Bioorganic & Medicinal Chemistry Letters 23 (2013) 3780–3784

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and anticancer activity of dichloroplatinum(II) complexes of podophyllotoxin

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ARTICLE INFO

Article history:
Received 26 January 2013
Revised 23 April 2013
Accepted 30 April 2013
Available online 9 May 2013

Keywords:
Cisplatin
Podophyllotoxin
Microtubules inhibition
DNA cleavage

ABSTRACT

A series of dichloroplatinum(II) complexes of podophyllotoxin (PPT) were prepared, and their cytotoxicity against sensitive (A-549, HeLa, HCT-8, Hep-G2, K562) and resistant (ADM/K562) cell lines were evaluated. Complex *cis*-[4α-*O*-(2″,3″-diaminopropanoyl)-podophyllotoxin] dichloride platinum(II) (**12**) displayed most potent cytotoxicity with IC_{50} value in the range 0.071–2.98 μM. Complex **12** induces cell cycle arrest in the G_2/M phase, and inhibits the formation of microtubules in HeLa cells. Furthermore, this complex exhibits potent DNA cleavage capabilities.

Anticancer drugs are rarely used singly to treat cancer, because only a few tumours are sensitive enough to be cured by single drugs. For a specific type of tumour, effective chemotherapy usually depends on suitable drugs combinations.[1] Cisplatin is a widely used chemotherapeutic agent for the treatment of testicular cancer, and it is used in combination regimens for a variety of other tumors, including ovarian, cervical, bladder, lung, and those of the head and neck.[2] However, cisplatin has several shortcomings that limit its application in clinic, such as toxicity (ototoxicity, neurotoxicity, gastrointestinal toxicity, renal and hepatic toxicity), low water-solubility, narrow spectrum of activity and intrinsic or acquired resistance.[3] Over the last 30 years, 23 other platinum-based drugs have entered clinical trials with only two (carboplatin and oxaliplatin) of these gaining international marketing approval, and another three (nedaplatin, heptaplatin and lobaplatin) (Fig. 1) gaining approval in individual nations.[4] Although 40 years have passed since the discovery of the anticancer activity of cisplatin, its mechanism of action is unclear.[5] Early investigations of the mechanism of action of cisplatin, suggested that the distortions in the DNA structure, caused by the covalent binding of cisplatin to two adjacent guanines on the same strand of the nuclear DNA, interfere with replication and trigger cellular events that lead to the death of the cancer cell.[6,7] Further study on non-DNA cellular interactions of platinum drugs suggested that the mitochondria might be the critical pharmacological target of cisplatin.[8] Several strategies have been used to increase tumor cell selectivity of platinum drugs or to overcome one or more resistance mechanisms, including their conjugation to potentially site-directing molecules, such as folate, porphyrins, adenine, terpenoids, peptides and many others.[9]

Podophyllotoxin (PPT, **1**) has cathartic, antirheumatic, and antiviral properties.[10] However, its antimitotic activity has proved to be of the greatest interest to researchers.[11] Because PPT is highly toxic, it is not used as an anticancer drug itself, but several semi-synthetic derivatives, including etoposide (VP-16, **2**), teniposide

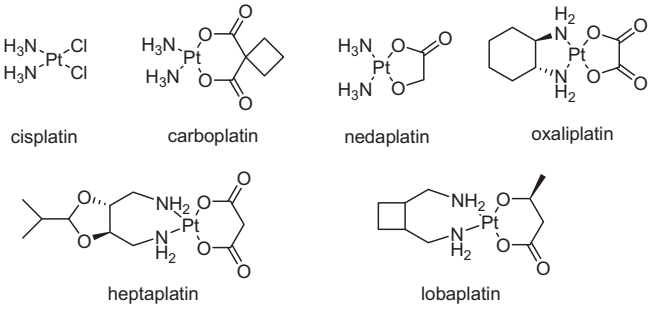

Figure 1. Structures of platinum-based anticancer drugs.

