Anticancer, antifungal and antibacterial potential of bis(β-ketoiminato)ruthenium(II) carbonyl complexes

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Herein we report a library of new ruthenium(II) complexes which incorporate a range of functionalised β-ketoiminate ligands. The complexes undergo an unusual reduction from Ru(III) to Ru(II), and consequently incorporate carbonyl ligands from the 2-ethoxyethanol solvent, forming ruthenium dicarbonyl complexes. In order to address the potential applications of these complexes, we have screened the library against a range of tumour cell lines, however, all compounds exhibit low cellular activity and this is tentatively assigned to the decomposition of the compounds in aqueous media. Studies to establish the antifungal and antibacterial potential of these complexes was addressed and show increased growth inhibitions for *C. neoformans* and *S. aureus species*.

Transition metal coordination complexes are some of the most promising anti-cancer drugs to date, with many complexes showing selective potency both *in vitro* and *in vivo*.[1,2] However, due to the potential of multiple isomers, there remains issues with such complexes in terms of their intracellular isomerisation and instability in aqueous media. This was highlighted during the clinical Phase trials of budotitane, *cis*-$[(EtO)_2(bzac)_2Ti]$ (bzac = benzoylacetone) (**Figure 1A**),[3] which exhibited high *in vivo* activity but Phase I trials were terminated due to severe adverse side-effects and issues with formulation.[4] We have also reported similar titanium complexes, $[(X)_2(bzacR)_2Ti]$ (**Figure 1B**), which undergo ligand exchange and more than one isomer is observed in solution. The cellular testing of the compounds has been terminated, due to issues with determining the active species.[5,6]

After platinum-based drugs, ruthenium complexes are the second most promising class of therapeutics. The first known ruthenium complexes to be investigate, were the analogues of cisplatin, in which Clarke et al. reported the anticancer activity of *fac*-$[Cl_3(NH_3)_3Ru]$ (**Figure 1C**).[7] However, further work was halted on this compound, due to poor solubility and formulation issues. Prior to this work, the first reported ruthenium halide complex containing DMSO was synthesised by James *et al.* in 1971, whereby they first described the synthesis of $[Cl_2(DMSO)_4Ru]$.[8] In 1983, Sava et al. highlighted the therapeutic importance of this complex, and reported the *cis*-$[Cl_2(DMSO)_4Ru]$ (**Figure 1D**) to have high *in vivo* potencies, which were 3-fold more active than cisplatin.[9–11] The results led to the synthesis of the *trans* analogue, *trans*-$[Cl_2(DMSO)_4Ru]$ (**Figure 1E**), which was found to be *ca.* 20-fold more active than the *cis* complex against Lewis lung carcinoma, a metastasizing murine tumour.[12] Unlike the *trans* analogue of cisplatin, transplatin, which remains non-toxic.[13]

During this period, Keppler *et al.* highlighted a ruthenium(III) complex, [IndH]*trans*-$[Cl_4(Ind)_2Ru]$ (KP1019, Ind = indazole, **Figure 1F**), which exhibited high cellular activity, especially against platinum-resistant colorectal autochthonous tumours.[14] This compound entered Phase I clinical trials, showing no serious side-effects and progressed towards Phase II trials to elucidate the therapeutic efficacy.[15–17] Alongside KP1019, the work of Sava et al. highlighted another *trans* ruthenium(III) complex, [ImH]*trans*-$[Cl_4(Im)(DMSO)Ru]$ (NAMI-A, Im = imidazole, **Figure 1G**), which is known for its antimetastatic properties.[18] The complex was able to inhibit the growth of *in vivo* pulmonary metastases solid tumours. NAMI-A was in Phase II clinical trials and tested in combination with gemcitabine, though the trials were recently terminated as the results did not show an improvement on using gemcitabine alone.[19]

