

# Substituted Coumarins and their Metal Complexes as Potential Antimicrobial Agents

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## Overview

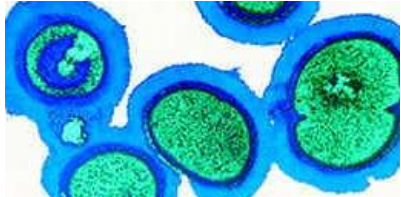
- Introduction to Hospital Acquired Infections (HAIs) and Drug Resistance
- General Introduction to Coumarins, Carboxylates and Metal Complexation
- Silver Coumarin Carboxylate Complexes with known Antimicrobial Activity
- Synthesis of Novel Coumarin Carboxylates and their Metal Complexes

# *Hospital Acquired Infections*

- HAI is an infection which is obtained subsequent to admission to hospital or after medical treatment
- Infections which become clinically evident after 48 hrs are considered 'hospital acquired'
- Caused by bacteria, fungi, viruses or parasites
- Most common HAIs are urinary tract infections, pneumonia and surgical wound infections
- Common causes include urinary catheterisation, respiratory procedures, surgery and wounds and IV procedures
- Overuse of antibiotics and the emergence of antibiotic resistant microorganisms have led to the rise in HAIs
- Community acquired Infections

# Major Types of Microorganisms which cause HAIs

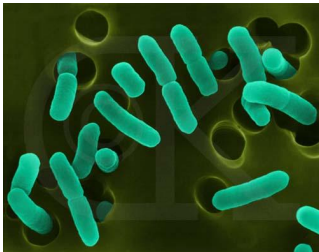
Methicillin-resistant *Staphylococcus aureus* (MRSA)



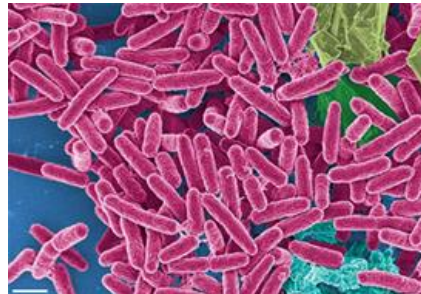
*Clostridium difficile*



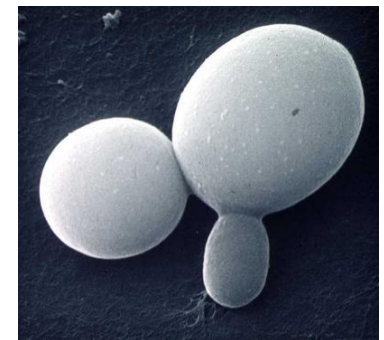
*Escherichia coli*



*Pseudomonas aeruginosa*



*Candida albicans*



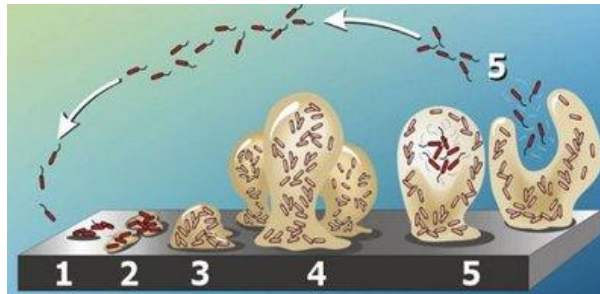
# MRSA

- Epidemic in hospitals in past 10-20 years
- Variety of *Staphylococcus aureus* (SA)
- SA exists on the skin and in the nose of about 30% of the population without any severe health effects
- 1940's Penicillin – Mutation of SA strains
- 1959 Methicillin – First case of MRSA within one year
- Glycopeptides such as Vancomycin are the last resort for treatment but resistance is beginning to appear



# Bacterial Resistance

- Resistance to antibiotics due to overuse, genetic evolution of bacteria and biofilm formation
- Certain bacteria adhere onto surfaces in multilayered cell clusters and secrete a polysaccharide coating (slime). The adherent bacteria and the coating are known as a biofilm.
- Exhibit extreme resistance as protected from the host's immune cells and action of antibiotics.



