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Potent anticancer activity of a new bismuth (III) complex against human lung cancer cells

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ABSTRACT

The aim of this work is experimental study of an interesting bismuth(III) complex derived from pentadentate 2,6-pyridinedicarboxaldehyde bis(^{4}*N*-methylthiosemicarbazone), [BiL$(NO_3)_2$]NO_3 {L = 2,6-pyridinedicarboxaldehyde bis(^{4}N-methylthiosemicarbazone)}. A series of *in vitro* biological studies indicate that the newly prepared [BiL$(NO_3)_2$]NO_3 greatly suppressed colony formation, migration and significantly induced apoptosis of human lung cancer cells A549 and H460, but did not obviously decrease the cell viability of non-cancerous human lung fibroblast (HLF) cell line, showing much higher anticancer activities than its parent ligands, especially with half maximum inhibitory concentration (IC_{50}) <3.5 μM. Moreover, *in vivo* study provides enough evidence that the treatment with [BiL$(NO_3)_2$]NO_3 effectively inhibited A549 xenograft tumor growth on tumor-bearing mice (10 mg kg^{-1}, tumor volume reduced by 97.92% and tumor weight lightened by 94.44% compared to control) and did not indicate harmful effect on mouse weight and liver. These results suggest that the coordination of free ligand with Bi(III) might be an interesting and potent strategy in the discovery of new anticancer drug candidates.

1. Introduction

Thiosemicarbazones with high *anti*-tumor potential [1–5] show good versatility as ligands [6], since the *p*-delocalisation of charge and the configurational flexibility of their molecular chain can give rise to a great variety of coordination modes. Triapine, 3-aminopyridine-2-carboxaldehyde-thiosemicarbazone, is a well-known representative compound acting as a promising anticancer agent under clinical trials due to its significant cytotoxicity against various cancer cell lines [7]. Considering that their biological activities mostly depend on the presence of different groups at N-4 position, more substituted thiosemicarbazones might present with improved biological activities [8].

The coordination of organic compounds with metal ions can cause drastic change in the biological properties of both the ligand and the metal ion moieties. Nitrogen containing heterocyclic molecules constitutes the largest portion of chemical entities, which is the vital part of many natural biologically active pharmaceuticals for the enhancement of the survivability [9]. There have been a large number of studies on metal based tridentate thiosemicarbazones complexes derived from α-N-heterocyclic carboxaldehydes due to their more significant biological activities [10–13]. Several mechanisms of anticancer action have been proposed for this class of chelating ligands and their complexes. For example, they could interact with DNA *via* forming a covalent or non-covalent bond between metal and DNA and thus interfer in DNA synthesis [14].

Bismuth is unusually of low toxicity and environmentally friendly [15,16] and bismuth-containing quadruple rescue therapy has, therefore, been widely selected to replace the traditional triple therapy for the treatment of gastropathy infected by *Helicobacter pylori* [17–22]. Bismuth has two biologically important oxidation states, Bi(III) and Bi(V). Recently, in a report published by Islam. A. et al., novel complexes of organobismuth(V) with heterocyclic 2-acetylbenzoic acid and 5-acetylsalicylic acid were proved to have good antitumor potential by evaluation of their *in vitro* cytotoxic activity against human chronic myelogenous leukemia (K562) and high metastatic murine melanoma (B16F10) cells and comparison of their activity in non-cancerous murine fibroblast (L929) and murine melanocytes (Melan-A) cells) [23]. However, compared with Bi(V), Bi(III) easily reacts with closely spaced thiols, yielding stable cyclic complexes. In addition, the element arsenic in the same main group as bismuth, is known to have the most effects of its compounds in cells through binding to thiols in cysteine residues [24]. Trivalent arsenic has proved to be potent inhibitor of the thioredoxin system and thus might be used as potential cancer chemotherapeutic agent [25]. Many protein-labeling agents have been

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developed indeed based on the high affinity of trivalent arsenicals with vicinal dithiols [26–29]. We accordingly assume that Bi(III) might have the same quality of anticancer, particularly the organometallic Bi(III) complexes. Actually, several bismuth based medicines have been developed and displayed good bioactivities [30–34].