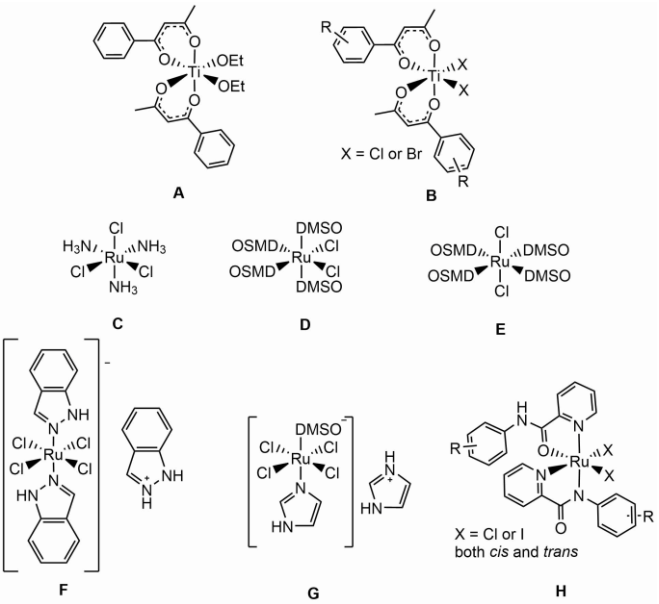


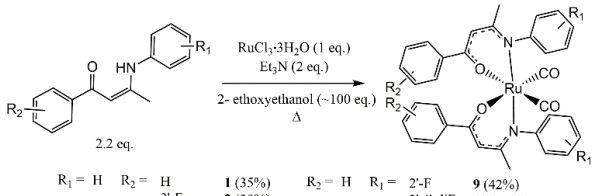
Figure 1 Range of ruthenium coordination compounds which have been shown to have high in vitro/ in vivo potency

To date there have been many promising ruthenium(II) coordination complexes and ruthenium(II) arene complexes which exhibit high micromolar potency towards cancerous cell lines.[20] We have previously reported a range of ruthenium(III) bis(picolinamide) dihalide complexes, $[X_2(L)_2Ru]$ (L = functionalised picolinamide ligand) (**Figure 1H**), and have shown that the cytotoxicity is dependent of the isomers present.[21] Additionally, we reported ruthenium and iridium arene complexes which incorporate functionalised β-ketoiminate ligands,[22–24] and have shown that these complexes exhibit low micromolar potency. Therefore, the

work discussed herein aims to combine coordination ruthenium complexes with β-ketoiminate ligands, to assess their ability to form single stable isomers and screen their cytotoxicity towards tumour cell lines, fungi and bacteria.

Synthesis of β-ketoiminato ruthenium(II) complexes

By treating a functionalised β-ketoiminate ligand (2 eq.), with triethylamine (2 eq.) and ruthenium(III) chloride trihydrate (1 eq.), whilst heating to reflux for 6 h in ethoxyethanol (~100 eq.), we attempted to synthesise ruthenium bis(β-ketoiminato)ruthenium(II) chloride complexes, $[Cl(L)_2Ru]$. However, from the reaction mixture the ruthenium dicarbonyl complexes **1**-**16** (**Scheme 1**) were isolated. This synthesis is characterised by the reduction of ruthenium in the metal precursor from Ru(III) to Ru(II), allowing for NMR analysis, and the usual incorporation of terminal carbon monoxide. This was initially not expected, and the formation of the carbonyl ligands is thought to be a result of the decarbonylation of the 2-ethoxyethanol acting as the solvent. When comparing to the literature, Ammermann *et al.* reported an iridium(III) complex which also incorporated a carbonyl ligand when using 2-ethoxyethanol.[25] Similar to our own conclusions, the research group noted that changes in the reagent ratios and solvent did not yield the desired ruthenium carbonyl complexes. This complex does not undergo a reduction to iridium(II), however, using labelled $H_2{}^{18}O$ experiments, the oxygen in the carbonyl ligand was assigned to that from water, whilst the carbon is tentatively assigned to the 2-ethoxyethanol solvent. Although unusual, the possibility of the formation of hydride-, carbonyl- or hydridocarbonyl-metal complexes when a transition metal complex is in contact with an alcoholic medium is well documented.[26] For example, Chatt *et al.* have shown that ruthenium phosphine complexes can form ruthenium carbonyl complexes in alcoholic solvents.[27] This synthetic pathway yields only moderate yields of 30-43%, which were slightly improved by using a slight excess of base. Column chromatography (dicholoromethane/hexane) was used to purify the crude bis(β-ketoiminate)ruthenium(II) dicarbonyl complexes, and yielded complexes **1**-**16** as yellow-green crystalline compounds which are air-stable.



