Cytotoxic derivatives of dichloroacetic acid and some metal complexes

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Abstract
This study outlines a number of studies of dichloroacetic acid (DCA) and some of its derivatives. Although DCA has low cytotoxic potencies, various structural modifications are described which result in potent cytotoxins. In particular, hybrid molecules created from DCA and other bioactive molecules whose modes of action differ from DCA are particularly promising as candidate anticancer agents. Considerable emphasis in this review is placed on various series of compounds that incorporate both platinum and DCA into their structures. In addition, the importance of the formulation of some of the bioactive compounds described herein is revealed.

KEYWORDS
anticancer, dichloroacetate, dichloroacetic acid, metal complexes, mitochondria

1 | INTRODUCTION

The therapeutic potential of dichloroacetate (DCA) was foreshadowed in 1960s for its interesting biological activities.[1] At that time, it was discovered that DCA can stimulate significant effects on carbohydrates and lipid metabolism in experimental diabetes[2] and soon thereafter, the historic report of its eliciting effect on the pyruvate dehydrogenase complex (PDC) was revealed.[3] These early findings led to the identification of DCA as a lipid-lowering and antidiabetic drug,[4] with the possibility of treating these diseases as well as myocardial and cerebrovascular ischemia[5] and acquired[6,7] and congenital[8,9] forms of lactic acidosis.

The anticancer properties of DCA were first discovered by Bonnet et al. They suggested that the metabolic alterations that occur in tumor cells can be manipulated to kill them.[10] Since then, more than 500 peer-reviewed publications have been published to establish DCA as a prototype of a new class of "metabolic modulators" acting to shift a cancer cell's metabolism away from cytoplasm and toward mitochondria, a consequence that, ironically, initiates the cancer cell's death.[11] To understand the interesting effects of DCA on cancer metabolism, the mechanism of action is briefly described below.

Over the last 10 years, a number of reviews have been written on DCA that provide further evidence of its potential usefulness in cancer chemotherapy.[12–14] Although an extensive body of literature demonstrates the antineoplastic properties of DCA, its effective clinical application for treating cancer is limited to clinical trials.[15] The clinical use of DCA is still restricted due to the existence of side effects such as neurotoxicity as well as the suspicion of carcinogenicity.[15,14] To avoid side effects that enhance drug bioavailability and efficacy, many researchers studied the synergistic effects of DCA with other potential or clinically used anticancer drugs.[17–20] In addition, over the last decade many scientists have designed and synthesized DCA derivatives that could be used as hybrids or prodrugs. A hybrid molecule is designed so that it can react at different receptor sites to increase cytotoxic potency while a prodrug is devoid of bioactivity until it releases one or more bioactive compounds. In this article, we have focused on compounds containing the DCA moiety in esters, amides, and metal...
complexes that showed antineoplastic properties and suggesting the employment of DCA in cancer therapy.

2 | MECHANISM OF ACTION OF DCA IN CANCER CELLS

Malignant cells have well-known properties including abnormal proliferation, deregulation of apoptosis, and cell cycle. Besides these properties, cancer cells also exhibit different metabolisms compared to normal cells. In fact, to achieve increased energetic and anabolic needs, cancer cells follow reprogramming metabolic pathways for ATP, reduced coenzymes (e.g., NADPH), lipids, proteins, and nucleotides. Therefore, one of the popular strategies for developing antineoplastic agents has been therapies that target the metabolic differences between cancers and normal cells. These therapeutic potentials arise from a discovery by Otto Warburg in the 1920s known as the "Warburg effect." According to the Warburg effect, for ATP production cancer cells incline to transform glucose into lactate via glycolysis even under aerobic conditions in place of the much more efficient oxidative phosphorylation (OXPHOS) pathway which is the preferred pathway for most other cells in the body. Although ATP production via cytoplasmic glycolysis is 100 times faster than the mitochondrial OXPHOS, the energetic yield of glycolysis is very small since ATP production through glycolysis is 18 times lower than OXPHOS. Because of this thermodynamic trade-off between the yield and rate of ATP production, cancer cells significantly increase glucose uptake by upregulating the glucose receptors in an attempt to "catch up." In fact, positron emission tomography imaging studies confirmed that most solid tumors showed markedly increased glucose uptake and metabolism, compared to noncancerous cells. This bioenergetic difference between malignant and normal cells may be targeted therapeutically due to its unique importance in cancer cells.

Before OXPHOS can occur, pyruvate must first be converted to acetyl-CoA in a series of reactions facilitated by the PDC, named after pyruvate dehydrogenase (PDH), the first enzyme in the complex. PDC inhibition can occur if the E1α subunit of the PDH is phosphorylated by pyruvate dehydrogenase kinase-1 (PDK1), an enzyme commonly overexpressed in malignant cells. PDC inhibition by PDK1’s stops acetyl-CoA formation from pyruvate, which prevents oxidation from occurring in the TCA cycle and inhibits OXPHOS (Figure 1).

A clear need is demonstrated for compounds which inhibit PDK activity since PDK inhibition can reverse the Warburg effect. A number of compounds such as DCA, a halogenated acetophenone (1), analogs of ATP, for example, adenosine 5’-[β,γ-imido]triphosphate (2), certain triterpenes such as 3, certain lactones, such as 4 and (R)−3,3,3-trifluoro-2-hydroxy-2-methylpropionamides such as 5 are known inhibitors that can inhibit PDK activity (Figure 2). Among these, DCA is the focus of this review. The molecular interactions between DCA and PDKs have been studied extensively. In general, DCA appears to bind to a hydrophobic pocket in the N-terminal domain of PDK1 which promotes conformational changes at the active site and prevents phosphorylation of the E1α subunit of the PDC. This frees up the PDH enzyme in the PDC and allows for full glucose oxidation through OXPHOS, inhibiting lactate fermentation (cytoplasmic glycolysis), and restoring apoptosis capability via mitochondrial depolarization (Figure 3).

This cascade of events increases the interest in the use of DCA for its antitumor properties. Many different studies have shown that DCA and its derivatives reverse the glycolytic phenotype by activating a metabolic switch from aerobic glycolysis to OXPHOS; they also show suppression of tumor proliferation. DCA combination with other chemotherapeutic drugs such as 5-fluorouracil or paclitaxel was more effective in killing resistant cancer cells than either chemotherapeutic drugs or DCA treatment alone. Apoptosis capability...
is restored. DCA has also been approved as a treatment method for lactic acidosis.[9]

3 | LIMITATIONS OF THE USE OF DCA

The clinical use of DCA has been related with reversible peripheral neurotoxicity, which is thought to be caused by uncompensated oxidative stress from increased reactive oxygen species (ROS) formed because of PDK inhibition by DCA.[15,39,40] In addition, it has been found that the catabolism of the amino acids phenylalanine and tyrosine and heme synthesis are interfered by the metabolism of DCA. As a result, the accumulation of reactive molecules capable of forming adducts with DNA and proteins are formed and resulted in oxidative stress.[15] Suspicion of carcinogenicity is also a restricting factor in the use of DCA and administration as an anticancer agent should be confined solely to clinical trials.

