

## SYNTHESIS, STRUCTURAL, AND BIOLOGICAL SCREENING OF SOME NEW ORGANOBISMUTH COMPOUNDS

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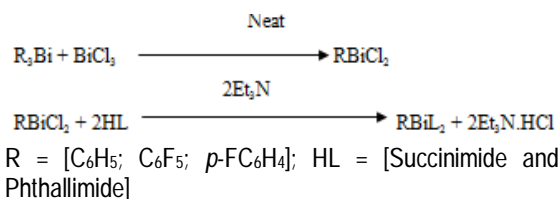
**Abstract-** In past few years the use of organometallics in medicinal purpose takes the full attention of researchers to work in this field. The present work deals the synthesis of some new organobismuth amides having a pyramidal geometry as resulted by their characterization with the help of different techniques along with spectral analysis. The compounds are also screened for their biological studies against different bacterial and fungal strains along with human breast adenocarcinoma and mammary cancer cell lines in-vitro and the results are surprising that these compounds plays significant role in biomedical activities.

### INTRODUCTION

Despite a voluminous work appearing in to literature concerning the synthesis and characterization of partially and fully halophenyl substituted Group 15 organometallics, (M = As, Sb and Bi) the studies are mainly centered on antimony compounds [1-5]. Organobismuth compounds have assumed pharmaceutical and biological significance in recent years, a tradition maintained over a period of 250 years, because they are non-toxic, biodegradable and are easily excreted from the body system. It may be noted that inorganic bismuth compounds e.g. bismuth subsalicylate, bismuth subcitrate and bismuth subcarbonate have been used over a long period for treatment of gastro entities, peptic ulcer and duodenal illness and frequently administered for stomach related ailments despite the prevalence of so many antibiotics [6-14]. The medicinal importance of organobismuth compounds due to their non-toxic nature coupled with the paucity of data in this field prompted the author to undertake a systematic study on fully and partially fluoro substituted phenylbismuth (III) compounds. The introduction of fluoro group in the organic moieties bound to the metal atom or in the ligand is known to enhance both hydrophilicity and lipophilicity and thereby make them better candidates for biological activity [15-17]. The present chapter describes the synthesis and characterization of some mono and diorganobismuth (III) amides for their biomedical aspects which was an untouched area till date.

### EXPERIMENTAL

The synthesis of mono and diorganobismuth (III) amides was performed in laboratory with the help of earlier reported methods [18], using following reactions.



The syntheses of some representative compounds are as follows.

#### Reaction of (C<sub>6</sub>H<sub>5</sub>)BiCl<sub>2</sub> with Succinimide (1)

Under nitrogen atmosphere, solution of phenylbismuth (III) dichloride (0.357gm; 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<sub>3</sub>N.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 mass which was further recrystallised in pet-ether.

#### Reaction of (C<sub>6</sub>H<sub>5</sub>)BiCl<sub>2</sub> with Phthallimide (2)

Under nitrogen atmosphere, solution of phenylbismuth (III) dichloride (0.357gm; 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<sub>3</sub>N.HCl formed (M.P. =240°C), 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<sub>6</sub>F<sub>5</sub>)BiCl<sub>2</sub> with Succinimide (3)

In nitrogen atmosphere the solution of pentafluorophenylbismuth (III) dichloride (0.447gm;

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  $\text{Et}_3\text{N.HCl}$  formed (M.P. =240°C), 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 $(\text{C}_6\text{F}_5)\text{BiCl}_2$ with Phthalimide (4)

In nitrogen atmosphere, a solution of pentafluorophenylbismuth (III) dichloride (0.447gm; 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  $\text{Et}_3\text{N.HCl}$  was formed (M.P. =240°C), 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 $(\text{C}_6\text{H}_4\text{F})\text{BiCl}_2$ with Succinimide (5)

Under nitrogen atmosphere a solution of (*p*-fluorophenyl)bismuth (III) dichloride (0.375gm; 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  $\text{Et}_3\text{N.HCl}$  formed (M.P. =240°C), was filtered off and the filtrate on evaporation in vacuum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

#### Reaction of $(\text{C}_6\text{H}_4\text{F})\text{BiCl}_2$ with Phthalimide (6)

In an oxygen free nitrogen atmosphere, a solution of (*p*-fluorophenyl) bismuth (III) dichloride (0.375gm; 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  $\text{Et}_3\text{N.HCl}$  formed (M.P. =240°C), was filtered off and filtrate on evaporation in vacuum gives an off-white colour crystalline solid mass which was further recrystallised in pet-ether.

