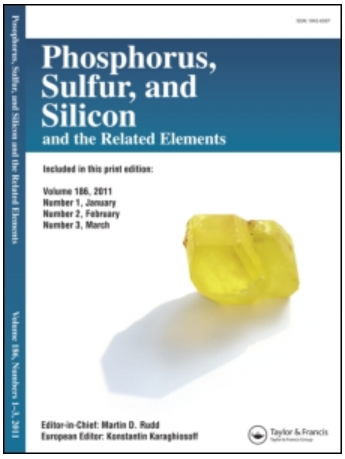

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Biological Studies of Some Perfluorophenyl Antimony Compounds

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Biological Studies of Some Perfluorophenyl Antimony Compounds

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Perfluoroorganoantimony(III) $(R_f)_nSbCl_{3-n}$ and -antimony(V) $(R_f)_nSbCl_{5-n}$ (n = 1,2,3, $R_f = C_6F_5$) compounds are screened for the first time for biological activity. The compounds exhibited significant in vitro antitumor activity against MCF-7 (human breast cancer) cell line and antibacterial activity against three pathogenic bacteria: Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae. They also showed antifungal activity against Aspergillus flavus and Aspergillus niger as well as insecticidal activity against cockroach (Periplanata americana), housefly (Musca domestica), Tobacco caterpillar (Spodoptera litura), and spider mite (Tetranychus urticae). These studies suggest a better biocidal activity for the pentafluorophenyl antimony halides compared to the corresponding phenyl analogues.

Keywords Antibacterial; antifungal; antitumor; insecticidal activity; perfluorophenyl-antimony(III) and -antimony(V) chlorides

INTRODUCTION

The medicinal importance of organoantimony compounds against trypanosomes and *Leishmania* is well established[1] and they are also used as antibacterial and antifungal agents in the textile industry.[2] In recent years, organoantimony compounds have also exhibited moderate

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to significant antiproliferative activity.[3–5] No toxicological studies have been reported so far, however. Antimicrobial, antitumor, and CNS activity of a variety of organoantimony compounds has been reported earlier.[6–9]

Organoantimony(III) compounds show inhibitory effects against Ehrlich ascites tumor[4,5] and also respond against P388 leukemia.[10] A mutagenic potential of some diphenylantimony(III) derivatives has also been evaluated.[11] Only recently have Chinese researchers discovered organoantimony(V) derivatives as potential cytotoxic agents, which in some cases surpass even cis-platin therapeutic activity.[12–14] However, these studies were mainly confined to symmetrical organoantimony(III) and -antimony(V) compounds based on hydrocarbon ligands[15] and no biological and toxicological activity was reported for hydrocarbon ligands. Recently a patent about the improved synthesis of $(C_6F_5)_2Sb(C_6H_5)$ and its insecticidal, acaricidal, and antimicrobial activity has been filed.[16] The above compound is reported to have high insecticidal and acaricidal activity against polyphagous pest of crops, *S. litura* (Lepidoptera: Noctudae) and *T. urticae* (Tetranychidal Acari), respectively. However, antimicrobial and cytotoxic activity of symmetrical perfluorinated organoantimony compounds has not been reported to date.

It may be noted that potency of an organometallic compound increases[17] both *in vivo* and *in vitro* with the increase of hydrophilic (to facilitate acceptance by water rich cells) and lipophilic (essential for crossing the cell membrane) characters. Thus, the introduction of OH groups and the partial substitution of hydrogen atoms by fluorine atoms in case of organotin compounds increase their *in vitro* activity. Fluorine-containing compounds are more soluble in water as well as in nonpolar solvents compared to hydrocarbon-based analogues.[17] The *in vivo* testing is also affected by the limited water solubility of the compounds in question. As in case of perfluoroalkyl group containing organotin compounds, pentafluorophenyl antimony compounds appear to be a possible way to solve the water-solubility problem encountered with almost all the organoantimony compounds and in turn could enhance the *in vitro* activity.

