Organotin (IV) based Rabeprazole and Pregabalin Complexes Formation and Biocidal Investigation

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Abstract:
New organotin (IV) complexes with NaL1 (sodium salt of 2-[(4-(3-methoxy-propoxy)-3-methylpyridin-2-yl)methyl]sulfinyl|benzimidazol-1-ide) and NaL2 (sodium salt of 3-aminomethyl-5-methylhexanoic acid) were synthesized by the reaction of diorganotin (IV) and triorganotin (IV) salt (Bu3SnCl, SnCl4,Bu3SnCl2, Me3SnCl2) using the solvent (dry toluene) by constant stirring and refluxing. All the organotin (IV) complexes were characterized by different diagnostic techniques such as FT-IR (Infra-red) and UV-visible spectroscopy. The results exhibited that ligand NaL1 (sodium salt) is attached to tin metal by a nitrogen atom of benzimidazole ring and the oxygen atom of the sulfonyl group. While ligand NaL2 (sodium salt) coordinate with tin(IV) moiety through oxygen atom of the carboxylate group. The newly synthesized complexes 1 & 2 of ligand NaL1 (sodium salt) showed trigonal bipyramidal geometry while complexes 3 & 4 octahedral geometry around tin(IV) centre. The organotin(IV) complexes 5-7 of ligand NaL2 (sodium salt) have the tetrahedral geometry around tin(IV) centre. The synthesized complexes (1-7) were tested for antifungal and antibacterial microbial activities. All the complexes showed significant antibacterial and anti-fungal activities against tested bacterial and fungal strains.

Keywords: Organotin carboxylate, Organotin benzimidazole, antibacterial activity, antifungal activity.

1.0. Introduction:
Organotin (IV) complexes having a variety of substituted ligands possess a lot of applications such as catalysis, coating, cytotoxicity, biological properties, agricultural and industrial products [1-7]. The complex structure & coordination no. of the tin metal significantly prejudiced the biochemical potential of organotin (IV) complexes [8,9]. Therefore, acknowledgement of importance between the structure of organotin (IV) complexes and biotic properties encouraged the study of organotin (IV) complexes [10-13]. Carboxylate and benzimidazole group based ligands have lipophilic properties in nature, plays a significant role to enter into the cell having a lipid layer through the cell membrane [14-16]. The literature reveals that a lot of drugs are coordinated with metals to enhance their therapeutic activity [17-20]. The nitrogen and oxygen donor ligands plays important role in many kinds of bioactivities such as antifungal, anticancer, anti-histaminic, anti-neuropathic, antibacterial activities [21, 22].

In keeping view the biological importance of benzimidazole and carboxylate group containing ligands, we have synthesized and characterized organotin (IV) complexes of rabeprazole and pregabalin. The prepared complexes were screened for antifungal and antibacterial activities to check their potential as antimicrobial agents.

2.0. Experimental
2.1. Materials, reagents and instrumentation:
The ligand NaL1 known as sodium salt of 3-aminomethyl-5-methylhexanoic acid, known as Pregabalin (3-aminomethyl-5-methylhexanoate) commercially, were purchased from local medical store while the precursors, organotin (IV) chlorides (Bu3SnCl, SnCl4, Bu2SnCl2, Me3SnCl2) were bought from international supplier company Sigma-Aldrich and used as received. Organotin (IV) based complexes of NaL1 and NaL2 were prepared by using dry toluene as solvent. While different other solvents i.e Chloroform, Acetone, Chloromethane, DMSO, Ethanol, n-Hexane, Dichloromethane, Methanol, Pyridine, Benzene, were used for checking their solubilites. The solvents were obtained from Merck, German made company. All solvents were of analytical grade [23]. Shimadzu 8400 FT-IR spectrophotometer was used to record spectrum of organotin (IV) complexes in the range 4000-400 cm-1. Shimadzu 1800 UV-Visible spectrophotometer was used to record UV-Visible spectrum of synthesized complexes.

