2016

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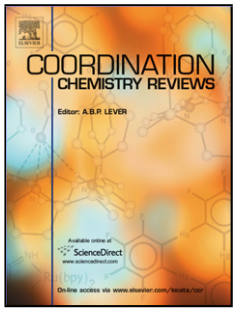

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PII: S0010-8545(16)30052-2
DOI: http://dx.doi.org/doi:10.1016/j.ccr.2016.03.014
Reference: CCR 112227

To appear in: *Coordination Chemistry Reviews*

Received date: 31-1-2016
Revised date: 27-3-2016
Accepted date: 28-3-2016

Please cite this article as: C.-H. Leung, L.-J. Liu, K.-H. Leung, D.-L. Ma, Epigenetic modulation by inorganic metal complexes, *Coordination Chemistry Reviews* (2016), http://dx.doi.org/10.1016/j.ccr.2016.03.014

Epigenetic modulation by inorganic metal complexes

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Abstract

Epigenetic mechanisms have emerged as key targets for therapeutic intervention. However, while a number of organic small-molecular “epidrugs” have been approved for clinical use, no

metal-based epigenetic modulator have been successfully approved. Metal complexes exhibit a variety of distinct properties that make them viable alternatives to organic molecules as therapeutic agents. In this review, we highlight recent examples of epigenetic modulation by metal complexes, with a view towards showcasing the different mechanisms exploited by metal complexes for targeting either epigenetic marks or epigenetic pathways for potential therapeutic applications.

Keywords

Transition metal complexes; Epigenetic; Epidrugs; Histone; Chromatin-modifying enzymes

Abbreviations

DNMTs DNA methyltransferase enzymes; R arginine; K lysine; S serine; T threonine; HATs histone acetyl transferases; HDACs histone deacetylases; lncRNAlong noncoding RNA; aza-dC5-aza-20-deoxycytidinedecitabine or Dacogen; PCAsN-substituted 2-pyridinecarbothioamides; NCPnucleosome core particle; SW480human colon carcinoma cell; CH1human ovarian cancer cell; A549human lung cancer cell; QED_w^{mo}maximum information content; RAPTA-C[(η^6-*p*-cymene)Ru(1,3,5-triaza-7-phosphaadamantane)Cl_2]; RAED-C[(η^6-*p*-cymene)Ru(ethylene-diamine)Cl]PF_6; ICP-MSinductively-coupled plasma mass spectrometry; SECsize exclusion chromatography; SHL superhelix location; QM/MM quantum mechanical/molecular mechanical; KP1019 indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III); HRPC hormone refractory prostate cancer; VA valproic acid; ACHP cis cis trans-diaminedichlorodihydroxy-platinum(IV); AA ascorbic acid; SAHA suberoylanilide hydroxamic acid; HDACis HDAC inhibitors; mal malonic acid malSAHA malonic acid-derivatized SAHA; A2780P cisplatin-sensitive ovarian cancer cell lines; NHDF normal human dermal fibroblast cells; JAHA Jay Amin hydroxamic acid; SubH suberoyl-bis-hydroxamic acid; *t*Bu_2bpy 4 4'-di-*tert*-butyl-22'-bipyridine; UV ultraviolet; Vis visible A2780cisR cisplatin-resistant A2780 cells; Ph phenyl; Cp cyclopentadiene; MCF7 human breast adenocarcinoma cell; A431 epidermoid carcinoma cell; HeLa human cervical cancer cells; A375 human malignant melanoma cell; B16F1mouse musculus skin

melanoma cell; TSA trichostatin A; MDA-MB-231 human caucasian breast adenocarcinoma cell; BRDs bromodomains; BET bromodomain and extra-terminal domain; P-TEFb positive transcription elongation factor; H4AcK4 tetra-acetylated lysine histone 4 peptide; ECM extracellular matrix; JMJD Jumonji C domain-containing; H3K9me3histone H3 trimethyl lys9; piq 1-phenylisoquinoline; dmobpy 4 4'-dimethoxy-2 2'-bipyridine.

1. Introduction

Epigenetic modulation has emerged as a promising strategy for the treatment of human diseases, particularly cancer.

Recently, a number of metal-based epigenetic modulators have been identified.

Metal complexes can target histones or epigenetic modification enzymes.

Many transition metal complexes have been used throughout history for the diagnosis and treatment of a wide variety of disorders [1-5]. The unique properties of transition metal complexes, such as their structural diversity, interesting photochemical and photophysical properties, and their ability to form specific interactions with biomolecules, make them versatile alternatives to organic small molecules for drug discovery [6-10]. Alternatively, metal complexes have also been used as functional scaffolds for the development of sensory probes [11-15].

Structurally, metal complexes consist of a metal center surrounded by a number of organic or inorganic ligands via coordination bonds. Metal complexes can adopt a large range of structural

types, including octahedral or square-planar geometries, which are unavailable to purely organic small molecules. This feature can allow metal complexes to sample regions of chemical space within biomolecules that cannot be accessed by organic compounds. Additionally, metal complexes can perform ligand exchange reactions with biological molecules, forming covalent adducts that can perturb cellular function. Importantly, the lability of metal-ligand bonds can be tuned by the choice of metal ion, its oxidation state, and auxiliary ligands. Finally, metal complexes can coordinate ligands that are themselves biologically active, leading to dual function or even synergistic effects between the metal ion and the ligand.

The most famous examples of metal-based drugs are the platinum anticancer compounds [16], such as cisplatin and its analogues, which have been approved for the treatment a number of human cancers. The archetypical platinum drugs target DNA via covalent interactions, forming DNA cross-links that trigger cellular apoptosis. However, this relatively non-specific mode of action can lead to side effects due to damage to normal cells; furthermore, resistance to platinum drugs can often be developed. This has stimulated the development of other types of metal complexes that act via alternative modes of action, in order to both reduce adverse effects as well as to bypass existing mechanisms of anticancer drug resistance.

Epigenetic modulation refers to changes in chromatin structure that affect how genes are expressed, but are independent of DNA sequence. In recent years, the discovery that epigenetic dysfunction may be implicated in the pathogenesis of various human diseases has opened up new avenues for potential therapeutic intervention. However, only a few examples of metal complexes targeting epigenetic marks or pathways have been reported. In this review, we

highlight recent examples of epigenetic modulation by metal complexes to illustrate the potential that inorganic compounds can have in this area. This is an emerging discipline, and to our knowledge, no review of this nature has yet been published in the literature. This review aims not to be exhaustive, but intends to showcase recent examples of metal complexes that can target epigenetic pathways via various mechanisms for potential therapeutic applications. Broadly speaking, the metal complexes in this review target epigenetic pathways via three distinct mechanisms. In the first mechanism, the metal complex acts as a "chaperone" for a bioactive ligand that is subsequently released upon activation within the cell. Having released the ligand, the metal moiety itself may also possess bioactivity. In the second approach, a metal complex with labile ligands is able to form covalent adducts with DNA or proteins involved with epigenetic regulation. In the third mechanism, kinetically-inert metal complexes interact non-covalently with epigenetic targets. Broader reviews in the general area of inorganic medicinal chemistry have been recently published by the groups of Sadler [17], Megger [18], Sheldrick [19], Che [20], Gasser [21], Hartinger [22], Ott [23], Barton [24] and others [25-27].