Because biological activity of pentafluorophenylantimony compounds has remained an untouched area so far, we initiated the present study to address structure-function relationship of fully fluorine-substituted aryl antimony compounds against selected fungal and bacterial species as well as against the MCF-7 tumor cell line. We also wanted to investigate the insecticidal activity of the perfluorinated antimony compounds $(C_6F_5)_3Sb$ (**1**), $(C_6F_5)_2SbCl$ (**2**),

$(C_6F_5)SbCl_2$ (**3**), $(C_6F_5)_3SbCl_2$ (**4**), $(C_6F_5)_2SbCl_3$ (**5**), $(C_6F_5)SbCl_4$ (**6**), and $(C_6F_5)_3C_2H_5SbCl$ (**7**) against *S. litura*, *P. Americana*, and *M. domestica*.

RESULTS AND DISCUSSION

In Vitro Antitumor Activity

The antitumor activity of perfluorinated antimony compounds (**1–7**) against the human breast adenocarcinoma cell line (MCF-7) was studied. Compounds **1**, **2**, **3**, **6**, and **7** showed moderate antiproliferative activity against the MCF-7 cell line. They inhibit the growth of about 30–35% of the tumor cell line. The remaining compounds do not show significant activity. Organoantimony(III) compounds are apparently found to be more active because of their stereoselectivity. Antimony(V) compounds **4** and **5** are inactive, whereas compounds **6** and **7** are active. The former has a symmetrical trigonal bipyramidal structure (TBP) and the latter has a square pyramidal geometry (SP). In addition, the high chlorine content of compound **6** affects its activity and also in compound **7** the presence of a C_2H_5 group probably affects the activity. This is not surprising, because in the case of organotin compounds, enhanced biological activity has been found in ethyl derivatives. Compounds **1**, **2**, and **3** have a pyramidal structure where antimony is in the oxidation state +3 with a lone pair of electrons at the antimony atom. A variety of organoantimony(III) compounds have been reported for cytotoxic activity.[15,16] It was found that the pentafluorophenyl antimony compounds generally interact with nitrogenous bases of nucleotides of nucleic acid and inhibit the cell division by interfering with the replication and transcription of DNA molecules.[18] The organoantimony compounds may also affect the multienzyme complexes responsible for replication and transcription of DNA thus causing a stop of proliferation of the cells[19] (Table I).

Antimicrobial Activity

Antibacterial Activity

Antibacterial activity of perfluorinated antimony compounds (**1–7**) was studied against three human pathogenic bacteria: *P. aeruginosa*, *S. aureus*, and *K. pneumoniae*, using 10 μg/mL concentrations of the test compounds (Table II). It was found that compounds **1**, **2**, **4**, **6**, and **7** show moderate activity against *P. aeruginosa*. Compound **5** shows the highest activity against these bacteria, whereas compound **3**

TABLE I *In Vitro* Antitumor Activity

	Cell no. $\times 10^4$	Activity
$(C_6F_5)_3Sb$	9.35 ± 061	+
$(C_6F_5)_2SbCl$	9.17 ± 0.90	+
$(C_6F_5)SbCl_2$	9.17 ± 0.87	+
$(C_6F_5)_3SbCl_2$	12.34 ± 1.05	−
$(C_6F_5)_2SbCl_3$	11.89 ± 1.05	−
$(C_6F_5)SbCl_4$	9.25 ± 0.86	+
$(C_6F_5)_3(C_2H_5)SbCl$	5.79 ± 0.55	+
Negative control	10.21 ± 1.01	−
Positive control	40.26 ± 3.23	−

exhibits the lowest activity. It has been reported that variation in activity is affected because of (1) change in oxidation state of the metal (antimony) and (2) change of the organic moiety as well as the nature of the ligand. Against *S. aureus*, compounds **2**, **4**, and **6** show moderate activity, whereas compounds **3** and **7** show high activity. Compounds **1** and **5** show the lowest activity. There is no regular trend of the activity shown by these compounds. In the case of compound **7**, introduction of an ethyl group enhances the antibacterial activity. The activity of perfluorinated antimony compounds (**1–7**) against *K. pneumoniae* does not differ much from that in the former cases. Compounds, **1**, **3**, **5**, and **6** are moderately active, whereas compound **2** shows the lowest activity. Compounds **4** and **7** show the highest activity against these bacteria. It was found that the compounds may damage the cell wall of the bacteria by reacting with peptides of the cell wall, but the exact mechanism is yet to be established.

