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Synthesis, crystal structures and in vitro antitumor activities of some organoantimony arylhydroxamates

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Abstract

A series of novel organoantimony arylhydroxamates with the formulae $[Ar_3SbL_2]^-[HNEt_3]^+$ (LH = arylhydroxamic acid; Ar = C_6H_5, 4-$CH_3C_6H_4$, 3-$CH_3C_6H_4$, 4-ClC_6H_4, 4-FC_6H_4) and $(4\text{-}CH_3C_6H_4)_4SbL$ were synthesized and characterized by elemental analysis, IR, ^1H NMR and mass spectroscopy. The crystal structures of $(4\text{-}CH_3C_6H_4)_4SbL$ and $[Ph_3SbL_2]^-[HNEt_3]^+$ were determined by X-ray diffraction. The in vitro antitumor activities of all compounds against three human cancer cells are reported.

Keywords: Organoantimony; Arylhydroxamic acid; Crystal structures; Antitumor activity

1. Introduction

A great number of references describing synthesis and biological activities of organoantimony carboxylates with the general formula $Ar_nSbX_{(5-n)}$ ($n = 3, 4$; X = carboxylate) have already appeared in the literature [1–8]. However, the published data on the antitumor activity of these compounds are relatively limitied [9,10]. In recent years some organoantimony (III) derivatives have been reported to exhibit marginal antitumor activities [11–14], at the same time we have found that some organoantimony (V) derivatives exhibit high in vitro antitumor activity against human tumor cell lines and often higher than cisplatin [15–17]. Arylhydroxamic acids are important ligands, because they are often included in the active sites of some biological enzymes [18] and have a wide range of biological activity, including antitumor activity [19–22]. Therefore, we have synthesized a series of arylantimony derivatives of arylhydroxamic acid in order to investigate whether including arylhydroxamate groups in organoantimony (V) derivatives can improve their antitumor properties. In addition, we are also interested in studying the nature of bonding and the structure of these compounds.

2. Results and discussion

2.1. Preparations

The title compounds are synthesized under anhydrous condition. All compounds are white crystals and stable under ordinary conditions. They are soluble in organic solvents such as THF, dichloromethane, chloroform, acetone, methanol and dimethyl sulfoxide, but not soluble in benzene, hexane and petroleum ether. The general reaction is shown as follows:

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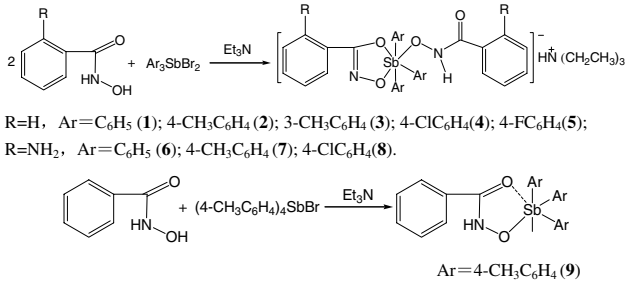


R=H, Ar=C_6H_5 (**1**); 4-$CH_3C_6H_4$ (**2**); 3-$CH_3C_6H_4$ (**3**); 4-ClC_6H_4(**4**); 4-FC_6H_4(**5**);
R=NH_2, Ar=C_6H_5 (**6**); 4-$CH_3C_6H_4$ (**7**); 4-ClC_6H_4(**8**).

Ar=4-$CH_3C_6H_4$ (**9**)

2.2. IR

The IR spectra of these compounds have been recorded in the range of 4000–400 cm^{-1}. The important IR data of the free ligands and the title compounds are summarized in Table 1. The IR spectroscopic data provide further support for the molecular constitution of the title compounds. In majority of organoantimony (V) compounds the antimony has generally a coordination number of five. Because the vacant 5d orbital of antimony atom can accept lone electron pairs of ligands, in some cases the antimony may have a coordination number of six [23,24] or seven [9,25]. When there are interactions between the antimony atom and the carbonyl oxygen atom of the hydroxamate group, the absorption vibration frequencies [ν(C=O)] decrease. In the title compounds, the very strong stretching vibrations ν(C=O) is displaced to lower frequency between 1615 and 1628 cm^{-1}, so we can assume that there are coordination interactions between the antimony atom and the carbonyl oxygen atom of the hydroxamate group (see the crystal structures of compounds **1** and **9**). In addition, the frequencies of Sb–C deformations appear between 453 and 504 cm^{-1} which is consistent with the literature [6].

