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

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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\textsubscript{80} = 0.63 \mu M).

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

1. Introduction

Coumarin (2H-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\textsubscript{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...
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).](image-url)
were synthesised by the same method as that employed to prepare I and using the appropriate substituted aldehyde. All compounds were isolated as solids and the purity of each precursor was confirmed by ¹H and ¹³C 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 ¹H and ¹³C 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 ¹H and ¹³C 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-carboxylic acid [8-EtOCcaH] (17) was refluxed for 6 h leaving a yellow solution. Upon cooling, a yellow 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, 8-ethoxycoumarin-3-carboxylic acid [8-EtOCcaH] (17), and all the remaining coumarin carboxylate derivatives synthesised are given in Table 1. The ¹H and ¹³C 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 ¹H and ¹³C NMR spectra of the coumarin-based carboxylic acids (14–26) is shown in Fig. 1.

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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.
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, 1H and 13C 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 1H and 13C 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 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) (0.15 g, 1.00 mmol) and sodium hydroxide (0.03 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,
The yellow solid was then dried in the dark in a vacuum oven at 60 °C for 7 days. The silver(I) complexes 27–40 were synthesised on a similar scale by the procedure outlined in Section 2.4.

Table 4

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

<table>
<thead>
<tr>
<th>Complex/molecular formula</th>
<th>Colour</th>
<th>Yield (%)</th>
<th>MP (°C)</th>
<th>Calc. (found)</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="27">Ag(Cca)</a>/C10H5AgO4</td>
<td>white</td>
<td>76</td>
<td>294–296</td>
<td>40.44 (39.88)</td>
</tr>
<tr>
<td><a href="28">Ag(6-NO2Cca)</a>/C10H5AgO4</td>
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<td>65</td>
<td>270–272</td>
<td>38.37 (37.16)</td>
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<tr>
<td><a href="29">Ag(6,8-diClCca)</a>/C10H5AgO4</td>
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<td>83</td>
<td>248–252</td>
<td>38.37 (38.98)</td>
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<tr>
<td><a href="30">Ag(7-MeOCca)</a>/C10H5AgO4</td>
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<td>74</td>
<td>274–276</td>
<td>38.37 (37.86)</td>
</tr>
<tr>
<td><a href="31">Ag(8-EtOCca)</a>/C10H5AgO4</td>
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<td>28</td>
<td>246–248</td>
<td>42.26 (42.61)</td>
</tr>
<tr>
<td><a href="32">Ag(6-diClCca)</a>/C10H5AgClO4</td>
<td>yellow</td>
<td>82</td>
<td>&gt;300</td>
<td>36.24 (36.75)</td>
</tr>
<tr>
<td><a href="33">Ag(6-BrCca)</a>/C10H5AgClO4</td>
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<td>79</td>
<td>&gt;300</td>
<td>31.95 (32.28)</td>
</tr>
<tr>
<td><a href="34">Ag(8-OHCca)</a>/C10H5AgClO4</td>
<td>yellow/orange</td>
<td>36</td>
<td>277–280</td>
<td>32.82 (32.87)</td>
</tr>
<tr>
<td><a href="35">Ag(6-BrCca)</a>/C10H5AgClO4</td>
<td>yellow/orange</td>
<td>28</td>
<td>233–236</td>
<td>52.83 (52.16)</td>
</tr>
</tbody>
</table>

Values shown in brackets are OH signals, s, singlet; d, doublet; t, triplet; q, quartet; dd, double–doublet; J value in Hz.

<table>
<thead>
<tr>
<th>Complex/molecular formula</th>
<th>Colour</th>
<th>Yield (%)</th>
<th>MP (°C)</th>
<th>Calc. (found)</th>
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<tr>
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<td>36</td>
<td>277–280</td>
<td>32.82 (32.87)</td>
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<tr>
<td><a href="37">Ag(6-NO2Cca)</a>/C10H5AgClO4</td>
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<td>28</td>
<td>233–236</td>
<td>52.83 (52.16)</td>
</tr>
<tr>
<td><a href="38">Ag(8-OHCca)</a>/C10H5AgClO4</td>
<td>yellow/orange</td>
<td>36</td>
<td>277–280</td>
<td>32.82 (32.87)</td>
</tr>
<tr>
<td><a href="39">Ag(6,8-diClCca)</a>/C10H5AgClO4</td>
<td>yellow/orange</td>
<td>28</td>
<td>233–236</td>
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</tbody>
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<tr>
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<th>Colour</th>
<th>Yield (%)</th>
<th>MP (°C)</th>
<th>Calc. (found)</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="40">Ag(6,8-diClCca)</a>/C10H5AgClO4</td>
<td>yellow/orange</td>
<td>28</td>
<td>233–236</td>
<td>52.83 (52.16)</td>
</tr>
</tbody>
</table>

Values shown in brackets are OH signals, s, singlet; d, doublet; t, triplet; q, quartet; dd, double–doublet; J value in Hz.

