Journal of Inorganic Biochemistry 191 (2019) 77–84

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Review article

Multi-action Pt(IV) anticancer agents; do we understand how they work?

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ARTICLE INFO

Keywords:
Platinum(IV)
Multi-action
Anticancer agents

ABSTRACT

Pt(IV) complexes act as prodrugs that are activated inside cancer cells releasing cytotoxic Pt(II) drugs such as cisplatin as well as two axial ligands. These ligands can be used to confer favorable pharmacological properties to the prodrug. They can be innocent spectators, targeting agents or bioactive moieties. When the ligands are bioactive moieties such as enzyme inhibitors or antiproliferative agents, the prodrug attacks several cellular targets at the same time acting as a multi-action prodrug. These compounds are very potent and often overcome resistance to cisplatin. Despite solid rationalization and careful design, often there is no correlation between the ability of the bioactive ligand to inhibit the target enzyme and the cytotoxicity. This might be because most bioactive ligands affect several cellular functions and not only the ones they were designed to inhibit. Thus, even "dual action" prodrugs might in reality be multi-action prodrugs. This class of multi-action Pt(IV) prodrugs seems to have great potential in the attempts to overcome resistance.

1. Introduction

The fortunate accidental discovery of the anticancer activity of cisplatin by Rosenberg triggered decades of extensive research aimed at the development of novel therapeutics based on metal complexes. Platinum complexes are the most successful class of metal-based anticancer agents. To date, seven platinum complexes have been approved for use in humans (Fig. 1). Three (Cisplatin, Carboplatin and Oxaliplatin) were approved by the FDA and are in worldwide use [1,2]. In addition, Nedaplatin, is used in Japan for the treatment of non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), esophageal cancer and head and neck cancers [3,4]. In China, Lobaplatin is used to treat inoperable metastatic breast cancer, chronic myelogenous leukemia (CML), and SCLC and Heptaplatin is approved in Korea for the treatment of gastric cancer [5,6]. More recently, Miriplatin was approved in 2009 in Japan for treating hepatocellular carcinoma.

Although it may seem that decades of research yielded only a handful of clinically approved platinum drugs, they have proven to be invaluable. A platinum drug is used in 50% of all chemotherapeutic regimens administered in the clinic [1].

In 1973, based on a rather limited set of experimental data, Cleare and Hoeschele formulated the structure–activity relationships (SAR) for platinum anticancer agents [7]. They suggested that for platinum complexes to have anticancer activity they should be neutral square planar Pt(II) complexes with two cis oriented inert ammine or chelating diamine ligands and two semi-labile cis oriented ligands such as chlorides or chelating ligands that are bound to the platinum via oxygen donors. Surprisingly, despite decades of intensive research in the field, to date, all the platinum drugs approved for use in humans conform to the original SAR (Fig. 1). They enter the cancer cell via passive diffusion or transporters [8]. Once inside the cell they are activated, presumably by aquation, losing the non am(m)ine ligands yielding reactive aqua species, some of which bind covalently to two adjacent guanines on the same strand of the nuclear DNA, causing structural distortion of the double stranded DNA that triggers cellular responses that lead to apoptosis [9].

Although they have been quite successful, there are still some major drawbacks associated with the platinum anticancer drugs. Two of the major problems are the ability of the tumors to develop resistance to the drugs and the side effects associated with the chemotherapy [10]. The side effects, low bioavailability and the inability to administer these drugs orally (they are administered intravenously) are attributed to the reactivity of the Pt(II) drugs with biological nucleophiles prior to reaching the tumor. Many attempts were made to develop new platinum-based drugs that will overcome these drawbacks [11].

Over the years, thousands of Pt(II) complexes were prepared and screened for anticancer activity. Many were Pt(II) complexes that conform to the original SAR but many others were specifically designed to modify the DNA in a manner fundamentally different than cisplatin in order to trigger different cellular responses in the hope of overcoming resistance. Among them were trans oriented compounds, monofucnctional compounds, polynuclear compounds and non-covalent compounds that have been described in an excellent recent review [11] Fig. 2.

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https://doi.org/10.1016/j.jinorgbio.2018.11.008
Received 22 August 2018; Received in revised form 12 November 2018; Accepted 13 November 2018
Available online 15 November 2018
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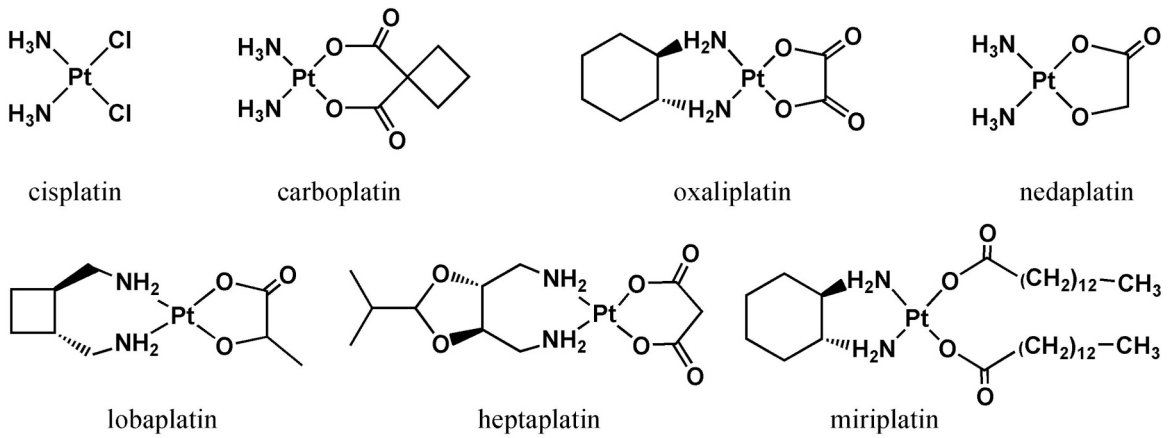


Fig. 1. Platinum anticancer drugs approved for use in humans. All are square planar Pt(II) complexes conforming to the classic structure-activity relationships.

