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Docking Study of Stannane of Menthol as Human Peroxisome Proliferator Activated Receptor Gamma (PPAR γ) Agonists

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Abstract—*The pharmaceutical industry is paying more attention to the research of PPAR agonists, as it controls the gene expression of different diseases like cancer, diabetes, atherosclerosis and obesity. The main aim of the present research work is to synthesis stannane of menthol. It was further characterized by spectroscopic techniques, like UV–Visible and ^1H NMR method. Molecular docking was carried out using computational software iGemDock v2.1. Effect of complex on glycaemic and lipid parameter was studied using Protein Data Bank (PDB) files (4HEE, 2XKW, 3FEI and 2Q6S). The result was analyzed based on the interaction of Hydrogen bond, van der Waal interaction and interacting residues and the binding energy. The better interaction was chosen based on the minimum binding energy with amino acid residue at the receptors' active site. Further, pharmacokinetic study was carried out using admetSAR software, the complex has the potential to cross Blood Brain Barrier, pass human intestine, $CaCO_2$ permeable, Non AMES toxic and non-carcinogenic. The pharmacokinetic properties study was validated by comparing the study with standard reference anti-diabetic drug 'Saroglitazar'. The result shows that the compound could be agonist potential to activate the PPAR gamma.*

Keywords*: PPARgamma, stannane, molecular docking, pharmacokinetic properties.*

1. INTRODUCTION

Peroxisome Proliferator Activated Receptors (PPARs) represent a family of ligand-activated nuclear hormone receptors (NRs) belonging to the steroid receptor superfamily that regulate the gene expression of proteins involved in energy, glucose and lipid metabolism, the proliferation and differentiation of adipocytes and the sensitivity of insulin [1]. They function as cellular sensors that activate transcription in response to the binding of natural or synthetic ligands. Binding of the ligands results in conformational changes of the receptors that facilitate their interaction with coactivator proteins in the nucleus [2].The resulting protein complexes activate the transcription of specific target genes, resulting in the induction of intracellular signalling cascades that mediate the physiological effects of the ligands.

They exist in three is forms: PPAR α, PPAR β/δ and PPAR γ. PAR α mainly influences fatty acid metabolism and its activation lowers lipid levels, while PPAR γ is mostly involved in the regulation of the adipogenesis, energy balance, and lipid biosynthesis. PPAR β/δ participates in fatty acid oxidation, mostly in skeletal and cardiac muscles, but it also regulates blood glucose and cholesterol levels. Many ligands bind with PPAR gamma and activates it are called agonists. Peroxisome proliferator activated receptor (PPAR) agonists favourably influence glycaemic and lipid parameters in patients with Type 2 diabetes and a dual PPAR agonist is expected to have favourable effect on both parameters The activation of PPAR gamma by ligands may be responsible for inhibiting the growth of cultured human breast, gastric, lung, prostate and other cancer cell lines.Recent research suggests that PPAR γ has a therapeutic potential to treat inflammatory diseases and certain cancers.[3] Agonists of PPARα and PPARγ are currently approved for use in treating dyslipidemia and T2DM, respectively.[4]

2. MATERIAL AND METHOD

2.1 Material

Analytical Grade chemicals and solvents were used for carrying out synthesis work. In silico study was carried out using software iGEMDOCK at ARSD College, University of Delhi.

2.2 Experimental method

Synthesis of stannane of menthol was done by using aziotropic removal of water in benzene-ethanol medium in a 6 hours reaction time.[5] After synthesis the complex was

characterized by UV visible spectra studies and ^{1}H NMR spectral. The characteristic peaks observed confirmed the complex formation.

2.3 Molecular docking using software (IGEMDOCK)

2.3.1 Preparation of Binding site.

A literature survey was done for the selection of Binding site. All the protein structure files were acquired from Protein Data Bank (http://www.rcsb.org/) (PDB ID: 4HEE, 2XKW, 3FEI and 2Q6S).The best binding pocket was selected considering the site score and site's hydrophobic/hydrophilic areas, which bears better cavity.

