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Synthesis, characterization and antimicrobial activity of a series of substituted coumarin-3-carboxylatosilver(I) complexes

Bernadette S. Creaven [a,*], Denise A. Egan [a], Kevin Kavanagh [b], Malachy McCann [c], Andy Noble [a], Bhumika Thati [a], Maureen Walsh [a]

[a] Department of Applied Science, Institute of Technology, Tallaght, Dublin 24, Ireland
[b] Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland
[c] Department of Chemistry, National University of Ireland, Maynooth, Co. Kildare, Ireland

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Abstract

A series of new coumarin-derived carboxylate ligands and their silver(I) complexes have been synthesized, characterized and screened for their in vitro antibacterial activity against a range of Gram-positive and Gram-negative bacteria as well as for their antifungal activity against a clinical isolate of *Candida albicans*. The ligands were synthesised by either acid or base hydrolysis of their corresponding esters, which in turn were synthesised via the Knoevenegal reaction. The reaction of silver(I) nitrate with the coumarin carboxylate ligands in either aqueous or aqueous/ethanol solutions allowed the isolation of a series of novel Ag(I) carboxylate complexes. Whilst none of the ligands showed any antimicrobial activity, a number of the Ag(I) complexes exhibited potent activity. In particular, Ag(I) complexes of hydroxy-substituted coumarin carboxylates demonstrated potent activity against the clinically important methicillin-resistant *Staphylococcus aureus* (MRSA) bacterium ($MIC_{80} = 0.63$ μM).

Keywords: Coumarin; Carboxylate; Silver(I); Antimicrobial

1. Introduction

Coumarin (2*H*-1-benzopyran-2-one), a naturally occurring plant constituent, has been used in the treatment of cancer [1] and oedemas [2], and many of its derivatives have also shown biological activity. Biological effects observed include antibacterial [3], anti-thrombotic and vasodilatory [4], anti-mutagenic [5] and anti-tumourigenic [6–9] effects as well as acting as lipoxygenase and cyclooxygenase inhibitors [10,11]. A number of recent studies have highlighted the antimicrobial activity of naturally derived and synthetic coumarins [12–14]. Lately, a number of metal complexes of coumarins have been synthesised and their biological activity determined. Kostova et al. have shown the cytotoxic potential of coumarins complexed with cerium, lanthanum, zirconium and neodymium [15–19]. We have previously been concerned with two main areas of coumarin chemistry, namely the chemotherapeutic [20–26] and antimicrobial [27] activity of functionalised coumarins. In the latter work a series of copper(II) and silver(I) complexes of hydroxynitrocoumarins were prepared and their antimicrobial activity assessed against a series of Gram-positive and Gram-negative bacterial strains and also against a clinical isolate of *C. albicans*. While none of the coumarin-based ligands or the simple copper(II) perchlorate salt showed any significant antimicrobial activity, $AgNO_3$ and its coumarin complexes effectively inhibited the growth of the clinically important methicillin-resistant *Staphylococcus aureus* (MRSA) bacterium. These complexes also demonstrated good activity, comparable to that of the commercial fungicides clortrimazole and ketoconazole, against the fungal pathogen *C. albicans*. Both of these human pathogenic

* Corresponding author. Tel.: +353 1 4042889; fax: +353 1 4042700.
E-mail address: bernie.creaven@it-tallaght.ie (B.S. Creaven).

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organisms are of increasing importance with the development of resistance to current drug therapies. A recent study showed that ca. 44% of *S. aureus* bacteremia isolates in Britain and in the Republic of Ireland were resistant to methicillin [28]. Also of concern is the growth in the population of immunosuppressed individuals and the increase in the numbers and types of fungal infections noted in these patients. Candidemia is a serious complication in patients undergoing treatment for cancer [29,30].

It is well known that silver ions and silver-based compounds are highly toxic to microorganisms [31,32] showing strong biocidal effects. Therefore as an advancement of our previous studies, we have now prepared a series of Ag(I) complexes of coumarin-3-carboxylic acid (2-oxo-2*H*-benzo pyran-3-carboxylic acid) and investigated their antimicrobial activity. Carboxylate ligands are known to form a range of complexes with Ag(I) and some of these have proven anti-*Candida* activity [33].

