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Halogenated Pt(IV) complexes from *N*-halosuccinimide oxidation of Pt(II) antitumor drugs: Synthesis, mechanistic investigation, and cytotoxicity

Zoufeng Xu,[a,b] Cai Li,[a,b] Zixuan Tong,[a] Lili Ma,[a,b] Man-Kit Tse,[a] and Guangyu Zhu*[a,b]

Abstract: Compared with square-planar Pt(II) drugs, Pt(IV)-based anticancer prodrugs are relatively inert under physiological conditions and can be activated after entering cells. The two axial ligands in Pt(IV) complexes provide an opportunity to further functionalize these prodrugs. In recent years, much effort has been devoted to developing new Pt(IV)-based anticancer prodrugs with higher cytotoxicity but fewer side effects. New synthetic methods, however, are intensely desired for structurally diversified and biologically distinct Pt(IV) complexes. Here we utilize *N*-halosuccinimides as oxidants and carry out the oxidation reaction on Pt(II) drugs including cisplatin, carboplatin, and oxaliplatin to obtain halogenated Pt(IV) complexes. The related mechanism on the reaction of *N*-bromosuccinimide (NBS) with cisplatin in ethanol is thoroughly investigated. *N*-chlorosuccinimide is also utilized to obtain respective dichlorinated Pt(IV) complexes. When *N*-iodosuccinimide reacts with Pt(II) drugs, novel Pt(IV) compounds containing iodide and succinimide ligands in the axial position are formed. Cytotoxicity test shows some of the complexes are active against cisplatin-resistant human ovarian cancer cells. Our study provides a detailed understanding of the reaction mechanism between NBS and cisplatin and offers a more convenient strategy to obtain dichlorinated and novel iodinated Pt(IV) anticancer prodrugs.

Introduction

The discovery of the antitumor activity of cisplatin in 1969 opened a new era of cancer treatment.[1] Later on, platinum drugs including cisplatin, carboplatin, and oxaliplatin were approved by FDA and gradually became one of the most important classes of antineoplastic agents.[2] Even though, these Pt(II)-based drugs suffer from severe side effects such as neurotoxicity and neutropenia. Much effort has been made in order to overcome these disadvantages.[3] In the development of the next generation of platinum-based anticancer drugs, Pt(IV) prodrugs are quite promising and some of them are now undergoing clinical trials.[4]

A widely applied method to synthesize Pt(IV) complexes is to use Pt(II) compounds as precursors and react them with appropriate oxidizing agents such as peroxides or halogens.[5-7] For example, when hydrogen peroxide is used as an oxidizing agent and the reaction is carried out in water, cisplatin is oxidized to oxoplatin {*c,c,t*-[$Pt(NH_3)_2Cl_2(OH)_2$]}, a representative starting material to obtain potential Pt(IV) anticancer agents.[8-11] The use of coordinating solvents instead of water leads to the formation of asymmetric Pt(IV) complexes. For instance, oxidizing cisplatin in ethanol or acetic acid with H_2O_2 yields *c,c,t*-[$Pt(NH_3)_2Cl_2(OH)(OCH_2CH_3)$] or *c,c,t*-[$Pt(NH_3)_2Cl_2(OH)(OOCCH_3)$], respectively.[12,13] More recently, a novel asymmetric Pt(IV)-acetamidato complex was synthesized from cisplatin using a mixture of H_2O_2, acetonitrile, and methanol.[14,15] Even though, with the aim of developing the next generation of Pt(IV) anticancer prodrugs, new synthetic methods are still demanded to promote the discovery and investigation of these complexes.[8,9]

In 2010, a French patent from Sanofi Aventis claimed a new method to synthesize asymmetric Pt(IV) complexes by using *N*-halosuccinimides including *N*-chlorosuccinimide (NCS), *N*-bromosuccinimide (NBS), and *N*-iodosuccinimide (NIS).[16] In their pioneering work, *N*-halosuccinimides were used to oxidize Pt(II) compounds in ethylene glycol and finally a new series of asymmetric Pt(IV) compounds containing two different axial ligands were obtained. The inventors also tested the biological activity of these compounds and some complexes were cytotoxic in cancer cells. The further investigation of these complexes, however, was not reported. In 2014, Osella and co-workers used different types of Pt(II) compounds and the corresponding asymmetric Pt(IV) complexes containing axial chlorine and ethane-1,2-diol ligands were successfully synthesized by using NCS. Furthermore, the reaction was performed in different solvents such as water or acetic acid to obtain chlorinated and asymmetric Pt(IV) complexes bearing axial hydroxides or acetates.[17]

