International Journal of Global Science Research **ISSN: 2348-8344 (Online)**
Vol. 12, Issue. 2, October 2025, pp. 2856-2863 **DOI: https://doi.org/10.26540/ijgsr.v12.i2.2025.324**
***Available Online* at www.ijgsr.com**

Research Paper

Antimicrobial and antitumor studies of some new organobismuth compounds

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Received: 22/09/2025
Revised: 30/09/2025
Accepted: 10/10/2025

Abstract: Some organobismuth (III) amides of the type Ar_2BiL, (Ar=C_6H_5, C_6F_5, C_6H_4F, L = succinimide and phthallimide) assayed first time for their biological activity, exhibited significant in vitro antitumor activity against human breast adenocarcinoma cell line (MCF-7), mammary cancer (EVSA-7), antibacterial activity against human pathogenic bacteria viz.-*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae*. They also exhibited remarkable antifungal activity against *Aspergillus flavus* and *Aspergillus niger* strains.

Keywords: Organobismuth (III) amides, Antitumor, Antibacterial, Antifungal activity

Introduction:

Metals have played an important role in medicine for years, ever since humans have walked the planet, although people have only recently realized their significance. The importance of metal ions lies in the fact that they are essential components for various physico-chemical processes occurring in living system and has potential use as metalopharmaceuticals (Nath *et. al.*, 2024).

All oncologists know introduction of antitumor studies of ligands and the existence of relationship between cancer and metal. However, many ignore the various aspects about their relationship. It is surprising to observe that metals are able to do the best and the worst, i.e. metals are able to induce the disease and also to treat the infectious disease, and some of them are able to perform the both i.e. they act as paradox (Colley *et. al.*, 2023)

It is well known that almost all metals are able to generate reactive oxygen species (ROS), and this property explains a great part of the treatment of cancer. Organometallics of both transition and non-transition metals plays important role in the treatment of cancer and other infectious diseases (Tyagi *et. al*., 2004).

In search of antiproliferative properties of a variety of organobismuth compounds, especially thiolates and carboxylate have been synthesized and tested in vitro for their antitumor activity along with their antimicrobial activity (Tiekink, 2002; Socacin et. al., 1991;

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Socacin *et. al*., 1994; Ying *et. al*., 2002; Charles and Charles, 2005 and Li *et. al*., 2002). A combination of organobismuth and-germanium compounds, aryl bismuth triphenylpropionate, has been found to show much higher activity against MCF-7 cell line compared to well-known cis-platin complex Li *et. al*., 2002) . To enhance the hydrophilic (to facilitate acceptance by water rich cells) as well as lipophilic (essential for crossing the cell membrane) character of these compounds, fluorine-containing compounds were synthesized and characterized. It is well known that the compound containing fluorine and other polar groups enhances the biological activity *in vitro* (Agarwal and Mishra 1989) .

A comprehensive structure-activity relationship on the reported potentially bioactive organotin compounds reveal that the activity of an organometallic compound is highly affected by, the nature and number of organic groups, nature of ligands, presence of fluoro substituents for hydrophilic and lipophilic character and the hydrolytic stability of metal-carbon bond (Gielen et. al., 1997).

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

Result and Discussion:

The diarylbismuth (III) amides (R_2 BiL) can easily be prepared by the reaction of diarylbismuth (III) chloride with suitable amide in presence of triethylamine as a hydrogen chloride acceptor.

$$R_2BiCl + HL \xrightarrow{Et_3N} R_2\,BiL + Et_3N.\,HCl$$

R= (C_6H_5), (C_6F_5), (C_6H_4F)

HL Succinimide, Phthallimide

All the reactions were proceeds under inert condition.