- Need for effective alternatives and the use of metal based drugs is a promising approach

## *Antimicrobial Effects of Metals*

- Silver**
- Romans used silver coins to preserve beverages
  - 18th Century used to treat wounds
  - 1960s – silver nitrate used to treat burn victims (silver sulfadiazine)
- $\text{Ag}^+$  disrupts critical cellular functions and is active in small quantities
  - Incorporated into many household and medical items including paints, furniture, wound dressings, catheters etc.
- Copper** – known to inhibit the growth of microorganisms
- Fixtures and fittings made of copper or copper alloy are used in homes and hospitals (Selly Oak) to create hygienic surfaces
- Zinc** – complexes used in various commercially available products
- Zinc acetate is used in lozenges to treat the common cold
  - zinc pyrithione used to treat dandruff (Head and Shoulders®) and skin conditions such as eczema, ringworm and athlete's foot

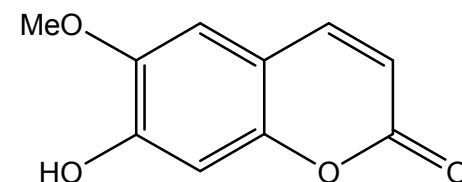
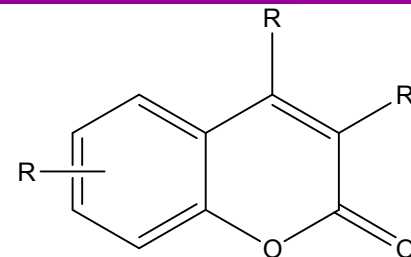
# Coumarins

Coumarin occurs naturally in many plants and essential oils.

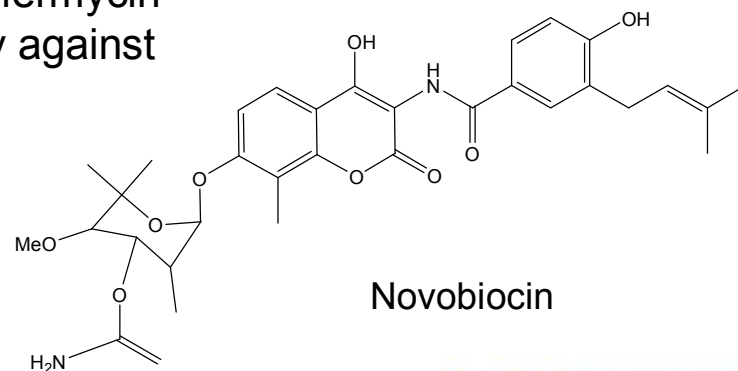
- Belongs to a class of compounds called benzopyrones which consist of a benzene ring fused to pyrone ring
- Biological activity includes antimicrobial, chemotherapeutic, anticoagulant and anti-inflammatory

Scopoletin and umbelliferone have shown antimicrobial activity

Novobiocin and its analogues, Clorobiocin and Coumermycin A<sub>1</sub> are aminocoumarins with antimicrobial activity against Gram-positive strains in particular *Staphylococci*