2.3. ^1H NMR

The ^1H NMR data of the title compounds are listed in Table 2. The chemical shifts of the protons of CH_3 appear between 0.92 and 1.05 ppm. The protons of CH_2 appear between 2.61 and 2.82 ppm. The protons of Ar show a complex multiplet between 6.51 and 8.00 ppm. The amino hydrogen is so active that its displacement cannot be assigned. All the protons in the compounds have been identified and the total number of protons calculated from the integration curve tallies with what was expected from the molecular formula.

Table 1
IR data of the compounds (cm^{-1})

Compound	ν(C=O)	ν(N–O)	ν(Sb–C)
1	1622	907	460
2	1622	906	487
3	1629	909	471
4	1624	905	489
5	1628	907	510
6	1619	907	464
7	1621	907	485
8	1615	907	491
9	1595	907	489
PhCONHOH	1639	898	
2-$NH_2C_6H_4$CONHOH	1632	903	

2.4. Mass spectra

The main mass spectra data of compound **1** are listed in Table 3. Although there is no molecular ion peak, the fragment ions found are in agreement with the expected structure of the compound. The $PhCO^+$ (m/z = 105) is the base peak. The breakdown of Sb–O and Sb–C bonds are the principle breakdown patterns for the compound.

2.5. Crystal structures

2.5.1. Crystal structure of compound *1*

The colorless crystals of compound **1** were obtained from CH_2Cl_2–petroleum ether. One of the approximate dimensions $0.18 \times 0.14 \times 0.10$ mm was mounted in a glass capillary and used for data collection. Fig. 1 shows the molecular structure of compound **1** and gives the atom numbering scheme. The selected bond distances and angles are listed in Table 4.

Hydroxamates are versatile ligands which can be either unidentate or bidentate. Antimony–oxygen bond lengths in organoantimony compounds are extremely

Table 2
^1H NMR data of the compounds

Compound	Ar	CH_2	CH_3
1	7.31–8.00 (25 H, m)	2.70–2.78 (6 H, q)	0.99–1.04 (9 H, t)
2	7.15–7.96 (22 H, m) 2.32 (9 H, s)	2.67–2.74 (6 H, q)	0.96–1.01 (9 H, t)
3	7.15–7.95 (22 H, m) 2.27 (9 H, s)	2.72–2.80 (6 H, q)	0.98–1.03 (9 H, t)
4	7.33–7.96 (22 H, m)	2.75–2.82 (6 H, q)	0.99–1.03 (9 H, t)
5	7.04–7.97 (22 H, m)	2.74–2.81 (6 H, q)	0.98–1.03 (9 H, t)
6	6.51–7.90 (23 H, m)	2.71–2.78 (6 H, q)	1.00–1.05 (9 H, t)
7	6.64–7.90 (20 H, m) 2.37 (9 H, s)	2.61–2.69 (6 H, q)	0.98–1.03 (9 H, t)
8	6.52–7.81 (20 H, m)	2.70–2.77 (6 H, q)	0.92–0.97 (9 H, t)
9	7.03–7.45(21 H, m) 2.28 (12 H, s)		

