Monomeric and Dimeric Coordinatively Saturated and Substitutionally Inert Ru(II) Polypyridyl Complexes as Anticancer Drug Candidates

Anna Notaro, Gilles Gasser

► To cite this version:

Anna Notaro, Gilles Gasser. Monomeric and Dimeric Coordinatively Saturated and Substitutionally Inert Ru(II) Polypyridyl Complexes as Anticancer Drug Candidates. *Chemical Society Reviews*, 2017, 46 (23), pp.7317-7337. 10.1039/C7CS00356K . hal-01624345

HAL Id: hal-01624345

<https://hal.science/hal-01624345>

Submitted on 26 Oct 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Monomeric and Dimeric Coordinatively Saturated and Substitutionally Inert Ru(II) Polypyridyl Complexes as Anticancer Drug Candidates

Anna Notaro,^a Gilles Gasser^{a,*}

Due to the increasing impact of cancer on worldwide mortality, more and more attention is being devoted to the investigation of novel anticancer strategies. Among these, chemotherapy plays a key role in fighting cancer. This explains the increasing engagement of both pharmaceutical industry and academia towards the discovery of new chemotherapeutic agents. Over the recent years, metal-based drugs have attracted much attention due to their atypical physico-chemical properties compared to organic molecules. After the approval of cisplatin as a chemotherapeutic agent in 1978, several types of metal-based drugs have been explored. Among them, Ru-based anticancer drug candidates have become a central subject in this research field. However, most of the Ru-based compounds investigated over the last two decades express their cytotoxicity with a mechanism of action involving, among others, a ligand-exchange mechanism. In this Review, we give a complete overview of a specific class of antiproliferative ruthenium complexes, namely coordinatively saturated and substitutionally inert Ru(II) polypyridyl complexes. This implies that the cytotoxicity observed comes from the entire complex and not from a ligand-exchange. In this Review, we are presenting monomeric and dimeric Ru(II) polypyridyl complexes, which have been found to be toxic to cancer cells. More specifically, the monomeric Ru(II) polypyridyl complexes are analysed considering their direct interaction or not with DNA as cause of cell death, while dimeric Ru(II) polypyridyl complexes, are classified according to their biological targets. Very importantly, the cellular targets of these complexes are discussed in detail. Indeed, several targets were identified and different mechanisms of action were suggested.

1. Introduction

Cancer is a prominent cause of death worldwide. In high-income countries, it is already the main cause of mortality, a trend which is becoming more and more similar in low- and middle-income countries.^[1,2] The latter increase is a consequence of the economic transition, which intensifies pollutants exposure due to the higher mechanisation in transports and labor.² The adoption of cancer-associated lifestyle choices in these countries (e.g. smoking, physical inactivity, and “westernised” diets) is another cause of this rise.¹ Furthermore, it has to be noted that other factors playing a key role in this increasing phenomena are population aging and growth, which are once again correlated with economic development.^[2,3] On the other hand, from data estimated by GLOBOCAN, a project from the World Health Organization to estimate cancer incidence, mortality and prevalence worldwide,^[4,5] it is interesting to note that even if the incidence rate for all cancers in developing countries is about half when compared with developed ones, the mortality rate is quite similar. This disparity between incidence and mortality can be related with the lack of prevention as much as with the shortage of appropriate therapeutic facilities and drug availability occurring in developing countries.¹

To date, surgery is the most efficient treatment when used as a single form of cancer therapy since it can completely remove cancer from the organism.³ On the other hand, radiotherapy and chemotherapy are considered complementary techniques since they are able to kill only part of cancer cells in each treatment.³ However, surgery itself is only working for localised primary cancers. For all other cases such as metastatic cancers, a combination therapy is required.³ Due to these considerations, in recent years, more and more attention has been dedicated to the discovery of new chemotherapeutic agents.

Among the different agents investigated, metal-based drug candidates have been intensively studied since they present atypical physico-chemical properties compared to organic compounds. Among others, metal complexes offer a greater structural variety and the possibility of ligand exchange, which allows for the covalent interaction with biological molecular targets.^[6–12] Moreover, thanks to their precise three-dimensional configuration, they can recognise and interact with a defined molecular target, like, for example, the metal-based kinase inhibitors of Meggers.¹³ Since metals have access to different stable oxidation states, they can also react with the biological redox chemistry of cells.¹²

Cytotoxic chemotherapeutic agents damage proliferating cells.¹⁴ In some cases, as for cisplatin (**1**, Figure 1), this is achievable by inhibiting replication or by blocking biological pathways involved in DNA synthesis and cell cycle.⁷ Platinum-based drugs were investigated, for the first time, during the 1960s by Rosenberg and co-workers.^[15–17] These researchers discovered the ability of platinum complexes to inhibit cell divisions in bacteria.^[15–17] This activity gave the basis for the investigation of such complexes as a chemotherapeutic agents.¹⁸ The mechanism of action of cisplatin is believed to be linked to DNA damage, which involves the inhibition of replication, transcription or a combination of the two.¹⁹ Inter alia, cytotoxicity of cisplatin is due to the covalent crosslinks generated by its interaction with DNA.⁷ Its cytotoxicity is enhanced by the saturation of the cellular capacity to repair platinum adducts of DNA. *Trans*-adducts are repaired more easily than *cis*-adducts, explaining the 30 times difference in toxicity between cisplatin and transplatin.²⁰ Although cisplatin is, to date, one of the most widely used and successful chemotherapeutic drugs, it exhibits severe side effects such as kidney damage, hearing loss, peripheral nerve damage, severe nausea and vomiting, and bone marrow suppression.¹⁹ These side effects as well as the phenomena of tumour resistance hampered cisplatin efficacy, limiting its clinical uses. In order to find a balance between the potential of cisplatin as a drug and its side effects, several different platinum-based compounds have been investigated like carboplatin and oxaliplatin (**2** and **3**, respectively in Figure 1), which achieved worldwide approval and use.^[8,9]

Due to the discovery of the potential of cisplatin and its derivatives, an impressive amount of metal complexes have been explored as chemotherapeutic agents.^[6,12,21–30] Among these, ruthenium complexes play a key role since they present several advantages compared to other metal complexes.^[31,32] They have multiple stable oxidation states under physiological conditions and were found to possess unique properties during pre-clinical trials.^[31,32] They were indeed found to enter more selectively cancer cells than healthy cells, therefore reducing the toxic effects on healthy cells.³² The

Fig. 1. Structures of metal-based drugs or drug candidates.

reason given for this observation was that they can mimic iron, which binds to transferrin proteins, whose receptors are largely overexpressed on malignant cells surface. This results in an increased uptake in cancer cells.³³ However, this latter point is currently under

strong debate, as recently pointed out by Alessio in a nice review.^[33,34] Importantly, compared to Pt(II) complexes, Ru complexes have a larger structural variety due to their octahedral geometry.^[22,27] In addition, Ru(III) and Ru(II) complexes usually have ligand exchange kinetics similar to those of Pt(II) complexes.³¹ They also own a well-developed synthetic chemistry.³⁵ Nevertheless, structurally similar ruthenium compounds can behave differently in a biological setting and have therefore different biological targets.³⁴ In fact, such compounds have an extensive diversity concerning their mechanisms of action, activities and toxicities.³⁴ To date, two Ru(III) complexes, namely NAMI-A (**4** in Figure 1) and NKP-1339 (**5** in Figure 1) have entered into clinical trials.^[36–39] Chemistry speaking, their mechanisms of action are comparable to that of cisplatin with the involvement of ligand exchange but any other detailed explanation about this is beyond of the scope of this Review. We send the interested readers to excellent Reviews.^[8,10,11,32,35,40–48,]

In this article, we are reviewing a specific type of ruthenium complexes, namely cytotoxic, coordinatively saturated and substitutionally inert Ru(II) polypyridyl complexes. Thus, since covalent binding is not possible for this type of metal complexes, their cytotoxicity must come from different mechanisms than those of cisplatin. Interestingly, as explained in detail below, they exert their cytotoxicity by inducing cell death through pathway activations, which often seem to be DNA independent. In this Review, we only present monomeric and dimeric Ru(II) polypyridyl complexes, which have been found to be toxic to cancer cells. More specifically, the monomeric Ru(II) polypyridyl complexes are

analysed considering their direct interaction or not with DNA as cause of cell death. Dimeric Ru(II) polypyridyl complexes, on the other hand, are classified considering their biological targets. Decisively, only Ru(II) complexes fully characterised and whose biological activity was tested on cancer cell lines are reviewed. We also try to rationalise the antiproliferative activity of the complexes taking into account the experimental data (i.e. cellular uptake and cellular localization). However, we would like to highlight that the role of the counter-anions in the cytotoxicity of the Ru(II) complexes reported in this Review is not completely clear. It is known that the counter-anion can influence the solubility of a complex (i.e. KP1019 vs NKP1339).³⁹ However, to the best of our knowledge, there has not been a study comparing the cytotoxicity of a Ru(II) polypyridyl complexes depending on its counter-anion. Very interestingly, a recent paper by Zhu presented a new method for delivering DNA 'light-switching' Ru(II) polypyridyl complexes into the nucleus of living cells.⁴⁹ In this work, Zhu and co-workers demonstrated that the cell-impermeable Ru(II) polypyridyl cationic complexes might form novel lipophilic and relatively stable ion-pair complexes with three structurally unrelated hydrophobic weak acids (i.e. pentachlorophenol (PCP), carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) and tolfenamic acid (TA)) leading to a remarkably enhanced nuclear uptake.⁴⁹ From this work, scientists speculate that ion-pair formation might be a general, promising live-cell delivery method for cell-impermeable metal complexes and potentially bio-medically important.⁴⁹ However, it is unclear if this has an impact on the cytotoxicity. In this Review, we will therefore make sure to mention every time the counter-anion employed in the biological studies. It is important to note that most of the studies reported in this Review are based on a continuation of previous works (i.e. researchers are trying to improve the cytotoxicity of a complex with the help of a structure-activity relationship study based on previous results which have shown that a complex was toxic to cancer cells). However, there are not, to the best of our knowledge, many examples of inert Ru(II) polypyridyl complexes, which have been rationally designed to aim for a specific target, like the Ru-based kinase inhibitors of Meggers, which are not discussed herein since this field was reviewed in detail.^[50–53] Recently, Ru(II) cyclometalated complexes have been also investigated as anticancer agents.^[54–57] However, this topic together with the Ru(II) polypyridyl complexes activated by light or the non-coordinatively saturated Ru(II) polypyridyl complexes are

not reviewed here. We send the interested readers to an excellent, very recent Review of Zeng and co-workers.⁵⁸ Over the years, many scientists focused their attention on reviewing the current state in medicinal chemistry of Ru complexes,^[24,26,32,35,45,46,59–61] in some cases considering only Ru(II) polypyridyl complexes and the possible applications.^[62–64] However, to the best of our knowledge, this is the first Review of the last 6 years focused only on the cytotoxicity of Ru(II) polypyridyl complexes.

2. Historical overview of coordinatively saturated and substitutionally inert Ru(II) polypyridyl complexes

Coordinatively saturated and substitutionally inert Ru(II) polypyridyl complexes such as [Ru(phen)₃](ClO₄)₂ (**6**) and [Ru(bpy)₃](ClO₄)₂ (**7**) shown in Figure 2 were biologically investigated for the first time by the Australian scientist Dwyer and his co-workers in the 1950s.^[65–67] The first study focused on the interaction of such complexes with enzymes as well as their bactericidal and bacteriostatic properties and toxicity to mice. Their first results showed that Ru(II) polypyridyl complexes exerted some bacteriostatic activity and were toxic for mice (toxic dose > 3mg/kg). The latter was associated with the inhibition of acetylcholinesterase, which is an enzyme mainly involved in neurotransmitter metabolism.⁶⁵ Further studies of the acetylcholinesterase inhibition and *in vivo* evaluation of toxicity in mice suggested that this kind of neurotoxicity was charge-related.⁶⁶ This observation was based on the inactivity showed by some neutral, analogue complexes such as Co(NH₃)₃(NO₂)₃. The interaction accountable for the inhibitory activity, in fact, occurs between the positively charged complex and the negatively charged sites of the enzyme.⁶⁶ The approval of penicillin in 1943 brought the pharmaceutical industry to mostly focus their attention on the development of new organic drugs.⁶⁸ Therefore, historical reasons as much as the detection of severe side effects like neurotoxicity mentioned above, shadowed the biological investigation of Ru(II) polypyridyl complexes for more than fifty years. In the 1990s, Ru(II)

Fig. 2. Structures of [Ru(phen)₃](ClO₄)₂ (**6**), [Ru(bpy)₃](ClO₄)₂ (**7**).

polypyridyl complexes once again attracted much attention in the diagnostic field as DNA probes because of their interaction ability.^[49,69–81] From these studies, it was concluded that these complexes may interact with DNA through three different modes, namely electrostatic, non-intercalative (“surface” interaction) and intercalative binding mode with a quite consistent enantiomeric preference for the Δ form respective to the Λ form.^[82–88] On the other hand, the potential anticancer activity of such compounds started to be reconsidered in 2000 when Liu and co-workers reported the *in vitro* cytotoxicity of some Ru(II) polypyridyl complexes.⁸⁹ In this work, the toxicity of several Ru(II)/Co(III) polypyridyl complexes were tested against HL-60, BEL-7402, KB, and HeLa cells. The compounds, such as for $[\text{Ru}(\text{bpy})_2(\text{dppz})](\text{BF}_4)_2$ (**8**, Figure 3), showed no cytotoxicity even though they were shown to be DNA intercalators and to strongly bind to DNA in some cases.⁷⁴ On the other hand, it was pointed out that structurally similar compounds such as $[\text{Ru}(\text{bpy})_2(\text{pztp})](\text{ClO}_4)_2$ and $[\text{Ru}(\text{phen})_2(\text{pztp})](\text{ClO}_4)_2$ (**9** and **10** in Figure 3, respectively) express very different cytotoxicity against all the cancer cell lines tested (IC_{50} = 10 μM and IC_{50} $\geq$ 100 μM , respectively).⁸⁹ These results gave the indication that the cytotoxicity of such complexes was strongly dependent on the metal, the ligand, and the shape of the complex.⁸⁹

3. Mononuclear Ru(II) polypyridyl complexes

In this part of the Review, mononuclear Ru(II) polypyridyl complexes are analysed considering their direct interaction(s) or not with DNA as cause of cell death (Sections 3.1 and 3.2, respectively). However, as can be seen below, mechanisms of action for Ru(II) polypyridyl complexes are often multiple or unclear, which have rendered this subdivision somewhat complicated. Caution is therefore to be taken

Fig. 3. Structures of $[\text{Ru}(\text{bpy})_2(\text{dppz})](\text{BF}_4)_2$ (**8**), $[\text{Ru}(\text{bpy})_2(\text{pztp})](\text{ClO}_4)_2$ (**9**), $[\text{Ru}(\text{phen})_2(\text{pztp})](\text{ClO}_4)_2$ (**10**).

about the complete appropriateness of this subdivision.