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http://dx.doi.org/10.1016/j.bmcl.2013.04.089

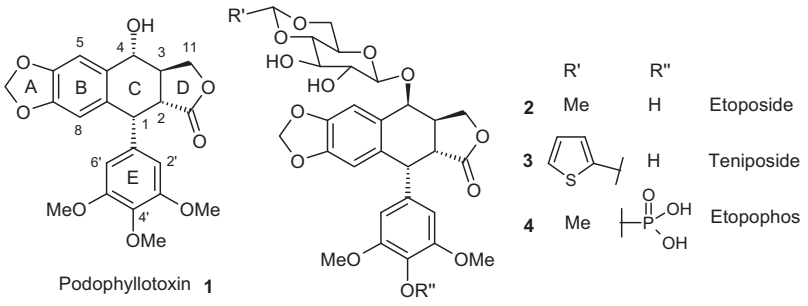


Figure 2. Podophyllotoxin (**1**) and its based clinical drugs.

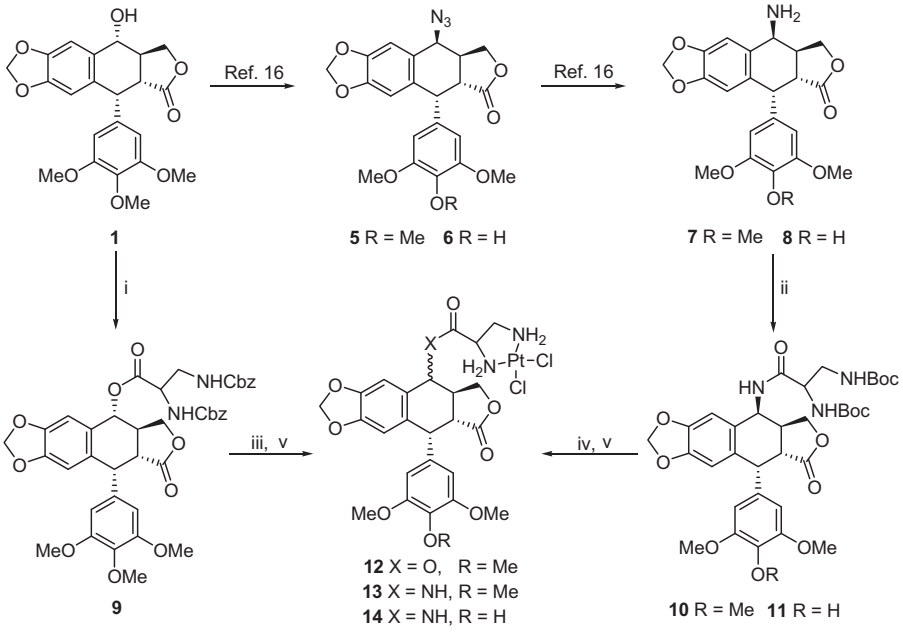
(**3**), and etopophos (**4**) (Fig. 2), are in clinical use against various cancers, including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma.[12] The mechanism of action of etoposide involves the inhibition of topoisomerase-II (TOP-II), unlike the parent compound which inhibits mitosis.[13] With the goal of developing more-potent analogues and overcoming drug resistance, several potential drug candidates based on PPT, such as NK-611, GL-331, tafluposide and F14512, are being evaluated in clinical trials.[14]

Combination chemotherapy with cisplatin and VP-16 is always applied for non-small-cell lung carcinoma, malignant lymphoma and other cancer.[15] In this Letter, which is part of our continuing effort to find new natural product-based compounds with potent activities,[16–21] we prepared a series of dichloroplatinum(II) conjugated podophyllotoxin complexes and evaluated their cytotoxicities against a panel of cancer cell lines. The most active complex, *cis*-[4α-*O*-(2″,3″-diaminopropanoyl)-podophyllotoxin] dichloride platinum(II) **12**, was evaluated for its effect on cell cycle progression, inhibition of microtubules formation, and DNA cleavage.

