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Synthesis, characterization, activities, cell uptake and DNA binding of trinuclear complex: [{*trans*-PtCl(NH_3)}$_2$$\mu$-{*trans*-Pt($NH_3$)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]$Cl_4$

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Abstract

The trinuclear complex: [{*trans*-PtCl(NH_3)}$_2$$\mu$-{*trans*-Pt($NH_3$)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]$Cl_4$ (code named CH9) has been synthesized and characterized. The activity of the compound against human ovarian cancer cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R}, cell up take, level of binding with DNA and nature of its interaction with pBR322 plasmid DNA have been determined. Although the compound is found to be less active (about a half time as active as cisplatin) against the parent ovary cell line A2780, it is found to be more active than cisplatin against resistant cell lines: A2780^{cisR} (3.6 times more) and A2780^{ZD0473R} (3.4 times more). The higher activity of CH9 against the resistant cell lines suggests that the compound has been able to overcome multiple mechanisms of resistance operating in A2780^{cisR} and A2780^{ZD0473R} cell lines. Like other multicentered complexes, the compound is believed to form a range of interstrand GG adducts with duplex DNA that induces permanent global changes in the DNA conformation. This binding is different from that of cisplatin and ZD0473 that form mainly intrastrand adducts, inducing a local kink in a DNA strand. Increasing prevention of BamH1 digestion of form I and form II pBR322 plasmid DNA with the increase in concentration of CH9 provides support to the idea that global changes in DNA conformation are induced as a result of its interaction with the compound.

Keywords: Platinum; Anticancer activity; Cell culture; Cell uptake; DNA binding; Gel electrophoresis

1. Introduction

Although cisplatin is a widely used anticancer drug [1,2], it has a limited spectrum of activity due to the development of drug resistance [3,4] and a number of side effects including neurotoxicity, nephrotoxicity, ototoxicity, myelosuppression, nausea and vomiting [5,6]. The success of cisplatin and its drawback have stimulated the search for newer and better tumor active platinum compounds with improved pharmacological properties [7]. Although thousands of platinum compounds have been synthesized in the past 30 years, only over 28 have entered clinical trials [8]. Currently attention is given to rule breaker platinum compounds primarily with the aim of widening the spectrum of activity [9,10]. One such class of compounds are the polynuclear platinum complexes [11] that contain two or more platinum units linked together by diaminoalkane chains [12]. A notable example is BBR3464 that has been found to circumvent the inherent or acquired cisplatin-resistance in vitro and in vivo in a panel of human adult tumor models [13]. It consists of three *trans*-platinum units joined together by two 1,6-diaminohexane chains. Only the two terminal platinum units in

Abbreviations: AAS, atomic absorption spectrophotometry; CH9, [*trans*-PtCl(NH_3)}$_2$$\mu$-{*trans*-Pt($NH_3$)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]$Cl_4$ Salmon sperm DNA; Cisplatin, *cis*-dichlorodiammineplatinum(II); DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, ethylene diamine tetraacetic acid; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; 1 × TAE buffer, 0.05 M Tris base + 0.05 M glacial acetic acid + 1 mM EDTA, pH 8.0; PBS, phosphate-buffered saline.

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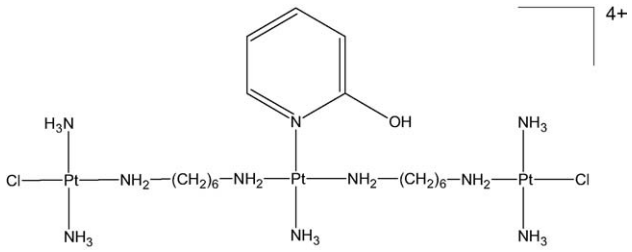


Fig. 1. Structure of CH9: [{*trans*-PtCl(NH_3)}$_2\mu$-{*trans*-Pt(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4. Only the tetrapositive ion is shown, the valencing anions (4 Cl^-) are not included.

