Review

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Regulating Histone Deacetylases and Carbonic Anhydrases by Metal Complexes: A Potent Strategy for Treating Cancers

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Utilizing metal complexes to inhibit histone deacetylases (HDACs) and carbonic anhydrases (CAs) highlights their therapeutic potential, particularly in anticancer strategies. The metal complexes, with their unique three-dimensional structures, fit adequately into the active sites of the enzymes, not only improving selectivity but also providing facile coordination with amino acid residues to enhance their inhibitory ability. This review emphasizes the role of metal complexes in the selective inhibition of HDACs and CAs along with details of their mechanism of action. Additionally, we summarize the inhibition ability and cytotoxicity of metal complexes targeting HDACs and CAs, as well as the therapeutic implications that can lead to the invention and development of metal complexes as potent anticancer agents.

Keywords: metal complexes; metalloenzymes; histone deacetylases; carbonic anhydrases; cancers

Introduction

Over the last few decades, drugs based on metal complexes have been extensively studied and utilized in the treatment of human diseases, especially cancers [1,2]. Among these, platinum-based drugs (*e.g.*, **cisplatin**, **carboplatin**, and **oxaliplatin**) have been applied in the treatment of about 50–70% of cancers [3,4]. However, these drugs elicit significant side effects and may increase the likelihood of drug resistance development [5]. In further advancements, non-platinum drugs based on ruthenium [6–8], iron [9,10], and gold [11,12] were also developed as advanced therapeutic agents. Notably, some compounds such as **NAMI-A** [13], **KP1019** [14], and **NKP1339** [15], have advanced to clinical trials. Platinum drugs typically target DNA, a strategy that, while effective, has revealed limitations, particularly collateral damage to normal cells [3,16]. Thus, alternative targets, such as enzymes involved in cancer-related metabolic pathways, have been suggested for metal-based drug design [17]. Recent studies have highlighted the safety and efficacy of metal complex-based inhibitors targeting a broad range of therapeutically relevant enzymes, strengthening the acceptance of metal complexes for therapy [18–21]. Numerous types of metal complexes have found utility in medicinal chemistry, including platinum coordination complexes, metallocenes, half-sandwich metallocenes [22], metal carbenes, metal carbonyls, and metal-arene compounds [23,24]. The mechanisms of action of these metal-based inhibitors are diverse and offer advantages over organic compounds. These include (i) a wide range of stereochemistry, allowing them to fit into the hydrophobic pocket of the enzyme; (ii) kinetic stability and lipophilicity, prompting cellular uptake; and (iii) the ability to incorporate functional ligands to bind to specific enzyme sites [25–28]. These properties make metal complexes ideal bases for the development of enzyme inhibitors, providing a promising avenue for developing more selective and less harmful cancer treatments.

Zinc-dependent metalloenzymes, including histone deacetylases (HDACs) and carbonic anhydrases (CAs), play pivotal roles in numerous biological processes, underscoring their importance in the maintenance of cellular homeostasis and human health [29,30]. The catalytic sites of HDAC7 and CA II are shown in Fig. 1. HDACs, which belong to a family of enzymes that remove acetyl groups from the ε-N-acetyl lysine amino acid of histones, are important in regulating gene expression by altering chromatin structure, consequently influencing cell cycle progression, differentiation, and apoptosis [31–33]. Their dysregulation has been implicated in the development of various diseases such as cancers, neurological disorders, and inflammatory diseases, highlighting their potential as therapeutic targets [34–37]. CAs could catalyze the conversion of CO_2 and water into bicarbonate and protons, a fundamental reaction in various physiological processes such as respiration, acid-base balance, and ion transport [38,39]. Abnormal CA activity has been linked to diseases including glaucoma [40], epilepsy [41], and especially cancer [42–

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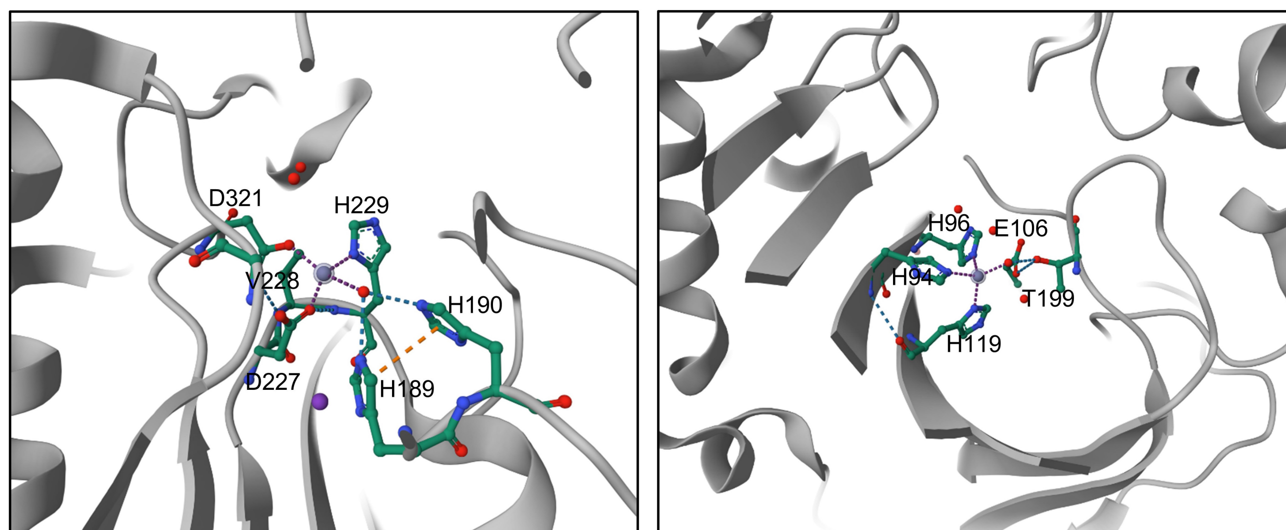

Fig. 1. Crystal structures of the active sites of HDAC7 (left, PDB: 3C0Y), and human CA II (right, PDB: 1XEV). Abbreviation: HDAC, histone deacetylase; CA, carbonic anhydrase.

Table 1. Classes, localization, catalytic sites, and function of HDACs.

Class	HDACs	Subcellular location	Catalytic domain	Target protein	Functions	Refs.
I	HDAC1,2,3,8	nucleus	Zn(II)-dependent	histone	promote cell proliferation and inhibit apoptosis	[48–50]
IIa	HDAC4,5,7,9	nucleus and cytoplasm	Zn(II)-dependent	histone and non-histone	promote angiogenesis	[51]
IIb	HDAC6,10				promote angiogenesis and cell migration	[52–55]
III	SIRT1,6,7	nucleus	NAD^+-dependent	histone and non-histone	regulate cell metabolism and cell growth	[52–54]
	SIRT3,4,5	mitochondrial				
IV	HDAC11	nucleus and cytoplasm	Zn(II)-dependent	non-histone	inhibit cell migration	[55]

Abbreviation: HDAC, histone deacetylase; SIRT, Sirtuin.

44], further emphasizing the therapeutic relevance of these enzymes. Therefore, these have contributed to an expanding interest in developing selective inhibitors, particularly metal complexes, as therapeutic agents.

The existing metal complex-based inhibitors targeting HDACs and CAs are mainly developed using two approaches: (i) modifying existing anticancer metal drugs with enzymatic inhibitor moieties, such as sulfonamides for CA inhibition and molecules including 4-phenylbutyrate (**PhB**) and valproate (**VPA**) for HDAC inhibition; and (ii) enhancing existing organic inhibitors with metallocenes and/or metal arene complexes. These approaches enhance the lipophilicity of these complexes [45,46], neutralize the negative charge of the inhibitor groups [27], and increase their synergistic effects in targeting cancer cells [23,47].

This review focuses on the latest progress in the generation of metal-based complexes as inhibitors of zinc-containing metalloenzymes, HDACs, and CAs. Recognizing the crucial roles these enzymes play in cancer progression and the significant antitumor potential of their inhibition, the review delves into the design of metal complexes and their relevance in cancer therapy.

Histone Deacetylases

HDACs can regulate gene expression and chromatin structure. HDACs function by catalyzing the removal of acetyl groups (O=C–CH_3) from lysine residues of histone proteins, leading to a more condensed chromatin structure and reduced gene transcription [31]. HDACs play a key role in the dynamic regulation of chromatin structure and influence cellular processes such as differentiation, proliferation, and apoptosis [32]. The HDAC enzyme family is categorized into four classes based on their homology to yeast counterparts and their dependence on cofactors. Each class has a unique structure, enzymatic activity, and subcellular localization, which determines their specific role in the cell (Table 1, Ref. [48–55]). Under normal conditions, HDACs are distributed to various cell compartments: Class I HDACs (1, 2, 3, and 8) are found primarily in the nucleus and regulate gene expression and cell cycle progression [32]. Class II HDACs shuttle between the nucleus and cytoplasm and participate in tissue-specific gene expression and cell differentiation [45,46]. Class III HDACs play a role in aging, metabolism, and stress resistance [47]. Class IV, consisting only of HDAC11, exhibits characteristics of both Class I and II enzymes. HDAC11's role is not yet fully

understood, but it has recently been reported that it shares the same function as a lysine defattyacylase [56,57].

The mechanism of action of HDACs involves the interaction between histone acetylation and deacetylation mediated by histone acetyltransferases (HATs) and HDACs, respectively [32]. Acetylation of histones by HATs leads to an open chromatin structure, which facilitates access of transcriptional machinery to the DNA and results in gene activation [58,59]. Conversely, HDAC-mediated deacetylation results in a closed chromatin structure, which suppresses gene transcription [59]. This dynamic balance between acetylation and deacetylation is important for precise control of gene expression patterns that govern cellular function and identity [32]. HDACs are not limited to histones but can deacetylate a variety of non-histone proteins, affecting their stability, localization, and function. These proteins include transcription factors and transcription-related proteins, such as p53 tumor protein, peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), damage-specific DNA binding protein 1 (DDB1), FK506-binding protein 51 (FKBP51), forkhead box O3 (FOXO3) [60].

HDACs have been implicated in the pathogenesis of a wide range of diseases, including cancers, neurodegenerative disorders, cardiovascular diseases, and inflammatory conditions [61]. Dysregulation of HDAC activity can result in abnormal gene expression patterns that contribute to the development and progression of cancer [32]. Class I HDACs interact with transcription factors that relate to cancer progression [48]. The expression of HDACs, especially Class I HDACs, has been found to be elevated in multiple cancer cell lines, such as colorectal cancer, lung cancer, prostate cancer, breast cancer, and liver cancer [62]. For example, HDAC1 and HDAC2 are associated with Sin3A, CoREST, and Nucleosome Remodeling and Deacetylase (NuRD) protein complexes and participate in the regulation of transcription, DNA replication, and DNA repair [34]. In particular, they negatively regulate acetylation and transcriptional activity of nuclear factor kappa B (NF-κB), and signal transducer and activator of transcription 1 and 3 (STAT1 and STAT3) [63]. HDAC1 and HDAC2 promote tumorigenesis by interacting with metastasis-associated protein 1 (MTA-1), a cancer-related protein of the NuRD complex [35]. HDAC3 could regulate transcription factors I and II to inhibit nuclear receptor transporting activity and promote cancer cell proliferation [64]. HDAC8 promotes stemness, proliferation, migration, and invasion of cancer cells by activating the transforming growth factor beta (TGF-β) signaling [65]. Class II HDACs bind classical transcription factors that participate in tumorigenesis and progression [66]. Also, HDAC4 and HDAC6 levels have been found to be elevated in glioblastoma, correlating with poor patient survival [36,67]. Separately, upregulation of HDAC9 can enhance cell proliferation and drug resistance in lymphoblastic leukemia [37].

