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Novel tetranuclear triarylantimony(v) complexes with (±)-mandelic acid ligands: synthesis, characterization, *in vitro* cytotoxicity and DNA binding properties†

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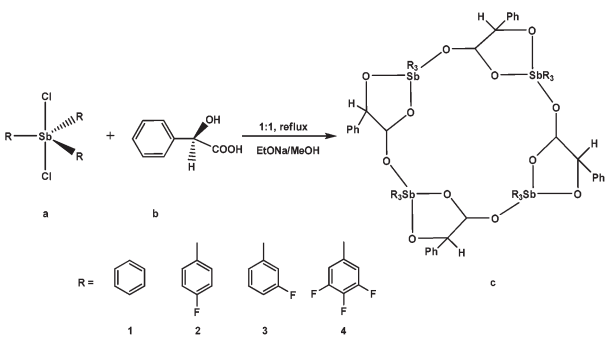
Four novel tetranuclear organoantimony(v) complexes $[R_3SbL]_4$, in which LH = (±)-mandelic acid and R = phenyl (1), 4-fluorophenyl (2), 3-fluorophenyl (3), 3,4,5-trifluorophenyl (4), were synthesized and characterized. The complexes displayed rapid, low micromolar *in vitro* cytotoxicity against a range of epithelial tumour cells and efficient CT-DNA binding.

The clinical success of cisplatin in the treatment of several human malignant tumours has motivated major research efforts toward the discovery of alternative metal complexes with potential as anticancer drugs,[1] which are expected to overcome the remaining problems such as severe side effects and resistance phenomena. Besides platinum, complexes of other transition metals, such as gold[2] and ruthenium,[3] have been reported to exhibit promising anticancer activities. In contrast, the reports on the anticancer properties of the main-group metal complexes are limited. Recent studies have shown that a series of main-group metal complexes displays potent *in vitro* or *in vivo* antitumour activities,[4] in some cases being more effective than cisplatin in *in vitro* tests.[5,6]

The synthesis, crystal structure and cytotoxic activity of compounds containing multiple coordination with heavier Group 15 (such as Sb, Bi) elements have been among the most exciting targets in the chemistry of main-group elements.[7,8] As a contribution towards understanding the stereochemistry of nucleophilic substitution at tetrahedral Group 15 centers,[9] the solid-state geometries of all the known five or six coordinated dioxo cyclometallates are presented. Thereby motivated, we herein make use of a chiral (±)-mandelic acid ligand to coordinate the triarylantimony ion in the +V oxidation state and have prepared a series of novel tetranuclear triarylantimony(v) complexes with $(Sb_4(\mu\text{-}OCO)_4)$ geometry. These complexes are found to exhibit prominent cytotoxic activities *in vitro*. They are designed to have three bound aryl groups with or without fluoro-substituents, which are subsequently found to be crucial for their cytotoxic activities. In addition, the DNA-binding properties of these complexes with calf thymus DNA (CT-DNA) have also been investigated by fluorescence spectroscopy.

The triarylantimony complexes used in this study are shown in Scheme 1, which were synthesized by reactions of triarylantimony dichlorides (denoted as **a**: $\mathbf{a_1}$–$\mathbf{a_4}$ hereafter) and the (±)-mandelic acid ligand (denoted as **b** hereafter) in the presence of sodium ethoxide, and the fluoro-substituted triarylantimony dichloride was prepared according to the modified literature method (see Scheme S1 and Fig. S1 in ESI†).[10] All the titled complexes (denoted as **c**: $\mathbf{c_1}$–$\mathbf{c_4}$ hereafter) were characterized by ^{1}H and ^{13}C NMR, elemental analysis. The ^{1}H and ^{13}C NMR spectra of $\mathbf{c_1}$–$\mathbf{c_4}$ revealed one set of sharp signals corresponding to ligand **b**, showing the presence of only one species in $CDCl_3$ solution. However, the electrospray ionization mass spectra (ESI-MS) of $\mathbf{c_1}$–$\mathbf{c_4}$ show that complexes **1**–**4** split into several fragments and the ligands depart from $(R3)_3Sb$



Scheme 1 Preparation of the compounds studied.