R_1 = H, R_2 =		R_2 = H, R_1 =	
H	**1** (35%)	2'-F	**9** (42%)
3'-F	**2** (30%)	2',4'-diF	**10** (29%)
4'-F	**3** (39%)	4'-F	**11** (30%)
2',4'-diCl	**4** (21%)	2',4'-diCl	**12** (42%)
4'-Cl	**5** (31%)	4'-Cl	**13** (31%)
3'-Br	**6** (36%)	3'-Br	**14** (37%)
4'-Br	**7** (33%)	3'-Me	**15** (37%)
4'-Me	**8** (34%)	2',3'-diMe	**16** (37%)

Scheme 1 Synthesis of bis(β-ketoiminato)ruthenium(II) dicarbonyl complexes **1**-**16**

Analysis of β-ketoiminato ruthenium(II) complexes

Complexes **1**-**16** have been fully characterised by infrared spectroscopy, ^1H, $^{13}C\{^1H\}$, COSY and HMQC spectroscopy, mass spectrometry, elemental analysis and single crystal X-ray crystallography where appropriate. All complexes show the characteristic CO stretches between 1900-2100 cm^{-1}, which are consistent with other reported ruthenium carbonyls (**Figure S1** and **Table S6**).[28] ^1H NMR spectroscopy was used to follow the progress of the reaction, with the loss of the β-ketoiminate ligand NH being the most characteristic change, followed by the shift to lower frequencies of the methine resonance, from approximately 5.70 ppm (free ligand) to 5.50 ppm (complex) (**Figure S2**).

Single crystals suitable for X-ray diffraction were obtained for complexes **1**-**4**, **6**-**11**, **13** and **15** (CCDC numbers: 1940927-1940938, **Table S1**-**S3**), by either vapour diffusion of dichloromethane/pentane, or concentrated acetonitrile at < 4°C. The complexes crystallised in either a monoclinic (**1**, **4**, **9**-**11** and **12**), triclinic (**2**, **3** and **6**-**8**) or orthorhombic (**15**) space group, with molecular structures shown in **Figure 2**. The complexes exhibit *pseudo* octahedral structures, with the ligands' bond angles in the ranges of 83–96° (*cis*) and 170-185° (*trans*). The Ru-N(amine) and Ru-O(phenolate) bond lengths are within the ranges 2.08-2.10 Å and 2.04-2.10 Å, respectively, and are consistent with Ru(II) β-ketoiminate complexes reported in the literature.[22] The Ru-C(carbonyl) bond lengths, in the range 1.86-1.88 Å, are slightly longer than reported Ru-C bond lengths.[29] Characteristic short bond lengths, in the range 1.13-1.14 Å are observed for C≡O in all complexes and are within reported literature values (**Table S4** (lengths) and **Table S5** (angles)).[30,31] Unlike our previously reported ruthenium(III) bis(picolinamide) complexes, the complexes presented herein only crystallise in a *cis* isomer (with respect to the ancillary ligand), with all solid state structures showing a *cis(CO)-cis(O)-trans(N)* arrangement.[21] In order to address the isomers present in solution, ^1H NMR spectra were recorded for the complexes between 333K and 278K (**Figure S3** and **S4**), and show no changes or broadening of the resonances at all temperatures. The evidence of single stable *cis* isomers is contrary to our previously published work, and is thought to be due to the backdonation of the carbon monoxide ligands, which helps to stabilise the *cis* arrangement. The elimination of multiple isomers is a significant step forward in producing drug candidates which are stable and have fewer issues during formulation, and we are conducting additional studies to further understand these observations.

Chemosensitivity Assays under Normoxic Conditions

The cytotoxicity of complexes **1**-**16** was evaluated against human pancreatic carcinoma (MIA PaCa-2), human colon carcinoma (HCT116 $p53^{+/+}$) and normal human retinal pigment epithelial cells (ARPE-19). All of the results show that these complexes are either non-toxic or moderately cytotoxic, therefore structure activity relationships cannot be fully determined (**Table 1**). There is a slight trend observed, whereby the *para* mono-substituted halide complexes **3** (4'-F), **6** (4'-Cl) and **8** (4'-Br) have higher potency than other complexes in the library. Our previously reported work has highlighted the *meta* fluoro β-ketominate ligand to be the

