Chemistry
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European Chemical Societies Publishing

Accepted Article

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To be cited as: *Chem. Eur. J.* 10.1002/chem.202004024

Link to VoR: https://doi.org/10.1002/chem.202004024

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10.1002/chem.202004024

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FULL PAPER

Bis(bipyridine)ruthenium(II) ferrocenyl β-diketonate complexes: exhibiting nanomolar potency against human cancer cell lines

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Supporting information for this article is given via a link at the end of the document. Ligands and complexes have been submitted to the CCDC, with deposition numbers 1991794-1991801 and 2015048-2015057, respectively.

Abstract: The synthesis and characterisation of new bis(bipyridine)ruthenium(II) ferrocenyl β-diketonate complexes, $[(bpy)_2Ru(Fc\text{-}acac)][PF_6]$ (bpy = 2,2'-bipyridine; Fc-acac = functionalized ferrocenyl β-diketonate ligand) are reported. Alongside clinical platinum drugs, these bimetallic ruthenium-iron complexes have been screened for their cytotoxicity against MIA PaCa-2 (human pancreatic carcinoma), HCT116 $p53^{+/+}$ (human colon carcinoma, *p53*-wild type) and ARPE-19 (human retinal pigment epithelial) cell lines. With the exception of one complex, the library exhibit nanomolar potency against cancerous cell lines, and their relative potencies are up to 40x, 400x and 72x more cytotoxic than cisplatin, carboplatin and oxaliplatin, respectively. Under hypoxic conditions, the complexes remain cytotoxic (sub-micromolar range), highlighting their potential in targeting hypoxic tumour regions. The Comet assay was used to determine their ability to damage DNA, and results show dose dependent damage which correlates well with the cytotoxicity results. Their potential to treat bacterial and fungal strains has been determined, and highlight complexes have selective growth inhibition of up to 87-100% against *Staphylococcus aureus* and *Candida albicans*.

Introduction

Ruthenium (Ru) complexes have been frequently assessed as potential cancer therapeutics due to their air-stable synthesis, kinetic and thermodynamic stability, and the ease of modifying their ligand environments.[1,2] NAMI-A, (ImH)[*trans*-$RuCl_4$(DMSO)(Im)] (Im = imidazole, **Figure 1A**) was the first Ru compound to be studied in humans[3–5] and this drug was based on the findings by Keppler and co-workers, who highlighted that Ru(III)-azoles, e.g. KP1019 (IndH)[*trans*-$RuCl_4(Ind)_2$] (Ind = indazole, **Figure 1B**), displayed anticancer activity in several animal models.[6,7] Unlike the clinical Pt-based drugs, NAMI-A is a active against Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma, with increased cytotoxicity against xenograft studies.[8,9] It was highlighted that such complexes can disturb the redox balance and cause cell cycle arrest, blocking DNA synthesis and ultimately leading to apoptosis.[7] Although the high activity and selectivity of these Ru(III) complexes is promising, the majority of Ru research has been directed towards organometallic Ru(II) "piano-stool" complexes of the type $[(arene)Ru(L)X]^{0/n+}$ (L = bidentate ligand, e.g. **Figure 1C**),[10] which is in part due to the ease of synthesis and characterisation. This research has since moved forward to include heterobimetallic Ru compounds,[11,12] Ru cluster,[13,14] Ru DNA intercalators[15–21] and supramolecular 'Trojan Horses', which contain a cytotoxic payload that is released upon entry to the cancer cell.[22,23] It has also been well-documented that the overall charge of the complex (monocationic > dicationic > neutral) and the nature of the bidentate ligands, L, can have a significant effect on the cytotoxicity of the complexes.[24–26]

A significant amount of research has been conducted on Ru(II)-polypyridyl complexes, specifically those which incorporate functionalized ligands derived from 2,2'-bipyridine (bpy) or 1,10-phenanthroline (phen). Their potential as photo-induced cytotoxic compounds (**Figure 1D**),[27] DNA intercalators (**Figure 1E**),[15,16,28] and use in photodynamic therapy (PDT) have been widely assessed.[29,30] Where these compounds can intercalate with DNA, Barton et al. have revealed the preference of metallo-insertion was dependent on the binding mode of the polypyridyl ligand dppz (dipyrido[3,2-a:2',3'-c]phenazine). Yano et al. have expanded the knowledge of hetero-bimetallic Ru-polypyridyl complexes, by introducing a Pt(II) centre (**Figure 1F**). However, this bimetallic species was in fact less cytotoxic than the mono-metallic species, yet the phototoxic index (PI) increased by >22 fold.[17] More recently, these types of Ru-polypyridyl complexes have been reported to bind to i-motif and G-quadruplex DNA, where the binding was also driven by the dppz ligand.[18–20] It was later shown that the photophysical properties of the Ru-polypyridyl complex, $[(bqp)_3Ru]^{2+}$ (bqp = 2,6-bi(quinolin-8-yl)pyridine, **Figure 1G**), changes upon binding to DNA. The single enantiomer Λ-*cis*

