PLATINUM COMPLEXES WITH EDDA (ETHYLENEDIAMINE-*N*, *N* - DIACETATE) LIGANDS AS POTENTIAL ANTICANCER AGENTS

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KOMPLEKSI PLATINE SA EDDA (ETILENDIAMIN-*N*, *N'*-DIACETAT) LIGANDIMA KAO POTENCIJALNI ANTITUMORSKI AGENSI

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ABSTRACT

The design of platinum based drugs is not a new field of interest. Platinum complexes are widely used as anticancer agents and currently, approximately 30 platinum(II) and platinum(IV) entered into some of the phases of clinical trials. A special place in today's research belongs to platinum complexes with diammine ligands. A large number of edda (ethylenediamine-N, N'-diacetate)-type ligands and their corresponding metal complexes has been successfully synthesized. This article summarizes recent progress in research on edda-type-platinum complexes. Some of these agents achieves better effect compared to the gold standard (cisplatin). It has been shown that there is a possible relationship between the length of the ligand ester group carbon chain and its cytotoxic effect. In most cases the longer the ester chain is the greater is the antitumor activity. Of particular interest are the noticeable effects of some new platinum compound with edda-type ligand on cell lines that are known to have a high level of cisplatin-resistance. Exanimate complexes appear to have a different mode of mechanism of action compared with cisplatin which includes apoptotic and necrotic cell death. There are indications that further investigations of these compounds may be very useful in overcoming the problems associated global cancer statistic.

Key words: *platinum complexes, edda ligand, cytotoxicity*

SAŽETAK

Kompleski platine koriste se kao osnova za dizajn novih lekova. Oni su u širokoj upotrebi kao antitumorski agensi i do danas je oko 30 komplesa platine(II) i platine(IV) u nekoj od faza kliničkog ispitivanja. Posebno mesto u današnjim istaživanjima zauzimaju kompleksi metala sa edda ligandima. Uspešno je sintetisan veliki broj novih edda liganda i odgovarajućih kompleksa. Neki od ovih agensa pokazuju bolju aktivnost od zlatnog standarda, cisplatine. Pokazano je da postoji moguća veza između dužine ugljovodiničnog lanca estraske grupe liganda i citotoksičnog efekta. U većini slučajeva dužina lanca direktno korelira sa antitumorskom aktivnošću. Zabeležena je efikasnija citotoksična aktivnost određenih kompleska platine sa edda ligandima na ćelijskim linijama tumora koji pokazju odgovarajući stepen rezistencije na cisplatinu. Ispitivani komleksi imaju različit mehanizam dejstva od cisplatine, koji uključuje elemente nekrotične i programirane ćelijske smrti. Postoje nagoveštaji da dalja istraživanja ovih agensa mogu biti značajna za prevazilaženje globalnog problema sa kojim se svet danas suočava, a koji se odnosi na stalni porast osoba obolelih od karcinoma.

Ključne reči: *kompleksi platine, edda ligandi, citotoksičnost*

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INTRODUCTION

The era of modern medical chemistry, which includes drugs based on metals, began with discovery of cisplatin (*cis*-diaminedichloroplatinum(II)) (1). It appears that metal complexes are a solid basis for the design of new drugs. A vast number of geometric isomers and different coordination numbers of metallic ions enable fine-tuning of both kinetic (ligands substitution rate) and thermodynamic (strength of metal-ligand bonds, electrode potential) parameters during synthesis of metal complexes – a potential drug (2-5). Ligands play a significant role in design and synthesis of novel complexes, both due to their ability to recognize sites where a complex should bind in a target cell, and the redox processes involved when a ligand that may be released in the cell (6-10).

Platinum complexes are widely used as anticancer agents and currently, approximately 30 platinum(II) and platinum(IV) complexes have entered into some phase of clinical trial (11). A special place in current research belongs to platinum complexes with diamine ligands. A large number of edda (ethylenediamine-*N, N'*-diacetate)-type ligands and their corresponding metal complexes (platinum, ruthenium, cobalt and palladium) have been successfully synthesized (12). Among them, platinum and ruthenium complexes stand out due to their anticancer effects, which have been confirmed on a large panel of different tumour cell lines.

DESIGN AND BIOLOGICAL EVOLUTION OF PLATINUM BASED DRUG

Cisplatin is the first platinum-based drug with anticancer effect approved by the FDA (Food and Drug Administration) and it is most efficient in treatment of many tumors including testicular, ovarian, kidney and neck cancer (2,13). After intravenous administration, cisplatin remains structurally unaltereddue to the high concentration of hloride in blood plasma (100 mM). It reaches tumor cells by either simple diffusion through cell membrane or active transport by copper transporter CTR1 (14-16). Due to much lower concentration of chloride ions in cytosol (3-20 mM) compared to extracellular fluid, there is rapid hydrolysis and substitution of chloride ligands by modified water molecules. After hydrolysis, the platinum cationic complex ($[Pt(NH_3)_2(H_2O)_2]^{2+}$) enters nucleus where it forms a coordinative bond with nitrogen atoms of nucleic bases of DNA, usually guanine (17,18). A bifunctional GG macrochelate is formed through coordination with guanine nitrogen atoms from adjacent DNA chains. $[Pt(NH_3)_2(H_2O)_2]^{2+}$ represents a chain between DNA strands. The modified DNA is permanently damaged and impossible to be used for transcription and replication, resulting cell cycle arrest and consequently apoptosis (18-21).

Despite extraordinary success of cisplatin, this drug has a number of drawbacks (22). For example cisplatin does not show sufficient selectivity towards tumor cells and cause nephrotoxicity, ototoxicity, or anemia (23,24). However, from the chemical standpoint, the platinum(II) complex is highly reactive. It may react with sulphur-containing amino acids (Cys and Met), such as metallothionein and albumin. In the cell, in the $[Pt(NH_3)_2(H_2O)_2]^{2+}$ form, it can react with different chemical classes, carbonate ions, phosphates, methionine, glutathione or metallothioneins. All this greatly reduces efficiency and utilization of this drug (3,19,24-27). It has therefore been necessary to synthesize more selective and less reactive molecule. This led to platinum complexes of the second and third generation (28-30). Complexes of the second generation are structural analogs of cisplatin, designed to overcome the toxicity of cisplatin, while the third generation complexes were created as even more advanced analogues with the main task to act on tumor cells resistant to cisplatin. Since the FDA approved cisplatin as drug, seven more platinum(II) complexes have been introduced to clinical use: 2 of them worldwide (carboplatin and oxaliplatin) and 5 of them in certain countries (nedaplatin, loboplatin, heptaplatin, miriplatin and cycloplatin) (31-33).