TABLE II Antibacterial Activity

	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
$(C_6F_5)_3Sb$	−	++	+	++
$(C_6F_5)_2SbCl$	−	++	++	+
$(C_6F_5)SbCl_2$	−	+	+++	++
$(C_6F_5)_3SbCl_2$	−	++	++	+++
$(C_6F_5)_2SbCl_3$	−	+++	+	++
$(C_6F_5)SbCl_4$	−	++	++	++
$(C_6F_5)_3(C_2H_5)SbCl$	−	++	++	+++

+ = 6–10 mm diameter; ++ = 10–14 mm diameter; +++ =>14 mm diameter; − = Inactive (control).

TABLE III Antifungal Activity

	Conc. (μg/mL)	*Aspergillus flavus* (dia. mm)	% Inhibition	*Aspergillus niger* (dia. mm)	% Inhibition
$(C_6F_5)_3Sb$	10	1.2	60.0	1.0	50.0
	20	1.0	66.6	1.0	50.0
	50	0.6	80.0	0.5	75.0
	100	0.4	86.7	0.2	90.0
$(C_6F_5)_2SbCl$	10	1.4	53.3	1.5	25.0
	20	1.0	66.6	1.0	50.0
	50	0.7	76.6	0.6	70.0
	100	0.4	86.7	0.1	95.0
$(C_6F_5)SbCl_2$	10	1.4	53.3	1.0	50.0
	20	1.2	60.0	0.8	60.0
	50	1.0	66.6	0.5	75.0
	100	0.8	73.3	0.2	90.0
$(C_6F_5)_3SbCl_2$	10	1.2	60.0	1.4	30.0
	20	1.0	66.6	1.0	50.0
	50	0.8	73.3	0.8	60.0
	100	0.5	83.3	0.4	80.0
$(C_6F_5)_2SbCl_3$	10	1.2	60.0	1.5	25.0
	20	0.7	76.6	1.2	40.0
	50	0.5	83.3	0.8	60.0
	100	0.1	96.7	0.5	75.0
$(C_6F_5)SbCl_4$	10	0.8	73.3	1.4	30.0
	20	0.6	80.0	1.2	40.0
	50	0.4	86.7	0.5	75.0
	100	0.2	93.3	0.2	90.0
$(C_6F_5)_3(C_2H_5)SbCl$	10	1.0	66.6	0.8	60.0
	20	0.8	73.3	0.5	75.0
	50	0.5	83.3	0.4	80.0
	100	0.1	96.7	0.1	95.0
Control		3.0	—	2.0	—

Antifungal Activity

Antifungal activity of perfluorinated antimony compounds (**1–7**) was tested against the fungal strains *A. flavus* and *A. niger* at concentrations of 10, 20, 50, and 100 μg/mL (Table III). At 10 μg/mL the compounds **1**, **4**, **5**, **6**, and **7** show higher inhibition against *A. flavus* while the remaining compounds show moderate activity. In the case of *A. niger* the compounds **1**, **3**, and **6** show high activity, whereas the compounds **2**, **4**, **5**, and **7** show moderate activity. The variation in fungicidal activity might be due to the variability in the oxidation state of antimony, the presence of an ethyl group, and the number of chlorine atoms at the antimony. At 20 μg/mL concentration, compounds **1**, **4**,

and **6** show higher inhibition against *A. flavus* and compounds **1**, **3**, **5**, **6**, and **7** shows higher inhibition against *A. niger*, whereas the remaining compounds are moderately active. At 50 and 100 μg/mL almost all the compounds show high inhibition against fungal strains. It may be noted that the pentafluorophenyl derivatives show higher activity compared to the phenyl analogues. Again, compounds in which the oxidation state of antimony is +3 and compounds having an ethyl group are relatively more active.

