Copper(II) complexes of coumarin-derived Schiff bases and their anti-*Candida* activity

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ABSTRACT

The condensation of 7-amino-4-methyl-coumarin (1) with a number of substituted salicylaldehydes yielded a series of Schiff bases (2a–2k) in good yields. Subsequent reaction of these ligands with copper(II) acetate yielded Cu(II) complexes (3a–3k) and some were characterised using X-ray crystallography. All of the free ligands and their metal complexes were tested for their anti-*Candida* activity. A number of the ligands and complexes exhibited anti-*Candida* activity comparable to that of the commercially available antifungal drugs, ketoconazole and Amphotericin B.

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1. Introduction

Fungal pathogens represent the major eukaryotic agents of serious infection in European countries [1]. Infections due to *Candida albicans* and *Aspergillus fumigatus* are the most common and clinically important pathogens and indeed *Candida* now ranks as the fourth most common cause of nosocomial bloodstream infections [2]. In some cases, such as patients with malignant haematological disease and in bone-marrow transplant recipients, candidosis is the most common invasive fungal infection [3]. The development of azole-based antifungal drugs has considerably impacted on the fight against fungal infections, but the necessity to use high doses or combinations of drug therapies results in considerable side effects in patients and resistance to these drugs has also been reported [4–6]. Indeed, the repertoire of available antifungal chemotherapeutic agents is inadequate to treat life-threatening infections that are characterised by morbidities that exceed those due to the most important bacterial and viral diseases [7–13]. Therefore, there is an urgent need to generate new, efficacious, non-toxic compounds with broad-spectrum antifungal activity.

Derivatives of coumarin are known to possess significant antifungal as well as antibacterial properties, and there are a number of commercially available coumarin-based antibiotics such as Novobiocin, Clorobiocin and Coumermycin A_1. Many of the coumarins present in plants, and also their synthetic analogues, have been reported to be good antifungal and antibacterial agents [14–21]. Preliminary structure–activity relationship studies have shown that the presence of hydroxyl or carboxylic groups on the coumarin nucleus are necessary for antimicrobial activity [22]. We have previously published a number of studies on both carboxylate- and hydroxy-substituted coumarin ligands, and while the ligands themselves were not active against fungal species a number of their Ag(I) and Cu(II) complexes showed good antimicrobial activity [23–25]. Indeed, the silver carboxylate complexes showed excellent activity against MRSA but they were less promising antifungal agents [24]. A series of Cu(II) coumarin dioxyacetic acetate complexes displayed promising antibacterial activity but also showed reduced activity against fungal strains [25].

Schiff bases are a class of compound which is known to exhibit antifungal activity [26,27]. Schiff bases derived from 5-nitrosalicyl-aldehyde and amines, such *o*- and *p*-aminophenols, were prepared by Murthy et al. [28] and a number of metal complexes of the resulting ligands were tested for their antibacterial activity. Raman et al. have recently reviewed a series of transition metal complexes of Schiff bases derived from 4-aminoantipyrine and reported their

antimicrobial activity [29]. These workers found that the complexes were active against bacterial strains but not particularly effective against *C. albicans.* Coumarin-derived Schiff bases are well known compounds and several reports have been written about their applications as dye and fluorescent agents [30,31]. Iminocoumarins have also been shown to exhibit anti-inflammatory, antibacterial and antifungal activities [32–35].

Cu(II) complexes of some organic drugs have been the subject of a number of studies aimed at establishing the presumed synergy between the Cu(II) ion and the drug [36–39]. In more recent years there have been numerous reports highlighting the significant biological activity of Cu(II) Schiff base complexes [40–46]. Patil et al. detailed the preparation of Co(II), Ni(II) and Cu(II) complexes with 1,2,4-triazole-derived coumarin Schiff bases and assessed their biological activity [47]. In this work, we have prepared a series of coumarin-derived Schiff bases and their Cu(II) complexes and the anti-*Candida* activity of the metal-free ligands and the complexes were assessed. Included, are the first crystal structures of Cu(II) Schiff-base coumarin complexes.

2. Experimental

2.1. Materials/instrumentation

All chemicals purchased from Sigma–Aldrich were reagent grade and used without purification. Infrared spectra were recorded in the region of 4000–400 cm^{-1}, on a Nicolet Impact 410 Fourier-Transform Infrared spectrophotometer using Omnic software. Melting point values were measured using a Stuart scientific SMP1 melting point apparatus (up to 320 °C). ^1H NMR spectra were recorded in the region of −5 to 15 ppm from TMS with a resolution of 0.0006 ppm. ^{13}C NMR spectra were recorded in the region −33 to 233 ppm from tetramethyl silane (TMS) with a resolution of 0.008 ppm. All of the NMR spectra were run on a JEOL JNM-LA300 FT–NMR (300 MHz ^1H and 75 MHz ^{13}C) in d$_6$-DMSO. Atomic absorption spectroscopy (AAS) measurements were taken on a Perkin–Elmer 460 AAS instrument (emission wavelength 324.8 nm). UV–visible (UV–vis) spectra were recorded on a Hitachi U-2001 Spectrophotometer. Microanalytical data were provided by the Microanalytical Laboratory, National University of Ireland Dublin, Belfield, Dublin 4. Solid state magnetic susceptibility measurements were carried out at room temperature using a Johnson Matthey Magnetic Susceptibility Balance with Hg[Co(SCN)4] being used as a reference standard.