Very recently, in order to synthesize a new series of asymmetric Pt(IV) anticancer prodrugs, we used NBS as an oxidizing agent and carried out the reaction of NBS with Pt(II) complexes in different solvents including water, acetic acid, and ethylene glycol.[18] As a result, we successfully obtained asymmetric Pt(IV) compounds with bromine in the axial position and OH, $OOCCH_3$, or OCH_2CH_2OH on the opposite side of bromine. Intriguingly, for the reaction of NBS with oxaliplatin in ethanol, when 1.2 equivalents of NBS were used, we were not able to get the expected *trans*-[Pt(DACH)(ox)(OCH_2CH_3)Br] [DACH = *trans*-(1R, 2R)-1,2-cyclohexanediammine, ox = oxalate]. Instead, the products were 50% of *trans*-[Pt(DACH)(ox)Br_2] and 50% of unreacted oxaliplatin. When 2.4 equivalents of NBS were used, all the oxaliplatin turned into *trans*-[Pt(DACH)(ox)Br_2]. For cisplatin and carboplatin, the same oxidation reaction occurred using 2.4 equivalents of NBS, and the corresponding *cis,cis,trans*-[$Pt(NH_3)_2Cl_2Br_2$] and *cis,trans*-[Pt(CBDCA)$(NH_3)_2Br_2$] (CBDCA=1,1-cyclobutanedicarboxylate) were finally formed (Figure 1). This discovery offers us a more convenient way to synthesize mono- and di-brominated Pt(IV)

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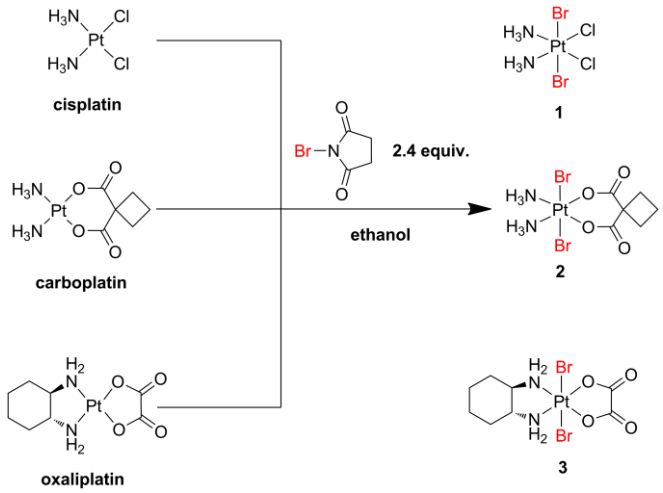
complexes by avoiding the use of more dangerous liquid Br_2. Although we discussed the possible mechanism of these reactions, the detailed mechanism especially the formation of dibrominated Pt(IV) complexes from NBS is still unclear.

Figure 1. Synthesis of dibrominated Pt(IV) complexes with 2.4 equivalents of NBS in ethanol.

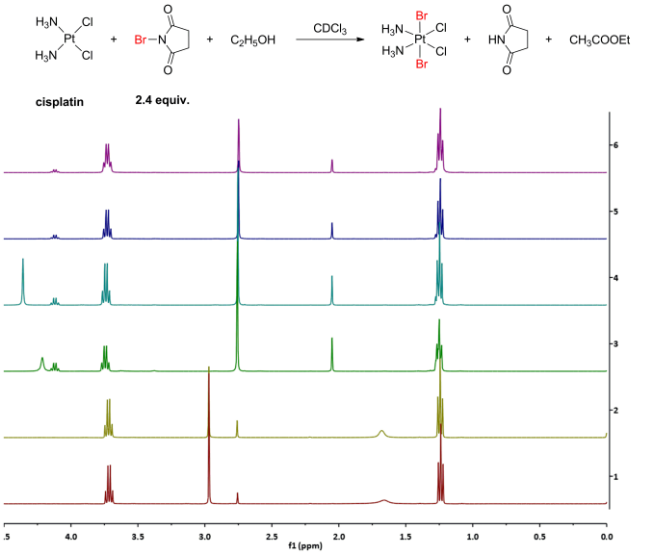


Here we investigated the possible mechanism of the reaction between Pt(II) complexes and an excess amount of NBS in ethanol. Experiments in different conditions were carried out to corroborate our hypothesis and a reasonable mechanism was proposed. The reaction scope was further expanded by utilizing NCS and NIS, the analogs of NBS, to synthesize dichlorinated and diiodinated symmetric Pt(IV) anticancer prodrugs. Furthermore, the cytotoxicity of these complexes against human ovarian cancer cells was revealed.

Results and Discussion

NBS is widely used as a source of "positive bromine" and 1 equivalent of NBS can get two electrons from Pt center which is enough to oxidize Pt(II) to Pt(IV). In this brominating reaction, another 1 equivalent of "negative bromine" is needed to balance the charge in order to form dibrominated Pt(IV) compounds. Thus, the formation of "negative bromine" is the key step. Since ethanol could be used as a reducing agent to reduce NBS and subsequently produce "negative bromine" in a form of HBr,[19] we assume that the reaction of NBS with ethanol or Pt(II) complexes could occur at the same time and finally the reaction results in dibrominated Pt(IV) compounds.