3.1 Mechanisms of action involving direct interactions with DNA

For many years, scientists have tried to correlate DNA-binding properties to the cytotoxicity expressed by Ru(II) polypyridyl complexes.^[81,90–100] In 2005 Hotze and co-workers were the first ones to evaluate the biological activity of some Ru(II) polypyridyl complexes since the report of Liu in 2000 (see above).⁸⁹ Hotze *et al.* analysed the cytotoxicity of a series of Ru(II) polypyridyl complexes, i.e. $\text{mer-}[\text{Ru}(\text{azpy})_3](\text{PF}_6)_2$ (**11**), $\alpha\text{-}[\text{Ru}(\text{azpy})_2(\text{bpy})](\text{PF}_6)_2$ (**12**), $\beta\text{-}[\text{Ru}(\text{azpy})_2(\text{bpy})](\text{PF}_6)_2$ (**13**), $[\text{Ru}(\text{azpy})(\text{bpy})_2](\text{PF}_6)_2$ (**14**) against A498, EVSTA-T, H226, IGROV, M19 MCF-7 and WIDR cell lines (Figure 4).⁹⁰ In general, all the complexes showed moderate antiproliferative activity. In more detail, **11**, **12** and **13** expressed greater cytotoxicity in M19 cells with IC_{50} = 4.47 μM , IC_{50} = 13.3 μM and IC_{50} = 19.5 μM , respectively for **11**, **12** and **13** (Cisplatin IC_{50} = 1.9 μM) while **14** was more active against H226 cells with IC_{50} = 17.7 μM and IC_{50} = 10.9 μM for **14** and cisplatin, respectively. Intercalative properties were suggested to be responsible for the cell death observed.⁹⁰ In 2007, Ma and co-workers tested the cytotoxicity of $[\text{Ru}(\text{tBu}_2\text{bpy})_2(2\text{-appt})](\text{PF}_6)_2$ (**15** in Figure 4), against KB-3-1, KB-V-1, HepG-2 and HeLa cell lines.⁹¹ The complex was found to express moderate cytotoxicity in KB-3-1, HepG-2 and HeLa showing IC_{50} = 52.3 ± 3.4 μM , IC_{50} = 30.2 ± 6 μM and IC_{50} = 59.7 ± 0.5 μM , respectively (cisplatin IC_{50} = 22.1 ± 3.6 μM , IC_{50} = 10.5 ± 0.5 μM and IC_{50} = 11.6 ± 0.2

Fig. 4. Structures of $\text{mer-}[\text{Ru}(\text{azpy})_3](\text{PF}_6)_2$ (**11**), $\alpha\text{-}[\text{Ru}(\text{azpy})_2(\text{bpy})](\text{PF}_6)_2$ (**12**), $\beta\text{-}[\text{Ru}(\text{azpy})_2(\text{bpy})](\text{PF}_6)_2$ (**13**), $[\text{Ru}(\text{azpy})(\text{bpy})_2](\text{PF}_6)_2$ (**14**) and $[\text{Ru}(\text{tBu}_2\text{bpy})_2(2\text{-appt})](\text{PF}_6)_2$ (**15**).

μM , respectively). In this case, spectroscopic titrations and viscosity experiments indicated that complex **15** interacts with DNA via groove binding. In more detail, modelling studies suggested the minor groove as the preferential binding site.⁹¹

In 2010, from the investigation of the interaction between $[\text{Ru}(2,9\text{-dmp})_2\text{PMIP}](\text{ClO}_4)_2$ (**16**, Figure 5) and calf thymus DNA (CT DNA) using several biophysical methods, Zhang and co-workers suggested that the antitumor activity observed for **16** against A549 cell line was related with its intercalation in CT DNA.⁹² One year later, Gill and co-workers examined the cytotoxicity of complexes $[\text{Ru}(\text{bpy})_2(\text{tpphz})](\text{ClO}_4)_2$ and $[\text{Ru}(\text{phen})_2(\text{tpphz})](\text{ClO}_4)_2$ (**17** and **18** shown in Figure 5, respectively).⁸¹ Both complexes bind DNA *in vitro* and act as DNA probes.^[81,93] In this work, these scientists demonstrated that these compounds were taken up by cells and displayed cytotoxicity towards MCF-7 and A2780 cells comparable with cisplatin. In both cases, complex **18** was found to be more cytotoxic than **17** and A2780 cells to be more sensitive to the complexes compared with MCF-7.⁸¹ Inter alia, $\text{IC}_{50} = 36 \pm 6 \mu\text{M}$, $\text{IC}_{50} = 11 \pm 6 \mu\text{M}$ and $\text{IC}_{50} = 12 \pm 2 \mu\text{M}$ for **17**, **18** and cisplatin, respectively were determined against the A2780 cell line. Unlike cisplatin, both complexes retained their cytotoxicity against A2780-CP70 cisplatin resistant cell lines ($\text{IC}_{50} = 47 \pm 6 \mu\text{M}$, $\text{IC}_{50} = 20 \pm 5 \mu\text{M}$ and $\text{IC}_{50} = 55 \pm 8 \mu\text{M}$ for **17**, **18**, and cisplatin, respectively).⁸¹ Microscopy studies clearly showed internalisation of complex **18** but limited nuclear accumulation. These scientists speculated that such complexes could affect numerous cellular processes, such as DNA replication and translation. However, further studies are needed to clarify the exact mechanism of cytotoxicity.⁸¹

In 2012, $[\text{Ru}(\text{bpy})_2(\text{H}_2\text{IIP})](\text{ClO}_4)_2$ (**19** in Figure 6) was designed and synthesised by Liu and co-workers.⁸⁵ In this case, the higher binding of an enantiomer to DNA/RNA was confirmed for both CT-DNA and

yeast tRNA, with **19** being a preferential binder for tRNA. Cytotoxicity tests were performed against HL-60 and HepG-2 cell lines using cisplatin as a control.⁸⁵ In both cases **19** was found to be less cytotoxic than cisplatin with $\text{IC}_{50} = 18.36 \pm 1.35 \mu\text{M}$ and $\text{IC}_{50} = 5.85 \pm 0.44 \mu\text{M}$ in HL-60 and $\text{IC}_{50} = 20.45 \pm 1.53 \mu\text{M}$ and $\text{IC}_{50} = 11.18 \pm 0.84 \mu\text{M}$ in HepG-2 for **19** and cisplatin, respectively. In this case, the scientists speculated that the cytotoxicity observed was related to other factors apart from DNA insertion, such as molecular shape of complex **19** and nature and chemical structure of the inserted ligand.⁸⁵

Another kind of mechanism proposed for coordinatively saturated and substitutionally inert Ru(II) polypyridyl complexes is the inhibition of topoisomerases I and II, which are essential enzymes that control and modify the topological states of DNA.^[101–103] In 2012, Kou and co-workers suggested the inhibition of topoisomerases I and II for the cytotoxicity observed against HeLa, MCF-7, HepG2 and BEL-7402 tumour cell lines for two chiral Ru(II) anthraquinone complexes, Δ - and Λ - $[\text{Ru}(\text{bpy})_2(\text{ipad})](\text{ClO}_4)_2$ (Δ/Λ -**20** in Figure 6).⁹⁴ Both complexes demonstrated higher *in vitro* cytotoxicity against selected tumour cell lines than 5-fluorouracil, a widely used clinical antitumor drug, but relatively lower cytotoxicity than cisplatin. Similar findings were already discovered in 2011 for complexes **21** and **22** shown in Figure 6 by Du and co-workers.⁹⁵ However, in the work of Kou and co-workers, the two enantiomers displayed small differences in antitumor activity against the tested cancer cell lines. This was suggested to be due to differences in DNA binding, hence leading to different topoisomerases inhibition.⁹⁴ These scientists suggested that both complexes may block the DNA strand passage event of the enzyme and serve as catalytic inhibitors of topoisomerases. Experimental data suggested that complexes Δ - and Λ - $[\text{Ru}(\text{bpy})_2(\text{ipad})](\text{ClO}_4)_2$ may bind to the DNA by intercalative mode with Λ -enantiomer intercalating less deeply than Δ -complex.⁹⁴ Enantioselective DNA-binding is in accordance with previous data on similar complexes (i.e. Δ - and Λ - $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$).^[84,88] The enantioselectivity was found to be more consistent after the introduction of the anthraquinone group. This can be attributed to the formation of specific hydrogen bond between the intercalated anthraquinone group and DNA.⁹⁴ The same mechanism was re-proposed two years later in 2014 by Wang and co-workers. The dual inhibition of topoisomerase I and II was suggested to be the base of

Fig. 5. Structures of $[\text{Ru}(2,9\text{-dmp})_2\text{PMIP}](\text{ClO}_4)_2$ (**16**), $[\text{Ru}(\text{bpy})_2(\text{tpphz})](\text{ClO}_4)_2$ (**17**) and $[\text{Ru}(\text{phen})_2(\text{tpphz})](\text{ClO}_4)_2$ (**18**).

Fig. 6. Structures of $[\text{Ru}(\text{bpy})_2(\text{H}_2\text{IIP})](\text{ClO}_4)_2$ (**19**), Δ - and Λ - $[\text{Ru}(\text{bpy})_2(\text{ipad})](\text{ClO}_4)_2$ (**20**), $[\text{Ru}(\text{bpy})_2(\text{bfipH})](\text{ClO}_4)_2$ (**21**) and $[\text{Ru}(\text{phen})_2(\text{bfipH})](\text{ClO}_4)_2$ (**22**), Δ/Λ - $[\text{Ru}(\text{bpy})_2(\text{pscl})](\text{ClO}_4)_2$ (**23**) and Δ/Λ - $[\text{Ru}(\text{bpy})_2(\text{psbr})](\text{ClO}_4)_2$ (**24**).

the cytotoxicity exerted by two new enantiomeric pair of complexes such as Δ/Λ - $[\text{Ru}(\text{bpy})_2(\text{pscl})](\text{ClO}_4)_2$ and Δ/Λ - $[\text{Ru}(\text{bpy})_2(\text{psbr})](\text{ClO}_4)_2$ (Δ/Λ -**23** and Δ/Λ -**24**, respectively in Figure 6).⁹⁶ The cytotoxicity of these complexes was tested against HeLa, A549, HepG2 and BEL-7402 cell lines using NAMI-A and cisplatin as controls. The two enantiomers display a different cytotoxicity in agreement with the cellular uptake results obtained, with the Δ enantiomers being the most active.⁹⁶ They displayed higher cytotoxicity when compared to NAMI-A but relatively lower compared to cisplatin. For example, in the case of HeLa cells (cell line against which the complexes were found to be the most active), $\text{IC}_{50} = 41.8 \pm 2.3 \mu\text{M}$, $\text{IC}_{50} = 54.6 \pm 2.9 \mu\text{M}$, $\text{IC}_{50} = 515.7 \pm 17.4 \mu\text{M}$, $\text{IC}_{50} = 18.3 \pm 1.4 \mu\text{M}$ for Δ -**23**, Δ -**24**, NAMI-A and cisplatin, respectively were determined.⁹⁶ The V annexin stain method and flow cytometry analysis showed that complexes Δ -**23** and Δ -**24** induced apoptosis in HeLa cells. Moreover, topoisomerase inhibition studies and DNA relegation assays revealed that all four Ru(II) complexes acted as dual poisons of topoisomerase I and II and caused DNA damage. This could lead to cell cycle arrest and/or induction of apoptosis.⁹⁶

In 2013, Wang and co-workers proposed a new anticancer mechanism of action for Ru(II) polypyridyl complexes targeting DNA.⁹⁷ In this work, the *in vitro* cytotoxicity of $[\text{Ru}(\text{bpy})_2(\text{tip})](\text{PF}_6)_2$ (**25**, Figure 7) and $[\text{Ru}(\text{phen})_2(\text{tip})](\text{PF}_6)_2$ (**26**, Figure 7) against HeLa

cells was investigated. Experimental data suggested the interaction of such complexes with G-quadruplex DNA, which is a secondary structure formed in specific guanine-rich sequences.¹⁰⁴ Interestingly, DNA sequences that can form G-quadruplexes have been found in the upstream promoter of protooncogenes.¹⁰⁵ Metal complexes are usually utilised as gene expression regulators promoting the formation and/or stabilising G-quadruplexes.^[62,106]

Examples of Ru(II) polypyridyl complexes which preferentially target G-quadruplexes DNA over duplex DNA and are able to stabilise such structures are already present in the literature.^[107–109] Wang and co-workers demonstrated through *in vitro* experiments that both complexes (**25** and **26**) could induce and stabilise the formation of G-quadruplex in promoter of bcl-2 gene (B cell lymphoma gene 2). Cytotoxicity studies revealed antiproliferative activity on HeLa cells, ($\text{IC}_{50} = 22.10 \pm 1.01$ and $18.56 \pm 0.67 \mu\text{M}$, respectively for **25** and **26**). The highest cytotoxicity of complex **26** is consistent with its DNA-binding and ability to stabilise G-quadruplexes.⁹⁷ In order to have more insights on the mechanism of cell-growth inhibition, further experiments were performed. Both apoptosis and cell-cycle arrest were found to be involved. Apoptosis was suggested to be related to the regulation of the bcl-2 gene expression, dependent on the G-quadruplex stabilisation.⁹⁷ However, the apoptotic pathway activation and the binding mode with G-quadruplex DNA still need further elucidations. Similar findings were reported the following year, in 2014, by Zhang and co-workers.⁹⁸ In this work, they observed a greater inhibitory activity against MDA-MB-231 expressed by complex **27** (showed in Figure 7) compared with cisplatin, with $\text{IC}_{50} = 16.3 \pm 2.6 \mu\text{M}$ and $\text{IC}_{50} = 36.1 \pm 1.9 \mu\text{M}$ for complex **27** and cisplatin, respectively. Further studies prompted these scientists to suggest a correlation between the induced apoptosis observed and the stabilisation of G-quadruplex conformation of c-myc oncogene promoter via groove binding mode which led to the down-regulated expression of c-myc.⁹⁸ A detailed mechanism of action is still under investigation but these findings suggest complex **27** as a potential apoptosis inducer characterized by a low toxicity against healthy HAcet cells.⁹⁸

Moreover, during the same year, Srishailam and co-workers explored three new Ru(II) polypyridyl complexes, namely $[\text{Ru}(\text{phen})_2\text{ptip}](\text{ClO}_4)_2$ (**28**), $[\text{Ru}(\text{bpy})_2\text{ptip}](\text{ClO}_4)_2$ (**29**) and $[\text{Ru}(\text{dmb})_2\text{ptip}](\text{ClO}_4)_2$ (**30**).¹¹⁰ These complexes showed considerable antibacterial activity against *S. aureus* and *E. coli* at 1 mg/mL and 0.5 mg/mL concentrations. The *in vitro* cytotoxicity was

also

Fig. 7. Structures of $[\text{Ru}(\text{bpy})_2(\text{tip})](\text{PF}_6)_2$ (**25**), $[\text{Ru}(\text{phen})_2(\text{tip})](\text{PF}_6)_2$ (**26**) and $[\text{Ru}(\text{phen})_2(\text{p-tFMPIP})](\text{ClO}_4)_2$ (**27**), $[\text{Ru}(\text{phen})_2\text{ptip}](\text{ClO}_4)_2$ (**28**), $[\text{Ru}(\text{bpy})_2\text{ptip}](\text{ClO}_4)_2$ (**29**) and $[\text{Ru}(\text{dmb})_2\text{ptip}](\text{ClO}_4)_2$ (**30**).

against HeLa tumour cells. The compounds were found to be toxic and the authors suggested that the observed toxicity was related to DNA interaction.¹¹⁰ Confocal microscopy studies demonstrated that complexes **28-30** gradually penetrated the nucleus.