2.2. Synthesis of 7-amino-4-methylcoumarin (**1**)

7-Amino-4-methyl-coumarin (**1**) was synthesised *via* a one step von Pechmann reaction using a modified literature procedure [48].
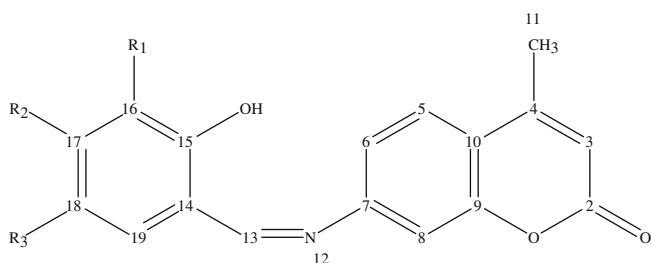

Fig. 1. Structure of the Schiff bases (**2a–2k**) showing the numbering system used in the assignment of ^1H and ^{13}C NMR spectra.

Table 1
Starting aldehyde and identification of functional groups on Schiff bases **2a–2k**.

Starting aldehyde	Schiff base	R_1	R_2	R_3
2-Hydroxybenzaldehyde	**2a**	–H	–H	–H
2,3-Dihydroxybenzaldehyde	**2b**	–OH	–H	–H
2,4-Dihydroxybenzaldehyde	**2c**	–H	–OH	–H
2-Hydroxy-3-methoxybenzaldehyde	**2d**	–OCH$_3$	–H	–H
2-Hydroxy-3-ethoxybenzaldehyde	**2e**	–OCH$_2$CH$_3$	–H	–H
2-Hydroxy-4-methoxybenzaldehyde	**2f**	–H	–OCH$_3$	–H
3,5-Dichloro-2-hydroxybenzaldehyde	**2g**	–Cl	–H	–Cl
3,5-Dibromo-2-hydroxybenzaldehyde	**2h**	–Br	–H	–Br
3,5-Diiodo-2-hydroxybenzaldehyde	**2i**	–I	–H	–I
2-Hydroxy-5-nitrobenzaldehyde	**2j**	–H	–H	–NO$_2$
2-Hydroxy-3-methoxy-5-nitrobenzaldehyde	**2k**	–OCH$_3$	–H	–NO$_2$

2.3. Synthesis of Schiff base ligands

All of the Schiff bases **2a–2k** (Fig. 1 and Table 1) were synthesised using the following general procedure. A solution of the appropriate aldehyde (3 mmol) in ethanol (10 mL) was added slowly to a solution of **1** (0.52 g, 3 mmol) in ethanol (50 mL) (see Table 2). Glacial acetic acid (0.25 mL) was then added and solution was heated under reflux for 2 h. After cooling, the product was isolated by filtration, washed with cold methanol and allowed to dry in air. The Schiff bases **2a–2g** were recrystallised from ethanol (100 mL). Assignments of NMR spectra of the ligands are based on the numbering scheme shown in Fig. 1. Substituents R_1, R_2, and R_3 of the aldehyde moiety are given in Table 1. All of the ligands were fully characterised by ^1H, ^{13}C NMR, IR, and UV–vis spectroscopies as well as by melting point and elemental analysis (Tables 3–6).

Table 2
Crystal data and structure refinement for the complexes **3a** and **3h**.

Complex	3a	3h
Empirical formula	C$_{68}$H$_{48}$Cu$_2$N$_4$O$_{12}$	C$_{34}$H$_{20}$Br$_4$CuN$_2$O$_6$
Formula weight	1240.18	935.70
Temperature	89(2) K	89(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Triclinic	Monoclinic
Space group	$P-1$	$P2_1/n$
Unit cell dimensions	$a = 8.9076(5)$ Å	$a = 12.2159(18)$ Å
	$\alpha = 103.436(3)°$	$\alpha = 90°$
	$b = 12.2384(7)$ Å	$b = 9.5253(13)$
	$\beta = 102.099(3)°$	$\beta = 92.623(8)°$
	$c = 13.5807(8)$ Å	$c = 27.093(4)$ Å
	$\gamma = 105.233(3)°$	$\gamma = 90°$
Volume	1330.35(13) Å^3	3149.2(8) Å^3
Z	1	4
Density (calculated)	1.548 Mg/m^3	1.974 Mg/m^3
Absorption coefficient	0.875 mm^{-1}	5.820 mm^{-1}
Crystal size	0.52 × 0.06 × 0.06 mm^3	0.40 × 0.10 × 0.02 mm^3
Index ranges	−11 <= h <= 11	−15 <= h <= 15
	−15 <= k <= 15	−11 <= k <= 12
	−16 <= l <= 33	−33 <= l <= 33
Theta range for data collection	1.81–26.42°	1.50–27.12°
Reflections collected	34,790	44,071
Independent reflections	5452 [R(int) = 0.0353]	6419 [R(int) = 0.1314]
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	5452/0/390	6419/0/426
Goodness-of-fit on F^2	1.038	1.150
Final R indices [$I > 2\text{sigma}(I)$]	$R_1 = 0.0261$, w$R_2 = 0.0635$	$R_1 = 0.0604$, w$R_2 = 0.1340$
R indices (all data)	$R_1 = 0.0317$, w$R_2 = 0.0661$	$R_1 = 0.1058$, w$R_2 = 0.1587$
Largest diff. peak and hole	0.322 and −0.359 e.Å^{-3}	1.620 and −1.115 e.Å^{-3}

Table 3
Physical data for the Schiff base ligands **2a–2k** and their corresponding Cu(II) complexes **3a–3k**.

