Dalton Transactions

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Dalton Trans.*, 2023, **52**, 11958

Synthesis, characterisation and antibacterial activity of novel Ga(III) polypyridyl catecholate complexes†

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Received 7th June 2023,
Accepted 4th August 2023

DOI: 10.1039/d3dt01761c

rsc.li/dalton

Ga(III) polypyridyl catecholate complexes of type $[Ga(bipy)_2(O,O)](NO_3)$ or $[Ga(phen)_2(O,O)](NO_3)$ respectively were readily synthesised on reaction of $Ga(NO_3)_3$ in methanol with 1 equivalent of catecholate ligand (2,3-DHBA, 3,4-DHBA, 2,3,4-THBA or CafA) and then 2 equivalents of either bipy or phen. The complexes were characterised in full including by X-ray crystallography, which established that the catecholate ligands coordinate the Ga(III) centres in a bidentate manner *via* the two deprotonated hydroxy groups. All Ga(III) complexes exhibited good *in vitro* antibacterial activity against the Gram-negative pathogenic bacteria *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The complexes were inactive against the Gram-positive pathogenic bacteria *Staphylococcus aureus* including against a methicillin-resistant *Staphylococcus aureus* strain (MRSA). $[Ga(bipy)_2(2,3\text{-}DHBA_{-2H})](NO_3)\cdot 1.5H_2O$ (**1**) was shown to be non toxic *in vivo* in larvae of *Galleria mellonella* at doses up to 2000 μg mL^{-1} and to offer protection at doses of 100 and 250 μg mL^{-1} at 48 and 96 h to larvae infected with *P. aeruginosa*.

Introduction

The World Health Organisation clearly states that antibacterial resistance is a major global health and development threat.[1] The emergence and spread of drug-resistant bacterial pathogens, so-called superbugs, that have acquired new resistance mechanisms and cause infections that are not treatable with existing medicines is of grave concern. Significantly the pipeline of new innovative antibacterial drugs is remarkably dry.[1] There is therefore an urgent need for the development and advancement of new classes of antibacterial drugs.

Metal-based drugs play very important and well-established roles in the clinic as both therapeutic and diagnostic agents. They represent a unique class of chemotherapeutics with many metal-based complexes under investigation and development as anticancer and antimicrobial agents for instance.[2–4] Though bismuth- and silver-based anti-bacterials are currently in clinical use as antimicrobial agents, there is much recent interest in the role that gallium-based compounds can play in the fight against antibacterial resistance.[5–8]

Gallium (Ga) in its +3 oxidation state, Ga(III), is an effective Fe(III) mimic given it has a similar size and the same charge.[9] Significantly Ga(III) is redox inert under physiological conditions and therefore cannot be reduced. It consequently inhibits critical physiological Fe redox activity and in turn Ga(III)-substituted proteins disrupt important metabolic pathways.[9] Fe for example is found as a co-factor in numerous important enzymes such as oxidoreductases, which play roles in electron transfer, DNA synthesis and oxidative stress.[5,9]

Ga-based compounds are believed to hold promise in targeting antibacterial resistance as Fe is an essential nutrient for bacteria, particularly pathogenic bacteria, and it is difficult for biological systems to distinguish between Fe(III) and Ga(III).[5] Bacteria have evolved numerous strategies to acquire iron, including the use of low molecular weight iron sequestering agents, siderophores. It is believed siderophores facilitate the cellular uptake of Ga(III) through Fe-siderophore uptake pathways.[5,7,9]

Ga(III) is readily hydrolysed under physiological conditions to produce insoluble Ga(III) hydroxides. The bioavailability and antibacterial activity of Ga(III) can therefore be enhanced by developing Ga(III) coordination compounds with increased water solubility.

Gallium compounds such as Ga(III) citrate, Ga(III) maltolate Fig. 1, Ga(III) tartrate, tris(8-quinolinolato) Ga(III) (KP46), and

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† Electronic supplementary information (ESI) available. CCDC 2253969–2253972. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d3dt01761c

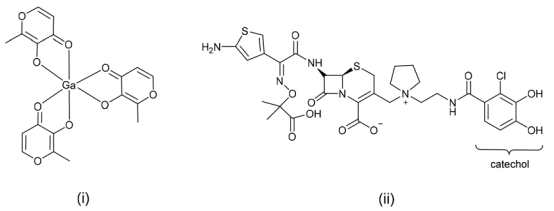

Fig. 1 Structure of (i) Ga(III) maltolate and (ii) cefiderocol.