2. Mechanisms of epigenetic modulation

In eukaryotes, nuclear DNA and histone proteins are intimately intertwined in a macromolecular structure known as chromatin. The basic functional module of chromatin is the nucleosome, which is structure consisting of a 147-base pair long DNA sequence wrapped around a heterooctameric histone complex. Two of each histone proteins H2A, H2B, H3, and H4 constitute an octamer. Histone proteins play a vital role in the organization and packaging of eukaryotic DNA [28].

The term “epigenetics” loosely describes heritable modifications to chromatin that affect gene expression without involving changes in the DNA sequence, although no consensus definition of epigenetics has been universally agreed upon [29]. Modifications to both DNA and histones are continually added (by epigenetic “writer” enzymes) and removed (by epigenetic “eraser” enzymes) in a tightly choreographed manner. The major epigenetic mechanisms are DNA methylation and hydroxymethylation, as well as various histone modifications [29-32]. In differentiated mammalian cells, the principal epigenetic tag found in DNA is the covalent attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotides [33, 34]. Cytosines other than those in CpG can also be methylated in undifferentiated stem cells, and that this methylation is also crucial for gene regulation [35]. A family of DNA methyltransferase enzymes (DNMTs) are implicated in de novo DNA methylating activity and differential maintenance. DNMT1 functions in the replication complex to recognize hemimethylated DNA and to add methyl groups in the corresponding CpG site to newly formed daughter DNA strands [33, 36].

Histone modifications include the methylation of arginine (R), the methylation, acetylation, ubiquitination and sumoylation of lysine (K), and the phosphorylation of serine (S) and threonine (T) [37]. Recent advances in the field have determined that lysine acetylation and methylation are key epigenetic modulator marks for transcriptional activation or repression [38]. Histone acetylation has been implicated in the control of chromatin condensation, DNA repair and replication [39]. Levels of acetylation are controlled by the competing activities of two enzymatic families: the histone acetyl transferases (HATs) that ‘write’ acetylation sites and the histone deacetylases (HDACs) that ‘erase’ acetylation sites. Recently, long noncoding RNAs

(lncRNAs, defined as RNAs > 100 nucleotides in length) have become a new paradigm for epigenetic regulation [30, 40] due to their roles in imprinting and X-chromosome inactivation [41].

Many diseases, including cancer, heart disease, diabetes, and mental illnesses are influenced by epigenetic mechanisms, and epigenetic therapy offers a potential way to influence those pathways directly [42-44]. A number of these so-called “epidrugs” have reached clinical trials or even been approved for the treatment of cancer. Notable examples include 5-azacytidine (Vidaza) and 5-aza-20-deoxycytidine (known as aza-dC, decitabine or Dacogen), which are DNMT1 inhibitors approved for the treatment of myelodysplastic syndromes (MDS), and suberoylanilide hydroxamic acid (SAHA, also known as vorinostat or Zolinza) or romidepsin, HDAC inhibitors approved for the treatment of advanced cutaneous T-cell lymphoma. However, to our knowledge, no metal-based epigenetic modulator has yet been approved for clinical use.

The following sections discuss examples of metal complexes that have been reported to either target histones directly, or enzymes that modify chromatin. We intend to showcase the various mechanisms that have been exploited by researchers in the design of metal-based epigenetic modulators in order to achieve their desired biological functions.

3. Metal complexes targeting histones

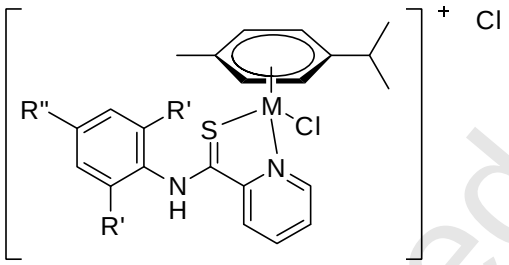
Ruthenium complexes have emerged as promising alternatives to platinum-based drugs due to their potent activities against various cancers and favorable pharmacokinetic properties [45]. In 2013, a series of organometallic anticancer compounds based on ruthenium(II) and osmium(II)

complexes bearing *N*-substituted 2-pyridinecarbothioamides (PCAs) were developed by Hartinger and co-workers (Fig. 1) [46]. Interestingly, while the isolated ligands have shown gastric activity as mucosal protectants in vivo [47], the coordination of these ligands into a metal complex led to the formation highly active anticancer compounds. The ruthenium(II) complex **1A** hydrolyzed more rapidly in aqueous solution compared with osmium complex **1B**, which is consistent with the osmium center's greater kinetical stability compared with ruthenium. Once hydrolyzed, both complexes were stable over pH 1.74–11.62. Notably, their stability to hydrochloric acid suggested that they could withstand the acidic milieu of the stomach.

Crystallographic studies of complexes **1A**, **3A**, **1B**, and **3B** with the nucleosome core particle (NCP) were conducted, which showed that the Os(II) complexes **1B** and **3B** possessed identical binding sites with the histone octamer. Both complexes formed adducts with His-106 on H2B in a similar fashion to RAPTA-C. H2B His-79 was the second binding site that was situated in a cleft between two halves of the nucleosome with substantial numbers of hydrophobic histone groups, and the third binding site was located at the histone dimer-dimer and dimer-tetramer interfaces of the octamer and may thus influence the dynamics of chromatin. Meanwhile, the two Ru(II) complexes **1A** and **3A** exclusively formed adducts on the histone proteins at the second and third binding sites.

The cytotoxicity of complexes **1A**–**6B** was further evaluated in human colon carcinoma SW480, human ovarian cancer CH1 and human lung cancer A549 cell lines. Notably, the complexes were most active in the cisplatin-resistant, P-glycoprotein-overexpressing SW480 cells as well as in the CH1 cell line. Complexes containing the *N*-phenyl, *N*-4-fluorophenyl and *N*-mesityl ligands

showed the strongest antiproliferative activity towards CH1 and SW480 cells with IC_{50} values lower than 8 μM, but also moderate cytotoxic effect in A549 cells with IC_{50} values between 14 to 30 μM. Comparison of Ru(II) and Os(II) congeners showed that neither series were consistently superior. Furthermore, the cytotoxicity activity of the complexes was associated with the lipophilicity of complexes. Finally, computational assessment of the druglikeness of the synthesized complexes using the weighted quantitative estimate of drug-likeness based on maximum information content (QED_w^{mo}) methodology suggested that these compounds displayed similar druglikeness compared with known anticancer drugs.



1A: M = Ru, R' = H, R" = H;
2A: M = Ru, R' = H, R" = OH;
3A: M = Ru, R' = H, R" = F;
4A: M = Ru, R' = CH_3, R" = CH_3;
5A: M = Ru, R' = H, R" = morpholinyl;
6A: M = Ru, R' = H, R" = benzoyl;
1B: M = Os, R' = H, R" = H;
2B: M = Os, R' = H, R" = OH;
3B: M = Os, R' = H, R" = F;
4B: M = Os, R' = CH_3, R" = CH_3;
5B: M = Os, R' = H, R" = morpholinyl;
6B: M = Os, R' = H, R" = benzoyl;

Fig. 1. Chemical structures of ruthenium complexes **1A–6B** and osmium complexes **1B–6B** [46].