Between 2015 and 2016, Deepika and co-workers investigated several types of Ru(II) polypyridyl complexes.^[99,100] They first designed a new ligand named CNPFIP. Then, three complexes carrying this ligand, namely $[\text{Ru}(\text{phen})_2\text{CNPFIP}](\text{ClO}_4)_2$ (**31**), $[\text{Ru}(\text{bpy})_2\text{CNPFIP}](\text{ClO}_4)_2$ (**32**) and $[\text{Ru}(\text{dmb})_2\text{CNPFIP}](\text{ClO}_4)_2$ (**33**) shown in Figure 8 were investigated as antimicrobial and antiproliferative agents.⁹⁹ The *in vitro* antibacterial activity was tested against *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) bacteria. All three complexes showed higher activity against *S. aureus* than *E. coli* with complex **31** being the most active. Increasing the lipophilicity of the complexes, in fact, led to better permeation through the lipid layer of the bacterial membrane and thus higher activity.⁹⁹ None of these complexes showed higher activity than the positive control Ampicillin. In addition, the *in vitro* cytotoxicity of the complexes was tested against HeLa cells using cisplatin as positive control.⁹⁹ The same trend observed before (during the antimicrobial experiments) was found leading to compound **33** being the least active. In this case too, the positive control cisplatin was the most active compound tested ($\text{IC}_{50} = 35.51 \mu\text{M}$, $36.74 \mu\text{M}$, $40.38 \mu\text{M}$ and $4.52 \mu\text{M}$, respectively for **31**, **32**, **33** and cisplatin).⁹⁹ Later, the same scientists examined two new complexes, namely $[\text{Ru}(\text{phen})_2\text{bPPP}](\text{ClO}_4)_2$ and $[\text{Ru}(\text{phen})_2\text{7-Br-}$

tested

$\text{dppz}](\text{ClO}_4)_2$ (**34** and **35** in Figure 8, respectively).¹⁰⁰ The *in vitro* antimicrobial activity was tested against the same bacteria mentioned above. In this case, the complexes showed higher activity against *E. coli* than *S. aureus* but in both cases still lower than ampicillin.¹⁰⁰ Microscopy studies revealed that complexes **34** and **35** can be up taken from HeLa cells and accumulate in the nuclei. The *in vitro* toxicity on cancer cell lines, on the other hand, was tested against HeLa, Du145 and A549 cell lines. Both complexes displayed moderate cytotoxic activity against all tumour cell lines.¹⁰⁰ More precisely, they were found to be slightly more effective against the HeLa cell line with **34** ($\text{IC}_{50} = 32.0 \pm 1.20 \mu\text{M}$) showing relatively higher cytotoxicity than **35** ($\text{IC}_{50} = 35.0 \pm 1.56 \mu\text{M}$). In all cases, the IC_{50} values were found to be higher than cisplatin.¹⁰⁰

3.2 Mechanisms of action not involving direct interactions with DNA

To the best of our knowledge, the first study since the work by Dwyer and co-workers suggesting the exclusion of direct interactions of Ru(II) polypyridyl complexes with DNA in cell death was reported in 2008 by Schatzschneider and co-workers.¹¹¹ In this study, these scientists investigated the cell growth inhibitory potency against two human cancer cell lines (HT-29 and MCF-7) of a series of Ru(II) polypyridyl complexes with varying degrees of extension of the aromatic ligand in order to correlate the lipophilicity of a compound with its cytotoxicity.¹¹¹ This work showed that the cellular uptake was directly correlated with the size of the ligand. Inter alia, more lipophilic ligands led to greater Ru(II) concentration levels in cells.¹¹¹ This statement was in complete agreement with that previously

Fig. 8. Structures of $[\text{Ru}(\text{phen})_2\text{CNPFIP}](\text{ClO}_4)_2$ (**31**), $[\text{Ru}(\text{bpy})_2\text{CNPFIP}](\text{ClO}_4)_2$

(**32**), $[\text{Ru}(\text{dmb})_2\text{CNPPIP}](\text{ClO}_4)_2$ (**33**), $[\text{Ru}(\text{phen})_2\text{bPPP}](\text{ClO}_4)_2$ (**34**) and $[\text{Ru}(\text{phen})_2\text{7-Br-dppz}](\text{ClO}_4)_2$ (**35**).

demonstrated on structurally similar Ru(II) polypyridyl complexes.¹¹² Moreover, the authors pointed out that greater cellular accumulation resulted in greater cytotoxicity on both cell lines. In particular, $[\text{Ru}(\text{bpy})_2(\text{dppn})]\text{Cl}_2$ (**36**, Figure 9), which was the complex carrying the ligand with the greater aromatic surface area considered in this work, showed the most significant antiproliferative activity with $\text{IC}_{50\text{s}} < 10 \mu\text{M}$, values which are comparable to that of cisplatin under identical conditions.¹¹¹ The mechanism of action was suggested to be related to the modification of cell membrane function and cell adhesion properties.¹¹¹ This observation was in contrast with the accustomed focus on the DNA intercalative properties of such compounds.

One year later, Gao and co-workers showed that the antitumor activity of Ru(II) polypyridyl complexes must be related to different mechanisms than only DNA intercalation.¹¹³ In this work, these researchers designed and synthesised different complexes in order to improve the DNA-binding capability of their Ru(II) complexes by introducing electropositive pendants to the ancillary ligand or by increasing their intercalative aromatic surface (complexes **37-40** in Figure 9). The authors then tested the activity of these compounds as inhibitors of DNA transcription, which is involved in the mechanism of action for modifications mentioned above. They improved the DNA binding ability of their complexes, which led to a direct, positive correlation with the inhibition of DNA transcription.¹¹³ However, no clear trend was observed between the DNA binding ability of the complexes and their antitumor activity,

$[\text{Ru}(\text{bpy})_2(\text{pdppz})](\text{ClO}_4)_2$ (**38**), $[\text{Ru}(\text{R}_2\text{bpy})_2(\text{dppz})](\text{ClO}_4)_6$ (**39**), $[\text{Ru}(\text{R}_2\text{bpy})_2(\text{pdppz})](\text{ClO}_4)_6$ (**40**).

indicating that DNA transcription inhibition must not be the only process involved.¹¹³ The unlikely DNA involvement in the mechanism of action of certain Ru(II) polypyridyl complexes was also confirmed by Zava and co-workers.¹¹⁴ They explored the cellular uptake mechanism and the cytotoxicity of a class of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ complexes, where the bpy group was derivatised with functionalities to provide different lipophilicities. Figure 6 shows complexes **41-45** which carry respectively bpy, dmb, dmab, dmcb and dmb ligands. Microscopy studies showed that subcellular localisation varies greatly (i.e., plasma membrane, cytoplasmic organelles). Since accumulation in the cell nucleus was not observed for any of the compounds, the DNA damage associated with cell death was strongly questioned for these types of compounds.¹¹⁴ Over the years, different types of mechanism of cytotoxic action started to be proposed. Among them, the apoptotic cell death through the activation of the mitochondrial dysfunctional pathway started to arise as a main cause of cytotoxicity exerted by Ru(II) polypyridyl complexes.^[115-119] In 2010, combinatorial coordination chemistry followed by cytotoxicity screening allowed the discovery of a new antiproliferative complex ($[\text{Ru}(\text{tBu}_2\text{bpy})_2(\text{phox})]\text{PF}_6$ (**46**, Figure 10).¹¹⁷ After a brief structure-activity relationship, Meggers and co-workers demonstrated that complex **46** showed the highest cytotoxicity when compared with the other tris-heteroleptic complexes tested.

Fig. 9. Structures of $[\text{Ru}(\text{bpy})_2(\text{dppn})](\text{Cl})_2$ (**36**), $[\text{Ru}(\text{bpy})_2(\text{dppz})](\text{ClO}_4)_2$ (**37**),

Fig. 10. Structures of $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ and its derivatives (**41-45**) and $[(\text{Ru}(\text{tBu}_2\text{bpy})_2(\text{phox}))\text{PF}_6]$ (**46**).

Its cytotoxicity was tested in HeLa cells and displayed LC_{50} values of 1.3 μM and 0.3 μM after incubation for 24 and 72 h respectively, where the LC_{50} is the lethal concentration required to kill 50% of the cell population.¹¹⁷ Afterwards, the antiproliferative and apoptotic properties of the complex were confirmed in BJAB cells. The strong reduction of the mitochondrial membrane potential registered is a clear sign of a programmed cell death pathway activation.¹²⁰ Interestingly, complex **46** was able to affect the viability of BJAB cells after 24 h incubation in a dose-dependent manner starting from very low concentrations (100 nM).¹¹⁷ It is worth noting that Ru(II) complexes with chelating ligands containing both nitrogen and oxygen or nitrogen and sulphur as donor atoms were already investigated presenting interesting biological activities as anticancer and antibacterial agents.^[121,122]

During the same year Tan and co-workers demonstrated, for the first time, that Ru(II) polypyridyl complexes with β -carboline alkaloids could simultaneously induce autophagy and apoptosis in HeLa cells through a ROS-mediated mechanism (ROS= reactive oxygen species), which led to mitochondrial dysfunction.¹¹⁶ However, DNA could be the primary target of this nuclear permeable complex since the cytotoxicity of the complexes directly correlated with their DNA binding affinity.¹¹⁶ Once again, the antiproliferative character of the complexes was in agreement with their intracellular uptake, which was itself dependent on the lipophilicity. In this case, the $[\text{Ru}(\text{DIP})_2(1\text{-Py-}\beta\text{C})](\text{PF}_6)_2$ (**47**, Figure 11) was the complex carrying the most extended π -system and, as expected, showed the strongest cytotoxicity.¹¹⁶

A year later, in 2011, the same scientists investigated the cytotoxicity of three different Ru(II) polypyridyl complexes of the general formula $[\text{Ru}(\text{N-N})_2(\text{Norharman})_2](\text{SO}_3\text{CF}_3)_2$, where N-N = bpy (**48**), phen (**49**), DIP (**50**), respectively, as shown in Figure 11. Norharman is a naturally occurring β -carboline alkaloid.¹¹⁹ The *in vitro* cytotoxicity of these complexes was evaluated against three cancer cell lines HepG2, HeLa, MCF-7 using cisplatin as a control. The greatest cytotoxicity was exhibited by complex **50** which had an $\text{IC}_{50} = 0.92 \pm 0.1 \mu\text{M}$, $\text{IC}_{50} = 0.74 \pm 0.21 \mu\text{M}$ and $\text{IC}_{50} = 2.30 \pm 0.5 \mu\text{M}$ for HepG2, HeLa and MCF-7, respectively.¹¹⁹ These values were found to be much lower than that of cisplatin ($\text{IC}_{50} = 20.2 \pm 3.6 \mu\text{M}$, $\text{IC}_{50} = 16.7 \pm 2.5 \mu\text{M}$ and $\text{IC}_{50} = 35.2 \pm 4.2 \mu\text{M}$, respectively) and of Norharman itself ($\text{IC}_{50} = 178.3 \pm 9.1 \mu\text{M}$, $\text{IC}_{50} = 185.3 \pm 7.9 \mu\text{M}$ and

$\text{IC}_{50} = 167.7 \pm 8.3 \mu\text{M}$, respectively) indicating that the coordination to the Ru(II) polypyridyl moiety significantly improves the anticancer activity. Moreover, consistently with their previous work,¹¹⁶ they observed a direct correlation between lipophilicity, cellular uptake efficiency and cytotoxicity.¹¹⁹ It was shown that the apoptosis induced by complexes **48-50** was related to mitochondrial dysfunction and ROS accumulation. However, as discussed above,¹¹⁶ DNA could also be the primary target of the compounds.¹¹⁹ As demonstrated by confocal microscopy studies the nuclear permeability was also confirmed for complex **50**.¹¹⁹ This phenomenon was speculated to be related to β -carboline since other complexes containing this moiety were already found to pass through the nuclear envelope.^[116,123]

Taking into account the above-mentioned studies, in 2012, our group in collaboration with the teams of Ferrari and Spiccia reported a detailed study of the *in vitro* behaviour of four complexes, namely $[\text{Ru}(\text{bpy})_2(\text{CppH})](\text{PF}_6)_2$ (**51**), $[\text{Ru}(\text{bpy})_2(\text{Cpp-NH-Hex-COOH})](\text{PF}_6)_2$ (**52**), $[\text{Ru}(\text{dppz})_2(\text{CppH})](\text{PF}_6)_2$ (**53**), and $[\text{Ru}(\text{dppz})_2(\text{Cpp-NH-Hex-COOH})](\text{PF}_6)_2$ (**54**) (Figure 12).¹²⁴ Their cytotoxicity was investigated against the cancerous HeLa, MCF-7, U2OS, A2780, A2780-CP70 cell lines and the non-cancerous MRC-5 cell line as a control. In this case, the exclusion of nuclear DNA from the mechanism of action responsible for the cytotoxicity was demonstrated.¹²⁴ Specifically, the bpy-containing Ru(II) polypyridyl complexes (**51** and **52**) were found to be non-cytotoxic contrary to the dppz-containing Ru(II) polypyridyl complexes (**53** and **54**) which were found to be cytotoxic towards all the six cell lines tested. Inter alia, complex $[\text{Ru}(\text{dppz})_2(\text{CppH})](\text{PF}_6)_2$ (**53**) expressed the best therapeutic profile

Fig. 11. Structures of, $[\text{Ru}(\text{DIP})_2(1\text{-Py-}\beta\text{C})](\text{PF}_6)_2$ (**47**),

$[\text{Ru}(\text{bpy})_2(\text{Norharman})_2](\text{SO}_3\text{CF}_3)_2$ (**48**), $[\text{Ru}(\text{phen})_2(\text{Norharman})_2](\text{SO}_3\text{CF}_3)_2$ (**49**), $[\text{Ru}(\text{DIP})_2(\text{Norharman})_2](\text{SO}_3\text{CF}_3)_2$ (**50**).

Fig. 12. Structures of $[\text{Ru}(\text{bpy})_2(\text{CppH})](\text{PF}_6)_2$ (**51**), $[\text{Ru}(\text{bpy})_2(\text{Cpp-NH-Hex-COOH})](\text{PF}_6)_2$ (**52**), $[\text{Ru}(\text{dppz})_2(\text{CppH})](\text{PF}_6)_2$ (**53**), and $[\text{Ru}(\text{dppz})_2(\text{Cpp-NH-Hex-COOH})](\text{PF}_6)_2$ (**54**).

with IC_{50} values comparable with cisplatin in cancerous cell lines and lower toxicity toward the healthy cell line.¹²⁴ Moreover, cell localisation studies demonstrated the accumulation of compound **53** in mitochondria. On the contrary, complex **54** was found to accumulate in the cell nucleus. Further experimental studies brought our team to exclude the presence of ROS involvement in the mode of cell death of **53** and to confirm apoptosis as the mode of cell death induced by a mitochondria related pathway.¹²⁴

In 2013, Jiang and co-workers revived the same mechanism of action involving the production of ROS species, as already described above with the compounds of Tan and co-workers.^[116,125] In this work, they demonstrated that complexes $[\text{Ru}(\text{phen})_2(\text{adppz})](\text{ClO}_4)_2$ and $[\text{Ru}(\text{DIP})_2(\text{adppz})](\text{ClO}_4)_2$ (**55** and **56**, Figure 13) can enter into the cytoplasm and accumulate in the nucleus.¹²⁵ They exerted their *in vitro* cytotoxicity against BEL-7402 cell line through activation of caspase 7, upregulation of proapoptotic protein and ROS-mediated mitochondrial dysfunction pathways (IC_{50} = 23.5 ± 2.3 μM , IC_{50} = 12.7 ± 1.1 μM and IC_{50} = 11.5 ± 1.2 μM for **55**, **56** and cisplatin respectively).¹²⁵

More or less the same observations were made by Chao and co-workers during the same year.¹²⁶ In this work, they analysed the cytotoxicity of four different complexes, namely $[\text{Ru}(\text{bpy})_2(\text{PAIDH})](\text{ClO}_4)_2$ (**57** in Figure 13), $[\text{Ru}(\text{phen})_2(\text{PAIDH})](\text{ClO}_4)_2$ (**58** in Figure 13), $[\text{Ru}(4,7\text{-dmp})_2(\text{PAIDH})](\text{ClO}_4)_2$ (**59** in Figure 13), and $[\text{Ru}(\text{DIP})_2(\text{PAIDH})](\text{ClO}_4)_2$ (**60** in Figure 13).¹²⁶ Compounds **57** and **58** were already reported in a previous study by the same authors, where they focused their

attention on DNA-binding properties of Ru complexes,¹²⁷ while complexes **59** and **60** were investigated here for the first time. Cytotoxicity studies were performed against HeLa, A549, BEL-7402, HepG2 and LO2 cells using cisplatin as a control.¹²⁶ From these studies, a consistency between the cytotoxicity and the cellular uptake properties of the compounds was unveiled. Complex **60**, which carries the most lipophilic ligand, expresses the highest cellular uptake and cytotoxicity.¹²⁶ It induces HeLa cells apoptosis through the activation of mitochondrial dysfunctional pathway with IC_{50} = 7.9 ± 0.7 μM , while cisplatin has IC_{50} = 17.8 ± 2.6 μM . All the complexes express better selectivity than cisplatin against the healthy cell line LO2 (IC_{50} = 77.4 ± 4.3 μM and IC_{50} = 11.2 ± 0.6 μM , respectively for complex **60** and cisplatin).¹²⁶ Moreover, complex **60** was shown to accumulate preferentially in mitochondria. These findings suggest complex **60** as a potent and selective chemotherapeutic agent.

