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Synthesis and Spectroscopic Characterization of Biologically Active Triarylantimony(V) Carboxylates Containing Germanium

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A new series of bimetallic (Ge and Sb) compounds (1-7) was synthesized and characterized by elemental analyses, FT-IR, multinuclear NMR (^{1}H, ^{13}C) spectroscopy and mass spectrometry. These techniques not only confirmed the formation of the compounds, but also revealed their trigonal bipyramidal geometry around antimony(V). Selected compounds were screened against different pathogens and showed promising antibacterial and antifungal activities.

Key Words: Bimetallic carboxylates, antimony(V), germanium, biological activities.

Organoantimony compounds have a diversity of applications in both biological and non-biological fields[1−3]. Bajpai and coworkers considered that the biological activity of organoantimony compounds is not significantly affected by the nature of the aryl group at the antimony[4]. However, Singhal and coworkers found that the affect of the nature of 'R' group on the activity of organoantimony compounds was relatively complex[5]. It has been reported that the organoantimony(V) carboxylates possess potential invitro activity against certain cancer cells[6]. In order to study the nature of the bonding as well as antibacterial and antifungal activity of these compounds, we prepared a series of new bis-triphenylgermyl (substituted) propionato triarylantimony(V) derivatives and characterized them by elemental analyses, FT-IR, multinuclear NMR (^{1}H,^{13}C), and mass spectrometry.

Experimental

Chemicals

Substituted cinnamic acids and antimony(III) chloride were purchased from Aldrich Chemicals (USA) and were used as received, since the melting points agreed with the values in the literature. Germanium dioxide

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(99.9% purity) was purchased from the People's Republic of China and was used as received. Solvents were dried over sodium benzophenone before use according to standard methods[7].

Instrumentation

Elemental analyses were carried out at Midwest Micro-Lab, Indianapolis, USA. Melting points were determined with Mitamura Riken Kogyo (Japan) and are uncorrected. IR was recorded on a Bio-Rad Excalibur FT-IR Model FTS 3000 MX using KBr disc. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 spectrometer, using deuterated solvents and TMS as a reference, operating at 300 and 75.5 MHz, respectively.

General Synthetic Procedure of Precursors

3-(Triphenylgermyl)propionic acid was synthesized according to the reported procedure[8]. Typically, phenyl magnesium bromide was prepared by the reaction between magnesium (0.176 mol) and bromobenzene (0.16 mol) in THF (30 cm^3) under nitrogen. The resulting phenylmagnesium bromide was added dropwise to 3-(trichlorogermyl) propionic acid (0.04 mol) in THF at 0 °C. After the addition, the mixture was refluxed for 4 h and then 10% aqueous hydrochloric acid was added. The water phase was extracted 3 times with chloroform. The chloroform phase was dried with anhydrous magnesium sulfate and filtered. The solvent was removed under vacuum to give a white solid. The solid was then recrystallized from chloroform and petroleum ether (3:1) to obtain a colorless crystalline solid.

The tri-*o*-tolylantimony dibromide was prepared by the method reported by Lice and coworkers[9]; dissolving (0.05 mol) freshly distilled $SbCl_3$ in dry diethyl ether, tri-*o*-tolylantimony magnesium bromide was added dropwise at 0 ^oC over 1 h, with regular stirring. The temperature was allowed to rise slowly and the mixture was subsequently refluxed for 1 h. The reaction mixture was cooled and hydrolyzed with cold distilled water. The organic layer was separated and dried over anhydrous $MgSO_4$; the solvent was evaporated under reduced pressure. The solid tri-*o*-tolylantimony(III) was recrystallized in hot petroleum ether. The resulting tri-*o*-tolylantimony(III) was converted to tri-*o*-tolylantimony antimony dibromide by direct bromination, and the solid product was recrystallized from toluene-petroleum ether mixture (3:1).

Synthesis of Tri (*o*-tolyl) antimony(V) bis-triphenylgermyl (substituted) propionates

Bis-[3-triphenylgermyl(substituted)propionato]tri-*o*-tolylantimony(V) derivatives were synthesized under mild conditions as reported[9]. Typically, 3-triphenylgermyl (substituted) propionic acid (1 mol) and triethylamine (0.8 cm^3) in toluene (50 cm^3) was added (o-$CH_3C_6H_4)_3SbBr_2$ (0.5 mol). The reaction mixture was stirred at room temperature for 8 h and then filtered. The filtrate was evaporated under reduced pressure. The obtained solid was crystallized from CH_2Cl_2- Pet-ether mixture (1:3).

