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Study of Pt(II) and Pt(IV) derivatives, functionalized with antitumor active principles: Multitarget anticancer agents with several action mechanisms

Estudio de derivados del Pt(II) y Pt(IV), funcionalizados con principios activos antitumorales: Agentes anticancerígenos multiobjetivo con varios mecanismos de acción

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El camino del progreso no es ni rápido, ni fácil.
Maria Salomea Skłodowska-Curie

Primero, agradecer a mi familia la oportunidad que me han dado con la carrera de Química y por el apoyo durante esta etapa. A Laura por ser capaz de comprenderme y ayudarme cuando las cosas se han puesto difíciles. Al grupo de amigos que hemos hecho, sin duda ellos son parte del proceso.

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REPORT

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1. ABSTRACT

Over the past 50 years, antitumor platinum complex such as cisplatin oxaliplatin and carboplatin have played relevant roles in the treatment of solid tumors.

However, many side effects, drug resistance and poor selectivity limit their application encouraging the search for new platinum-based antitumor drugs with new and more effective action mechanisms.

In this final degree project, a bibliographic research is carried out of the most novel platinum antitumour complexes, as well as their mechanisms of action and strategies. We also investigate a method of drug monitoring using a conjugated fluorescent probe.

The search for information was carried out mainly with the SciFinder platform and using the University's access to a multitude of scientific journals. After this search, the most relevant information obtained was selected, organized and synthesised.

The idea of conjugating several biomolecules with a specific anticancer activity to the platinum complex provides several anticancer mechanisms acting simultaneously improving the efficiency and the selectivity of platinum antitumour complexes against cancer cells versus normal cells, and may bypass the resistance of cancer cells.

Concerning the strategy, we envision the possible application of multitarget Pt(IV) complexes as prodrugs instead of the classical Pt(II) ones. They are more stable, thus avoiding undesired reactions.They can also carry on more ligands than Pt(II) complexes, increasing the possibilities to combine different mechanisms of action or to combine them with ligands that increase their specificity and thus reducing toxicity.

For the study and monitoring of such complexes, fluorescence testing is being used, which is a technique capable of distinguishing the oxidation state of platinum as well as being able to track its location within the cell or the specific target tissue and organ in the body.

Keywords: Anticancer agents, platinum complex, multi-target anticancer agents, cancer cells resistance.

2. RESUMEN

En los últimos 50 años, los complejos antitumorales de platino, como el cisplatino, el oxaliplatino y el carboplatino, han desempeñado un papel relevante en el tratamiento de los tumores sólidos. Sin embargo, los numerosos efectos secundarios, la resistencia a los fármacos y la escasa selectividad limitan su aplicación, lo que fomenta la búsqueda de nuevos fármacos antitumorales basados en el platino con mecanismos de acción nuevos y más eficaces.

En este trabajo de final de grado, realizamos una investigación bibliográfica de los complejos antitumorales de platino más novedosos, así como de sus mecanismos de acción y estrategias. También investigamos un método de monitorización del fármaco mediante una prueba de fluorescencia conjugada.

La búsqueda de información se realizó principalmente con la plataforma SciFinder y utilizando el acceso de la Universidad a multitud de revistas científicas. Tras esta búsqueda, se seleccionó, organizó y sintetizó la información más relevante obtenida.

La idea de conjugar varias biomoléculas con una actividad anticancerígena específica a los complejos de platino proporciona diversos mecanismos de acción anticancerígenos que actúan simultáneamente, mejorando la eficiencia y la selectividad de los complejos antitumorales de platino sobre las células cancerígenas versus células normales, y pueden evitar la resistencia de las células cancerígenas

En cuanto a la estrategia, contemplamos la posible aplicación de complejos de Pt(IV) multiobjetivo como profármacos en lugar de los clásicos de Pt(II). Son más estables, por lo que evitan reacciones indeseadas. Además, pueden llevar más ligandos, aumentando las posibilidades de combinar diferentes mecanismos de acción o combinarlos con ligandos que aumenten su especificidad y así reducir la toxicidad.

Para el estudio y seguimiento de estos complejos, se está utilizando la prueba de fluorescencia, que es una técnica capaz de distinguir el estado de oxidación del platino sin ser

destructivo para células vivas, además de poder seguir su localización en el interior de la célula o en un tejido objetivo y órgano del cuerpo.

Palabras clave: Agentes anticancerígenos, complejos de platino, agentes anticancerígenos multiobjetivo, resistencia de células cancerígenas.

3. INTRODUCTION

Cancer is a group of diseases characterised by the division, development and spread of abnormal cells, losing control of their growth and ability to die.
The fight against cancer is in constant development. The advent of chemotherapy was a major breakthrough, adapting treatments to different types of cancer.

Platinum-based drugs are the most widely used chemotherapy drugs due to their wide range of anticancer activities, cost-effectiveness and anticancer mechanism. ***(Figure 1)***

These drugs first took part in history with cisplatin or CDDP ***(Figure 1)***, which was described in 1845 by M.Peyrone and then called Peyrone Salt [1] and its structure determined in 1893 by Alfred Werner. [2] But it was not until 1964 that, serendipitously, its anticancer properties were discovered by B. Rosenberg. [3]

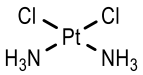


Figure 1: Cisplatin.

This new drug initiated a new line of research on antitumor platinum complexes.
Subsequently, carboplatin ***(Figure 2)*** and oxaliplatin ***(Figure 3)*** were discovered, but were not approved by the U.S. Food and Drug Administration (FDA) until 1993 and 2002 respectively.

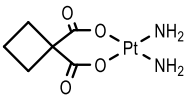


Figure 2: Carboplatin.

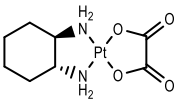


Figure 3: Oxaliplatin.

Nowadays, numerous platinum-related anticancer agents are in development or are in preclinical or clinical trials. In China, South Korea and Japan, some drugs such as lobaplatin ***(Figure 3.1)***, heptaplatin ***(Figure 3.2)*** and nedaplatin ***(Figure 3.3)*** have been approved.

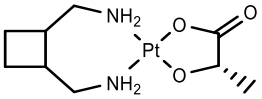


Fig. 3.1. Lobaplatin.

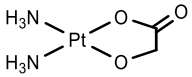


Fig. 3.3. Nedaplatin.

