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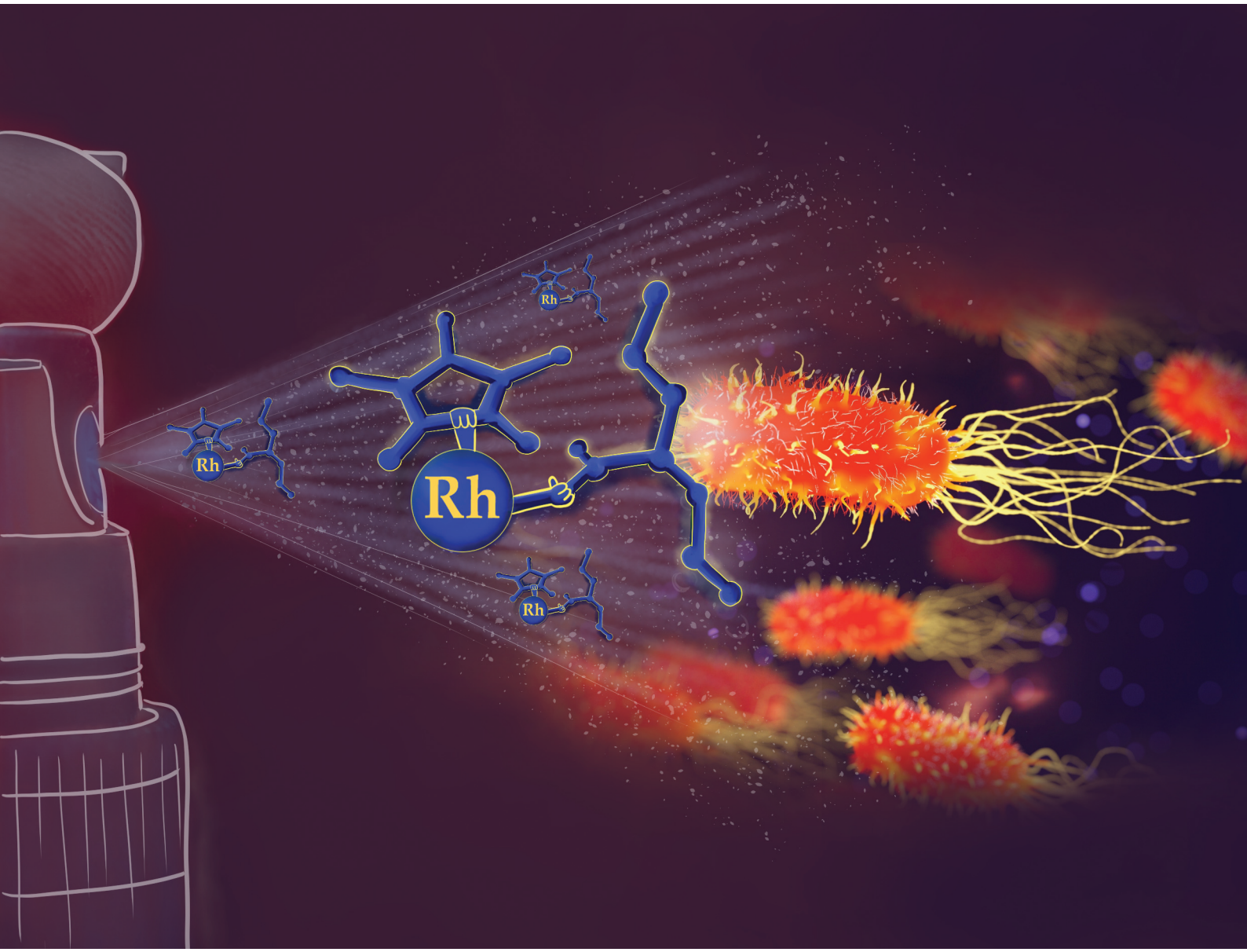

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Novel valproate half-sandwich rhodium and iridium conjugates to fight against multidrug-resistant Gram-positive bacteria†

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New valproate Ir(III) and Rh(III) half-sandwich conjugates containing a C,N-phenylbenzimidazole chelated ligand have been synthesized and characterized. The valproic acid conjugation to organometallic fragments seems to switch on the antibacterial activity of the complexes towards *Enterococcus faecium* and *Staphylococcus aureus* Gram-positive bacteria.

