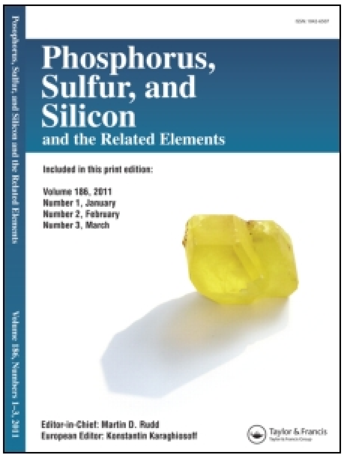

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Synthesis and Biological Activity on Some Organoantimony (III) Compounds

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Synthesis and Biological Activity on Some Organoantimony (III) Compounds

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Organoantimony (III) amides of the type Ar_2SbL; ($Ar = C_6H_5, C_6F_5, C_6H_4F$; L = succinimide and phthallimide) were synthesized and assayed for their biological activities. These compounds exhibited significant in-vitro antitumor activity against human breast adenocarcinoma cell line (MCF-7), mammary cancer cell line (EVSA-7), and antibacterial activity against human pathogenic bacteria viz. Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiela pneumoniae. These compounds also exhibited remarkable antifungal activity against Aspergillus flavus and Aspergillus niger strains.

Keywords Antibacterial; antifungal activity; antitumor; organoantimony (III) amides

INTRODUCTION

Metals have played an important role in medicine for last many years, however their significance has been realized recently. The importance of metal ions lies in the fact that they are essential components for various physicochemical processes of living system and has potential use as metalopharmaceuticals.[1]

However, oncologists knew about the antitumor activities of ligands and the existence of relationship between cancer and metal. However, many of them ignored the various aspects about their relationship. It is also surprising to observe that metals may acts as the best and the

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worst, i.e., metals are able to induce the disease and also used to treat the infectious disease; and some of them are able to perform both and acts as paradox.[2]

It is well known that almost all metals are able to generate reactive oxygen species (ROS); and this property is mainly used for the treatment of cancer. Organometallics of both transition and non-transition metals plays important role in the treatment of cancer and other infectious diseases.[3]

In search of antiproliferative properties of a variety of organoantimony compounds, especially thiolates and carboxylate have been synthesized and tested in-vitro for their antitumor activity along with their antimicrobial activity.[4–9] A combination of organobismuth and-germanium compounds, aryl bismuth triphenylpropionate has been found to show much higher activity against MCF-7 cell line compared to well known cis-platin complex.[10] To enhance the hydrophilic (to facilitate acceptance by water rich cells) as well as lipophilic (essential for crossing the cell membrane) character of these compounds, fluorine containing compounds were synthesized and characterized as it is well known that the compound containing fluorine and other polar groups enhances the biological activity in vitro.[11]

A comprehensive structure—activity relationship on the reported potentially bioactive organotin compounds reveal that the activity of an organometallic compound is highly affected by; the nature and number of organic groups, nature of ligands, presence of fluoro substituents for hydrophilic and lipophilic character and the hydrolytic stability of metal-carbon bond.[12] A patent has been recently filed on the fluoro based organoantimony compounds as a potent biologically active compound.[13]

In sharp contrast to the well documented organoantimony (III) compounds,[14–20] those containing Sb(III)-N bonds are limited to aminostabines and prepared by the reaction of lithium diethylamide with halo or dihalostibine at low temperature.[21] Besides this, the organoantimony (V) nitrogen derivatives have been studied extensively in past few years,[22–24] but there are no studies reported earlier on biological activities of such kinds of organoantimony compounds.

The present communication deals with the synthesis and biological activity of the new organoantimony compounds. The compounds were characterized for antibacterial, antifungal and antitumor activity and were found to be potentially active against (MCF-7), (EVSA-7), cell line and human pathogenic fungal and bacterial strains.

EXPERIMENTAL

The synthesis of organoantimony (III) amides was carried out by the reported method.[25] The diarylorganoantimony (III) chloride R_2SbCl was prepared by the redistribution reaction of R_3Sb and $SbCl_3$ in neat and crystallized in dichloromethane in inert atmosphere.

$$2\,R_3Sb + SbCl_3 \xrightarrow{\text{Neat}} 3R_2SbCl$$

$$(R = C_6H_5, C_6F_5, C_6H_4F) \tag{1}$$

The organic moieties were prepared by the standard techniques and purified by crystallization before use. Molecular weights were determined cryoscopically in benzene. The IR spectra of new organoantimony compounds were recorded in a Perkin –Elmer spectrophotometer in 4000–4200 cm^{-1} range. Further characterization of these compounds on the basis of ^1H, ^{13}C, and ^{19}F NMR spectral analysis along with their normal coordination analysis using computational programming is in progress for the determination of structure of these compounds. The representative methods of preparation of organoantimony (III) amides are as follows.