To validate our hypothesis, a certain amount of cisplatin (50 mg) was reacted with 2.4 equivalents of NBS in different solvents for 8 h at room temperature. Firstly, cisplatin and NBS were added into 1 mL of chloroform ($CHCl_3$). Chloroform is a weak coordinating solvent comparing with ethanol and it can't be oxidized by NBS.[20] As expected, dibrominated Pt(IV) compound was not obtained in this inert solvent. When the reaction was carried out in a mixture of 50:50 $CHCl_3$/EtOH (v/v), NBS did oxidize cisplatin and the dibrominated Pt(IV) product was obtained in a high yield. Based on these observations, we assume that only a small amount of ethanol is required for the reaction. Most of the ethanol just serves as a solvent that does not participate in the reaction and can be replaced by other inert solvents such as DCM or chloroform. Therefore, we gradually decreased the amount of ethanol in this reaction, aiming to determine the minimum amount of ethanol needed. Deuterated chloroform ($CDCl_3$) was used as a solvent in the following

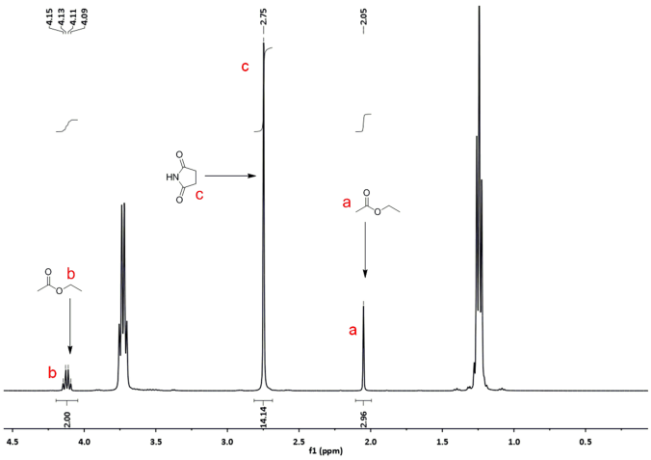


reactions which were monitor by ^{1}H NMR. The results are shown in Figure 2.

Figure 2. ^{1}H NMR spectra of the reaction mixture from the synthesis of *c,c,t*-$[Pt(NH_3)_2Cl_2Br_2]$ in $CDCl_3$ with different amounts of ethanol.

The peak at δ=2.95 ppm in the NMR spectra is the signal of protons in NBS and the one at δ=2.75 ppm belongs to succinimide, the byproduct from NBS. We also found the signal of ethyl acetate (EA) at 4.12 ppm (q) and 2.05 ppm (s), and the peak at 1.26 ppm (t) from EA overlaps with that from ethanol. The appearance of ethyl acetate is likely due to the oxidation of ethanol.[19] When 3.6 equivalents of ethanol or more were used, this reaction occurred and there was no significant difference in the yield. In contrast, when 2.4 or 1.2 equivalents of ethanol were used, only a small amount of the byproduct succinimide was produced and the oxidizing product of ethanol (EA) couldn't even be observed from the ^{1}H NMR spectra. It seems that these reactions with minimum amount of ethanol proceed at a very slow rate and then NBS still cannot totally react even when the reaction time is extended to 16 h.

Theoretically, among 2.4 equivalents of NBS, 1 equivalent will oxidize Pt(II) complex, and the excess 1.4 equivalents will react with ethanol to produce 0.7 equivalent of ethyl acetate. At the same time, 2.4 equivalents of succinimide will be produced



as the byproduct. The ratio of succinimide to ethyl acetate should be 2.4:0.7, or 3.43:1. Since succinimide and ethyl acetate dissolve well in $CDCl_3$, we calculated the average ratio of succinimide to ethyl acetate in the reactions mentioned above based on the ^1H NMR spectra. The average ratio is 3.53:1, which is identical to the theoretical value (Figure 3). These experiments clearly prove that the oxidation of ethanol by NBS occurs and a certain amount of ethanol is essential to trigger this reaction. It is worth noting that although theoretically 1.4 equivalents of ethanol is enough to reduce 1.4 equivalents of NBS, in the real situation, only 1.4 equivalents of ethanol will not trigger this reaction, suggesting that the concentration of ethanol is also an important factor which may directly affect the formation of *cis,cis,trans*-$[Pt(NH_3)_2Cl_2Br_2]$.

Figure 3. The ratio of succinimide to ethyl acetate after the reaction.