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complex shows a phosphorescent "switch-on" effect in the presence of i-motif DNA from the promoter region of DAP in a mixture of other DNA secondary structures.[21]

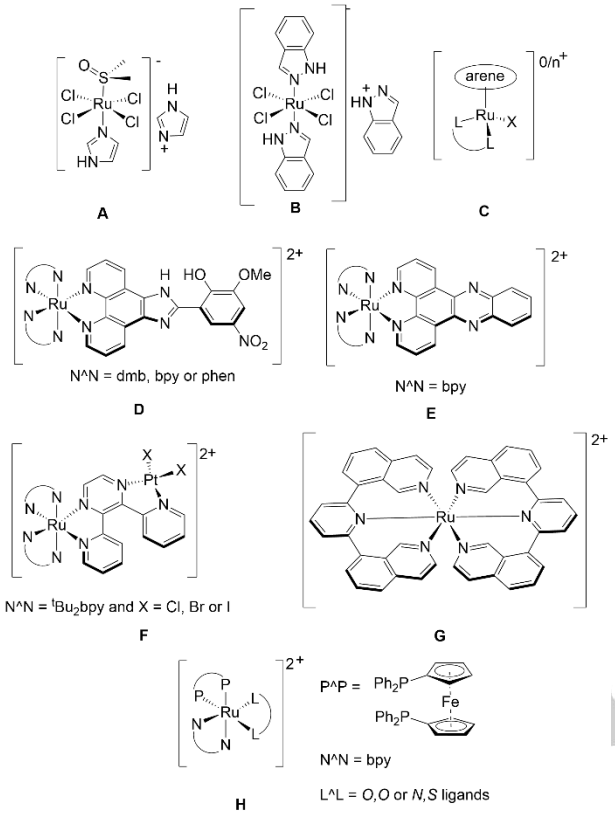


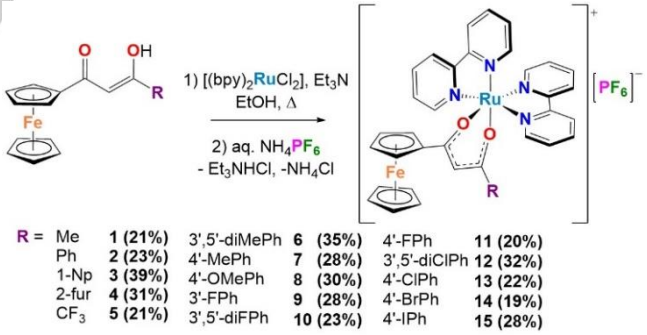
Figure 1. A range of Ru-based complexes which have been designed as anticancer agents.

The incorporation of a ferrocenyl (Fc) moiety into potential drug candidates has shown to increase cancer cell potency and extends the scope of their therapeutic effects.[31–41] These compounds were able to act as "redox antennas" in cancer cell lines, aiding in the formation of ROS through a redox activation mechanism which ultimately leads to DNA damage in the cells.[42–45] D'Sousa Costa et al. highlighted that the incorporation of 1,1'-bis(diphenylphosphino) ferrocene (dppf) into a Ru(II)-bpy complex (**Figure 1H**, *O,O* ligands) yielded nanomolar potency, induced caspase-dependent and mitochondrial intrinsic apoptosis in human colorectal carcinoma (HCT116), in a ROS-mediated pathway.[11] Whilst Guedes et al. synthesized similar heterobimetallic Ru-Fe complexes (**Figure 1H**, *N,S* ligands) and highlighted their high activity and high selectivity in breast cancer cells (MDA-MB-231).[12] Evidence suggested multiple modes of action, through DNA binding and mitochondria damage, with high levels of apoptosis in sub-G1 phase.

In light of these results, we were interested in observing the cytotoxic potential of hetero-bimetallic complexes, with incorporation of our recently published ferrocenyl β-diketonate compounds (Fc-acac). As we have reported that the addition of the Fc moiety into the β-diketonate compounds increased the cytotoxicity by up to 18-fold.[46] Ru(II) β-diketonate compounds, i.e. $[(bpy)_xRu(\beta\text{-diketonato})_{3-x}]$ have also previously been prepared to act as ferrocene mimics,[47] therefore, we have synthesized fifteen new cationic Ru(II)-bpy complexes which incorporate a functionalized Fc β-diketonate ligand (Fc-acac), $[(byp)_2Ru(Fc\text{-}acac)][PF_6]$. Single crystal X-ray diffraction for a range of ligands and complexes in presented, alongside the *in vitro* cytotoxicity against a range of cancerous and normal cell lines. Additionally, the complexes abilities to damage DNA have been measure via the Comet assay. As Ru-polypyridyl complexes have been shown to exhibit antibacterial properties,[48–54] and both Ru and ferrocenyl compounds have been shown to have antifungal properties,[32,54–59] we have conducted screening and HIT studies on the growth inhibition of bacterial and fungal strains after incubation with these hetero-bimetallic compounds.