Unlike Class I/II HDACs, Class III HDACs play opposite roles in various cancers [68]. Both Sirtuin 1 (SIRT1) and Sirtuin 2 (SIRT2) (members of Class III HDACs) contribute to tumor suppression by maintaining genomic stability, regulating chromatin dynamics, and deacetylating tumor suppressor proteins like p53, which governs cell cycle arrest and apoptosis [68]. The tumor-suppressive activity of SIRT1 and SIRT2 has been observed in prostate, bladder, and ovarian cancers [68–71]. SIRT1 has also been implicated in promoting cancer progression and drug resistance, particularly in cervical and lung cancers [72], while SIRT2 shows cancer-promoting activity through the deacetylation of proteins like α-tubulin and E1A binding protein p300 (EP300) [73]. Additionally, SIRT3, another member of III class HDACs, primarily acts as a tumor suppressor by regulating cellular metabolism and oxidative stress, showing antitumor effects against prostate cancer [74], hepatocellular carcinoma, and pancreatic cancer [75]. Conversely, SIRT3 acts as an oncogenic factor in cervical cancer by regulating fatty acid synthesis [76] and colon cancer [77].

HDAC inhibitors are recognized as powerful epigenetic therapies, with four main classes comprising 17 inhibitors [78]. Of these inhibitors, only five have been approved for clinical use by the U.S. Food and Drug Administration (FDA), including vorinostat (or superpolyamide hydroxamic acid (**SAHA**)), romidepsin, belinostat, panobinostat, and chidamide [78]. Despite their therapeutic potential, the broad specificity of these inhibitors poses challenges, resulting in off-target side effects and toxicity [29, 79]. This highlights the need to develop more selective inhibitors targeting specific HDACs and/or classes [80]. Combining HDAC inhibitors with other treatments, including DNA repair drugs, radiotherapy, topoisomerase inhibitors, epigenetic modifiers, and immune checkpoint inhibitors, has been shown to improve effectiveness [29]. Despite uncertainty about their comprehensive biological impacts, inhibiting HDACs remains a promising avenue for anticancer therapy.

Carbonic Anhydrase

Belong to a family of zinc metalloenzymes, CAs are essential for regulating pH, fluid homeostasis, and CO_2 transportation in various tissues throughout the body [38, 39]. These enzymes catalyze the reversible conversion of CO_2 and water into bicarbonate and protons, known as the zinc-hydroxide mechanism, a reaction that is fundamental to many physiological processes [38,39]. These enzymes are expressed in most living organisms and are encoded by eight evolutionary distinct gene families: α, β, δ, ε, γ, ι, τ, and ζ, α-CAs is predominantly expressed in vertebrates and is the only class observed in humans with 15 isoforms. Among these 15 isoforms, 12 isoforms are active (Table 2, Ref. [40,41,43,44,81–92]) [93,94]. Other isoforms (VIII, X, XI) that are devoid of Zn(II) active sites

Table 2. Characterizations, distribution, localization, and catalytic activity of 12 active human CA isoforms, along with their associated diseases.

Isoforms	Primary locations	Subcellular location	Known/Suspected functions	Associated diseases	Refs.
CA I	RBCs, eye	cytosol	gas exchange, pH balance	upregulation → hemolytic anemia	[81]
CA II	ubiquitous	cytosol	pH regulation, ion transport, CO_2 hydration	upregulation → glaucoma, epilepsy, cancer	[82,83]
CA III	skeletal muscle, adipose tissue	cytosol	Buffering, cell growth, and differentiation	downregulate CA III → oxidative stress	[84,85]
CA IV	kidney, lung	membrane-bound	ion transport, diffusion of CO_2 across cell membranes	upregulate CA IV → Retinitis pigmentosa, glaucoma	[40]
CA VA	liver	mitochondria	ureagenesis, gluconeogenesis	upregulation → obesity, insulin resistance	[86]
CA VB	heart, skeletal muscle, kidney	mitochondria			
CA VI	saliva, milk	secreted	maintain pH balance in the mouth and digestive tract	downregulation → dental caries	[87]
CA VII	brain, liver, colon, skeletal muscle	cytosol	neurological processes, pH regulation	upregulation → epilepsy	[41]
CA IX	stomach, certain cancers	transmembrane	pH regulation in tumors, cell proliferation	upregulation → cancer	[43,44]
CA XII	kidney, eye, cancers, reproductive epithelia	transmembrane	pH regulation, ion transport	upregulation → cancer, glaucoma	[88–90]
CA XIII	kidney, thymus, intestine, reproductive organs	cytosol	pH regulation, ion transport	downregulation → sterility	[91]
CA XIV	kidney, liver, bladder, eyes, brain	transmembrane	ion transport, pH regulation	upregulation → retinopathy, epilepsy	[92]

Abbreviations: CA, carbonic anhydrase; RBCs, red blood cells.

are referred to as CA-related proteins [95]. The 12 active isoforms differ in terms of cellular distribution and physiological functions, which are summarized in Table 2.

Under normal conditions, CAs are involved in a variety of physiological processes. These processes include respiratory activities (such as the transfer of CO_2 across membranes and the exchange of oxygen within red blood cells (RBCs), which are influenced by pH and Bohr effects) [96], movement of fluids through epithelial cells, regulation of acid-base balance across the epithelial barrier (*e.g.*, gastric acid secretion and bicarbonate released by the pancreas) [97,98], and acid secretion by osteoclasts during bone resorption [99]. Importantly, CA-mediated acid-base regulation by renal epithelial cells is essential for maintaining the body's overall acid-base equilibrium [100]. This equilibrium is essential for stabilizing cellular pH, which underlies numerous biochemical processes, including gluconeogenesis, ureagenesis, and lipogenesis, and is important for virtually all cellular functions [100].

CAs play an important role not only in the mentioned physiological processes but also in the pathophysiology of various diseases, especially cancers and neurological disorders [101]. Specific CA isoforms, particularly CA IX and CA XII isoforms, are overexpressed in various tumors and are associated with tumor acidosis and tumor microenvironment regulation [88,102,103]. These isoforms help cancer cells adapt to hypoxic conditions by promoting the removal of CO_2 produced during the metabolic switch to glycolysis (Warburg effect) [101,102]. Expression of CA IX isoform increases significantly in response to low oxygen levels, a process that is dependent on hypoxia-inducible factor (HIF-1) and occurs in cells with von Hippel-Lindau (VHL) syndrome deficiencies [82,104,105]. Similarly, the expression of CA XII isoform is increased in VHL-deficient kidney cells and is influenced by estrogen receptor alpha (ERα) in breast cancer cells [82,83]. Expression of CA IX and CA XII isoforms is often associated with poor prognosis, tumor progression, and metastasis [88,106–108]. Thus, CA IX and CA XII have been validated as essential targets for the treatment of hypoxic tumors and metastasis.

The main strategy to treat the diseases by targeting CAs is inhibiting their enzymatic activity. The active site within α-CAs consists of hydrophobic and hydrophilic residues lined up on the opposite sides of the cavity. At the bottom of this cavity is attached a metal ion, which is important for catalysis, where a water molecule activated by Zn(II) promotes nucleophilic attack on the substrates [109]. Additionally, CAs have a large active pocket, allowing them to be inhibited by various classes of inhibitors, especially sulfonamides and their isosteres [110,111]. Developing CA inhibitors have been recognized as a promising strategy for treating human diseases, including cancers [103]. Sulfonamides and their isosteres are recognized as potential inhibitors, particularly effective in the deprotonated form; these inhibitors bind with high affinity to the Zn(II) of the enzyme and can affect several CA isoforms in vertebrates, including humans [112]. Among the existing sulfonamides, several have shown promise in clinical settings, such as acetazolamide (**AAZ**), methazolamide (MZA), ethoxzolamide (EZA), saccharin (SAC), brinzolamide (BRZ), and dorzolamide (DRZ) [111]. However, they lack selectivity toward different CA isoforms [111]. Of note, new types of inhibitors (non-sulfonamides) have been invented with different blocking mechanisms [113]. These mechanisms include attaching to Zn-coordinated water molecules, blocking the entrance of the active site, and/or binding outside the active site [113]. Despite challenges in isoform selectivity, CA inhibitors remain a promising strategy for cancer therapy, and this underscores the need to discover highly selective inhibitors for specific isoforms (*e.g.*, CA IX and CA XII).

Inhibition of HDAC by Metal Complexes

Platinum Complexes

Platinum-based chemotherapeutic agents, including **cisplatin**, **oxaliplatin**, and **carboplatin** (Fig. 2), are commonly utilized in the treatment of various human cancers. Nevertheless, their application is limited by numerous shortcomings such as resistance (both endogenous and acquired), negative side effects, limited spectrum of activity, low bioavailability, and ineffectiveness against all types of cancer [114–116]. Extensive studies have been conducted on investigating the mechanism of action of these drugs, which were among the first to be approved. Their mechanisms primarily involve processes such as cellular uptake, activation through hydration, generation of DNA adducts that alter the DNA structure, and the activation of cellular processes leading to apoptosis [21]. Therefore, the development of new platinum-based anticancer drugs often focuses on modifying these processes to improve cellular uptake and altering the way these drugs interact with DNA to influence the processing of DNA adducts. One promising approach in this direction is targeting HDAC as part of a strategy to improve therapeutic outcomes.

Efforts to target HDAC have been proceeded by coordinating HDAC inhibitors into Pt(IV) complexes, a derivative of **cisplatin**, **oxaliplatin**, and **carboplatin**. HDAC inhibitors, such as **PhB** and **VPA**, are frequently utilized as axial ligands in Pt(IV) complexes [19,117–121]. This strategy was believed to promote platinum absorption by improving the lipophilicity of the complexes over **cisplatin** or **oxaliplatin** [121]. Additionally, this incorporation neutralizes the negative charge of **PhB**/**VPA**, resulting in increased accumulation of **PhB**/**VPA** [27]. In addition to the cellular accumulation of these compounds, the coordination of **PhB**/**VPA** into Pt(IV) complexes significantly improved their anticancer efficacy compared to free **PhB**/**VPA** and **cisplatin**/**oxaliplatin** [119,121].

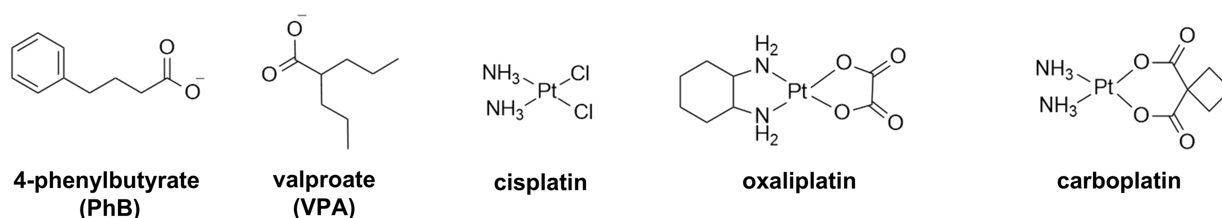

Fig. 2. Chemical structure of organic HDAC inhibitors and three traditional platinum drugs. The chemical structures were illustrated using ChemDraw Ultra 12.0 (CambridgeSoft, Waltham, MA, USA).