#### Antibacterial activity

The antibacterial activity of these organobismuth (III) compounds was determined by disc diffusion method [19]. In this technique, the filter paper (Whatman No. 1) sterile discs 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 discs were measured after 24 hrs. The activity was classified as 'highly active' (diameter > 14 mm); 'moderately active' (diameter = 10-14 mm) and 'slightly active' (diameter = 6-10). The diameter less than 6 mm was regarded as inactive.

#### Antifungal activity

The antifungal activity of these compounds was tested by agar diffusion method [20] using two concentrations of the test compound, viz, 50 and 100 µg/ml; against *Aspergillus flavus* and *Aspergillus niger*. The one ml of

each organobismuth compound was poured into a petri dish having about 20-25 ml of molten potato dextrose agar medium of. As the medium gets solidify, petri dishes were inoculated separately with the fungal isolates and kept at 26°C for 96 hrs in incubator. All the values (% inhibition) were recorded after 96 hrs. The % inhibition of these compounds was calculated by using following mathematical equation.

Percent (%) Inhibition =  $\frac{C-T}{C} \times 100$

Where: C = Diameter of fungus in control; T = Diameter of fungus in test compound

#### Anti-tumor activity

This method was carried out to estimate the effect of test compound on the growth of tumor cells. The human breast cancer (MCF-7) and mammary cancer (EVSA-7) cell lines were employed for valuation of this activity. The cell lines were co-incubated with the organobismuth compounds/test compounds at 1 µg/ml doses for 96 hrs and the cell growth count was measured by MTT assay [21]. The basic principle involved in this assay depends upon the reduction of tetrazoleum salt. The yellow colored tetrazoleum MTT, [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoleumbromide] is reduced by metabolically active cells in part by the action of dehydrogenase enzymes 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 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 the 100 µl culture along with test compound and incubated at 37°C for 4 hrs. The medium was then removed carefully without disturbing the purple colored formazon crystals. Then, 50 ml of dimethylsulfoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals of the formazon. The plates were then read on ELISA plate reader at a wavelength of 570 nm. The readings were presented as optical density/ cell count to evaluate the activity.

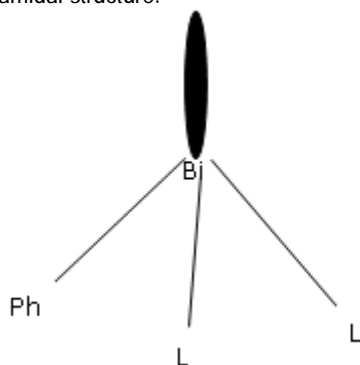
#### RESULTS AND DISCUSSION

All the newly synthesized organobismuth amides 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 possess pyramidal structure as per results obtained by further analysis.

#### IR and NMR Spectral Analysis

The IR spectra of new organobismuth compounds were recorded in Perkin-Elmer spectrophotometer in 4000-200  $\text{cm}^{-1}$  range. The IR spectra of compound show absorption bands due to phenyl, *p*-fluorophenyl and pentafluorophenyl groups. The absorption frequencies due to carbonyl group in amide 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  $\text{cm}^{-1}$ . The  $^1\text{H}$ NMR spectra of the representative organobismuth compounds showed a multiplet in the range  $\delta$  7.82ppm to  $\delta$  8.12ppm which could be assigned to aromatic protons. The  $^{19}\text{F}$ NMR spectra of the compound was carried out at room temperature and the compounds showed peaks appearing in the range -108.30ppm to -112.30ppm and is consistent with the presence of *p*-fluorophenyl groups. Thus on the basis of above discussions the newly synthesized organobismuth (III) compounds assigned a pyramidal structure.



#### Antibacterial activity

All the organobismuth (III) amides were tested for antibacterial activity against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* using 10  $\mu\text{g}/\text{ml}$  concentration of test compound. All the compounds show higher to moderate activity against the bacterial strains. 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 metalloenzymes, 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. 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 was tested against *Aspergillus flavus* and *Aspergillus niger* using 50 and 100  $\mu\text{g}/\text{ml}$  concentration. The activity of these compounds was found variable at 50 $\mu\text{g}/\text{ml}$  concentration but at higher concentration compounds show high activity against fungal strains. Presence of nitrogen, phenyl and pentafluorophenyl ring along with bismuth in +3 oxidation state are considered for fungal activity. These 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.

