Synthesis, isomerisation and biological properties of mononuclear ruthenium complexes containing the bis[4(4′-methyl-2,2′-bipyridyl)]-1,7-heptane ligand†

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A series of mononuclear ruthenium(II) complexes containing the tetradentate ligand bis[4(4′-methyl-2,2′-bipyridyl)]-1,7-heptane have been synthesised and their biological properties examined. In the synthesis of the [Ru(phen)(bb)]2+ complexes (where phen = 1,10-phenanthroline and its 5-nitro-, 4,7-dimethyl- and 3,4,7,8-tetramethyl- derivatives), both the symmetric cis-α and non-symmetric cis-β isomers were formed. However, upon standing for a number of days (or more quickly under harsh conditions) the cis-β isomer converted to the more thermodynamically stable cis-α isomer. The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) of the ruthenium(II) complexes were determined against six strains of bacteria: Gram-positive Staphylococcus aureus (S. aureus) and methicillin-resistant S. aureus (MRSA); and the Gram-negative Escherichia coli (E. coli) strains MG1655, APEC, UPEC and Pseudomonas aeruginosa (P. aeruginosa). The results showed that the [Ru(5-NO2phen)2(bb)]2+ complex had little or no activity against any of the bacterial strains. By contrast, for the other cis-α-[Ru(phen)(bb)]2+ complexes, the antimicrobial activity increased with the degree of methylation. In particular, the cis-α-[Ru(Me4phen)(bb)]2+ complex showed excellent and uniform MIC activity against all bacteria. By contrast, the MBC values for the cis-α-[Ru(Me4phen)(bb)]2+ complex varied considerably across the bacteria and even within S. aureus and E. coli strains. In order to gain an understanding of the relative antimicrobial activities, the DNA-binding affinity, cellular accumulation and water–octanol partition coefficients (log P) of the ruthenium complexes were determined. Interestingly, all the [Ru(phen)2(bb)]2+ complexes exhibited stronger DNA binding affinity (Kd ≈ 1 × 10^7 M⁻¹) than the well-known DNA-intercalating complex [Ru(phen)2(dpdpz)]2+ (where dpdpz = dipyrido[3,2-a:2′,3′-c]phenazine).

Introduction

Due to a developing resistance to antimicrobial drugs, bacterial diseases are becoming a significantly greater threat to humans. Of note, the World Health Organisation has stated that antimicrobial resistance is one of the most important problems currently affecting global health, food security and development.1 As a consequence, there is considerable interest in the development of new classes of antimicrobial agents, and while the development of new drugs based upon organic compounds continues, transition metal-based antimicrobial agents in general have been attracting attention – and ruthenium(II) complexes in particular.2–14 A range of ruthenium(II) complexes have been studied, and generally have shown good activity against Gram-positive bacteria, but variable and often poor activity against Gram-negative species.10–12

Dwyer and co-workers were the first to study the biological activity of mononuclear tris(bidentate) inert metal complexes...
with ligands such as 1,10-phenanthroline (phen) and its
derivatives \( \text{e.g.} \ 3,4,7,8\)-tetramethyl-1,10-phenanthroline
\((\text{Me}_4\text{phen})\) and 5-nitro-1,10-phenanthroline \((5-\text{NO}_2\text{phen})\).\(^9\)

More recently, a wide range of mononuclear \{\text{e.g.} \ \{\text{Ru}(2,9-
\text{Me}_2\text{phen})_2(dppz)\}_2^{2+}\} \ (2,9-\text{Me}_2\text{phen} = 2,9-dimethyl-1,10-phen-
anthroline; dppz = dipyrido[3,2-\alpha:2',3'-\beta]phenazine\)\(^10\) and di-
and oligo-nuclear polypyridylruthenium(II)\(^12,14,15\) complexes
have been examined. In particular, earlier studies from our lab-
oratories have examined the antimicrobial properties of di-, tri-
and tetra-nuclear polypyridylruthenium(II) complexes in which
the metal centres are linked by the bis\{4(4'-methyl-2,2'-bipyrid-
yl)-1,\(n\)-alkane ligand \(\text{"bb}_{n}\); see Fig. 1\}.\(^14\) While these oligo-
nuclear ruthenium complexes showed excellent activity against
drug-sensitive Gram-positive bacterial strains, and maintained
the activity against drug-resistant strains, they exhibited rela-
tively poor activity against some Gram-negative strains.\(^16\)

More recently, we synthesised mononuclear ruthenium complexes
that contained the \(\text{bb}_{n}\) moiety (for \(n = 10\) and 12), but as a tetra-
dentate ligand,\(^17\) rather than as a ligand linking metal centres.
While the \(\text{cis-}\alpha-\{\text{Ru}(\text{phen})(\text{bb}_{12})\}_2^{2+}\) species showed better activity
towards the clinically-important Gram-negative \textit{Pseudomonas}
aeruginosa, its activity against other bacteria was only equivalent
to \([\text{Ru}(\text{Me}_4\text{phen})_3]^{2+}\),\(^8\) the most active of the ruthenium com-
plexes prepared by Dwyer and co-workers sixty years ago. The
\(\text{cis-}\alpha-\{\text{Ru}(\text{phen})(\text{bb}_{10})\}_2^{2+}\) complex was less active than \(\text{cis-}\alpha-\{\text{Ru-}
(\text{phen})(\text{bb}_{12})\}_2^{2+}\) and \([\text{Ru}(\text{Me}_4\text{phen})_3]^{2+}\) against most of the
bacterial strains.\(^17\) The antimicrobial activity of the \([\text{Ru}(\text{phen})-
(\text{bb}_{n})]^{2+}\) complexes could be potentially improved through an
increase of the lipophilicity, for example by utilising the \(\text{bb}_{16}\) ligand.
However, an alternative approach is to decrease the lipoi-
philicity of the \(\text{bb}_{n}\) chain but increase the electron density at
the ruthenium centre through the incorporation of electron-
donating groups on the 1,10-phenanthroline ligand. It has been
established that the antimicrobial activity of the metal com-
plexes is significantly affected by the cellular uptake,\(^15\) which in
turn is presumably sensitive to the charge on the metal centre
as well as the overall lipophilicity. Consequently, the modu-
lation of the electron density at the metal centre could provide
higher uptake and greater ability to modulate the differential
uptake between bacterial and eukaryotic cells.

