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EFFECT OF GLY 16 ARG SINGLE NUCLEOTIDE POLYMORPHISM ON AGONIST BINDING TO BETA 2 ADRENERGIC RECEPTOR – A STRUCTURAL PHARMACOGENOMIC APPROACH

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Keywords:

β_2 sympathomimetics, Gly16Arg, Single nucleotide polymorphism, molecular docking

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ABSTRACT: Asthma is obstructive disease characterized by bronchoconstriction and inflammation for which beta 2 (β_2) agonists that act through the β_2 adrenoceptor are the first line agents of choice. Glycine16Arginine (Gly16Arg) is a common single nucleotide polymorphism (SNP) of β_2 adrenoceptor whose effects on treatment response remain inconclusive. Hence the study aims to understand and determine the effect of the Gly16Arg SNP on β_2-agonist binding and treatment responsiveness. Structure guided mutagenesis was performed with discovery studio tool, energy minimization was performed using Chemistry at Harvard Macromolecular Mechanics(CHARMM) force field, protein confirmation was studied using Ramachandran plot, Molecular docking analysis was performed using Autodock 4.2 and statistical analysis was performed using Graph pad prism 6.0. Statistically significant difference was observed between the binding energies of Glycine16 (Gly16) and Arginine16 (Arg16) groups. Further, the binding energies of β_2 agonists were found to be comparatively less in Arg16 group than the Gly16 group suggesting that the Arg16 variant carriers may be poor responders of β_2 – sympathomimetic therapy. Presence of functional non synonymous single nucleotide polymorphisms in the β_2 adrenoceptor significantly alters $\beta_2$sympathomimetic binding. Patients with Arg16 variant may therefore be poor or non-responders of conventional bronchodilator therapy.

INTRODUCTION: Asthma is a chronic pulmonary disease characterized by inflammation and hyper responsiveness of the tracheobronchial smooth muscle resulting in obstruction of the lower respiratory tract and structural changes such as airway remodeling. Obstruction of the airways in asthma occurs due to bronchospasm, inflammation and mucus formation [1, 2].

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Bronchoconstriction occurs as a result of airway smooth muscle contraction and mucus secretion from the sub mucosal glands caused by increased parasympathetic tone [3]. Hence bronchodilators play the central role in the symptomatic management of acute as well as chronic asthmatic episodes. The bronchodilators are generally β_2-adrenergic agonists that cause smooth muscle relaxation, resulting in dilation of bronchial passages [4].

The human β_2 adrenoceptor is a 413 amino acid G-protein coupled receptor encoded by the adrenergic receptor beta 2 (ADRB2) intronless genes located on chromosome 5q31.32[5]. Binding of β_2-sympathomimetics to the extracellular ligand binding domain causes displacement of

boundguanosine diphosphate (GDP) by guanosine triphosphate (GTP) followed by the dissociation of the α-subunit from the βγ dimer. The separated α subunit activates the effector, adenyl cyclase resulting in intracellular accumulation of cyclic adenosine monophosphate (cAMP). Increased cAMP formation in the bronchial smooth muscle cell results in relaxation through cAMP-dependent protein kinase a mediated phosphorylation [6]. The β_2-adrenergic agonists used are broadly classified as short acting and long acting β_2 agonists on the basis of their duration of action. The basic structural unit of the β_2 agonists is a benzene nucleus attached to an ethyl amine group. For instance, isoprenaline is a synthetic catecholamine whereas salbutamol and terbutaline are non – catecholamine derivatives and structural changes in these ligands renders longer halve lives by conferring resistance to catechol-o-methyl transferases [7, 8].

The common bronchodilators used include Salbutamol, Levosalbutamol, Terbutaline, Isoprenaline, Procaterol etc. Variations in bronchodilator response have been reported due to several single nucleotide polymorphisms associated with the ADRB2 gene. Pharmacogenetic screens have identified approximately more than 50 SNPs within the coding and promoter regions of ADRB2, some of which are known to alter response to β_2-adrenergic agonists [9-11]. The common non synonymous SNPs of the β_2 adrenoceptor that tend to alter the protein conformation are Gly16Arg, Glutamate 27 Glutamine (Glu27Gln), Valine 34 Methionine (Val34Met) and Theonine 164 Isoleucine (Thr164Ile). The most prominent coding SNP is Gly16Arg and Gly16 receptor has been reported to be associated with increased agonist-promoted down regulation than the Arg16 variant, but there is discrepancy in the existing data [12-14].