Herein, we showed the synthesis of a new organometallic Bi(III) complex derived from pentadentate 2,6-pyridinedicarboxaldehyde bis(^{4}*N*-methylthiosemicarbazone), [BiL$(NO_3)_2$]NO_3, {L = 2,6-pyridinedicarboxaldehyde bis(^{4}N-methylthiosemicarbazone)}, and the investigation of its *in vitro* biological activities such as cytotoxicity, colony formation rate, cell migration rate as well as cellular apoptosis in human lung cancer cells of A549 and H460 as well as *in vivo* anticancer effects on mice bodies. The results demonstrate that the newly developed organometallic Bi(III) complex can be used as highly effective anticancer agent, based on the high cytotoxicity, low colony formation rate, slow cell migration, significant induction of cellular apoptosis and effective inhibition of tumor growth in living body for cancer treatment.

The possible mechanism of [BiL$(NO_3)_2$]NO_3 inhibiting the growth of cancer cells A549 and H460 was described as follows [14,35]: firstly, deoxy nucleotide triphosphate (dNTP) is known to be composed of the human genetic material DNA. Normally, nucleoside monophosphate (NMP) is first converted to nucleoside diphospate (NDP) under the action of kinase which may be then changed to be deoxy-ribonucleoside diphosphate (dNDP) by ribonucleoside diphosphate reductase (RDR), finally forming dNTP. During the [BiL$(NO_3)_2$]NO_3 treatment, the activity of RDR is inhibited, preventing the conversion from NDP to dNDP. The synthesis of DNA is accordingly inhibited, causing the apoptosis of cancer cells; secondly, Bi(III) in the prepared drug may form covalent or non-covalent bond between Bi(III) and DNA and thus interferes with DNA replication or translation, leading to the apoptosis of cancer cells as well; thirdly, Bi(III) ions probably insert to DNA structure to disturb the normal function of DNA by causing mutations or other mismatches.

2. Experimental section

2.1. Synthesis of L

L was prepared *via* a previously reported method [10]. Briefly, 2,6-Pyridinedicarboxaldehyde (PDA, 0.5 g, 3.7 mmol) and ^{4}*N*-methylthiosemicarbazide (0.778 g, 7.4 mmol) was mixed in ethanol (150 mL) and refluxed at 85 °C for 5.5 h. The resulting solution was then concentrated with a Dean Stark trap to *ca.* 15 mL and cooled for 12 h at 4 °C, finally forming a yellowish precipitate, the ligand L. The produced L was thoroughly washed with diethyl ether during the filtration and dried *in vacuo*.

L ($C_{13}H_{17}N_5S_2$, MW = 307.44): yellowish solid; ^{1}H NMR (600 MHz, dimethyl sulfoxide, DMSO) δ 11.81 (d, *J* = 115.2 Hz, 2H), 8.66 (m, 2H), 8.22 (m, 2H), 8.09 (m, 2H), 7.89 (t, *J* = 7.9 Hz, 1H), 3.04 (d, *J* = 4.6 Hz, 6H); ^{13}C NMR (151 MHz, DMSO) δ177.94 (C2), 153.72 (*C*4), 140.60 (C3), 136.81 (C6), 120.07 (C5), 30.98 (*C*1). Anal. (%) Calcd: C, 50.79; H, 5.57; N, 22.78; S, 20.86. Experimental Anal. (%): C, 50.83; H, 5.59; N, 22.81; S, 20.81.

2.2. Synthesis of [BiL$(NO_3)_2$]NO_3

A $Bi(NO_3)_3 \cdot 5H_2O$ solution was first prepared by dissolving 0.157 g solid (0.323 mmol) in methanol with the help of a few drops of nitric acid and then added dropwise to a methanol solution (30 mL) containing 0.1 g L^{-1} (0.323 mmol) of the prepared L [10]. After being refluxed for 2 h at 85 °C under stirring, the resultant mixture was filtered to collect the yellow precipitate which was further purified with methanol and dried *in vacuo*.