In the reaction of NBS with cisplatin, an orange suspension was formed after 8 hours. The suspension was centrifuged and *cis,cis,trans*-$[Pt(NH_3)_2Cl_2Br_2]$ was collected as the precipitate. The pH value of the supernatant was around 1, proving the formation of acid in this reaction. When the supernatant was added into a solution of silver nitrate, a light yellow precipitate appeared, the color of which is the same as that of silver bromide (AgBr), and the precipitate could be dissolved in ammonia hydroxide. These phenomena strongly supported our assumption that HBr was generated in this reaction and subsequently utilized as a source of "negative bromine". Conversely, when water, which can't reduce NBS, was chosen as a solvent, even with 2.4 equivalents of NBS, the reaction mixture was still neutral.

Based on these results, the possible mechanism of the synthesis of dibrominated Pt(IV) compounds by more than 2 equivalents of NBS is as follows. In the presence of enough ethanol, 1 equivalent of NBS will oxidize ethanol to ethyl acetate and result in HBr (negative bromine). Another 1 equivalent of NBS will directly react with Pt(II) compound. Since ethanol is a weak coordinating solvent compared to Br^-, the Br^- is more easily added to the Pt core, finally leading to dibrominated Pt(IV) compounds (Figure 4).

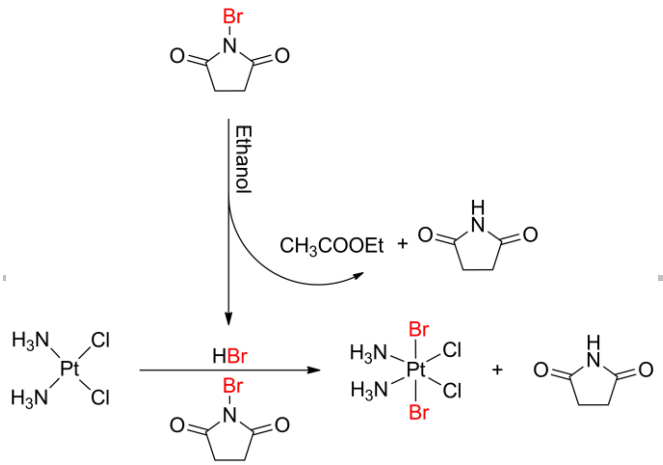
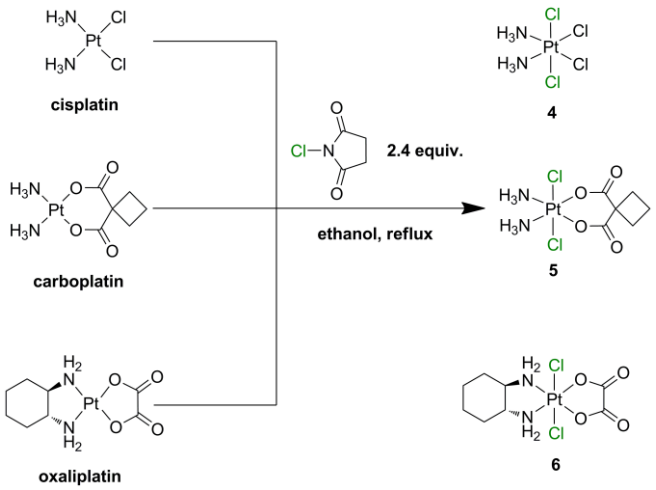


Figure 4. A proposed mechanism of the reaction between cisplatin and 2.4 equivalents of NBS in ethanol.

NCS and NIS are the analogs of NBS, and we anticipated that the similar oxidation reaction on Pt(II) may occur, resulting in dichlorinated and diiodinated Pt(IV) complexes. The well-known method for the synthesis of dichlorinated Pt(IV) is to use chlorine gas as an oxidant. In these reactions, Pt(II) complexes are oxidized via oxidative addition and subsequently converted into dichlorinated Pt(IV) complexes.[9] Chlorine gas is a yellow-green gas with a pungent smell. Its low boiling point (−34 ˚C), high causticity, and high reactivity make it difficult to be operated in the laboratory. In order to avoid these issues, several electrophilic chlorinating reagents including iodobenzene dichloride ($PhICl_2$), *N,N*-dichlorobenzenesulfonamide ($PhSO_2NCl_2$), and *N,N*-dichlorotosylamide have been utilized to obtain dichlorinated Pt(IV) complexes.[21-23] These approaches, however, suffer from difficulties in the preparation of chlorinating reagents, harsh reaction conditions, and high price, limiting their further use. In the previous reports, adding excess NCS to *cis*-$[PtL_2X_2]$ in methanol typically generated *cis,cis,trans*-$[PtL_2X_2(OCH_3)Cl]$, where L is the N-donor ligand and X is the leaving group.[24] In 2008, Sanford and co-workers have already carried out the reaction of NCS with $[Pt(phpy)_2]$ in DCM. In their study, instead of getting the desired Pt(IV) complexes, the major oxidized products are the Pt(III) complexes.[25] We believe the possible reason is that DCM cannot be oxidized by NCS and no additional Cl^- is produced to balance the charge. Therefore, we assume that if ethanol is used as a solvent, a similar reaction as NBS with Pt(II) complexes may occur and finally the reaction may lead to the formation of dichlorinated Pt(IV). Based on this assumption, the reaction of 2.4 equivalents of NCS with cisplatin was carried out in ethanol at room temperature. To our supprise, the final product was a mixture of *cis*-$[Pt(NH_3)_2Cl_4]$ and *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OCH_2CH_3)Cl]$, and the latter is the major product (Figure S1, ESI). When carboplatin or oxaliplatin instead of cisplatin was used in the same reaction condition, the final product was still a mixture of dichlorinated and monochlorinated Pt(IV) complexes. These results are confusing since ethanol is a weak coordinating solvent compared to Cl^-. Theoretically, the major oxidation product of cisplatin should be *cis*-$[Pt(NH_3)_2Cl_4]$ rather than *cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OCH_2CH_3)Cl]$.