Compound	Mw (g/mol)	μ_{eff} (B.M.)	Yield (%)	Empirical formula	Found (calc) (%)				m.p. (°C)	Λ_M (S cm^2/mol)
					C	H	N	Cu		
2a	279.29	–	78	$C_{17}H_{13}NO_3$	73.34 (73.13)	4.77 (4.66)	5.05 (5.01)	10.3 (10.0)	178–180	–
3a	1240.18	1.63(2.28*)	80	$C_{34}H_{24}CuN_2O_8$	65.31 (65.85)	3.77 (3.90)	4.38 (4.52)		230–232	10.71
2b	295.3	–	89	$C_{17}H_{13}NO_4$	68.87 (69.15)	4.34 (4.44)	4.78 (4.74)	9.7 (10.5)	238–240	–
3b	652.11	1.77	83	$C_{34}H_{24}CuN_2O_8$	62.49 (62.62)	3.70 (3.71)	4.15 (4.30)		300–304	21.4
2c	295.3	–	60	$C_{17}H_{13}NO_4$	68.62 (69.17)	4.40 (4.44)	4.67 (4.74)	8.35 (8.6)	219–220	–
3c	652.09	1.81	76	$C_{34}H_{24}CuN_2O_8$	59.25 (59.26)	3.63 (3.93)	3.52 (3.64)		>320	8.45
2d	309.18	–	92	$C_{18}H_{15}NO_4$	69.48 (69.8)	4.71 (4.89)	4.43 (4.53)	9.3 (10.0)	200–202	–
3d	680.16	1.97	70	$C_{36}H_{28}CuN_2O_8$	63.26 (63.57)	4.14 (4.15)	3.81 (4.12)		304–306	3.68
2e	323.36	–	77	$C_{19}H_{17}NO_4$	70.40 (70.58)	5.33 (5.30)	4.48 (4.33)	9.0(7.6)	150–152	–
3e	708.21	–	37	$C_{38}H_{32}CuN_2O_8$	62.67 (64.44)	4.95 (4.55)	3.85 (3.96)		232–238	–
2f	309.32	–	86	$C_{18}H_{15}NO_4$	69.81 (69.89)	4.77 (4.89)	4.30 (4.53)	9.3 (8.9)	194–197	–
3f	680.16	1.98	86	$C_{36}H_{28}CuN_2O_8$	62.99 (60.19)	4.15 (4.29)	3.72 (3.51)		>320	2.59
2g	348.18	–	66	$C_{17}H_{11}NO_3Cl_2$	58.37 (58.64)	3.17 (3.18)	3.52 (4.02)	8.4 (8.5)	260–262	–
3g	757.89	1.76	94	$C_{34}H_{20}Cl_2CuN_2O_6$	53.76 (53.88)	2.75 (2.66)	3.73 (3.70)		>320	14.9
2h	437.08	–	96	$C_{17}H_{11}NO_3Br_2$	46.82 (46.71)	2.55 (2.54)	3.01 (3.20)	6.8 (6.5)	270–273	–
3h	935.69	1.76	91	$C_{34}H_{20}Br_2CuN_2O_6$	43.44 (43.64)	2.27 (2.15)	2.98 (2.99)		316–318	15.32
2i	531.08	–	97	$C_{17}H_{11}NO_3I_2$	38.37 (38.45)	2.04 (2.09)	2.64 (2.64)	5.67 (4.9)	274–276	–
3i	1123.69	2.18	81	$C_{34}H_{20}I_2CuN_2O_6$	36.17 (36.34)	1.93 (1.79)	2.33 (2.49)		304–306	9.92
2j	324.29	–	81	$C_{17}H_{12}N_2O_5$	63.27 (62.96)	3.58 (3.73)	8.82 (8.64)	9.0 (8.6)	310–312	–
3j	710.11	1.76	95	$C_{34}H_{22}CuN_4O_{10}$	56.28 (57.51)	3.17 (3.12)	7.53 (7.89)		>320	7.19
2k	354.31	–	91	$C_{18}H_{14}N_2O_6$	59.28 (61.02)	3.91 (3.98)	7.67 (7.91)	7.1 (7.3)	292–294	–
3k	770.16	1.73	94	$C_{36}H_{26}CuN_4O_{12}$	55.20 (56.14)	3.59 (3.40)	7.12 (7.27)		318–320	21.1

* Value for 2 Cu(II) atoms.

Table 4
Characteristic ^1H NMR signals (ppm), peak multiplicity and coupling constants J (Hz) for the Schiff base ligands recorded in d$_6$-DMSO.

Schiff base ligand	H$_3$(vinyl)	H$_{11}$(CH$_3$)	H$_{13}$(CH=N)	H$_{15}$(OH)	R$_1$	R$_2$	R$_3$
2a	6.39, s	2.44, s	9.03, s	12.63, s	6.99, d, $J = 8.43$	7.47, t, $J = 17.3$	7.01, t, $J = 16.65$
2b	6.37, s	2.45, s	9.01, s	12.69, s	9.32, s (–OH)	6.99, dd, $J = 9.54$	6.82, t, $J = 7.68$
2c	6.31, s	2.27, s	8.90, s	13.13, s	6.36, s	10.42, s (–OH)	6.44, d, $J = 10.42$
2d	6.34, s	2.40, s	9.01, s	12.54, s	3.89, s (–OCH$_3$)	7.16, dd, $J = 7.39$	6.93, t, $J = 15..75$
2e	6.38, s	2.45, s	9.04, s	12.81, s	4.09, q (–OCH$_2$–)1.36, t (–CH$_3$)	7.15, dd, $J = 8.05$	6.90, t, $J = 15.72$
2f	6.32, s	2.44, s	8.93, s	13.13, s	6.56, s	3.83, s (–OCH$_3$)	6.60, d, $J = 2.37$
2g	5.90, s	1.73, s	8.39, s	8.10, s	–Cl	na*	–Cl
2h	5.88, s	2.44, s	9.06, s	8.10, s	–Br	na*	–Br
2i	6.42, s	2.50, s	9.61, s	9.88, s	–I	na*	–I
2j	5.90, s	2.27, s	8.72, s	9.19, s	na*	na*	–NO$_2$
2k	6.41, s	2.25, s	8.34, s	9.22, s	8.87, s (–OCH$_3$)	na*	–NO$_2$

s – singlet, d – doublet, t – triplet, dd – double doublet, q – quatet.
* na – not assigned as signals overlapped.

