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IN SILICO PHARMACOLOGICAL AND *IN VITRO* BIOLOGICAL STUDY OF NOVEL ORGANOTINSORBATE

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Keywords:

Stannane, PPAR γ agonist, Antibacterial study, Multi-target drug, Lipinski's rule of five, Computational drug design

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ABSTRACT: In this research work, a well established method was used to synthesize of stannane of 2, 4-hexadienoic acid and its characterization was carried using ultraviolet (UV)-visible and ^{1}H NMR (nuclear magnetic resonance). Our research aimed at developing a potent multi-target drug which could act as antibacterial agent and also peroxisome proliferator-activated receptor gamma (PPAR γ) agonists that is having the potential to activate the PPAR γ. *In vitro* antibacterial study was carried out against, *Staphylococcus aureus* (gram positive) and *Escherichia coli* 1610 (gram negative) using 'well diffusion method'. It was followed by *in silico* docking studies, using computational software iGemDock v2.1 tool and its effect on glycemic and lipid parameter was also studied. Pharmacokinetic properties were studied using pkCSM software developed by the University of Cambridge. Drug likeness study and all the specifications were tested with the help of Molinspiration Molecule Viewer software available online. It was observed that the stannane of 2, 4-hexadienoic acid inhibited the bacterial growth of *Staphylococcus aureus* (gram positive) and *Escherichia coli* 1610 (gram negative). The complex has shown good docking results on almost all the receptors, with interaction supporting the fitting of the drug to the target molecules. The novel complex has shown good antibacterial activity both *in vitro* and in *in silico* studies. It was also found to be a potent PPAR γ agonist. The Organotinsorbate did not contravene the 'Lipinski's rule of five' hence it has the prospective for ultimate development as oral agents.

INTRODUCTION: Stannanes are attaining great interest because of its wide application in biological and potential activity in the area of inorganic and metal organic chemistry. It is utilized widely in industries such as marine anti-biofouling paints, preservatives, fungicides, bactericides, acaricides and fire retardants.

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Advances in the use of stannane in pharmacological as antibacterial, antifungal, anti-tuberculosis and cytotoxic agents [1, 2] and better biological action as an anticancer drug as compared to other traditional heavy metals, have gained relevant interest. Tin in +4 coordination forms most stable complexes with a unique structure and physicochemical properties that are used in the synthesis of organic compounds, used as heat stabilizers, catalyst in drug development, as biologically active agents, and in other areas. Identification of novel chemical entities possessing desirable biological activity holds key interest in drug discovery.

For an effective drug development, proper pharmacokinetics and toxicity profile along with efficacy act as major factors [3]. Poor absorption, distribution, metabolism and excretion (ADME) along with the toxicity (T) are the leading causes of exorbitant last-stage failures in drug development [4, 5]. *In silico* implementations in drug-discovery prove to be beneficial [3, 6] as they have accelerated the progression of bringing a drug to the market by reducing production cost and time, necessary to conduct these *in vitro* screens.

Keeping in mind the various applications of stannane, this research work reports the synthesis, characterization, *in silico* pharmacological and *in vitro* biological study of stannane of 2, 4-hexadienoic acid, *i.e.* a step towards development of a novel multi-target drug.

MATERIALS AND METHOD:

Chemicals: All the experimental works were carried out using analytical grade chemicals and solvents, they were procured from commercial sources and we have followed standard procedure for solvent drying process [6].

Instruments: The instrument used for the sterilizing purpose in antimicrobial study was Khera laboratory autoclave, Khera instrumentation and incubator used was an orbital shaker, PSN instrumentation Pvt. Ltd. The UV-visible spectra study was carried using UV instrument SHIMADZU UV 1800, at wavelength 200–600nm in ethanol medium. The ^{1}H NMR spectra were recorded using dimethyl sulfoxide (DMSO)–d 6 solution and TMS (tetramethylsilane**)** as internal standard at 25 °C on a JEOL ECX-400P NMR spectrophotometer at 400 MHz and 100 MHz. NMR spectra was processed using software JEOL Delta™.

Experimental Method: The synthesis was carried out using the well established method of azeotropic removal of water [6]. 2 mmol of ligand (2, 4-hexadienoic acid) was added into 1 mmol dibutyltin oxide in benzene: ethanol medium (**Fig. 1**). The entire reaction mixture was refluxed for 15 minutes using dean stark attachment. The process of refluxing was further continued for 6 hours till there was complete removal of water from the reaction mixture. Excess solvent left was then removed using a rotator evaporator. The final product obtained was characterized by UV-visible spectra studies and ^{1}H NMR spectra.

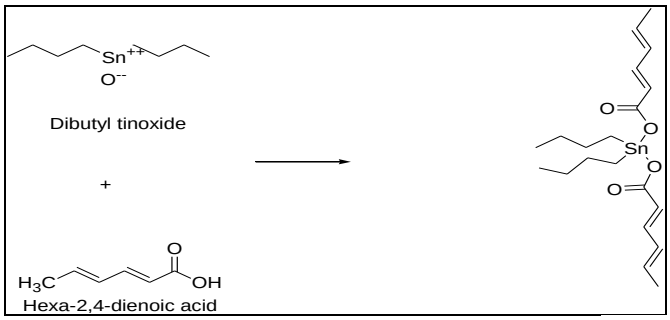

FIG. 1: SYNTHESIS OF STANNANE OF 2, 4-HEXADIENOIC ACID

***In vitro* Antibacterial Test:** The ligand 2, 4-hexadienoic acid and its synthesized stannane were screened *in vitro* for their antibacterial activity using the agar-well diffusion method [6]. *Staphylococcus aureus* (gram-positive) and *Escherichia coli* 1610 (gram-negative) bacterial strains were cultivated independently in nutrient agar plates. The 250mL bacterial inoculums [0.168 OD (optical density)] was prepared and incubated in an incubator for 24 hours. Samples of stannane of 2, 4-hexadienoic acid with different concentrations (0.001 g/100mL, 0.0005 g/100mL, 0.00025 g/100mL and 0.000125 g/100mL in ethanol) were introduced into different wells.