Table 3
Fragment ions observed for compound **1**

m/z	Fragment	Intensity (%)
490	$[Ph_3Sb(ONHOCPh)]^+$	4.2
488	$[Ph_3Sb(ONHOCPh)]^+$	5.8
489	$[(PhCONO)SbPh_3]^+$	16.6
487	$[(PhCONO)SbPh_3]^+$	21.4
354	Ph_3Sb^+	9.6
352	Ph_3Sb^+	13.4
277	Ph_2Sb^+	8.0
275	Ph_2Sb^+	17.0
200	$PhSb^+$	63.6
198	$PhSb^+$	79.0
105	$PhCO^+$	100

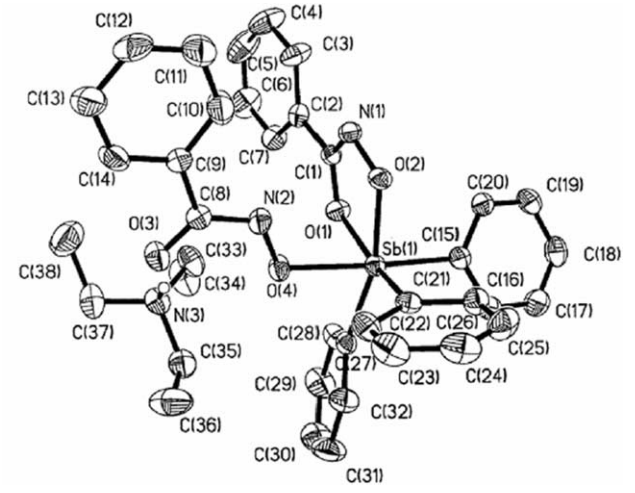


Fig. 1. The molecular structure of compound **1**.

Table 4
Selected bond distances (Å) and bond angles (°) of compound **1**

Bond	Distance (Å)	Bond	Angle (°)
Sb(1)–O(1)	2.099(3)	O(1)–Sb(1)–O(2)	76.73(14)
Sb(1)–O(2)	2.050(2)	O(1)–Sb(1)–O(4)	82.93(13)
Sb(1)–O(4)	2.143(3)	O(1)–Sb(1)–C(15)	87.97(17)
Sb(1)–C(15)	2.165(6)	O(1)–Sb(1)–C(27)	91.01(14)
Sb(1)–C(21)	2.147(5)	O(1)–Sb(1)–C(21)	162.12(16)
Sb(1)–C(27)	2.144(4)	O(2)–Sb(1)–O(4)	86.00(12)
N(1)–C(1)	1.294(5)	O(2)–Sb(1)–C(21)	86.48(18)
N(1)–O(2)	1.421(4)	O(2)–Sb(1)–C(15)	95.18(17)
N(2)–C(8)	1.307(6)	O(2)–Sb(1)–C(27)	163.93(12)
N(2)–O(4)	1.372(4)	O(4)–Sb(1)–C(27)	82.16(14)
N(2)–H(2)	0.860(6)	O(4)–Sb(1)–C(21)	89.89(16)
O(1)–C(1)	1.317(5)	O(4)–Sb(1)–C(15)	170.31(17)
O(3)–C(8)	1.244(5)	C(15)–Sb(1)–C(21)	99.8(2)
		C(15)–Sb(1)–C(27)	94.72(19)
		C(21)–Sb(1)–C(27)	104.25(18)
		C(1)–N(1)–O(2)	111.7(3)
		C(8)–N(2)–O(4)	121.6(4)
		C(1)–O(1)–Sb(1)	111.1(3)
		N(1)–O(2)–Sb(1)	115.2(2)
		N(2)–O(4)–Sb(1)	116.9(3)

variable [26], ranging from 1.935 Å in $(Ph_3SbO)_2$ [27] to 2.506 Å in Ph_4SbOSO_2Ph [3], and the Sb(1)–O(1), Sb(1)–O(2) and Sb(1)–O(4) distances [2.099(3),