2. Experimental

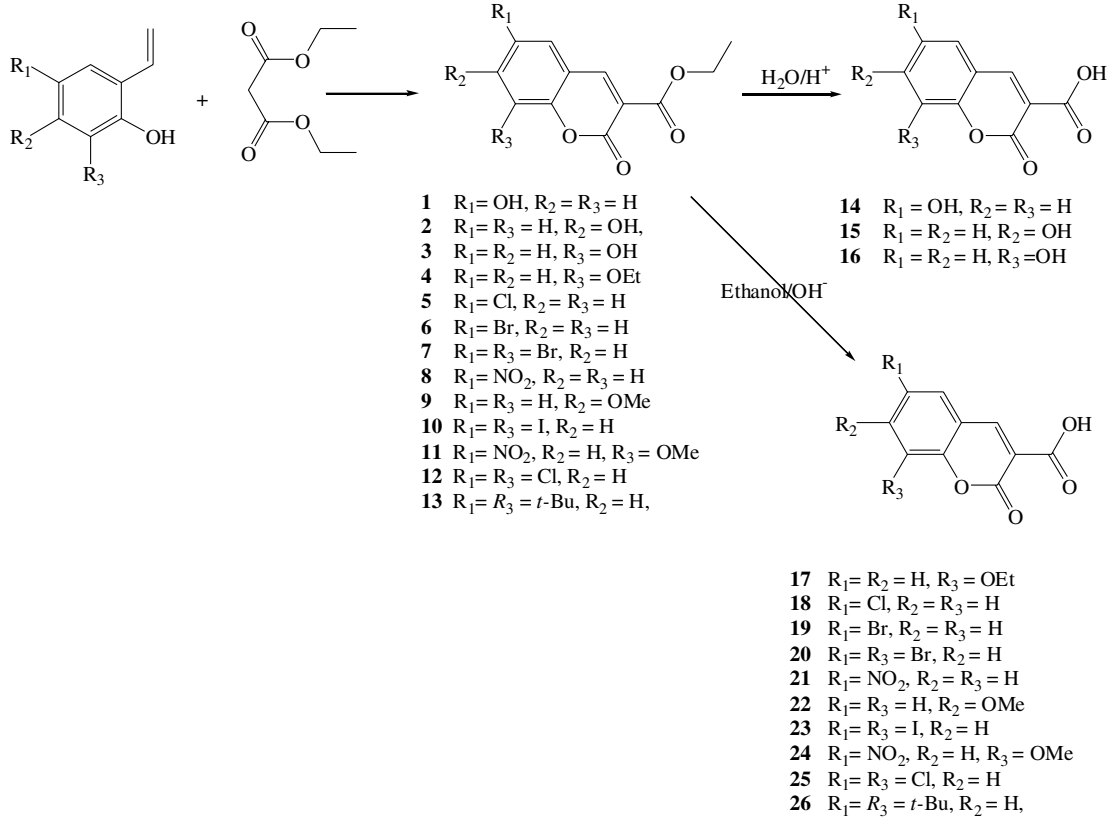
2.1. General methods

Chemicals and solvents were purchased from Sigma-Aldrich Co. (Dorset, UK) and used without further purification. Infrared spectra of solids (in a KBr matrix) were recorded in the region 4000–400 cm^{-1} on a Nicolet Impact 410 Fourier-Transform Infrared Spectrophotometer. Melting points were recorded on a Stuart Scientific SMP-1 apparatus (up to 300 °C). A JEOL JNM-LA300 FT-NMR spectrometer was used to record ^{1}H NMR spectra (−5 to 15 ppm from TMS) and ^{13}C NMR spectra (−33 to 233 ppm from TMS) as solutions in d_6-DMSO. Microanalytical data were provided by the Microanalytical Laboratory, National University of Ireland, Dublin, Belfield, Dublin 4.

2.2. Syntheses of ligands

2.2.1. Synthesis of ethyl 6-hydroxycoumarin-3-carboxylate [6-OHCcaEt] (*1*) and the substituted esters (*2*–*13*) given in Scheme 1

2,5-Dihydroxybenzaldehyde (0.50 g, 3.6 mmol) and diethyl malonate (0.64 g, 0.70 ml, 4.0 mmol, 10 mol% excess) were heated with stirring in ethanol (95%, 20 ml) until dissolution occurred. Addition of piperidine (0.4 ml, 0.34 g, 4.0 mmol) to the solution resulted in a colour change from green to brown. The solution was refluxed for 6 h and on cooling a green crystalline solid formed. Crystals of ethyl 6-hydroxycoumarin-3-carboxylate (**1**) were isolated by filtration and washed with cold ethanol (0 °C). The solid was recrystallised from ethanol, filtered and washed with cold ethanol again. The crystals were dried in a vacuum oven at 50 °C for 2 days. The ester derivatives (**2**–**13**) shown in Scheme 1, which were the precursors used for the synthesis of the carboxylate ligands (**14**–**26**),



Scheme 1. General reaction scheme for the synthesis of coumarin-based esters (**1**–**13**) and acids (**14**–**26**).

were synthesised by the same method as that employed to prepare **1** and using the appropriate substituted aldehyde. All compounds were isolated as solids and the purity of each precursor was confirmed by ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy, m.p. and TLC analysis (given as supplementary data in Tables S1–S3).

2.2.2. Synthesis of 6-hydroxycoumarin-3-carboxylic acid [6-OHCcaH] (14), 7-hydroxycoumarin-3-carboxylic acid [7-OHCcaH] (15) and 8-hydroxycoumarin-3-carboxylic acid [8-OHCcaH] (16)

A solution of ethyl 6-hydroxycoumarin-3-carboxylate (**1**) (2.50 g, 10.7 mmol) in water (50 ml) containing concentrated hydrochloric acid (37%, 5 ml) was refluxed for 6 h leaving a green/yellow solution. Upon cooling, a green precipitate formed. The solid was isolated by filtration and washed with water and ethanol and then placed in a vacuum oven at 50 °C for three days. The analytical data for this compound, 6-hydroxycoumarin-3-carboxylic acid [6-OHCcaH] (**14**), and all the remaining coumarin carboxylate derivatives synthesised are given in Table 1. The ^{1}H and ^{13}C NMR spectral data for this compound and all of the following carboxylic acid derivatives of coumarin are given in Tables 2 and 3. The atom numbering system used for the assignment of the ^{1}H and ^{13}C NMR spectra of the coumarin-based carboxylic acids (**14**–**26**) is shown in Fig. 1.

The carboxylate derivatives 7-hydroxycoumarin-3-carboxylic acid [7-OHCcaH] (**15**) and 8-hydroxycoumarin-3-carboxylic acid [8-OHCcaH] (**16**) were synthesised by the same method as that employed to prepare (**14**) except compounds (**2**) and (**3**), respectively, were used as the precursor ester.