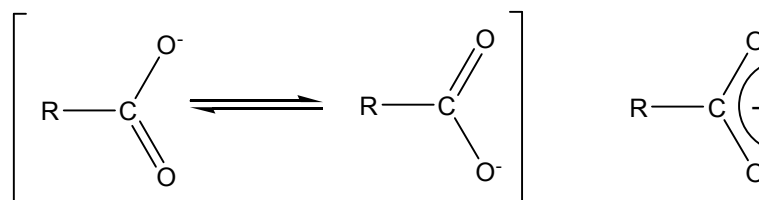
Scopoletin



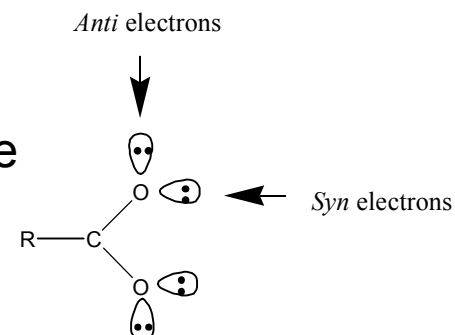
Novobiocin

# Carboxylates

- Carboxylic Acids - formation of carboxylate ion upon deprotonation with base

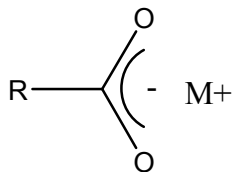


- Carboxylate anions ( $\text{RCOO}^-$ ) are versatile ligands capable of binding in a number of coordination modes
- Availability of lone pairs of electrons localised around two oxygen atoms
- Considering these sets of electrons available for donation and their relative geometries (*syn* and *anti*) a number of possible coordination modes arise
- Characterisation can be difficult

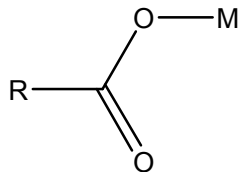


## Coordination Modes

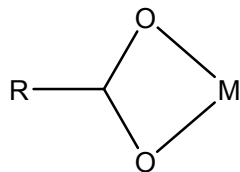
- Coordination modes are (i) ionic, (ii) unidentate (iii) bidentate (symmetrical and asymmetrical and (iv) bridging



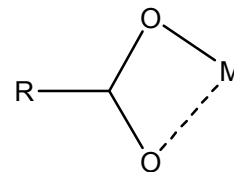
Ionic



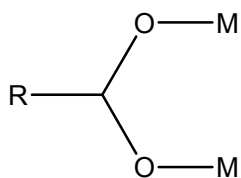
Unidentate



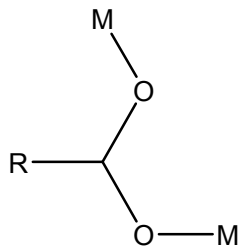
Symmetrical bidentate



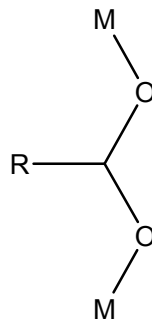
Asymmetrical bidentate



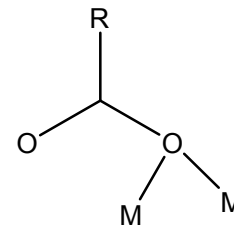
*syn-syn* bridging



*syn-anti* bridging



*anti-anti* bridging



Monatomic bridging

# Metal Complexation

## Silver – Ag(I)

- Forms good complexes with nitrogen, sulphur and halogen donor ligands. Forms complexes via oxygen in carboxylate ligands
- Common coord modes – Linear, Trigonal Planar, Tetrahedral and Octahedral
- Unidentate mode is also possible e.g. Silver(I) carboxylates

## Copper – Cu(II)

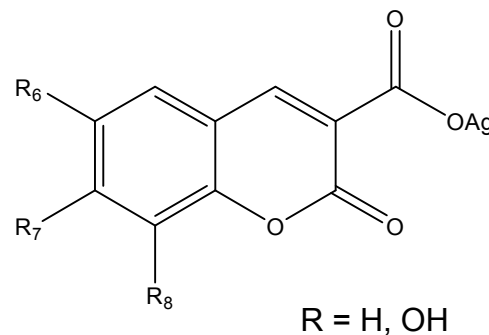
- Common coord. modes – Tetrahedral, Square Planar, Trigonal Bipyramidal and Octahedral
- Copper(II) carboxylates form mostly bridged dimers however mononuclear complexes are reported

## Zinc – Zn(II)

- Common coord. modes – Tetrahedral, trigonal bipyramidal, square pyramidal and octahedral
- Zinc(II) carboxylates can form complexes with unidentate, bridging and chelating modes.