Bioassays

The selected numbers of compounds (1-5) were evaluated for their antibacterial activity against 6 different types of bacteria by the agar well diffusion method[10]. The bacteria cultures used were *Escherichia coli, Bacillus subtilis, Shigella flexenari*, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi.*

Imipenum was used as the standard antibiotic. The 24-hours-old culture containing approximately 10^4-10^6 colony forming unit (CFU) was spread on the surface of Muller-Hinton Agar (MHA) plates. Wells were created in the medium with the help of a sterile metallic borer. Test samples of different concentrations were added in their respective wells. Experimental plates were incubated at 37 °C for 24 h and zones of inhibition (%) were measured and compared with the standard antibiotic imipenum with zone inhibition of 20 and 22 mm, respectively.

The agar tube dilution protocol[11]was applied to study the activity of the compounds against various fungal strains like *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canes, Fusarium solani,* and *Candida glaberata.* The standard antifungal drugs used for comparison testing were Amphotericin B and Miconazole. The tubes containing sterile sabour and dextrose agar were incubated with the test compound at different concentrations and solidified at room temperature. Test fungal cultures were inoculated on the slant, and growth inhibition (%) was observed after incubation for 7 days.

Shrimp larvae were applied as a tool to monitor the cytotoxicity of samples using Etoposide as the standard cytotoxic drug [12]. Brine shrimp eggs (50 mg) were placed in a hatching tray half filled with brine solution and incubated for 2 days at 27 °C. Test samples (20 mg) were dissolved in DMSO and diluted to 1000μ, 100μ, and 10 μg/ mL in 500μ, 50μ, and 5 μL vials using a Pasteur pipette. In each vial, 30 larvae were placed and a volume of 5 mL was made by adding sea water. The contents were incubated at 25-27 °C for 24 h under illumination. The numbers of survivors were counted and compared with the standard cytotoxic drug.

Results and Discussion

The compounds (1-7) were prepared by the reaction of $Ar_3SbBr_2$with the equivalent of triphenylgermyl (substituted) propionic acid in the presence of triethyl amine as a hydrogen halide acceptor in toluene, under mild conditions as shown (Eq. 1).

$$2Ph_3GeCHRCH_2CO_2H + Ar_3SbBr_2 \xrightarrow[\text{Toluene}\ \text{r.t.8h}]{Et_3N} (Ph_3GeCHRCH_2CO_2)_2SbAr_3 + Et_3N.\ HBr \quad \text{(Eq. 1)}$$

Ar = *o*-$CH_3C_6H_4$

R = *p*-$CH_3C_6H_4$(1), n-C_3H_7(2), $(CH_3)_2$ (3), C_6H_5(4), *p*-$CH_3OC_6H_4$ (5) *o*-$CH_3OC_6H_4$(6), CH_3(7).

The yield is generally 63% -85%. All compounds are white powders. They are unaffected by atmospheric moisture and easily soluble in organic solvents, e.g., benzene, dichloromethane, chloroform, and dimethylsulfoxide. The physical data and elemental analyses results of the synthesized compounds are shown in Table 1.

Table 1. Physical data of triarylantimony derivatives of general formula, $[(C_6H_5)_3GeCR^1(R^2)CH_2COO]_2Sb[o\text{-}CH_3C_6H_4]$.

Comp. No.	R^1	R^2	Molecular Formula	M.P. °C	Yield %	Elemental Analysis Found (Calc.)	
						C %	**H %**
(1)	H	*p*-$CH_3C_6H_4$	$C_{77}H_{71}O_4Ge_2Sb$	206-209	63	69.65 (69.63)	5.31 (5.35)
(2)	H	n-C_3H_7	$C_{69}H_{71}O_4Ge_2Sb$	195-198	79	67.21 (67.26)	5.77 (5.76)
(3)	CH_3	CH_3	$C_{67}H_{67}O_4Ge_2Sb$	230-232	76	66.84 (66.83)	5.54 (5.56)
(4)	H	C_6H_5	$C_{75}H_{67}O_4Ge_2Sb$	179-181	65	69.25 (69.28)	5.11 (5.15)
(5)	H	*p*-$CH_3OC_6H_4$	$C_{77}H_{71}O_6Ge_2Sb$	214-216	85	67.71 (67.99)	5.20 (5.22)
(6)	H	*o*-$CH_3OC_6H_4$	$C_{77}H_{71}O_6Ge_2Sb$	201-203	68	67.88 (67.99)	5.24 (5.22)
(7)	H	CH_3	$C_{65}H_{63}O_4Ge_2Sb$	164-166	71	66.34 (66.38)	5.31 (5.36)

