ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Synthesis, characterization, biological activities and computational anticancer study of Dibutylbis [(2-isopropyl-5-ethylcyclohexyl) oxy] stannane

Rohit Babu Aniyery[1*], Dr Anita Gupta[2], Dr Prashant Singh[3], Chitra Khatri[2], Ankita Pathak[4]
[1]Zlife education, Gulmohar Park, New Delhi, India.
[2]Amity University, Noida-125, India.
[3]ARSD college, University of Delhi.
[4]Jamia Hamdard University,Hamdard Nagar, New Delhi, India
***Corresponding author: E.mail: rohit7rba1992@gmail.com, Contact number: +918376898863**

ABSTRACT

In this work, we report, the synthesis of a novel organotin (IV) complex Dibutyl bis [(2-isopropyl-5-methyl cyclohexyl) oxy] stannane, which was characterized by UV –Visible and ^{1}H NMR. The computaional study of the complex was carried out using computational software using iGEMDOCK. The synthesized novel organotin complex was found to inhibit the B-RAF Kinase activity and therefore it could be used as potential pharmaceutical drug for melanoma patients. The ligands and its organotin complex were also tested in vitro against bacterial strains, *Staphylococus aureus* (gram-positive) and *Escherichia coli* (gram-negative), its in vitro antifungal activity was also studied by using two fungal strains *Fusarium oxysporum* and *Aspergillus niger*. It was observed that synthesized organotin (IV) complex showed better antibacterial and antifungal activities.

KEY WORDS: Stannane, synthesis, spectroscopy, antibacterial activity, synthetic chemistry, Computational study, iGEMDOCK.

1. INTRODUCTION:

Organotin (IV) complexes have always been the subject of great interest for many years due to their biomedical and extensive commercial applications (especially in the field of PVC stabilization) (Singh and Varshney, 2001). Many organotin complexes were found to be an effective antimicrobial, antiviral and antifouling agents (generally used for protecting materials). The expanding application of metal complexes in the treatment of numerous human diseases is increasing in the field of biomedical and inorganic chemistry. They are chemical compounds containing tin bonded to the hydrocarbons. Despite the fact that carbon to tin bond is weaker than carbon-carbon or carbon-silicon bond, the organotin complex is non-polar having high stability in the air, moisture as well as in the presence of many nucleophilic species. (Tabassum and Pettinari, 2006; Beltran, 2007; Muhammad, 2009; Gleeson, 2008). The variation in coordination number, their geometries, thermodynamic, and kinetic characteristics, also the intrinsic properties of the metal ion are some special characteristics of organo tin complexes that offer the pharmacologist or scientists to employ different strategies for their exploitation.

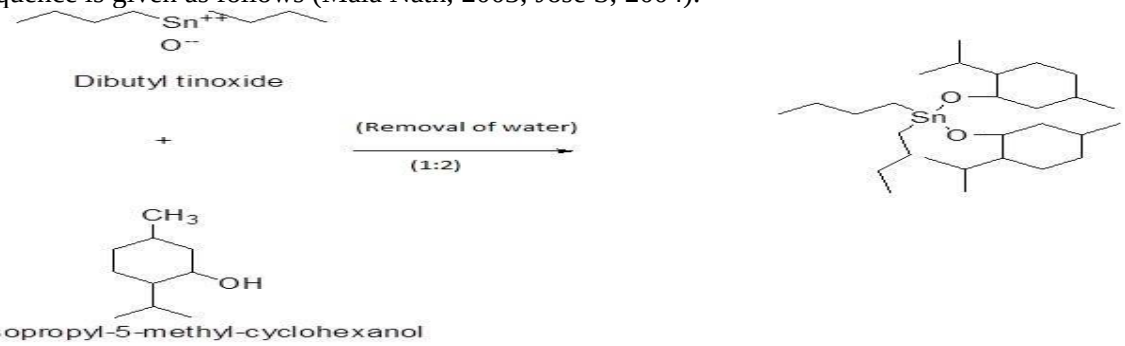
Oxides of Organotin normally react with inorganic or organic acids, alcohols, mercaptanes etc. by ligand exchange method .According to a proposed mechanism; it involves the coordination of tin atom with –OH containing compounds. Tin catalysts are mainly used in the field of condensation reactions for preparing vulcanized silicones at room temperature. The tin catalyst forms an intermediate with cross-linker to form a -Sn-O-Si- bond. Other applications include biocidal activity in antifouling paints, wood preservatives and agriculture (Biunden, 1984; Cima, 2003). Stanannes are also advantageous in the form of tin oxide coatings; used on bottles to for breakage on a line and on window-panes as heat reflective coatings (Yousif, 2009; Win, 2012).

