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Unusual saccharin-N,O (carbonyl) coordination in mixed-ligand copper(II) complexes: Synthesis, X-ray crystallography and biological activity

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ABSTRACT

Three tridentate Schiff bases containing N and S donor atoms were synthesized *via* the condensation reaction between S-2-methylbenzyldithiocarbazate with 2-acetyl-4-methylpyridine (**S2APH**); 4-methyl-3-thiosemicarbazide with 2-acetylpyridine (**MT2APH**) and 4-ethyl-3-thiosemicarbazide with 2-acetylpyridine (**ET2APH**). Three new, binuclear and mixed-ligand copper(II) complexes with the general formula, **[Cu(sac)(L)]**$_2$ (sac = saccharinate anion; L = anion of the Schiff base) were then synthesized, and subsequently characterized by IR and UV/Vis spectroscopy as well as by molar conductivity and magnetic susceptibility measurements. The Schiff bases were also spectroscopically characterized using NMR and MS to further confirm their structures. The spectroscopic data indicated that the Schiff bases behaved as a tridentate NNS donor ligands coordinating *via* the pyridyl-nitrogen, azomethine-nitrogen and thiolate-sulphur atoms. Magnetic data indicated a square pyramidal environment for the complexes and the conductivity values showed that the complexes were essentially non-electrolytes in DMSO. The X-ray crystallographic analysis of one complex, [Cu(sac)(S2AP)]$_2$ showed that the Cu(II) atom was coordinated to the thiolate-S, azomethine-N and pyridyl-N donors of the S2AP Schiff base and to the saccharinate-N from one anion, as well as to the carbonyl-O atom from a symmetry related saccharinate anion yielding a centrosymmetric binuclear complex with a penta-coordinate, square pyramidal geometry. All the copper(II) saccharinate complexes were found to display strong cytotoxic activity against the MCF-7 and MDA-MB-231 human breast cancer cell lines.

1. Introduction

Saccharin (*o*-sulfobenzoimide or 1,2-benzothiazole-3(2H)-one 1,1-dioxide) has been known as a popular non-caloric artificial sweetening agent since 1885 [1]. However in the 1970s, saccharin was found to cause urinary bladder cancer in mice and was subsequently banned as a food additive [2]. Further research refuted these earlier findings and the ban was subsequently lifted by the United States Food and Drug Administration (US FDA) in the 1980s and as a result, saccharin still remains a widely-used artificial sweetener to date [3,4].

Saccharin is also of interest to synthetic chemists because of its ability to act as a polyfunctional ligand [5]. The coordination chemistry of the saccharin anion is very interesting as its donor atoms can chelate to a metallic centre in different modes, *via* the imino-nitrogen, carbonyl- or sulfonyl-oxygen atoms and can generate either N– or O– or S– monodentate or (N,O)-bidentate chelating complexes [4]. Mixed-ligand complexes that contain the saccharinate anion and metallic cations usually result in the coordination of the metal centre to the imino-nitrogen of saccharin [6]. Mixed-ligand complexes are formed when the metal ion binds to two different ligand moieties. However, there have also been published reports on the coordination of metal cations with the carbonyl- or sulfonyl-oxygen of saccharin [5]; although the latter is

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less basic and is rarely involved in bonding. A comparatively rare (N,O)-bidentate bridging coordination mode, that is reported for one of the complexes in this study, has been found in only three examples in the literature [7–9]. Mixed-ligand metal complexes also play important roles in biological processes, galactose oxidase, vitamin B12, chlorophyll and haemoglobin are examples of such complexes [10].

Dithiocarbazates and thiosemicarbazides are among the most studied O–, N– or S– donor ligands [11]. The use of different aldehydes or ketones that are reacted with these ligands produce a variety of Schiff bases with a combination of donor atoms such as bidentate NS and tridentate NNS or ONS Schiff bases, to name a few [12]. These versatile Schiff bases exhibit interesting physical and chemical properties and the interactions of dithiocarbazate or thiosemicarbazide derived Schiff bases with many transition metal ions form complexes with different geometries and properties [13]. The donor atoms determine the coordination structure of the complexes and subsequently impact their biological properties [14]. In studies that investigated the effect of structurally-related copper(II) complexes of the 2-acetylpyridine Schiff base of S-benzyldithiocarbazate in phytopathogenic fungi *A. solanyi, F. Equisetti* and *M. Phaseolina* and pathogenic bacteria *E. coli* and *S. Aureas*, chelation of the tridentate NNS Schiff base to the copper(II) ion was found to enhance the anti-fungal and anti-bacterial activities, an observation that was ascribed to the chelation effect of the metal complex [13–15]. Thiosemicarbazones derived from 2-acetylpyridine have also been reported to have anti-tuberculosis activity [16]. A series of related 2-acetylpyridinethiosemicarbazone, Haptsc ligands and their vanadium complexes were tested against *Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294 and their biological properties varied correspondingly with variation of substituents on the N4 atom of the thiosemicarbazones moiety. The non-substituted thiosemicarbazone, Haptsc itself was mildly active but upon the substitution of one hydrogen linked to N4 with a phenyl or methyl group, the biological activities were found to increase significantly. Coordination of vanadium (V) and (IV) to the ligands resulted in a further enhancement of their anti-tubercular properties [17].