Results and Discussion

Synthesis. The ferrocenyl β-diketonate (Fc-acac) ligands were prepared using previously published literature methods (**Scheme S1**).[46,60] All ligands were purified by column chromatography and fully characterized by ^{1}H NMR (**Figures S1-S10**) and $^{13}C\{^1H\}$ NMR spectroscopy, mass spectrometry, elemental analysis and single crystal X-ray diffraction, where possible. The bis(bipyridine)ruthenium(II) ferrocenyl β-diketonato complexes, $[(bpy)_2Ru(Fc\text{-}acac)][PF_6]$ (bpy = bis(2,2'-bipyridine) and Fc-acac = a functionalized ferrocenyl β-diketonato ligand) (**1**-**15**), were prepared from an adapted literature procedure.[47] $[(bpy)_2RuCl_2]$ was dissolved in ethanol, and with stirring, a functionalized ferrocenyl β-diketonate ligand (1 eq.) and triethylamine (1 eq) were added, before refluxing for 48 hours (**Scheme 1**). Aqueous NH_4PF_6 was used to precipitate the crude products as a dark red solids, which were further purified by column chromatography to obtain red powders (19-39%). All complexes were fully characterized by ^{1}H NMR (**Figure S11-S25**) and $^{13}C\{^1H\}$ NMR spectroscopy, mass spectrometry, elemental analysis and single crystal X-ray diffraction, where possible.



Scheme 1. Synthetic pathway for the synthesis of $[(bpy)_2Ru(Fc\text{-}acac)][PF_6]$ complexes **1**-**15**

Complexes **1**-**15** show clear NMR shifts towards lower frequencies for all Fc-acac resonances when compared to the free ligand (e.g. **Figure S26**). The protons on the bottom Cp ring are shifted by approximately 0.5 ppm in the ^{1}H NMR spectra, whilst the top Cp ring is split into three broad singlets due to the loss in symmetry down the plane of the Fc-acac ligand. Red single crystals for ligands **L2-L7**, **L8**, **L10** and **L15,** were obtained from slow evaporation of acetonitrile, and crystallized in monoclinic ($P2_1/c$ **L2**, **L6**, **L8** and **L15**; $P2_1/n$ **L3**, **L5** and **L7**), orthorhombic

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($P2_12_12_1$ **L4**) or triclinic (P-1 **L10**) cells (**Figure 2** and **Table S1**-**S3**). Bond lengths and angles (**Table S4**-**S5**) show similarities to our previously reported ligands, where the Cp ligands are eclipsed (energetically favourable) and have planar central angles (O1-C21-C22-O2) in the range of 119-122°.[46] Red single crystals of complexes **1**, **2**, **5**-**6** and **10**-**15** were obtained from the slow evaporation of acetone. The complexes crystallized in monoclinic (I_2/a **1**; P21/n **6**; $P2_1$/c **11** and **12**) or triclinic (P-1 **2**, **5**, **10**, **13**-**15**) cells (**Figure 3**, **Tables S6**-**S8**). The Ru-N and Ru-O bond lengths (**Table S9**) are similar to those observed in other reported Ru(II)-bpy complexes,[61,62] with the expected octahedral geometry bond angles ranging from 79-97° (**Table S10**). The slightly distorted geometry around the metal centre is due to the three bidentate ligands restraining the bond angles, which is similar to our previously reported Ru(II)-picolinamide complexes.[63]

Hydrolytic Stability. All of the Ru(II) complexes were found to be highly stable under hydrolysis conditions (9:1 *v/v* MeCN:H_2O), when analysing both the NMR and UV-vis spectra.[64] Higher amounts of water could not be used due to low solubility, and this has also been predicted using Swiss ADME (**Table S12**).[65] No notable changes in the spectra were seen over a 4 day observation window. Complex **2** was additionally analysed after 35 days, with no further changes observed, confirming their high hydrolytic stability. In general, all UV-Vis absorption spectra displayed an intense band at 245 nm (bpy π→π2*) and 295 nm (π→π1*) intra-ligand transitions. A broad Ru(dπ)→bpy(π*) MLCT band is observed at around 500 nm,[37,41,42] whilst the peaks at 205 and 330 nm likely arise from a ligand based absorbance and MLCT transition from the ferrocene β-diketonate ligand (**Figure S27**).

Chemosensitivity studies

The cytotoxic potential of complexes **1**-**15**, cisplatin (**CDDP**), carboplatin (**CARB**) and oxaliplatin (**OXA**) were determined using the MTT assay over a 96 h period. All compounds were initially screened against MIA PaCa-2 (human pancreatic carcinoma) and HCT116 *p53*$^{+/+}$ (human colorectal carcinoma, *p53*-wild type) cancerous cell lines, and the results are presented as IC_{50} values in **Table 1** (**Figure S28**). Overall, the library of complexes exhibits nanomolar cytotoxicity against the tested cell lines. The compounds are statistically more active ($p < 0.05$) than the clinical platinum complexes. Complex **3**, which contains a naphthyl (Np) functionalized Fc-acac ligand, is the most cytotoxic of this library, with IC_{50} values of 0.09 ± 0.02 μM and 0.11 ± 0.03 μM against MIA PaCa-2 and HCT116 *p53*$^{+/+}$, respectively. Against MIA-PaCa2, complex **3** has relative potency (RP) values which are >40x, 400x and >72x more cytotoxicity than **CDDP**, **CARB** and **OXA**, respectively (**Figure S29** and **Table S13**).