2.2.3. Synthesis of 8-ethoxycoumarin-3-carboxylic acid [8-EtOCcaH] (17) and the substituted carboxylic acids (18–26)

A solution comprising sodium hydroxide (2 M, 30 ml), ethanol (95%, 10 ml) and ethyl 8-ethoxycoumarin-3-carbox-

Table 1
Physical, spectral and analytical data for the ligands (**14**–**26**)

Ligand/molecular formula	Colour	Yield (%)	R_f[a]	MP (°C)[b]	Calc. (found)			IR (cm^{-1})[c]	
					%C	%H	%N	ν_{O-H}	$\nu_{C=O}$ (lactone)
6-OHCcaH (**14**)/$C_{10}H_6O_5$	green	88	0.13	>300	58.26 (58.22)	2.93 (2.88)		3163	1739
7-OHCcaH (**15**)/$C_{10}H_6O_5$	orange	58	0.11	262–266	58.26 (58.20)	2.93 (2.88)		3122	1710
8-OHCcaH (**16**)/$C_{10}H_6O_5$	yellow	94	0.14	>300	58.26 (58.18)	2.93 (2.85)		3112	1735
8-EtOCcaH (**17**)/$C_{12}H_{10}O_5$	white	90	0.17	192–195	61.54 (61.29)	4.30 (4.04)		3426	1747
6-ClCcaH (**18**)/$C_{10}H_5ClO_4$	white	95	0.13	186–188	53.47 (53.36)	2.24 (2.32)		3442	1752
6-BrCcaH (**19**)/$C_{10}H_5BrO_4$	white	87	0.10	213–216	44.64 (44.50)	1.87 (1.59)		3434	1764
6,8-diBrCcaH (**20**)/$C_{10}H_4Br_2O_4$	white	92	0.13	233–236	34.52 (34.38)	1.16 (1.02)		3443	1771
6-NO_2CcaH (**21**)/$C_{10}H_5NO_6$	brown	85	0.11	244–246	51.08 (51.06)	2.14 (2.14)	5.96 (5.72)	3429	1720
7-MeOCcaH (**22**)/$C_{11}H_8O_5$	off-white	90	0.20	208–210	60.00 (59.69)	3.66 (3.34)		3434	1732
6,8-diICcaH (**23**)/$C_{10}H_4I_2O_4$	yellow	92	0.14	240–242	27.18 (27.16)	0.91 (0.77)		3436	1742
8-MeO-6-NO_2CcaH (**24**)/$C_{11}H_7NO_7$	brown	87	0.17	224–227	49.82 (49.76)	2.66 (2.53)	5.28 (5.08)	3437	1746
8-MeO-6-NO_2CcaH (**25**)/$C_{10}H_4Cl_2O_4$	white	96	0.12	220–224	46.37 (46.28)	1.56 (1.58)		3470	1778
6,8-di-*t*-butylCcaH (**26**)/$C_{18}H_{22}O_4$	white	58	0.71	106–108	71.50 (71.43)	7.33 (7.45)		3417	1745

[a] Mobile phase 70:30, ethyl acetate:hexane.
[b] MP's are uncorrected.
[c] All IR spectra were run as KBr disc.

Table 2
^{1}H NMR data for substituted coumarin-3-carboxylic acids **14**–**26** recorded in d_6 DMSO

Coumarin acid	^{1}H NMR signal: δ, multiplicity, J value (Hz)					
	H_4	H_5	H_6	H_7	H_8	COO*H*
6-OHCcaH (**14**)	8.66, s	7.21, d, $J = 8.8$	(9.9)	7.16, dd, $J = 2.9$, 8.8	7.30, d, $J = 2.7$	(13.2)
7-OHCcaH (**15**)	8.69, s	7.76, d, $J = 8.6$	6.83, dd, $J = 2.2$, 8.8	(11.07)	6.74, d, $J = 2.2$	(11.1)
8-OHCcaH (**16**)	8.69, s	7.21, pt, $J = 8.2$	7.32, q, $J = 8.6$, 9.3	7.20, t, $J = 8.2$	(10.4)	(13.2)
8-EtOCcaH (**17**)	8.72, s	7.43, dd, $J = 1.5$, 7.5	7.31, *pt*, J= 7.7, 8.1	7.39, dd, $J = 1.3$, 8.1	OEt	(13.3)
6-ClCcaH (**18**)	8.70, s	8.04, d, $J = 2.6$	Cl	7.75, dd, $J = 2.6$, 9.0	7.47, d, $J = 9.0$	(13.4)
6-BrCcaH (**19**)	8.69, s	8.16, pt, $J = 2.0$	Br	7.86, dd, $J = 2.0$, 8.8	7.41, dd, J=1.5, 8.8	(13.4)
6,8-diBrCcaH (**20**)	8.68, s	8.18, d, $J = 2.4$	Br	8.24, d, $J = 2.4$	Br	(13.5)
6-NO_2CcaH (**21**)	8.89, s	8.90, d, $J = 2.7$	NO_2	8.49, dd, $J = 2.7$, 9.2	7.64, d, $J = 9.0$	(13.5)
7-MeOCcaH (**22**)	8.72, s	7.83, d, $J = 8.4$	7.00, dd, $J = 2.4$, 8.2	OMe	7.03, d, $J = 2.4$	(13.0)
6,8-diICcaH (**23**)	8.60, s	8.28, d, J= 2.0	I	8.42, d, $J = 2.0$	I	(13.5)
8-MeO-6-NO_2CcaH (**24**)	8.83, s	8.47, pt, $J = 2.4$, 1.6	NO_2	8.03, *pt*, $J = 2.4$, 1.5	OMe	(13.5)
8-MeO-6-NO_2CcaH (**25**)	8.71, s	8.04, d, $J = 2.6$	Cl	8.02, d, $J = 2.6$	Cl	(13.5)
6,8-di-*t*-butylCcaH (**26**)	8.22, s	7.13, d, $J = 2.2$	*t*-butyl	7.28, d, $J = 2.2$	*t*-butyl	

Atom numbering for assignment of NMR signals is given in Fig. 1.
Values given in brackets are OH signals, s, singlet; d, doublet; t, triplet; q, quartet; dd, double–doublet; *pt*, pseudo-triplet.