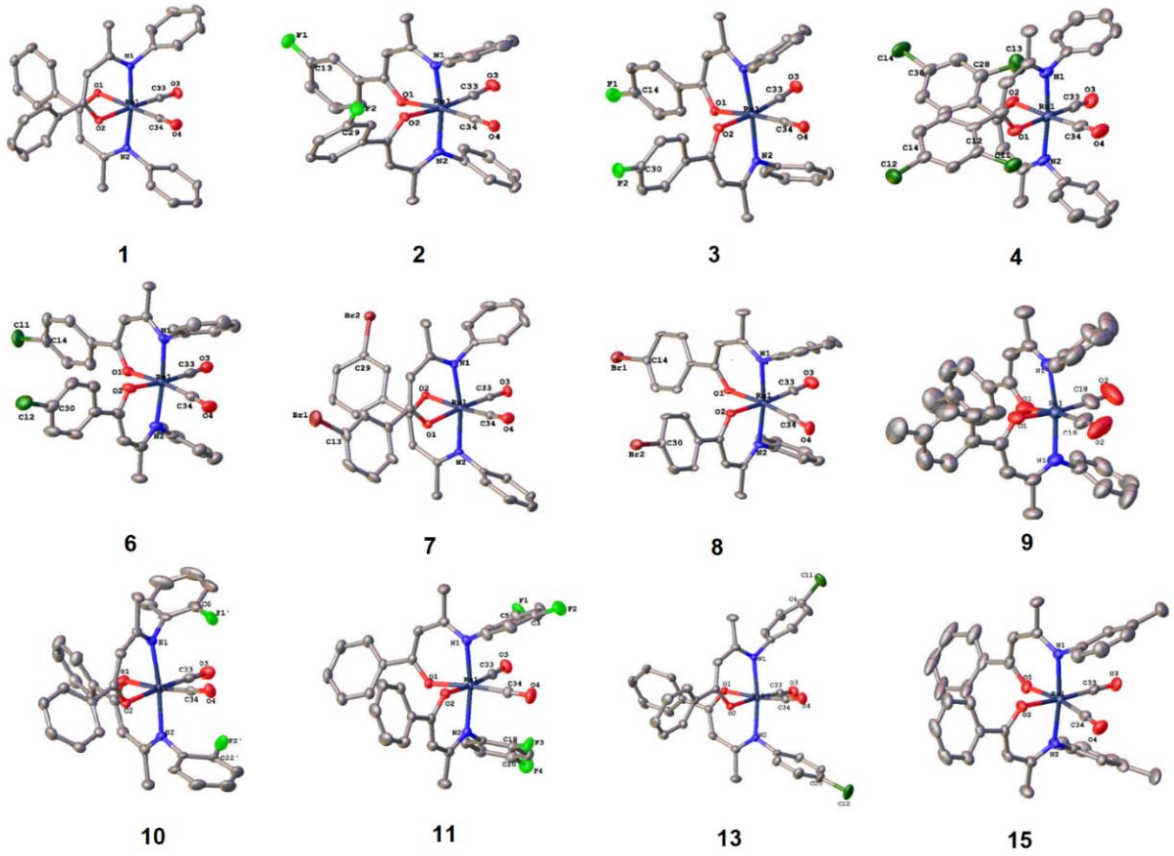


Figure 2 Molecular structures of bis(β-ketoiminato)ruthenium(II) carbonyl complexes **1**-**4**, **6**-**11**, **12** and **15**. Displacement ellipsoids are at the 50% probability level and hydrogen atoms and disordered parts are omitted for clarity.

most promising when complexed to ruthenium, however, the results shown herein highlight the *para* fluoro complex **3** to be almost 3 times as potent. Interestingly, our previously reported organometallic ruthenium *p*-cymene complexes with these β-ketoiminate ligands have cytotoxicity values ranging from 3.5-22.0 μM (against HT-29), whilst the activity decreases by up to 24-fold when the *p*-cymene ring is removed and replaced by another equivalent of the ligand.[22,23]

The cytotoxicity of complex **3** is significantly reduced when the aniline ring is functionalised with a *para* fluoro substituent (**12**), and the phenyl ring connected to the keto group remains non-functionalized. Complex **12** exhibits a 3-fold decrease in potency, when compared to complex **3**, highlighting for this example that a functionalisation of the benzoyl moiety may lead to more potent drugs candidates than a functionalisation of the aniline ring. The LogP values of all complexes were predicted using ALOGPS (**Table S9**) and were all found to be hydrophobic,[32,33] however, no structure activity relationships could be determined between LogP and cytotoxicity.