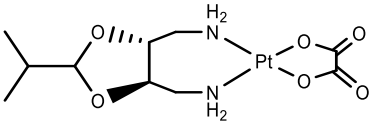


Fig. 3.2. Heptaplatin.

Although cisplatin, carboplatin and oxaliplatin still are the most widely used chemotherapy drugs, they have some shortcomings such as bad aqueous solubility, side effects and drug resistance that restrict their clinical aplications.

These problems encourage new research to overcome these limitations.

Nowadays, the main challenge is to overcome drug resistance through new action mechanisms and new targets.

4. OBJECTIVES

The aim of this final degree thesis is to carry out a literature review of the most current platinum complexes with antitumor activity.

In particular this study is centered in both for Pt(II) complexes and Pt(IV) complexes conjugated to bioactive ligands. In addition we will focus on platinum based multitarget complexes, classifying them by mechanism of action and type of target.

5. METHODS

1) For the literature review of Pt(II) and Pt(IV) derivatives conjugated to antitumor active principles, we have consulted the SciFinder on-line database in order to find articles related to our work.

2) We have divided our work method for the search and synthesis of results in four parts:

a) In the first part we performed a general search with the keywords Anticancer or antitumor

Platinum complexes and then refined the search with the following keywords:

-Multitarget,

-Multipurpose,

-With several action mechanisms (multi-action mechanism),

-Bypass resistance,

-Conjugated,

-Multidrug resistance,

-Inhibitors of resistance,

-Fluorescent probes.

It should be noted that in addition to the refinement with these new keywords, we have filtered the results in the period 2006-2021, due to the fact that we are interested in the most recent investigations.

b) In the second part, we have downloaded the articles of interest of each search and we have divided them by topics, before studying them.

c) In the third part, we studied each article carefully, selecting the most relevant ideas and associating them with their corresponding reference.

d) Finally, we have written the report according to the selected topics in part two and combining ideas and concepts highlighted in part three.

6. RESULTS AND DISCUSSION

6.1. GENERAL ACTION MECHANISM OF PLATINUM DRUGS

The general mechanism of action of Pt drugs can be divided into four essential parts: cellular uptake, aquation, Pt-DNA binding and induction of cellular apoptosis.

Cellular uptake can occur via passive diffusion or active transport via transporters such as copper transporter 1 (CTR1).[4]

In the aquation phase, ligands are shifted by water molecules. In the case of cisplatin, one or two of chloride ligands are displaced to form monoquo complexes $[Pt(NH_3)_2Cl(H_2O)]^+$ or diaquo complexes $[Pt(NH_3)_2(H_2O)_2]^{2+}$.[5]

Then, those positively charged complexes act by binding Pt to the N7 positions of guanine and/or adenine bases to form platinum-DNA adducts via ligand exchange.
This causes an intrastrand cross-linkage if it occurs whithin the same strand or interstrand if it occurs between opposite strands.

Finally, these Pt-DNA adducts are detected and initiate the apoptosis cell proces.[6] For example, mismatch repair proteins (MMR) detect the adduct and activate tumour suppressor p53 gene, which binds to DNA and from here is detected by target genes resulting in activation of proapoptotic genes that initiate cell apoptosis. Fail in DNA repair can also activate p53 gene.[7,5]

We illustrate this mechanism in (***Figure 4***) using cisplatin as example.

6.2. GENERAL RESISTANCE MECHANISM OF PLATINUM DRUGS

There are several mechanisms of resistance, which may be classified according to the point at which they act.

1) The first would be to reduce the concentration of the drug inside the cell.

This is carried out by decreasing the absorption and increasing the excretion of the drug out of the cell. For example, by downregulating CTR1 or removing subunits of VRACs we reduce drug absorption.

On the other hand, increasing the number of ATP7A and ATP7B enhances drug transport

out of the cell. [8]

There are also nucleophilic biomolecules such as glutathione (GSH) that bind to the complexes in their aquo form. The Pt-Nucleophile adduct can be excreted by ABC transporters.[9]

2) The second mechanism of resistance would be the repair of DNA damaged by the drug. Apart from the proteins that detect the Pt-DNA adduct and initiate the apoptosis process, there are other proteins that could detect it and repair the DNA, such as nucleotide excision repair proteins (NER).[10]

3) Thirdly, there are mechanisms related to defects in the apoptotic process caused by the Pt-DNA adduct. For example, inactivation of p53 or, in the case of cisplatin, activation of the AKT pathway, which is a mechanism that promotes cell survival, can result in drug resistance.[11]

4) We can also find survival pathways not related to the Pt-DNA adduct such as the increase of epidermal growth factor receptor (EGFR).[12]

We illustrate these resistance mechanisms in (***Figure 4***) using cisplatin as example.