The first step of this reaction is the two electron oxidation of Pt(II) complexes by Cl^+. Afterward, it may form a cationic intermediate $[PtL_2X_2Cl]^+$.[25] Since this intermediate is not stable and will prefer to trap Cl^- rather than the solvent in the solution, a reasonable explanation for the formation of monochlorinated Pt(IV) complexes bearing ethoxy group in the axial position as the major products might be that there is no enough Cl^- in the solution. We assume that NCS could quickly react with Pt(II) complexes to form a large amount of $[PtL_2X_2Cl]^+$. For the reaction of NCS with ethanol, NCS might have a lower reaction rate and it could not produce enough Cl^- at the same time. In fact, NCS has a stronger oxidizing ability than NBS, even though, only 65% of ethanol could be oxidized by NCS at a high

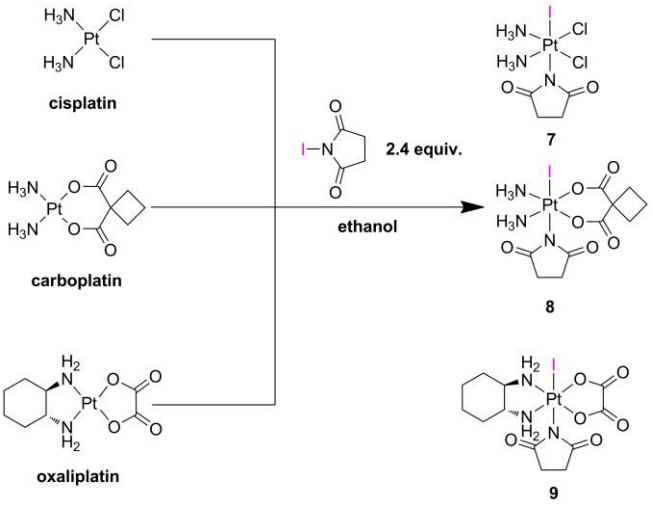
temperature after 2 hours.[19] Without enough Cl^-, the only choice for the intermediate $[PtL_2X_2Cl]^+$ is to trap the solvent in the solution and finally form *cis,cis,trans*-$[PtL_2X_2(OCH_2CH_3)Cl]$ complexes. In order to solve this issue, a higher temperature was used to accelerate the formation of Cl^-, and this reaction was carried out in a reflux condition. Indeed, after increasing the reaction temperature from room temperature to 78 °C, most of



the Pt(II) complexes turned into dichlorinated Pt(IV) compounds with high yields by using 2.4 equivalents of NCS in ethanol (Figure 5). Since NCS is safer, cheaper, and easier to be handled, the application of NCS as a chlorinating reagent is among the best choices to form not only monochlorinated but also dichlorinated Pt(IV) compounds.[17]

Figure 5. Synthesis of dichlorinated Pt(IV) compounds with 2.4 equivalents of NCS in ethanol.

Compared to chlorine gas and liquid bromine, iodine is solid and less toxic, and these properties make it easier to be handled in the laboratory. Probably due to these reasons, previous methods to synthesize diiodinated Pt(IV) complexes directly utilized iodine.[21] In spite of these advantages, iodine could easily sublimate at room temperature and the formation of iodine gas will still be a potential concern. NIS is a widely used electrophilic iodinating reagent which has the similar properties with NBS and NCS. We believe that the reaction of NIS with Pt(II) complexes would occur through the same process. The reactions of Pt(II) complexes with 2.4 equivalents of NIS were subsequently carried out in ethanol at room temperature. Surprisingly, instead of forming diiodinated Pt(IV) complexes, novel platinum compounds containing iodide and succinimide ligands in the axial position finally formed (Figure 6). To the best of our knowledge, there are only few examples in which succinimide was able to be directly added to a metal center.[26] For platinum, compounds **7**, **8** and **9** are the first examples of



directly oxidative addition of NIS to the platinum center. Further investigation on the mechanism to form complexes **7, 8** and **9** is still on going.