According to the World Health Organization (WHO), drug-resistant infections cause nearly 5 million deaths yearly, and are projected to increase to 10 million deaths by 2050.[1] The increasing global spread of multidrug-resistant (MDR) bacteria (commonly referred to as "superbugs"),[2] which cause diseases that cannot be treated with conventional antibiotics, is threatening the world. Especially alarming is a list of pathogens designated by the acronym ESKAPE: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*.[3] This group of pathogenic bacteria encompasses Gram-positive and Gram-negative species capable of "escaping" the biocidal action of normally used antimicrobial agents. ESKAPE pathogens are responsible for some of the deadliest hospital-acquired infections (HAIs) and are a leading cause of mortality and morbidity worldwide.[4] What is truly worrying is that the clinical pipeline of new antibiotics is dry. In 2019, the WHO identified 32 antibiotics in clinical development that address the priority pathogen list, of which only six were classified as innovative.[5] Therefore, new classes of antibacterial compounds are urgently needed.

Organometallic complexes offer rich versatility for the design of new antimicrobial agents.[6–10] Unlike organic molecules, which have simple one- or two-dimensional shapes, metal compounds can access a great variety of three-dimensional structures, becoming ideal scaffolds for the development of new antibiotics.[7,11]

Despite the fact that rhodium and iridium half-sandwich complexes have been less studied than other half-sandwich ruthenium complexes,[12] recent reports have highlighted the promising antibacterial properties of these compounds, particularly those featuring pentamethylcyclopentadienyl (Cp*), which provides stability and hydrophobicity, along with neutral chelating ligands[13–15] or carbene ligands.[16,17] However, there are no examples in the literature about Rh and Ir half-sandwich complexes incorporating C,N-chelating ligands that have antimicrobial activity against ESKAPE pathogens.

Valproic acid (VPA) is a branched short-chain fatty acid that is commonly used in the clinic to treat epilepsy.[18] This small molecule has been reported as a histone deacetylase (HDAC) inhibitor, resulting in an increased interest for its use in chemotherapy.[19,20] Recent studies have demonstrated that VPA and derivatives also have antimicrobial properties.[21–24]

Herein we report the synthesis of new half-sandwich rhodium(III) and iridium(III) complexes containing chelated benzimidazole ligands derivatized with valproic acid (Scheme 1A). We have screened them for *in vitro* biological activity against various multidrug-resistant bacteria of clinical interest: two Gram-positive (vancomycin-resistant *Enterococcus faecium* and methicillin-resistant *Staphylococcus aureus*) and two Gram-negative (*Acinetobacter baumanii* and *Pseudomonas aeruginosa*) strains. Remarkably, the valproic acid conjugation to organometallic fragments switched on the activity of the complexes towards the Gram-positive bacteria, especially for

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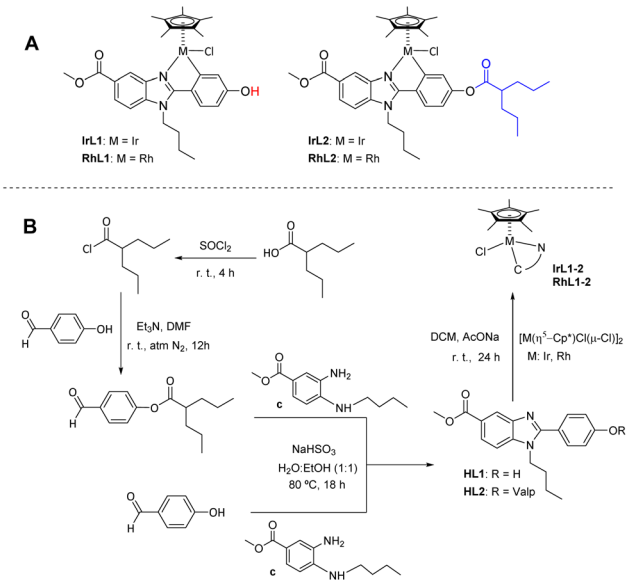
†Electronic supplementary information (ESI) available: Details of general procedure for the synthesis of Ir(III) and Rh(III) complexes, figures for the characterization and stability of the new compounds (NMR, MS, UV-Vis), additional figures of X-ray diffraction, biological methods. CCDC 2266047. For ESI and crystallographic data in CIF or other electronic format see DOI: **https://doi.org/10.1039/d3dt01678a**