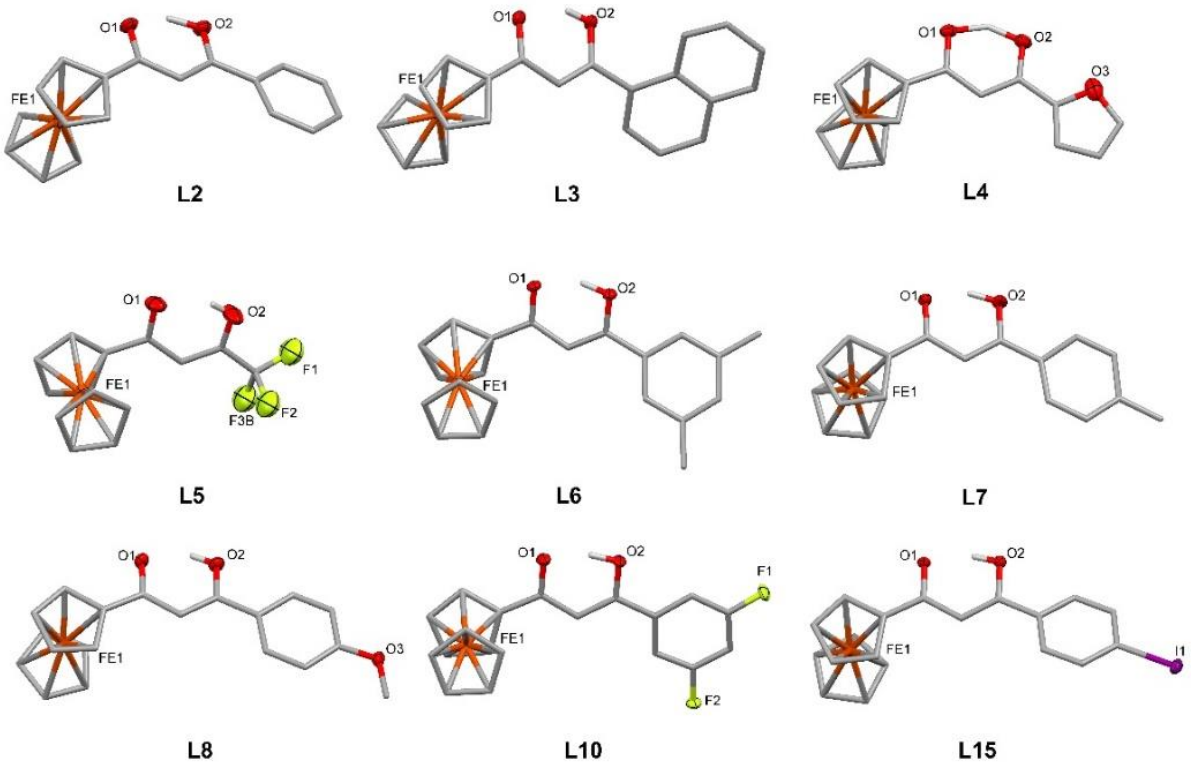

Figure 2. Molecular structure of ligands **L2**-**L7**, **L8**, **L10** and **L15**. Hydrogen atoms are omitted for clarity and thermal ellipsoids (shown for heteroatoms only) are at the 50% probability level.