It has been found that each ligand has a role in the structure-activity relationship of synthesized complex compound. L-ligands, permanent ligands, form the strongest bond with platinum and remain intact in the final compound of the complex and DNA (34,35). The resistance of tumor cells to the drug mainly depends on these ligands. Oxaliplatin is an analogue of cisplatin, which has a more voluminous and hydrophobic diaminocyclohexyl ligand that "fits" in a major DNA groove thus preventing access to enzymes which "fix" DNA. The main advantage of oxaliplatin compared to cisplatin is that it acts on tumor cells resistant to cisplatin. Currently, it is the drug of choice for colorectal cancer (21,36). Pt-X bond (X is an outgoing ligand) is the weakest, and this is the place of possible hydrolysis in the cell. Therefore, this ligand directly affects the kinetics of reaction between the drug and DNA (34). Modification of these X-ligands can be achieved by reducing the number of side reactions in the cell. Both L and X ligand groups affect lipophilicity and solubility of the complex. Carboplatin (*cis*-diammine-1,1-cyclobutanedicarboxylateplatinum(II)) has in its structure bidentate cyclobutane dicarboxylate ligand which has impact on reduction of number of side reactions of this drug in the cell. These changes eliminated nephrotoxicity of carboplatin (23).

Due to the many side reactions of cisplatin and its analogues in cells, QSAR (quantitative structure activity-relationship) assessments of platinum(IV) complex are beginning. These complexes, $5d^6$ low spin electron configurations of Pt(IV) ion have octahedral geometry, which compared to platinum(II) complexes provides two new axial ligands, thereby increasing the kinetic stability and reducing the reactivity of these complexes compared to platinum(II) complexes. These ligands should be lipophilic to facilitate easier complex passage through the membrane

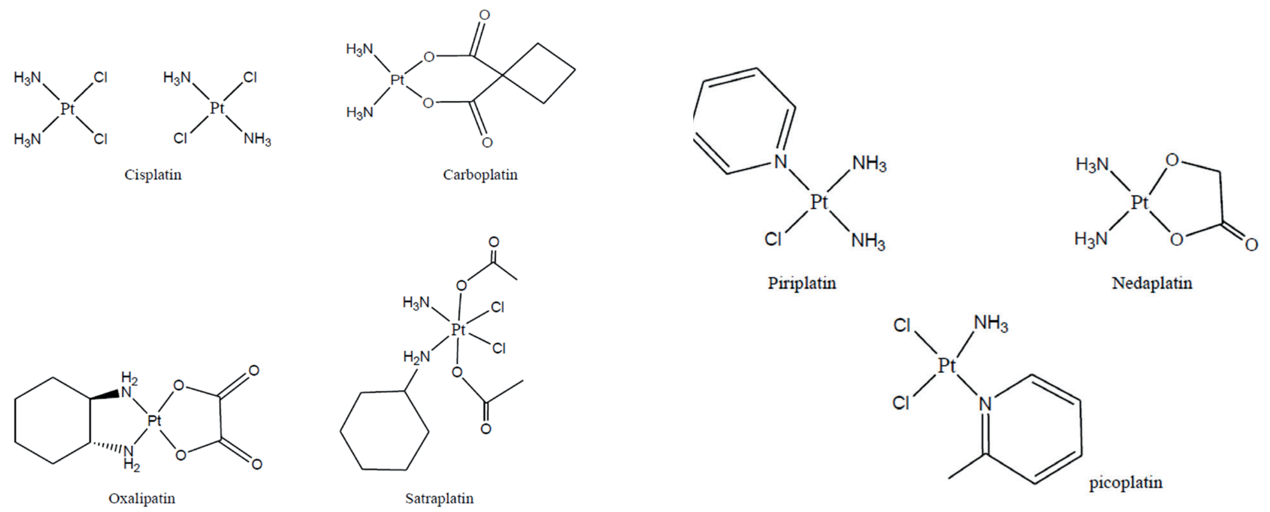

Figure 1. Structural formulae of platinum drug

and to make Pt-ligand bond stronger, so there would not be any hydrolysis and side reactions. Furthermore, these ligands are potential binding sites for so-called carriers in cells, nanoparticles that allow smooth passage of the drug to target site in a cell. It is assumed that this structure of platinum(IV) complex affects stability of the complex, which is the basis for their potential oral use (34, 37).

It was believed that octahedral platinum(IV) complexes are more inert in blood circulation and that they will be activated when they enter the cell. By cell entering Pt(IV) complexes will lose Pt(II) species which are responsible for cytotoxicity (38). It was believed that this fact will allow platinum(IV) complexes to be superior over the platinum(II) complexes regarding the degree of resistance, side effects and possible oral administration. The first attempt to synthesize a whole new drug platinum(IV) based line, in context of prodrugs, has been made by Rosenberg (6). Platinum(IV) complexes *cis*-[$Pt(NH_3)_2Cl_4$], *trans*-[$Pt(NH_3)_2Cl_4$] and [$Pt(en)Cl_4$] were soon abandoned because they showed less anticancer activity then cisplatin. By today, two most prosperous agents are satraplatin (JM216) and LA-12 (Figure 1) (39, 40). The greatest success was with complex of JM216 - satraplatin. Satraplatin is a lipophilic molecule, easily enters the cell, it is inert and stable, and because of all this has potential for oral administration. It is reduced within the cell by the cytochrome C, and then by hemoglobin as well in the presence of NADH. Satraplatin is characterized by a comfort drug use (can be used orally) for patient unlike other Pt(II) drugs that can only be used intravenously. The presence of intracellular agents as glutathione, ascorbic acid and others is required for reduction of satraplatin and therefore for its activation (41). Also satraplatin can be used for treatment of prostate, lung and ovarian tumors with little signs of nephro-, neuro- and ototoxicity. LA12 is a satraplatin analogue and future investigation will probably demonstrate that it may be used for ovarian carcinoma resistant on cisplatin, or even colorectal tumors (42,43).