A few initial studies have reported that Arg16 homozygotes have increased bronchodilator response to short acting β_2-agonist than Gly16 homozygotes[15-17]. However, further studies have reported the Gly16 allele to be associated with increased bronchodilator response while a few studies have reported no significant association between Gly16Arg functional SNP and drug responsiveness. Thus the results remain inconclusive [18-21]. Hence, in order to understand and determine the effect of the Gly16Arg SNP on β_2-agonist binding and responsiveness, a receptor – ligand interaction study was carried out with both the wild type and Arg16Gly mutant variants of β_2 adrenergic receptor.

MATERIALS AND METHODS:

Retrieval of Crystal Structure and Target Preparation:

The high resolution crystal structure of human β_2-adrenergic G protein coupled receptor was retrieved from the protein data bank. FASTA sequence of the retrieved structure revealed the presence of glycine at position 16 [22]. Hence the Arg16 variant was prepared by structure guided mutagenesis using the Discovery Studio tool. Both the wild type and mutant targets were pre-processed by standard methods prior to binding analysis.

Energy minimization:

CHARMM is a general and flexible program for macromolecular energy minimization and dynamics calculations that utilizes both classical and quantum mechanical energy functions for molecular systems [23]. Energy minimization of both the wildtype and mutant targets were carried out under CHARMM27 force field. Gradient was set to 0.05.

Structural Assessment of Proteins:

Three dimensional structure and conformational stability of the protein was analyzed by means of Ramachandran plot. Ramachandran plot analysis was carried out individually for the energy minimized Gly16 and Arg16 receptors.

Active site prediction:

The ligand binding domain of the human beta-2 adrenergic receptor and its variant bearing the Gly16Arg single nucleotide polymorphism were individually predicted using the Site Finder module of Molecular Operating Environment.

Molecular Docking Analysis:

In order to understand the difference in binding conformation and affinity, salbutamol was individually docked to both the wild type and mutant variants of human beta 2 adrenergic

receptor. Autodock 4.2 tool was used for molecular docking analysis. Both the receptor and ligands were prepared by addition of hydrogen's and gasteiger charges. A grid defining the active site was constructed before running the docking simulation. Genetic algorithm was adopted for conformer search while docking [24].

Statistical Analysis:

Presence of statistically significant difference between the binding affinities of β_2 - agonists with wild type Gly16 and mutant Arg16 variants groups was analyzed using Graph pad Prism 6.0 statistics package. A two sample paired t – test was carried out at 95%percentile confidence interval.

RESULTS AND DISCUSSION:

Energy minimization of the Gly16 and Arg16 variants was carried out. The initial and post minimization potential energies of both the allelic variants are listed in **Table 1**.

TABLE 1: ENERGY MINIMIZATION OF GLY16 AND ARG16 VARIANTS

S. No	Type of Variant	Pre-Minimization Potential Energy	Post-Minimization Potential Energy
1	Gly16 variant	5150.8530 kcal/mol	-744.0501 kcal/mol
2	Arg16 variant	5246.0517 kcal/mol	-918.6189 kcal/mol

* Force field: CHARMM27, Gradient: 0.05, H and LP adjusted

The conformation and stability of the Gly16 and Arg16 variants were analyzed in terms of their dihedral angles phi and psi using a ramachandran plot. A typical ramachandran plot consists of a favored, allowed and disallowed region. The ramachandran plots of Gly16 and Arg16 variants are shown in **Fig. 1** and **2** respectively.