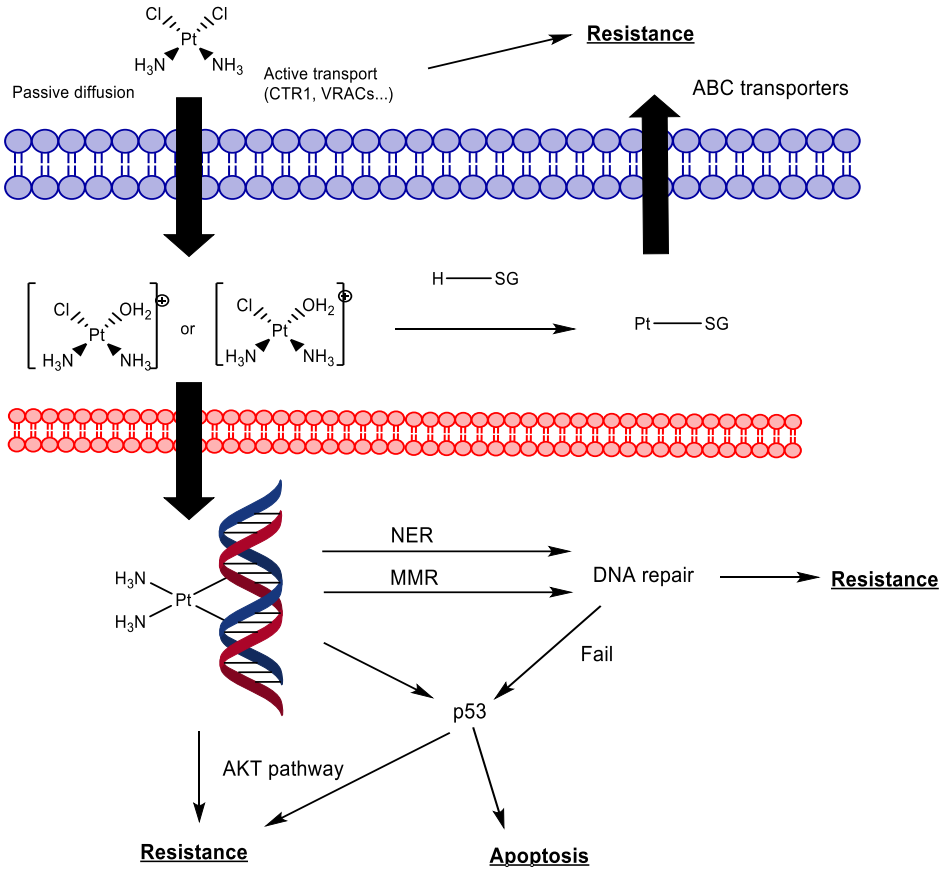


Figure 4 : Mecanism of anticancer action and drug resistance of cisplatin.

6.3. CONJUGATED BIOMOLECULES

In previous sections, we have seen various problems due to biomolecules deactivate in vivo the platinum complexes, when entering the body and disturb the mechanism of action of Pt antitumor drugs.

Nowadays, the option of conjugating bioactive molecules to Pt antitumor drugs is being studied so that they can somehow help the complex by increasing its cytotoxicity or reducing its toxicity, favouring at some point the mechanism of action of the Pt antitumour drug. [13]

6.3.1. Glucose transporters

Cancer cells are in a hypoxic environment, which causes them to use anaerobic pathways to obtain energy. These pathways are less efficient than aerobic pathways.[14] Because of this, they have a higher demand for glucose, resulting in an overexpression of glucose transporters on the cell surface, which only allow the passage of glucose-like structures into the cell.

Several studies have been developed in which a glucose-type ligand is added so that the cancer cells absorb the drug specifically and more easily than a healthy cell.[15]
It can also make it more soluble in water. In a seminal investigation, a platinum complex with a glucose-functionalized malonate moiety ***(Figure 5)*** was synthesised and it was approximately 150 times more soluble than oxaliplatin.[16]
Moreover,in a paper published in 2017[17], two mannose-conjugated platinum(II) complexes were studied. ***(Figure 6) (Figure 7).***

In terms of solubility, compounds **7** and **6** showed water solubility of 58(mg/mL) and 639(mg/mL) respectively while cisplatin, oxaliplatin and carboplatin showed values of 1.0(mg/mL), 6.1(mg/mL) and 17.3(mg/mL) respectively.

Both showed, for HT29, H460, DU145, A549, SKOV3 and MCF-79 cancer cells, comparable or in some cases superior cytotoxicity than oxaliplatin in several *in vitro* assays. However, only compound **7** shows better activity *in vivo* tan oxaliplatin.

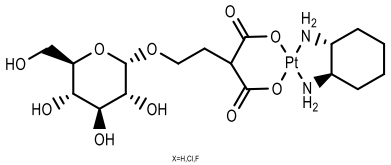


Figure 5: Platinum complex with a glucose-functionalized malonate

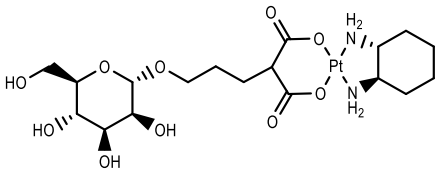


Figure 6: Mannose-conjugated platinum(II) complex

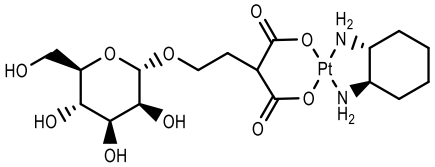


Figure 7: Mannose-conjugated platinum(II) complex

6.3.2. Peptides

On the surface of cancer cells there are usually a large number of receptors for folates, somatostatin, etc.

There are studies that have tried to combine peptides with drugs for photodynamic therapies, taking advantage of the specificity provided by the peptide-receptor relationship.[18 19] ***(Figure 8)***. However, it is true that the conjugation of a peptide to the complex is somewhat difficult. In addition, since they are weakly linked, the peptide can be irreversibly substituted by another ligand, causing us to lose the target effect.

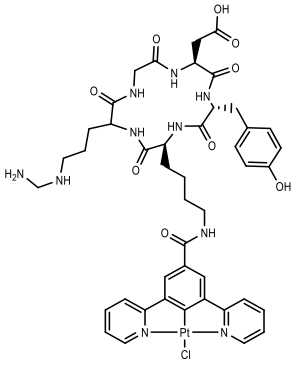


Figure 8: Chemical structure of a platinum(II)-peptide conjugate for integrin-targeted photodynamic therapy

6.3.3. Sex hormones

The involvement of sex hormones in cancer progression in hormone-sensitive tissues is well-established.[20] This is based on the fact that there are a high number of hormone receptors on the cell surface of certain cancer tissues (breast, testicle, ovary, etc.). This is why by conjugating steroids to the Pt complex we would take advantage of this fact as a target, providing specificity and reducing toxicity.[21] ***(Figure 9).***

These complexes showed greater in vitro cytotoxicity to MCF-7 (ER+) and MDA-MB-231 (ER-) cells compared to oxaliplatin, carboplatin and cisplatin.[10]

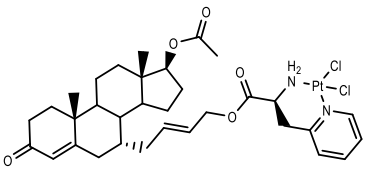


Figure 9: Chemical structure of testosterone-linked platinum(II) conjugate

6.3.4. Biotin

Cancer cells generally have a large number of biotin receptors on their surface, which prompts the idea of conjugating biotin to the complex to take advantage of this fact and use it as a target.[22] In several studies biotin was conjugated to photoactivatable Pt(II) complexes, making it a target for cancer cells.[23] [24] ***(Figure 10).*** It is a breakthrough because they combine photoactivation with a wide range of photoactivation and a great specificity.

