Recent progress in the development of organometallics for the treatment of cancer

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Abstract

From their early successes in medicine, organometallic compounds continue to attract interest as potential chemotherapeutics to treat a range of diseases. Here, we show from recent literature selected largely from the last 2 years that organometallics offer unique opportunities in medicine and, increasingly, a mechanistic-based approach is applied to their development, which has not always been the case.

Introduction

Organometallic compounds, i.e. compounds with a direct metal-carbon bond, have a long history in medicinal chemistry. Indeed, the first reported drug discovered from screening a library of compounds identified an organometallic as a potent antimicrobial. We refer here to the historically important discovery of the organoarsenic compound, Salvasan, by Paul Ehrlich via a mechanism-free approach [1]. Other organometallics, and metal-based drugs more generally, were subsequently identified with no or only limited mechanistic insights and, today, most have been superseded due to side-effects associated with their use [2]. Based on considerably more stringent therapeutic indexes required, combined with the incompatibility of many organometallics with high-throughput screening methods, mechanism-based organometallic drug design should now be considered as essential [3]. Importantly, organometallics offer certain structural characteristics [4, 5] or mechanistic pathways [6] that are inaccessible to other classes of compounds, and it is these unique pathways where there is most promise for new organometallic drugs with alternative or superior properties to other classes of compounds. Here, we have selected organometallics from the last two years which fulfil these criteria. Notably, these recently discovered compounds have not reached a level of maturity to enter the clinic, but we believe the approaches hold considerable promise and will lead to new therapeutics.

Ruthenium organometallics – continuing to yield promising lead compounds

Organometallic complexes based on ruthenium have emerged as some of the most promising alternatives to platinum-based anticancer agents, with half-sandwich structures being a major focus of this class. The RAED and RAPTA series of compounds (Fig. 1(a)) sparked the development of a wide range of ruthenium-arene anticancer agents, with the half-sandwich structure permitting wide-ranging structural variation through modulation of arene substituents and/or the remaining ligands occupying the ruthenium coordination sphere [7, 8].

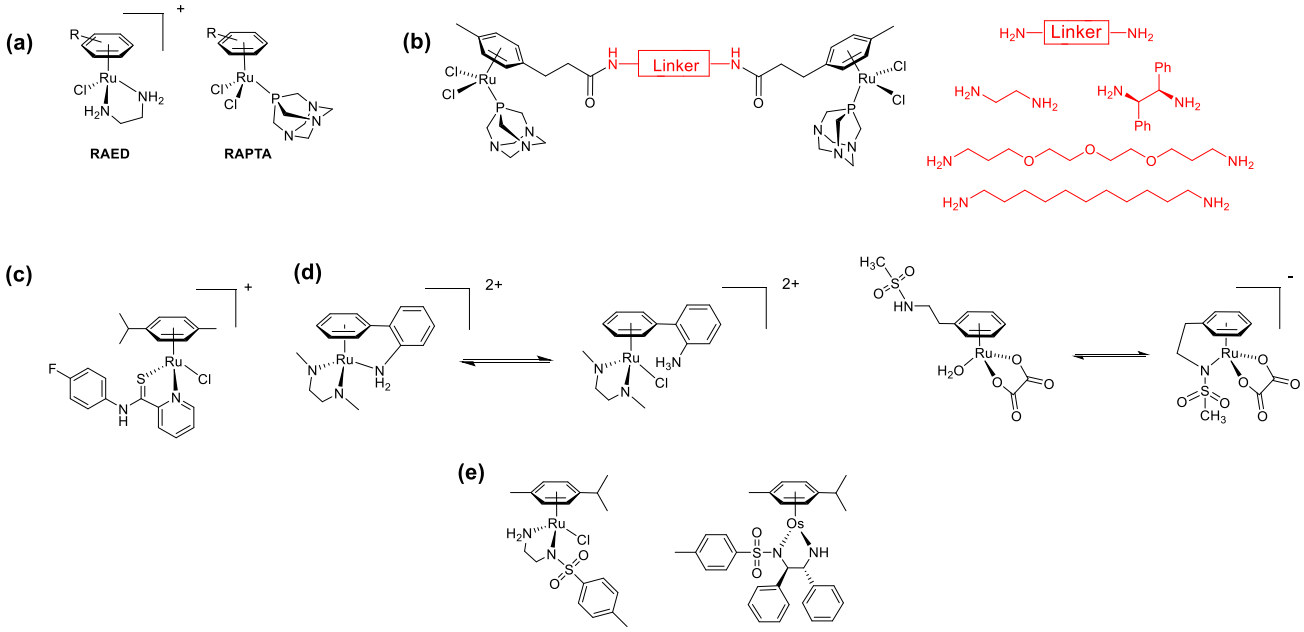

Figure 1 Chemical structures of half-sandwich metallodrugs; (a) the mononuclear RAED and RAPTA structures, (b) dinuclear RAPTA analogues which bind to the histone H2A–H2B dimer, (c) a plectin-targeting complex, (d) complexes exhibiting pH-dependent reactivity towards 5'-GMP, (e) catalytic metallodrugs.