Table 5
Characteristic ^{13}C NMR signals (ppm) for the Schiff base ligands recorded in d$_6$-DMSO.

Schiff base ligand	C2 (lactone)	C3 (vinyl)	C4	C7	C9	C10	C11 (methyl)	C13 (imine)	C14	C15 (phenol)
2a	159.80	113.63	153.0	152.95	151.41	119.27	18.10	165.08	118.08	160.29
2b	159.82	113.63	153.88	152.96	151.94	118.09	18.12	165.62	119.38	149.36
2c	159.89	108.11	153.01	153.97	151.51	117.54	18.11	164.23	112.03	163.19
2d	154.93	108.92	149.24	143.38	na	113.41	13.24	160.27	114.75	148.06
2e	154.79	113.65	152.95	153.86	151.07	118.14	18.11	165.36	119.25	150.88
2f	159.78	113.41	152.88	154.04	151.36	117.77	18.03	164.12	113.09	163.25
2g	160.9	112.5	152.8	na	151.50	na	21.20	160.10	157.90	na
2h	na	94.01	na	na	na	na	25.78	158.45	na	na
2i	na	94.01	na	na	na	na	25.78	160.10	na	na
2j	160.9	112.5	152.59	152.8	na	na	21.20	167.2	na	na
2k	158.95	152.8	158.79	153.8	na	na	17.36	162.36	156.2	na

na – not assigned.

2.4. Synthesis of Cu(II) complexes (**3a–3k**)

The Cu(II) complexes were prepared by the following general method: 2.4 mmol of the appropriate ligand (**2a–2k**) was dissolved in ethanol (40 mL) and a solution of copper(II) acetate (0.22 g, 1.2 mmol) in ethanol (10 mL) was added. The resulting mixture was heated under reflux for up to 4 h. After cooling, the precipitated product was collected by filtration, washed with water and dried in air. **3a** and **3h** were recrystallised by vapour diffusion of ethanol into acetonitrile and dimethylformamide (DMF), respectively. Despite repeated attempts to recrystallise the other Cu(II) complexes good quality crystals of the remaining complexes were not obtained.

Table 6
Selected IR data (cm^{-1}) for the Schiff base ligands **2a–2k**.

Schiff base	ν(CH=N)	ν(C=O)	ν(C–O)	ν(O–H)
2a	1619	1710	1270	3435
2b	1606	1722	1237	3289
2c	1599	1688	1239	3243
2d	1598	1728	1251	3248
2e	1599	1727	1246	3432
2f	1610	1733	1250	3437
2g	1596	1727	1269	3435
2h	1591	1727	1224	3430
2i	1580	1716	1325	3431
2j	1602	1720	1277	3432
2k	1599	1716	1268	3432

2.5. X-ray crystallography

X-ray crystallographic studies were performed at the University of Otago, New Zealand on a Bruker Kappa Apex II diffractometer with a CCD area detector. All of the crystal structures were solved using SHELXS-97. All structures were refined against F^2 using all data by full-matrix least-squares techniques with ShelX-86 and SHELXL-97 [49,50]. Details of the data collections, solutions and refinements for **3a** and **3h** are given in Table 2. CCDC numbers: 707548 and 707549 for **3a** and **3h**, respectively.

### 2.6. Anti-Candida susceptibility testing

The ligands, complexes and commercially available drugs, ketoconazole and Amphotericin B, were tested against a clinical isolate of the fungal strain, *C. albicans* (ATCC 10231). The anti-*Candida* activities were determined using a broth microdilution susceptibility protocol (NCCLS) [51]. The screening protocol included the following steps: Isolates were grown for 24 h on Sabouraud dextrose agar (SDA) plates at 37 °C. Cell suspensions were prepared in sterile phosphate buffered saline (PBS) to a density equal to a 1.0 McFarland standard yielding a concentration of 1×10^6 cells/mL and then further diluted (1:100) with minimal media. Ligands, complexes and the commercial drug were prepared as 1% solutions or suspensions where appropriate in DMSO. All compounds were tested at a range of concentrations of 20, 10, 5 and 1 µg/mL, and the plates were incubated for 24 h at 37 °C. Each compound was assessed in triplicate and three independent experiments were performed. As most of the synthesised complexes were only soluble in DMSO, the effect of the solvent on the growth of *C. albicans* was also studied.

3. Results and discussion

3.1. Synthesis and characterisation of the Schiff base ligands (**2a–2k**)

Condensation of the amino coumarin **1** with a number of substituted salicylaldehydes yielded a series of Schiff bases in good yield (Table 3). The products were obtained by refluxing ethanolic solutions of starting materials in the presence of a catalytic amount of acetic acid (Scheme 1). Physical data and empirical formulae are presented in Table 3. All of the ligands had good solubility in a range of organic solvents.