Table 3
^{13}C NMR data for substituted coumarin-3-carboxylic acids recorded in d_6-DMSO

Coumarin acid	^{13}C NMR signal in ppm									
	Carbon no.									
	2	3	4	5	6	7	8	9	10	*C*OOH
6-OHCcaH (**14**)	157	118	148	113	153	117	118	147	122	164
7-OHCcaH (**15**)	164	114	149	132	110	157	101	157	112	164
8-OHCcaH (**16**)	156	118	144	120	124	120	148	143	118	164
8-EtOCcaH (**17**)	156	117	148	121	124	118	145	143	118	164
6-ClCcaH (**18**)	156	119	147	128	128	133	118	153	119	163
6-BrCcaH (**19**)	156	119	146	131	116	136	118	153	119	163
6,8-DiBrCcaH (**20**)	155	120	146	131	116	138	110	150	120	163
6-NO_2CcaH (**21**)	158	120	147	126	143	128	117	155	118	163
7-MeOCcaH (**22**)	164	111	149	131	113	156	100	157	113	164
6,8-DiICcaH (**23**)	155	119	146	138	89	149	86	153	120	163
8-MeO-6-NO_2CcaH (**24**)	155	120	147	109	146	116	143	148	118	163
6,8-DiClCcaH (**25**)	155	120	146	128	128	132	120	148	120	163
6,8-Di-*t*-ButylCcaH (**26**)	156	117	151	124	135	128	146	149	118	163

Atom numbering for assignment of NMR signals is given in Fig. 1.
All spectra were run in d_6-DMSO.

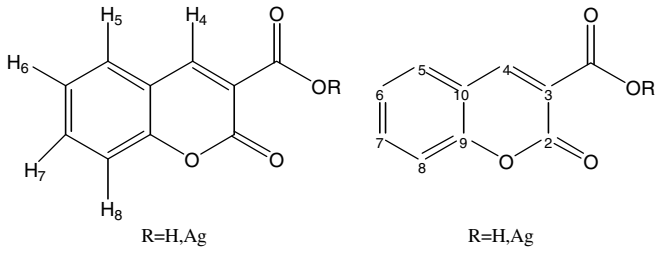


Fig. 1. ^{1}H and ^{13}C NMR atom labels for the analysis of the coumarin-3-carboxylic acids and their Ag(I) complexes.

ylate (**4**) (0.75 g, 3.0 mmol) was refluxed for 2 h. On cooling, hydrochloric acid was added to the yellow solution until a white precipitate formed which was isolated by filtration, washed with water and cold ethanol and then placed in an oven at 50 °C for 3 days. Ligands **18**–**26** were all prepared by this method using compounds **5**–**13**, respectively, as the precursor esters. Analytical data for all of the ligands are given in Tables 1–3.

2.3. Syntheses of substituted coumarin-3-carboxylatosilver(I) complexes

Syntheses of the Ag(I) complexes were conducted in the absence of light and all complexes were stored in the dark. Microanalytical, ^{1}H and ^{13}C NMR spectral data for the following Ag(I) complexes (**27**–**40**) are given in Tables 4–6. The atom numbering system used for the assignment of ^{1}H and ^{13}C NMR spectra of the complexes are shown in Fig. 1. The main IR spectral bands for the complexes are given in Table 7.

*2.3.1. Synthesis of coumarin-3-carboxylatosilver(I) (**27**) [Ag(Cca)]*

A solution of silver(I) nitrate (0.170 g, 1.00 mmol) in water (10 ml) was added to a heated solution of coumarin-3-carboxylic acid (0.191 g, 1.00 mmol) in hot methanol (10 ml) over a period of 10 min resulting in the formation of a white precipitate. The suspension was left to stir for 1 h and the solid product isolated by filtration, washed with hot methanol and then with cold water and then dried in the dark in a vacuum oven at 50 °C for 7 days.

*2.3.2. Synthesis of 6-hydroxycoumarin-3-carboxylatosilver(I) [Ag(6-OHCca)] (**28**), 7-hydroxycoumarin-3-carboxylatosilver(I) [Ag(7-OHCca)] (**29**) and 8-hydroxy-coumarin-3-carboxylatosilver(I) [Ag(8-OHCca)] (**30**)*

A solution of 6-hydroxycoumarin-3-carboxylic acid (**14**) (0.150 g, 1.00 mmol) and sodium hydroxide (0.030 g, 1.00 mmol) in water (10 ml) was added to a solution of silver(I) nitrate (0.124 g, 1.00 mmol) in water (10 ml) over a period of 10 min at room temperature, resulting in the formation of a yellow precipitate. The suspension was left to stir for 1 h and was the resulting precipitate isolated by filtration. The resulting solid, 6-hydroxycoumarin-3-carboxylatosilver(I) [Ag(6-OHCca)] (**28**), was washed with hot methanol and then with cold water. The solid was then dried in the dark in a vacuum oven at 50 °C for 7 days. 7-Hydroxy-coumarin-3-carboxylatosilver(I) [Ag(7-OHCca)] (**29**) and 8-hydroxy-coumarin-3-carboxylatosilver(I) [Ag(8-OHCca)] (**30**) were synthesised by the same method except that acids **15** and **16**, respectively, were used as ligands.