2. MATERIAL AND PHYSICAL MEASUREMENTS

All the chemicals used for this research work were of Analytical Grade and obtained from commercial sources (Merck specialitics,Spectrochem, Qualigen and Fischer scientific). Solvents used for the synthesis were dried and purified by standard procedures (Armarego and Perrin, 1996)]the instrument used was Rotatory Vaccum Evaporator (Khera Instruments Pvt Ltd,pressure range 0-30 in Hg/0 to -760 mmHg . The novel organotin complex was synthesized according to the conventional method. The UV visible spectra studies were done using UV instrument SHIMADZU UV 1800 ,200 -600 in ethanol at Amity university NOIDA. The ^{1}H NMR spectra (in DMSO –d6 solution) of Organotin (IV)complexes were recorded on JEOL ECX-400P NMR at 400 MHz and 100 MHz, respectively at USIC, University of Delhi. The NMR spectra were processed by JEOL Delta^{TM}NMR data processing software.Computational study was carried out using software iGEMDOCK (Generic Evolutionary Method for molecular Docking) at ARSD College, University of Delhi.

2.1. Experiment: For the preparation of Dibutylbis [(2-isopropyl-5-methylcyclohexyl) oxy] stannane ,a mixture of dry benzene (30 ml), absolute ethanol (10 ml) and dibutyltin (IV) oxide (1mmol) was prepared in which 2 mmol of ligand (menthol) was dissolved in order to maintain the molar ratio of 1:2 (metal: ligand) . The reaction

mixture was then further refluxed azeotropically using a Dean Stark separator over the heating mantle. Within 10-15 min Dibutyltin (IV) oxide dissolves and gave a clear solution, refluxing was further continued for 5-6 hours and the contents were then cooled. The overall reactions maintained equilibrium which are driven to completion by complete removal of water, by azeotropic distillation. Excess of solvent left was removed under further reduced under pressure by using a rotator evaporator to leave behind a solid complex. The solid was filtered and washed using chloroform and dried in vacuum. Recrystallisation was done using ethanol. The general reaction sequence is given as follows (Mala Nath, 2003; Jose S, 2004).



Reaction pathway for the formation of Dibutylbis((2-isopropyl-5-methylcyclohexyl)oxy)stannane

The product so obtained with the above mentioned method was found to be very good with a high yield of 0.4457g or 82% o,it was off white coloured viscous semisolid , soluble in dimethyl sulphoxide and ethanol. Analysis (%), (calculated) for [$C_{28}H_{36}O_2Sn$] (544.453): C, 61.88; H, 10.39; O, 5.89; Sn, 21.84.

2.2. Antibacterial test: The ligand and its organotin (IV) complex were screened for their antibacterial activity using the agar well diffusion method (Rahman, 2001). The wells were dug (6mm) in the plate containing the broth using a sterile metallic borer and 18-24 h bacterial inoculums containing 0.1658 OD was spread on the surface of the nutrient agar using a sterile cotton swab. In one of these wells ,we filled ethanol and the reference antibacterial drug which served as negative and positive controls respectively and in the other wells the sample of different concentrations were introduced.The plates were incubated immediately at 37 ℃ for 24 h. (Mala Nath, 1997; 1999; Tushar, 2008). The antibacterial activity was measured by measuring the diameter of the inhibition zone (in mm). The results were then compared with the reference drug.

2.3. Antifungal activity: Above synthesized Complex was then checked for its antifungal activity using Agar well diffusion method. The plates were prepared using the nutrient Czapak medium. The 6 mm well was dug in the media using sterile metallic borer. The fungal strains of *Fusarium oxysporum and Aspergillus niger* were streaked on the different plates and the samples having different concentrations in ethanol was introduced into the respective wells. Immediately the plates were incubated at 32℃ for 72 h. (Ruzicka, 2002; Aniyery Rohit Babu, 2015).