Infrared spectroscopy

The infrared spectra of these compounds have been recorded in the range of 4000-400 cm^{-1} The bands of interest are assigned on the basis of earlier publications[13,14]. Important data are listed in Table 2. IR data support the molecular constitution of the title compounds by the absence of a broad band of ν(OH) in the region of 3500-3300 cm^{-1} by deprotonation and coordination of 3-triphenylgermyl(substituted) propionic acid with triarylantimony. The IR stretching vibration frequencies of carbonyl groups in triorganoantimony(V) carboxylates containing germanium are helpful in elucidating the structures and bonding behavior of the ligand. The coordination number of antimony affects the absorption vibrational frequency of the carbonyl group. Therefore, the mode of coordination of the carboxylate group has been related to the magnitude of difference, ($\Delta\nu$), between $\nu(CO_2)_{asy}$ and $\nu(CO_2)_{sym}$ vibrations[15]. The $\Delta\nu$ value is indicative of the coordination number around the antimony and it is > 300 $cm^{-1}$for 5 coordinated compounds. As is evident from Table 3, the values of $\Delta\nu$ lie between 303 and 332 cm^{-1}, suggesting a trigonal bipyramidal geometry of the compounds around the 5-coordinated antimony. In addition, the frequencies of ν(Sb−C) and ν(Sb−O) appeared between 459 and 484 cm^{-1}, and 561 and 585 cm^{-1}, respectively, which is consistent with the literature[8].

NMR Spectroscopy

^{1}H NMR spectroscopy

The ^{1}H NMR data of synthesized compounds (1-7) in non-coordinated solvent are shown in Table 3. All protons in the compounds have been identified and are in agreement with those calculated from the expected molecular formula. The signal of aromatic protons shows a multiplet at 6.34-7.50 ppm due to phenyl, and a well-defined sharp singlet at 2.15-2.85 ppm due to ortho-methyl substituent at the aryl group of antimony.

The characteristic feature of these compounds is that GeCH is a chiral center, CH_2 is a pro chiral center, and the 3 hydrogens is $GeCHCH_2$ unit comprises an ABX system, which appears as 2 multiplets at 3.15-3.82 and 2.12-2.63 ppm, respectively. Sub-spectral analysis of the ABX spectrum revealed that the 8 lines portion of the spectrum consists of 2 AB sub-spectra, whereas the X part of the spectrum consists of only 4 detectable lines giving 3 chemical shifts, νA, νB, and νX, and 3 coupling constants, JAB, JAX, and$J$BX [16].

Table 2. Characteristic IR frequencies (cm^{-1}) for tri-*o*-tolylantimony(V) derivatives containing germanium.

Comp.	$\nu(COO)_{asym}$	$\nu(COO)_{sym}$	$\Delta\nu$	ν(Ge−C)	ν(Sb−C)	ν(Sb−O)
(1)	1661	1329	332	653	469	585
(2)	1664	1335	329	674	468	574
(3)	1663	1360	303	645	467	578
(4)	1666	1341	325	645	468	571
(5)	1646	1328	318	658	467	561
(6)	1655	1330	325	648	469	569
(7)	1661	1329	332	653	461	585

Table 3. ^{1}H NMR data[(a−d)] of tri-*o*-tolylantimony(V) derivatives with general formula, $[(C_6H_5)_3GeCR^1R^2CH_2COO]_2$ $Sb[o\text{-}CH_3C_6H_4]_3$.