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 agglomerans (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/cm³. 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.
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, $^1$H 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 ligand:silver formulation for all complexes. 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.

3.1.1. IR spectra

The $\nu_{\text{sym}}$ (OCO) and $\nu_{\text{asym}}$ (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 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>
<thead>
<tr>
<th>Complex</th>
<th>$\nu_{\text{sym}}$ (OCO)</th>
<th>$\nu_{\text{asym}}$ (OCO)</th>
<th>$\Delta\nu$(OCO)</th>
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<tbody>
<tr>
<td>[Ag(Cca)] (27)</td>
<td>1733</td>
<td>1596</td>
<td>1384</td>
</tr>
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<td>[Ag(6-OHICca)] (28)</td>
<td>1707</td>
<td>1586</td>
<td>1385</td>
</tr>
<tr>
<td>[Ag(7-OHICca)] (29)</td>
<td>1708</td>
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<td>1376</td>
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<td>[Ag(6,8-di-t-butylCca)] (40)</td>
<td>1709</td>
<td>1583</td>
<td>1394</td>
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Table 7

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

<table>
<thead>
<tr>
<th>Complex</th>
<th>Carbon no.</th>
<th>$^{13}$C NMR signal in ppm</th>
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<tr>
<td>[Ag(Cca)] (27)</td>
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<td>157</td>
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<tr>
<td>[Ag(6-OHICca)] (28)</td>
<td>2</td>
<td>158</td>
</tr>
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<td>[Ag(7-OHICca)] (29)</td>
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<td>162</td>
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<tr>
<td>[Ag(8-OHICca)] (30)</td>
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<td>156</td>
</tr>
<tr>
<td>[Ag(6,8-diClCca)] (39)</td>
<td>2</td>
<td>157</td>
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<tr>
<td>[Ag(6,8-di-t-butylCca)] (40)</td>
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</table>

Atom numbering for assignment of NMR signals is given in Fig. 1.
>200 cm$^{-1}$, suggesting undentate 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$(\mu_7$-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 carboxylic acid ligands, which is attributed to $v_{\text{C}=\text{O}}$ 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$_2$Cca)] [35] and [Ag(8-MeO-6-NO$_2$Cca)] (38), the frequency of the $v_{\text{C}=\text{O}}$ group of the lactone ring increased by about 20 cm$^{-1}$ upon complex formation. We have previously isolated a number of nitratated 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, $v_{\text{asym}}$ (NO$_2$) was shifted to lower frequency upon formation of a Ag(I) complex. The $v_{\text{asym}}$ (NO$_2$) band of the free 6-NO$_2$CcaH ligand at 1535 cm$^{-1}$ increased in frequency by about 20 cm$^{-1}$ upon formation of the Ag(I) complex 35 whilst $v_{\text{asym}}$ (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 $v_{\text{asym}}$ (NO$_2$) band shifts observed are likely due to an inductive effect within the coumarin nucleus. It should be noted that the $v_{\text{sym}}$ (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 $^1$H 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$_6$ and C$_5$–C$_8$, respectively) showed a distinct downfield shift upon complex formation in both the $^1$H NMR and $^{13}$C NMR spectra. However, the most pronounced shifts (~0.5 ppm in $^1$H NMR and ~10 ppm for $^{13}$C NMR) were attributed to the vinyl proton and carbon, respectively (H$_4$, C$_4$) which are $\alpha$ 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 phenoxyl type bonds to metal centres [36,37]. The $^1$H NMR spectra of the present complexes indicated that the signal assigned to the acid proton (ca. $\delta$ 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) (ca. 10–11 ppm) was still present. It was also noted that while the chemical shift of C$_6$ in 6-NO$_2$CcaH did move slightly upfield by 4 ppm upon complex formation, the corresponding signal in the complex formed by 8-MeO-6-NO$_2$CcaH 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 monodentate carbonylate bond although it is possible that the lactone carboxyl 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 $\mathrm{H}$-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 (MI) 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 $\mu$M. 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 $\mu$M) 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 $\mu$M) against the pathogenic MRSA bacterium. This MIC$_{80}$ value is particularly relevant when compared to the value observed for S. aureus (71.9 $\mu$M). 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
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 sulphhydryl 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<sub>50</sub> = 34.1 µM) was more active than AgNO<sub>3</sub> (MIC<sub>50</sub> = 66.8 µM) and was comparable in activity to the commercially available fungicide ketoconazole (MIC<sub>50</sub> = 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


References