‡These authors contributed equally to this work.



Scheme 1 A) General structure of Ir(III) and Rh(III) half-sandwich complexes synthesized in this study. (B) Route of synthesis of ligands and complexes.

Rh(III) complex against *S. aureus*, a bacterium that has a quickly rising incidence and causes 11 000 death in the United States each year.[25]

In this work, we have designed two different HC^N ligands based on benzimidazole moiety for developing the new metal compounds. Benzimidazole core was selected for the wide range of pharmacological properties reported by this organic molecule[26–28] and its metal derivatives.[13,29–31] Moreover, benzimidazole ligand is very versatile and can be easily derivatized to modulate the final properties of the complexes.

The first ligand (**HL1**) is similar to previous benzimidazole ligands described by some of us,[29,30] but includes a hydroxyl group in the phenyl ring that allows further functionalization. The second ligand (**HL2**) contains the same benzimidazole scaffold conjugated to valproic acid, with the aim of exploring the effect of this conjugation on the activity of the new Rh(III) and Ir(III) complexes.

HL1 and **HL2** were synthesized by following the method in the literature.[32,33] Briefly, methyl 3-amino-4-(butylamino)benzoate **c** (Scheme 1B) and the corresponding aldehyde were stirred in ethanol–water (1 : 1, v : v) in the presence of $NaHSO_3$ overnight at 80 °C. For **HL2**, the synthetic procedure included the previous formation of valproic acid chloride and its reaction with 4-hydroxybenzaldehyde.[34] The final complexes (**IrL1–2** and **RhL1–2**) were synthesized as previously reported[29] by reaction of the corresponding rhodium or iridium dimer $[(\eta^5\text{-Cp}^*)MCl_2]_2$ and **HL1** or **HL2** in dichloromethane in the presence of sodium acetate at room temperature for 24 h.

All compounds were characterized by 1D and 2D NMR spectroscopy (Fig. S1–S25†), ESI-MS and elemental analysis. The ^{1}H NMR spectra of all ligands and complexes showed the characteristic signals of this type of benzimidazole ligand: the ester group is observed as a singlet with integration of three protons in the region near 4 ppm, and the butyl group shows three different signals at δ 4.7–4.2 ppm, 1.9–1.7 ppm and 1.5–1.2 ppm respectively, and a triplet with integration of three protons in the region near to 0.9 ppm. On the other hand, the ^{1}H NMR spectra of **HL2** and the complexes with this ligand (**IrL2**, **RhL2**) also showed the signals corresponding to the valproate group: a multiplet with integration of one proton near to 2.6 ppm, two multiplets with integration of four protons at δ 1.8–1.6 ppm and 1.5–1.3 ppm and a triplet with integration of six protons at δ 1.0–0.9 ppm. In addition, in all complexes, a singlet is observed at δ 1.8–1.7 ppm corresponding to the methyl protons of the Cp* ring. High resolution positive-ion ESI-MS confirmed the identity of the complexes, with a base peak corresponding to the molecular $[M - Cl]^+$ for all complexes (Fig. S26–S31†). The new complexes were shown to be at least 95% pure by both elemental analysis and reverse-phase RP-HPLC (Fig. S32†).