2.2. Synthesis of complexes (1-7)
2.2.1. Synthesis of [Bu3SnL1](1)
Bu3SnCl (0.34g, 1.048 mmol) was taken in double neck round bottom flask containing dry toluene up to 100 mL. NaL1 (0.4g, 1.048 mmol) was added to the solution in one to one (1:1) molar ratio & well mixed. The mixture was stirred & refluxed for 10 hrs. Afterward the solution was left uninterrupted for 24 hrs. The settled solid (NaCl) was filtered out and the filtrate was rotary evaporated to yield a solid product; M.P. 90-92°C. FT-IR (KBr, cm-1): 1558 ν(C=N), 1320 ν(C=O), 1299 ν(S=O), 599 ν(Sn-O), 425 ν(Sn-N); UV-Visible (Chloroform): 1697 nm.

2.2.2. Synthesis of [Ph3SnL1](2)
Complex 2 was prepared in similar way as complex 1 by taking Ph3SnCl (0.404 g, 1.048 mmol) and NaL1 (0.4g, 1.048 mmol) in 1: 1 molar ratio. M.P. 66-69°C. FT-IR (KBr, cm-1): 1558 ν(C=N), 1301 ν(C=O), 1026 ν(Sn-O), 430 ν(Sn-N), UV-Visible (Chloroform): λmax 275 nm.

2.2.3. Synthesis of [Bu2SnL2](3)
Complex 3 was prepared in the same way as complex 1 by using Bu2SnCl2 (0.159g, 0.524 mmol) and NaL1 (0.4g, 1.048 mmol) in 1:2 molar ratio. M.P. 57-59°C. FT-IR (KBr, cm-1): 1558 ν(C=N), 1360 ν(C=O), 1030 ν(Sn-O), 617 ν(Sn-N), 420 ν(Sn-N), UV-Visible (Chloroform): λmax 277 nm.

2.2.4. Synthesis of [Me3SnL2](4)
Complex 4 was prepared in the same way as 1 by using Me3SnCl2 (0.115g, 0.524 mmol) and NaL1 (0.4g, 1.048 mmol) in 1:2 molar ratio. M.P. 60-62°C. FT-IR (KBr, cm-1): 1558 ν(C=N), 1288 ν(C=O), 1023 ν(Sn-O), 614 ν(Sn-N), 418 ν(Sn-N), UV-Visible (Chloroform): λmax 276 nm.

2.2.5. Synthesis of [Bu2SnL3](5)
Complex 5 was prepared in the same way as 1 by using Bu2SnCl2 (0.899g, 2.762mmol) and NaL2 (0.5g, 2.762mmol) in 1:1 molar ratio. The complex was semi-solid. FT-IR (KBr, cm-1): 1684 ν(OCO) asym, 1463 ν(OCO) sym, (Δν=221 cm-1), 593 ν(Sn-C), 527 ν(Sn-O), UV-Visible (Chloroform): λmax 276 nm.

2.2.6. Synthesis of [Ph3SnL3](6)
Complex 6 was prepared in the same way as complex 1 by using Ph3SnCl (2.128g, 5.524 mmol) and NaL2 (1.0g, 5.524 mmol) in 1:1 molar ratio. The complex was semi-solid. FT-IR (KBr, cm-1): 1658 ν(OCO) asym, 1430 ν(OCO) sym, (Δν=228 cm-1), 454 ν(Sn-O), UV-Visible (Chloroform): λmax 277 nm.
2.2.7. Synthesis of [Bu₂SnL₄]⁻ [7]
Complex 7 was prepared in the same way as complex 1 by using Bu₂SnCl (0.838g, 2.762) and NaL⁻ (1.0g, 5.524mmol) in 1:2 molar ratio. The complex was semi-solid. FT-IR (KBr, cm⁻¹): 1670 ν(OCO) asym, 1441 ν(OCO) sym, (δo=229 cm⁻¹), 599 ν(Sn-C), 512 ν(Sn-O). UV-Visible (Chloroform): λₘₐₓ, 276 nm.

2.2.8. Antibacterial studies
The well adopted method (Agar Well Diffusion) process was used to evaluate the antibacterial activities [24, 25] of the newly prepared organotin(IV) complexes against Klebsiella pneumoniae and Escherichia Coli growth. Erythromycin was used as standard drug. Four to six hours old broth culture consisting 10⁻¹⁻¹⁰ CFU (colony forming units)/mL was poured in 65mL nutrient agar, mixed well keeping the temperature at 45 °C and then dispensed into a pasteurized (sterilized) petri dishes. The wells were dug with germ-free metallic tool having centers at least 24mm away from each other. The suggested concentration of the trial samples of organotin(IV) complexes 1-7 (1 mg/mL in DMSO) was added into the corresponding wells. The wells were filled with Erythromycin standard drug and DMSO, serving as a positive and negative control respectively. Now the petri dishes were incubated at 37 °C for 24 hours. The antibacterial activity was found out by calculating the diameter of region presenting entire inhibition in millimeters. Positive control was used as a reference to calculate the inhibition zone.