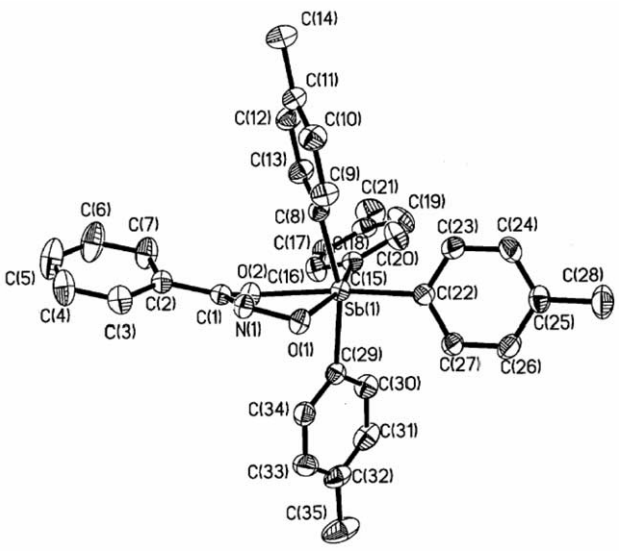


Fig. 2. The molecular structure of compound **9**.

2.050(2) and 2.143(3) Å, respectively] in compound **1** both lie within this rang. However, the Sb(1)–O(3) distance [4.831(4) Å] is rather longer than the sum of van der Waals' radii of antimony and oxygen atom (2.2 and 1.4 Å, respectively) [28]. This indicates that there is no interaction between Sb(1) and O(3), while there is a bond between Sb(1) and O(1) which makes the coordination geometry of antimony to be converted from a trigonal-bipyramid to a distorted octahedron. The atoms Sb(1), O(1), O(2), C(21) and C(27) are coplanar within 0.0561 Å, the atoms O(1), O(2), C(21) and C(27) occupy the equatorial positions. The O(4)–Sb(1)–C(15) angle is 170.31(17)°, the atoms O(4) and C(15) occupy the axial positions. The antimony atom of the compound is six-coordinate. Therefore, the coordination geometry of antimony can be described as a distorted octahedron.

2.5.2. Crystal structure of compound **9**

A colorless crystals of compound **9** was obtained from CH_2Cl_2–petroleum ether solution. The molecular structures of compound **9** with the atom numbering scheme is depicted in Fig. 2. The selected bond distances and angles of the compound are listed in Table 5.

The crystal structure of compound **9** can be reported as monomer. The most important feature in this structure is the very strong secondary interaction between antimony atom and the formally non-bonded oxygen of the carbonyl group. This is the very interaction which makes the coordination geometry of antimony to be converted from a trigonal-bipyramid to a distorted octahedron.

The Sb(1)–O(1) and Sb(1)–O(2) distances [2.201(2) and 2.299(2) Å, respectively] are different from those in $CH_3CO_2SbPh_4$ [2.235(4) and 2.585(5) Å, respectively] [29]. This indicates that there is very strong coordination interaction between the carbonyl oxygen and the

Table 5
Selected bond distances (Å) and bond angles (°) of compound **9**

Bond	Distance (Å)	Bond	Angle (°)
Sb(1)–O(1)	2.201(2)	C(22)–Sb(1)–O(2)	167.37(9)
Sb(1)–O(2)	2.299(2)	C(22)–Sb(1)–O(1)	95.26(9)
Sb(1)–C(22)	2.148(3)	C(29)–Sb(1)–C(8)	162.15(11)
Sb(1)–C(15)	2.157(3)	C(15)–Sb(1)–C(29)	93.97(11)
Sb(1)–C(29)	2.168(3)	C(15)–Sb(1)–C(8)	94.23(11)
Sb(1)–C(8)	2.178(3)	C(22)–Sb(1)–C(8)	97.40(11)
N(1)–O(1)	1.370(3)	C(22)–Sb(1)–C(15)	102.34(12)
N(1)–C(1)	1.319(3)	C(22)–Sb(1)–C(29)	96.29(11)
N(1)–H(1)	0.860(3)	C(8)–Sb(1)–O(1)	82.57(10)
C(1)–O(2)	1.271(3)	C(29)–Sb(1)–O(1)	84.82(10)
C(1)–C(2)	1.473(4)	C(15)–Sb(1)–O(1)	162.38(10)
C(11)–C(14)	1.506(5)	C(8)–Sb(1)–O(2)	82.43(9)
C(25)–C(28)	1.495(5)	C(15)–Sb(1)–O(2)	90.26(10)
C(32)–C(35)	1.509(5)	C(29)–Sb(1)–O(2)	81.71(10)
		O(1)–Sb(1)–O(2)	72.16(7)
		N(1)–O(1)–Sb(1)	109.66(14)
		C(1)–O(2)–Sb(1)	111.06(17)
		C(1)–N(1)–O(1)	121.0(2)
		O(2)–C(1)–N(1)	119.4(3)
		O(2)–C(1)–C(2)	119.9(3)
		N(1)–C(1)–C(2)	120.6(3)