The low stability of DCA in a biological environment and also its low cellular membrane permeability are other major issues that are principally associated with the clinical use of DCA for cancer treatment.[11,41] In fact, due to its anionic charge DCA is unable to cross the cell membrane through passive diffusion and is unable to
enter inside mitochondria by the classical mitochondrial pyruvate carrier. Therefore, high dosages of DCA are required to reach therapeutic effectiveness and clinical results.

4 | DERIVATIVES OF DICHLOROACETIC ACID

Because of neurotoxicity, the clinical effectiveness, and toxic properties of DCA as an antineoplastic agent need to be carefully investigated in clinical trials. Nonetheless, promising clinical and translational research suggests a wide therapeutic window for DCA, provided it can be administered with adequate tolerability and safety. Thus, there is increasing interest in the development of new strategies aimed to engineer DCA derivatives with increased tolerability and safety and that will be able to cross the cellular membranes, enabling the cellular uptake. Toward this goal over the last decade many scientists designed and synthesized derivatives of dichloroacetic acid as potent cytotoxic agents. Therefore, in this study, we have focused on various types of compounds containing the dichloroacetyl group that showed promising anticancer activity. A major emphasis is given on the structure–activity relationships of these dichloroacetic acid derivatives in this study. Different compounds are grouped together as derivatives of natural, synthetic organic and metal complexes, and their potencies as anticancer agents are discussed.

4.1 | DCA derivatives of natural compounds

Historically natural products and their derivatives play a significant role in drug development, especially in the area of cancer therapy. A diverse source of new medicinal leads have been obtained from natural products and their derivatives. Among them, triterpenes are a significant class of compounds for their anticancer properties. Recently, a dichloroacetamide (6b) of a triterpenoid saponin albizia bioside A (6a) (Figure 4) was reported to show selective cytotoxicity against PDK-medium and PDK-high-expressed human cancer cells. Previously, it was found that albizia bioside A (isolated from Albizia inundata) could induce apoptosis in A375 cells by adjusting mitochondrial function, involving a caspase cascade with an IC₅₀ value of 5.47 μM. When the dichloroacetyl moiety was attached to albizia bioside A (6a) as an amide, the resultant compound 6b displayed greater cytotoxicity compared with 6a and DCA alone against all tested cancer cells (MCF-7, HCT116, A375, and 4T1). Most importantly, the dichloroacetamide 6b exhibited the best cytotoxicity against the PDK-high-expressed human breast cancer cells (MCF-7) with IC₅₀ value 0.43 μM, which is enhanced 47.5-fold compared to albizia bioside A. This enhanced activity can be attributed to the more efficient PDK inhibition capacity of 6b. The compound 6b could inhibit PDK activity and impact the energy metabolism reprogramming of tumor cells and turn cellular metabolism away from aerobic glycolysis to OXPHOS. It was also reported that compound 6b induced a significant increase in intracellular ROS and reduced the accumulation of lactic acid in the tumor microenvironment (TME). Importantly, compound 6b caused apoptosis via a caspase dependent pathway, inhibited the glutathione peroxidase IV (GPX4) pathway and induced ferroptosis by the accumulation of lipid peroxidation. Additionally, in an antitumor experiment on nude mice containing MCF-7 tumors, compound 6b was found to be an effective and safe agent. It was also reported that 6b could inhibit both primary and distal tumor progression in a dual-4T1 tumor model in female BALB/c mice by remolding the tumor immunosuppression microenvironment via eliminating M2-TAMs (tumor-associated macrophages).

Tormentic acid (7a) (Figure 4), a triterpenoid, can be isolated from different plants including Myrianthus serratus, Perilla frutescens, Cotoneaster simonsii, and Rubus sieboldii. Tormentic acid (7a) possesses many biological activities such as in vitro inhibition of platelet aggregation, reduction of vascular smooth muscle cell proliferation, anti-inflammatory activity, and inhibition of α- and β-DNA polymerases. Although only a weak cytotoxic activity has been established against different cancer cell lines for 7a, the
derivatives 7b–7d containing monochloroacetyl groups showed excellent cytotoxic activities against 518A2 (melanoma), B505C (anaplastic thyroid), A253 (head), A2780 (ovarian), A549 (lung), DLD1 (colon), MCF7 (mamma). Among all the tormentic acid derivatives, compound 7b exhibited excellent antitumor activity with IC50 values ranging 1.1–1.6 μM and this anticancer property was found due to the induction of an apoptosis pathway.

Doxorubicin (8a) (Figure 5) is a natural compound isolated from a soil born bacteria, Streptomyces peucetius. It has been used as an efficient chemotherapeutic drug for many cancers, including bladder cancer, Kaposi’s sarcoma, breast cancer, lymphoma, and acute lymphocytic leukemia. However, because of several side effects such as vomiting and nausea, hepatotoxicity, cardiotoxicity, and myelosuppression, its clinical application is limited. To reduce side effects, enhance antitumor efficiency, and increase drug loading content, Yang et al. synthesized the amide 8b by coupling doxorubicin (8a) and DCA. It was reported that compound 8b can be easily self-assembled into NPs with a small amount of PEGylated lipid DSPE-PEG2000 (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)−2000]). The nanoformulation of amide 8b exhibited a high drug loading content (71.8%, w/w) that can significantly reduce the side effects produced by the excipient. The NPs showed high maximum tolerated dose of by 75 mg/kg of 8b, which was 15-fold higher than that of free doxorubicin and undetectable systemic toxicity. Additionally, compound 8b displayed enhanced antitumor activity and great tumor targeting capability in a murine melanoma model.

Phenanthroindolizidines, a family of alkaloids, are isolated from various species of Asclepiadaceae. Phenanthroindolizidines, for example, tylocrebrine and tylophorine, display excellent cytotoxicity against various human cancerous cell lines. They are also known to exhibit excellent cytotoxicity against multidrug-resistant (MDR) cancer cell lines. To increase the potency of these compounds, Omran et al. developed a codrug of 14-hydroxytylophorine and dichloroacetic acid by combining the two molecules through an ester linkage. The resultant DCA esters 9 (Figure 5) were evaluated against two reference cell lines: human embryonic kidney cells (HEK293) and Chinese hamster ovary cells (HEK293) and against four cancer cell lines: neuroblastoma (SK-N-DZ), Burkitt’s lymphoma, hepatocarcinoma (HepG2), and colorectal adenocarcinoma (DLD-1). The cytotoxic data of esters 9 were compared with the parent 14-hydroxytylophorine and with the reference chemotherapeutic drug, doxorubicin. Although the developed codrugs 9 maintained the cytotoxicity of the parent alkaloids, the codrug strategy did not improve their potency or selectivity.