3. RESULTS AND DISCUSSION

3.1. Electronic absorption spectra: The λ_{max} of ligand (menthol) was found to be 220nm and absorbance 0.010000 which is generally assigned to π-π* transition. After complexation the λ_{max} of organotin complex shifted to 249 nm and absorbance to 1.150000, this was actually due to the coordination of ligand with the Dibutyl Tin Oxide. The shift in the peak from 220nm to 249nm (Redshift) is attributed to the n--$\boldsymbol{\pi}$* transition which indicates the ligand metal charge transfer (LCMT).Shifting of the UV peaks to higher wavelength shows 'Bathochromic shift or Red shift'. This gives an evidence of complex formation of ligand with dibutyl tin oxide (Leovacet, 2007; Norrihen San, 2012). Refer Table.1.for Electronic absorption data (UV spectra) and figure.1.UV graph for ligand,its organotin complex.

3.2. ^{1}H NMR spectra: The Organotin complex showed resonance signals δ(ppm):3.16(CH, Cyclohexane), 1.50(CH cyclohexane), 1.61(CH, cyclohexane), 1.68, 1.42(CH2, cyclohexane), 1.52, 1.27(CH2, cyclohexane), 1.3(CH2, methylene), 1.82(CH, methane), 0.96(CH3, methyl), 1.26(CH2, methyl), 1.31(CH2, methyl), 0.91(CH3, methyl). The absence of -OH proton signal in the ^{1}H NMR spectra of the organotin(IV) complexes (Jose S and Casas, 2004) indicated that the phenolic oxygen is coordinated to the Sn(IV) atom after de-protonation. (Uche B. Eke, 2010). Refer figure.4 for ^{1}H NMR spectra.

3.3. Antibacterial studies: Since the complex inhibited the growth of microorganisms, it has been assumed that the production of an enzyme is being affected; hence, the organisms were less able to metabolize nutrients and, consequently, growth ceased. Those enzymes that require free sulfydryl groups (-SH) for activity, appear to be especially susceptible to deactivation by ions of the complex. Refer Table 2 for Antibacterial activity of the ligand and its organotin (IV) complex**,** figure.6 for the Schematic representation of Antibacterial action of the synthesized organotin complex and figure.5 for developed plates of *S. aureus* and *E.coli* loaded with the ligand and its Organotin complex

3.4. Antifungal activity: In in vitro antifungal studyresults against the fungal strains *Fusarium oxysporium* and *Aspergillus niger* , it was observed that all the synthesized complex exhibited a good antifungal potency higher than those of the corresponding ligand .The MIC was found to be 10 ppm to 20 ppm. Refer figure.7 for the developed plates of *Fusarium oxysporum* and *A.niger* .

3.5. Computational software study:

3.6. iGEMDOCK : It is Generic Evolutionary Method for molecular DOCKing (Yang and Chen, 2004) which is an automatic program for computing a ligand's conformation and orientation relative to the active site of target protein, using an empirical scoring functions and an evolutionary approach. This computational tool was developed by Jinn-Moon Yang.

The coordinates of target protein atoms from the PDB is specified first (ligand binding area, atom formal charge and atom types). After that from the prepared ligand base and target protein, GEMDOCK software sequentially read the atom coordinates of a ligand. Executes flexible docking for each ligand, re-ranks and sorts all docked ligand conformations for the post-docking analysis. The interaction forces between a ligand and a biomolecule (protein) may involve hydrophobic forces, electrostatic interactions, van der Waals interactions, and hydrogen bonds. Different organic small molecules have different types of interactions toward proteins. The negative value of binding energy change (ΔG) reveals that the binding process is spontaneous. Ross has characterized the sign and magnitude of the thermodynamic parameter, which are associated with various individual kinds of interactions that take place in protein association process. (Aniyery Rohit Babu, 2015).

2XRG: Stands for Autotoxin which is in a complexed form. It is generally abbreviated as ATX, also called ecto nucleotide pyrophosphatase/phosphodiesterase-2, ENPP2) which is secreted lysophospholipase that creates the lipid mediator lysophosphatidic acid (LPA) which is a mutagen and chemo attractant for many cell. ATX –LPA signalling are involved in various pathologies like tumor progression and inflammation. Autotaxin was originally identified as a tumor cell-motility-stimulating factor; later it was shown to be LPA (which signals through Lysophospholipid receptors), the lipid product of the reaction catalyzed by autotaxin, which is responsible for its effects on cell-proliferation. (Aniyery Rohit Babu, 2015). The protein encoded by this gene functions as both a phosphodiesterase, which cleaves phosphodiester bonds at the 5' end of oligonucleotides, and as a phospholipase, which catalyzes production of lysophosphatidic acid (LPA) in extracellular fluids. LPA promotes the growth factor-which includes responses, stimulation of cell proliferation and chemotaxis. This gene express the motility of tumor cells, has angiogenic properties, and its expression is upregulated in several kinds of carcinomas.