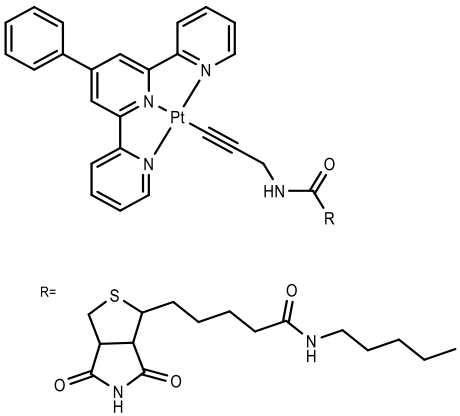


Figure 10: Chemical structure of biotinylated conjugated platinum(II) complex

6.4. ANTIBODY-DRUG CONJUGATES

As discussed in previous sections, several methods have been studied in recent years to improve the targeting specificity of antitumour drugs. These include the use of specific antibodies conjugated to the drug, taking advantage of antigen-antibody specificity.

The general function of an antibody is to bind to a specific molecule (antigen) on the surface of a problematic cell. When an antibody binds to the antigen, it serves as a marker to attract disease-fighting molecules or as a trigger that promotes cell destruction by other immune system processes.

Because of an antibody's ability to connect with a cancer cell, the antibody can be designed as a delivery vehicle for other treatments. These antibodies are produced in laboratories and are known as monoclonal antibodies.

Some monoclonal antibodies bind to a chemotherapy drug to deliver the treatment directly to cancer cells and spare healthy cells.

6.4.1. Immunotargeting carriers

In one particular study [25], cisplatin was combined with a carboxymethyldextran (CM-dex) carrier to avoid drug loss by excretion. They also complex, using avidin as an intermediate, with a monoclonal antibody 108 (mAb-108), taking advantage of the overexpression of epidermal

growth factor receptor (EGF-R), typical of cancer cells, to carry out what is known as immunotargeting. In addition to its specificity, this complex has a high molecular weight, which means that the drug stays longer in the tumour site than the free drug.

This complex has shown considerable inhibition of tumor growth upon intratumor injection. Tests with mAb-108 and free drug gave lower results. On the other hand, they also found that the complexed antibody must be tumour-specific.

6.4.2. Nanobodies

In recent years, a type of antibody that is much smaller than the usual antibodies because it is composed of relatively simple proteins has been studied. Also, they are fully functional and possess the full antigen binding activity. These are known as nanobodies because they have only a few nanometres in size. Despite the advantages of their smaller size, they are also more easily removed.

Their effectiveness was evaluated in a study [26] in which a fusion protein, termed NGC, that was composed of a biparatopic anti-EGFR nanobody (N, 7D12- 9G8) for targeting to EGFR positive tumour cells, a high-affinity gadolinium-binding domain (G, ProCA32) for MRI and a C3-tag (C) for drug conjugation to a maleimide-functionalized Pt(IV) prodrug (Mal-Pt) ***(Figure 11).***

Two cell lines of EGFR negative cells A375 and EGFR positive cells A431 were tested. Drug accumulation was higher in EGFR positive than in EGFR negative cells due to the presence of N, 7D12- 9G8.

On the other hand, accumulation was tested with cisplatin on the same cell lines. The accumulation was higher because it uses other pathways for uptake different from the nanobody-drug conjugates pathways. Cisplatin uses passive diffusion facilitated by copper transport proteins while nanobody-drug conjugates are taken up by induced endocytosis. However, with cisplatin, specificity regarding EGFR-positive and negative cells was lost.The same happens with the cytotoxicity assays. Cisplatin had a higher cytotoxicity than nanobody-drug conjugate but nanobody-drug conjugate had a higher differentiation between EGFR-positive and EGFR-negative cells. To avoid the problem, anti-albumin nanobody was added to the nanobody-drug conjugate to make it more dificult to remove due to its size. In *in vivo* assays, it showed much slower clearance than cisplatin when anti-albumin nanobody was added. On the other hand, cisplatin showed greater dispersion in the body due to its lack of

specificity. The slower clearance of the nanobody-drug conjugate resulted in increased accumulation in EGFR-negative cells due to longer drug exposure time.

The antitumor efficacy was evaluated by measuring the tumor volume and again Pt-NGCA inhibited the growth of A431 tumours but not A375 tumors. Cisplatin inhibited the growth of both tumor types. This confirms the high specificity of Pt-NGCA with fewer side-effects. This new type of multifunctional nanobody-drug conjugate (NDC) for the targeted-delivery of the platinum(IV) has shown a higher specificity by decreasing the amount of drug in different organs and increasing it in tumors resulting in less toxicity.

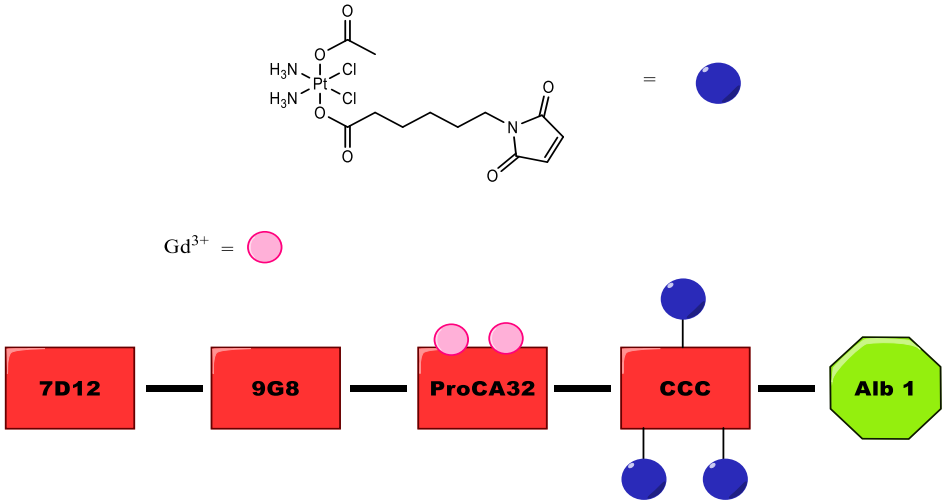


Figure 11: The conjugation of the Mal-Pt prodrug and the loading of Gd3+ to NGCA.

6.4.3. Antibodies as drugs

Certain monoclonal antibodies may attack the cell more directly, even though they are designed for another purpose. When some of these antibodies bind to a cell, a series of events within the cell can cause the cell death. This type of complex have the problem that the classical linker that binds the antibody is relatively weak and can lead to premature loss of the antibodies.[27]

Generally, cysteines via maleimide chemistry are used as linkers between antibodies. However, this approach affords a high heterogeneity of antibody-conjugated drugs.[28]

Recently, several methods have been proposed to solve this problem. Unnatural amino acids, additional cysteines and transglutaminase are some examples with good results, but they require genetic engineering and complex processes.