The natural compound honokiol (10a) (Figure 6) is isolated from the plant Magnolia grandiflora which has been used in traditional Chinese medicine for its anti-inflammatory, muscle relaxant, antiallergic,
antioxidative, and antibacterial activities.\textsuperscript{[54]} Recently, honokiol (10a) has also been found as a promising anticancer agent in preclinical studies because of its nontoxic properties.\textsuperscript{[55]} However, honokiol has poor solubility and bioavailability.\textsuperscript{[54]} To increase its cellular uptake and lipophilicity, honokiol (10a) was transformed into the corresponding bis-DCA ester derivative (10b). It was reported that both honokiol (10a) and its DCA derivative 10b could inhibit androgen receptor (AR) activity in prostate cancer cells,\textsuperscript{[57]} showed in vivo activity against vemurafenib-resistant melanoma,\textsuperscript{[58]} and are selective allosteric inhibitors of the mitochondrial chaperone TNF receptor-associated protein 1 (TRAP1).\textsuperscript{[59]}

Both honokiol (10a) and its DCA derivative 10b were reported to inhibit the growth of an androgen-responsive prostate cancer cell line with wildtype p53 (LNCaP) and its androgen-independent variant (C4-2) in a time and dose-dependent manner.\textsuperscript{[57]} The bis-DCA ester 10b was more potent than the parent compound 10a and other tested analogs. Like the cell viability data, compound 10b was relatively more efficient in reducing AR protein level than the parent compound 10a in prostate cancer cells. It was also reported that both proteasomal degradation and transcriptional repression mechanisms likely cause honokiol-mediated downregulation of the AR protein expression. In addition, the reasons for showing different effects of 10 on mRNA and AR protein between LNCaP and C4-2 cells were not yet clear.

Honokiol bis-DCA (10b) and a hexafluoro analog 11 (Figure 6) were studied against aggressive melanoma such as LM36R and A375, and less aggressive LM36 cells in vivo.\textsuperscript{[58]} Both 10b and 11 showed activity against A375 melanoma in vivo, but compound 10b was more effective. Honokiol 10b exhibited activity against LM36R (vemurafenib resistant) in vivo but not toward parental LM36. Both 10b and 11 inhibited the phosphorylation of dynamin-related protein 1 (DRP1), which stimulates a phenotype suggesting respiration via mitochondrial normalization. It was suggested that 10b possibly acts in vemurafenib-resistant melanomas by increasing both respiration and ROS production, which leads to show its activity against aggressive melanoma in vivo.

Honokiol bis-DCA (10b) was also found to display allosteric inhibition of TRAP1 chaperone activity which makes it a potential lead compound for a new generation of antineoplastic agents.\textsuperscript{[59]} The molecular chaperones TRAP1 are the mitochondrial paralog of the heat shock protein 90 (Hsp90). It is necessary for tumor growth found in several tumor cell models. TRAP1 induces bioenergetic rewiring, maintenance of redox homeostasis, and orchestration of a hypoxia-inducible factor 1-alpha (HIF1α)-mediated pseudohypoxic program by inhibiting succinate dehydrogenase (SDH) activity. It was reported that compound 10b can bind to the allosteric site in TRAP1, which inhibited TRAP1 but not Hsp90 ATPase activity. In neoplastic cells, 10b was able to revert TRAP1-dependent downregulation of SDH, decrease proliferation rate, increase mitochondrial superoxide levels, and abolish tumorigenic growth.\textsuperscript{[59]}

A series of new DCA derivatives of phenstatin were reported recently that focused on the use of a single compound acting on two different biological targets thereby enhancing potency and lowering the toxicity of the chemotherapeutic agent.\textsuperscript{[60]} The first point of action was inhibition of the tubulin which is involved in cell proliferation due to its ability to polymerize and form microtubules which are key components of the cytoskeleton. The second point of action was the inhibition of mitochondrial pyruvate dehydrogenase kinases (PDK1-4) that activate the PDC. The dual-targeted compounds 12 and 13 (Figure 7) were designed and synthesized by combining antitubulin benzophenones and benzothiophenones derived from phenstatin, which is a known potent tubulin

\begin{center}
\textbf{FIGURE 7 Structures of compounds 12 and 13}
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\text{12a-h} \\
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\begin{itemize}
\item \textbf{a}: R\textsuperscript{1}=R\textsuperscript{5}=H, R\textsuperscript{2}=R\textsuperscript{3}=R\textsuperscript{4}=OCH\textsubscript{3}, R\textsuperscript{6}=COCH\textsubscript{2}Cl
\item \textbf{b}: R\textsuperscript{1}=H, R\textsuperscript{2}=R\textsuperscript{3}=R\textsuperscript{4}=R\textsuperscript{5}=OCH\textsubscript{3}, R\textsuperscript{6}=COCH\textsubscript{2}Cl
\item \textbf{c}: R\textsuperscript{1}=R\textsuperscript{5}=H, R\textsuperscript{2}=R\textsuperscript{4}=CH\textsubscript{3}, R\textsuperscript{3}=OCH\textsubscript{3}, R\textsuperscript{6}=COCH\textsubscript{2}Cl
\item \textbf{d}: R\textsuperscript{1}=R\textsuperscript{5}=H, R\textsuperscript{2}=R\textsuperscript{3}=R\textsuperscript{4}=OCH\textsubscript{3}, R\textsuperscript{6}=COCHCl\textsubscript{2}
\item \textbf{e}: R\textsuperscript{1}=R\textsuperscript{5}=H, R\textsuperscript{2}=R\textsuperscript{3}=R\textsuperscript{4}=OCH\textsubscript{3}, R\textsuperscript{6}=COCl\textsubscript{2}
\item \textbf{f}: R\textsuperscript{1}=R\textsuperscript{3}=R\textsuperscript{4}=F, R\textsuperscript{2}=OCH\textsubscript{3}, R\textsuperscript{5}=R\textsuperscript{6}=COCH\textsubscript{2}Cl
\item \textbf{g}: R\textsuperscript{1}=R\textsuperscript{3}=R\textsuperscript{4}=F, R\textsuperscript{2}=OCH\textsubscript{3}, R\textsuperscript{5}=OH, R\textsuperscript{6}=COCH\textsubscript{2}Cl
\item \textbf{h}: R\textsuperscript{1}=R\textsuperscript{3}=R\textsuperscript{4}=F, R\textsuperscript{2}=OCH\textsubscript{3}, R\textsuperscript{5}=R\textsuperscript{6}=COCl\textsubscript{2}
\end{itemize}
polymerization inhibitor, with mono-, di- and tri-chloroacetate groups targeting PDK1. Based on biological studies, all the synthesized compounds were grouped as: (i) inhibitors of tubulin polymerization, classic cytotoxic agents, for example, compounds 12a, 12b, 12d, 12e; (ii) inhibitors of PDK1, which are cytostatic, such as compounds 12f, 12g, 12h; (iii) dual inhibitors of tubulin/PDK1, for example, compounds 12c and 13; (iv) highly cytotoxic compounds.