Figure 6. The reaction of 2.4 equivalents of NIS with Pt(II) complexes in ethanol.

Cytotoxicity of the Pt(IV) complexes along with cisplatin, carboplatin, and oxaliplatin was measured by MTT assay. Cisplatin-sensitive A2780 human ovarian carcinoma cells were used. The half maximal inhibitory concentration (IC_{50}) values of cisplatin, carboplatin, and oxaliplatin are in the typical micromolar range upon 72 h drug treatment. Cisplatin-based Pt(IV) complexes **1**, **4**, and **7** bearing different halogen axial ligands have similar IC_{50} values compared to that of cisplatin (Table 1). For complexes **2**, **5**, and **8**, which are the Pt(IV) complexes based on carboplatin, higher IC_{50} values than the parent Pt(II) drug are observed. Among oxaliplatin-based Pt(IV) complexes, compound **3** is more active than oxaliplatin. We also tested the complexes in cisplatin-resistant A2780cisR cells, and the resistant factors (RF) are calculated. Cisplatin, carboplatin, and oxaliplatin have RF values of greater than 10. Several Pt(IV) compounds display reduced cross-resistance. For example, carboplatin-based dichlorido Pt(IV) complex **5** has a dramatically decreased RF value of 0.3, although the complex is not significantly active in the sensitive cells. The RF value of oxaliplatin-based dichlorido Pt(IV) complex **6** decreases from 10 (for oxaliplatin) to 1.4. In addition, cisplatin-based diiodino Pt(IV) complex **7** shows improved cytotoxicity in the resistant cells with a decreased RF value compared with its parent Pt(II) drug.

Table 1. Cytotoxicity of different complexes in cisplatin-sensitive (A2780) and -resistant (A2780cisR) cancer cells. Cells were treated with the complexes for 72 h prior to MTT test. Cytotoxicity data are presented as IC_{50} in μM. R_1 and R_2 are the axial ligands.

Compound	Oxidation state	Pt(II) moiety	R_1	R_2	A2780	A2780cisR	RF[a]
1	Pt(IV)	cisplatin	Br	Br	1.1±0.2[b]	27±1[b]	25
2	Pt(IV)	carboplatin	Br	Br	9.5±1.2[b]	>50[b]	>5.3
3	Pt(IV)	oxaliplatin	Br	Br	0.9±0.2[b]	16±4[b]	17
4	Pt(IV)	cisplatin	Cl	Cl	3.1±0.7	106±17	34
5	Pt(IV)	carboplatin	Cl	Cl	54±8	16±5	0.3
6	Pt(IV)	oxaliplatin	Cl	Cl	40±6	56±4	1.4
7	Pt(IV)	cisplatin	I	$C_4H_4NO_2$	3.0±0.9	4.1±0.8	1.4
8	Pt(IV)	carboplatin	I	$C_4H_4NO_2$	>14	>14	----
9	Pt(IV)	oxaliplatin	I	$C_4H_4NO_2$	2.4±0.2	48±3	20
cisplatin	Pt(II)		---	---	1.6±0.1[b]	22±2[b]	14
carboplatin	Pt(II)		---	---	1.4±0.3[b]	15±3[b]	10
oxaliplatin	Pt(II)		---	---	1.2±0.1[b]	12±2[b]	10

[a] resistant factors (RF), defined as IC_{50} in A2780cisR/IC_{50} in A2780; [b] results from reference [18].

Conclusion

In conclusion, we scrutinized the mechanism of the formation of dibrominated Pt(IV) complexes from Pt(II) drugs with excess NBS. In the reaction of Pt(II) drugs with NBS, ethanol serves as a reducing agent to reduce NBS and ethanol itself is oxidized into ethyl acetate. The resulting "negative bromine" is subsequently added to the metal center. The reaction of Pt(II) drugs with NCS or NIS are also carried out in similar conditions, and dichlorinated Pt(IV) complexes are obtained as well. Surprisingly, when NIS reacts with Pt(II) drugs, the direct oxidative addition into the N-I bond occurs, forming a new type of Pt(IV) compound bearing succinimide ligand in the axial position. Most of the synthesized compounds are active against the proliferation of cisplatin-senstive human ovarian cancer cells, and some of them are also cytotoxic in cisplatin-resistant cells. Our work provides a new and convenient synthetic method for dihalogenated Pt(IV) complexes.