Ga(III) complexes of α-N-heterocyclic thiosemicarbazones, exhibit encouraging antibacterial activity against Gram-negative and Gram-positive bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Mycobacterium tuberculosis*, and methicillin-resistant *Staphylococcus aureus* (MRSA).[7] We recently used quantitative proteomics to reveal Ga(III) maltolate induces an iron-limited stress response and reduces quorum-sensing in *P. aeruginosa*.[10] These processes are a fundamental component of bacterial virulence and dissemination and therefore point to a potential role for Ga(III) maltolate in the treatment of *P. aeruginosa* infection.[10]

Cefiderocol, Fig. 1 is a recently approved siderophore cephalosporin-based antibiotic, which exhibits antibacterial activity against Gram-negative pathogens including multidrug-resistant (MDR) enterobacteriaceae and non-fermenting organisms.[11] Significantly the catechol-type siderophore was incorporated to bind to extracellular free iron and utilise the bacterial active iron transport channels to enhance uptake and antibacterial potency of the antibiotic.

We surmised that Ga(III) complexes of catecholates would represent interesting antibacterial drug candidates. We therefore sought to develop water soluble heteroleptic Ga(III) compounds as antibacterial agents. We describe within the development of a novel class of Ga(III) polypyridyl catecholate complexes of 2,3-dihydroxybenzoic acid (2,3-DHBA), 3,4-dihydroxybenzoic acid (3,4-DHBA), 2,3,4-trihydroxybenzoic acid (2,3,4-THBA) and caffeic acid (CaffA), their activity against a panel of Gram-positive and Gram-negative pathogenic bacteria *in vitro* and *in vivo* in larvae of *Galleria mellonella* infected with *P. aeruginosa.*

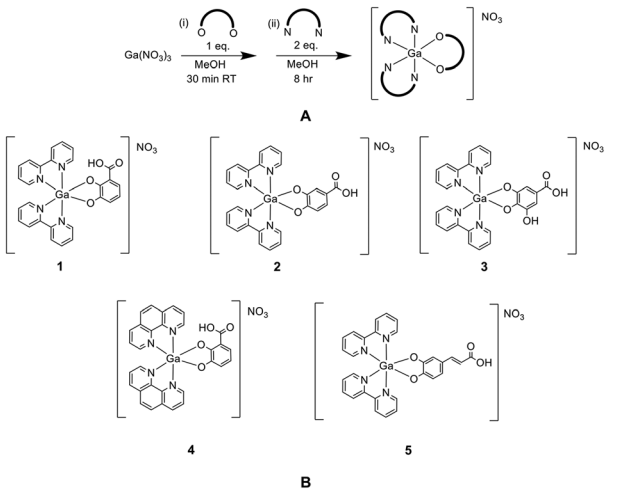

Fig. 2 (A) General synthetic route to compounds of type $[Ga(bipy)_2(O,O)](NO_3)$ or $[Ga(phen)_2(O,O)](NO_3)$. (B) Structures of $[Ga(bipy)_2(2,3\text{-}DHBA_{-2H})](NO_3)$ (**1**), $[Ga(bipy)_2(3,4\text{-}DHBA_{-2H})](NO_3)$ (**2**), $[Ga(bipy)_2(2,3,4\text{-}THBA_{-2H})](NO_3)$ (**3**), and $[Ga(phen)_2(2,3\text{-}DHBA_{-2H})](NO_3)$ (**4**), $[Ga(bipy)_2(CaffA_{-2H})](NO_3)$ (**5**).

Results and discussion

Synthesis of Ga(III) complexes 1 to 5

Reaction of $Ga(NO_3)_3$ hydrate in methanol with 1 equivalent of catecholate ligand (2,3-DHBA, 3,4-DHBA, 2,3,4-THBA or CafA) and then 2 equivalents of either bipy or phen gave the corresponding complexes of general formula $[Ga(bipy)_2(O,O)](NO_3)$ or $[Ga(phen)_2(O,O)](NO_3)$ respectively, Fig. 2.

All Ga(III) complexes were fully characterised by elemental analysis, ^{1}H NMR, ^{13}C NMR and IR spectroscopy and mass spectrometry and the structures of **1** to **4** elucidated by X-ray crystallography (Fig. S1–S21†).

Briefly with regards to the characterisation of $[Ga(bipy)_2(2,3\text{-}DHBA_{-2H})](NO_3)\cdot 1.5H_2O$ (**1**) as a representative example, the elemental analysis is fully consistent with two bipy ligands, one doubly deprotonated 2,3-DHBA ligand, one nitrate counterion and one and a half water molecules per Ga(III) centre. In the ^{1}H NMR spectrum of **1** (CD_3CN, Fig. S1†) the free and uncoordinated carboxylic acid proton signal is found at 13.01 ppm. The sixteen aromatic protons associated with the two bipy ligands are observed across ten signals ranging from 7.63 to 8.98 ppm. The three signals associated with the $2,3\text{-}DHBA_{-2H}$ ligand are found at 6.54, 6.72 and 7.04 ppm. These signals have shifted from 6.77, 7.06 and 7.34 ppm in the free ligand, indicative of coordination of the catecholate group. In the ^{13}C NMR spectrum (Fig. S2†) 25 unique signals are observed for the 27 carbons. In the IR spectrum (Fig. S3†) the ν(C=O) for the free carboxylic acid is observed at 1701 cm^{-1}.