Chemosensitivity Assays under Hypoxic Conditions

Due to the abnormal vasculature and microenvironment of solid tumours, the use of chemotherapy and radiation cancer treatments becomes difficult, as some tumour cells are often resistant to treatment.[34] An advantage of some inorganic complexes is the ability of the metal and/or redox active ligands to be activated in low oxygen (reducing) conditions, therefore, we have tested the moderately active complex **4** under hypoxic conditions. This complex was tested alongside cisplatin, after 96 hours incubation with the HCT116 $p53^{+/+}$ cell line at 0.1% O_2. The results show that the activity of complex **4** decreases by 2-fold when tested under hypoxic conditions (IC_{50} = 21.6 μM (21% O_2) and 50.5 μM (0.1% O_2)), whereas the activity of cisplatin decreases by 26-fold under the same conditions (IC_{50} = 3.3 μM (21% O_2) and 95.5 μM (0.1% O_2). The decrease in activity of cisplatin has been associated with the activation of autophagy and mediated cisplatin resistance;[35] therefore, complexes with higher activity than cisplatin under hypoxic conditions are promising and can provide an understanding towards smart synthesis when designing new compounds as potential drug candidates. Though the ruthenium is highly unlikely to reduce *in vitro*, these studies under low O_2 concentration can help to identify complexes which remain cytotoxic or can be used in hypoxia targeting.[36,37]

Stability Studies in Aqueous Media

In order to address the stability of the complexes in aqueous conditions, initial samples were set up in 10% DMSO:90% H_2O

or D_2O to analyse both the UVvis spectra and NMR spectra,[38] however the complexes precipitate out of solution at such high water content (**Figures 5A** and **5B**). Samples were then made up at varying concentrations of water, and found to only remain in solution at 10% H_2O. ^1H NMR samples were prepared in 90% d_3-acetonitrile:10% D_2O to give a final concentration of 8 mg mL^{-1}, and spectra were recorded every 24 hours over a period of 4 days (**Figure S6**). Minor changes in the ^1H NMR spectra are observed from day 0 to day 4, whereby the intensity of the resonances decreases, particularly in the aromatic (7-8 ppm) and methine β-ketoiminate proton (5.7-5.9 ppm) regions. The resonance corresponding to the methine proton disappears completely by day 4, with no broadening of resonances or paramagnetic shifts. This suggests the potential hydrolysis of the β-ketoiminate ligands over this period of time, however, there are no peaks in the ES-MS which can be assigned to the free ligand. The hydrolysis of these β-ketominate ligands has already been reported by the group when bound to ruthenium(II) p-cymene or iridium(III) Cp*.[22,24]

Table 1 Chemosensitivity results of complexes **1**-**16**, cisplatin and oxaliplatin against MIA-PaCa-2, HCT116 *p53*$^{+/+}$ and ARPE-19. Values are stated as inhibition concentrations (IC_{50}) ± Standard Deviation (SD) and are triplicate repeats.

Complex	IC_{50} ± SD (μM)		
	MIA-PaCa-2	HCT116 *p53*$^{+/+}$	ARPE-19
1	89 ± 9	86 ± 22	92 ± 14
2	>100	65 ± 19	>100
3	>100	22 ± 4	38 ± 9
4	>100	>100	>100
5	>100	>100	>100
6	96 ± 7	43 ± 6	51 ± 3
7	>100	96 ± 7	>100
8	93 ± 12	68 ± 11	82 ± 21
9	>100	54 ± 14	52 ± 12
10	61 ± 9	60 ± 7	78 ± 20
11	81 ± 12	63 ± 8	79 ± 25
12	92 ± 14	67 ± 7	89 ± 20
13	>100	72 ± 6	>100
14	84 ± 19	82 ± 8	91 ± 17
15	>100	65 ± 16	>100
16	>100	>100	>100
cisplatin	3.6 ± 0.7	3.3 ± 0.4	6 ± 1
oxaliplatin	6 ± 1	0.9 ± 0.1	6 ± 3