2.2.9. Antifungal activities
The reported method known as the Agar tube dilution method was used to test the antifungal activities of newly prepared organotin (IV) complexes 1-7 using three fungal strains Aspergillus fumigatus, Aspergillus flavus and Aspergillus niger [24, 25]. Terbinafine was used as standard drug. Sabouraud dextrose agar (SDA) medium was prepared by mixing of glucose agar 4%, Sabouraud agar 32.5g & agar-agar 4% steamed dissolved in distilled water (500 mL). 4mL of the SDA was dispensed in screw-capped test tubes & autoclaved for 15 minutes at 120 °C. The final concentration was made by adding 66 μL compounds (3 mg/mL in DMSO) into non-solidified SDA at 45 °C. The tubes were solidified at room temperature. Then 7 days old fungal culture of three different fungal strains was injected into respective tubes and incubated at 28 °C for 7-8 days. Terbinafine used as positive control and DMSO acted as negative control. The formula of % growth inhibition is given below:

\[
\% \text{ Growth inhibition} = \frac{\text{linear growth in control (mm) } - \text{linear growth in test sample (mm)}}{\text{linear growth in control (mm)}} \times 100
\]

3.0. Results and Discussion
Organotin (IV) complexes were prepared by the reaction of corresponding salt of metals (R₄SnCl) and the sodium salt of ligand. The stoichiometric amount of reactants are 1:1 molar ratio in complex 1, 2, 5 & 6 while it is 1:2 molar ratio in complex 3, 4 & 7 as in the following scheme 3.1.

\[
\text{NaL} + \text{R}_n\text{SnCl}_{4-n} \xrightarrow{\text{Dry Tolene Reflux 10 hrs}} \text{R}_n\text{SnL}_{4-n} + \text{NaCl}
\]

Where
L=Sodium salt of Rabeprazole/Pregabalin
R= Butyl/phenyl/methyl

Scheme 3.1 Preparation of organotin(IV) based complexes of rabeprazole/pregabalin

3.1. FT-IR Studies of Complexes
The IR spectral studies of newly synthesized complexes 1-4 indicate the bidentate mode (chelate nature) of coordination of ligand with metal through the nitrogen of C=N bond and via oxygen of the sulfinyl group. The sulfoxide group vibrational band appeared in the range at 1030-1070 cm⁻¹ in complexes 1-4 which is in consistent with the literature values reported elsewhere [26,27]. The appearance of new bands in the range 430-418 and 620-599 cm⁻¹ for complexes 1-4 are assigned to ν(Sn-N) and ν(Sn-O), respectively which confirm the formation of complexes. Another new band appeared in the range 1378-1375 cm⁻¹ in complexes 1-4 which might be because of the formation of chelate ring. Our findings are in consistent with the literature values reported elsewhere [17,18]. Whereas the formation of complexes 5-7 is confirmed by the absence of broad peak in the region of 3650-3200 cm⁻¹, which shows the deprotonation of carboxylic acid group. The binding way of carboxylate oxygen either monodentate or bidentate can be inferred by Δo, the difference between carboxylate asymmetric and symmetric stretching vibrations. The Δo value more than 200 cm⁻¹ show monodentate nature, while less than 200 cm⁻¹ show bidentate mode of coordination of carboxylate moiety [25]. The observed Δo values for complexes 5-7 are 221, 228, 229 cm⁻¹ respectively which shows bonding of uninegative oxygen (monodentate mode of coordination) of carboxylate moiety. The Sn-O bond formation is also confirmed by the appearance of a new band at 527, 454, 512 cm⁻¹ in complexes 5-7 respectively. The results are in accordance with the literature [17,18,25]. The representative FT-IR spectra are given in supplementary material Figure S1-S3. On the basis of above findings, the proposed structures of complexes 1-7 are represented in scheme 3.2.