The UV–visible spectra of the new complexes were recorded in acetonitrile and water (1% DMF) solution (Fig. S33†). All compounds showed intense absorption bands at 250–450 nm due to intraligand (IL) $\pi \rightarrow \pi^*$ transitions and metal-to-ligand charge transfer transitions (^{1}MLCT and ^{3}MLCT).[35]

Single crystals suitable for X-ray diffraction analysis were obtained for **RhL2** by slow diffusion of hexane into a saturated solution of the complex in CH_2Cl_2. Crystallographic data are listed in Table S2.† **RhL2** adopt a pseudo-octahedral geometry with a "piano-stool" shape (Fig. 1) typical of half-sandwich complexes,[13,29,36] with the pentamethylcyclopentadienyl group displaying the common π-bonded η^5 coordination mode, and the 1-butyl-2-phenylbenzimidazole carboxylate assuming a bidentate-chelate coordination mode (κ^2-C,N), where the two rings of the benzimidazole and phenyl moieties not being strictly coplanar. The Rh–Cl bond distance [2.3942(4) Å] is similar to the previously observed in some related Rh(III) C^N complexes.[37]

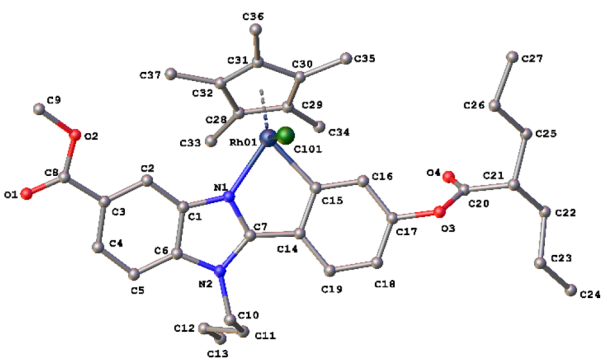


Fig. 1 The X-ray crystal structure of complex **RhL2**. Selected bond lengths [Å]: Rh(01)–C(15): 2.0252(13); Rh(01)–N(1): 2.0865(11); Rh(01)–C(30): 2.1480(13); Rh(01)–C(28): 2.1496(14); Rh(01)–C(29): 2.1590(14); Rh(01)–C(31): 2.2421(14); Rh(01)–C(32): 2.2694(14); Rh(01)–Cl(01): 2.3942(4). Selected angles [°]: N(1)–Rh(01)–C(32): 105.87(5); C(15)–Rh(01)–Cl(01): 87.02(4); N(1)–Rh(01)–Cl(01): 92.02(3).

The stability of complexes **IrL1** and **RhL1** was studied first in DMSO-d_6 by ^{1}H NMR, but we observed changes in the spectra (Fig. S34 and S35†) probably due to the exchange of the chloride ligand by the solvent.[31] Strong affinity of iridium(III) complexes to bind dimethyl sulfoxide is well known.[38] Then, the stability was checked in DMF-d_7, and no changes were observed after 48 h (Fig. S36–39†). Therefore, this solvent was selected for further experiments. Regarding the stability of the complexes in aqueous solutions, it was observed that they undergo straightforward aquation, leading to the replacement of chloride by water (Fig. S41†). The stability was confirmed in other biologically relevant conditions using a 5% of DMF (Fig. S40†), showing **IrL2** and **RhL2** the best stability in that medium. Accordingly, the presence of the valproate group clearly improves the stability of these type of complexes.

The ability of the new Rh(III) and Ir(III) half-sandwich complexes to inhibit the growth of two Gram-positive (vancomycin-resistant *Enterococcus faecium* and methicillin – resistant *Staphylococcus aureus*) and two Gram-negative (*Acinetobacter baumanii* and *Pseudomonas aeruginosa*) strains was studied using the broth microdilution method and compared to the cyclometalating ligands, valproic acid and the broad-spectrum fluoroquinolone antibiotic Norfloxacin as positive control. The minimum inhibitory concentration (MIC) values, that is, the lowest concentration of the compounds under study that prevents bacterial growth, are collected in Table 1. As observed, **HL1**, **HL2** and valproic acid did not exhibit any activity against the bacterial strains studied (MIC > 60 μM). All complexes were inactive against Gram-negative bacteria probably due to the neutral nature of these compounds. By contrast, complexes **IrL2** and **RhL2** (with the valproate group in the cyclometalating ligand) displayed antimicrobial activity against Gram-positive strains, being the rhodium derivative, **RhL2**, the most active complex, even more effective than the broad-spectrum antibiotic Norfloxacin in both bacterial strains. Thus, the role played by the metal centre in this series of complexes is secondary since the functionalization with a valproate group in the cyclometalating ligand is responsible for the antimicrobial activity of these complexes. This antimicrobial activity may be related to the ability of valproate to inhibit histone deacetylase and to affect fatty acid biosynthesis in bacteria and yeast.[39]