Between 2013 and 2014, Xie and co-workers focused their attention on the cytotoxicity of Ru(II) polypyridyl complexes with ligands containing nitro-, amino- or hydroxyl group.^[128–130] First, they considered four Ru(II) polypyridyl complexes, namely $[\text{Ru}(\text{bpy})_2(\text{NHPIP})](\text{ClO}_4)_2$ (**61**), $[\text{Ru}(\text{phen})_2(\text{NHPIP})](\text{ClO}_4)_2$ (**62**), $[\text{Ru}(\text{bpy})_2(\text{AHPIP})](\text{ClO}_4)_2$ (**63**), and $[\text{Ru}(\text{phen})_2(\text{AHPIP})](\text{ClO}_4)_2$ (**64**) shown in Figure 14.¹²⁹ Fluorescence microscopy studies demonstrated that these complexes ultimately accumulated in the nucleus after being taken up by the cells. The *in vitro* cytotoxicity of these complexes was then tested against four cancer cell lines, namely BEL-7402, HeLa, MG-63, and MCF-7.¹²⁹ It was confirmed once again that the complex carrying the more lipophilic ancillary ligands is more cytotoxic. Moreover, the results suggested that the amino group could also enhance the cytotoxicity leading to complex **64** being the most active.¹²⁹

Fig. 13. Structures of $[\text{Ru}(\text{phen})_2(\text{adppz})](\text{ClO}_4)_2$ (**55**), $[\text{Ru}(\text{DIP})_2(\text{adppz})](\text{ClO}_4)_2$

(56), [Ru(bpy)₂(PAIDH)](ClO₄)₂ (57), [Ru(phen)₂(PAIDH)](ClO₄)₂ (58), [Ru(4,7-dmp)₂(PAIDH)](ClO₄)₂ (59), and [Ru(DIP)₂(PAIDH)](ClO₄)₂ (60).

These researchers then investigated two other analogues with a more lipophilic ancillary ligand. Complexes [Ru(2,9-dmp)₂(NHPIP)](ClO₄)₂ (65) and [Ru(2,9-dmp)₂(AHPIP)](ClO₄)₂ (66, Figure 14) were tested towards the same cell lines and a small improvement in cytotoxicity was noted (IC₅₀ = 13.6 ± 1.2 μM (64) and IC₅₀ = 5.4 ± 0.5 μM (66) in BEL-7402).¹²⁸ A reduction of the mitochondrial potential and an increase of ROS species upon treatment of the cells with the Ru(II) complexes were detected, indicating activation of apoptosis through the mitochondrial pathway.¹²⁸ Further studies on complexes carrying either more lipophilic or functionalised ligands, unfortunately, led to no significant improvement in cytotoxicity towards A549, BEL-7402, HeLa and MG-63 cells.^[130,131] In 2014, Du and co-workers showed that similar complexes such as [Ru(phen)₂(*p*-tFPIP)](ClO₄)₂ (67 in Figure 14) accumulated in mitochondria and were able to induce mitochondria-mediated apoptosis in MG-63 cells by mitochondrial fragmentation.¹³² In 2014, Jiang and co-workers investigated the biological potential of Ru(II) polypyridyl complexes carrying new ligands, namely dmdppz and addppn to prepare [Ru(phen)₂(dmdppz)](ClO₄)₂ and its derivatives (68-71), and [Ru(phen)₂(addppn)](ClO₄)₂ (72).^[133,134] In the case of complexes 68-71, no improvement in cytotoxicity compared to cisplatin was

[Ru(phen)₂(AHPIP)](ClO₄)₂ (64), [Ru(2,9-dmp)₂(NHPIP)](ClO₄)₂ (65), [Ru(2,9-dmp)₂(AHPIP)](ClO₄)₂ (66) and [Ru(phen)₂(*p*-tFPIP)](ClO₄)₂ (67).

recorded on BEL-7402 cells.¹³³ On the contrary, higher cytotoxicity than cisplatin was demonstrated for 72.¹³⁴ All the complexes (68-72) increased the level of ROS and induced a decrease of the mitochondrial membrane potential.^[133,134] These results suggested the intrinsic ROS-mediated mitochondrial pathway as the determinant for the apoptosis of BEL-7402 cells. Moreover, programmed cell death induction was accompanied by the regulation of Bcl-2 family proteins.^[133,134]

Concurrently, Yu and co-workers proposed two new Ru(II) polypyridyl complexes with hydrophobic ancillary ligands which affect multiple targets simultaneously.¹³⁵ In this work, these scientists investigated the anti-cancer effects and molecular mechanisms of complexes (shown in Figure 16), [Ru(bpy)₂(5-idip)](ClO₄)₂ (73) and [Ru(phen)₂(5-idip)](ClO₄)₂ (74). The cytotoxicity of these compounds was tested against HeLa, A549, A375, HepG2, Hep-2 and normal cell NIH/3T3 using cisplatin as a control.¹³⁵ Complex 74 was found to have a good therapeutic profile since it has IC₅₀ values comparable with cisplatin, especially against HeLa (IC₅₀ = 11.7 ± 1.2 μM and IC₅₀ = 7.6 ± 0.4 μM for 74 and cisplatin, respectively). Both complexes can induce apoptosis through the activation of two different mechanisms.¹³⁵ On one hand, the activation of the mitochondrial dysfunctional pathway and on the other the stabilisation of telomeric DNA through the formation of G-quadruplexes which denies the telomerase access, leading to apoptosis induction.¹³⁵ Complex 74 accumulates first in lysosomes and then relocates with time within more sensitive organelles such as nucleus and chondriosome.

Fig. 14. Structures of [Ru(bpy)₂(NHPIP)](ClO₄)₂ (61), [Ru(phen)₂(NHPIP)](ClO₄)₂ (62), [Ru(bpy)₂(AHPIP)](ClO₄)₂ (63),

Fig. 15. Structures of [Ru(phen)₂(dmdppz)](ClO₄)₂ and its derivatives (68-71), [Ru(phen)₂(addppn)](ClO₄)₂ (72).

Fig. 16. Structures of $[\text{Ru}(\text{bpy})_2(5\text{-idip})](\text{ClO}_4)_2$ (**73**) and $[\text{Ru}(\text{phen})_2(5\text{-idip})](\text{ClO}_4)_2$ (**74**).

Between 2014 and 2015, Mazuryk and co-workers proposed a new Ru(II) polypyridyl complex containing a nitroimidazole unit as an optical imaging, chemotherapeutic and anti-metastatic agent.^[136,137] Firstly, they found out that $[\text{Ru}(\text{DIP})_2(\text{bpy-2-nitroIm})]\text{Cl}_2$ (**75** in Figure 17) targeted preferentially hypoxic cells.¹³⁶ The rationale of this behaviour seems to be the trapping of the compound inside the cells as a consequence of the interaction between proteins and/or DNA and a derivative formed by the irreversible reduction of a nitroimidazole moiety under hypoxic conditions.^[136,138] Preliminary studies showed the applicability of complex **75** as an optical imaging and chemotherapeutic agent. In more detail, the complex can be used as a dye at concentrations lower than 1 μM , while at higher concentrations the growth inhibition starts to be consistent.¹³⁶ The cytotoxicity of compound **75** was tested under normoxia and hypoxia. Preliminary data was obtained on A549 cell line showing a clear increment of the cytotoxicity under hypoxia ($\text{LC}_{50} = 11.8 \pm 1.2 \mu\text{M}$ under normoxic conditions and $\text{LC}_{50} = 7.7 \pm 0.4 \mu\text{M}$ under 1% of oxygen).¹³⁶ In a following paper, the scientists confirmed the cytotoxicity of **75**. It was tested against 4T1 and A549 under normoxia and hypoxia, with or without serum leading to IC_{50} values from 1.5 to 18.8 μM .¹³⁷ Cisplatin was used as a control and showed lower cytotoxicity under the same conditions. Apoptosis was suggested to be a consequence of ROS generation.¹³⁷ Confocal microscopy studies demonstrated accumulation of complex **75** in mitochondria. More interestingly, a distinct impact on cell adhesion and migration as well as an interesting effect on endothelial cell vasculature was recorded.¹³⁷ On one hand, it was found that the compound tested favoured the adhesion of attached cells and disfavoured it in the case of unattached ones thus leading to promising anti-metastatic activity.¹³⁷ On the other hand, it was suggested that Ru(II) polypyridyl complexes could affect the formation of new capillaries through the re-arrangement of endothelial cells leading to an improvement in blood perfusion. These findings gave the basis to consider Ru(II) polypyridyl complexes as interesting compounds for angiogenesis-based

treatments.¹³⁷ Regulation of angiogenesis process (new blood vessels formation) is in fact a known powerful supporting strategy in cancer treatment.¹³⁹

In 2015, Lai and co-workers examined the potential antiproliferative activity of $[\text{Ru}(2,9\text{-dmp})_2(\text{pddppn})](\text{ClO}_4)_2$ (**76**, Figure 17).¹⁴⁰ Fluorescence microscopy studies showed that **76** accumulated in the nucleus. The *in vitro* cytotoxicity of the complex was evaluated against BEL-7402, HeLa, A549 and MG-63 cell lines. Complex **76** showed higher cytotoxicity than cisplatin in the cell lines tested except for HeLa where the IC_{50} value was found to be relatively similar ($9.0 \pm 0.8 \mu\text{M}$ (**76**) and $7.3 \pm 1.4 \mu\text{M}$ (cisplatin)).¹⁴⁰ Further experiments showed that **76** could induce apoptosis of cancer cells, enhance the ROS levels and reduce the potential of mitochondrial membrane. Moreover, complex **76** was found to up-regulate the levels of different caspases and to down-regulate the expression of some proteins belonging to the Bcl-2 family.¹⁴⁰ These findings suggested that $[\text{Ru}(2,9\text{-dmp})_2(\text{pddppn})](\text{ClO}_4)_2$ induced apoptosis through an intrinsic ROS-mediated mitochondrial dysfunction pathway, which was accompanied by the regulation of Bcl-2 family proteins.¹⁴⁰

One year later, Zhang and co-workers studied four new Ru(II) polypyridyl complexes, namely $\text{Ru}(\text{dmb})_2(\text{DQTT})](\text{ClO}_4)_2$ (**77**), $\text{Ru}(\text{bpy})_2(\text{DQTT})](\text{ClO}_4)_2$ (**78**), $\text{Ru}(\text{phen})_2(\text{DQTT})](\text{ClO}_4)_2$ (**79**) and $[\text{Ru}(2,9\text{-dmp})_2(\text{DQTT})](\text{ClO}_4)_2$ (**80**) shown in Figure 18.¹⁴¹ The *in vitro* cytotoxicity was evaluated against human BEL-7402, A549, HeLa, HepG-2 and MG-63 cancer cell lines. All complexes exhibited moderate cytotoxicity against BEL-7402 cell line ($\text{IC}_{50} = 31.8 \pm 1.0 \mu\text{M}$, $\text{IC}_{50} = 35.8 \pm 1.6 \mu\text{M}$, $\text{IC}_{50} = 29.0 \pm 0.8 \mu\text{M}$, $\text{IC}_{50} = 25.0 \pm 5.9 \mu\text{M}$, respectively for **77-80**).¹⁴¹ On the other hand, complexes **77-79**

Fig. 17. Structures of $[\text{Ru}(\text{DIP})_2(\text{bpy-2-nitroIm})]\text{Cl}_2$ (**75**) and $[\text{Ru}(2,9\text{-dmp})_2(\text{pddppn})](\text{ClO}_4)_2$ (**76**).

showed high cell growth inhibition in A549 cells ($\text{IC}_{50} = 15.9 \pm 1.0 \mu\text{M}$, $\text{IC}_{50} = 41.8 \pm 2.1 \mu\text{M}$, $\text{IC}_{50} = 16.4 \pm 0.7 \mu\text{M}$, $\text{IC}_{50} = 96.6 \pm 14.8 \mu\text{M}$,

respectively for **77-80**). Unexpectedly, complexes **77**, **79**, and **80** were found to be completely ineffective against HeLa and HepG2 cells, and complexes **79** and **80** were found to not be cytotoxic to MG-63 cells.¹⁴¹ Further studies in BEL-7402 cells showed that the complexes induced apoptosis through a ROS-mediated mitochondrial dysfunction pathway, which was accompanied by regulation of the expression of Bcl-2 family proteins.¹⁴¹ Fluorescence microscopy studies demonstrated that complexes entered the cells and accumulated in a region around the nucleus. Almost the same finding was concurrently discovered by Liu and co-workers on two different complexes.¹⁴² Indeed, $\text{Ru}(\text{dmb})_2(\text{NMIP})](\text{ClO}_4)_2$ (**81**) and $\text{Ru}(\text{phen})_2(\text{NMIP})](\text{ClO}_4)_2$ (**82**) shown in Figure 18 were tested as chemotherapeutic agents against six different cancer cell lines, namely A549, BEL-7402, HeLa, PC-12, SGC-7901 and SiHa.¹⁴² The complexes were found to have a moderate cytotoxicity against BEL-7402 cells with IC_{50} values of $53.9 \pm 3.4 \mu\text{M}$ and $39.3 \pm 2.1 \mu\text{M}$, respectively for **81** and **82**. Unfortunately, these complexes were found to be less active than cisplatin under the same conditions ($\text{IC}_{50} = 10.8 \pm 1.3 \mu\text{M}$).¹⁴² Moreover, complex **81** had no cytotoxicity toward A549, HeLa, SGC-7901 and SiHa cells, and complex **82** was inactive against PC-12, SGC-7901 and SiHa cells. Interestingly, the mechanism of action was investigated also in this case; the authors suggested that the complexes induced apoptosis in BEL-7402 cells through a ROS-mediated mitochondrial dysfunction pathway.¹⁴² Meanwhile, four other Ru(II) polypyridyl complexes (Figure 19) were found to induce apoptosis of HepG2 cells through ROS-mediated

Fig. 18. Structures of $\text{Ru}(\text{dmb})_2(\text{DQTT})](\text{ClO}_4)_2$ (**77**), $\text{Ru}(\text{bpy})_2(\text{DQTT})](\text{ClO}_4)_2$ (**78**), $\text{Ru}(\text{phen})_2(\text{DQTT})](\text{ClO}_4)_2$ (**79**), $[\text{Ru}(\text{2,9-dmp})_2(\text{DQTT})](\text{ClO}_4)_2$ (**80**), $\text{Ru}(\text{dmb})_2(\text{NMIP})](\text{ClO}_4)_2$ (**81**) and $\text{Ru}(\text{phen})_2(\text{NMIP})](\text{ClO}_4)_2$ (**82**).
mitochondrial dysfunction pathway.¹⁴³ Zeng and co-workers examined four complexes containing the dhbn ligand, namely

$[\text{Ru}(\text{N-N})_2(\text{dhbn})](\text{ClO}_4)_2$ where N-N = dmb (**83**); bpy (**84**); phen (**85**); 2,9-dmp (**86**). The cytotoxicity of these complexes was then tested against HepG-2, HeLa, MG-63, and A549.¹⁴³ In all cases, cytotoxic activity induced by the complexes was found to be lower than the control (cisplatin) under the same experimental conditions. Further experiments demonstrated that the complexes could induce apoptosis in Hep-G-2 cells by the activation of the ROS mediated mitochondrial dysfunctional pathway.¹⁴³ Cellular uptake and localization studies showed the ability of the complexes to enter the cells and to accumulate in the nucleus. No clear trend was found to directly correlate the planarity of the ancillary ligands and the cytotoxicity, suggesting that the latter is influenced by other factors.¹⁴³

To find more effective anticancer reagents and obtain more insights into the anticancer activity of Ru(II) polypyridyl complexes, Wan and co-workers between 2016 and 2017 proposed three new dppz-like ligands, namely bddp, PTPP and FFTP.^[144-146] In the first place they investigated complexes $[\text{Ru}(\text{N-N})_2(\text{bddp})](\text{ClO}_4)_2$ **87-90** (N-N=dmb (**87**), bpy (**88**), phen (**89**) and 2,9-dmp (**90**)) shown in Figure 20. The cytotoxicity *in vitro* was evaluated against BEL-7402, A549, HeLa and MG-63 cell lines.¹⁴⁴ Complexes **87-90** were found to be highly cytotoxic against all the cell line tested (IC_{50} between $5.3 \pm 0.6 \mu\text{M}$ and $15.7 \pm 3.6 \mu\text{M}$) while the ligand itself was completely non-cytotoxic. Fluorescent microscopy studies revealed that complexes **87-90** can be taken up by A549 cells and accumulate in the nucleus.¹⁴⁴ Moreover, they induce apoptosis in the cell line mentioned above through ROS-mediated mitochondrial dysfunctional pathway accompanied by the regulation of the expression of Bcl-2 family proteins.¹⁴⁴

Fig. 19. Structures of $[\text{Ru}(\text{N-N})_2(\text{dhbn})](\text{ClO}_4)_2$ (**83-86**).