# Antimicrobial Activity of Silver Coumarin Carboxylates

- Our group previously synthesised a series of Silver complexes of substituted Coumarin-3-Carboxylic acids with unidentate coordination
- Several compounds found to have significant antimicrobial activity against a range of bacteria

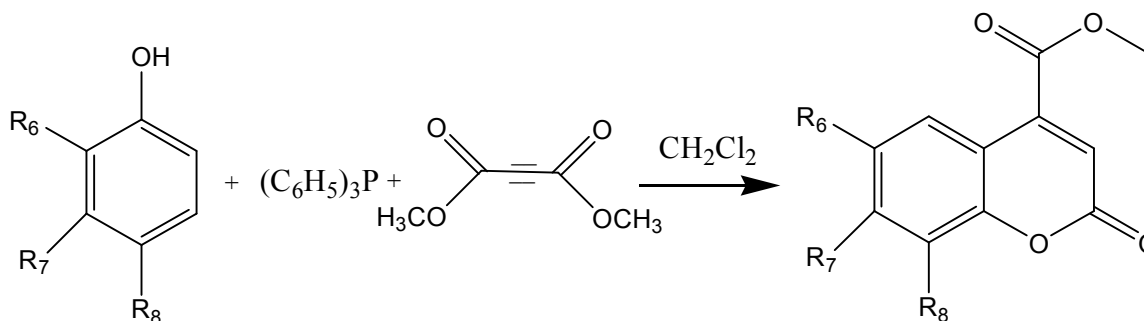


## MIC<sub>80</sub> values in µg/ml for selected ligands and their complexes

Compound	SA	MRSA	S. Sim	ML	E. Coli	BO	PA	CAN
Ligands	>500	>500	>500	>500	>500	>500	>500	>500
[Ag(Cca)]	71.9	0.630	123.4	100.2	17.5	10.5	55.3	163
[Ag(6-OHCca)]	72.0	76.2	87.9	66.3	114	94.0	77.6	34.1
[Ag(7-OHCca)]	34.5	35.6	17.3	28.9	36.1	32.1	23.3	69.3
[Ag(8-OHCca)]	140.9	137.6	137.7	114.1	50.0	33.8	38.8	270

## Synthesis of Coumarin-4-Carboxylates

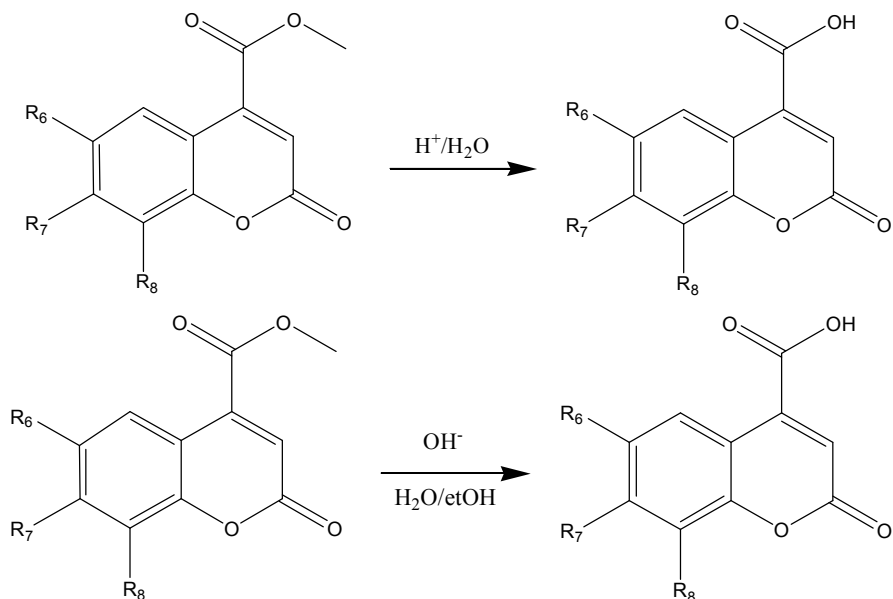
- Based on **Wittig Reaction** - carbonyl compound is reacted with a phosphonium ylide to produce an alkene (conversion of  $C=O$  to  $C=C$ )
- Coumarin synthesis – reaction between the conjugate base of a phenol and a vinyl triphenylphosphonium salt