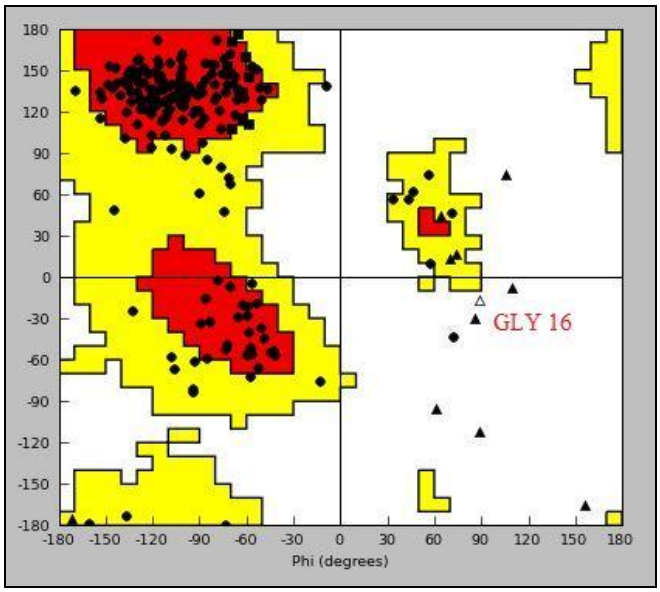

FIG. 1: RAMACHANDRAN PLOT OF GLY16 VARIANT

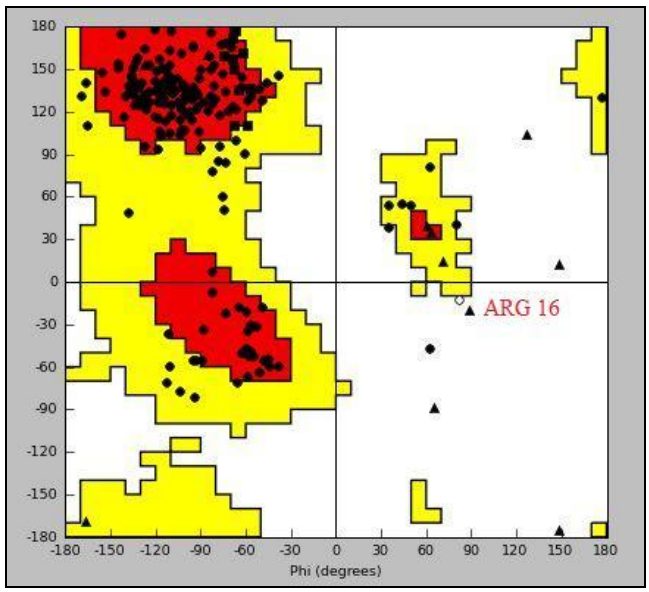

FIG. 2: RAMACHANDRAN PLOT OF ARG16 VARIANT

A shift in the position of the residues and difference in dihedral angles is noted between the proteins bearing glycine and arginine at position 16 respectively. The difference in the amino acid pattern as observed in the ramachandran plot is suggestive of Arg16 single nucleotide polymorphism to be non-synonymous that alters the conformation of the wild type variant. Thus the ligand binding domain of the ADRB2 gene product is altered and β_2 – agonist binding to the altered active site is ultimately affected. This finding correlates with the results of the active site analysis. The active site residues of the gly16 and arg16 variants differ to a considerable extent suggesting a conformational difference of the ligand binding region. The active site residues of the two variants are listed in **Table 2.**

TABLE 2: ACTIVE SITE ANALYSIS OF GLY16 AND ARG16 VARIANT TARGETS

S. No	Type of Variant	Active Site Residues
1	Gly16 variant	GLN37 GLN38 LYS39 LYS42 SER43 PRO44 LYS45 LEU47 VAL58 PRO59 ARG61 PHE62 ILE75 ALA81 ASP82 TYR86
2	Arg16 variant	GLN37 GLN38 LYS39 SER43 PRO44 LYS45 LEU47 GLY57 VAL58 PRO59 ARG61 PHE62 ALA81 ASP82 TYR86

The primary active site of the gly16 variant is formed of sixteen amino acids whereas that of arg16 variant is formed only of fifteen amino acids. The active site of arg16 variant lacks Lys42, Ile75 and instead bears Gly57.

To gain better insight for the interactions between β_2-sympathomimetics and the considered dual variants of the $\beta_2$adrenergic receptor, molecular docking studies were carried out. The interactions of the ligands with the active site residues of the target are analyzed in terms of the following parameters: Binding energy, number of hydrogen bonds established by the ligand with residues of the active site, π-π interactions, conformation oriented by the ligand within the active site and root mean square deviation (RMSD) of the active site residues. The dock score of Autodock is reported in kcal/mol. Autodock uses the following empirical formula to calculate the free energy of binding:

Binding energy (ΔG) = Intermolecular energy + Vanderwaal's hydrogen bond desolvation energy (VHBDE) + Electrostatic energy + Total internal energy + Torsional energy – Unbound energy of the system.

Desolvation energy is a prime parameter that decides a molecules interaction with its pharmacodynamic target. In the biological environment, all drug binding pockets of a target protein remain solvated and hence a ligand cannot as such occupy the active site unless it dislodges the water molecules. The similarity of docked structures is measured by computing the root mean square deviation and clusters are created based on the comparison of conformations and estimated RMSD values. The docking score of selected ligands with the Gly16 variant and Arg16 variant receptors are shown in **Table 3** and **4** respectively.