Afterwards, the same scientists investigated complexes **91-94** shown in Figure 20 carrying PTPP and FFTP ligands and bpy and phen as ancillary ligands.¹⁴⁵ The *in vitro* cytotoxicity of these complexes was tested against BEL-7402, HeLa, A549, HepG2 and

HOS cancer cells. Complex **92** exhibited the highest cytotoxic activity against BEL-7402 and A549 cells, while complexes **93** and **94** were found to be more effective against HOS and HepG2 cells, respectively.¹⁴⁵ As previously discussed, these researchers also observed that larger hydrophobicity led to higher cytotoxicity. Experimental data, in fact, demonstrated that the more lipophilic ancillary ligand such as phen (contained in complexes **92**, **94**) confers relatively higher cytotoxic activity when compared with complexes containing bpy (more hydrophilic) as ancillary ligands.¹⁴⁵ This trend was true for all the selected cell lines with the exception of the HOS cell line.¹⁴⁵ Complexes **91**, **92** and **94** showed higher cytotoxicity than cisplatin against HepG2 cells (IC_{50} = $10.4 \pm 1.2 \mu M$, IC_{50} = $9.3 \pm 0.6 \mu M$, IC_{50} = $5.6 \pm 1.2 \mu M$, IC_{50} = $20.3 \pm 2.2 \mu M$, for **91**, **92**, **94** and cisplatin respectively). Wan and co-workers suggested that the examined complexes (**91-94**) induced apoptosis of HepG2 cells through ROS-mediated mitochondrial dysfunction pathway and could regulate the expression of Bcl-2 family proteins.¹⁴⁵ One year later, the same scientists investigated complexes **95-100** carrying more lipophilic ancillary ligands such as dmb, 2,9-dmp and ttbpy.¹⁴⁶ The cytotoxicity of these complexes was tested against the same cell lines used in the previous study and mentioned above with the addition of the non-cancerous LO2 cell lines. From the IC_{50} values, it is possible to observe that complexes with the same ancillary ligands are in general more toxic when they carry PTPP ligand instead of FTTP.¹⁴⁶ In more detail, complex **96** was found to be more active than cisplatin against HeLa, BEL-7402 and HepG-2 with IC_{50} values going from $4.1 \pm 0.3 \mu M$ to $2.0 \pm 0.2 \mu M$. Complex **97** exerted its highest activity against BEL-7402 cells with IC_{50} = $1.5 \pm 0.1 \mu M$ while complex **99** was more active against A549 and HOS cell lines.¹⁴⁶ On the other hand, complex **100** was moderately active against all the cell line tested with IC_{50} values in the range from $12.8 \pm 0.4 \mu M$ to $3.2 \pm 0.1 \mu M$. Unfortunately, all the complexes were not highly selective in the expression of their toxicity since they were found to be also very toxic against healthy LO2 cells.¹⁴⁶ This work confirmed that by increasing the lipophilicity of the complex, the cytotoxicity of the complex increases. Apoptosis was found to be induced by the activation of a ROS-mediated mitochondrial dysfunction pathway accompanied by the regulation of Bcl-2 family proteins.¹⁴⁶

Fig. 20. Structures of $[Ru(dmb)_2(bddp)](ClO_4)_2$ (**87**), $[Ru(bpy)_2(bddp)](ClO_4)_2$ (**88**), $[Ru(phen)_2(bddp)](ClO_4)_2$ (**89**), $[Ru(2,9-dmp)_2(bddp)](ClO_4)_2$ (**90**), $[Ru(bpy)_2(FTTP)](ClO_4)_2$ (**91**), $[Ru(phen)_2(FTTP)](ClO_4)_2$ (**92**), $[Ru(bpy)_2(PTTP)](ClO_4)_2$ (**93**), $[Ru(phen)_2(PTTP)](ClO_4)_2$ (**94**), $[Ru(dmb)_2(PTTP)](ClO_4)_2$ (**95**), $[Ru(2,9-dmp)_2(PTTP)](ClO_4)_2$ (**96**), $[Ru(ttbp)_2(PTTP)](ClO_4)_2$ (**97**), $[Ru(dmb)_2(FTTP)](ClO_4)_2$ (**98**), $[Ru(2,9-dmp)_2(FTTP)](ClO_4)_2$ (**99**), $[Ru(ttbp)_2(FTTP)](ClO_4)_2$ (**100**).

In 2014, Luo and co-workers suggested a completely new mechanism of action for their Ru(II) polypyridyl complexes by proposing mitochondrial thioredoxin reductase (TrxR) as a target for their Ru(II) complexes.¹⁴⁷ The thioredoxin system, which is composed of thioredoxin (Trx), TrxR and NADPH, has been intensively considered as an interesting target in anticancer research over the last five years. It has a key role in regulating redox balance and intracellular signalling pathways. TrxR is a homodimeric selenoenzyme characterised by a broad substrate specificity. Moreover, the propensity of the active site selenolate group to react with “soft” metal ions, makes it a good target for metal-based drugs. So far, only complexes with labile ligands have been investigated (i.e. cisplatin, gold complexes, Ru(II)-arene compounds, Ru(II) N-heterocyclic carbene complexes) because the inhibition of such enzyme is believed to be due to the covalent binding between the metal and the selenium.^[148–152] In this specific article, Ru(II) polypyridyl complexes were considered, for the first time, as inhibitors of mitochondrial TrxR. Inter alia, $[Ru(bpy)_3](ClO_4)_2$ (**101**), $[Ru(phen)_3](ClO_4)_2$ (**102**), $[Ru(ip)_3](ClO_4)_2$ (**103**), $[Ru(pip)_3](ClO_4)_2$ (**104**) (Figure 21) were tested against various cancer cell lines, namely, A375, MCF-7, PC-12, HepG-2 and A549 cells in order to evaluate their *in vitro* cytotoxicity.¹⁴⁷ Afterwards, the interaction of

the complexes with TrxR and their lipophilicity were investigated. Experimental data showed that increasing the lipophilicity led to an increased cytotoxicity. On the other hand, the most lipophilic compound (**104**) had the greatest TrxR inhibitory potency.¹⁴⁷ The mechanism of action suggested by these researchers considered TrxR as the first target of the complex **104**. In order to obtain more insights about the possible interactions, these scientists used mass spectrometry. In particular, they employed the selenocysteine-containing model peptide Ala-Gly-Sec-Val-Gly-Ala-Gly-Leu-Ile-Lys (AGUVGAGLIK) for the study of the possible interaction with the active site of TrxR.¹⁵³ After 12 h of treatment with the complex **104**, the molecular peak of the selenopeptide completely vanished. This was accompanied by the appearance of a peak with the mass corresponding to the fragment (H-UVGA-OH) attached to **104**.¹⁴⁷ This data made the scientists suggest that the Ru(II) polypyridyl complexes might form a covalent adduct with the selenol of the selenocysteine, thus affecting the TrxR activity. To confirm this hypothesis, a photophysical study of complex **104** was carried out in the presence of the selenopeptide under physiological conditions (at 37°C in PBS buffer).¹⁴⁷ The results showed a concomitant disappearance of the absorption band of complex **104** and the rise of a new one centred at 497 nm. In addition, no luminescence was found when **104** was incubated with the selenopeptide.¹⁴⁷ The authors could also demonstrate that the inhibition of TrxR then promoted the generation and accumulation of ROS, leading to cell apoptosis through the activation of mitochondrial pathway.¹⁴⁷ However, since the complex is substitutionally inert, coordination of the metal seems impossible. Unfortunately, no further information is available about the suggested covalent adduct which must occur between the ligand of complex **104** and the selenol.

Fig. 21. Structures of $[\text{Ru}(\text{bpy})_3](\text{ClO}_4)_2$ (**101**), $[\text{Ru}(\text{phen})_3](\text{ClO}_4)_2$ (**102**), $[\text{Ru}(\text{ip})_3](\text{ClO}_4)_2$ (**103**), $[\text{Ru}(\text{pip})_3](\text{ClO}_4)_2$ (**104**).

3.3 Short conclusions

Overall, as shown with the many examples presented above, the general trend observed for monomeric Ru(II) polypyridyl complexes is that the more lipophilic complexes are generally more cytotoxic due to a greater cellular uptake. However, it is extremely difficult to rationalise all results reported to date and to allow for a general trend of their biological activity since targets for these complexes can be different. It is also interesting to note that the cellular localisation of Ru(II) polypyridyl complexes is difficult to predict since many parameters (i.e. size, charge, presence of hydrogen bonds, structural features, etc.) are playing a role.

4. Dinuclear Ru(II) polypyridyl complexes

Below, we review the cytotoxic dinuclear Ru(II) polypyridyl complexes reported to date by considering their biological targets.

4.1 Dinuclear Ru(II) complexes targeting DNA

The interest towards the cytotoxicity of dinuclear Ru(II) polypyridyl complexes targeting DNA arose around 2007.¹⁵⁴ At that time, Hotze and co-workers studied a triple-stranded ruthenium cylinder (showed in Figure 22) and demonstrated that such a complex binds and coils DNA.¹⁵⁴ Interestingly the cytotoxicity was found to be only 2-5 times lower than cisplatin ($\text{IC}_{50}=22\ \mu\text{M}$ and $\text{IC}_{50}=4.9\ \mu\text{M}$ for the dimeric complex and cisplatin, respectively against HBL100 cells). One year later, they investigated a class of compounds of the type $[\text{Ru}_2(\text{LL})_4\text{L}^1](\text{PF}_6)_4$ where LL =bpy or phen and $\text{L}^1 = \text{C}_{25}\text{H}_{20}\text{N}_4$ a bis(pyridylimine) ligand containing a diphenylmethane spacer (**105** in Figure 23 is an example of such structures).¹⁵⁵ The cytotoxicity of these complexes was tested against HBL100 and SKOV-3 cell lines.

Figure 22. Crystal Structure of $[\text{Ru}_2\text{L}'_3]^{4+}$ cation.¹⁵⁴ Ru green, N violet, C grey.

Hydrogen atoms, anions ($4 \times \text{PF}_6^-$), and co-crystallized solvent molecules (H_2O and benzene) are omitted for clarity.

Unfortunately, it was found that they hardly inhibited cell proliferation. The slight activity combined with the low solubility of the compound avoided the determination of absolute IC_{50} values.¹⁵⁵ In 2007, MacDonnell and co-workers found out that the ruthenium dimer $[(phen)_2Ru(tatpp)Ru(phen)_2](Cl)_4$ (**106** Figure 24) could induce DNA cleavage *in vitro* and that this cleavage was enhanced under anaerobic conditions.¹⁵⁶ Complex **106** is known to intercalate and to bind DNA tightly, but as already mentioned above, this is not a sufficient condition to induce DNA damage.^[74,156] Experimental data showed that DNA damage was caused by *in situ* reduction of complex **106** with glutathione (GSH). Moreover, exposure to air, even in a large excess of reductive agent, attenuated the cleavage activity.¹⁵⁶ The active species was found to be the doubly protonated complex **H₂106** shown in Figure 24. As previously suggested by Yamaguchi and co-workers, DNA cleavage can occur via oxygen-dependent and independent pathways.^[157,158] On these bases, the authors suggested that the **H₂106** compound was directly related to the production of radical carbon species responsible for DNA cleavage.¹⁵⁶ This latter statement was demonstrated by the attenuation of DNA cleavage in the presence of TEMPO (carbon radical scavenger) and not in the presence of DMSO (ROS scavenger).¹⁵⁶ This was the first example reported in the literature of a metal complex with potentiated DNA cleavage activity under hypoxia conditions. This interesting finding suggested the possible application of such complexes as a target for tumour cells, which are often characterised by a low-oxygen environment.¹⁵⁹

With these results in hand, in 2013, MacDonnell and co-workers demonstrated, for the first time, the *in vivo* anticancer activity of such Ru(II) polypyridyl complexes.¹⁶⁰ These researchers discovered that the enhanced biological activity of their complexes was associated with the tatpp ligand. From this study, in fact, **106** in

Figure 23. Structures of $[Ru(bpy)_2 L'^1](PF_6)_4$ (**105**) and ligand L'.

Fig. 24. Structures of $[(phen)_2Ru(tatpp)Ru(phen)_2](Cl)_4$ (**106**) and of its reduced form **H₂106**.

Figure 24 and **107** in Figure 25 (complexes containing the tatpp ligand) were found to be more efficient and selective for inducing apoptosis in cultured NSCLC cell lines when compared to other complexes containing different ligands (such as $[Ru(phen)_2(dppz)](Cl)_2$ (**108**), $[Ru(phen)_2(tpphz)](Cl)_2$ (**109**), $[Ru(phen)_3](Cl)_2$ (**110**), $[(phen)_2Ru(tpphz)Ru(phen)_2](Cl)_4$ (**111**), shown in Figure 25).¹⁶⁰ The authors showed a significant tumour growth repression and a doubling of the survival time of mice with H358 human lung cancer xenograft. MacDonnell and co-workers hypothesised the likely mechanism of DNA damage: the tatpp carbon radical in the reduced species abstracts a H-atom from the deoxyribose unit.¹⁶⁰ The concentration of the key intermediate is

Fig. 25. Structures of $[Ru(phen)_2(tatpp)](Cl)_2$ (**107**), $[Ru(phen)_2(dppz)](Cl)_2$ (**108**), $[Ru(phen)_2(tpphz)](Cl)_2$ (**109**), $[Ru(phen)_3](Cl)_2$ (**110**), $[(phen)_2Ru(tpphz)Ru(phen)_2](Cl)_4$ (**111**), ligand.¹⁶¹

dependent on the O_2 and reducing agent concentrations. Moreover, since tatpp can be reduced again, the concentration of the key carbon radical species is controlled by the O_2 and reducing agent concentrations. Interestingly, complexes **106** and **107** showed significant selectivity against malignant cells. This observation could be explained by the differences in transport mechanisms.¹⁶⁰ Complexes **108**, **110**, **111** were generally shown to enter cells via passive diffusion,^[162,163] while it seems reasonable to assume that complexes **106** and **107** enter cells via active transport as already demonstrated for Ru(II) polypyridyl complexes containing the tpphz ligand. In order to obtain more insights into this class of metal complexes, MacDonnell and co-workers investigated a number of derivatives in which the terminal 1,10-phenantroline ligands were replaced with more lipophilic ligands, such as DIP or tmphen.¹⁶⁴ In this work, they carried out a structure-activity relationship study examining the cytotoxicity of Ru(II) polypyridyl complexes against three malignant (MCF7, CCL228, and H358) and one non-malignant (MCF10) human cultured cell lines. All the Ru(II) complexes carrying non-redox active ligands were found to be slightly active against the cell lines tested.¹⁶⁴ Complexes **112** and **113** (shown in Figure 26) are the most lipophilic complexes containing the tatpp ligand examined in this work and showed consistent cytotoxicity against malignant cell lines ($IC_{50} \approx 10 \mu M$).¹⁶⁴ At the same time, they were also well-tolerated by non-

Fig. 26. Structures of $[Ru(DIP)_2(tatpp)](Cl)_2$ (**112**), $[(DIP)_2Ru(tatpp)Ru(DIP)_2](Cl)_4$ (**113**).

malignant MCF10 cells. This data is in complete agreement with the trend found during the *in vitro* AChE inhibition studies.¹⁶⁴ It is clear, that the lipophilicity is inversely proportional to the AChE inhibition ability. Moreover, *in vivo* toxicity studies suggested that the tatpp ligand might be responsible for lowering the observed toxicity.¹⁶⁴ In 2017, MacDonnell and co-workers proposed a mechanistic model to explain the dependency of the DNA cleavage on oxygen concentration.¹⁶⁵ In more detail, they performed *cell-free* experiments, whose data supported the mechanistic pathway shown in Figure 27. The mechanism exhibits a multistep pathway by which Ru(II) polypyridyl complexes **106** and **107** undergo a redox-cycling mediated by the [GSH] and $[O_2]$ which activate O_2 to produce superoxide and hydrogen peroxide.¹⁶⁵ H_2O_2 has a central role since it is an indispensable intermediate in the cleavage mechanism.^[166,167] Moreover, it can be directly produced in a single step via a 2-electron, 2-proton transfer to O_2 by **H₂106** (or **H₂107**). Since, the steady state concentration of **H₂106** increases under hypoxic conditions, H_2O_2 is

Fig. 27. Redox cycling pathway for **106**.¹⁶⁵

produced more efficiently and DNA cleavage is enhanced.¹⁶⁵ Furthermore, the *in vitro* cytotoxicity of **106** and **107** was examined against H358, HCC2998, HOP-62, Hs766t cell lines under normoxia (18 % O₂) and hypoxia (1.1 % O₂). Unlike many O₂ activating drugs which are less effective under hypoxic conditions, **106** showed no differences in cytotoxicity going from normoxia to hypoxia.¹⁶⁵ Complex **107** is two-times more cytotoxic under hypoxic conditions in HOP-62, Hs766t when compared to normoxia. From the results discussed above, it seems that the role of ruthenium is mostly structural, so it would be very interesting to investigate analogues of these complexes containing different metals (e.g. osmium).