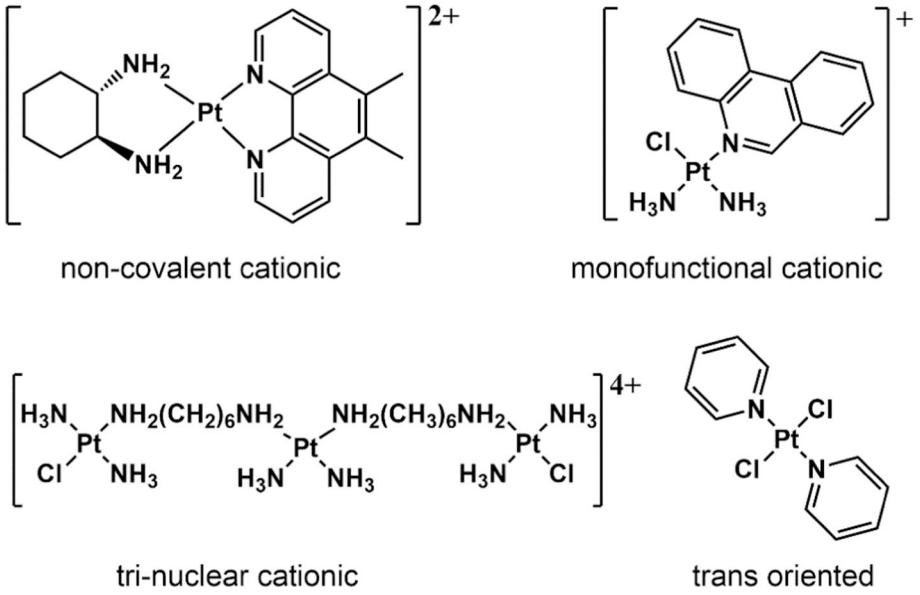
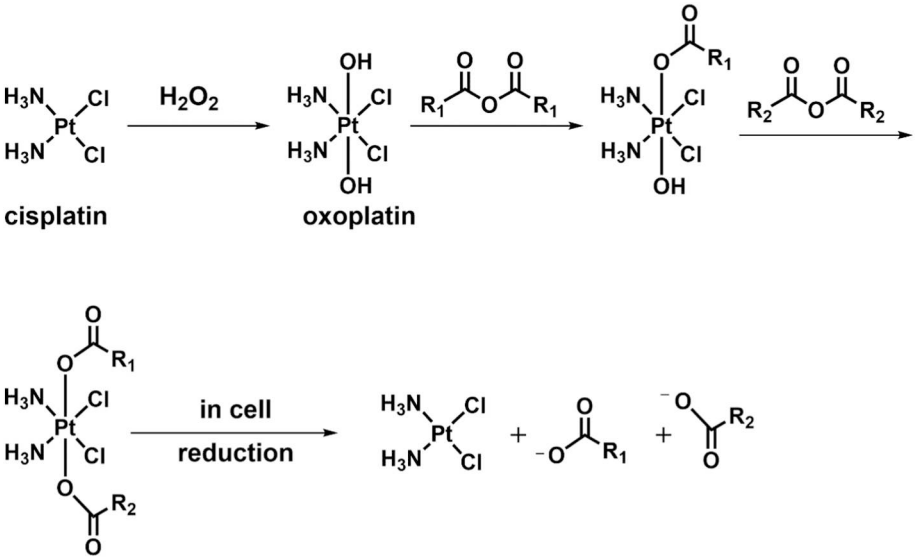


Fig. 2. Cytotoxic square planar Pt(II) complexes that do not conform to the classic SAR. The non-covalent di-cationic Pt56MeSS (top left), phenanthriplatin (top right), BBR3464 (bottom left) and the trans bipyridine (bottom right).



Scheme 1. The square planar cisplatin is oxidized to oxoplatin that can then be modified to introduce the same or two different ligands in the axial positions. Inside the cell, activation by reduction releases the original cisplatin as well as the two axial ligands.

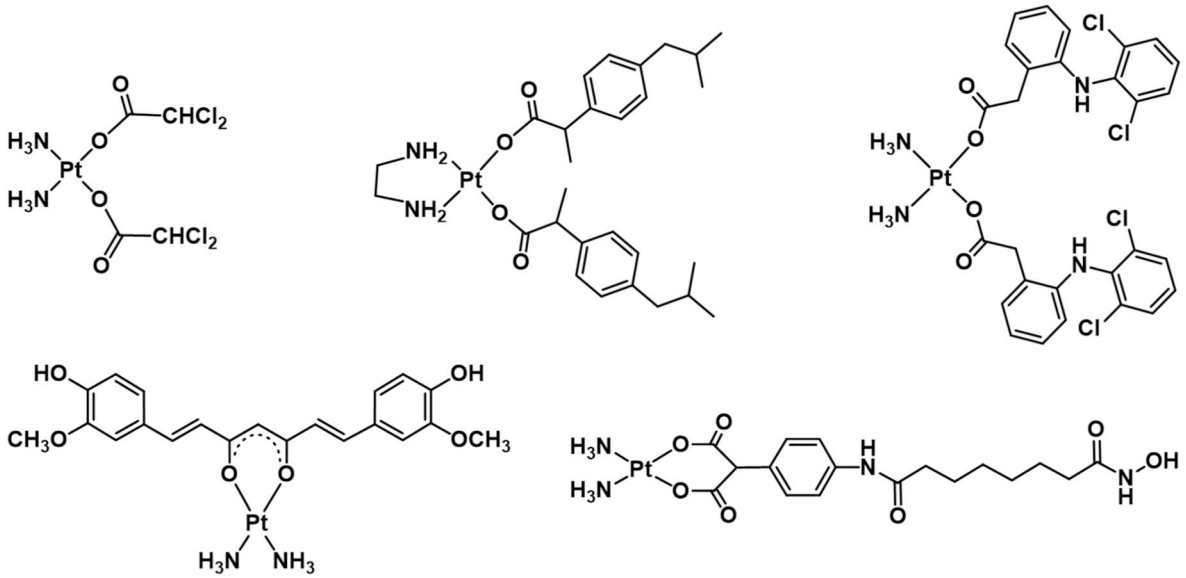


Fig. 3. "Dual action" Pt(II) compounds that upon aquation release in the cancer cell the bioactive ligands. Top – DCA, ibuprofen and diclofenac, bottom – curcumin and Vorinostat.