IR Spectra

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

In-vitro Antitumor Activity

The antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell line. The compounds showed moderate to higher activity against the tumor cell line and inhibit the growth of about 45-50% of tumor. It was found that all these compounds are in +3 oxidation state of bismuth. The slight variation in their activity is due to presence of different kinds of amides as ligands along with presence of fluorine in the main moiety of the compounds. These compounds generally interact with the receptor site of enzyme complex, which are responsible for the cytostatic and cytotoxic conditions for a cell. The bismuth compounds in +3 oxidation state can easily binds with the receptor site Besides this one molecular based mechanism of inhibition of growth of tumor cell line. It may be possible that the organobismuth compounds generally

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binds with nitrogen 7 position of nucleotide bases of DNA molecules, where they reacts with a labile hydrogen and form a complex with DNA strands and affected the replication and transcription of the DNA, and therefore stop the division of cell in some extent along with the protein synthesis.

Antibacterial Activity

The organobismuth compounds were tested for antibacterial activity against three human pathogenic bacterial strains, viz, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiela pneumoniae*, using 10 µg/ml concentration of the test compounds. All compounds showed moderate to higher activity against these three bacterial strains The activity of the compound 4 and 6 was found highest against all the three strains. The rest of the compounds are moderately active. It is found that organometallic compounds containing fluoro and pentafluorophenyl ring are more effective against microbes because of their water and lipid solubility. These fluorine containing compounds may generally form complexes with metaloenzymes, particularly those that are in responsible in basic physiology such as cytochrome oxidase. All these compounds may react with peptidoglycan layer of bacterial cell wall and damage it by penetrating in such a manner that the two phenyl ring gets entered inside the cell by puncturing it and ultimately causing death of bacterial cell. Some times all these compounds in lower concentration may cause the bacteriostatic condition by slow down the growth of bacteria.

Antifungal Activity

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

Experimental:

The synthesis of organobismuth (III) amides was carried out by the reported method (Saxena et. al., 1985). The diarylorganobismuth (III) chloride R_2 BiCl was prepared by the redistribution reaction of R_3Bi and $BiCl_3$ in neat and crystallized in dichloromethane in inert atmosphere.

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

(R = C_6H_5, C_6F_5, C_6H_4F)

The organic moieties were prepared by the standard techniques and purified by crystallization before use. Molecular weights were determined cryoscopically in benzene. The IR spectra of new organobismuth compounds were recorded in a Perkin-Elmer spectrophotometer in 4000-200 cm^{-1} range. The representative methods of preparation of organobismuth (III) amides are as follows.

Reaction of $(C_6F_5)_2$ BiCl with succinimide

In an inert atmosphere, a solution of bis (pentafluorophenyl) bismuth (111)

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chloride (0.578 gm, 1 mmol) in benzene and succinimide (0.099 gm, 1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3-4 hr Et_3N HCI formed was filtered off and the filtrate on evaporation and concentration in vacuo gives a white colour crystalline solid which was recrystalized from pet ether (60-80 °C) to give bis (pentafluorophenyl) bismuth (III) succinimide.

Reaction of $(C_6F_5)_2$ BiCl with phthalimide

In an inert atmosphere, a solution of bis (pentafluorophenyl) bismuth (III) chloride (0.578 gm; 1 mmol) in benzene and phthalimide (0.147 gm; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3-4 hr. Et_3N HCI formed was filtered off and the filtrate on evaporation and concentration in vacuo gives a red /pink colour crystalline solid which was recrystalized from pet-ether (40-60 °C) to give bis (pentafluorophenyl) bismuth (III) phthalimide.

Reaction of $(C_6H_5)_2$ BiCl with succinimide

In an inert atmosphere, a solution of diphenylbismuth (III) chloride (0.398 gm; 1 mmol) in toluene and succinimide (0.099 gm; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3-4 hr. Et_3N HCI formed was filtered off and the filtrate on evaporation and concentration in vacuo gives a white colour crystalline solid which was recrystalized from pet-ether (40-60^oC) diphenylbismuth (III) succinimide.

Reaction of $(C_6H_4F)_2$ BiCl with succinimide

In an inert atmosphere, a solution of bis (*p*-fluorophenyl) bismuth (III) chloride (0.434 gm; 1 mmol) in toluene and succinimide (0.099 gm; 1 mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 3-4 hr. Et_3 N HCI formed was filtered off and the filtrate on evaporation and concentration in vacuo gives a white colour crystalline solid which was recrystalized from pet-ether (40-60 °C) to give bis (*p*-fluorophenyl) bismuth (III) succinimide.