- Substituted phenol is reacted with dimethyl acetylenedicarboxylate (DMAD) in the presence of triphenylphosphine in DCM for 100 hrs. Recrystallised from ethanol
- All compounds were analysed by NMR, IR, CHN, TLC, Solubility and Melting point

# Synthesis of Coumarin-4-Carboxylic Acids

- Hydrolysis of coumarin esters gave the corresponding carboxylic acids
- Both base and acid hydrolysis was used



Coumarin	R <sub>6</sub>	R <sub>7</sub>	R <sub>8</sub>
6-methoxycoumarin-4-carboxylic acid	OCH <sub>3</sub>	H	H
7-methoxycoumarin-4-carboxylic acid	H	OCH <sub>3</sub>	H
8-methoxycoumarin-4-carboxylic acid	H	H	OCH <sub>3</sub>
6-chlorocoumarin-4-carboxylic acid	Cl	H	H
6-bromocoumarin-4-carboxylic acid	Br	H	H
8-chloro-6-methoxycoumarin-4-carboxylic acid	OCH <sub>3</sub>	H	Cl

- All compounds were analysed by NMR, IR, UV, CHN, TLC, Solubility and Melting point

# Synthesis of Metal Complexes

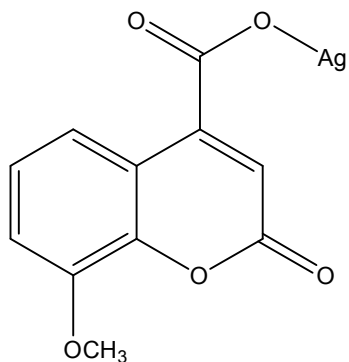
## Silver Complexes – 1:1 metal:ligand ratio

- Coumarin ligands were deprotonated with a stoichiometric amount of sodium hydroxide and the silver nitrate salt was added dropwise over 10 mins. Suspension was stirred for 1 hr

## Copper and Zinc Complexes – 1:2 metal:ligand ratio

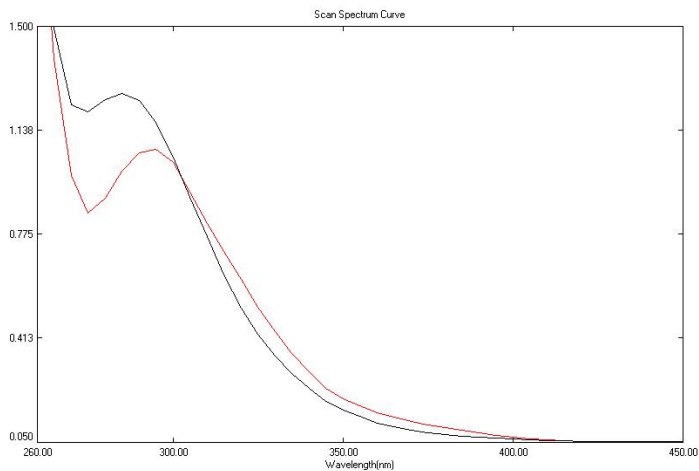
- Coumarin ligands and metal salt were dissolved separately in hot methanol and then the metal salt was added dropwise to the coumarin solution over 10 mins. Suspension was stirred for 2 hours.
- All complexes were analysed by NMR, IR, UV, CHN, TLC, Solubility and Melting point