Herein, we describe the synthesis of a series of
complexes involving 1,10-phenanthroline and some of its deriva-

Fig. 1 The \(\text{bb}_{n}\) ligand and the \(\text{Rubb}_{n}\), \(\text{Rubb}_{n}\)-tri and \(\text{Rubb}_{n}\)-tetra complexes.
tives – [Ru(phen')(bb2)]2+ {where phen’ = phen; 4,7-dimethyl-1,10-phenanthroline (Me2phen); Me2phen; and 5-NO2phen} – and an examination of their DNA binding, cellular uptake and antimicrobial activity against a range of bacteria. Synthetically, the results indicate that although the cis-\(\alpha\)-[Ru(phen')(bb2)]2+ complexes are formed initially they are unstable and are converted to the cis-\(\alpha\) isomer and, to a very small extent, the corresponding [Ru(phen')(Me2bpy)]2+ species. The cis-\(\alpha\)-[Ru(Me4phen)(bb2)]2+ complex showed uniformly high activity against Gram-positive and Gram-negative bacteria. Furthermore, the results demonstrate the effect of the electron density at the ruthenium centre on the cellular uptake of the metal complexes.

**Results**

**Synthesis of cis-\(\alpha\)-[Ru(phen')(bb2)]2+ complexes**

The synthesis of the cis-\(\alpha\) and cis-\(\beta\) isomers of [Ru(phen')(bb12)]2+ was carried out as previously reported.\(^\text{17}\)

The synthesis of a series of mononuclear cis-\(\alpha\)-[Ru(phen')(bb7)]2+ complexes was achieved with good yields, as shown in Scheme 1, by heating cis,cis-[RuCl2(DMSO)2(phen')] \(^\text{17}\) to 130–140 °C with the bb7 ligand in ethylene glycol: the 2+ complexes were isolated from higher-charged species using cation-exchange chromatography on a SP Sephadex C-25 column with sodium toluene-4-sulfonate as the eluent, and the resulting solid products recrystallised from acetonitrile/diethyl ether. Subsequent cation-exchange chromatography on a SP Sephadex C-25 column (1 metre) with sodium toluene-4-sulfonate as the eluent realised two bands – the cis-\(\alpha\) isomer and corresponding [Ru(phen')(Me2bpy)]2+ species as a minor product. All the complexes were characterised by microanalysis, NMR spectroscopy and high-resolution electrospray ionisation (ESI) mass spectrometry. The cis-\(\alpha\)-[Ru(Me4phen)(bb7)]2+ complex was also characterised by X-ray crystallography (see Table 1 and Fig. 2).

In our recent synthetic study\(^\text{17}\) of the analogous [Ru(phen')(bb10)]2+ and [Ru(phen')(bb12)]2+ complexes, in both cases the cis-\(\alpha\) isomers and cis-\(\beta\) isomers (see Fig. 3) were isolated and individually characterised. In the present case, only the cis-\(\alpha\)-[Ru(phen')(bb2)]2+ isomer was isolated, and consequently the cis,cis-[RuCl2(DMSO)2(Me2phen)] reaction with the bb2 ligand was examined in detail. When samples were taken from the reaction mixture during the course of the synthesis and studied by \(^1\)H NMR, both the cis-\(\alpha\) and cis-\(\beta\) isomers were unambiguously identified in COSY spectra of the reaction mixture. However, after either heating the reaction mixture to 200 °C (see Fig. 4) or allowing the mixture of the isolated isomers stand at room temperature for several weeks, only the cis-\(\alpha\) isomer was observed. In addition, during the purification process for the cis-\(\alpha\) isomer for all the [Ru(phen')(bb2)]2+ cases, a small amount (<5% yield based upon starting material) of [Ru(phen')(Me2bpy)]2+ was observed: for phen’ = Me2phen and Me2phen, the products were isolated and the \(^1\)H NMR and TOF-MS (ESI+) characterisation data are provided in the ESI.† For [Ru(Me4phen)(Me2bpy)]2+, the structure confirmed by X-ray crystallography (see Fig. S1: ESI†).

The same observations were made for reactions conducted under normal laboratory light or in the dark. As the cis-\(\beta\)-[Ru(Me4phen)(bb7)]2+ complex appeared to be less stable than the corresponding cis-\(\alpha\) isomer, it was of interest to compare the relative energies of the isomers through DFT calculations. In addition, the differences in energies between the cis-\(\alpha\) and cis-\(\beta\) isomers for the bb7 complex were compared to those for the corresponding bb12 complex. As shown in Table 2, there was good agreement between the X-ray and DFT-optimised structures based upon the cis-\(\alpha\)-[Ru(Me4phen)(bb7)]2+ N–Ru–N angles for the corresponding chelate rings. While there were only small differences in the N–Ru–N bond angles between the cis-\(\alpha\)-[Ru(Me4phen)(bb12)]2+ (designated \(\alpha\)-Me4phen-7) and the cis-\(\alpha\)-[Ru(Me4phen)(bb12)]2+ (designated \(\alpha\)-Me4phen-12) complexes, significant differences were observed between the corresponding cis-\(\beta\) isomers (designated \(\beta\)-Me4phen-7 and \(\beta\)-Me4phen-12, respectively); see Table 3. Furthermore, the \(\beta\)-Me4phen-12 complex was determined to be 5.6 kJ mol\(^{-1}\) less stable than the \(\alpha\)-Me4phen-12, whereas by contrast \(\beta\)-Me4phen-7 was 76.8 kJ mol\(^{-1}\) less stable than the \(\alpha\)-Me4phen-7 isomer. It is observed that the calculated difference in the N1–Ru–N5 angle between the \(\beta\)-Me4phen-7 and \(\beta\)-Me4phen-12 species is significant (Table 3), and this may well be the origin of the instability of the \(\beta\)-Me4phen-7 species relative to both the \(\alpha\)-Me4phen-7 isomer and the \(\beta\)-Me4phen-12 analogue. However, no significant differences in the calculated Ru–N bond lengths were observed between the \(\alpha\)-Me4phen-7 and \(\beta\)-Me4phen-7 isomers.

**Scheme 1** Synthesis of [Ru(phen')(bb2)]2+ complexes containing the bb2 ligand.
Antimicrobial activity

The minimum inhibitory concentrations (MIC) for the ruthenium complexes against six bacterial strains (methicillin-resistant Staphylococcus aureus (MRSA) and a methicillin-sensitive strain of S. aureus, both avian pathogenic (APEC) and uropathogenic (UPEC) strains of Escherichia coli as well as a laboratory strain (E. coli MG1655), and Pseudomonas aeruginosa PAO1) were determined and the results are summarised in Table 4. Interestingly, the \([\text{Ru}(\text{5-NO}_2\text{phen})(\text{bb7})]^{2+}\) complex (designated \(\alpha\text{-NO}_2\text{phen}-7\)) showed very little or no activity against any of the bacterial strains. By contrast, for the other \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{phen})'(\text{bb})]^{2+}\) complexes, the antimicrobial activities increased with the degree of methylation. Most importantly, \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{Me}_4\text{phen})(\text{bb7})]^{2+}\) displayed very similar activity against all bacterial strains: for ruthenium( II)-based complexes, the observed similarity in activity against Gram-positive and Gram-negative is rare. In absolute terms, the \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{Me}_4\text{phen})(\text{bb7})]^{2+}\) complex exhibited comparable or better activities to the Gram-negative species compared to \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{Me}_4\text{phen})(\text{bb12})]^{2+}\) and \([\text{Ru}(\text{Me}_4\text{phen})_3]^{2+}\) (designated \(\text{Me}_4\text{phen}_3\)). The dinuclear complexes Rubb7 and Rubb12 (see Fig. 1) were also examined, and the results indicated that the \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{Me}_4\text{phen})(\text{bb7})]^{2+}\) complex was more active than Rubb7, but less active than Rubb12.