Compounds 12 and 13 (Figure 7) have been found to exhibit excellent anticancer activity against NCI-60 cancer cell lines as dual tubulin/PDK1 inhibitors and antimitotic agents.[60] Colon cancer (KM12 and HCT-15), melanoma (specifically MDA-MB-435 and M14), and leukemia (HL-60(TB) and K-562) cell lines were found to be most sensitive to these molecules. It was reported that the dual tubulin/PDK1 inhibitor 13 displayed enhanced anticancer activity compared to the analog 12a which showed only antitubulin efficacy on renal A498 and non-small-cell lung cancer (NSCLC; A549/ATCC, NCI-H522, and NCI-H322M) cells. This result was probably due to the significant over expression of PDK1 found in NSCLC cells including A549 cells. The benzophenones 12c and the analog without the monochloroacetyl group showed the same tendency. The monochloroacetylated benzophenone 12c, is a dual inhibitor of tubulin PDK1 and exhibited greater anticancer potential on A549/ATCC/NSCLC cells and on A498 renal cancer cells compared to non-monochloroacetylated benzophenone containing only a free phenolic group.[60]

4.2 | DCA derivatives of synthetic organic compounds

For improving the potentiality of DCA in clinical applications, Trapella et al. designed and synthesized DCA containing molecules 14 and 15 (Figure 8) having a tertiary amine scaffold and the corresponding quaternary ammonium salts.[61] Compounds 14 and 15 are able to deliver multiple DCA molecules with the goal to achieve appropriate therapeutic concentrations of the drug into tumor cells. They reported that compound 14 displayed antitumor activity in vitro at very low (>30-fold) concentrations compared to DCA against a panel of leukemic cell lines (MEC-1, MEC-2, JVM-2, MAVER, and HL-60) and bcl patient derived cell cultures. In contrast, the quaternary ammonium salt 15 was inactive against all cell lines employed which is probably due to the high hydrophilic property of compound 15 that restricts crossing the cell membrane. The DCA-loaded compound 14 was less toxic toward human healthy primary cells with IC50 values approximately 3-fold greater suggesting that normal PBMCs are less sensitive to compound 14. The antileukemic activity of compound 14 was found to be due to the combined result of induction of apoptosis, cell cycle arrest, and induction of the p21 molecular mediator. In addition, oxygen consumption rate (OCR) measurements demonstrated that pretreatment with the mitochondria-targeted DCA-loaded compound 14, but not 15, significantly affected the mitochondrial respiratory capacity of leukemic cells which suggested that the compound 14 was able to deliver DCA to the mitochondria.

As very high doses of DCA are required to overcome the immunosuppressive nature of a glycolytic tumor, DCA was engineered for efficient cellular and mitochondrial uptakes with the objective of inhibiting glycolysis, demonstrating anticancer properties, and enhancing the effects of antitumor immunity at pharmacologically relevant doses.[62] Consequently, a mitochondria-targeted DCA derivative 16 (Figure 9) was reported containing multiple DCA groups and a lipophilic triphenylphosphonium (TPP) cation. Compared to DCA alone, the compound increased potency and showed threefold cancer cell selectivity. Compound 16 can target mitochondria because of its lipophilic TPP cation linker which is also biodegradable. Compound 16

FIGURE 8 Structures of compounds 14 and 15

FIGURE 9 Structure of compound 16
caused dysfunctional mitochondria in tumor cells whereas it did not affect normal cells. It shifts the metabolic process of tumor cells from glycolysis to glucose oxidation and subsequent apoptosis cell death. Compound 16 allowed effective delivery of DCA to the mitochondria. As a result, the lactate levels were found to be significantly reduced in 16 treated cancer cells. In addition, 16 played important roles in regulating the dendritic cell (DC) phenotype demonstrated by the release of interleukin-12 from DCs upon stimulation with tumor antigens.

To efficiently deliver compound 16 inside the mitochondria in cancer cells, a biodegradable nanoparticle (NP) was constructed from poly-(lactic-co-glycolic acid) (PLGA)-block(b)-polyethylene glycol (PEG) functionalized using a terminal TPP cation. The NP formulation of compound 16 was reported to reduce glycolysis by targeting PDK1 only in cancer cells, not affecting the immune cells. A syngeneic tumor model was used that stimulates pathways to instruct inhibitory effects on multiple immune checkpoint proteins while improving cytotoxic T cell infiltration.

3,5-Bis(benzylidene)-4-piperidones are known to possess promising cytotoxic potencies. These compounds were designed to undergo sequential interactions with cellular thiols leading to greater toxicity to tumors than normal cells. To enhance tumor-selective cytotoxicity of this piperidine scaffold, the dichloroacetyl group was attached to the cytotoxic warheads, 3,5-bis(benzylidene) -4-piperidones, to create a series of 15 novel hybrid molecules having the general structure 17 (Figure 10). In general, these new hybrid molecules exhibit excellent antineoplastic activity against human HCT116 colon cancer cells. A number of lead compounds particularly 17d, 17e, 17g, and 17h emerged having IC50 values in the micromolar range (0.03–0.04 μM). Most of these compounds were less toxic to human CRL1790 nonmalignant colon cells and huge selectivity index figures (SI > 300-fold) were observed for most of the compounds. Some of the compounds displayed >100-fold higher cytotoxic potencies than the reference drug 5-fluorouracil (5-FU). The potencies of the compounds increased as the magnitude of the Hammett σ and Taft σ* values of the aryl substituents were elevated. Mode of action studies showed that the potencies of the cytotoxins in series 17 included the lowering of the mitochondrial membrane potential (MMP) and the generation of ROS in HCT116 cells.

A variety of N-phenyl dichloroacetamide derivatives 18 (Figure 10) have been designed, synthesized, and evaluated for antineoplastic activity. The cytotoxicity of all synthesized compounds was determined using human tumor cell lines derived from oral epidermoid carcinoma (KB), gastric carcinoma (BGC-823), non-small-cell lung cancer (A549), and liver carcinoma (BEL-7402). N-Phenyl dichloroacetamide derivatives 18 exhibited great potencies in controlling the growth of A549 cells as well as BEL-7402 and KB neoplasms. The IC50 values against BEL-7402, KB, and A549 were almost 5- to 10-times lower than against BGC-823. structure–activity relationship (SAR) studies suggested that the nature and positions of the substituents on the benzene ring determine the cytotoxic activities of N-phenyl-2,2-dichloroacetamide analogs 18. For example, compounds with substituents in the meta and para locations are more potent against A549 lung cancer cells than the analogs possessing an ortho group. Thus, the IC50 value of 18c and 18d was lower than that of 18b. It was suggested that the ortho-compound may block the interaction between the pharmacophore and the receptor as the ortho-substituent is close to the pharmacophore (2,2-dichloroacetyl). The best potencies to inhibit A549 cells was found to be the meta-substituted structures, followed by the ones with para-substituents. For example, the IC50 value of dichloroacetamide 18e is 4.76 μM, whereas it is 12.54 μM for 18f. Among the meta-substituted compounds, the compounds containing strong electron-withdrawing substituents (e.g., 18j–18l) showed higher activities, while those containing electron-donating substituents (e.g., 18h, 18i) had low potencies. Compound 18e can also stimulate apoptosis in cancer cells and displayed lower toxicity in mice (LD50: 1117 mg/kg).

To further explore and enhance the cytotoxic activity of N-phenyl dichloroacetamides 18, compounds 18e and 18g were
further modified by substituting the iodo groups with phenyl or heteroaryl groups to synthesize series 19 [67] (Figure 11). The cytotoxicity of all the synthesized compounds were determined using a human non-small-cell lung cancer (A549), large cell lung cancer cell line NCI-H460 (H460) and epidermoid carcinoma cell line (KB-3-1) and their SAR data were evaluated. Most of the compounds containing a heteroaryl group showed low potency, while dichloroacetamide (19a) was the most potent compound against all of the cancer cell lines examined. Therefore, a series of compounds 19 as well as 20 (Figure 11) were synthesized and their anticancer activities were assessed. The substituted N-([1,1′:3′,1″-terphenyl]-5″-yl)-2,2-dichloroacetamide derivatives 20 exhibited potent cytotoxic activity toward A549 cells and also toward H460 and KB-3-1 cells. Particularly, compound 20g showed anticancer activity with IC_{50} values 1.04 μM against H460 cells, 1.73 μM against A549 cells, and 2.40 μM against KB-3-1 cells.