The plates were then incubated instantly at 37 °C for 24 hours [7]. Inhibition zone was then measured in mm, to determine the antibacterial activity. The experiential results were further compared with the control (chloramphenicol) and also the minimum inhibition concentration (MIC) was also determined after 24 hours of incubation [6]. The data on the growth responses of the bacterial strains to various concentrations of the stannane following a 24-hour incubation period were analyzed, using the percentage inhibition of diameter growth (PIDG) against chloramphenicol. PIDG was determined [8] using the below mentioned to the equation:

$$\text{PIDG (\%)} = \frac{\text{Diameter of sample} - \text{Diameter of Control}}{\text{Diameter of Control}} \times 100$$

***In silico* Antimicrobial Studies Using Molecular Docking Software (IGEMDOCK):** iGemDock v2.1 software gives protein interaction profiles of van der Waal's (V) interactions, electrostatic (E) and hydrogen-bonding. It ranks and forms a perception of the screening compound by the

combination of the energy-based scoring function and pharmacological interactions. The empirical scoring function of iGemdock was calculated using the formula:

Energy = vdW + Hbond + Elec.

Where, the vdW refers to van der Waal energy; Hbond refers to hydrogen bonding energy and Elect refer to electro statistic energy. This software is useful in the structure-based virtual screening and post-screening analysis for the drug discovery. The software maintained by Drug Design and Systems Biology Laboratory of National Chiao Tung University, Taiwan. The accurateness of molecular docking and the screening efficacy were better as compared to other docking methods, and results have been published [9, 10].

Preparation of the Binding Site: A literature survey was done prior the selection of Binding site. All the protein structure files [PDB (Protein Data Bank)] for docking were downloaded from http://www.rcsb.org/. The co-crystal ligand in the PDB was detached, and bond orders were checked.

Ligand Preparation and Docking: The structure of stannane of 2, 4-hexadienoic acid was drawn using Chem Draw software. The structure was optimized prior docking using Gaussian software, and the method followed was Energy (Ground state) Hartree-Fork. Ligand file in CDX (compound index) format was converted into MOL 2 format by Openbabel software. Docking with each PDB files was done using accurate docking function (slow docking) [10]. Lastly, the post analysis was done, a tool which works by using K-means, visualized and ranked the screening compound by conglomerating the pharmacological interactions and energy-based scoring function. Step-wise energy optimization was carried out in the process of docking by hydrogen, side chains and finally the backbone of the receptor [11, 12]. After that, the optimized complex was then checked for different interaction with receptor like hydrogen bonding, hydrophobic bonding and van der Waal's interaction.

***In silico* Pharmacokinetic Properties Study:** Absorption, distribution, metabolism, excretion, and toxicity known as 'ADMET' are important factors / properties to be considered in the discovery / development of novel drugs [13]. Pharmacokinetic properties study was done using online-based software pkCSM developed by Douglas E. V. Pires *et al.*, [13] of University of Cambridge, which mainly works on graph-based signatures to build up predictive models of central ADMET properties for the drug development.

***In silico* Drug Likeness Study Using Molinspiration Molecule Viewer:** Molinspiration Molecule Viewer software permits perception of molecules by employing sophisticated Bayesian statistics, which match the structures and properties of representative compound active on the specific target with the structures of inactive molecules, to recognize substructure features typical for active molecules.

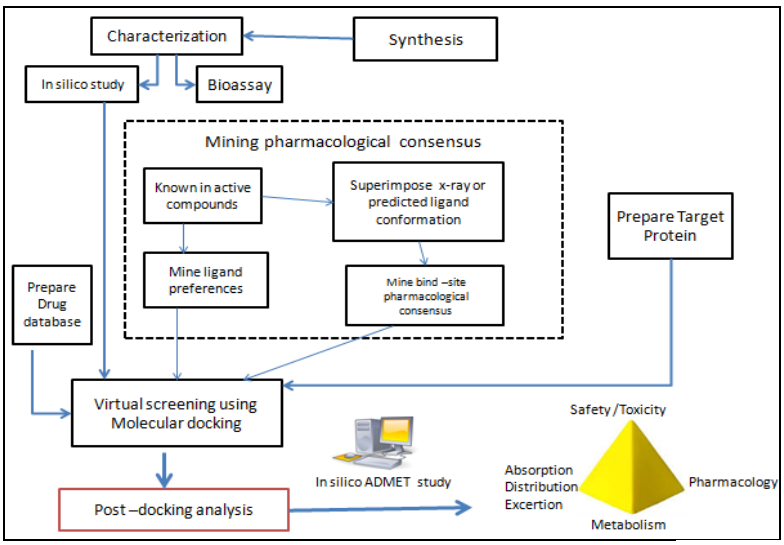

FIG. 2: SCHEMATIC REPRESENTATION OF METHODOLOGY FOLLOWED IN *IN-SILICO* PHARMACOLOGICAL AND *IN VITRO* BIOLOGICAL STUDY OF NOVEL ORGANOTINSORBATE

Prediction of Xenobiotic Metabolism Using MetaPrint2D: MetaPrint2D online server (http://www-metaprint2d.ch. cam.ac.uk/) was used to calculate the xenobiotic metabolism by means of data-mining and statistical analysis of identified metabolic transformations reported in scientific literature. Model used was 'Human (Metabolite 2010.2)' and setting was 'default'.