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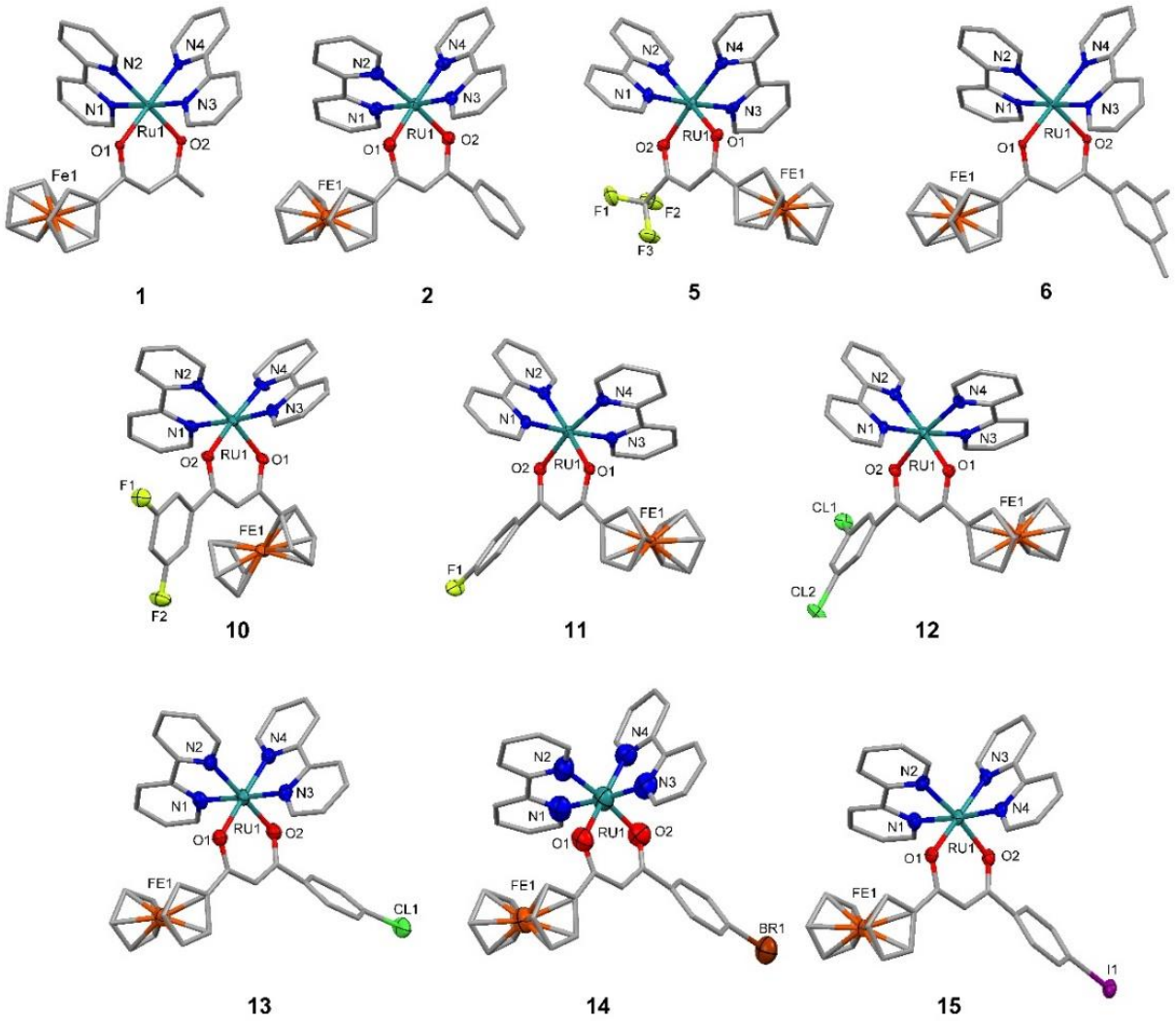

Figure 3. Molecular structure of complexes **1**, **2**, **5**, **6** and **10**-**15**. Hydrogen atoms and counter ions are omitted for clarity. Thermal ellipsoids (shown for heteroatoms only) are at the 50% probability level.

Complex **5**, which contains a trifluoromethyl (-CF_3) functionalized Fc-acac ligand, is the least cytotoxic of the library, yet it remains more cytotoxic than the clinical platinum drugs, with RP values of 1.5 (**CDDP**), 14.8 (**CARB**) and 2.7 (**OXA**) (**Figure 4** and **Table S11**). A clear correlation can be seen between the increasing aromaticity of the R substituent on ferrocenyl β-diketonato ligand and the cytotoxicity of the complexes follows the trend: **1** < **2** < **3** against both cell lines tested. This could be due to the increasing hydrophobicity (**Table S11**), which may aid in an easier passive transport. Generally, there are very few trends observed, as the IC_{50} values are all in the nanomolar range. However, when considering the para halide substituted complexes against both MIA PaCa-2 and HCT116 *p53*$^{+/+}$, the 4-Br (**14**) and 4-I (**15**) complexes are more cytotoxic than the 4-F (**11**) and 4-Cl (**13**), following the order: **11** < **13** < **14** = **15**. The most important trends are comparison of the cytotoxicity of the free ligand when compared to the complex. As we have previously reported,[46] the free ligands have low to moderate IC_{50} values, however the cytotoxicity significantly increases when the ligand is bound to the Ru center. **Table S12** highlights the increases in cytotoxicity, with complex **4** exhibiting an IC_{50} value >147 that of the free ligand, when screened against HCT116 *p53*$^{+/+}$. Since the *cis*-$[Ru(bpy)_2Cl_2]$ precursor and Fc-acac exhibit lower cytotoxicity, we are confident the nanomolar potencies of the $[Ru(bpy)_2(Fc\text{-}acac)]$ complexes are due to the combination of both the Fc-acac ligand and Ru(II)-bpy precursor.[66,67]

A limitation of many existing anti-cancer drugs is poor selectivity towards cancer cells, which can restrict the drug dosage and increase the harmful side effects for patients. The cell viability has been determined for complexes **1**-**15**, **CDDP**, **CARB** and **OXA** against human retinal pigment epithelial cell line, ARPE-19, which is used to indicate their cancer selectivity (**Table 1**). The results have been expressed as a selectivity index (SI) and defined as the ratio of the mean IC_{50} for the normal ARPE-19 cells divided by the mean IC_{50} for each individual cancer cell line tested (**Table S14** and **Figure S30**). However, all complexes equitoxic with SI values ~1-2, and further modifications are now required to increase this selectivity.