TABLE 3: MOLECULAR DOCKING ANALYSIS OF β_2– AGONISTS WITH GLY16 RECEPTOR

Drug	ΔG (kcal/mol)	kI (mM)	Intermolecular Energy	VHBDE	Electrostatic Energy	Total Internal Energy	Torsional Energy
Salbutamol	-3.97	1.23	-6.35	-6.62	0.26	-1.17	2.39
Formeterol	-4.13	0.94	-7.41	-7.83	0.42	-0.73	3.28
Isoprenaline	-4.25	0.76	-6.34	-6.53	0.19	-0.23	2.09
Terbutaline	-3.67	2.05	-5.75	-5.9	0.15	-0.01	2.09
Procaterol	-5.22	0.14	-7.31	-7.56	0.26	-0.13	2.09
Salmeterol	-1.67	59.24	-7.34	-7.71	0.37	-1.03	5.67

TABLE 4: MOLECULAR DOCKING ANALYSIS OF β_2– AGONISTS WITH ARG 16 RECEPTOR

Drug	ΔG (kcal/mol)	kI (mM)	Intermolecular Energy	VHBDE	Electrostatic Energy	Total Internal Energy	Torsional Energy
Salbutamol	-3.61	2.27	-5.59	-6.52	0.53	-0.6	2.39
Formeterol	-2.72	10.13	-6.0	-6.55	0.55	-0.95	3.28
Isoprenaline	-3.46	2.91	-5.55	-5.62	0.07	-047	2.09
Terbutaline	-3.04	5.88	-5.13	-5.76	0.63	-0.49	2.09
Procaterol	-2.87	7.93	-4.95	-5.55	0.6	-0.66	2.09
Salmeterol	1.0	N/A	-4.67	-4.85	0.18	0.09	5.67

*N/A = Not applicable. kI of salmeterol was not calculated since the free binding energy of salmeterol was found to be +1.0

The hydrogen bonding interactions established by the ligands with the active site of both Gly16 and Arg16 receptors are shown in **Table 5**. The number of hydrogen bonds and the residues involved are significantly different between the two groups due to the conformational change induced by the non-synonymous single nucleotide polymorphism.

TABLE 5: HYDROGEN BONDING INTERACTIONS OF β_2 – AGONISTS WITH GLY AND ARG 16 RECEPTORS

S. No	Drug	Gly16 variant		Arg16 variant	
		No. of H-bonds	Residues	No. of H-bonds	Residues
1.	Salbutamol	2	Ser 43, Lys 45	4	Gln 37, Gly57
2.	Formeterol	1	Ser 43	2	Gly57, Arg 61
3.	Isoprenaline	2	Ser 43	3	Ser 43, Lys 45, Gln 37
4.	Terbutaline	2	Gln 37, Ser 43	1	Gln37
5.	Procaterol	3	Ser 43, Lys 45	0	-
6.	Salmeterol	1	Ser 43	1	Gln 37

Statistically significant difference was observed between the binding energies of Gly16 and Arg16 groups. A p-value of 0.017 was observed at a confidence interval of 95% which clearly indicates that presence of Arg16 single nucleotide polymorphism alters β_2 – sympathomimetic binding and activity. Further, the binding energies of β_2 agonists were found to be comparatively less in Arg16 group than the Gly16 group suggesting that the Arg16 variant carriers may be poor responders of β_2 – sympathomimetic therapy.

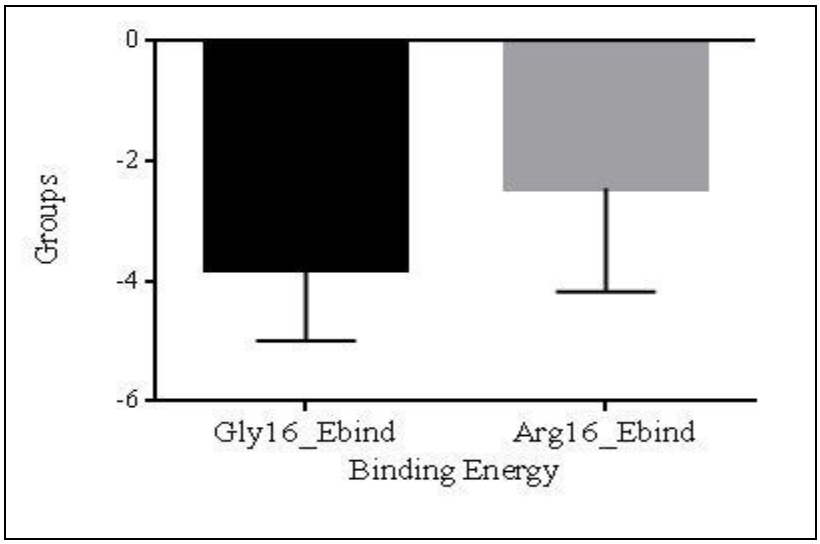

FIG. 3: PAIRED t-TEST PLOT (p value <0.05, CI=95%)