A series of platinum(IV) complexes similar to JM216 has also been synthesized, with aliphatic, aromatic and alicyclic amines, with straight and branched chain, which showed higher activity compared to cisplatin. Despite major efforts and detailed studies and predictions, none of platinum(IV) complexes, including JM216, has not been approved for clinical use. Very good results of biological tests of oxaliplatin and satraplatin encouraged the idea of synthesis of platinum(II) and platinum(IV) complex with edda type ligands as their analogues, in order to obtain better anti-cancer agents (42,43).

R_2EDDA-TYPE LIGAND

Since the beginning of platinum derivates exploration, less attention has been given to aminocarboxylate ligand complexes. Liu (44) was the first who showed the coordination of ethylenediamine-*N,N'*-diacetate with platinum(II). Unfortunately, he obtained [$Pt(H_2edda)Cl_2$] complex in which both carboxylate groups were protonate, mainly due to synthesis conditions, which left platinum(II) coordination sphere the same as in [$Pt(en)Cl_2$]. Over the coming years, investigations of complexes with edda and related ligands have attracted the attention, chiefly because the good chelating ability of the ligands which may indicate a variety of complexes' stereochemical and physical properties. Ethylenediamine-*N-N'*-diacetic acid (edda) contains two nitrogens and also two oxygens as donor atoms. It acts as a tetradentate ligand in the case of complete coordination. R_2 edda ligand type belongs to the dialkil esters group of ethylenediamine-*N-N'*-diacetic acid (H_2edda), di(izo)propionic acid (H_2eddp, H_2eddip), di-2-(3-cyclohexyl)-propanoic acid (H_2eddch), di-2-(3methyl)-butanoic adic (H_2eddv), di-2-(4-methyl)-pentanoic acid (H_2eddl), as well as propilendiamine-*N-N'*-diacetic acid (H_2pdda) (Figure 2). Edda ligands can very easily be esterified, and during the complete coordination edda- ligands type esters mainly behave as

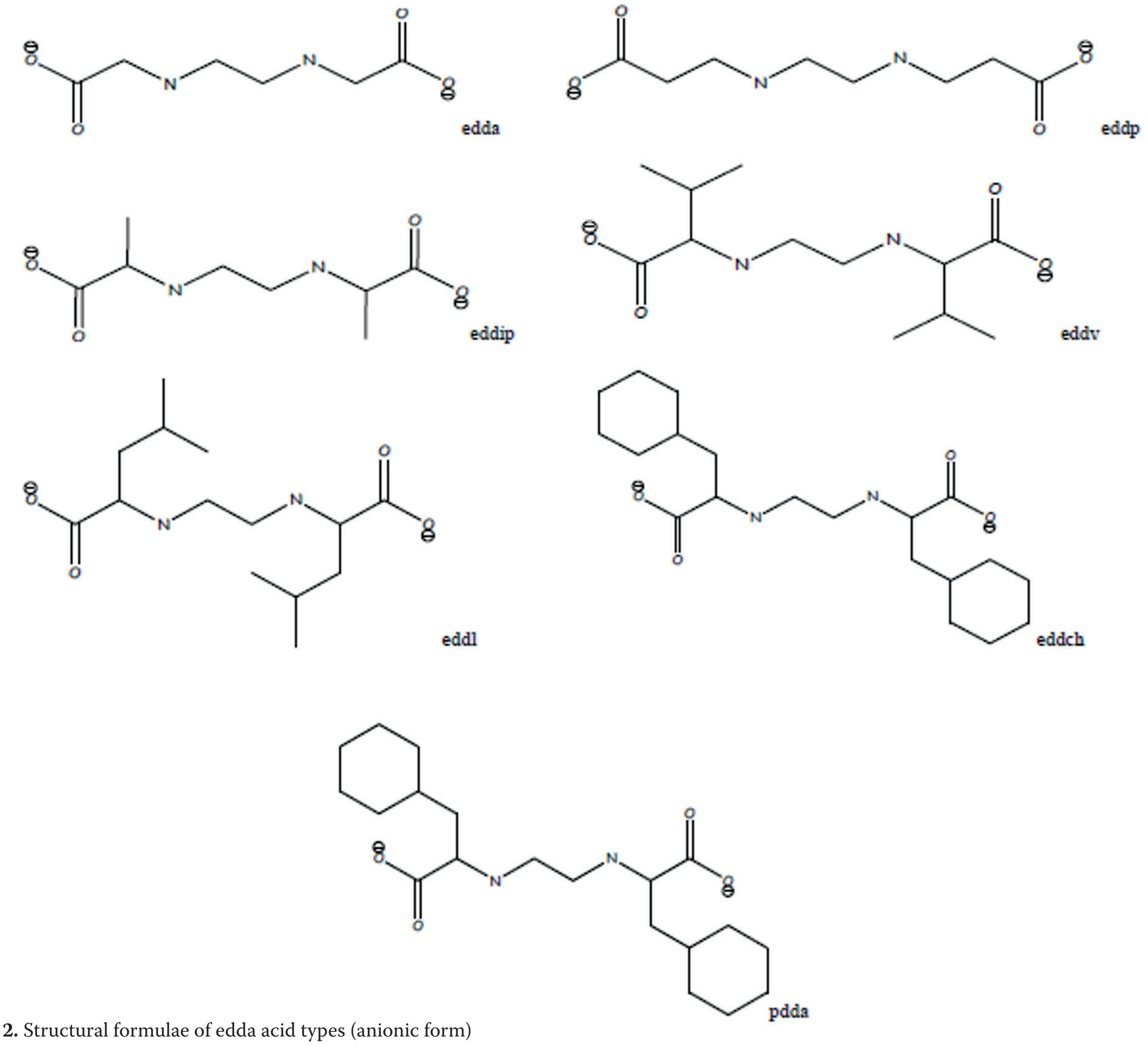

Figure 2. Structural formulae of edda acid types (anionic form)

bidentate ligands. In some cases, hydrolysis of one or both of ester groups occurs, thus the ligands may behave as bidentate or tridentate, respectively (45).