In 2014, Davey and co-workers studied the biological mechanisms of two prototypical ruthenium-arene compounds: the non-cytotoxic antimetastasis compound [(η^6-*p*-cymene)Ru(1,3,5-triaza-7-phosphaadamantane)Cl_2] **7** (RAPTA-C) and the relatively cytotoxic anti-primary tumor compound [(η^6*p*-cymene)Ru(ethylene-diamine)Cl]PF_6 **8** (RAED-C) (Fig. 2) [48]. **7** forms adducts with histone proteins, while **8** preferentially targets the DNA of chromatin

[49]. An inductively-coupled plasma mass spectrometry (ICP-MS) study of **7** and **8** in cancer cells showed that of the chromatin-bound adducts, most of **7** was located with the protein component, while the majority of **8** was associated with the DNA component. In size exclusion chromatography (SEC)-ICP-MS experiments, **7** and **8** were revealed to react with the NCP to yield stable adducts with association constants of 2.3 X 10^5 M^{-1} and 6.7 X 10^5 M^{-1}, respectively. However, **8** reacted three times faster than **7** and formed 71% of adducts with DNA, whereas **7** formed 85% adducts with histone proteins.

A nuclease digestion DNA footprinting assay with both naked DNA and NCPs revealed that **8** selectively formed adducts with guanine nucleotides with a preference for GG sites, suggesting that electrostatic attraction might be a contributing factor for reactivity, as observed for simple metal hydrates. On the other hand, the weak binding of **7** to DNA prevented the identification of strong footprints except at the termini and a single site at SuperHelix Location (SHL) ±1.5. X-ray crystallography analysis of NCP-**7** complexes revealed that **7** adducted to the NCP at three sites, involving bivalent coordination to glutamate, histidine and lysine side chains of the histone proteins. Treatment of NCP crystals with **8** also resulted in adduct formation at two different histone sites. The first site (histone glutamate) is also common to **7**, but the second involved adducts with DNA. Hybrid quantum mechanical/molecular mechanical (QM/MM) calculations indicated that bulky phosphaadamantane ligand of **7** was a key factor that determined the histone/DNA site selectivity preference between the two complexes.

Interestingly, the apoptosis and cell cycle profiles between cells treated cisplatin, **7** or **8** were markedly distinct, as demonstrated by a multivariate analysis method. Surviving cells recover more rapidly after treatment with **7** or **8** compared with cisplatin. **8** was more toxic towards

A2780 cells (IC_{50} = 4.5 μM) and cisplatin-resistant crA2780 (IC_{50} = 6.8 μM) cells than **7** (IC_{50} = 247 and 507 μM, respectively). Additionally, **7** was more effective than **8** at inducing G2/M-phase arrest. Importantly, while cisplatin was 14-fold less potent against cisplatin-resistant crA2780 cells relative to A2780 cells, **7** and **8** displayed only a slight degree of cross-resistance, at 2.1-fold and 1.5-fold, respectively.

This study was important because it demonstrated that control over the biomolecular targets of ruthenium-arene agents can be tuned simply by replacement of a single ligand. Switching the ethylenediamine ligand of the [Ru-cymene-Cl]$^{+}$ scaffold to phosphaadamantane (and Cl^{-}) transformed selectivity from primarily targeting DNA to targeting chromatin proteins. Additionally, the ability of **7** and **8** to recognize both chemical and structural features of nucleosomes could potentially allow them to target epigenetic weak points of cancer cells.

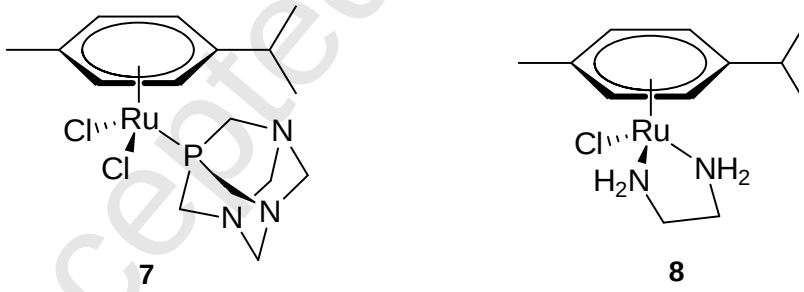


Fig. 2. Chemical structures of **7** (RAPTA-C) and **8** (RAED-C) [48].

KP1019 [indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] **9** (Fig. 3) shows promising anticancer activity against colorectal tumor cells both *in vitro* and *in vivo* [50]. The mechanism of action of KP1019 has been linked with DNA damage, induction of cell cycle delay, and

apoptosis [51, 52]. However, the exact molecular targets(s) of KP1019 had been unclear. Recently, Tomar and co-workers utilized *Saccharomyces cerevisiae* as a model organism to elucidate the molecular target of KP1019 [53]. KP1019 was revealed to eject histones from nucleosomal DNA and interact with histone H3, but without affecting acetylated H4K8 *in vitro*. Furthermore, various histone H3 mutants, particularly histone tail mutants, showed enhanced sensitivity to the antiproliferative activities of KP1019. As histone tails play an important role in maintaining nucleosome structure, these results suggest that KP1019 exerts its effect by further destroying nucleosome structure in these mutants. In normal cells, H3K56 is acetylated during DNA damage repair [54]. The markedly increased sensitivity of the H3K56A mutant to KP1019 suggests that this drug causes DNA damage, consistent with previous reports. This study is significant because it provides a possible epigenetic mechanism by which the well-known anticancer drug KP1019 can exert its antiproliferative effects.

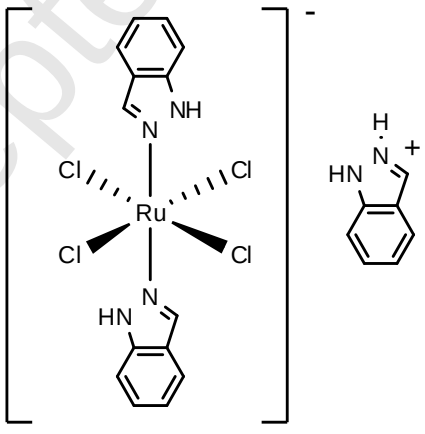


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Fig. 3. Chemical structure of **9** (KP1019) [53].

4. Metal complexes targeting chromatin-modifying enzymes

4.1. Histone deacetylases (HDACs)

Despite its use as a first-line antitumor agent against different types of cancer, cisplatin can exert severe toxic side effects to normal cells [55]. Octahedrally-coordinated platinum(IV) compounds have lower toxicity but can be reduced intracellularly to platinum(II) to recover their cytotoxicity [56-58]. The kinetic inertness of the Pt(IV) center allows those complexes to bypass many of the problems associated with cisplatin and its analogues [59]. Furthermore, carboxylate ligands can be easily incorporated into the octahedral Pt(IV) scaffold to further modulate the lipophilicity, stability, redox behavior, and biological activity of the prodrugs [60]. Satraplatin, an analogue of cisplatin that contains two acetate ligands in the axial positions, has entered Phase III clinical trials for the treatment of the patients with hormone refractory prostate cancer (HRPC) [61].