Another strategy that gained popularity recently is the use Pt(IV) complexes as prodrugs. Pt(IV) complexes are prepared by oxidative addition of the square planar Pt(II) complexes to yield the octahedral complex that retains the original Pt(II) equatorial coordination sphere (Scheme 1). Most often, the oxidation is performed using hydrogen peroxide resulting in two hydroxidos in the axial position that can be modified to tether to the axial positions a variety of ligands designed to improve the pharmacological properties of the complex. Because platinum(IV) complexes have a low-spin, d^6 octahedral geometry, they are more resistant to substitution than Pt(II) complexes and hence can be administered orally and are likely to have higher bioavailability and reduced/different toxicity compared to the Pt(II) complexes. This was demonstrated by Satraplatin, ctc-[$Pt(NH_3)$(c-hexylamine)$(OAc)_2Cl_2$], that completed phase III clinical trials by oral administration [12]. Once inside the cell, the Pt(IV) complexes undergo a two electron reduction regenerating the original square planar Pt(II) drug and releasing the two axial ligands. (Scheme 1). Since the activation by reduction of the Pt(IV) complexes is believed to take place primarily inside the cancer cell, resulting in the release the original Pt(II) drug, Pt(IV) complexes are considered prodrugs. The use of Pt(IV) complexes as prodrugs was pioneered by Johnson Matthey in the 1990s and has gained momentum and attention ever since [13,14].

The axial ligands of the Pt(IV) complexes can be viewed as cargos that the cytotoxic Pt(II) moiety unloads inside the cell. The axial ligands can be relatively innocent spectators (devoid of significantly biological activity – such as acetatos or hydroxidos), or they can be designed to achieve specific goals.

The axial ligands can be lipophilic moieties that can enhance passive uptake, or they can be cancer cell targeting agents (such as folates), subcellular targeting agents (such as nuclear localizing peptides) to target to the nucleus or triphenylphosphonium to the mitochondria. The axial ligands can be used to tether the prodrugs to delivery systems such as polymers, nanoparticles etc. in order to increase bioavailability and attain specificity [11]. They can also be bioactive moieties such as drugs, enzyme inhibitors, pathway activators or suppressors, epigenetic modifiers, antimetabolites etc. that might work in synergy with the Pt (II) moiety to improve the pharmacological properties.

In this review, we will not discuss the topics of targeting or delivery by polymers or nanoparticles, but will focus on multi-action Pt(IV) prodrugs, examining the working hypotheses, the basic chemistry of these complexes and the interpretations of the biological data.

2. Multi-action platinum prodrugs

In this review we will use the term "multi-action" prodrugs to describe platinum complexes that release inside the cancer cell at least one bioactive ligand in addition to the cytotoxic platinum moiety. We often associate the terms "dual action" or "dual threat" that are used in the literature, primarily with Pt(IV) derivatives of cisplatin with "bioactive" axial ligands that are activated by reduction inside the cancer cells. But these terms also apply to Pt(II) complexes that release a bioactive moiety inside the cancer cell.

3. "Dual action" Pt(II) prodrugs

Although we rarely discuss cisplatin in these terms, cisplatin should be considered a prodrug since it requires intracellular activation in order to bind to the nuclear DNA. The chlorido ligands of cisplatin can be directly replaced by sulfur donors but not by nitrogen donors. In order to bind to the guanines in the DNA, the chlorides (innocent spectators) must first be replaced by the more reactive aqua ligand. Here we will use the term "Pt(II) dual action prodrugs" to describe only those Pt(II) compounds that release a bioactive ligand that can act independently from the platinum moiety, on a different cellular target. Therefore, this refers to compounds that are activated by aquation where the bioactive ligands are usually bound to the Pt via carboxylate groups. Some examples are depicted in Fig. 3. The three compounds in the top row release the bioactive ligands dichloroaceate (DCA) [15,16], ibuprofen [17] or diclofenac [18] and those in the bottom row curcumin [19] or a derivative of vorinostat [20]. Although we will focus on the Pt(IV) "multi-action" prodrugs it should be emphasized the Pt(II) complexes can give rise to "dual action" prodrugs.

4. Multi- action Pt(IV) prodrugs

When discussing "dual action" Pt(IV) drugs it is important to define what "dual action" means. In this context we distinguish three type of axial ligands; "innocent" ligands such as hydroxido or acetato, targeting ligands such as folate, and "bioactive" such as ibuprofen. "Dual action" Pt(IV) complexes, can have two identical bioactive ligands or one bioactive in an axial position while the other is occupied by an "innocent" ligand. We will refer to a Pt(IV) compounds with one bioactive and one targeting ligand as "targeted dual action" compounds and

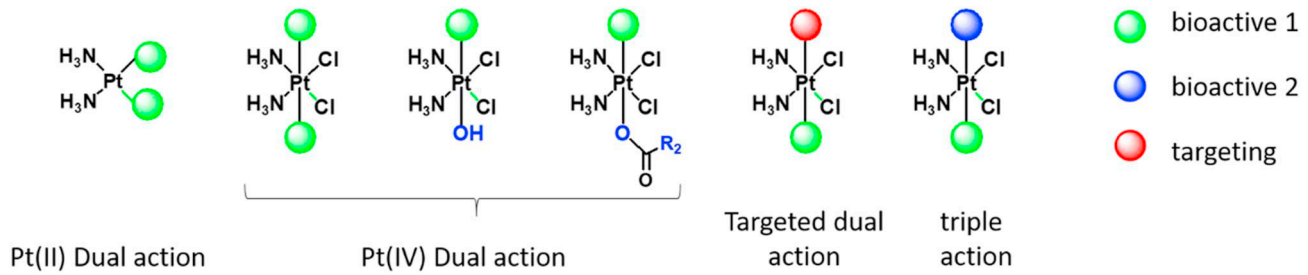


Fig. 4. Examples of the core structures for Pt(II) and Pt(IV) "dual action" compounds, targeted "dual action" Pt(IV) compounds and "triple action" compounds.

those with two different bioactive ligands as "triple action" compounds (Fig. 4).