HRMS in the positive mode (Fig. S5†) was also used to verify the complex cation of **1**, $[Ga(bipy)_2(2,3DHBA_{-2H})]^+$ (533.0743 a.m.u). The analysis outlined for **1** is also supported by X-ray crystallography.

A UV-Vis, HRMS and ^{1}H NMR study was undertaken to demonstrate that **1** is stable in solution over 24 hours (Fig. S22–S24†). In the UV-Vis spectrum of **1** in water at 37 °C over the course of 24 h, there is no reduction in the absorbance at λ_{max} at 283 nm. The HRMS spectrum of the same solution also verifies the presence of the complex cation of **1**, $[Ga(bipy)_2(2,3DHBA_{-2H})]^+$ (533.0747 a.m.u). In the ^{1}H NMR study in D_2O : CD_3CN (50 : 50), a solvent system that solubilises **1**, bipy and 2,3-DHBA, it is clear that there is no release of bipy or 2,3 DHBA ligands in this timeframe (Fig. S24†).

All Ga complexes **1–5** are isolated as racemic mixtures of Λ and Δ enantiomers. Racemic mixtures are optically inactive and no CD spectra of solutions of complexes **1–5** were recorded exhibiting noteworthy signals. Furthermore no

rotation of plan polarised light was observed on investigation of the optical properties of solutions of complexes **1–5** by polarimetry.

X-ray crystal structures

1, **2**, **3** and **4** were readily converted to [Ga(bipy)$_2$(2,3-DHBA$_{-2H}$)](PF$_6$)·MeOH (**1b**), [Ga(bipy)$_2$(3,4-DHBA$_{-2H}$)](PF$_6$)·MeOH (**2b**), [Ga(bipy)$_2$(3,4,5-THBA$_{-2H}$)](PF$_6$)·MeOH (**3b**) and [Ga(phen)$_2$(2,3-DHBA$_{-2H}$)](PF$_6$)·EtOH (**4b**) on stirring parent complexes with 1.5 eq. of KPF$_6$ in water. Slow evaporation of solutions of the resultant precipitates from MeOH (**1b**, **2b**, **3b**) or EtOH (**4b**) produced single crystals suitable for X-ray crystallography, Fig. 3 and Fig. S25–31 (ESI†). In each complex the catecholate ligands 2,3-DHBA, 3,4-DHBA, and 3,4,5-THBA as expected coordinate the Ga(III) centres in a bidentate manner *via* two deprotonated hydroxy groups.

Antimicrobial activity

***In vitro* susceptibility assays.** *In vitro* susceptibility assays were undertaken to investigate the activity of **1** to **5** against three Gram-negative bacteria; *Escherichia coli*, *Klebsiella pneumoniae* and *P. aeruginosa* and two strains of the Gram-positive bacteria *Staphylococcus aureus*, where one is methicillin resistant *S. aureus* (MRSA). The Gram-positive *S. aureus* and MRSA (Fig. S32, ESI†) showed no susceptibility to the complexes and investigations into the activity of this class of Ga complexes against Gram-positive bacteria was discontinued. The Gram-negative bacteria *E. coli*, *K. pneumoniae* and *P. aeruginosa* are particularly sensitive to the five complexes tested and IC$_{50}$ values were determined for **1** to **5**, Fig. S33 (ESI†) and Table 1.

On average the five complexes were slightly more active against *E. coli*. **4** was most active with **1** being the second most active of the Ga(III) polypyridyl catecholate complexes against all three bacteria. These results support the promising activity previously reported for Ga(III) compounds against the Gram-negative bacteria *E. coli*, *K. pneumoniae* and *P. aeruginosa*.[12–14]

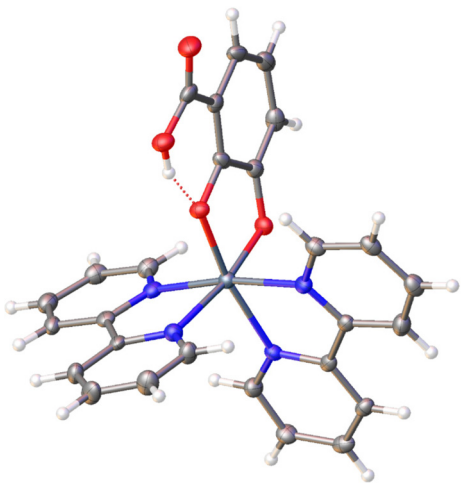

Fig. 3 Structure of the complex cation of **1** only with displacement shown at 50% probability. The molecular structure is completed by a PF$_6$ anion and methanol solvate, not shown. See ESI Fig. S22† for the complete asymmetric unit.