Valproic acid (VA), a commonly-used antiepileptic and anticonvulsant drug in the clinic, has recently been identified as one of the short-chain fatty acid class of HDAC inhibitors [62, 63]. Similar with other HDAC inhibitors, VA exerts its anticancer effects in the control of biological processes in cell cycle arrest, cell apoptosis, metastasis, angiogenesis, differentiation, and senescence [64, 65]. Based on the structure of satraplatin, Tang, Shen and co-workers proposed in 2012 that VA could be used as ligand of a Pt(IV) complex to form a satraplatin-like Pt(IV)−VA (VAAP, complex **10**) prodrug (Fig. 4) [66]. In the design of this complex, VA are used as axial ligands, trans to one another, so that these ligands are released upon reduction to Pt(II). VAAP was synthesized by the reaction of cis,cis,trans-diaminedichlorodihydroxy-platinum(IV) [$Pt(NH_3)_2Cl_2(OH)_2$, ACHP] with VA. A HDAC inhibition activity assay revealed that the inhibition

of HDAC activity by VAAP could be dramatically increased when VAAP was first treated with the reducing agent ascorbic acid (AA). This indicates that the reduction of VAAP to platinum(II) by AA results in the release of free VA, leading to the inhibition of HDAC activity. Additionally, this result also suggests that the Pt(II) ion does not interfere with HDAC inhibition by VA.

VAAP showed low micromolar cell growth inhibition rate against a panel of four tumor cell lines, human lung carcinoma A549 (IC_{50} = 0.15 μM), human breast cancer BCap37 (IC_{50} = 0.20 μM), human ovarian carcinoma SKOV-3 (IC_{50} = 0.17 μM) and human hepatocellular carcinoma HepG2 cell lines (IC_{50} = 0.14 μM). Importantly, the potency of VAAP was superior to ACHP, VA or a mixture of ACHP and VA, indicating that the combination of the Pt(IV) complex with the VA ligands was essential for enhanced cytotoxicity. Notably, VAAP was even more potent compared with cisplatin. Cellular distribution studies suggested that VAAP could efficiently bind to a cell membrane owing to its hydrophobicity, and then further enter into the cytosol. In the nucleus, the increase of histone acetylation by VA could loosen histone−DNA interactions, allowing the binding of the Pt(II) complex to DNA. Therefore, the synergy of the Pt(II) complex and VA could explain VAAP's high cytotoxicity. *In vivo*, VAAP significantly inhibited tumor growth in an A549 tumor xenograft model as compared with ACHP, and also caused no effects on mice body weights and nephrotoxicity, in contrast to ACHP. This study illustrates an important advantage of metal complexes, in that bioactive ligands can be easily attached to the metal scaffold, leading to the possibility of additive or even synergistic effects in the cell. One aspect of this study was that a direct comparison between the cytotoxicity of VAAP and a mixture of VAAP's reduction products, i.e. cisplatin and VA, was not performed, so the actual extent of synergism contained within the molecule seems to be unclear. Additionally, there was

no direct evidence in the study that showed that VAAP was reduced in the intracellular environment. The fact that VAAP showed significantly less HDAC inhibitory activity in nuclear extracts compared to AA-pretreated VAAP suggests that the crucial reduction process may represent rate-limiting step in the activation of this compound in cells.

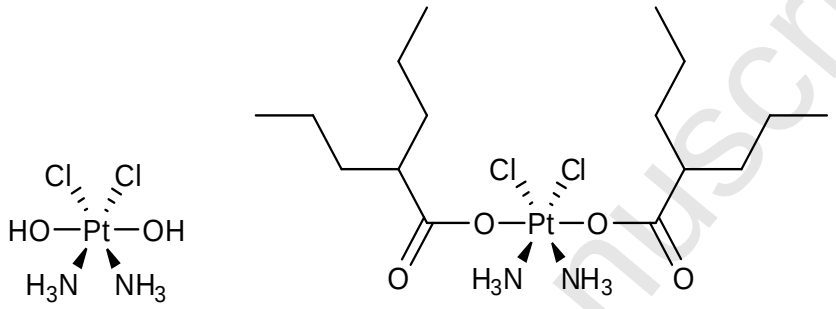

Fig. 4. Chemical structures of ACHP and **10** (VAAP) [66].

SAHA is a HDAC inhibitor approved for clinical use by FDA to treat numbers of hematological and solid tumors [67, 68]. Crystal structures of human HDACs with SAHA bound show that the hydroxamic acid moiety coordinates with the active-site zinc ion [69]. Several HDAC inhibitors (HDACis) have thus been designed that are comprised of a metal-binding group, a linker domain that occupies a narrow channel and a cap group which interacts with residues on the enzyme surface.

In 2009, Marmion and co-workers attached a malonic acid (mal)-derivatized SAHA analogue, malSAHA, to a Pt(II) complex (Fig. 5) [64]. The *cis*-[Pt(II)$(NH_3)_2$(malSAHAH_{-2})] **11** (where malSAHAH_{-2} is the doubly-deprotonated form of malSAHA) was found to bind to and unwind

DNA as determined by electrophoresis in a similar fashion to cisplatin. Moreover, **11** exhibited HDAC1 inhibitory activity at low micromolar concentrations, albeit with reduced potency compared with SAHA and malSAHA. Finally, **11** showed comparable cytotoxicity to cisplatin against the cisplatin-sensitive ovarian cancer cell lines A2780P, but was less towards non-tumorigenic, normal human dermal fibroblast cells, NHDF. Additionally, the cis-[$Pt^{II}(NH_3)_2(malH_{-2})$] (where $malH_{-2}$ is the doubly-deprotonated form of mal) was nearly twofold less cytotoxic compared with *cis*-[$Pt^{II}(NH_3)_2(malSAHAH_{-2})$], indicating that the incorporation of the malSAHA moiety in **11** enhances cytotoxicity. This study demonstrates that the toxicity of platinum drugs may be potentially reduced by conjugation with bioactive moieties, such as SAHA, that are known to be specific for cancer cells over normal cells.