Fereidoonnezhad et al. further studied a series of novel N-aryl-2,2-dichloroacetamides (21) and aryl-2,2-DCAs (22) (Figure 11) as a continuing effort to identify and screen more active DCA analogs. [68] Series 21 and 22 were evaluated for anticancer activity using various human cancer cell lines including MCF-7 (breast adenocarcinoma), MDA-MB-231 (breast adenocarcinoma), A549 (human lung adenocarcinoma epithelial cell line), HCA-7 (human colon cancer cell line), SKOV3 (ovarian cancer cell line) and KB (human oral epidermoid carcinoma cell line). The results showed that compounds 21 and 22 had satisfactory potencies, and compounds 21a, 21b, and 21c had the highest cytotoxic effects. 21a can also induce apoptosis in the A549 cell line. The binding site of these compounds to four PDKs isoenzymes and the important amino acids of the binding site were also determined using molecular docking studies.

In addition, a comparative quantitative structure–activity relationship (QSAR) analysis, molecular docking as well as protein ligand interaction fingerprints (PLIF) studies of compounds in series 18–22 were investigated by Fereidoonnezhad et al. [68, 69] A collection of chemometric methods such as multiple linear regression (MLR), factor analysis-based multiple linear regression (FA-MLR), principal component regression (PCR), and partial least squares combined with a genetic algorithm for variable selection (GA-PLS) were applied to obtain relationships between structural features and cytotoxic activities of a variety of DCA analogs 18–22. Based on these models, in silico screening was used to design the new DCA derivatives 21 with potential cytotoxic activity by substituting different bio-isosteric groups (O, S) in the place of a –NH group. Among different designed
molecules, the compounds 21a–21f showed the best cytotoxic activity (pIC_{50} > 5.25) and were suggested for synthesis.

A series of new piperidine and piperazine derivatives 23 and 24 (Figure 12) containing the dichloroacetyl group were designed and investigated using molecular docking analysis.\textsuperscript{70,71} Compounds 23a–23c and 24a–24f containing the lowest binding energies and better interaction with PDK isoenzymes were selected based on the docking results. These selected compounds were synthesized and evaluated against MCF7 and HT-29 human cancer cell lines. It was reported that the piperazine derivatives 24 were more potent than the piperidine derivatives 23. Both 23 and 24 displayed moderate potency and much higher cytotoxic activity than DCA. In general, the anticancer activities toward HT-29 were better than those for the MCF7 cancer cell line. The most active compound of the series was found to be 24a which contained two dichloroacetyl groups and possesses an IC_{50} value of 7.79 \mu M against the HT-29 cell line.

### 4.3 Metal complexes of DCA

Metal complexes have been used in cancer chemotherapy for many years. Because of their unique molecular geometries as well as their catalytic, ligand exchange, photophysical, and redox reactions, metal complexes have the ability to react and interact with biomolecules in distinctive ways and by unique mechanisms of action.\textsuperscript{72} Since the discovery of cisplatin as an anticancer drug in 1967 by Rosenberg, platinum-based metal complexes particularly have played an important role in chemotherapy.\textsuperscript{73} Since then, more than 30 platinum-based complexes have entered clinical trials. Among them, only cisplatin, carboplatin, and oxaliplatin have become clinical drugs and are used globally while some of them have been approved regionally.

The mechanisms of action of cisplatin and other platinum-based anticancer drugs have been extensively investigated.\textsuperscript{74–76} A 4-step mechanism of action has been proposed generally (Figure 13) which includes: (i) cellular uptake, (ii) ligand displacement with water called aquation or activation, (iii) coordination of a metal with DNA, and (iv) formation of DNA lesions leading to cell death.\textsuperscript{75,76}

Passive diffusion and/or active transport are the two predominant mechanisms known for cellular uptake for cisplatin and related compounds.\textsuperscript{77} After cellular uptake, a ligand substitution event occurs in the cytoplasm in which a chloride ligand is replaced by a water molecule in cisplatin to form cis-\{Pt(NH\textsubscript{3})\textsubscript{2}Cl(H\textsubscript{2}O)\}\textsuperscript{+}, known as aquation or activation.\textsuperscript{76} Due to the positive charge on the platinum complex, it can be attracted to the negatively charged nuclear DNA, and subsequently coordinatively interact with DNA to form DNA adducts. The N-7 atoms of the purine residues of guanine and adenine are the most nucleophilic sites of DNA, and these are preferentially coordinated with the cis-\{Pt(R-NH\textsubscript{2})\textsubscript{2}Cl\} fragment. Following this event, the remaining chloride ligand is substituted for a second guanine base, forming either an intrastrand or interstrand DNA cross-links. The formation of cross-link adducts distort the structure of DNA in a substantial manner leading to an impairment of DNA expression and stability of G quadruplexes, which are thought to be the crucial lesion for its biological effectiveness as an antitumor agent.\textsuperscript{73,78,79} Besides platinum-based metal complexes, complexes with other metals such as Cu, Ru, Os, and Rh are also known in the literature that can act as anticancer agents.

#### 4.3.1 Platinum complexes of dichloroacetic acid

Although platinum-based complexes have entered clinical trials, the poor selectivity, severe side effects, drug resistance, and systemic toxicity have restricted the application to clinical use of these drugs. Therefore, a considerable amount of effort has been dedicated for developing new platinum-based antineoplastic agents having equal or higher antitumor activity but lower toxicity.\textsuperscript{73} To obtain novel platinum antineoplastic drugs with ideal therapeutic effects and pharmacological properties, dual-targeting strategies are undertaken in recent years. Toward this end, many scientists designed and synthesized platinum complexes with the DCA moiety that can attack both nuclear DNA with Pt(II) and mitochondria with DCA in cancer cells. Based on the chemical structures, these complexes can be divided into three groups: (i) octahedral Pt(IV) complexes where DCA moiety is an axial ligand, (ii) square planar Pt(III) complexes where DCA is directly coordinated with Pt(II), and (iii) square planar Pt(II) complexes where DCA is a part of a ligand of the complexes.

One of the most popular strategies was to design and synthesize octahedral Pt(IV) complexes as prodrugs with dual-targeted sites of action. The Pt(IV) complexes are appealing prodrugs for overcoming the disadvantages of Pt(II) complexes, because they possess low systemic toxicity and are kinetically inert. Moreover, they show less...
reactivity as compared to their Pt(II) precursors and are suitable for oral administration. The prodrugs Pt(IV) complexes can be reduced inside cancer cells by cellular reductants, releasing two axial ligands and a cytotoxic Pt(II) moiety which forms Pt-DNA adducts and interferes with DNA replication and transcription. Moreover, it is possible to make the prodrugs as a dual-targeted agent by incorporating additional bioactive moieties such as DCA at the axial positions of the Pt(IV) complexes. A number of such complexes having a dual targeting strategy with DCA axial ligand have been reported over the last 10 years which are discussed below.