Table 6
Antitumor activity of all compounds in vitro

Compound	Inhibition ratio (%) (10 μM)[a]		
	HL-60	BGC-823	MDA-MB-435
1	17.1	32.2	26.1
2	20.1	17.6	34.7
3	11.0	25.1	40.3
4	9.4	17.9	33.4
5	12.6	15.5	22.0
6	24.0	39.1	25.1
7	29.7	38.5	31.0
8	33.3	55.8	37.6
9	82.3	85.1	89.5
PhCONOH	28.3	34.5	30.3
2-$NH_2C_6H_4CONOH$	30.1	34.0	26.2
$(4\text{-}CH_3C_6H_4)_3SbBr_2$	31.4	31.5	31.4
Cisplatin[b]	45.4	90.6	57.5

[a] Inhibition ratio (%) = $(A_1 - A_2)/A_1 \times 100\%$. Drug is active when inhibition ratio at 10 μM concentration is $\geqslant 50\%$. A_1: the mean optical density of untreated cells. A_2: the mean optical density of drug-treated cells. Negative values indicate that the mean optical density of drug-treated cells (A_2) is greater than that of untreated cells (A_1), i.e. the drug promoted growth of some tumor cells.
[b] Whose concentration is 33 μM.

antimony atom. This interaction leads to a large variation of the three equatorial angles of compound **9**, the C(8)–Sb(1)–C(29) angle is increased to 162.15(11)°, while the C(8)–Sb(1)–C(15) and C(15)–Sb(1)–C(29) angles are decreased to 94.23(11)° and 93.97(11)°, respectively. The Sb(1) is displaced by 0.0986 Å towards C(22) from the plane defined by the equatorial carbon atoms C(8), C(15) and C(29). The atoms Sb(1), O(1), O(2), C(15) and C(22) are coplanar within 0.0156 Å, the atoms O(1), O(2), C(15) and C(22) occupy the epuatorial positions. The C(8)–Sb(1)–C(29) angle is 162.15(11)°, the atoms C(8) and C(29) occupy the axial positions. The antimony atom of the compound is six-coordinate. Therefore, the coordination geometry of antimony can be described as a distorted octahedron.

2.6. Antitumor activity

The antitumor activity data of all compounds are listed in Table 6. The results of bioassay show that these compounds exhibit antitumor activities against the three human cancer cells in vitro. When the arylhydroxamate is 2-$NH_2C_6H_4CONO$, compounds **6**–**8** have relatively higher antitumor activities against HL-60 and BGC-823 cells than compounds **1**–**5**. The antitumor activities are also affected by the nature of the aryl at Sb, for example, when Ar is 4-ClC_6H_4, compound **8** is rather more potent against BGC-823 cells. In addition, the tetraarylantimony benzohydroxamate, namely compound **9**, has much higher antitumor activity against the three human cancer cells than the triarylantimony benzohydroxamates. When comparing with cisplatin, compound **9** has also higher antitumor activity.