Antitumor Activity

The *in vitro* antitumor activity of these compounds was carried out by MTT-Method (Mosmann, 1983). This method was carried out to estimate the effect of compounds on the growth of cells. The human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell line was used here. The principle behind this assay depends upon the reduction of tetrazolium salts. The yellow colored tetrazoleum MTT [3(4.5-dimethylthiazolyl-2)-2,5-diphenyl tetrazoleum bromide] gets reduced by metabolically active cells partially by the action of dehydrogenase enzyme to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple colour zones was solubilized and quantified by spectrophotometer method. The MTT was first dissolved in PBS (Phosphate buffer saline) at a concentration of 5 mg/ml. Then 50 µl of the MTT solution was added to each well of the 96 well culture plate, containing 100 µl culture medium along with test compound, and incubated at 37°C for 4 hrs. The medium was then removed carefully without disturbing the purple colored zone crystals. Then 50 ml of DMSO was added to each well and mixed thoroughly to dissolve the crystals of the zones. The plates were then read on a micro plate reader at a wavelength of 570 nm. The readings were presented as optical density, which gives the cell count value.

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Antibacterial Activity

Antibacterial activity of these compounds was carried out by disc diffusion method (Verma and Imam, 1973). In this technique, the filter paper (Whatmann No. 1) sterile disc of 5 mm diameter, impregnated with the test compounds (10 µg/ml of ethanol) were placed on the nutrient agar plate at 37°C for 24 hrs. The inhibition zones around the dried impregnated disc were 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 compounds was tested by agar plate diffusion method (Horsfall, 1945) using four concentrations of the test compounds viz., 10, 20, 50 and 100 µg/ml against two human pathogenic fungal strains, *Aspergillus flavus* and *Aspergillus niger*. The one ml of each compound was poured into a Petri dish having about 20-25 ml of molten potato dextrose- agar medium. As the medium gets solidify. Petri dishes were inoculated at the center of plate separately with the fungal isolates and kept at 37°C for 96 hrs. All the value (% inhibition) was recorded by known methods (Giri and Khare, 1976).

Table-1 : Physicochemical properties of organobismuth compounds

S.N.	Molecular Formula	Elemental Analysis			IR (cm^{-1})		Colour
		C (%)	H (%)	N (%)	υ_{asym}(CO)	υ_{sym}(CO)	
1	$C_{16}H_{14}NO_2Bi$	41.64	3.03	3.03	1706vs	1307ms	White
2	$C_{20}H_{14}NO_2Bi$	47.15	2.75	2.75	1761vs	1310ms	White
3	$C_{16}F_2H_{12}NO_2Bi$	38.63	2.41	2.81	1732ms	1331ms	White
4	$C_{20}F_2H_{12}NO_2Bi$	44.03	2.20	2.56	1729vs	1329ms	White
5	$C_{16}F_{10}H_4NO_2Bi$	29.95	0.62	2.18	1730vs	1330ms	White
6	$C_{16}F_{10}H_4NO_2Bi$	34.83	0.58	2.03	1732ms	1332m	Pink/Red

Table-2 : Analytical data of organobismuth compounds

S.N.	Compounds	Formula	Formula Weight	Melting Point (C)	Yield (%)	Solvent for Crystallization
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)	$C_{16}F_{10}H_4NO_2Bi$	689	114	75	Pet-ether (40-60°C)

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Table-3 : *In vitro*- Antitumor activity

S.N.	Compounds	Cell No. x 10^4 (MCF-7)	Activity	Cell No. x 10^4 (EVSA-7)	Activity
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

Table-4 : Antibacterial activity

S.N.	Compounds	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klaebsiela pneumoniae*
1	$C_{16}H_{14}NO_2Bi$	-	+ +	+	++
2	$C_{20}H_{14}NO_2Bi$	-	+ +	+ +	+
3	$C_{16}F_2H_{12}NO_2Bi$	-	+	+ + +	++
4	$C_{20}F_2H_{12}NO_2Bi$	-	++	+ +	+++
5	$C_{16}F_{10}H_4NO_2Bi$	-	+++	+	++
6	$C_{20}F_{10}H_4NO_2Bi$	-	++	++	++