From the outset the RAPTA compounds displayed a vastly different cytotoxicity profile to typical platinum chemotherapeutic agents; in general limited cytotoxicity observed within reasonable (0-200 µM) dosing ranges in 2D cell culture experiments, but strong antimetastatic and antiangiogenic activity was observed in *in vivo* models, alongside activity against primary tumours when appropriate dosing regimens were employed [9]. While the RAED compounds appear to be abandoned, preclinical development of RAPTA compounds continues due to their unique pharmacological profiles, with RAPTA-T recently shown to modulate the tumor microenvironment allowing effective treatment of chemoresistant mesothelioma when combined with a cytotoxic platinum agent (which is essentially inactive in the absence of RAPTA-T) [10]. Although the full mechanism of action of RAPTA-T is complicated, like other RAPTA compounds, binding to the 'acidic patch' on the histone H2A–H2B dimer in the nucleosome, impacting on chromatin dynamics, has been observed, and even leads to major structural rearrangements that illicit allosteric binding [11]. More recently, a compound that bridges these allosteric sites was reported [12]. The binding constants of RAPTA-T are low and to strengthen binding dimeric compounds (Fig. 1(b)) were produced that bind considerably more strongly to the histone H2A–H2B dimer (Fig. 2) [13].

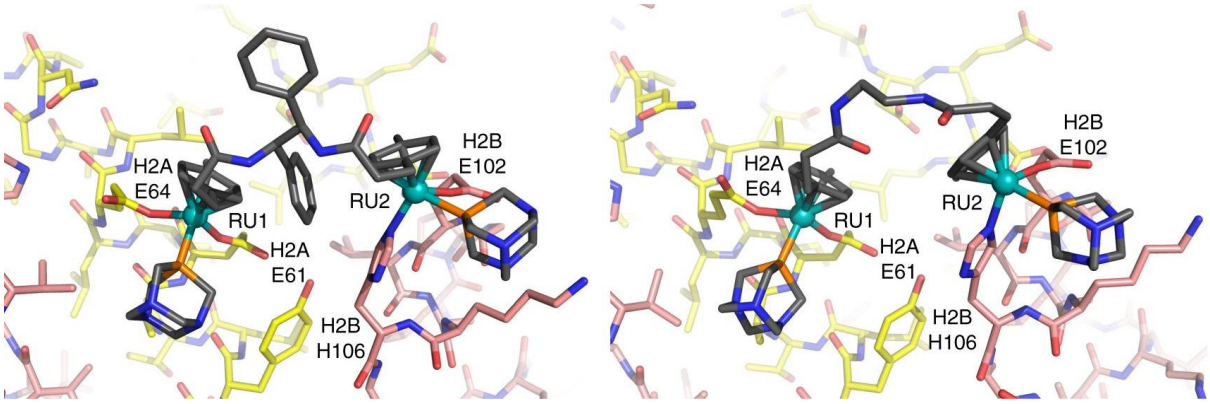

Figure 2: Binuclear RAPTA compounds were found to form interprotein crosslinks in chromatin – crosslinking the H2A-H2B dimer. Images correspond to close-up views of the histone interactions of the complexes (see figure 1(b)) constructed from a

(1R,2R)-(+)-1,2-diphenylethylenediamine linker (left) and an ethylenediamine linker (right). Figure adapted from ref. [13] under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/).

New families of ruthenium-arene complexes are emerging that also exert unique activity through selectivity in their protein binding profile. Meier *et. al.* have identified a family of ruthenium compounds with plectin, a cytolinker protein, as the major cellular target (Fig. 1(c)) [14]. Plectin targeting leads to G0/G1 arrest in HCT116 cells, with large changes to the cytoarchitecture. In a 3D HCT116 tumour spheroid model, treatment resulted in reduced invasiveness, and also to reduced tumour volumes in murine colon carcinoma (CT-26) and B16 melanoma tumor models. These results indicate the significant potential of this new family of ruthenium-arene compounds in the development of anticancer chemotherapeutics operating via novel mechanisms of action through interaction with a well-defined target.

