SYNTHESIS AND CHARACTERIZATION OF NOVEL ORGANOBISMUTH FOR ANTIMICROBIAL AND ANTITUMOR STUDIES

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ABSTRACT

Objectives: The major objective of this manuscript is to present synthesis and biomedical screening of some organic derivatives of bismuth having general formula [R\(_3\)BiL\(_2\)] by the method reported and characterized with the help of M.P., elemental, I.R., and NMR spectral analysis along with their antimicrobial and in vitro antitumor activity against human breast (MCF-7) and mammary cancer (EVSA-7) cell line.

Methods: All the newly organobismuth having general formula [R\(_3\)BiL\(_2\)] were synthesized by the method reported especially using oxidative addition and complexation reactions.

Results: It was found that organobismuth compounds have trigonal bipyramidal structure as per their elemental and spectral analysis and show potentiality as antimicrobial and antitumor agents.

Conclusion: The newly synthesized organobismuth(V) substituted carboxylates were fully characterized chemically to ascertain their structure by sophisticated instrumental and spectral analysis resulted as trigonal bipyramidal structure. The compounds were also screened 1st time for antitumor and antimicrobial studies. The observations clearly indicated that organobismuth carboxylates show potent antimicrobial and antitumor activity.

Keywords: Organobismuth, Antimicrobial, Antitumor.

INTRODUCTION

It is well reported that bismuth compounds have attracted considerable interest due to their biological and medicinal utility [1-5]. They have been utilized from more than 2 centuries in the treatment of gastrointestinal disorders such as dyspepsia, diarrhea, and peptic ulcer [6-9]. Bismuth salts such as colloidal bismuth sub-citrate, bismuth sub-salicylate, and ranitidine bismuth citrate are common agents used for Helicobacter pylori eradication therapy and therefore promoted these compounds as antimicrobials [10,11]. The utility of bismuth formulations has motivated many studies into their possible mechanism of action and to the discovery of their biological targets. In search of antiproliferative studies, a variety of organobismuth compounds has been synthesized and tested in vitro for their antitumor activity along with their antimicrobial activity [12-15]. The present manuscript describes the synthesis, structural, antimicrobial, and antitumor studies of some novel fluorine-based organic derivatives of bismuth. The compounds were synthesized by the method reported earlier and characterized with the help of M.P., elemental, I.R., and NMR 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.

METHODS

All the newly organobismuth having general formula [R\(_3\)BiL\(_2\)] were synthesized by the method reported especially using oxidative addition and complexation reactions.

RESULTS AND DISCUSSION

The synthesis of tris(pentafluorophenyl)bismuth(III)dicarboxylates was performed in laboratory with the help of the following reactions:

\[ \text{R}_3\text{BiCl}_2 + \text{HCN} \rightarrow \text{R}_3\text{BiNCN} \]

\[ \text{R}_3\text{BiNCN} + \text{H}_2\text{L}_2 \rightarrow \text{R}_3\text{BiL}_2 + \text{N}_{2} \]

Here:

- $\text{R} = (\text{C}_6\text{F}_5)_3$
- $\text{HL} = (\text{Respective carboxylic acids})$

All the newly synthesized tris(pentafluorophenyl)bismuth(V) dicarboxylates were synthesized and characterized chemically to ascertain their structure by infrared, and NMR spectroscopy to ascertain their structures and explore their biological properties. The new compounds have sharp melting points and possess trigonal bipyramidal structure as per results obtained by further analysis.

IR and NMR spectral analysis

The IR spectra of new tris(pentafluorophenyl)bismuth(V) dicarboxylates were recorded in PerkinElmer spectrophotometer in 4000-200/cm range. The IR spectra of these compounds show absorption bands due to pentafluorophenyl groups. The absorption frequencies have been fully assigned. The Bi-C vibration in case of pentafluorophenyl dicarboxylates 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 trigonal bipyramidal structure as per results obtained by further analysis.
Here, 
\( R = \{(C\_F\_3)\}; \ L = \text{Respective carboxylate as ligands.} \)

**Antibacterial activity**

Antibacterial activity of these compounds was studied against three human pathogenic bacteria, namely, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. It was found that the respective compounds may damage the cell wall of bacteria by reacting with peptides of cell wall of bacteria.