More recently, the DNA binding and cytotoxic assay studies of Cu(II), Ni(II), Zn(II) and Cd(II) complexes derived from S-substituted dithiocarbazate Schiff bases have been reported, but interestingly, only Cu(II) complexes showed promising biological activities and this was supported by the DNA binding affinity of the Cu(II) complexes which was postulated to be due to the ability of the complexes to block the enzymatic binding to the nitrogen bases of DNA or RNA of the cancer cells [18,19]. Several other copper complexes have been reported to have effective anti-inflammatory, antirheumatic and anticancer activities, which was associated with their superoxide dismutase-like activity [20]. In addition, copper is also part of the redox active metalloenzymes which consists of cytochrome *c* oxidase, tyrosinase or Cu,Zn-superoxide [21].

In this report, the tridentate NNS Schiff bases derived from dithiocarbazate and thiosemicarbazide were reacted with copper(II) saccharinate. Studies on this subject have been motivated by the findings that mixed-ligand complexes are more active than their constituent ligands or the corresponding homo-ligated bis-complexes. Mixed-ligand complexes also find use in industrial reactions such as hydrogenation, hydroformation and oxidative hydrolysis of olefins and carboxylation of methanol where the mixed-ligand complexes act as active catalysts in those reactions [22].

This new series of complexes are a part of our on-going research into copper(II) saccharinate complexes and their biological properties. Therefore in this work, we report the synthesis, characterization and cytotoxic activity of three tridentate NNS Schiff bases and their corresponding copper(II) complexes that contain the saccharinate anion (sac) as well as the single crystal X-ray diffraction analysis that highlights an unusual N,O- bridging mode of saccharinate in binuclear $[Cu(sac)(S2AP)]_2$.

2. Experimental

2.1. Instrumentation and materials

Melting points were determined using an Electrothermal digital melting point apparatus. CHNS analysis was carried out using a LECO CHNS-932 instrument. IR spectra were recorded using PerkinElmer Spectrum 100 with Universal ATR Polarization in the range of 4000-280 cm^{-1}. Copper content determinations were performed on a Perkin-Elmer Plasma 1000 Emission Spectrometer. Molar conductance of 10^{-3} M solutions of the metal complexes in DMSO were measured using a Jenway 4310 conductivity meter and a dip-type cell with a platinised electrode. Magnetic susceptibilities were measured using a Sherwood Scientific MSB-AUTO magnetic susceptibility balance at room temperature. The UV–Vis spectra were recorded using a Shimadzu UV–Vis 1650 PC recording spectrophotometer over the range of 1000–200 nm. ^1H NMR and ^{13}C NMR of the Schiff bases were analysed using **a** Jeol NMR ECA-500 Nuclear Magnetic Resonance Spectrophotometer using dimethylsuphoxide (DMSO) as the solvent and tetramethylsilane (TMS) as the internal standard. The mass spectra were performed by a Shimadzu GCMS QP5050A mass spectrometer using the direct insertion (DI-MS) technique.

All chemicals and solvents were of analytical grade and were used as supplied. Chemicals: 2-methylbenzylchloride, potassium hydroxide, hydrazinium hydroxide, carbon disulphide, 4-methyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide, 2-acetylpyridine, 2-acetyl-4-methylpyridine, copper(II) acetate monohydrate, sodium saccharinate and nitric acid (65%).

Solvents: absolute ethanol (99.8%), ethanol (95%), acetonitrile (Baker) and dimethyl sulfoxide (DMSO).

2.2. Synthesis

2.2.1. S-2-methylbenzyldithiocarbazate (S2MBDTC)

The ligand was prepared according to the method reported by Ali et al. [23]. Potassium hydroxide (0.2 mol, 11.2 g) was dissolved in absolute ethanol (70 ml). Hydrazine hydrate (100%, 0.2 mol, 10.0 g) was added and the mixture was cooled in an ice-salt bath to 0 °C. Carbon disulphide (0.2 mol, 12.1 ml) was added drop-wise with vigorous stirring for 1 h. The resulting two layers were separated using a separating funnel. 40% ethanol (60 ml) was added to the lower, brown organic layer. 2-Methylbenzyl chloride (0.2 mol, 26.1 ml) was added drop-wise while stirring and the resulting white product was filtered off, washed with cold ethanol and dried in a desiccator over anhydrous silica gel. Yield: 70%, m.p: 170–171 °C.

2.2.2. Schiff bases

The Schiff bases were prepared according to a published method [24]. A solution of dithiocarbazate or thiosemicarbazide was reacted with an equimolar amount of ketone to synthesize all Schiff bases. The resulting solution was then left overnight to precipitate.

2.2.2.1. 2-Methylbenzyl-(2-acetylpyridin-4-yl)methylenedithiocarbazate (S2APH). S2MBDTC (0.02 mol, 4.25 g) in hot acetonitrile (50 ml) was reacted with 2-acetyl-4-methylpyridine (0.02 mol, 2.70 g) in absolute ethanol (25 ml). The mixture was heated and stirred until a yellow precipitate formed which was filtered off, washed with acetonitrile and dried over anhydrous

silica gel. Yield: 70%, m.p: 155–156 °C.

2.2.2.2. 2-Acetylpyridine-4-methyl-3-thiosemicarbazone (MT2APH) [25]. 4-Methyl-3-thiosemicarbazide (0.01 mol, 1.05 g) dissolved in hot ethanol (50 ml) was reacted with an equimolar amount of 2-acetylpyridine (1.12 ml) in ethanol (25 ml). The mixture was heated and stirred until a yellow precipitate formed which was filtered off, washed with cold ethanol and dried over anhydrous silica gel. Yield: 85%, m.p: 146–147 °C.