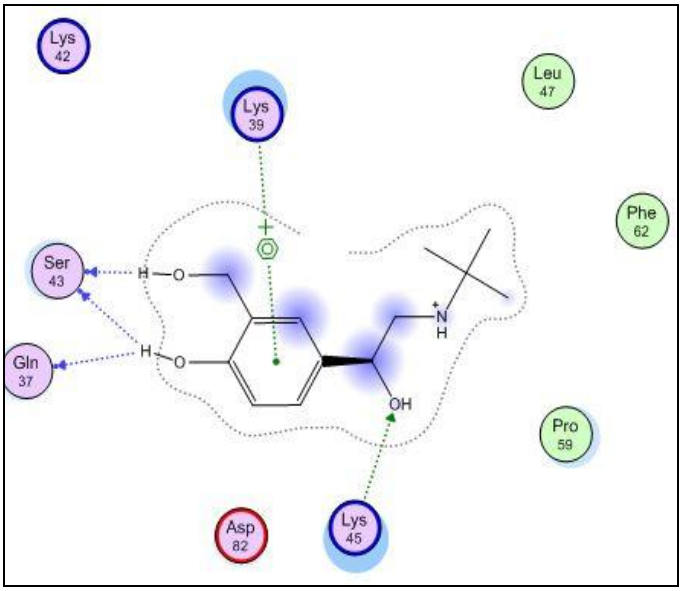

FIG. 4a: SALBUTAMOL WITH Gly 16 VARIANT

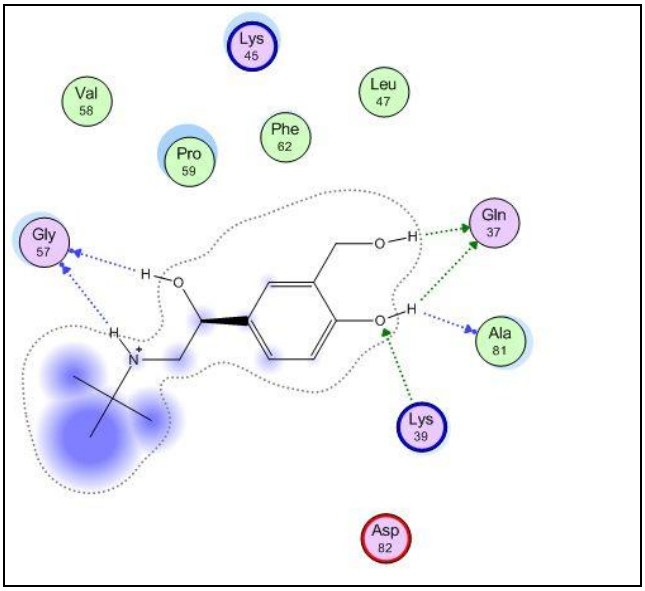

FIG 4b: SALBUTAMOL WITH Arg16 VARIANT

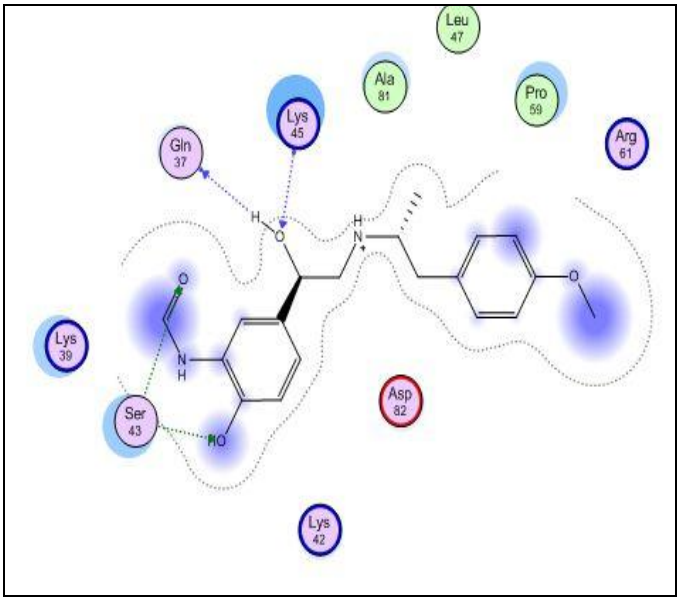

FIG 4c: FORMETEROL WITH Gly16 VARIANT