**Antifungal activity**

Antifungal activity of these compounds was tested against two fungal strains, namely, *Aspergillus flavus* and *Aspergillus niger* at different concentrations, namely, 10 mg/ml, 20 mg/ml, 50 mg/ml, and 100 mg/ml of the test compounds. At 10 mg/ml conc., the compounds show better inhibition (%) against *A. flavus* and *A. niger*. At 20 mg/ml concentration of test compounds, the compounds show higher percentage inhibition while at 50 mg/ml and 100 mg/ml concentration, approximately all the compounds show higher percentage of inhibition against fungal strains.

**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 the 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 forms complexes with DNA strands affecting replication and transcription of DNA molecule and stops the cell division along with protein synthesis.

**Experimental**

The fluorine-based triorganobismuth(V) dichloride was synthesized by the methods reported earlier [16]. The ligands were recrystallized before use while the reactions were performed under inert/nitrogen atmosphere. Preparation of representative organobismuth compounds is discussed below.

**Reaction of (C\_F\_3)\_BiCl\_2** and (HOOC.C\_H\_5.N(OH).CH\_2)\_2

In an oxygen-free nitrogen atmosphere, solution of tris(pentafluorophenyl)bismuth(V) dichloride (1 mmol) in benzene and 2-nitrobenzoic acid (2 mmol) in same solvent was stirred together in the presence of triethylamine at room temperature for 6 h. The off-white color Et\_3NHCl 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 recrystallized in petroleum ether.

**Reaction of (C\_F\_3)\_BiCl\_2** and (HOOC.C\_H\_5.NO)\_2

In an inert atmosphere, solution of tris(pentafluorophenyl)bismuth(V) dichloride (1 mmol) in benzene and 4-nitrobenzoic acid (2 mmol) in same solvent was stirred together in the presence of triethylamine at room temperature for 6 h. The off-white color Et\_3NHCl 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 recrystallized in petroleum ether.

**Reaction of (C\_F\_3)\_BiCl\_2** and (HOOC.C\_H\_5.Cl)\_2

In an oxygen-free nitrogen atmosphere, solution of tris(pentafluorophenyl)bismuth(V) dichloride (1 mmol) in benzene and 2-chlorobenzoic acid (2 mmol) in same solvent was stirred together in the presence of triethylamine at room temperature for 6 h. The off-white color Et\_3NHCl 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 recrystallized in petroleum ether.

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6 h. The off-white color Et,NHCl 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 recrystallized in petroleum ether.

Reactions of (C₆F₅)₂BiCl₄ with [HOOC.C₆H₄(NH)₂]Cl₂

In an inert atmosphere, solution of tris(pentafluorophenyl)bismuth(V) dichloride (1 mmol) in benzene and 3-methoxy 4-hydroxybenzoic acid (2 mmol) in same solvent was stirred together in the presence of triethylamine at room temperature for 7 h. The off-white color Et,NHCl 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 recrystallized in petroleum ether.

Table 1: Physicochemical activity of triorganobismuth (V) dicarboxylates

| S. No. | Compounds | Yield % | M.P °C | Solvent | IR (cm⁻¹) | \( \nu_{\text{asym}} \) (CO) | \( \nu_{\text{sym}} \) (CO) |
|--------|-----------|---------|--------|---------|-----------|----------------|----------------|
| 1.     | (C₆F₅)₂Bi(OOCC₆H₄NO₂)₂ | 68      | 62     | Petroleum ether | 1724 vs 1326 ms |
| 2.     | (C₆F₅)₂Bi(OOCC₆H₄NO₂)₂ | 66      | 60     | Petroleum ether | 1730 vs 1334 ms |
| 3.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | 70      | 63     | Petroleum ether | 1732 ms 1330 ms |
| 4.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | 72      | 61     | Petroleum ether | 1709 vs 1306 ms |
| 5.     | [(C₆F₅)₂Bi(OC₆H₄(OH))OCH₃]₂ | 66      | 64     | Petroleum ether | 1758 vs 1356 ms |
| 6.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | 65      | 66     | Petroleum ether | 1726 ms 1325 ms |
| 7.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | 65      | 67     | Petroleum ether | 1729 vs 1327 ms |
| 8.     | (C₆F₅)₂Bi(OC₆H₄N(CH₃))₂ | 62      | 60     | Petroleum ether | 1752 vs 1350 ms |
| 9.     | (C₆F₅)₂Bi(OC₆H₄N(C₆H₅))₂ | 60      | 58     | Petroleum ether | 1727 ms 1325 ms |

