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SYNTHESIS AND CHARACTERIZATION OF NOVEL ORGANOBISMUTH COMPOUNDS: ANTIMICROBIAL AND ANTITUMOR STUDIES

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Abstract-The present manuscript deals the design and synthesis of some novel organobismuth compounds which are synthesized by the method reported earlier and characterized with the help of M.P., elemental and I.R. spectral analysis along with their antimicrobial studies, against different pathogenic bacterial and fungal strains and *in-vitro* antitumor activity against human breast (MCF-7) and mammary cancer (EVSA-7) cell line and found that compounds have potentiality as antitumor and antimicrobial agents.

Keywords- Organobismuth, Antimicrobial, Antitumor.

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Introduction

The study of metals in biology is a rapidly expanding field, especially the subfield of bioorganometallic chemistry, which explores the role of metal complexes containing direct metal–carbon bonds. It offers a potentially rich field for the development of new medicinal agents with novel mechanisms of action [1,2]. The major advantage of organometallic compounds, as compared with organic or inorganic compounds, is their high reactivity. The range of potential commercial applications of organometallic compounds appear very promising, whether for environmental applications, bioanalysis, enzymology and biomimetic catalysis, in which the models are increasingly efficient, or indeed the development of novel therapies. Bismuth compounds have attracted considerable interest owing to their biological and medicinal utility [3-5].They have been utilized from 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 common agents used for *Helicobacter pylori* eradication therapy [10,11] and therefore promoted these compounds as antimicrobials [12-20]. 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, a variety of organobismuth compounds have been synthesized and tested *in-vitro* for their antitumor activity along with their antimicrobial activity [22-27]. The present manuscript deals the design and synthesis of some novel organobismuth compounds and their characterization with the help of M.P., elemental and I.R. spectral analysis along with their antimicrobial studies, against different pathogenic bacterial and fungal strains and *in-vitro* antitumor activity against human breast (MCF-7) and mammary cancer (EVSA-7) cell lines.

Materials and Methods

The diphenylbismuth (III) chloride was synthesized by the methods reported earlier [28,29]. The ligands were recrystallised before use. The reactions were performed under inert/nitrogen atmosphere. Preparation of representative organobismuth compounds are discussed below.

Reaction of $(C_6H_5)_2BiCl$ with $(OOC.C_6H_4.NO_2)$

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 2-nitrobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with $(OOC.C_6H_4.NO_2)$

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 4-nitrobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with $(OOC.C_6H_4.Cl)$

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 2-chlorobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with $(OOC.C_6H_4.Cl)$

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 4-chlorobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with[$(OOC.C_6H_3(OH)(OCH_3)$]

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 3-methxy4-hydroxybenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with $(OOC.C_6H_4.NH_2)$

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 2-aminobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with $(OOC.C_6H_4.NH_2)$

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 4-aminobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with [$(OOC.C_6H_4.N(CH_3)_2)$]

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 3-dimethylaminobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Reaction of $(C_6H_5)_2BiCl$ with [$(OOC.C_6H_4.N(C_2H_5)_2)$]

Under nitrogen atmosphere, solution of diphenylbismuth (III) chloride (1mmol) in benzene and 4-diethylaminobenzoic acid (1mmol) in same solvent were stirred together in presence of triethylamine at room temperature for 4-5 hours. The off-white color Et3N.HCl was formed (M.P. =240°C), which was filtered off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallised in pet-ether.

Antibacterial Activity

Antibacterial activity of the synthesized compound was carried out by disc diffusion method [30] 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.

Antifungal Activity

The antifungal activity of the compound was tested by agar plate diffusion method [31], using ampicilin as standard. Four 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 1 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 incubated 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 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 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 then 50 ml of DMSO was 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.

Results and Discussion

The synthesis of diorganobismuth (III) carboxylates was performed in laboratory with the help of following reactions.

$$R3Bi + BiCl3 \xrightarrow{\text{Neat}} R2BiCl$$

$$R2BiCl + HL \xrightarrow{Et3N} R2BiL + Et3N.HCl$$

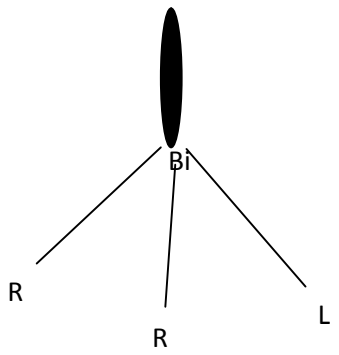
Here:

R = [C6H5] ; HL = [Respective carboxylic acids]

All the newly synthesized diorganobismuth (III) carboxylates were crystalline solids, air stable and soluble in common organic solvents. The compounds were further characterized by their melting points and analytical techniques such as elemental analysis, infrared and NMR spectroscopy to ascertain their structures and explore their biological properties. The new compounds have sharp melting points and posses pyramidal structure as per results obtained by further analysis.