2.2.2.3. 2-Acetylpyridine-4-ethyl-3-thiosemicarbazone (ET2APH) [26]. 4-Ethyl-3-thiosemicarbazide (0.01 mol, 1.19 g) dissolved in hot ethanol (50 ml) was reacted with an equimolar amount of 2-acetylpyridine (1.12 ml) in ethanol (25 ml). The mixture was heated and stirred until a yellow precipitate that formed was filtered off, washed with cold ethanol and dried over anhydrous silica gel. Yield: 90%, m.p: 150–151 °C.

2.3. Copper(II) saccharinate, $[Cu(sac)_2(H_2O)_4]\cdot 2H_2O$

The synthesis was carried out using the method reported by Haider et al. [27]. Sodium saccharinate (0.02 mol, 4.46 g) was dissolved in water (25 ml) and was added to a solution of copper(II) acetate monohydrate (0.01 mol, 2.01 g) in water (25 ml). The mixture was heated and stirred to reduce the volume to half. On standing overnight, blue crystals of $[Cu(sac)_2(H_2O)_4]\cdot 2H_2O$ formed which were filtered off, washed with cold ethanol and dried in a desiccator over anhydrous silica gel. Yield: 90%, m.p: 223–224 °C.

2.4. Synthesis of Cu(II) saccharinate complexes

The synthesis was carried out using the method outlined by Ravoof et al. [28]. $[Cu(sac)_2(H_2O)_4]\cdot 2H_2O$ (0.001 mol, 0.536 g) dissolved in 25 ml of boiling water was mixed with 0.001 mol of the appropriate Schiff base (S2APH, 0.33 g; MT2APH, 0.21 g; ET2APH, 0.22 g) in ethanol (50 ml). The mixture was heated and stirred until a precipitate formed. On standing overnight, green crystalline complexes formed which were filtered off, washed with ethanol and dried in a desiccator over anhydrous silica gel. Yield: *ca.* 70–75%. Crystals of the dithiocarbazate complex, $[Cu(sac)(S2AP)]_2$, suitable for X-ray diffraction analysis were obtained from the slow evaporation of its acetonitrile solution. Analytical, physical and spectral data are given in Tables 1 and 3.

2.5. Single crystal X-ray structure determination

A crystal of $[Cu(sac)(S2AP)]_2$ was mounted on a fibre loop using perfluoropolyether oil and rapidly cooled to 100 K in a stream of cold N_2 using an Oxford Cryosystems Cobra unit. The diffraction data were measured using an Oxford Diffraction Gemini E CCD diffractometer and the intensity data were processed with CrysAlis Pro software suite [29]. The structure was solved by direct methods [30] and full-matrix least-squares refinement on F^2 was performed using SHELXL-2014/7 [31] integrated in WinGX [32]. The C-bound H atoms were placed on stereochemical grounds and refined in the riding model approximation with $U_{iso} = 1.2–1.5U_{eq}$(carrier atom). The maximum and minimum residual electron density peaks of 1.49 and 0.59 e Å^{-3}, respectively, were located 0.85 and 0.66 Å from the S1 atom. A weighting scheme of the form $w = 1/[\sigma^2(F_o^2) + (0.044P)^2 + 1.308P]$ where $P = (F_o^2 + 2F_c^2)/3$ was employed in the final cycles of refinement. Crystallographic diagrams were drawn with ORTEP-3 for Windows [32] and DIAMOND [33]; PLATON [34] was also used in the study. Crystallographic data and refinement details are tabulated in Table 2.

Table 2
Crystallographic data and structure refinement details for $[Cu(sac)(S2AP)]_2$.

Empirical formula	$C_{96}H_{88}Cu_4N_{16}O_{12}S_{12}$
Formula weight	2296.74
Crystal system	Triclinic
Space group	*P*1
Unit cell dimensions	a = 9.1855(7) Å; α = 95.339(6)°
Unit cell dimensions	b = 11.5972(7) Å; β = 110.410(7)°
Volume	c = 12.3874(9) Å; γ = 99.705(6)°
	1202.46(16) Å^3
Z/Z′	1/0.5
Calculation density	1.586 g cm^{-3}
Absorption coefficient	1.204 mm^{-1}
F(000)	590
Crystal size	0.10 × 0.20 × 0.30 mm
Theta range for data collection	2.3-28.9°
Reflections collected/unique	10,520/5437
Reflections collected/unique	(R_{int} = 0.038)
Reflections with $I > 2\sigma(I)$	4626
Data/restraints/parameters	5418/0/319
Final R indices [$I > 2\sigma(I)$]	R = 0.043, R_w = 0.103
Final R indices [all data]	R = 0.053, R_w = 0.110

2.6. Cytotoxicity assay

The MCF-7 (estrogen receptor-positive human breast cancer cells) and MDA-MB-231 (estrogen receptor-negative human breast cancer cells) cells were obtained from the National Cancer Institute, USA. The cells were cultured in 25 cm × 25 cm x 25 cm flasks containing RPMI 1640 culture medium supplemented with 10% foetal bovine serum (FBS) and 1% penicillin. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of these complexes [35]. The cytotoxicity levels were expressed as IC_{50} values, the concentration in which the test compound inhibited the growth of cancer cells by 50%. Tamoxifen was used as the positive control and DMSO was used as the negative control.

Table 1
Analytical data and physical properties of the Schiff bases and their copper(II) saccharinate complexes.