The ^{1}H- and ^{13}C NMR spectra of the ligands were recorded in d$_6$-DMSO and the data are reported in Tables 4 and 5. The most characteristic signal in the ^{1}H NMR spectrum of each Schiff base was due to the imine hydrogen singlet found in the range 8.8–9.2 ppm. The singlet for the phenolic proton of the *ortho*-hydroxy group was present in the range 10–13 ppm, the downfield shift resulting from the intramolecular hydrogen bonding to the imine nitrogen. If the Schiff base ligands contained other hydroxyl sub-
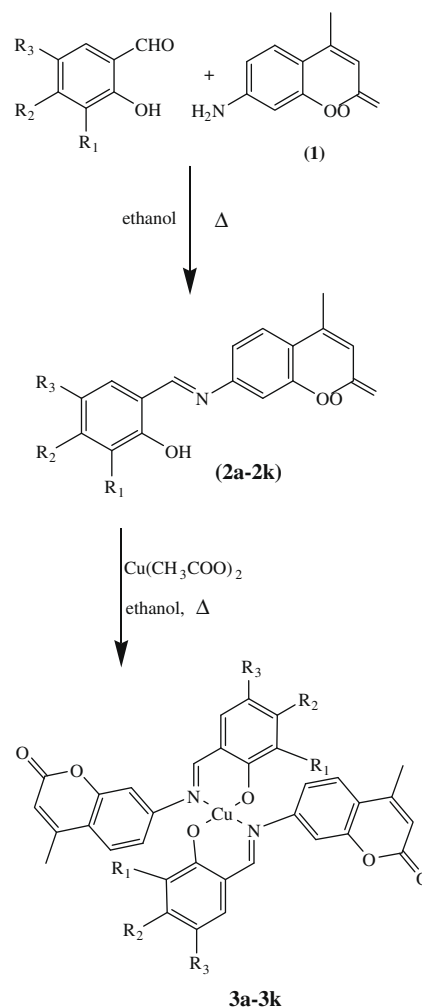
Scheme 1. Synthesis of coumarin-derived Schiff base ligands (**2a–2k**) and their corresponding Cu(II) complexes (**3a–3k**) (proposed structure of the complexes **3b–3k**).

stituents, then additional singlets appear in the range 9–11 ppm. Where possible, the ^{13}C NMR signals of the Schiff base ligands were assigned with the help of CHCHiFt cross peaks and DEPT experiments.

As expected the IR spectra of the Schiff base ligands exhibited a strong $\nu_{C=N}$ stretching vibration in the range 1619–1591 cm^{-1} (Table 6). The medium intensity bands in the region 1224–1277 cm^{-1} were assigned to the phenolic ν_{C-O} stretch. A broad band at ca. 3400 cm^{-1} was assigned to the ν_{OH} stretch. The breadth of this band can be explained as the effect of intramolecular hydrogen bonding between the imine nitrogen and the hydrogen from the *ortho*-hydroxyl group [52,53].

The UV–vis spectra of the Schiff bases contained hypochromic absorption bands in the ultraviolet region of the spectra corresponding to the $\pi \rightarrow \pi^*$ transitions of the aromatic rings and of the conjugated system arising from the imine and coumarin moieties. Low intensity, broad bands, with absorption maxima at ca. 430 nm, were assigned to forbidden $n \rightarrow \pi^*$ transitions associated with the azomethine group [30,52,53].

3.2. Synthesis and characterisation of the Cu(II) complexes (**3a–3k**)

The appropriate ligand (**2a–2k**) was reacted with copper(II) acetate in a ratio of 2 ligand:1 Cu(II) in refluxing ethanol to give the corresponding copper(II) complexes (**3a–3k**) (Scheme 1). With

the exception of **3e** and **3f** the microanalytical data corresponded to an empirical formulation of 1 Cu^{2+} and 2 deprotonated Schiff base ligands (Table 3). Somewhat unsatisfactory results for several other complexes (**3b**, **3d**, **3i**, **3j** and **3k**) may well be due to the poor solubility of these compounds generally which made recrystallisation of the complexes difficult. Despite repeated attempts to prepare a pure Cu(II) complex of **2e**, we were unable to do so and no antimicrobial screening of **3e** was carried out. Most of the resulting complexes were soluble only in DMSO or DMF. The poor solubility of complexes (**3d**, **3g**, **3j** and **3k**) prevented the recording of reliable UV–vis and conductivity measurements and it also meant that the antimicrobial screening of these complexes had to be carried out as suspensions.

3.2.1. IR spectra of Cu(II) complexes

In the IR spectra of almost all the complexes the ν_{CN} stretching vibration associated with the imine functional group of the ligand was shifted to a lower wavelength compared to that in the corresponding free ligand, indicating that coordination to the Cu(II) ion occurs *via* the imine nitrogen (Table 7) [54,55]. The $\nu_{C=O}$ stretching vibration of the carbonyl function in the lactone ring of the free ligands appeared as a strong sharp band in the range 1688–1733 cm^{-1}. In the spectrum of most of the complexes, the position of this band remained largely unchanged, suggesting that the lactone carbonyl oxygen is not involved in coordination to the metal [56]. Upon complexation, the phenolic $\nu_{C–O}$ stretching vibration shifted to lower frequency, suggesting coordination of this oxygen atom. Several new bands present in the region 400–600 cm^{-1} in the spectra of the complexes were assigned to $\nu_{Cu–N}$ and $\nu_{Cu–O}$ stretching vibrations [52–55].

3.2.2. UV–vis spectra of Schiff bases and their Cu(II) complexes

UV–vis spectra of only two complexes could be reliably recorded over the full range due to solubility problems. Compared to the UV–vis spectra of the free ligands, significant changes in the wavelengths of absorption maxima were observed in those of the corresponding complexes (Table 8). All the spectra of the complexes contained broad bands in the UV region, with tailing into the visible region. The high energy bands at ca. $\lambda = 340$ nm were assigned to a ligand to metal change transfer (LMCT) transition, and the lower energy band in the region of ca. $\lambda = 420$ nm were assigned to a metal to ligand change transfer (MLCT) transition [52]. A low intensity absorption band in the visible region of the spectra of concentrated solutions of **3b** and **3c** correspond to a $d \rightarrow d^*$ transition, but solubility problems at high concentrations meant that the extinction coefficients at these wavelengths of other compounds are probably not accurate.

