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Synthesis, characterization and *in vitro* cytotoxicity screening of some triarylbismuth(V) di(*N*-salicylidene)amino carboxylates and the crystal structure of $(2\text{-}HOC_6H_4CH{=}NCH_2CO_2)_2Bi(C_6H_5)_3$

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Four novel triarylbismuth(V) di(*N*-salicylidene)amino carboxylates were synthesized. Their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. The crystal structure of $(2\text{-}HOC_6H_4CH{=}NCH_2CO_2)_2BiPh_3$ was determined by X-ray diffraction. The *in vitro* cytotoxicity of all compounds against three human cancer cells (HL-60, BGC-823 and MDA-MB-435) at 10 μM are reported. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: triarylbismuth; *N*-salicylideneamino carboxylates; crystal structure; cytotoxicity

INTRODUCTION

Bismuth compounds have attracted considerable interest owing to their biological activity and medicinal utility.[1–3] Descriptions of the synthesis, structures and applications of R_3BiX_2 (R = alkyl, aryl; X = carboxylate) have appeared in the literature.[4–10] The use of bismuth in medicine has been reviewed by Tiekink.[11] Bismuth salts, such as colloidal bismuth subcitrate (CBS), bismuth subsalicylate (BSS) and ranitidine bismuth citrate (RBC) are now common agents for the treatment of gastrointestinal disorders, such as dyspepsia, diarrhea and peptic ulcer.[12–16] The recent demonstration that these salts are useful for *Helicobacter pylori* eradication therapy has promoted antibacterial and antitumor studies of various bismuth compounds.[17–23] The organism implicated as the pathogen leading to gastric complaint can be eliminated by bismuth therapy.[24–27] Studies have shown that there may be a connection established between antitumor activity and bismuth compounds.[11] Moreover, the potassium salt of *N*-salicylideneamino acid has a wide range of biological activities.[28–30] Therefore, we have prepared four novel triarylbismuth(V) di(*N*-salicylidene)amino carboxylates in order to study the influence of the organic ligands at bismuth on cytotoxicity. In addition, we are also interested in studying the nature of the bonding ability and the structure of these compounds.

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EXPERIMENTAL

General

Elemental analyses were determined on a Yanaco CHN Corder MT-3 elemental analyzer. IR spectra were recorded on a Bruker Equinox 55 spectrophotometer in KBr discs. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC-300 spectrometer in $CDCl_3$ solution with tetramethylsilane as internal standard.

The potassium salts of *N*-salicylideneamino acid (see Fig. 1) were synthesized by the method reported by Heinert and Martell.[31] Ar_3BiCl_2 was prepared by the method reported by Challebger.[5] The solid product was recrystallized from chloroform and methanol mixture.

Synthesis of the title compounds

The potassium salt of *N*-salicylideneamino acid (2 mmol) in 15 ml methanol was dropped into Ar_3BiCl_2 (1 mmol) in 15 ml tetrahydrofuran (THF). The reaction mixture was stirred at room temperature for 6 h and then evaporated to

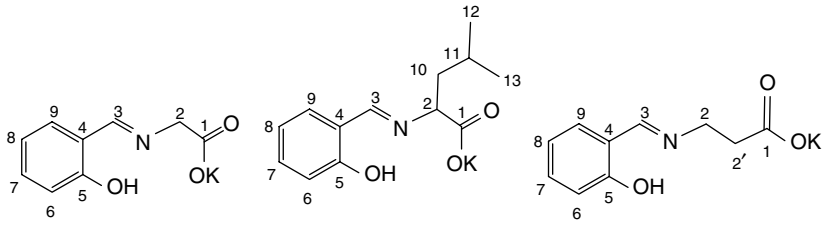
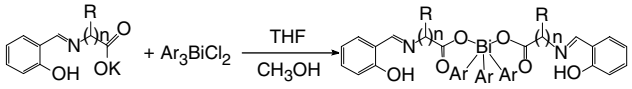


Figure 1. Ligands used in the present work.

dryness *in vacuo*. The solid obtained was recrystallized from CH_2Cl_2–petroleum ether solution. The yields, melting points and elemental analyses of the compounds prepared are given in Table 1.



n=1, R=H, Ar=Ph (1), 4-$CH_3C_6H_4$ (2); R=$CH_2CH(CH_3)_2$, Ar=Ph (3)
n=2, R=H, Ar=Ph (4),

