J M C L
Journal of Medicinal Chemistry Letters
ISSN: 2230–9314 & E-ISSN: 2230–9322, Volume. 1, Issue 2, 2011, PP-10-19
Online available at http://www.bioinfo.in/contents.php?id=64

STUDIES ON SYNTHETIC AND BIOLOGICAL ACTIVITY OF SOME NEW TRIORGANOTIN (IV) CARBOXYLATES

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Received: October 31, 2011; Accepted: November 14, 2011

Abstract-The present manuscript contains a series of new triorganotin (IV) carboxylates of the type R_3SnL, which are synthesized by the modified method and characterized first time for their biological activity. These compounds show remarkable antitumor, antimicrobial activity against various microbial strains along with insecticidal activity respectively.
Key words: Triorganotin (IV) carboxylates, antitumor, antibacterial, antifungal and insecticidal activity.

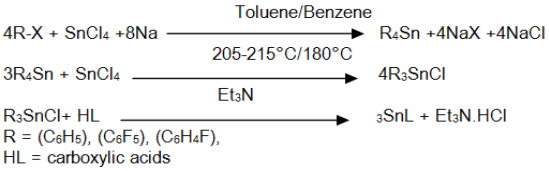
Introduction

The organotin (IV) compounds have wide applications as catalysts, as biocides, as antifouling agents, and for wood preservers [1-10]. In recent years, investigations have been carried out to test their antitumor activity and it has been found that several di and triorganotin (IV) species were found to be active against various types of cancer [11-13]. Biological activity of organotin complexes is believed to be independent on the structure of molecule and coordination number of the metal [4-6]. It has also been noted that many di and triorganotin (IV) carboxylates display interesting antitumor activities [13-20]. However their solubility in water and other polar solvents is poor, therefore many organotin (IV) complexes with carboxylate ligands containing polar substituents have been prepared and studied [13-20] recently. Polar substituents, like fluorine or polyoxaalkyl moieties, improve the water solubility problem of the compounds.

EXPERIMENTAL

The organotin compounds were synthesized by the earlier reported method [21]. The tetraorganotin compound as a base material can be synthesized by the reaction of respective haloarene with tin tetrachloride and sodium metal in inert atmosphere. The synthesis of base material triorganotin (IV) chloride was carried out by cleavage of the base material, tetraorganotin, with metal halides at 205-215^{o}C for one hour by fixing an air condenser and then the temperature was maintained at 180^{o}C for a period of one and half hour. The semisolid mass was extracted with hot pet-ether (40-60^{o}C) and recrystallised with same solvent.

The preparation of triorganotin (IV) carboxylates was carried out by the reaction of R_3SnCl and suitable carboxylic acid in presence of triethylamine, as HCl acceptor, under room temperature and nitrogen atmosphere. The method of preparation of some representative compounds is as follows.



Reaction of $(C_6H_5)_3SnCl$ with CH_3COOH (1)

In an oxygen free nitrogen atmosphere, a solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and acetic acid (0.060gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives a white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_3SnCl$ with $CH_2ClCOOH$ (2)

In an oxygen free nitrogen atmosphere, a solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and chloroacetic acid (0.095gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_3SnCl$ with $CHCl_2COOH$ (3)

In an oxygen free nitrogen atmosphere, a solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and dichloroacetic acid (0.129gm; 1mmol) in same

solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_3SnCl$ with CCl_3COOH (4)
In an oxygen free nitrogen atmosphere, a solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and trichloroacetic acid (0.164gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_3SnCl$ with CF_3COOH (5)
In an oxygen free nitrogen atmosphere, a solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and trifluoroacetic acid (0.114gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives a white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_3SnCl$ with CH_3COOH (6)
In an oxygen free nitrogen atmosphere, a solution of tris(pentafluorophenyl)tin (IV) chloride (0.655gm; 1mmol) in benzene and acetic acid (0.060gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_3SnCl$ with $CH_2ClCOOH$ (7)
In an oxygen free nitrogen atmosphere, a solution of tris(pentafluorophenyl)tin (IV) chloride (0.655gm; 1mmol) in benzene and chloroacetic acid (0.095gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_3SnCl$ with $CHCl_2COOH$ (8)
In an oxygen free nitrogen atmosphere, a solution of tris(pentafluorophenyl)tin (IV) chloride (0.655gm; 1mmol) in benzene and dichloroacetic acid (0.129gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_3SnCl$ with CCl_3COOH (9)
In an oxygen free nitrogen atmosphere, a solution of tris(pentafluorophenyl)tin (IV) chloride (0.655gm; 1mmol) in benzene and trichloroacetic acid (0.164gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6F_5)_3SnCl$ with CF_3COOH (10)
In an oxygen free nitrogen atmosphere, a solution of tris(pentafluorophenyl)tin (IV) chloride (0.655gm; 1mmol) in benzene and trifluoroacetic acid (0.114gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_4F)_3SnCl$ with CH_3COOH (11)
In an oxygen free nitrogen atmosphere, a solution of tris(*p*-fluorophenyl)tin (IV) chloride (0.439gm; 1mmol) in benzene and acetic acid (0.060gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_4F)_3SnCl$ with $CH_2ClCOOH$ (12)
In an oxygen free nitrogen atmosphere, a solution of tris(*p*-fluorophenyl)tin (IV) chloride (0.439gm; 1mmol) in benzene and chloroacetic acid (0.095gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_4F)_3SnCl$ with $CHCl_2COOH$ (13)
In an oxygen free nitrogen atmosphere, a solution of tris(*p*-fluorophenyl)tin (IV) chloride (0.439gm; 1mmol) in benzene and dichloroacetic acid (0.129gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_4F)_3SnCl$ with CCl_3COOH (14)
In an oxygen free nitrogen atmosphere, a solution of tris(*p*-fluorophenyl)tin (IV) chloride (0.439gm; 1mmol) in benzene and trichloroacetic acid (0.164gm; 1mmol) in same solvent were stirred together in presence of

triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives a white colour crystalline solid mass which was further recrystallised in pet-ether.

Reaction of $(C_6H_4F)_3SnCl$ with CF_3COOH (15)

In an oxygen free nitrogen atmosphere, a solution of tris(*p*-fluorophenyl)tin (IV) chloride (0.439gm; 1mmol) in benzene and trifluoroacetic acid (0.114gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

Antitumor Activity

The *in-vitro* antitumor activity of these compounds was carried out by MTT-Method [22]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used for this purpose. The principle behind this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT [3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazoleum bromide] was reduced partially by metabolically active cells by the action of dehydrogenase enzyme to generate NADH and NADPH as reducing equivalents. The resulting intracellular purple Colour zone was solubilized and quantified by spectrophotometer. The MTT was first dissolved in Phosphate buffer saline at a concentration of 5 mg/ml. The MTT solution (50 μl) was added to each well of 96 well culture plate containing 100 μl of culture medium and incubates at 37°C for 4 hrs. The medium was then removed carefully without disturbing the crystals of purple colored zone. 50 ml of DMSO was then added to each well and mixed thoroughly to dissolve the crystals of the zone. The plate was then read on a micro ELISA plate reader at a wavelength of 570 nm to find out the optical density and cell count value.

Antibacterial Activity

Antibacterial activity of the synthesized triorganotin compound was carried out by disc diffusion method [23] using ampicilin as standard. The filter paper (Whatmann No.1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 μg/ml of ethanol) along with standard were placed on the nutrient agar plate at 37°C for 24 hrs in BOD incubator. The inhibition zone around the dried impregnated disc was measured after 24 hrs. 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 compound was tested by agar plate diffusion method [24], using ampicilin as standard. Two concentrations of the test compounds viz., 50 and 100 μg/ml were prepared and tested against two pathogenic fungal strains, *Aspergillus flavus* and *Aspergillus nigar*. The one ml of each compound was poured into a Petri dish containing 20-25 ml of molten potato dextrose - agar medium. As the medium solidify, Petri dishes were inoculated at 37°C for 96 hrs in BOD incubator. After 96 hrs the colony diameter was measured and % inhibition was calculated using standard method.

Contact Toxicity against Insect

The contact toxicity of these compounds was carried out by topical application method [25] against larvae of *Spodoptera litura*, which is harmful for Indian crops. First the given compounds were dissolved in acetone and different concentrations were prepared *viz*., 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. Now each concentration was applied on the dorsal surface of the larvae of insect. About 10 μl of each concentration was applied on each larvae. Some of the larvae of insect was treated by acetone alone, were works as control. Now the mortality data was recorded after 24 hrs, and the treated mortality was corrected with control morality. These corrected mortality data was used for calculation of LC_{50}/LD_{50}.

Stomach Toxicity against Insect

The stomach toxicity of these compounds was carried out by leaf dip method [26]. In this method we used fourth instars larvae of *Spodoptera litura* of an insect which is responsible for the damage of Indian agricultural crops. Ten larvae were used for each replication and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared *viz*. 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. The leaf disc were prepared out of caster leaf and dipped in various concentrations of the test compounds for thirty seconds. Now air dried the leaf discs to evaporate the excess acetone. (The leaf disc dipped only in acetone was served as control). The mortality data was recorded after 24 hrs, and the treatment mortality was corrected with control mortality. These mortality data was used for calculation of LC_{50}/LD_{50}.

Antifeedant Toxicity against Insect

The antifeedant activity of these compounds was also carried out by leaf dip method [26] using fourth instars larvae of *Spodoptera litura*, an insect responsible for the damage of Indian agricultural crops. There are ten larvae were used for each replications and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared *viz*. 0.06%, 0.12%, 0.25%, 0.50% and 1.00%. The leaf discs of about 25 cm^2 were prepared and dipped for thirty seconds in various concentrations of the test compounds. Air dried the leaf discs to evaporate the excess acetone and the leaf discs offered for feeding. The insects were allowed to feed for 24 hrs. After 24 hrs leaf area uneaten was measured by

using leaf area meter. The differences between leaf area provided and the leaf area uneaten is taken as amount of leaf area consumed. The feeding inhibition was calculated and used for calculation of effective concentration (EC_{50}/LD_{50}).

Acaricidal Toxicity against Mites

The acaricidal activity of these compounds was carried out by leaf dip method [26]. Compounds was dissolved in Acetone and different concentrations were prepared *viz*. 0.001%, 0.005%, 0.05%, 0.1%, 0.5% using 0.2% tween 20 as emulsifier. Leaf discs of Mulberry (5 cm^2 diameter) were dipped in different concentration for 30 seconds. Now air dried the leaf discs to remove the excess of acetone and placed over wet cotton in Petri plate. The adult female mites were released on treated leaf discs and mortality data were recorded after 48 hrs. Mites released on leaf treated only with Acetone and tween 20 emulsifier served as control. The mortality data was used for calculation of LC_{50}/LD_{50}.

RESULTS AND DISCUSSION

All the newly synthesized compounds were crystalline solids, air stable and soluble in common organic solvents. The compounds were further characterized by using analytical techniques such as elemental analysis, infrared, NMR spectrometry.

Infrared Spectroscopy

The Infrared spectra of the carboxylic acids and synthesized compounds have been recorded from their KBr pellets in range 4000-400 cm^{-1}. The coordinating mode of the carboxylic acids towards the triorganotin (IV) moieties can be compared by the infrared spectra of free acids and synthesized triorganotin compounds. Frequencies assigned to $\nu_{asym}(COO)$ and $\nu_{sym}(COO)$ have been identified in free acids along with synthesized compounds. The main feature observed in the spectra of these compound is the absence of the broadband in range 2504-3034 cm^{-1}, which appears in free acid as ν(O-H)-position, indicating the metal-acid bond formation through this site. The values of IR stretching vibration frequencies of carboxyl groups [$\nu_{asym}(COO)$ and $\nu_{sym}(COO)$] in triorganotin(IV) carboxylate are helpful in the elucidation of the structures and bonding behavior of the ligands. Therefore, attempts have been taken to correlate the values of characteristic vibration frequencies with their precursor one.

^{1}H NMR Spectroscopy

^{1}H NMR spectra for triorganotin(IV)carboxylates and the free acids have been recorded in $CDCl_3$ and DMSO solution. The data are consistent with those reported earlier. ^{1}H NMR signals of the proton attached to the phenyl, *p*-fluorophenyl moieties have been fully assigned for determination of structure of the compounds.

^{19}F NMR Spectral Studies

In fluorine containing triorganotin compounds, as for the F-4, two signals appeared at δ143.72 and δ144.70 ppm for pentafluorophenyl rings respectively. The coupling of F4 with F2, 6 could not be observed though it was expected. Similarly for F3, 5, two signals appeared at δ155.48 and δ157.8 ppm which are double the intensity of F4 signals. The F2, 6 also showed two signals at δ124.8 and δ128.10ppm. The coupling due to F4 is not observed.

Antitumor Activity

The antitumor activity of triorganotin (IV) carboxylate was studied against the human breast cancer (MCF-7) and mammary cancer cell lines (EVSA-7). Compound shows moderate to high antiproliferative activity against the cell lines. They inhibit the growth of about 40-45% of tumor. The variation in activity is due to variable kind of carboxylate as ligand. The carboxylate having fluorine contents show higher efficacy. It was found that the compounds generally interact with nitrogenous bases of nucleotides of nucleic acid and inhibit the cell division by interfering the replication and transcription of DNA molecules. The compounds may also affect the multienzyme complexes responsible for replication and transcription of DNA thus causing a stop of proliferation of the cells.

Antibacterial Activity

The antibacterial activity of these compounds was tested against three human pathogenic bacteria: *Pseudomonas auruginosa, Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 μg/ml concentration of the test compound. It was found that compound shows high activity against *pseudomonas auruginosa*, *Klebsiela pneumoniae* and against *Staphylococcus aureus*. The variability in the bacterial activity is due to presence of different kinds of carboxylate group as ligand. The chloride containing carboxylate ligands are more effective than the simple carboxylate ligands.

Antifungal Activity

The antifungal activity of these triorganotin compounds was tested against two fungal strains: *Aspergillus flavus* and *Aspergillus niger* at 50 μg/ml and 100 μg/ml respectively of the test compounds. It was so amazing that these compound so much higher efficacy against the fungal strains. Again the activity is due to presence of different kinds of carboxylate which shows higher activity against different fungal strains. The presence of chloride group in carboxylate molecule enhances the activity. At 100 μg/ml concentration, all the compounds show high activity against *Aspergillus flavus* and *Aspergillus niger.* The carboxylate ligand definitely play important role in controlling the fungal infections.

Contact Toxicity against Insects

The contact activity of triorganotin (IV) carboxylate was also tested against the larvae of *Spodoptera litura* insect using different concentration of the compounds. The corrected mortality was calculated to find out the LC_{50} value of the compounds. It was found that the compounds show better activity against the larvae of

insects and shows low value of LC_{50}. It was found that compounds having chlorine and fluorine based ligands show higher activity against insects.

Stomach Toxicity against Insects

The stomach toxicity of these compounds was also tested against the larvae of *Spodoptera litura* using different concentration of the compounds: 0.06%, 0.12%, 0.25%, 0.50% and 1.00%.The corrected mortality was calculated for the calculation of lethal concentration/lethal dose (LC_{50}). It was found that compounds show good activity against the larvae of insect and are much effective. The variation in activity was due to presence of different kinds of carboxylate group in the molecule. The presence of chlorine group in carboxylate ligand increases the activity.

Antifeedant Activity against Insects

The antifeedant activity of these compounds was tested against the insect *Spodopters litura* larvae using different concentration of the compound and the corrected mortality was calculated to find out the effective concentration (EC_{50}). It was found that compound shows high antifeedant activity. It was found that compound having acetate, dichloroacetate; trichloroacetate moieties are more effective against the insects.

Acaricidal Activity against Mites

Acaricidal activity of these compounds was tested against *Tetranychus urticae* using different concentrations 0.001%, 0.005%, 0.05%, 0.1% and 0.5%. The percentage of corrected mortality was calculated to find out the LC_{50} of these compounds. The results were very surprising that all the compounds show high acaricidal activity against the mite. The presence of different kind of carboxylate group as ligand in compounds enhances the activity.