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†Electronic supplementary information (ESI) available: Synthesis and characterization of complexes **c1**–**c4**; cytotoxicity experiments and competitive ethidium displacement experiments; ESI figures/table; references. CCDC 888519, 931285, 905087, 931642, 915546, 915547 and 915548. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3dt50221j

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and leave vacant coordinative positions in methanol compared to those in the crystal structures of $\mathbf{c_1}$–$\mathbf{c_4}$. In addition, molecular structures of $\mathbf{c_1}$–$\mathbf{c_4}$ were unambiguously determined by the single-crystal X-ray diffraction analysis (Fig. 1–4) together with the partial atomic numbering scheme. The compounds crystallized in different space groups although with similar geometry structures.

Usually, both triaryl antimony dicarboxy esters[11] and cyclometallate compounds[12] could be prepared by the metathetical reaction of the dihalide $SbAr_3X_2$ (X-Br or Cl) with the corresponding α-hydroxy carboxylic ligands under the different reaction conditions. Excitingly, by the common method we obtain compounds $\mathbf{c_1}$–$\mathbf{c_4}$, which contain two interactions at the same time, that is, di-carboxy esters and 1,3-transannular interactions, with the central Sb atoms hexa-coordinated. So far, the vast majority of the Sb(III and V) complexes tested are mononuclear. Although tetranuclear or polymeric species have also been investigated,[13,14] no examples of organoantimony have been reported ($Sb_4(\mu\text{-}OCO)_4$).

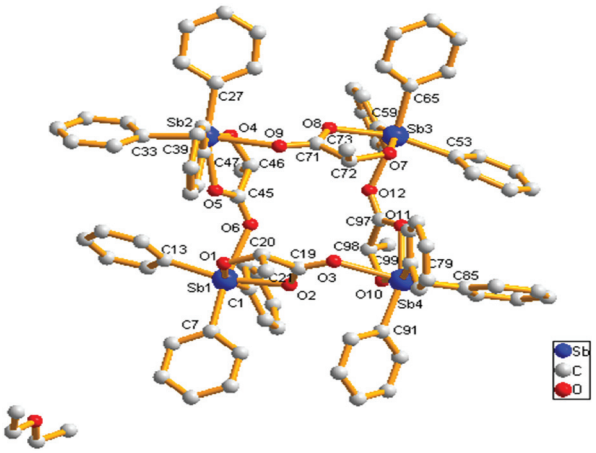

Fig. 1 Molecular structure of complex $\mathbf{c_1}$. Hydrogen atoms are omitted and the phenyl group of ligand **b** is replaced by a carbon atom for clarity.

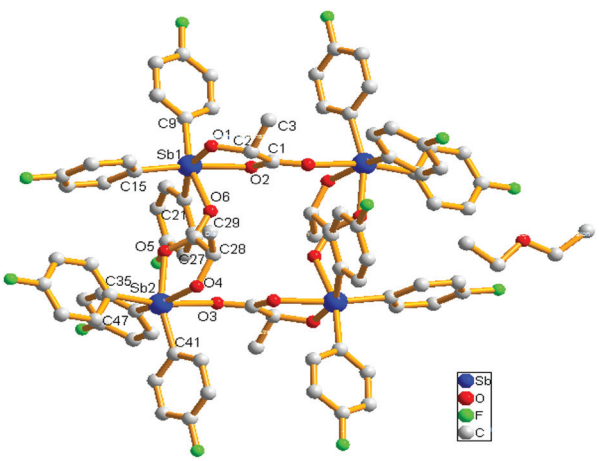

Fig. 2 Molecular structure of complex $\mathbf{c_2}$.

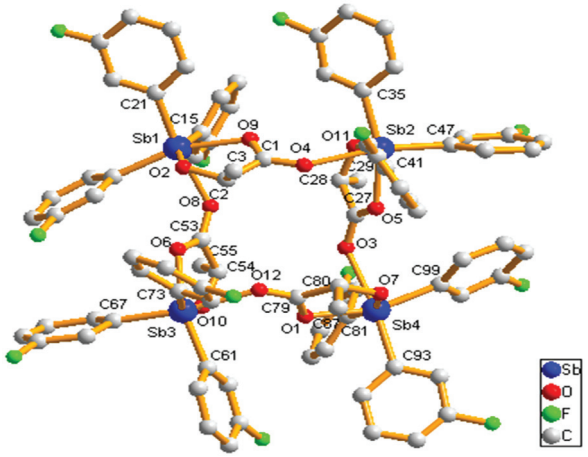

Fig. 3 Molecular structure of complex $\mathbf{c_3}$.