The synthesis of complexes **12–14** was carried out according to the procedure outlined in Scheme 1. Firstly, the intermediates 4β-amino-4-deoxylpodophyllotoxin (**7**) and 4β-amino-4′-demethyl-4-deoxylpodophyllotoxin (**8**) were obtained from PPT as described in previous publication.[17] Compound **9** was prepared by PPT reacted with 2,3-bis(benzoxycarbonylamino)propanoic acid in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) at room temperature.[21] Compounds **10** and **11** were easily obtained though intermediates **8** and **9** reacted with 2,3-bis(*t*-butoxycarbonylamino)propanoic acid under 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of *N*-hydroxybenzotriazole (HOBT) in dichloromethane.[19] Then, the protection group Cbz in compound **9** were removed by catalytic hydrogenation over Pd/C; and the protection group Boc in compounds **10** and **11** were easily removed using trifluroacetic acid. Subsequently, these products were without further purified, and used directly for complexation reaction with potassium tetrachloroplatinate in a solution of THF and water (1:1) affording target complexes **12–14** in moderate yields.[22]

The structures of complexes **12–14** were identified by ^{1}H NMR spectra, ^{13}C NMR spectra, IR spectra and HRMS (SI). In infrared spectrum of complexes **12–14**, the fact that the amino groups are coordinated to the platinum atom is confirmed by the presence of ν (Pt–N) in the range 3250–3260 cm^{-1}. The strongest peaks of complexes **12–14** solution in dimethylsulfoxide are the [M–Cl+DMSO]$^{+}$ in the mass spectrum (ESI) as reference reported.[23]

The in vitro cytotoxicity of complexes **12–14** and intermediates **9–11** along with the clinically used drug cisplatin and VP-16 as references were evaluated on the six human cancer cell lines (A-549, lung carcinoma; HeLa, cervical carcinoma; HCT-8, colon carcinoma; Hep-G2, hepatic carcinoma; K562, human chronic



Scheme 1. Synthesis of complexes **12–14**. Reagents and conditions: (i) 2,3-bis (benzoxycarbonyl amino)propanoic acid, DMAP, DCC, CH_2Cl_2, (ii) 2,3-bis(*t*-butoxycarbonylamino)propanoic acid, EDCI, HOBt, CH_2Cl_2, (iii) H_2, 10% Pd/C, MeOH, (iv) TFA, CH_2Cl_2, (v) K_2PtCl_4, THF/H_2O, rt.

Table 1
In vitro cytotoxicity (IC_{50}, μM)[a] of compounds **9**–**14**

Compounds	A-549[b]	HeLa[b]	HCT-8[b]	Hep-G2[b]	K562[c]	ADM/K562[c]
9	2.78	2.35	4.64	10.2	0.15	0.20
10	27.0	55.2	38.8	39.9	29.5	55.6
11	29.6	37.4	28.1	27.2	12.3	21.2
12	0.59	0.27	2.98	0.38	0.071	0.091
13	85.4	48.4	30.7	71.6	33.8	29.2
14	26.0	8.30	25.2	26.2	12.4	9.56
Cisplatin	16.3	17.8	10.0	12.5	3.11	4.55
VP-16	23.9	12.2	11.4	6.00	0.354	NA

[a] Data are the mean of three independent experiments.
[b] MTT method with 72 h drug exposure.
[c] CCK-8 method with 72 h drug exposure.

myelogenous leukemia; and ADM/K562, K562 resistant to ADM) used the standard MTT or CCK-8 method.[21] The results expressed as IC_{50} values are summarized in Table 1.

As illustrated in Table 1, after a 72 h exposure, complex **12** showed excellent in vitro cytotoxicities against various experimental cell lines compared to clinical drug VP-16 and Cisplatin. And intermediate **9** also exhibited moderate in vitro cytotoxicities to these six cell lines. Especially to ADM/K562 resistant cell line, the cytotoxicity of **12** is 50 times more potent than those of VP-16 and Cisplatin. In Table 1, we also found that the 4′-OH series of podophyllotoxin derivatives were more cytotoxic than the corresponding 4′-OMe analogues (**14** vs **13**, **11** vs **10**), the result confirmed the assumption that free hydroxyl group at the C-4′ position in derivatives of PPT was favorable for their antitumor activity.[13]

PPT and its analogues are reported to induce apoptosis and cell cycle arrest in the G_2/M phase.[24] To determine whether complexes of PPT and cisplatin act in a similar way, the effects of **12** on cell cycle progression were determined by FACS analysis in propidium iodide-stained HeLa cells.[21] As shown in Figure 3, treatment with 0.25 μM **12** lead to a time-dependent accumulation of cells in the G_2/M phase with a concomitant decrease in the population of G_1 phase cells. 48.8% and 94.0% of the cells were in G_2/M phase after **12** exposed for 12 h (Fig. 3B) and 24 h (Fig. 3C), respectively, compared with 15.0% in untreated cultures (Fig. 3A). These results demonstrate that **12** have similar effects on the cell cycle arrest with that of parent compound PPT.