CYTOTOXICITY OF PLATINUM COMPLEXES WITH EDDA-TYPE LIGANDS

Platinum complexes with edda-type ligands

Platinum(II) and platinum(IV) complexes with ehtylenediamine ligands, *N*-(2-hydroxyethyl)ethylenediamine (heen), *N-N'-bis*(2-hydroxyethyl)ehtylenediamine (he2n), ehtylenediamine-*N, N'*-diacetic acid (H_2edda) and ehtylenediamine-*N*-monoacetic acid (hedma) were examined in order to reveal their cytotoxicity on different cell lines of human ovarian carcinoma (Table 1) (45). These complexes have temperate cytotoxic effects, through they were significantly lower than those of cisplatin and JM-216. It has been proven that platinum(II)/(IV) complexes with multidentate ligands *N*-(2-hydroxyethyl)ethane-1,2-diamine (NNOH) and ethylenediamine-*N,N'*-diacetic acid (H_2edda) have a different influence on CH1, 41M and Skov-3 cell line than on cisplatin resistant cell lines (Table 1) (46). While platinum(IV) complex with *NN'* donor set is 2-5 five times more potent against cisplatin sensitive/resistant cell lines, in comparison with platinum(II) complex, but with complexes with edda ligand situation is entirely different - platinum(II) complexes are far more active. Platinum(IV) complexes with ligands dialkyl esters of ethylenediamine-*N, N'*-diacetic acid (R_2edda) [$PtCl_4$(R_2edda)] x H_2O (R = Me, Et, *n*-Pr) were also investigated primarily to clarify influence at length of carbon chain in ester group on antiproliferative effect *in vitro* (Figure 3) (47). Examinations were performed on severe human tumor cell lines (Table 1). It was shown that by replacing methyl group in ester chain by ethyl or propyl group cytotoxic effect will be increased - the longer the ester chain is, the greater is the antitumor activity. The absence of this trend is observed on DLD-1 cell line. Complexes [$PtCl_4$(Et_2edda)] and [$PtCl_4$(Pr_2edda)] achieved highest cytotoxic activity on cisplatin-resistant