*2.3.3. Synthesis of 8-ethoxy-coumarin-3-carboxylatosilver(I) [Ag(8-EtOCca)] (**31**) and the silver(I) complexes (**32**–**40**)*

A solution of silver(I) nitrate (0.758 g, 4.46 mmol) in water (20 ml) was added over a period of 10 min at room temperature to a heated solution of 8-ethoxycoumarin-3-carboxylic acid (**17**) (1.00 g, 4.46 mmol) and sodium hydroxide (0.196 g, 4.91 mmol) in methanol/water (1:1,

Table 4
Physical, spectral and analytical data for the Ag(I) complexes (**14**–**26**)

Complex/molecular formula	Colour	Yield (%)	MP (°C)[a]	Calc. (found)		
				%C	%H	%N
[Ag(Cca)] (**27**)/$C_{10}H_5AgO_4$	white	76	294–296	40.44 (39.88)	1.70 (1.56)	
[Ag(6-OHCca)] (**28**)/$C_{10}H_5AgO_5$	yellow	65	270–272	38.37 (37.16)	1.61 (2.08)	
[Ag(7-OHCca)] (**29**)/$C_{10}H_5AgO_5$	red/black	83	248–252	38.37 (38.98)	1.61 (1.65)	
[Ag(8-OHCca)] (**30**)/$C_{10}H_5AgO_5$	red/black	74	274–276	38.37 (37.86)	1.61 (1.30)	
[Ag(8-EtOCca)] (**31**)/$C_{12}H_9AgO_5$	yellow/orange	28	246–248	42.26 (42.61)	2.66 (2.22)	
[Ag(6-ClCca)] (**32**)/$C_{10}H_4AgClO_4$	yellow	82	>300	36.24 (36.75)	1.22 (1.09)	
[Ag(6-BrCca)] (**33**)/$C_{10}H_4AgBrO_4$	yellow/brown	79	>300	31.95 (32.28)	1.07 (1.02)	
[Ag(6,8-diBrCca)] (**34**)/$C_{10}H_3AgBr_2O_4$	yellow	62	268–270	26.41 (26.26)	0.66 (0.38)	
[Ag(6-NO_2Cca)] (**35**)/$C_{10}H_4AgNO_6$	yellow	63	293–296	35.12 (35.02)	1.18 (0.91)	4.10 (3.39)
[Ag(7-MeOCca)] (**36**)/$C_{11}H_7AgO_5$	yellow/orange	77	272–274	40.40 (39.82)	2.16 (1.55)	
[Ag(6,8-diICca)] (**37**)/$C_{10}H_3AgI_2O_4$	yellow	89	277–280	21.89 (21.59)	0.55 (0.29)	
[Ag(8-MeO-6-NO_2Cca)] (**38**)/$C_{11}H_6AgNO_7$	yellow/orange	75	275–278	35.51 (35.42)	1.63 (1.38)	3.76 (3.52)
[Ag(6,8-diClCca)] (**39**)/$C_{10}H_3AgCl_2O_4$	yellow/orange	89	277–280	32.82 (32.87)	0.83 (0.57)	
[Ag(6,8-di-*t*-butylCca)] (**40**)/$C_{18}H_{21}AgO_4$	white	70	233–236	52.83 (52.16)	5.17 (4.92)	

[a] MP's are uncorrected.

Table 5
^{1}H NMR data for substituted coumarin-3-carboxylatosilver(I) complexes (**27**–**40**) recorded in d_6-DMSO

Complex	^{1}H NMR signals: δ, multiplicity, J value in Hz,				
	H_4	H_5	H_6	H_7	H_8
[Ag(Cca)] (**27**)	8.21, s	7.77, dd, $J = 1.4$, 7.7	7.60, dd, $J = 1.6$, 7.4	7.30, td, $J = 7.3$, 7.8	7.35, dd, $J = 1.1$, 7.7
[Ag(6-OHCca)] (**28**)	8.14, s	7.08, d, $J = 2.9$	(9.81)	7.03, dd, $J = 3.0$, 8.8	7.21, d, $J = 8.8$
[Ag(7-OHCca)] (**29**)	8.35, s	7.62, d, $J = 8.6$	6.78, dd, $J = 2.2$, 8.4	not observed	6.69, d, $J = 2.2$
[Ag(8-OHCca)] (**30**)	8.24, s	7.20, dd, $J = 2.6$, 8.8	7.13, dd, $J = 6.8$, 7.9	7.12, *p*t, $J = 8.1$	(10.29)
[Ag(8-EtOCca)] (**31**)	8.14, s	7.31, dd, $J = 2.4$, 6.8	7.24, *p*t, $J = 6.6$, 8.0	7.26, dd, $J = 7.7$	OEt
[Ag(6-ClCca)] (**32**)	7.96, s	7.86, d, $J = 2.6$	Cl	7.56, dd, $J = 2.6$, 8.8	7.37, d, $J = 8.8$
[Ag(6-BrCca)] (**33**)	8.01, s	8.00, d, $J = 2.4$	Br	7.69, dd, $J = 2.4$, 8.8	7.31, d, $J = 8.8$
[Ag(6,8-diBrCca)] (**34**)	8.13, s	8.06, d, $J = 2.2$	Br	8.09, d, $J = 2.2$	Br
[Ag(6-NO_2Cca)] (**35**)	8.31, s	8.78, d, $J = 2.8$	NO_2	8.37, dd, $J = 2.8$, 9.2	7.58, d, $J = 9.0$
[Ag(7-MeOCca)] (**36**)	8.23, s	7.68, d, $J = 8.6$	6.92, dd, $J = 2.4$, 8.4	OMe	6.97, d, $J = 2.4$
[Ag(6,8-diICca)] (**37**)	8.04, s	8.16, d, $J = 1.6$	I	8.29, d, $J = 1.6$	I
[Ag(8-MeO-6-NO_2Cca)] (**38**)	8.27, s	7.95, d, $J = 2.4$	NO_2	8.38, d, $J = 2.6$	OMe
[Ag(6,8-diClCca)] (**39**)	8.11, s	7.90, d, $J = 2.4$	Cl	7.90, d, $J = 2.8$	Cl
[Ag(6,8-di-*t*-butylCca)] (**40**)	8.18, s	7.63, d, $J = 2.2$	*t*-butyl	7.57, d, $J = 2.4$	*t*-butyl

Atom numbering for assignment of NMR signals is given in Fig. 1.
Values shown in brackets are OH signals, s, singlet; d, doublet; t, triplet; q, quartet; dd, double–doublet; *p*t, pseudo-triplet; td, triple doublet.