Reaction of $(C_6F_5)_2SbCl$ with Succinimide

In an inert atmosphere, a solution of bis (pentafluorophenyl) antimony (III) chloride (0.490 g; 1 mmol) in benzene and succinimide (0.099 g; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3–4 h. $Et_3N.HCl$ formed was filtered off and the filtrate on evaporation and concentration in vacuum gives a white color crystalline solid, which was recrystallized from pet.-ether (60–80°C) to give bis (pentafluorophenyl)antimony (III)succinimide.

Reaction of $(C_6F_5)_2SbCl$ with Phthalimide

In an inert atmosphere, a solution of bis (pentafluorophenyl) antimony (III) chloride (0.490 g; 1 mmol) in benzene and phthalimide (0.147 g; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3–4 h. $Et_3N.HCl$ formed was filtered off and the filtrate on evaporation and concentration in vacuum gives a red/pink color crystalline solid which was recrystallized from pet.-ether (40–60°C) to give bis (pentafluorophenyl)antimony (III) phthalimide.

Reaction of $(C_6H_5)_2SbCl$ with Succinimide

In an inert atmosphere, a solution of diphenylantimony (III) chloride (0.311 g; 1 mmol) in toluene and succinimide (0.099 g; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3–4 h. $Et_3N.HCl$ formed was filtered off, and the filtrate on evaporation and concentration in vacuum gives a white color crystalline solid which was recrystallized from pet.-ether (40–60°C) to give diphenylantimony (III) succinimide.

Reaction of $(C_6H_4F)_2SbCl$ with Succinimide

In an inert atmosphere, a solution of bis (*p*-fluorophenyl) antimony (III) chloride (0.346 g; 1 mmol) in toluene and succinimide (0.099 g; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3–4 h. $Et_3N.HCl$ formed was filtered off and the filtrate on evaporation and concentration in vacuo gives a white color crystalline solid which was recrystallized from pet.-ether (40–60°C) to give bis (*p*-fluorophenyl) antimony (III) succinimide.

Antitumor Activity

The in-vitro antitumor activity of these compounds was carried out by MTT-Method.[19] This method was carried out to estimate the effect of compounds on the growth of cells. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used here. The principle behind this assay depends upon the reduction of tetrazolium salts. The yellow colored tetrazoleum MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazoleum bromide] reduced by metabolically active cells partially by the action of dehydrogenase enzyme to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple color zones was solubilized and quantified by spectrophotometer method. The MTT was first dissolved in PBS (Phosphate buffer saline) at a concentration of 5 mg/ml. Then 50 μl of the MTT solution was added to each well of the 96-well culture plate, containing 100 μl of culture medium and incubated at 37°C for 4 h. The medium was then removed carefully without disturbing the purple colored zone crystals. Then 50 ml of DMSO was added to each well and mixed thoroughly to dissolve the crystals of the zones. The plates were then read on a micro plate reader at a wavelength of 570 nm. The readings were presented as optical density, which gives the cell count value.

Antibacterial Activity

Antibacterial activity of these compounds was carried out by disc diffusion method (20) using ampicillin as standard. In this technique, the filter paper (Whatman No.1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 μg/ml of ethanol) along with standard was placed on the nutrient agar plate at 37°C for 24 h in BOD incubator. The inhibition zones around the dried impregnated disc were measured after 24 h. The activity was classified as highly active (dia => 15 mm), moderately active (dia = 10–15 mm) and slight active (dia = 5–10 mm). The diameter less than 5 mm was regarded as inactive.

Antifungal Activity

The antifungal activity of these compounds was tested by agar plate diffusion method,[21] using ampicilin as standard. Four concentrations of the test compounds viz., 10, 20, 50, and 100 μg/ml were prepared and tested against two human pathogenic fungal strains, *Aspergillus flavus,* and *Aspergillus niger*. The one ml of each compound was poured into a Petri dish having about 20–25 ml of molten potato dextrose-agar medium. As the medium gets solidify, Petri dishes were inoculated at the center of the plate, separately, with the fungal isolates and kept at 37°C for 96 h in BOD incubator. After 96 h, the colony diameter was measured and % inhibition was calculated by known mathematical calculation[22] in comparison to control.

