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Synthesis, characterization and anti-proliferative activity of heterocyclic hypervalent organoantimony compounds

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ABSTRACT

Three heterocyclic hypervalent organoantimony chlorides $RN(CH_2C_6H_4)_2SbCl$ (**2a** R = *t*-Bu, **2b** R = Cy, **2c** R = Ph) and their chalcogenide derivatives $[RN(CH_2C_6H_4)_2Sb]_2O$ (**3a** R = *t*-Bu, **3b** R = Cy, **3c** R = Ph) were synthesized and characterized by techniques such as ^1H NMR, ^{13}C NMR, X-ray diffraction, and elemental analysis. It is found that the anti-proliferative activity detected over these compounds can be attributed to the coordination bond between the antimony and nitrogen atoms of these compounds. Moreover, a preliminary study on mechanistic action suggests that the inhibition effect is ascribable to cell cycle arrest and cell apoptosis.

1. Introduction

Cancer is a major health problem. Many types of cancer are incurable, and mortality decline is mainly based on early detection and appropriate treatments [1]. In the fighting against cancers, new metal compounds are synthesized as antitumor agents [2,3]. Antimony is a group-15 element. It shows remarkable therapeutic efficacy on patients who suffer from leishmaniasis and acute promyelocytic leukemia [4–7]. Despite antimony compounds are used clinically for quite a number of diseases, they are rarely used as antitumor agents. In the 1990's, Silvestru and coworkers reported for the first time the antitumor activity of organoantimony(III) compounds [4,8,9]. Fifteen years later Wang et al. [10] and Ludmila et al. [11] reported the relatively high antitumor activity of organoantimony(V) compounds. So far the most studied antimony compounds in the context of antitumor activity are organometallic, and they are compounds with antimony-carbon bonds having ligands such as arylhydroxamates [10], lapachol [11], thioamides [12], and hydrazones [13]. The antitumor performance of these compounds, however, is unsatisfactory. It is hence meaningful to explore the chemical and pharmacological properties of new organoantimony compounds for the purpose of developing anticancer drugs.

In the present study, we investigated the anti-proliferative activity of organoantimony compounds that are heterocyclic and hypervalent in nature. Through the use of different nitrogen substituent groups, we controlled the steric and substitution patterns of the organoantimony compounds. It is demonstrated that this kind of compounds can be used for the fabrication of anticancer drugs.

2. Results and discussion

2.1. Chemistry

Dilithiation of tertiary amines $(2\text{-Br-}C_6H_4CH_2)_2NR$ (**1a** R = *t*-Bu, **1b** R = Cy, **1c** R = Ph) with *n*-BuLi, followed by subsequent reaction

Abbreviations: *t*-Bu, tertiary butyl; Cy, cyclohexyl; Ph, phenyl; PI, propidium iodide; μM, μmol/L; mM, mmol/L; RT, room temperature.

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with $SbCl_3$, produces the organoantimony chlorides $RN(CH_2C_6H_4)_2SbCl$ (**2a** R = *t*-Bu, **2b** R = Cy, **2c** R = Ph) (Scheme 1). Treatment of these chlorides with KOH gives the organoantimony chalcogenide derivatives $[RN(CH_2C_6H_4)_2Sb]_2O$ (**3a** R = *t*-Bu, **3b** R = Cy, **3c** R = Ph).