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Table 1-Physicochemical Properties of triorganotin(IV)carboxylate

S.N.	Compounds	Formula	M.P (°C)	Yield (%)	Color	Solvent
1	$(C_6H_5)_3Sn(OOC.CH_3)$	$C_{20}H_{18}O_2Sn$	128-130	62	white	Pet.Ether
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	$C_{20}H_{17}O_2SnCl$	122-125	65	Off-white	Pet.Ether
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	$C_{20}H_{16}O_2SnCl_2$	119/120	70	Off-white	Pet.Ether
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	$C_{20}H_{15}O_2SnCl_3$	115-117	75	Off-white	Pet.Ether
5	$(C_6H_5)_3Sn(OOC.CF_3)$	$C_{20}H_{15}O_2SnF_3$	118-120	82	white	Pet.Ether
6	$(C_6F_5)_3Sn(OOC.CH_3)$	$C_{20}F_{15}H_3O_2Sn$	126-129	65	Off-white	Pet.Ether
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	$C_{20}F_{15}H_2O_2SnCl$	114-116	68	Off-white	Pet.Ether
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	$C_{20}F_{15}HO_2SnCl_2$	110-115	80	Off-white	Pet.Ether
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	$C_{20}F_{15}O_2SnCl_3$	102-106	65	Off-white	Pet.Ether
10	$(C_6F_5)_3Sn(OOC.CF_3)$	$C_{20}F_{18}O_2Sn$	103-105	80	Off-white	Pet.Ether
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	$C_{20}H_{15}F_3O_2Sn$	122-124	55	Off-white	Pet.Ether
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	$C_{20}H_{14}F_3O_2SnCl$	116-120	60	Off-white	Pet.Ether
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	$C_{20}H_{13}F_3O_2SnCl_2$	115-117	65	Off-white	Pet.Ether
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	$C_{20}H_{12}F_3O_2SnCl_3$	110-115	65	white	Pet.Ether
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	$C_{20}H_{12}F_6O_2Sn$	108-110	75	Off-white	Pet.Ether

Table 2- Analytical data of triorganotin(IV)carboxylate

S.N.	Compounds	Formula	Formula Wieght	Elemental Analysis	
				C(%)	H(%)
1	$(C_6H_5)_3Sn(OOC.CH_3)$	$C_{20}H_{18}O_2Sn$	409	58.67	4.40
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	$C_{20}H_{17}O_2SnCl$	443.5	54.11	3.83
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	$C_{20}H_{16}O_2SnCl_2$	478	50.20	3.34
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	$C_{20}H_{15}O_2SnCl_3$	512.5	46.82	2.92
5	$(C_6H_5)_3Sn(OOC.CF_3)$	$C_{20}H_{15}O_2SnF_3$	463	51.83	3.23
6	$(C_6F_5)_3Sn(OOC.CH_3)$	$C_{20}F_{15}H_3O_2Sn$	679	35.34	0.44
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	$C_{20}F_{15}H_2O_2SnCl$	713.5	33.63	0.28
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	$C_{20}F_{15}HO_2SnCl_2$	748	32.08	0.13
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	$C_{20}F_{15}O_2SnCl_3$	782.5	30.69	-
10	$(C_6F_5)_3Sn(OOC.CF_3)$	$C_{20}F_{18}O_2Sn$	733	32.74	-
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	$C_{20}H_{15}F_3O_2Sn$	463	51.83	3.23
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	$C_{20}H_{14}F_3O_2SnCl$	497.5	48.24	2.81
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	$C_{20}H_{13}F_3O_2SnCl_2$	532	45.11	2.44
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	$C_{20}H_{12}F_3O_2SnCl_3$	566.5	42.36	2.11
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	$C_{20}H_{12}F_6O_2Sn$	517	46.42	2.32

Table 3-Antitumor activity of triorganotin (IV) carboxylate

S. No.	Compounds	MCF-7 Cell No. x 10^4	EVSA-7 Cell No. x 10^4	Activity
1	$(C_6H_5)_3Sn(OOC.CH_3)$	9.19±0.92	9.29±0.88	Positive
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	9.17 ± 0.90	8.6 7 ± 0.69	Positive
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	11.59±1.06	11.29±1.02	Negative
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	9.29±0.88	9.89±0.92	Positive
5	$(C_6H_5)_3Sn(OOC.CF_3)$	8.95±0.67	8.55±0.62	Positive
6	$(C_6F_5)_3Sn(OOC.CH_3)$	8.79 ± 0.52	8.42 ± 0.46	Positive
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	11.52±1.02	11.82±1.06	Negative
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	9.19±0.92	9.29±0.88	Positive
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	12.31±1.02	12.39±1.03	Negative
10	$(C_6F_5)_3Sn(OOC.CF_3)$	8.79 ± 0.52	8.42 ± 0.46	Positive
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	9.19±0.92	9.29±0.88	Positive
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	8.95±0.67	8.55±0.62	Positive
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	12.79±1.20	12.69±1.16	Negative
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	11.52±1.02	11.82±1.06	Negative
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	9.19±0.92	9.29±0.88	Positive
16	Negative control	10.21±1.01	10.22±1.01	–
17	Positive control	40.26±3.23	41.23±3.28	–

Table 4-Antibacterial Activity of triorganotin (IV) carboxylate

S. N.	Compounds	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiela pneumoniae*
1	$(C_6H_5)_3Sn(OOC.CH_3)$	–	+++	+++	++
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	–	++	++	++
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	–	++	++	++
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	–	++	++	++
5	$(C_6H_5)_3Sn(OOC.CF_3)$	–	++	++	+++
6	$(C_6F_5)_3Sn(OOC.CH_3)$	–	+++	++	++
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	–	++	++	++
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	–	++	++	+++
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	–	+	+++	++
10	$(C_6F_5)_3Sn(OOC.CF_3)$	–	+++	++	++
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	–	++	+	++
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	–	++	++	++
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	–	+	++	+
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	–	+++	+	++
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	–	++	++	++

+ = 6-10 mm; ++ = 10-14 mm; +++= >14 mm; – = Inactive

Table 5-Antifungal Activity of triorganotin (IV) carboxylate at 50 μg/ml conc.

S. N.	Compounds	*Aspergillus flavus* Col. Dia. (mm)	% Inhibition	*A. niger* Col. Dia.(mm)	% Inhibition
1	$(C_6H_5)_3Sn(OOC.CH_3)$	0.2	93.3	0.7	65.0
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	0.2	93.3	0.7	65.0
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	0.4	86.7	0.6	70.0
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	0.7	76.63	0.6	70.0
5	$(C_6H_5)_3Sn(OOC.CF_3)$	0.2	93.3	0.7	65.0
6	$(C_6F_5)_3Sn(OOC.CH_3)$	0.2	93.3	0.7	65.0
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	0.7	76.6	0.7	65.0
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	0.8	73.3	0.8	60.0
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	0.8	73.3	0.8	60.0
10	$(C_6F_5)_3Sn(OOC.CF_3)$	0.7	76.63	0.6	70.0
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	0.7	76.6	0.5	75.0
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	0.5	83.3	0.4	80.0
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	0.5	83.3	0.4	80.0
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	0.6	80.0	0.7	65.0
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	0.7	76.6	0.7	65.0
16	Control	3.0	–	2.0	–

Table 6- Antifungal Activity of triorganotin (IV) carboxylate at 100 μg/ml conc.

S. N.	Compounds	*Aspergillus flavus* Col. Dia. (mm)	% Inhibition	*Aspergillus niger* Col. Dia. (mm)	% Inhibition
1	$(C_6H_5)_3Sn(OOC.CH_3)$	0.1	96.7	0.4	80.0
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	0.2	93.3	0.3	75.0
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	0.1	96.7	0.3	75.0
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	0.1	96.7	0.1	95.0
5	$(C_6H_5)_3Sn(OOC.CF_3)$	0.2	93.3	0.3	85.0
6	$(C_6F_5)_3Sn(OOC.CH_3)$	0.1	96.7	0.3	75.0
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	0.2	93.3	0.3	75.0
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	0.1	96.7	0.2	90.0
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	0.2	93.3	0.1	95.0
10	$(C_6F_5)_3Sn(OOC.CF_3)$	0.1	96.7	0.1	95.0
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	0.4	86.7	0.2	90.0
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	0.2	93.3	0.2	90.0
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	0.1	96.7	0.4	80.0
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	0.1	96.7	0.2	90.0
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	0.2	93.3	0.3	85.0
16	Control	3.0	–	2.0	–

Table 7-Contact Toxicity of triorganotin (IV) carboxylate

S. N.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1	$(C_6H_5)_3Sn(OOC.CH_3)$	1.61-9.30	1.07±0.17	0.67 (3)	2.83
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	0.72-1.46	1.71±0.18	3.32 (3)	0.97
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	1.87-12.07	1.09±0.19	1.63 (3)	3.52
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	0.56-1.05	1.32±0.15	0.63 (3)	0.73
5	$(C_6H_5)_3Sn(OOC.CF_3)$	0.43-0.75	1.63±0.6	2.94 (3)	0.58
6	$(C_6F_5)_3Sn(OOC.CH_3)$	1.42-3.89	1.32±0.16	2.37 (3)	2.12
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	1.87-12.07	1.09±0.19	1.62 (3)	3.53
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	1.57-9.32	1.07±0.17	0.72 (3)	2.83
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	0.28-0.40	1.96±0.16	4.39 (3)	0.33
10	$(C_6F_5)_3Sn(OOC.CF_3)$	0.39-0.59	1.67±0.15	5.62 (3)	0.46
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	1.87-12.07	1.09±0.19	1.63 (3)	3.52
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	0.56-1.05	1.32±0.15	0.63 (3)	0.73
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	1.42-3.89	1.32±0.16	2.37 (3)	2.12
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	0.72-1.46	1.71±0.18	3.32 (3)	0.97
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	1.87-12.07	1.09±0.19	1.63 (3)	3.52

Table 8-Stomach Toxicity of triorganotin (IV) carboxylate

S. N.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1	$(C_6H_5)_3Sn(OOC.CH_3)$	0.74-1.32	1.62±0.18	3.24 (3)	0.94
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	0.85-1.82	1.22±0.16	0.72 (3)	1.12
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	0.55-0.97	1.32±0.15	0.69 (3)	0.73
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	1.33-3.99	1.42±0.20	2.38 (3)	2.01
5	$(C_6H_5)_3Sn(OOC.CF_3)$	0.55-0.90	1.48±0.16	3.37 (3)	0.67
6	$(C_6F_5)_3Sn(OOC.CH_3)$	0.56-0.97	1.33±0.15	0.63 (3)	0.75
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	0.55-0.97	1.32±0.15	0.69 (3)	0.73
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	1.61-9.55	1.45±0.17	0.68 (3)	2.97
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	0.86-1.99	1.28±0.16	0.80 (3)	1.20
10	$(C_6F_5)_3Sn(OOC.CF_3)$	0.49-0.76	1.57±0.16	2.78 (3)	0.60
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	0.55-0.90	1.48±0.16	3.37 (3)	0.67
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	0.56-0.97	1.33±0.15	0.63 (3)	0.75
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	0.85-1.82	1.22±0.16	0.72 (3)	1.12
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	1.33-3.99	1.42±0.20	2.38 (3)	2.01
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	1.62-9.39	1.01±0.17	0.69 (3)	2.93

Table 9-Antifeedant Toxicity of triorganotin (IV) carboxylate

S. N.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1	$(C_6H_5)_3Sn(OOC.CH_3)$	0.45-1.09	0.87±0.13	1.71 (3)	0.64
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	0.49-0.76	1.52±0.16	2.59 (3)	0.58
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	0.72-2.41	0.93±0.14	0.22 (3)	1.13
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	0.30-0.47	1.28±0.14	3.42 (3)	0.39
5	$(C_6H_5)_3Sn(OOC.CF_3)$	0.33-0.61	1.00±0.13	0.68 (3)	0.43
6	$(C_6F_5)_3Sn(OOC.CH_3)$	0.83-2.33	1.08±0.15	0.79 (3)	1.24
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	0.30-0.47	1.28±0.14	3.42 (3)	0.39
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	0.33-0.61	1.00±0.13	0.68 (3)	0.43
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	0.33-0.61	1.00±0.13	0.68 (3)	0.43
10	$(C_6F_5)_3Sn(OOC.CF_3)$	0.82-3.41	1.81±0.14	0.43 (3)	1.35
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	0.68-1.72	1.03±0.14	0.66 (3)	0.98
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	0.43-0.87	1.03±0.14	0.34 (3)	0.58
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	0.62-1.42	1.06±0.14	1.07 (3)	0.86
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	0.83-2.33	1.08±0.15	0.79 (3)	1.24
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	0.72-2.41	0.93±0.14	0.22 (3)	1.13

Table 10- Acaricidal Toxicity of triorganotin (IV) carboxylate

S. N.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1	$(C_6H_5)_3Sn(OOC.CH_3)$	0.08-0.23	0.65±0.07	6.12 (3)	0.13
2	$(C_6H_5)_3Sn(OOC.CH_2Cl)$	0.05-0.10	0.97±0.07	13.23 (3)	0.07
3	$(C_6H_5)_3Sn(OOC.CHCl_2)$	0.07-0.22	0.76±0.06	5.63 (3)	0.14
4	$(C_6H_5)_3Sn(OOC.CCl_3)$	0.05-0.10	0.78±.0.06	4.64 (3)	0.06
5	$(C_6H_5)_3Sn(OOC.CF_3)$	0.10-0.23	0.88±0.08	2.14 (3)	0.15
6	$(C_6F_5)_3Sn(OOC.CH_3)$	0.07-0.22	0.76±0.06	5.63 (3)	0.14
7	$(C_6F_5)_3Sn(OOC.CH_2Cl)$	0.05-0.10	0.78±.0.06	4.64 (3)	0.06
8	$(C_6F_5)_3Sn(OOC.CHCl_2)$	0.10-0.23	0.88±0.08	2.14 (3)	0.15
9	$(C_6F_5)_3Sn(OOC.CCl_3)$	0.05-0.09	0.16±0.09	12.67 (3)	0.07
10	$(C_6F_5)_3Sn(OOC.CF_3)$	0.14-0.31	0.96±0.09	7.52 (3)	0.20
11	$(FC_6H_4)_3Sn(OOC.CH_3)$	0.12-0.30	0.78±0.08	1.70 (3)	0.18
12	$(FC_6H_4)_3Sn(OOC.CH_2Cl)$	0.14-0.31	0.96±0.09	7.52 (3)	0.20
13	$(FC_6H_4)_3Sn(OOC.CHCl_2)$	0.05-0.10	0.93±0.08	13.22 (3)	0.06
14	$(FC_6H_4)_3Sn(OOC.CCl_3)$	0.04-0.09	0.69±0.06	4.64 (3)	0.05
15	$(FC_6H_4)_3Sn(OOC.CF_3)$	0.05-0.09	0.16±0.09	12.67 (3)	0.07

I J C R
International Journal of Chemical Research
ISSN: 0975-3699 & E-ISSN: 0975–9131, Vol. 3, Issue 3, 2011, pp-61-65
Available online at http://www.bioinfo.in/contents.php?id=23

STUDIES ON ANTIMICROBIAL AND ANTITUMOR EFFICACY OF SOME NEW DIORGANOTIN (IV) DICARBOXYLATES

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Received: October 11, 2011; Accepted: November 09, 2011

Abstract-In this manuscript a series of new diorganotin (IV) dicarboxylates were screened out first time for their bio medicinal activity, like antimicrobial and *in-vitro* antitumor activity against different pathogenic bacterial and fungal strains along with human breast and mammary cancer cell line. It was found that the compounds show remarkable antitumor efficacy and moderate antimicrobial activity against pathogenic microbial strains.
Key words: Diorganotin (IV) dicarboxylates, antitumor, antibacterial, antifungal activity.

INTRODUCTION

The spectrum of the biomedicinal aspects of organotin compounds has been expanded as they have found their place among a class of potential biologically active compounds [1-4] exhibiting antimicrobial activity against different kinds of microbial strains [5-14]. They also show anti-inflammatory and cardiovascular activity [15], trypanosomal activity [16, 17] along with anti-herpes [18] and anti-tubercular activity [19]. The present work deals the bio medicinal activity, like antimicrobial and *in-vitro* antitumor activity against different pathogenic bacterial and fungal strains along with human breast and mammary cancer cell line. Fluorine based compounds were synthesized because of their higher biological efficacy due to higher water and lipid solubility.