Compound	R^1	R^2	CH_2	*o*-$CH_3C_6H_4Sb$	C_6H_5Ge
(1)	3.31 [q, 2H,^3J(7.65)]	6.63-6.97 (m, 8H) 2.2 (s, 6H)	2.57 [dd, 2H, ^3J(14.12,7.63)] 2.63 [dd, 2H, ^3J(14.12, 5.71)]	6.34-6.60 (m, 12H) 2.54 (s, 9H)	7.18-7.50 (m, 30H)
(2)	3.15 [q, 2H,^3J(7.38)]	0.81 (t,6H,^3J(7.44) 2.93-3.17 (m, 8H)	2.37 [dd, 2H, ^3J(15.91,6.83)] 2.51 [dd, 2H, ^3J(15.91,5.11)]	7.23-7.30 (m, 12H) 2.46 (s, 9H)	7.38-7.43 (m, 30H)
(3)	1.06 (s, 6H)	1.06 (s, 6H)	2.63 (s, 4H)	7.23-7.30 (m, 12H) 2.46 (s, 9H)	7.34-7.41 (m, 30H)
(4)	3.3 [q, 2H,^3J(6.83)]	6.15-6.72 (m, 10H)	2.41 [dd, 2H, ^3J(15.74,6.62)] 2.59 [dd, 2H, ^3J(15.74,5.90)]	6.44-6.46 (m, 12H) 2.14 (s, 9H)	7.17-7.21 (m, 30H)
(5)	3.24 [q, 2H,^3J(7.15)]	7.09-7.16 (m, 8H) 3.64 (s, 6H)	2.12 [dd, 2H, ^3J(13.93,7.31] 2.42 [dd, 2H,^3J(13.93,6.12)]	6.67-6.99 (m, 12H) 2.15 (s, 9H)	7.20-7.23 (m, 30H)
(6)	3.80-3.82 (m, 2H)	6.81-6.90 (m, 8H) 2.88 (s, 6H)	2.48-2.63 (m, 14H)	6.81-6.90 (m, 12H) 2.88 (s, 9H)	7.34-7.41 (m, 30H)
(7)	3.58-3.62 (m, 2H)	0.89 [d, 6H,^3J(7.06)]	2.38-2.63 (m, 6H)	6.43-6.52 (m, 12H) 2.40 (s, 9H)	7.10-7.23 (m, 30H)

[a] In $CDCl_3$ at 297 K.
[b] Chemical shifts in ppm. $^3J(^1H-^1H)$ in Hz.
[c] Multiplicity is given as s = singlet, t = triplet, m = multiplet, d = doublet, dd = doublet of double.
[d] R^1 = H (1, 2, 4-7), CH_3 (3)
[e] R^2 = *p*-$CH_3C_6H_4$ (1), n-C_3H_7 (2), CH_3 (3, 7), C_6H_5 (4), *p*-$CH_3OC_6H_4$ (5), *o*-$CH_3OC_6H_4$ (6).

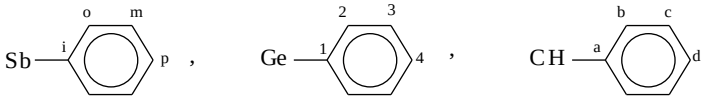
^{13}C NMR spectroscopy

The results obtained from ^{13}C NMR are provided in Table 4. The ^{13}C NMR studies in non-coordinated solvent ($CDCl_3$) make it possible to assign the magnetically non-equivalent carbon atoms. The position of carboxylate carbon moved to a lower field and was absorbed at 176.89-179.91 ppm as compared to 3-triphenylgermyl(substituted) propanoic acid in all synthesized compounds, indicating the participation of the carboxylate group in coordination to antimony. The signal of the substituent on the phenyl ring of the ligand acid is represented as 's' and is absorbed at the value that is expected, based on the literature, according to the nature of the substituent. For example, when the methyl group attached to the phenyl group in both the ligand acid as well as in the antimony(V) group, it showed a signal at 21.56- 24.04 ppm, while the methoxy group attached to the phenyl group and resolved at 54.52- 55.69 ppm due to a strong electron withdrawing effect[17].

Table 4. ^{13}C NMR data$^{(a-c)}$ of tri-*o*-tolylantimony(V) derivatives of general formula, $[(C_6H_5)_3GeCR^1R^2CH_2COO]_2$ $Sb[o\text{-}CH_3C_6H_4]_3$.