20 ml) resulting in the formation of a yellow/orange precipitate. The suspension was stirred for 1 h, filtered, and the solid washed with hot methanol followed by cold water. The yellow solid was then dried in the dark in a vacuum oven 50 °C for 7 days. The silver(I) complexes **32**–**40** of ligands **18**–**26** were synthesised on a similar scale by the method used to prepare **31**.

2.4. Antimicrobial studies

Bacterial and fungal isolates: All bacterial isolates were obtained clinically: *S. aureus* (urinary track infection), methicillin resistant *S. aureus* (MRSA) (wound infection), *S. simulans* (facial skin), *Micrococcus luteus* (facial skin), *Escherichia coli* (gastro-intestinal tract), *Bacillus oleronius* (facial skin), *Pantonea agglumerans* (facial skin).

The fungal isolate *C. albicans* ATCC 10231 was obtained from the American Type Culture Collection (MD, USA).

2.4.1. Assessment of antibacterial activity

Ligands **14**–**26**, the commercially available ligand coumarin-3-carboxylic acid(coumarin-3-carboxylic acid, CcaH) and the Ag(I) complexes **27**–**40**, were tested against four Gram-positive and three Gram-negative strains to determine their antibacterial activity. Bacterial strains were grown overnight in nutrient broth medium at 30 °C and 200 rpm in an orbital incubator. The absorbance of these cultures was measured at 660 nm and cultures were diluted to an optical density of 0.1. The cell suspension (100 μl) was added to the wells of a 96 well plate containing test compound dissolved in nutrient broth medium in serial dilutions from 100–0.25 μg/cm^{-3}. Plates were incubated at 30 °C for 24 h and the optical density was measured spectrophotometrically (Dynex Technology) at 450 nm.

2.4.2. Antifungal susceptibility testing

Antifungal susceptibility testing was performed using broth microdilution assays according to the National

Committee for Clinical Laboratory standards (Document M27-A2) protocol with slight modifications. M27-A2 method was altered by substituting antibiotic medium 3 for RPMI 1640 medium. Using this method MIC_{80}'s were determined spectrophotometrically at 405 nm by comparing the turbidity of growth in each well. MIC_{80} is defined as the lowest concentration of drug that inhibits fungal growth by 80%.

3. Results and discussion

3.1. Synthesis and characterisation of the complexes

The coumarin carboxylate ligands **14**–**26** were isolated by first preparing their corresponding esters **1**–**13** using the Knoevenagal reaction (Scheme 1). This reaction, which involved refluxing the appropriate substituted salicylaldehyde with diethyl malonate in ethanol in the presence of a catalytic base piperidine, allowed the isolation of the series of esters in high yields. In general, the solutions were refluxed for ca. 2 days and the precipitated products were subsequently recrystallised from ethanol and characterized by standard techniques. Isolation of the acid derivatives **14**–**16** was then achieved by refluxing the corresponding esters **1**–**3** for 6 h in distilled water with a catalytic amount of hydrochloric acid. Upon cooling, the products precipitated and were recrystallised from ethanol. The acid derivatives **17**–**26** could be synthesized in the same manner but gave higher yields when made by base hydrolysis. In all cases, the acids were recrystallised from ethanol. All of the carboxylate ligands **14**–**26** were fully characterised by elemental analysis, m.p., TLC and by IR, ^1H and ^{13}C NMR spectroscopy (Tables 1–3).

The silver(I) complex of commercially available coumarin-3-carboxylic acid (CcaH), [CcaAg] (**27**), was synthesised by a metathesis reaction with silver nitrate in a 1:1 ratio. Isolation of the silver(I) complexes of 6-, 7- and 8-hydroxy-coumarin-3-carboxylic acids (**28**–**30**) by this method proved problematic. These silver(I) complexes were ultimately synthesised in aqueous solution by deprotonation of the acid using NaOH, followed by the addition of silver nitrate. The remaining silver(I) complexes were isolated in a similar fashion, except that ethanol:water was used as the reaction solvent. In all cases the products precipitated out of solution, and when dried and stored in the dark they appeared to be air and moisture stable. The complexes were insoluble in water and common organic solvents with the exception of DMSO. Elemental analyses were in agreement with the proposed 1:1 ligand:silver formulation for all complexes.

Table 7
Selected IR data (cm^{-1}) for the Ag(I) coumairn-3-carboxylic acid complexes (**27**–**40**)

Complex	ν_{CO} (lactone)	ν_{asym} (OCO)	ν_{sym} (OCO)	$\Delta\nu$ (OCO)
[Ag(Cca)] (**27**)	1733	1596	1384	212
[Ag(6-OHCca)] (**28**)	1707	1586	1385	201
[Ag(7-OHCca)] (**29**)	1708	1577	1376	201
[Ag(8-OHCca)] (**30**)	1718	1592	1382	210
[Ag(6-ClCca)] (**31**)	1711	1590	1378	212
[Ag(8-EtOCca)] (**32**)	1742	1575	1375	200
[Ag(6-BrCca)] (**33**)	1741	1589	1376	223
[Ag(6,8-diBrCca)] (**34**)	1738	1604	1371	234
[Ag(6-NO_2Cca)](**35**)	1739	1599	1377	222
[Ag(7-MeOCca)] (**36**)	1718	1597	1378	219
[Ag(6,8-diICca)] (**37**)	1729	1589	1374	222
[Ag(8-MeO-6-NO_2Cca)] (**38**)	1762	1604	1370	234
[Ag(6,8-diClCca)] (**39**)	1730	1595	1369	226
[Ag(6,8-di-*t*-butylCca)] (**40**)	1709	1583	1394	199