The minimum bactericidal concentrations (MBC) of the ruthenium complexes were also determined, and the results are summarised in Table 5. Consistent with previous results, the MBC values for Rubb12 and \([\text{Ru}(\text{Me}_4\text{phen})_3]^{2+}\) were generally \(\leq 2 \times \text{MIC}\), indicating that the two ruthenium complexes are bactericidal. By contrast, \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{phen})'(\text{bb})]^{2+}\) is borderline bactericidal/bacteriostatic. Surprisingly, the MBC values for \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{Me}_4\text{phen})(\text{bb7})]^{2+}\) show considerable variation, e.g. S. aureus compared to MRSA, or APEC to UPEC. Based upon the MBC/MIC ratios, \(\text{cis}\text{-}\alpha\text{-}[\text{Ru}(\text{Me}_4\text{phen})(\text{bb7})]^{2+}\) is clearly bactericidal against MRSA and APEC, but bacteriostatic against S. aureus and UPEC. These significant differences within both the Gram-positive and Gram-negative classes of bacteria indi-
cate that cis-α-[Ru(Me₄phen)(bb₇)]²⁺ has significant specific toxicity to some bacteria and suggests that the mechanism of the activity is considerably different compared to [Ru(Me₄phen)(bb₁₂)]²⁺, [Ru(Me₄phen)₃]²⁺ and Rubb₁₂.

Lipophilicity (log \( P \))
Lipophilicity is a factor that affects the biological activity of any metal complex because it is often correlated with the capacity of the drug to penetrate through the cell membrane. The standard octanol/water partition coefficients (log \( P \)) were determined for the [Ru(phen′)(bb₇)]²⁺ and [Ru(Me₄phen)₃]²⁺ complexes, and the results are summarised in Table 6. As expected, cis-α-[Ru(Me₄phen)(bb₇)]²⁺ was more lipophilic than the other cis-α-[Ru(phen′)(bb₇)]²⁺ analogues. The introduction of the nitro-substituent on 1,10-phenanthroline ligand in cis-α-[Ru(5-NO₂phen)(bb₇)]²⁺ did not decrease the lipophilicity compared with the cis-α-[Ru(phen)(bb₇)]²⁺ analogue. More significantly, the cis-α-[Ru(Me₄phen)(bb₇)]²⁺ isomer is more lipophilic than the Rubb₁₂ complexes, but of similar lipophilicity to [Ru(Me₄phen)₁]²⁺, and less lipophilic than their [Ru(phen)-(bb₁₂)]²⁺ analogues.

Cellular accumulation
The cellular accumulations of the cis-α-[Ru(phen′)(bb₇)]²⁺ analogues (and Rubb₁₂ and [Ru(Me₄phen)₁]²⁺) in MRSA, UPEC and

Table 2 Comparison of angles (in degrees) in cis-α-[Ru(Me₄phen)(bb₇)]²⁺ X-ray crystal and DFT-optimised structures. Nomenclature is defined in Fig. 5

| Angle         | α-Me₄phen-7 (crystal structure) | α-Me₄phen-7 (DFT) | % deviation |
|---------------|-------------------------------|------------------|-------------|
| N1–Ru–N2      | 78.9                          | 77.5             | 1.77        |
| N1–Ru–N3      | 94.6                          | 95.5             | 0.95        |
| N1–Ru–N5      | 97.1                          | 97.3             | 0.21        |
| N1–Ru–N6      | 88.9                          | 89.5             | 0.67        |
| N4–Ru–N2      | 94.6                          | 95.6             | 1.06        |
| N4–Ru–N3      | 78.5                          | 77.4             | 1.40        |
| N4–Ru–N5      | 89.4                          | 89.9             | 0.56        |
| N4–Ru–N6      | 98.2                          | 97.8             | 0.41        |
| N2–Ru–N6      | 100.6                         | 99.5             | 1.09        |
| N2–Ru–N3      | 82.1                          | 83.7             | 1.95        |
| N3–Ru–N6      | 98.4                          | 99.1             | 0.71        |
| N3–Ru–N6      | 176.0                         | 174.6            | 0.80        |

Table 3 Comparison of angles (in degrees) in all DFT-optimised structures. Nomenclature is defined in Fig. 5

| Angle         | α-Me₄phen-7 | α-Me₄phen-12 | β-Me₄phen-7 | β-Me₄phen-12 | [Ru(Me₂bpy)₂-(Me₄phen)]²⁺ |
|---------------|-------------|-------------|-------------|-------------|--------------------------|
| N1–Ru–N2      | 77.5        | 77.3        | 76.9        | 77.4        | 77.2                     |
| N1–Ru–N3      | 95.5        | 98.1        | 89.2        | 88.8        | 89.6                     |
| N1–Ru–N5      | 97.3        | 96.5        | 103.9       | 99.1        | 97.5                     |
| N1–Ru–N6      | 89.5        | 88.4        | 96.5        | 97.2        | 96.4                     |
| N4–Ru–N2      | 95.6        | 98.0        | 96.2        | 96.7        | 97.3                     |
| N4–Ru–N3      | 77.4        | 77.3        | 97.2        | 96.5        | 96.5                     |
| N4–Ru–N5      | 89.9        | 88.4        | 84.2        | 87.3        | 88.4                     |
| N4–Ru–N6      | 97.8        | 96.4        | 77.6        | 77.9        | 78.0                     |
| N2–Ru–N6      | 99.5        | 97.6        | 94.2        | 89.3        | 88.1                     |
| N2–Ru–N5      | 83.7        | 87.3        | 91.0        | 96.6        | 98.2                     |
| N3–Ru–N5      | 99.1        | 97.7        | 75.8        | 77.3        | 77.2                     |
| N3–Ru–N6      | 174.6       | 172.6       | 173.0       | 172.3       | 172.1                    |
Table 4 MIC values (µg mL\(^{-1}\)) for the ruthenium complexes against Gram-positive and Gram-negative bacteria