EXPERIMENTAL

The organotin compounds were synthesized by the earlier reported method [20]. Tetraorganotin compound as base material can be synthesized by the reaction of respective haloarene with tin tetrachloride and sodium metal in inert atmosphere. The synthesis of base material diorganotin (IV) dichloride was carried out by cleavage of the base material, tetraorganotin, with metal halides at 180°C for two hour by fixing an air condenser. The semisolid mass was extracted with hot pet-ether (40-60°C) and recrystallised with same solvent.

$$4R\text{-}X + SnCl_4 + 8Na \xrightarrow{\text{Toluene/Benzene}} R_4Sn + 4NaX + 4NaCl$$

$$R_4Sn + SnCl_4 \xrightarrow{180^\circ C} 2R_2SnCl_2$$

$$R_2SnCl_2 + 2HL \xrightarrow{Et_3N} R_2SnL_2 + 2Et_3N.HCl$$

R = (C_6H_5), (C_6F_5), (C_6H_4F), HL = Respective carboxylic acid

The preparation of diorganotin (IV) dicarboxylates was carried out by the reaction of R_2SnCl_2 and suitable carboxylic acid in presence of triethylamine, as HCl acceptor, under room temperature and nitrogen atmosphere. The biomedicinal screening of the entire newly synthesized compound was performed by the standard reported methods. The experimental details are as follows.

Antitumor Activity

The *in-vitro* antitumor activity of these compounds was carried out by MTT-Method [21]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used for this purpose. The principle behind this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT [3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazoleum bromide] was reduced partially by metabolically active cells by the action of dehydrogenase enzyme to generate NADH and NADPH as reducing equivalents.

The resulting intracellular purple Colour zone was solubilized and quantified by spectrophotometer. The MTT was first dissolved in Phosphate buffer saline at a concentration of 5 mg/ml. The MTT solution (50 μl) was added to each well of 96 well culture plate containing 100 μl of culture medium and incubates at 37°C for 4 hrs. The medium was then removed carefully without disturbing the crystals of purple colored zone. 50 ml of DMSO was then added to each well and mixed thoroughly to dissolve the crystals of the zone. The plate was then read on a micro ELISA plate reader at a wavelength of 570 nm to find out the optical density and cell count value.

Antibacterial Activity

Antibacterial activity of the synthesized compound was carried out by disc diffusion method [22] using ampicilin as standard. The filter paper (Whatmann No.1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 μg/ml of ethanol) along with standard were placed on the nutrient agar plate at 37°C for 24 hrs in BOD incubator. The inhibition zone around the dried impregnated disc was measured after 24 hrs. 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 the compound was tested by agar plate diffusion method [23], using ampicilin as standard. Two concentrations of the test compounds viz., 50 and 100 μg/ml were prepared and tested against two pathogenic fungal strains, *Aspergillus flavus* and *Aspergillus nigar*. The one ml of each compound was poured into a Petri dish containing 20-25 ml of molten potato dextrose - agar medium. As the medium solidify, Petri dishes were inoculated at 37°C for 96 hrs in BOD incubator. After 96 hrs the colony diameter was measured and % inhibition was calculated using standard method.

RESULTS AND DISCUSSION

All the newly synthesized compounds were crystalline solids, air stable and soluble in common organic solvents. The compounds were further characterized by using analytical techniques such as elemental analysis, infrared, NMR spectrometry, to ascertain their structures and explore other properties but in this manuscript we focused only on their biomedicinal characterization.

Antitumor Activity

The antitumor activity of diorganotin (IV) dicarboxylate was studied against the human breast cancer (MCF-7) and mammary cancer cell lines (EVSA-7). Compound shows moderate to high antiproliferative activity against the cell lines. They inhibit the growth of about 35-40% of tumor. The variation in activity is due to variable kind of carboxylate as ligands. The carboxylate having fluorine contents show higher efficacy. It was found that the compounds generally interact with nitrogenous bases of nucleotides of nucleic acid and inhibit the cell division by interfering the replication and transcription of DNA molecules. The compounds may also affect the multienzyme complexes responsible for replication and transcription of DNA thus causing a stop of proliferation of the cells.

Antibacterial Activity

The antibacterial activity of these compounds was tested against three human pathogenic bacteria: *Pseudomonas auruginosa, Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 μg/ml concentration of the test compound. It was found that compound shows high activity against *pseudomonas auruginosa*, *Klebsiela pneumoniae* and against *Staphylococcus aureus*. The variability in the bacterial activity is due to presence of different kinds of carboxylate group as ligand. The chloride containing carboxylate ligands are more effective than the simple carboxylate ligands.

Antifungal Activity

The antifungal activity of these compounds was tested against two fungal strains: *Aspergillus flavus* and *Aspergillus niger* at 50 μg/ml and 100 μg/ml respectively of the test compounds. It was so amazing that these compound so much higher efficacy against the fungal strains. Again the activity is due to presence of different kinds of carboxylate which shows higher activity against different fungal strains. The presence of chloride group in carboxylate molecule enhances the activity. At 100 μg/ml concentration, all the compounds show high activity against *Aspergillus flavus* and *Aspergillus niger.* The carboxylate ligand definitely play important role in controlling the fungal infections.

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Table 1-Antitumor activity of diorganotin (IV) dicarboxylate

S. No.	Compounds	MCF-7 Cell No. x 10^4	EVSA-7 Cell No. x 10^4	Activity
1	$(C_6H_5)_2Sn(OOC.CH_3)_2$	11.69 ± 1.04	11.82 ± 1.06	Negative
2	$(C_6H_5)_2Sn(OOC.CH_2Cl)_2$	9.17 ± 0.90	8.6 7 ± 0.69	Positive
3	$(C_6H_5)_2Sn(OOC.CHCl_2)_2$	8.79 ± 0.52	8.42 ± 0.46	Positive
4	$(C_6H_5)_2Sn(OOC.CCl_3)_2$	12.31±1.02	12.39±1.03	Negative
5	$(C_6H_5)_2Sn(OOC.CF_3)_2$	8.95±0.67	8.55±0.62	Positive
6	$(C_6F_5)_2Sn(OOC.CH_3)_2$	11.59±1.06	11.29±1.02	Negative
7	$(C_6F_5)_2Sn(OOC.CH_2Cl)_2$	9.29±0.88	9.89±0.92	Positive
8	$(C_6F_5)_2Sn(OOC.CHCl_2)_2$	12.79±1.20	12.69±1.16	Negative
9	$(C_6F_5)_2Sn(OOC.CCl_3)_2$	11.52±1.02	11.82±1.06	Negative
10	$(C_6F_5)_2Sn(OOC.CF_3)_2$	9.19±0.92	9.29±0.88	Positive
11	$(FC_6H_4)_2Sn(OOC.CH_3)_2$	9.17 ± 0.90	8.6 7 ± 0.69	Positive
12	$(FC_6H_4)_2Sn(OOC.CH_2Cl)_2$	8.95±0.67	8.55±0.62	Positive
13	$(FC_6H_4)_2Sn(OOC.CHCl_2)_2$	8.79 ± 0.52	8.42 ± 0.46	Positive
14	$(FC_6H_4)_2Sn(OOC.CCl_3)_2$	11.52±1.02	11.82±1.06	Negative
15	$(FC_6H_4)_2Sn(OOC.CF_3)_2$	9.19±0.92	9.29±0.88	Positive
16	Negative control	10.21±1.01	10.22±1.01	–
17	Positive control	40.26±3.23	41.23±3.28	–

Table 2-Antibacterial Activity of diorganotin (IV) dicarboxylate

S. N.	Compounds	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiela pneumoniae*
1	$(C_6H_5)_2Sn(OOC.CH_3)_2$	–	+++	++	++
2	$(C_6H_5)_2Sn(OOC.CH_2Cl)_2$	–	++	+	++
3	$(C_6H_5)_2Sn(OOC.CHCl_2)_2$	–	++	+	++
4	$(C_6H_5)_2Sn(OOC.CCl_3)_2$	–	++	++	++
5	$(C_6H_5)_2Sn(OOC.CF_3)_2$	–	+	++	+
6	$(C_6F_5)_2Sn(OOC.CH_3)_2$	–	+++	+	++
7	$(C_6F_5)_2Sn(OOC.CH_2Cl)_2$	–	++	+	++
8	$(C_6F_5)_2Sn(OOC.CHCl_2)_2$	–	++	+	+++
9	$(C_6F_5)_2Sn(OOC.CCl_3)_2$	–	+	+++	++
10	$(C_6F_5)_2Sn(OOC.CF_3)_2$	–	+++	++	++
11	$(FC_6H_4)_2Sn(OOC.CH_3)_2$	–	++	+	++
12	$(FC_6H_4)_2Sn(OOC.CH_2Cl)_2$	–	++	++	++
13	$(FC_6H_4)_2Sn(OOC.CHCl_2)_2$	–	+	++	+
14	$(FC_6H_4)_2Sn(OOC.CCl_3)_2$	–	+++	+	++
15	$(FC_6H_4)_2Sn(OOC.CF_3)_2$	–	++	+	++

+ = 6-10 mm; ++ = 10-14 mm; +++= >14 mm; – = Inactive

Table 3-Antifungal Activity of diorganotin (IV) dicarboxylate at 50 μg/ml conc.

S. N.	Compounds	*Aspergillus flavus* Col. Dia. (mm)	% Inhibition	*Aspergillus niger* Col. Dia. (mm)	% Inhibition
1	$(C_6H_5)_2Sn(OOC.CH_3)_2$	0.7	76.6	0.5	75.0
2	$(C_6H_5)_2Sn(OOC.CH_2Cl)_2$	0.5	83.3	0.4	80.0
3	$(C_6H_5)_2Sn(OOC.CHCl_2)_2$	0.5	83.3	0.4	80.0
4	$(C_6H_5)_2Sn(OOC.CCl_3)_2$	0.6	80.0	0.7	65.0
5	$(C_6H_5)_2Sn(OOC.CF_3)_2$	0.7	76.63	0.6	70.0
6	$(C_6F_5)_2Sn(OOC.CH_3)_2$	0.8	73.3	0.8	60.0
7	$(C_6F_5)_2Sn(OOC.CH_2Cl)_2$	0.7	76.6	0.7	65.0
8	$(C_6F_5)_2Sn(OOC.CHCl_2)_2$	0.2	93.3	0.7	65.0
9	$(C_6F_5)_2Sn(OOC.CCl_3)_2$	0.2	93.3	0.7	65.0
10	$(C_6F_5)_2Sn(OOC.CF_3)_2$	0.4	86.7	0.6	70.0
11	$(FC_6H_4)_2Sn(OOC.CH_3)_2$	0.7	76.63	0.6	70.0
12	$(FC_6H_4)_2Sn(OOC.CH_2Cl)_2$	0.8	73.3	0.8	60.0
13	$(FC_6H_4)_2Sn(OOC.CHCl_2)_2$	0.7	76.6	0.7	65.0
14	$(FC_6H_4)_2Sn(OOC.CCl_3)_2$	0.2	93.3	0.7	65.0
15	$(FC_6H_4)_2Sn(OOC.CF_3)_2$	0.2	93.3	0.7	65.0
16	Control	3.0	–	2.0	–

Table 4-Antifungal Activity of diorganotin (IV) dicarboxylate at 100 μg/ml conc.

S. N.	Compounds	*Aspergillus flavus* Col. Dia. (mm)	% Inhibition	*Aspergillus niger* Col. Dia. (mm)	% Inhibition
1	$(C_6H_5)_2Sn(OOC.CH_3)_2$	0.1	96.7	0.2	90.0
2	$(C_6H_5)_2Sn(OOC.CH_2Cl)_2$	0.2	93.3	0.1	95.0
3	$(C_6H_5)_2Sn(OOC.CHCl_2)_2$	0.1	96.7	0.1	95.0
4	$(C_6H_5)_2Sn(OOC.CCl_3)_2$	0.4	86.7	0.2	90.0
5	$(C_6H_5)_2Sn(OOC.CF_3)_2$	0.2	93.3	0.2	90.0
6	$(C_6F_5)_2Sn(OOC.CH_3)_2$	0.1	96.7	0.4	80.0
7	$(C_6F_5)_2Sn(OOC.CH_2Cl)_2$	0.2	93.3	0.3	75.0
8	$(C_6F_5)_2Sn(OOC.CHCl_2)_2$	0.1	96.7	0.3	75.0
9	$(C_6F_5)_2Sn(OOC.CCl_3)_2$	0.1	96.7	0.2	90.0
10	$(C_6F_5)_2Sn(OOC.CF_3)_2$	0.2	93.3	0.3	85.0
11	$(FC_6H_4)_2Sn(OOC.CH_3)_2$	0.1	96.7	0.4	80.0
12	$(FC_6H_4)_2Sn(OOC.CH_2Cl)_2$	0.2	93.3	0.3	75.0
13	$(FC_6H_4)_2Sn(OOC.CHCl_2)_2$	0.1	96.7	0.3	75.0
14	$(FC_6H_4)_2Sn(OOC.CCl_3)_2$	0.1	96.7	0.1	95.0
15	$(FC_6H_4)_2Sn(OOC.CF_3)_2$	0.2	93.3	0.3	85.0
16	Control	3.0	–	2.0	–

International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975–9107, Vol. 3, Issue 3, 2011, PP-121-127
Online available at http://www.bioinfo.in/contents.php?id=26

STUDIES ON SYNTHETIC, BIOLOGICAL AND INSECTICIDAL ASPECTS OF SOME DIORGANOTIN (IV) DIAMIDES

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Received: October 31, 2011; Accepted: November 14, 2011

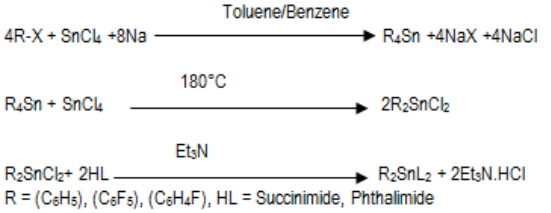
Abstract- The present manuscript contains a series of new diorganotin (IV) diamides of the type R_2SnL_2; (R= C_6H_5, C_6F_5, C_6H_4F; L = succinimide and phthalimide), which are synthesized and tested first time for their biological and insecticidal activity. These compounds show valuable antimicrobial activity against various microbial strains as seen in their inhibition zone value along with higher to moderate insecticidal activity respectively.
Key words: Diorganotin (IV) diamides, Antibacterial, Antifungal and insecticidal activity.

INRODUCTION

The complexes of metals are used routinely in several biomedical and commercial applications like agricultural biocides, disinfectants, antitumor agents, wood preservatives, antioxidants, stabilizers for polyvinylchloride, marine antifouling coating, anti-herpes agents, flame retardants, smoke suppressants, anti-wear agents, homogenous catalysts and recycling agents[1-9]. The organotin compounds emerged as potential biologically active compounds in last 15-20 years. The studies of organotin compounds has been expanded as they have found their place among a class of potential biologically active compounds [1] exhibiting antimicrobial activity against different kinds of microbial strains [2]. They also show anti-inflammatory and cardiovascular activity [3], trypanosomal activity [4] along with anti-herpes [5] and anti-tubercular activity [6]. The first organotin compound, for which the anti-tumor activities were examined, was formally similar to cisplatin [7] or its analogous carboplatin and paraplatin [8]. These compounds show borderline activities against leukemia P388 and L1210 [9]. Many diorganotin compounds, R_2SnX_2, were investigated in context of their antimicrobial and antitumor activity [10-16]. They appear to contain pronounced *in-vitro* antitumor activity but their solubility still remains drawbacks [16], which affect the tumor activity. In order to make this kind of compounds more soluble in water, less complicated structure was designed which contains polar moieties [17]. The introduction of polar groups in the organotin molecules leads to some improvement in the solubility and *in-vitro* activity. Fluorine containing organotin compounds were synthesized to check the effect of compounds on tumor cell line [18]. It was found that fluorine containing compounds are more soluble in water and still very soluble in non-polar solvents [19]. The solubility can also be increased by preparing salts of organotin compounds [20]. In present manuscript a series of new diorganotin (IV) diamides are synthesized, characterized and tested first time for their antitumor, antimicrobial and insecticidal activity.

EXPERIMENTAL

The organotin compounds were synthesized by the earlier reported method [21]. The tetraorganotin compound as a base material can be synthesized by the reaction of respective haloarene with tin tetrachloride and sodium metal in inert atmosphere. The synthesis of base material diorganotin (IV) dichloride was carried out by cleavage of the base material, tetraorganotin, with metal halides at 180°C for two hour by fixing an air condenser. The semisolid mass was extracted with hot pet-ether (40-60°C) and recrystallised with same solvent.



The preparation of diorganotin (IV) diamides was carried out by the reaction of R_2SnCl_2 and suitable amide in presence of triethylamine, as HCl acceptor, under room temperature and nitrogen atmosphere. The method of preparation of some representative compounds is as follows.