3OG7: Stands for B-Raf kinase and is the most frequently mutated protein kinase in human cancers. The finding that oncogenic mutations in BRAF are common in melanoma, followed by the demonstration that these tumors are dependent on the RAF/MEK/ERK pathway, offered hope that inhibition of B-RAF Kinase activity could benefit melanoma patients

On the basis of docking of the complex with PDB 2XRG (Refer Table.3 for Total energy, Vander-Waal interaction, Hydrogen bonding, electrostatic energy of the novel organotin complex interaction with PDB 2XRG and figure.2 for graph showing interaction curves for Amino acid involved in the docking with PDB 2XRG) and 3OG7(Refer Table.3 for Total energy, Vander-Waal interaction, Hydrogen bonding, electrostatic energy complex on interaction with PDB 3OG7 ,figure.3 for graph showing interaction curves for Amino acid involved in the docking with PDB 3OG7)., the synthesized complex gave minimum energy. Therefore, it was proven to be an inhibitor for RNAse and B-Raf Kinase. The complex also has the ability to affect the antisense effect and inhibit the destroying of mRNA strand. 2XRG is involved in various pathologies including tumor progression and inflammation so via the inhibition this enzyme using complex. Further, complex can also inhibit the B-RAF Kinase activity and therefore, thus it could benefit melanoma patients.

Table.1.Electronic absorption data(UV spectra)

Sample	Wavelength (nm)	Absorbance
Ligand	220 .00	0.010000
Organotin Complex	249.00	1.150000

Table.2.Total energy, Vander-Waal interaction, Hydrogen bonding, electrostatic energy of the novel organotin complex interaction with PDB 2XRG and PDB 3OG7

PDB	Total Energy	VDW	H-Bond	Electrostatic
PDB 2XRG	-84.6307	-79.941	-4.68966	0
PDB 3OG7	-89.5806	-89.5806	0	0

Table 3: Antibacterial activity of the ligand and its organotin (IV) complex

Sample	Inhibition zone (mm) *E. coli* 1610	Conclusion	Inhibition zone (mm) *S. aureus*	Conclusion
Control Chloramphenicol	21	Active	16	moderate
Ligand	5	Weak	10	Weak
Complex CD1	9	weak	16	Moderate
CD2	-	Nil	-	Nil
CD3	-	Nil	-	Nil
CD4	-	Nil	-	Nil
CD5	-	Nil	-	Nil

*CD1-Complex Dilution 1-(0.002 g/100 mL), CD2 Complex Dilution 2-(0.001 g/100 mL), CD3 -Complex Dilution 3-(0.0005g/100 mL), CD4- Complex Dilution- 4(0.00025g/100mL) ,CD 5- Complex Dilution 5-(0.000125g/100mL)

Table.4.Amino acid involved in the docking of the complex on interaction

with PDB 2XRG		**with PDB 3OG7**	
Energy	-84.6	**Energy**	-89.6
H-S-SER-224	0	**H-S-LYS-483**	0
H-S-ASN-524	-3.5	**H-M-CYS-532**	0
H-M-NAG-1860	0	**H-M-ASP-594**	0
V-S-TYR-221	-4.0631	**V-S-VAL-471**	-4.58254
V-M-PRO-522	-4.104	**V-S-LYS-483**	-2.39936
V-M-ASN-523	-6.7546	**V-S-LEU-505**	0
V-S-ASN-524	-5.7877	**V-S-TRP-531**	-6.84907
V-S-LEU-745	-7.0492	**V-M-CYS-532**	-6.375
V-S-HIS-831	-11.09	**V-S-PHE-583**	-19.186
V-M-NAG-1860	-5.9138	**V-M-GLY-593**	-1.5333
V-M-NAG-1860	-10.477	**V-M-ASP-594**	-1.6970