Table 1. Cytotoxic effect of Platinum complexes with edda ligand type

Ligand type	Pt complex	Carbon chain in ester group (R)	Cell line	Cytotoxic effect comparing to cisplatin		Study
edda	IV	Me, Et, *n*-Pr	testicular germ cell tumors (1411HP, H12.1), colon carcinoma (DLD-1), melanoma (518A2), liposarcoma and lung carcinoma (A549)	lower	stronger cytotoxic efficacy in cisplatin-resistant 1411HP cells (compared to other cell lines) [ca. 35–40 μM] (vs. cisplatin[IC_{50} 2.7 μM])	Kaludjerovic et al (2008) [47]
	II	Me, Et, *n*-Pr	melanoma (5182A), human thyroid carcinoma (8505C), head and neck tumor (A253), cervix (A431), lung (A549), ovarian (A2780), breast (MCF-7) and all colon (HT-29, HCT-8, DLD-1, SW1736)	lower	*n*-Pr complex showed the highest action against ovarian (A2780) cells [IC_{50} value of 51 μM] (vs. cisplatin [IC_{50} 0.55 μM])	Kaludjerovic et al (2014) [48]
eddp	IV	Br,J	human ovarian cancer (A2780/A2780cisR)	lower	A2780 cell line: Pt complexes[IC_{50} value ca. 30-90 μM] (vs. cisplatin [IC_{50} 0.2 μM]) A2780cisR cell line: Pt complexes[IC_{50} value ca. 90-270 μM] (vs. cisplatin [IC_{50} 3.5 μM])	Sabo et al (2004) [49]
		Et and *n*-Pr	ovarian (A2780), cervix (A431), melanoma (518A2), lung (A549), head and neck (FaDu), colon (HT-29, HCT-8, DLD-1, 8505C, SW480)	lower	$PtCl_4$(*n*-Pr_2eddp)] has highest effect on A2780, 518A2 and A549 cell lines[IC_{50} value 8.6 μM / 17.99 μM / 20.81 μM] (vs. cisplatin [IC_{50} 0.5 μM / 1.5 μM / 1.5 μM])	Kaludjerovic et al (2009) [52]
		n-Bu	melanoma (B16)	more potently	N/A	Maksimovic-Ivanic et al (2012) [53]
	II and IV	*n*-Bu, *n*-Pe/ Br,J	human cervix adenocarcinoma (HeLa), human myelogenous leukemia (K562)	lower	Pt(dveddp)Cl_4 effect on K562 cell line is closest to cisplatin [IC_{50} value 5.87 μM] (vs. cisplatin [IC_{50} 5.0 μM])	Kaludjerovic et al (2005) [50]
eddp and pdda	IV	*n*-Bu, *n*-Pe/ Br	mouse fibrosarcoma (L929), human astrocytoma (U251)	comparable	best results were gained with platinum(IV) complexes [$PtCl_4$(R_2eddp) (K562 cell line [IC_{50} value 5.87 μM] vs. cisplatin [IC_{50} 5.0 μM])	Kaludjerovic et al (2005) [51]
(S,S) eddp	II and IV	*i*-Pr, *i*-Bu;	human cervix adenocarcinoma(HeLa), human myelogenous leukemia (K562), malignant melanoma (Fem-x cell)	lower	Pt(IV) isopropyl (*S,S*)eddp complex is most active [IC_{50} value 30.48μM / 12.26 μM / 13.68 μM] (vs. cisplatin [IC_{50} 4.47 μM / 5.77 μM / 4.7 μM])	Krajcinocic et al (2008) [54]
	IV	Br,J	rat glioma cell line(C6), human glioma cell line(U251), mouse fibrosarcoma cell line (L929)	lower	IC_{50} value of cisplatin 9.8 μM / 23.6 μM / 19.3 μM vs. IC_{50} values of Pt(IV) complexes were over then 100 μM	Djinovic et al (2010) [55]
		n-Pr, *n*-Bu, *n*-Pe	the colon cancer adnocarcinoma cell line (HTC-116), breast cancer cell line (MDA-MB-231)	more potently (*n*-Pr and *n*-Pe)	*n*-Pr effect on HCT-116 cells - [IC_{50} value of 77.68 μM] (vs. cisplatin [IC_{50} 263.66 μM]) and on MDA-MB-231 cells (72h) [IC_{50} value of 64.21 μM] (vs. cisplatin [IC_{50} 114.11 μM]) *n*-Pe effect on HCT-116 cells - [IC_{50} value of 96.08 μM] (vs. cisplatin [IC_{50} 263.66 μM]) and on MDA-MB-231 cells (24h) [IC_{50} value of 238.60 μM] (vs. cisplatin [IC_{50} 425.32 μM])	Stojkovic et al (2014) [56]
eddp and *(S,S)* eddp	II and IV	*i*-Pr, *i*-Bu; cyclopentyl	mouse colon cancer (CT26CL25), colon cancer (HTC116 and SW620), prostate cancer (PC3 and LNCaP), glioblastoma (U251), human melanoma (A375), and murine melanoma (B16))	more potently (Platinum(IV) complexes)	IC_{50} value of platinum(IV) complexes is up to 3 times lower than that of the corresponding platinum(II) complexes Platinum(IV) complexes on CT26CL25, HCT116, SW620, and B16 cell lines) [IC_{50} ca. 35–100 μM] (vs. cisplatin[IC_{50} ca. 45-120 μM])	Kaludjerovic et al (2012) [57]
eddip	II	Et, *n*-Pr, *n*-Bu, *n*-Pe	human colon cancer cell lines (HCT116, SW480 and CaCo-2)	more potently	*n*-Bu Pt(II) complex has highest effect [IC_{50} value 11.23 μM / 5.09 μM / 4.02 μM] (vs. cisplatin [IC_{50} 161.25 μM / 51.64 μM / 64.74 μM])	Volarevic et al (2013) [58]
meddch	IV	H, Me, Et, *n*-Pr and *n*-Bu	Glioma (C6 and U251), fibrosarcoma (L929) and melanoma (B16)	more potently	IC_{50} value 1.9–8.7 μM compare to cisplatin (IC_{50} 10.9–67.0 μM)	Lazić et al (2010) [59]
		Me, Et, *n*-Pr and *n*-Bu	human melanoma (A375), human glioblastoma (U251), human prostate cancer (PC3), human colon cancer (HCT116), mouse melanoma (B16) and mouse colon cancer (CT26CL25) cells	more potently	IC_{50} value of Pt complexes ca. 2.9-21.3 μM (vs. cisplatin [IC_{50} ca. 12.5-120 μM])	Mihajlovic et al (2012) [63]
eddl	II	Et, *n*-Pr, *n*-Bu, *n*-Pe	chronic lymphocytic leukemia (CLL)	more potently	IC_{50} value (form Et to *n*-Pe) 22.35 μM / 9.85 μM / 5.39 μM / 10.37 μM (vs. cisplatin [IC_{50} 263.75 μM])	Vujic et al (2011) [60]
	IV	Et, *n*-Pr, *n*-Bu, *n*-Pe	human breast cancer (MDA-MB-361 and MDA-MB-453), T-leukemia (Jurkat), chronic myelogenous leukemia (K562), colorectal cancer (SW480) and CLL lymphocytes	more potently only for SW480 cells, for other cell types lower or comparable	SW480 cells: IC_{50} value of Pt complexes (form Et to *n*-Pe) 5.09 μM / 2.32 μM / 3.95 μM / 0.74 μM (vs. cisplatin [IC_{50} 31.92 μM])	Vujic et al (2012) [61]
eddch	IV	Me, Et, *n*-Pr, *n*-Bu	human glioblastoma (U251), mouse melanoma (B16)	more potently	U251 cells: IC_{50} value of Pt complexes ca. 1.9-17.5 μM (vs. cisplatin [IC_{50} ca. 20.0 μM]) B16 cells: IC_{50} value of Pt complexes ca. 3.1-21.3 μM (vs. cisplatin [IC_{50} ca. 94.3 μM])	Mihajlovic et al (2013) [64]
(S,S)-1,3-propanediamine-*N,N'*-di-2-(3-cyclohexyl) propanoic acid	II	*i*-Bu; *n*-Pe and *i*-Pe/J	human neoplastic cell lines (HeLa, A549, MDA-MB-231, LS-174, EA.hy 926), and human fetal lung fibroblast cell line (MRC-5)	more potently (*n*-Pe Pt(II) complex)	*n*-Pe Pt(II) complex IC_{50} value 5.4 μM / 11.1 μM / 11.9 μM / 12.2 μM/ 5.9 μM (vs. cisplatin [IC_{50} 6.9 μM/ 17.2 μM / 15.4 μM/ 21.9 μM/ 22.4 μM])	Savic et al (2014) [65]
(*S,S*)-eddba	IV	Et, *n* - Pr, *n*-Bu	chronic lymphocytic leukemia (CLL)	more potently	IC_{50} value of Pt complexes (form Et to *n*-Bu) 5.04 μM / 6.08 μM / 25.28 μM (vs. cisplatin [IC_{50} 331.61 μM])	Dimitrijevci et al (2013) [66]

