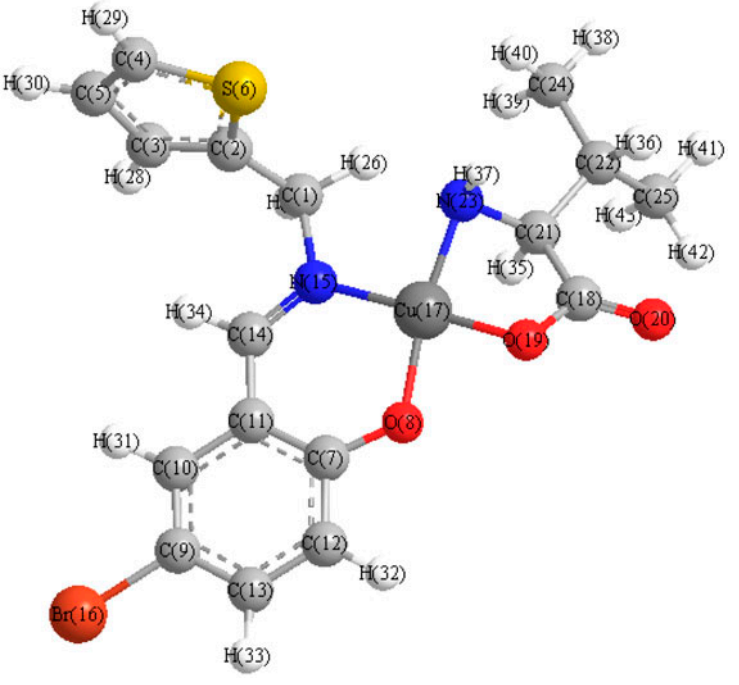


Figure 4. The optimized geometry of [Cu(ATS)(Val)] (**3**) along with the atom numbering scheme.

softness (σ), global electrophilicity (ω) [93–96], global softness (S), and additional electronic charge (ΔN_{max}), have been calculated [97]. The parameters χ and Pi are related to each other. The inverse of the global hardness is designated as the softness σ [98]. From the obtained data, provided as Supplementary material (see online supplemental material at http://dx.doi.org/10.1080/00958972.2014.900549), we can deduce that:

(1) The reactivity index measures the stabilization in energy when the system acquires an additional electronic charge (ΔN_{max}) from the environment. The electrophilicity index is positive and the direction of the charge transfer is completely determined by the electronic chemical potential (Pi) of the molecule. The electronic chemical potential must be negative, as supported by the values given as Supplementary material.
(2) The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) determine the interaction for the molecule with other species. The energies of the HOMO and LUMO are negative, which indicate the stability of Cu(II) complexes [99].
(3) From the calculations of the binding energy there is an increase in the value of the calculated binding energy of complexes from **1** to **3,** which indicates that the stability of the formed metal complexes is [Cu(ATS)(Gly)] < [Cu(ATS)(Ala)] < [Cu(ATS)(Val)].

Understanding the role of chemical structure on biological activity is very important. Thus, an attempt to correlate the biological potency of the synthesized compounds with their chemical structure was undertaken. Therefore, SAR calculations were performed in order to investigate physico-chemical properties that may be related to the antimicrobial action of the studied compounds. Properties of interest in this study were the HOMO and LUMO energies, the dipole moment, and lipophilicity value (log *P*), which were correlated to the determined zone of inhibition (Supplementary material). [Cu(ATS)(Val)] (**3**), which presented the lowest value of LUMO energy among all Cu(II) complexes, showed the highest activity against all selected types of bacteria and fungi.

Studies on the structure–activity relationship have shown the importance of the lipophilic nature of biologically active molecules [100, 101]. The computer program QSAR implemented in HYPERCHEM 7.5 predicts the lipophilicity values of compounds (log *P*) using the atom-additive method [102]. The program lists the atom contributions of each atom type and calculates the log *P* value by summing up all the atom contributions. The calculated log *P* values given in the supplementary data are in agreement with the bacterial inhibition activities observed with [Cu(ATS)(Val)] having higher lipophilicity log *P* value. Lipophilicity of compounds usually increases with increase in alkyl groups in the compounds, consistent with the higher log *P* values of [Cu(ATS)(Val)].

The dipole moment may give insight into the degree of hydrophobicity/hydrophilicity of the compounds, i.e. dipole moment is a very useful parameter for determining the penetration through cell membrane and for the speed of excretion. SAR studies suggest an inverse correlation between the dipole moment and the activity of the complexes against the studied bacterial and fungal species. As dipole moment decreases, the polarity decreases and in turn the lipophilic nature of the compound increases [103]. From the provided supplementary data, **3** has a lower dipole moment ($\mu = 8.46$), consistent with its higher lipophilicity. [Cu(ATS)(Val)], which presented the lowest value of HOMO energy among all Cu(II) complexes, showed the highest biological activity against all selected types of bacteria and fungi. The results obtained from this study can be used as guidelines for further improvement of antibacterial agents.

4. Conclusion

The present paper reports synthesis, characterization, and electronic absorption spectra of complexes of Cu(II) with ATS and amino acids in the molar ratio (1 : 1 : 1). The new Schiff base participated in bonding to copper as a monobasic bidentate ligand through the azomethine nitrogen and phenolic oxygen via deprotonation, forming a stable six-membered heterocyclic ring. The amino acids also act as monobasic bidentate ligands via amino and ionized carboxylate groups. Similar coordination of amino and carboxylate groups was also exhibited by copper(II) complexes of aspartic acid [17]. Thus, the charge of the metal ion was neutralized by deprotonation of the Schiff base phenolic OH group and the amino acid carboxylate (scheme 1) with a total of four coordination sites around the metal. The Cu-ATS-AA complexes have square-planar geometry. From conductance measurements, the complexes are non-electrolytes. The ionization constants of the investigated ligands have been determined potentiometrically. Geometry optimization and conformational analysis have been performed and the agreement with the spectral studies suggests the structures of the complexes. The present investigation described the formation equilibria of Cu(II) with ATS and amino acids. The protonation of amino acids and their complex formation equilibria in DMSO–water mixture will be a contribution to mechanistic studies in biological media. The antimicrobial study reveals that some of the complexes show better or comparable activity to the standard antibiotic. The relationship between structural and biological properties has been explored to help in designing more potent antibacterial agents. SAR studies suggested an inverse correlation between the dipole moment and the activity of the complexes against the studied bacterial and fungal species, and direct relation between lipophilicity (log *P*) and biological activity of complexes. [Cu(ATS)(Val)] shows significant activity against both colon carcinoma (HCT116) and larynx carcinoma (HEP2) tumor cells.

Supplementary material

The calculated quantum chemical parameters of Cu(II) complexes and bond distances, and angles for [Cu(ATS)(Val)] have been reported as supplementary data, see online supplemental material at http://dx.doi.org/10.1080/00958972.2014.900549.

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