Insecticidal Activity

Contact Toxicity

Contact toxicity of perfluorinated antimony compounds (**1–7**) was tested against the fourth instar larvae of *S. litura* using different concentrations of the test compounds in acetone and adding Tween 20 as emulsifier. The mortality data of each compound was used for calculating the respective LC_{50}/LD_{50} value. It was found that the compounds **1–3** showed higher activity against the larvae. The activity again depends upon the oxidation state of antimony. Antimony compounds in the oxidation state +3 are found to be more active than those in the oxidation state +5 because of lower Lewis acidity in the oxidation state +3.

Stomach Toxicity

Stomach toxicity of perfluorinated antimony compounds (**1–7**) was checked against the same larvae of insect (*S. litura*) using different concentrations in acetone and adding Tween 20 as emulsifier. The mortality data were used to calculate the LC_{50}/LD_{50} value. The highest mortality was found for compounds **1–3**. Evidently, compounds with antimony in the oxidation state +3 are more active.

Antifeedant Activity

Antifeedant activity of perfluorinated antimony compounds (**1–7**) was evaluated against fourth instar larvae of *S. litura* using different concentrations in acetone and adding Tween 20 as emulsifier. Mortality data were used to calculate the LC_{50}/LD_{50} value of the respective compounds. The compounds with an oxidation state of antimony of +3 show higher activity compared to those with an oxidation state of +5.

Acaricidal Activity

The acaricidal activity of perfluorinated antimony compounds (**1–7**) was assayed against mites *T. urticae*. Different concentrations of

the compounds were prepared in acetone and Tween 20 was added as emulsifier. All perfluorinated antimony compounds (**1–7**) show moderate to higher activity against mites. Again, the Sb(III) compounds are more active than the Sb(V) compounds.

Structure-Activity Relationship

It is found that organometallic compounds containing a pentafluorophenyl ring are more water and lipid soluble, which is responsible for crossing out the cell membrane of microorganisms and insects. The compounds containing a pentafluorophenyl ring together with chlorine generally cause death of the insects because they form complexes with metalloenzymes, and particularly with those involved in respiratory pathways, such as cytochrome oxidase. Perfluorinated antimony compounds produce spasm (excessive muscular contraction and sudden convulsive movement), regurgitation, flaccid paralysis, etc. The organoantimony compounds containing a pentafluorophenyl ring may also act similarly to DDT, blocking the axonal membrane in the nervous system and forming a perfect shape to block the nerve impulse.

EXPERIMENTAL

Tris(pentafluorophenyl) antimony was obtained by a modification of the published method.[16] Bis(pentafluorophenyl) antimony(III) chloride and pentafluorophenyl antimony(III) dichloride were obtained by a redistribution reaction and exhibited melting points and IR spectra as reported.[20] Bis(pentafluorophenyl) antimony trichloride and pentafluorophenyl antimony tetrachloride were obtained by the direct chlorination of $(C_6F_6)_2SbCl$ and $(C_6F_5)SbCl_2$ as reported earlier.[20] All the compounds were recrystallized before being subjected to biological activity. The experimental techniques followed for assaying the biological activity are given below.

Antitumor Activity

These experiments were carried out to estimate the effect of the test compound on the growth of tumor cells. The human breast cancer cell line (MCF-7) was employed. The human breast cancer cell line (MCF-7) was co-incubated with the test compounds at 1 μg/mL doses for 96 h and the cell growth count was measured by MTT assay.[21] The basic principle involved in this assay depends upon the reduction of a tetrazolium salt. The yellow-colored tetrazolium MTT

[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] is reduced in part by metabolically active cells by the action of dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple color zones were solubilized and quantified by spectrophotometric method. The MTT was dissolved in PBS at a concentration of 5 mg/mL. Then 50 μL of the MTT solution was added to each well of the 96-well culture plates, containing the 100 μL culture along with the test compound, and was incubated at 37°C for 4 h. The medium was then removed carefully without disturbing the purple-colored formazon crystals. Then, 50 mL of dimethylsulfoxide was added to each well and mixed thoroughly to dissolve the crystals of the formazon. The plates were then read on an ELISA plate reader at a wavelength of 570 nm. The readings were presented as optical density/cell count (Table I).