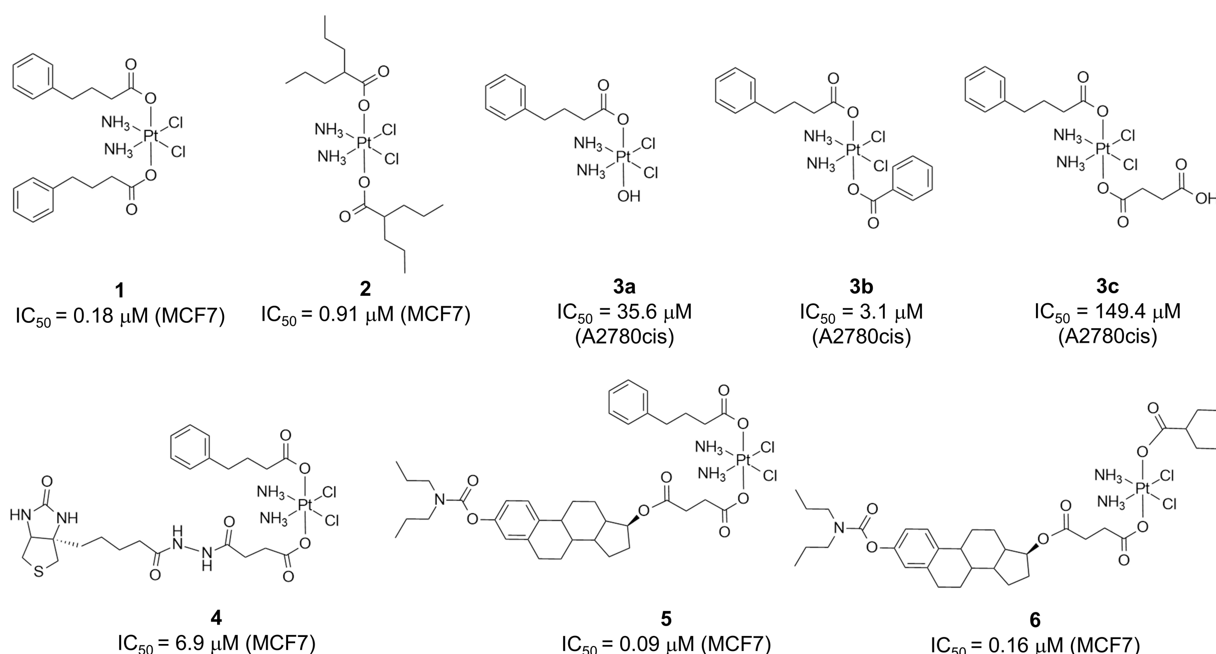

Fig. 3. HDAC inhibitors were developed based on cisplatin. The chemical structures were illustrated using ChemDraw Ultra 12.0. Notes: MCF7 is a cell line of human breast cancer; A2780cis is a cell line of **cisplatin**-resistant ovarian cancer. Abbreviations: IC_{50}, half-maximal inhibitory concentration.

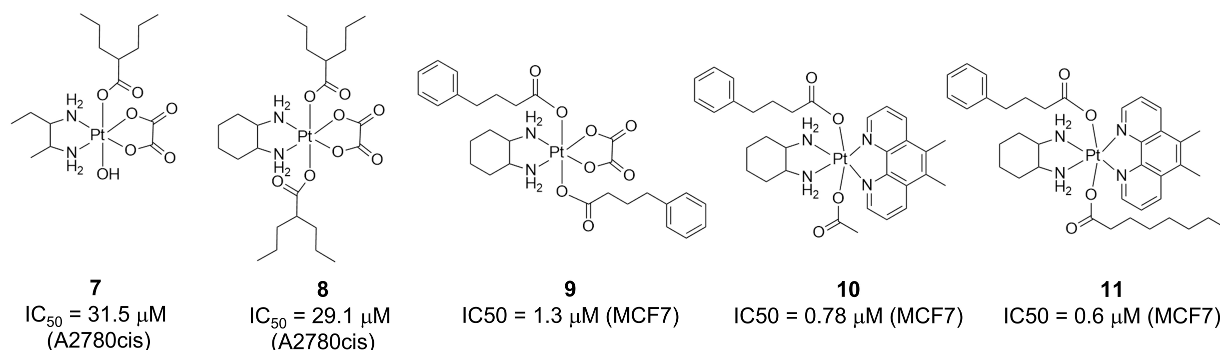

Fig. 4. HDAC inhibitors were developed based on oxaliplatin. The chemical structures were illustrated using ChemDraw Ultra 12.0. Notes: MCF7 is a cell line of human breast cancer; A2780cis is a cell line of **cisplatin**-resistant ovarian cancer. Abbreviations: HDAC, histone deacetylase; IC_{50}, half-maximal inhibitory concentration.

HDAC Inhibitors Based on **Cisplatin**'s Derivatives

The Pt(IV) compounds (compounds **1** and **2**, in Fig. 3), derivatives of **cisplatin**, have been developed as highly potent cytotoxic agents against a variety of human cancer cell lines, showing up to 100-fold higher potency than **cisplatin** [118]. It also inhibited HDAC activity by 60–70% in breast cancer MCF-7 cells after 24 hours of treatment at small half-maximal inhibitory concentration (IC_{50}) values (compound

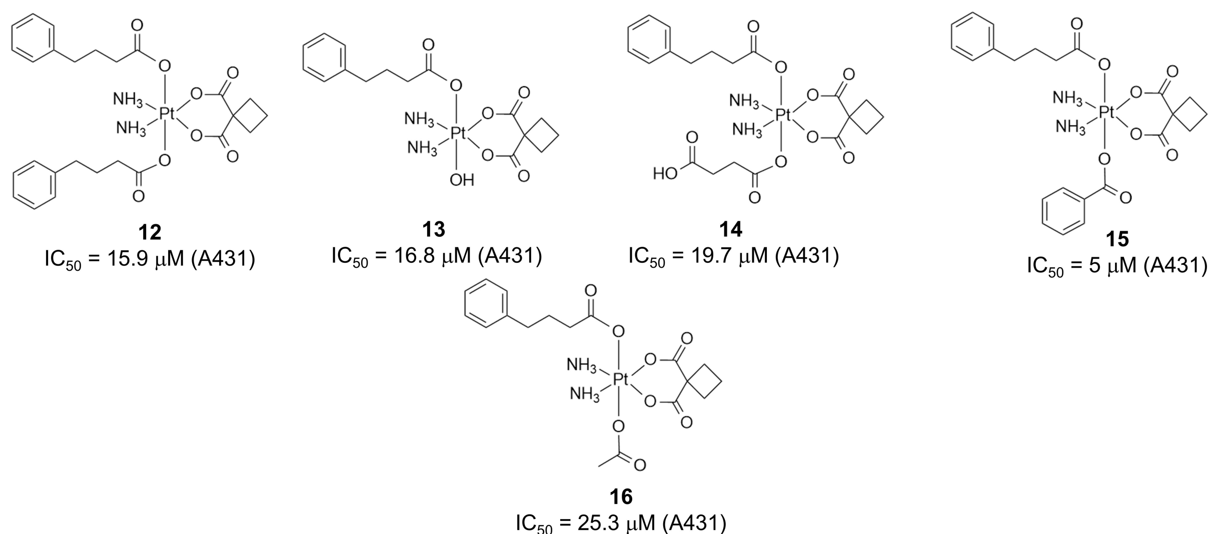

Fig. 5. HDAC inhibitors were developed based on carboplatin. The chemical structures were illustrated using ChemDraw Ultra 12.0. Note: A431 is a cell line of epidermal cancer.

1: 0.18 μM; and compound 2: 0.91 μM). In contrast, the *in vitro* IC_{50} values of free **PhB** in inhibiting Class I HDACs (HDAC1, HDAC2, HDAC3, and HDAC8) range from 64 to 260 μM, and are notably less against Class IIa HDACs (HDAC4, HDAC5, HDAC7, and HDAC9) with values exceeding 2000 μM, as well as 240 μM for HDAC6 [122]. Similarly, free **VPA** shows IC_{50} values for HDAC inhibition ranging from 39 to 161 μM for Class I and >2000 μM for all other classes [122]. These findings suggest that the outstanding anticancer efficacy of these Pt(IV) complexes may be due to their synergistic effects when combined with platinum. In another study, compound **2** was observed to significantly reduce the expression of HDAC2 and HDAC7 in ovarian cancer cells A2780 [119]. Remarkably, compound **2** inhibited HDAC activity in A2780 cells at a concentration approximately 34,000-fold lower than the concentration required for free **VPA** to exhibit a similar inhibitory effect on HDAC activity. This again supports the synergistic effect between the platinum component and the **VPA/PhB** ligands [119]. After the investigation of compound **1**, Almotairy *et al*. [123] examined the inhibitory activity of a series of Pt(IV) complexes (compounds **3a-c**) with **PhB** (Fig. 3). Studies have confirmed that these compounds could interact with their intended targets, DNA and HDAC. In particular, compound **3b** was found to be the most cytotoxic among compounds **3a** to **3c**, significantly increasing cellular reactive oxygen species (ROS) levels and inducing apoptosis independent of DNA damage in both **cisplatin**-sensitive A2780 and **cisplatin**-resistant A2780cis ovarian cancer cells. These findings suggest that compound **3b** operates through multiple mechanisms to cause DNA damage and induce apoptosis in an effective manner even in the presence of **cisplatin** resistance.

Another study on platinum prodrugs developed Pt(IV) complex, compound **4**, which contains the HDAC inhibitor (**PhB**), along with a tumor-targeting moiety (biotin) [19]. This compound demonstrated antiproliferative effects against a variety of cancer cell lines, including human breast cancer MCF-7, human colorectal cancer HCT-116, and hepatocellular carcinoma HepG-2, with results comparable to or even surpassing those of **cisplatin**. Importantly, the toxicity of this compound to normal human liver LO2 cells was significantly lower than that of **cisplatin**, highlighting its potential for safer cancer treatment. Unlike traditional platinum-based prodrugs such as **cisplatin**, compound **4** also exhibited HDAC inhibitory activity in HepG-2 cells, providing a distinct and potentially more targeted mechanism of action.

Two Pt(IV) prodrugs compounds **5** and **6**, which are **cisplatin** derivatives containing HDAC inhibitors (*i.e.*, **PhB** and **VPA**), have been utilized as promising multi-action anticancer agents for the treatment of prostate cancer cells [121]. The mechanism of action for these two compounds is attacking multiple targets in the cancer cells simultaneously in a concerted fashion. It was also shown that the activity of HDAC decreased with the increasing intracellular level of acetylated histone H3 in human prostate LNCaP cells. This effect occurred through the reduction of the Pt(IV) complexes with DNA-damaging Pt(II) agent (*i.e.*, **cisplatin**) and HDAC inhibitor ligands [121].

HDAC Inhibitors Based on **Oxaliplatin**'s Derivatives

Platinum-based prodrugs (compounds **7** and **8**), which are **oxaliplatin** derivatives linked to **VPA**, have been examined for their potential as anticancer agents (Fig. 4) [124]. Antiproliferative tests showed that these new compounds were less cytotoxic than **oxaliplatin** in cultured cells. Nonetheless, compounds **7** and **8** showed cytotoxic effects in **cisplatin**-sensitive and -resistant ovarian tumor (A2780cis) cells due to increased intracellular accumula-

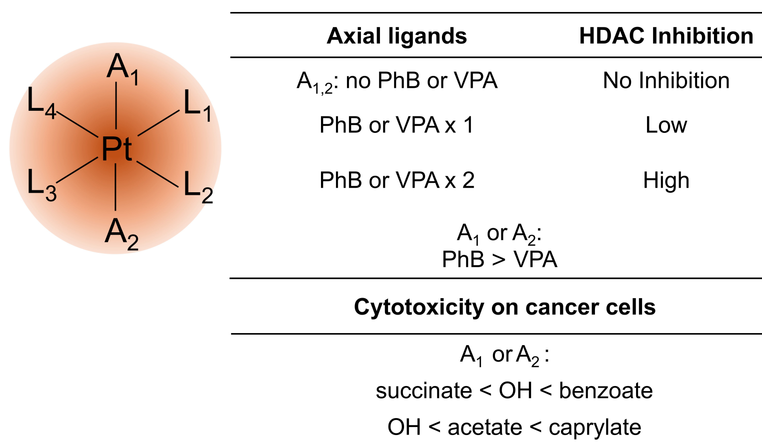

Fig. 6. Relationship of axial ligand design with PhB and VPA ligands and their effects on HDAC inhibition activity. This schematic diagram was created using Microsoft PowerPoint. Abbreviations: **PhB**, 4-phenylbutyrate; **VPA**, valproate.

tion. Moreover, these conjugates simultaneously acted on two targets: genomic DNA and HDAC in A2780 cancer cells. The findings indicate the potential of these compounds in creating more effective and promising dual-targeting agents for the treatment of various types of cancer, especially those resistant to traditional **cisplatin**-based chemotherapy [124].

