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

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**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 | – |