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Synthesis and cytotoxic activities of chloropyridylimineplatinum(II) and chloropyridyliminecopper(II) surface-functionalized poly(amidoamine) dendrimers

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ABSTRACT

The preparations of novel platinum and copper metallodendrimers are reported. Surface modified first generation (G0) poly(amidoamine) (PAMAM) dendritic Schiff base, prepared via a condensation reaction was coordinated with platinum chloride and copper chloride yielding [G0-Py_4-$[PtCl_2]_4$] (4D) and [G0-Py_4-$[CuCl_2]_7$] (7E) respectively. These functionalized hyper-branched complexes were characterized by IR spectroscopy and CHN analysis. 4D was further characterized through ^1H and ^{13}C spectroscopy, while 7E was characterized using matrix-assisted laser desorption ionization time-of-flight (MALDI/TOF) Mass Spectrometer. The cytotoxic effects of the compounds against cells of neoplastic origin (MOLT-4, MCF-7) and cells of benign origin (Chang Liver) were studied. Their cytotoxicities were then compared to their mono-nuclear analogues, [$(MeCONHCH_2CH_2N{=}CHPy)(PtCl_2)$] (1D) and [$(MeCONHCH_2CH_2N{=}CHPy)(CuCl_2)$] (1E). The multi-nuclear complexes showed increased cytotoxic activities as compared to their respective mono-nuclear compounds. Most notably, significant inhibitions were observed for 7E on all cell lines, in which its IC_{50} values were 11.1 ± 0.6, 10.2 ± 1.5 and 8.7 ± 0.7 μM against MOLT-4, MCF-7 and Chang Liver cells respectively. The multi-nuclear copper-based complexes (7E) are therefore most effective against a cancer cell line (MOLT-4) and a cisplatin-resistant cell line (MCF-7).

1. Introduction

Since the discovery of cisplatin, much effort has been undertaken to find new platinum-based and other metal-based complexes as anticancer drugs. Such efforts are spurred on ironically by both the successes as well as limitations of cisplatin as an anticancer drug [1–5]; where one of its limitations is the ability of tumor cells to develop drug resistance against it [6]. These newly formulated metal-based anticancer drugs should therefore have improved properties (e.g. water solubility) over cisplatin and have to be effective against cancer cells that have intrinsic or acquired resistance against cisplatin [3,7].

It has been previously reported [2] that an increased number of interstrand crosslinks is a key reason for the high activity of di-nuclear compounds against cisplatin-resistant cell lines. Towards this end, efforts are made to develop molecules that will give rise to an increased number of interstrand adducts. It is therefore pertinent to investigate compounds with multi-metal centers that have more than one DNA binding moiety. Synthesizing multi-nuclear metallic (i.e. platinum and copper) compounds will therefore be a viable approach in developing anticancer drugs that will be effective against cisplatin-resistant cancer cells.

Dendritic polymers, or dendrimers, are hyper-branched polymers that would make ideal scaffolds for the connection of multiple nuclear centers. Dendrimers can also be synthesized to be mono-dispersed with controllable size, and having high surface functionality to allow for the rapid attachment of various anticancer agents [8]. Poly(amidoamine) (PAMAM) dendrimers appear particularly well suited for this application because of additional advantages such as their biocompatibility, high water solubility, lack of immunogenicity and possess terminal-modifiable amine functional groups for binding various targeting or guest molecules.

Following up our previous work [9] on the synthesis of platinum (Pt) and copper (Cu) coordinated mono-nuclear compounds [$(MeCONHCH_2CH_2N{=}CHPy)(PtCl_2)$] (1D) and [$(MeCONHCH_2CH_2N{=}CHPy)(CuCl_2)$] (1E), we now report the synthesis, characterization and cytotoxicity of two novel multi-nuclear Pt and Cu complexes. In order to provide a good metal chelating ligand, first generation PAMAM dendrimers will be modified by reacting with

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pyridine-2-carboxaldehyde to afford the corresponding terminally modified PAMAM Schiff base. This Schiff base was then further reacted with four platinum chloride and seven copper chloride moieties to yield [G0-Py_4-$[PtCl_2]_4$] (4D) and [G0-Py_4-$[CuCl_2]_7$] (7E) respectively. The *in vitro* cytotoxicities of these compounds were then tested on cisplatin sensitive cancer cell line (MOLT-4), cisplatin-resistant breast cancer cell line (MCF-7) [10–12] and cells of benign origin (Chang Liver). In this study, cisplatin was used as a reference against these multi-nuclear complexes. For comparison purposes, the cytotoxicity of Pt and Cu mono-nuclear compounds are cited [9]. The differences in cytotoxicity on these cell lines induced by the variation of metals (i.e. Pt vs. Cu) and the number of metal centers (i.e. mono-nuclear vs. multi-nuclear) are reported in this paper.

2. Experimental

2.1. Materials

All reagents were purchased from commercial sources and were used without further purification unless otherwise stated. Solvents were dried and distilled under nitrogen before use. The ligand [G0-Py_4] (4L) was prepared according to methods reported previously [13]. All syntheses were carried out in a purified nitrogen or argon atmosphere, employing standard Schlenk techniques.