A new series of Pt(IV) anticancer prodrugs (compounds **9–11**) featuring **PhB** as the axial ligand has been reported [120]. These prodrugs demonstrated remarkable efficacy against cancer cells, especially the MDA-MB-231 breast cancer cell line. The observed anticancer activity results from multiple mechanisms, including significant HDAC inhibition, as evidenced by a 28%, 17%, and 23% reduction upon treatment with compounds **9**, **10**, and **11**, respectively. The modes of action proposed include altering nuclear DNA, reducing mitochondrial membrane potential, triggering epigenetic processes, and modifying the structure of the cytoskeleton network [120].

HDAC Inhibitors Based on **Carboplatin's** Derivatives

A series of Pt(IV) complexes combining **carboplatin** and **PhB**, designated as compounds **12–16**, have been developed as well (Fig. 5) [125]. Their cytotoxic effects were evaluated across a variety of cancer cell types, including melanoma (A375), pancreatic (BxPC3), colon (LoVo), and epidermal (A431) cancer cells. Among these derivatives, compound **15** has been reported as a promising HDAC inhibitor, showing 10-fold greater potency than **carboplatin**, and decreasing basal cellular HDAC activity by approximately 18% in A431 human cervical cancer cells [125]. It also exhibited significant cytotoxicity against the A431 cell line with an IC_{50} value of 5 μM and demonstrated levels of intracellular platinum accumulation similar to that of **cisplatin**.

Brief Summary of Platinum Complexes

In summary, treating cancers with platinum complexes involves multiple mechanisms, including HDAC inhibition and DNA interaction. Like the parent platinum complexes (*i.e.*, **cisplatin**, **oxaliplatin**, and **carboplatin**), complexes **1–16** also can bind to DNA to show anticancer ability. These complexes, however, demonstrated significantly stronger cytotoxicity against cancer cells while remaining harmless to normal cells. Furthermore, complexes **1–16** are stronger HDAC inhibitors than free **PhB** and **VPA**. The modifications of the axial ligands in these platinum complexes significantly enhance their HDAC inhibitory activity and cytotoxic effects on cancer cells (Fig. 6).

Ferrocene Complexes

Efforts to develop ferrocene-based HDAC inhibitors are mainly focused on integrating ferrocene with super-polyamide hydroxamic acid (**SAHA**) and its derivatives to increase synergistic effects (Fig. 7). **SAHA** is one of the six basic categories of HDAC inhibitors and is one of the most extensively studied drugs [126]. The mechanism of action of **SAHA** involves binding of the hydroxamate group to a Zn(II) located in the HDAC cavity [127,128]. The binding of **SAHA** to Zn(II) in the HDAC active site is primarily driven by the coordination to the catalytic Zn(II) by the deprotonated oxygen of the hydroxamate group, which effectively displaces the Zn(II)-bound water molecule in the resting state of the enzyme (Fig. 7). Besides, hydroxamate moiety also forms two hydrogen bonds with His139 and Tyr331 [128]. So far, multiple studies have focused on developing new HDAC inhibitors inspired by the **SAHA**. These efforts have primarily concentrated on modifying the cap, functional unit, and spacer components of the **SAHA** molecule, as depicted in Fig. 7. Any structural modifications in the compounds could result in changes to the inhibitory activity.

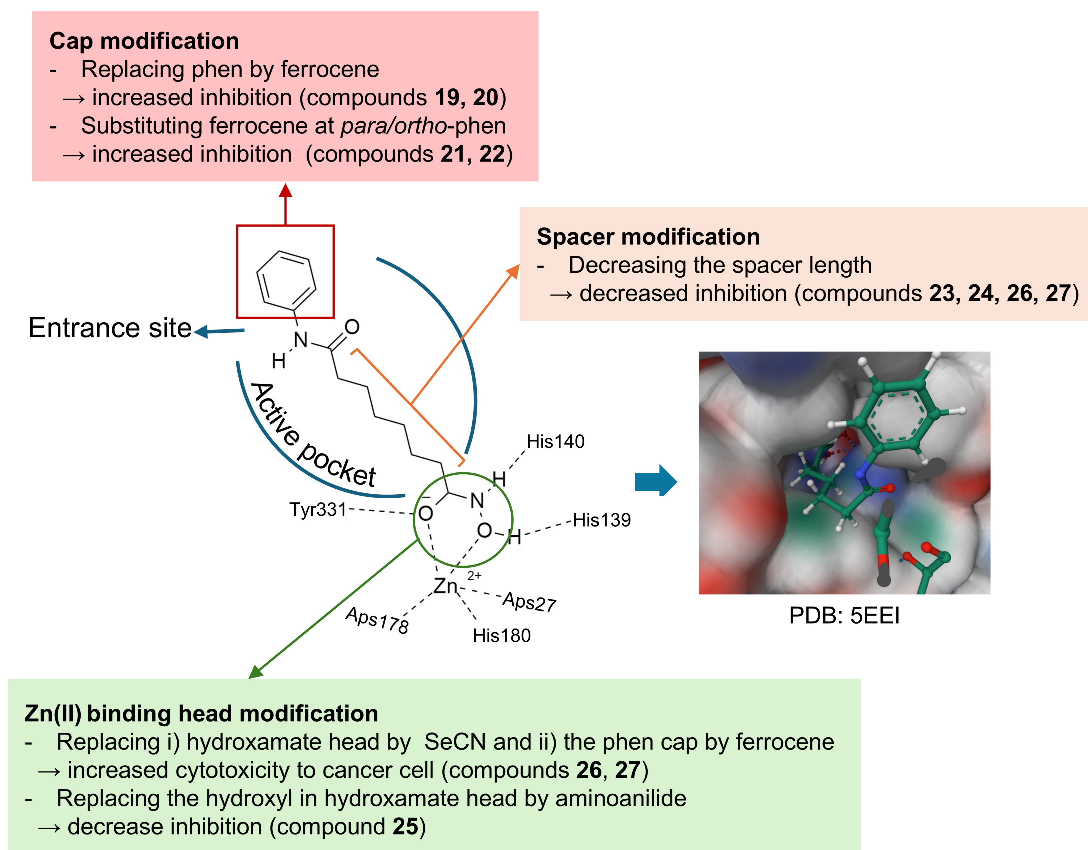

Fig. 7. Zn(II) binding mechanism of superpolyamide hydroxamic acid (SAHA) in the active site of HDACs and different approaches in developing new HDAC inhibitors by incorporating ferrocene to SAHA. This schematic diagram was created using Microsoft PowerPoint and the chemical structure of SAHA was illustrated using ChemDraw Ultra 12.0.

Marinero *et al*. [129] employed compounds **17** and **18**, which were developed by combining ferrocifen with **SAHA** (Fig. 8), for HDAC inhibition and antiproliferative effects. The integration of ferrocifen and **SAHA** led to the development of HDAC inhibitors with enhanced cytotoxicity against triple-negative MDA-MB-231 breast cancer cell line. Compound **17** showed a significant improvement of cytotoxicity (IC_{50} = 0.7 µM) compared to **SAHA** (IC_{50} = 3.6 µM) [129]. Additionally, compound **18** was effective in suppressing the proliferation of both MDA-MB-231 and MCF-7 cells, with IC_{50} values of 0.5 µM and 1.8 µM, respectively. Compound **17** demonstrated significant enzymatic inhibition of HDAC, which was lower than that of **SAHA**. Despite the relatively strong antiproliferative effects, compound **18** could not inhibit HDAC activity. The hydroxamate head of **SAHA** and compound **17** could chelate metal ions such as Fe(III) and Zn(II) to form tris- and/or bis-hydroxamato complexes. Thus, they can coordinate Zn(II) from the active site of HDAC. However, this chelation was not observed upon treatment with compound **18**, which contains an amide head. Therefore, the antiproliferative activity of these compounds could be attributed to specific properties of the organometallic structure [129].

Spencer *et al*. [130] introduced a series of Jay Amin hydroxamic acids (**JAHA**), ferrocene-based Class I HDAC inhibitors (compounds **19–23**), by replacing a sandwich ferrocene with the phenyl cap of **SAHA** to evaluate inhibition of HDAC1, 2, 3, 6, and 8. The compounds (except compound **23**) showed inhibition of HDAC activity at nanomolar dose, with notably stronger inhibition of HDAC6 compared to **SAHA** [130]. Compounds **19–23** exhibited strong anticancer ability against MCF-7 cells with an IC_{50} value from 1.9 to 5.08 µM. Docking studies of compound **19** to HDAC8 suggested that the ferrocenyl group effectively replaces the aryl cap of **SAHA**, fitting well into the specific pocket formed by Phe152, Tyr100, and Tyr306.

Two HDAC inhibitors derived from **JAHA**, namely compounds **24a** and **24b**, which exhibited pharmacokinetic improvement, were also introduced [131]. Compound **24b** appeared to be a more effective HDAC inhibitor, achieving a low IC_{50} value slightly better than **SAHA** in biochemical *ex vivo* and *in vivo* tests. Additionally, to determine the ability of compound **24b** to inhibit HDAC6 *in vivo*, the authors exposed *Xenopus laevis* embryos to compound **24b** at a concentration of 100 µM. The effect on HDAC6 was measured by measuring the acetylation levels of α-tubulin, showing that compound **24b** effectively inhibited deacety-