N/A- not applicable

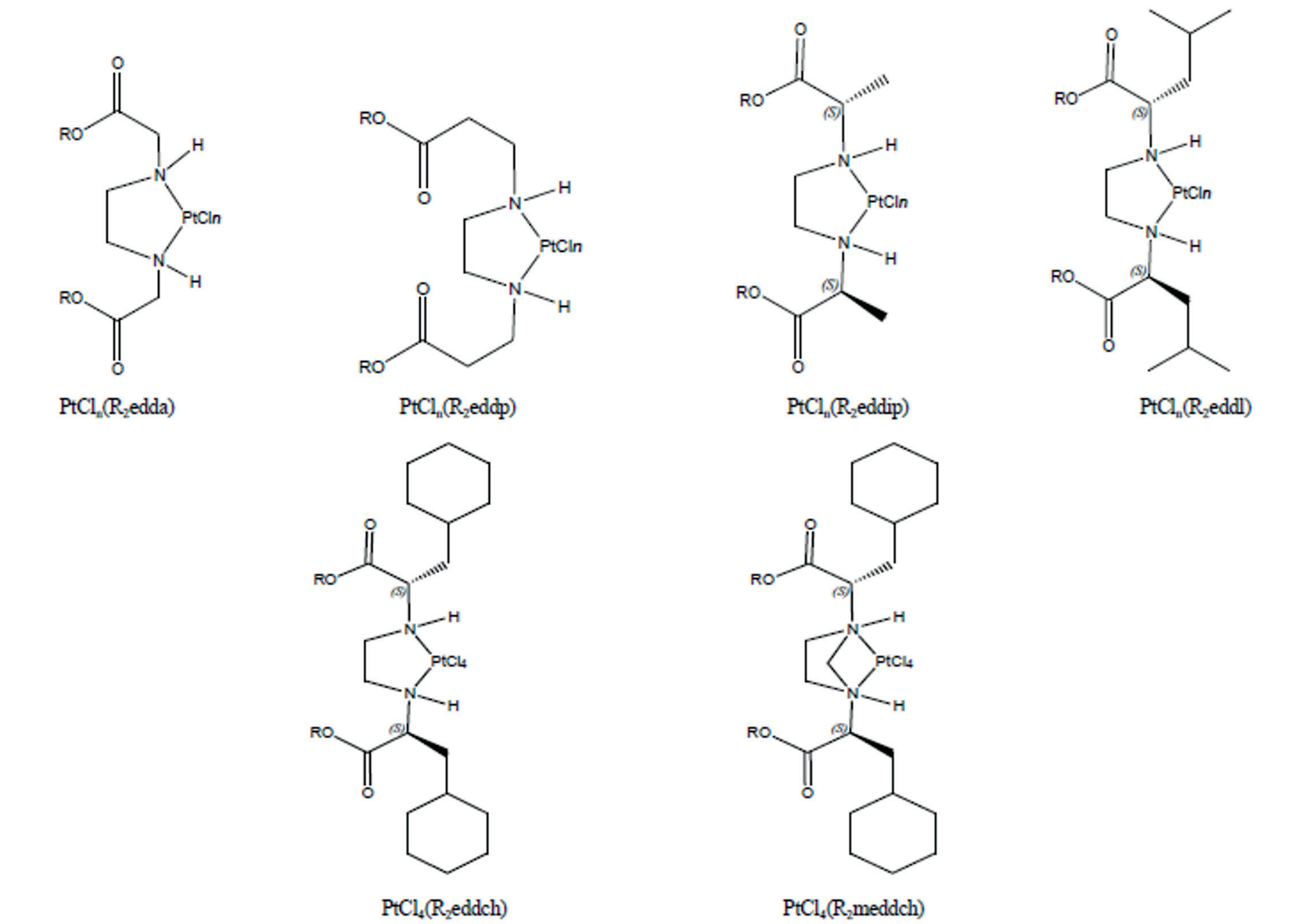

Figure 3. Structural formulae of platinum compounds with R2edda ligand type

1411HP cell line. All of them induce apoptosis and their effect is dose-dependent. Platinum(II) complexes with bidentate edda ligand type [PtCl$_2$(R$_2$edda)] (R = Me, Et, *n*-Pr; edda = ethylenediamine-*N, N'*-diacetate) were also questioned (48). *In vitro* cytotoxic effect was studied on various cell lines to generate new evidence regarding the cytotoxic effect of these complexes, (Table 1). The aforementioned trend of the impact of alkyl chain (in ester group) length on antitumor activity can also be applied in this case, except of HTC-8 and HT-29 cells. Thus, the highest cytotoxic effect has been achieved by [PtCl$_2$(*n*-Pr$_2$edda)] against A2780 cells. However, the activities of all these new platinum (II) compounds is lower compared to the appropriate platinum(IV) complexes, as well as cisplatin.