4.2 Dinuclear Ru(II) complexes targeting mitochondria

Between 2010 and 2011 Keane, Collins and co-workers investigated a class of dinuclear Ru(II) polypyridyl complexes where the metals are linked by a flexible bridge ($\Delta\Delta/\Lambda\Lambda$ - $\{Ru(phen)_2\}_2\{\mu-bb_n\}\}(Cl)_4$ **114** in Figure 28).^[168,169] They demonstrated that increasing the lipophilicity of the complexes by increasing the length of the alkane chain significantly improved the cytotoxic properties.¹⁶⁸ The cytotoxicity of this family of Ru(II) complexes was analysed in the L1210 cell line. It was found that $\Delta\Delta$ isomers were more active than their counterpart $\Lambda\Lambda$. This observation is in agreement with that found for other dimeric complexes.¹⁶⁴ The predominant site of accumulation of **114** are mitochondria and the cytotoxicity is positively direct correlated with cellular accumulation. Changes in cell morphology were observed after treatment with the series of complexes, indicating the induction of apoptosis.¹⁶⁸ Subsequently, a time-course experiment using annexin V450 showed that the level of apoptosis induced by $\Delta\Delta$ -Rubb₁₆ in the L1210 cell line is time dependent.¹⁶⁹ Flow cytometric studies demonstrated that $\Delta\Delta$ -Rubb₁₆ was taken up by both L1210 cells and primary B cells to different degrees, despite the close relationship of the two cell types. In more detail, a greater uptake by L1210 was recorded, suggesting a selective accumulation

Fig. 28. Structure of $\{Ru(phen)_2\}_2\{\mu-bb_n\}\}(Cl)_4$ (**114**).

in cancer cells.¹⁶⁹ Further studies revealed that the introduction of polyether or polyamine in the linking chains lowered the cytotoxicity, while the trinuclear and tetranuclear species exerted higher activity when compared with their dinuclear analogues.¹⁷⁰ Moreover, this class of complexes showed interesting results as potential antimicrobial agents.¹⁷¹ Inter alia, they expressed a selective *in vitro* toxicity against gram positive and gram negative bacteria while they remain non-toxic for eukaryotic cells at concentrations significantly greater than the corresponding MIC value (minimal inhibitory concentration).^[171,172] Interestingly, a later study on $\Delta\Delta$ -Rubb₁₂ and its derivatives revealed a high selectivity for nucleus accumulation in BHK, HEK-293 and Hep-G2 cell lines.¹⁷² These results showed a potential level of cell type selectivity.

4.3 Dinuclear Ru(II) complexes targeting biological membranes

In 2013, Gill and co-workers examined other dimeric Ru(II) polypyridyl complexes, namely $[(Ru(DIP)_2)_2(tpphz)](Cl)_4$ and $[(Ru(phen)_2)_2(tpphz)](Cl)_4$ (**115** and **116** in Figure 29, respectively).¹⁷³ They previously reported the cellular uptake properties of the dinuclear Ru(II) complex **116** demonstrating that this luminescent light switch complex functions as a DNA imaging agent.¹⁶¹ On the other hand, the most lipophilic analogue **115** was found to have possible applications as an *in cellulo* imaging agent targeting mainly lipid-dense endoplasmic reticulum (ER).¹⁷³ Cellular localisation studies showed that the targets of this compound are biological membranes with the major accumulation recorded in the ER.¹⁷³ Eukaryotic cell's secretory, endocytic, and degradative processes involve membrane-enclosed transport carriers to move diverse molecules among membrane-bound compartments.¹⁷⁴ As a result, membrane-enclosed intracellular structures and organelles

Fig. 29. Structures of $[(Ru(DIP)_2)_2(tpphz)](Cl)_4$ (**115**) and $[(Ru(phen)_2)_2(tpphz)](Cl)_4$ (**116**).

interaction and regulation within eukaryotic cells is of vital relevance within cell biology and medicine.¹⁷⁴ The *in vitro* cytotoxicity of the Ru(II) complex was tested towards MCF-7 and HeLa cells and was found to be comparable to cisplatin. Cellular morphology studies suggested necrosis as the main cause of cell death since no evidence of apoptosis was found.¹⁷³ However, future studies are needed to provide more detailed insights about the bioactivity of **115**.

4.4 Short conclusions

Overall, as can be seen with the studies reported to date with dinuclear Ru(II) complexes, the main targets of these compounds are DNA, mitochondria or biological membranes. Among the complexes targeting DNA, dimeric complex **106** showed the greatest potential due to its peculiar properties (i.e. great selectivity and high cytotoxicity under hypoxia). In addition, promising *in vivo* results were obtained which showed relatively low acute animal toxicity and demonstrable tumour regression in murine tumour models. Other similar complexes were investigated and it is interesting to note that the complexes containing non-redox ligands showed much lower activity, while no clear trend in respect to lipophilicity was found. In fact, the most lipophilic analogue of compound **106**, namely **112** showed IC₅₀ between 1.5 µM and 100 µM depending on the cell line studied, while complexes **106** and **107** (which are one order of magnitude less lipophilic) were found to be some of the complexes presenting the broadest activity. On the contrary, in the case of all the other dinuclear Ru(II) polypyridyl complexes reported here, the general trend stating that more lipophilic complexes lead to highest cytotoxicity was confirmed.

5. Conclusions

Over the years, Ru(II) polypyridyl complexes were shown to have promising bactericidal, bacteriostatic and anti-cancer activity. Unfortunately, not much *in vivo* data is currently publicly available, rendering it difficult to assess the full potential of this class of compounds. A highlight is undoubtedly the dinuclear complex **106** ((phen)₂Ru(tatpp)Ru(phen)₂](Cl)₄) discovered by MacDonnell and co-workers. This complex exerts its cytotoxicity, targeting directly DNA with a carbon radical generated by the *in situ* reduction of a special ligand, namely tatpp. Moreover, *in vivo* studies suggested that the tatpp ligand might be responsible for a decrease in toxicity.

Very interestingly, complex **106** showed a great selectivity towards cancer cells and its cytotoxicity improves under hypoxia (condition usually found in cancer cells). In our opinion, these properties associated with the promising *in vivo* data reported make complex **106** the most promising compound presented in this Review. However, further investigations are needed to validate the full potential of this compound as well as the correlation between the lowered toxicity observed and the AChE inhibition.

Overall, we hope that this Review will be beneficial for scientists aiming to improve the clinical profile of this class of compounds in terms of cytotoxicity and *in vivo* tolerability. We are extremely impatient to read new developments in this field of research in the near future.

Ligand abbreviation

2-appt= 2-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine
 2,9-dmp = 2,9-dimethyl-1,10-phenanthroline
 4,7-dmp = 4,7-dimethyl-1,10-phenanthroline
 5-idip = 2-indole-[4,5- f][1,10]phenanthroline
 7-Br-dppz = 7-bromo-dipyrido[3,2-a:2'',3''-c]
 addppn = acenaphtheno[1,2-b]-1,4-diazabenz[o]dipyrido[3,2-
 a:2',3'c]phenazine
 adppz = dipyrido[3,2-a:2',3'-c]phenazin-11-amine
 AHPIP = 2-(3-amino-4-hydroxyphenyl)imidazo[4,5-
 f][1,10]phenanthroline
 azpy = 2-phenylazopyridine
 bb_n = 1,*n*-bis[4(4'-methyl-2,2'-bipyridyl)]-*n*-ane (*n* = 2, 5, 7, 10, 12 or 16)
 bddp=benzilo[2,3-*b*]-1,4-diazabenz[o]dipyrido[3,2-*a*:2',3'-
 c]phenazine
 bppp = 11-bromo-pyrido[2',3':5,6]pyrazino[2,3-
 f][1,10]phenanthroline
 bpy = 2,2'-bipyridine
 bpy-2-nitroIm = 4-[3-(2-nitro-1H-imidazol-1-yl)propyl]
 CNPFIP = 2-(5(4-chloro-2-nitrophenyl)furan-2-yl)-1H-
 imidazo[4,5f][1,10]phenanthroline
 CppH = 2-(2'-pyridyl)pyrimidine-4-carboxylic acid
 Cpp-NH-Hex-COOH = 6-(2-(pyridin-2-yl)pyrimidine-4-
 carboxamido)hexanoic acid
 dhbn = 12,13-diphenylpyrazino[2,3-*i*]dipyrido[3,2-*a*:2',3'-
 c]phenazine
 DIP = 4,7-diphenyl-1,10-phenanthroline

dmab = 4,4'-bis(N,N-dimethylamino)-2,2'-bipyridine
 dmb = 4,4'-dimethyl-2,2'-bipyridine
 dmcb = 4,4'-dimethoxycarbonyl-2,2'-bipyridine
 dmdppz = 5,8-dimethoxypyrido[3,2-*a*:2',3'-*c*]phenazine
 dmob = 4,4'-dimethoxy-2,2'-bipyridine
 dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine
 DQTT = 12-(1,4-dihydroquinoxalin-6-yl)-4,5,9,14-tetraazabenzotriphenylene, dmb = 4,4'-dimethyl-2,2'-bipyridine
 FFTP = 2-(3-fluoronaphthalen-2-yl)-1,4,8,9-tetraazatriphenylene
 H₂IIP = 2-(indole-3-yl)-imidazo[4,5-*f*][1,10]phenanthroline
 Hphox = 2-(2'-hydroxyphenyl)oxazoline
 ip = imidazole[4,5-*f*][1,10]phenanthroline,
 ipad = 2-(anthracene-9,10-dione-2-yl)imidazo[4,5-*f*][1,10]phenanthroline)
 NHPIP = 2-(3-nitro-4-hydroxyphenyl)imidazo[4,5-*f*][1,10]phenanthroline)
 NMIP = 2'-(2"-nitro-3",4"-methylenedioxyphenyl)imidazo[4',5'-*f*][1,10]-phenanthroline
 Norharman = 9H-pyrido[3,4-*b*]indole
 PAIDH = 2-Pyridyl-1H-anthra[1,2-*d*]imidazole-6,11-dione
 pddppn: phenanthro[1,2-*b*]-1,4-diazabenz[*i*]dipyrido[3,2-*a*:2',3'-*c*]phenazine
 phen = 1,10 phenanthroline
 pip = 2-phenylimidazo[4,5-*f*][1,10]phenanthroline)
 psbr = 2-(5-bromothiophen-2-yl)imidazo[4,5-*f*][1,10]phenanthroline
 pscl = 2-(5-chlorothiophen-2-yl)imidazo[4,5-*f*][1,10]phenanthroline
 ptip = (2-(5-phenylthiophen-2-yl)-1H-imidazo[4,5-*f*][1,10]phenanthroline
 PTTP = 2-phenoxy-1,4,8,9-tetraazatriphenylene
 pztp = 3-(pyrazin-2-yl)-[1,2,4]triazino[5,6-*f*][1,10]phenanthroline
 R₂bpy = 5,5'-di[1-(triethylammonio)methyl]-2,2'-dipyridine
 tatpp = 9,11,20,22-tetraazatetrapyrido[3,2-*a*:2',3'-*c*:3'',2''-1:2''',3'''-*n*]pentacene
 tBu₂bpy = 4,4'-di-*tert*-butyl-2,2'-bipyridine
 tFIIP = 2-(2-trifluoromethanophenyl)imidazole[4,5-*f*][1,10]phenanthroline)
 tip = 2-(thiophen-2-yl)-1H-imidazo[4,5-*f*][1,10]phenanthroline)
 tmphen = 3,4,7,8-tetramethyl-1,10-phenanthroline
 tpphz = tetrapyrido[3,2-*a*:2',3'-*c*:3'',2''-h:2''',3'''-*j*]phenazine
 ttbpy = 4,4'-ditertiary butyl-2,2'-bipyridine

Cell lines explanation

4T1 = mouse breast cancer cell line
 A2780 = human ovarian carcinoma cell line
 A2780-CP70 = cisplatin-resistant ovarian carcinoma cell line
 A375 = human melanoma cell line
 A549 = human lung adenocarcinoma cell line
 B = B lymphocytes cell line
 BEL-7402 = human hepatocellular carcinoma cell line
 BHK = baby hamster kidney cell line
 BJAB = Burkitt-like lymphoma cell line
 CCD-19Lu = human noncancerous normal lung fibroblasts cell line
 Du145 = human prostate cancer cell line
 EVSA-T = human breast carcinoma cell line
 HBL100 = human breast cancer cell line
 HCC2998 = human colon adenocarcinoma cell line
 HaCaT = aneuploid immortal keratinocyte cell line
 HeLa = human adenocarcinoma of the cervix cell line
 HEK-293 = human embryonic kidney cell line
 HepG-2 = human hepatocarcinoma cell line
 HL-60 = human leucocytoma cell line
 HOP-62 = human lung adenocarcinoma cell line
 Hs766t = human pancreatic adenocarcinoma cell line
 HT-29 = human colon cancer cell line
 H226 = human squamous cell lung carcinoma cell line
 H358 = human non-small cell lung cancer cell line
 IGROV = human ovarian endometrioid adenocarcinoma cell line
 LO2 = human hepatic cell lines
 KB = human cervical adenocarcinoma cell line
 KB-3-1 = human cervical adenocarcinoma cell line
 KB-V-1 = human cervical adenocarcinoma cell line
 MCF-7 = human breast carcinoma cell line
 MDA-MB-231 = human breast adenocarcinoma
 MG-63 = human osteosarcoma cell line
 MRC-5 = human lung fibroblasts
 M19 = human mesothelioma cell line
 NIH/3T3 = Mouse fibroblast
 NSCLC = human non-small cell lung cancer cell line
 PC12 = rat pheochromocytoma cell line
 SGC-7901 = human gastric cancer cell line
 SiHa = human cervical cancer cell line
 U2OS = human osteosarcoma cell line
 WIDR = human rectosigmoid adenocarcinoma cell line

Biographies

Anna Notaro

Anna Notaro (left) is from a small town near Naples (Italy). She studied at the Università Degli Studi di Napoli "Federico II" where she obtained her master degree in chemical sciences in 2016. During her master thesis, she worked on carbohydrate chemistry under the supervision of Dr. Annalisa Guaragna and Dr. Daniele D'Alonzo. Part of this work was performed at the National University of Ireland (Galway, Ireland) in the frame of an Erasmus for traineeship program under the supervision of Prof. V. Paul Murphy. At the beginning of 2017, Anna started a PhD thesis in the Gasser Group at Chimie ParisTech, PSL Research University (France). She is currently working on the preparation of novel Ru(II) polypyridyl complexes as chemotherapeutic agents.