RESULTS AND DISCUSSION:

Physical Study: The complex was obtained in good yield (92.9%) and was yellow colored viscous semi solid, soluble in DMSO and ethanol with molecular formula $C_{20}H_{32}O_4Sn$ and molecular weight 455.18 $g \cdot mol^{-1}$. Analysis (%), calculated was found to be Carbon: 52.77 %; Hydrogen: 7.09 %; Oxygen: 14.00 %; and Tin: 26.08%.

Characterization:

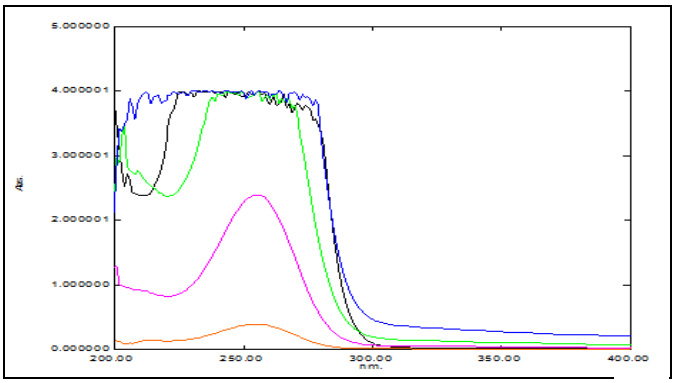

FIG. 3: UV SPECTRAL STUDIES OF LIGAND (2, 4-HEXADIENOIC ACID) AND STANNANE OF 2, 4-HEXADIENOIC ACID

TABLE 1: UV DATA FOR LIGAND (2, 4-HEXADIENOIC ACID) AND STANNANE OF 2, 4-HEXADIENOIC ACID

Sample	Wavelength (nm)	Absorbance
Ligand(2,4-hexadienoic acid)	238	4.000
Stannane of 2,4-hexadienoic acid (dilution 1*)	244.00	3.996338
Stannane of 2,4-hexadienoic acid (dilution 2*)	244.00	1.898987
Stannane of 2,4-hexadienoic acid (dilution 3*)	244.00	0.290000

(*Dilution 1-(0.002 g/100mL), Dilution 2-(0.001 g/100mL), Dilution 3-(0.0005g/100mL)

The peaks of 2, 4-hexadienoic acid were reported at λ_{max} 238nm (absorbance 4.000) which is assigned to π-π* transition. Further peaks of stannane of 2, 4-hexadienoic acid peak was reported at λ_{max} 244.00nm (absorbance 3.996338) (**Fig. 3**, **Table 1**) which is assigned to n--π* transition. This shifting of absorption maxima to longer wavelength, known as "Bathochromic / Red shift" is due to the presence of non-bonding electron pair as they become available for interaction with metal ion.

^{1}H NMR: The stannane of 2, 4-hexadienoic acid showed resonance signals δ (ppm): 1.63 (CH_2, attached to Sn-O, methylene of n-butyl group), 1.26 (CH_2, methylene of n-butyl group), 2.05 (CH_3, methyl of n butyl group), 0.91 (CH_3, methyl of n-butyl group), the other peaks for the 1-ethylene group were observed at δ: 5.32, 7.50, 6.27, 5.72 respectively.

In-vitro Antibacterial Test:

TABLE 2: DIAMETER OF INHIBITION ZONE AND ITS INTERPRETATION FOR BACTERIAL STRAIN-*ESCHERICHIA COLI*

S. no	Sample	Inhibition zone (mm)	Interpretation	PIDG (%)
1	Control -Chloramphenicol	21 ± 1.8	Inhibition zone greater than 16 mm hence it is an active antimicrobial agent	-
2	2,4-hexadienoic acid	6 ± 0.5	Nil/No antimicrobial activity	-71.429
3	Stannane of 2,4-hexadienoic acid Dilution (0.002 g/100mL)	19 ± 1.5	Inhibition zone greater than 10 mm but less than 16 mm, hence it is a moderate antimicrobial agent	-9.524
4	Stannane of 2,4-hexadienoic acid Dilution (0.001 g/100mL)	12 ± 1.2	Inhibition zone greater than 10 mm but less than 16 mm, hence it is a moderate antimicrobial agent	-42.857
5	Stannane of 2,4-hexadienoic acid Dilution (0.0005g/100mL)	-	Nil/No antimicrobial activity	-
6	Stannane of 2,4-hexadienoic acid Dilution (0.00025g/100mL)	-	Nil/No antimicrobial activity	-
7	Stannane of 2,4-hexadienoic acid Dilution (0.000125g/100mL)	-	Nil/No antimicrobial activity	-