Compound	Colour	m.p (°C)	Λ[a]	μ[b] (B.M)	Elemental Analysis[c] (%)			
					C	H	N	Cu
S2APH	Yellow	155–156	–	–	61.6 (61.9)	5.7 (5.8)	12.1 (12.7)	–
$[Cu(sac)(S2AP)]_2$	Dark-green	196–197	1.17	1.73	50.5 (50.2)	3.3 (3.9)	9.6 (9.7)	11.4 (11.1)
MT2APH	Yellow	146–147	–	–	52.5 (52.0)	5.3 (5.8)	26.1 (26.9)	–
$[Cu(sac)(MT2AP)]_2$	Dark-green	198–199	2.35	1.75	43.1 (42.4)	3.5 (3.4)	14.9 (15.4)	13.6 (14.0)
ET2APH	Yellow	150–151	–	–	54.9 (54.0)	6.4 (6.4)	25.3 (25.2)	–
$[Cu(sac)(ET2AP)]_2$	Dark-green	188–189	5.29	1.68	43.5 (43.7)	3.4 (3.6)	14.6 (15.0)	13.3 (13.6)

[a] Molar conductance ($\Omega^{-1}cm^2mol^{-1}$) of 10^{-3} M solutions in DMSO.
[b] Magnetic moments at 298 K.
[c] Calculated values are given in parentheses.

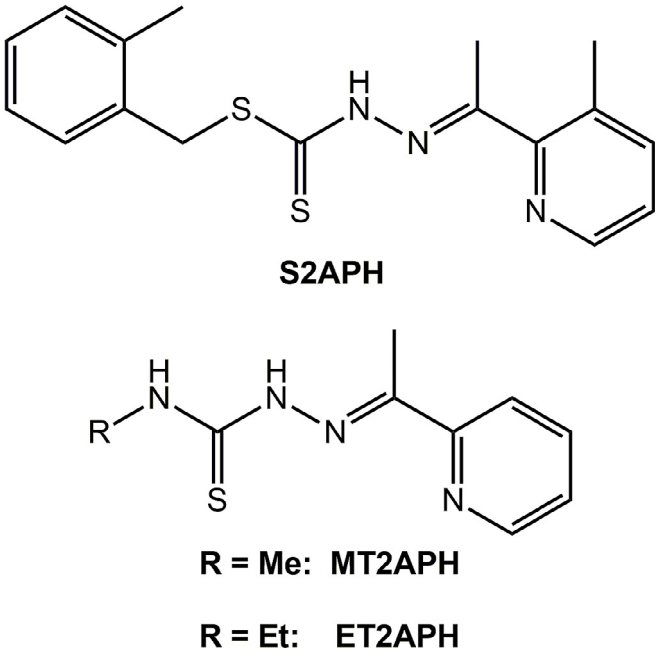

Fig. 1. Structure of the Schiff bases S2APH, MT2APH and ET2APH.

3. Results and discussion

The structures of the Schiff bases are shown in Fig. 1 while the physical properties and analytical data of the Schiff bases and the copper(II) saccharinate complexes are given in Table 1. Elemental analyses indicated good agreement with the proposed formulae. The Schiff bases were soluble in most organic solvents while the complexes were soluble in acetonitrile, DMSO and tetrahydrofuran (THF).

3.1. IR spectral studies

The IR spectra of the Schiff bases and their corresponding metal complexes are summarised in Table 3. The Schiff bases exhibited ν(C═N), ν(N–N) and ν(C–S) bands at 1535-1595 cm^{-1}, 1035-1054 cm^{-1} and 832-854 cm^{-1}, respectively which were shifted during complexation proving the coordination to the copper(II) ion through the azomethine-nitrogen and thiolate-sulphur atoms [13]. The coordination of thiosemicarbazone and dithiocarbazate ligands *via* the azomethine nitrogen to the metal was indicated by the shift of the ν(C═N) and ν(N–N) bands to higher frequencies, as reported in previous work [36]. The absence of a ν(N–H) band in the spectra of the complexes also indicated the deprotonation of the Schiff base upon coordination [37]. The strong $\nu_{sym}(SO_2)$, $\nu_{asy}(SO_2)$ and ν(C═O) bands in the spectra of the Cu(II) complexes confirmed the presence of the saccharinate anion [38].

3.2. NMR spectral studies

^{1}H NMR spectra of Schiff bases were recorded in DMSO-d_6. All the data are tabulated in Table 4. A well-defined singlet at 12.5 ppm (S2APH), 11.6 ppm (MT2APH) and 10.2 ppm (ET2APH) attributable to the –NH proton indicated that the Schiff bases remained in the thione form even in solution. The absence of a singlet due to –SH at around δ 4.00 ppm also supported the findings that the Schiff bases were in thione form even in polar solvents like DMSO [39]. The upfield-shifted resonance from 2.3 ppm to 3.5 ppm was assigned to the protons of the methyl group of the ketone moiety (═C–CH_3) for the Schiff bases. The methyl group attached to the pyridine ring for S2APH Schiff base was observed at 2.40 ppm. The distinct presence of the S–CH_2 protons in S2APH was observed at 4.4 ppm. The remaining resonances observed were those expected for the molecular formula proposed.

The ^{13}C NMR spectra of the Schiff bases showed a downfield resonance attributable to the thioamide carbon (C═S) at *ca.* δ 199.1 ppm, 178.2 ppm and 178.1 ppm. This resonance appeared as the most downfield peak due to the presence of the electronegative sulphur and nitrogen atoms adjacent to the carbon atom.