RESULTS AND DISCUSSION

The diarylantimony (III) amides (R_2SbL) can easily be prepared by the reaction of diarylantimony (III) chloride with suitable amide in

TABLE I Physicochemical Properties of Organoantimony Compounds

S. No.	Molecular formula	Elemental analysis C (%)	H (%)	N (%)	IR (cm^{-1}) V_{asym}(CO)	V_{sym}(CO)	Color
1	$C_{16}H_{14}NO_2Sb$	51.47	3.75	3.75	1706 vs	1307 ms	White
2	$C_{20}H_{14}NO_2Sb$	57.00	3.32	3.32	1761 v	1310 ms	White
3	$C_{16}F_2H_{12}NO_2Sb$	46.94	2.93	3.42	1732 ms	1331 ms	White
4	$C_{20}F_2H_{12}NO_2Sb$	52.51	2.62	3.06	1729 vs	1329 ms	White
5	$C_{16}F_{10}H_4NO_2Sb$	34.71	0.72	2.53	1730 vs	1330 ms	White
6	$C_{20}F_{10}H_4NO_2Sb$	39.93	0.66	2.32	1732 ms	1332 m	Pink/Red

TABLE II Analytical Data of Organoantimony Compounds

S. No.	Compounds	Formula	Formula weight	Melting point (°C)	Yield (%)	Solvent for crystallization
1	$(C_6H_5)_2Sb$(succinimide)	$C_{16}H_{14}NO_2Sb$	373	156	80	Pet.-ether (40–60°C)
2	$(C_6H_5)_2Sb$(phthalimide)	$C_{20}H_{14}NO_2Sb$	421	122	85	Pet.-ether (40–60°C)
3	$(C_6H_4F)_2Sb$(succinimide)	$C_{16}F_2H_{12}NO_2Sb$	409	119	65	Pet.-ether (60–80°C)
4	$(C_6H_4F)_2Sb$(phthalimide)	$C_{20}F_2H_{12}NO_2Sb$	457	110	70	Pet.-ether (40–60°C)
5	$(C_6F_5)_2Sb$(succinimide)	$C_{16}F_{10}H_4NO_2Sb$	553	65	70	Pet.-ether (60–80°C)

presence of triethylamine as a hydrogen chloride acceptor.

$$R_2SbCl + HL \xrightarrow{Et_3N} R_2SbL + Et_3N.HCl \qquad (2)$$

$R = (C_6H_5), (C_6F_5), (C_6H_4F)$
HL = Succinimide, Phthallimide
All the reactions occurred under inert conditions.

IR Spectra

The IR spectra of all the synthesized compounds show absorption bands due to phenyl, *p*–fluorophenyl and pentafluorophenyl groups. The absorption frequencies, which have diagnostic values, are given in Tables I and II. The absorption frequencies due to carbonyl groups (symmetric as well as asymmetric) in the amide derivative have been assigned. The Sb-C vibrations in case of phenyl and pentafluorophenyl derivatives corresponding to the y mode and appears in the range of 448–460 cm^{-1}.

In-vitro Antitumor Activity

The antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compounds showed moderate to higher activity against the tumor cell line and inhibit the growth of tumor (Table III). It was found that all these compounds are in +3 oxidation state of antimony. The slight variation in their activity is due to presence of different kinds of amides as ligands along with presence of fluorine in the main moiety

TABLE III In vitro Anti-tumor Activity

S. No.	Compounds	Cell No. × 10^4 (MCF-7)	Activity	Cell No. × 10^4 (EVSA-7)	Activity
1.	$C_{16}H_{14}NO_2Sb$	12.34 ± 1.05	–	11.74±1.22	–
2.	$C_{20}H_{14}NO_2Sb$	11.69 ± 1.02	–	10.68±1.08	–
3.	$C_{16}F_2H_{12}NO_2Sb$	9.17 ± 0.87	+	9.69±0.92	+
4.	$C_{20}F_2H_{12}NO_2Sb$	9.34 ± 0.65	+	9.66±0.90	+
5.	$C_{16}F_{10}H_4NO_2Sb$	9.89 ± 0.85	+	8.28±0.46	+
6.	$C_{20}F_{10}H_4NO_2Sb$	9.25 ± 0.86	+	8.22±0.42	+
7.	Negative control	10.21 ± 1.01	–	10.23±1.03	–
8.	Positive control	40.26 ± 3.23	–	42.24±4.22	–

*Negative control: culture medium only; **Positive control: 17 β estradiol.

of the compounds. These compounds generally interact with the receptor site of enzyme complex, which are responsible for the cytostatic and cytotoxic conditions for a cell. The antimony compounds in +3 oxidation state can easily binds to the receptor site. Besides this one, molecular-based mechanisms for the inhibition of growth of tumor cell lines may also be considered. It may be possible that the organoantimony compounds generally binds with nitrogen 7 position of purine bases of DNA molecules, where they react with a labile hydrogen and form a complex with DNA strands and affected the replication and transcription of the DNA, and therefore, stop the division of cell in some extent along with the protein synthesis.