Mitaplatin (25) (Figure 14) was the first reported compound in which two DCA units are attached to the axial positions of an octahedral Pt(IV) center. Due to a negative intracellular redox potential, the Pt(IV) of mitaplatin (25) is reduced to cisplatin, a Pt(II) compound, and releases two equivalents of DCA. As a result, mitaplatin (25) interacts with both mitochondria with DCA and nuclear DNA with cisplatin in cancer cells. This dual-killing mode of actions of mitaplatin (25) allowed it to kill cancer cells selectively when they were cocultured with normal fibroblasts and to partially overcome cisplatin resistance cells. The anticancer activity of 25 against different cancer cell lines (MCF-7, NTERA-2, U2OS, HeLa, and A549) is equal or better than that of all known Pt(IV) compounds and is similar to that of cisplatin. Xue et al. reported that mitaplatin induced more apoptosis in cisplatin-resistant (CP-r) human epidermoid adenocarcinoma KB-CP 20 and hepatoma BEL 7404-CP 20 cancer cells than that of cisplatin, DCA and cisplatin/DCA when compared on an equal molar basis. Platinum accumulation was higher in mitaplatin-treated CP-r cells due to its greater lipophilicity enhancing transmembrane permeability. Mitaplatin (25) also showed special targeting to mitochondria through a dramatic collapse of MMPs (∆Ψm) and modulation of abnormal glycolysis by inhibiting the phosphorylation of PDH.

Because of these encouraging results, many researchers designed and synthesized mitaplatin analogs and evaluated their biological activity against various cancer cells. Recently, four Pt(IV) complexes 26a–27b (Figure 14) with DCA/TFA and chloride ions as axial ligands have been reported to be potential dual-targeting anticancer agents. These four complexes exhibited potent anticancer activity against BGC803 (IC50: 7.63–57.73 μM), HepG-2 (IC50: 1.35–17.54 μM), MCF7 (IC50: 7.96–27.08 μM), and NCI-H460 (IC50: 1.94–200 μM) cancer cell. Among them, compound 27a displayed the highest anticancer activity against four cancer cell lines. 27a also showed much better activity than the positive controls (cisplatin, oxaliplatin, and carboplatin). It was reported that the Pt(IV) complex 27a was able to induce apoptosis in HepG-2 cells through cell cycle arrest at the S phase and interrupt the membrane potential of mitochondria by releasing both the corresponding Pt(II) complex and DCA.

A hybrid Pt(IV) prodrug 28 (Figure 14) with dual-targeting ability have been synthesized by Xiao et al. Compound 28 was further attached to an amphiphilic and biodegradable block carrier copolymer, methoxypoly(ethylene glycol)-block-poly(ε-caprolactone)-block-poly(ε-lysine) by the reaction between the carboxylic acid group of 28.
and the amino groups of the copolymer. The resultant polymer-Pt(IV) conjugate can self-assemble to form micelles to enable intracellular delivery. It was reported that the polymer conjugate of 28 rapidly and directly released cisplatin and DCA under the simulated intracellular conditions and displayed much higher anticancer activity against the human ovarian cancer cells (SKOV-3) than its precursors. The order of uptake of different compounds in 2h by SKOV-3 cancer cells was: micelles of polymer-Pt(IV) conjugate (95.4 ng Pt/mg protein) > cisplatin (33.8 ng Pt/mg protein) > compound 28 (15.9 ng Pt/mg protein). These results suggested that drugs inside the micelles can enter the cells via endocytosis more effectively than small molecule drugs via simple diffusion. In addition, the micelles of the polymer-Pt(IV) conjugate was most effective for inducing mitochondrial membrane depolarization in SKOV-3 cells.

To investigate the effects of axial ligands on the bioactivity of the complex, Jin et al. designed and synthesized a novel Pt(IV) complex 29 (Figure 15) containing DCA and biotin moiety as axial ligands.[80] It was reported that the addition of DCA significantly inhibited the growth of those cancer cells having active glycolysis by increasing the reactivity and lipophilicity of the complex. On the other side, the addition of biotin moiety improved the tumor-targeting ability of the complex. The complex 29 displayed much higher cytotoxic property toward variety of cancer cell lines as compared to the Pt(IV) complex without the axial DCA group. 29 disrupted the mitochondrial...
morphology by altering the MMP. The mitochondrial and cellular ROS levels were reduced too. In addition, the mitochondrial function of tumor cells was reduced by 29, due to the inhibition of both glycolysis and glucose oxidation which finally causes the death of cancer cells through mitochondria-mediated apoptosis. These results demonstrated that 29 inhibits the growth of cancer cells primarily by modifying metabolic pathways and emphasizes the importance of dual-targeting for the efficiency of anticancer drugs.

The transformation of a Pt(II) complex, oxaliplatin, to its Pt(IV) derivatives 30 and 31 (Figure 15) containing axial DCA ligands was accomplished by Zajac et al.[85] Compounds 30, 31, oxaliplatin and its derivative Pt(IV) complex with two axial hydroxyl ligands were evaluated against five human cancer cell lines. These cells included an inherent cisplatin resistant breast cancer cell line (MCF-7), cisplatin-resistant (A2780cisR), and cisplatin-sensitive (A2780) ovarian cancer cell lines and two colon cancer cell lines (HCT116 and SW480). When two biologically inactive hydroxyl ligands were added at the axial positions of oxaliplatin to convert it into a Pt(IV) derivative, the complex became more inactive than the parent oxaliplatin. However, when DCA was added in place of axial hydroxyl groups, the toxicity of the resultant complexes was enhanced significantly in all the cells. This enhancement was most noticeable for 31, that is, when both axial positions were occupied by DCA. In addition, it was demonstrated that these Pt(IV) prodrugs showed different mechanisms of biological action as compared to oxaliplatin and altered mitochondrial function, and glucose metabolism and involved autophagy. These differences in biological actions from those of oxaliplatin can be attributed to the DCA ligands that were carried in cancer cells as the axial ligand of the Pt(IV) derivatives of oxaliplatin. Remarkably, the cytotoxicity of the oxaliplatin derivatives containing axial DCA ligands can be enhanced further by administering them in combination with 5-FU.

Savino et al. synthesized and characterized two novel platinum (IV) derivatives (32 and 33; Figure 16) of kiteplatin containing two DCA groups in the axial positions and tested them against a series of cancer cell lines in vitro and in vivo.[84] Cell lines that were used include cervical (A431), breast (MCF-7), lung (A549), pancreatic (BxPC3), and colon (HCT-15) cancers, as well as melanoma (A375). Both compounds 32 and 33 displayed a better cytotoxicity toward neoplastic cells in comparison to nontumor cells. In particular, compound 32 exhibited the best cytotoxicity against human pancreatic carcinoma cells (BxPC3) among all the tested cells. Generally, the presence of oxalate leaving ligand appears to decrease the cytotoxicity of kiteplatin derivatives. It was reported that the most active Pt(IV) complex 32 underwent very rapid hydrolysis in a solution at pH = 7.4 to release the axial DCA ligands. In addition, both complexes underwent rapid reduction (half times of minutes) by ascorbic acid to form the Pt(II) complex (kiteplatin). As a result, DNA-platination was observed due to the presence of Pt(II) and a significant increase of ROS generation, inhibition of OXPHOS, hypopolarization of the mitochondrial membrane, and caspase-3/7-mediated apoptotic cell death were also observed due to the presence of DCA which was released by the Pt(IV) compounds. Finally, complex 32 was able to reduce the tumor mass of murine LLC solid tumor found in an in vivo study which was similar to that of cisplatin but it is less cytotoxic.