5. "Dual action" Pt(IV) prodrugs

"Dual action" compounds were reviewed previously [21,22]. There are many examples of "dual action" compounds, making it impossible to discuss or describe all of them and hence we have chosen to discuss only several examples (and there are many more) that allow us to demonstrate the rationale behind their design and to compare the experimental results to the expectations. Some examples are; Ethacraplatin, Mitaplatin, Asplatin/Platin-A, Neri-Pt(IV) and the Pt(IV) derivatives of cisplatin and oxaliplatin with ibuprofen, indomethacin, valproate or phenylbutyrate as the axial ligands (Fig. 5).

5.1. Targeting the resistance pathways to platinum drugs

Ethacraplatin (Fig. 5A), ctc-[Pt(NH$_3$)$_2$(EA)$_2$Cl$_2$]-EA = ethacrynic acid, represents an early attempt to rationally design a Pt(IV) prodrug that will overcome resistance to cisplatin. One of the mechanisms of resistance to cisplatin is the intracellular inactivation of cisplatin by a thiol containing tripeptide (glutathione- GSH) that is present in cells in mM concentrations, that covalently binds to cisplatin preventing it from binding to the DNA and facilitating its excretion from the cell [23]. The binding of GSH to cisplatin is catalyzed by glutathione-S-transferase (GST) and hence, inhibiting GST might help overcome this mechanism of resistance. EA is an inhibitor of GST and hence, Ethacraplatin, that releases two equivalents of ethacrynic acid in the cell, is a bona fide "dual action" prodrug.

Although Ethacraplatin reduced the GST cellular activity and exhibited better potency than cisplatin after short incubation times, it had only a moderate cytotoxic effect after 72 h [24,25]. Later on, the authors demonstrated that combining ethacrynic acid (EA) and cisplatin can reverse MGST1(microsomal glutathione transferase 1) dependent drug resistance most likely by inhibition of MGST1 [26]. Osella and coworkers examined the ability of Pt(II) and Pt(IV) conjugates of EA to overcome resistance in mesothelioma and concluded that decreasing intrinsic resistance by targeting GST with EA or its bifunctional

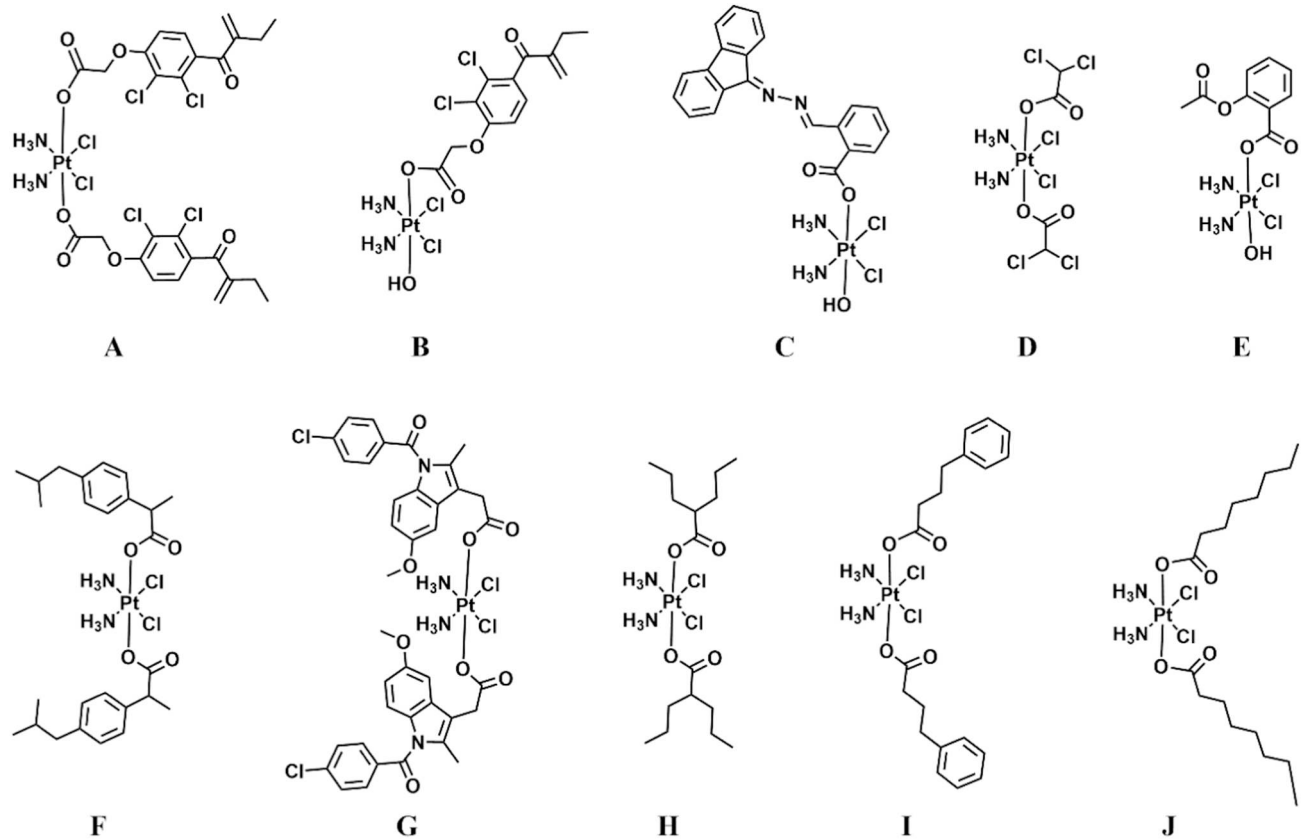


Fig. 5. "Dual action" Pt(IV) prodrugs. A) ethacraplatin, B) mono hydroxy- ethacraplatin, C) Neri-Pt(IV), D) Mitaplatin, E) Asplatin/Platin-A, F) ctc-[Pt(NH$_3$)$_2$(ibuprofen)$_2$Cl$_2$], G) ctc-[Pt(NH$_3$)$_2$(indomethacin)$_2$Cl$_2$], H) ctc-[Pt(NH$_3$)$_2$(VPA)$_2$Cl$_2$], I) ctc-[Pt(NH$_3$)$_2$(PhB)$_2$Cl$_2$], J) ctc-[Pt(NH$_3$)$_2$(Oct)$_2$Cl$_2$].