Antibacterial Activity

The organoantimony compounds were tested for antibacterial activity against three human pathogenic bacterial strains, viz., *Pseudomonas*

TABLE IV Antibacterial Activity (Zone of Inhibition (mm) dia. ± S.E)

S. No.	Compounds	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiela pneumoniae*
1.	$C_{16}H_{14}NO_2Sb$	11.00 ± 0.57	8.10 ± 0.16	12.00 ± 1.15
2.	$C_{20}H_{14}NO_2Sb$	11.33 ± 0.66	11.00 ± 0.57	8.5 ± 0.29
3.	$C_{16}F_2H_{12}NO_2Sb$	8.0 ± 0.28	19.00 ± 0.57	13.00 ± 0.50
4.	$C_{20}F_2H_{12}NO_2Sb$	17.33 ± 0.6	19.00 ± 0.57	15.00 ± 0.57
5.	$C_{16}F_{10}H_4NO_2Sb$	18.66 ± 0.66	7.83 ± 0.44	10.5 ± 0.76
6.	$C_{20}F_{10}H_4NO_2Sb$	15.66 ± 0.33	16.00 ± 0.57	17.00 ± 0.57
7.	Untreated Control	No inhibition	No inhibition	No inhibition
8.	Ampicilin (standard)	18.0 ± 0.21	12.66 ± 0.50	16.26 ± 0.30

aeruginosa, *Staphylococcus aureus* and *Klebsiela pneumoniae*; using 10 μg/ml concentration of the test compounds. All compounds showed moderate to high activity against these three bacterial strains. The activities of the compounds 4 and 6 were found to be the highest against all the three strains. The rest of the compounds are moderately active (Table IV). It is found that organometallic compounds containing fluoro and pentafluorophenyl ring are more effective against microbes because of their water and lipid solubility. These fluorine-containing compounds may generally form complexes with metaloenzymes, particularly those that are in responsible in basic physiology such as cytochrome oxidase. All these compounds may react with peptidoglycan layer of bacterial cell wall and damage it by penetrating in such a manner that the two phenyl ring gets entered inside the cell by puncturing it and ultimately causing death of bacterial cell. Sometimes, all these compounds in lower

TABLE V Antifungal Activity

S. no.	Compounds	Con. μg/ml.	*Aspergillus flavus* (dia.mm)	% Inhibition	*Aspergillus niger* (dia.mm)	% Inhibition
1.	$C_{16}H_{14}NO_2Sb$	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
2.	$C_{20}H_{14}NO_2Sb$	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
3.	$C_{16}F_2H_{12}NO_2Sb$	10	1.4	5.3	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
4.	$C_{20}F_2H_{12}NO_2Sb$	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
5.	$C_{16}F_{10}H_4NO_2Sb$	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	0.01	96.7	0.5	75.0
6.	$C_{20}F_{10}H_4NO_2Sb$	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
7.	Control		3.0	—	2.0	—

concentration may cause the bacteriostatic condition by slow down the growth of bacteria.

Antifungal Activity

The antifungal activity of all these organoantimony compounds were tested against two fungal strains viz. *Aspergillus flavus* and *Aspergillus niger* using concentrations 10, 20, 50, and 100 μg/ml. The activity of these compounds was found variable at lower concentration but at higher concentration of 50 and 100 μg/ml, all the compounds showed very high activity against the two fungal strains (Table V). The presence of nitrogen, oxygen, phenyl, and pentafluorophenyl ring, along with antimony in +3 oxidation state is the major cause of fungal activity. These compounds generally damage the cell of fungal strains by puncturing the cell wall as same as in case of bacteria. It is well know that the Lewis acidic nature of antimony in +3 oxidation state is also affect the fungal activity. The presence of fluorine also increases the activity due to it water and lipid solubility.

CONCLUSION

The organoantimony (III) amides of the type Ar_2SbL; (Ar = C_6H_5,C_6F_5,C_6H_4F; L = succinimide and phthallimide) have great potential as an anticancer and antimicrobial compounds. These compounds may be exploited for the development of new drugs for the treatment of diseases like cancer and can also be exploited for the treatment of diseases caused by various microorganisms.

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