2.3.2 Ligand Preparation and Docking.

The structure of stannane of menthol 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. The ligand file in CDX (compound index) format was converted into MOL 2 format by Openbabel software. Docking with each PDB(Protein Data Bank)files was done using accurate docking function (slow docking) [6]. Lastly, the post analysis 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 done in this work by first hydrogen, second side chains and finally the backbone of the receptor [7].After that, the optimized complexes were then checked for various interaction of ligand with receptor like hydrogen bonding, hydrophobic bonding and van der Waal's interaction.

2.4 In Silico Pharmacokinetic Properties Study

ADMET (Absorption, Distribution, Metabolism and Toxicity) properties was calculated using admetSAR database which provides latest and most inclusive manually created data for various chemicals with known ADMET properties. [6,8]

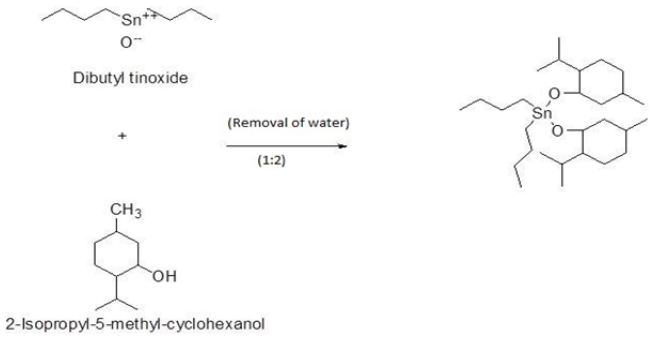

Fig. 1: Synthesis of stannane of menthod.

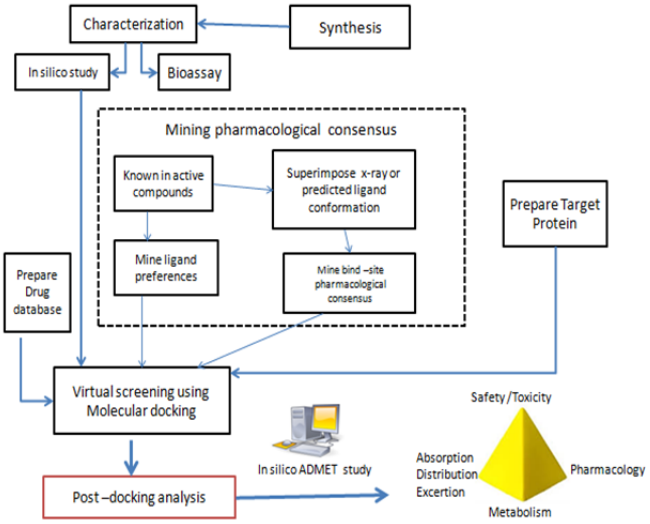

Fig. 2: Schematic representation of methodology followed.

3. RESULTS AND DISCUSSION

3.1 Physical study

A white coloured viscous semisolid soluble in Dimethyl Sulphoxide (DMSO) and ethanol with molecular weight 455.18 g mol^{-1}was obtained in good yield (0.4457 g), 82%.

3.2 UV absorption spectra

The λ_{max} for ligand (menthol) was found to be 220 nm and absorbance 0.010000 which is generally assigned to π-π* transition. The λ_{max} of stannane of menthol was recorded at 249 nm and absorbance 1.150000. The shift in the peak from 220 nm to 249 nm (Redshift) is attributed to the n-π^* transition transition which indicates the ligand metal charge transfer (LCMT).That corresponds to coordination or attaching of ligand with the dibutyl tin oxide. Shifting of the UV peaks to higher wavelength shows 'Bathochromic shift or Red shift'. [9]