A series of recent reports have also outlined the development of ruthenium-arene compounds that display reactivity that is dependent on the local chemical environment (Fig. 1(d)). Towards exploiting the known extracellular pH differences between healthy (7.2-7.4) and cancerous (6.5-6.9) tissue, pH-dependent coordination profiles have been demonstrated in complexes containing arene-tethered functionality able to reversibly form an intramolecular chelate in a pH-dependent manner. One set of complexes exploit the reversible formation of an η^6:κ^1-arene/N chelate to control the coordination of guanosine 5′-monophosphate (5′-GMP, used as a model for DNA) to the central ruthenium ion – with the open form able to readily coordinate to the nucleobase whereas the closed form remained unreactive [15]. However, under physiologically relevant conditions the closed form of these complexes predominates (ligand NH_3^+ pK_a values of ~2.5) meaning they were unreactive towards 5′-GMP and inactive toward the A2780 ovarian cancer cell line.

Control of 5′-GMP coordination to ruthenium across the physiologically relevant pH range has been achieved using complexes bearing a pendant sulfonamide functionality (Fig. 1(d)) [16]. Through variation of the sulfonamide substituent, the basicity of the sulphonamide nitrogen could be modulated, enabling tuning of the pH-region over which the intramolecular chelate form of the complex dominates and allowing pH-dependent regulation of 5′-GMP coordination to the central metal ion. Further work is required to achieve complete control over ligand coordination as a function of pH as L-histidine coordination to the ruthenium was not controlled in a pH-dependent manner. However, this work opens the way for the development of new organometallics able to selectively coordinate to a single class of biomolecular target. Other approaches to enhance the selectivity of ruthenium-arene chemotherapeutics based on light activated ruthenium-arene complexes have also been reported recently [17, 18].

Catalytic organometallics – promising low-dose chemotherapeutic agents

Catalytic metal-based drugs that mimic metalloenzymes have made considerable advances towards clinical approval for a number of different conditions [19]. The use of metal complexes to catalyse biorthogonal transformations in living cells also represents a potentially powerful strategy toward the development of drugs with unique modes of action. Recent examples of organometallic complexes catalysing biorthogonal transformations include ruthenium-cyclopentadienyl complexes for the catalytic deprotection of alloc- and allyl-protected amines in HeLa or 4T1 (mouse mammary carcinoma) cells [20-22], and olefin metathesis in E. Coli., utilising an artificial metalloenzyme based around a biotinylated Hoveyda–Grubbs second-generation catalyst [23].

In the development of catalytic metallodrugs with anticancer activity, complexes able to catalyse transfer hydrogenation have emerged as promising prototypes. Noyori-type ruthenium-arene complexes (Fig. 1(e)) were used to catalyse the reduction of NAD^+ to NADH in A2780 cells using

formate as the hydride donor. The mechanism of cell death was linked to reductive stress induced by modulation of the NAD^+/NADH redox couple [24]. While the Noyori-type ruthenium-arene catalysts provide outstanding enantioselectivites, for this reaction non-chiral catalysts are sufficient, and since these catalysts suffer from low catalytic rates, it is likely that more efficient transfer hydrogenation catalysts (with respect to turnover frequencies) would be even more effective.

Related organometallic osmium(II)-arene complexes (Fig. 1(e)) were subsequently shown to enantioselectively reduce pyruvate to lactate in A2780 cells, utilising sodium formate as a hydride source [25]. Interestingly, the resulting antiproliferative effect observed on complex and formate administration to A2780 cells was found to be significantly reduced in MRC-5 lung fibroblasts and human ovarian fibroblasts (HOF), indicative of selectivity toward the cancer cell line. Although the specific cause of cell death was not identified, and could be due to interactions of the catalyst with DNA or proteins, this study highlights the potential of catalytic metallodrugs as a strategy by which high efficacy may be achieved, and where drug resistance may possibly be overcome through multiple unprecedented mechanisms of action. While small molecule organometallic catalytic drugs have been most extensively studied, artificial metalloenzyme drugs hold considerable promise and could potentially offer even greater benefits in the long-term [26].