#### Anti-tumor activity

The antitumor activity of these compounds was studied against the human breast adenocarcinoma (MCF-7) and mammary cancer (EVSA-7) cell lines. The compounds show moderate to higher 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 amides group as ligand along with presence of fluorine on main moiety of the compound. The compounds generally interact with the receptor site of multienzyme complex responsible for the cytostatic and cytotoxic conditions. The 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.

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Table 1- Physicochemical properties of organobismuth (III) amides

| S.N. | Compounds   | Formula  | Formula Weight | M.P. (°C) | Yield (%) |
|------|---|--|----------------|-----------|-----------|
| 1    | (C <sub>6</sub> H <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | C <sub>14</sub> H <sub>13</sub> O <sub>4</sub> N <sub>2</sub> Bi               | 482            | 65        | 66        |
| 2    | (C <sub>6</sub> H <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | C <sub>22</sub> H <sub>13</sub> O <sub>4</sub> N <sub>2</sub> Bi               | 578            | 59        | 62        |
| 3    | (C <sub>6</sub> F <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | C <sub>14</sub> F <sub>5</sub> H <sub>8</sub> O <sub>4</sub> N <sub>2</sub> Bi | 572            | 62        | 68        |
| 4    | (C <sub>6</sub> F <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | C <sub>22</sub> F <sub>5</sub> H <sub>8</sub> O <sub>4</sub> N <sub>2</sub> Bi | 668            | 60        | 64        |
| 5    | (C <sub>6</sub> H <sub>4</sub> F)Bi(succinimide) <sub>2</sub> | C <sub>14</sub> FH <sub>12</sub> O <sub>4</sub> N <sub>2</sub> Bi              | 500            | 56        | 65        |
| 6    | (C <sub>6</sub> H <sub>4</sub> F)Bi(phthalimide) <sub>2</sub> | C <sub>22</sub> FH <sub>12</sub> O <sub>4</sub> N <sub>2</sub> Bi              | 596            | 58        | 70        |

Table 2-Analytical data of organobismuth (III) amides

| S.N. | Molecular Formula  | Elemental Analysis |       |       | IR (cm <sup>-1</sup> ) |                       |
|------|--|--------------------|-------|-------|------------------------|-----------------------|
|      |  | C (%)              | H (%) | N (%) | V <sub>asym</sub> (CO) | V <sub>sym</sub> (CO) |
| 1    | C <sub>14</sub> H <sub>13</sub> O <sub>4</sub> N <sub>2</sub> Bi               | 34.85              | 2.69  | 5.80  | 1758vs                 | 1354ms                |
| 2    | C <sub>22</sub> H <sub>13</sub> O <sub>4</sub> N <sub>2</sub> Bi               | 45.67              | 2.25  | 4.84  | 1750vs                 | 1350ms                |
| 3    | C <sub>14</sub> F <sub>5</sub> H <sub>8</sub> O <sub>4</sub> N <sub>2</sub> Bi | 29.37              | 1.40  | 4.90  | 1725ms                 | 1325ms                |
| 4    | C <sub>22</sub> F <sub>5</sub> H <sub>8</sub> O <sub>4</sub> N <sub>2</sub> Bi | 39.52              | 1.20  | 4.19  | 1724vs                 | 1324ms                |
| 5    | C <sub>14</sub> FH <sub>12</sub> O <sub>4</sub> N <sub>2</sub> Bi              | 33.60              | 2.40  | 5.60  | 1730vs                 | 1330ms                |
| 6    | C <sub>22</sub> FH <sub>12</sub> O <sub>4</sub> N <sub>2</sub> Bi              | 44.29              | 2.01  | 4.69  | 1732ms                 | 1332ms                |

Table 3- Antibacterial activity of organobismuth (III) amides

| S. N. | Compounds   | Control | <i>Pseudomonas aeruginosa</i> | <i>Staphylococcus aureus</i> | <i>Klebsiela pneumoniae</i> |
|-------|---|---------|-------------------------------|------------------------------|-----------------------------|
| 1     | (C <sub>6</sub> H <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | -       | ++                            | ++                           | ++                          |
| 2     | (C <sub>6</sub> H <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | -       | ++                            | ++                           | +++                         |
| 3     | (C <sub>6</sub> F <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | -       | +                             | +++                          | ++                          |
| 4     | (C <sub>6</sub> F <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | -       | +++                           | ++                           | ++                          |
| 5     | (C <sub>6</sub> H <sub>4</sub> F)Bi(succinimide) <sub>2</sub> | -       | ++                            | +                            | ++                          |
| 6     | (C <sub>6</sub> H <sub>4</sub> F)Bi(phthalimide) <sub>2</sub> | -       | ++                            | ++                           | ++                          |