Antibacterial Activity

Antibacterial activity of perfluorinated antimony compounds (**1–7**) was determined by disc diffusion method.[22] In this technique, filter paper (Whatman No. 1) sterile discs of 5 mm diameter, impregnated with the test compounds (10 μg/mL in ethanol), were placed on the nutrient agar plate at 37°C for 24 h. The inhibition zones around the dried impregnated discs were measured after 24 h. The activity was classified as "highly active" (diameter > 14 mm), "moderately active" (diameter = 10–14 mm), and "slightly active" (diameter = 6–10 mm). The diameter less than 6 mm was regarded as inactive (Table II).

Antifungal Activity

Antifungal activity of perfluorinated antimony compounds (**1–7**) was tested by agar diffusion method[23] using four concentrations of the tested compound, viz., 10, 20, 50, and 100 μg/mL against *Aspergillus flavus* and *Aspergillus niger*. One milliliter of each compound was poured into a Petri dish having about 20–25 mL of molten potato dextrose agar medium. As the medium became solid, the Petri dishes were inoculated separately with the fungal isolates and kept at 26°C for 96 h. All the values (% inhibition) were recorded. The percentage inhibition of perfluorinated antimony compounds (**1–7**) was calculated using the following mathematical equation:

$$\text{Percentage (\%) Inhibition} = \text{C} - \text{T/C} \times 100$$

where C = diameter of fungus in control and T = diameter of fungus in tested compound. The results are presented in Table III.

TABLE IV Stomach Toxicity Data After 24 h

	Fiducially limits	Slope	X^2	LC_{50} after 24 h
$(C_6F_5)_3Sb$	0.57–1.05	1.32 ± 0.15	0.63 (3)	0.74
$(C_6F_5)_2SbCl$	054–0.90	1.49 ± 0.16	3.39 (3)	0.68
$(C_6F_5)SbCl_2$	0.49–0.77	1.57 ± 0.16	2.79 (3)	0.60
$(C_6F_5)_3SbCl_2$	0.86–1.99	1.28 ± 0.16	0.80 (3)	1.20
$(C_6F_5)_2SbCl_3$	1.61–9.55	1.01 ± 0.17	0.68 (3)	2.97
$(C_6F_5)SbCl_4$	0.82–1.67	1.45 ± 0.17	0.65 (3)	1.10
$(C_6F_5)_3(C_2H_5)SbCl$	0.56–1.05	1.32 ± 0.15	0.62 (3)	0.73

Insecticidal Studies

Stomach Toxicity

Stomach toxicity of perfluorinated antimony compounds (**1–7**) was tested by the leaf dip method.[24] In this technique, the leaf discs of about 25 cm^2 were prepared out of caster leafs and were dipped for 30 s in various concentrations of the test compounds (the compounds were dissolved in acetone at various concentrations). The leaf discs were air-dried to evaporate the excess acetone. The leaf discs dipped in acetone alone served as control. The fourth instar larvae of *S. litura* were used for this purpose; ten larvae were used for each replication and three replications were maintained for each concentration. The dried leaf discs were now offered for feeding. The mortality was recorded after 24 h and treatment mortality was corrected with control mortality. The mortality data were used for calculating LC_{50} (Table IV).

Contact Toxicity

Contact toxicity of perfluorinated antimony compounds (**1–7**) was tested by the topical application method.[25] Fourth instar larvae of *S. litura* were used for this purpose. About 30 larvae were used for each concentration. The compounds were dissolved in acetone and different concentrations were prepared. Then each concentration was applied on the dorsal surface of the larvae (about 10 μL for each larvae). Insects treated only with acetone served as control and were left for 24 h. After 24 h the mortality was recorded and treatment mortality was corrected with the control mortality. These mortality data were used for calculating LC_{50} (Table V).