As above mentioned, PPT induce apoptosis of tumor cells in a time-dependent manner,[24] we did not find obvious apoptosis in FACS analysis of **12**-treated HeLa cells, to confirm the effect of

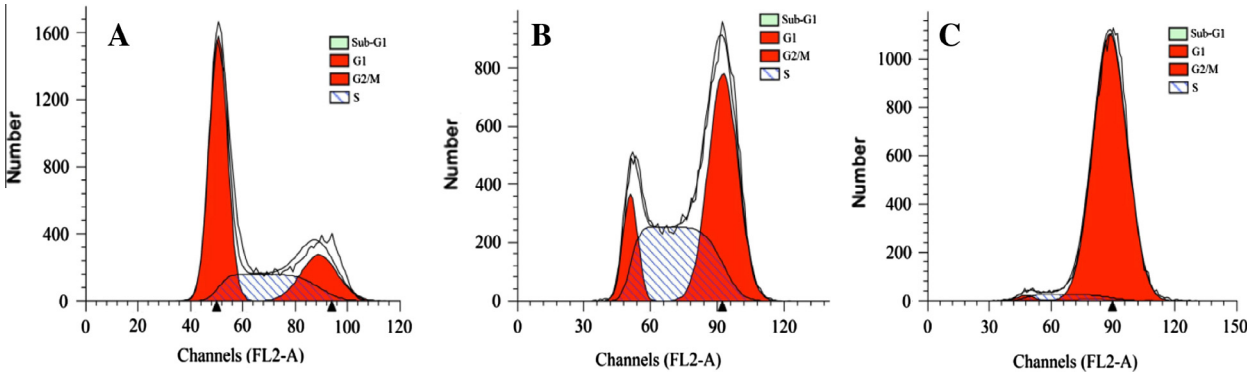


Figure 3. Effects of **12** on cell cycle progression. (A) Control HeLa cells; (B) HeLa cells treated with 0.25 μM **12** for 12 h; (C) HeLa cells treated with 0.25 μM **12** for 24 h.

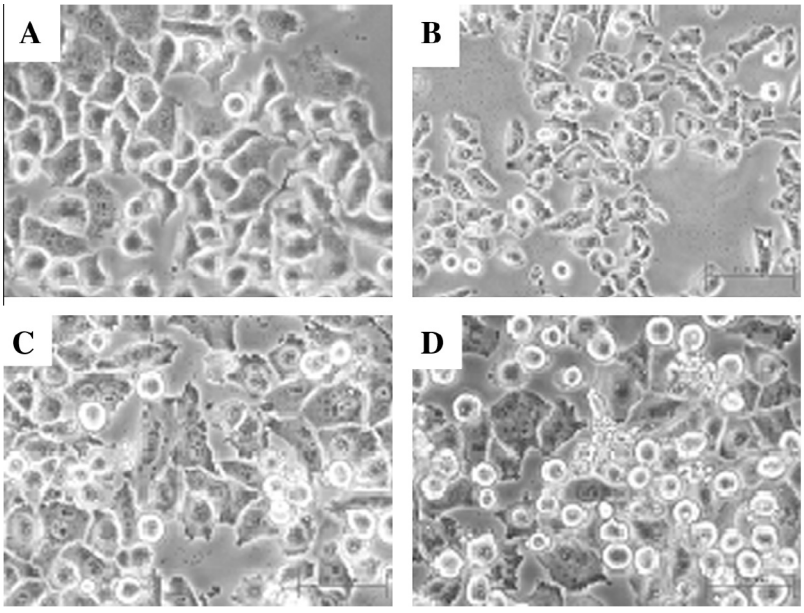


Figure 4. Effects of **12** on cell morphologic. (A) Control HeLa cells; (B) HeLa cells treated with 0.25 μM **12** for 6 h; (C) HeLa cells treated with 0.25 μM **12** for 12 h; (D) HeLa cells treated with 0.25 μM **12** for 24 h.