3.2.3. Magnetic properties of Cu(II) complexes

Values of magnetic susceptibility (μ_{eff}) for the Cu(II) complexes are given in Table 3. A mononuclear Cu(II) structure is assigned to

Table 7
Selected IR data (cm^{-1}) for the Cu(II) complexes **3a–3k**.

Complex	$\nu(CH=N)$	$\nu(C=O)$	$\nu(C–O)$
3a	1607	1697	1266
3b	1592	1719	1264
3c	1590	1701	1233
3d	1590	1725	1245
3e	1586	1727	1219
3f	1592	1726	1236
3g	1598	1735	1263
3h	1592	1734	1219
3i	1588	1735	1313
3j	1600	1719	1248
3k	1595	1719	1257

Table 8
Selected UV–vis data for the Schiff base ligands and their Cu(II) complexes recorded in DMSO.

Schiff base		Complex	
λ_{max} (nm)	ε ($M^{-1}\,cm^{-1}$)	λ_{max} (nm)	ε ($M^{-1}\,cm^{-1}$)
(**2a**)		(**3a**)	
300	65,520	343	129,000
353	37,650	422	51,400
(**2b**)		(**3b**)	
290	63,000	339	135,600
354	36,030	421	44,900
		686	1090
(**2c**)		(**3c**)	
280	96,930	351	59,650
354	112,300	436	67,870
		672	1900

complexes **3b–3k** as the μ_{eff} value for these complexes varied from 1.73 to 2.18 B.M., and is characteristic for this type of Cu(II) complex [57–63]. The μ_{eff} value for complex **3a** was 1.63 B.M. and is characteristic of a binuclear complex in which there is anti-ferromagnetic coupling between the Cu(II) centres [59,60].

3.2.4. Molar conductivity

Determination of molar conductivities was carried out on those complexes that had good solubility in DMSO and the results obtained are given in Table 3. The molar conductivity values determined for the complexes were in the range of 2.50–21.4 S cm^2/mol, and were consistent with those recorded for other Cu(II) Schiff base complexes and would suggest their non-electrolytic character [64,65].

3.2.5. Crystal structure analysis

The structures of the copper(II) complexes **3a** and **3h** were determined by X-ray crystallography. The structure of complex **3a** is shown in Fig. 2 and to our knowledge this is a first example of a binuclear Cu(II) complex of a coumarin Schiff base. Selected bond distances and angles are given in Table 9. The complex **3a** is centrosymmetric and the coordination about each Cu(II) centre is completed with one imine nitrogen and one phenolic oxygen from a single Schiff base ligand, a bridging phenolic oxygen from an additional ligand which also provides a further imine nitrogen to coordinate singly to the metal centre. The final coordination site for each Cu(II) ion is filled by a phenolic oxygen atom from the second bridging Schiff base ligand. Therefore, each Cu(II) centre is bound to three Schiff base ligands with a different coordination mode to each ligand. Each Cu(II) ion is primarily in a coordination environment that may be considered as intermediate between distorted trigonal bipyramidal and a square pyramidal [66,67]. The copper ions are bridged by the two phenolic oxygen atoms with a short copper–copper distance of 2.61 (5) Å and a Cu–O6–Cu angle of 103.69 (5)°. The rigid structure of the aromatic rings of *ortho*-substituted phenolic moieties usually favours square planar or square pyramidal coordination modes but the bulkiness of the ligand in this case causes a distortion of the trigonal bipyramidal structure. The known binuclear complex of *bis*(N-phenyl-5-chloro-salicylideneaminato)copper(II), has a coordination geometry around the Cu(II) ion which is similar to that of **3a** [68].

The crystal structure of **3h** (Fig. 3) shows a mononuclear Cu(II) centre bound to two Schiff base ligands. The bond lengths and angles (Table 9) suggest a distorted square planar coordination environment for the metal [67]. Coordination of the Cu(II) centre to both ligands is *via* the imine nitrogen and phenolic oxygen atoms. Bond lengths between the Cu(II) centre and the donor atoms are consistent with previously reported compounds of similar structures [54,67]. There is no centre of symmetry in the molecule
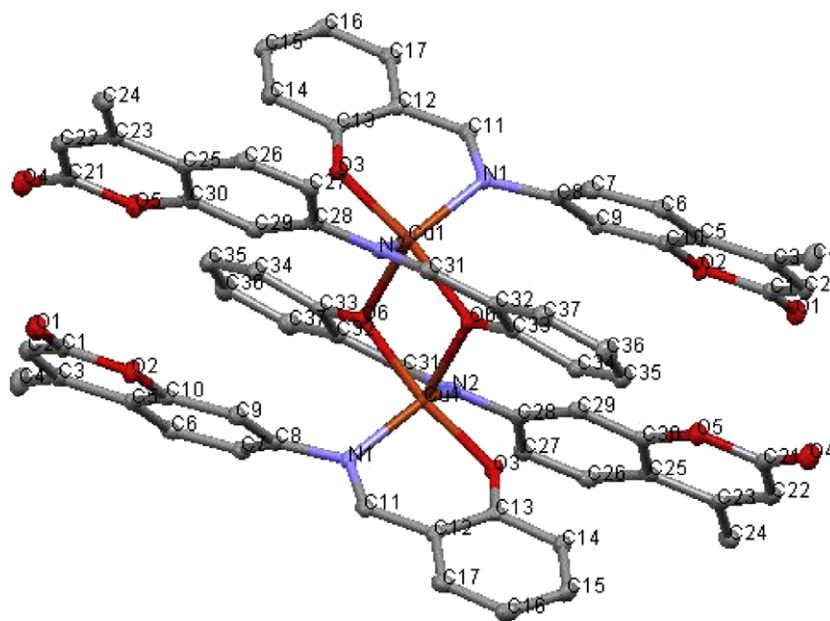
Fig. 2. Crystal structure of **3a**.