Additionally, UV-vis spectra were recorded every 24 hours for 4 days in 90% acetonitrile and 10% water to give a final concentration of 50 μM (**Figure S7** and **Table S7**). The final products were analysed by ESI-MS. Slow darkening of the initial colour, from yellow to brown, was observed for all complexes between days 0 to day 4, with complex **6** showing the slowest colour change, with changes observed in the UV-vis spectra. All complexes convert to a species which is likely to be the same in all experiments, whereby the peak at 350-400 nm has both a bathochromic and hypochromic shift, suggestive of a structurally different complex. As observed in **Table 1**, the complexes only have moderate to low anticancer activity, and with the complexes studied for hydrolysis, the order of anticancer activity is inversely proportional to the rate of hydrolysis; **3** > **1** > **6** > **12** > **13** > **9** (**Table S8**). The hydrolysis rates are relatively similar for the unsubstituted complex **1** and the electron withdrawing substituted complex **3** suggesting that addition of the electron withdrawing substituents on the ligand has no significant effect on the rate of hydrolysis. Contrary to this, electron donating substituents, such as the para methyl group on complex **9**, significantly lower the rate of hydrolysis. Additionally, the nature of the phenyl ring also affects the rate of hydrolysis, whereby complex **3** (*para* fluoro phenyl) is completely hydrolysed by within 24 hours, while **11** (*para* fluoro aniline) is only completely hydrolysed by day 4. Analogues complexes, **6** (*para* chloro phenyl) and **13** (*para* chloro aniline) also show comparable results.

Antifungal and Antibacterial Properties of β-ketoiminato ruthenium(II) Complexes

To date there have been few reports on the use of ruthenium complexes as anti-fungal agents,[39] though activities against *Aspergillus flavus* and *fusarium* species have been reported for ruthenium Schiff base complexes. In collaboration with the CO-ADD (Community for Antimicrobial Drug Discovery, The University of Queensland, Australia), we have evaluated the antifungal activities of complexes **1**-**16** against *Candida albicans* (*C. albicans*) and *Cryptococcus neoformans var. grubii* (*C. neoformans*) (**Table S10**). The complexes showed selectivity towards the *C. albicans* fungal strain as shown by the positive growth inhibition values when compared to the negative values obtained for *C. neoformans*, with complex **7** having a growth inhibition of 44.1 %, and a selectivity ratio > 18.5. Comparing these results to those obtained by Dyson *et al.* on the inhibition properties of ruthenium(II) arene RAPTA-like (RAPTA = ruthenium arene 1,3,5-triaza-7-phosphatricyclo-[3.3.1.1]decane) complexes, our complexes exhibited inhibition of *C. neoformans* which are several orders of magnitude higher.[40] Though we have not yet identified the mechanism of inhibition, we are investigating these complexes as carbon monoxide-releasing molecules (CORMs).[41]

One of the major advances in the medical field has been the development and widespread use of antimicrobials, with transition metal complexes receiving significant interest for the development of metal based antimicrobial agents.[42] The ability of fine-tuning the coordination sphere, the oxidation state and the possibility of simultaneous multiple mechanisms of action, may help to overcome drug resistance.[43] As CORMs are known to have a different mode of action in their biological and therapeutic applications when compared to other transition metal based molecules, it has prompted investigations into their potential application for the treatment of antibiotic-resistant bacteria.[44] To assess the potential of our complexes, we collaborated with the CO-ADD and screened complexes **1**-**16** against five different antibiotic-resistant bacterial strains. Though most of the complexes are inactive (**Table S11**), complex **10** is partially active against Gram-positive *S. aureus* species, with a growth inhibition of 58%, and

inactive against the other four bacterial strains, which is again an order of magnitude higher than recently reported ruthenium(II) arene complexes,[40] and similar to other reported metallocene complexes.[45]

Conclusions

In this study we have introduced a range of new bis(β-ketominato) ruthenium(II) carbonyl complexes which have an unusual reaction pathway. We are currently conducting mechanistic work on the understanding of these reactions and products. The complexes were screened for their anticancer, antimicrobial and antifungal activities, whereby the position of the different substituents on the β-diketoiminate ligand has a significant effect on the complexes' activity. Though the anticancer activities are only moderate, the antifungal and antibacterial results are promising for complexes **7** and **10**, which have increased growth inhibitions for *C. neoformans* and *S. aureus* species, respectively. The recorded inhibition values are several orders of magnitude higher than previously reported metal-based complexes.

Conflicts of interest

There are no conflicts to declare.

Notes and references

The authors would like to thank Dr Markus Zegke for crystallography support, Mr. Stephen Boyer for conducting elemental analysis (London Metropolitan University Elemental Analysis Service) and Dr Stuart Warriner for providing mass spectrometry analysis (University of Leeds). They would also like to acknowledge Dr Samantha Shepherd (University of Huddersfield) for cell culture training. The work was kindly supported by the Schlumberger Foundation-Faculty for the Future. The authors also kindly thank the Community for Antimicrobial Drug Discovery, The University of Queensland, Australia, for providing antifungal and antibacterial studies.