Antifeedant Activity

Antifeedant activity of perfluorinated antimony compounds (**1–7**) was tested by the leaf dip method.[24] In this method the leaf discs of

TABLE V Contact Toxicity After 24 h

	Fiducially limits	Slope	X^2	LC_{50} after 24 h
$(C_6F_5)_3Sb$	0.48–0.75	1.61 ± 0.16	2.94 (3)	0.59
$(C_6F_5)_2SbCl$	0.40–0.59	1.66 ± 0.15	5.66 (3)	0.48
$(C_6F_5)SbCl_2$	0.29–0.39	1.97 ± 0.16	4.39 (3)	0.34
$(C_6F_5)_3SbCl_2$	1.87–12.08	1.09 ± 0.19	1.60 (3)	3.53
$(C_6F_5)_2SbCl_3$	1.33–3.99	1.32 ± 0.20	2.38 (3)	2.01
$(C_6F_5)SbCl_4$	1.61–9.55	1.01 ± 0.17	0.68 (3)	2.97
$(C_6F_5)_3(C_2H_5)SbCl$	0.74–1.32	1.62 ± 0.18	3.24 (3)	0.94

about 25 cm^2 were prepared out of caster leaf and were dipped for 30 s in various concentrations of the test compounds. The leaf discs were air-dried to evaporate the excess of acetone. The leaf discs dipped only in acetone served as control. The fourth instar larvae of *S. litura* were used for this purpose. Ten larvae were used for each replication and three replications were used for each concentration. The dried leaf discs were offered for feeding and allowed to feed for 24 h; after 24 h the leaf area uneaten was measured using a leaf area meter. The difference between leaf area provided and the leaf area left over was taken as amount of leaf area consumed. The feeding inhibition was calculated and was used for calculating the EC_{50} (Table VI).

Acaricidal Activity

Acaricidal activity of perfluorinated antimony compounds (**1–7**) was tested by leaf dip method.[24] In this method, the compound was dissolved in distilled water or acetone and different concentrations were prepared using 0.02% Tween 20 as emulsifier. The leaf discs of mulberry (5 cm^2 diameter) were dipped in different concentrations for 30 s, air-dried, and placed over wet cotton in a Petri plate. Adult female

TABLE VI Antifeedant Activity After 24 h

	Fiducially limits	Slope	X^2	LC_{50} after 24 h
$(C_6F_5)_3Sb$	0.62–1.46	1.05 ± 0.46	1.09(3)	0.87
$(C_6F_5)_2SbCl$	0.84–2.34	1.06 ± 0.15	0.70 (3)	1.24
$(C_6F_5)SbCl_2$	0.43–0.87	1.03 ± 0.14	0.34 (3)	0.58
$(C_6F_5)_3SbCl_2$	−0.30 to 0.48	1.25 ± 0.14	3.48 (3)	0.37
$(C_6F_5)_2SbCl_2$	0.71–2.21	0.89 ± 0.14	0.20 (3)	1.08
$(C_6F_5)SbCl_4$	0.49–1.25	0.87 ± 0.13	0.89 (3)	0.71
$(C_6F_5)_3(C_2H_5)SbCl$	0.49–0.77	1.57 ± 0.16	2.79 (3)	0.60

TABLE VII Acaricidal Activity after 24 h

	Fiducially limits	Slope	X^2	LC_{50} after 24 h
$(C_6F_5)_3Sb$	0.05–0.09	1.16 ± 0.09	12.57 (3)	0.07
$(C_6F_5)_2SbCl$	0.05–0.10	0.87 ± 0.07	20.01 (3)	0.07
$(C_6F_5)SbCl_2$	0.04–0.09	0.70 ± 0.06	4.61 (3)	0.05
$(C_6F_5)_3SbCl_2$	0.05–0.10	0.97 ± 0.08	13.22 (3)	0.07
$(C_6F_5)_2SbCl_3$	0.12–0.26	0.89 ± 0.08	8.52 (3)	0.17
$(C_6F_5)SbCl_4$	0.08–0.20	0.75 ± 0.07	5.53 (3)	0.12
$(C_6F_5)_3(C_2H_5)SbCl$	0.05–0.10	0.97 ± 0.08	13.22 (3)	0.07

mites (*T. species*) were released on treated leaf discs and mortality was recorded after 24 h of treatment. These mortality data were used for the calculation of the LC_{50} (Table VII).

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