Crystallography

Diffraction measurements for a $0.12 \times 0.16 \times 0.20$ mm^3 sample of **1** was carried out at 298 K on a Bruker Smart 1000 diffractometer (graphite-monochromatized Mo Kα radiation, $\lambda = 0.71073$ Å). Crystal data: $C_{36}H_{31}BiN_2O_6$, $M = 796.61$, monoclinic, $C2/c$, $a = 12.877(4)$, $b = 9.741(3)$, $c = 25.098(7)$ Å, $\alpha = 90$, $\beta = 95.646$, $\gamma = 90°$, $V = 3132.9(16)$ Å^3, $Z = 4$, 2770 unique data (θ_{max} 25.0°), 2652 data with $I \geq 2\sigma(I)$, $R = 0.033$ (obs. data), $wR = 0.060$ (all data). Programs used: SADABS, SHELXL-97, ORTEP. CCDC deposition no.: 240115.

Cytotoxicity screening

The cell lines human immature granulocyte leukemia (HL-60), human gastric carcinoma (BGC-823) and human mammary carcinoma (MDA-MB-435) were used for screening. All cell lines were grown in PRMI 1640 medium with 10% fetal bovine serum, in 5% CO_2 atmosphere.

The cytotoxicity of these compounds was assayed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT)[32] or sulforhodamine B (SRB)[33] methods. All cell lines were seeded into 96-well plates at a all concentration of about 50 000 ml^{-1} and were incubated in 5% CO_2 atmosphere at 37 °C for 24 h. Then, 20 µl of the sample (organobismuth complex) was 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 a wavelength of 570 nm (MTT) or 540 nm (SRB). The cytotoxicity was determined three times in independent experiments, using three replicate wells per toxicant concentration (10, 1, 0.1 µM), and the mean optical densities obtained for drug-treated cells at each concentration as a percentage of that of untreated cells.

RESULTS AND DISCUSSION

The title compounds were prepared under mild conditions. All compounds are buff-colored crystals and stable under ordinary conditions. They are soluble in organic solvents, such as acetone, dichloromethane, chloroform, and dimethyl sulfoxide, but not soluble in ether, hexane, or petroleum ether.

IR

The IR spectra of these compounds have been recorded in the range of 4000–400 cm^{-1}. The absorption bands can be assigned on the basis of earlier publications, and the important data are listed in Table 2.

The IR spectroscopic data provide further support for the molecular constitution of the title compounds. When there are interactions between the bismuth atom and the carbonyl oxygen atom of the carboxylate groups, the asymmetric absorption vibration frequencies ($\nu_{asy}(CO_2)$) of carbonyl

Table 1. Yields and elemental analyses of the compounds

Compound	Yield (%)	M.p. (°C)	Elemental analysis, Found (calc.) (%)			Formula for Calc.
			C	H	N	
1	50.0	140–142	54.21 (54.28)	4.05 (3.92)	3.62 (3.52)	$C_{36}H_{31}BiN_2O_6$
2	63.2	75 dec.	55.72 (55.85)	4.49 (4.45)	3.42 (3.34)	$C_{39}H_{37}BiN_2O_6$
3	86.6	120–122	58.16 (58.15)	5.11 (5.21)	3.26 (3.08)	$C_{44}H_{47}BiN_2O_6$
4	40.0	133–135	55.27 (55.34)	4.46 (4.28)	3.54 (3.40)	$C_{38}H_{35}BiN_2O_6$

Table 2. Important IR data of the compounds (cm^{-1})

Compound	$\nu_{asy}(CO_2)$	$\nu_{sym}(CO_2)$	$\Delta\nu(CO_2)$	ν(Bi–C)
1	1634	1352	282	472
2	1636	1392	244	469
3	1633	1355	278	473
4	1634	1398	236	480

groups decrease and the symmetric absorption vibration frequencies ($\nu_{sym}(CO_2)$) increase. Therefore, their differences ($\Delta\nu(CO_2)$) decrease.[7–9] In the IR spectra of the title compounds the carboxylate bands are observed in the characteristic regions: $\nu_{asy}(CO_2)$ between 1636 and 1633 cm^{-1} and $\nu_{sym}(CO_2)$ between 1398 and 1352 cm^{-1}. On the basis of the whole $\Delta\nu(CO_2)$, these compounds show low $\Delta\nu(CO_2)$ values (between 236 and 282 cm^{-1}). To this we can assume that there are relatively strong interactions between the carbonyl oxygen atoms of the carboxylate groups and the bismuth atom (see Fig. 2). In addition, the vibration frequencies of Bi–C deformations appear between 469 and 480 cm^{-1}, which is consistent with the literature.[34,35]