[BiL(NO3)2]NO3 (C13H17BiN8O9S2, MW = 702.43): yellow solid, ^{1}H NMR (600 MHz, DMSO) δ 11.75 (s, 2H), 8.69 (d, *J* = 3.7 Hz, 2H), 8.22 (d, *J* = 7.8 Hz, 2H), 8.06 (s, 2H), 7.90 (s, 1H), 3.01 (s, 6H); ^{13}C NMR (151 MHz, DMSO) δ 177.91 (C2), 152.47 (*C*4), 140.59 (C3), 137.01 (C6), 120.16 (C5), 31.01 (*C*1). Anal. (%) Calcd: C, 22.23; H, 2.44; Bi, 29.75; N, 15.95; O, 20.50; S, 9.13. Experimental Anal. (%): C, 22.28; H, 2.40; Bi, 29.79; N, 15.98; O, 20.58; S, 9.11. MALDI-TOF-MS (m/z): 640.93 $[M]^+$.

2.3. Cytotoxicity assay

The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to evaluate the cytotoxicity. Human lung cancer cells A549, H460 and normal human lung fibroblasts (HLF) were placed in 96-well plates at a density of 1×10^4 cells/well and then incubated with the tested compounds. After 24 h incubation, cultures were incubated in 100 μL of the medium containing 10 μL 5 mg mL^{-1} MTT solution for 4 h at 37 °C. Afterwards, the medium with MTT was removed, and 100 μL of DMSO was added to each well to dissolve the formazan. The absorbance at 570 nm was measured with a microplate reader (Bio-Tek ELX800, USA). The inhibitory percentage of each compound at various concentrations was calculated so as to determine the half maximum inhibitory concentration (IC_{50}) value.

2.4. In vitro inhibition rate of [BiL$(NO_3)_2$]NO_3

A549, H460 and HLF cells were seeded in a 96-well plate at a density of 0.5×10^4 cells/well at 37 °C in 5% CO_2 to allow the cells to adhere. The cells were then incubated in a complete medium containing [BiL$(NO_3)_2$]NO_3 with 5 different concentrations of 0, 2, 4, 8 and 16 μM according to $IC_{50,}$ respectively. MTT assay was tested to evaluate the cell viabilities in each group through 0, 12, 24, 48 and 72 h incubations.

2.5. In vitro colony formation assay

A549, H460 and HLF cells were seeded in a 6-well plate at a density of 400 cells/well at 37 °C in 5% CO_2 for the adherence of cells. The cells were then incubated in a complete medium containing 1% DMSO and 8 μM of PDA, L and [BiL$(NO_3)_2$]NO_3. The colony was counted only if it contained more than 50 cells, and the number of colonies was counted on the 7th day after seeding. Colony formation rate was calculated with the equation:

$$\text{colony formation rate} = (\text{number of colonies}/\text{number of seeded cells}) \times 100\%.$$

Each treatment was carried out three times.

2.6. In vitro cell migration assay

A549, H460 and HLF cells were seeded in a 6-well plate at a density of 3×10^5 cells/well at 37 °C in 5% CO_2, and each well contained 2 mL medium solution. After cells formed monolayer cell, the scratch line was made through the monolayer cell vertically with 200 μL tips. The culture medium was then removed from the 6-well plate which washed with PBS for three times to remove the dislodged cells. The cultured cells were incubated in 1% fetal bovine serum medium containing 1% DMSO, PDA, L and [BiL$(NO_3)_2$]NO_3 at an equivalent concentration of 8 μM at 37 °C in 5% CO_2, respectively. The cell migration was captured at the moments of 0, 6, 12 and 24 h with the corresponding cell-uncovered line width recorded, respectively. The cell migration rate was calculated according to the equation below:

$$\text{cell migration rate} = S_c/S_a \times 100\%$$

where S_a is the control group migration width and S_c the assay group migration width.