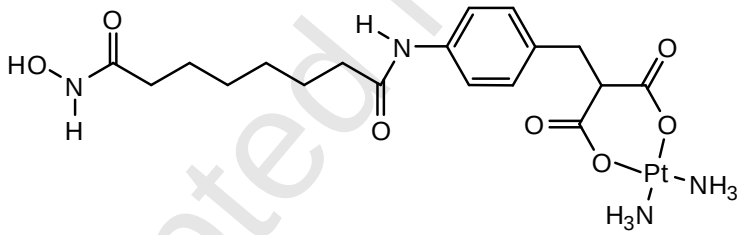

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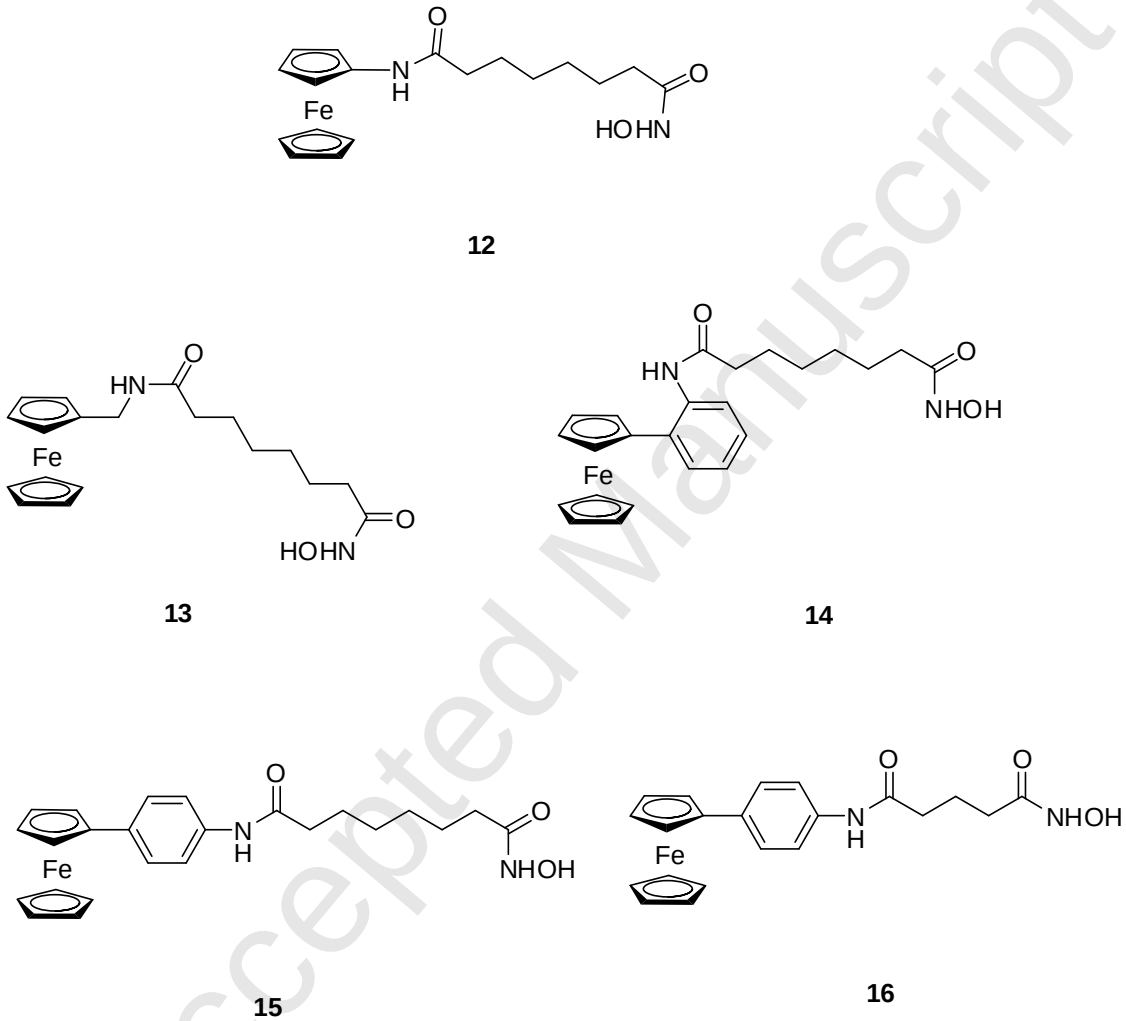
Fig. 5. Chemical structure of **11** [70].

Later, Spencer and co-workers designed a novel iron-based SAHA derivative to study the impact of a nonplanar-phenyl bioisostere, the ferrocene unit, on HDAC inhibitory activity (Fig. 6) [71]. The combination of protein-binding ligands with organometallic moieties can explore regions of chemical space unavailable to simple aromatic motifs or alicyclic ring scaffolds. Complex **12**, termed Jay Amin hydroxamic acid (JAHA) was suggested by molecular docking to bind in a similar fashion to SAHA. JAHA formed the archetypal interaction between the hydroxamate

moiety and the catalytic zinc ion, as well as a hydrogen bond between the ligand amide group and Asp101. Moreover, the ferrocenyl group of **12** was situated in a shallow pocket formed by Tyr100, Phe152, and Tyr306.

Class I HDACs (HDACs 1, 2, 3, and 8) are predominantly nuclear proteins that are ubiquitously expressed in most tissues and cell lines, while class II HDACs (which are further subdivided into class IIa, containing HDACs 4, 5, 7, and 9, and class IIb, containing HDACs 6 and 10) shuttle between the nucleus and the cytosol and may have tissue-specific functions. Early HDAC inhibitors such as SAHA act unselectively, a feature that may be responsible for their adverse side effects. Therefore, research efforts have been devoted towards the elucidation of biological roles played by specific HDACs in cells, as well as the identification of inhibitors that are selective for only a subset of HDAC members [72]. In this study, JAHA **12** and a library of JAHA analogues **13–16** were screened for HDAC inhibitory activity against a variety of HDACs. **12** had a similar, broad HDAC inhibitory profile to SAHA. Complex **13**, which contains an extra methylene linker compared with **12**, showed similar inhibition on HDACs 1-3 but was also four-fold more potent against HDAC8 and 10-fold less potent against HDAC6. Complex **15** exhibited the most potent inhibitory activity towards HDACs 1, 2, and 6, while complex **14** showed the highest potency towards HDAC8. Complex **16**, containing a shorter alkyl chain length, displayed poor HDAC inhibition. None of **12–16** displayed any significant inhibition of class IIa HDACs. However, these compounds were less toxic to MCF7 breast cancer cells than SAHA, which was attributed to their lower cellular permeability as a result of the ferrocene group. Interestingly, flow cytometry experiments revealed that **14** and **15** promoted both bulk chromatin acetylation (the endogenous substrate of class I HDACs) and the acetylation of α-tubulin (a substrate of

HDAC6) with comparable potency to SAHA. This study illustrates that modification of the aryl "cap" of SAHA can be achieved without greatly affecting its HDAC inhibitory activity.

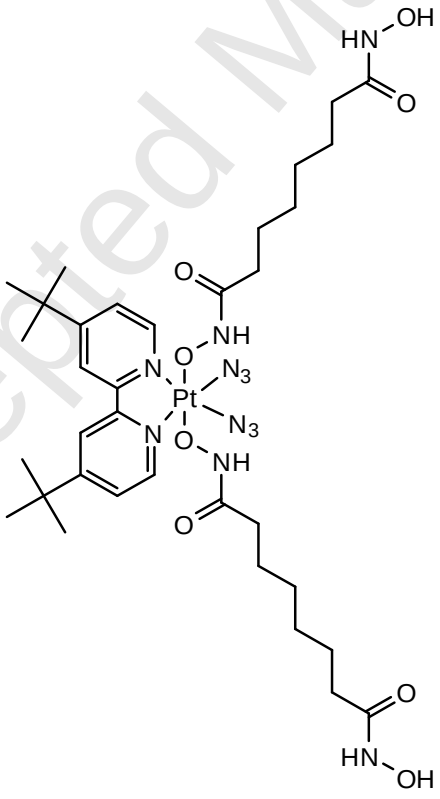


12 **13** **14** **15** **16**

Fig. 6. Chemical structures of JAHA **12** and JAHA analogues **15–16** [71].