| Compound | Gram-positive | Gram-negative |
|----------|---------------|---------------|
|          | S. aureus     | MRSA          | E. coli MG1655 | E. coli APEC | E. coli UPEC | P. aeruginosa PAO1 |
| α-phen-7 | 8             | 8             | >128           | >128         | >128         | >128           |
| α-Me-phen-7 | 8          | 8             | 32             | 64           | 128          | >128           |
| α-Me-phen-7 | 4          | 4             | 8              | 8            | 8            | 8              |
| α-NO-phen-7 | 128         | 128           | >128           | >128         | >128         | >128           |
| β-phen-12 | 2             | 2             | 8              | 16           | 16           | 32             |
| β-phen-12 | 2             | 2             | 16             | 4            | 8            | 8              |
| Rubb\(_b\) | 8             | 8             | 16             | 4            | 16           | 128            |
| Rubb\(_b\) | 2             | 2             | 2              | 2            | 2            | 16             |
| Gentamicin | 0.25          | 0.25          | 0.5            | 0.5          | 1            | 0.25           |

Fig. 5 Atom label nomenclature for the ruthenium complexes in this work, shown for [Ru(phen\(_{bb7}\))\(_{2+}\)] and [Ru(Me\(_4\)phen)\(_{3}\)]\(_{2+}\). Carbon chain lengths of \(n\) = 7 and 12 were calculated for cis-α- and cis-β-[Ru(Me\(_4\)phen)\(_{bb7}\)]\(_{2+}\) families.

Table 5 MBC values (µg mL\(^{-1}\)) for the ruthenium complexes against Gram-positive and Gram-negative bacterial strains

| Compound | Gram-positive | Gram-negative |
|----------|---------------|---------------|
|          | S. aureus     | MRSA          | MG1655         | APEC         | UPEC         | PAO1          |
| α-phen-7 | 64            | 16            | >128           | >128         | >128         | >128           |
| α-Me-phen-7 | 64          | 16            | ≥128           | ≥128         | >128         | >128           |
| α-Me-phen-7 | 64          | 4             | ≥128           | ≥128         | >128         | >128           |
| α-NO-phen-7 | 128         | 128           | >128           | >128         | >128         | >128           |
| α-phen-12 | 4             | 2             | 8–16–32        | 32           | 32           | 64             |
| β-phen-12 | 4             | 8             | 16–32          | 32           | 32           | 128            |
| β-phen-12 | 1             | 1             | 8              | 8            | 16–32-64     | 64             |
| Rubb\(_b\) | ≥32          | ≥16           | 32             | 32           | 64           | >128           |
| Rubb\(_b\) | 2             | 2             | 2              | 2–4          | 64           |                 |
| Gentamicin | 2             | 1             | 1              | 1            | 4            | 1              |

Table 6 Octanol/water partition coefficients (log \(P\)) for the ruthenium complexes

| Metal complex | Charge | \(\log P\) |
|---------------|--------|------------|
| α-phen-7      | 2      | -1.83 ± 0.04 |
| α-Me-phen-7   | 2      | -1.41 ± 0.07 |
| α-Me-phen-7   | 2      | -1.33 ± 0.03 |
| α-NO-phen-7   | 2      | -1.58 ± 0.09 |
| α-phen-12\(^a\) | 2       | -0.9       |
| β-phen-12\(^a\) | 2       | -1.0       |
| (Me-phen)\(_b\) | 2       | -1.35      |
| Rubb\(_b\) \(^b\) | 4      | -3.4       |
| Rubb\(_b\) \(^b\) | 4      | -2.7       |
| Rubb\(_b\) \(^b\) | 4      | -3.3       |

\(^a\) Results from ref. 10. \(^b\) Results from ref. 7.
being two- to four-fold higher than for the other three complexes.

Interaction with DNA

As previous studies showed that the di-, tri- and tetra-nuclear ruthenium complexes linked by the bb\textsubscript{n} ligand preferentially localise with DNA and RNA in bacterial and eukaryotic cells\textsuperscript{18,19}, it was of interest to examine the DNA binding affinity of the [Ru(phen)(bb\textsubscript{n})\textsuperscript{2+}] complexes to DNA. The binding of the ruthenium complexes to calf-thymus (CT)-DNA was examined by UV/Vis spectroscopy and the intrinsic binding constants \(K\) to DNA calculated by a non-linear least-square method using eqn (1)\textsuperscript{20}.

\[
\frac{(\varepsilon_a - \varepsilon_t)}{(\varepsilon_b - \varepsilon_t)} = \frac{(b - (b^2 - 2K^2C_t[DNA]/s)^{1/2})}{2KC_t} \quad (1a)
\]

\[
b = 1 + KC_t + K[DNA]/2s \quad (1b)
\]

where [DNA] is the concentration of DNA in M (nucleotide); \(\varepsilon_a\) is the molar absorption coefficient observed for the \(1\) MLCT absorption band at a given DNA concentration; \(\varepsilon_t\) is the molar absorption coefficient of the free complex without DNA; \(\varepsilon_b\) is the molar absorption coefficient of the complex fully bound to DNA; \(K\) is the equilibrium-binding constant in M\(^{-1}\); and \(C_t\) is the total metal complex concentration and \(s\) is the binding site size.

All [Ru(phen)(bb\textsubscript{n})\textsuperscript{2+}] complexes showed strong binding to CT-DNA, with binding constants of approximately 1 × 10\(^7\) M\(^{-1}\) (see Table 7). As the DNA binding affinities were surprisingly strong, the well-known high affinity metallointercalator complex [Ru(phen)\textsubscript{2}(dppz)]\textsuperscript{2+} (ref. 21) was examined as a control. The observed binding constant, 2 × 10\(^6\) M\(^{-1}\), was comparable with previously reported values\textsuperscript{20,21}. The \(cis\)-\(\alpha\)-[Ru(5\textsuperscript{-}NO\textsubscript{2}phen)(bb\textsubscript{7})\textsuperscript{2+}] complex exhibited the strongest binding affinity. However, no significant differences in the DNA binding affinities between the \(cis\)-\(\alpha\)-[Ru(phen)(bb\textsubscript{12})\textsuperscript{2+}] and \(cis\)-\(\alpha\)-[Ru(Me\textsubscript{4}phen)(bb\textsubscript{7})\textsuperscript{2+}] isomers were observed. In addition, the binding constants of \(cis\)-\(\alpha\)-[Ru(phen)(bb\textsubscript{12})\textsuperscript{2+}] isomers are higher than for the [Ru(phen)(bb\textsubscript{10})\textsuperscript{2+}] and [Ru(phen)(bb\textsubscript{12})\textsuperscript{2+}] analogues\textsuperscript{17}.