Another problem is that they make use of C-S bonds with the risk of retro-Michael reactions by replacing the linker-drug with thiol-containing molecules such as albumin.Some studies [29, 30, 31] proposes to use platinum as a linker for antibodies taking advantage of the strong Pt-S bond to overcome the problems of stability and heterogeneity of the more classical linkers with great results. ***(Figure 12).*** Most of them use [ethylenediamineplatinum(II)]$^{2+}$ as a linker having great results in *in vivo* tests in terms of stability.[32]

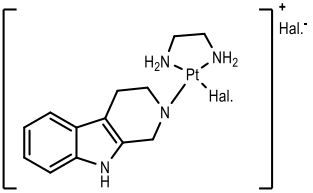


Figure 12: Example of a Lx-Hal. ADCs complex

6.4.4. Combination

Some studies [33] are developing the idea of combining classical carboplatin treatments with folate receptor alpha (FRα)-targeting antibody-drug conjugate (ADC) to treat platinum-sensitive ovarian cancer.

The results showed a considerable reduction in hematological toxicity compared to the free carboplatin drug. Alopecia was also not observed in the tested patients.Overall the results showed great potential for future research in the field of ADCs.

6.5. MULTI-ACTION MECHANISM

6.5.1. Pt(IV) complexes

Platinum(IV) complexes are used as prodrugs, their oxidation state is only used for the better transport of the drug, which will then act as Pt(II).

Pt(IV) complexes have low spin d^6 have a saturated octahedral geometry instead of the typical square-planar geometry of Pt(II) complexes. This is why they exhibit less unwanted reactivity with biomolecules, thus reducing drug toxicity.

They are also orally available because they are resistant to hydrolysis by gastric juices.[34]

Although it is true that they are very inert, studies have shown that their ability to be inert depends on the ligands they possess in their structure.[35]

As mentioned above, they act as prodrugs, and their activation takes place by the reduction of Pt(IV) to its activated form Pt(II) by losing two electrons ***(Figure 13).*** The resulting complex retains the equatorial ligands, but releases the two axial ligands.

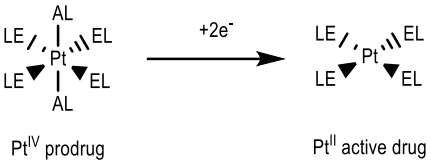


Figure 13 Platinum (IV) complexe reduction.

Axial ligands are usually used for the reduction of Pt(IV), but recently axial ligands are being proposed that not only reduce, but also assist in the targeting properties of the complex. Such as targeting of glucose receptors, hormone or biotin receptors, etc.

On the other hand, axial ligands may give the possibility to add new functions to overcome the resistance of cancer cells with multi-action mechanism.

But mostly the activity/selectivity of the Pt(II) complexes depends on the equatorial ligands. The axial ligands are usually more related to the lipophilicity and the redox properties of the complex.

6.5.2. Pt(IV) as a base for multi-action mechanism

In most cases, multi-action treatments are carried out by combination therapy. The intention is to overcome cellular resistance and reduce side effects. However, this can lead to pharmacokinetics or drug-drug interaction problems.

The idea of preparing a complex containing several drugs could be a breakthrough and could overcome the problems of combination therapies.

The Pt(IV) complexes are reduced by releasing their axial ligands, leaving the active molecule in the Pt(II) form together with four ligands. This gives us a wide variety of possibilities to conjugate different bioactive molecules with different action mechanisms, achieving the so-called multi-action mechanism.

6.5.3. Mitochondria-targeted complexes

As mentioned in previous sections, cancer cells use anaerobic pathways to obtain energy instead of aerobic pathways in what is known as the Warburg effect.

The Wardburg hypothesis states that this leads to a suppression of apoptosis.

Dichloroacetate (DCA) reverses the Wardburg effect by inhibiting pyruvate dehydrogenase (PDK) by keeping the pyruvate dehydrogenase complex (PDC) dephosphorylated and promoting activation of the aerobic pathway.

This results in the release of pro-apoptotic agents from within the mitochondria such as cytochrome c, again activating the intrinsic or mitochondrial apoptosis process.

In an interesting study, a Pt(IV) complex called mitaplatin ***(Figure 14)*** that has two DCA as axial ligands, was developed and its cytoroxicity evaluated. It was observed that the complex induced intrinsic apoptosis. Cytotoxicity was superior to that of cisplatin on NTera-2, HeLa, U-2 OS, A549, MCF-7, A2780 cell lines and also on the cisplatin-resistant subline A2780/CP70 cells. In addition, it induces intrinsic apoptosis in cancer cells but not in healthy cells.

The problem it presents is that it can be hydrolysed by hydroxide ions, so, It could be prematurely hydrolysed outside the cell. For this reason, the complex was encapsulated in polymer nanoparticles, increasing the time it remains in the blood. However, with encapsulation, the effectiveness of the drug is slightly reduced.

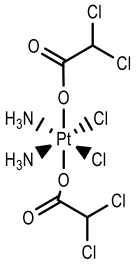


Figure 14: Mitaplatin.

Mitaplatin-derived compounds have also been prepared [36] by substituting a DCA for a succinate, ***(Figure 15),*** in order to be linked to polymers and form micelles.

The micelles show a higher mitochondrial depolarization than free DCA.

The same was done with a carboplatin derivative in which DCA was as equatorial leaving ligand and hydroxide and succinate were axial ligands ***(Figure 16)***. This gave a higher cytotoxicity than carboplatin. Both compounds showed increased accumulation in tumors, endocytosis and a longer blood circulation.

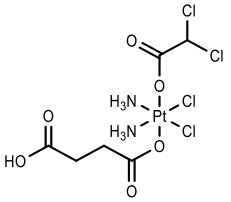


Figure 15: Mitaplatin derivated.

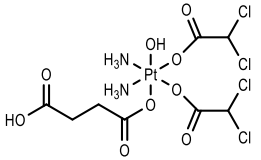


Figure 16: Carboplatin derivated.

Lippard et al. synthesised other mitochondria targeting compounds.[37]

In ***(Figure 17)*** it is illustrated a complex conjugated with tocopherol succinate (α-TOS) a vitamin E analog that inhibits Bcl-2 and Bcl-xL which are two anti-apoptotic proteins inducing to the apoptotic process. This α-TOS conjugated complex showed a far superior potency in comparison to cisplatin or the combination of cisplatin with α-TOS in many cancer cell types.