Compound	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**
R	*p*-$CH_3C_6H_4$	n-C_3H_7	$(CH_3)_2$	C_6H_5	*p*-$CH_3OC_6H_4$	*o*-$CH_3OC_6H_4$	CH_3
a	135.98	23.97		135.87	136.09	136.80	
b	134.55	22.45		133.05	135.71	135.74	
c	129.05	14.73	25.59	129.10	113.74	110.14	16.60
d	131.57	–		131.80	157.51	156.77	
s	23.86	–		131.80	55.69	54.52	
H	32.71	34.98	28.05	30.23	32.13	30.21	30.29
CH_2	39.50	39.20	46.95	39.27	39.58	38.15	40.49
C_6H_5-Ge 1	136.16	137.21	136.72	139.79	139.90	140.04	136.79
2	135.87	135.75	136.36	135.08	136.22	136.95	136.59
3	129.22	129.21	129.03	129.43	129.34	128.18	129.38
4	132.38	134.53	134.65	134.36	134.26	131.80	134.78
o-$CH_3C_6H_4Sb$ i	134.64	135.23	136.56	135.94	135.92	135.06	135.81
o	132.38	134.81	135.35	134.59	134.82	134.74	135.21
m	128.66	128.60	128.61	128.77	128.48	128.98	128.71
p	129.64	132.05	132.60	132.10	131.86	131.64	132.13
s	21.56	23.41	24.27	23.81	23.81	23.71	24.08
COO	179.43	178.06	177.95	179.91	177.0	177.61	176.89

[a]In $CDCl_3$ at 297 K, [b]Chemical shifts in ppm, [c]s = substituent on phenyl ring



Mass spectrometry

Mass spectrometry has been used for structure elucidation and interpretation of triarylantimony(V) carboxylates containing germanium. The monoisotopic mass fragmentation pattern of representative compounds (1 and 7) with relative abundance is presented in Table 5. The fragment ions formed are in agreement with the expected structure and proposed molecular formula of all synthesized compounds. The bond dissociation energies for these compounds are relatively low, so the parent molecular ion peak is not observed

Table 5. Mass spectrometric data of triarylantimony(V) derivatives containing germanium

Fragment Ion	**(1)** m/z(%)	**(2)** m/z(%)	**(3)** m/z(%)	**(4)** m/z(%)	**(5)** m/z(%)	**(6)** m/z(%)	**(7)** qm/z(%)
$((C_6H_5)_3GeCR^1R^2CH_2CO_2)_2SbAr_3$	1328(n.o)	1232(n.o)	1204(n.o)	1300(n.o)	1360(n.o)	1360(n.o)	1176(n.o)
$((C_6H_5)_3GeCR^1R^2CH_2CO_2)SbAr_3$	861(29)	813(6)	799(65)	847(26)	877(16)	877(13)	785(16)
$((C_6H_5)_3GeCR^1R^2CH_2CO_2)SbAr_2$	770(16)	722(21)	708(60)	756(20)	786(22)	786(6)	694(16)
$((C_6H_5)_3GeCR^1R^2CH_2CO_2)$	467(21)	419(17)	405(46)	453(19)	483(30)	483(25)	391(15)
Ar_3Sb^+	394(19)	394(56)	394(89)	394(36)	394(30)	394(51)	394(70)
Ar_2Sb^+	303(94)	303(63)	303(72)	303(86)	303(79)	303(97)	303(98)
$ArSb^+$	212(16)	212(26)	212(89)	212(100)	212(100)	212(100)	212(23)
Sb^+	121(5)	121(6)	121(11)	121(4)	121(7)	121(3)	121(7)
$(C_6H_5)_3Ge^+$	305(100)	305(100)	305(100)	305(92)	305(94)	305(98)	305(100)
$(C_6H_5)_2Ge^+$	228(28)	228(48)	228(45)	228(11)	228(55)	228(16)	228(46)
$[(C_6H_5)_2Ge\text{-}H]^+$	227(30)	227(50)	227(41)	227(25)	227(47)	227(20)	227(49)
$(C_6H_5)Ge^+$	151(29)	151(45)	151(20)	151(16)	151(39)	151(24)	151(42)

and the molecule suffers considerable fragmentation[18,19]. The decarboxylation and dephenylation from the metal atom are the 2 main breakdown patterns for synthesized compounds. Thus, the mass spectral data and fragmentation pattern support the proposed molecular structure and molecular formulae of synthesized [3-triphenylgermyl(substituted) propionato] tri-*o*-tolylantimony(V) compounds.