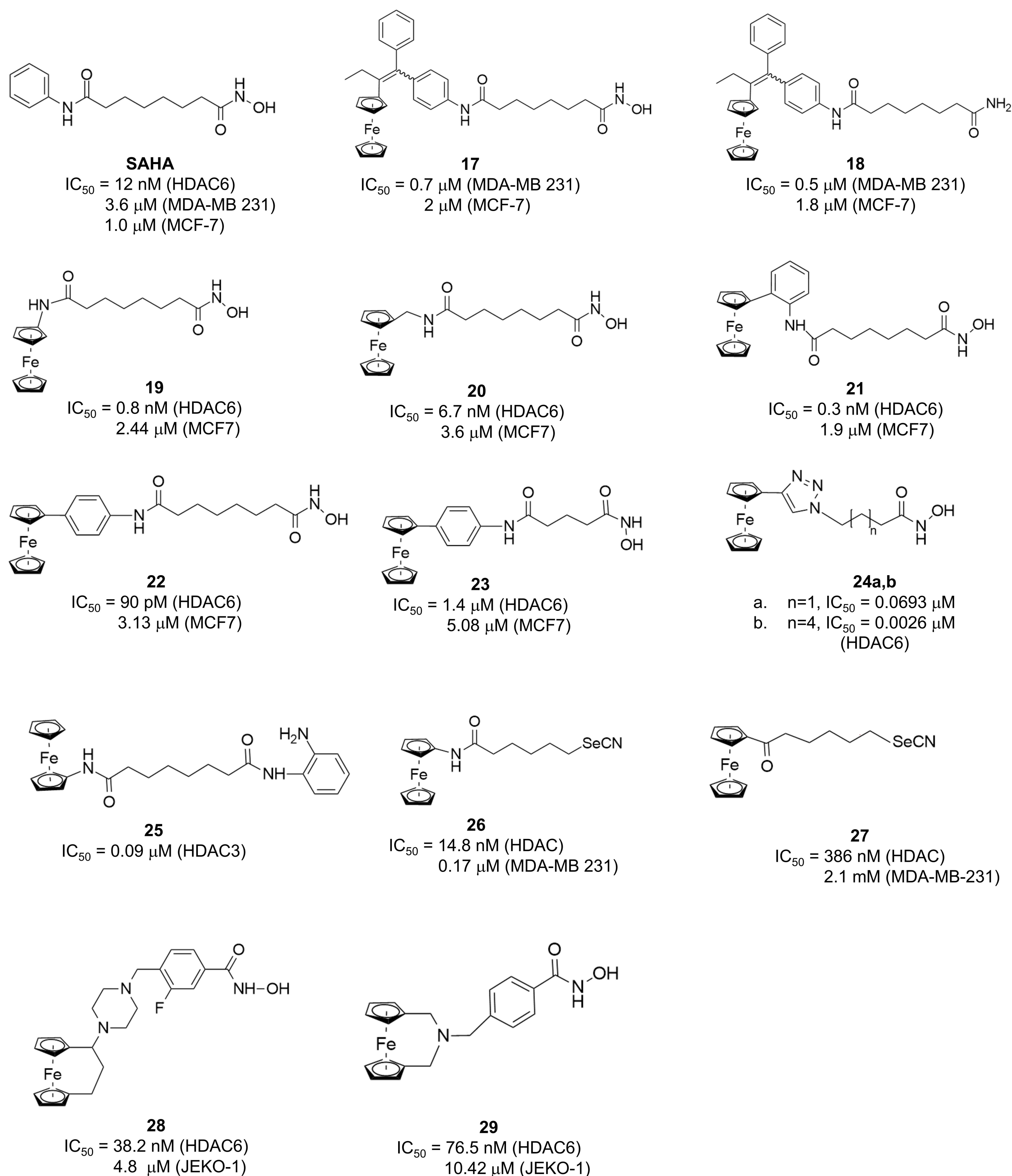

Fig. 8. Ferrocene-containing HDAC inhibitors. The chemical structures were illustrated using ChemDraw Ultra 12.0. Notes: MCF7 is a cell line of human breast cancer; MDA-MB 231 is a cell line of triple-negative breast cancer; JEKO-1 is a cell line of mantle cell lymphoma.

lation in a similar manner to **SAHA**. Another **JAHA** derivative, compound **25**, is highlighted as an HDAC inhibitor [132]. This compound demonstrated remarkable potency against HDAC3, with an IC_{50} value of 0.09 μM. Compared to RGFP966, a selective HDAC3 inhibitor, compound **25** displayed superior efficacy in inhibiting the invasion of HCT116 cells. Furthermore, compound **25**'s redox pharmacology was explored by treating HCT116 cells with the

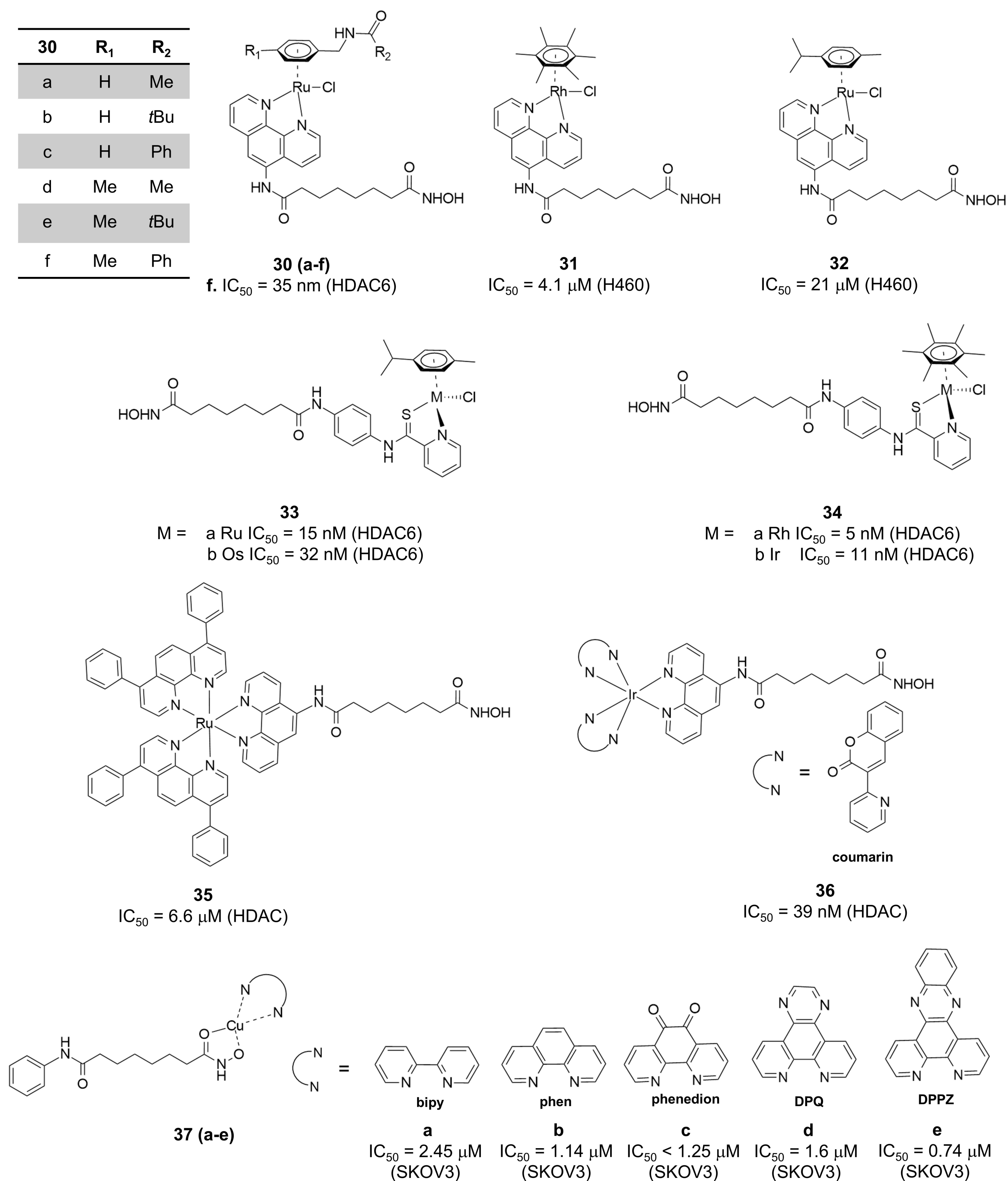

Fig. 9. Other metal complexes as HDAC inhibitors. The chemical structures were illustrated using ChemDraw Ultra 12.0. Notes: H460 is a cell line of human lung cancer; SKOV3 is a cell line of human ovarian cancer.

compound. Upon treatment with compound **25**, sodium nitroprusside, and glutathione, cytotoxicity and DNA damage, likely attributed to the activation of a Fe(III) species, were significantly heightened. Compound **25** has also been proposed as the inhibitor of triple-negative breast cancer (TNBC) cell viability and growth in culture, which operates by inducing an impairment of cell autophagic process and mitochondrial function [133].

Designed by incorporating a ferrocenyl group and a selenocyanide as a zinc-binding motif (Fig. 8), compounds

26 and **27** are effective against TNBC cells [134]. Both *in vitro* and *in vivo* studies showed that compound **26** increased ERα expression in TNBC cells and made MDA-MB-231 cells more responsive to standard endocrine treatments by reactivating ERα. It was also found that compound **26** blocked HDAC activity with an IC_{50} value of 14.8 nM. Additionally, using free SeISA as a reference standard, orally administering compound **26** at a dose of 20 mg/kg daily for 21 days resulted in a 58.6% reduction in tumor xenograft volume in a mouse model bearing MDA-MB-231 tumors. Importantly, treatment with compound **26** did not significantly change the body weight of tumor-bearing mice and no adverse effects appeared throughout the treatment period. Consequently, compound **26** is recognized as a potent HDAC inhibitor with potential as a therapeutic option for TNBC. Compounds **28** and **29** exhibited inhibitory activity against HDAC6, with IC_{50} values in the nanomolar range [135]. Both compounds demonstrated moderate antiproliferative effects across various cancer cell lines. Notably, compound **28** showed superior antiproliferative activity in JEKO-1 cells, with an IC_{50} of 4.80 µM [135].

Other Metal Complexes

Efforts to develop new metal complexes as HDAC inhibitors, inspired by **SAHA**, have extended beyond ferrocene complexes to include half-sandwich arene compounds (Fig. 9). For example, Cross *et al.* [136] introduced a variety of ruthenium compounds combined with **SAHA**. These complexes, designated as complexes **30a–f** (Fig. 9), exhibited significant HDAC inhibitory activity, reducing the activity of HDAC1 and HDAC6 to less than 25% at a concentration of 1 µM. In particular, complex **30f** showed very low IC_{50} values, at 80 nM and 35 nM for HDAC1 and HDAC6, respectively. Most of these ruthenium complexes showed cytotoxic effects similar to **SAHA** against the MCF-7 cell line. It has been reported that Ru(II) and Rh(III) piano-stool complexes (complexes **31** and **32**) exhibited HDAC inhibitory and antiproliferative properties against H460 lung cancer cells [137]. At a concentration of 0.1 µM, both complexes **31** and **32** inhibited approximately 85% of HDAC activity, which was slightly lower than that of **SAHA** (94% inhibition). The use of these 3D piano-stool structures as HDAC inhibitors was expected to improve binding efficiency by targeting previously inaccessible regions on the HDAC enzyme surface [137]. Briefly, the complexes **30**, **31**, and **32** demonstrated anticancer effects similar to **SAHA**, with HDAC inhibition identified as the primary mechanism of action, without any interaction with DNA [137].

A series of organometallic compounds featuring transition metal centers (*i.e.*, Ru, Ir, Rh, Os) and a **SAHA** moiety, compounds **33a–b** and **34a–b** were developed (Fig. 9) [138]. These compounds exhibited inhibitory activity in the nanomolar range against HDAC6, particularly compounds **34a** and **34b** demonstrated stronger inhibition than **SAHA**. Moreover, compound **34a** showed high cytotoxicity in human cancer cell lines such as HCT116 (colon), NCI-H460 (non-small cell lung), SiHa (cervix), and SW480 (colon), while showing low cytotoxicity in hemolysis studies and in zebrafish [138]. This emphasizes the influence of the metal center that can significantly enhance target specificity and potency toward HDACs and differentiate between cancerous and normal cells, which can be leveraged for reducing side effects. Additionally, compound **34a** slightly reduced the expression of vascular endothelial growth factor receptor 2 (VEGFR2), which could be upregulated by **SAHA**. These findings indicate that the new organometallic compounds exhibit different modes of action compared to their bioactive components [138].

The fluorescent Ru(II) complex containing **SAHA** moiety (compound **35** in Fig. 9) was investigated by Rui-Rong *et al.* [21]. Compound **35** has shown to be a broad-spectrum antiproliferative agent against a variety of human cancer cell lines, including HeLa (cervical), A549 (lung), HepG2 (liver), and LO2 (liver), while exhibiting significantly low toxicity toward normal cells. Additionally, a detailed *in vitro* study on the inhibition of HDAC activity in HeLa nuclear extract identified compound **35** as an effective HDAC inhibitor. Induction of apoptosis by compound **35** was associated with activation of mitochondria-related pathways and increased ROS production [21]. The same research group also introduced a cyclometalated Ir(III) complex (compound **36**) using a pyridyl framework that has a cancer-fighting mechanism as shown in Fig. 9 [139]. Specifically, compound **36** was shown to induce apoptosis in HeLa cells by inhibiting HDACs, increasing ROS levels, and causing mitochondrial damage. The therapeutic impact of compound **36** was further amplified when exposed to ultraviolet light. The study also highlighted that combining HDAC inhibition with the photodynamic therapy (PDT) properties of luminescent Ir(III) complexes could provide a powerful approach to metal-based cancer therapy [139].