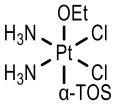
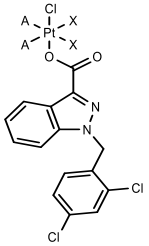


Figure 17: α-TOS conjugated complex.

Lonidamine acts by suppressing the activity of mitochondrial complex and hexokinase by blocking aerobic glycolysis ***(Figure 18)***. It also acts by increasing intracellular acidity in the tumor by blocking mitochondrial pyruvate carrier and carboxylate transporter. Finally, it may also act by altering the mitochondrial transmembrane potential. As a result, they show better activity than their analogues against several types of cancer cells but especially against human prostate adenocarcinoma cells.

However, in the case of the cisplatin-based prodrug, the drug shows a lower cellular uptake. Despite this disadvantage, it is important to note that it has a better overcoming of resistance.



A = NH_3

16.1 X = Cl^-
16.2 X = cyclobutane-1,1-dicarboxylate
16.3 X = 3-oxocyclobutane-1,1-dicarboxylate

A x 2 = 1,2-Diaminocyclohexane

16.4 X = cyclobutane-1,1-dicarboxylate
16.3 X = 3-oxocyclobutane-1,1-dicarboxylate

Figure 18: Pt(IV) prodrugs containing lonidamine as an axial ligand.

6.5.4. GSH-S-Transferase-targeted complexes

In the section "General resistance mechanism of platinum drugs", the resistance mechanism based on nucleophilic biomolecules such as glutathione (GSH) was discussed. The reaction linking GSH to Pt is catalysed by enzyme GSH-S-transferase.

Ethacrynic acid (EA) ***(Figure 19)*** is an inhibitor of enzyme GSH-S-transferase.

In some studies. [38] [39] In some studies, EA was used as axial ligands in order to overcome this resistance mechanism, ***(Figure 20).***

Ethacraplatin showed superior potential to cisplatin in T47D cell lines. It also reduced GSH-S-transferase activity to 10% and its high lipophilicity increased cellular accumulation.

However, tests on malignant pleural mesothelioma (MPM) cell lines were not as positive. In this case, the cells reinforced the resistance mechanism by increasing the concentration of GSH. This may be due to this type of cancer has an overexpression of anti-oxidative pool.

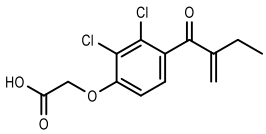


Figure 19: Ethacrynic acid.

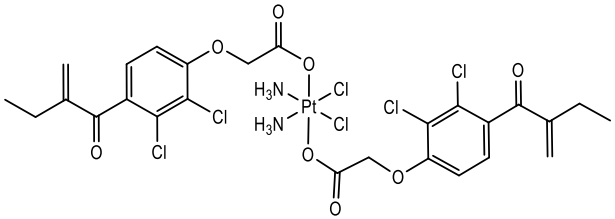


Figure 20: Ethacraplatin.

In addition, there is an interesting cisplatin based complex called Platin-B ***(Figure 21)*** which has 6-bromohexanoate as its axial groups.[40] This ligand prevents excretion of the drug via GSH and acts as an alkylating agent for DNA bases. It has also been found to act on mitochondria by causing a loss of mitochondrial mass in resistant cells.

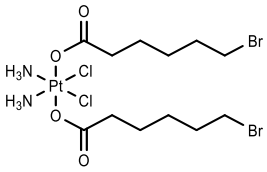


Figure 21: Platin-B.

In malignant bone tumors called osteosarcoma, the use of cisplatin was limited due to its side effects. In order to overcome this problem, Hong Chen et al. [41] studied a cisplatin-based complex with the GST inhibitor 6-(7-nitro-2,1,3-benzoxadiazole-4-ylthio) hexanol as axial ligand, ***(Figure 22)***. It showed higher anticancer activity compared to cisplatin and the free inhibitor.

Moreover, also showed higher selectivity than cisplatin. In addition, this complex was also tested against cisplatin-sensitive gastric carcinoma SGC-7901 and showed a lower resistance factor.

It was also found that the aforementioned complex causes more DNA damage inducing cell apoptosis.It also is worth to mention that the complex inhibits cell migration, giving it the potential to prevent metastasis.

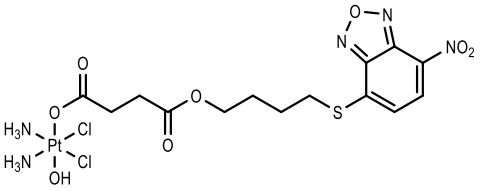


Figure 22: Cisplatin-based complexe with 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio) hexanol

6.5.5. Triple action mechanism

A “triple action” cisplatin-based Pt(IV) complex containing inhibitors of histone deacetylase (PhB) ***(Figure 23.1)*** and pyruvate dehydrogenase kinase (DCA) ***(Figure 23.2)*** as axial ligands ***(Figure 24)*** were studied.[42] They were much more cytotoxic than cisplatin. In addition, they showed high activity against oxaliplatin and cisplatin-resistant cell lines. Moreover, they have a high selectivity in front of many different cell lines.

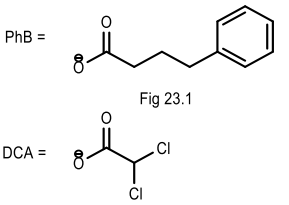


Fig 23.1

Fig. 23.2

Figure 23: Phenylbutyrate and dichloroacetate.

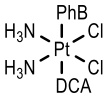


Figure 24: Cisplatin-based Pt(IV) complexe with PhB and DCA.

Another option for Pt(IV) prodrugs is to incorporate two Pt(IV) centres to increase the amount of bioactive ligands. Thus, E. Petruzzella et al. [43] studied a complex with two Pt(IV) centres, one of the centres had DCA as an axial and Pt56MeSS-based ligand, and the other an axial PhB, ***(Figure 25).***

This complex showed IC_{50} values lower than that of cisplatin or oxaliplatin, in nine cancer cell lines an also against KRAS mutated cell lines and against HCT-15 and PSN-1, among others. It proved to be more potent than its analogue without the axial ligands.