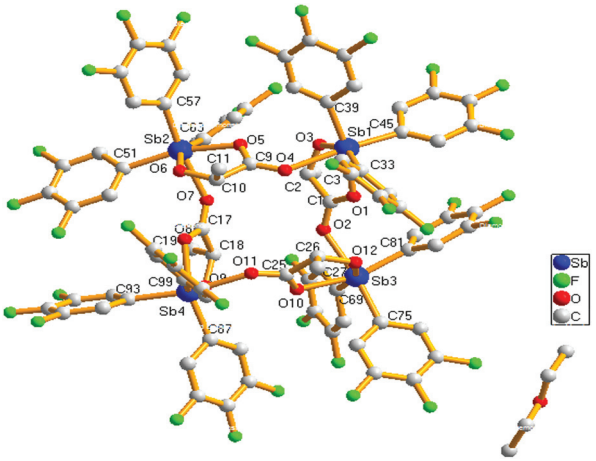

Fig. 4 Molecular structure of complex $\mathbf{c_4}$.

The unit cell of the complexes, except for $\mathbf{c_3}$, contains one independent molecule of **c** (Scheme 1) and one ethyl ether molecule. We take complex $\mathbf{c_1}$ as an example to describe their structural characteristics. It is composed of four antimony metal centers, in which each one is bonded to three phenyl ligands and coordinated to the tridentate $O_2CC(O)(H)Ph$ moiety. Through the 1,3-transannular interaction, it forms four similar cyclometallations (Scheme 1). The central part of the structure remains essentially planar (the coordination environment of Sb2 for example), as the antimony atom is only slightly bent out of the plane defined by O4–C46–C45–O5 atoms (0.4257 (0.0118) Å). During the di-carboxy ester interaction, it forms a chelate ring, which contains sixteen members (Scheme 2). Representing the μ-OCO group as M, the stereostructure of the chelate ring, that is, boat conformation, is presented evidently.[14] The outside of the compact chelate ring is protected by phenyl groups. Therefore, there are no close intermolecular contacts with other cyclic molecules or with ethyl ether molecules. The high solubility and the relatively high stability of $\mathbf{c_1}$ may be explained by the coordinative

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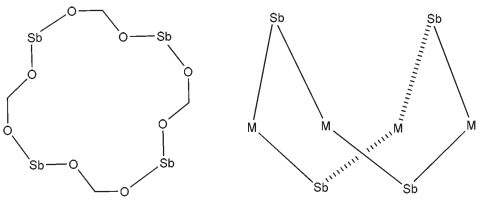

Scheme 2 The chelate ring structure of compounds $\mathbf{c_1}$–$\mathbf{c_4}$.

saturation of the atoms in the ring and their shielding by the phenyl groups.

In many cases, the polynuclear backbones of molecular organometallic compounds can indeed be regarded as fragments that are cut off from a solid-state network.[15] The chelate ring framework structure of compound $\mathbf{c_1}$ is dictated by the bonding abilities of the oxygen atoms located at the corners of the metal-centered polyhedral building blocks shown in Scheme 2. In the formation of the solid-state structure, oxygen plays a structure-directive role. The final outcome of the structure is dictated by the Sb–M–Sb bond angles that determine how the polyhedral units can be assembled into polynuclear structures. Since the Sb–M–Sb bond angles are quite flexible, a great structural diversity is possible.