Table 2: Antibacterial activity

| S. No. | Compounds | Control | Pseudomonas aeruginos | Staphylococcus aureus | Klebsiella pneumonia |
|--------|-----------|---------|-----------------------|-----------------------|---------------------|
| 1.     | (C₆F₅)₂Bi(OC₆H₄NO₂)₂ | ++       | ++                    | ++                    | ++                  |
| 2.     | (C₆F₅)₂Bi(OC₆H₄NO₂)₂ | ++       | ++                    | ++                    | ++                  |
| 3.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | ++       | ++                    | ++                    | ++                  |
| 4.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | ++       | ++                    | ++                    | ++                  |
| 5.     | [(C₆F₅)₂Bi(OC₆H₄(OH))OCH₃]₂ | ++       | ++                    | ++                    | ++                  |
| 6.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | ++       | ++                    | ++                    | ++                  |
| 7.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | ++       | ++                    | ++                    | ++                  |
| 8.     | (C₆F₅)₂Bi(OC₆H₄N(CH₃))₂ | ++       | ++                    | ++                    | ++                  |
| 9.     | (C₆F₅)₂Bi(OC₆H₄N(C₆H₅))₂ | ++       | ++                    | ++                    | ++                  |

Table 3: Antifungal activity of 10 µg/ml conc. of compound

| S. No. | Compounds | Aspergillus flavus col. dia. (mm) | % inhibition | Aspergillus niger col. dia. (mm) | % inhibition |
|--------|-----------|----------------------------------|--------------|---------------------------------|--------------|
| 1.     | (C₆F₅)₂Bi(OC₆H₄NO₂)₂ | 1.2 | 60.0 | 1.0 | 50.0 |
| 2.     | (C₆F₅)₂Bi(OC₆H₄NO₂)₂ | 1.4 | 53.3 | 1.5 | 25.0 |
| 3.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | 1.4 | 53.3 | 1.0 | 50.0 |
| 4.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | 1.2 | 60.0 | 1.4 | 30.0 |
| 5.     | [(C₆F₅)₂Bi(OC₆H₄(OH))OCH₃]₂ | 1.2 | 60.0 | 1.5 | 25.0 |
| 6.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | 0.8 | 73.3 | 1.4 | 30.0 |
| 7.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | 1.0 | 66.6 | 0.8 | 60.0 |
| 8.     | (C₆F₅)₂Bi(OC₆H₄N(CH₃))₂ | 1.2 | 60.0 | 1.0 | 50.0 |
| 9.     | (C₆F₅)₂Bi(OC₆H₄N(C₆H₅))₂ | 0.8 | 73.3 | 1.2 | 40.0 |
| 10.    | Control   | 3.0 | –     | 2.0 | –   |