3.3. Mass spectral studies

The mass spectra of S2APH, MT2APH and ET2APH were consistent with the proposed formula (molecular ion peaks at *m*/*z* 329, 208, 222). The base peak for S2APH observed at *m*/*z* 105 was assigned to the C_8H_9 fragment and peaks observed at *m*/*z* 138, 91 and 77 corresponded to fragments $C_8H_9S^{\bullet}$, $C_7H_7^{\bullet}$ and $C_6H_4^{\bullet}$ [18], respectively. Both MT2APH and ET2APH also had similar fragmentation patterns with a base peak at *m*/*z* 105 but displayed fragmentation corresponding to $C_2H_2N_3S^{\bullet+}$ and $C_6H_5N2^{+}$.

3.4. Magnetic susceptibility measurements

The magnetic moments of the copper(II) saccharinate complexes at room temperature were slightly lower than the range expected for $3d^9$ copper ions (Table 2) [40]. Cu(II) complexes with square pyramidal geometries have been reported to have magnetic moments in the range of 1.6–1.9 B M [23,41]. For example, the reaction of Cu(II) with the *S*-methyl-β-*N*-(6-methylpyrid-2-yl) methylenedithiocarbazate (HNNS) and saccharin yielded a square pyramidal Cu(II) complex [Cu(NNS)(sac)(H_2O)] which had an effective magnetic moment close to 1.59 μ_B at room temperature [28]. The molar conductance values of the complexes were in the range of 1.17–5.29 Ω^{-1} cm^2 mol^{-1} indicating that they were essentially non-electrolyses in DMSO [42]. This indicated that both the Schiff base and the saccharinate anion were coordinated to the

Table 3
Selected IR bands and electronic spectral bands for the Schiff bases and their copper(II) saccharinate complexes.

Compound	IR bands (cm^{-1})[a]							Electronic spectra λ_{max} (log ε_{max})[b]
	ν (N–H)	ν (C═N)	ν (N–N)	ν (CS_2)	ν_{sym} (SO_2)	ν_{asym} (SO_2)	ν (C═O)	
S2APH	3163 (w)	1587 (m)	1039 (s)	854 (m)	–	–	–	260 (3.38), 330 (4.20)
[Cu(sac)(S2AP)]$_2$	–	1597 (m)	1048 (s)	878 (m)	1143 (s)	1290 (s)	1656 (s)	270 (3.97), 344 (4.30), 410 (3.94), 650 (2.55)
MT2AP	3287 (s)	1535 (s)	1035 (s)	832 (s)	–	–	–	263 (3.30), 320 (4.30)
[Cu(sac)(MT2AP)]$_2$	3350 (m)	1590 (m)	1052 (w)	823 (s)	1139 (w)	1278 (s)	1648 (s)	260 (3.86), 302 (4.08), 407 (3.94), 628 (2.13)
ET2AP	3341 (s)	1595 (s)	1054 (s)	830 (s)	–	–	–	246 (3.60), 321 (4.30)
[Cu(sac)(ET2AP)]$_2$	3326 (s)	1627 (s)	1052 (w)	891 (m)	1161(m)	1297 (s)	1663 (s)	259 (3.93), 304 (4.18), 408 (4.07), 617 (2.13)

[a] s = strong intensity, m = medium intensity, w = weak intensity.
[b] Units of λ_{max} are in nm and units of log ε_{max} are in L mol^{-1} cm^{-1}.

Table 4
^{1}H NMR and ^{13}C NMR data for S2APH, MT2APH, and ET2APH Schiff bases.

Schiff base	^{13}C NMR Assignment, δ (ppm)					^{1}H NMR Assignment, δ (ppm)			
	−C=S	−C=N	−S−CH_2	−CH_3	Aromatic carbons	−NH	−S−CH_2	=C−CH_3	Aromatic Protons
S2APH	199.1	154.1	37.2	13.9, 19.4, 21.3	121.3–153.5	12.5 (1H, s)	4.4 (2H, s)	2.3 (3H, s)	7.1–8.4 (1H, s)
MT2APH	178.2	155.2	–	12.9, 15.6	121.9–149.5	8.4 (1H, m) 11.6 (1H,s)	–	3.5 (3H, s)	7.1–7.6 (4H, m)
ET2APH	178.1	155.2	39.0	12.6, 14.9	121.3–148.5	8.6 (1H, t) 10.2 (1H,s)	–	2.4 (3H, s)	7.3–8.5 (4H, m)

central Cu(II) ion as uninegatively charged ligands and did not dissociate in DMSO [37,43]. The conductance values obtained for the copper(II) saccharinate complexes were found to be comparable with previously reported related work [44].

3.5. Electronic spectral studies

The UV/Vis spectra of the complexes in DMSO are given in Table 3. The absorption bands at 259–270 nm and 321–344 nm were attributed to the intra-ligand $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively. A weak DMSO-d_6 transition at 617–650 nm agreed well with the position of the DMSO-d_6 band in the electronic spectra of other related square-pyramidal copper(II) complexes. For example, the electronic spectrum of a five-coordinate copper(II) complex containing the 2-acetylpyridine Schiff base of S-benzyldithiocarbazate whose structure was established by X-ray diffraction, displayed DMSO-d_6 bands at *ca* 640 nm [45]. S → Cu(II) charge transfer bands were also observed at 407–410 nm which supported the coordination of the Schiff base to the Cu(II) ion *via* the thiolate-sulphur atom [46]. This band is common in the electronic spectra of copper(II) complexes of related tridentate NNS thiosemicarbazone ligands [13].