By means of MTT assays,[16,17] the *in vitro* cytotoxicities of complexes $\mathbf{c_1}$–$\mathbf{c_4}$ along with their metal salts $\mathbf{a_1}$–$\mathbf{a_4}$, the free ligand **b** and the clinical antitumour agent cisplatin towards a panel of established human cancer cell lines including lung carcinoma (A549), colon carcinoma (HCT-116) and colon adenocarcinoma (Caco-2), which are well-known cisplatin-resistant cancer cell lines, were measured. Because most of the compounds showed inhibition activity at the concentration of >10 μM, herein biological results were merely listed at the concentration of 10 μM in Fig. S2 in ESI.† In the meantime, the IC_{50} (half maximal inhibitory concentration) values for compounds $\mathbf{c_1}$–$\mathbf{c_4}$ obtained after 48 h of drug treatment were calculated from the dose–survival curves, which are summarized in Table 1. The viability assay shows that the cell proliferation can also be inhibited by Sb(v) chloride salts to some extent, which exhibit a relatively weak activity, and for the ligand, it is non-active absolutely to the determined cancer cells. However, the cytotoxic activities of complexes $\mathbf{c_1}$–$\mathbf{c_4}$ are markedly improved, possibly due to the cooperative interaction of their Sb(v) precursors and the ligand. In contrast, cisplatin is less cytotoxic to these cells (IC_{50} > 100 μM).

Additionally, we chose four aryl groups, phenyl, 4-fluorophenyl, 3-fluorophenyl and 3,4,5-trifluorophenyl, as organic ligands bound with antimony centers in Sb(v) complexes to investigate the electronic influence on their cytotoxic activities. As we can see, complex $\mathbf{c_4}$ exhibits the highest activity compared with other complexes, and complex $\mathbf{c_2}$, which has fluorine atoms in the *para*-position, shows better activity than complex $\mathbf{c_3}$ with fluorine atoms in the *meta*-position. This means that the cytotoxicity tends to increase as the quantity and the electron-withdrawing ability of F atom increase, that is, 3,4,5-3F-substituted > 4-F-substituted > 3-F-substituted.

Using the rat hepatocytes cell line (BRL), the cytotoxicity of all complexes and their precursors to non-cancerous originated cells were also examined. Among all the complexes, only $\mathbf{c_1}$ is less cytotoxic towards the rat hepatocytes cells than towards the examined cancerous cells. Meanwhile, for $\mathbf{c_1}$, the cytotoxicity is decreased after the formation of the organoantimony(v) compound compared to their Sb(v) salt precursor. On the other hand, the participation of fluorine atoms exacerbates the lethality of the complexes to normal cells. Fluoro-substituted complexes $\mathbf{c_2}$–$\mathbf{c_4}$ all exhibit higher cytotoxicity to the normal cells. Therefore, to reduce their toxicity towards normal cells, and to ensure high cytotoxic activity of fluorinated complexes, will be the focus of future research.

DNA is a key biological target for many metal-based antitumour drugs, and distortions and damage of DNA structures are often associated with anticancer activity.[18] Thus, it is important to understand the DNA binding of complexes and their possible relationship to cytotoxicity in tumour cell lines.[19] Polynuclear organoantimony(v) species may exhibit a higher cytotoxic activity than related mononuclear compounds, possibly due to their ability to interact with DNA. The interaction of compounds $\mathbf{c_1}$–$\mathbf{c_4}$ with DNA has been evaluated by the ethidium bromide (EB)–DNA system with limited EB bound to excess of DNA, which can be used to distinguish intercalating and non-intercalative ligands.[20] Competitive binding of other intercalators leads to a loss of fluorescence because of the depletion of the EB–DNA complex.[21] As shown in Fig. 5 and Fig. S3 in ESI,† the fluorescent intensity of EB in the bound form is remarkably quenched upon adding $\mathbf{c_1}$–$\mathbf{c_4}$, respectively. The Stern–Volmer DNA binding constant (K_{SV}) is evaluated to be 0.58, 1.50, 0.47, and 0.18 for complexes $\mathbf{c_4}$, $\mathbf{c_3}$, $\mathbf{c_2}$, and $\mathbf{c_1}$, respectively, which are in accordance with the extent of displacement of EB by the complexes. Complexes $\mathbf{c_2}$–$\mathbf{c_4}$ show higher K_{SV} values. These results show that the position and quantity of fluoro-substituents of the metal salts play an important role in the DNA binding ability of complexes besides their molecular structures and the Sb atom. In addition, we also determined the DNA binding properties of four organoantimony salts ($\mathbf{a_1}$–$\mathbf{a_4}$) and the free ligand **b** (see Fig. S3 in ESI†). $\mathbf{a_3}$ followed the same trend as the four complexes. Especially, the fluorescence intensity of EB–DNA after adding $\mathbf{a_1}$, $\mathbf{a_2}$, $\mathbf{a_4}$ and **b** became intricate unexpectedly. On the basis of all of the fluorescence studies, we conclude that