Table 1 MIC values (μM) of the ligands and complexes. Valproic acid (VPA) and Norfloxacin were included as a control

MIC, μM	Gram-positive		Gram-negative	
	E. faecium	*S. aureus*	*A. baumanii*	*P. aeruginosa*
HL1	>60	>60	>60	>60
HL2	>60	>60	>60	>60
IrL1	>60	>60	>60	>60
RhL1	>60	>60	>60	>60
IrL2	7.5	3.8	>60	>60
RhL2	3.8	2	>60	>60
VPA	>60	>60	>60	>60
Norfloxacin	6	6	20	3

Table 2 MBC values of the most promising complexes

MBC, μM	Gram-positive	
	E. faecium	*S. aureus*
IrL2	60	30
RhL2	15	7.5

In order to clarify if these complexes were bactericide (kill bacteria) or bacteriostatic (prevent bacterial growth) agents, the minimum bactericidal concentration (MBC), that is, the lowest concentration of the compound that reduces the viability of the initial bacterial inoculum by ≥99.9% was also evaluated. A bactericide agent is formally defined as an antimicrobial compound for which the ratio MBC-to-MIC is ≤4, while a bacteriostatic agent is one for which the MBC-to-MIC ratio is >4. According to the results collected in Table 2, **RhL2** is a bactericide complex whereas **IrL2** is a bacteriostatic agent. Thus, the metal is governing the magnitude of the antimicrobial effect.

Accumulation of **IrL1–2** and **RhL1–2** in bacteria was also investigated by ICP-MS experiments. Although iridium complexes were internalised more than the rhodium analogues, they barely manage to enter Gram-negative strains (Fig. 2). These results are expectable since the presence of the extra outer membrane in Gram-negative bacteria hinders drug internalization.[9] The type of metal centre and the bacterial uptake were not directly correlated with the antimicrobial activity (Table 1). Thus, while the iridium complex accumulates to a greater extent in *S. aureus*, the Rh complex shows greater activity in said bacterial strain. A similar behaviour has been observed previously,[16] and it could be due to the fact that the metal complexes have a different mechanism of action. On the other hand, complexes bearing L2 were more accumulated than complexes with L1. Therefore, valproate clearly enhanced bacterial internalization due to its lipophilic character.

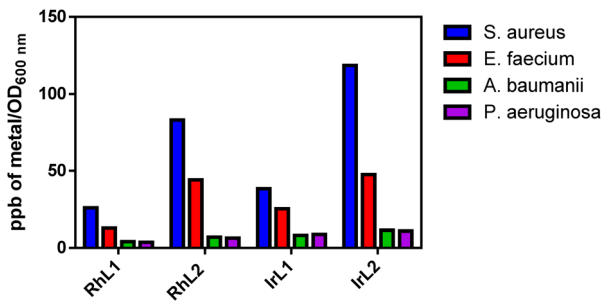


Fig. 2 Metal accumulation in bacteria treated with 1 μM of the complexes under study for 4 h.

Conclusions

In summary, we report the synthesis, characterization and antimicrobial activity of two novel valproate half-sandwich Ir(III) and Rh(III) conjugates and their parent compounds. The stability study in different media showed that the presence of the valproate group improved the stability of **IrL2** and **RhL2**.