Table 1 IC$_{50}$ values determined for **1** to **5** and gallium nitrate (GaN) against 3× Gram-positive bacteria; *E. coli*, *K. pneumoniae* and *P. aeruginosa*

Complex	*E. coli* IC$_{50}$ (μg mL^{-1})	*K. pneumonia* IC$_{50}$ (μg mL^{-1})	*P. aeruginosa* IC$_{50}$ (μg mL^{-1})
1	37	60	52
2	53	77	73
3	62	62	64
4	12	18	26
5	65	81	71
GaN	nd	nd	100

We are particularly interested in the activity of our complexes against the cystic fibrosis (CF)-related pathogen *P. aeruginosa.* All five complexes had lower IC$_{50}$ values as compared to gallium nitrate, Ga(NO$_3$)$_3$ (GaN, 100 μg mL^{-1}, Fig. S34, ESI†) and gallium maltolate, [Ga(Mal$_{-1H}$)$_3$] (GaM, 62.5 μg mL^{-1})[10] against *P. aeruginosa.*

Toxicity evaluation in *G. mellonella*. Given our recent interest in the effects of GaM on the bacterial pathogen, *P. aeruginosa*,[10] we investigated the *in vivo* activity of the Ga(III) polypyridyl catecholate complexes using the *G. mellonella* infection model. *G. mellonella* larvae have been extensively employed in toxicity assays to investigate the *in vivo* toxicity and efficacy of novel drugs and *G. mellonella*-based models have provided comparable results as those generated using mammalian models.[15,16]

Larvae of *G. mellonella* were administered doses of 250, 500, 1000 and 2000 μg mL^{-1} of **1** to **5** in order to measure toxicity in the host. Larvae administered **1**, **2**, **3** and **5** showed no reduction in viability (Fig. S35†) whereas larvae administered **4** did experience toxic effects with a loss of viability observed at 1000 but in particular 2000 μg mL^{-1} (Fig. S35†).

Utilisation of *G. mellonella* larvae to assess the *in vivo* efficacy of 1 against *P. aeruginosa*. The ability of **1** as a representative non-toxic Ga(III) polypyridyl catecholate complex to act in the host and prolong survival of larvae infected with *P. aeruginosa* was assessed. Treatment of larvae infected with 1.5 × 10^1 CFU mL^{-1} of *P. aeruginosa* with doses of 60, 100 and 250 μg mL^{-1} of **1** 1 hour post-infection was investigated. Concentrations were selected based on the approximate IC$_{50}$, IC$_{70/80}$ and 5 × IC$_{50}$ concentrations where 250 μg mL^{-1} was also the highest tested dose in the *in vitro* susceptibility assay. Treatment increased larval survival at the 48 and 96 hours time points with the effects most pronounced at the highest dose of 250 μg mL^{-1}. 250 μg mL^{-1} treatment with **1** increased survival from 26.6% (8/30 larvae) to 40% (12/30 larvae) at 48 and 96 h, Fig. 4.

Experimental

Materials and instrumentation

GaNO$_3$·*x*H$_2$O, 1,10 phenanthroline (phen), 2,2 bipyridine (bipy), 2,3-dihydroxy benzoic acid (2,3-DHBA), 3,4-dihydroxy

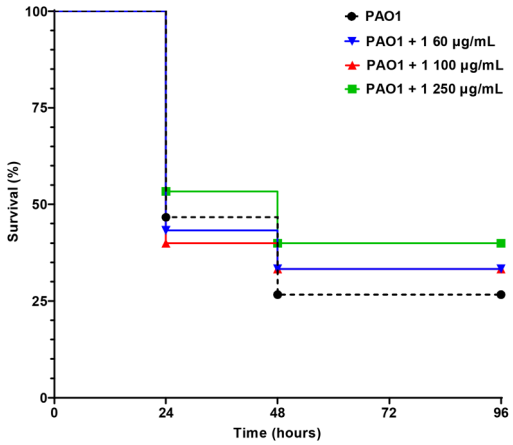

Fig. 4 Kaplan Meier plot for treatment of *G. mellonella* larvae infected with 1.5 × 10^1 CFU mL^{-1} of *P. aeruginosa* with **1**, n = 3.

benzoic acid (3,4-DHBA), 3,4,5-trihydroxy benzoic acid (3,4,5-THBA) and caffeic acid (CafA) were used as received from Sigma Aldrich (Sigma Aldrich Ireland Ltd, Arklow, Co Wicklow). All other commercially available reagents and solvents, including deuterated solvents, were also purchased from Sigma-Aldrich and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer. The spectra were analysed using MestReNova software. The residual undeuterated solvent signals were used as internal references.[17] Mass spectrometry experiments were performed on an Advion Expression Compact Mass Spectrometer where 10 μL of the samples were injected in 300 μL of methanol : isopropanol : water : formic acid (80 : 10 : 10 : 1 v/v). The mass spectrometry data were acquired in positive ion mode and the spectra analysed using the Advion Mass Express software programme. Elemental analysis (C, H, N) was performed at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University College Dublin, Ireland.