Table 1 IC_{50} (μM) of all compounds against three human tumour cell lines and one normal cell line

	A549	HCT-116	Caco-2	BRL
$\mathbf{c_1}$	3.7 ± 0.4	6.4 ± 0.6	17.0 ± 0.7	30.7 ± 1.1
$\mathbf{c_2}$	3.7 ± 0.5	3.0 ± 0.5	4.2 ± 0.6	6.6 ± 0.5
$\mathbf{c_3}$	7.2 ± 0.6	3.7 ± 0.5	11.6 ± 0.5	7.0 ± 0.5
$\mathbf{c_4}$	1.8 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	3.7 ± 0.3
$\mathbf{a_1}$	36.6 ± 2.9	45.0 ± 3.2	40.5 ± 3.2	18.0 ± 2.2
$\mathbf{a_2}$	25.0 ± 2.4	24.0 ± 2.1	26.6 ± 2.7	22.8 ± 2.0
$\mathbf{a_3}$	26.3 ± 2.6	20.0 ± 2.0	29.8 ± 2.3	19.1 ± 1.9
$\mathbf{a_4}$	25.6 ± 2.0	12.2 ± 1.4	26.5 ± 2.0	11.4 ± 1.6
b	>100	>100	>100	>100
Cisplatin	>100	>100	>100	>100

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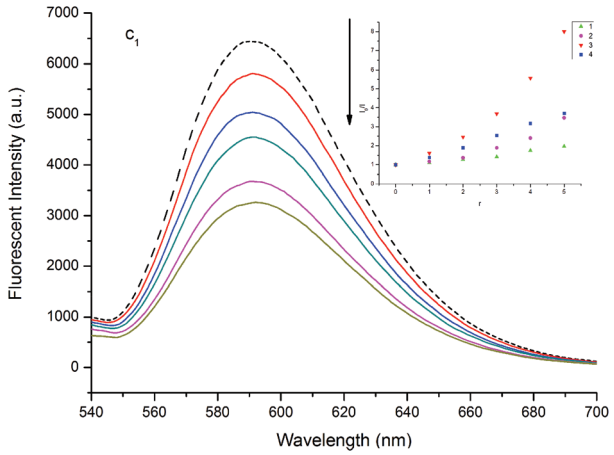

Fig. 5 Emission spectra from EB bound DNA in the absence (---) and in the presence of [complex $\mathbf{c_1}$] 0–125 μM concentration, [DNA] 25 μM and [EB] 3 μM. The arrow shows changes in the emission intensity upon addition of increasing concentration of the complex. Inset: plots of I_0/I *vs.* r (r = [Complex]/[DNA]) with experimental data points. λ_{ex} = 258 nm.

organoantimony(v) complexes $\mathbf{c_1}$–$\mathbf{c_4}$ and metal salt $\mathbf{a_3}$ can bind to CT-DNA in an intercalative mode, but the interactions between DNA and $\mathbf{a_1}$, $\mathbf{a_2}$, $\mathbf{a_4}$ and **b** are more complicated.

In short, the present article describes the syntheses and structural characterization of four new complexes of the type $[Sb_4(\mu\text{-}OCO)_4]$ containing (±)-mandelic acid ligands. Preliminary *in vitro* cytotoxic studies reveal that they display remarkably high cytotoxicity toward platin-resistant cancer cell lines. In addition, this is a significant report on the DNA-binding properties of organoantimony(v) complexes, which could bind with CT-DNA *via* an intercalative mode, and it supports the conclusion that DNA is the target for the antimony(v) complexes $\mathbf{c_1}$–$\mathbf{c_4}$ and the interaction intensity is influenced by the position and quantity of fluoro-substitutents on antimony(v) complexes. These results may provide the grounds for establishing new structure–pharmacological activity relationships for DNA-targeting complexes as novel anticancer drugs.

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