# Characterisation of Ag Complexes

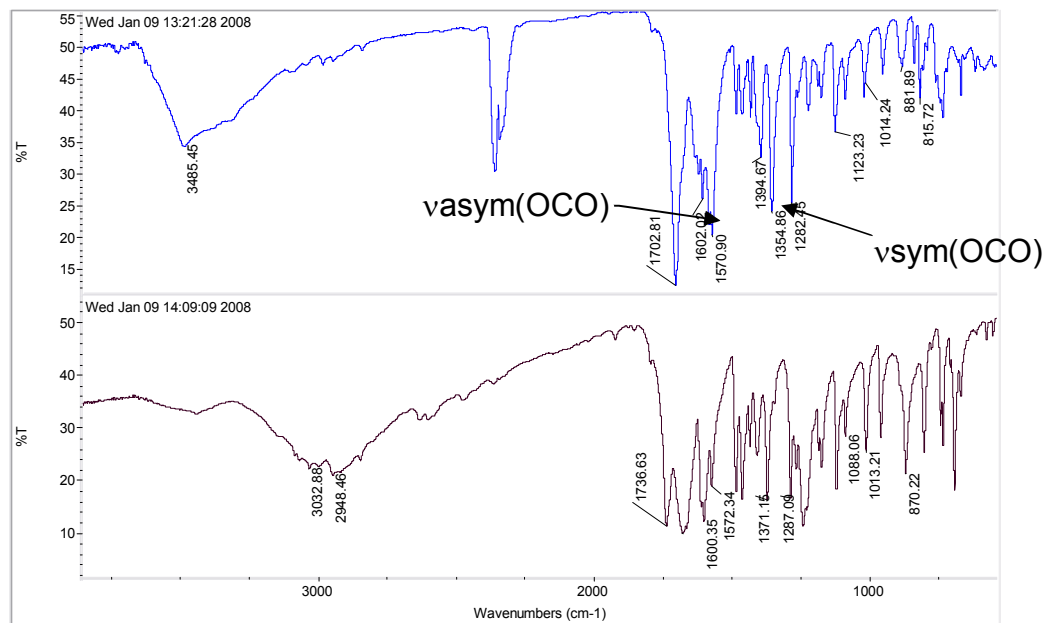


## Unidentate Coordination

8-methoxycoumarin-4-carboxylate silver(I)



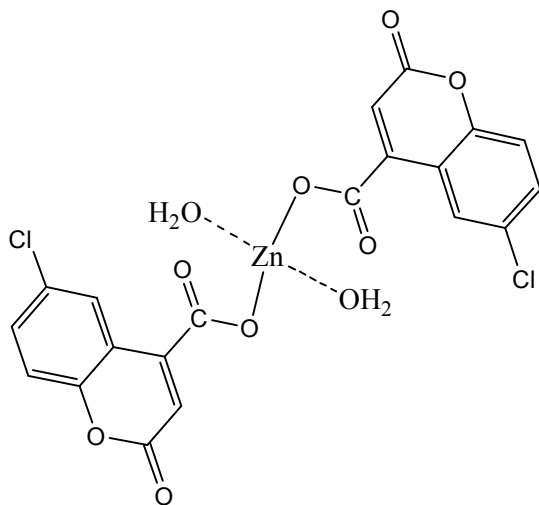
UV spectrum of 8-methoxycoumarin-4-carboxylic acid and its silver complex