1 K. B. Garbutcheon-Singh, M. P. Grant, B. W. Harper, A. M. Krause-Heuer, M. Manohar, N. Orkey and J. R. Aldrich-Wright, *Curr. Top. Med. Chem.*, 2011, **11**, 521–542.
2 U. Ndagi, N. Mhlongo and M. E. Soliman, *Drug Des. Devel. Ther.*, 2017, **11**, 599–616.
3 B. K. Keppler, M. E. Heim, H. Flechtner, F. Wingen and B. L. Pool, *Arzneimittelforschung.*, 1989, **39**, 706–709.
4 T. Schilling, B. K. Keppler, M. E. Heim, G. Niebch, H. Dietzfelbinger, J. Rastetter and A. R. Hanauske, *Invest New Drugs*, 1996, **13**, 327–333.
5 R. M. Lord, J. J. Mannion, A. J. Hebden, A. E. Nako, B. D. Crossley, M. W. McMullon, F. D. Janeway, R. M. Phillips and P. C. McGowan, *ChemMedChem*, 2014, **9**, 1136–1139.
6 R. M. Lord, J. J. Mannion, B. D. Crossley, A. J. Hebden, McMullon, J. Fisher, R. M. Phillips and P. C. McGowan, *ChemistrySelect*, 2016, **1**, 6598–6605.
7 M. J. Clarke, F. Zhu and D. R. Frasca, *Chem. Rev.*, 1999, **99**, 2511–2534.
8 B. R. James, E. Ochiai and G. L. Rampel, *Inorg. Nucl. Chem. Lett.*, 1971, **7**, 781–784.
9 G. Sava, T. Giraldi, G. Mestroni and Grazia. Zassinovich, *Chem. Biol. Interact.*, 1983, **45**, 1–6.
10 G. Mestroni, E. Alessio, G. Sava, S. Pacor and M. Coluccia, VCH, Weinheim, Germany, Ed. B. K. Keppler., 1993, p. 157.
11 G. Mestroni, E. Alessio, G. Sava, S. Pacor, M. Coluccia and A. Boccarelli, *Met.-Based Drugs*, 1994, **1**, 41–63.
12 E. Alessio, *Chem. Rev.*, 2004, **104**, 4203–4242.
13 K. S. Blisard, D. A. Harrington, D. A. Long and J. E. Jackson, *J. Comp. Pathol.*, 1991, **105**, 367–375.
14 B. K. Keppler, K.-G. Lipponer, B. Stenzel and F. Kratzin, *Metal Complexes in Cancer Chemotherapy*, VCH, Weinheim, Germany, Ed. B. K. Keppler,., 1993.
15 C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas and B. K. Keppler, *J. Inorg. Biochem.*, 2006, **100**, 891–904.
16 C. G. Hartinger, M. A. Jakupec, S. Zorbas-Seifried, M. Groessl, A. Egger, W. Berger, H. Zorbas, P. J. Dyson and B. K. Keppler, *Chem. Biodivers.*, 2008, **5**, 2140–2155.
17 U. Golla, S. Swagatika, S. Chauhan and R. S. Tomar, *Oncotarget*, 2017, **8**, 98426–98454.
18 G. Sava, I. Capozzi, K. Clerici, G. Gagliardi, E. Alessio and G. Mestroni, *Clin. Exp. Metastasis*, 1998, **16**, 371–379.
19 S. Leijen, S. A. Burgers, P. Baas, D. Pluim, M. Tibben, E. van Werkhoven, E. Alessio, G. Sava, J. H. Beijnen and J. H. M. Schellens, *Invest. New Drugs*, 2015, **33**, 201–214.
20 L. Zeng, P. Gupta, Y. Chen, E. Wang, L. Ji, H. Chao and Z.-S. Chen, *Chem. Soc. Rev.*, 2017, **46**, 5771–5804.
21 A. M. Basri, R. M. Lord, S. J. Allison, A. Rodríguez-Bárzano, S. J. Lucas, F. D. Janeway, H. J. Shepherd, C. M. Pask, R. M. Phillips and P. C. McGowan, *Chem. - Eur. J.*, 2017, **23**, 6341–6356.
22 R. M. Lord, A. J. Hebden, C. M. Pask, I. R. Henderson, S. J. Allison, S. L. Shepherd, R. M. Phillips and P. C. McGowan, *J. Med. Chem.*, 2015, **58**, 4940–4953.
23 S. J. Lucas, R. M. Lord, R. L. Wilson, R. M. Phillips, V. Sridharan and P. C. McGowan, *Dalton Trans.*, 2012, **41**, 13800–13802.
24 R. Lord, M. Zegke, I. R. Henderson, C. M. Pask, H. J. Shepherd and P. C. McGowan, *Chem. – Eur. J.*, 2019, **25**, 495–500.
25 S. Ammermann, C. Daniliuc, P. G. Jones, W.-W. du Mont, W. Kowalsky and H.-H. Johannes, *Dalton Trans*, 2008, 4095–4098.
26 Y.-Z. Chen, W. C. Chan, C. P. Lau, H. S. Chu, H. L. Lee and G. Jia, *Organometallics*, 1997, **16**, 1241–1246.
27 J. Chatt, B. L. Shaw and A. E. Field, *J. Chem. Soc. Resumed*, 1964, **0**, 3466–3475.
28 O. A. M. Ali, A. K. Abu Al-Nasr and R. M. Ramadan, *J. Taibah Univ. Sci.*, 2014, **8**, 258–264.
29 J. G. Małecki and A. Maroń, *Transit. Met. Chem.*, 2012, **37**, 727–734.
30 J. Niesel, A. Pinto, H. W. P. N'Dongo, K. Merz, I. Ott, R. Gust and U. Schatzschneider, *Chem. Commun.*, 2008, **0**, 1798–1800.
31 M. R. Churchill, R. A. Lashewycz and F. J. Rotella, *Inorg. Chem.*, 1977, **16**, 265–271.
32 I. V. Tetko and V. Yu. Tanchuk, *J. Chem. Inf. Comput. Sci.*, 2002, **42**, 1136–1145.
33 I. V. Tetko, H. P. Varbanov, M. Galanski, M. Talmaciu, J. A. Platts, M. Ravera and E. Gabano, *J. Inorg. Biochem.*, 2016, **156**, 1–13.
34 N. Rohwer and T. Cramer, *Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother.*, 2011, **14**, 191–201.
35 Q. Guo, F. Lan, X. Yan, Z. Xiao, Y. Wu and Q. Zhang, *Oncol. Lett.*, 2018, **16**, 801–808.