platinum conjugates is not a viable strategy in MPM (malignant pleural mesothelioma) chemotherapy [27]. More recently, Ang and coworkers redesigned their original compound and replaced one of the EA ligands by a hydroxido (Fig. 5B). This compound is more cytotoxic than Ethacraplatin even though it is a much weaker inhibitor of GST. This might be due to the very slow reduction of Ethacraplatin. Ctc-[Pt$(NH_3)_2$(EA)(OH)Cl_2] has in vivo activity almost as good a cisplatin [28].

Another prominent mechanism of resistance to platinum drugs is the nuclear excision repair (NER) that removes the platinum-DNA lesions formed by cisplatin [29]. Zhu and co-workers designed a Pt(IV) derivative of cisplatin with an inhibitor of the NER that blocks the interaction between the DNA excision repair proteins ERCC1 (excision repair cross-complementation group 1) and XPF (xeroderma pigmentosum, complementation group F). This compound (Fig. 5C), targets the DNA repair as well as platinating the nuclear DNA. This a classic example for the design of a "dual action" prodrug. The compound was more cytotoxic than cisplatin and exhibited reduced DNA damage repair compared to cisplatin [30].

5.2. Targeting the hallmarks of cancer

Hallmarks of cancer comprise biological capabilities acquired during the development of human tumors, such as the ability to sustain proliferative signaling, enable replicative immortality, induce angiogenesis, circumvent growth suppressors etc. [31,32]. Cancer cells can alter their glucose metabolism and even in the presence of oxygen, they limit the energy metabolism to "aerobic glycolysis" that is approximately 18 fold less effective than oxidative phosphorylation in term of production of ATP. DCA and phenylbutyrate (PhB) are inhibitors of aerobic glycolysis, reversing the Warburg effect. They do so by inhibiting pyruvate dehydrogenase kinase (PDK) that plays a key role in enabling glycolysis [33]. Lippard reported on a "dual action" Pt(IV) derivative of cisplatin with two axial dichloroacetatos (DCA), ctc-[Pt$(NH_3)_2$(DCA)$_2Cl_2$] (Fig. 5D). Following reduction in the cell, DCA and cisplatin are released, resulting in simultaneous attack on both the DNA and mitochondria [34]. The cytotoxicity of this compound was comparable to that of cisplatin and when co-cultured with normal fibroblasts it preferentially killed the cancer cells [35].

Another of the hallmarks of cancer is the tumor promoting inflammation. Hence, non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, indomethacin or flubiorifen, are potential anticancer agents. Most of these NSAIDs inhibit cyclooxygenase-2 (COX-2) that converts arachidonic acid to prostaglandins that induce inflammation. COX, is involved in tumorigenesis and is associated with tumor cell resistance against platinum-based drugs. Platin-A and Asplatin (Fig. 5E) are two names given to the same compound, ctc-[Pt$(NH_3)_2$(aspirin)(OH)Cl_2] that upon activation releases one equivalent of cisplatin and one of aspirin. It showed no particular advantage over cisplatin in cytotoxic studies against PC3, DU145 and LaNCap cancer cells [36]. It was however somewhat more potent than cisplatin in MCF-7, HepG2, A549, and A549R cancer cells [37].

The Pt(IV) derivatives of cisplatin or oxaliplatin with the COX inhibitors such as ibuprofen (Fig. 5F) or indomethacin (Fig. 5G) were studied by Hey-Hawkins and coworkers. Interestingly, there was no correlation between the ability to inhibit COX-1 or COX-2 and the cytotoxicity. The ibuprofen derivatives are significantly more cytotoxic than the indomethacin derivatives against HCT-116 and MDA-MB-231 cancer cell lines even though they are much weaker inhibitors of COX-1 and COX-2 compared with their indomethacin analogs. These complexes seem to exert their cytotoxicity via COX-independent mechanisms [17,38]. There are reports suggesting that NSAIDs may act through mechanisms other than inhibition of the activity of COX. There are NSAIDs that do no inhibit COX activity yet effectively inhibit colon carcinogenesis [39]. Mechanisms such as induction of apoptosis by release from the mitochondria of cytochrome C and subsequent activation of caspase-9 and -3, and/or interference with cell-cycle progression were described [40]. Interestingly, the cisplatin derivative with ibuprofen was nearly 5 fold more potent than its oxaliplatin analog [17,38].

Another interesting approach was described by Gou and coworkers who prepared a targeted dual action Pt(IV) prodrug where the Pt(IV) derivative of cisplatin had an axial indomethacin ligand and a targeting biotin ligand on the other [41]. Although the compound inhibited the activity of both COX-1 and COX-2, its cytotoxic IC50 values against six cancer lines were significantly inferior to cisplatin and only in one cell line resistant to cisplatin it was more effective. This further corroborates the results of Hey-Hawkins who demonstrated the lack of correlation between COX inhibition and cytotoxicity.

5.3. Combining platinum drugs with epigenetic modifiers

Epigenetics denotes the factors that affect gene activity and expression, but unlike mutations does not involve any changes in the DNA sequence. The development and progression of cancer is closely associated with epigenetics. Epigenetic mechanisms can silence tumor suppression genes or activate oncogenes in cancer cells thereby promoting cancer progression. Two of the main epigenetic mechanisms include DNA methylation and histone modification [42].

Pt(IV) derivatives of cisplatin or oxaliplatin with axial histone deacetylate (HDAC) inhibitors are another type of "dual action" prodrugs. HDAC inhibitors act as epigenetic agents and several, like Vorinostat and Belinostat, were approved as anticancer drugs [43]. Inhibition of HDAC activity causes hyperacetylation of the histones that prevents tight association with the nuclear DNA leaving the DNA in an open form that facilitates transcription but also makes it more susceptible to platination [44,45].