Synthetic procedures

[Ga(bipy)$_2$(2,3-DHBA$_{-2H}$)](NO$_3$)·1.5H$_2$O (1). 2,3-DHBA (0.186 g, 1.37 mmol, 1eq.) dissolved in methanol (10 mL) was added to Ga(NO$_3$)$_3$ hydrate (0.35 g, 1.37 mmol) in methanol (15 mL). After 30 min stirring at RT, the reaction was brought to reflux and a solution of bipy (0.43 g, 2.74 mmol) in methanol (10 mL) of was added dropwise. The yellow reaction mixture was stirred at reflux for 8 hours, cooled, filtered and the volume of the filtrate was reduced *in vacuo* to *ca.* 10 mL and subsequently stored at 4 °C for 24 h. The yellow solid that precipitated was collected *via* filtration and washed with small amounts of isopropanol and diethyl ether before being recrystallized from MeOH/H$_2$O to give 1 as a yellow solid. Yield: 0.62 g (73%). ^{1}H NMR (400 MHz, CD$_3$CN): δ/ppm = 13.01 (s 1H, COOH) 8.98 (d, 1H, Ar*H*), 8.90 (d, 1H, Ar*H*), 8.69 (m, 2H, Ar*H*), 8.62 (d, 2H, Ar*H*), 8.48 (t, 2H, Ar*H*), 8.33 (t, 2H, Ar*H*), 7.94 (m, 2H, Ar*H*), 7.79 (d, 1H, Ar*H*), 7.73 (d, 1H, Ar*H*), 7.63 (m, 2H, Ar*H*), 7.04 (d, 1H, Ar*H*), 6.72 (d, 1H, Ar*H*), 6.54 (t, 1H, Ar*H*). ^{13}C NMR (100 MHz, CD$_3$CN, 25 °C): δ_C 169.07, 154.47, 153.84, 149.34, 149.23, 148.96, 147.53, 147.34, 147.05, 146.97, 146.83, 145.19, 145.09, 144.75, 144.72, 130.09, 129.91, 129.57, 129.54, 125.04, 124.93, 124.76, 118.65, 118.39, 115.25 ppm. IR (cm^{-1}): 1701, 1602, 1585, 1562, 1468, 1444, 1348 (*NO$_3^-$*), 1309, 1264, 1205, 1150, 1062, 1031, 841, 752, 731, 639. Anal. required for C$_{27}$H$_{20}$GaN$_5$O$_7$·1.5H$_2$O: C, 52.03, H, 3.72, N, 11.19%. Found: C, 52.38, H, 3.37, N, 11.21%. MS (ESI, positive ion): m/z: 533.2 [Ga(bipy)$_2$(2,3DHBA$_{-2H}$)]$^+$. HRMS (ESI, positive ion): m/z: 533.0743 [Ga(bipy)$_2$(2,3DHBA$_{-2H}$)]$^+$ (required – 533.0735).

[Ga(bipy)$_2$(3,4-DHBA$_{-2H}$)](NO$_3$)·1.75H$_2$O (2). 3,4-DHBA (0.186 g, 1.37 mmol) dissolved in methanol (10 mL) was added to Ga(NO$_3$)$_3$ hydrate (0.35 g, 1.37 mmol) in methanol (15 mL). After 30 min stirring at RT, the reaction was brought to reflux and a solution of bipy (0.43 g, 2.74 mmol) in methanol (10 mL) of was added dropwise. The orange reaction mixture was stirred at reflux for 8 hours, cooled, filtered. The filtrate was reduced *in vacuo* to *ca.* 20 mL and subsequently stored at 4 °C for 24 h. The orange solid that precipitated was collected *via* filtration and washed with small amounts of isopropanol and diethyl ether before being recrystallized from MeOH/H$_2$O to give 2 as an orange solid. Yield: 0.59 g (69%). ^{1}H NMR (400 MHz, DMSO-d^6): δ/ppm = 11.68 (s, 1H, COOH) 8.91–8.85 (m, 4H, ArH), 8.68 (d, 2H, ArH), 8.47 (t, 2H, ArH), 8.38 (d, 2H, ArH), 7.98–7.92 (m, 2H, ArH), 7.43 (m, 2H, ArH), 7.15 (s, 1H, ArH), 7.10 (dd, 1H, ArH), 6.52 (dd, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d^6, 25 °C): δ_C 168.38, 159.01, 155.24, 152.83, 149.31, 147.01, 145.06, 144.97, 143.12, 137.37, 128.15 124.24, 123.13, 120.49, 119.99, 117.82, 112.81, 111.78. IR (cm^{-1}): 1679, 1614, 1603, 1579, 1493, 1477, 1444, 1315, 1262 (*NO$_3^-$*), 1029, 941, 807, 769, 731, 639. Anal. required for C$_{27}$H$_{20}$GaN$_5$O$_7$·1.75H$_2$O: C, 51.66, H, 3.77, N, 11.16%. Found: C, 51.56, H, 3.32, N, 11.08%. MS (ESI, positive ion): m/z: 533.2 a.m.u. [Ga(bipy)$_2$(3,4DHBA$_{-2H}$)]$^+$.