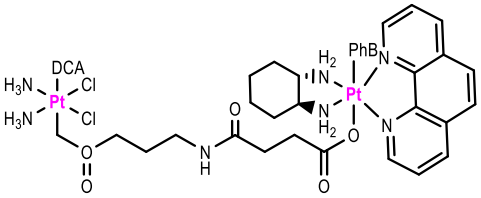


Figure 25: Complexe with two Pt(IV) centers with DCA and PhB.

6.6. MULTITARGET COMPLEX

Many single-target drugs with high specificity have been studied for years. However, the studies did not get very far as cancer cells used compensatory ways in order to carry out the cell-resistance. As seen in the "Multi-action" section, the idea of using a cocktail of drugs is very efficient to overcome resistance, but presents many side-effects.

Due to that, multi-targeting complexes are being proposed lately.When we talk about the concept of multitargeting, we are referring to a drug that will target different parts of the cell in order to reach it specifically or to eliminate it in many different ways.

6.6.1. Pt(II) multitarget complexes

In 2018 Yang Zhang et al. studied a series of Pt(II) dual-targeting anticancer drugs.[44] These drugs have the formula [(CNN)Pt(X)]]}+ in which (CNN) is a tridentate ligand and (X) a monodentate ligand. See ***(Figures 26 to 29).***

Specifically, the tridentate ligand may be 4,6-diphenyl-2,20-bipyridine (dpbp) or 6-(2-naphthalenyl)-2,20-bipyridine (nabp) and the monodentate ligand would be 4-anilinoquinazoline derivatives. The subunit 4-anilinoquinazoline is an inhibitor of the epithermal growth factor receptor (EGFR) which is key in cell growth process.On the other hand, (CNN)Pt is the nucleic acid targeting moiety.

With these complexes, we would have a potential dual-targeting drug, EGFR and nucleic acid targeting.

Drugs **26-29** showed high EGFR-inhibiting activity and nucleic acid binding. In particular **26, 27** and **28** reduced cell proliferation more than their respective precursors **30.1** and **30.2** against A549, HeLa, A431 and MCF-7 cells. In the case of MCF-7 and A549 cells, **26** and **27** showed greater anti-proliferative activity than cisplatin.

Fluorescence assays showed a majority accumulation in the cell membrane and in the nucleus, confirming the dual-targeting character of this type of drugs.

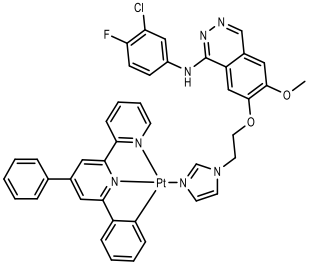


Figure 26

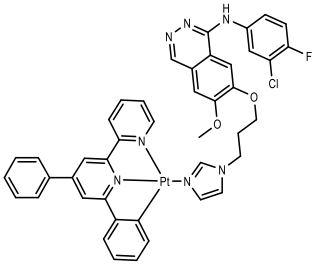


Figure 27

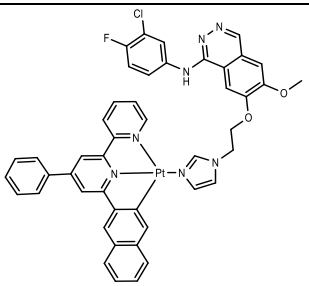


Figure 28

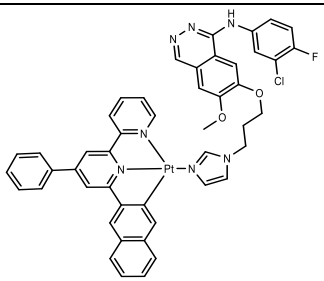


Figure 29

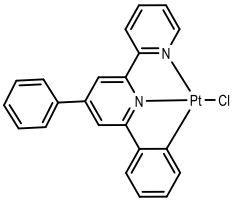


Fig. 30.1

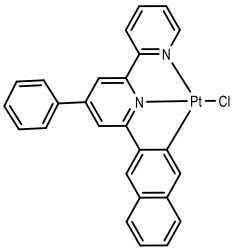


Fig. 30.2

Figure 30

6.6.2. Pt(IV) multitarget complexes

As we have seen above, Pt(IV) complexes act as prodrugs. They have an octahedral spatial arrangement which in an axial plane contains the typical plano-square structure of Pt(II) complexes. The release of the axial ligands leads to the reduction of platinum and thus activates the drug. Upon entry into the cell this reductive elimination process takes place, these axial ligands may be bioactive molecules.

A dual-targeting platinum(IV) prodrug named Asplatin ***(Figure 31)*** has recently been studied.[45] It has Aspirin® as an axial ligand, which is released upon entering the cell. This complex works by inhibiting COX-1 and COX-2 by preventing inflammation due to the tumor and altering the cellular response to platinum drugs.

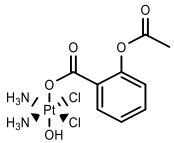


Figure 31: Asplatin.

It is known that cell surface glycoprotein CD44 is overexpressed in many types of cancer cells. CD44 plays an important role in metastasis, which makes it an interesting target. [46] C. Sun et al. studied a nanocomplex ***(Figure 32)*** with hyaluronic acid (HA), which interacts with CD44, ethylenediamine as an intermediate between HA and Pt(IV). It manages to be recognised by HA receptors and taken up by the cell. In several *in vitro* assays, it showed higher cytotoxicity against cancer cells overexpressing CD44, such as melanoma B16-F10, liver carcinoma Hep

G2 or Kidney HEK-293 and lower toxicity due to drug specificity. *In vivo* assays also showed minimal toxicity.[47]

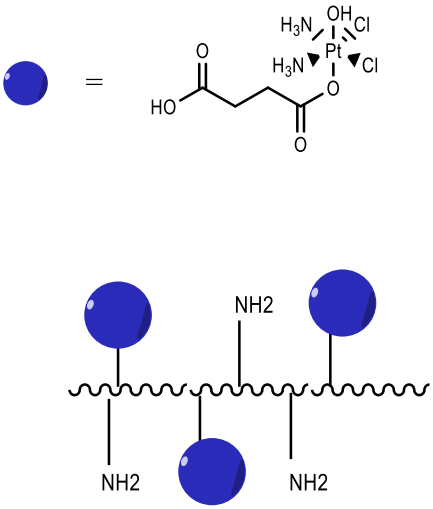


Figure 32: HA-EDA-Pt(IV) nanoconjugated.