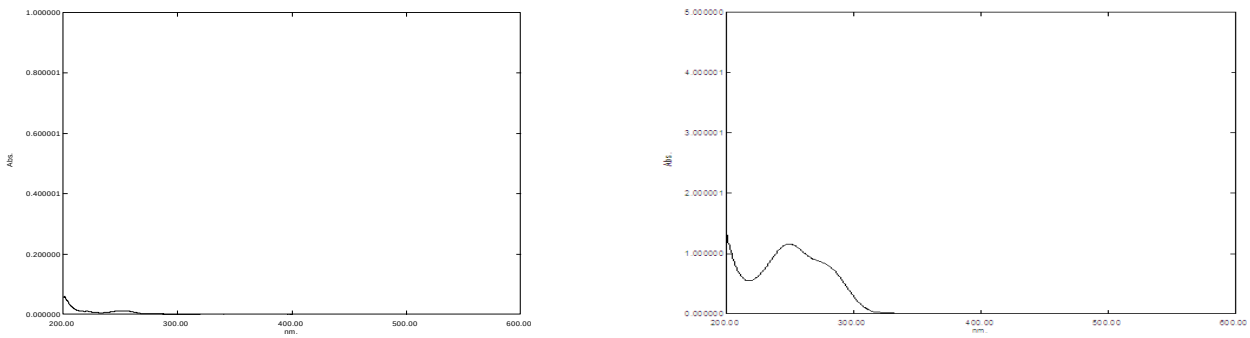


Figure.1.UV graph for ligand menthol and UV graph for Dibutylbis ((2-isopropyl-5-methylcyclohexyl)oxy)stannane

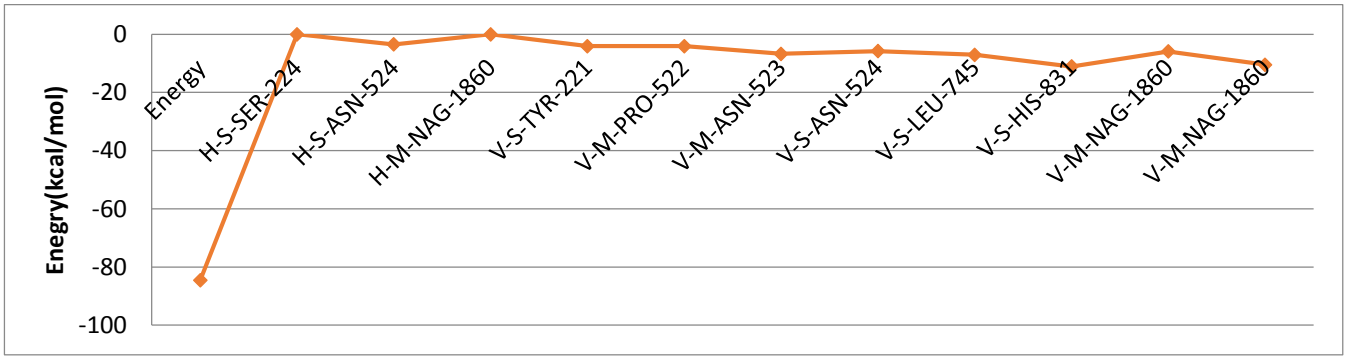


Figure.2.Graph showing interaction curves for Amino acid involved in the docking with PDB 2XRG

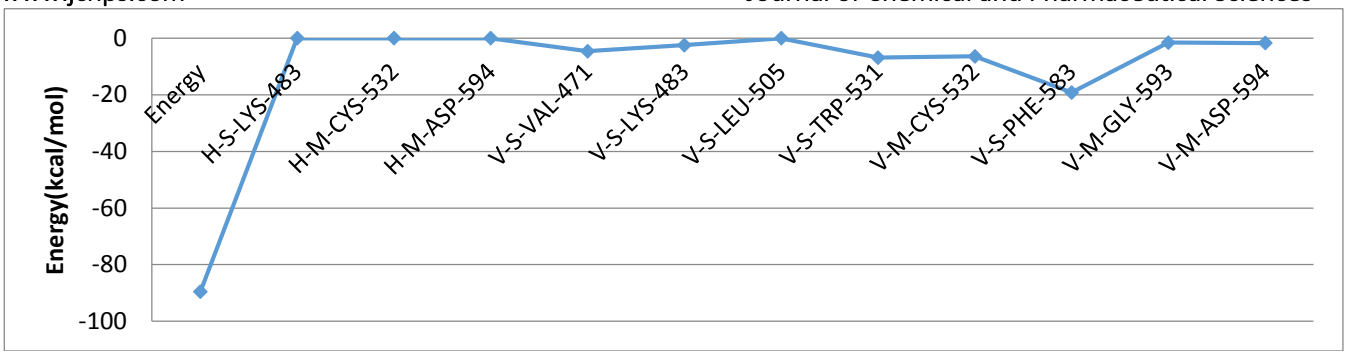


Figure.3.Graph showing interaction curves for Amino acid involved in the docking with PDB 3OG7