Shen and Osella prepared the Pt(IV) derivative of cisplatin with two valproate ligands in the axial positions (Fig. 5H) [46,47]. In contrast to Vorinostat or Belinostat whose IC50 values for cellular inhibition of HDAC activity are in the nanomolar range, the IC50 of valproate is in the mM range making valproate (VPA) a very weak extracellular HDAC inhibitor. Yet, ctc-[Pt$(NH_3)_2$(VPA)$_2Cl_2$] is significantly more cytotoxic than cisplatin in several cancer cell lines. Neither Shen nor Osella examined the ability of the compound to inhibit HDAC activity in cancer cells, leaving unanswered the question whether cisplatin and valproate act synergistically by DNA platination and HDAC inhibition, to kill the cancer cells. Brabec demonstrated that the compound reduced the cellular HDAC activity by inhibiting the expression of the enzyme. In addition to hyperacetylation of H3 and decondensation of chromatin resulting in higher levels of DNA platination, GSH levels in the cells were depleted suggesting that other mechanisms in addition to HDAC inhibition might be involved in killing the cancer cells [48]. Valproate was chosen to specifically target HDAC. While it did reduced HDAC activity, other reports show that once inside the cells, valproate also protects cells from ER (endoplasmic reticulum) induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase 3 [49]. It also regulates GLAST (glutamate aspartate transporter)/EAAT1(excitatory amino acid transporter 1) expression [50], inhibits production of IL-6 (interleukin) and TNF-α (tumor necrosis factor) and the activation of NF-kB [51] (nuclear factor kappa-light-chain-enhancer of activated B cells) that has been correlated with enhanced apoptosis [52]. Clearly, it can have several different cellular targets and most probably acts as a multitasking agent rather than as specific inhibitor. We do not know if any of these additional cellular activities triggered by valproate, contribute to the killing of the cancer cells in conjunction or in synergy with the DNA platination.

Phenylbutyrate (PhB) is also a weak extracellular inhibitor of HDAC activity. We prepared the Pt(IV) derivatives of cisplatin or oxaliplatin with PhB (Fig. 5I) and demonstrated that ctc-[Pt$(NH_3)_2$(PhB)$_2Cl_2$] was significantly more potent than ctc-[Pt$(NH_3)_2$(VPA)$_2Cl_2$] having submicomolar IC50 values against a panel of cancer cells [53]. As in the case of valproate, PhB also affects many cellular processes in addition to

inhibiting HDAC activity (see below) [54–57]. Interestingly, like DCA, PhB inhibits PDK increasing the activity of PDHC (pyruvate dehydrogenase complex) and that in combination with DCA, the activity of PDHC is increased beyond the sum of the two [58]. PhB not only inhibits HDAC activity but also can act to reverse the Warburg effect.

Octanoic acid is the linear isomer of valproic acid but it is not an inhibitor of HDAC. When we tried to assess the contribution of an HDACi (VPA) to the cytotoxicity, we prepared ctc-[Pt$(NH_3)_2$(octanoate)$_2Cl_2$] as a negative control to ctc-[Pt$(NH_3)_2$(VPA)$_2Cl_2$] (Fig. 5J). We were quite surprised to see that ctc-[Pt$(NH_3)_2$(octanoate)$_2Cl_2$] was significantly more potent than ctc-[Pt$(NH_3)_2$(VPA)$_2Cl_2$]. Osella screened both compounds against a panel of 9 cancer cells where ctc-[Pt$(NH_3)_2$(VPA)$_2Cl_2$] had a range of IC50 values of 11–1400 nM while ctc-[Pt$(NH_3)_2$(octanoate)$_2Cl_2$] had values of 2.3–91 nM [47]. Through the efforts of Osella, Brabec and Gandin, it was shown that the high potency of ctc-[Pt$(NH_3)_2$(octanoate)$_2Cl_2$] can be attributed to a combination of several factors such as extremely effective cellular accumulation, enhanced DNA platination, reduction of the mitochondrial membrane potential and most interestingly, hypermethylation of the DNA (an epigenetic event). These effects were attributed to the octanoate that was released inside the cell [59].

6. "Triple action" Pt(IV) prodrugs

Surprisingly, there are hardly any reports on "triple action" compounds. We recently described the synthesis and cytotoxic properties of Pt(IV) derivatives of cisplatin, with two different bioactive axial ligands that exert different cellular activities. We chose to study "triple action" compounds where the bioactive axial ligands are inhibitors of cyclooxygenase (COXi), histone deacetylase (HDACi) or pyruvate dehydrogenase kinase (PDKi) because their "dual action" Pt(IV) prodrugs were reported to act synergistically with cisplatin. These "triple action" compounds, ctc-[Pt$(NH_3)_2$(COXi)(PDKi)Cl_2], ctc-[Pt$(NH_3)_2$(COXi)(HDACi)Cl_2] and ctc-[Pt$(NH_3)_2$(HDACi)(PDKi)Cl_2], where COXi = aspirin or ibuprofen, PDKi = dichloroacetate and HDACi = valproate or phenylbutyrate (Fig. 6), were significantly more potent than cisplatin against a panel of human cancer cells. The average IC50s for the eight triple action compounds over the six cancer cell lines ranged from 0.37–1.46 μM compared to 12.5 μM for cisplatin [60]. They were exceptionally effective against pancreatic and thyroid cancer cells where the average IC50 for the eight compounds against pancreatic cancer was 0.26 μM vs. 18.25 μM for cisplatin and their average IC50 against thyroid cancer was 0.14 μM vs. 7.38 μM for cisplatin. The compounds were also screened against non-cancerous HEK293 cells and the selectivity indices were calculated against three different cancer cell lines. Several compounds have very high SI (14–82) compared to cisplatin (1.1–2.6). Remarkably, in 3D spheroid cancer cell cultures, that are supposed to be more predictive for in vivo studies, some of these compounds were 50-fold more potent than cisplatin against the KRAS mutated pancreatic cancer cell line (PSN-1 cells). We did not find any correlations between potency and cellular uptake, DNA platination or inhibition of HDAC, or COX or changes in mitochondrial parameters. It is noteworthy, that all eight complexes, despite having axial ligands with very different biological properties, were significantly more potent than cisplatin. Even though each class of compounds presumably works by a combination of different mechanisms, regardless of the combination, all are very effective.