A variety of Cu(II) prodrugs based on **SAHA** (compounds **37a–e** as shown in Fig. 9) containing phenanthrene ligands known for their DNA intercalation and HDAC inhibition abilities have been developed as well [140]. These complexes demonstrated effective DNA binding and induced DNA damage through ROS generation. These compounds also showed enhanced antiproliferative effects against three cancer cell lines: SKOV-3 (ovarian), MCF-7, and DU145 (prostate). Confocal microscopy and gene expression studies revealed that cytotoxic actions are primarily carried out through apoptosis [140].

Moreover, selenium complexes have shown promising results in the development of cancer drugs due to their potential safety profile and therapeutic effects [141,142]. **Ebselen**, a clinically safe selenium complex, has been approved for treating neurological and psychiatric disorders, as well as ototoxicity [141,142]. **Ebselen** and its oxide form have been identified as inhibitors of several HDACs, with

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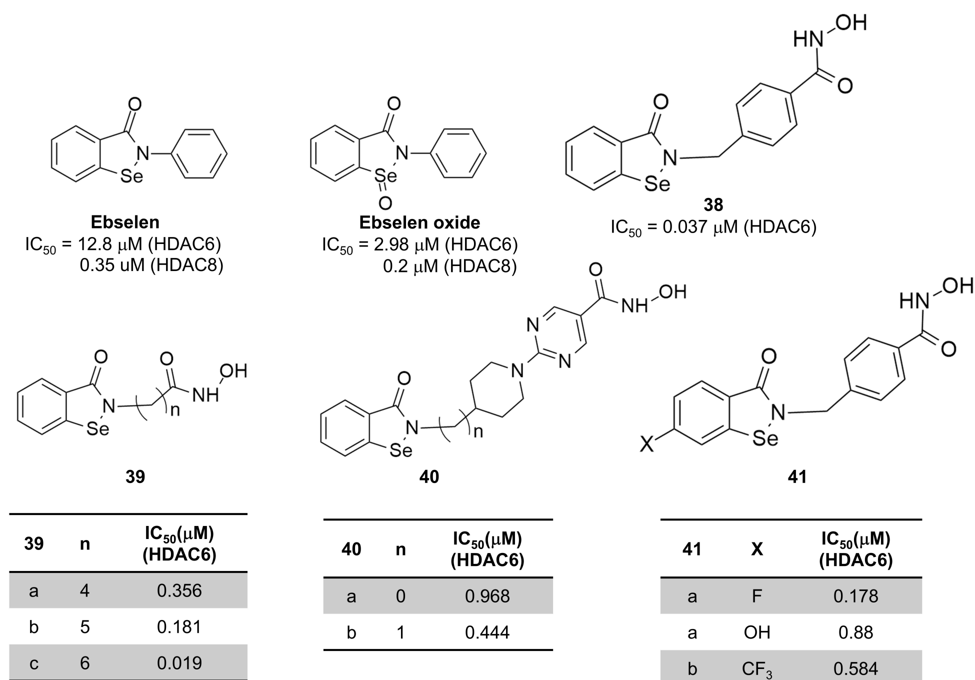


39	n	IC_{50}(μM) (HDAC6)
a	4	0.356
b	5	0.181
c	6	0.019

40	n	IC_{50}(μM) (HDAC6)
a	0	0.968
b	1	0.444

41	X	IC_{50}(μM) (HDAC6)
a	F	0.178
a	OH	0.88
b	CF_3	0.584

Fig. 10. Selenium complexes as HDAC inhibitors. The chemical structures were illustrated using ChemDraw Ultra 12.0.

IC_{50} values in the single-digit micromolar range (as depicted in Fig. 10) [141,142]. A series of **Ebselen** derivatives (compounds **38–41**) were developed by hybridizing **Ebselen** with **SAHA**, functioning as multitarget-directed ligands for treating Alzheimer's disease [143]. These complexes exhibited an inhibitory effect on HDAC6 at IC_{50} lower than 1 μM. In particular, compound **38** was found to be a potent HDAC6 inhibitor (IC_{50} = 0.037 μM). Furthermore, compound **38** demonstrated significant protective effects against hydrogen peroxide-induced damage and the ability to prevent ROS accumulation in pheochromocytoma PC12 cells [143].

A Brief Summary of Metal Complex-Based HDAC Inhibitors

The metal complex-based HDAC inhibitors can be broadly divided into two classes:

(i) *Single-target anticancer compounds.* This class of compounds includes metal-complexed compounds developed from **SAHA**, such as ferrocene-based HDAC inhibitors and arene-metal complexes (compounds **30–32**). These compounds exhibited remarkable inhibitory activity against the low nanomolar range of HDAC, surpassing the inhibition levels of their parent inhibitors like **SAHA**. Moreover, these compounds showed significantly greater cytotoxicity against cancer cells compared to **SAHA**, and traditional platinum-based anticancer drugs such as **cisplatin**, **oxaliplatin**, and **carboplatin**.

(ii) *Multitarget anticancer compounds*. This class of compounds contains all platinum complexes (compounds **1–16**) and some of the other metal complexes (compounds **34–41**). These complexes, besides targeting HDAC, also targeted receptors and enzymes, including VEGFR2 (compounds **33** and **34**) and matrix metalloproteinase (MMP; compounds **1, 2, 35**), and promoted Bax/Bcl-2 modulation (compound **4**). They also induced biological effects such as p53 activation (compounds **1**, **2**, **37**), ROS production and mitochondrial damage (compounds **1–16**, and **35–37**), and DNA intercalation (compounds **1–16**, **37**). Additionally, compounds **39–41** targeted multiple enzymes (*e.g.*, lipoxygenases, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, protein kinase C, H^+/K^+-ATPase, and IMPase) [136,143]. These compounds exhibited significant toxicity to cancer cells compared to their parent platinum-based drugs. This enhanced cytotoxicity is believed to result from the synergistic and multiple actions of the ligand and the parent coordination complex.

Inhibition of CA by Metal Complexes

Solid tumors thrive in hypoxic and acidic environments, which help them grow, spread, and resist treatments [144]. This condition leads to impairment of the DNA repair machinery and increased mutations while reducing the effectiveness of chemotherapeutic drugs such as **cisplatin** [82]. Efforts to utilize hypoxia-activated drugs were not found to significantly improve anticancer effectiveness [145]. Treatments targeting hypoxia and acidity often have limited efficacy because they can also impact healthy tissues, leading to side effects and limiting the therapeutic window [145]. Therefore, targeting tumor-associated CAs (CA IX and CA XII isoforms) offers a promising strategy

to specifically address the tumor microenvironment and disrupt metabolism, potentially improving treatment outcomes [83]. In the development of CAs inhibitors, the sulfonamide (–SO_2NH_2) moiety plays an important role in binding to Zn(II) in the active site (Fig. 11) [111,146]. The binding of sulfonamide to Zn(II) is primarily driven by two mechanisms. First, the deprotonated sulfonamide nitrogen coordinates with the catalytic Zn(II), leading to the displacement of a Zn(II)-bound water molecule (Fig. 11). Second, the sulfonamide moiety forms two hydrogen bonds with Thr199 [146]. In this binding process, the nature of the R substituent in the sulfonamide also results in further interactions with either the hydrophilic or the hydrophobic regions of the active site [146]. The strategy of developing CA inhibitors based on metal complexes encompasses two approaches that significantly change the nature of the R group in sulfonamide structure: (i) coordination complexes, where the sulfonamide acts as a ligand to the metal center, and (ii) sandwich and half-sandwich complexes, where the metal is coordinated between cyclic organic ligands, potentially including sulfonamide derivatives. Besides, linear or near-linear metal complexes of silver or gold-containing sulfonamide moiety were also introduced as potent strategies for targeting CAs.

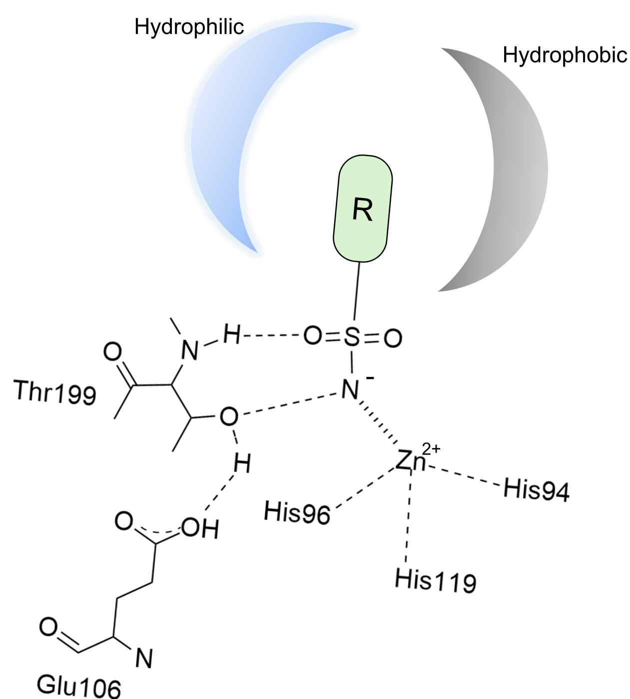


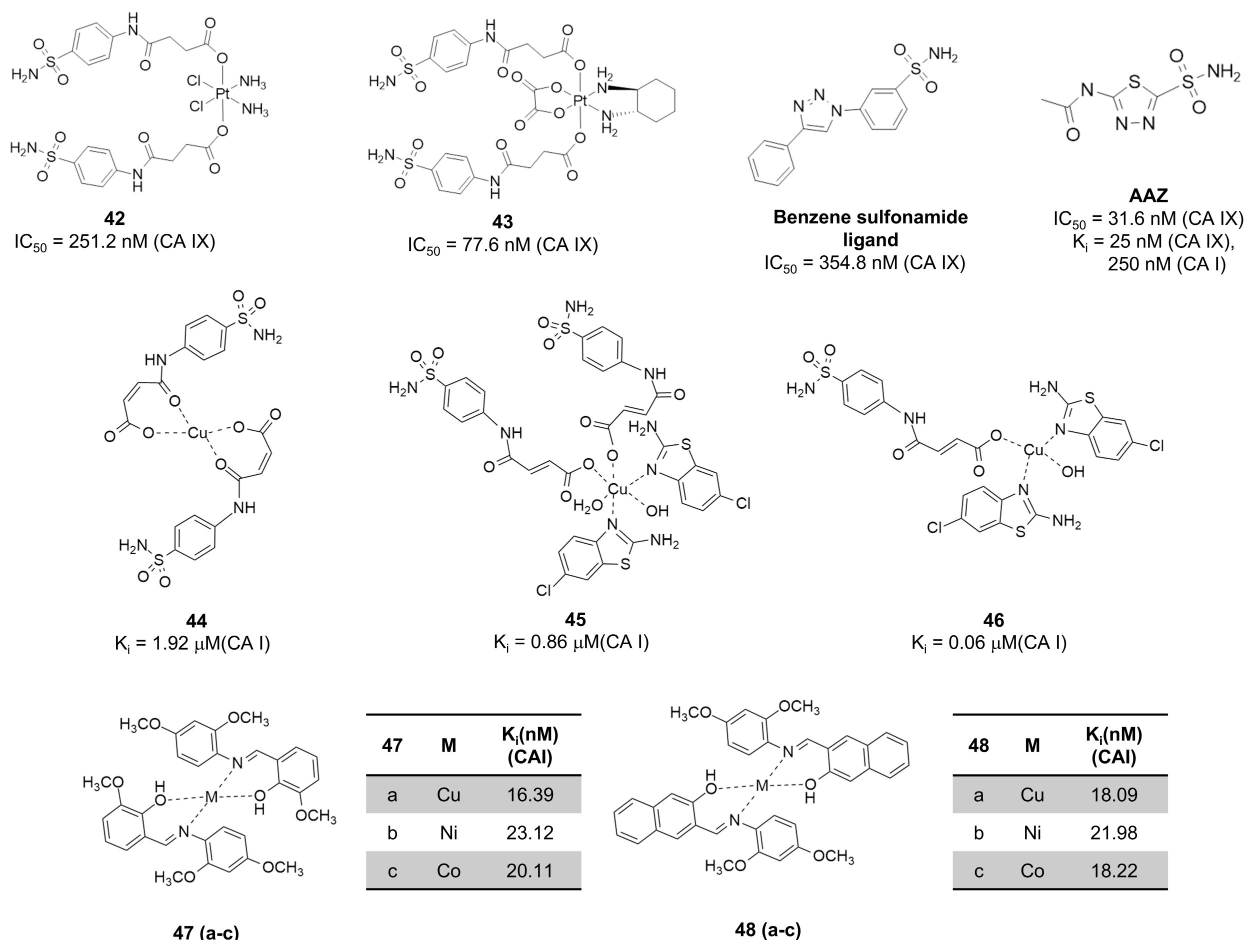
Fig. 11. Schematic illustration of the key interactions between a sulfonamide inhibitor and the CA II isoform's active site. The schematic diagram was created using PowerPoint and ChemDraw Ultra 12.0.