Table 4: Antifungal activity of 20 µg/ml conc. of compound

| S. No. | Compounds | Aspergillus flavus col. dia. (mm) | % inhibition | Aspergillus niger col. dia. (mm) | % inhibition |
|--------|-----------|----------------------------------|--------------|---------------------------------|--------------|
| 1.     | (C₆F₅)₂Bi(OC₆H₄NO₂)₂ | 1.0 | 66.6 | 1.0 | 50.0 |
| 2.     | (C₆F₅)₂Bi(OC₆H₄NO₂)₂ | 1.0 | 66.6 | 1.0 | 50.0 |
| 3.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | 1.2 | 60.0 | 0.8 | 60.0 |
| 4.     | (C₆F₅)₂Bi(OC₆H₄Cl)₂  | 1.0 | 66.6 | 1.0 | 50.0 |
| 5.     | [(C₆F₅)₂Bi(OC₆H₄(OH))OCH₃]₂ | 0.7 | 76.6 | 1.2 | 40.0 |
| 6.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | 0.6 | 80.0 | 1.2 | 40.0 |
| 7.     | (C₆F₅)₂Bi(OC₆H₄NH₂)₂ | 0.8 | 73.3 | 0.5 | 75.0 |
| 8.     | (C₆F₅)₂Bi(OC₆H₄N(CH₃))₂ | 1.0 | 66.6 | 1.0 | 50.0 |
| 9.     | (C₆F₅)₂Bi(OC₆H₄N(C₆H₅))₂ | 0.6 | 80.0 | 0.8 | 60.0 |
| 10.    | Control   | 3.0 | –     | 2.0 | –   |
Table 5: Antifungal activity of 50 µg/ml conc. of compound

| S. No. | Compounds               | Aspergillus flavus col. dia. (mm) | % inhibition | Aspergillus niger col. dia. (mm) | % inhibition |
|--------|-------------------------|----------------------------------|--------------|---------------------------------|--------------|
| 1.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.6                              | 80.0         | 0.3                             | 75.0         |
| 2.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.7                              | 79.6         | 0.3                             | 70.0         |
| 3.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 0.8                              | 66.5         | 0.5                             | 75.0         |
| 4.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 0.7                              | 79.3         | 0.5                             | 60.0         |
| 5.     | [(C,F₃) Bi (OOC₃H₂OH) OCH₃]₂ | 0.5                        | 83.3         | 0.5                             | 60.0         |
| 6.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.4                              | 86.7         | 0.5                             | 75.0         |
| 7.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 0.5                              | 83.3         | 0.4                             | 80.0         |
| 8.     | (C,F₃) Bi (OOC₃H₂N (CH₃)₂ | 0.5                         | 83.3         | 0.4                             | 80.0         |
| 9.     | (C,F₃) Bi (OOC₃H₂N (CH₃)₂ | 0.4                         | 86.7         | 0.6                             | 70.0         |
| 10.    | Control                 | 3.0                              | –            | 2.0                             | –            |

Table 6: Antifungal activity of 100 µg/ml conc. of compounds

| S. No. | Compounds               | Aspergillus flavus col. dia. (mm) | % inhibition | Aspergillus niger col. dia. (mm) | % inhibition |
|--------|-------------------------|----------------------------------|--------------|---------------------------------|--------------|
| 1.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.2                              | 86.7         | 0.2                             | 90.0         |
| 2.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.4                              | 86.7         | 0.1                             | 95.0         |
| 3.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 0.5                              | 73.3         | 0.2                             | 90.0         |
| 4.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 0.5                              | 83.3         | 0.4                             | 80.0         |
| 5.     | [(C,F₃) Bi (OOC₃H₂OH) OCH₃]₂ | 0.1                        | 96.7         | 0.5                             | 75.0         |
| 6.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.2                              | 93.3         | 0.2                             | 90.0         |
| 7.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 0.1                              | 96.7         | 0.1                             | 95.0         |
| 8.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 0.4                              | 86.7         | 0.5                             | 75.0         |
| 9.     | (C,F₃) Bi (OOC₃H₂N (CH₃)₂ | 0.2                         | 93.3         | 0.4                             | 80.0         |
| 10.    | Control                 | 3.0                              | –            | 2.0                             | –            |