A series of novel Pt(IV) prodrugs with triple action capacity has been developed and synthesized by Petruzzella et al. for evaluating the hypothesis that compounds with multi-action capacity may have more cytotoxic effect than a single action compound for killing cancer cells.[87] The rationale behind this hypothesis was based on the assumption that different bioactive moieties may interact with different cellular processes. Thus, they developed Pt(IV) derivatives of cisplatin with "triple action" ability where they introduced PDK, inhibitors of cyclooxygenase,[25] or histone deacetylase (HDAC). The inhibitors were aspirin or ibuprofen as COX, DCA as PDK, and valproate or phenylbutyrate as HDAC. All complexes (only four, 34–37, are shown in Figure 17), [Pt(NH3)2][PDCl2], [Pt(NH3)2][HDAC][Cl2], and [Pt(NH3)2][HDAC][PDK][Cl2] exhibited higher cytotoxicity than cisplatin against all the tested human cancer cell lines including PSN-1 pancreatic, HCT-15 colorectal, LoVo colon, and BCPAP thyroid. In addition, two cisplatin-resistant tumor cell lines (ovarian adenocarcinoma cells 2008/C13) were used in this study. In particular, thyroid and pancreatic cancer cells were most sensitive to all the designed compounds. Among all the tested compounds, the most promising compound was 36, which was not only active against oxaliplatin and cisplatin-resistant cell lines but also showed high selectivity toward PSN-1, LoVo, and BCPAP cancer cell lines. Structure–activity relationships of these triple action drugs were determined by employing classical standard bio-assays data which revealed that there was no linear correlations between cellular

![FIGURE 16 Structures of compounds 32 and 33](image-url)
uptake and the overall cytotoxicity data or between the potential biological targets (mitochondria, DNA, COX, and HDAC) and the cytotoxicity data. Since all compounds are cytotoxic, most likely, a common single mechanism of action is not involved for all compounds. The potency of these Pt(IV) prodrugs do not necessarily just depend on the amount of the drug accumulating in the cell but possibly depends mainly on the specific cellular interactions of each of the components and on probable synergistic effects to which the cell interacts.

Recently, mono- and di-axial functionalized photoactive diazido Pt(IV) complexes \( 38 \)–\( 41 \) (Figure 18) containing the antineoplastic agent coumarin-3-carboxylate, the PDK inhibitor DCA, and/or PDK and histone deacetylase (HDAC) inhibitor 4-phenylbutyrate have been designed and synthesized for cytotoxic evaluation.\[88\] These complexes were reported to produce Pt(II) moieties which coordinate to 5’-GMP as detected by LC-MS, and release hydroxyl and azidyl radicals as identified by EPR. Upon irradiation, these complexes can release both axial ligands. However, significant differences were observed for mono- and di-functionalized complexes in their photobiological and photochemical properties. Mono-functionalized complexes, for example, \( 38 \) had higher negative reduction potentials and aqueous solubility whereas di-functionalized complexes \( 39 \)–\( 41 \) showed much lower aqueous solubility. In compared to mono-functionalized analogs, compounds \( 39 \)–\( 41 \) displayed considerably higher accumulation of cellular Pt (about 3–17-fold) and photo-induced cellular ROS levels when treated with cells. All compounds showed higher photocytotoxicity in presence of blue light (1 h, 465 nm, 4.8 mW cm\(^{-2}\)) than the parent dihydroxido complex, \( \text{trans,trans,trans} \)\[-\text{[Pt(py)2(N3)2(OH)2]}\] in A2780 human ovarian cancer cells (IC\(_{50}\) 0.9–2.9 \( \mu \)M for \( 38 \) and other mono-functionalized complexes and 0.11–0.39 \( \mu \)M for \( 39 \)–\( 41 \)) and in A549 human lung cancer cells (IC\(_{50}\) 5.4–7.8 \( \mu \)M for \( 38 \) and other mono-functionalized complexes and 1.2–2.6 \( \mu \)M for \( 39 \)–\( 41 \)). Importantly, all complexes showed no apparent cytotoxicity in healthy lung MRC-5 fibroblasts.

In addition to a prodrug strategy of octahedral Pt(IV) complexes, square-planar hybrid DCA-platinum(II) complexes have also been studied by a number of research groups. Zhang et al. synthesized a hybrid DCA-platinum(II) complex \( 42 \) (Figure 19) that possesses dual-targeting mode of action and has the ability to inhibit the growth of cisplatin drug resistance ovarian cancer cells.\[89\] They have found that the mode of action of \( 42 \) is different from the present clinically used Pt(II) drugs. The complex \( 42 \) showed significant anticancer activity against two ovarian cancer cells: A2780 (cisplatin-sensitive) and A2780DDP (cisplatin resistant). In compared to cisplatin,
carboplatin, and some other drugs, compound 42 showed less cellular uptake and fewer Pt-DNA adduct formation when treated with cells. However, 42 exhibited superior cytotoxicity among all the tested compounds against two ovarian cancer cell lines. The complex 42 induced much more mitochondrial dysfunction than analogs through G2/M phase arrest along with caspase-3 and caspase-9 cleavage. As a result, it induced more apoptosis in both sensitive and resistant cells lines. Interestingly, disruption of mitochondria due to 42 was observed more in cisplatin-resistant A2780DDP cells than in cisplatin sensitive A2780 cell lines.

The antiproliferative activity of complexes 43–47 (Figure 19) were investigated against both Pt sensitive A2780 and Pt resistant SKOV-3 human cancer cell lines. The amine complexes 42–43 were slightly more active against Pt sensitive A2780 cells than the complexes 45–47. However, the amine complexes 42–43 were noticeably less cytotoxic toward Pt resistant SKOV-3, which resembles cisplatin behavior, while 45–47 displayed the same anticancer activity as A2780. These results suggested that the amine complexes 45–47 had different mechanisms of action as compared to complexes 42–43 and cisplatin, while complexes 42–43 acted through a similar mechanism of action to that of cisplatin. Their further investigations suggested that the introduction of DCA for complexes 42–43 had little effect on the anticancer activity against Pt sensitive A2780, and Pt-resistant SKOV-3. On the other hand, the introduction of DCA for complexes 45–47 generated a substantial antiproliferative property on both cell lines.