Table 9
Selected bond lengths (Å) and angles (°) for **3a** and **3h**.

Cu(1)–O(3)	1.8820 (11)	Cu(1)–O(6)	1.895 (4)
Cu(1)–O(6)	1.9152 (11)	Cu(1)–O(3)	1.897 (5)
Cu(1)–N(1)	2.0379 (14)	Cu(1)–N(2)	1.973 (5)
Cu(1)–N(2)	2.0674 (14)	Cu(1)–N(1)	2.001 (5)
Cu(1)–O(6)#1	2.2785 (12)	N(1)–C(11)	1.291 (9)
		N(1)–C(8)	1.441 (8)
		O(3)–C(13)	1.308 (8)
O(3)–Cu(1)–O(6)	169.13 (5)	O(6)–Cu(1)–O(3)	154.7 (2)
O(3)–Cu(1)–N(1)	91.02 (5)	O(6)–Cu(1)–N(2)	93.5 (2)
O(6)–Cu(1)–N(1)	93.98 (5)	O(3)–Cu(1)–N(2)	89.1 (2)
O(3)–Cu(1)–N(2)	94.04 (5)	O(6)–Cu(1)–N(1)	93.0 (2)
O(6)–Cu(1)–N(2)	89.52 (5)	O(3)–Cu(1)–N(1)	92.6 (2)
N(1)–Cu(1)–N(2)	133.42 (5)	N(2)–Cu(1)–N(1)	161.0 (2)
O(3)–Cu(1)–O(6)#1	92.91 (5)	C(11)–N(1)–Cu(1)	123.2 (4)
O(6)–Cu(1)–O(6)#1	76.37 (5)	C(8)–N(1)–Cu(1)	121.6 (4)
N(1)–Cu(1)–O(6)#1	108.19 (5)	C(13)–O(3)–Cu(1)	128.6 (4)
N(2)–Cu(1)–O(6)#1	117.72 (5)	C(31)–N(2)–Cu(1)	124.2 (5)
		C(28)–N(2)–Cu(1)	120.8 (4)
		C(33)–O(6)–Cu(1)	128.9 (4)

which is reflected particularly in the bond length differences between copper and each of the nitrogen atoms of the two ligands coordinated to it; 1.973(5) Å for Cu(1)–N(2) and 2.001(5) Å for Cu(1)–N(1). The low symmetry in the molecule is also reflected in the relative OCuN bond angles; O(6)–Cu(1)–N(2) is 93.5(2)° and O(3)–Cu(1)-N(1) is 92.6(2)°. The crystal packing diagram (Fig. 4) indicates aryl–aryl stacking interactions between the ring systems of adjacent molecules.
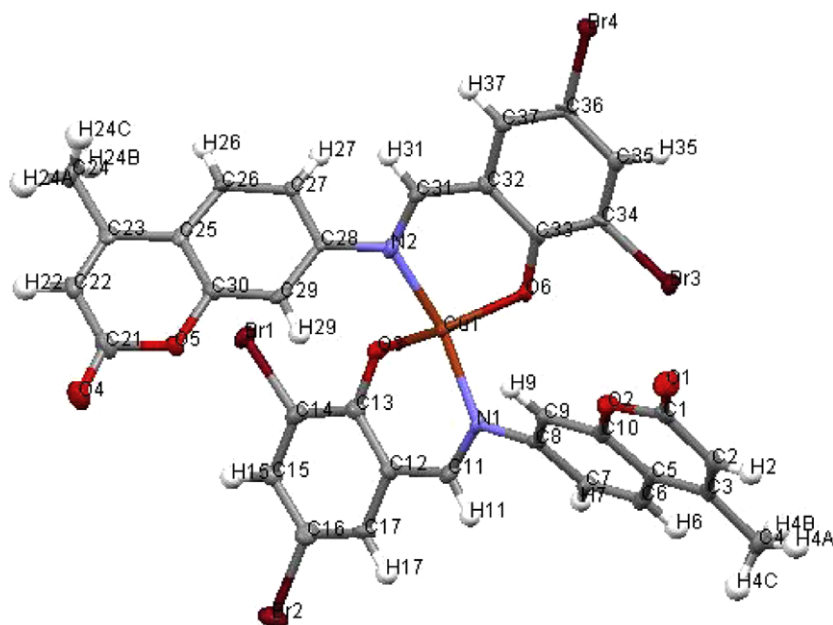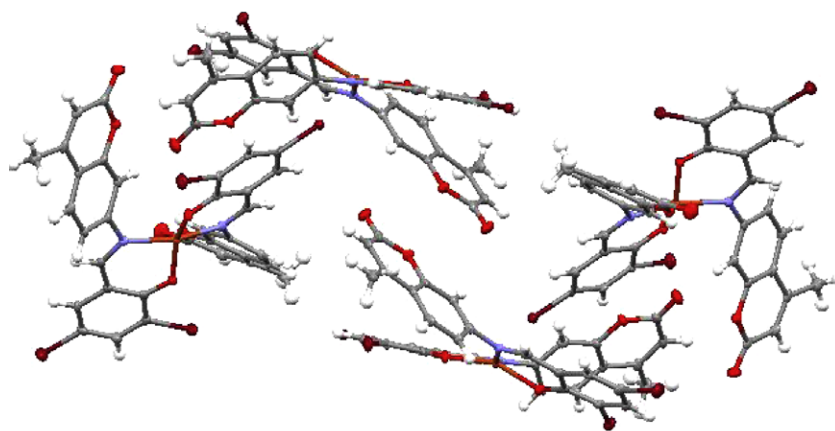
3.2.6. Anti-Candida activities of Schiff Base ligands and Cu(II) complexes