\+ = 6-10 mm (dia)
++ = 10-14 mm
+++ = >14 mm
' -' = Inactive; (control)

Table-5 : Antifungal activity of 10 µg/ml conc. of compounds

S.N.	Compounds	*Aspergillus flavus* Col. dia. (mm)	% Inhibition	*Aspergillus niger* Col. dia. (mm)	% Inhibition
1	$C_{16}H_{14}NO_2Bi$	1.2	60.0	1.0	50.0
2	$C_{20}H_{14}NO_2Bi$	1.4	53.3	1.5	25.0
3	$C_{16}F_2H_{12}NO_2Bi$	1.4	5.3	1.0	50.0
4	$C_{20}F_2H_{12}NO_2Bi$	1.2	60.0	1.4	30.0
5	$C_{16}F_{10}H_4NO_2Bi$	1.2	60.0	1.5	25.0
6	$C_{20}F_{10}H_4NO_2Bi$	0.8	73.3	1.4	30.0
7.	Control	3.0	-	2.0	-

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Table-6 : Antifungal activity of 20µg/ml conc. of compounds

S.N.	Compounds	*Aspergillus flavus* Col. dia. (mm)	% Inhibition	*Aspergillus niger* Col. dia. (mm)	% Inhibition
1	$C_{16}H_{14}NO_2Bi$	1.0	66.6	1.0	50.0
2	$C_{20}H_{14}NO_2Bi$	1.0	66.6	1.0	50.0
3	$C_{16}F_2H_{12}NO_2Bi$	1.2	60.0	0.8	60.0
4	$C_{20}F_2H_{12}NO_2Bi$	1.0	66.6	1.0	50.0
5	$C_{16}F_{10}H_4NO_2Bi$	0.7	76.6	1.2	40.0
6	$C_{20}F_{10}H_4NO_2Bi$	0.6	80.0	1.2	40.0
7.	Control	3.0	-	2.0	-

Table-7 : Antifungal activity of 50µg/ml conc. of compounds

S.N.	Compounds	*Aspergillus flavus* Col. dia. (mm)	% Inhibition	*Aspergillus niger* Col. dia. (mm)	% Inhibition
1	$C_{16}H_{14}NO_2Bi$	0.6	80.0	0.5	75.0
2	$C_{20}H_{14}NO_2Bi$	0.7	76.6	0.6	70.0
3	$C_{16}F_2H_{12}NO_2Bi$	1.0	66.6	0.5	75.0
4	$C_{20}F_2H_{12}NO_2Bi$	0.8	73.3	0.8	60.0
5	$C_{16}F_{10}H_4NO_2Bi$	0.5	83.3	0.8	60.0
6	$C_{20}F_{10}H_4NO_2Bi$	0.4	86.7	0.5	75.0
7.	Control	3.0	-	2.0	-

Table-8 : Antifungal activity of 100µg/ml conc. of compounds

S.N.	Compounds	*Aspergillus flavus* Col. dia. (mm)	% Inhibition	*Aspergillus niger* Col. dia. (mm)	% Inhibition
1	$C_{16}H_{14}NO_2Bi$	0.4	86.7	0.2	90.0
2	$C_{20}H_{14}NO_2Bi$	0.4	86.7	0.1	95.0
3	$C_{16}F_2H_{12}NO_2Bi$	0.8	73.3	0.2	90.0
4	$C_{20}F_2H_{12}NO_2Bi$	0.5	83.3	0.4	80.0
5	$C_{16}F_{10}H_4NO_2Bi$	0.01	96.7	0.5	75.0
6	$C_{20}F_{10}H_4NO_2Bi$	0.2	93.3	0.2	90.0
7.	Control	3.0	-	2.0	-

Acknowledgements:
The authors are thankful to the Head, Department Chemistry, Principal and Managing committee of D.B.S.P.G. College, Kanpur, and D.G. College, Kanpur for providing facilities in the college, Director, Hygia Institute of Pharmaceutical Education & Research (HIPER), Lucknow for providing necessary facilities.

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