Gilles Gasser

Gilles Gasser (right) was born, raised and educated in Switzerland. After a PhD thesis in supramolecular chemistry with Prof. Helen Stoeckli-Evans (University of Neuchâtel, Switzerland), Gilles performed two post-docs, first with the late Prof. Leone Spiccia (Monash University, Australia) in bioinorganic chemistry and then as an Alexander von Humboldt fellow with Prof. Nils Metzler Nolte (Ruhr-University Bochum, Germany) in bioorganometallic chemistry. Gilles then started his independent scientific career at the University of Zurich in 2010 first as a Swiss National Science

Foundation (SNSF) Ambizione Fellow and then as a SNSF Assistant Professor in 2011. In 2016, Gilles moved to Chimie ParisTech, PSL Research University (France), thanks among other to a PSL Chair of Excellence Program Grant. Gilles was the recipient of several fellowships and awards including the Alferd Werner Award from the Swiss Chemical Society, an ERC Consolidator Grant, the Thieme Chemistry Journal Award and recently the Jucker Award for his contribution to cancer research. Gilles's research group aims at utilizing the specific physico-chemical properties of metal complexes in different areas of medicinal chemistry.

Acknowledgements

We thank Patrick Felder (Chimie ParisTech) for his essential contribution to the graphics. This work was financially supported by an ERC Consolidator Grant PhotoMedMet to G.G. (GA 681679) and has received support under the program *Investissements d'Avenir* launched by the French Government and implemented by the ANR with the reference ANR-10-IDEX-0001-02 PSL (G.G.).

Notes

The authors declare no competing financial interest.

References

- 1 A. Jemal, F. Bray and J. Ferlay, *CA. Cancer J. Clin.*, 2011, **61**, 69–90.
- 2 L. A. Torre, R. L. Siegel, E. M. Ward and A. Jemal, *Cancer Epidemiol. Biomarkers Prev.*, 2015, **25**, 16–27.
- 3 A. Urruticoechea, R. Alemany, J. Balart, A. Villanueva, F. Viñals and G. Capellá, *Curr. Pharm. Des.*, 2010, **16**, 3–10.
- 4 J. Ferlay, H.-R. Shin, F. Bray, D. Forman, C. Mathers and D. M. Parkin, *Int. J. Cancer*, 2010, **127**, 2893–2917.
- 5 L. A. Torre, F. Bray, R. L. Siegel, J. Ferlay, J. Lortet-tieulent and A. Jemal, *CA Cancer J. Clin.*, 2015, **65**, 87–108.
- 6 C. X. Zhang and S. J. Lippard, *Curr. Opin. Chem. Biol.*, 2003, **7**, 481–489.
- 7 E. Jamieson and S. Lippard, *Chem. Rev.*, 1999, **99**, 2467–2498.
- 8 E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451–2466.
- 9 D. Lebwohl and R. Canetta, *Eur. J. Cancer*, 1998, **34**, 1522–1534.
- 10 E. Alessio, G. Mestroni, A. Bergamo and G. Sava, *Curr. Top. Med. Chem.*, 2004, **4**, 1525–35.
- 11 V. Singh, G. K. Azad, A. Reddy M., S. Baranwal and R. S.

- Tomar, *Eur. J. Pharmacol.*, 2014, **736**, 77–85.
- 12 S. P. Fricker, *Dalton Trans.*, 2007, **43**, 4903–4917.
- 13 K. S. M. Smalley, R. Contractor, N. K. Haass, A. N. Kulp, G. E. Atilla-Gokcumen, D. S. Williams, H. Bregman, K. T. Flaherty, M. S. Soengas, E. Meggers and M. Herlyn, *Cancer Res.*, 2007, **67**, 209–217.
- 14 P. G. Corrie, *Medicine (Baltimore)*, 2007, **36**, 24–28.
- 15 B. Rosenberg, L. Van Camp and T. Krigas, *Nature*, 1965, **205**, 698–699.
- 16 B. Rosenberg, L. van Camp, E. B. Grimley and A. J. Thomson, *J. Biol. Chem.*, 1967, **242**, 1347–52.
- 17 B. B. Rosenberg, *Platin. Met. Rev.*, 1971, **15**, 42–51.
- 18 B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansour, *Nature*, 1969, **222**, 385–386.
- 19 P. Pieter and S. J. Lippard, *Encycl. Cancer*, 1997, **1**, 525–543.
- 20 J. A. Mello, S. J. Lippard and J. M. Essigmann, *Biochemistry*, 1995, **34**, 14783–91.
- 21 M. J. Clarke, F. Zhu and D. R. Frasca, *Chem. Rev.*, 1999, **99**, 2511–2533.
- 22 M. a Jakupec, M. Galanski, V. B. Arion, C. G. Hartinger and B. K. Keppler, *Dalton Trans.*, 2008, 183–194.
- 23 P. C. Brujininx and P. J. Sadler, *Curr. Opin. Chem. Biol.*, 2008, **12**, 197–206.
- 24 T. Gianferrara, I. Bratsos and E. Alessio, *Dalton Trans.*, 2009, 7588–7598.
- 25 G. Gasser, I. Ott and N. Metzler-Nolte, *J. Med. Chem.*, 2011, **54**, 3–25.
- 26 E. Reisner, V. B. Arion, B. K. Keppler and A. J. L. Pombeiro, *Inorg. Chim. Acta*, 2008, **361**, 1569–1583.
- 27 M. J. Clarke, *Coord. Chem. Rev.*, 2003, **236**, 209–233.
- 28 P. J. Dyson and G. Sava, *Dalton Trans.*, 2006, 1929–33.
- 29 D. Chen, V. Milacic, M. Frezza and Q. Dou, *Curr. Pharm. Des.*, 2009, 777–791.
- 30 A. C. Komor and J. K. Barton, *Chem. Commun.*, 2013, **49**, 3617–3630.
- 31 B. C. S. Allardyce and P. J. Dyson, *Platin. Met. Rev.*, 2001, **45**, 62–69.
- 32 E. S. Antonarakis and A. Emadi, *Cancer Chemother. Pharmacol.*, 2010, **66**, 1–9.
- 33 M. Pongratz, P. Schluga, M. A. Jakupec, V. B. Arion, C. G. Hartinger, G. Allmaier and B. K. Keppler, *J. Anal. At. Spectrom.*, 2004, **19**, 46–51.
- 34 E. Alessio, *Eur. J. Inorg. Chem.*, 2017, **55**, 1549–1560.
- 35 A. Bergamo and G. Sava, *Dalton Trans.*, 2007, 1267–1272.
- 36 G. Sava, I. Capozzi, K. Clerici, G. Gagliardi, E. Alessio and G. Mestroni, *Clin. Exp. Metastasis*, 1998, **16**, 371–379.
- 37 F. Lentz, A. Drescher, A. Lindauer, M. Henke, R. a Hilger, C. G. Hartinger, M. E. Scheulen, C. Dittrich, B. K. Keppler and U. Jaehde, *Anticancer. Drugs*, 2009, **20**, 97–103.
- 38 R. Trondl, P. Heffeter, C. R. Kowol, M. a. Jakupec, W. Berger and B. K. Keppler, *Chem. Sci.*, 2014, **5**, 2925–2932.
- 39 C. G. Hartinger, S. Zorbas-Seifried, M. A. Jakupec, B. Kynast, H. Zorbas and B. K. Keppler, *J. Inorg. Biochem.*, 2006, **100**, 891–904.
- 40 V. Brabec, *Prog. Nucleic Acid Res. Mol. Biol.*, 2002, **71**, 1–68.
- 41 P. Heffeter, K. Böck, B. Atil, M. A. Reza Hoda, W. Körner, C. Bartel, U. Jungwirth, B. K. Keppler, M. Micksche, W. Berger and G. Koellensperger, *J. Biol. Inorg. Chem.*, 2010, **15**, 737–748.
- 42 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.
- 43 F. Kratz, M. Hartmann, B. K. Keppler and L. Messori, *J. Biol. Chem.*, 1994, **269**, 2581–2588.
- 44 G. Sava and A. Bergamo, *Int. J. Oncol.*, 2000, **17**, 353–365.
- 45 A. Levina, A. Mitra and P. a Lay, *Metallomics*, 2009, **1**, 458–470.
- 46 A. Bergamo, C. Gaiddon, J. H. M. Schellens, J. H. Beijnen and G. Sava, *J. Inorg. Biochem.*, 2012, **106**, 90–99.
- 47 G. Natarajan, R. Malathi and E. Holler, *Biochem. Pharmacol.*, 1999, **58**, 1625–1629.
- 48 I. Bratsos, S. Jedner, T. Gianferrara and E. Alessio, *Chim. Int. J. Chem.*, 2007, **61**, 692–697.
- 49 B.-Z. Zhu, X.-J. Chao, C.-H. Huang and Y. Li, *Chem. Sci.*, 2016, **7**, 4016–4023.
- 50 M. Dörr and E. Meggers, *Curr. Opin. Chem. Biol.*, 2014, **19**, 76–81.
- 51 E. Meggers, *Chem. Commun.*, 2009, 1001–1010.
- 52 M. Patra and G. Gasser, *ChemBioChem*, 2012, **13**, 1232–1252.
- 53 E. M. P. Mulcahy, *Top Organomet Chem*, 2010, **32**, 141–153.
- 54 H. Huang, P. Zhang, B. Yu, Y. Chen, J. Wang, L. Ji and H. Chao, *J. Med. Chem.*, 2014, **57**, 8971–8983.
- 55 H. Huang, P. Zhang, H. Chen, L. Ji and H. Chao, *Chemistry*, 2015, **21**, 715–725.
- 56 L. Zeng, Y. Chen, H. Huang, J. Wang, D. Zhao, L. Ji and H. Chao, *Chemistry*, 2015, **21**, 15308–15319.
- 57 H. Rico Bautista, R. O. Saavedra Diaz, L. Q. Shen, C. Orvain, C. Gaiddon, R. Le Lagadec and A. D. Ryabov, *J. Inorg. Biochem.*, 2016, **163**, 28–38.
- 58 L. Zeng, P. Gupta, Y. Chen, E. Wang, L. Ji, H. Chao, Z.-S. Chen, J. Mai, H. Zhang, Z. Li, M. Guevara, Z.-W. Mao, H. Shen, G. Superti-Furga, B. K. Keppler and C. G. Hartinger, *Chem. Soc. Rev.*, , DOI:10.1039/C7CS00195A.
- 59 H. A. Wee and P. J. Dyson, *Eur. J. Inorg. Chem.*, 2006, 4003–4018.
- 60 D. Chatterjee, A. Mitra and G. S. De, *Platin. Met. Rev.*, 2006, **50**, 2–12.
- 61 V. Brabec and O. Nováková, *Drug Resist. Updat.*, 2006, **9**, 111–122.
- 62 M. R. Gill and J. A. Thomas, *Chem. Soc. Rev.*, 2012, **41**, 3179–3192.
- 63 G. Li, L. Sun, L. Ji and H. Chao, *Dalton Trans.*, 2016, **45**, 13261–13276.
- 64 L. Salassa, *Eur. J. Inorg. Chem.*, 2011, 4931–4947.
- 65 F. P. Dwyer, E. C. Gyarfas, W. P. Rogers and J. H. Koch, *Nature*, 1952, **170**, 190–191.
- 66 F. P. D. J. H. Koch, C. Gyarfas, 1956, **9**, 371–381.
- 67 J. H. Koch, E. C. Gyarfas and F. P. Dwyer, *Aust. J. Biol. Sci.*, 1956, **9**, 371–381.
- 68 Rustam I. Aminov, *Front. Microbiol.*, 2010, **1**, 1–7.

- 69 Y. Jenkins, A. E. Friedman, N. J. Turro and J. K. Barton, *Biochemistry*, 1992, **31**, 10809–10816.
- 70 J.-G. Liu, B.-H. Ye, H. Li, Q.-X. Zhen, L.-N. Ji and Y.-H. Fu, *J. Inorg. Biochem.*, 1999, **76**, 265–271.
- 71 K. E. Erkkila, D. T. Odom and J. K. Barton, *Chem. Rev.*, 1999, **99**, 2777–2796.
- 72 Y. Xiong, X. He, X. Zou, J. Wu, X. Chen, L. Ji, R. Li, J. Zhou and K. Yu, *J. Chem. Soc. Dalt. Trans.*, 1999, 19–23.
- 73 L. N. Ji, X. H. Zou and J. G. Liu, *Coord. Chem. Rev.*, 2001, **216–217**, 513–536.
- 74 A. E. Friedman, J.-C. Chambron, J.-P. Sauvage, N. J. Turro and J. K. Barton, *J. Am. Chem. Soc.*, 1990, **112**, 4960–4962.
- 75 E. J. C. Olson, D. Hu, a Ho, a M. Jonkman, M. R. Arkin, E. D. a Stemp, J. K. Barton and P. F. Barbara, *J. Am. Chem. Soc.*, 1997, **7863**, 11458–11467.
- 76 C. A. Puckett and J. K. Barton, *J. Am. Chem. Soc.*, 2009, **131**, 8738–8739.
- 77 J. K. Barton, E. D. Olmon and P. A. Sontz, *Coord. Chem. Rev.*, 2011, **255**, 619–634.
- 78 H. Song, J. T. Kaiser and J. K. Barton, *Nat. Chem.*, 2012, **4**, 615–20.
- 79 C. R. Cardoso, M. V. S. Lima, J. Cheleski, E. J. Peterson, T. Venâncio, N. P. Farrell and R. M. Carlos, *J. Med. Chem.*, 2014, **57**, 4906–4915.
- 80 Z.-P. Zeng, Q. Wu, F.-Y. Sun, K.-D. Zheng and W.-J. Mei, *Inorg. Chem.*, 2016, **55**, 5710–5718.
- 81 M. R. Gill, H. Derrat, C. G. W. Smythe, G. Battaglia and J. A. Thomas, *ChemBioChem*, 2011, **12**, 877–880.
- 82 M. Eriksson, M. Leijon, C. Hiort, B. Norden and A. Graeslund, *Biochemistry*, 1994, **33**, 5031–5040.
- 83 G. Yang, J. Z. Wu, L. Wang, L. N. Ji and X. Tian, *J. Inorg. Biochem.*, 1997, **66**, 141–144.
- 84 J. P. Hall, P. M. Keane, H. Beer, K. Buchner, G. Winter, T. L. Sorensen, D. J. Cardin, J. A. Brazier and C. J. Cardin, *Nucleic Acids Res.*, 2016, **44**, 9472–9482.
- 85 J. Liu, L. F. Tan, L. H. Jin and F. Luan, *DNA Cell Biol.*, 2012, **31**, 250–258.
- 86 C. J. Cardin, J. M. Kelly and S. J. Quinn, *Chem. Sci.*, 2017, **8**, 4705–4723.
- 87 J. K. Barton, A. T. Danishefsky and J. M. Goldberg, *J. Am. Chem. Soc.*, 1984, **106**, 2172–2176.
- 88 P. Lincoln, A. Broo and B. Norden, *J. Am. Chem. Soc.*, 1996, 2644–2653.
- 89 J. Liu, X.-H. Zou, Q.-L. Zhang, W.-J. Mei, J.-Z. Liu and L.-N. Ji, *Met. Based Drugs*, 2000, **7**, 343–348.
- 90 A. C. G. Hotze, E. P. L. Van Der Geer, H. Kooijman, A. L. Spek, J. G. Haasnoot and J. Reedijk, *Eur. J. Inorg. Chem.*, 2005, **2**, 2648–2657.
- 91 D. Ma, C. Che, F. Siu, M. Yang and K. Wong, *Inorg. Chem.*, 2007, **46**, 740–749.
- 92 P. Zhang, J. Chen and Y. Liang, *Acta Biochim. Biophys. Sin.*, 2010, **42**, 440–449.
- 93 D. A. Lutterman, A. Chouai, Y. Liu, Y. Sun, C. D. Stewart, K. R. Dunbar and C. Turro, *J. Am. Chem. Soc.*, 2008, **130**, 1163–1170.
- 94 J. F. Kou, C. Qian, J. Q. Wang, X. Chen, L. L. Wang, H. Chao and L. N. Ji, *J. Biol. Inorg. Chem.*, 2012, **17**, 81–96.
- 95 K. J. Du, J. Q. Wang, J. F. Kou, G. Y. Li, L. L. Wang, H. Chao and L. N. Ji, *Eur. J. Med. Chem.*, 2011, **46**, 1056–1065.
- 96 Y. C. Wang, C. Qian, Z. L. Peng, X. J. Hou, L. L. Wang, H. Chao and L. N. Ji, *J. Inorg. Biochem.*, 2014, **130**, 15–27.
- 97 C. Wang, Q. Yu, L. Yang, Y. Liu, D. Sun, Y. Huang, Y. Zhou, Q. Zhang and J. Liu, *BioMetals*, 2013, **26**, 387–402.
- 98 Z. Zhang, Q. Wu, X. H. Wu, F. Y. Sun, L. M. Chen, J. C. Chen, S. L. Yang and W. J. Mei, *Eur. J. Med. Chem.*, 2014, **80**, 316–324.
- 99 P. Venkat Reddy, M. Rajender Reddy, S. Avudoddi, Y. Praveen Kumar, C. Nagamani, N. Deepika, K. Nagasuryaprasad, S. Satyanarayana Singh and S. Satyanarayana, *Anal. Biochem.*, 2015, **485**, 49–58.
- 100 N. Deepika, C. Shobha Devi, Y. Praveen Kumar, K. Laxma Reddy, P. Venkat Reddy, D. Anil Kumar, S. S. Singh and S. Satyanarayana, *J. Photochem. Photobiol. B Biol.*, 2016, **160**, 142–153.
- 101 J. C. Wang, *Annu. Rev. Biochem.*, 1996, **65**, 635–692.
- 102 J. J. Champoux, *Annu. Rev. Biochem.*, 2001, **70**, 369–413.
- 103 J. C. Wang, *Nat. Rev. Mol. Cell. Biol.*, 2002, **3**, 430–440.
- 104 T. Simonsson, *Biol. Chem.*, 2001, **382**, 621–628.
- 105 L. H. Hurley, *Biochem. Soc. Trans.*, 2001, **29**, 692–696.
- 106 C. Metcalfe and J. a Thomas, *Chem. Soc. Rev.*, 2003, **32**, 215–224.
- 107 X. Chen, J.-H. Wu, Y.-W. Lai, R. Zhao, H. Chao and L.-N. Ji, *Dalton Trans.*, 2013, **42**, 4386–4397.
- 108 Q. Yu, Y. Liu, C. Wang, D. Sun, X. Yang, Y. Liu and J. Liu, *PLoS One*, 2012, **7**, 1–13.
- 109 S. Shi, J. Xu, X. Gao, H. Huang and T. Yao, *Chem. - A Eur. J.*, 2015, **21**, 11435–11445.
- 110 A. Srishailam, Y. P. Kumar, P. Venkat Reddy, N. Nambigari, U. Vuruputuri, S. S. Singh and S. Satyanarayana, *J. Photochem. Photobiol. B Biol.*, 2014, **132**, 111–123.
- 111 U. Schatzschneider, J. Niesel, I. Ott, R. Gust, H. Alborzinia and S. Wölfl, *ChemMedChem*, 2008, **3**, 1104–1109.
- 112 S. Schäfer, I. Ott, R. Gust and W. S. Sheldrick, *Eur. J. Inorg. Chem.*, 2007, 3034–3046.
- 113 F. Gao, X. Chen, J. Q. Wang, Y. Chen, H. Chao and L. N. Ji, *Inorg. Chem.*, 2009, **48**, 5599–5601.
- 114 O. Zava, S. M. Zakeeruddin, C. Danelon, H. Vogel, M. Grätzel and P. J. Dyson, *ChemBioChem*, 2009, **10**, 1796–1800.
- 115 T. Chen, Y. Liu, W. J. Zheng, J. Liu and Y. S. Wong, *Inorg. Chem.*, 2010, **49**, 6366–6368.
- 116 C. Tan, S. Lai, S. Wu, S. Hu, L. Zhou, Y. Chen, M. Wang, Y. Zhu, W. Lian, W. Peng, L. Ji and A. Xu, *J. Med. Chem.*, 2010, **53**, 7613–7624.
- 117 S. P. Mulcahy, K. Gründler, C. Frias, L. Wagner, A. Prokop and E. Meggers, *Dalton Trans.*, 2010, **39**, 8177–82.
- 118 T. Chen, W.-J. Mei, Y.-S. Wong, J. Liu, Y. Liu, H.-S. Xie and W.-J. Zheng, *Med. Chem. Comm.*, 2010, **1**, 73–75.
- 119 C. Tan, S. Wu, S. Lai, M. Wang, Y. Chen, L. Zhou, Y. Zhu, W. Lian, W. Peng, L. Ji and A. Xu, *Dalton Trans.*, 2011, **40**, 8611–8621.
- 120 K. M. Debatin, *Toxicol. Lett.*, 2000, **112–113**, 41–48.
- 121 U. K. Mazumder, M. Gupta, S. S. Karki, S. Bhattacharya, S. Rathinasamy and S. Thangavel, *Chem. Pharm. Bull.*