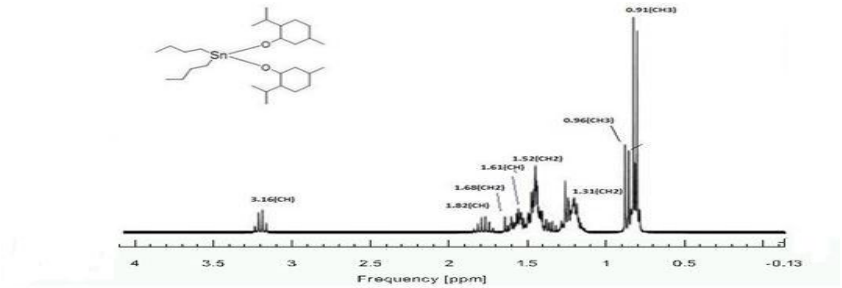


Figure.4.[1]H NMR SPECTRA OF Dibutylbis((2-isopropyl-5-methylcyclohexyl)oxy)stannane

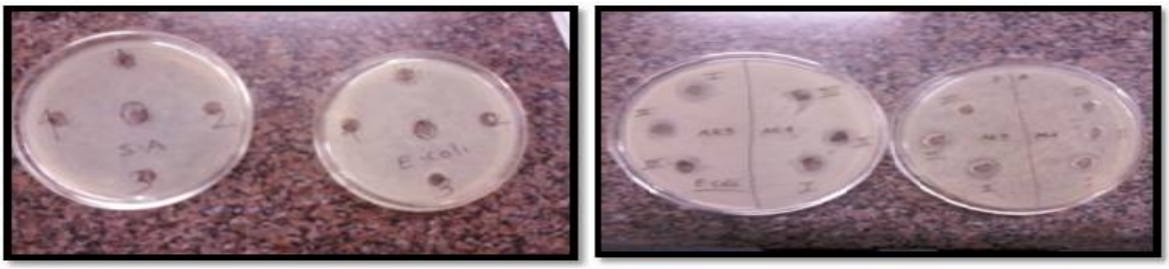


Figure.5.Developed plates of S. aureus and E.coli loaded with the ligand and its Organotin complex

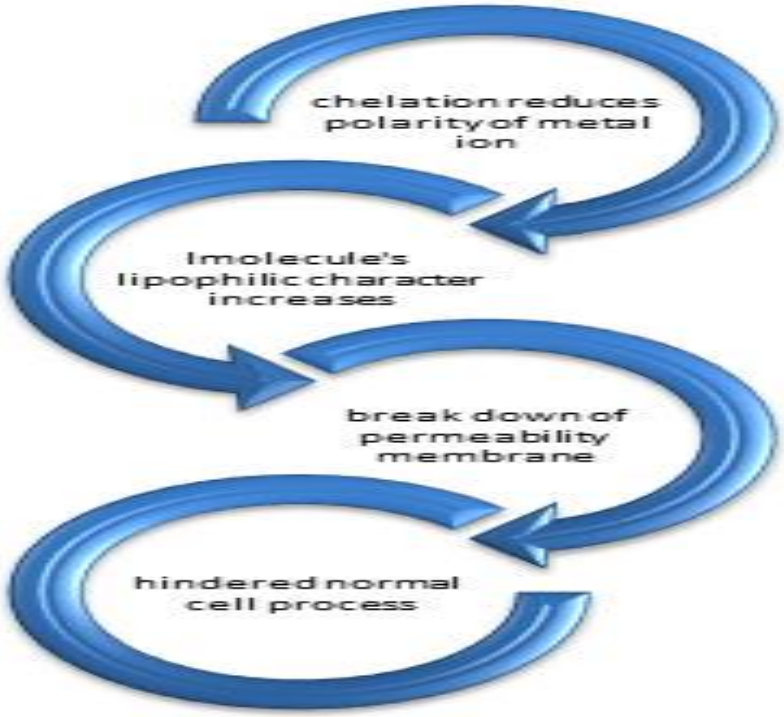


Figure.6.Schematic diagram showing Antibacterial action of the complex

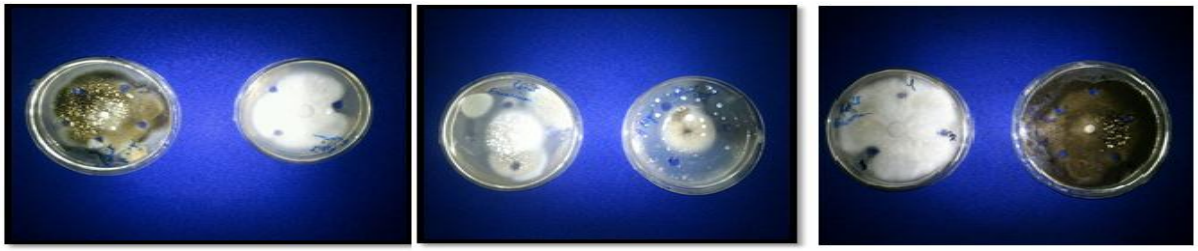


Figure.7.Developed plates of Fusarium and A.niger loaded with Ligands and its organotin complex