All compounds had no effect on Gram-negative bacteria, probably due to their neutral nature and negligible internalization. In contrast, the valproate conjugates (**IrL2** and **RhL2**) demonstrated antibacterial action against Gram-positive pathogens. The bactericidal rhodium complex, **RhL2**, was the most effective compound. These results demonstrate the functionalization with a valproate group in the cyclometalating ligand is responsible for the antimicrobial activity whereas the metal is governing the magnitude of the antimicrobial effect.

This appears to be the first report of active Ir(III) and Rh(III) antibacterial complexes containing a C,N-chelating ligand against some ESKAPE pathogens. The preliminary data of antimicrobial activity shown here are very encouraging for further investigation of this class of complexes functionalized with valproate, including elucidation of their mechanism of action.

Author contributions

A. M. and G. V. contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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References

1 I. A. Joshua, M. Bobai and C. S. Woje, in *The Global Antimicrobial Resistance Epidemic*, ed. G. Tellez-Isaias, IntechOpen, Rijeka, 2022, ch. 4.
2 S. N. Khan and A. U. Khan, *Front. Microbiol.*, 2016, **7**, 174.
3 H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg and J. Bartlett, *Clin. Infect. Dis.*, 2009, **48**, 1–12.
4 Y.-X. Ma, C.-Y. Wang, Y.-Y. Li, J. Li, Q.-Q. Wan, J.-H. Chen, F. R. Tay and L.-N. Niu, *Adv. Sci.*, 2020, **7**, 1901872.
5 World Health Organization, *2019 antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline*, World Health Organization, Geneva, 2019.
6 M. Patra, M. Wenzel, P. Prochnow, V. Pierroz, G. Gasser, J. E. Bandow and N. Metzler-Nolte, *Chem. Sci.*, 2015, **6**, 214–224.
7 A. Frei, J. Zuegg, A. G. Elliott, M. Baker, S. Braese, C. Brown, F. Chen, C. G. Dowson, G. Dujardin, N. Jung, A. P. King, A. M. Mansour, M. Massi, J. Moat, H. A. Mohamed, A. K. Renfrew, P. J. Rutledge, P. J. Sadler, M. H. Todd, C. E. Willans, J. J. Wilson, M. A. Cooper and M. A. T. Blaskovich, *Chem. Sci.*, 2020, **11**, 2627–2639.
8 S. M. Cooper, C. Siakalli, A. J. P. White, A. Frei, P. W. Miller and N. J. Long, *Dalton Trans.*, 2022, **51**, 12791–12795.
9 N. Busto, G. Vigueras, N. Cutillas, B. García and J. Ruiz, *Dalton Trans.*, 2022, **51**, 9653–9663.
10 A. Jain, N. T. Garrett and Z. P. Malone, *Photochem. Photobiol.*, 2022, **98**, 6–16.
11 F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752–6756.
12 F. Li, J. G. Collins and F. R. Keene, *Chem. Soc. Rev.*, 2015, **44**, 2529–2542.
13 A. M. Mansour and K. Radacki, *Dalton Trans.*, 2020, **49**, 4491–4501.
14 P. Chellan, V. M. Avery, S. Duffy, J. A. Triccas, G. Nagalingam, C. Tam, L. W. Cheng, J. Liu, K. M. Land, G. J. Clarkson, I. Romero-Canelón and P. J. Sadler, *Chem. – Eur. J.*, 2018, **24**, 10078–10090.
15 F. Chen, J. Moat, D. McFeely, G. Clarkson, I. J. Hands-Portman, J. P. Furner-Pardoe, F. Harrison, C. G. Dowson and P. J. Sadler, *J. Med. Chem.*, 2018, **61**, 7330–7344.
16 C. M. Bernier, C. M. DuChane, J. S. Martinez, J. O. I. Falkinham and J. S. Merola, *Organometallics*, 2021, **40**, 1670–1681.
17 Y. Yang, L. Guo, Z. Tian, Y. Gong, H. Zheng, S. Zhang, Z. Xu, X. Ge and Z. Liu, *Inorg. Chem.*, 2018, **57**, 11087–11098.
18 J. Bruni and B. J. Wilder, *Arch. Neurol.*, 1979, **36**, 393–398.
19 A. R. Blaheta, H. Nau, M. Michaelis and J. Cinatl Jr., *Curr. Med. Chem.*, 2002, **9**, 1417–1433.
20 W. Feng and S. C. Kenney, *Cancer Res.*, 2006, **66**, 8762–8769.
21 L. Dong, L. Fang, X. Dai, J. Zhang, J. Wang and P. Xu, *Drug Dev. Res.*, 2022, **83**, 131–141.
22 C. Zutz, M. Bacher, A. Parich, B. Kluger, A. Gacek-Matthews, R. Schuhmacher, M. Wagner, K. Rychli and J. Strauss, *Front. Microbiol.*, 2016, **7**, 510.
23 L. Tabrizi, P. McArdle, M. Ektefan and H. Chiniforoshan, *Inorg. Chim. Acta*, 2016, **439**, 138–144.