- (Tokyo)., 2004, **52**, 178–185.
- 122 S. S. Karki, S. Thota, S. Y. Darj, J. Balzarini and E. De Clercq, *J. Enzym. Inhib. Med. Chem.*, 2010, **25**, 513–519.
- 123 L. C. Tu, C. S. Chen, I. C. Hsiao, J. W. Chern, C. H. Lin, Y. C. Shen and S. F. Yeh, *Chem. Biol.*, 2005, **12**, 1317–1324.
- 124 V. Pierroz, T. Joshi, A. Leonidova, C. Mari, J. Schur, I. Ott, L. Spiccia, S. Ferrari and G. Gasser, *J. Am. Chem. Soc.*, 2012, **134**, 20376–20387.
- 125 G.-B. Jiang, Y.-Y. Xie, G.-J. Lin, H.-L. Huang, Z.-H. Liang and Y.-J. Liu, *J. Photochem. Photobiol. B.*, 2013, **129**, 48–56.
- 126 C. Qian, J.-Q. Wang, C.-L. Song, L.-L. Wang, L.-N. Ji and H. Chao, *Metallomics*, 2013, **5**, 844–854.
- 127 Y. Yuan, A. Y. Wang, A. L. Jiang, A. F. Gao, A. S. Liang, C. Su, A. H. Chao and L. J. A, *Aust. J. Chem.*, 2008, **61**, 732–739.
- 128 Y. Y. Xie, Z. Z. Li, G. J. Lin, H. L. Huang, X. Z. Wang, Z. H. Liang, G. Bin Jiang and Y. J. Liu, *Inorganica Chim. Acta*, 2013, **405**, 228–234.
- 129 G. J. Lin, Z. Z. Li, J. H. Yao, H. L. Huang, Y. Y. Xie and Y. J. Liu, *Aust. J. Chem.*, 2013, **66**, 555–563.
- 130 B.-J. Han, G.-B. Jiang, J. Wang, W. Li, H.-L. Huang and Y.-J. Liu, *RSC Adv.*, 2014, **4**, 40899–40906.
- 131 W. Li, G.-B. Jiang, J.-H. Yao, X.-Z. Wang, J. Wang, B.-J. Han, Y.-Y. Xie, G.-J. Lin, H.-L. Huang and Y.-J. Liu, *J. Photochem. Photobiol. B.*, 2014, **140**, 94–104.
- 132 Y. Du, X. Fu, H. Li, B. Chen, Y. Guo, G. Su, H. Zhang, F. Ning, Y. Lin, W. Mei and T. Chen, *ChemMedChem*, 2014, **9**, 714–718.
- 133 G. Bin Jiang, X. Zheng, J. H. Yao, B. J. Han, W. Li, J. Wang, H. L. Huang and Y. J. Liu, *J. Inorg. Biochem.*, 2014, **141**, 170–179.
- 134 G.-B. Jiang, J.-H. Yao, J. Wang, W. Li, B.-J. Han, Y.-Y. Xie, G.-J. Lin, H.-L. Huang and Y.-J. Liu, *New J. Chem.*, 2014, **38**, 2554–2563.
- 135 Q. Yu, Y. Liu, L. Xu, C. Zheng, F. Le, X. Qin, Y. Liu and J. Liu, *Eur. J. Med. Chem.*, 2014, **82**, 82–95.
- 136 O. Mazuryk, M. Maciuszek, G. Stochel, F. Suzenet and M. Brindell, *J. Inorg. Biochem.*, 2014, **134**, 83–91.
- 137 O. Mazuryk, F. Suzenet, C. Kieda and M. Brindell, *Metallomics*, 2015, **7**, 553–566.
- 138 S. Kizaka-Kondoh and H. Konse-Nagasawa, *Cancer Sci.*, 2009, **100**, 1366–1373.
- 139 C. Kieda, B. El Hafny-Rahbi, G. Collet, N. Lamerant-Fayel, C. Grillon, A. Guichard, J. Dulak, A. Jozkowicz, J. Kotlinowski, K. C. Fylaktakidou, A. Vidal, P. Auzeloux, E. Miot-Noirault, J. C. Beloeil, J. M. Lehn and C. Nicolau, *J. Mol. Med.*, 2013, **91**, 883–899.
- 140 S. H. Lai, G. Bin Jiang, J. H. Yao, W. Li, B. J. Han, C. Zhang, C. Zeng and Y. J. Liu, *J. Inorg. Biochem.*, 2015, **152**, 1–9.
- 141 C. Zhang, B.-J. Han, C.-C. Zeng, S.-H. Lai, W. Li, B. Tang, D. Wan, G.-B. Jiang and Y.-J. Liu, *J. Inorg. Biochem.*, 2016, **157**, 62–72.
- 142 S. Liu, J. Zhu, H. Xu, Y. Wang, Y. Liu, J. Liang, G. Zhang, D. Cao, Y. Lin, Y. Wu and Q. Guo, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2016, **161**, 77–82.
- 143 C.-C. Zeng, S.-H. Lai, J.-H. Yao, C. Zhang, H. Yin, W. Li, B.-J. Han and Y.-J. Liu, *Eur. J. Med. Chem.*, 2016, **122**, 118–126.
- 144 D. Wan, S.-H. Lai, H.-H. Yang, B. Tang, C. Zhang, H. Yin, C.-C. Zeng and Y.-J. Liu, *J. Photochem. Photobiol. B Biol.*, 2016, **161**, 295–303.
- 145 D. Wan, S.-H. Lai, H.-H. Yang, B. Tang, C. Zhang, H. Yin, C.-C. Zeng and Y.-J. Liu, *J. Photochem. Photobiol. B Biol.*, 2016, **165**, 246–255.
- 146 D. Wan, S.-H. Lai, C.-C. Zeng, C. Zhang, B. Tang and Y.-J. Liu, *J. Inorg. Biochem.*, 2017, **173**, 1–11.
- 147 Z. Luo, L. Yu, F. Yang, Z. Zhao, B. Yu, H. Lai, K.-H. Wong, S.-M. Ngai, W. Zheng and T. Chen, *Metallomics*, 2014, **6**, 1480–1490.
- 148 I. Ott, X. Qian, Y. Xu, D. H. W. Vlecken, I. J. Marques, D. Kubutat, J. Will, W. S. Sheldrick, P. Jesse, A. Prokop and C. P. Bagowski, *J. Med. Chem.*, 2009, **52**, 763–770.
- 149 M. Yamada, A. Tomida, H. Yoshikawa, Y. Taketani and T. Tsuruo, *Clin. Cancer Res.*, 1996, **2**, 427–432.
- 150 S. Prast-Nielsen, M. Cebula, I. Pader and E. S. J. Arnér, *Free Radic. Biol. Med.*, 2010, **49**, 1765–1778.
- 151 A. Casini, C. Gabbiani, F. Sorrentino, M. P. Rigobello, A. Bindoli, T. J. Geldbach, A. Marrone, N. Re, C. G. Hartinger, P. J. Dyson and L. Messori, *J. Med. Chem.*, 2008, **51**, 6773–6781.
- 152 L. Oehninger, M. Stefanopoulou, H. Alborzinia, J. Schur, S. Ludewig, K. Namikawa, A. Muñoz-Castro, R. W. Köster, K. Baumann, S. Wölfl, W. S. Sheldrick and I. Ott, *Dalton Trans.*, 2013, **42**, 1657–66.
- 153 A. Meyer, C. P. Bagowski, M. Kokoschka, M. Stefanopoulou, H. Alborzinia, S. Can, D. H. Vlecken, W. S. Sheldrick, S. Wölfl and I. Ott, *Angew. Chemie Int. Ed.*, 2012, **51**, 8895–8899.
- 154 G. I. Pascu, A. C. G. Hotze, C. Sanchez-Cano, B. M. Kariuki and M. J. Hannon, *Angew. Chemie Int. Ed.*, 2007, **46**, 4374–4378.
- 155 U. McDonnell, J. M. C. A. Kerchoffs, R. P. M. Castineiras, M. R. Hicks, A. C. G. Hotze, J. Hannon and A. Rodger, *Dalton Trans.*, 2008, 667–675.
- 156 T. K. Janaratne, A. Yadav, F. Ongeri and F. M. MacDonnell, *Inorg. Chem.*, 2007, **46**, 3420–3422.
- 157 T. Yamaguchi, H. Nomura, K. Matsunaga, S. Ito, J. Takata and Y. Karube, *Biol. Pharm. Bull.*, 2003, **26**, 1523–1527.
- 158 T. Yamaguchi, S. Matsumoto and K. Watanabe, *Tetrahedron Lett.*, 1998, **39**, 8311–8312.
- 159 G. L. Semenza, *J. Mol. Med.*, 2007, **85**, 1301–1307.
- 160 A. Yadav, T. Janaratne, A. Krishnan, S. S. Singhal, S. Yadav, A. S. Dayoub, D. L. Hawkins, S. Awasthi and F. M. Macdonnell, *Mol. Cancer Ther.*, 2013, **12**, 643–653.
- 161 M. R. Gill, J. Garcia-Lara, S. J. Foster, C. Smythe, G. Battaglia and J. a Thomas, *Nat. Chem.*, 2009, **1**, 662–667.
- 162 C. A. Puckett and J. K. Barton, *J. Am. Chem. Soc.*, 2007, **129**, 46–47.
- 163 C. A. Puckett and J. K. Barton, *Biochemistry*, 2008, **47**, 11711–11716.
- 164 N. Alatrash, E. S. Narh, A. Yadav, M.-J. Kim, T. Janaratne, J. Gabriel and F. M. MacDonnell, *ChemMedChem*, 2017, **12**, 1055–1069.
- 165 C. A. Griffith, A. Dayoub, T. Janaratne, N. Alatrash, K. Abayan, A. Mohamedi, Z. Breitbach, D. W. Armstrong and F. M. MacDonnell, *Chem. Sci.*, 2017, **8**, 3726–3740.
- 166 R. Franco, M. I. Panayiotidis and J. A. Cidlowski, *J. Biol.*

- Chem.*, 2007, **282**, 30452–30465.
- 167 A. R. Giandomenico, G. E. Cerniglia, J. E. Biaglow, C. W. Stevens and C. J. Koch, *Free Radic. Biol. Med.*, 1997, **23**, 426–434.
- 168 M. J. Pisani, D. K. Weber, K. Heimann, J. G. Collins and F. R. Keene, *Metallomics*, 2010, **2**, 357–432.
- 169 M. J. Pisani, P. D. Fromm, Y. Mulyana, R. J. Clarke, H. Körner, K. Heimann, J. G. Collins and F. R. Keene, *ChemMedChem*, 2011, **6**, 848–858.
- 170 Y. Mulyana, D. K. Weber, D. P. Buck, C. A. Motti, J. G. Collins and F. R. Keene, *Dalton Trans.*, 2011, **40**, 1510.
- 171 F. Li, Y. Mulyana, M. Feterl, J. M. Warner, J. G. Collins and F. R. Keene, *Dalton Trans.*, 2011, **40**, 5032.
- 172 X. Li, A. K. Gorle, T. D. Ainsworth, K. Heimann, C. E. Woodward, J. Grant Collins and F. Richard Keene, *Dalt. Trans.*, 2014, **44**, 3594–3603.
- 173 M. R. Gill, D. Cecchin, M. G. Walker, R. S. Mulla, G. Battaglia, C. Smythe and J. a Thomas, *Chem. Sci.*, 2013, **4**, 4512–4519.
- 174 J. Lippincott-Schwartz and R. D. Phair, *Annu. Rev. Biophys.*, 2010, **39**, 559–578.