Reaction of $(C_6H_5)_2SnCl_2$ with Succinimide (1)

Under nitrogen atmosphere, solution of diphenyltin (IV) dichloride (0.344gm; 1mmol) in benzene and succinimide (0.198gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ was formed which

was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid mass which was further recrystallised in pet. ether.

Reaction of $(C_6H_5)_2SnCl_2$ with Phthallimide (2)
Under nitrogen atmosphere, solution of diphenyltin (IV) dichloride (0.344gm; 1mmol) in benzene and phthalimide (0.294gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vacuum gives an off-white color crystalline solid mass which was further recrystallised in pet. ether.

Reaction of $(C_6F_5)_2SnCl_2$ with Succinimide (3)
In an oxygen free nitrogen atmosphere, solution of bis(pentafluorophenyl)tin (IV) dichloride (0.524gm; 1mmol) in benzene and succinimide (0.198gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vacuum gives an off white colour crystalline solid mass which was further recrystallised in pet. ether.

Reaction of $(C_6F_5)_2SnCl_2$ with Phthalimide (4)
In an oxygen free nitrogen atmosphere, solution of bis(pentafluorophenyl)tin (IV) dichloride (0.524gm; 1mmol) in benzene and phthalimide (0.294gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ was formed which was filtered off and filtrate on evaporation in vacuum gives an off white colour crystalline solid mass which was further recrystallised in pet. ether.

Reaction of $(C_6H_4F)_2SnCl_2$ with Succinimide (5)
In an oxygen free nitrogen atmosphere, solution of bis(*p*-fluorophenyl)tin (IV) dichloride (0.380gm; 1mmol) in benzene and succinimide (0.198gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vacuum gives an off-white colour crystalline solid mass which was further recrystallised in pet. ether.

Reaction of $(C_6H_4F)_2SnCl_2$ with Phthalimide (6)
In an oxygen free nitrogen atmosphere, solution of bis(*p*-fluorophenyl)tin (IV) dichloride (0.380gm; 1mmol) in benzene and phthalimide (0.294gm; 2mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vacuum gives an off-white colour crystalline solid mass which was further recrystallised in pet. ether.

Antitumor Activity
The *in-vitro* antitumor activity of these compounds was carried out by MTT-Method [22]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used for this purpose. The principle behind this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT [3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazoleum bromide] was reduced partially by metabolically active cells by the action of dehydrogenase enzyme to generate NADH and NADPH as reducing equivalents. The resulting intracellular purple Colour zone was solubilized and quantified by spectrophotometer. The MTT was first dissolved in Phosphate buffer saline at a concentration of 5 mg/ml. The MTT solution (50 μl) was added to each well of 96 well culture plate containing 100 μl of culture medium and incubates at 37°C for 4 hrs. The medium was then removed carefully without disturbing the crystals of purple colored zone. 50 ml of DMSO was then added to each well and mixed thoroughly to dissolve the crystals of the zone. The plate was then read on a micro ELISA plate reader at a wavelength of 570 nm to find out the optical density and cell count value.

Antibacterial Activity
Antibacterial activity of the synthesized compound was carried out by disc diffusion method [23] using gentamycin as standard. The filter paper (Whatmann No.1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 μg/ml of ethanol) along with standard were placed on the nutrient agar plate at 37°C for 24 hrs in BOD incubator. The inhibition zone around the dried impregnated disc was measured after 24 hrs. 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 the compound was tested by agar plate diffusion method [24], using gentamycin as standard. Two concentrations of the test compounds viz. 50 and 100 μg/ml were prepared and tested against two pathogenic fungal strains, *Aspergillus flavus* and *Aspergillus niger*. The one ml of each compound was poured into a Petri dish containing 20-25 ml of molten potato dextrose - agar medium. As the medium solidify, Petri dishes were inoculated at 37°C for 96 hrs in BOD incubator. After 96 hrs the colony diameter was measured and % inhibition was calculated.

Contact Toxicity
The contact toxicity of these compounds was carried out by topical application method [25] against larvae of *Spodoptera litura*, which is harmful for Indian crops. First the given compounds were dissolved in acetone and different concentrations were prepared *viz*., 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. Now each concentration was applied on the dorsal surface of the larvae of insect. About 10 μl of each concentration was applied on each larvae. Some of the larvae of insect was

treated by acetone alone, were works as control. Now the mortality data was recorded after 24 hrs, and the treated mortality was corrected with control morality. These corrected mortality data was used for calculation of LC_{50}/LD_{50} using Maximum Likelihood programme MLP 3.01.

Stomach Toxicity

The stomach toxicity of these compounds was carried out by leaf dip method [26]. In this method we used fourth instar larvae of *Spodoptera litura* of an insect which is responsible for the damage of Indian agricultural crops. Ten larvae were used for each replication and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared *viz*. 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. The leaf disc were prepared out of caster leaf and dipped in various concentrations of the test compounds for thirty seconds. Now air dried the leaf discs to evaporate the excess acetone. (The leaf discs dipped only in acetone were served as control). The mortality data was recorded after 24 hrs, and the treatment mortality was corrected with control mortality. These mortality data was used for calculation of LC_{50}/LD_{50} using maximum likelihood programm, MLP 3.01.

Antifeedant Activity

The antifeedant activity of these compounds was also carried out by leaf dip method [26] using fourth instar larvae of *Spodoptera litura*, an insect responsible for the damage of Indian agricultural crops. There are ten larvae were used for each replications and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared *viz*. 0.06%, 0.12%, 0.25%, 0.50% and 1.00%. The leaf discs of about 25 cm^2 were prepared and dipped for thirty seconds in various concentrations of the test compounds. Now air dried the leaf discs to evaporate the excess acetone and the leaf discs offered for feeding. The insects were allowed to feed for 24 hrs. After 24 hrs leaf area uneaten was measured by using leaf area meter. The differences between leaf area provided and the leaf area uneaten is taken as amount of leaf area consumed. The feeding inhibition was calculated and used for calculation of effective concentration (EC_{50}/LD_{50}) using Maximum likelihood programme, MLP 3.01.

Acaricidal Activity

The acaricidal activity of these compounds was carried out by leaf dip method [26]. Compounds was dissolved in Acetone and different concentrations were prepared *viz*. 0.001%, 0.005%, 0.05%, 0.1%, 0.5% using 0.2% tween 20 as emulsifier. Leaf discs of Mulberry (5 cm^2 diameter) were dipped in different concentration for 30 seconds. Now air dried the leaf discs to remove the excess of acetone and placed over wet cotton in Petri plate. The adult female mites were released on treated leaf discs and mortality data were recorded after 48 hrs. Mites released on leaf treated only with Acetone and tween 20 emulsifier served as control. The mortality data was used for calculation of LC_{50}/LD_{50} using Maximum Likelihood Programme (MLP). 3.01.

RESULTS AND DISCUSSION

IR Spectra

The IR spectra of new organotin compounds were recorded in a Perkin–Elmer spectrophotometer in 4000-200 cm^{-1}range. The IR spectra of compound show absorption bands due to phenyl, *p*-fluorophenyl and pentafluorophenyl groups. The absorption frequencies due to carbonyl group (both symmetric and asymmetric) in amide have been fully assigned. The Sn-C vibration in case of phenyl and pentafluorophenyl derivatives corresponding to the y mode and appears in the range of 448-460 cm^{-1}.

Antitumor Activity

The antitumor activity of diorganotin (IV) diamides was studied against the human breast cancer (MCF-7) and mammary cancer cell lines (EVSA-7). Compound shows moderate to high antiproliferative activity against the cell lines. They inhibit the growth of about 35-40% of tumor. The variation in activity is due to variable kind of carboxylate as ligands. The carboxylate having fluorine contents show higher efficacy. It was found that the compounds generally interact with nitrogenous bases of nucleotides of nucleic acid and inhibit the cell division by interfering the replication and transcription of DNA molecules. The compounds may also affect the multienzyme complexes responsible for replication and transcription of DNA thus causing a stop of proliferation of the cells.

Antibacterial Activity

The organotin compounds were tested for antibacterial activity against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 μg/ml concentration of test compound. All the compounds show moderate to higher activity against the bacterial strains. The activity of compounds 4 and 6 was found higher in three bacterial strains. The rest of compounds were moderately active. It was found that compounds having fluoro and pentafluorophenyl ring are more effective because of their water and lipid solubility. The fluorine-containing compounds may generally form complexes with metaloenzymes, particularly those which responsible in basic physiology such as cytochrome oxidase. 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 followed by death of bacterial cell. Some times these compounds in low concentration may cause bacteriostatic condition by slow down the growth of bacteria.

Antifungal Activity

The antifungal activity of these compounds were tested against *Aspergillus flavus* and *Aspergillus niger* using

concentrations 50 and 100 μg/ml. Activity of the compound was found variable at lower concentration but at higher concentration compounds show high activity against fungal strains. Presence of nitrogen, phenyl and pentafluorophenyl ring are considered for fungal activity. Compounds generally damage the fungal strains by puncturing the cell wall similarly as in case of bacteria. Water and lipid solubility also increases the activity due to presence of fluorine.

Stomach Toxicity

The stomach toxicity of these compounds was tested against the larvae of *Spodoptera litura* using different concentration of the compounds, 0.06%, 0.12%, 0.25%, 0.50% and 1.00% .The corrected mortality was calculated for the calculation of lethal concentration/lethal dose (LC_{50}). It was found that the compounds show good activity against the larvae of insect pest. The variation in activity was due to presence of different kinds of amide group as a ligand in the molecule. The presence of chlorine and bromine group in ligand increases the activity.

Contact Toxicity

The contact activity of these compounds was also tested against the larvae of same insect, *Spodoptera litura,* using different concentration of the compounds. The corrected mortality was calculated to find out the LC_{50} value of the compounds. It was found that the compounds show better activity against the larvae of insects and shows low value of LC_{50}.

Antifeedant Activity

The antifeedant activity of these compounds was tested against the insect *Spodopters litura* larvae using different concentration of the compound and the corrected mortality was calculated to find out the effective concentration (EC_{50}). It was found that compound shows higher to moderate antifeedant activity. It was found that compound having chlorine and bromine groups are more effective against the insects.

Acaricidal Activity

Acaricidal activity of these compounds was tested against *Tetranychus urticae* using different concentrations, 0.001 %, 0.005 %, 0.05 %, 0.1 % and 0.5 %. The percentage of corrected mortality was calculated to find out the LC_{50} of these compounds. The results were very surprising that all the compounds show high acaricidal activity against the mite. The presence of different kind of amide group as ligand in organotin compounds enhances the activity.

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Table 1- Physicochemical properties of diorganotin (IV) diamide

S.N.	Compounds	Formula	Formula Weight	M.P. (°C)	Yield (%)	Solvent
1	$(C_6H_5)_2Sn(succinimide)_2$	$C_{20}H_{18}O_4N_2Sn$	469	82	65	Pet-ether (40-60 ºC)
2	$(C_6H_5)_2Sn(phthalimide)_2$	$C_{28}H_{18}O_4N_2Sn$	565	76	85	Pet-ether(40-60 ºC)
3	$(C_6F_5)_2Sn(succinimide)_2$	$C_{20}F_{10}H_8O_4N_2Sn$	649	82	70	Pet-ether(60-80 ºC)
4	$(C_6F_5)_2Sn(phthalimide)_2$	$C_{28}F_{10}H_8O_4N_2Sn$	745	80	65	Pet-ether(40-60 ºC)
5	$(C_6H_4F)_2Sn(succinimide)_2$	$C_{20}F_2H_{16}O_4N_2Sn$	505	90	60	Pet-ether(60-80 ºC)
6	$(C_6H_4F)_2Sn(phthalimide)_2$	$C_{28}F_2H_{16}O_4N_2Sn$	601	86	70	Pet-ether(40-60 ºC)

Table 2-Analytical data of diorganotin (IV) diamide

S.N.	Molecular Formula	Elemental Analysis			IR (cm^{-1})	
		C (%)	H (%)	N (%)	$V_{asym}(CO)$	$V_{sym}(CO)$
1	$C_{20}H_{18}O_4N_2Sn$	51.17	3.83	5.97	1706 vs	1308ms
2	$C_{28}H_{18}O_4N_2Sn$	59.46	3.18	4.95	1758vs	1354ms
3	$C_{20}F_{10}H_8O_4N_2Sn$	36.97	1.23	4.31	1726ms	1326ms
4	$C_{28}F_{10}H_8O_4N_2Sn$	45.10	1.07	3.75	1729vs	1329ms
5	$C_{20}F_2H_{16}O_4N_2Sn$	47.52	3.16	5.54	1732vs	1332ms
6	$C_{28}F_2H_{16}O_4N_2Sn$	55.90	2.66	4.65	1740ms	1338ms

Table 3-Antitumor activity of diorganotin (IV) diamide

S. N.	Compounds	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiela pneumoniae*
1.	$(C_6H_5)_2Sn(succinimide)_2$	11.00±0.57	08.10±0.16	12.00±1.15
2.	$(C_6H_5)_2Sn(phthalimide)_2$	11.33±0.66	11.00±0.57	08.5±0.29
3.	$(C_6F_5)_2Sn(succinimide)_2$	08.0±0.28	19.00±0.57	13.00±0.50
4.	$(C_6F_5)_2Sn(phthalimide)_2$	17.33±0.6	19.00±0.57	15.00±0.57
5.	$(C_6H_4F)_2Sn(succinimide)_2$	18.66±0.66	07.83±0.44	10.5±0.76
6.	$(C_6H_4F)_2Sn(phthalimide)_2$	15.66±0.33	16.00±0.57	17.00±0.57
7.	Ampicilin (standard)	18.72±0.68	18.11±0.32	18.08±0.28

Table 4-Antibacterial activity (Zone of Inhibition (mm) dia. ± S.E)

S. N.	Compounds	MCF-7 Cell No. x 10^4	EVSA-7 Cell No. x 10^4	Activity
1	$(C_6H_5)_2Sn(succinimide)_2$	12.31±1.02	12.39±1.03	Negative
2	$(C_6H_5)_2Sn(phthalimide)_2$	9.17 ± 0.90	8.6 7 ± 0.69	Positive
3	$(C_6F_5)_2Sn(succinimide)_2$	8.79 ± 0.52	8.42 ± 0.46	Positive
4	$(C_6F_5)_2Sn($ phthalimide$)_2$	8.95±0.67	8.55±0.62	Positive
5	$(C_6H_4F)_2Sn(succinimide)_2$	11.59±1.06	11.29±1.02	Negative
6	$(C_6H_4F)_2Sn(phthalimide)_2$	9.19±0.92	9.29±0.88	Positive
7	Negative control	10.21±1.01	10.22±1.01	–
8	Positive control	40.26±3.23	41.23±3.28	–

Table 5-Antifungal activity

S. N.	Compounds	*Con.* µg/ml.	*Aspergillus flavus* (dia.mm)	% Inhibition	*Aspergillus niger* (dia.mm)	% Inhibition
1	$(C_6H_5)_2Sn(succinimide)_2$	50	0.6	80.0	0.5	75.0
		100	0.4	86.7	0.2	90.0
2.	$(C_6H_5)_2Sn(phthalimide)_2$	50	0.7	76.6	0.6	70.0
		100	0.4	86.7	0.1	95.0
3.	$(C_6F_5)_2Sn(succinimide)_2$	50	1.0	66.6	0.5	75.0
		100	0.8	73.3	0.2	90.0
4.	$(C_6F_5)_2Sn($ phthalimide$)_2$	50	0.8	73.3	0.8	60.0
		100	0.5	83.3	0.4	80.0
5.	$(C_6H_4F)_2Sn(succinimide)_2$	50	0.5	83.3	0.8	60.0
		100	0.01	96.7	0.5	75.0
6.	$(C_6H_4F)_2Sn(phthalimide)_2$	50	0.4	86.7	0.5	75.0
		100	0.2	93.3	0.2	90.0
7.	Control		3.0	-	2.0	-

Table 6- Contact Toxicity

S. N.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50}
1.	$(C_6H_5)_2Sn(succinimide)_2$	1.87-12.07	1.09±0.19	1.62 (3)	3.53
2.	$(C_6H_5)_2Sn(phthalimide)_2$	1.57-9.32	1.07±0.17	0.72 (3)	2.83
3.	$(C_6F_5)_2Sn(succinimide)_2$	0.28-0.40	1.96±0.16	4.39 (3)	0.33
4.	$(C_6F_5)_2Sn($ phthalimide$)_2$	0.39-0.59	1.67±0.15	5.62 (3)	0.46
5.	$(C_6H_4F)_2Sn(succinimide)_2$	0.43-0.75	1.63±0.6	2.94 (3)	0.58
6.	$(C_6H_4F)_2Sn(phthalimide)_2$	1.87-12.07	1.09±0.19	1.63 (3)	3.52