^{1}H NMR spectra: The complex showed resonance signals (ppm):3.16 (-CH, Cyclohexane), 1.50 (-CH cyclohexane), 1.61 (-CH, cyclohexane), 1.68, 1.42 (-CH_2, cyclohexane), 1.52, 1.27 (-CH_2, cyclohexane), 1.3 (-CH_2, methylene), 1.82 (-CH, methane), 0.96 (-CH_3, methyl), 1.26 (-CH_2, methyl), 1.31 (-CH_2, methyl), 0.91 (-CH_3, methyl). The absence of -OH proton signal in the 1H NMR spectra of the organotin (IV) complexes indicated that the phenolic oxygen is coordinated to the Sn (IV) atom after deprotonation.

Molecular Docking

Table 1: Summary of Total energy, Vander-Waal interaction, Hydrogen bonding, electrostatic energy of stannane of menthol on interaction with PDB files.

PDB	Total Energy (kcal/mol)	Vander-Waal (kcal/mol)	Hydrogen Bond (kcal/mol)	AverCon Pair (kcal/mol)
2Q6S	-52.4618	-49.672	-2.78957	13.2581
3FEI	-63.5563	-59.767	-3.78981	20.9677
2XKW	-68.0185	-68.019	0	16.3548
4HEE	-68.1339	-68.134	0	15.5161

Table 2: Pharmacological interactions and Residues involved in the binding site.

PDB 2Q6S		PDB 2XKW	
Amino acid residue (predicted pharmacologic interactions)	**Energy (kcal/mol)**	**Amino acid residue (predicted pharmacologic interactions)**	**Energy (kcal/mol)**
H-M-ILE-262	-2.78957	H-M-SER-342	0
H-M-PHE-264	0	V-M-GLY-258	-5.8704
V-S-ILE-262	-6.01871	V-M-GLY-284	-1.51698
V-M-PHE-264	0	V-S-PHE-287	-0.174541
V-S-PHE-264	-0.0056407	V-S-ILE-341	-6.33265
PDB 3FEI		PDB 4HEE	
Amino acid residue (predicted pharmacologic interactions)	Energy (kcal/mol)	Amino acid residue (predicted pharmacologic interactions)	Energy (kcal/mol)
H-S-THR-279	-2.33464	V-M-GLY-284	-3.11946
V-M-CYS-275	-5.97957	V-M-PHE-287	-4.52837
V-M-CYS-276	-4.0012	V-S-PHE-287	-7.36631
V-M-THR-279	-4.25968	V-M-ARG-288	-3.52578
V-S-THR-279	4.22423	V-S-ARG-288	-4.57842
V-M-VAL-332	-5.22494	V-M-ILE-341	-0.583597
V-S-TYR-334	-10.2597	V-S-ILE-341	-3.92817
		V-S-GLU-343	-1.19622