[Ga(bipy)$_2$(3,4,5-THBA$_{-2H}$)](NO$_3$)·H$_2$O (3). 3,4,5-THBA (0.233 g, 1.37 mmol) dissolved in methanol (10 mL) was added to Ga(NO$_3$)$_3$ hydrate (0.35 g, 1.37 mmol) in methanol (15 mL). After 30 min stirring at RT, the reaction was brought to reflux and a solution of bipy (0.43 g, 2.74 mmol) in methanol (10 mL) of was added dropwise. The orange reaction mixture was stirred at reflux for 8 hours, cooled, filtered. The filtrate was reduced *in vacuo* to *ca.* 20 mL and subsequently stored at 4 °C for 24 h. The orange solid that precipitated was collected *via* filtration and washed with small amounts of isopropanol and diethyl ether before being recrystallized from MeOH/H$_2$O to give **3** as an orange solid. Yield: 0.58 g (67%). ^{1}H NMR (400 MHz, DMSO-d^6): δ/ppm = 11.64 (s, 1H, COOH) 9.10 (s, 1H, OH), 8.86 (dd, 3H, ArH), 8.69 (dd, 2H, ArH), 8.51 (t, 2H, ArH), 8.38 (d, 2H, ArH), 7.97–7.90 (m, 5H, ArH), 7.47–7.44 (m, 2H, ArH), 6.84 (s, 1H, ArH), 6.74 (s, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d^6, 25 °C): δ_C 168.53, 155.21, 152.83, 149.30, 146.09, 143.38, 143.14, 137.37, 124.25, 123.08, 120.45, 116.07, 107.16, 106.91 ppm.

IR (cm^{-1}): 1698, 1655, 1604, 1570, 1476, 1444, 1311 (*NO$_3^-$*), 1270, 1201, 1109, 1081, 941, 886, 874, 851. Anal. required for C$_{27}$H$_{20}$GaN$_5$O$_8$·H$_2$O: C, 51.46, H, 3.52, N, 11.11%. Found: C,

51.34, H, 3.12, N, 11.00%. MS (ESI, positive ion): *m*/*z*: 550.2 $[Ga(bipy)_2(3,4,5THBA_{-2H})]^+$.

$[Ga(phen)_2(2,3DHBA_{-2H})](NO_3)$ (4)

2,3,-DHBA (0.186 g, 1.37 mmol) dissolved in methanol (10 mL) was added to $Ga(NO_3)_3$ hydrate (0.35 g, 1.37 mmol) in methanol (15 mL). After 30 min stirring at RT, the reaction was brought to reflux and a solution of 1,10′-phenantroline (0.49 g, 2.74 mmol) in methanol (10 mL) of was added dropwise. The yellow reaction mixture was stirred at reflux for 8 hours, cooled, filtered. The filtrate was reduced *in vacuo* to *ca.* 15 mL and subsequently stored at 4 °C for 24 h. The yellow solid that precipitated was collected *via* filtration and washed with small amounts of isopropanol and diethyl ether before being recrystallized from $MeOH/H_2O$ to give **5** as an orange solid. Yield: 0.59 g (67%). ^{1}H NMR (400 MHz, CD_3CN): δ/ppm = 13.07 (s, 1H, COOH) 9.36 (d, 1H, ArH), 9.30 (d, 1H, ArH), 9.06 (m, 2H, ArH), 8.82 (dd, 2H, ArH), 8.36 (m, 2H, ArH), 8.31–8.26 (m, 4H, ArH), 8.01 (d, 1H, ArH), 7.93 (d, 1H, ArH), 7.80–7.75(m, 2H, ArH), 7.06 (dd, 1H, ArH), 6.76 (dd, 1H, ArH). 6.55 (t, 1H, ArH). ^{13}C NMR (100 MHz, CD_3CN, 25 °C): δ_C 168.97, 154.77, 154.28, 150.27, 150.22, 148.65, 148.41, 143.73, 143.28, 138.56, 138.32, 128.95, 128.93, 128.22, 128.14, 127.48, 118.78, 118.58, 115.32. IR (cm^{-1}): 1726, (s, C═O) 1586, 1566, 1471, 1428, 1279 (NO_3^-), 1206, 1142, 1107, 1031, 851, 720. Anal. required for $C_{31}H_{20}GaN_5O_7$: C, 57.79, H, 3.13, N, 10.87%. Found: C, 57.41, H, 3.24, N, 10.77%. MS (ESI, positive ion): *m*/*z*: 581.2 $[Ga(phen)_2(2,3DHBA_{-2H})]^+$.

$[Ga(bipy)_2(CafA_{-2H})](NO_3)\cdot 0.5H_2O$ (5). cafA (0.23 g, 1.37 mmol) dissolved in methanol (10 mL) was added to $Ga(NO_3)_3$ hydrate (0.35 g, 1.37 mmol) in methanol (15 mL). After 30 min stirring at RT, a solution of bipy (0.43 g, 2.74 mmol) in methanol (10 mL) of was added dropwise. The yellow mixture was left to stir at room temperature for 24 hours. The reaction mixture was then placed in the fridge at 4 °C for 24 h.