A photoactivatable platinum(IV)-diazido complex conjugated with suberoyl-bis-hydroxamic acid (SubH) as axial ligands has been recently reported to target genomic DNA and HDACs by K asparkova and co-workers (Fig. 7) [73]. cis,*trans*-[$Pt(N_3)_2(Sub)_2(tBu_2bpy)$] (where tBu_2bpy = 4,4'-di-*tert*-butyl-2,2'-bipyridine) **17** could be selectively activated in tumor cells by low

intensity ultraviolet (UV) or visible (Vis) irradiation, causing the simultaneous release of the cytotoxic Pt(II) species and the HDAC inhibitor SubH. HDAC activity in human ovarian carcinoma A2780 cells was decreased when the cells irradiated with either UVA or Vis light in the presence of **17**, while the total levels of genome-wide histone H3 acetylation were increased. In addition, the photoactivated complex **17** was more highly toxic to cisplatin-resistant A2780cisR cells than its photoactivatable Pt(IV) analogues containing biologically inactive axial ligands, or the Pt(II) anticancer drug cisplatin. This result was attributed to the ability of **17** to inhibit HDAC activity via SubH, which increases histone acetylation levels and enhances the accessibility of chromatin DNA to damage induced by the platinum moiety.



17

Fig. 7. Chemical structure of **17** [73].

Previous studies had indicated that highly potent selective estrogen receptor inhibitors could be synthesized by replacing the phenyl (Ph) ring in tamoxifen by [$Re(CO)_3$(Cp-R)] (Cp = cyclopentadiene) [74]. Inspired by this concept, Alberto and co-workers reported the synthesis and biological activity of new organometallic analogs of SAHA by replacing the terminal phenyl cap of SAHA with [$Re(CO)_3$(Cp-R)] (Fig. 8) [75]. The antitumor activity of the organometallic HDAC complexes **18**–**20** were then evaluated towards five carcinoma cell lines, MCF7, A431, HeLa, A375 and B16F1 cells. Complexes **18**–**20** were slightly less active compared with SAHA, indicating that the bulkier [$Re(CO)_3$(Cp)] moiety is disfavored over a simple planar Ph ring, possibly as a result of cellular penetration. Moreover, no significant effect on cytotoxicity was observed by altering the position of the amide linker at the Cp. In a more recent approach, Tan, Mao and co-workers have conjugated a SAHA derivative to a Re(I) tricarbonyl complex **21** also bearing a 4,7-diphenyl-1,-10-phenanthroline N^N ligand to generate a phosphorescent HDAC inhibitor that targeted mitochondrial and induced paraptosis [76].

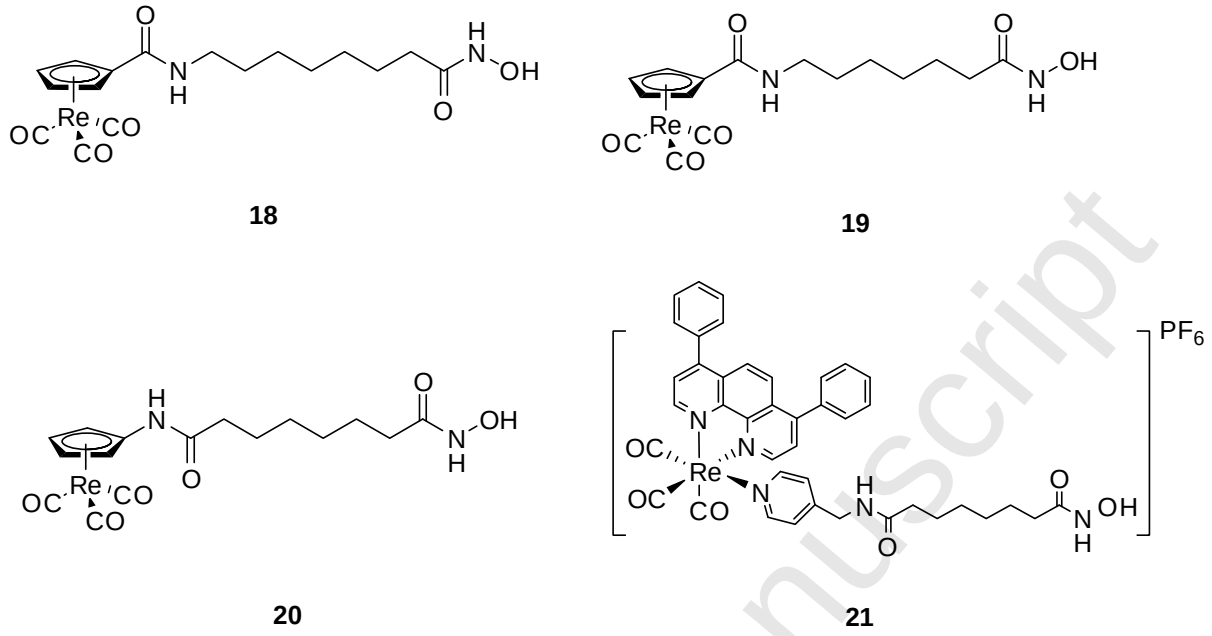
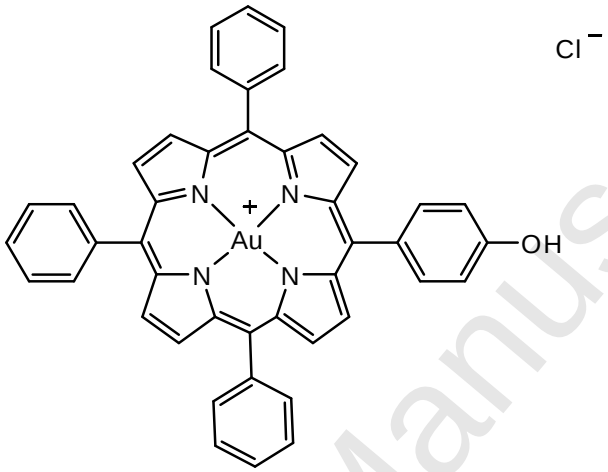


Fig. 8. Chemical structures of **18**–**21** [75, 76]**.**

Gold(III) complexes have shown promise as antitumor agents [77, 78]. A novel gold(III) porphyrin analogue [5-hydroxyphenyl-10,15,20-triphenylpor-phyrinato gold(III) chloride)] **22** developed by Yang, Che and co-workers selectively inhibited Wnt/β-catenin signaling through modulating HDAC activities (Fig. 9) [79]. Complex **22** significantly increased DNA fragmentation and apoptosis in MDA-MB-231 cells, and also inactivated Wnt/β-catenin signaling by decreasing the protein levels of β-catenin through transcriptional regulation. **22** was enriched in the nucleoid fractions, where it potently inhibited all class I HDACs, including HDAC1, HDAC2, HDAC3, and HDAC8, with comparable potency to positive control compound trichostatin A (TSA). Furthermore, **22** enhanced the binding of acetylated histone H4 to related Wnt signaling molecules promoters, leading to transcriptional inactivation. Molecular docking studies indicated that **22** interacted with the binding pocket at the surface of HDAC8 with a favorable

binding energy of −9.67 kcal/mol. Finally, **22** attenuated mammary MDA-MB-231 tumor growth in nude mice. This study suggested that gold(III) porphyrins could potentially be developed as HDAC inhibitors with promising *in vivo* anticancer activities.