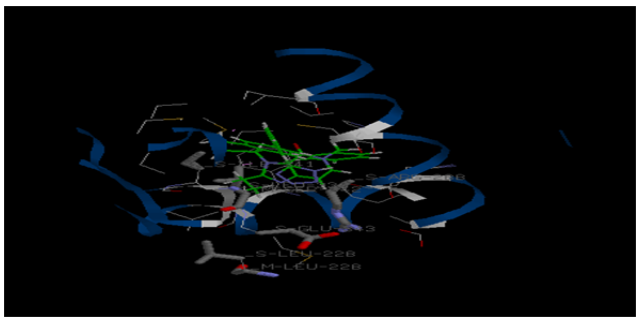

Fig. 3A: Docking pose with PDB 4HEE

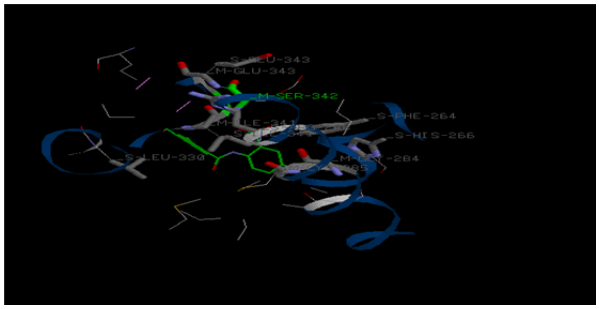

Fig. 3B. Docking pose with PDB 2Q6S

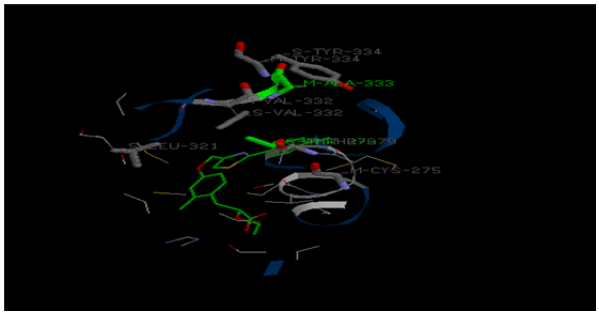

Fig. 3C. Docking pose with PDB 3FEI

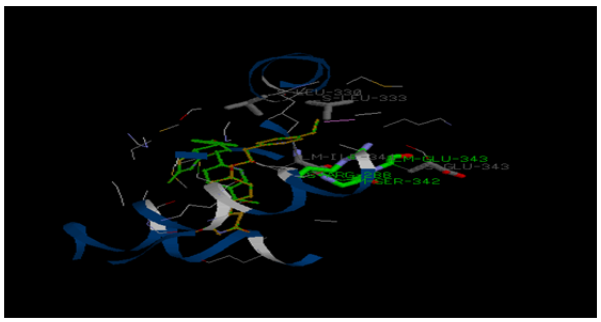

Fig. 3D. Docking pose with PDB 2XKW

Fig. 3A to D. Docking pose of stannane of menthol with PDB files , showing interaction of ligand binding with different amino acid of the receptor.*

*The green and grey colour represents the amino acids involved in(H)hydrogen bonding and(V) van der Waals are interaction types M and S are main chain and side chain respectively.

3.3 Interaction profile

3.3.1 PDB 2Q6S stands for 2.4 angstrom crystal structure of PPAR gamma complexed to BVT.13 without co-activator peptides

The complex interacted with the PDB file with total fitness value of -52.4618kcal/mol, which comprises of van der waal interaction energy value of -49.672 kcal/mol and Hydrogen

bonding with energy value -2.78957 kcal/mol. The stannane of menthol interacted with basic amino acid residue:

Hydrogen bonding with main chain of Isoleucine at position 262 with binding energy value -2.78957 kcal/mol and van der waal interaction with side chain of Isoleucine at position 262 and Phenylalanine at position 264 with binding energy value -6.01871kcal/mol and -0.00564077 kcal/mol respectively.

3.3.2 PDB 3FEI stands for Design and biological evaluation of novel, balanced dual PPARa/g agonists

The complex interacted with the PDB file with total fitness value of -63.5563 kcal/mol which comprises van der waal interaction energy value of -59.767 kcal/mol and Hydrogen bonding with energy value -3.78981 kcal/mol. The stannane of menthol interacted with basic amino acid residue:

Hydrogen bonding with side chain of Threonine at position 279 with binding energy value -2.33464 kcal/mol. Van der waal interaction with main chain of Cysteine at position 276 and 275 with binding energy value of -4.0012 kcal/mol and -5.97957 kcal/mol. Van der waal interaction with side chain of Tryosine at position 334 with binding energy value -10.2597kcal/mol and main chain of Valine at position 332 with binding energy value -5.22494 kcal/mol, Threonine at position 279 with binding energy value of -4.25968 kcal/mol.