Table 7-Stomach Toxicity

S. N.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$(C_6H_5)_2Sn(succinimide)_2$	1.61-9.55	1.45±0.17	0.68 (3)	2.97
2.	$(C_6H_5)_2Sn(phthalimide)_2$	0.86-1.99	1.28±0.16	0.80 (3)	1.20
3.	$(C_6F_5)_2Sn(succinimide)_2$	0.49-0.76	1.57±0.16	2.78 (3)	0.60
4.	$(C_6F_5)_2Sn($ phthalimide$)_2$	0.55-0.90	1.48±0.16	3.37 (3)	0.67
5.	$(C_6H_4F)_2Sn(succinimide)_2$	0.56-0.97	1.33±0.15	0.63 (3)	0.75
6.	$(C_6H_4F)_2Sn(phthalimide)_2$	0.85-1.82	1.22±0.16	0.72 (3)	1.12

Table 8- Antifeedant Activity

S. No.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$(C_6H_5)_2Sn(succinimide)_2$	0.82-3.41	1.81±0.14	0.43 (3)	1.35
2.	$(C_6H_5)_2Sn(phthalimide)_2$	0.68-1.72	1.03±0.14	0.66 (3)	0.98
3.	$(C_6F_5)_2Sn(succinimide)_2$	0.43-0.87	1.03±0.14	0.34 (3)	0.58
4.	$(C_6F_5)_2Sn($ phthalimide$)_2$	0.62-1.42	1.06±0.14	1.07 (3)	0.86
5.	$(C_6H_4F)_2Sn(succinimide)_2$	0.83-2.33	1.08±0.15	0.79 (3)	1.24
6.	$(C_6H_4F)_2Sn(phthalimide)_2$	0.72-2.41	0.93±0.14	0.22 (3)	1.13

Table 9-Acaricidal Activity

S. No.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$(C_6H_5)_2Sn(succinimide)_2$	0.12-0.30	0.78±0.08	1.70 (3)	0.18
2.	$(C_6H_5)_2Sn(phthalimide)_2$	0.14-0.31	0.96±0.09	7.52 (3)	0.20
3.	$(C_6F_5)_2Sn(succinimide)_2$	0.05-0.10	0.93±0.08	13.22 (3)	0.06
4.	$(C_6F_5)_2Sn($ phthalimide$)_2$	0.04-0.09	0.69±0.06	4.64 (3)	0.05
5.	$(C_6H_4F)_2Sn(succinimide)_2$	0.05-0.09	0.16±0.09	12.67 (3)	0.07
6.	$(C_6H_4F)_2Sn(phthalimide)_2$	0.07-0.22	0.76±0.06	5.63 (3)	0.14

International Journal of Agriculture Sciences, ISSN: 0975-3710, Volume 2, Issue 1, 2010, pp-05-10

Studies on insecticidal and pesticidal activity of some organotin compounds

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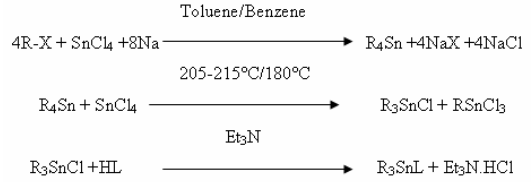
Abstract- A series of new triorganotin compounds were synthesized and tested first time for their insecticidal and pesticidal activity against *Periplanata americana, Musca domestica* and *Spodoptera litura* and *Tetranichus urticae* of a pest crop mite and were found highly active against *Spodoptera litura*. It was found that these insects and pest highly damage the Indian crops and ultimately economy also.
Key words: Organotin(IV)amide, insecticidal, pesticidal, antifeedant and acaricidal activity.

Introduction

It is well known that the studies on insect and pest behavior and their control are collectively proceeds in entomology [1]. Insects are found in almost all types of environment. They affect man's interest in a number of ways; insects like mosquitoes and housefly spread large number of diseases like malaria, dengue and cholera in addition to painful bites. However, all insects are not harmful; some are beneficial such as honey bee which give us honey and silk worm which provide us silk. Insecticide is mainly a substance intended for killing, repelling or otherwise preventing treated surface from insects [2]. The use of insecticides started with the discovery of Paris green [3], which was a synthetic compound of arsenic, in 1867 to control the colorado potato beetle. Until 1939, most of the insecticides were inorganic chemicals [4]. There are a large number of organic [5] and metal organic [6] compounds as insecticides which have made revolutionary change in the field of chemical control. The phenolics [7] and carbamates [8] are the major organic derivatives used as potent insecticides. But later on the introduction of metal based organic derivatives as insecticides made a new revolution in entomology. Organotin derivatives have emerged as potential biologically active compounds in last 15-20 years. The spectrum of the chemotherapeutic values of organotin compounds has been expanded as they have found their place among a class of potential biologically active compounds [9] exhibiting antimicrobial activity against different kinds of microbial strains [10]. They also show anti-inflammatory and cardiovascular activity [11], trypanosomal activity [12] along with anti-herpes [13] and anti-tubercular activity [14]. In case of control of insect and pest organotin compounds are not much explored because of their solubility reasons. In order to make this kind of compounds more soluble in water, less complicated structure was designed which contains polar moieties [15]. The introduction of polar groups in the organotin molecules leads to some improvement in the solubility and efficacy of activity. Fluorine containing compounds are more soluble in water and still very soluble in non-polar solvents [16]. The solubility can also be increased by preparing salts of organotin compounds [17]. In the present communication a series of new triorganotin compounds were synthesized and tested first time for their insecticidal and pesticidal activity against *Periplanata americana, Musca domestica and Spodoptera litura and Tetranichus urticae* of a pest crop mite and were found highly active against *Spodoptera litura*.

Experimental

The synthesis of organotin compounds was carried out by the earlier reported method [18]. The tetraorganotin compound as a base material can be synthesized by the reaction of respective haloarene with tin tetra chloride and sodium metal in inert atmosphere. The preparation of triorganotin (IV) chloride was takes place by cleavage of the base material with anhydrous tin tetra chloride by fixing an air condenser without using any solvent and the reaction temperature is maintained at 205-215°C for one hour and then 180°C temperature was maintained for a period of one and half hour. The compounds isolated in pet-ether and recrystalized with the same solvents.



R = (C6H5), (C6F5), (C6H4F), HL = Succinimide, Phthalimide

The preparation of triorganotin (IV) amides was carried out by the reaction of R3SnCl and suitable amide in presence of triethylamine, as HCl acceptor, at room temperature under nitrogen atmosphere. The method of preparation of some representative compounds is as follows.

Reaction of (C6H5)3SnCl with Succinimide

In an oxygen free nitrogen atmosphere, solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and succinimide (0.099gm; 1mmol) in same solvent were stirred together in presence of

triethylamine at room temperature for 4-5 hours. The off white color Et3N.HCl formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of (C6H5)3SnCl with Phthalimide
In an oxygen free nitrogen atmosphere, solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and phthalimide (0.147gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color Et3N.HCl formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of (C6F5)3SnCl with Succinimide
In an oxygen free nitrogen atmosphere, solution of tris (pentafluorophenyl) tin (IV) chloride (0.655gm; 1mmol) in benzene and succinimide (0.099gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color Et3N.HCl formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of (C6F5)3SnCl with Phthalimide
In an oxygen free nitrogen atmosphere, solution of tris (pentafluorophenyl) tin (IV) chloride (0.655gm; 1mmol) in benzene and phthalimide (0.147gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color Et3N.HCl formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of (C6H4F)3SnCl with Phthalimide
In an oxygen free nitrogen atmosphere, solution of tris (p-fluorophenyl) tin (IV) chloride (0.439gm; 1mmol) in benzene and phthalimide (0.147gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color Et3N.HCl formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of (C6H4F)3SnCl with Succinimide
In an oxygen free nitrogen atmosphere, solution of tris (p-fluorophenyl) tin (IV) chloride (0.439gm; 1mmol) in benzene and succinimide (0.099gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color Et3N.HCl formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Insecticidal Activity
The insecticidal activity of these compounds was determined against male and female cockroaches (*Periplanata americana*) and housefly (*Musca domestica*) following the method of Nash [19], using acetone as a standard. The 0.1% and 0.5% acetone solution of the compounds were injected between 4th and 5th abdominal segment on the ventral side of the body in cockroaches and sprays on the colony of housefly using microsyrings. A 0.02 ml of acetone was alone injected for control (standard). The treated insects were kept under observation for 48 hrs at room temperature (No food was given in this period and their knock down value is calculated.

Contact Toxicity
The contact toxicity of these compounds was carried out by topical application method [20] against larvae of *Spodoptera litura*, which is harmful for Indian crops. First the given compounds were dissolved in acetone and different concentrations were prepared viz., 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. Now each concentration was applied on the dorsal surface of the larvae of insect. About 10 □l of each concentration was applied on each larvae. Some of the larvae of insect was treated by acetone alone, were works as control. Now the mortality data was recorded after 24 hrs, and the treated mortality was corrected with control morality. These corrected mortality data was used for calculation of LC50/LD50 using Maximum Likelihood programme MLP 3.01.

Stomach Toxicity
The stomach toxicity of these compounds was carried out by leaf dip method [21]. In this method we used fourth instar larvae of *Spodoptera litura* of an insect which is responsible for the damage of Indian agricultural crops. Ten larvae were used for each replication and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared viz. 0.06%, 0.12%, 0.25%, 0.50%, and 1.00%. The leaf disc were prepared out of caster leaf and dipped in various concentrations of the test compounds for thirty seconds. Now air dried the leaf discs to evaporate the excess acetone. (The leaf discs dipped only in acetone were served as control). The mortality data was recorded after 24 hrs, and the treatment mortality was corrected with control mortality. These mortality data was used for calculation of LC50/LD50¬ using maximum likelihood programm, MLP 3.01.

Antifeedant Activity
The antifeedant activity of these compounds was also carried out by leaf dip method [21] using fourth instar larvae of *Spodoptera litura*, an insect

responsible for the damage of Indian agricultural crops. There are ten larvae were used for each replications and three replications were maintained for each concentration. The given compounds were dissolved in acetone and different concentrations were prepared viz. 0.06%, 0.12%, 0.25%, 0.50% and 1.00%. The leaf discs of about 25 cm2 were prepared and dipped for thirty seconds in various concentrations of the test compounds. Now air dried the leaf discs to evaporate the excess acetone and the leaf discs offered for feeding. The insects were allowed to feed for 24 hrs. After 24 hrs leaf area uneaten was measured by using leaf area meter. The differences between leaf area provided and the leaf area uneaten is taken as amount of leaf area consumed. The feeding inhibition was calculated and used for calculation of effective concentration (EC50/LD50) using Maximum likelihood programme, MLP 3.01.

Acaricidal Activity
The acaricidal activity of these compounds was carried out by leaf dip method [21]. Compounds was dissolved in Acetone and different concentrations were prepared viz. 0.001%, 0.005%, 0.05%, 0.1%, 0.5% using 0.2% tween 20 as emulsifier. Leaf discs of Mulberry (5 cm2 diameter) were dipped in different concentration for 30 seconds. Now air dried the leaf discs to remove the excess of acetone and placed over wet cotton in petriplate. The adult female mites were released on treated leaf discs and mortality data were recorded after 48 hrs. Mites released on leaf treated only with Acetone and tween 20 emulsifier served as control. The mortality data was used for calculation of LC50/LD50 using Maximum Likelihood Programme (MLP). 3.01.

Result and Discussions
IR Spectra
The IR spectra of new organotin compounds were recorded in a Perkin–Elmer spectrophotometer in 4000-200 cm-1range. The IR spectra of compound show absorption bands due to phenyl, p-fluorophenyl and pentafluorophenyl groups. The absorption frequencies due to carbonyl group (both symmetric and asymmetric) in amide have been fully assigned. The Sn-C vibration in case of phenyl and pentafluorophenyl derivatives corresponding to the y mode and appears in the range of 448-460 cm-1. Further characterization of these compounds is in progress for confirmation of structure of the compounds.

Insecticidal Activity
Insecticidal activity of these compounds was checked against male and female cockroaches (*Periplanata americana*) and housefly (*Musca domestica*) using parathion as a standard. The compound shows higher to moderate activity. The variation in insecticidal activity was due to variation in amide group present in the molecule. The presence of chlorine and bromine groups in ligands enhances the activity. They generally affect on nervous system of the insects and causes unconsciousness resulting to death.

Pesticidal Activity
Stomach Toxicity
The stomach toxicity of these compounds was tested against the larvae of *Spodoptera litura* using different concentration of the compounds, 0.06%, 0.12%, 0.25%, 0.50% and 1.00% .The corrected mortality was calculated for the calculation of lethal concentration/lethal dose (LC50). It was found that the compounds show good activity against the larvae of insect pest. The variation in activity was due to presence of different kinds of amide group as a ligand in the molecule. The presence of chlorine and bromine group in ligand increases the activity.

Contact Toxicity
The contact activity of these compounds was also tested against the larvae of same insect, *Spodoptera litura*, using different concentration of the compounds. The corrected mortality was calculated to find out the LC50 value of the compounds. It was found that the compounds show better activity against the larvae of insects and shows low value of LC50.

Antifeedant Activity
The antifeedant activity of these compounds was tested against the insect *Spodopters litura* larvae using different concentration of the compound and the corrected mortality was calculated to find out the effective concentration (EC50). It was found that compound shows higher to moderate antifeedant activity. It was found that compound having chlorine and bromine groups are more effective against the insects.

Acaricidal Activity
Acaricidal activity of these compounds was tested against Tetranychus urticae using different concentrations, 0.001 %, 0.005 %, 0.05 %, 0.1 % and 0.5 %. The percentage of corrected mortality was calculated to find out the LC50 of these compounds. The results were very surprising that all the compounds show high acaricidal activity against the mite. The presence of different kind of amide group as ligand in organotin compounds enhances the activity.

Acknowledgement
The authors are thankful to the University Grants Commission, New Delhi(India) for providing fund to our research group(Sanction Letter No.F.6-1(29)/2008(MRP/NRCB), 26 Feb.2008),Director, NBRI, Lucknow; Director IMTECH, Chandigarh

for biological activity and Director, RSIC, CDRI for analysis of the compounds.