Figure S1. FT-IR Spectrum of [Bu₂SnL⁻]⁻ (1)
Figure S2. FT-IR Spectrum of [Bu₂SnL₂] (5)

Figure S3. FT-IR Spectrum of [Ph₃SnL₂] (6)
Scheme 3.2. Proposed structures of the complexes 1-7

3.2. UV-Visible Spectroscopy
In electron absorption spectroscopy of complexes 1-7, two common bands were observed, one band was appeared in the range 275-277 nm which is due to n→π* electronic transitions and other band appeared in the range 330-333 nm which is due to charge transfer transitions. The representative UV-Visible spectra are shown in supplementary materia as Figure S4 & S5.
3.3. Anti-microbial investigations

The newly synthesized organotin(IV) complexes 1-7 were screened for anti-bacterial and antifungal activities because anti-microbial activities increase on complexation with metal.

3.3.1. Antibacterial Screening

The prepared complexes were first subjected for In Vitro antibacterial screening against two different bacterial strains. To perform the experiment disc agar diffusion method was used. The interpreted results are given in Figure 1. All the activity performed established on inhibition zone in the disc agar well diffusion method [25]. If the zone inhibited is less than 14 mm, it is considered as insignificant, if the inhibition zone range is 14-20 mm, considered as significant, 21-29 mm, considered as more significant, 30-39 mm, then it is considered as most significant [25]. Erythromycin was used as standard drug & acting as positive control. All the complexes showed significant activity against Escherichia Coli & Klebsiella Pneumoniae growth. The complexes 1-7 showed significant activity against Klebsiella Pneumoniae having an inhibition zone (24 mm to 30 mm) more than the standard drug used (14 mm). The complexes 1 & 6 having highest value (30 mm) inhibition zone against the Escherichia Coli while other complexes 2-5 & 7 showed significant activity against Escherichia Coli having an inhibition zone from 19 mm to 25 mm, more than the standard drug used.

3.3.2. Antifungal investigations

In in vitro antifungal activity, testing was made for organotin (IV) complexes 1-7 against three different fungal strains. Agar tube dilution method was used to perform the experiment. The results are shown in Figure 2. All the activity performed based on percentage of inhibition [18]. If percentage (%) inhibition is less than 50%, it is considered as insignificant, within the range of 50-60% considered as moderate while in the range of 60-70%, considered as good, and more than 70% percentage of inhibition considered as significant [18]. According to this criteria, all the newly synthesized complexes are effective as antifungal activity. Complexes 2-4, 6 & 7 showed significant inhibition activity against all three types of fungus named as Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus while complexes 1 & 5 showed moderate antifungal activity. Complex 2 has highest antifungal activity having 84 % & 87 % inhibition potential against Aspergillus niger & Aspergillus fumigatus respectively. Complex 4 has highest antifungal activity having 81 % & 80 % inhibition activity against Aspergillus niger and Aspergillus flavus respectively.
4.0. Conclusion

New seven organotin (IV) complexes were synthesized successfully and then characterized via various analytical techniques like FT-IR and UV-Visible spectroscopy. The synthesis of organotin (IV) complexes 1-4 having benzimidazole group was confirmed by the appearance of Sn-O & Sn-N bands in the range of 620-599 and 430-418 cm \(^{-1}\) respectively using FT-IR spectroscopy. These complexes exhibited the coordination of oxygen of sulfoxide group and nitrogen of benzimidazole present in NaL\(^+\) to the Sn-metal. The proposed geometry for complexes 1 & 2 is trigonal bipyramidal while octahedral geometry for complexes 3 & 4. The formation of organotin(IV) carboxylates complexes 5-7, were confirmed by the appearance of new Sn-O band in the range 527-454 cm \(^{-1}\) and the value of Δ\(\nu\) (the difference between asymmetric & symmetric CO\(^{-}\) absorption bands) more than 200 cm \(^{-1}\) showed the monodentate mode of coordination. So the proposed geometry in complexes 5-7 is tetrahedral. In electron absorption spectroscopy of organotin (IV) complexes 1-7, a band observed in the range 275nm-277nm was due to \(n\rightarrow\pi^*\) electronic transitions and another band in the range 330-333 nm appeared due to charge transfer transitions. The in vitro biological studies of newly synthesized complexes exhibited significant to most significant antibacterial & antifungal activity as compared to standard drugs with few exceptions.

5.0. Acknowledgment

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6.0. Conflict of interests

The authors certify that there is no conflict of interests with any financial organization regarding the materials discussed in the paper.

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