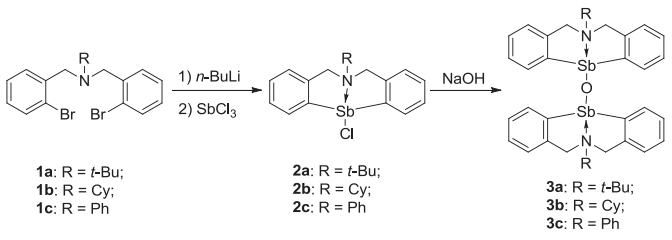
The molecular structures of **2a**–**c** and **3a**–**c** are confirmed by elemental analyses and NMR techniques (^1H and ^{13}C NMR). Due to stronger electron-attracting ability of Sb, the protons linked with the carbon atom adjacent to Sb atom shift downfield (8.25 ppm, **2a**; 8.26 ppm, **2b**; 8.24 ppm, **2c**) in comparison with those of the corresponding starting materials (7.55 ppm, **1a**; 7.59 ppm, **1b**; 7.60 ppm, **1c**). Meanwhile, the ^{13}C NMR data of the carbon atoms adjacent to Sb shift downfield (145.11 ppm, **2a**; 144.01 ppm, **2b**; 147.96 ppm, **2c**) in comparison with those of the corresponding starting materials (139.10 ppm, **1a**; 139.68 ppm, **1b**; 136.27 ppm, **1c**). On the contrary, compared with the ^1H NMR spectra of the corresponding starting materials (8.25 ppm, **2a**; 8.26 ppm, **2b**; 8.24 ppm, **2c**), stronger electron-donating ability of O counteracts the electron-attracting ability of Sb, leading to upfield shift of the protons linked with the carbon atoms adjacent to Sb atom (8.12 ppm, **3a**; 8.21 ppm, **3b**; 8.12 ppm, **3c**).

Crystal structures of **2a** and **3a** (Fig. 1) were determined by single-crystal X-ray diffraction analysis, and selected bond lengths and angles are listed in Table 1. One can see that the coordination polyhedron around the centre Sb of hypervalent compounds **2a** and **3a** can be best described as a strongly distorted pseudo-trigonal bipyramid. The N(1), Cl(1) and O(1) atoms are located at the apical positions, while the C(1), C(10) and C(14) atoms are situated at the equatorial positions along with an electron lone pair of Sb. The Sb(1)-N(1) distance (2.4638(14) Å) in **2a** and that (2.6546(17) Å) in **3a** is longer than that (2.397(3) Å) in 12-chloro-6-cyclohexyl-5,6,7,12-tetrahydrodibenzo[c,f] [1,5]-azastibocine [14]. The results suggest that the N → Sb coordination in **2a** and **3a** is weaker than that of the latter. Furthermore, the two N–Sb distances in **2a** and **3a** are slightly longer than the sum of the covalent radii (2.11 Å) [15] but much shorter than the sum of the van der Waals radii (3.74 Å) [16], indicating that there is coordination bonding between the antimony and the nitrogen atoms. According to Musher's idea of hypervalent molecules [17], compounds **2a**–**c** and **3a**–**c** with high Sb valences can be considered as hypervalent. The Sb–O bond length (2.0055(14) Å) in **3a** is similar to those of organoantimony oxides such as $[\{2\text{-}(Me_2NCH_2)C_6H_4\}_2Sb]_2O$ (1.986(3) Å), $(Ph_2Sb)_2O$ (1.978(3) Å), and $(Me_2Sb)_2O$ (2.099(6) Å) [18–20]. Due to constrain imposed by intramolecular N → Sb coordination, the N–Sb–Cl angle in compound **2a** (161.36(3)°) and N–Sb–O angle in compound **3a** (159.029(43)°) are significantly deviated from the ideal case of 180°.