Experimental Section

Materials and physical measurements

Unless otherwise noted, all the reactions were carried out under normal atmospheric conditions with protection form light. Pt(II) drugs and other chemicals were purchased from commercial suppliers. All the solvents were used as received without additional drying or purification.

Physical measurements of the compounds are shown in Figures S1-S19. ^{1}H, ^{13}C and ^{195}Pt NMR spectra were measured by a Bruker AVANCE III 300 or 400 MHz spectrometer or a Bruker Ascend AVANCE III 600 MHz spectrometer at room temperature. All NMR chemical shifts (δ) are reported in parts per million (ppm) and referenced as described below. ^{1}H and ^{13}C NMR spectra were referenced internally to residual solvent peaks using deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated dimethylformamide (DMF-d_7) as the solvent. Electrospray ionization mass spectrometry (ESI-MS) was carried out on an Agilent API 150EX mass spectrometer. Elemental analysis was performed using a Vario Micro elemental analyzer. IR spectra were recorded on a Perkin Elmer Spectrum 2000 by using an OMNIC software at room temperature.

Synthesis

Synthesis of complexes 4 to 6. Pt(II) drug (0.25 mmol, which is 75 mg of cisplatin, or 93 mg of carboplatin, or 99 mg of oxaliplatin) was added into 4 mL of ethanol. *N*-Chlorosuccinimide (0.6 mmol, 80 mg) was directly added into the suspension. The mixture was heated to reflux with stirring for 8 h. After the reaction completed, the suspension was cooled to room temperature and the precipitate of the final product was collected by centrifugation. The solid was dissolved in minimal DMF and precipitated with dichloromethane (30 mL) to get the pure product.

***cis*-[Pt(NH$_3$)$_2$Cl$_4$] (4).** Yield: 64% 60 mg (0.16 mmol). ^{1}H NMR (400 MHz, DMSO) δ 5.84 (m, 6H). ^{195}Pt NMR (129 MHz, DMSO) δ -112.8. IR (KBr, cm^{-1}): 3262, 3177, 1638, 1560, 1319. ESI-MS (Negative ion mode): 369.2 m/z $[M-H]^-$; calcd for $Cl_4H_5N_2Pt$ 368.9 m/z $[M-H]^-$.

***cis,trans*-[Pt(CBDCA)(NH$_3$)$_2$Cl$_2$] (5).** Yield: 78% 86 mg (0.19 mmol). ^{1}H NMR (400 MHz, DMF) δ 6.52 (m, 6H), 2.82 (t, *J* = 7.9 Hz, 4H), 2.21 – 1.93 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 176.9, 56.3, 32.3, 16.1. ^{195}Pt NMR (86 MHz, DMSO) δ 770.4. IR (KBr, cm^{-1}): 3218, 2953, 1622, 1356, 1228. ESI-MS (positive ion mode): 443.0 m/z $[M+H]^+$; calcd for $C_6H_{13}Cl_2N_2O_4Pt$ 443.0 m/z $[M+H]^+$.

***trans*-[Pt(DACH)(ox)Cl$_2$] (6)**. Yield: 89% 104 mg (0.22 mmol). ^{1}H NMR (400 MHz, DMSO) δ 8.21 (d, *J* = 8.6 Hz, 2H), 7.60 (s, 2H), 2.57 (s, 2H), 2.02 (d, *J* = 12.1 Hz, 2H), 1.69 – 1.37 (m, 4H), 1.08 (t, *J* = 9.6 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 163.5, 62.4, 31.0, 24.1. ^{195}Pt NMR (86 MHz, DMSO) δ 383.1. IR (KBr, cm^{-1}): 3456, 3148, 3051, 2961, 2939, 2871, 1689, 1664, 1383. ESI-MS (positive ion mode): 469.1 m/z $[M+H]^+$; calcd for $C_8H_{15}Cl_2N_2O_4Pt$ 469.0 m/z $[M+H]^+$.

Synthesis of complex 7 to 9. Pt(II) complex (0.25 mmol, which is 75 mg of cisplatin, or 93 mg of carboplatin, or 99 mg of oxaliplatin) was added into 4 mL of ethanol. *N*-iodosuccinimide (0.6 mmol, 135 mg) was directly added into the suspension. The mixture was stirred at room temperature for 8 h. After the reaction completed, the precipitate of the final product was collected by centrifugation. The pure product was obtained by washing with ethanol (3 X 10 mL) and then dichloromethane (3 X 30 mL).