TABLE 3: DIAMETER OF INHIBITION ZONE AND ITS INTERPRETATION FOR BACTERIAL STRAIN-*STAPHYLOCOCCUS AUREUS*

S. no	Sample	Inhibition zone (mm)	Interpretation	PIDG (%)
1	Control-Chloramphenicol	16 ± 1.5	Inhibition zone greater than 16 mm hence it is an active antimicrobial agent	-
2	2,4-hexadienoic acid	10 ± 1.0	No activity	-37.5
3	Stannane of 2,4-hexadienoic acid Dilution (0.002 g/100mL)	15 ± 1.4	Inhibition zone greater than 10 mm but less than 16 mm, hence it is a moderate antimicrobial agent	-6.25
4	Stannane of 2,4-hexadienoic acid Dilution (0.001 g/100mL)	-	Moderate,	-
5	Stannane of 2,4-hexadienoic acid Dilution (0.0005g/100mL)	-	Nil/No antimicrobial activity	-
6	Stannane of 2,4-hexadienoic acid Dilution (0.00025g/100mL)	-	Nil/No antimicrobial activity	-
7	Stannane of 2,4-hexadienoic acid Dilution (0.000125g/100mL)	-	Nil/No antimicrobial activity	-

TABLE 4: MINIMUM INHIBITION CONCENTRATIONS (MIC*)

Bacterial Strain	Sample concentration (g/100mL)	ppm
Escherichia coli 1610	0.001	10
Staphylococcus aureus	0.002	20

*MIC refers to the lowest conc. of antibacterial agent required to inhibit the visible growth of microbes

The antimicrobial activity of the synthesized compounds (**Table 2** and **3**) may be due the presence of a functional pharmacophore, which maximize the lipophilic character of the molecules and thus ease its movement across the cell membrane of the microorganism.

The antibacterial property of metal complexes can be described as follows: (1) normal cell process of the bacteria being affected by the metal, (2) metal ions polarity are totally decreased on chelation due to the partial distribution of its positive charge with a donor groups and (3) π-electron delocalization over the whole molecule increases the lipophilic character of the metal complex which results in the breakdown of the permeability barrier of the cells and thus its interference with normal cell process [14]. Chelation theory [15] could better explain the activities of the metal complexes, which explains that a decrease in polarizability of the metal could intensify the lipophilicity of the complexes [16].

FIG. 4A AND B: DEVELOPED PLATE: (A) *STAPHYLOCOCCUS AUREUS* AND (B) *ESCHERICHIA COLI* 1610 LOADED WITH LIGAND (2, 4-HEXADIENOIC ACID) AND STANNANE OF 2, 4-HEXADIENOIC ACID

Abbreviations: C, Chloramphenicol; L, ligand (2,4-hexadienoic acid); S1, stannane of 2,4-hexadienoic acid, dilution 1 (0.002 g/100mL); S2, stannane of 2,4-hexadienoic acid, dilution 2 (0.001 g/100mL); S3, stannane of 2,4-hexadienoic acid, dilution 3 (0.0005g/100mL); S4, 2,4-hexadienoic acid dilution 4(0.00025g/100mL); S5, 2,4-hexadienoic acid, dilution 5 (0.000125g/100mL

In silico Antimicrobial Studies Using Molecular Docking Software (iGEMDOCK):

TABLE 5: SUMMARY OF TOTAL ENERGY, VAN DER-WAAL INTERACTION, HYDROGEN BONDING, AND ELECTROSTATIC ENERGY OF STANNANE OF 2, 4-HEXADIENOIC ACID ON INTERACTION WITH DIFFERENT PDB FILES

PDB File	Total Energy (kcal/mol)	van der-Waal (kcal/mol)	Hydrogen Bond (kcal/mol)	AverConPair (kcal/mol)
3D2U	-73.3106	-56.781	-16.5301	17
3D2Y	-77.6204	-63.279	-14.3419	20.68
3EOO	-61.9395	-48.08	-13.8592	14.16

2I42	-87.2901	-71.51	-15.7801	18.04
2XKW	-76.7184	-73.218	-3.5	17.56
4HEE	-73.3876	-69.888	-3.5	17.36

TABLE 6: INTERACTION PROFILE OF STANNANE OF 2, 4-HEXADIENOIC WITH DIFFERENT PDB FILES

PDB 3D2U		**PDB 4HEE**	
Amino Acid Residue	**Energy(kcal/mol)**	**Amino Acid Residue**	**Energy(kcal/mol)**
H-S-NAG-811	-2.39677	H-M-SER-342	-3.5
H-M-FUC-814	-8.06424	V-M-GLY-284	-6.47798
V-S-ASN-54	-9.9308	V-M-PHE-287	-4.53641
V-M-GLY-18	-2.04561	V-S-PHE-287	-5.33629
V-S-LYS-19	-5.74387	V-M-ARG-288	-4.04513
V-M-NAG-811	-4.00316	V-S-ARG-288	-7.73395
V-S-NAG-811	-15.5322	V-S-LEU-330	-0.991015
V-M-FUC-814	1.08745	V-M-ILE-341	-2.57361
		V-S-ILE-341	-4.37733
		V-S-GLU-343	-7.4957
PDB 3EOO		PDB 3D2Y	
Amino Acid Residue	Energy(kcal/mol)	Amino Acid Residue	Energy(kcal/mol)
H-M-ILE-428	-6.85923	V-M-TRP-110	-4.96686
H-M-GLY-429	-7	V-S-TRP-110	-10.2233
V-S-GLU-307	-11.66	V-M-LYS-159	-4.48214
V-S-LEU-353	-4.00741	V-S-LYS-159	-7.19159
V-M-GLY-429	-4.33431	V-S-ASP-160	-4.13974
PDB 2XKW		PDB 2I42	
Amino acid residue	Energy(kcal/mol)	Amino Acid Residue	Energy(kcal/mol)
H-M-SER-342	-3.5	V-S-ARG-255	-10.4357
V-M-GLY-258	-1.13717		
V-M-GLY-284	-12.7548		
V-S-PHE-287	-7.79959		
V-S-ILE-341	-7.83098		

Abbreviations: H, hydrogen bonding; V, van der Waals; M, main chain; S, side chain; ALA, alanine; LYS, lysine; TRP, tryptophan; ASP, aspartic acid. ASN, asparagine; NAG, N-Acetyl–Glucosamine; LYS, lysine; GLY, glycine; FUC, fucose; ILE, isoleucine; GLU, glutamic acid; LEU, leucine; ARG, Arginine; PHE, Phenylalanine; SER, Serine.