3. Experimental

Elemental analyses were determined on a Yanaco CHN Corder MT-3 elemental analyzer. IR spectra were recorded on a Bruker Equinox 55 spectrometer in KBr discs. ^1H NMR spectra were measured on a Bruker AC-300 spectrometer in $CDCl_3$ solution with TMS as

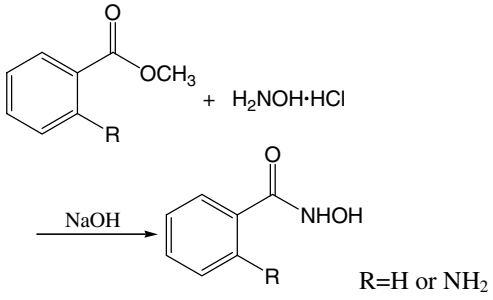
Table 7
Yields and elemental analyses of the compounds

Compound	Yield (%)	M.p. (°C)	Elemental analysis: Found (Calc.) (%)			
			C	H	N	Formula for calculated
1	82.8	148–150	62.61 (62.82)	5.35 (5.83)	6.01 (5.78)	$C_{38}H_{42}N_3O_4Sb$
2	58.9	100–102	61.42 (61.45)	5.43 (6.10)	5.27 (5.18)	$C_{41}H_{48}N_3O_4Sb \cdot 0.5CH_2Cl_2$
3	57.3	118–120	63.66 (64.07)	5.63 (6.29)	6.30 (5.47)	$C_{41}H_{48}N_3O_4Sb$
4	78.4	124–126	54.92 (55.00)	4.81 (4.74)	5.22 (5.06)	$C_{38}H_{39}Cl_3N_3O_4Sb$
5	65.4	162–164	58.42 (58.48)	4.95 (5.04)	5.49 (5.38)	$C_{38}H_{39}F_3\ N_3O_4Sb$
6	66.2	102–104	56.92 (56.74)	5.83 (5.59)	8.53 (8.54)	$C_{38}H_{44}N_5O_4Sb \cdot 0.75CH_2Cl_2$
7	69.8	99–100	61.77 (61.66)	6.40 (6.31)	8.78 (8.77)	$C_{41}H_{50}N_5O_4Sb$
8	39.6	112–114	53.10 (53.08)	4.88 (4.81)	8.08 (8.14)	$C_{38}H_{41}Cl_3N_5O_4Sb$
9	86.8	194–196	67.63 (67.54)	5.60 (5.51)	2.29 (2.25)	$C_{35}H_{34}NO_2Sb$

internal standard. Mass spectra were recorded on a TRACE DSQ mass spectrometer (EI-MS). All the reactions involving metal halides were carried out under anhydrous. Solvents were purified, dried, and stored by the literature methods.

3.1. Reagents

The arylhydroxamic acids were prepared from the corresponding esters via the following reaction [21]. Ar_3SbBr_2 was prepared by the method reported by Lile and Menzies [7], and the solid product was recrystallized from toluene–petroleum ether mixture. To prepare Ar_4SbBr, an adaptation of the method of McEwen et al. [8] was used.



3.2. Synthesis of the title compounds

The title compounds were synthesized by more convenient method. Ar_3SbBr_2 (0.5 mmol) or Ar_4SbBr (1 mmol) was added to a solution of arylhydroxamic acid (1 mmol) in 30 ml THF and 1 ml Et_3N. The reaction mixture was stirred at r.t. for 8 h, cooled and filtered. The filtrate was evaporated in vacuo and the petroleum was added. The obtained solid was recrystallized from CH_2Cl_2–petroleum ether to afford the title compounds. The yields, melting points and elemental analysis of the prepared compounds are given in Table 7.