A number of Pt(II) complexes have been reported for their anticancer activities where DCA was not directly coordinated with the Pt(II); rather it was a part of the ligands of the complexes. For example, a number of mixed-NH$_2$/amine platinum(II) complexes of 3-dichloroacetoxycyclobutane-1, 1-dicarboxylate (48a–48c, 49–51; Figure 20) have been synthesized and evaluated for in vitro cytotoxic profiles in various cancer cells by Liu et al. Compounds 48a–48b, and 49 were reported to go through hydrolysis in the aqueous phase, producing two active species, DCA and the platinum (II) pharmacophores. Among all the synthesized complexes, 48a–48b and 49 exhibited significant cytotoxicity toward two human ovarian cancer cell lines (SK-OV-3, SK-OV-3/DDP) and one lung cancer cell line (A549). Compounds 48a–48b and 49 also selectively induced apoptosis in cancer cells and displayed slight effect on human BEAS-2B normal cells. It was reported that due to possible synergistic effect of the DCA and platinum(II) pharmacophores, complexes 48a–48b showed the ability to overcome the resistance of cancer cells found with cisplatin. In addition, it was expected that complexes 48a–48b may cross the cell membrane more easily to enter the cells because of better lipophilicity of the molecules and increase the uptake of DCA into cells as compared to free DCA alone.

A new amido-phosphine ligand 52 (Figure 21) was designed and synthesized to transport both proapoptotic DCA and a cytotoxic metal ion. Thus, nine new complexes 53–59 (Figures 21 and 22) were prepared through coordination of 52 (acting as a P-donor) and a variety of cytotoxic metal ions, (Pt$^{2+}$, Pd$^{2+}$, Ru$^{2+}$, Re$^{2+}$, Au$^+$. These nine complexes were evaluated for their in vitro antiproliferative activity against ovarian cisplatin-resistant A2780cis, cisplatin-sensitive A2780, and erythroleukemic K562 cancer cell lines, and for comparison cisplatin was used as positive standard. These Pt(II) complexes exhibited selective cytotoxicity toward different cancer cells and these antiproliferative activity were comparable to cisplatin.
FIGURE 20  Structures of compounds 48–51

FIGURE 21  Structures of compounds 52–56
but higher than the free ligand \( \text{52} \). For example, complex \( \text{55a} \) showed better selectivity toward A2780 cells, \( \text{54a}, \text{55a}, \text{and 56} \), toward A2780cis cells and \( \text{56} \) for K562. The best performances were observed probably due to the concurrent presence of \( \text{52} \) (containing two residues of DCA) and the Pt(II) pharmacophore. Moreover, the most active Pt(II) complexes were found to induce apoptosis which causes the observed antiproliferative activity. In contrast, other active metal ions did not show good results when they were coordinated with \( \text{52} \), with the exception of the complexes containing Pd (\( \text{54b} \) and \( \text{55b} \)), Ru (\( \text{57} \)), and Au (\( \text{59} \)) (Figure 22), that displayed a significant proapoptotic activity on the K562 cell line.

4.3.2 | Other metal complexes of dichloroacetic acid

Beside platinum, osmium and ruthenium complexes have been studied extensively as potential anticancer agents.\(^{[94,95]}\) Their mechanisms of actions are different than the platinum-based drugs. Therefore, their cytotoxicity profiles are different and they have the potency to overcome both the intrinsic and acquired resistance of various tumors against platinum-based drugs. Two half-sandwich complexes of Ru (\( \text{60a} \)) and Os (\( \text{60b} \)) (Figure 23) containing a releasable DCA ligand have been reported to show good in vitro anticancer property against A2780 human ovarian carcinoma cells with IC\(_{50} = 3.5\) and 2.6 \( \mu \)M respectively, slightly higher than that of the clinically used platinum-based drug cisplatin (IC\(_{50} = 5.9\) \( \mu \)M).\(^{[96]}\) However, they were much less toxic toward healthy human hepatocytes. Upon hydrolysis, the complexes \( \text{60a} \) and \( \text{60b} \) released the DCA ligand which is probably the reason for this cytotoxicity at least in part. The complexes \( \text{60a} \) and \( \text{60b} \) did not bind with the model proteins lysozyme and cytochrome c. The flow cytometry experiments suggested that both the complexes altered the MMP, cell cycle, and mitochondrial cytochrome c release by a different way. That means, the two complexes possess different mechanisms of action.

Novohradsky et al., recently found that the Os complex \( \text{60b} \) has the potency to efficiently and selectively kill cancer stem cells (CSCs) in heterogeneous populations of two human breast cancer cells.
(SKBR-3 and MCF-7). Importantly, 60b displayed remarkable submicromolar cytotoxicity for killing CSCs, which is considerably more efficient than that of its Ru analog 60a and salinomycin, known for one of the most selective CSC-targeting compounds. Additionally, Os complex 60b has been reported to reduce the size, formation, and existence of three-dimensional mammospheres which is more closely related to the tumor microenvironment than cells in traditional two-dimensional cultures. Complex 60b can kill human breast CSCs primarily by a programmed form of necrosis, known as necroptosis. In contrast, Ru(II) complex 60a and salinomycin displayed different modes of action which involved blocking of both apoptosis and necroptosis in CSCs. Os complex 60b undergoes hydrolysis slower than the Ru complex 60a, therefore, it is possible that 60b has better ability to carry the biologically active DCA into the cell as compared to the Ru(II) complex and salinomycin, and thereby, they have differences in their biological effects.

A multifunctional rhenium (Re) complex 61a (Figure 23) containing DCA has been developed and synthesized as an anticancer agent. Complex 61a can alter cancer cell metabolism from glycolysis to glucose oxidation because of its ability to efficiently diffuse into cancer cells and selectively accumulate in mitochondria as pharmacologically relevant DCA doses. Mechanism studies suggested that complex 61a can induce metabolic reversal at the early stage of drug treatment in NCI-1229 cells by inhibiting PDK. As a result, the Re complex 61a selectively killed cancer cells cocultured with normal cells, significantly inhibited cancer cell metastasis and invasion, along with demonstrating remarkable antiangiogenesis activities in zebrafish embryos. In contrast, although DCA-free Re analog 61b exhibited anticancer activity due to the Re(I) pharmacophore, it was not able to induce metabolic reversal and displayed much lower antimetastasis activity as compared to 61a, suggesting the synergistic effect of Re(I) pharmacophore and DCA moiety on cancer cells. In addition, the in vivo studies suggested that 61a could efficiently inhibit the tumor growth of nude mice without affecting its body weight, and the therapeutic effect was much better than the DCA-free analog 61b.

5 | CONCLUSION

The data presented in this review establishes the value of attaching DCA to a number of bioactive organic compounds which led to some potent cytotoxins. The formation of organometallic compounds, which contain both platinum as well as DCA led to a variety of antineoplastic agents. In the future, a number of ways of creating novel cytotoxins based on this study should be implemented. These investigations should include the formation of compounds containing two or more ester groups of DCA in different chemical environments. These molecules will be able to undergo sequential release of DCA whereby the first release of DCA chemosensitizes neoplasms to a further interaction with DCA. The mitochondriotropic properties of some of the compounds should be examined by replacing the positively charged nitrogen atom with other positively charged atoms such as sulfur and phosphorous. The ability of these compounds to chelate with metals other than platinum and their antineoplastic properties should be evaluated. Further studies have as their goals finding compounds which are useful in inhibiting the growth of MDR cell lines as well as undertaking some formulation studies.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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