Inhibition of CA by Coordination Metal Complexes

The CA inhibitor development strategy that includes multitarget anticancer drugs involves incorporating sulfonamide moiety into coordination compounds, with a transition metal (such as Pt, Cu, Ni, and Co) coordinated at the center (compounds **42–48**). Two Pt(IV) complexes, known as CAIXplatins (compounds **42**, **43**), have been developed to target hypoxic and aggressive tumors by specifically targeting the CA IX isoform (Fig. 12) [20]. This achievement was realized by attaching benzene sulfonamide, the active moiety of CA IX inhibitors, to **cisplatin** and **oxaliplatin**. These complexes effectively inhibited the activity of CA IX isoform at low IC_{50} value (compound **42**: 251.2 nM; and compound **43**: 77.6 nM). Compounds **42** and **43** exhibited markedly low toxicity to normal cell lines (IC_{50} >100 µM, over 72 hours), with the cancer selectivity index (SI) being 70–90 times greater than **cisplatin** or **oxaliplatin** under hypoxic conditions. This selective action is due to their ability to bind CA IX isoform and utilize CA IX-mediated active transport and endocytosis [20]. Three Cu(II) complexes derived from proton transfer salts of sulfonamide-modified maleic acid, specifically compounds **44**, **45**, and **46** were developed [147]. These compounds demonstrated inhibition of esterase and hydratase activities in both CA I and CA II isoforms. Notably, compounds **45** and **46** interacted robustly with the active sites of carbonic anhydrase, effectively engaging both hydrophilic and hydrophobic sites. Compound **45** inhibited the CA I isoform with a K_i value of 0.86 µM and the CA II isoform with a K_i value of 0.71 µM, while compound **46** showed even lower K_i values of 0.06 µM for the CA I isoform and 0.02 µM for the CA II isoform [147].

A series of transition metal complexes (*i.e.*, Cu, Ni, Co) featuring bidentate Schiff-base ligands was developed, specifically compounds **47a–c** and **48a–c** [148]. These complexes exhibited potent inhibitory activity against CA. The inhibitory properties of these compounds were systematically evaluated against the cytosolic CA isozymes, CA I and CA II. Generally, all compounds demonstrated significant inhibition potency toward CA I with K_i values ranging from 16.39–23.12 nM [148].

In summary, the aforementioned metal complexes were developed with at least one sulfonamide serving as the zinc-binding group, where the metal center and the coordinating ligand function as the R-group, as shown in Fig. 11. In most cases, the R-groups were bulkier than those of **AAZ**, yet these compounds still managed to enter the active pocket of CA and coordinate with the Zn(II) at the active center. Additionally, compounds **42** and **43** exhibited a binding mode similar to **AAZ**, forming additional hydrogen bonds with amino acids (His64, Gln92, Asn62, Gln67, and Trp5) [20]. However, the CA inhibition ability of these compounds (**42–46**) was lower or similar to that of **AAZ**, but greater than that of the benzene sulfonamide ligands (compounds **42, 43, 46**). This could be at-



47	M	K_i(nM) (CAI)
a	Cu	16.39
b	Ni	23.12
c	Co	20.11

48	M	K_i(nM) (CAI)
a	Cu	18.09
b	Ni	21.98
c	Co	18.22

Fig. 12. Metal complexes as CA inhibitors. The chemical structures were illustrated using ChemDraw Ultra 12.0.

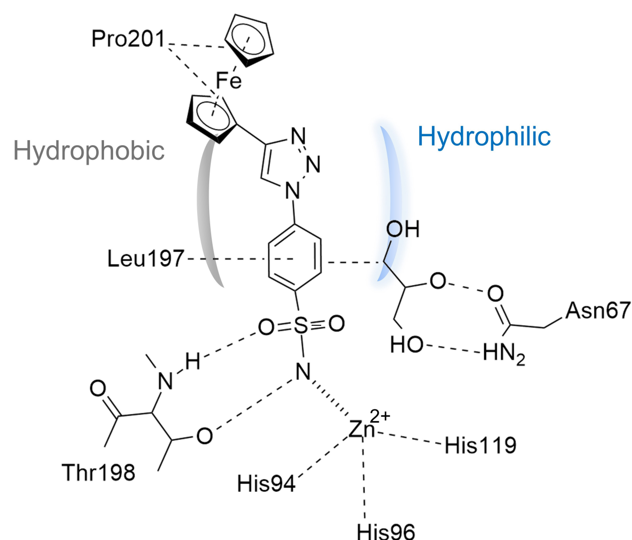


Fig. 13. Interaction of the compound 49 with Zn(II) active site of CA II isoform. The chemical structure was illustrated using PowerPoint and ChemDraw Ultra 12.0.

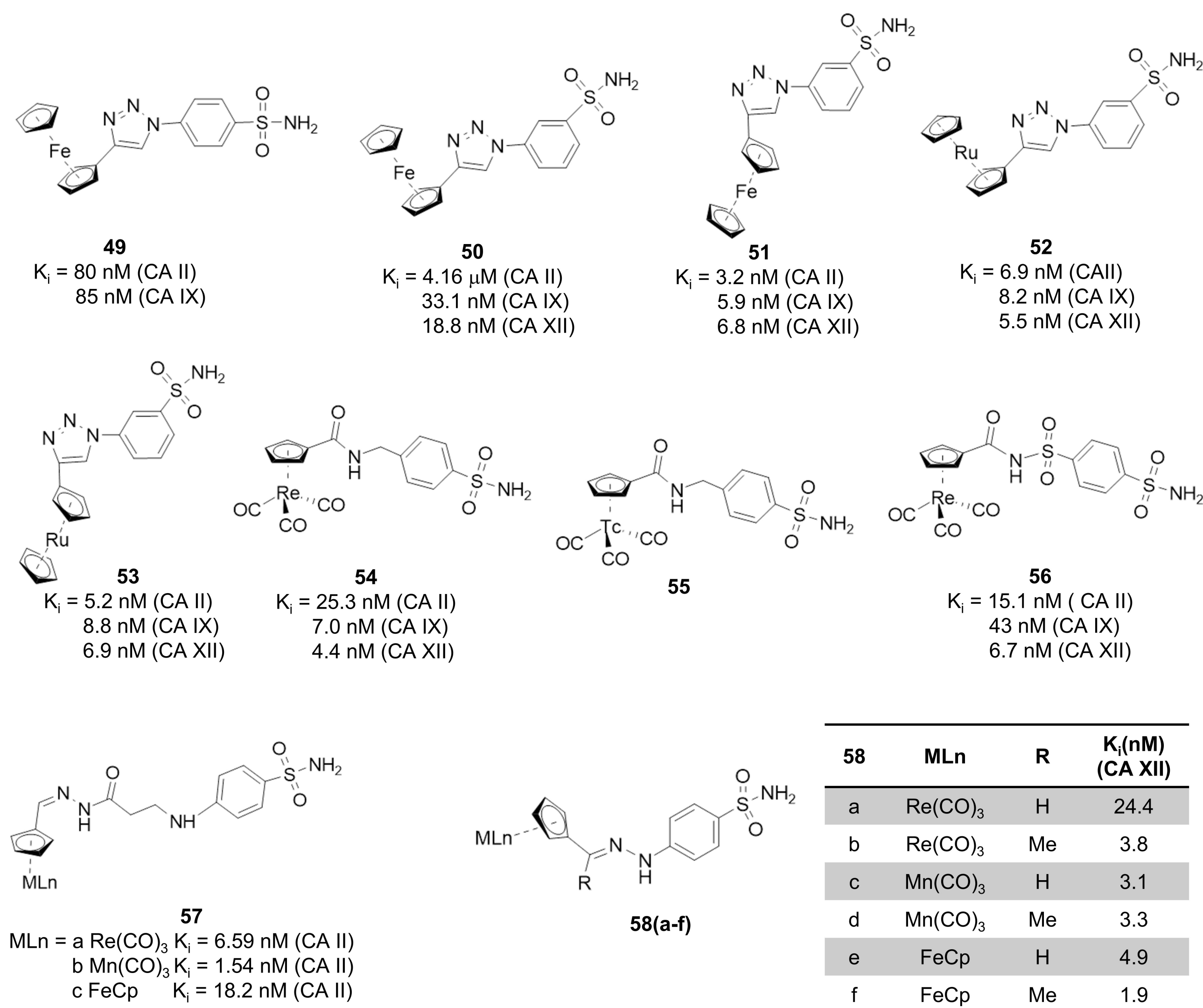
tributed to the unique properties of **AAZ**'s structure, where the heterocyclic ring facilitates easier access to the pocket site and enables more extensive bonding within the active site [149]. It was also observed that compounds **42** and **43** underwent reduction to Pt(II), releasing benzene sulfonamide ligands. This instability and consequent reduction might restrict their ability to effectively enter and act within the active site [20].

Inhibition of CA by Sandwich/Half-Sandwich Metal Complexes

In the development of new CA inhibitors based on sandwich/half-sandwich complexes, multiple studies incorporated metallocene or metal arene into benzene sulfonamides and/or into **AAZ** [150–154]. This approach significantly raised inhibition activity over **AAZ** and coordination metal complexes containing sulfonamides. It will be discussed in detail in this section.

Sandwich/Half-Sandwich Metallocene Complexes

The mechanism of action of sandwich/half-sandwich complexes also involves binding of the sulfonamide head to a Zn(II) located in the CA cavity. While the metallocene-triazole tail groups could occupy the space in the active site and interact with further sites in the active cavity, as pre-



58	MLn	R	K_i(nM) (CA XII)
a	$Re(CO)_3$	H	24.4
b	$Re(CO)_3$	Me	3.8
c	$Mn(CO)_3$	H	3.1
d	$Mn(CO)_3$	Me	3.3
e	FeCp	H	4.9
f	FeCp	Me	1.9

Fig. 14. Sandwich and half-sandwich complexes as CAs inhibitors. The chemical structures were illustrated using ChemDraw Ultra 12.0.

sented in Fig. 13 [151]. Any modifications of metallocene or metal arene could lead to changes in inhibition activity.