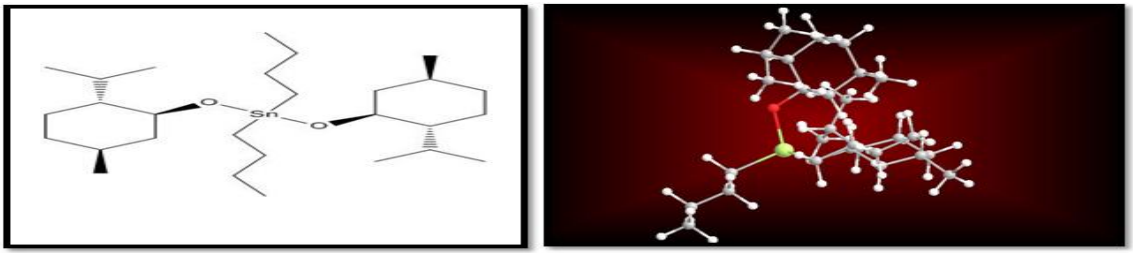


Fig.8..Organotin complex of menthol and its proposed 3D structure (software used Chem Draw 3D)
Green – tin atom Red –oxygen atom Grey –carbon atom White –hydrogen atom

4. CONCLUSION

In view of the antibacterial showed by the synthesized complex, it can have potential uses in the field of anti-bio fouling agents, bactericides and other fields of synthetic chemistry. The study conducted against the fungus showed that the new novel complexes can be used as antifungal agent in agriculture these complexes were found to be better fungicides as compared to that of its ligand .

5. ACKNOWLEDGEMENTS

The authors were grateful towards Amity University Noida -125, Delhi University and Jamia Hamdard University for their kind support in carrying out the research work successfully.

REFERENCES

Aniyery Rohit Babu, Organotin Bis-Complexes: Synthesis, Characterization, antimicrobial activity and Anticancer (computational) studies, Int. J. of Adv. Res., 3 (7), 2015, 262-273.

Armarego W.L.F, and Perrin D.D, Purification of Laboratory Chemicals, Butterworth Heinemann, Oxford, UK, 4th edition, 1996.

Beltran H.I, Synthesis and characterization of di-phenyl-tin IV- salicyliden-ortho-aminophenols: Analysis of *in vitro* antitumor/antioxidant activities and molecular structures, J. Inorg. Biochem, 101, 2007, 1070-85.

Biunden SJ, The environmental Chemistry of Organotin compounds, Environmental chemistry, The Royal Society of Chemistry, London, 1984, 49-77.

Cima F, Organotin Compounds in the Environment, in Organometallic compounds in the Environment, Wiley, Chichester, 2003, 101-141.

Crowe AJ, Metal Complexes in Cancer Chemotherapy, Keppler B.K.(ed.), VCH:Weinheim, 1993, 369-379.

Davies A.G, Tin Chemistry: Fundamentals, Frontiers, and Applications, Chapter 4, Wiley & Sons, Chichester, 2008.

Farina Y, Synthesis, structural and fungicidal studies of some diorganotin(IV) with benzamidoleucine, Aust.J. Basic Appl. Sci., 3, 2009, 1670–1673.

Gielen M, and Tiekink E.R.T, In Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: The Use of Metals in Medicine, M.Gielen, E.R.T.Tiekink (Eds), Wiley & Sons, Chichester, United Kingdom, 2005, 421-428.

Gleeson B, Novel organotin antibacterial and anticancer drugs, Polyhedron, 27, 2008, 3619-24.

Hawkey P, and Lewis D, Medical Bacteriology—A Practical Approach, Oxford University Press, Oxford, 1994.

Jose S, Diorganotin (IV) complexes of diprotonated pyridoxine (PN,Vitamin B6), The crystal structure of [SnEt2(PN-2H).CH3OH,[SnEt2(PN-2H)(DMSO)] and [SnBu2(PN-2H)], Elsevier; Journal of Organometallic Chemistry , 2004, 620-626.