In an alternative approach, an organoiridium(III) photocatalyst, $[Ir(ttpy)(pq)Cl]^+$, photocatalytically oxidizes 1,4-dihydronicotinamide adenine dinucleotide (NADH) to generate $NAD^\bullet$ radicals, a process that has been shown to take place in the mitochondria of cells upon irradiation with blue light, as the compound accumulates in the mitochondria [27]. Metal containing photocatalysts (photosensitizers) employed in the clinic [28], or under clinical evaluation [29], are usually activated by red light as this penetrates tissue most effectively. The classical mechanism of action of photosensitizers involves the photocatalytic generation of $HO_2^\bullet$ or $O_2^{\bullet-}$ from O_2 (Type I mechanism) and generation of $^1O_2$ from $^3O_2$ (Type II mechanism) in endothelial cells, which close the blood vessels around the tumor via oxidative damage to cut off the supply of nutrients [30], whereas $[Ir(ttpy)(pq)Cl]^+$ functions in hypoxic environments directly inside cancer cells and potentially opens the way to new phototherapy strategies.

Rhenium tricarbonyl compounds – an emerging class of metallodrug

The anticancer activity of organometallic rhenium compounds is much less explored than that of group 8 and 10 metal compounds, but recent reports illustrate the progress achieved with this element. Notably, a series of rhenium(I) tricarbonyl complexes were discovered that induce cell death in a manner distinct to that exerted by cisplatin, and which circumvent cisplatin resistance in KBCP20, A2780CP70, A549CisR and H460 cell lines [31, 32]. Studies on one of the complexes (Fig. 3) revealed its ability to coordinate to 9-ethylguanine, *N*-acetylcysteine and *N*-acetylhistidine, that it localises within the mitochondria and induces cell death in a manner that is caspase-independent. The biodistibution of this complex was examined in naïve C57B16 mice using both ^{99m}Tc and Re analogues – non-specific uptake was not detected in the examined organs and rapid hepatic and renal clearance was observed (over 120 minutes post-injection). Antitumour activity was evaluated in NOD scid gamma mice using ovarian cancer patient-derived zenografts. Dosing at 10 mg/kg twice per week led to reduced tumour volume without loss in body weight over 30 days, although tumour weights were unexpectedly no different to that of untreated controls and this was attributed to an increase in the density of treated tumours. No side effects were observed during the treatment regime although the rhenium content in the tumours was lower than in most organs (kidneys, heart, lung, liver). It is clear that while this compound can significantly influence tumour size, its inability to decrease tumour weight coupled with off-target accumulation of rhenium could limit future prospects for this particular family of compounds.

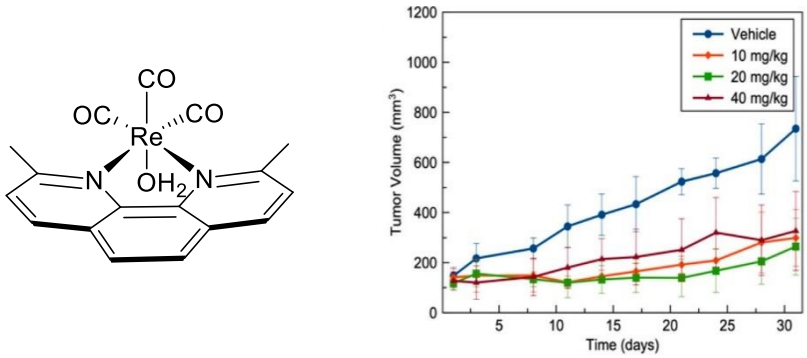

Figure 3. A recently reported rhenium(I) tricarbonyl complex (left) that causes a significant reduction in tumour volume (right) but not weight when administered in the range 10-40 mg/kg. Right-hand image adapted with permission from Konkankit CC, King AP, Knopf KM, Southard TL, Wilson JJ: **In Vivo Anticancer Activity of a Rhenium(I) Tricarbonyl Complex.** *ACS Med Chem Lett* 2019, **10**: 822−827. Copyright 2019 American Chemical Society.