Table 1. IC_{50} values (μM) ± SD for complexes **1**-**15**, **CDDP**, **CARB** and **OXA** against MIA PaCa-2, HCT116 *p53*$^{+/+}$ and ARPE-19 cell lines after 96 h and 48 h (MIA PaCa-2 only) exposure. Selectivity index (SI) values are shown in parenthesis.

Compounds	IC_{50} values (μM) ± SD			
	MIA PaCa-2	HCT116 p53+/+	ARPE-19	MIA PaCa-2, 48 h
1	0.4 ± 0.1 (1.7)	0.34 ± 0.03 (2.2)	0.74 ± 0.05	0.39 ± 0.02
2	0.13 ± 0.04 (2.5)	0.30 ± 0.04 (1.1)	0.32 ± 0.07	0.112 ± 0.005
3	0.09 ± 0.02 (1.2)	0.11 ± 0.03 (1.0)	0.11 ± 0.03	0.109 ± 0.005
4	0.11 ± 0.01 (0.9)	0.23 ± 0.07 (0.4)	0.10 ± 0.03	--
5	2.4 ± 0.3 (1.1)	3.0 ± 0.1 (0.9)	2.7 ± 0.5	--
6	0.12 ± 0.01 (2.0)	0.30 ± 0.04 (0.8)	0.25 ± 0.08	--
7	0.13 ± 0.03 (1.6)	0.21 ± 0.03 (0.7)	0.21 ± 0.02	--
8	0.22 ± 0.03 (0.6)	0.25 ± 0.06 (0.5)	0.13 ± 0.03	--
9	0.33 ± 0.01 (0.8)	0.72 ± 0.05 (0.4)	0.27 ± 0.03	--
10	0.35 ± 0.02 (1.0)	0.82 ± 0.07 (0.4)	0.35 ± 0.06	--
11	0.25 ± 0.03 (0.7)	0.32 ± 0.04 (0.6)	0.18 ± 0.06	--
12	0.10 ± 0.02 (1.4)	0.11 ± 0.01 (1.2)	0.13 ± 0.01	--
13	0.16 ± 0.04 (0.8)	0.32 ± 0.04 (0.4)	0.13 ± 0.02	--
14	0.12 ± 0.01 (1.1)	0.30 ± 0.07 (0.4)	0.13 ± 0.03	--
15	0.12 ± 0.02 (0.9)	0.19 ± 0.04 (0.6)	0.11 ± 0.02	--
CDDP	3.6 ± 0.7 (1.8)	3.3 ± 0.4 (2.0)	6 ± 1	4.3 ± 0.2
CARB	36 ± 8 (2.2)	32 ± 11 (2.4)	77 ± 11	--
OXA	6 ± 1 (0.5)	0.9 ± 0.1 (3.5)	3 ± 0.3	--

Influence of Hypoxia

Metal complexes are particularly affected by the reducing environment of the hypoxic cells, as a change in the oxidation state of a complex can lead to alterations in their structure, binding mode, cellular drug uptake, metabolism and the mechanism of action.[68,69] Using a 96 h MTT assay under severe hypoxic conditions (0.1% O_2), the compounds' cytotoxic abilities under low O_2 conditions was assessed (**Figure 4** and **Table S15**). Complexes **1**-**3**, **6**, **7** and **12** were screened and results highlight that lowering the oxygen concentration decreases the activity for all complexes against both cancerous cell lines, although to a lesser degree against MIA PaCa-2 (*cf.* HCT116 *p53*$^{+/+}$). It should be noted that the Pt-based complexes were rendered completely inactive at the maximum tested threshold (> 50 μM). The cytotoxicity trends are the same under both normoxic and hypoxic condition: **1** < **2** < **3**, although for reasons yet unknown complex **1** experienced a greater loss of activity in comparison to the others complexes. Complex **3** has sub-micromolar activity under hypoxic conditions (IC_{50} = 2.8 ± 0.7 μM) and is within error of **CDDP** in normoxic conditions (IC_{50} = 3.6 ± 0.7 μM), highlighting the potential of this compound against tumors with a high degree of hypoxia.

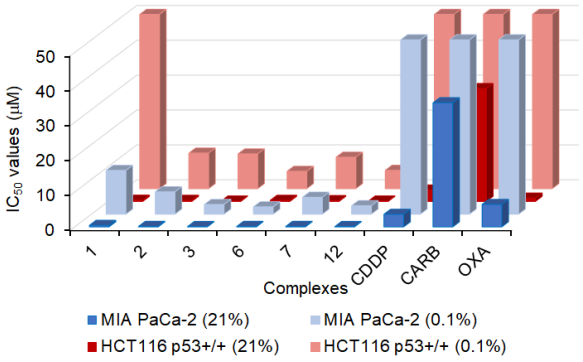

Figure 4. Bar-charts showing the IC_{50} values of complexes **1**-**3**, **6**, **7**, **12**, **CDDP**, **CARB**, **OXA** and against MIA PaCa-2 and HCT116 *p53*$^{+/+}$, under both 21% O_2 (normoxia) and 0.1% O_2 (hypoxia) conditions.