3.3.3 PDB 2XKW stands for ligand binding domain of human PPAR-gamma in complex with the agonist pioglitazone.

The complex interacted with the PDB file with total fitness Value -68.0185 kcal/mol which comprises mainly of Van der waal interaction. The stannane of menthol interacted with basic amino acid residue:

Vander waal interaction with side chain of Isoleucine at position 341 with binding energy value -6.33265 kcal/mol and Phenylalanine at position 287 with binding energy value -0.174541 kcal/mol. Vander waal interaction with main chain of Glycine at position 258 and 284 with binding energy -5.8704 kcal/mo l and -1.51698 kcal/mol.

3.3.4 PDB 4HEE stands for Crystal structure of PPARgamma in complex with imidazo[4,5-c]pyridin-4-one derivatives.

The complex has shown binding energy of -68.1339 kcal/mol which comprises mainly of Van der waal interaction. Vander waal interaction with main chain of:

Glycine at position 284 with binding energy value -3.11946 kcal/mol, Phenylalanine at position 287 with binding energy value -4.52837 kcal/mol, Arginine at position 288 with binding energy value -3.52578 kcal/mol, Isoleucine at position 341 with binding energy value -0.583597 kcal/mol

Vander waal interaction with side chain of: Glutamic acid at position 343 with binding energy value -1.19622 kcal/mol, Arginine at position 288 with binding energy value -4.57842 kcal/mol, Phenylalanine at position 287 with binding energy value -7.36631kcal/mol.

3.4 In silico pharmacokinetic properties study

The pharmacokinetic properties study was validated by comparative study of stannane of menthol with standard reference drug Saroglitazar.[10]

Table 3: ADMET Predicted Profile-Classification

Model	Result Saroglitazar	Probability	Stannane of Menthol	Probability
	(Reference drug)	Saroglitazar		Stannane of Menthol
Absorption				
Blood-Brain Barrier	BBB+	0.6706	BBB+	0.6307
Human Intestinal	HIA+	0.9616	HIA+	0.7500
Absorption				
$CaCo_2$-Permeability	Caco2-	0.5148	Caco2+	0.5971
P-glycoprotein Substrate	Substrate	0.6179	Substrate	0.7404
P-glycoprotein Inhibitor	Non-inhibitor	0.5847	Inhibitor	0.5076
	Inhibitor	0.8061	Non-inhibitor	0.9508
Renal Organic Cation	Inhibitor	0.5297	Non-inhibitor	0.8669
Transporter				
Distribution Metabolism				
CYP450 2C9 Substrate	Non-substrate	0.6579	Non-substrate	0.7689
CYP450 2D6 Substrate	Non-substrate	0.7014	Non-substrate	0.7908
CYP450 3A4 Substrate	Substrate	0.6618	Substrate	0.5000
CYP450 1A2 Inhibitor	Non-inhibitor	0.6333	Non-inhibitor	0.7530
CYP450 2C9 Inhibitor	Non-inhibitor	0.5448	Non-inhibitor	0.7921
CYP450 2D6 Inhibitor	Non-inhibitor	0.6880	Non-inhibitor	0.8712
CYP450 2C19 Inhibitor	Inhibitor	0.5651	Non-inhibitor	0.7538
CYP450 3A4 Inhibitor	Inhibitor	0.9171	Non-inhibitor	0.6270
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity	0.9249	Low CYP Inhibitory	0.8807

			Promiscuity	
Excretion Toxicity				
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9876	Weak inhibitor	0.6433
	Inhibitor	0.6975	Inhibitor	0.6671
AMES Toxicity	Non AMES toxic	0.7719	Non AMES toxic	0.6875
Carcinogens	Non-carcinogens	0.9157	Non-carcinogens	0.8676
Fish Toxicity	High FHMT	0.9815	High FHMT	0.9884
Tetrahymena Pyriformis Toxicity	High TPT	0.9371	High TPT	0.9937
Honey Bee Toxicity	Low HBT	0.6087	High HBT	0.5782
Biodegradation	Not ready biodegradable	0.9855	Not ready biodegradable	0.9888
Acute Oral Toxicity	III	0.6567	Ill	0.5255
Carcinogenicity (Three-class)	Non-required	0.5829	Non-required	0.5759