2.2. Biological activity

2.2.1. Anti-proliferative activity

Using CCK-8 assay, the anti-proliferative effect of compounds **1a**–**c**, **2a**–**c** and **3a**–**c** on A549 cells was examined. The concentrations of compounds required to inhibit 50% of cell growth (i.e. IC_{50}) are shown in Table 2. It is observed that **2a**–**c** and **3a**–**c** show much higher anti-proliferative activity than their starting materials **1a**–**c**. The compounds with the same nitrogen substituent show anti-proliferative effects that follow the order: **3a** (IC_{50} = 3.5 μM) > **2a** (IC_{50} = 6.6 μM) > **1a** (IC_{50} > 30 μM), **3b** (IC_{50} = 5.5 μM) > **2b** (IC_{50} = 31.4 μM) > **1b** (IC_{50} > 30 μM, inhibition ratio is 9.39% at 30 μM). With IC_{50} above 30 μM, compounds **1c**, **2c** and **3c** are all weak in anti-proliferative activity. In other words, the order of anti-proliferative effect of these compounds can also be arranged according to the nitrogen substituents: *t*-Bu > Cy > Ph. Since compared to Cy and Ph groups, *t*-Bu group is stronger in electron-donating ability but weaker in steric effect, it is hence deduced that the anti-proliferative activity towards A549 cells can be related to the coordination bonding between the antimony and nitrogen atoms of these compounds.



Scheme 1. Synthesis of compounds **2a**–**c** and **3a**–**c**.

In view that compounds **2a** and **3a** (both with same nitrogen substituent) showed stronger anti-proliferative activity, they were adopted to examine the time course at various concentrations (Fig. 2) as well as to examine the dose effect on anti-proliferative activity (Fig. 3). The results suggest that the anti-proliferative activity is concentration as well as time dependence. Based on the results of optimization, we adopted compound concentration of 10 μM and incubation period of 48 h for further investigation.

To assess the possible side effects of administrating these compounds, the anti-proliferation activity of them on normal human bronchial epithelial cells (HBEC) was evaluated. After 48 h incubation, the IC_{50} values of compounds **2a** and **3a** on HBEC are 18.7 and 11.2 μM, giving IC_{50} (HBEC)/IC_{50}(A549) ratio of 2.83 and 3.20, respectively. When the commercial anticancer drug cisplatin was adopted, the IC_{50} value on A549 cells under the same experimental conditions is above 30 μM. With anti-proliferation activity towards A549 cells stronger than that of cisplatin, the heterocyclic hypervalent compounds **2a** and **3a** can be further studied as anti-tumor drugs.

2.2.2. Effect of the compounds on the cell cycle

There are five stages of the cell cycle: (1) the G_1 phase that follows mitosis, a period for the synthesis of enzymes needed for DNA replication; (2) the S phase, a period of DNA replication; (3) the G_2 phase where the cell continues to grow and produce new proteins; (4) the M phase where the cell divides into two daughter cells; and (5) the quiescent G_0 phase where the cell remains stable until it begins the cell cycle again. To determine the possible effect of the heterocyclic hypervalent compounds on the progression of cell cycle, we performed flow cytometric analysis to quantify the percentage of A549 cells after cell permeabilization and propidium iodide (PI) labeling. In the analysis, the amount of bound dye is correlated with the DNA content of a given cell. In other words, DNA fragmentation in apoptotic cells is translated to fluorescence intensity, giving fluorescence peak that is lower in intensity than that of G_0/G_1 cells, i.e. a sub-G_0/G_1 peak.

In A549 cells incubated with compounds **2a** and **3a** (10 μM for 48 h), the proportion of cells in the sub-G_0/G_1 phase increased to 4.86% and 8.01%, respectively, a large increase compared to 0.74% of the untreated control (Fig. 4). This increase in sub-G_0/G_1 phase was accompanied by an increase in cell number of the G_2/M phase (compared with that of untreated cells), showing values of 28.76 versus 6.06% and 20.06 versus 6.06% (Fig. 4), indicating inhibiting effect of compounds **2a** and **3a** on cell mitosis. In other words, the increased proportion of cells in the sub-G_0/G_1 phase confirms that the apoptosis of A549 cells is a result of DNA degradation induced by compounds **2a** and **3a**. On the other hand, after treatment with compounds **2a** and **3a**, the cells in S phase decreased from the control value of 23.38% to 15.02% and 14.92%, individually, indicating the inhibiting effect of compounds **2a** and **3a** on DNA replication.