Analysis of cellular DNA damage by the Comet assay

Accumulation of cellular DNA damage can lead to programmed cell death, therefore, the ability of complexes **1**-**3** to induce double strand breakage (DSB) of DNA has been assessed using the Comet assay.[70,71] These complexes were chosen, as they exhibit

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cancer cell potency which increases as the aromaticity of the complex increases: Np (**3**) > Ph (**2**) > Me (**1**). Prior to the 48 h incubation period required for the Comet assay, the cell viability via MTT assay were measured for complexes **1**-**3**, showing the compounds are highly cytotoxic after only 48 h (**Table S16**), whereas the cytotoxicity of CDDP decreases by >27-fold. For the preparation of the Comet assay, the complexes were incubated with MIA PaCa-2 cells for 48 h before harvesting, and quantification of the DSB was determined via single cell gel electrophoresis. The complexes all induce DSB in DNA (**Figure 5**), showing the same trends observed for the chemosensitivity assays. The naphthyl substituted complex **3** is the most cytotoxic and also causes the highest degree of double strand damage (**Table S17**), following the trend: **1** < **2** < **3**. Even though high levels of damage were observed at the lowest concentration of 1.25 μM, generally the degree of damage increases slightly with increasing exposure concentration. However, CDDP exhibited low DSB, and complex **3** exhibits >3x more DNA damage at 5 μM incubation concentrations.

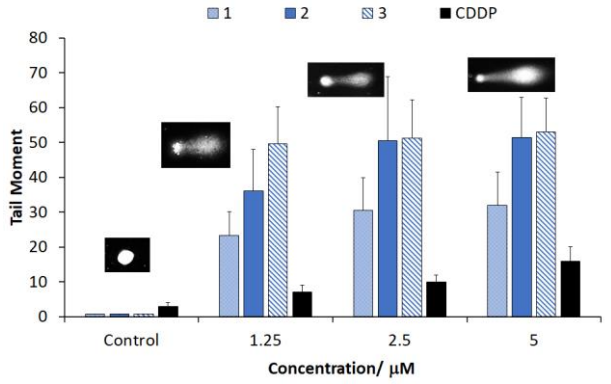

Figure 5. Bar-chart showing the Double Strand Break (DSB) Comet assay results for complexes **1**-**3** and CDDP after 48 h incubation with MIA PaCa-2.

Antimicrobial and Antifungal Agents

As Ru agents[48–54,72,73] and ferrocene compounds[74] are well-known in literature to have antibacterial properties, complexes **1-15** were screened for their potential to inhibit the growth of several bacterial strains (**Table S18**). The results highlight these ruthenium-iron complexes are more active towards the Gram positive *S. aureus* (66.35% (**7**) to 87.15% (**8**)), when compared to the other Gram negative strains. Whilst several of the complexes are partially active against *A. baumannii*. Our results are in agreement with other Ru(II)-polypyridyl complexes, which also have preferential inhibition of the Gram positive strains.[73,75] Few trends are observed, however, a slight decrease in inhibition is observed with a decrease in electronegativity of the *para*-halogenated Fc-acac ligands: **11** (4'-F) > **13** (4'-Cl) > **14** (4'-Br) > **15** (4'-I). The active complexes were also screened for HIT confirmation, and their minimum inhibitory concentrations (MICs) were determined as the lowest concentration at which the growth was fully inhibited. Complexes with MIC < 16.0 μg·mL^{-1} are classed as confirmed active HITs (**Table S19**). To assess the complexes potency towards normal cell types, screening was also conducted against human embryonic kidney cell line, HEK293, and hemolysis assays conducted against human whole blood. These complexes gave very low MIC values, ranging from 0.5 μg·mL^{-1} (**4**) to 8.0 μg·mL^{-1} (**1** and **5**) against *S. aureus*. Additionally, complexes **2** and **13** exhibit low MIC against the Gram negative *A. baumannii,* however, the toxicity against HEK293 normal cells (CC_{50} = 2.7-4.4 μg·mL^{-1}) and human blood cells (HC_{10} = 2.6-9.1 μg·mL^{-1}) are high. Importantly, complexes **1** and **5** are considered non-toxic in human blood cells (HC_{10} > 32 μg·mL^{-1}).