3.6. Crystal and molecular structure of [Cu(sac)(S2AP)]$_2$

The molecular structure of [Cu(sac)(S2AP)]$_2$ is shown in Fig. 2 and selected geometric parameters are collected in Table 5. The Cu(II) atom is coordinated by the thiolate-sulphur, azomethine-nitrogen and pyridyl-nitrogen donors of the uninegative S2AP anion and the saccharinate-nitrogen atom from one anion, and a carbonyl-oxygen atom from a symmetry related saccharinate anion. It is noted the Cu−O1^i interaction of 2.301(2) Å is considerably longer than the other bond lengths about the Cu(II) centre, even the Cu−S bond length, Table 5; symmetry operation i: 2-x, 1-y, 1-z. [Cu(sac)(S2AP)]$_2$ is a centrosymmetric binuclear complex with the Cu(II) centre within a N_3OS donor set; the Cu … Cu^i separation within the dimer is 4.9433(6) Å. The pentacoordinate geometry is based on a square pyramid as indicated by the value of $\tau = 0.03$, cf. 0.0 and 1.0 for ideal square pyramidal and trigonal pyramidal geometries, respectively [7]. In this description, the weakly bound O1^i atom occupies the axial position and the Cu(II) atom lies 0.1973(10) Å above the basal plane defined by the N_3S donor atoms (r.m.s. deviation = 0.0723 Å) in the direction of the O1^i atom. The central eight-membered $\{CuNCO\}_2$ ring is non-planar and is based on a chair conformation with the outer CN links being above and below the central Cu_2O_2 plane. The tridentate ligand comprises two planar regions hinged at the methylene-C8 atom. The 15 non-hydrogen and non-tolyl atoms define one plane (r.m.s. deviation = 0.0288 Å) and the tolyl atoms the other, with the latter orientated almost perpendicular to the first plane: dihedral angle = 75.23(6)°. The planarity of the ligating portion of S2AP is consistent with delocalisation of π-electron density over the backbone of the anion as seen for example in the relatively long and short of the N1−N2 and C2−C3 bond lengths, Table 5. The 10 non-hydrogen and non-sulphonate-oxygen atoms of the saccharinate anion are coplanar (r.m.s. deviation = 0.0352 Å) and this plane forms a dihedral angle of 70.32(4)° with the chelating residue of the S2AP ligand.

The N,O(carbonyl) bridging mode for saccharin is quite unusual in the structural chemistry of copper-saccharinate complexes. A search of the Cambridge Structural Database [8] revealed over 80 hits for copper complexes containing saccharin. Of these, only three featured a bridging mode as observed in [Cu(sac)(S2AP)]$_2$. The first of the structures is formulated as [Cu(sac)(μ-sac)(imidazole)$_2$]$_2$ [9]; this structure has attracted additional crystallographic interest owing to positional disorder associated with the N-bound saccharinate ligands [47]. Only bridging saccharinate anions are found in a structure most closely related to that of [Cu(sac)(S2AP)]$_2$, i.e. [Cu(6-methylpyridine-2-carbaldehyde thiosemicarbazonato)(μ-sac)]$_2$ [44]. While the aforementioned literature structures contain copper in the +2 oxidation state, the third example is a copper(I) complex, i.e. [Ph_3PCu(μ-sac)] [6]. The common feature of the first two cited structures along with that of [Cu(sac)(S2AP)]$_2$ reported herein, is that they are centrosymmetric binuclear complexes. While the central eight-membered ring in [Cu(6-methylpyridine-2-carbaldehyde thiosemicarbazonato)(μ-sac)]$_2$ [44] is as for [Cu(sac)(S2AP)]$_2$, it is strictly planar in [Cu(sac)(μ-sac)(imidazole)$_2$]$_2$ [9]. By contrast, in the copper(I) complex, the central ring is a butterfly with the two pairs of N,O donor atoms lying to one side of the Cu···Cu hinge; the Cu···Cu separation is 2.9978(5) Å.

In the molecular packing, the sulphonate-O atoms play a pivotal role in forming methyl- and pyridyl-C−H…O contacts leading to supramolecular layers in the ab-plane [48]. Connections between layers, along the c-axis, are of the type pyridyl-C−H … π(tolyl) [48] so that a three-dimensional architecture is formed, Fig. 3.

3.7. Cytotoxicity analysis

The cytotoxic activity of the Schiff bases and their mixed-ligand Cu(II) saccharinate complexes are presented in Table 6. Copper is a biologically essential trace element that is present in many proteins and metalloenzymes that are needed for the proper functioning of organs and metabolic processes in the human body.

The Schiff bases in this study were found to be inactive against both the MDA-MB-231 and MCF-7 cell lines but all three of the mixed-ligand Cu(II) complexes demonstrated cytotoxic activity against the two breast cancer cells lines tested. The IC_{50} values were lower than that of the positive control, Tamoxifen. In addition, the Cu(II) saccharinate salt itself was found to be potent against both the cell lines tested although not as potent as the mixed-ligand Cu(II) complexes.

These observations were consistent with previously published data [49] where it was reported that the cytotoxicity of metal complexes that contained sulphur-nitrogen tridentate chelates were found to be enhanced upon complexation. Besides the mixed-ligand complexes reported in this work, similar mixed-ligand