Platinum complexes with eddp and eddip-type ligands

Grow inhibition which had been provided by influence of two new platinum(IV) complexes ([PtX$_2$(eddp)] x *n* H$_2$0; X= Cl/Br; n = 1 or 1,24; eddp=ethylenediamine-*N, N'*-di-3-propionate) on A2780/A2780cisR pair of human ovarian cancer cell lines, showed that trans-[PtX$_2$(eddp)] (x= Cl$^-$, Br$^-$) complexes have far less cytotoxic affinity compared to cisplatin and complexes with edda ligands (*cis*-[PtCl$_2$(edda)]) (49). There is a high probability that difference in effects stems from otherwise complex geometry. These two complexes will obtain dissimilar adducts with cell molecules (DNA nucleic bases) by direct interaction. However, if reduction process (Pt(IV) to Pt(II)) occurs before interaction with nucleic bases, *trans*-[PtX$_2$(eddp)] complexes will form tetracoordinated platinum(II) complexes where eddp ligand occupies all four coordination positions preventing in that way reaction with DNA nucleic bases (49). In the contrary, reduction of *cis*-[PtCl$_2$(edda)] complex, by formatting tetracoordinated platinum(II) complexes which contain biscoordinated edda ligand and leaving two chloro ligands which could easily be replaced and make a link with DNA (49). Also, some studies of platinum(II)/(IV) complexes ([PtCl$_4$(Bu$_2$eddp)], [PtBr$_3$Cl(Bu$_2$eddp)], [PtCl$_2$I$_2$(Bu$_2$eddp)], [PtCl$_4$(Pe$_2$eddp)], [PtCl$_2$(Bu$_2$eddp)]) have demonstrated that these complexes had five times weaker cytotoxic effect on HeLa (human cervix adenocarcinoma) cell line compared to cisplatin, but the effect on K562 (human myelogenous leukemia) cell line was almost equal to the effect of cisplatin (Table 1) (50). It was concluded that the exchange of two chloro ions for two iodo ions in present complexes will only decrease the antitumor activity of the complexes. It has been shown that these complexes induce apoptosis, but in some cells secondary necrosis was detected (50). Several complexes of platinum(II)/(IV) were investigated in the light of *in vitro* antitumor activity against some mouse and human cell lines (Table 1) (51). Of all of the complexes whose efficiency was studied (*trans*-$[PtCl_2(pdda)]$, *trans*- $[PtBr_2(pdda)]$, *trans*- $[PtCl_2(eddp)]$, *trans*- $[PtBr_2(eddp)]$, $[PtCl_2(H_2eddp)]$, $[PtCl_4(Pe_2eddp)]$, $[PtCl_2(Bu_2eddp)]$, $[PtCl_4(Bu_2eddp)]$), best results were gained with platinum(IV) complexes $[PtCl_4(R_2eddp)]$ (R= Bu or Pe). These two complexes showed the cytotoxic activity was dose-depended and comparable with cisplatin, but also that they archive cytotoxic effect more rapidly than cisplatin. Further examination of toxicity of $[PtCl_4(Pe_2eddp)]$ and $[PtCl_4(Bu_2eddp)]$ pointed out that these complexes cause ROI (reactive oxygen intermediates)-dependent, ERK (extracellular signal-regulated kinase)-independent induction of tumor cell necrosis as opposed to cisplatin - it induced ROI-independent apoptotic death of tumor cells (48). Cytotoxic effect of two more platinum(IV) complexes, $[PtCl_4(Et_2eddp)]$ and $[PtCl_4(n\text{-}Pr_2eddp)]$, has been investigated on severe cell lines but each one of them showed less activity *in vitro* in regard to cisplatin (Table 1) (52). Kaludjerovic et al. (52) also established that there is an interaction between plasmid pBR322 DNA and platinum(II)/(IV) complexes, in the presence or absence of ascorbic acid. From all of the platinum (eddp) complexes, $[PtCl_4(n\text{-}Bu_2eddp)]$ is the only one which has *in vivo* anti-tumor activity demonstrated (53). Cytotoxic effect of $[PtCl_4(n\text{-}Bu_2eddp)]$ is dose and time dependent, and this complex shows its effect faster then cisplatin against B16 melanoma cells. Investigation on mice examined platinum(IV) complex demonstrated greater efficiency then cisplatin in terms of reducing volume of a tumor in its appropriate doses. The greater advantage over the cisplatin is reflected in the absence of kidney damage; $[PtCl_4(n\text{-}Bu_2eddp)]$ did not show any sign of nephrotoxicity (53). A new modification of platinum(II/IV) complexes with R_2(*S,S*)eddp ligand type (*O,O'*-di-isopropyl or *O,O'*-di-isobutyl- (*S,S*)-ethylenediamide-*N, N'*-di-2-propionate) were synthetized (Table 1) (54). The results showed that the Pt(IV)complexes were followed with better cytotoxic activity. Also, it has been noted that if in platinum(II) complexes with this ligand type occur the interchange isopropyl group for isobutyl group, cytotoxic activity will be increased. On the other hand, if this exchange occurs in platinum(IV) complexes, the cytotoxic activity will be decreased, regardless of the type of cell line. However, the best activity has been demonstrated by platinum(IV) complex with *O,O'*-di-isopropyl-(*S,S*)-ethylenediamide-*N, N'*-di-2-propionate ligand against K526 and Fem-x cell lines, unfortunately each one of these complexes had lower cytotoxic activity (2-5 times) in comparison to the corresponding cisplatin. Complexes $[PtCl_2\{(S,S)\text{-}iBu_2eddip\}]$, $[PtCl_4\{(S,S)\text{-}iPr_2eddip\}]$ and $[PtCl_4\{(S,S)\text{-}iBu_2eddip\}]$ induce apoptosis. Complex $[PtCl_2\{(S,S)\text{-}iPr_2eddip\}]$ led to chromatin condensation in HeLa cells, but contrary to previous mentioned complexes which cause rounding of cells, this complex caused more irregular cell shapes which may indicate that disruption of cytoskeleton and/or plasma membrane may be occurred. Two new platinum(IV) complexes, and there *in vitro* activities, were demonstrated - $[PtX_2(S,S\text{-}eddp)] \times nH_2O$ (*S,S*-eddp = ethylenediamine-*N, N'*-di-*S,S*-2-propanoate ion, X = chlorido or bromido, n = 4, 0) (55). The complexes displayed significantly lower cytotoxicity on severe cell lines and have a quite different mechanism of action compared to cisplatin (Table 1). Also exchange of tetradentate eddp ligand with bidentate eddp ester ligand will lead to enhancement in cytotoxicity of platinum(IV) complexes. Stojkovic et al. (56) synthesized three new platinum(IV) complexes with bidentate *N, N'*-ligands, $[PtCl_4(R_2\text{-}S,S\text{-}eddp)]$ (R=*n*-Pr, *n*-Bu, *n*-Pe). It has been shown that all three new complexes have a dose and time-dependent grow-inhibition effect. $[PtCl_4(n\text{-}Pr_2\text{-}S,S\text{-}eddp)]$ and $[PtCl_4(n\text{-}Pe_2\text{-}S,S\text{-}eddp)]$ had much higher antiproliferative activity in comparison with cisplatin. Also, MDA-MD-231 cell line showed to be less sensitive to the treatment with all these complexes, including cisplatin. The greatest cytotoxic effect on HTC-116 cell line was demonstrated by $[PtCl_4(n\text{-}Pe_2\text{-}S,S\text{-}eddp)]$ and on the other side greatest effect on a MDA-MB-231 was made by $[PtCl_4(n\text{-}Pr_2\text{-}S,S\text{-}eddp)]$. All this compounds induced a type of programmed cell death, but the third complex ($[PtCl_4(n\text{-}Pe_2\text{-}S,S\text{-}eddp)]$) had highest proapoptotic effect. Cytotoxic effect of two platinum(IV) complexes ($[PtCl_4(R_2eddp)]$) (R= *i*Pr (isopropyl) or *i*Bu (isobutyl)) and three platinum(II)/(IV) complexes ($[PtCl_{2/4}(R_2eddip)]$) (R= *i*Pr (isopropyl), *i*Bu (isobutyl) or *c*-Pe (cyclopentyl)) was examined on various cancer cell lines (Table 1) (57). Increasing the number of hydrophobic alkyl side chains appears to result in enhancement of cytotoxicity, in fact complexes with isopropyl group had less activity then those with isobutyl or cyclopentyl group. Platinum(II) (eddip) complexes with isobutyl group have proven to be more effective on HCT116 and SW620 cells then cisplatin, as was the effect of these types of complexes with cyclopentyl group on CT-26CL25, HCT116, SW6220 and B16 cells (Table 1). No signs of toxicity on normal primary cells (fibroblasts and keratinocytes) of complexes was found. All of these new platinum compounds induce caspase-dependent apoptosis. Moreover, ROS (reactive oxygen species) and RNS (reactive nitrogen species) are not being singled out as the main mediators of toxicity. On the same human colon cancer cell lines Volarevic et al. (58) examined the antiproliferative effect of four new platinum(II) complexes with *O,O'*-dialkyl esters of (*S,S*)-ethylenediamine- *N, N'*-di-2-(4-methyl) pentanoic acid (alkyl, ethyl, propyl, *n*-butyl, *n*-pentyl). In comparison to cisplatin all these new complexes have shown a higher cytotoxic activity. Conclusion of this study indicated that the shorter the ester chain in complex is, the complex will show less cytotoxic activity. Thus, the greatest impact was expected from platinum(II) complex with *O,O'*-dipentyl esters. Still, highest impact on human colon cancer cells (especially on HTC116) has been made by platinum(II) complex with *O,O'*-dibutyl esters. It is thought that the reason for this is the superior intercellular accumulation (59).