3.1.1. IR spectra

The ν_{asym} (OCO) and ν_{sym} (OCO) vibrational frequencies, together with the $\Delta\nu$(OCO) values for the carboxylate group of the silver(I) complexes (**27**–**40**), are listed in Table 7. All of the complexes produced a $\Delta\nu$(OCO) value of

Table 6
^{13}C NMR data for substituted coumarin-3-carboxylatosilver(I) complexes (**27**–**40**) recorded in d_6-DMSO

Complex	^{13}C NMR signal in ppm									
	Carbon no.									
	2	3	4	5	6	7	8	9	10	*C*OOH
[Ag(Cca)] (**27**)	157	125	142	128	115	132	124	153	118	166
[Ag(6-OHCca)] (**28**)	158	126	141	112	153	119	118	146	120	167
[Ag(7-OHCca)] (**29**)	162	119	144	130	113	157	101	155	110	166
[Ag(8-OHCca)] (**30**)	157	126	142	118	124	118	144	142	119	167
[Ag(8-EtOCca)] (**31**)	157	126	142	119	124	119	145	142	115	166
[Ag(6-ClCca)] (**32**)	157	128	138	127	127	131	117	151	120	166
[Ag(6-BrCca)] (**33**)	157	128	139	130	115	133	118	152	120	166
[Ag(6,8-diBrCca)] (**34**)	156	129	139	130	115	135	109	149	121	165
[Ag(6-NO_2Cca)] (**35**)	157	124	143	126	139	129	117	156	119	166
[Ag(7-MeOCca)] (**36**)	162	118	142	129	114	155	100	157	112	165
[Ag(6,8-diICca)] (**37**)	157	128	139	130	118	133	115	152	120	166
[Ag(8-MeO-6-NO_2Cca)] (**38**)	156	129	143	107	146	115	139	146	119	165
[Ag(6,8-diClCca)] (**39**)	157	128	139	127	120	130	120	142	121	166
[Ag(6,8-di-*t*-butylCca)] (**40**)	157	123	145	125	135	126	142	150	118	166

Atom numbering for assignment of NMR signals is given in Fig. 1.

>200 cm^{-1}, suggesting unidentate carboxylate coordination to the silver(I) centre [34]. A similar monodentate carboxylate coordination mode has been reported for the structurally characterised Ag(I) salicylate complexes [Ag(μ_3-hexamethylenetetramine)(salH)$_2$] [35] and [$Ag_2(NH_3)_2$-(salH)$_2$] [33]. The band located at between 1770 and 1710 cm^{-1} in all of the carboxylate ligands, which is attributed to $\nu_{C=O}$ of the lactone ring, is shifted in all of the complexes by between 10 and 40 cm^{-1} upon formation of the silver(I) complex except for complexes **35** and **38** whose coumarin ligand contain nitro groups on the aromatic. For [Ag(6-NO_2Cca)] (**35**) and [Ag(8-MeO-6-NO_2Cca)] (**38**), the frequency of the $\nu_{C=O}$ group of the lactone ring increased by about 20 cm^{-1} upon complex formation. We have previously isolated a number of nitrated coumarin Ag(I) complexes where binding of the coumarin ligand to the metal centre via the oxygen atoms of the nitro group was confirmed by both NMR spectroscopy and X-ray crystallography [27]. In these complexes, ν_{asym} (NO_2) was shifted to lower frequency upon formation of a Ag(I) complex. The ν_{asym} (NO_2) band of the free 6-NO_2CcaH ligand at 1535 cm^{-1} increase in frequency by about 20 cm^{-1} upon formation of the Ag(I) complex **35** whilst ν_{asym} (NO_2) at 1534 cm^{-1} in the free ligand 8-MeO-6-NO_2-CcaH is virtually unchanged upon formation of its silver complex (**38**). Thus the ν_{asym} (NO_2) band shifts observed are likely due to an inductive effect within the coumarin nucleus. It should be noted that the ν_{sym} (NO_2) is more difficult to assign unambiguously as there are many overlapping bands in this spectral region. Repeated attempts to recrystallise the present Ag(I) complexes were unsuccessful due largely to their lack of solubility in common solvents.

The IR data, taken together with the insolubility of the complexes, suggests that they exist in the solid state as polymeric structures with bonding of Ag(I) likely to both the deprotonated carboxylate oxygen and lactone carbonyl oxygen of neighbouring ligands.

3.1.2. NMR spectra

Peak assignments for the ^1H and ^{13}C NMR spectra of all of the Ag(I) complexes (**27**–**40**) in DMSO solution are given in Tables 5 and 6 and were carried out using standard 2D correlation techniques. The signals for the aromatic hydrogens and carbons of the ligand (H_5–H_8 and C_5–C_8, respectively) showed a distinct downfield shift upon complex formation in both the ^1H NMR and ^{13}C NMR spectra. However, the most pronounced shifts (~0.5 ppm in ^1H NMR and ~10 ppm for ^{13}C NMR) were attributed to the vinyl proton and carbon, respectively (H_4, C_4) which are α to the carboxylate group. The ^{13}C NMR signal for the lactone carbonyl, C_2, appears to be largely unaffected by complex formation. This pattern in chemical shift values was consistent even amongst complexes having alternative complexation sites; for example complexes (**28**–**30**) which could form phenoxy type bonds to metal centres [36,37]. The ^1H NMR spectra of the present complexes indicated that the signal assigned to the acid proton (ca. δ 13.4 ppm), which was present in all of the free ligands, was absent in all cases while the signal assigned to the hydroxyl group of complexes (**28**–**30**) (δ ca. 10–11 ppm) was still present. It was also noted that while the chemical shift of C_6 in 6-NO_2CcaH did move slightly upfield by 4 ppm upon complex formation, the corresponding signal in the complex formed by 8-MeO-6-NO_2CcaH was not affected by complexation to Ag(I).