Leovac, Synthesis and Charecterization of salicylaldehyde Girad-T hydrazine complexes, Structural Chemistry, 18, 2007, 113-119.

Mala Nath, Characteristic spectral studies and *in vitro* Antimicrobial and *in vivo* Multi-Infection Antifungal Activities in Mice of New Organotin(IV) Derivatives of Heterocyclic Amino acid, Applied Organometallic Chemistry, 13, 1999, 29-37.

Mala Nath, New organotin (IV) derivatives of dipeptides as models for metal–protein interactions: *in vitro* anti-tumour activity**,** Appl. Organometal. Chem., 17, 2003, 305–314.

Mala Nath, Spectral studies and *in vitro* antimicrobial Activity of New Organotin (IV) Complexes of Schiff Bases Derived from Aminoacids, The Chemical Society of Japan, 70, 1997, 1331-1337.

Mala Nath, Synthesis, Characteristic Spectral studies and *in vitro* antimicrobial and antitumour activities of Organotin(IV) Complexes of Schiff bases Derived from Amino Acids, Applied Organometallic Chemistry, 2, 1997, 727-736.

Ming Li, Synthesis, crystal structure and *in vitro* anti-tumour activity of dibutyltin complex of 2, 4-dichloro-5-flurobenzoic acid, Commun. Inorg. Synth., 1, 2013, 32-38.

Mohammed N.I, and Sheriff S.A, Synthesis, Characterization and Uses of Schiff Bases as Fluorimeric Analytical Reagents, e Journal of Chemistry, 4(4), 2009, 531 – 535.

Norrihen San, Synthesis, spectral characterization and biological activities of Organotin (IV) complexes with Ortho-Vanillin -2-hydrazinopyridine (VHP), Open Journal of Inorganic Chemistry, 2, 2012, 22-27.

Rahman A, BioassayTechniques for Drug Development, Harwood Academic, Amsterdam, The Netherlands, 2001.

Ruzicka Ales, Structure and *in vitro* antifungal activity of [2,6-bis9dimethylaminomethyl)phenyl] diphenyl tin (IV) compounds, Appl.Organometal.chem., 16, 2002, 315-322.

Sari N, Antibacterial Activities of Some new Amino acid Schiff bases, G.U. J. Sci., 16(2), 2003, 283 – 288.

Singh H.L, and Varshney A.K, Synthesis and characterization of coordination compounds of organotin (IV) with nitrogen and sulfur donor ligands, Applied Organometallic Chemistry, 15(9), 2001, 762–768.

Srivastava R.S, Pseudo tetrahedral Co (II), Ni (II) and Cu (II) Complexes of N^1-(O-chloro phenyl)-2-(2',4'-dihydroxyphenyl)-2-benzyazomethine, Inorg. Chim. Acta, 56, 1981, L65.

Tabassum S and Pettinari C, Chemical and biotechnological developments in Organotin cancer chemotherapy, J. Organomet. Chem, 691, 2006, 1761-68.

Tenover F, Mechanisms of antimicrobial resistance in bacteria, Am. J. Med., 119, 2006, 62–70.

Tian L.L, Synthesis, characterization and biological activity of tri organ-otin 2-phenyl-1,2,3-triazole-4-carboxylates, J. Inorg. Biochem., 99, 2005, 1646–1652.

Tushar S Basu Baul, Antimicrobial activity of Organotin(IV) compounds, Appl. Organometal. Chem., 22, 2008, 195-204.

Uche B Eke, The Synthesis of some organotin (IV) compounds in the melt –phase, J. Iran. Chem. Res., 3, 2010, 237-243.

Warren T Piver, Organotin Compounds: Industrial Application and Biological Investigation, Environmental Health perspective, 1973, 61-77.

Win Y, Preliminary *in vitro* cytotoxic assay of human liver carcinomacells (HepG2) of organotin(IV) complexes: synthesis and characterization of organotin(IV) complexes of 2,4-dinitrobenzoicand 3,5-dinitrobenzoic acids, Afr. J. Biotechnol., 11, 2012, 13140–13146.

Yeaman R, and Yount N, Mechanisms of antimicrobial peptide action and resistance, Pharmacol. Rev., 55, 2003, 27–55.

Yousif E, Synthesis, characterization and fungicidal activity of some diorganotin(IV) with 2-thioacetic-5-phenyl-1,3,4-oxadiazole, J. Fundam. Sci., 5, 2009, 94–98.