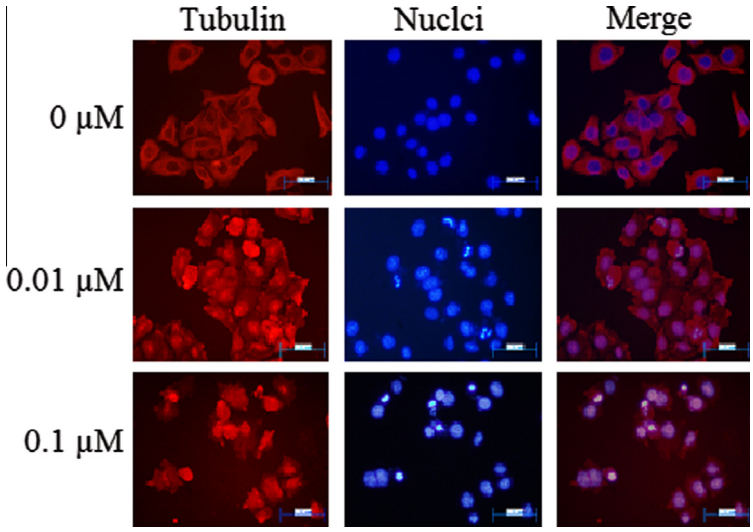


Figure 5. HeLa cells grown on coverslips were treated with **12** (0.01 μM and 0.1 μM) for 24 h. Microtubules (red) were stained by incubation with anti-α-tubulin antibody for 90 min and then with Rhodamine (TRITC)-conjugated secondary antibody for 30 min. Chromosomal DNA (blue) was stained with DAPI.

complex **12** on induction of apoptosis, the morphology of HeLa cells, which were treated with 0.25 μM **12** for 6 h, 12 h and 24 h, respectively, were examined using Hoechst staining (Fig. 4). As shown in Figure 4, untreated HeLa cells exhibited excellent growth characteristic (Fig. 4A). Interestingly, we found mitotic catastrophe in the HeLa cells treated with 0.25 μM **12** for 6 h, the typical features is large cytoplasmic vacuoles, abnormal mitotic figures, multinucleation, and formation of large cells (Fig. 4B).[25] However, HeLa cells treated with 0.25 μM **12** for 12 h or 24 h evoked typical apoptotic features, such as membrane blebbing, cell shrinkage and detachment, and nuclear condensation even fragmentation (Fig. 4C and D).

To determine whether **12** was able to depolymerize cellular microtubules as PPT does,[26] we examined the morphology of untreated and **12**-treated HeLa cells by means of indirect immunofluorescence with antitubulin antibody staining of microtubules and fluorescent staining of nuclei (Fig. 5). The untreated cells were elongated, and staining of thin bundles of microtubules was observed throughout the cytoplasm. In contrast, 24 h after treatment with **12**, the cells were round and contained short, dense microtubule networks; in addition we observed cell shrinkage and nuclear fragmentation and condensation dispersed throughout the cytoplasm, which are hallmarks of apoptosis. These effects increased dose dependently.

The mechanism of action of many platinum-based chemotherapeutic agents relies upon DNA damage.[27] In order to study whether complex **12** enables DNA cleavage, different concentrations (50 and 100 μM) of **12** were incubated with 0.5 μg pBR322 DNA for 24 h in 20 μL Tris–Cl buffer (pH 7.5). The samples were analyzed on 1% native agarose gel electrophoresis followed by ethidium bromide staining of the DNA bands.[28] As shown in Figure 6, the intensity of DNA bands decrease with concomitant increase in concentration of **12**, the result suggesting **12** induces degradation of pBR322 DNA similar to cisplatin.[29]