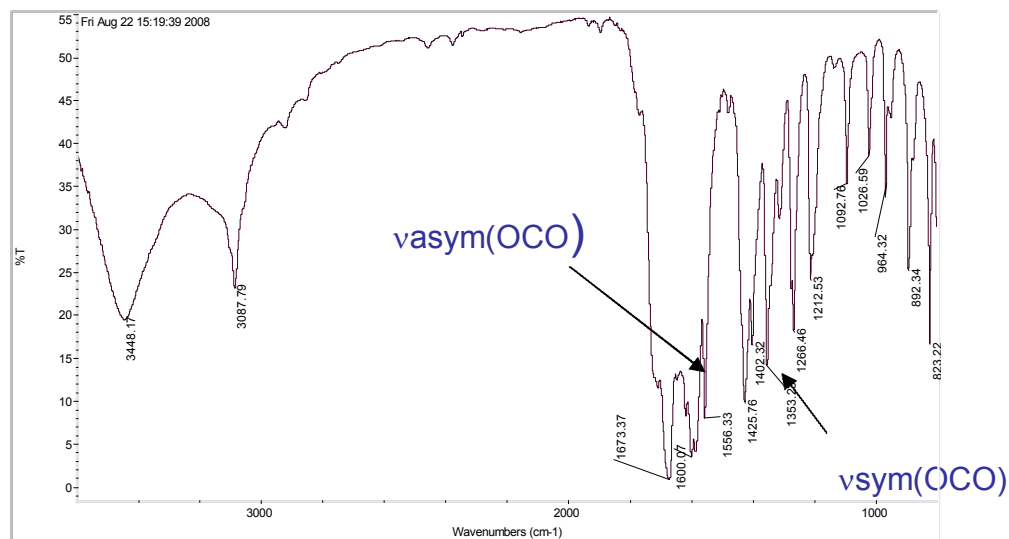
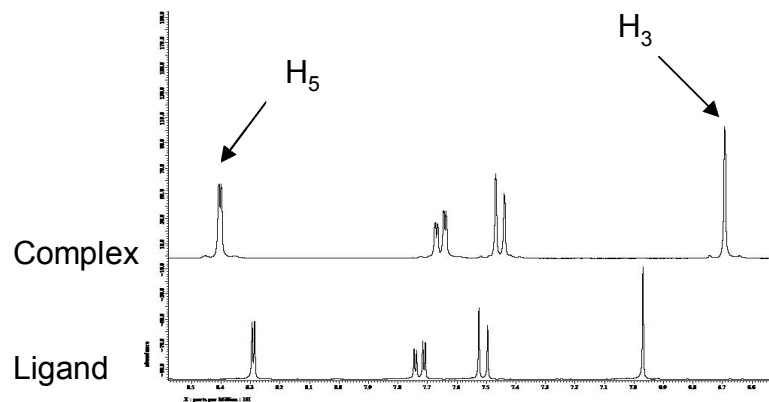
IR spectra of ligand (bottom) and silver complex (top)

# Characterisation of Cu and Zn Complexes

Based on CHN and IR data it was proposed that the Cu and Zn complexes are mononuclear with binding through the oxygen of two ligands and two water molecules to give **square planar geometry**

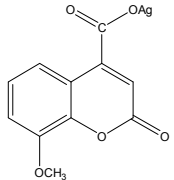
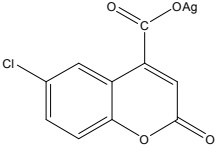


bis(6-chlorocoumarin-4-carboxylato)zinc(II)dihydrate



# Antimicrobial Activity of Tested Compounds

- To date only 2 silver complexes have had their antimicrobial activity tested against *Staphylococcus auerus*, *Pseudomonas Aeruginosa* and *Escherichia coli*
- Complexes show some activity against *Escherichia coli*

Compound	Structure	Activity MIC <sub>90</sub> (µM)		
		S.A	E.coli	P.A
8-methoxycoumarin-4-carboxylatosilver(I)		<b>188 (± 4.9)</b>	<b>47.3 (± 0.2)</b>	<b>93.2 (± 0.4)</b>
6-chlorocoumarin-4-carboxylatosilver(I)		<b>181.2 (± 2.3)</b>	<b>46.7 (± 0.2)</b>	<b>91.7 (± 1.0)</b>

## *Future Work*

- Synthesis of further substituted coumarin-4-carboxylic acids
- Synthesis of hydroxy substituted coumarin-4-carboxylic acids by demethylation of the methoxy substituted coumarin-4-carboxylic acids
- Synthesis of silver, zinc and copper complexes of all synthesised ligands
- Full characterisation
- Antimicrobial activity of all synthesised ligands and complexes to be tested

## Conclusions

- Coumarin carboxylic acids form metal complexes easily
- Carboxylates have a wide variety of coordination modes making characterisation of complexes difficult
- Silver complexes of coumarin-3-carboxylic acids have antimicrobial activity it is hoped that by SAR, coumarin-4-carboxylic acid complexes will also exhibit this activity
- HAIs due to bacterial resistance and overuse of antibiotics
- Need for effective alternative therapeutics

## *Acknowledgements*

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Thank you for listening!