24 N. Esiobu and N. Hoosein, *Antonie van Leeuwenhoek*, 2003, **83**, 63–68.
25 K. E. Thorpe, P. Joski and K. J. Johnston, *Health Aff.*, 2018, **37**, 662–669.
26 Y. Bansal and O. Silakari, *Bioorg. Med. Chem.*, 2012, **20**, 6208–6236.
27 D. Song and S. Ma, *ChemMedChem*, 2016, **11**, 646–659.
28 M. Boiani and M. Gonzalez, *Mini-Rev. Med. Chem.*, 2005, **5**, 409–424.
29 G. S. Yellol, A. Donaire, J. G. Yellol, V. Vasylyeva, C. Janiak and J. Ruiz, *Chem. Commun.*, 2013, **49**, 11533–11535.
30 J. Yellol, S. A. Pérez, A. Buceta, G. Yellol, A. Donaire, P. Szumlas, P. J. Bednarski, G. Makhloufi, C. Janiak, A. Espinosa and J. Ruiz, *J. Med. Chem.*, 2015, **58**, 7310–7327.
31 M. I. Acuña, A. R. Rubio, M. Martínez-Alonso, N. Busto, A. M. Rodríguez, N. Davila-Ferreira, C. Smythe, G. Espino, B. García and F. Domínguez, *Cancers*, 2023, **15**, 107.
32 Y. Jiang, S. Jia, X. Li, Y. Sun, W. Li, W. Zhang and G. Xu, *Chem. Pap.*, 2018, **72**, 1265–1276.
33 M. Betti, E. Genesio, G. Marconi, S. Sanna Coccone and P. Wiedenau, *Org. Process Res. Dev.*, 2014, **18**, 699–708.
34 R.-R. Ye, J.-J. Cao, C.-P. Tan, L.-N. Ji and Z.-W. Mao, *Chem. – Eur. J.*, 2017, **23**, 15166–15176.
35 L. Tabrizi, *Dalton Trans.*, 2017, **46**, 7242–7252.
36 W.-Y. Zhang, S. Banerjee, G. M. Hughes, H. E. Bridgewater, J.-I. Song, B. G. Breeze, G. J. Clarkson, J. P. C. Coverdale, C. Sanchez-Cano, F. Ponte, E. Sicilia and P. J. Sadler, *Chem. Sci.*, 2020, **11**, 5466–5480.
37 J. P. Cerón-Carrasco, J. Ruiz, C. Vicente, C. de Haro, D. Bautista, J. Zúñiga and A. Requena, *J. Chem. Theory Comput.*, 2017, **13**, 3898–3910.
38 A. C. Carrasco, V. Rodríguez-Fanjul, A. Habtemariam and A. M. Pizarro, *J. Med. Chem.*, 2020, **63**(8), 4005–4021.
39 P. Poolchanuan, P. Unagul, S. Thongnest, S. Wiyakrutta, N. Ngamrojanavanich, C. Mahidol, S. Ruchirawat and P. Kittakoop, *Sci. Rep.*, 2020, **10**, 9300.