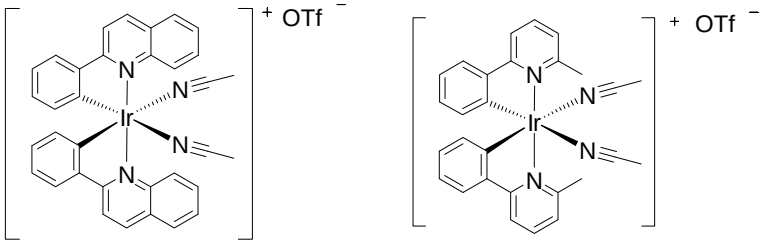
22

Fig. 9. Chemical structure of **22** [79].

4.2. BRD4

Bromodomains (BRDs) are protein-interaction modules that recruit chromatin-modifying enzymes to specific sites by recognizing acetylated lysine residues on histone tails [80-82]. In order words, BRDs act as epigenetic 'reader' modules [83]. BRD4, as a member of the bromodomain and extra-terminal domain (BET) family, regulates transcription in postmitotic cells by recruiting the positive transcription elongation factor (P-TEFb) to target promoters in response to extracellular signals [84]. Recently, BRD4 has become a potential target for epigenetic therapy because of its roles on cell cycle progression and sustaining the proliferation of tumor cells [85]. Recently, our group has discovered the first iridium(III)-based, irreversible

inhibitor of BRD4. Preliminary screening of a diverse set of complexes identified the iridium(III) organometallic compound **23**, possessing two 2-phenylpyridine C^N ligands and two acetonitrile ligands, which inhibited the protein–protein interaction between tetra-acetylated lysine histone 4 peptide (H4AcK 4) and BRD4 (Fig. 10) [86]. Structure-based optimization furnished compound **24**, containing two 2-phenyl-6-methyl-pyridine C^N ligands and two acetonitrile ligands, which was 10-fold more active than **23** in the *in vitro* assay and comparably potent to (+)-JQ1, a potent and highly specific inhibitor of the BET BRDs. Electrospray-ionization mass spectrometry analysis indicated that **24** bound irreversibly to BRD4, presumably via coordination to a histidine residue. While the kinetics or sequence of the ligand exchange process were not determined, the ease at which the acetonitrile ligands were replaced by DMSO after incubation in DMSO solution suggests that in an aqueous environment, complex **24** will be hydrolyzed to the corresponding *aquo* complex before reaction with its biomolecular target. Compound **24** also exhibited selectivity for BRD4(1) over BRD4(2). Additionally, **24** was able to suppress transcription of the MYC promoter and the c-*myc* expression both *in vitro* and *in vivo*. Importantly, **24** significantly repressed A375 melanoma xenograft growth *in vivo* without causing visible toxicity to the mice. Microarray analysis revealed that **24** could up-regulate the expression of genes involved in the extracellular matrix (ECM) pathway and down-regulate the expression of genes related to the VEGF signaling pathway, which was attributed to the attenuation in BRD4-directed transcriptional activity *in vivo.*



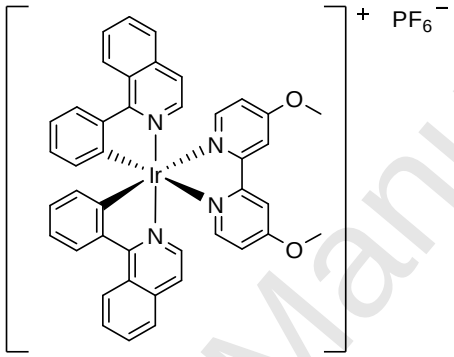
23 **24**

Fig. 10. Chemical structures of **23** and **24** [86]**.**

4.3. JMJD2

Lysine methylation has substantial roles in chromatin dynamics [87]. Changes in the regulation of histone lysine methylation have been linked to the dysregulation of histone lysine methyltransferases or demethylases [88, 89]. JMJD (Jumonji C domain-containing) proteins, the second family of histone demethylases requiring Fe^{2+}, O_2, and 2-oxoglutarate to function, have been considered as candidate oncogenes for tumor formation [90]. The JMJD2 proteins, composed of members JMJD2A-E, are one of the largest and most extensively studied JMJD subfamilies [91]. Recently, our group have identified a kinetically-inert organometallic compound $[Ir(piq)_2(dmobpy)](PF_6)$ (where piq = 1-phenylisoquinoline and dmobpy = 4,4'-dimethoxy-2,2'-bipyridine) **24** as a potential epigenetic modulator through its inhibition of JMJD2 activity (Fig. 11) [91]. Compound **25** inhibited the activity of JMJD2D with an IC_{50} value of 15 μM, as revealed by a decrease of the demethylation of a trimethylated peptide substrate, histone H3 trimethyl lys9 (H3K9me3). Besides JMJD2D, compound **25** also inhibited JMJD2A, JMJD2B, JMJD2C, and JMJD2E, **25** at 50 μM. Notably, **25** electively inhibited JMJD2 activity over other Jumonji domain-containing proteins including JMJD3 or JARID, as well as HDACs. Moreover, **25** potently blocked the trimethylation of the *p21* promoter on H3K9me3 and

disrupted the interaction of JMJD2D-H3K9me3 in human lung cancer A549 cells. Finally, **25** suppressed cancer cell progression and induced cancer cell apoptosis with IC_{50} values of about 0.15 μM. This study suggests that iridium(III) complexes could potentially be developed as epigenetic modulators through their ability to inhibit JMJD2 activity. However, the precise mechanism of action of this complex was not determined.



25

Fig. 11. Chemical structure of **25** [91].