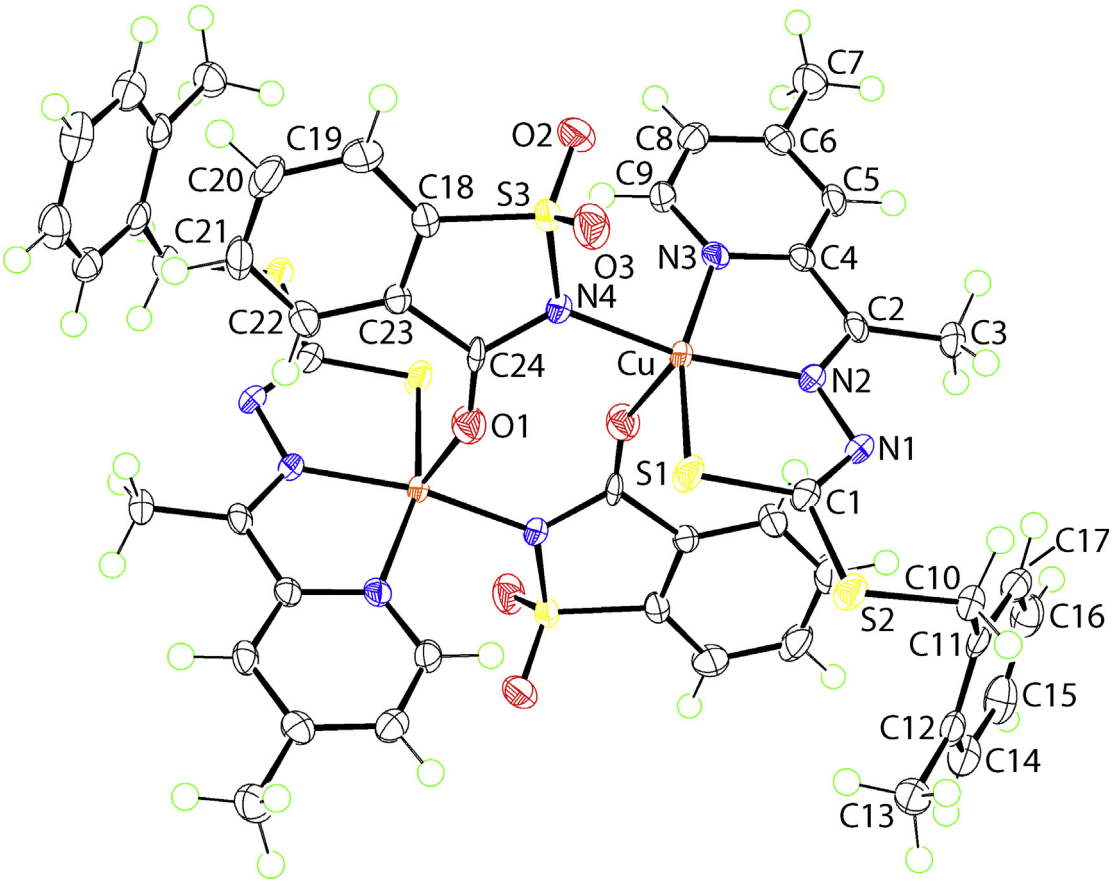

Fig. 2. Molecular structure of $[Cu(sac)(S2AP)]_2$ showing atom labelling and 70% probability displacement ellipsoids. Unlabelled atoms are related by the symmetry operation i: 2-x, 1-y, 1-z.

Table 5
Selected bond lengths (Å) and bond angles (°) for $[Cu(sac)(S2AP)]_2$.

Bond lengths		Bond angles	
Cu–S1	2.2711(8)	S1–Cu–N2	83.86(7)
Cu–N2	1.978(2)	S1–Cu–N3	162.60(7)
Cu–N3	2.042(2)	S1–Cu–N4	95.76(7)
Cu–N4	2.000(2)	S1–Cu–O1^{i}	102.54(6)
Cu–O1^{i}	2.301(2)	N2–Cu–N3	79.29(9)
S1–C1	1.733(3)	N2–Cu–N4	164.44(9)
S2–C1	1.749(3)	N2–Cu–O1^{i}	102.59(8)
N1–C1	1.309(4)	N3–Cu–N4	99.29(9)
N1–N2	1.391(3)	N3–Cu–O1^{i}	85.50(8)
C2–N2	1.298(3)	N4–Cu–O1^{i}	92.69(8)
C2–C3	1.471(4)		

Symmetry operation i: 2-x, 1-y, 1-z.

copper(II) complexes containing imidazole have also been reported to have potent anticancer activity. The tridentate Schiff base derived from salicylaldehyde and thiosemicarbazide was found to be inactive against both MCF-7 and MDA-MB-231 cancer cells but the mixed ligand Cu(II) complex containing the Schiff base and imidazole had notably enhanced cytotoxicity [50]. The enhanced bioactivities of metal complexes as compared to free ligands can be due to the chelation effect, whereby metal ions bind with multi-donor chelating ligands to create compounds that are more lipophilic and enter the cell more easily as compared to the chelating ligand itself [51].

Complexes derived from thiosemicarbazide ligands, $[Cu(sac)(MT2AP)]_2$ and $[Cu(sac)(ET2AP)]_2$ were found to have better cytotoxic activity when compared to the complex derived from the dithiocarbazate ligand, $[Cu(sac)(S2AP)]_2$. This could be due to the presence of an extra benzene ring in the Schiff base, S2APH. The bulkier S2APH ligand is expected to result in greater steric hindrance, which hampers the creation of available sites for association with biological targets in the cancer cells [37]. The planar portions of Cu(II) complexes also create a higher potency to interact effectively with the DNA of the cancer cells leading in turn to an enhanced cytotoxicity [18].

The complex with the methyl-substituted ligand, MT2AP was found to be slightly more cytotoxic than that of the ethyl-substituted ligand, ET2AP. The presence of a smaller hydrophobic methyl group is likely to promote the interaction of the complex with the lipophilic plasma membrane that will facilitate entry into the aqueous cytoplasm of the cell; thus reaching the site of action more efficiently [52]. Furthermore, by being smaller in size, the steric hindrance is reduced thus allowing the complex to be more effective in entering the cells and causing cell death [53].