Discussion

Due to the emergence of drug-resistant bacteria there is a need to develop new classes of antimicrobial agents. We have previously shown\textsuperscript{17} that ruthenium(II) complexes containing bis-[4(4\textsuperscript{-}methyl-2,2\textsuperscript{-}bipyridyl)]-\(n\)-alkane (bb\textsubscript{n}; for \(n = 10\) and 12) as a tetradeutate ligand in a mononuclear complex (rather than as a bridging ligand in oligonuclear complexes) exhibit excellent antimicrobial activity in terms of MIC values, against both Gram-positive and Gram-negative bacteria. In this study

![Cellular accumulation of the cis-\(\alpha\)-[Ru(phen)(bb)]\textsuperscript{2+}, [Ru(Me\textsubscript{4}phen)\textsubscript{3}\textsuperscript{2+} and Rubb\textsubscript{12} complexes into bacteria after incubation for 15, 30, 60, 90 and 120 minutes.](image)
we have extended the [Ru(phen)(bb₆)]²⁺ family of complexes by altering both the bb₆ and phen ligands, and report their anti-
microbial activities in terms of both MIC and MBC values.

In the syntheses of the [Ru(phen')(bb₇)]²⁺ complexes, both the cis-α and cis-β isomers were initially observed. However, in contrast to the bb₁₀ and bb₁₂ homologues of [Ru(phen)bb₇]²⁺, the cis-β isomer was not stable and converted predominantly to the cis-α isomer over time or in harsh conditions. It is concluded that the cis-β is a kinetic product but the cis-α is the thermodynamic product. The DFT calculations demonstrated that the cis-β complex is highly strained and considerably less stable than the corresponding cis-α isomer. As ruthenium(n) complexes with three bidentate chelating ligands groups (as is essentially the case in [Ru(phen')(bb₇)]²⁺ - but with an additional chelate ring involving the polymethylene chain in the tetradendate bb₂) are considered to be “kinetically inert” the conversion from the cis-β to the cis-α is unusual, although isomerisation of ruthenium(n) complexes with monodentate ligands, e.g. pyridine, is well known. Examples of photoactivated isomerisation, ligand loss and degradation of tris(bidentate) complexes, e.g. [Ru(bpy)(bb₇)]²⁺, are known and it has been suggested that ruthenium(n) complexes with distorted octahedral geometry photodecompose through ligand dissociation. The present DFT calculations have indicated that the cis-β-[Ru(Me₄phen)(bb₁₂)]²⁺ complex is unstable and the octahedral geometry is distorted: consequently, the cis-β and cis-α isomers can interconvert but demonstrably by a thermal mechanism in this case, as the presence of light does not affect the process.

The MIC/MBC values presented in this study demonstrate that apart from cis-α-[Ru(5-NO₂phen)(bb₁₀)]²⁺, the cis-α- [Ru(phen')(bb₁₀)]²⁺ complexes are active against Gram-positive bacteria but exhibit variable activity towards Gram-negative species. However, the cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ complex shows good activity (MICs ≤ 8 μg mL⁻¹) against both Gram-positive and Gram-negative bacteria, including the notoriously drug-resistant P. aeruginosa. Ruthenium(n) complexes, like most antimicrobial agents, have generally not shown good activity against P. aeruginosa. Recently we reported that the cis-α-[Ru(phen)(bb₁₂)]²⁺ complex does readily accumulate in P. aeruginosa and exhibits good activity against this bacterial strain. The results of the current study indicate that cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ is slightly more active (in terms of MIC) against P. aeruginosa than cis-α-[Ru(phen)(bb₁₂)]²⁺. However, a sixteen-fold difference between the MIC and MBC values for cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ against P. aeruginosa was observed, compared to the two-fold difference for [Ru(Me₄phen)]²⁺ and a four-fold difference for cis-α-[Ru(phen)(bb₁₂)]²⁺. This suggests the cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ complex may exert its antimicrobial activity through a different mechanism. Furthermore, and of note, of all the mono-, di-, tri- and tetra-
nuclear ruthenium complexes that incorporate the bb₁₀ ligand that we have examined, the cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ complex displays the most similar MIC values but the most varied MBC values across a range of Gram-positive and Gram-
negative bacteria. This is also consistent with the cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ complex exerting its antimicrobial activity through a different mechanism. Alternatively, the highly variable MBC values observed for cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ could be due to different resistance mechanisms - e.g. the up-regulation of efflux pumps and plasmid-mediated resistance mechanisms - that exist between bacteria, even closely related bacteria such as the E. coli strains UPEC and APEC.

While we have previously reported the antimicrobial activities of mono- and oligo-nuclear ruthenium complexes that incorporate the bb₉ ligand, the present study also examined the effects of substituents on the 1,10-phenanthroline ligand. The antimicrobial activities were examined in relationship to the electron-withdrawing/donating effect of substituents on the phenanthroline ligand through the analyses of their respective cellular accumulations and DNA binding. The results clearly demonstrate the importance of both cellular accumulation and DNA binding. The cis-α-[Ru(5-NO₂phen)-(bb₁₂)]²⁺ complex bound DNA with the highest affinity but displayed no antimicrobial activity because it was not taken up by the bacterial cells. Given that cis-α-[Ru(5-NO₂phen)(bb₁₂)]²⁺ is slightly more lipophilic than cis-α-[Ru(phen)(bb₁₂)]²⁺, the lack of uptake and antimicrobial activity of the nitro-substituted complex suggests that relatively minor changes of the charge density on the ruthenium centre play a critical role in the diffusion of the ruthenium complex across the bacterial membrane. The introduction of methyl substituents on the 1,10-
phenanthroline progressively increased the cellular accumulation and antimicrobial activities of the cis-α-[Ru(phen')-(bb₁₂)]²⁺ complexes, especially against Gram-negative bacteria.