7. "Quadruple action" Pt(IV) prodrugs

Recently we described the design, synthesis and cytotoxic properties of a "quadruple action" Pt(IV) prodrug (Fig. 7) [61]. It is comprised of two different octahedral Pt(IV) centers that are linked through the axial ligands forming a dinuclear compound. Following reduction, the two Pt (IV) centers disengage from the axial ligands releasing four different bioactive moieties in the cancer cell; cisplatin, dichloroacetate (DCA), phenylbutyrate (PhB), and [Pt(1*S*,2*S*-diaminocyclohexane)(5,6-dimethyl-1,10-phenanthroline)]$^{2+}$ (Pt56MeSS) and the linker. We already described the modes of action of cisplatin (DNA modifier), DCA (PDKi) and PhB (HDACi and PDKi). Pt56MeSS is a very potent cytotoxic

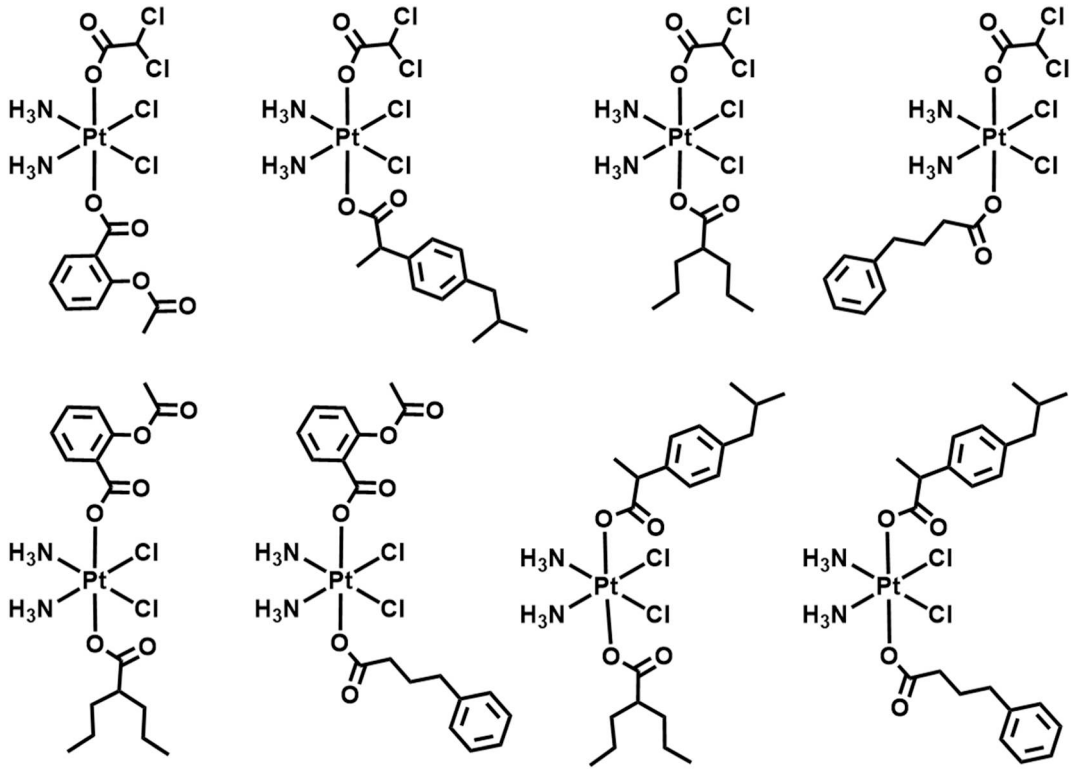


Fig. 6. "Triple action" Pt(IV) prodrugs; the DCA (red) is a DPK inhibitor, Asp or Ibu (purple) are COX inhibitors and Val or PhB (blue) are HDAC inhibitors.

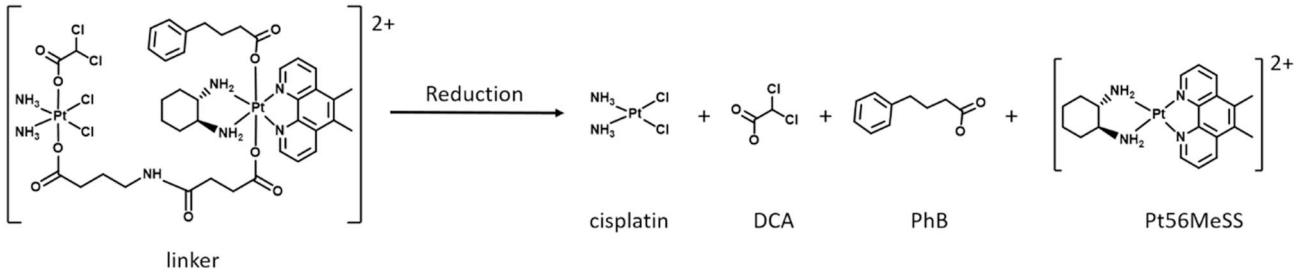


Fig. 7. The quadruple action prodrug is comprised of two Pt(IV) complexes tethered through the axial positions and each has one bioactive axial ligands. Upon reduction, the axial bonds are severed releasing the four active moieties (cisplatin, DCA, PhB and Pt56MeSS) inside the cell.