IR and NMR Spectral Analysis

The IR spectra of new diorganobismuth (III) carboxylates were recorded in Perkin–Elmer spectrophotometer in 4000-200 cm-1 range. The IR spectra of these compounds show absorption bands due to phenyl and pentafluorophenyl groups. The absorption frequencies have been fully assigned. The Bi-C vibration in case of phenyl and pentafluorophenyl derivatives corresponding to the y mode appears in the range of 448-460 cm-1. The IR data suggested a monodentate coordination mode of the carboxylate ligands. The 1HNMR spectra of the representative diorganobismuth (III) carboxylate showed a multiplet in the range δ7.82ppm to δ8.12ppm which could be assigned to aromatic protons. The 19FNMR spectra of the compound was carried out at room temperature and the compounds showed peaks appearing in the range -108.30 ppm to -112.30 ppm consistent with the presence of fluorophenyl groups. Thus on the basis of above discussions the newly synthesized diorganobismuth (III) compounds assigned a pyramidal structure.



R = (C6H5)

L = Respective Carboxylate as Ligands

Suggested structure of diorganobismuth (III) carboxylate

Antibacterial activity:

All the compounds show higher to moderate activity against the bacterial strains. It was found that compounds having water and lipid solubility are more effective. The compounds 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 phenyl ring gets entered inside the cell by puncturing it followed by death of bacterial cell. Sometimes these compounds in low concentration may cause bacteriostatic condition by slow down the growth of bacteria.

Table-1 *Physicochemical Analysis of diorganobismuth (III) carboxylates*

S.N.	Compounds	Formula	Formula Weight	Yield %	M.P °C	Solvent	IR (cm^{-1})	
							$\nu_{asym}(CO)$	$\nu_{sym}(CO)$
1	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	$C_{19}H_{14}NO_4Bi$	529	70	79	Pet-Ether	1706 vs	1309ms
2	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	$C_{19}H_{14}NO_4Bi$	529	68	78	Pet-Ether	1758vs	1354ms
3	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	$C_{19}H_{14}O_2ClBi$	518.5	72	81	Pet-Ether	1726ms	1325ms
4	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	$C_{19}H_{14}O_2ClBi$	518.5	70	80	Pet-Ether	1729vs	1327ms
5	$[(C_6H_5)_2Bi(OOCC_6H_3(OH)OCH_3]$	$C_{20}H_{17}O_4Bi$	530	66	76	Pet-Ether	1732vs	1334ms
6	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	$C_{19}H_{16}NO_2Bi$	499	60	89	Pet-Ether	1740ms	1338ms
7	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	$C_{19}H_{16}NO_2Bi$	499	60	90	Pet-Ether	1758vs	1354ms
8	$(C_6H_5)_2Bi(OOCC_6H_4N(CH_3)_2)$	$C_{21}H_{20}NO_2Bi$	527	62	79	Pet-Ether	1752vs	1350ms
9	$(C_6H_5)_2Bi(OOCC_6H_4N(C_2H_5)_2)$	$C_{23}H_{24}NO_2Bi$	555	60	75	Pet-Ether	1725ms	1323ms

Table-2 *Antibacterial Activity of diorganobismuth (III) carboxylates*

S. N.	Compounds	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiela pneumoniae*
1	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	–	+++	+++	++
2	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	–	++	++	++
3	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	–	+++	++	++
4	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	–	++	++	++
5	$[(C_6H_5)_2Bi(OOCC_6H_3(OH)OCH_3]$	–	++	++	+++
6	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	–	+++	++	++
7	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	–	++	++	++
8	$(C_6H_5)_2Bi(OOCC_6H_4N(CH_3)_2)$	–	++	++	+++
9	$(C_6H_5)_2Bi(OOCC_6H_4N(C_2H_5)_2)$	–	+++	+++	++

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

Antifungal Activity:

The activity of these compounds was found variable at 50µg/ml concentration but at higher concentration all the organobismuth (III) compounds show high activity against fungal strains. Presence of nitrogen, phenyl ring along with bismuth in +3 oxidation state are considered for fungal activity. The role of different carboxylates as ligands was also commendable. These compounds generally damage the fungal strains by puncturing the cell wall similarly as in the case of bacteria. Water and lipid solubility also increases the activity.