It is likely in the solution phase, that binding of the carboxylate ligands to the Ag(I) centre is via a unidentate carboxylate bond although it is possible that the lactone carbonyl may also be coordinated to the silver(I) centre in a chelate fashion. A comparison of the ^{13}C NMR spectra of the free ligands and their silver(I) complexes (Tables 3 and 6) indicated that the carbons of the carboxylate and lactone functionalities experience only a slight deshielding effect upon complex formation. However, in DMSO solution, the hydroxyl group of the acid of the free ligand is likely to be H-bonded to the lactone carbonyl oxygen and causing a deshielding effect on the respective carbonyl carbon atoms similar to that which would be observed in the ligand upon formation of a Ag(I) chelate complex.

3.2. Antimicrobial results

The Ag(I) complexes (**27**–**40**) and the metal-free ligands (**14**–**26**) were screened for their ability to inhibit the growth of a number of Gram-positive and Gram-negative bacterial strains. The Gram-positive strains studied were clinical isolates of *S. aureus* (SA), methicillin-resistant *S. aureus* (MRSA), *S. simulans* (S. Sim.) and *M. luteus* (Ml) whilst the Gram-negative strains were *E. coli* (E.Coli), *B. olenius* (BO) and *P. agglumerans* (PA). Whilst a number of coumarin-based compounds have previously shown good antimicrobial activity [12–14] none of the current metal-free coumarin ligands showed any antibacterial activity over the test concentration range (data not shown). The growth inhibition results for the Ag(I) complexes that showed antimicrobial activity are given in Table 8 as MIC_{80} values in μM. The MIC_{80} value was the minimum concentration required to inhibit 80% of the growth of the microbe.

The simple silver salt $AgNO_3$ displayed moderate activity against most of the bacterial strains and good activity ($MIC_{80} = 16.9$ μM) against *S. simulans*. However, the Ag(I) complexes of the commercial ligand CcaH (**27**) and the hydroxylated derivatives [Ag(6-OHCCa)] (**28**), [Ag(7-OHCCa)] (**29**) and [Ag(8-OHCCa)] (**30**) all showed good activity against a broad spectrum of bacterial strains. Of particular note was the potent activity of [Ag(Cca)] (**27**) ($MIC_{80} = 0.63$ μM) against the pathogenic MRSA bacterium. This MIC_{80} value is particularly relevant when compared to the value observed for *S. aureus* (71.9 μM). Whilst the hydroxylated derivatives had very good antibacterial activity it was surprising that the other Ag(I) complexes were essentially inactive against all of the microbial species tested. Although the range of functionalities on the aromatic ring of the coumarin nucleus is considerable

Table 8
Antimicrobial activity, given as the concentration of compound required to inhibit cell growth by 80% [MIC_{80} (μM)]

Compound	MIC_{80} for test organism (μM)							
	SA	MRSA	SSim	Ml	E.Coli	BO	PA	Can
$AgNO_3$	69.5	123.9	16.9	64.6	69.5	31.4	67.8	66.8
CcaH	>2500	>2500	>2500	>2500	>2500	>2500	>2500	331.6
[Ag(Cca)] (**27**)	71.9	0.63	122.4	100.2	17.5	10.5	55.3	163.4
[Ag(6-OHCca)] (**28**)	72	76	87.9	66.3	114	94.0	77.6	34.1
[Ag(7-OHCca)] (**29**)	34.5	35.6	17.3	28.9	36.1	32.1	32.3	69.3
[Ag(8-OHCca)] (**30**)	140.9	137.6	137.7	114.2	50.0	33.8	38.8	270.0

(SA) *Staphylococcus aureus*: (MRSA) methicillin-resistant *Staphylococcus aureus*; (SSim.) *Staphylococcus simulans*; (Ml) *Micrococcus luteus*; (E.Coli) *Escherichia coli*; (BO) *Bacillus olenius*; (PA) *Pantonea agglumerans*; (Can) *Candida albicans*.

only the presence of a hydroxyl group on the aromatic ring confers antimicrobial activity on the subsequent Ag(I) complexes. The role of the hydroxyl group in this activity is difficult to determine but the metal complexes of other hydroxylated derivatives of coumarin have been previously shown to have good antimicrobial activity. Examples include Cu(II) and Ni(II) complexes of 4-hydroxycoumarins [36,37]. In a previous study on the antimicrobial activity of catechols, the position and number of hydroxyl groups on the aromatic ring were thought to be related to their relative toxicity towards microorganisms, with evidence that increasing hydroxylation results in an increase in antimicrobial activity [38]. The mechanism suggested to be responsible for catechol toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through sulphydryl groups or by non-specific interactions with proteins. The results presented here would indicate that substitution of the hydroxyl groups on the aromatic ring of the coumarin ligand was also essential for conferring antimicrobial activity onto the Ag(I) complexes.

The anti-*Candida* activity of each of the complexes and their respective ligands was also determined using a clinical isolate of *C. albicans* (Table 8). Whereas the free ligands, with the exception CcaH, were ineffective in preventing the growth of the organism, the Ag(I) complexes of the hydroxylated coumarin acids displayed moderate activity. Only [Ag(6-OHCca)] (**28**) ($MIC_{80} = 34.1$ μM) was more active than $AgNO_3$ ($MIC_{80} = 66.8$ μM) and was comparable in activity to the commercially available fungicide ketoconazole ($MIC_{80} = 25$ μM) [27].

4. Conclusions

A number of new Ag(I) coumarin–carboxylate complexes exhibit potent antibacterial and anti-*Candida* activity. Of particular note is the high potency of the complexes against the clinically important MRSA bacterium. Whilst in general hydroxylation of the aromatic ring of the coumarin ligand appears to be important for conferring antimicrobial activity, the most active Ag(I) complex MRSA was that of the unsubstituted coumarin carboxylate ligand, CcaH. Currently, studies are underway to determine the mechanism of action of these complexes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ica.2006.04.006.

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