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Table 1- Physicochemical properties of triorganotin compounds

S.N.	Compounds	Formula	Formula Weight	Melting Point (°C)	Yield (%)	Solvent for Crystallization
1	$(C_6H_5)_3Sn$(succinimide)	$C_{22}H_{19}NO_2Sn$	447.71	88	80	Pet.-ether (40-60 ºC)
2	$(C_6H_5)_3Sn$(phthalimide)	$C_{26}H_{19}NO_2Sn$	494.71	80	85	Pet.-ether(40-60 ºC)
3	$(C_6H_4F)_3Sn$(succinimide)	$C_{22}H_{16}NO_2F_3Sn$	500.71	90	65	Pet.-ether(60-80 ºC)
4	$(C_6H_4F)_3Sn$(phthalimide)	$C_{26}H_{16}NO_2F_3Sn$	548.71	85	70	Pet.-ether(40-60 ºC)
5	$(C_6F_5)_3Sn$(succinimide)	$C_{22}H_4NO_2F_{15}Sn$	716.71	95	70	Pet.-ether(60-80 ºC)
6	$(C_6F_5)_3Sn$(phthalimide)	$C_{26}H_4NO_2F_{15}Sn$	764.71	90	75	Pet.-ether(40-60 ºC)

Table 2- Analytical data of triorganotin compounds

S.N.	Molecular Formula	Elemental Analysis			IR (cm^{-1})	
		C (%)	H (%)	N (%)	$V_{asym}(CO)$	$V_{sym}(CO)$
1	$C_{22}H_{19}NO_2Sn$	59.09	4.25	3.13	1706 vs	1308ms
2	$C_{26}H_{19}NO_2Sn$	63.06	3.84	2.82	1758vs	1354ms
3	$C_{22}H_{16}NO_2F_3Sn$	52.72	3.19	2.79	1726ms	1326ms
4	$C_{26}H_{16}NO_2F_3Sn$	56.86	2.91	2.55	1729vs	1329ms
5	$C_{22}H_4NO_2F_{15}Sn$	36.83	0.55	1.95	1732vs	1332ms
6	$C_{26}H_4NO_2F_{15}Sn$	40.79	0.52	1.83	1740ms	1338ms

Table 3- Stomach Toxicity

S. No.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$C_{22}H_{19}NO_2Sn$	1.61-9.55	1.45±0.17	0.68 (3)	2.97
2.	$C_{26}H_{19}NO_2Sn$	0.86-1.99	1.28±0.16	0.80 (3)	1.20
3.	$C_{22}H_{16}NO_2F_3Sn$	0.49-0.76	1.57±0.16	2.78 (3)	0.60
4.	$C_{26}H_{16}NO_2F_3Sn$	0.55-0.90	1.48±0.16	3.37 (3)	0.67
5.	$C_{22}H_4NO_2F_{15}Sn$	0.56-0.97	1.33±0.15	0.63 (3)	0.75
6.	$C_{26}H_4NO_2F_{15}Sn$	0.85-1.82	1.22±0.16	0.72 (3)	1.12

Table 4- Contact Toxicity

S. No.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$C_{22}H_{19}NO_2Sn$	1.87-12.07	1.09±0.19	1.62 (3)	3.53
2.	$C_{26}H_{19}NO_2Sn$	1.57-9.32	1.07±0.17	0.72 (3)	2.83
3.	$C_{22}H_{16}NO_2F_3Sn$	0.28-0.40	1.96±0.16	4.39 (3)	0.33
4.	$C_{26}H_{16}NO_2F_3Sn$	0.39-0.59	1.67±0.15	5.62 (3)	0.46
5.	$C_{22}H_4NO_2F_{15}Sn$	0.43-0.75	1.63±0.6	2.94 (3)	0.58
6.	$C_{26}H_4NO_2F_{15}Sn$	1.87-12.07	1.09±0.19	1.63 (3)	3.52

Table 5- Antifeedant Activity

S. No.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$C_{22}H_{19}NO_2Sn$	0.82-3.41	1.81±0.14	0.43 (3)	1.35
2.	$C_{26}H_{19}NO_2Sn$	0.68-1.72	1.03±0.14	0.66 (3)	0.98
3.	$C_{22}H_{16}NO_2F_3Sn$	0.43-0.87	1.03±0.14	0.34 (3)	0.58
4.	$C_{26}H_{16}NO_2F_3Sn$	0.62-1.42	1.06±0.14	1.07 (3)	0.86
5.	$C_{22}H_4NO_2F_{15}Sn$	0.83-2.33	1.08±0.15	0.79 (3)	1.24
6.	$C_{26}H_4NO_2F_{15}Sn$	0.72-2.41	0.93±0.14	0.22 (3)	1.13

Table 6- Acaricidal Activity

S. No.	Compounds	Fiducial limits	Slop ± S.E.	Chi. Square	LC_{50}/LD_{50} at 24 hrs.
1.	$C_{22}H_{19}NO_2Sn$	0.12-0.30	0.78±0.08	1.70 (3)	0.18
2.	$C_{26}H_{19}NO_2Sn$	0.14-0.31	0.96±0.09	7.52 (3)	0.20
3.	$C_{22}H_{16}NO_2F_3Sn$	0.05-0.10	0.93±0.08	13.22 (3)	0.06
4.	$C_{26}H_{16}NO_2F_3Sn$	0.04-0.09	0.69±0.06	4.64 (3)	0.05
5.	$C_{22}H_4NO_2F_{15}Sn$	0.05-0.09	0.16±0.09	12.67 (3)	0.07
6.	$C_{26}H_4NO_2F_{15}Sn$	0.07-0.22	0.76±0.06	5.63 (3)	0.14

International Journal of Pharmaceuticals Analysis, ISSN: 0975-3079, Volume 2, Issue 1, 2010, pp-09-14

Antimicrobial, antitumor and gastroprotective studies of some new water soluble organic derivatives of bismuth

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Abstract- Some triorganobismuth (V) amide, which are soluble in water, were synthesized by method reported earlier and characterized by their elemental and I.R. spectral analysis along with their antimicrobial, antitumor and gastro protective activity against different pathogenic microbial strains, human breast and mammary cancer cell line and gastric ulcer.
Key words: - Antimicrobial, antitumor, gastro protective, triorganobismuth.

Introduction

There is an enormous potential for the application of metals in medicine [1] and the selection of metal ions offer the possibility for the discovery of metallodrugs with novel mechanism of action [2]. Metal containing compounds may offer certain advantages over pure organic compound in drug therapy i.e., the metal complexes may acts as a pro-drug [3]. The organobismuth compounds have also attracted the attention owing to their microbiological and material utility [4-8] from more than 200 yrs. It was found that organobismuth compounds were active against the treatment of gastrointestinal disorders like dyspepsia, diarrhea and in peptic ulcers by inhibiting *E. coli*. [9-18]. Recently a group of Japanese workers synthesized a series of organobismuth compounds which show potent antimicrobial activity against fungus and bacterial culture responsible for human pathogenic disease[19]. The salts of organobismuth compounds, such as colloidal bismuth sub-salicylate (CBS), bismuth sub-citrate (BSC) and ranitidine bismuth citrate (RBC) are now common for controlling bacterial and fungal infections[20]. The recent demonstrations has shown that these salts are useful for *Helicobacter pylori* eradication therapy, (*Helicobacter pylori* now well known for the formation of gastrointestinal ulcer in Human beings and organobismuth compounds are the only cure against this bacteria) and has promoted the antibacterial and antifungal studies of various organobismuth compounds[21-29]. There are a lot's of thiabismuth heterocyclic compounds were synthesized and characterized as potent antimicrobial agents [30]. Some investigators have to synthesize a lots number of organobismuth compounds which might have highest antimicrobial activity [31-44]. The existence of relationships between, tumor (cancer) and metal is known to all oncologists. However various aspects about these relationships are ignored by many. It is surprising to observe that metals are able to do the best and the worst i.e. metal are able to induce cancer and also to treat the cancer, some are able to perform both. Basically both, transition and non-transition metals plays important role in the treatment of tumors. The synergic administration of cis-platin and bismuth compounds are known to reduce the toxic side effects of cis-platin; an effect that may be traced to the increased production of metallothionein induced by bismuth compounds [45-48]. The organobismuth compounds are extremely potent cytotoxic agent when attached to a monoclonal antibody as these can target leukemia, lymphoma and other tumors [49].

Experimental

The tris(Pentafluorophenyl) bismuth (V) dichloride was synthesized by the method reported earlier [50]. The substituted amides were recrystalised before use. The reactions were performed under nitrogen atmosphere. Preparation of some representative organobismuth compounds are discussed below.

Reaction of tris (Pentafluorophenyl) bismuth (V) dichloride with 5-bromoisatin

In oxygen free nitrogen atmosphere a solution of tris (Pentafluorophenyl) bismuth (V) dichloride (1mmol) in methanol (40ml) and 5-bromoisatin (2mmol) in the same solvent was stirred together in presence of triethylamine at room temperature for 5 hrs. The $Et_3N.HCl$ formed was filtered off under nitrogen atmosphere and the filtrate on concentration in vacuum gives a off-white color crystalline solid which was further recrystalised in pet. ether (40-60^{0}C).

Reaction of tris (Pentafluorophenyl) bismuth (V) dichloride with 6-chloro-5-methoxyisatin

In oxygen free nitrogen atmosphere a solution of tris (Pentafluorophenyl) bismuth (V) dichloride (1mmol) in methanol (40ml) and 6-chloro-5-methoxyisatin (2mmol) in the same solvent was stirred together in presence of triethyl amine at room temperature for 5 hrs. The $Et_3N.HCl$ formed was filtered off under nitrogen atmosphere and the filtrate on concentration in vacuum gives an off-white color crystalline solid which was further recrystalised in pet. ether (40-60^{0}C).

Reaction of tris (Pentafluorophenyl) bismuth (V) dichloride with 6-methoxy-5-bromoisatin

In oxygen free nitrogen atmosphere a solution of tris (Pentafluorophenyl) bismuth (V) dichloride (1mmol) in methanol (40ml) and 6-methoxy-5-bromoisatin (2mmol) in the same solvent was

stirred together in presence of triethyl amine at room temperature for 5 hrs. The $Et_3N.HCl$ formed was filtered off under nitrogen atmosphere and the filtrate on concentration in vacuum afforded yellow color crystalline solid which was further recrystalised in pet. ether (40-60^{0}C).

Reaction of tris (Pentafluorophenyl) bismuth (V) dichloride with 7-chloroisatin

In oxygen free nitrogen atmosphere a solution of tris (Pentafluorophenyl) bismuth (V) dichloride (1mmol) in methanol (40ml) and 7-chloroisatin (2mmol) in the same solvent was stirred together in presence of triethylamine at room temperature for 5 hrs. The $Et_3N.HCl$ formed was filtered off under nitrogen atmosphere and the filtrate on concentration in vacuum afforded off-white color crystalline solid which was further recrystalised in pet. ether (40-60^{0}C).

Antimicrobial Activity

The antimicrobial activity of all these compounds was performed by disc diffusion method [51]. In this techniques the filter paper (whatmann No-1) sterile disc of 5 mm diameter impregnated with the test compounds (10ug/ml ethanol) were placed on the nutrient agar plate at 37^{0}C for 24 hrs. The inhibition zone around the dried impregnated discs were measured and reported after 24 hrs.

Antitumor Activity

The *in-vitro* antitumor activity of these compounds was carried out by MTT-method [52]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used for this purpose. The principle behind this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazolium MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazoleum bromide] was reduced partially by metabolically active cells by the action of dehydrogenase enzyme to generate NADH and NADPH as reducing equivalents. The resulting intracellular purple color zone was solubilized and quantified by spectrophotometer. The MTT was first dissolved in Phosphate buffer saline at a concentration of 5 mg/ml. The MTT solution (50 µl) was added to each well of 96 well culture plate containing 100 µl of culture medium and incubates at 37°C for 4 hrs. The medium was then removed carefully without disturbing the crystals of purple colored zone. 50 ml of DMSO was then added to each well and mixed thoroughly to dissolve the crystals of the zone. The plate was then read on a micro ELISA plate reader at a wavelength of 570 nm to fine out the optical density and cell count value.

Anti-Ulcer screening

Aspirin (ASP) Induced Ulcers:-Aspirin in dose of 200mg/ kg (20mg/ml) was administered to the animals on the day of the experiment and ulcers were scored after four hrs. The animals were sacrificed and the stomach was then excised and cut along the greater curvature, washed carefully with 5 ml of 0.9% NaCl and ulcers were scored by a person unaware by the experimental protocol in the glandular portion of the stomach. Ulcer index was calculated by adding the total number of ulcers/ stomach and total severity of ulcers/stomach. The pooled group ulcer score was then calculated by reported method [53].

Ethanol (EtOH) induced Ulcers:-The gastric ulcers were induced in rats by administering ethanol (1ml/200gm/kg for 1 hr) and the animals were sacrificed by cervical dislocation and the stomach was incised along the greater curvature and examined for ulcers. The ulcer index was scored, based upon the product of length and width of the ulcers present in the glandular portion of the stomach (mm^2/rats).

Results and discussion

The tris (pentafluorophenyl) bismuth(V) amides can be easily obtained by using metathetical reaction where a respective isatin reacted with tris(pentafluorophenyl) bismuth(V) dichloride in an appropriate ratio in presence of triethylamine which behaves as a hydrogen chloride acceptor.

$$R_3BiCl_2+2HL \xrightarrow{Et3N} R_3BiL_2+2Et_3N.HCl$$

$R=C_6F_5$, HL = HNR_2 = 6-Chloro-5Methoxy, 6-Methoxy-5 Bromo, 7-Chloro 6-Bromo-5Methoxy, 5-bromo and 5 methoxy isatin

All the reactions were performed in room temperature and under nitrogen atmosphere. The organobismuth compounds which were obtained have sharp melting point and stable towards air and moisture. These compounds were also characterized on the basis of their elemental analysis, I.R. spectra and their antimicrobial and antitumor activity in various pathogenic microbial strains along with human breast and mammary tumor cell line *in-vitro*.

Infrared Spectra

The I.R. spectra of these compounds show almost similar absorption bands due to presence of Pentafluorophenyl group. The position and pattern of these absorption bands do not differ much from the I.R. data of tris (Pentafluorophenyl) bismuth (V) halides. A remarkable features in the I.R. spectra of all these compound is the absence of V_{sym}(Bi-C) absorption corresponding to 't' mode which should be located in the region of 250-300 cm^{-1} . The absorption frequencies having diagnostic values are listed in table.

Antimicrobial Activity

The organobismuth compounds were tested for antibacterial activity against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 µg/ml concentration of test compound. All the compounds show moderate to higher activity against the bacterial strains. It was

found that organometallic compounds containing fluoro and pentafluorophenyl ring are more effective because of their water and lipid solubility. The fluorine-containing compounds may generally form complexes with metaloenzymes, particularly those which responsible in basic physiology such as cytochrome oxidase. 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 followed by death of bacterial cell. Some times these compounds in low concentration may cause bacteriostatic condition by slow down the growth of bacteria.

Antitumor Activity

Antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compounds show moderate to higher activity against tumor cell lines. It was found that the slight variation in their activity is due to presence of different amides as ligand along with presence of fluorine on main moiety of the compound. The compound generally interacts with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions for a cell. It may be noted that the organobismuth compound generally binds with nitrogen 7 position of purine bases in DNA molecule, where they reacted with labile hydrogen and form complex with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis.

Anti-Ulcer screening

Anti-ulcer activity was performed on Sprague-Dawley rats (140-180g). The compounds exhibit higher activity than the standard Ranitidine when the tests were carried out with Aspirin (ASP) induced and moderate activity was seen when the tests were done with Ethanol (EtOH) induced. It was known that aspirin caused mucosal damage by interrupting the synthesis of prostaglandin and increasing acid secretion and back diffusion of H^+ ions, which results in overproduction of leucotrienes and other products of 5-lipoxygenase pathways. Hence the protective action of these compounds against aspirin-induced gastric ulcer could possibly be due to its inhibitory effect on 5-lipoxygenase enzymes pathway. In case of ethanol induced ulcer which is predominantly occurs at glandular part of stomach was reported to stimulate the formation of leucotrienes C-4, mast cell secretary products and reactive oxygen species, which results in the damage of gastric mucosa of rat. These compounds could possibly play an important role in inhibition of these pathways.

Conclusion

Organobismuth (V) compounds have great potential as antiulcer, anticancer and antimicrobial agents. These compounds may be exploited for the development of new drugs for the treatment of diseases like ulcer, cancer and caused by various microorganisms.

Acknowledgement

The authors are thankful to the University Grants Commission, New Delhi(India) for providing fund to our research group(Sanction Letter No.F.6-1(29)/2008(MRP/NRCB), 26 Feb.2008),Director, HIPER, Lucknow for antimicrobial, antiulcer and antitumor activity and Director, RSIC, CDRI for analysis of the compounds.