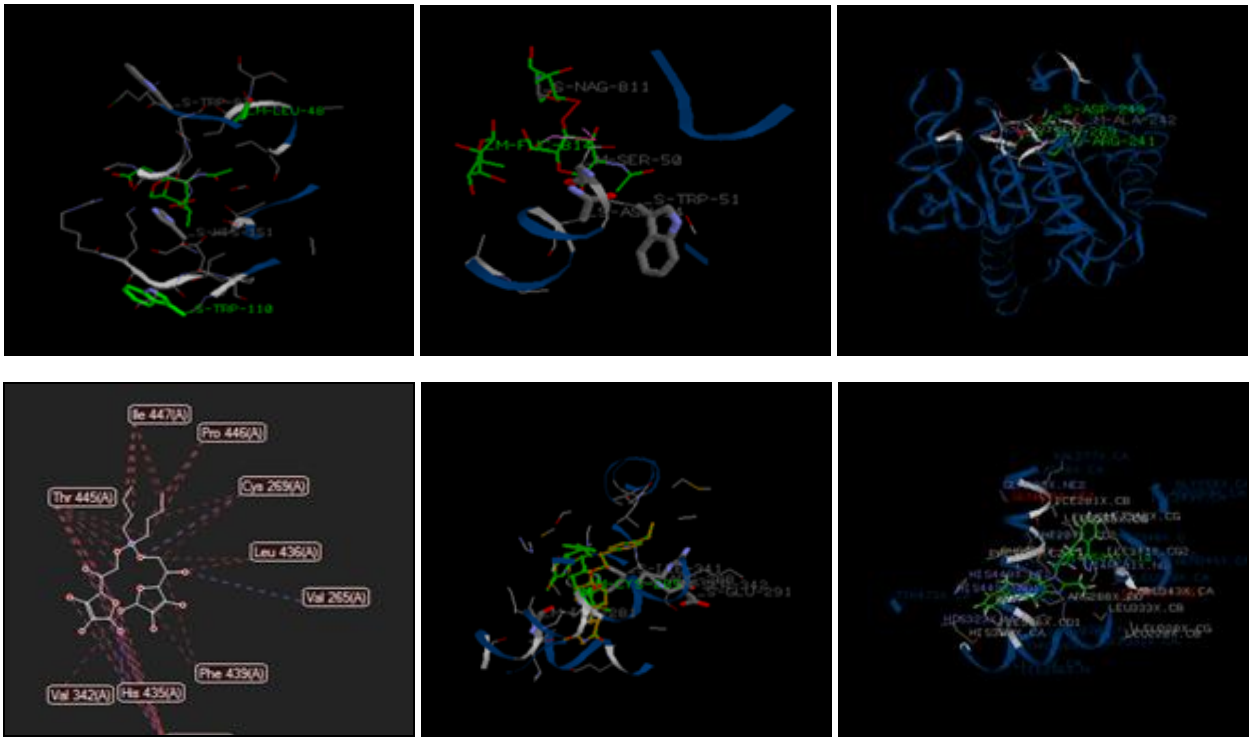

FIG. 5A-F: DOCKING POSES OF STANNANE OF 2, 4-HEXADIENOIC ACID WITH: (A) PDB 3D2Y; (B) PDB 3D2U; (C) PDB 2I42; (D) PDB 3EOO; (E) PDB 2XKW; (F) PDB 4HEE

Note: The green and grey colour represents the amino acids involved in hydrogen bonding and van der Waals respectively. The negative value of binding energy change (ΔG) reveals that the binding process is spontaneous; hence, it can fit well in the receptor cavity forming energetically most stable drug receptor. More the negative value of the binding energy, greater the compound acknowledged as a drug. During the process of the docking, the ligand and the protein bend their conformation to accomplish an overall "best-fit". The drug either acts as an agonist or antagonist with on a given receptor. An agonist binds to the receptor site and activates it, producing a biological response, whereas, the antagonists have affinity to the receptor site but no efficiency for their related receptors, and the binding will disrupt the interaction and inhibit the protein function [9, 10].

PDB 3D2Y is a Protein Data Bank File for Complex of the N-acetylmuramyl-L-alanine amidase AmiD from *E. coli* with the Substrate anhydro-N-acetylmuramic acid- L- Ala- D-gamma-Glu-L-Lys: The stannane of 2, 4-hexadienoic acid interacted with the PDB file (**Fig. 5A**) with total fitness value of -77.6204 kcal/mol which comprises of -63.279 kcal/mol van der Waal interactions and -14.3419 kcal/mol hydrogen bonding (**Table 5**). The complex snugly fits the active site, interacting with the amino acid residues including van der waal interaction with the side chain of tryptophan at position 110 with binding energy value -10.2233 kcal/mol and main chain with -4.96686 kcal/mol, van der waal interaction was observed with the side chain of lysine at position 159 with binding energy value -7.19159 kcal/mol and main chain with -4.48214 kcal/mol. Van der waal interaction with side chain of aspartate at position 160 with binding energy value -4.13974 kcal/mol was observed (**Table 6**).