Pt(II) agent developed by Aldrich-Wright [62], that does not covalently modify the DNA. Its mode of action has not been elucidated but some recent preliminary unpublished data we have suggests that if interferes with the cytoskeleton. The Pt(IV) derivative of Pt56MeSS with two axial PhB ligands was active both in vitro an in vivo [63]. The quadruple action compound is significantly more cytotoxic than cisplatin in both monolayer cultures (2D) and spheroid (3D) cancer cells. Significantly, it is 200–450-fold more potent than cisplatin against KRAS mutated pancreatic and colon cancers and is 40-fold more selective towards KRAS mutated cells compared to noncancerous cells. RAS proteins play a role in regulating cell differentiation, proliferation, and survival and KRAS is mutated in 90% of pancreatic adenocarcinomas,

45% of colorectal cancers, and 35% of lung adenocarcinomas and therefore compounds that are active against KRAS mutated cell are important. As in the case of the triple action compound, we did not observe any correlations with cell uptake, DNA platination or enzyme inhibition. This compound seems to act by several mechanisms being a true multi-action drug.

8. What do we know about the mode of action of multi-action Pt (IV) compounds?

The design of Pt(IV) anticancer prodrugs is predicated on the assumption that they will be reduced inside the cells releasing the axial ligands as well as the cytotoxic Pt(II) moiety. It is very difficult to obtain direct evidence to support this hypothesis since the concentrations of the drugs in the cell are very low and there are no easily accessible experimental methods to monitor the reduction.

Direct observation of the cellular reduction of Pt(IV) compounds can be obtained using XANES (X-ray absorption near edge structure) in synchrotron studies [64]. Model studies show the cancer cell extracts readily reduce Pt(IV) complexes [65].

The primary indirect evidence comes from observing the biological activity of the axial ligands inside the cells (inhibition of COX, HDAC etc.). If the Pt(IV) compounds are reduced outside the cells releasing cisplatin and the axial ligands, the bioactive axial ligands such as valproate, DCA or PhB, that are negatively charged, do not penetrate the cells and cannot exert their inhibitory effects. An increase in DNA platination relative to cisplatin provides further indirect evidence for accumulation and intracellular reduction.

We tend to categorize the Pt(IV) compounds as “dual action” or “triple action” etc. but do these terms accurately describe their mode of action? Dual action tends to imply that the prodrug acts only or primarily on two specific cellular targets. This however, is not usually the case. For instance, we designed the compound ctc-$[Pt(NH_3)_2(PhB)_2Cl_2]$ as a “dual action” prodrug where the cisplatin is supposed to target the nuclear DNA while the PhB is designed to inhibit HDAC activity. Because we related to PhB primarily as an HDAC inhibitor, we only performed biological experiments that assess the ability of the prodrug to inhibit HDAC activity and further downstream effects of the inhibition. PhB does not just inhibit HDAC activity; it acts on other cellular targets as well. Some examples include its inhibition of DPK [58], and its ability to activate receptors that are ligand-activated transcription factors that up-regulate the expression of several genes that code for lipid metabolizing enzymes [54]. This results in decreased conversion of mevalonic acid to farnesyl PPi [66], and decreased cholesterol production, and reduced prenylation of proteins and decreased activation of the p21ras target p42MAPK (mitogen-activated protein kinase)/ERK2(extracellular signal-regulated kinases) [55,67]. In addition, PhB can acts as a cisplatin sensitizer in head and neck cancer by inhibiting the FA(Fanconi's anaemia)/BRCA (breast cancer) pathway by down regulating BRCA1 as well as by an FA/BRCA-independent mechanism [56]. Clearly, once inside the cancer cell, PhB is multi-tasking and affects several cellular processes at the same time in addition to inhibiting HDAC activity.

Is it possible that some of the above listed cellular effects of PhB, rather than the inhibition of HDAC, are the major contributors to the potency of this compound? We cannot answer this question without performing many more biological studies. This of course is not practical but we should realize that our preconceptions, whether right or wrong, direct the design of the biological studies.

Similarly, there is a consensus that the critical biological target of cisplatin is the nuclear DNA. However, only 1–5% of the intracellular platinum is bound to the DNA. There are reports that non-DNA targets can contribute to cytotoxicity [68]. We do not know how the non-DNA bound cellular platinum affects cell viability and whether it can work in conjunction with the PhB to enhance cytotoxicity.

It is interesting to note that often we do not find correlations between the biological activity of the axial ligands and the cytotoxicity. Ethacraplatin, ctc-$[Pt(NH_3)_2(EA)_2(Cl_2)]$, is a more potent inhibitor of GST than ctc-$[Pt(NH_3)_2(EA)(OH)Cl_2]$ but less cytotoxic. Similarly, Hey-Hawkins and Gou found no correlation between COX inhibition and cytotoxicity. It is interesting that eight triple action Pt(IV) prodrugs with different bioactive axial ligands were all very potent against a panel of cancer cells from different origins. This suggests that attacking several intracellular targets at the same time is an efficient approach to killing the cancer cells without relying on a specific target or pathway and is a form of combination chemotherapy in a single molecule.

9. Conclusions

Many of the mutli-action platinum anticancer agents are very effective cytotoxic agents and in many cases, they are able to overcome acquired resistance to cisplatin and are also effective against cancers that are not responsive to cisplatin. Although the compounds are rationally designed to palatinate DNA and inhibit a certain key specific enzyme, in reality, each “targeted” ligand is often multitasking, and affects several cellular functions. As we cannot measure all these effects, it is hard to assess which of the various cellular activities of the bioactive ligands contributes to the cytotoxicity. In many cases, no correlation can be found between the ability of the compound to inhibit the target enzyme and its ability to kill the cancer cells. Even compounds that are designated as “dual action” may in fact be multi-action and the high potency of some of these compounds is probably the result

of a combination of interferences with several cellular processes. This might be one of the reasons that many of these compounds can overcome resistance to cisplatin or to other single drugs.

Multi-action platinum complexes represent a new approach to overcoming resistance and provide a sort of combination chemotherapy in a single molecule with a single pharmacokinetic profile. The disadvantage of this approach compared to conventional combination chemotherapy is that the ratios of the components are fixed and they are all delivered to the cancer cell at the same time. Optimal treatment may require stepwise administration and different dosages of the components. In addition, the multi-action drugs can also be used as a single agents in combinations chemotherapy. There is still a lot of room to explore platinum base multi-action drugs.

Acknowledgements

DG acknowledges the support of the Israel Science foundation (grants 1332/10, 1403/13, 1611/14).

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