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Table 1- Physicochemical and spectral data of organobismuth compounds

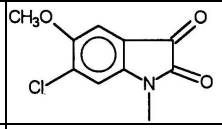
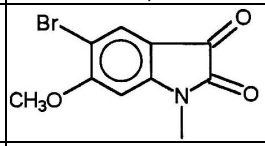
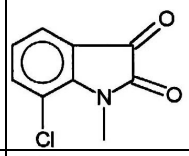
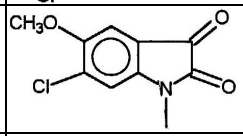
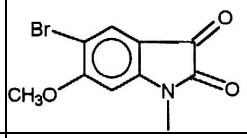
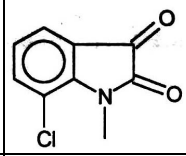
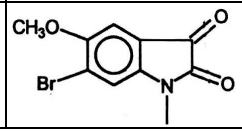
Sr.No.	Compound	M.P. (^{0}C)	Yield (%)	IR (in cm^{-1})		
				asym (CO)	sym (CO)	Bi-C
	$(C_6F_5)_3Bi(NR_2)_2$ $-NR_2=$					
1.		172	75	1738	1312	454
2.		185	70	1728	1322	462
3.		195	70	1740	1316	448
4.		176	65	1730	1316	448
5.		172	70	1726	1310	452
6.		182	75	1712	1314	460
7.		178	70	1740	1324	458

Table 2- Analytical data of organobismuth compounds

Compound No.	Empirical Formula	Elemental Analysis %		
		C	H	N
1.	$C_{36}H_{10}F_{15}N_2O_6Bi$	40.75	0.94	2.64
2.	$C_{36}H_{10}F_{15}Cl_2N_2O_6Bi$	38.19	0.88	2.47
3.	$C_{36}H_{10}Br_1N_2O_6Bi$	50.52	1.16	3.27
4.	$C_{36}H_6F_{15}N_2O_4Bi$	42.18	0.58	2.73
5.	$C_{36}H_{10}Br_2F_{15}N_2O_6Bi$	35.40	0.81	2.29
6.	$C_{33}H_5ClF_{20}NO_3Bi$	36.41	0.45	1.28
7.	$C_{33}H_5BrF_{20}NO_3Bi$	34.98	0.44	1.23

Table-3 Antimicrobial activity

S. No.	Compounds	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiela pneumoniae*
1.	$C_{36}H_{10}F_{15}N_2O_6Bi$	11.00±0.57	8.10±0.16	12.00±1.15
2	$C_{36}H_{10}F_{15}Cl_2N_2O_6Bi$	10.94±0.48	8.04±0.10	11.88±0.70
3.	$C_{36}H_{10}Br_1N_2O_6Bi$	11.33±0.66	11.00±0.57	8.58±0.29
4.	$C_{36}H_6F_{15}N_2O_4Bi$	11.24±0.60	8.70±0.26	12.06±0.77
5	$C_{36}H_{10}Br_2F_{15}N_2O_6Bi$	11.42±0.68	11.12±0.0.62	8.72±0.32
6	$C_{33}H_5ClF_{20}NO_3Bi$	11.33±0.66	11.00±0.57	8.54±0.22
7	$C_{33}H_5BrF_{20}NO_3Bi$	12.06±0.77	10.94±0.48	11.00±0.57
8	Ampicilin (standard)	18.0±0.21	12.66±0.50	16.26±0.30

Table 4- Antitumor activity

S. No.	Compounds	Cell No. x 10^4 (MCF-7)	Activity	Cell No. x 10^4 (EVSA-7)	Activity
1.	$C_{36}H_{10}F_{15}N_2O_6Bi$	9.69±0.92	+	10.68±1.08	-
2.	$C_{36}H_{10}F_{15}Cl_2N_2O_6Bi$	9.66±0.90	+	10.62±1.06	-
3.	$C_{36}H_{10}Br_1N_2O_6Bi$	8.28±0.46	+	9.69±0.92	+
4.	$C_{36}H_6F_{15}N_2O_4Bi$	8.22±0.42	+	9.68±0.88	+
5.	$C_{36}H_{10}Br_2F_{15}N_2O_6Bi$	9.62±0.52	+	9.62±0.90	+
6.	$C_{33}H_5ClF_{20}NO_3Bi$	9.67 ± 0.54	+	9.69 ± 0.92	+
7.	$C_{33}H_5BrF_{20}NO_3Bi$	9.69±0.92	+	9.66±0.90	+
8.	Negative Control	10.21±1.01	-	10.23±1.03	-
9.	Positive Control	40.26±3.23	-	42.24±4.22	-

***Negative Control- Culture Medium only, **Positive Control – 17 β estradiol**

Table 5- Antiulcer (Gastro protective) activity

S. N0.	Compounds	Aspirin Induced		Ethanol Induced	
		Ulcer Index (mm^2/rat)	Protective Ratio (%)	Ulcer Index (mm^2/rat)	Protective Ratio (%)
1	$C_{36}H_{10}F_{15}N_2O_6Bi$	7.2±0.58	61.68	19.9±5.4	18.21
2	$C_{36}H_{10}F_{15}Cl_2N_2O_6Bi$	7.2±0.58	61.68	19.7±5.2	18.17
3	$C_{36}H_{10}Br_1N_2O_6Bi$	7.1±0.54	61.21	19.8±5.5	18.18
4	$C_{36}H_6F_{15}N_2O_4Bi$	7.3±0.58	61.72	19.8±5.4	31.24
5	$C_{36}H_{10}Br_2F_{15}N_2O_6Bi$	6.2±0.28	62.16	14.4±2.2	34.70
6	$C_{33}H_5ClF_{20}NO_3Bi$	7.2±0.54	61.68	19.6±5.3	33.72
7	$C_{33}H_5BrF_{20}NO_3Bi$	7.2±0.56	61.70	19.6±5.2	31.20
8	Ranitidine	7.6±0.53	58.46	10.3±3.3	57.43
9	Aspirin	18.3±1.6	-	-	-
10	Ethanol	-		24.2±6.5	-

Antimicrobial and Antitumor Activity of Some New Triorganotin Compounds

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ABSTRACT

A series of new triorganotin compounds of the type R_3SnL; (R = C_6H_5, C_6F_5, C_6H_4F; L = succinimide and phthalimide) were synthesized and tested first time for their antimicrobial and antitumor activity. These compounds show valuable *in-vitro* antitumor activity against tumor cell lines and antimicrobial activity against various microbial strains as seen in their cell count and inhibition zone value respectively.

Keywords*:* Organotin (IV) amide, Antitumor, Antibacterial, Antifungal activity.

INTRODUCTION

There is an enormous potential for the application of metals in medicines [1] and the selection of metal ions offer the possibility for the discovery of metallodrugs with novel mechanism of action [2]. The importance of metal based drugs lies in the fact that they are essential components for various physico-chemical processes occurring in living system [3]. Organotin derivatives have emerged as potential biologically active compounds in last 15-20 years. The spectrum of the chemotherapeutic values of organotin compounds has been expanded as they have found their place among a class of potential biologically active compounds [4] exhibiting antimicrobial activity against different kinds of microbial strains [5]. They also show anti-inflammatory and cardiovascular activity [6], trypanosomal activity [7] along with anti-herpes [8] and anti-tubercular activity [9].

The first organotin compounds, for which the anti-tumor activities were examined, were formally similar to cisplatin [10] or to its analogous carboplatin and paraplatin [11]. These compounds show borderline activities against leukemia P388 and L1210 [12]. The antitumor activity of di-n-butyl tin dichloride towards Ehrlich ascites tumor, P388 lymphocyte leukemia and sarcoma 180 cell lines showed that this compound influences the DNA synthesis of proliferating cells. Many diorganotin compounds, R_2SnX_2, were investigated in context of their antitumor activity [13]. The di-n-butyltin analogue of carboplatin was synthesized and screened against MCF-7 and WiDr, tumor cell line of humans [14]. Besides this, series of organotin-derivatives of carboxylic and dicarboxylic acids were synthesized [15]. The organotin derivatives of pyridoxine and erythromycin were synthesized and tested against tumors cell line [16]. Some triorganotin compounds were also found active against various tumors cell line [17]. The steroid carboxylate series is one of the major early developments in this area [18]. They appear to contain pronounced *in-vitro* antitumor activity but their solubility still remains drawbacks [19], which affect the tumor activity. In order to make this kind of compounds more soluble in water, less complicated structure was designed which contains polar moieties [20]. The introduction of polar groups in the organotin molecules leads to some improvement in the solubility and *in-vitro* antitumor activity. Fluorine containing organotin compounds were synthesized to check the effect of compounds on tumor cell line [21].

Fluorine containing compounds are more soluble in water and still very soluble in non-polar solvents [22]. The solubility can also be increased by preparing salts of organotin compounds [23]. The most recent development in the field of antitumor active organotin compounds has been achieved by the synthesis and screening of compounds containing polyoxa alkyl moiety [24] which exhibit high antitumor activity. Study of the interaction of the antitumor active organotin compounds with DNA was recently undertaken using NMR study [25]. At around pH-7, a very weak hardly detectable interaction is observed in contrast with the results found in the case of platinum [26]. The interaction of DNA with dimethyltin dichloride was also studied very recently [27] indicating that by interfering the DNA replication they stop the growth of cell line [28].

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EXPERIMENTAL

The synthesis of organotin compounds was carried out by the earlier reported method [29]. The tetraorganotin compound as a base material can be synthesized by the reaction of respective haloarene with tin tetra chloride and sodium metal in inert atmoshphere.The preparation of triorganotin (IV) chloride was takes place by cleavage of the base material with anhydrous tin tetra chloride by fixing an air condenser without using any solvent and the reaction temperature is maintained at 205–215°C for one hour and then 180°C temperature was maintained for a period of one and half hour. The compounds isolated in pet-ether and recrystalized with the same solvents.

$$4R\text{-}X + SnCl_4 + 8Na \xrightarrow{\text{Toluene / Benzene}} R_4Sn + 4NaX + 4NaCl$$

$$R_4Sn + SnCl_4 \xrightarrow{\text{205-215°C / 180°C}} R_3SnCl + RSnCl_3$$

$$R_3SnCl + HL \xrightarrow{Et3N} R_3SnL + Et_3N.HCl$$

R = (C_6H_5), (C_6F_5), (C_6H_4F), HL = Succinimide, Phthalimide

The preparation of triorganotin (IV) amides was carried out by the reaction of R_3SnCl and suitable amide in presence of triethylamine, as HCl acceptor, at room temperature under nitrogen atmosphere. The method of preparation of some representative compounds is as follows.

Reaction of $(C_6H_5)_3SnCl$ with Succinimide

In an oxygen free nitrogen atmosphere, solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and succinimide (0.099gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of $(C_6H_5)_3SnCl$ with Phthalimide

In an oxygen free nitrogen atmosphere, solution of triphenyltin (IV) chloride (0.385gm; 1mmol) in benzene and phthalimide (0.147gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of $(C_6F_5)_3SnCl$ with Succinimide

In an oxygen free nitrogen atmosphere, solution of tris (pentafluorophenyl) tin (IV) chloride (0.655gm; 1mmol) in benzene and succinimide (0.099gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of $(C_6F_5)_3SnCl$ with Phthalimide

In an oxygen free nitrogen atmosphere, solution of tris (pentafluorophenyl) tin (IV) chloride (0.655gm; 1mmol) in benzene and phthalimide (0.147gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of $(C_6H_4F)_3SnCl$ with Phthalimide

In an oxygen free nitrogen atmosphere, solution of tris (*p*-fluorophenyl) tin (IV) chloride (0.439gm; 1mmol) in benzene and phthalimide (0.147gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

Reaction of $(C_6H_4F)_3SnCl$ with Succinimide

In an oxygen free nitrogen atmosphere, solution of tris (*p*-fluorophenyl) tin (IV) chloride (0.439gm; 1mmol) in benzene and succinimide (0.099gm; 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off white color $Et_3N.HCl$ formed was filtered off and filtrate on evaporation in vaccum gives an off white colour crystaline solid mass which was further recrystalised in pet.ether.

BIOLOGICAL STUDIES

Antibacterial Activity

Antibacterial activity of the synthesized compound was carried out by disc diffusion method [30] using gentamycin as standard. The filter paper (Whatmann No. 1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 µg / ml of ethanol) along with standard were placed on the nutrient agar plate at 37°C for 24 hrs in BOD incubator. The inhibition zone around the dried impregnated disc was measured after 24 hrs. 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 the compound was tested by agar plate diffusion method [31], using gentamycin as standard. Four concentrations of the test compounds viz., 10, 20, 50 and 100 µg / ml were prepared and tested against two pathogenic fungal strains, *Aspergillus flavus* and *Aspergillus niger*. The one ml of each compound was poured into a Petri dish containing 20-25 ml of molten potato dextrose - agar medium. As the medium solidify, Petri dishes were inoculated at 37°C for 96 hrs in BOD incubator. After 96 hrs the colony diameter was measured and % inhibition was calculated using standard method [32].

Antitumor Activity

The *in-vitro* antitumor activity of these compounds was carried out by MTT-Method [33]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used for this purpose. The principle behind this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT [3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyl tetrazoleum bromide] was reduced partially by metabolically active cells by the action of dehydrogenase enzyme to generate NADH and NADPH as reducing equivalents. The resulting intracellular purple colour zone was solubilized and quantified by spectrophotometer. The MTT was first dissolved in Phosphate buffer saline at a concentration of 5 mg / ml. The MTT solution (50 µl) was added to each well of 96 well culture plate containing 100 µl of culture medium and incubates at 37°C for 4 hrs. The medium was then removed carefully without disturbing the crystals of purple colored zone. 50 ml of DMSO was then added to each well and mixed thoroughly to dissolve the crystals of the zone. The plate was then read on a micro ELISA plate reader at a wavelength of 570 nm to fine out the optical density and cell count value.

RESULT AND DISCUSSIONS

IR Spectra

The IR spectra of new organotin compounds were recorded in a Perkin–Elmer spectrophotometer in 4000-200 cm^{-1} range. The IR spectra of compound show absorption bands due to phenyl, *p*-fluorophenyl and pentafluorophenyl groups. The absorption frequencies due to carbonyl group (both symmetric and asymmetric) in amide have been fully assigned. The Sn-C vibration in case of phenyl and pentafluorophenyl derivatives corresponding to the y mode and appears in the range of 448-460 cm^{-1}. Further characterization of these compounds is in progress for confirmation of structure of the compounds.

In-vitro Antitumor Activity

Antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compounds show moderate to higher activity against tumor cell lines. It was found that these compounds are in +4 oxidation state and slight variation in their activity is due to presence of different amides as ligand along with presence of fluorine on main moiety of the compound. The compound generally interacts with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions for a cell. The compounds in +4 oxidation state can easily binds with the receptor site [28].It may be noted that the organotin compound generally binds with nitrogen 7 position of purine bases in DNA molecule, where they reacted with a labile hydrogen and form complex with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis [28].

Antibacterial Activity

The organotin compounds were tested for antibacterial activity against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 µg / ml concentration of test compound. All the compounds show moderate to higher activity against the bacterial strains. The activity of compounds 4 and 6 was found higher in three bacterial strains. The rest of compounds were moderately active. It was found that compounds having fluoro and pentafluorophenyl ring are more effective because of their water and lipid solubility. The fluorine-containing compounds may generally form complexes with metaloenzymes, particularly those which responsible in basic physiology such as cytochrome oxidase. 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 followed by death of bacterial cell [34]. Some times these compounds in low concentration may cause bacteriostatic condition by slow down the growth of bacteria.

Antifungal Activity

The antifungal activity of these compounds were tested against *Aspergillus flavus* and *Aspergillus niger* using concentrations 10, 20, 50 and 100 µg / ml. Activity of the compound was found variable at lower concentration but at higher concentration compounds show high activity against fungal strains. Presence of nitrogen, phenyl and pentafluorophenyl ring along with bismuth in +4 oxidation state are considered for fungal activity. Compounds generally damage the fungal strains by puncturing the cell wall similarly as in case of bacteria. Water and lipid solubility also increases the activity due to presence of fluorine.

ACKNOWLEDGEMENT

The authors are thankful to the University Grants Commission, New Delhi(India) for providing fund to our research group(Sanction Letter No.F.6-1(29) / 2008(MRP / NRCB), 26 Feb.2008),Director, HIPER, Lucknow for antimicrobial and antitumor activity and Director, RSIC, CDRI for analysis of the compounds.