2.2. Synthesis

2.2.1. Preparation of [G0-Py_4] (4L)

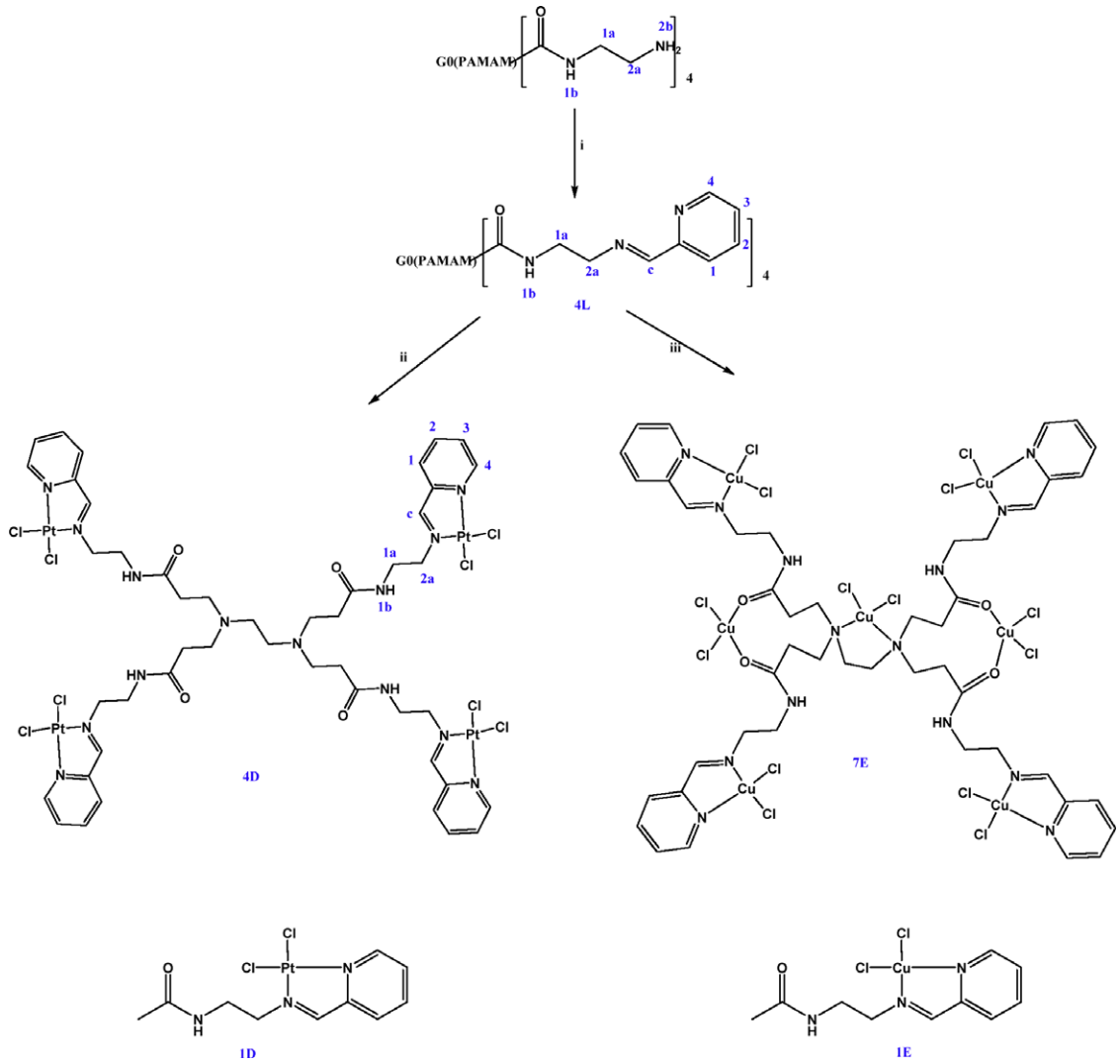
The ligand 4L was prepared through a condensation reaction using first generation PAMAM (G0) and pyridine-2-carboxaldehyde as described in our previous paper [13].

2.2.2. Preparation of [G0-Py_4-$(PtCl_2)_4$] (4D)

A CH_2Cl_2 (50 mL) solution of 4L (0.16 g, 0.18 mmol) was added to $[PtCl_2(cyclooctene)]_2$ [14] (0.40 g, 0.53 mmol) in CH_2Cl_2 (5 mL). The reaction mixture was stirred overnight under room temperature conditions. The crude products were subsequently filtered and washed three times each with 10 mL of dichloromethane, hexane and diethyl ether. Anal: Calcd for $C_{46}H_{60}N_{14}O_4Pt_4Cl_8$: C, 28.5%; H, 3.1%; N, 10.1%. Found: C, 27.6%; H, 3.3%; N, 9.5%. Although the analyses data for "C" and "N" for 4D are somewhat unsatisfactory, other analysis data (IR, ^1H NMR) reasonably supports the formula.

2.2.3. Preparation of [G0-Py_4-$(CuCl_2)_7$] (7E)

The reaction between 4L (0.11 g, 0.12 mmol) and excess $CuCl_2$ (0.14 g, 1.03 mmol) in methanol was conducted overnight under



Scheme 1. The first generation PAMAM dendrimer; 4L, the Schiff base [G0-Py_4]; 4D, [G0-Py_4-$(PtCl_2)_4$]; 1D, [$(MeCONHCH_2CH_2N{=}CHPy)(PtCl_2)$] and 7E, [G0-$Py_4$-$(CuCl_2)_7$]; 1E, [$(MeCONHCH_2CH_2N{=}CHPy)(CuCl_2)$]. The numbers refer to the assignment of ^1H NMR signals of the compounds (see Fig. 1).