All the ruthenium complexes showed better accumulation in the Gram-positive than the Gram-negative bacteria, suggesting a clear relationship between the cellular accumulation and MIC, but only to a point. The cis-α-[Ru(phen)(bb₁₂)]²⁺ exhibited similar antimicrobial activity but a higher level of cellular accumulation than the cis-α-[Ru(phen)(bb₁₀)]²⁺ across the bacteria, consistent with increasing lipophilicity (log P = ~0.9 and ~1.83 respectively). Furthermore, cis-α-[Ru(Me₄phen)(bb₁₀)]²⁺ has similar or even higher activity against Gram-negative bacteria than [Ru(Me₄phen)(bb₁₂)]²⁺ in terms of MIC, but cis-α-[Ru(Me₄phen)(bb₁₂)]²⁺ showed lower cellular accumulation in the Gram-negative bacteria compared to [Ru(Me₄phen)(bb₁₀)]²⁺. Notably, there was a different pattern of accumulation in P. aeruginosa between the cis-α-[Ru(Me₄phen)-(bb₁₀)]²⁺ and [Ru(Me₄phen)(bb₁₀)]²⁺ complexes compared with the cis-α-[Ru(phen)(bb₁₀)]²⁺ and Rubb₁₂ complexes. The uptake of the latter two complexes steadily increased over the two-hour time period; whereas for the former complexes, maximum accumulation was achieved at the first time point (15 minutes), with the cellular accumulation then remaining constant up to two hours. These results potentially suggest that P. aeruginosa may have an efflux pump against the cis-α-[Ru(Me₄phen)-(bb₁₀)]²⁺ and [Ru(Me₄phen)]²⁺ complexes, or that the ruthenium complexes incorporating the bb₁₂ ligand can permeabi-

The cis-α-[Ru(phen')(bb₁₀)]²⁺ complexes showed surprisingly high DNA binding affinity, even binding more strongly
than the established high-affinity metallointercalator \([\text{Ru(phen)}_2(dppz)]^{2+}\). The high DNA binding affinity of the cis-\(\text{Ru(phen)}(\text{bb})^{2+}\) complexes is likely to be due to an increased hydrophobic effect coupled with the decreased ability of the complexes to self-associate in water due to steric clashes between the methyl groups. The observations that cis-\(\text{Ru(Me}_4\text{phen})(\text{bb})^{2+}\) shows lower accumulation but exhibits the same or slightly better antimicrobial activities against the Gram-negative bacteria than the \([\text{Ru(Me}_4\text{phen})_2]\)^{2+} or cis-\(\text{Ru(phen)}(\text{bb})^{2+}\) complexes could suggest DNA (and RNA) binding is at least one aspect of the antimicrobial activity. Indeed, we have previously demonstrated that the dinuclear and oligonuclear ruthenium complexes target DNA and RNA in bacteria and eukaryotic cells. However, the mechanism of action for kinetically-inert polypyridylruthenium(II) complexes is still a matter of debate. In addition to nucleic acid binding, a variety of studies have proposed that these ruthenium complexes can permeabilise the bacterial membrane, inhibit the activity of important enzymes, or induce the production of reactive oxygen species (ROS) that could cause DNA damage and bacterial cell death. It is likely that for each ruthenium complex a number of these potential mechanisms are simultaneously active, with the balance between the various mechanisms modulated by the specific chemical structure.

**Conclusions**

The mononuclear cis-\(\text{Ru(Me}_4\text{phen})(\text{bb})^{2+}\) complex has significant potential as an antimicrobial agent. In this study, it has been shown that it is highly active against *P. aeruginosa*. Our results also suggest that the antimicrobial activity differences between the cis-\(\text{Ru(phen)}(\text{bb})^{2+}\) complex and the mononuclear \([\text{Ru(phen)}(\text{bb})^{2+}\) complexes previously studied are significant in terms of the electron density imposed by substituents on the 1,10-phenanthroline ligand. As a consequence, and given that the structure can be readily modified, it is possible that ruthenium(n) complexes can be customised to particular bacteria. Our studies continue on this project: the toxicity on eukaryotic cells of \([\text{Ru(phen)}(\text{bb})^{2+}\) species will be explored and a further probe of the working mechanisms of this class of ruthenium complexes is on the way.

**Experimental**

**Physical measurements**

\(1^H\) and \(13^C\) NMR spectra were recorded on a Varian Advance 400 MHz spectrometer at room temperature \([\text{CDCl}_3\ (99.8\%, \text{CIL}), \text{CD}_2\text{CN} (>99.8\%, \text{Aldrich}), \text{or CD}_2\text{OD} (>99.8\%, \text{Aldrich})]\). Luminescence was measured on a Cary Eclipse Fluorescence Spectrophotometer with \(\lambda_{ex} = 488\) nm: emission spectra were collected from \(\lambda_{em} = 500–800\) nm. Absorbance \((200–600)\) nm was measured on a VARIAN CARY 50 Probe UV-Visible spectrophotometer. Mass-spectroscopic analysis was performed by the RSC Mass-Spectrometry Facility (Research School of Chemistry, Australian National University, Canberra), and the Campbell Microanalytical Laboratory (Chemistry Department, University of Otago) performed the microanalyses.

**Materials and methods**

Potassium hexafluorophosphate (KPF\(_6\)), and 1,10-phenanthroline and its derivatives, were purchased from Aldrich and used as supplied. Amberlite IRA-402 (chloride form) anion-exchange resin and SP Sephadex C-25 cation exchanger were obtained from GE Health Care Bioscience. Cation-adjusted Mueller–Hinton broth (CAMHB), Mueller–Hinton Agar2 and the antibiotic gentamicin were purchased from Sigma-Aldrich (UK). LB broth was purchased from Formedium (UK).

The syntheses of ligands bb\(_n\) (\(n = 7, 12\)) and cis-\([\text{RuCl}_3(\text{DMSO})_4]\) were performed according to previously reported methods.

**Syntheses of metal complexes**

\(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\) complexes were synthesised using schemes based on previously reported methods. A suspension of 1 mmol of cis-\([\text{RuCl}_3(\text{DMSO})]\) and 1 mmol of the appropriate substituted 1,10-phenanthroline was refluxed in toluene (10 ml) for 2 h. The solid that separated was filtered and washed with toluene and then with diethyl ether. For \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\) and \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(5-\text{NO}_2\text{phen})]\), the pure product was realised after filtration. For \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\), after filtration the crude product was dissolved in a minimal volume of dichloromethane (DCM) and then loaded onto a silica gel column (230–400 mesh; 3 cm diam). The desired product and side products were gradient-eluted using 1–4% (v/v) methanol in DCM. The purity and contents of each fraction were determined by TLC (silica gel), using 5% (v/v) methanol in DCM as eluent. The purest fractions were combined and the solvent was removed in vacuo to give light yellow-green solids: yields: \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), yellow-green powder – 83% \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\), green-brown powder – 85% \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(5-\text{NO}_2\text{phen})]\), brown powder – 90%.

**cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})].** The \(1^H\) NMR and mass-spectral data were consistent with that reported previously for this complex. A small number of samples were measured on a MAT INCOS-50 mass spectrometer, using a Bremen source, and the spectra were reproducible. The syntheses of ligands bb\(_n\) (\(n = 7, 12\)) and cis-\([\text{RuCl}_3(\text{DMSO})_4]\) were performed according to previously reported methods. A suspension of 1 mmol of cis-\([\text{RuCl}_3(\text{DMSO})]\) and 1 mmol of the appropriate substituted 1,10-phenanthroline was refluxed in toluene (10 ml) for 2 h. The solid that separated was filtered and washed with toluene and then with diethyl ether. For \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\) and \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(5-\text{NO}_2\text{phen})]\), the pure product was realised after filtration. For \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\), after filtration the crude product was dissolved in a minimal volume of dichloromethane (DCM) and then loaded onto a silica gel column (230–400 mesh; 3 cm diam). The desired product and side products were gradient-eluted using 1–4% (v/v) methanol in DCM. The purity and contents of each fraction were determined by TLC (silica gel), using 5% (v/v) methanol in DCM as eluent. The purest fractions were combined and the solvent was removed in vacuo to give light yellow-green solids: yields: \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), yellow-green powder – 83% \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\), green-brown powder – 85% \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(5-\text{NO}_2\text{phen})]\), brown powder – 90%.

**cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})].** The \(1^H\) NMR and mass-spectral data were consistent with that reported previously for this complex. A small number of samples were measured on a MAT INCOS-50 mass spectrometer, using a Bremen source, and the spectra were reproducible. The syntheses of ligands bb\(_n\) (\(n = 7, 12\)) and cis-\([\text{RuCl}_3(\text{DMSO})_4]\) were performed according to previously reported methods. A suspension of 1 mmol of cis-\([\text{RuCl}_3(\text{DMSO})]\) and 1 mmol of the appropriate substituted 1,10-phenanthroline was refluxed in toluene (10 ml) for 2 h. The solid that separated was filtered and washed with toluene and then with diethyl ether. For \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\) and \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(5-\text{NO}_2\text{phen})]\), the pure product was realised after filtration. For \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\), after filtration the crude product was dissolved in a minimal volume of dichloromethane (DCM) and then loaded onto a silica gel column (230–400 mesh; 3 cm diam). The desired product and side products were gradient-eluted using 1–4% (v/v) methanol in DCM. The purity and contents of each fraction were determined by TLC (silica gel), using 5% (v/v) methanol in DCM as eluent. The purest fractions were combined and the solvent was removed in vacuo to give light yellow-green solids: yields: \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{phen})]\), yellow-green powder – 83% \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(\text{Me}_2\text{phen})]\), green-brown powder – 85% \(\text{cis,cis-[RuCl}_2(\text{DMSO})_2(5-\text{NO}_2\text{phen})]\), brown powder – 90%.
cis,cis-[RuCl2(DMSO)(5-NO2-phen)]·2CH2Cl2. Anal. calcld for C41H46N6PF6Ru: C, 49.9%; H, 4.28%; N, 8.1%. Found: C, 49.4%; H, 4.37%; N, 8.1%. 1H NMR (400 MHz, CD3CN): δ 8.45 (t, J = 5.6 Hz, 4H; H2/H9 & H5/H6); 8.27 (br s, 2H; bipy3); 8.13 (s, J = 1.2 Hz, 2H, bipy3); 7.57 (dd, J = 5.6, 0.8 Hz, 2H, H3/H8); 7.46 (d, J = 5.6 Hz, 2H, bipy6); 7.16 (d, J = 5.6 Hz, 2H, bipy6); 7.06 (dd, J = 1.6, 5.6 Hz, 2H, bipy5); 7.00 (dd, J = 1.2, 6.0 Hz, 2H, bipy5); 2.95 (s, 6H, CH3 Me2phen); 2.76–2.82 (m, 2H, CH2 bipy); 2.60–2.66 (m, 2H, CH2 bipy); 2.47 (s, 6H, CH3 bipy); 1.60–1.68 (m, 2H, CH2 bipy); 1.37–1.46 (m, 4H, 2 × CH2 bipy); 0.90–0.97 (m, 4H, 2 × CH2 bipy). TOF MS (ESI+): most abundant ion found for [M − 2PF6]+, m/z 339.12. Calc. for RuC41H40N6PF6+, m/z 359.12; most abundant ion found for (M − PF6−), m/z 863.20. Calc. for RuC41H40N4PF6+, m/z 863.21.

cis-[Ru(Me2phen)(bb7)](PF6)2. Anal. calcld for C43H44N6PF6Ru: C, 48.1%; H, 4.39%; N, 7.3%. Found: C, 48.2%; H, 4.44%; N, 7.7%. 1H NMR (400 MHz, CD3CN): δ 8.43 (s, 2H; H2/H9); 8.32 (s, 2H, H5/H6); 8.27 (br s, 2H; bipy3); 8.13 (s, J = 0.8, 5.2 Hz, 2H, bipy3); 7.45 (d, J = 6.0 Hz, 2H, bipy6); 7.16 (d, J = 5.6 Hz, 2H, bipy6); 7.06 (dd, J = 1.6, 4.4 Hz, 2H, bipy5); 7.00 (dd, J = 0.8, 5.2 Hz, 2H, bipy5); 2.83 (s, 6H, CH3 Me2phen); 2.77–2.79 (m, 2H, CH2 bipy); 2.60–2.66 (m, 2H, CH2 bipy); 2.48 (s, 6H, CH3 bipy); 1.60–1.69 (m, 2H, CH2 bipy); 1.38–1.47 (m, 2H, CH2 bipy); 0.92–0.98 (m, 2H, CH2 bipy); 0.67–0.83 (m, 4H, 2 × CH2 bipy). TOF MS (ESI+): most abundant ion found for [M − 2PF6]+, m/z 387.2. Calc. for RuC44H44N6RuPF6+, m/z 387.1. Most abundant ion found for (M − PF6−), m/z 919.3. Calc. for RuC44H44N4PF6+, m/z 919.3.

cis-[Ru(5-NO2-phen)(bb7)](PF6)2·1.5CH3Cl2. Anal. calcld for C41H42Cl2N2O2PF6Ru: C, 43.3%; H, 3.59%; N, 8.3%. Found: C, 43.2%; H, 3.49%; N, 8.3%.
Density functional theory calculations

The geometries of five ruthenium metal complexes – cis-α and cis-β-[Ru(Me₄phen)(bbn)]²⁺ (n = 7 and 12) and [Ru(Me₂bpy)₂(Me₄phen)]²⁺ were optimised with density functional theory (DFT) using QCHEM 4.4[14] at the B3LYP/6-311G(d,p)[35,36] level of theory with the LANL2DZ effective core potential[17] used for ruthenium atoms. Calculations were carried out for both low- and high-spin electronic configurations of the complexes, with the low-spin state (multiplicity 1 and net charge 2) found to have the lower energy in all five cases, as expected for d⁶ octahedral ruthenium complexes. Initial structures for cis-α-[Ru(Me₄phen)-(bbn)]²⁺ and [Ru(Me₂bpy)(Me₄phen)]²⁺ were obtained from crystal structures and were used to build the initial structures for cis-α-[Ru(Me₄phen)(bbn)]²⁺, cis-β-[Ru(Me₄phen)(bbn)]²⁺ and cis-β-[Ru(Me₄phen)(bbn)]²⁺ using Materials Studio 5.0.[38]