It was also found that the N,O-bonded saccharinate complex, $[Cu(sac)(S2AP)]_2$ had an overall increased cytotoxicity as compared to related N-bonded saccharinate complexes [37]. A copper(II) complex containing an N-bonded saccharin and a tridentate ligand derived from 2-acetylpyridine Schiff base of S-benzyldithiocarbazate, [Cu(sac)(NNS)] showed no significant cytotoxicity against MCF-7 cells [37].

4. Conclusions

The reaction of S-2-methylbenzyldithiocarbazate, 4-methyl-3-

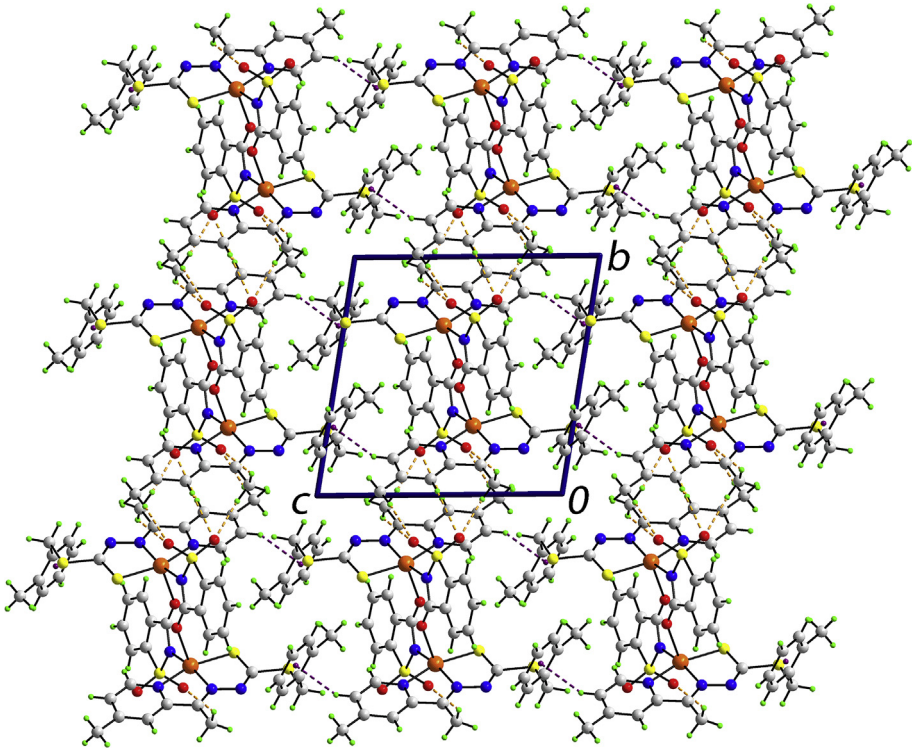

Fig. 3. A view in projection down the a-axis of the molecular packing in $[Cu(sac)(S2AP)]_2$. The C—H···O and C—H … π interactions are shown as orange and purple dashed lines, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Cytotoxic activities of the Schiff bases and their copper(II) saccharinate complexes.

Compound	IC_{50} (μM)	
	MCF-7[a]	MDA-MB-231[b]
S2APH	>5.0	>5.0
$[Cu(sac)(S2AP)]_2$	3.60	3.81
MT2AP	>5.0	>5.0
$[Cu(sac)(MT2AP)]_2$	0.30	0.41
ET2AP	>5.0	>5.0
$[Cu(sac)(ET2AP)]_2$	0.33	0.46
$[Cu(sac)_2(H_2O)_4]\cdot 2H_2O$	2.50	4.53
Tamoxifen (Positive Control)	4.04	13.46

IC_{50} values < 0.5 μM indicate that the complex is strongly active, whereas IC_{50} values of 0.5—5.0 μM and >5.0 μM indicate that the complex is moderately active and inactive, respectively.

[a] MCF-7 (estrogen receptor-positive human breast carcinoma).

[b] MDA-MB-231 (estrogen receptor-negative human breast carcinoma).

thiosemicarbazide and 4-ethyl-3-thiosemicarbazide with 2-acetylpyridine and 2-acetyl-4-methylpyridine yielded three tridentate Schiff bases which were reacted with Cu(II) saccharinate to form three new mixed-ligand Cu(II) saccharinate complexes. The Schiff bases coordinated to the Cu(II) ion as uninegatively charged tridentate NNS ligands. Crystallography showed that in one of the complexes, $[Cu(sac)(S2AP)]_2$, the saccharinate anion was bidentate bridging, binding *via* its carbonyl-oxygen and its imino-nitrogen leading to a centrosymmetric dimer with a distorted square-pyramidal geometry for the Cu(II) atom. All the complexes had enhanced cytotoxicity against MCF-7 and MDA-MB-231 cancer cells as compared to the Schiff bases alone. This was postulated to be due to several factors including the chelation effect, the planarity of the complexes and also that metal ions bound to multidonor chelating ligands create compounds that are more lipophilic thus enabling easier access into the cancer cells.

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Appendix A. Supplementary material

CCDC 1474933 contains the supplementary crystallographic data for $[Cu(sac)(S2AP)]_2$. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

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C8–H8…π(C9-C14) = 2.60 Å, C8…π(C11,C12-C14-C17)^{ii} = 3.454(3) Å with angle at H8 = 149°. Symmetry operation ii: 2-x, -y, 1-z; iii: 1+x, y, z; iv: x, y, 1+z.

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