Table 7: Antitumor activity

| S. No. | Compounds               | MCF-7 (cell no. × 10⁴) | EVSA-7 (cell no. × 10⁴) | Activity   |
|--------|-------------------------|------------------------|------------------------|------------|
| 1.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 8.79±0.52              | 8.42±0.46              | Positive   |
| 2.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 9.19±0.92              | 9.29±0.88              | Positive   |
| 3.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 8.95±0.67              | 8.55±0.62              | Positive   |
| 4.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 12.31±1.02             | 12.39±1.03             | Negative   |
| 5.     | [(C,F₃) Bi (OOC₃H₂OH) OCH₃]₂ | 8.79±0.52            | 8.42±0.46              | Positive   |
| 6.     | (C,F₃) Bi (OOC₃H₂NO₂)₂  | 9.2±0.88               | 9.89±0.92              | Positive   |
| 7.     | (C,F₃) Bi (OOC₃H₂Cl)₂   | 8.95±0.67              | 8.55±0.62              | Positive   |
| 8.     | (C,F₃) Bi (OOC₃H₂N (CH₃)₂ | 8.79±0.52             | 8.42±0.46              | Positive   |
| 9.     | (C,F₃) Bi (OOC₃H₂N (CH₃)₂ | 9.19±0.92             | 9.29±0.88              | Positive   |
| 10.    | Negative control         | 10.21±1.01             | 10.22±1.01             | –          |
| 11.    | Positive control          | 40.26±3.23             | 41.2±5.20             | –          |

*Negative control - Culture medium only, **Positive control – 17β estradiol

off and the filtrate on evaporation in vacuum gives an off-white color crystalline solid which was further recrystallized in petroleum ether.

Reaction of (C,F₃) BiCl₃ with (HOOC₃H₂NH₃)
In an oxygen-free nitrogen atmosphere, solution of tris(pentafluorophenyl)bismuth(V) dichloride (1 mmol) in benzene and 4-aminobenzoic acid (1 mmol) in same solvent was stirred together in the presence of triethylamine at room temperature for 6 h. The off-white color Et₃NHCl 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 recrystallized in petroleum ether.

Reaction of (C,F₃) BiCl₃ with [(HOOC₃H₂N(C₃H₃)]
In an oxygen-free nitrogen atmosphere, solution of tris(pentafluorophenyl)bismuth(V) dichloride (1 mmol) in benzene and 4-dimethylaminobenzoic acid (2 mmol) in same solvent was stirred together in the presence of triethylamine at room temperature for 6 h. The off-white color Et₃NHCl 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 recrystallized in petroleum ether.

Antibacterial activity
Antibacterial activity of these compounds was determined by disk diffusion method [17]. 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 h. The inhibition zones around the dried impregnated discs were measured after 24 h. The activity was classified as “highly active” (diameter >14 mm); “moderately active” (diameter = 10–14 mm), and “slightly active” (diameter = 6–10 mm). The diameter less than 6 mm was regarded as inactive.
Antifungal activity
The antifungal activity of these compounds was tested by agar diffusion method [18] using four concentrations of the test compounds, namely, 10, 20, 50, and 100 mg/ml; against the two human pathogenic fungi, A. flavus and A. niger. The 1 ml of each compound was poured into a Petri dish having about 20–25 ml of molten agar medium of potato dextrose. As the medium gets solidify, Petri dishes were inoculated separately with the fungal isolates and kept at 26°C for 96 h. All the values (% inhibition) were recorded. The % inhibition of these compounds was calculated using the following mathematical equation:

\[
\text{Percentage(\% inhibition) } = \frac{C \times T \times 100}{C - T}
\]

Here, \( C = \text{Diameter of fungus in control and } T = \text{Diameter of fungus in test compounds.} \)

Antitumor studies
The in vitro antitumor activity of these compounds was carried out by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) method [19]. This method was performed to estimate the effect of compounds on the growth of cell. The human breast adenocarcinoma (MCF-7) and mammary cancer (EWSA-7) cell lines were used for this purpose. The principle behind this assay depends on the reduction of tetrazolium salt. The yellow-colored tetrazolium MTT 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 h. 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.

CONCLUSION
The newly synthesized organobismuth(V) substituted carboxylates show trigonal bipyramidal structure as per elemental and spectral analysis. The compounds were also show potent antimicrobial and antitumor activity.

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One of the authors Dipti Mani Tripathi worked as research scholar under the supervision of rest two authors Ravi Kant and Krishna Srivastava to complete this research work.

CONFLICTS OF INTEREST
There are no conflicts of interest.

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