Biological activities

Antibacterial screening

The selected number of compounds (1-5) were evaluated for their antibacterial activity against the following 6 types of bacteria relative to the reference drug, imipenum, by the agar well diffusion method[10]: *E. coli, B. subtilis, S. flexenari, S. aureus, P. aeruginosa,* and *S. typhi.* The results are summarized in Table 6. Preliminary tests showed that these compounds exhibited good activity against *E. coli, B. subtilis,* and *S. typhi.*

Table 6. Antibacterial activity data[(a−c)] of organoantimony(V) derivatives containing germanium (in vitro).

Name of Bacteria	Zone of Inhibition (mm) Compounds					Zone of Inhibition of Std. Drug (mm)
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	
Escherichia coli	10	22	18	24	24	33
Bacillus subtilis	18	24	16	26	24	30
Shigella flexenari	16	–	21	–	18	35
Staphylococcus aureus	32	31	18	33	28	43
Pseudomonas aeruginosa	–	15	13	18	16	25
Salmonella typhi	34	36	28	32	35	40

[a]Concentration of sample = 5 mg/mL of DMSO

[b]Concentration of standard drug (Imipenum) = 10 μg/mL

[c](-) = No activity

Antifungal screening

The agar tube dilution protocol[11]was used to study the activity of the compounds against different types of fungi including *T. longifusus, C. albicans, A. flavus, M. canis, F. solani,* and *C. glaberata.* The results were compared with standard fungal agents with a minimum inhibitory concentration (MIC) of 95∼100 μg/mL and are shown in Table 7. Screening tests showed that the compound (2) had comparatively good activity against all tested fungi, which may be due to the presence of a n-propyl group in triphenylgermyl(substituted) propionic acid moiety[8].

Lethality bioassay

Bioactive compounds are often toxic to shrimp larvae. Shrimp larvae are used extensively to monitor the cytotoxicity of samples under study. Therefore, the cytotoxicity of selected synthesized compounds was determined by invivo lethality to shrimp larvae[12]. The results obtained with this method and their lethal doses (LD_{50}) are compared to the reference drug, Etoposide, and are provided in Table 8. The LD_{50} values

for these compounds were between 105.09 and 256.72 μg/mL. Although the compounds showed low toxicity, they demonstrated enhanced antibacterial and antifungal activity.

Table 7. Antifungal activity data[(a−c)] of organoantimony(V) derivatives containing germanium (invitro).

Name of Bacteria	Zone of Inhibition (mm) Compounds					Std. Drug MIC μg/mL
	(1)	**(2)**	**(3)**	**(4)**	**(5)**	
Trichophyton longifusus	25	61	–	40	28	Miconazole
Candida albicans	45	98	–	20	55	Miconazole
Aspergillus flavus	–	10	–	–	–	Amphotericin B
Microsporum canis	76	65	–	48	58	Miconazole
Fusarium solani	38	44	–	40	28	Miconazole
Candida glaberata	66	79	–	50	45	Miconazole

[a]Concentration of sample = 400 μg/mL of DMSO

[b]Incubation temperature (period) = 27 ± 1 °C (7 days)

[c](-) = No activity

Table 8. Cytotoxicity data[(a−b)] of organoantimony(V) derivatives containing germanium.

Comp. No.	LD_{50} (μg/mL)
(1)	157.21
(2)	256.72
(3)	105.09
(4)	120.51
(5)	118.80
Std. drug (Etoposide)	7.46

[a]Organism = Brine Shrimp (larvae)

[b]LD_{50} = Lethal dose at which 50% of organisms die

Conclusions

The elemental analyses results of all synthesized compounds were comparable with calculated and found values of carbon and hydrogen atoms, which confirmed the formation and purity of compounds. In all these the carboxylate group containing germanium in solid state acts as a monodentate, which has a trigonal bipyramidal geometry of compounds around the 5-coordinated antimony. Mass spectral data reveals that fragment ions formed are in agreement with the expected structure and proposed molecular formula of all synthesized compounds. Biological screening of the selected compounds demonstrated their promising activity against different microbes.

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