Compound **49–53**, a series of benzene sulfonamides containing ferrocenyl fragments were synthesized and investigated for their ability to inhibit the enzymatic activity of CA II isoforms, as well as tumor-associated CA IX and XII isoforms (Fig. 14) [150–152]. All compounds were nanomolar inhibitors of CA IX and XII, with K_i ranging from 5.9–85 nM and 5.5–18.8 nM, respectively [152]. In addition, most of these compounds (except compound **50**) also inhibited the CA II isoform with a K_i value in the nanomolar range. Ruthenocenyl derivatives (compounds **52** and **53**) provided superior CA inhibition compared to ferrocenyl compounds for all three CA isozymes. X-ray crystallography confirmed that the sulfonamide binds to the catalytic zinc in the active site of the CA II isoform whereas the metallocene located at the entrance does not interact with the enzyme [151,152]. The bulkiness of metallocene allows for better targeting within the active site of the enzyme compared to simpler compounds [151,152]. Can *et al*. [153,154] explored rhenium and technetium tricarbonyl fragments as metal-based CA inhibitors (compounds **54**, **55,** and **56**), attaching these fragments to the sulfonamide's tails. Similar to previous ferrocene/ruthenocene sulfonamides complexes, their crystal structures revealed that the sulfonamide group bound to Zn(II) of the enzyme without interaction between the metal-substituted tail and the protein [154]. These compounds displayed nanomolar affinity for specific CA isoforms, especially enhanced selectivity for CA II, IX, and XII isoforms (K_i ranged from 4.4 to 43 nM).

To obtain CA inhibitors containing organometallic moieties, a series of organometallic acylhydrazones (compounds **57a–c**) containing $Re(CO)_3$, $Mn(CO)_3$ and ferrocenyl moieties were reacted with amino-sulfonamides [155]. The resulting compounds demonstrated selective in-

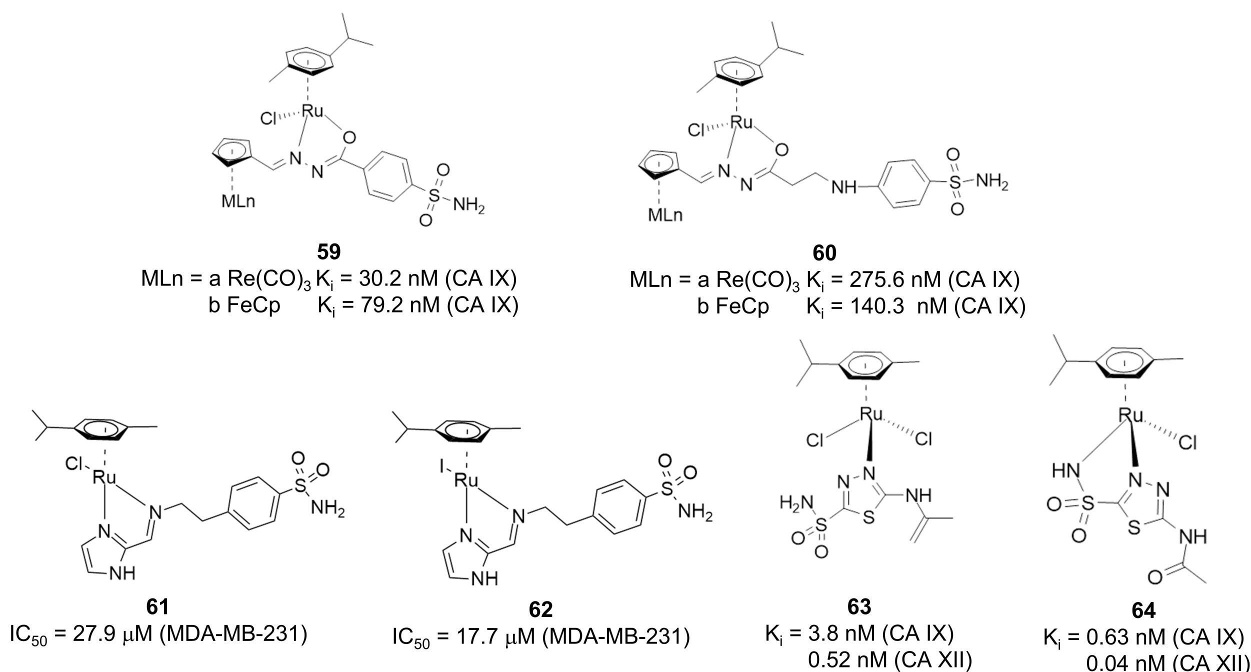


Fig. 15. Metal arene complexes as CA inhibitors. The compounds were prepared by using ChemDraw Ultra 12.0. Note: MDA-MB 23 is a cell line of triple-negative breast cancer.

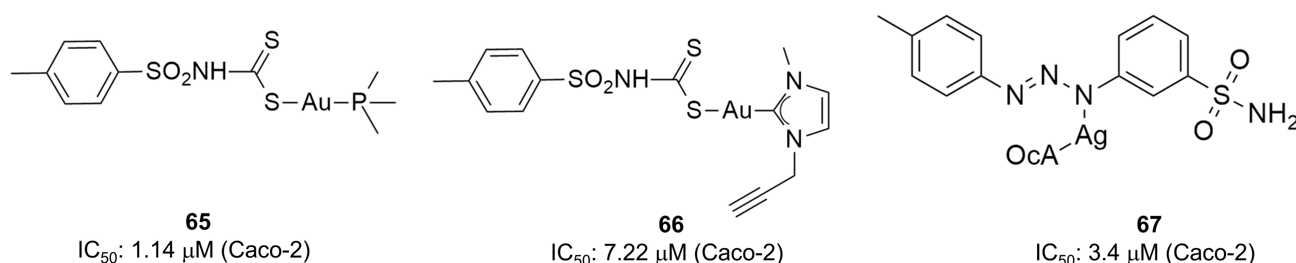


Fig. 16. Linear silver and gold complex as CA inhibitors. The chemical structures were illustrated using ChemDraw Ultra 12.0. Note: Caco-2 is a cell line of human colorectal andenocarcinoma.

hibition of CA II, VA, and VII isoforms with nanomolar K_i values, whereas the inhibitory effect on CA I isoform was much less potent, indicating much higher K_i values. Brichet *et al.* [156] introduced a series of bioorganometallic compounds featuring benzenesulfonamide with CA inhibitory properties (compounds **58a–f**). All six compounds showed significant inhibitory effects on CA XII in the K_i range from 1.9 to 24.4 nM. The cytosolic isoforms, CA I and II, were also effectively inhibited by almost all derivatives with K_i in the range of 1.7–22.4 nM. The inhibitory efficacy of these compounds was comparable to or even exceeded that of **AAZ**. This improved performance is potentially due to the presence of metal-organic or metal-carbonyl groups in the molecules of these CA inhibitors [156].

Half-Sandwich Metal Arene Complexes

Metal arene complex-based CA inhibitors, with a primary emphasis on ruthenium complexes, have been introduced (Fig. 15). For example, two Ru/Fe heterobimetallic complexes (**59a–b** and **60a–b**) containing sulfonamide were developed to act as selective inhibitors against tumor-related isoforms CA IX and XII [157]. These compounds selectively inhibit CA IX and XII isoforms at nanomolar value (K_i ranged from 30 to 275 nM), which was analyzed by a stopped-flow CO_2hydrase assay [157]. Two Ru(II) arene complexes (compounds **61** and **62**) containing sulfonamides have been developed. Their sulfonamides could serve as specific probes targeting cancer cells to enhance the uptake of these compounds into such cells [158]. These compounds show cytotoxicity toward cancer cells (MDA-MB-231 and MIA PaCa-2), while sparing non-cancerous CHO and MDCK cells. Enhanced toxicity under hypoxic conditions observed in MDA-MB-231 cells may be linked to higher expression of CA IX in these conditions. Both complexes **61** and **62** induce apoptosis by depolarizing mitochondrial membrane potential and arresting the cell cycle in the SubG1 phase [158].

In addition to the metal complexes containing benzene sulfonamides, complexes featuring **AAZ** were developed, exhibiting significantly higher inhibitory activity compared to **AAZ** alone and surpassing that of the benzene sulfonamides complex. For example, Seršen *et al.* [159] synthesized complexes **63** and **64** by combining **AAZ** with Ru(II) η^6-p-cymene chloride. These compounds demonstrated significant inhibitory effects on human CA I, IX, and XII isoforms, surpassing **AAZ**'s inherent inhibitory capabilities [159]. These compounds showed potent activity against CA I, with K_i values ranging from 8.5 to 23.4 nM, compared to the K_i value of 250 nM for **AAZ**. For the CA II isoform, its K_i values ranged between 0.48 and 4.2 nM. The inhibitory effect extended to CA IX and XII isoforms, with K_i value ranges of 0.63 to 3.8 nM and 0.04 to 0.52 nM, respectively, showing their remarkable potency against these enzymes [159].

In summary, developing CA inhibitors based on sandwich/half-sandwich metal complexes containing sulfonamide moieties involves single-target anticancer drugs. The compounds mentioned above (**49–64**) exhibit significant inhibitory effects toward CA IX and CA XII isoforms, with K_i values in the nanomolar range. While compounds **61** and **62** have demonstrated effective anticancer activity while sparing non-cancerous cells, most of the other compounds have not been examined for their anticancer activity and cytotoxicity. Detailed studies on the anticancer effect of these compounds on cancer cells should be processed.

Linear-Geometry Metal Complex

Linear metal complexes are characterized by their two-bond coordinated structures, commonly with Ag(I) and Au(I) as metal centers [160]. In developing CA inhibitors based on these metal complexes, a sulfonamide moiety is incorporated into Ag(I) and Au(I)-based anticancer drugs. This modification aims to enhance the efficacy of these complexes as multitarget anticancer agents [161]. Auranofin, an Au(I) complex, has garnered significant interest for its potential in cancer therapy [160]. A recent study has focused on derivatives of auranofin, specifically designed as CA IX inhibitors by incorporating dithiocarbamate gold(I) complexes with benzenesulfonamide (compounds **65** and **66**) (Fig. 16) [161]. Compounds **65** and **66** were developed as multitarget anticancer drugs that induced the apoptosis of colon cancer cells [161]. Both compounds are capable of inhibiting the CA IX isoform at lower concentrations. They exhibited strong toxicity on human colorectal adenocarcinoma Caco-2 cells, with IC_{50} values of 1.14 µM and 7.22 µM, respectively. Both complexes generate cellular stress that activates the p53 protein, leading to cell cycle arrest in the G1 phase. The inhibition of thioredoxin reductase causes a pro-oxidant effect, potentially augmented by the pro-oxidant action of p53 [161]. Briefly, the activity of CA IX isoform is compromised upon treatment with compounds **65** and **66**, indicating that both complexes are probably multitarget drugs [161]. A silver complex of 1,3-diaryltriazene-substituted sulfonamides was synthesized by Canakci *et al.* [162] (compound **67**), which showed one of the high cytotoxicity against all cancer cell lines (DLD-1, HeLa, MDA-MB-231, HT-29, ECC-1, DU-145, and PC-3) with IC_{50} values between 3.30 to 16.18 µM among other tested compounds.

A Brief Summary of Metal Complex-Based CA Inhibitors

Like HDAC inhibitors based on metal complexes, CA inhibitors based on metal complexes can be divided into two classes. Except for compounds **65** and **66**, which are multitarget anticancer complexes, most of the aforementioned CA inhibitors involve CA single-target compounds. These compounds, especially compounds **49–64**, exhibited remarkable inhibitory activity against CA within the very low nanomolar range, surpassing the inhibition levels of benzenesulfonamide and **AAZ**. However, the cytotoxicity and anticancer activity of these compounds have not been studied in detail. Therefore, future studies should investigate the broader impacts of these drugs on cancer cells.

Conclusions

In summary, this paper reviews the role of metal complexes as potent inhibitors of HDACs and CAs, with an emphasis on their superiority in selectivity and efficiency over traditional organic inhibitors. This review also presents the mechanisms of action and clarifies the relationship between molecular design, inhibitory efficiency, and cytotoxic effects, offering insights that could inform the design of future inhibitors. Future research should focus on designing new inhibitors based on metal complexes by incorporating relatively non-toxic metals with effective ligands (*i.e.*, organic inhibitors).

Author Contributions

YTN and HJL conceptualized the review. YTN, NK, and HJL performed the literature searches and YTN prepared the figures and tables in the manuscript. YTN, NK, and HJL prepared the first draft and critically revised the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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