The orange solid that precipitated was collected *via* filtration and washed with small amounts of isopropanol and diethyl ether before being recrystallized from $MeOH/H_2O$ to yield **4** as a yellow solid. Yield: (0.47 g, 53%). ^{1}H NMR (400 MHz, DMSO-d^6): δ/ppm = 11.77 (s, 1H, COOH) 8.85 (m, 4H, Ar*H*), 8.68 (d, 2H, Ar*H*), 8.48 (t, 2H, Ar*H*), 8.38 (d, 2H, Ar*H*), 7.99–7.93 (m, 4H, Ar*H*), 7.47–7.44(m, 2H, Ar*H*), 7.35 (dd, 1H, Ar*H*), 6.88 (s, 1H, Ar*H*), 6.65 (d, 1H, ArH), 6.52 (d, 1H, ArH), 5.98 (d, 1H, ArH) ppm. ^{13}C NMR (100 MHz, DMSO-d^6, 25 °C): δ_C 168.45, 157.68, 155.23, 153.74, 149.29, 146.97, 146.60, 144.89, 143.07, 137.32, 128.12, 124.20, 123.11, 122.46, 120.43, 112.28, 111.25, 109.87.

IR (cm^{-1}): 3067, 1615, 1604, 1578, 1555, 1478, 1446, 1398, 1313, 1260, 1159, 1118, 1032, 769, 732, 661, 540. Anal. required for $C_{29}H_{22}GaN_5O_7\cdot 0.5H_2O$: C, 55.18, H, 3.67, N, 11.09%. Found: C, 54.78, H, 3.60, N, 11.52%. MS (ESI, positive ion): *m*/*z*: 559.2 a.m.u. $[Ga(bipy)_2(CafA_{-2H})]^+$.

X-Ray crystallography

Data for **1**, **3** and **4** were measured on a Bruker D8 Quest ECO, using Mo Kα radiation (λ = 0.71073 Å) and data for **2** was measured on a Bruker APEX DUO. Each sample was mounted on a MiTeGen cryoloop and data collected at 100(2) K using and Oxford Cryosystems Cobra or Cryostream low temperature device. Bruker APEX[18] software was used to collect and reduce data. Absorption corrections were applied using SADABS.[19] Structures were solved with the SHELXT structure solution program[20] using Intrinsic Phasing. All were refined using Least Squares method on F^2 with SHELXL.[21] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned to calculated positions using a riding model with appropriately fixed isotropic thermal parameters. Molecular graphics were generated using OLEX2.[22] Crystal data, details of data collection and refinement are given in Table S1.†

In **1**, donor O–H hydrogen atoms were located and refined semi-free using restraints (DFIX). In **2**, disorder in one Ga bipy was modelled in two locations Ga1, N13–N24 65 : 35% with restraints (SIMU, DFIX). Disorder in the other Ga2-3,4-DHBA moiety was modelled in two locations, O60–O70, 52 : 48% with restraints (SADI, SIMU). Both PF_6 anions were modelled disordered over two locations using rigid groups, P1 83 : 17%, with restraints (SIMU) and P2, 53 : 47% with restraints also (SIMU).

In **3**, a weak high angle data lead to a high *R*(int). There was also mixed anion with disorder, with the PF_6 anion only 50% occupied and the remaining Cl anion modelled over three locations (22 : 20 : 8%) and both modelled with displacement restraints (ISOR, RIGU, SUMP) and constraints (EADP). Donor hydrogen atoms on the Ga complex were refined 'semi-free' with geometric restraints (DFIX). There are water and MeOH solvent molecules in the void. There are a total of 2.08 water molecules in 4 locations (100 : 50 : 33 : 25%) and refined with displacement restraints (ISOR). Hydrogen bonding networks were arranged manually to provide optimum contacts. The MeOH molecule is 20% occupied and modelled with geometric (DFIX) and displacement (ISOR) restraints. Methyl torsion angles did not refine and the CH_3 group was restrained in place (AFIX3). One water molecule hydrogen (O3s–H3sb) does not have any optimal hydrogen bonding contacts.

In **4**, the 2,3-DHBA was disordered (90 : 10%) and modelled in two locations using a rigid group, restraints (SIMU) and constraints (EADP). The partially occupied (75%) EtOH was modelled in two locations with a rigid group with restraints (SIMU).

Crystallographic data for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers 2253969–2253972.†

Ga complex stock solutions

Stock solutions of complexes **1–5** were dissolved (1 mg mL^{-1}) in sterile deionised water.

Bacterial culture conditions

Escherichia coli (clinical isolate), Methicillin-resistant *Staphylococcus aureus* (MRSA, clinical isolate), *Staphylococcus aureus* ATCC 33591, *Klebsiella pneumoniae* NCTC 13443 NDM-1

and *Pseudomonas aeruginosa* PAO1 were grown in nutrient broth (Oxoid) at 37 °C in an orbital shaker at 200 rpm. Bacterial stocks were maintained on nutrient agar at 4 °C.