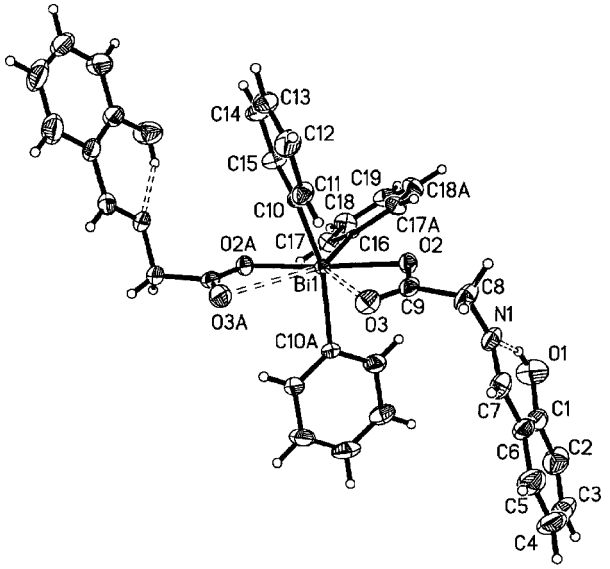


Figure 2. The molecular structure of (2-$HOC_6H_4CH{=}NCH_2CO_2)_2Bi(C_6H_5)_3$.

NMR

The ^1H and ^{13}C NMR data of the title compounds are shown in Tables 3 and 4 respectively. The ^1H and ^{13}C NMR spectra of **1–4** show the expected resonances and integration. The ^1H and ^{13}C NMR chemical shift of these compounds are assigned by the multiplicity patterns and/or resonance intensities.[36] The protons of OH have been observed in the range of δ 13.23–13.47 ppm because of the intramolecular hydrogen bonds O–H···N, and the protons of CH=N are between 8.15 and 8.21 ppm. The protons of Ar show a complex multiplet between 6.82 and 8.18 ppm. All of the protons in the compounds have been identified and the total number of protons calculated from the integration curve tallies with what has been expected from the molecular formula.

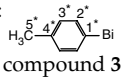
Table 3. ^1H NMR data of the compounds (ppm)

Compound	OH	CH=N	Ar	CH (R)	R
1	13.35 (2H, s)	8.19 (2H, s)	6.85–8.16 (23H, m)	4.17 (4H, s)	H
2	13.40 (2H, s)	8.18 (2H, s)	6.84–8.04 (20H, m), 2.37 (9H, s)	4.15 (4H, s)	H
3	13.47 (2H, s)	8.15 (2H, s)	6.83–8.14 (23H, m)	3.87–3.91 (2H, t)	$(CH_3)_2CHCH_2$ 0.73–1.59 (18H, m)
4	13.23 (2H, s)	8.21 (2H, s)	6.82–8.18 (23H, m)	3.66–3.70 (4H, t)	H
				2.49–2.53 (4H, t)	

Table 4. ^{13}C NMR data of the compounds (ppm)[a]

	Ligand skeleton[b]										Bi–Ar skeleton[c]				
Compound	C1	C2	C2′	C3	C4	C5	C6	C7	C8	C9	C1*	C2*	C3*	C4*	C5*
1	174.2	61.3		166.9	118.4	161.3	118.9	134.2	117.1	131.5	158.5	132.3	131.1	131.4	
2	174.0	61.4		166.9	118.3	161.4	118.9	134.0	117.1	132.0	155.1	141.5	131.4	132.3	21.4
3[d]	176.6	70.7		164.8	118.3	161.4	118.9	134.1	117.1	131.4	158.7	132.2	130.9	131.3	
4	177.5	55.7	36.1	165.6	118.2	161.3	118.8	133.7	116.9	131.1	160.3	132.0	130.6	131.3	

[a] Solvents: saturated $CDCl_3$ solutions were for the compounds.
[b] Refer to Fig. 1 for numbering scheme.
[c] Numbering schemes for Bi–Ar skeleton:



[d] The ^{13}C NMR data of $CH_2CH(CH_3)_2$ of compound **3** are 42.6 (C10), 24.6 (C11), 22.9 (C12) and 21.7 ppm (C13).