BBR3464 undergo covalent binding with DNA whereas the central platinum unit undergoes only non-covalent interactions such as electrostatic interaction and hydrogen bonding [14–16]. Based on the idea that the replacement of the central platinum unit with other suitable metal units may not significantly alter the covalent interactions of the terminal platinum units (although it could have a subtle effect on the non-covalent interactions), Daghriri et al. [17] synthesized a number of Pt–Pd–Pt trinuclear complexes all of which were found to display significant activity against human ovarian cancer cell lines (one being about twenty times as active as cisplatin against A2780^{cisR} cell line). The compounds are believed to overcome multiple mechanisms of resistance operating in A2780^{cisR} and A2780^{ZD0473R} cell lines. Since the central metal unit in trinuclear complexes takes part only in non-covalent interactions, Huq et al. hypothesized that the presence of one or more planaramine ligands bonded to the central metal ion would introduce additional types of non-covalent interactions such as stacking interaction with nucleobases in DNA that may influence the level and spectrum of activity of the compounds. Thus, they prepared the compound: [{*trans*-PtCl(NH_3)}$_2\mu$-{*trans*-Pd(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4 (code named CH25) which was found to exhibit significant anticancer activity against ovarian cancer cell lines: A2780, A2780^{cisR} and A2780^{ZD0473R}—about 45 times as active as cisplatin against A2780 cell line, about 76 times as active as cisplatin against A2780^{cisR} cell line and about seven times as active as cisplatin against A2780^{ZD0473R} cell line [18]. In this paper, we report on the synthesis, characterization, activity, cell uptake, and level and nature of binding with DNA of [{*trans*-PtCl(NH_3)}$_2\mu$-{*trans*-Pt(NH_3)(2-hydroxypyridine)-($H_2N(CH_2)_6NH_2)_2$]Cl_4 denoted as CH9 (Fig. 1).

2. Materials and methods

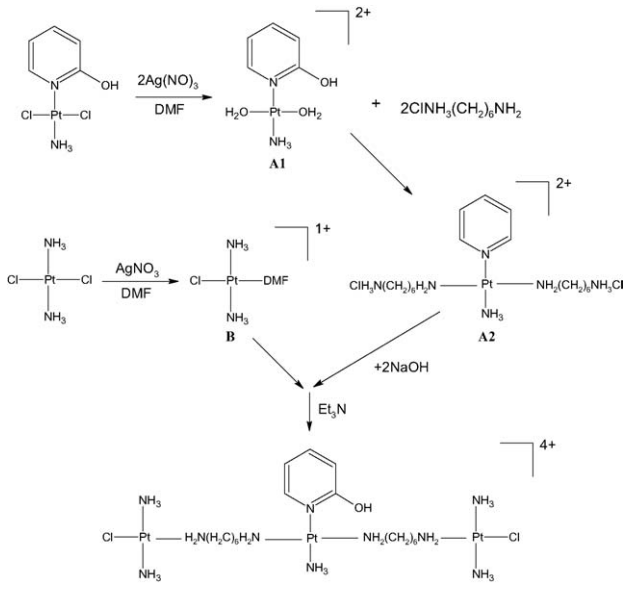
2.1. Materials

Potassium tetrachloroplatinate $K_2[PtCl_4]$, *N*,*N*-dimethylformamide (DMF) [C_3H_7NO], 2-hydroxypyridine and 1,6-diaminohexane were obtained from Sigma Chemical Company, St. Louise, USA; acetone [$(CH_3)_2CO$] and silver nitrate ($AgNO_3$) were obtained from Ajax Chemicals, Auburn, NSW, Australia; methanol [CH_3OH], ethanol [C_2H_5OH], dichloromethane [CH_2Cl_2] were obtained from Merck Pty. Limited, Kilsyth, Vic., Australia. pBR322 plasmid DNA was purchased from ICN Biomedicals, Ohio, USA. Foetal calf serum, 5 × RPMI 1640, 200 mM L-glutamine and 5.6% sodium bicarbonate were obtained from Trace Biosciences Pty Ltd., Australia. Other reagents were obtained from Sigma-Aldrich Pty Ltd., NSW, Australia. Commercially available JETQUICK Blood DNA Spin Kit/50 used to isolate high molecular weight DNA from cell pellet was obtained from Astral Scientific, Australia.

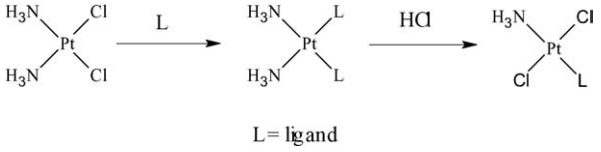
2.2. Synthesis

CH9 was prepared using step-up method of synthesis starting with the compound *trans*-(2-hydroxypyridine)(ammine)dichloroplatinum(II) corresponding to the central platinum unit, as shown in Scheme 1.

The first step in the synthesis of CH9 was the preparation of *trans*-(2-hydroxypyridine)(ammine)dichloroplatinum(II) to serve as the starting material for CH9. The compound was prepared according to previously reported procedure [19] described below. The method used is based on the difference in *trans* effect of halide and amine ligands in platinum(II) complexes, allowing selective substitution and hence control of the stereochemistry [20] (Scheme 2).



Scheme 1. Steps in the synthesis of CH9.



Scheme 2. Synthesis of *trans*-platinum complexes-L stands for a ligand.