Table 1
Physicochemical Properties of Triorganotin Compounds

S. N.	Compounds	Formula	Formula Weight	Melting Point (°C)	Yield (%)	Solvent for Crystal-lization
1	$(C_6H_5)_3Sn$ (succinimide)	$C_{22}H_{19}NO_2Sn$	447.71	88 (40-60 °C)	80	Pet.-ether
2	$(C_6H_5)_3Sn$ (phthalimide)	$C_{26}H_{19}NO_2Sn$	494.71	80 (40-60 °C)	85	Pet.-ether
3	$(C_6H_4F)_3Sn$ (succinimide)	$C_{22}H_{16}NO_2F_3Sn$	500.71	90 (60-80 °C)	65	Pet.-ether
4	$(C_6H_4F)_3Sn$ (phthalimide)	$C_{26}H_{16}NO_2F_3Sn$	548.71	85 (40-60 °C)	70	Pet.-ether
5	$(C_6F_5)_3Sn$ (succinimide)	$C_{22}H_4NO_2F_{15}Sn$	716.71	95 (60-80 °C)	70	Pet.-ether
6	$(C_6F_5)_3Sn$ (phthalimide)	$C_{26}H_4NO_2F_{15}Sn$	764.71	90 (40-60 °C)	75	Pet.-ether

Table 2
Analytical Data of Triorganotin Compounds

S. N.	Molecular Formula	Elemental Analysis			IR (cm^{-1})	
		C (%)	H (%)	N (%)	V_{asym} (CO)	V_{sym} (CO)
1	$C_{22}H_{19}NO_2Sn$	59.09	4.25	3.13	1706 vs	1308ms
2	$C_{26}H_{19}NO_2Sn$	63.06	3.84	2.82	1758vs	1354ms
3	$C_{22}H_{16}NO_2F_3Sn$	52.72	3.19	2.79	1726ms	1326ms
4	$C_{26}H_{16}NO_2F_3Sn$	56.86	2.91	2.55	1729vs	1329ms
5	$C_{22}H_4NO_2F_{15}Sn$	36.83	0.55	1.95	1732vs	1332ms
6	$C_{26}H_4NO_2F_{15}Sn$	40.79	0.52	1.83	1740ms	1338ms

Table 3
In vitro-Anti Tumor Activity

S. No.	Compounds	Cell No.×10^4 Activity (MCF-7)		Cell No.×10^4 Activity (EVSA-7)	
1.	$C_{22}H_{19}NO_2Sn$	13.43 ± 1.52	–	12.47±1.22	–
2.	$C_{26}H_{19}NO_2Sn$	12.42 ± 1.22	–	11.28±1.12	–
3.	$C_{22}H_{16}NO_2F_3Sn$	9.71 ± 0.82	+	9.89±0.85	+
4.	$C_{26}H_{16}NO_2F_3Sn$	9.32 ± 0.65	+	9.42±0.68	+
5.	$C_{22}H_4NO_2F_{15}Sn$	9.90 ± 0.86	+	8.82±0.48	+
6.	$C_{26}H_4NO_2F_{15}Sn$	9.22 ± 0.62	+	8.32±0.44	+
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

Table 4
Antibacterial Activity (Zone of Inhibition (mm) dia. ± S.E)

S. No.	Compounds	Pseudomonas aeruginosa	Staphylococcus aureus	Klebsiela pneumoniae
1.	$C_{22}H_{19}NO_2Sn$	11.00±0.57	08.10±0.16	12.00±1.15
2.	$C_{26}H_{19}NO_2Sn$	11.33±0.66	11.00±0.57	08.5±0.29
3.	$C_{22}H_{16}NO_2F_3Sn$	08.0±0.28	19.00±0.57	13.00±0.50
4.	$C_{26}H_{16}NO_2F_3Sn$	17.33±0.6	19.00±0.57	15.00±0.57
5.	$C_{22}H_4NO_2F_{15}Sn$	18.66±0.66	07.83±0.44	10.5±0.76
6.	$C_{26}H_4NO_2F_{15}Sn$	15.66±0.33	16.00±0.57	17.00±0.57
7.	Gentamycin (standard)	18.72±0.68	18.11±0.32	18.08±0.28

Table 5
Antifungal Activity

S. No.	Compounds	Con. µg/ml.	Aspergillus flavus (dia.mm)	% Inhi-bition	Aspergillus niger (dia.mm)	% Inhi-bition
1.	$C_{22}H_{19}NO_2Sn$	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.	$C_{26}H_{19}NO_2Sn$	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.	$C_{22}H_{16}NO_2F_3Sn$	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.	$C_{26}H_{16}NO_2F_3Sn$	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.	$C_{22}H_4NO_2F_{15}Sn$	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.	$C_{26}H_4NO_2F_{15}Sn$	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	–

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Biological Activity of Some New Organobismuth Compounds

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ABSTRACT

Some new organic derivatives of bismuth (III) of the type Ar_2BiL; (Ar = C_6H_5, C_6F_5, C_6H_4F; L = succinimide and phthallimide) were synthesized and assayed first time for biological activity. Compounds exhibit significant *in-vitro* antitumor activity against human breast adenocarcinoma cell line (MCF-7), mammary cancer cell line (EVSA-7); antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae* along with antifungal activity against *Aspergillus flavus* and *Aspergillus niger*.

Keywords: Bismuth (III) amide, Antitumor, Antibacterial, Antifungal activity.

INTRODUCTION

It is well known that metal played an important role in medicinal purposes for last many years, ever since humans have walked in the planet; but their significance has been realized recently[1]**.** Metals are essential component for various physico-chemical processes of living system and use as metalopharmaceuticals [2]. Oncologists knew about the relationship between cancer and metal. It is surprising to observe that metals are able to induce and to treat the disease and some of them are able to perform both and acts as paradox [2].

Bismuth compounds have attracted considerable interest owing to their biological and medicinal utility [3-5]. They have been utilized for more than two centuries in the treatment of gastrointestinal disorders such as dyspepsia, diarrhea and peptic ulcer[6-9]. Bismuth salts such as colloidal bismuth sub-citrate (CBS), bismuth sub-salicylate (BSS), and ranitidine bismuth citrate (RBC) are now common agents used for such purpose [10]. The recent demonstration found that these salts are useful for *Helicobacter pylori* eradication therapy [10, 11] and promoted antibacterial [12-20] or antitumor [21] studies of various bismuth compounds. Despite the long history of the use of bismuth as bio-medicinal agents, the mechanism of the biological action of bismuth is not fully understood [3, 10, 11, 20]. It is an important issue for us to know that how bismuth compound act against microorganisms.

It is known that metals are able to generate reactive oxygen species (ROS) which easily explain the treatment of cancer [21]. In search of antiproliferative studies of a variety of organobismuth compounds, thiolates and carboxylate have been synthesized and tested *in-vitro* for their antitumor activity along with their antimicrobial activity [22-27]. The combination of organobismuth and-germanium compound, aryl bismuth triphenyl-propionate has been found to exhibit higher activity against MCF-7 cell line in comparison to cis-platin [28]. To increase the hydrophilic (to facilitate acceptance by water rich cells) and lipophilic (essential for crossing the cell membrane) character of these compounds, fluorine containing compounds were synthesized and characterized [29]. It is well known that the compound containing fluorine and other polar groups enhances the biological activity in vitro [30].

The structure–activity relationship on the reported organometallic compounds show that the biological activity is highly affected by the nature and number of organic groups, nature of ligands, presence of fluoro constituents for hydrophilic and lipophilic nature and hydrolytic stability of metal-carbon bond [31].

The present communication deals with the synthesis and characterization of organobismuth(III) compounds for antibacterial, antifungal and antitumor activity and were found to be active against (MCF-7), (EVSA-7) cancer cell line along with pathogenic fungal and bacterial strains.

EXPERIMENTAL

The synthesis of organobismuth (III) amides was performed by the earlier reported method [29]. The diorganobismuth (III) chloride was prepared by the

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redistribution reaction of R_3Bi and $BiCl_3$ in absence of solvent and crystallized in dichloromethane under N_2 atmosphere. The ligands were purified by crystallization before use. The reaction of diarylbismuth (III) chloride with suitable amide in presence of triethylamine as hydrogen chloride acceptor forms the respective product.

$$2\ R_3Bi + BiCl_3 \xrightarrow{\text{Neat}} 3\ R_2BiCl$$

$$R_2BiCl + HL \xrightarrow{Et_3N} R_2BiL + Et_3N.HCl$$

R = (C_6H_5), (C_6F_5), (C_6H_4F), HL = Succinimide, Phthallimide

Molecular weights of the compounds were determined cryoscopically in benzene. The IR spectra of new organobismuth compounds were recorded in a Perkin–Elmer spectrophotometer in 4000-200 cm^{-1} range. Further characterization of these compounds is in progress for confirmation of structure of the compounds.

Table 1
Physicochemical Properties of Organobismuth Compounds

S. N.	*Compounds*	*Formula*	*Formula Weight*	*Melting Point (°C)*	*Yield (%)*	*Solvent for Crystal-lization*
1	$(C_6H_5)_2Bi$ (succinimide)	$C_{16}H_{14}NO_2Bi$	461	126	80	Pet.-ether (40-60 °C)
2	$(C_6H_5)_2Bi$ (phthalimide)	$C_{20}H_{14}NO_2Bi$	509	122	85	Pet.-ether (40-60 °C)
3	$(C_6H_4F)_2Bi$ (succinimide)	$C_{16}F_2H_{12}NO_2Bi$	497	119	65	Pet.-ether (60-80 °C)
4	$(C_6H_4F)_2Bi$ (phthalimide)	$C_{20}F_2H_{12}NO_2Bi$	545	110	70	Pet.-ether (40-60 °C)
5	$(C_6F_5)_2Bi$ (succinimide)	$C_{16}F_{10}H_4NO_2Bi$	641	119	70	Pet.-ether (60-80 °C)
6	$(C_6F_5)_2Bi$ (phthalimide)	$C_{20}F_{10}H_4NO_2Bi$	689	114	75	Pet.-ether (40-60 °C)

BIOLOGICAL STUDIES

Antitumor Activity

The *in-vitro* antitumor activity of these compounds was carried out by MTT-Method [32]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines were used for this purpose. The principle behind this assay depends upon the reduction of tetrazolium salt. The yellow colored tetrazoleum MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazoleum bromide] was reduced partially by metabolically active cells by the action of dehydrogenase enzyme to generate NADH and NADPH as reducing equivalents. The resulting intracellular purple colour zone was solubilized and quantified by spectrophotometer. The MTT was first dissolved in Phosphate buffer saline at a concentration of 5 mg/ml. The MTT solution (50 ml) was added to each well of 96 well culture plate containing 100 ml of culture medium and incubates at 37°C for 4 hrs. The medium was then removed carefully without disturbing the crystals of purple colored zone. 50 ml of DMSO was then added to each well and mixed thoroughly to dissolve the crystals of the zone. The plate was then read on a micro ELISA plate reader at a wavelength of 570 nm to fine out the optical density and cell count value.

Antibacterial Activity

Antibacterial activity of the synthesized compound was carried out by disc diffusion method [33] using ampicilin as standard. The filter paper (Whatmann No. 1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 mg/ml of ethanol) along with standard were placed on the nutrient agar plate at 37°C for 24 hrs in BOD incubator. The inhibition zone around the dried impregnated disc was measured after 24 hrs. 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 the compound was tested by agar plate diffusion method [34], using ampicilin as standard. Four concentrations of the test compounds viz., 10, 20, 50 and 100 mg/ml were prepared and tested against two pathogenic fungal strains, *Aspergillus flavus* and *Aspergillus niger*. The one ml of each compound was poured into a Petri dish containing 20-25 ml of molten potato dextrose-agar medium. As the medium solidify, Petri dishes were inoculated at 37 °C for 96 hrs in BOD incubator. After 96 hrs the colony diameter was measured and % inhibition was calculated using standard method [35].

RESULTS AND DISCUSSION

IR Spectra

The IR spectra of compound show absorption bands due to phenyl, *p*-fluorophenyl and pentafluorophenyl groups. The absorption frequencies having diagnostic values are listed in table-2. The absorption frequencies due to carbonyl group (symmetric as well as asymmetric) in amide derivatives have been assigned. The Bi-C vibration 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

Antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and

Table 2
Analytical Data of Organobismuth Compounds

S. N.	Molecular Formula	Elemental Analysis C (%)	H (%)	N (%)	IR (cm^{-1}) V_{asym} (CO)	V_{sym} (CO)
1	$C_{16}H_{14}NO_2Bi$	41.64	3.03	3.03	1708 vs	1310ms
2	$C_{20}H_{14}NO_2Bi$	47.15	2.75	2.75	1762v	1314ms
3	$C_{16}F_2H_{12}NO_2Bi$	38.63	2.41	2.81	1729ms	1329ms
4	$C_{20}F_2H_{12}NO_2Bi$	44.03	2.20	2.56	1730vs	1330ms
5	$C_{16}F_{10}H_4NO_2Bi$	29.95	0.62	2.18	1735vs	1335ms
6	$C_{20}F_{10}H_4NO_2Bi$	34.83	0.58	2.03	1738ms	1339m

mammary cancer (EVSA-7) cell lines. The compounds show moderate to higher activity against tumor cell lines. It was found that these compounds are in +3 oxidation state and slight variation in their activity is due to presence of different amides as ligand along with presence of fluorine on main moiety of the compound. The compound generally interacts with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions for a cell. The compounds in +3 oxidation state can easily binds with the receptor site [29]. It may be noted that the organobismuth compound generally binds with nitrogen 7 position of purine bases in DNA molecule, where they reacted with a labile hydrogen and form complex with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis [36].

Table 3
***In vitro* - Anti Tumor Activity**

S. No.	Compounds	Cell No.×10^4 Activity (MCF-7)		Cell No.×10^4 Activity (EVSA-7)	
1.	$C_{22}H_{19}NO_2Sn$	13.43 ± 1.52	–	12.47±1.22	–
1.	$C_{16}H_{14}NO_2Bi$	12.34 ± 1.05	–	11.74±1.22	–
2.	$C_{20}H_{14}NO_2Bi$	11.69 ± 1.02	–	10.68±1.08	–
3.	$C_{16}F_2H_{12}NO_2Bi$	9.17 ± 0.87	+	9.69±0.92	+
4.	$C_{20}F_2H_{12}NO_2Bi$	9.34 ± 0.65	+	9.66±0.90	+
5.	$C_{16}F_{10}H_4NO_2Bi$	9.89 ± 0.85	+	8.28±0.46	+
6.	$C_{20}F_{10}H_4NO_2Bi$	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

Antibacterial Activity

The organobismuth compounds were tested for antibacterial activity against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae* using 10 mg / ml concentration of test compound. All the compounds show moderate to higher activity against the bacterial strains. The activity of compounds 4 and 6 was found higher in three bacterial strains. The rest of compounds were moderately active (table 4). It was found that organometallic compounds containing fluoro and pentafluorophenyl ring are more effective because of their water and lipid solubility. The fluorine-containing compounds may generally form complexes with metaloenzymes, particularly those which responsible in basic physiology such as cytochrome oxidase. 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 followed by death of bacterial cell. Some times these compounds in low concentration may cause bacteriostatic condition by slow down the growth of bacteria.

Table 4
Antibacterial Activity (Zone of Inhibition (mm) dia. ± S.E)

S. No.	Compounds	Pseudomonas aeruginosa	Staphylococcus aureus	Klebsiela pneumoniae
1.	$C_{16}H_{14}NO_2Bi$	11.00±0.57	8.10±0.16	12.00±1.15
2.	$C_{20}H_{14}NO_2Bi$	11.33±0.66	11.00±0.57	8.5±0.29
3.	$C_{16}F_2H_{12}NO_2Bi$	8.0±0.28	19.00±0.57	13.00±0.50
4.	$C_{20}F_2H_{12}NO_2Bi$	17.33±0.6	19.00±0.57	15.00±0.57
5.	$C_{16}F_{10}H_4NO_2Bi$	18.66±0.66	7.83±0.44	10.5±0.76
6.	$C_{20}F_{10}H_4NO_2Bi$	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

Antifungal Activity

The antifungal activity of these compounds were tested against *Aspergillus flavus* and *Aspergillus niger* using concentrations 10, 20, 50 and 100 mg / ml. Activity of the compound was found variable at lower concentration but at higher concentration compounds show high activity against fungal strains (table 5). Presence of nitrogen, phenyl and pentafluorophenyl ring along with bismuth in +3 oxidation state are considered for fungal activity. Compounds generally damage the fungal strains by puncturing the cell wall similarly as in case of bacteria. It is well know that the Lewis acidic nature of bismuth in +3 oxidation state is also affect the fungal activity. Water and lipid solubility also increases the activity due to presence of fluorine.

CONCLUSION

Organobismuth (III) compounds of the type Ar_2BiL; (Ar = C_6H_5, C_6F_5, C_6H_4F; L = succinimide and phthallimide)

have great potential as anticancer and antimicrobial agents. These compounds may be exploited for the development of new drugs for the treatment of diseases like cancer, gastrointestinal disorders and diseases caused by various microorganisms.

Table 5
Antifungal Activity

S. No.	*Compounds*	*Con. µg/ml.*	*Aspergillus flavus (dia.mm)*	*% Inhibition*	*Aspergillus niger (dia.mm)*	*% Inhibition*
1.	$C_{16}H_{14}NO_2Bi$	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.	$C_{20}H_{14}NO_2Bi$	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.	$C_{16}F_2H_{12}NO_2Bi$	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.	$C_{20}F_2H_{12}NO_2Bi$	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.	$C_{16}F_{10}H_4NO_2Bi$	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.	$C_{20}F_{10}H_4NO_2Bi$	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	–

ACKNOWLEDGEMENT

The authors are thankful to the University Grants Commission, New Delhi (India) for providing fund to our research group (Sanction Letter No. F.6-1 (29) / 2008 (MRP / NRCB), 26 Feb. 2008), Director, HIPER, Lucknow for antimicrobial and antitumor activity and Director, RSIC, CDRI for analysis of the compounds.

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