Platinum complexes with meddch-, eddl-, eddch and eddba- type ligands

Recently, platinum(II) and platinum(IV) complexes with (*S,S*)-R_2eddl ligand type have been synthetized (60,61). Highest activity had come from complexes with n- butyl group in ester chain [$PtCl_n$((*S,S*)-R_2eddl)] (R= Et, Pr, *n*-Bu or *n*-Pe; n=2 or 4; *O,O'*-diethyl-(*S,S*)-ethylenediamine-*N, N'*-di-2-(4-methyl)-pentanoate)platinum), although cytotoxic effect increases with the increase of ester chain length, as previously mentioned. This type of platinum(II) complexes were found to display much higher antitumor activities on CLL cells in comparison to cisplatin (60), especially [$PtCl_2$((*S,S*)-*n*-Bu_2eddl)], which is the bearer of the highest antitumor activity of them all. Platinum(IV) compounds with (*S,S*)-R_2eddl ligand type were appraised for their cytotoxic effect (Table 1) (61). Very potent complex was also the one with *n*-butyl group in ester chain [$PtCl_4$((*S,S*)-*n*-Bu_2eddl)]. It is interesting that CLL cells is the only cell line more sensitive on platinum(II) complex. Lazic et al. (59), synthesized a new platinum(IV) compound with tetradentate coordinated (*S,S*)-ethylenediamine-*N,N'*-di-2-(3-cyclohexyl) propanoate (cyclohexyl edda/eddch). The cytotoxic effect of these complexes [$PtCl_4$((*S,S*)-R_2eddch)] (R= Me, Et, *n*-Pr, *n*-Bu) were tested against various cell lines (Table 1). All compounds were clearly more cytotoxic than cisplatin, especially against cisplatin-resistant B16 cells. They also suggested that the length of alkyl chain has different effect than is the case with other platinum complexes. The longer the alkyl chain is, the poorer is the antitumor activity. They also cleared the difference which existed between mechanisms of action of these complexes and golden standard. Cisplatin brings about caspase-dependent apoptosis realized by an autophagy response. On the other hand, new octahedral platinum(IV) complexes induce necrosis like cell death. Soon after, there was a report of octahedral Pt(IV) complex with di-*n*-propyl-(*S,S*)-ethylenediamine-*N, N'*-di-2-(3-cyclohexyl)propanoato ligand and its effect on immune cells (SPC and LNC) (62). It has been shown that this platinum(IV) complex, in concentrations which have been proven effective on tumor cells, does not notably affect viability of immune cells. Also, this complex disenables synthesis of IFN-γ, IL-17 and NO in immune cells. The new prospective platinum(IV) drugs were synthesized with the novel *N,N'*-methylene modified cyclohexyl ethylenediamine-*N,N'*-diacetate (edda)-type ligands, [$PtCl_4$((*S,S*)-R_2meddch)] (R= Me, Et, *n*-Pr, *n*-Bu) (63). All of these compounds, with the exemption of [$PtCl_4$((*S,S*)-Me-2meddch)], demonstrated higher citotoxic activity then cisplatin on every cell line, especially on HCT116 and CT26CL25 cell lines resistant or poorly responsive to treatment with cisplatin (Table 1). These platinum(IV) complexes with eddch ligand type induce apoptosis, but in lower dose range, and it has been shown that they affect primary keratinocytes and fibroblasts less then cisplatin, which may be indicative of their selectivity. Furthermore, series of complex electrochemical tests were performed by cyclic voltammetry and differential pulse voltammetry (64). This study indicated that the reduction of these complexes is performed as two-electron process followed by the loss of axial chloride ligand and the length of the C atom chain in esters part affects the reduction potential. Correlation between redox potentials and IC_{50} (half maximal inhibitory concentration) values was not established. Pt(II)-iododo complexes with derivatives of ethylenediamine-*N, N'*-diacetate (edda)-type of ligands, (esters of (*S,S*)-1,3-propanediamine-*N, N'*-di-2-(3-cyclohexyl)propanoic acid) are also a new potential anticancer substance (65). Cytotoxic effect of isobutyl, *n*-pentyl and isopentyl esters of these compounds were examined against various human cell lines (Table 1). Although summary effect of all these compounds was better in regard to cisplatin, in LS-174 cells effect was 3 to 4 times higher than golden standard. However, exanimate complexes seem to have a different mode of mechanism which includes apoptotic and necrotic elements of cell death. These complexes also evince better affinity for DNA binding then cisplatin and enter cells efficiently, which may be an important advantage in respect of avoiding cell resistance. Better intracellular accumulation and DNA binding are probably the result of substitution kinetics of iodide ligands and proper lipophilicity of an edda ligand type. The cytotoxic effect against freshly isolated CLL cells is achieved by [$PtCl_4$(R_2-*S,S*-eddba)] (R= Et, Pr or Bu) (eddba-ethylenediamine-*N, N'*-di-*S,S*-(2,2'-dibenzyl)acetic acid) complex, as reported by Dimitrijevic et al. (66). The cytotoxic influence of [$PtCl_4$(Et_2-*S,S*-eddba)] and [$PtCl_4$(Pr_2-*S,S*-eddba)] was better than complex with *n*-butyl group in ester chain, but still, all of them have considerably higher antiproliferative ability against CLL cells then cisplatin (Table 1).

CONCLUSION

The design of platinum based drugs is not a new field of interest. This article summarizes recent research progress in research on edda-type-platinum complexes - new type of platinum based drugs. Some of these compounds achieves better effect compared with the gold standard (cisplatin). Of particular interest are the noticeable effect of some new platinum compound with edda ligand type on cell lines which are known to have a high level of cisplatin-resistance. There are indications that further investigations of these compounds may be very useful in overcoming the problems with global cancer statistic. Further preclinical and clinical researches might give some useful information which can help in overcoming the main problems related to platinum based drugs (including tumor resistance and less serious side effect).

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Conflicts of interest

The authors declare no financial or commercial conflicts of interest.

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