Table 4: ADMET predicted profile-regression

Model	Value Saroglitazar	Value Stannane of Menthol	Unit
Absorption			
Aqueous solubility	-3.5562	-4.6528	LogS
$CaCO_2$ Permeability	0.7173	1.2426	LogPapp, cm/s
Rat Acute Toxicity	2.5853	2.0812	LD50, mol/kg
Fish Toxicity	1.2694	0.9598	pLC50, mg/L
Tetrahymena Pyriformis Toxicity	0.5431	0.9022	pIGC50, ug/

$CaCO_2$ permeability: The Complex has shown positive result in CaCO2 permeability which shows that it is readily permeable. $CaCO_2$ cells are a human colon epithelial cancer cell line generally used as a model for human intestinal assimilation of drugs and other compounds.

Human intestinal absorption: Intestine is normally the primary site for absorption of drug from an orally administered solution. The complex has shown positive result, which shows that it, could be absorbed or assimilated through human intestine.

P-glycoprotein substrate: P-glycoprotein (P-gp) is one among the first members of the ATP-Binding Cassette (ABC) transporter which acts as a physiological barrier by ejecting toxins and xenobiotics out of cells it limits the bioavailability of orally administered drugs by pumping them back into the lumen .Drugs which induce or inhibit P-glycoprotein can interact with other drugs handled by the pump. Substrates of P-glycoprotein can potentially act as inhibitors or inducers of its function. Inhibition of P-glycoprotein can result in increased bioavailability of the susceptible drug. Induction of P-glycoprotein reduces the bioavailability. The complex was found to be P- glycoprotein substrate and non-inhibitor.

Renal organic cation transporter (OCT): The complex was found to be non-inhibitor of Renal Organic Cation Transporter.

Blood brain barrier (BBB) permeability: The brain is safeguarded from exogenous compounds by the BBB. The potential of a drug to cross into brain is an essential parameter to consider which help to reduce the side effects and toxicities or to improve the efficacy of drugs whose pharmacological activity is within the brain. The complex has shown positive result hence it can easily cross the blood–brain barrier. The result generated by admet SAR database was further validated by using BBB predictor (http://www.cbligand.org/BBB/predictor.php).

Cytochrome P450 inhibitors: Inhibitors could affect drug metabolism and are contraindicated. Hence it is important to access a compound's ability to inhibit the Cytochrome P450. It is important to identify whether a new drug is a substrate, inducer or inhibitor in drug development. Drugs that inhibit will predictably increase the plasma concentrations of the medication and in some cases adverse outcomes will occur.

AMES toxicity: 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 .In some toxicity models, some negative results were recorded (regression profiles) indicates that they have very low probability values, Fish Toxicity, Tetrahymena Pyriformis Toxicity and Honey Bee Toxicity were reported since complex contains central metal atom which is Tin.

Human ether-a-go-go related gene (hERG): Potassium (K+) channels play a vital role in cardiac action potential repolarization. Mutations could reduce hERG conductance or surface expression and it results in congenital fatal long QT syndrome (LQTS), this may cause loss of hERG function. The complex was found to be non-inhibitor for Human Ether-a-go-go-Related Gene.

4. CONCLUSION

On comparing the docking results of all the PDB files used it was found the complex interacted mainly with the basic amino acid residues like Glycine, Phenyl alanine Lysine and Isoleucine. The compound has shown affinity for PPARgamma. The highest negative value indicated that, the complex may have good affinity. Hence the compound can be

used as a novel PPARγ agonist and therapeutic agent for the cancers but further research is needed to formulate it as a drug.

5. ACKNOWLEDGEMENT

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