Silver and gold complexes targeting multiple cancer-related pathways

N-heterocyclic carbene (NHC) complexes based on silver and gold have emerged as an area of focus for the development of new anticancer chemotherapeutics [33, 34]. A recent report [35] outlined the activity of a silver-NHC complex [36] (Fig. 4(a)) able to exert anticancer activity via interactions with multiple targets. The complex was found to efficiently inhibit purified thioredoxin reductase 1 (TrxR1), human topoisomerase I and II and human PARP-1, all significant prospective intracellular targets. Cellular ROS levels increased on treatment with the complex and selective inhibition of glycolysis in cancer cells (A2780 and OVCAR3) was observed compared to non-cancer cell lines. The complex was well tolerated in mice and anticancer activity was observed against ovarian cancers using the hollow fibre assay where fibres were implanted intraperitoneally (note that anticancer activity was not observed with fibres implanted subcutaneously). Given the metallodrug was tolerated well *in vivo*, whilst exerting activity via multiple DNA-damaging mechanisms, this silver-NHC complex is a lead structure worth further investigation and may yield a new family of metallodrugs able to overcome resistance issues afflicting platinum-based drugs. However, it should be noted that many metal-based drugs are able to inhibit the proteins listed above to varying degrees, and they do not necessarily correspond to the actual targets. Indeed, certain metals have been described as promiscuous [37], able to bind to many isolated proteins, and the major intracellular targets should ideally be identified from proteomics studies [38]. The demonstration that rational targeting of multiple cancer-related pathways can lead to highly effective metallodrugs is an important principle and suggests those exhibiting promiscuous activity may hold a certain advantage over those designed to act on a single target, as long as cancer cell selectively can be maintained. In this respect, in the treatment of cancer targeted drugs, i.e. drugs that selectively bind to a single enzyme/protein, tend to be applied in combination with broad acting drugs such as the clinically approved platinum-based drugs [39]. Recent studies have also highlighted the considerable potential of platinum-based drugs combined with immunotherapy [40].

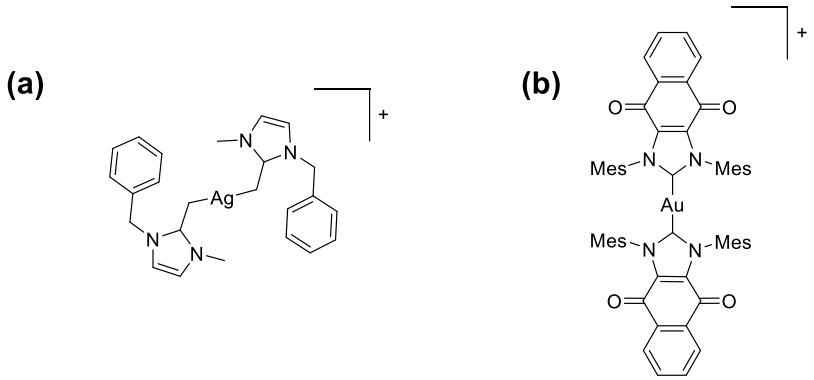

Figure 3. Recent examples of (a) silver and (b) gold organometallics assessed *in vivo*.

A dual targeting approach has been recently employed to rationally devise Au-NHC TrxR inhibitors that lead to the promotion of singlet oxygen generation [41]. The combination of a quinone-NHC moiety, as a redox cycling agent, and Au(I) centre (Fig. 4(b)), to coordinate within the TrxR active site, led to metallodrugs more active than doxorubicin against the A549 lung cancer cell line, and highly active against other cancer cell lines. In a zebrafish tumor xenograft model the Au-NHC complex was well tolerated and induced apoptosis in tumor xenografts.

Conclusions and future prospects

From a survey of recent literature, research encompassing the anticancer activity of organometallic compounds is flourishing and making significant progress [42-45]. Creative and novel strategies to enhance the efficacy and selectivity of small molecule organometallics show promise in animal models, with examples being well tolerated and doses well below maximum tolerated doses resulting in good antitumor activity. There is a clear trend for developing organometallics able to operate via multiple simultaneous mechanisms of action to exert anticancer activity, despite exciting prospects of organometallics designed to interact with a single target [46]. Multifactorial strategies offer benefits in overcoming cellular resistance and in, potentially, accessing low-dose therapeutic regimens, that are likely to be used in combination with targeted drugs, and the future of the field surely lies in this domain. There are still major challenges to be met to prevent off-target metallodrug accumulation and/or in the selective activation of a metallodrug in solely the target tumor environment. However, recent developments in the design of cancer cell specific prodrug activation and catalytic metallodrugs offer significant promise and are likely to inspire a new generation of prospective organometallotherapeutics. The most advanced organometallic compound, with respect to clinical development, is an antimalarial termed ferroquine, in which a ferrocenyl moiety replaces a phenyl ring in chloroquine to restore activity in resistant parasites. Although not covered in this review, ferrocene compounds have been extensively studied in medicinal chemistry [47]. Finally, whilst none of the compounds described in this review have yet reached the stage of clinical trials, organometallics have been evaluated on humans in relation to other diseases, and therefore the rapid progress of the field in recent years suggests these studies are not far away.

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