The stannane easily binds to protein structure which plays an important role in hydrolyzing the link between N-acetylmuramoyl residues and L-amino acid residues in certain cell-wall glycopeptides. This protein functions or helps in bacterial cell wall organization, peptidoglycan turnover and also peptidoglycan catabolic process; hence the entire activity of the protein could be hindered if the complex interacts with this protein. The peptidoglycan is an important part of the cell wall that provides the shape to bacterial cells and assures them against high internal osmotic pressures. AmiD is a lipoprotein which is attached to the outer membrane [17]. The complex binds with protein pocket which have the most favourable binding energy thus have the potency to inhibit the growth of *Escherichia coli.*

PDB 3D2U is Basically the Protein Data Bank File for Structure of UL18, a Peptide-Binding Viral MHC (Major Histocompatibility Complex) Mimic, Bound to a Host Inhibitory Receptor, Gene Names: H301, Glycoprotein UL18: From the results of docking, the complex was found to fit well with the binding sites of the target protein. The complex interacted with the PDB (**Fig. 5B**) with total fitness value of -73.3106 kcal/mol which comprises -56.781 kcal/mol Van der waal interaction and -16.5301 kcal/mol hydrogen (**Table 5**). After doing post analysis, it was found that the complex interacted with basic amino acid residues hydrogen bonding with the side chain of, N-Acetyl-Glucosamine at position 811 with binding energy value -2.39677 kcal/mol; main chain of FUC at position 814 with binding energy value -8.06424 kcal/mol.

Van der waal interaction with the: main chain of Glycine at position 18 with binding energy value -2.04561 kcal/mol; main chain of N-Acetyl - Glucosamine at position 811 with binding energy value -4.00316 kcal/mol; side chain of N-Acetyl - Glucosamine at position 811 with binding energy value -15.5322 kcal/mol; side chain of asparagine at position 54 with binding energy value -9.9308 kcal/mol; side chain of lysine at position 19 with binding energy value -5.74387 kcal/mol (**Table 6**).

The study reports that the complex having highest binding energy are very effectual in halting the undesirable effects of selected PDB which has biological process like antigen processing and presentation of peptide antigen by MHC class I; immune response of bacteria , negative regulation of interferon - γ production and biochemical function of glycoprotein binding. This complex binds with the protein pocket which has the most favourable binding energy and thus it could hinder the protein activity. The complex has the potency to efficiently inhibit the Human Cytomegalovirus' glycoprotein UL18.

Human cytomegalovirus or herpes viruses, express glycoprotein UL18 which is essentially an intensely glycosylated transmembrane bestowing approximately 25% sequence identity with class I MHC molecules [18] and helps to prevent host lysis. Based on the literature survey [19, 20] the interactions and fitness scores of the complex suggest that this compound can act as an antimicrobial drug against Human cytomegalovirus.

PDB 2I42 is Crystal Structure of Yersinia Protein Tyrosine Phosphate Complexed with Vanadate, Gene Names Yoph Yop51:

Interaction Profile: After post analysis it was found that the complex interacted with total fitness Value -87.2901kcal/mol which comprises -71.51 kcal/mol van der waal interaction and -15.7801 kcal/mol hydrogen bonding (**Table 5**). The complex interacted with amino acid residue arginine at position 255 with binding energy value -10.4357kcal/mol (**Table 6**). The complex interacted with the PDB file (**Fig. 5C**) which performs the biological function of dephosphorylation (process of removing one or more phosphoric ester or anhydride residue from a molecule), pathogenesis in the host cell and protein tyrosine phosphatase activity [catalysis of the reaction protein tyrosine phosphate (PTP) to protein tyrosine and phosphate].

PTP is involved in the regulation of several cell functions including growth, differentiation, motility, cell-cell interactions, metabolism, gene transcription, and the immune response of *Yersinia enterocolitica*. On contact with the host cell, the Yersinia has type III secretion system which transfers a set of proteins called Yops (Yersinia outer proteins) into the host cell. YopH, YopE, YopJ/P, YpkA/YopO, YopT, and YopM are the six Yops identified so far, and they inhibit the host immune response during infection [21, 22]. Five of the six Yops have catalytic activities which are essential for their pathogenic functions. Based on the literature survey [19, 20] the interactions and fitness scores of the complex suggest that this compound can act as an antimicrobial drug against *Yersinia enterocolitica.* Thus, it could a potent antibacterial agent for this microbe.

PDB 3E00 is 2.9A Crystal Structure of Methyl-isocitratelyase from *Burkholderia pseudomallei*:

Interaction Profile: The complex interacted with the PDB file (**Fig. 5D**) which control the lyase activity, propionate catabolic process and 2-methylcitrate cycle in the *Burkholderia pseudomallei* organism, with total fitness value -61.9395 kcal/mol which comprises van der waal interaction with energy value of -48.08 kcal/mol, hydrogen bonding with energy value -13.8592 kcal/mol (**Table 5**). Interaction was found with the basic amino acid residue. Hydrogen bonding with: main chain of glycine at position 429 with binding energy value -7 kcal/mol, isoleucine at position 428 with binding energy value -10.3414 kcal/mol.