Single crystal X-ray diffraction

Crystals of compounds [Ru(Me₄phen)(bbn)][PF₆]₂, 3CHCl₃ and [Ru(Me₄phen)(Me₂bpy)]₂[PF₆]₂ suitable for X-ray crystallographic studies were grown by slow diffusion of toluene into a chloroform solution of [Ru(Me₄phen)(bbn)][PF₆]₂ and hexane into a DCM solution of [Ru(Me₄phen)(Me₂bpy)]₂[PF₆]₂, respectively. [Ru(Me₄phen)(bbn)][PF₆], 3CHCl₃ crystallised as orange rod-shaped crystals and [Ru(Me₄phen)(Me₂bpy)]₂[PF₆]₂ as orange plate-like crystals. Single crystals were selected and mounted on a nylon loop in paratone-N cryo-protectant.

Single-crystal X-ray diffraction was performed at 100(2) K on the MX-1 beamline of the Australian Synchrotron (λ = 0.7107 Å).[29] Data sets were corrected for absorption using a multi-scan method, and structures were solved by direct methods using SHELXS-2014 and refined by full-matrix least squares on F² by SHELXL-2014,[40] interfaced through the program X-Seed.[41] In general, all non-hydrogen atoms were refined anisotropically and hydrogen atoms were included as geometrically estimated positions, unless specified otherwise.

CIF data have been deposited with the Cambridge Crystallographic Data Centre, CCDC reference numbers 1559396 and 1558934† (where [Ru(Me₄phen)(bbn)][PF₆]₂·3CHCl₃ = 1559396 and [Ru(Me₄phen)(Me₂bpy)]₂[PF₆]₂ = 1558934).

Special refinement details

Compound [Ru(Me₄phen)(bbn)][PF₆]₂ crystallises with three molecules of chloroform in the unit cell, two of which are disordered over 2 positions. The disorder modelled and both molecules were left to freely refine to give 70% and 76% occupancy for each main position.

Bacterial strains

Two *Staphylococcus aureus* (Gram-positive) isolates {a wild type *S. aureus* strain (SH 1000) and a clinical multidrug-resistant MRSA strain (USA 300 LAC JE2)}, and three Gram-negative *Escherichia coli* isolates {MG 1655, NCTC 12241 (APEC) and ST 131 (UPEC)} and a *Pseudomonas aeruginosa* strain PAO1 (WT), were used for *in vitro* antimicrobial studies.

MIC and MBC determination

The MIC tests were conducted by the broth micro-dilution method in duplicate as outlined in the CLSI guidelines.[42] The MBC tests were performed in duplicate according to the standard microbiological techniques protocol.[43] The bacteria were grown in LB media incubating at 37 °C for overnight. After washing and suspending in CAMHB, the bacteria were plated out on Mueller–Hinton agar, grown overnight and then suspended in growth medium CAMHB. Bacterial inocula were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and diluted to a final concentration of 4–8 × 10⁴ cfu mL⁻¹. Compounds tested were dissolved and serially diluted in CAMHB in sterile 96-well flat-bottom plates to a final volume of 100 μL in each well. An equal volume of inocula was added to each well, making a final concentration range of the compounds tested, including the control antibiotics gentamicin, of between 0.25 and 128 μg mL⁻¹. MICs were recorded after 16–18 h of incubation at 37 °C. Colony counts of the inocula were performed for determination of the MBC. After MIC results were noted, 10 μL from each well was plated out on Mueller–Hinton agar. MBCs were recorded after overnight incubation at 37 °C, and the concentration of compounds that produced a 99.9% kill relative to the starting inoculum was recorded as the MBC.

Cellular accumulation

The cellular accumulation of the ruthenium complexes was measured by monitoring the luminescence of the complexes remaining in the supernatant of the cultures after incubation for various periods. Bacterial inocula in log phase were adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and diluted to a final concentration of 4–8 × 10⁴ cfu mL⁻¹. The cell culture (24 mL) was placed in a 250 mL conical flask and 75 μL of stock solution (2.56 mg mL⁻¹) of the ruthenium complex was added to give a final concentration of 8 μg mL⁻¹. Control flasks containing 50 μL of each bacterial suspension were set up as blank samples to obtain fluorescence calibration curves for each complex. Culture flasks and control flasks were incubated with shaking (Incub-shake TL6-5, SciQuip Ltd, Newtown, Wem, Shropshire, UK) at 200 rpm at 37 °C for 15, 30, 60, 90 or 120 min. At each time point, 3.3 mL of
bacterial suspension was centrifuged (5500 rpm) at 4 °C for 10 min. Supernatants (3 mL) were carefully transferred to 5 mL tubes and the phosphorescence of the remaining ruthenium complex was measured on a Cary Eclipse Fluorescence Spectrophotometer with $\lambda_{ex} = 488$ nm. The emission spectra were collected at $\lambda = 500-800$ nm. Volumes (21, 39, 57, 75 and 93 µL) of a stock solution (320 µg mL$^{-1}$) of each complex were added to 3 mL aliquots of the supernatant from each control bacterial suspension (untreated with drug) to acquire a fluorescence-concentration linear correlation chart for calibration.

**Lipophilicity (log $P$) determination**

The partition coefficients (log $P$) were measured using the shake-flask technique. Each ruthenium complex (0.1 mM) was dissolved in the water phase (Milli-Q water) and an equal volume of $n$-octanol was added. The two phases were mutually saturated by shaking overnight at ambient temperature and allowed to separate on standing. The concentration of the metal complex in each phase was determined spectrophotometrically at $\lambda = 450$ nm.

**DNA-binding studies**

Experiments were carried out in phosphate-buffered saline (PBS) at pH 7.4. The ratio of the UV absorbance of a solution of CT-DNA at $\lambda = 260$ and 280 nm was greater than 1.8, thus indicating that the CT-DNA was sufficiently free from protein. The DNA concentration of the stock solution ($2.8 \times 10^{-10}$ M) was determined by UV absorbance by using a molar absorption coefficient of $13300$ M$^{-1}$ cm$^{-1}$ per base pair at $\lambda = 260$ nm. Absorption titration experiments were carried out by keeping the concentration of the ruthenium complex constant ($2 \times 10^{-5}$ M) and varying the CT-DNA concentration from 0 to fully binding. Absorbance values were recorded after each successive addition of the solution of CT-DNA.

**Conflicts of interest**

There are no conflicts to declare.

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