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

Table 4- Antifungal activity of organobismuth (III) amides at 50 µg/ml conc.

| S. N. | Compounds   | <i>Aspergillus flavus</i><br>Col. Dia.<br>(mm) | % Inhibition | <i>Aspergillus niger</i><br>Col. Dia.<br>(mm) | % Inhibition |
|-------|---|--|--------------|---|--------------|
| 1     | (C <sub>6</sub> H <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | 0.7  | 76.6         | 0.7   | 65.0         |
| 2     | (C <sub>6</sub> H <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | 0.8  | 73.3         | 0.8   | 60.0         |
| 3     | (C <sub>6</sub> F <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | 0.8  | 73.3         | 0.8   | 60.0         |
| 4     | (C <sub>6</sub> F <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | 0.7  | 76.6         | 0.6   | 70.0         |
| 5     | (C <sub>6</sub> H <sub>4</sub> F)Bi(succinimide) <sub>2</sub> | 0.7  | 76.6         | 0.5   | 75.0         |
| 6     | (C <sub>6</sub> H <sub>4</sub> F)Bi(phthalimide) <sub>2</sub> | 0.5  | 83.3         | 0.4   | 80.0         |
| 7     | Control   | 3.0  | –            | 2.0   | –            |

Table 5- Antifungal activity of organobismuth (III) amides at 100 µg/ml conc.

| S. N. | Compounds   | <i>Aspergillus flavus</i><br>Col. Dia.<br>(mm) | % Inhibition | <i>Aspergillus niger</i><br>Col. Dia.<br>(mm) | % Inhibition |
|-------|---|--|--------------|---|--------------|
| 1     | (C <sub>6</sub> H <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | 0.2  | 93.3         | 0.3   | 75.0         |
| 2     | (C <sub>6</sub> H <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | 0.1  | 96.7         | 0.2   | 90.0         |
| 3     | (C <sub>6</sub> F <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | 0.2  | 93.3         | 0.1   | 95.0         |
| 4     | (C <sub>6</sub> F <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | 0.1  | 96.7         | 0.1   | 95.0         |
| 5     | (C <sub>6</sub> H <sub>4</sub> F)Bi(succinimide) <sub>2</sub> | 0.4  | 86.7         | 0.2   | 90.0         |
| 6     | (C <sub>6</sub> H <sub>4</sub> F)Bi(phthalimide) <sub>2</sub> | 0.2  | 93.3         | 0.2   | 90.0         |
| 7     | Control   | 3.0  | –            | 2.0   | –            |

Table 6- Antitumor activity of organobismuth (III) amides

| S. No. | Compounds   | MCF-7<br>Cell No. x 10 <sup>4</sup> | EVSA-7<br>Cell No. x 10 <sup>4</sup> | Activity |
|--------|---|-------------------------------------|--------------------------------------|----------|
| 1      | (C <sub>6</sub> H <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | 11.52±1.02                          | 11.82±1.06                           | Negative |
| 2      | (C <sub>6</sub> H <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | 12.31±1.02                          | 12.39±1.03                           | Negative |
| 3      | (C <sub>6</sub> F <sub>5</sub> )Bi(succinimide) <sub>2</sub>  | 9.19±0.92                           | 9.29±0.88                            | Positive |
| 4      | (C <sub>6</sub> F <sub>5</sub> )Bi(phthalimide) <sub>2</sub>  | 8.79 ± 0.52                         | 8.42 ± 0.46                          | Positive |
| 5      | (C <sub>6</sub> H <sub>4</sub> F)Bi(succinimide) <sub>2</sub> | 9.19±0.92                           | 9.29±0.88                            | Positive |
| 6      | (C <sub>6</sub> H <sub>4</sub> F)Bi(phthalimide) <sub>2</sub> | 8.95±0.67                           | 8.55±0.62                            | Positive |
| 7      | Negative control  | 10.21±1.01                          | 10.22±1.01                           | –        |
| 8      | Positive control  | 40.26±3.23                          | 41.23±3.28                           | –        |