In vitro susceptibility assays

Sterile nutrient broth (100 μl) was added to all wells in a 96-well plate. For MRSA and *K. pneumoniae* susceptibility assays, nutrient broth was supplemented with ampicillin 25 μg mL^{-1} and 50 μg mL^{-1}, respectively, to maintain resistance. GaS stock solutions were added and serially diluted to obtain final concentrations of 0.98–250 μg mL^{-1}. Bacterial cultures grown overnight were measured by obtaining the optical density at 600 nm (OD_{600}) and adjusted to OD 0.1. Cell suspensions (100 μl) were added to serially diluted volumes of the tested complexes and plates were incubated in a static incubator at 37 °C for 24 h. Growth was measured at OD_{600}.

Galleria mellonella toxicity evaluation

Sixth instar larvae of the greater wax moth, *Galleria mellonella*, (Livefoods Direct Ltd, Sheffield, UK) displaying no signs of discolouration (melanisation) were each weighed (200–300 mg) and stored in 9 cm Petri dishes containing wood shavings. Ten healthy larvae were selected per sample group (total n = 30). Larvae were stored at 15 °C in the dark to prevent pupation between experiments.

Solutions of **1–5** (250–2000 μg mL^{-1}) were prepared in sterile deionised water. Larvae were injected *via* the last-left proleg using a U-100 insulin syringe (Terumo Europe, N.V., Belgium) and 25G × 5/8″ needle (BD Microlance™) to administer 20 μL. Larvae were incubated at 37 °C and monitored every 24 h for 96 h. Survival was determined based on the level of melanisation and response to touch.

Galleria mellonella viability evaluation

P. aeruginosa cells grown overnight were washed twice by centrifugation at 2500 rpm for 10 min and pellets were resuspended in sterile PBS. Bacterial cells were diluted in PBS to obtain a cell density of 1.5×10^1 CFUs mL^{-1}. Suspensions were injected into the last-left proleg as previously described and larvae were incubated at 37 °C for 1 h. Solutions of **1** were prepared in sterile deionised water and administered (20 μL) *via* the last-right proleg. Control samples were injected with PBS. Larvae were incubated at 37 °C and monitored up to 96 h to determine percentage survival.

Data analysis

All experiments were carried out independently in triplicate and error bars denote mean ± S.E. Graphs were constructed using GraphPad Prism Software v.9.5.1 (GraphPad Software Inc., San Diego, CA, USA).

Conclusions

There is increasing interest in the development of Ga-based antimicrobial drug candidates. We present a novel class of Ga(III) polypyridyl catecholate complexes of type $[Ga(bipy)_2(O,O)](NO_3)$ or $[Ga(phen)_2(O,O)](NO_3)$ respectively The complexes were fully characterised including by X-ray crystallography, which established that the catecholate ligands coordinate the Ga(III) centres in a bidentate manner *via* the two deprotonated hydroxy groups. All Ga(III) complexes exhibited good *in vitro* antibacterial activity against the Gram-negative pathogenic bacteria *E. coli*, *K. pneumoniae* and *P. aeruginosa*. This study builds on previous reports, which associated Ga-based compounds with good antibacterial activity against *P. aeruginosa*. Specifically our lead Ga(III) polypyridyl catecholate complexes, $[Ga(bipy)_2(2,3\text{-}DHBA_{-2H})](NO_3)$ (**1**), was shown to be non-toxic *in vivo* in larvae of *G. mellonella* at doses up to 2000 μg mL^{-1} and to offer protection at doses of 100 and 250 μg mL^{-1} at 48 and 96 h to larvae infected with *P. aeruginosa*. *P. aeruginosa* is a major cause of lung infections in people living with cystic fibrosis (CF). The activity of Ga-based compounds should potentially be investigated against other CF-related microbial pathogens.

Author contributions

Lewis More O'Ferrall: conceptualisation, methodology, investigation, writing – original draft preparation, funding acquisition. Magdalena Piatek: methodology, investigation, writing – original draft preparation. Brendan TwamLey: methodology, resources, writing – original draft preparation. Kevin Kavanagh: conceptualisation, methodology, resources, writing – original draft preparation. Christine O'Connor: conceptualisation, resources, methodology, writing – original draft preparation, supervision, project administration, funding acquisition. Darren M. Griffith: conceptualisation, resources, methodology, writing – original draft preparation, supervision, project administration, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

LMOF and COC sincerely thank TU Dublin Research Scholarship Fund for financial support. DMG, LMOF, KK and MP acknowledge funding received from the SSPC, the Science Foundation Ireland Research Centre for Pharmaceuticals, financed by a research grant from Science Foundation Ireland (SFI) and co-funded under the European Regional Development Fund under Grant Number 12/RC/2275_P2.

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