36 J. Zhao, W. Li, S. Gou, S. Li, S. Lin, Q. Wei and G. Xu, *Inorg. Chem.*, 2018, **57**, 8396–8403.
37 L. Zeng, Y. Chen, H. Huang, J. Wang, D. Zhao, L. Ji and H. Chao, *Chem. - Eur. J.*, 2015, **21**, 15308–15319.
38 A. Gatti, A. Habtemariam, I. Romero-Canelón, J.-I. Song, B. Heer, G. J. Clarkson, D. Rogolino, P. J. Sadler and M. Carcelli, *Organometallics*, 2018, **37**, 891–899.
39 A. I. Ramos, T. M. Braga and S. S. Braga, *Mini Rev. Med. Chem.*, 2012, **12**, 227–235.
40 Q. Laurent, L. K. Batchelor and P. J. Dyson, *Organometallics*, 2018, **37**, 915–923.
41 M. Tinajero-Trejo, K. J. Denby, S. E. Sedelnikova, S. A. Hassoubah, B. E. Mann and R. K. Poole, *J. Biol. Chem.*, 2014, **289**, 29471–29482.
42 F. Li, J. G. Collins and F. R. Keene, *Chem. Soc. Rev.*, 2015, **44**, 2529–2542.
43 A. Regiel-Futyra, J. M. Dąbrowski, O. Mazuryk, K. Śpiewak, A. Kyzioł, B. Pucelik, M. Brindell and G. Stochel, *Coord. Chem. Rev.*, 2017, **351**, 76–117.
44 J. L. Wilson, H. E. Jesse, B. Hughes, V. Lund, K. Naylor, K. S. Davidge, G. M. Cook, B. E. Mann and R. K. Poole, *Antioxid. Redox Signal.*, 2013, **19**, 497–509.
45 B. C. Hoffknecht, P. Prochnow, J. E. Bandow and N. Metzler-Nolte, *J. Inorg. Biochem.*, 2016, **160**, 246–249.