Van der waal interaction with: side chain of leucine at position 353 with binding energy value -5.90822 kcal/mol and glutamine at position 307 with binding energy value -6.95152 kcal/mol; main chain of gylcine at position 429 with binding energy value -4.33431kcal/mol (**Table 6**). The complex has shown binding affinity with this protein, it inhibits the methyl citrate cycle by interacting with methyl-isocitrate lyase. Based on the literature survey [23] the interactions and fitness scores of the complex suggest that this compound can act as an antimicrobial drug by inhibiting the function of this protein. Methylisocitratelyase plays a crucial role as it regulates the methylcitratecycle [24] in *Burkholderia pseudomallei*.

Effect on Glycaemic and Lipid Parameter:

PDB 2XKW Stands for Ligand Binding Domain of Human PPAR-Gamma in Complex with the Agonist Pioglitazone and PDB 4HEE Stands for Crystal Structure of Ppargamma in Complex With Imidazo[4,5-C]Pyridin-4-One Derivatives: Peroxisome Proliferator Activated Receptors (PPARs) denote a class of ligand-activated nuclear hormone receptors (NRs) belonging to the steroid receptor super family that controls the gene expression of proteins regulating the energy, glucose and lipid metabolism, propagation and differentiation of adipocytes and sensitivity of insulin. Similar to other nuclear receptors, Structure of PPARs are modular comprising of the following functional domains: an N-terminal region, a DNA-binding domain (DBD), a flexible hinge region, a ligand binding domain (LBD) and a C-terminal region. Ligands (synthetic/natural) attach to the LBD and activate the transcription and are termed as ‘agonists’.

In patients with type 2 diabetes, PPAR agonists constructively influence the glycaemic and lipid parameters. The ligand-stimulation of PPAR γ may be liable for inhibiting the growth of cultured human breast, gastric, lung, prostate and other cancer cell lines [25, 26].

In silico Drug likeness Study Using Molinspiration Molecule Viewer:

TABLE 7: MOLINSPIRATION BIOACTIVITY SCORE AND PROPERTY

Molinspiration bioactivity score v2014.03			Molinspiration property engine v2014.11	
Property	Molinspiration bioactivity score	Interpretation	Property	Molinspiration Score
GPCR ligand	0.05	(>0), it is active	miLogP	3.94
Ion channel modulator	0.03	(>0), it is active	TPSA	52.61
Kinase inhibitor	-0.23	(-5.0-0.0) , moderately active	natoms	25
Nuclear receptor ligand	0.18	(>0), it is active	MW	455.18
Protease inhibitor	0.09	(>0), it is active	nON	4
Enzyme inhibitor	0.11	(>0), it is active	nOHNH	0
			nviolations	0
			nrotb	14
			Volume	384.45

Abbreviations: nviolations, number of violations; natoms, number of atoms; miLog P, molinspiration predicted Log P; MW, molecular weight; nON, number of hydrogen bond acceptors; nOHNH, number of hydrogen bond donors; nrotb, number of rotatable bond.

Bioactivity of the drug can be evaluated by calculating the activity score of G-protein-coupled receptors (GPCR) ligand, kinase inhibitors (KI), nuclear receptor ligands (NRL), ion channel modulators (ICM), protease inhibitors (PI) and enzyme inhibitors (EI) and the results were retrieved as bioactivity scores (**Table 7**). The scores greater than 0.00 indicate high activity, between 0.00 to -0.5 indicate moderate activity and less than -0.5 indicate inactivity [27]. Nuclear receptors (NRs) are significant pharmaceutical targets, since, they are the main regulators of several metabolic and inflammatory diseases, including diabetes, dyslipidemia, cirrhosis and fibrosis [28].

Molinspiration Calculation of Properties for the Lipinsky Rule: Milog P was found below 5 that mean it shows good permeability across cell membrane. Topological polar surface area (TPSA) was 52.61 below 160 Å2. Molecular polar surface area basically calculates the sum of the surface of polar atoms and hence ensures the transport properties of the drug. It is a good descriptor, characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration [29]. The complex was checked for compliance to the Lipinsky rule of five [30], and the results are summarized in **Table 7**. According to Lipinsky rule, a given molecule could be developed as an orally active drug candidate if it does not show more than one violation of the following four criteria. It should not have: more than 5 hydrogen bond donors, more than 10 hydrogen bond acceptors, molecular weight greater than 500 Da, and octanol-water partition coefficient greater than 5 (a parameter used to check the permeability of the molecule across the cell membrane). The complex has not shown any violation of the above criteria. Therefore, it has a good potential for eventual development as oral agents and can be potentially active drug candidates.

MetaPrint2D:

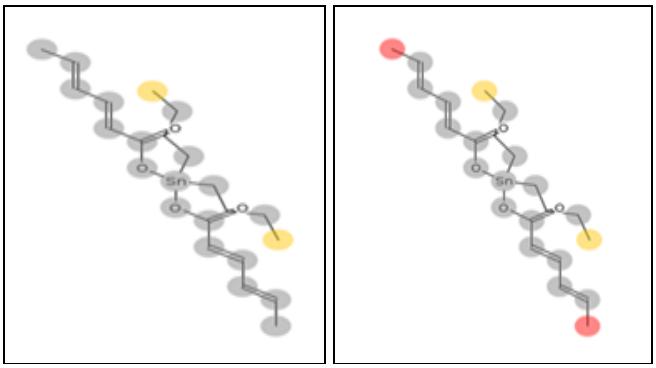

FIG. 6A AND B: METABOLIC PREDICTIONS USING, (A) METAPRINT2 AND (B) METAPRINT2D-REACT

Note: The atom highlighted by different colours indicate the normalized occurrence ratio (NOR). A

larger NOR indicates a more recurrently reported site of metabolism in the metabolite database. Atoms are coloured as per the probability of a metabolic site; high indicated by red (0.66 < = nor < = 1.00) medium by orange (0.33 < = nor < 0.66), low by green (0.15 < = nor < 0.33) very low is not coloured (0.00 < = nor < 0.15), and no data by grey. In **Fig. 6B** red indicates the site of oxidation (=o-oh), hydroxylation and yellow indicates hydroxylation.