6.6.3. Other approaches to bypass resistance

As we have seen in previous sections, cancer cells have a wide range of resistance mechanisms. Overcoming these cellular resistance mechanisms is one of nowadays objectives. For each resistance mechanism, a way of overcoming it has to be study.

Targeting Decreased Accumulation

The expulsion of the drug to the outside of the cell via ABC transporters and GSH biomolecules is one of the best known cellular mechanisms of resistance. The idea of using a synergistic attack strategy combining Pt antitumour drugs with other drugs in orther to overcome cell resistance is widely used.

Emodin (Figure 33) is a ROS generator that, combined with cisplatin, oxaliplatin or carboplatin, markedly increases cytotoxicity in bladder carcinoma cell lines SGC996. This is because emodin decreases the amount of GSH and causes a downregulation of one type of ABC transporters.[48]

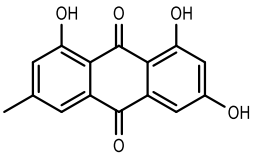


Figure 33: Emodin.

Another biomolecule that is very useful for overcoming cellular resistance is caffeic acid. ***(Figure 34).***Caffeic acid inhibits glutathione reductase and GSTs. As a result, it increases the cytotoxicity of cisplatin in ovarian carcinoma cell lines. In addition, it increases the amount of platinum bound to DNA.[49]

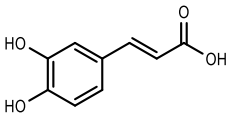


Figure 34: *Caffeic acid*

6.7. FLUORESCENT PROBE CONJUGATED TO PLATINUM COMPLEXES

To conjugate a platinum complex to a fluorescent probe is a very important approach to find potential targets within the cell, because the fluorescent site in the cell may be visualized by several microscopy techniques as confocal microscopy, among others.

Traditional methods such as atomic absorption spectrometry (GF-AAS) or inductively coupled plasma mass spectrometry (ICP-MS) cannot be used to study the accumulation of platinum complexes or the mechanisms of action because they are not able to distinguish Pt oxidation states.[50,51] X-ray near-edge adsorption spectroscopy (XANES) is able to distinguish platinum oxidation states but cannot be applied in living cells due to their destructive nature.[52]

Fluorescence microscopy is a very effective alternative for probing Pt species at the cellular level. Generally, the aim is to conjugate fluorophores to Pt complexes in order to follow their progress using fluorescene imaging.Pt(II) with conjugate fluorophores and Pt(IV) complexes with fluorophore groups as axial ligands have been tested.[53, 54] Despite the effectiveness of the test, conjugating such ligands to Pt presents problems in cell absorption and pharmacokinetic characteristics.[55]

Jun Xiang Ong et al. proposed a ratiometric probe using RDC1 ***(Figure 35)*** as Pt-selective fluorescent sensor.[56] When they tested with cisplatin, it gave a very good ratiometric response with a good linear working range (0-160μM) and a detection limit of 109 nM. The technique afforded generally good results for Pt(II) complexes but not for those with chelating ammine ligands such as oxaliplatin.

It also showed the ability to distinguish many Pt(IV) complexes from their Pt(II) analogues. This is key to the study of the reduction and accumulation of Pt(IV) complexes.

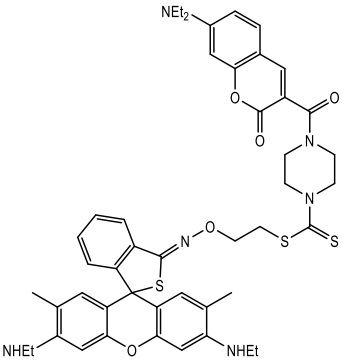


Figure 35: RDC1

Another study proposed the idea of preparing a specific fluorescent probe for Pt(II) drugs, taking advantage of the fact that they are more reactive than their Pt(IV) drug analogues.[57] As Pt-selective fluorescent sensors, they picked up Rho-DDTC. ***(Figure 36).***Tests to see if they could differentiate cisplatin from other metal ions were very positive.

In HeLa cells, cisplatin showed dispersion in the cytoplasm but not in the DNA because the binding of Pho-DDTC to cisplatin prevented it from reaching the nucleus. Oxaliplatin showed no response.

In the case of platinum (IV) complexes, the response took a few hours and was weaker because they had to be reduced first. It should be noted that in this case concentration was observed in the nucleus due to the hydrophobic nature of some Pt(IV) complexes.

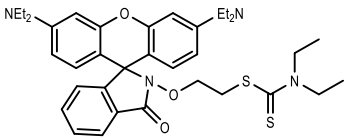


Figure 36: *Rho_DDTC*

In previous section, we mention a complex with bi- and tridentate ligands with the ability to perform dual-targeting. In that particular case, the tridentate ligands studied were 4,6-diphenyl-2,2'-bipyridine (dpbp) and 6-(2-naphthalenyl)-2,2'-bipyridine (nabp), which are π-conjugated ligands. These π-conjugated ligands have an enhanced photoluminescent character upon binding between Pt complexe and G4/DNA.[58] Good luminescence results were obtained in all cases. ***(***See, ***Figures 26 to 29).***

Because of this character of the π-conjugated ligands, they could know whether the drug can discriminate G4 DNA from duplex DNA.This gives a possible future application of luminescent probes for protein- and DNA-targeted platinum antitumour drugs in living cells.

7. CONCLUSIONS AND FUTURE PROSPECTS

Antitumor platinum complexes remain highly relevant in the treatment of cancer by chemotherapy. However, they still have many limitations that affect their efficacy, selectivity, toxicity and clinical application. Due to that fact, hundreds of antitumor platinum complexes have been developed. Research is still ongoing in order to overcome these limitations.

On the other hand, researchers are finding that antitumor platinum complexes with a multitarget or multi-action mechanism have greater efficacy and specificity than monotarget drugs. In addition, they are showing great potential to overcome the "cocktail" drug combination by reducing the side effects of such strategies. Even though, there are still some limitations such as to balance different target sites with accumulation of the specific drug. Due to this challenge, the research in this field is quite active nowadays.

Although classical drugs such as cisplatin, oxaliplatin or carboplatin remain the most widely used in clinics, cancer is a highly complex disease and as time goes by, new problems such as resistance to antitumor drugs are emerging. Considering the increasing demand of new anticancer drugs, multitarget platinum drugs have proven to be one of the most promising strategies in order to achieve efficacy and specificity against tumors.

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