In summary, *cis*-[4α-*O*-(2″,3″-diaminopropanoyl)-podophyllotoxin] dichloride platinum(II) exhibited promising in vitro cytotoxicities against not only VP-16 sensitive A-549, HeLa, HCT-8, Hep-G2 and K562 but also ADM resistant K562 cell lines. Fluorescence-activated cell sorting analysis indicated that **12** induced cell cycle arrest in the G_2/M phase accompanied by mitotic catastrophe in low-dose and apoptosis in high dose in HeLa cells. In addition, **12** inhibited the formation of microtubules in HeLa cells and caused cleavage of DNA. These results suggest that **12** induced apoptosis by means of multiple pathways.

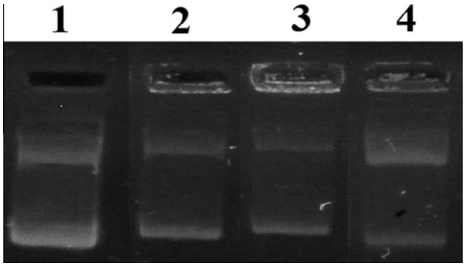


Figure 6. Cleavage of pBR322 plasmid DNA by complex **12**. Lane 1: control DNA; lane 2: DNA treated with 50 μM **12** for 24 h; lane 3: DNA treated with 100 μM **12** for 24 h; lane 4: DNA treated with 100 μM cisplatin for 24 h.

Acknowledgments

This work was financially supported by the Natural Science Foundation of Gansu Province (No. 1208RJZA247) and The Science and Technology Development Project of Lanzhou City (No. 08-1-57).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.089.

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22. Compound **9** (430 mg, 0.56 mmol) was dissolved in 30 mL dried methanol. 440 mg 10% Pd/C was added to the above solution and stirred under hydrogen at room temperature for overnight. Then the reaction mixture was filtered and the filtrate was evaporated to afford crude 4α-*O*-(2″,3″-diaminopropanoyl)-podophyllotoxin. The above crude product was dissolved in THF (5 mL) and H_2O (5 mL). Following K_2PtCl_4 (239 mg, 0.57 mmol) was added to the above solution, and the pH of the mixture keep at 5–6. Then the reaction mixture was stirred until the disappearance of the starting material as indicated by TLC. The reaction mixture was filtered and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography using a mixture of dichloromethane/methanol (92:8) as the eluent. Yield: 48%; white powder solid; mp: >250 °C; IR (KBr) 3441, 3260, 3118, 2935, 2837, 1779, 1589, 1505, 1484, 1239, 1126, 1037, 997, 869, 775 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.18 (s, 1H, H-5), 6.78 (br s, 1H, NH), 6.61 (s, 1H, H-8), 6.42 (br s, 1H, NH), 6.32 (s, 2 H, 2′,6′-H), 6.21 (m, 1H, NH), 6.03 (s, J = 9.6 Hz, 2H, OCH_2O), 5.93 (d, J = 8.6 Hz, 1H, H-4β), 5.56 (br s, 1H, NH), 4.57 (m, 1H, α-H of propanoic acid), 4.40 (d, J = 4.8 Hz, 1H, H-1), 4.21–4.12 (m, 1H, H-11_a), 3.88–3.76 (m, 1H, H-11_b), 3.65 (s, 6 H, 3′,5′-OMe), 3.61 (s, 3H, 4′-OMe), 3.45–3.38 (m, 2H, β-H of propanoic acid), 2.94–2.89 (m, 1H, H-2), 2.86–2.80 (m, 1H, H-3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 168.2, 152.0 (2C), 147.6, 146.9, 136.4, 135.6, 132.6, 127.8, 109.4, 107.9 (2C), 107.6, 101.5, 74.7, 70.8, 61.5, 60.6, 55.8 (2C), 48.7, 48.3, 44.0, 42.8; LR-MS (ESI): m/z 808.7 [M−Cl+DMSO] (100%); HR-MS (ESI): 814.1648 (calculated for 814.1644 of $C_{27}H_{28}D_6ClN_2O_{10}PtS^+$ [M−Cl+DMSO-d_6^+]).
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