Table 5. Cytotoxicity data of the title compounds *in vitro*

Compound	Inhibition ratio (10 μM)[a] (%)		
	HL-60	BGC-823	MDA-MB-435
1	27.8	12.1	44.7
2	59.6	29.1	93.5
3	82.1	43.4	57.6
4	5.5	25.8	27.4
2-$HOC_6H_4CH{=}NCH_2COOK$	1.0	20.4	50.3
Ph_3BiCl_2	−5.0	30.6	41.1
Cisplatin[b]	14.2	16.0	13.8

[a] Inhibition ratio (%) = $(A_1 - A_2)/A_1 \times 100\%$. Drug is active when inhibition ratio at 10 μM concentration is ≥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] Concentration is 3.3 μM.

Cytotoxicity

The cytotoxicity data of the title compounds are listed in Table 5. The results of bioassay show that these compounds exhibit cytotoxicities against the three cancer cells *in vitro*. Compounds **2** and **3** are more potent than 2-$HOC_6H_4CH{=}NCH_2COOK$ or Ph_3BiCl_2. The cytotoxicity data indicate the nature of the aryl potency, e.g. when Ar is 4-$CH_3C_6H_4$, **2** is rather more potent against MDA-MB-435 and HL-60 cells.

Crystal structure of $(2\text{-}HOC_6H_4CH{=}NCH_2CO_2)_2Bi(C_6H_5)_3$

Colorless crystals of $(2\text{-}HOC_6H_4CH{=}NCH_2CO_2)_2Bi(C_6H_5)_3$ were obtained from CH_2Cl_2–petroleum ether. The molecular structure with the atom numbering scheme is depicted in Fig. 2. The selected bond distances and angles are listed in Table 6.

The crystal structure of compound **1** comprises isolated molecules which have C_2 molecular symmetry, the Bi–C16–C19 atoms forming the C_2 axis. The Bi–C bond distances are 2.211(4) and 2.202(6) Å. The Bi–O bond distances are both 2.302(3) Å, and the non-bonded Bi–O3 distances are both 2.846(4) Å; this indicates that there are coordination interactions between the two carbonyl oxygen atoms and the bismuth atom.[10]

The C10–Bi–C10A angle, which is affected by the close approach of the O3 and O3A atoms, is increased to 148.0(3)°, and the C10–Bi–C16 and C10A–Bi–C16 angles are both decreased to 106.00(13)°. The atoms Bi, O2, O2A, O3, O3A and C16 are coplanar within 0.033(7) Å. The coordination geometry of bismuth can be described as a distorted pentagonal bipyramid with the plane being defined by four oxygen atoms from two chelating carboxylate groups and one carbon from one phenyl group associated with bismuth, while the other phenyl groups occupy the axial positions.

Table 6. Selected bond distances and bond angles of compound **1**

Bond	Distance (Å)	Bond	Angle (°)
Bi–C16	2.202(6)	O2A–Bi–O2	172.7(2)
Bi–C10	2.211(4)	C16–Bi–C10	106.00(13)
Bi–O2	2.302(3)	C10A–Bi–C10	148.0(3)
Bi–O3	2.846(4)	C16–Bi–O2	86.36(10)
O1–C1	1.355(8)	C10A–Bi–O2	88.79(15)
O1–N1	2.571(4)	C10–Bi–O2	93.22(15)
O2–C9	1.273(7)	C9–O2–Bi	106.1(3)
O3–C9	1.220(7)	C17–C16–Bi	120.4(3)
N1–C7	1.265(8)	C17A–C16–C17	119.3(6)
N1–C8	1.463(7)	C1–O1–H1	109.5(3)
C1–C6	1.375(9)	C7–N1–C8	118.2(6)
C1–C2	1.386(9)	O1–C1–C6	121.6(5)
C2–C3	1.360(12)	O1–C1–C2	118.4(7)
C8–C9	1.519(7)	O1–H1–N1	146.9(5)

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