Table-3 *Antifungal Activity of diorganobismuth(III)carboxylates at 50µg/ml conc.*

S. N.	Compounds	*Aspergillus flavus* Col. Dia. (mm)	% Inhibition	*Aspergillus niger* Col. Dia. (mm)	% Inhibition
1	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	0.7	76.6	0.6	70.0
2	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	0.2	93.3	0.7	65.0
3	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	0.2	93.3	0.7	65.0
4	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	0.5	83.3	0.4	80.0
5	$[(C_6H_5)_2Bi(OOCC_6H_3(OH)OCH_3]$	0.2	93.3	0.7	65.0
6	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	0.2	93.3	0.7	65.0
7	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	0.7	76.6	0.7	65.0
8	$(C_6H_5)_2Bi(OOCC_6H_4N(CH_3)_2)$	0.8	73.3	0.8	60.0
9	$(C_6H_5)_2Bi(OOCC_6H_4N(C_2H_5)_2)$	0.8	73.3	0.8	60.0
10	Control	3.0	-	2.0	-

***In-vitro* Antitumor Activity:**

The compounds show moderate to high activity against tumor cell lines. It was found that these compounds are in +3 oxidation state and the slight variation in their activity is due to presence of different carboxylate group as ligand. The compounds generally interact with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions. It was reported that compounds in +3 oxidation state can easily bind with the receptor site. It may be noted that the organobismuth compound generally binds with nitrogen 7 position of purine bases in DNA molecule and form complexes with DNA strands affecting replication and transcription of DNA molecule and stop the cell division along with protein synthesis.

Table-4 *Antifungal Activity of diorganobismuth(III)carboxylates at 100µg/ml conc.*

S. N.	Compounds	*Aspergillus flavus* Col. Dia. (mm)	% Inhibition	*Aspergillus niger* Col. Dia. (mm)	% Inhibition
1	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	0.1	96.7	0.4	80.0
2	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	0.2	93.3	0.3	75.0
3	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	0.2	93.3	0.1	95.0
4	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	0.1	96.7	0.1	95.0
5	$[(C_6H_5)_2Bi(OOCC_6H_3(OH)OCH_3]$	0.4	86.7	0.2	90.0
6	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	0.1	96.7	0.3	75.0
7	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	0.2	93.3	0.3	75.0
8	$(C_6H_5)_2Bi(OOCC_6H_4N(CH_3)_2)$	0.1	96.7	0.2	90.0
9	$(C_6H_5)_2Bi(OOCC_6H_4N(C_2H_5)_2)$	0.2	93.3	0.1	95.0
10	Control	3.0	--	2.0	--

Table-5: *Antitumor activity of diorganobismuth (III) carboxylates*

S. No.	Compounds	MCF-7 Cell No. x 10^4	EVSA-7 Cell No. x 10^4	Activity
1	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	9.19±0.92	9.29±0.88	Positive
2	$(C_6H_5)_2Bi(OOCC_6H_4NO_2)$	9.17 ± 0.90	8.6 7 ± 0.69	Positive
3	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	11.59±1.06	11.29±1.02	Negative
4	$(C_6H_5)_2Bi(OOCC_6H_4Cl)$	9.29±0.88	9.89±0.92	Positive
5	$[(C_6H_5)_2Bi(OOCC_6H_3(OH)OCH_3]$	8.95±0.67	8.55±0.62	Positive
6	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	8.79 ± 0.52	8.42 ± 0.46	Positive
7	$(C_6H_5)_2Bi(OOCC_6H_4NH_2)$	11.52±1.02	11.82±1.06	Negative
8	$(C_6H_5)_2Bi(OOCC_6H_4N(CH_3)_2)$	9.19±0.92	9.29±0.88	Positive
9	$(C_6H_5)_2Bi(OOCC_6H_4N(C_2H_5)_2)$	12.31±1.02	12.39±1.03	Negative
10	Negative control	10.21±1.01	10.22±1.01	–
11	Positive control	40.26±3.23	41.23±3.28	–

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

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