It has previously been reported that the amount of DMSO which is used to prepare samples for assessment of anti-Candida activity can affect the growth of the test strain used [69]. Therefore, before we tested the anti-Candida activity of the free ligands and their corresponding Cu(II) complexes we assessed the influence of the DMSO solvent on the growth of the Candida strain. The results indicated that the presence of 10% DMSO in the test mixture was highly toxic to the Candida cells, causing nearly 100% growth inhibition. 5% DMSO affected nearly 80% growth inhibition of the

Candida cells, and was also not suitable for the further studies. Reducing DMSO concentration to 2% resulted in approximately 55% growth inhibition. Finally it was found that the presence of 1% of DMSO in the test mixture did not affect the growth of the fungus. Therefore, it was decided that for the available strain of C. albicans, the maximal final concentration of DMSO that can be used in the assay cannot be higher than 1%.

The anti-Candida activity of the Schiff base ligands (**2a–2k**) and their Cu(II) complexes (**3a–3k, except 3e**) expressed as MIC_{50} values (the minimum concentration required to inhibit 50% of cells growth) is specified in Table 10. A number of the free ligands and complexes displayed anti-Candida activity comparable to those of the commercially available antifungal drugs, Amphotericin B and ketoconazole.

In our previous work on coumarin compounds, involving derivatised coumarin-3-carboxylic acids, 4-hydroxy-3-nitrocoumarin and coumarin dioxyacetic acid ligands, it was shown that none of the ligands had significant anti-Candida activity [23–25]. The results presented here show that a while a number of the coumarin Schiff base ligands have reasonable high MIC_{50} values, those of the halogenated and nitro derivatives are very low and are comparable to that of the commercially used antifungal agents, Amphotericin B and are indeed lower than that of the commercial drug, ketoconazole. In particular, the diiodo-substituted compound, **2i** (MIC_{50} = 1.2 μM), was particularly active. These results also agree with the findings of Guo et al. who demonstrated that Schiff bases containing 2-hydroxy-5-nitro and 5-chloro-2-hydroxy-substituted aromatic rings have good antifungal activities [71]. Because of solubility problems a few of the Cu(II) complexes were tested as DMSO suspensions. Cu(II) acetate did not show anti-Candida activity at any of the test concentrations. Overall, complexes with good solubility in DMSO showed higher activity than those with poor solubility, but almost all complexes showed considerably increased activity over their corresponding metal-free Schiff base ligand. Interestingly, the complexes with dichloro and dibromo substituents on the ligand (**3g** and **3h**) exhibited high anti-Candida activity even as a DMSO suspension in the case of **3g**. Complex **3i** was the most active of the series and maintained its significant activity (65% growth inhibition) even at a concentration of 1 μg/mL.

Fig. 3. Crystal structure of **3h**.

Fig. 4. Packing diagram for **3h**.

Table 10
Anti-*Candida* activity of Schiff base ligands and their Cu(II) complexes. See Fig. 1 for ligand structures and carbon and substituent numbering.

Ligand	MIC$_{50}$ (µM)	Cu(II) complex	MIC$_{50}$ (µM)	R_1	R_2	R_3
2a	92.3	**3a**	5.2	–H	–H	–H
2b	35.4	**3b**	10.4	–OH	–H	–H
2c	89.2	**3c**	16.7	–H	–OH	–H
2d	62.0	**3d**	8.2	–OCH$_3$	–H	–H
2e	92.6	**3e**	–	–OCH$_2$CH$_3$	–H	–H
2f	44.5	**3f**	14.5	–H	–OCH$_3$	–H
2g	17.1	**3g**	3.6	–Cl	–H	–Cl
2h	3.2	**3h**	4.4	–Br	–H	–Br
2i	1.2	**3i**	0.7	–I	–H	–I
2j	9.1	**3j**	9.8	–H	–H	–NO$_2$
2k	95.4	**3k**	12.6	–OCH$_3$	–H	–NO$_2$
Amphotericin B	0.7					
Ketoconazole[*]	4.7					

[*] Value recorded previously in our lab under the same conditions [70].

In general, it was found that Schiff base ligands with electron-withdrawing substituents such as –Cl, –Br, or –I, at the R_1 and R_3 positions of the salicylaldehyde moiety had greater anti-*Candida* activity than ligands with electron-donating substituents. Surprisingly, the complexation of these active ligands to Cu(II) ions resulted in only slight increases in the activity of the subsequent

complexes. This is in stark contrast to the behaviour of the less active ligands (**2a**–**2f**, **2k**), whose Cu(II) complexes (**3a**–**3d**, **3f**, **3k**) in all cases were significantly more active against *Candida*. It is difficult to attribute these trends to changes in solubility alone. All of the ligands were freely soluble in a range of organic solvents whereas only some of the metal complexes were soluble in DMSO or DMF. Some of the most active complexes, i.e. **3d**, **3g** and **3j** were tested as suspensions and their MIC$_{50}$ values were still lower than those of their corresponding free ligands. Previous work by Nair et al. reported a series of Mn(II) and Cu(II) complexes which exhibited activities lower than the activities of their corresponding free ligands when tested using separately 1,4-dioxane and DMF as solvent [72].

This present study has identified a series of coumarin-derived Schiff bases and their Cu(II) complexes who have displayed good antifungal activity against a clinical strain of *C. albicans*. We have also reported the first crystal structures of this type of metal complex.

Acknowledgements

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