Since Ru and ferrocenyl containing compounds have already been shown to exhibit antifungal properties,[54,56,57] complexes **1-15** were screened for the potential growth inhibition of fungal strains, *C. albicans* and *C. neoformans* (**Table S20**). All complexes (except complex **1**) show excellent growth inhibition of *C. neoformans*, with complexes **2**-**3**, **6**-**8** and **11**-**15** exhibiting between 89.80% (**8**) to 100.44% (**14**) growth inhibition. Complexes **1**, **4** and **5** are all inactive towards *C. albicans*, and these complexes lack an aromatic substituent on the Fc-acac ligand, suggesting that the aromatic group could be an important structural feature for *C. albicans* grow inhibition. Complexes **9** and **10** are slightly more active (*cf.* **1**, **4** and **5**) against *C. albicans*, and both complexes contain a fluoro-substituent phenyl substituent. Complexes which were classed as active underwent HIT confirmation to determine their MIC. Complexes with MIC < 16.0 μg·mL^{-1} are classed as confirmed active HITs (**Table S21**). Complexes were found to be more active *towards C. neoformans* than *C. albicans*, with only complexes **1**, **4** and **5**, showing MIC values > 32.0 μg·mL^{-1}. The most promising result is observed for complex **5**, which has >2-fold selectivity for *C. neoformans* and remains non-toxic towards human blood cells (HC_{10} > 32.0 μg·mL^{-1}).

Conclusions

We report 15 new bis(bipyridine)ruthenium(II) ferrocenyl β-diketonate complexes, $[(bpy)_2Ru(Fc\text{-}acac][PF_6]$, which have been fully characterized, including single crystal X-ray diffraction. The anticancer potential of the complexes was assessed by screening against MIA PaCa-2 and HCT116 *p53*$^{+/+}$ cell lines, and results shows a correlation between the increasing aromaticity of functionalized ferrocenyl β-diketonate ligands and their cytotoxicity. All of the complexes (except **5**) exhibit nanomolar potency and exceed the activity of **CDDP** by >40-fold and **CARB** by >400-fold. Under severe hypoxic conditions (0.1% O_2) the cytotoxicity of the complexes decreases slightly, however, the same trend in activity remains, and the complexes outperform **CDDP**. As a potential mode of action, the Comet assay was used to establish the amount of double strand DNA breakage (DSB) against MIA PaCa-2 cells, with complexes exhibiting dose-dependent DNA damage, and the degree of damage correlates to the cytotoxicity results. Additional assays were conducted to assess the complexes' abilities to inhibit the growth of bacteria and fungi strains. All complexes gave high inhibition against the Gram negative strain *S. aureus* and were selective over other Gram positive strains. Most of the complexes gave high growth inhibition of both fungal strains, *C. albicans* and *C. neoformans*, with complex **5** being the most promising due to its high MIC value against *C. neoformans* and lack of potency against human blood cells.

Experimental Section

All experimental details, characterization data, single crystal XRD data and biological protocols as stated in the Supporting Information.

Ligand Preparation. Ligands **L1**-**L15** were synthesized using adapted literature methods (**Scheme S1**).[46,60] A functionalized ethyl ester (13.0 mmol) was added to a stirred solution of acetyl ferrocene (7.2 mmol) and sodium ethoxide (13.0 mmol) in ether (20 mL). The solution was stirred at reflux for 24-72 hours after which time the product was isolated by one of two methods: **1)** the solid precipitate was isolated by filtration, dissolved in distilled water (150 mL) and acidified with 10% hydrochloric acid until pH 5 which caused a red solid to precipitate out in solution. The solid was filtered and dried overnight under vacuum before purification, or **2)** the solution was acidified with 10% hydrochloric acid until pH 5 and added to water (50 ml). The product was extracted with ether (3 x 20 mL) and the organic layers were combined, dried over $MgSO_4$ and filtered. Solvent was removed *in vacuo* to yield a red solid.

Complexes 1-15: The functionalized ferrocenyl β-diketonate ligand was dissolved in ethanol (20 mL) followed by addition of triethylamine (0.05 mL, 0.3 mmol) and bis(2,2'-bipyridine)dichlororuthenium (0.15 g, 0.30 mmol). The solution was stirred at reflux for 48 hours. The solvent was reduced *in vacuo* and added to aqueous NH_4PF_6 to yield a red solid. The mixture was filtered and the solid was washed with water and ether before being dried overnight in a desiccator. The crude product was then purified by column chromatography (see SI).

Acknowledgements

We would like to thank the Community for Open Antimicrobial Drug Discovery (CO-ADD) at The University of Queensland for the screening of our complexes against bacterial and fungal strains. We also thank Ms. Tanya Marinko-Covell (University of Leeds) and Mr. Stephen Boyer (London Metropolitan University) for elemental analysis services. We would like to acknowledge the University of Leeds (MA) and the Henry Ellison Scholarship (P. C.-M.) for PhD studentships, the University of Huddersfield and the Institute of Cancer Therapeutics (University of Bradford) both for cell culture facilities and the University of Bradford's research development fund which was awarded to RML.

Keywords: Bioinorganic • Cancer • Hetero-bimetallic • Iron • Ruthenium

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10.1002/chem.202004024

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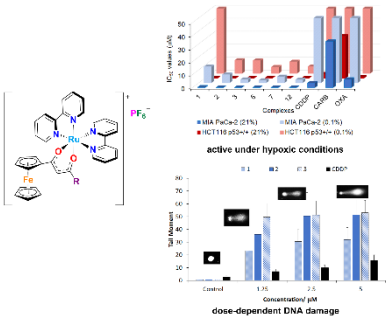

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