***In silico* Pharmacokinetic Properties Study:** The pharmacokinetic properties study was validated by comparing the study with standard reference antibacterial drug chloramphenicol and anti diabetic drug saroglitazar.

TABLE 8: *IN SILICO* PHARMACOKINETIC PROPERTIES STUDY USING PKCSM SOFTWARE

Property	Model Name	Predicted Value Saroglitazar	Predicted Value Chloramphenicol	Predicted Value Organotin complex of 2,4-hexadienoic acid	Unit
Absorption	Water solubility	-5.599	-3.213	-5.869	(log mol/L)
Absorption	Caco-2 permeability	1.106	-0.179	1.218	(log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	94.754	64.75	95.802	(% Absorbed)
Absorption	Skin Permeability	-2.787	-2.752	-2.657	(log Kp)
Absorption	P-glycoprotein substrate	Yes	Yes	Yes	(Yes/No)
Absorption	P-glycoprotein I inhibitor	Yes	No	Yes	(Yes/No)
Absorption	P-glycoprotein II inhibitor	Yes	No	Yes	(Yes/No)
Distribution	VDss (human)	-0.614	-0.595	0.191	(log L/kg)
Distribution	Fraction unbound (human)	0.054	0.391	0.141	(Fu)
Distribution	BBB permeability	-0.121	-1.022	-0.126	(log BB)
Distribution	CNS permeability	-2.269	-3.207	-2.666	(log PS)
Metabolism	CYP2D6 substrate	No	No	No	(Yes/No)
Metabolism	CYP3A4 substrate	Yes	No	No	(Yes/No)
Metabolism	CYP1A2 inhibitor	No	No	No	(Yes/No)
Metabolism	CYP2C19 inhibitor	Yes	No	Yes	(Yes/No)
Metabolism	CYP2C9 inhibitor	Yes	No	Yes	(Yes/No)
Metabolism	CYP2D6 inhibitor	No	No	No	(Yes/No)
Metabolism	CYP3A4 inhibitor	Yes	No	No	(Yes/No)
Excretion	Total Clearance	0.13	0.308	1.913	(log ml/min/kg)
Excretion	Renal OCT2 substrate	No	No	No	(Yes/No)
Toxicity	AMES toxicity	No	Yes	No	(Yes/No)
Toxicity	Max. tolerated dose (human)	0.766	1.262	1.174	(log mg/kg/day)
Toxicity	hERG I inhibitor	No	No	No	(Yes/No)
Toxicity	hERG II inhibitor	Yes	No	Yes	(Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.636	2.682	2.064	(mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	1.495	1.871	1.519	(log mg/kgbw/day)
Toxicity	Hepatotoxicity	Yes	No	No	(Yes/No)
Toxicity	Skin Sensitisation	No	No	No	(Yes/No)
Toxicity	*T. Pyriformis* toxicity	0.742	0.805	1.437	(log ug/L)
Toxicity	Minnow toxicity	-0.325	1.286	-2.045	(log mM)

The complex has shown high Caco-2 permeability (a model used for human intestinal absorption of drugs and other compounds), the predicted value was greater than 0.9010^{-6} cm/s, therefore is readily permeable. Intestine is generally the main site for absorption of drug from an orally administered solution. The predicted value for the compound absorbed through the human intestine was 95.802%

hence; the drug could be effortlessly absorbed or assimilated through human intestine [31]. P-glycoprotein (P-gp) acts as a physiological barrier by ejecting toxins and other xenobiotics out of cells to go through additional metabolism and clearance [32] resulting in failure of therapeutic potency of the drug because its concentration would be lowered than expected. Substrates of P-glycoprotein act as inhibitors or inducers of its function. Inhibition of P-glycoprotein results in better bioavailability of the liable drug.

The complex was found to be P- glycoprotein substrate and inhibitor. Inhibition of cytochrome P450 iso-forms results in drug-drug interactions where the co-administered drugs fail to be metabolized and thus enhance the plasma concentrations of the medication to toxic levels. Fortunately, any acute toxicity and mutagenic effect of the complex synthesized was not observed with respect to the AMES test data. A positive test shows that the compound is mutagenic and might be carcinogenic. The complex gave negative result in AMES toxicity test and also found to be a non-carcinogenic agent in carcinogenicity test.

CONCLUSION: On the basis of the above findings in the present research work, the novel complex was found to be a good antimicrobial agent *in vitro* [against *Staphylococcus aureus* (gram positive) and *Escherichia coli* 1610 (gram negative)] and *in silico* studies (*Escherichia coli, Yersinia enterocolitica*, *Burkholderia pseudomallei* and Human cytomegalovirus). The novel complex was also found to be a potent PPAR γ agonist having the potential to activate the PPAR γ.

Further *in vitro* studies of the *in silico* studies carried out in the present research work will lead to a new pathway to a novel multi-target drug discovery. The permeability properties for all compounds were found within the limit range stated for Lipinski′s rule of five hence, it has the prospective for ultimate development as oral agents.

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