***cis,cis,trans*-[Pt(NH$_3$)$_2$Cl$_2$(C$_4$H$_4$NO$_2$)I] (7).** Yield: 81% 106 mg (0.20 mmol). ^{1}H NMR (400 MHz, DMF) δ 6.68 – 5.69 (m, 6H), 2.58 (s, 4H). ^{13}C NMR (101 MHz, DMSO) δ 184.5, 30.6. ^{195}Pt NMR (86 MHz, DMSO) δ -654.8. IR (KBr, cm^{-1}): 3245, 3095, 1721, 1639, 1564, 1334, 1197. ESI-MS (positive ion mode): 548.0 m/z $[M+Na]^+$; calcd for $C_4H_{10}Cl_2IN_3O_2PtNa$ 547.9 m/z $[M+Na]^+$.

***cis,trans*-[Pt(CBDCA)(NH$_3$)$_2$(C$_4$H$_4$NO$_2$)I] (8).** Yield: 68% 100 mg (0.17 mmol). Anal. Calcd for: $C_{10}H_{16}IN_3O_6Pt$ (596.24): C, 20.14; H, 2,70; N, 7.05. Found: C, 20.18; H, 2.82; N, 6.96. ^{1}H NMR (400 MHz, DMSO) δ 5.79 (m, 6H), 2.67 (t, *J* = 8.0 Hz, 2H), 2.46 (s, 4H), 2.39 (t, *J* = 7.9 Hz, 2H), 2.00 – 1.75 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 184.1, 177.9, 56.7, 35.0, 30.4, 28.6, 15.9. ^{195}Pt NMR (86 MHz, DMSO) δ 304.2. IR (KBr, cm^{-1}): 3226, 3125, 2100, 2983, 2937, 1728,1637,1604, 1349,1222. ESI-MS (positive ion mode): 597.4 m/z $[M+H]^+$; calcd for $C_{10}H_{17}IN_3O_6Pt$ 597.0 m/z $[M+H]^+$.

***trans*-[Pt(DACH)(ox)(C$_4$H$_4$NO$_2$)I] (9).** Yield: 84% 130 mg (0.21 mmol). ^{1}H NMR (400 MHz, DMSO) δ 8.85 – 5.81 (m, 4H), 2.83 (s, 2H), 2.61 (s, 4H), 2.05 (t, *J* = 12.2 Hz, 2H), 1.48 (d, *J* = 10.0 Hz, 4H), 1.10 (d, *J* = 9.7 Hz, 2H). ^{13}C NMR (101 MHz, DMSO) δ 185.5, 163.3, 65.1, 60.8, 31.6, 30.5, 23.9, 23.8. ^{195}Pt NMR (86 MHz, DMSO) δ -3.8. IR (KBr, cm^{-1}): 3563, 3530, 3466, 3023, 2927, 1713, 1681, 1658, 1589, 1371, 1341, 1205. ESI-MS (positive ion mode): 644.9 m/z $[M+Na]^+$; calcd for $C_{12}H_{18}IN_3O_6PtNa$ 645.0 m/z $[M+Na]^+$.

Cytotoxicity test

Human ovarian carcinoma A2780 and cisplatin-resistant A2780cisR cells were kindly provided by Prof. Wee Han Ang at the National University of Singapore (NUS) and cultured in RPMI-1640 with 10% FBS, 100 units penicillin/streptomycin, and 2 mM L-glutamine at 37 °C in a 5% CO_2 incubator. For A2780cisR cells, 5 μM of cisplatin was added to the culture medium to maintain the resistance. Cells were seeded into 96-well plates at a density of 2500 cells per well and incubated for 24 h, followed by the treatment with medium containing various concentrations of compounds for 72 h at 37 °C in 5% CO_2. DMF was used as a supporting medium, and the final concentration of DMF was 1% (v/v). After the drug treatment, the compound-containing medium was replaced by FBS-free medium with 1 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). After 2 h incubation, the medium containing MTT was removed and DMSO was added to each well. The absorbance was measured at 570 and 630 nm by a Biotek microplate reader. Cells incubated with medium containing 1% DMF only were used as controls.

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Keywords: *N*-halosuccinimide • cisplatin • Pt(IV) prodrugs • oxidation

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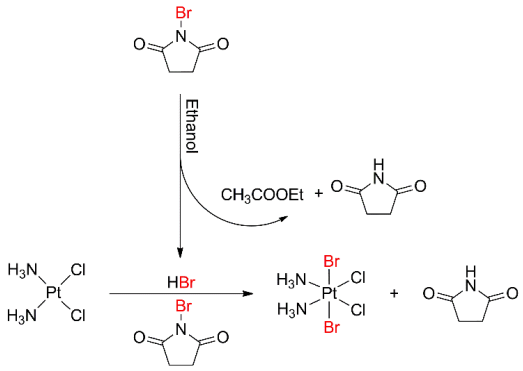

Pt(IV) complexes

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Halogenated Pt(IV) complexes from N-halosuccinimide oxidation of Pt(II) antitumor drugs: Synthesis, mechanistic investigation, and cytotoxicity