3.3. Crystal structure determination

Diffraction measurements of compounds **1** and **9** were carried out at 293 K on a Bruker Smart 1000 diffractometer (graphite-monochromatized Mo Kα radiation, $\lambda = 0.71073$ Å). The crystal class, orientation matrix and accurate unit-cell parameters were determined by standard procedures. The intensities were corrected for absorption using SADABS program. The structure was solved by heavy atom method and refined by a full-matrix least-square procedure based on F^2. Non-hydrogen atoms were

Table 8
Crystallographic data for compounds **1** and **9**

Compound	**1**	**9**
Formula	$C_{38}H_{42}N_3O_4Sb$	$C_{35}H_{34}NO_2Sb$
Formula weight	726.50	622.38
T (K)	293(2)	293(2)
λ (Å)	0.71073	0.71073
Crystal system	Monoclinic	Triclinic
Space group	$P2_1$	$P\bar{1}$
Unit cell dimensions		
a (Å)	9.478(3)	10.482(5)
b (Å)	18.489(7)	10.705(5)
c (Å)	10.446(4)	14.152(6)
α (°)	90	80.770(6)
β (°)	105.709(6)	78.125(7)
γ (°)	90	84.893(7)
V (Å^3)	1762.2(11)	1531.5(11)
Z	2	2
Density (Mg m^{-3})	1.369	1.350
Absorption coefficient (mm^{-1})	0.825	0.931
$F(000)$	748	636
Crystal size (mm)	$0.18 \times 0.14 \times 0.10$	$0.26 \times 0.20 \times 0.20$
θ Range for data collection (°)	2.20–26.43	1.49–26.48
Limiting indices	$-11 \leqslant h \leqslant 6$, $-22 \leqslant k \leqslant 23$, $-13 \leqslant l \leqslant 13$	$-8 \leqslant h \leqslant 13$, $-13 \leqslant k \leqslant 13$, $-17 \leqslant l \leqslant 17$
Reflections collected	10254	8894
Independent reflections (R_{int})	6864 (0.0222)	6222 (0.0210)
Completeness to θ	26.43° (99.6%)	26.48° (98.2%)
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Goodness-of-fit on F^2	1.039	1.013
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0340$, $wR_2 = 0.0699$	$R_1 = 0.0341$, $wR_2 = 0.0697$
R indices (all data)	$R_1 = 0.0463$, $wR_2 = 0.0744$	$R_1 = 0.0535$, $wR_2 = 0.0774$
Largest differential peak and hole (e Å^{-3})	0.253 and −0.500	0.543 and −0.326

refined with anisotropic thermal parameters. Crystal data are summarised in Table 8.

3.4. Antitumor activities

All cell lines were derived in the National Research Laboratories of Natural and Biomimetic Drugs of Peking University and grown in RPMI 1640 medium with 10% fetal bovine serum, in 5% CO_2 atmosphere.

The antitumor activity was assayed by the MTT or SRB methods [30,31]. The cell lines, human immature granulocyte leukemia (HL-60), human gastric carcinoma (BGC-823) and human mammary gland carcinoma (MDA-MB-435) were used for the screening. All cell lines were seeded into 96 well plates at a concentration of about 50 000 cells/ml and were incubated in 5% CO_2 atmosphere at 37 °C for 24 h. Then 20 μl of the sample (organoantimony complex) were added and further incubation was carried out at 37 °C for 48 h. 50 μl of 0.1% MTT or SRB (Sigma) was added to each well. After 4 h incubation, the culture medium was removed, and 150 μl of isopropanol was added to dissolve the insoluble blue formazan precipitates produced by MTT reduction. The plate was shaken for 20 min on a plate shaker to ensure complete dissolution. The optical density of each well was measured at 570 nm (MTT) or 540 nm (SRB) wavelength. The antitumor activity was determined three times in independent experiments, using three replicate wells per toxicant concentration (10, 1, 0.1 μg/ml) and obtained the mean optical densities for drug-treated cells at each concentration as a percentage of that of untreated cells.

4. Supplementary material

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 240981 for compound **1** and CCDC No. 240982 for compound **9**. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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