5. Conclusion

While many new compounds are highly successful during *in vitro* and preclinical studies, half of all drug candidates that enter clinical trials fail due to problems such as poor bioavailability, low efficacy and severe side effects. In this context, coordination of a drug to a metal complex can greatly improve aqueous solubility, passive cellular uptake and absorption, or nuclear localization [92-94]. In addition, the metal complex can itself be biologically active. A number of the examples in this review have conjugated a known epigenetic-modulating drug to a metal complex scaffold, which acts as a chaperone. For example, Tang, Shen and co-workers have incorporated VA, a HDAC inhibitor, into a Pt(IV) scaffold [66]. The reduction of the Pt(IV) prodrug VAAP inside the cell releases both VA and the cytotoxic Pt(II) moiety, leading to

simultaneous DNA damage and the impairment of DNA repair. Notably, VAAP was more potent than both cisplatin as well as the isolated metal complex and ligands, demonstrating that the assembly of the subunits into an intact complex was critical for biological activity. A similar principle was employed by Marmion and co-workers, who grafted a SAHA analogue onto a Pt(II) diammine core [70]. Such dual-action drugs may be more effective than the original parent drug(s), and may also be able to circumvent existing drug resistance mechanisms and/or achieve greater selectivity for cancer cells over normal cells. However, one drawback of the hybrid approach is that the attachment of bioactive ligands to metal scaffolds makes it more difficult to modulate the toxicity of the components, as it would be possible for combination therapies. Moreover, synthetic difficulties may restrict the types of ligands and metal scaffolds that can be conjugated together to form hybrid compounds.

Besides drug-metal complex conjugates, other metal complexes appear to directly target protein via either covalent or non-covalent modes of action. For example, RAPTA-C, a promising antimetastasis compound whose precise mechanism of action has not yet been fully elucidated, was recently shown by Davey and co-workers to bind to histone proteins at three different sites [48]. Notably, that study also showcased the fact that selectivity of a particular metal complex class for molecular targets (e.g. histones or DNA) can be switched simply by replacement of one ligand for another. The iridium(III) *solvato* complexes developed by our group also selectively and covalently interact with BRD4 in order to exert their epigenetic modulatory effects [86]. Kinetically-inert metal complexes, such as the JMJD2-inihbiting iridium(III) compound developed by our group, represent another means of inhibitor epigenetic modifying enzymes

[91]. A summary of the targets and mechanisms of the inorganic epigenetic modulators highlighted in this review is presented in Table 1.

Towards the future, we envision that as biochemical and cellular investigations into epigenetic regulation continue to progress, additional epigenetic molecular targets may be unveiled that could potentially be targeted by metal complexes. Indeed, we have highlighted recent mechanistic investigations into existing metal-containing drug candidates, such as RAED-C, RAPTA-C and KP-1019 that reveal that those compounds may be targeting epigenetic proteins in order to exert their antiproliferative activity. However, despite the promising preliminary studies that we have described here, more work needs to be done in order to further progress the field of inorganic epigenetic modulators. One key issue that has to be addressed is selectivity. Given that many of these complexes interact with biomolecules covalently, the unwanted binding of metal complexes to cellular machinery should be avoided as much as possible. Possible strategies include the prodrug approach discussed above, where Pt(IV) species are reduced to bioactive Pt(II) within the cell, or perhaps a photoactivatable release mechanism such as the SubH-linked Pt(II) complex described by Kasparkova and co-workers. An alternative mechanism for achieving selectivity may be in the use of kinetically-inert metal complexes, for which the group of Meggers has convincingly shown can display tremendous selectivity for target kinases despite using purely non-covalent interactions [18, 95, 96]. However, only one kinetically-inert epigenetic modulator (the JMJD2-inhibiting iridium(III) complex) appears to have been reported so far. Additionally, given that the ultimate goal of these compounds is human therapy, we encourage researchers to test their candidate complexes in *in vivo* animal models to ascertain whether or not the *in vitro* activities of the

compounds can be reproduced in a more complicated system. Finally, although no metal-based compound developed *a priori* as an epigenetic modulator has yet been approved for clinical use, we note that some metal-based compounds that have already entered human clinical trials, such as KP1019, or are progressing towards clinical trials, such as RAPTA-C, were found to act against epigenetic targets only after their anti-cancer activities were well-known. Therefore, it may well be possible that existing metal-based compounds in clinical use may actually also target epigenetic regulation as a potential mechanism of action. Given the promising studies that have been reported in the last few years, we are hopeful that it is only a matter of time before the first metal-based epidrug will be approved for clinical use.

Table 1. Transition metal-based epigenetic modulators, their targets and mechanisms, and efficacy against types of cancer.

Complex	Target	Mechanisms	Efficacy against	Reference
Ruthenium(II) and osmium(II) **1A–6B**	Histone proteins	Forms adducts with histone residues at the histone dimer-dimer and dimer-tetramer interfaces	Colon, ovarian and lung cancers	40
7 (RAPTA-C)	Histone proteins	Reacts with nucleosome core particle at three sites of specific adduct formation that entail bivalent coordination to glutamate, histidine and lysine side chains of the histone proteins	Ovarian cancer	42
8 (RAED-C)	Histone proteins and DNA	Reacts with the nucleosome core particle at histone glutamate and targets the DNA of chromatin	Ovarian cancer	42, 43
9 (KP1019)	Histone proteins	Ejects histones from nucleosomal DNA and interacts with histone H3	Colon cancer	47

10 (VAAP)	HDAC and DNA	VA ligand is released from **10** and inhibits HDAC activity, loosens histone−DNA interactions and facilitates the binding of Pt(II) to DNA	Lung, breast, ovarian and liver cancers	60
11	HDAC1 and DNA	Inhibits HDAC1 activity and delivers the DNA-binding Pt(II) to DNA	Ovarian cancer	64
JAHA **12** and analogues **13–16**	HDACs	JAHA forms the archetypal interaction between the hydroxamate moiety and the catalytic zinc ion, as well as a hydrogen bond between the amide moiety of the ligand and Asp101 in HDACs	Breast cancer	65
17	HDAC and DNA	SubH ligand is released from **17** and Inhibits HDAC activity, which increases histone acetylation levels and enhances the accessibility of chromatin DNA to damage induced by the platinum moiety	Ovarian cancer	67
18–21	HDAC	Inhibits HDAC activity	Melanoma, breast, epidermoid and cervical cancers	69, 70
22	Class I HDACs	Selectively inhibits Wnt/β-catenin signaling through modulating HDAC activities	Breast cancer	73
23 and **24**	BRD4	Inhibits the protein–protein interaction between tetra-acetylated lysine histone 4 peptide (H4AcK4) and BRD4	Melanoma	80
25	JMJD2	Selectively inhibits JMJD2 activity over other Jumonji domain-containing proteins including JMJD3 or JARID, as well as HDACs	Lung cancer	85

Acknowledgements

This work is supported by Hong Kong Baptist University (FRG2/14-15/004), the Health and Medical Research Fund (HMRF/13121482 and HMRF/14130522), the Research Grants Council (HKBU/201811, HKBU/204612 and HKBU/201913), the French National Research

Agency/Research Grants Council Joint Research Scheme (A-HKBU201/12), National Natural Science Foundation of China (21575121), Guangdong Province Natural Science Foundation (2015A030313816), Hong Kong Baptist University Century Club Sponsorship Scheme 2015, Interdisciplinary Research Matching Scheme (RC-IRMS/14-15/06), Inter-institutional Collaborative Research Scheme (RC-ICRS/15-16/02A), the Science and Technology Development Fund, Macao SAR (103/2012/A3), the University of Macau (MYRG091(Y3-L2)-ICMS12-LCH, MYRG2015-00137-ICMS-QRCM, MRG023/LCH/2013/ICMS and MRG044/LCH/2015/ICMS).

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