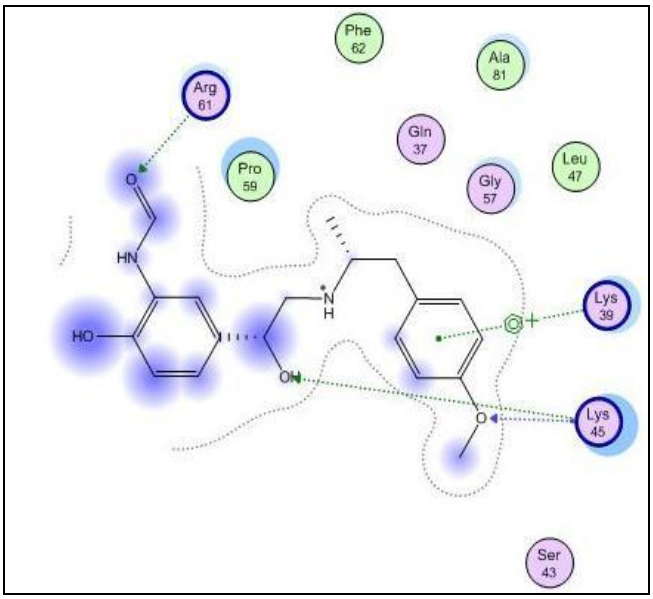

FIG. 4d: FORMETEROL WITH Arg16 VARIANT

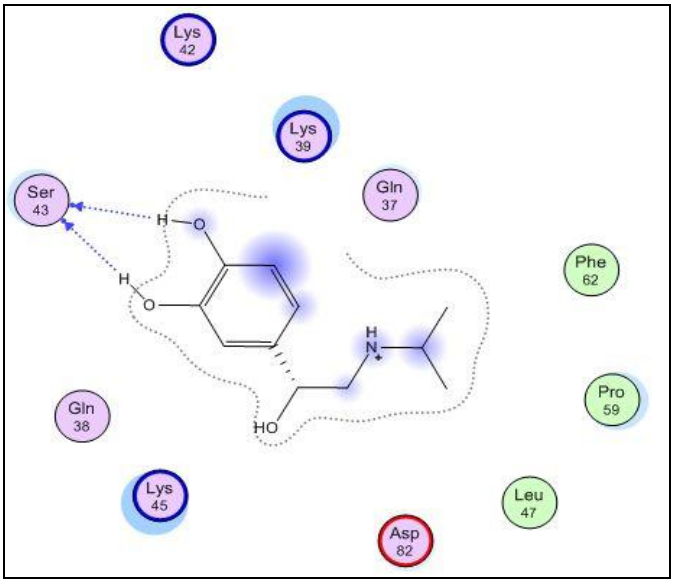

FIG 4e: ISOPRENALINE WITH Gly16 VARIANT

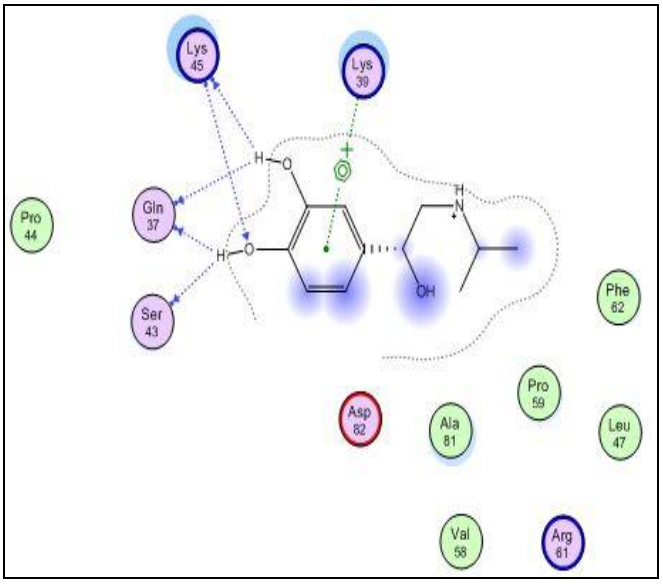

FIG 4f: ISOPRENALINE WITH Arg16 VARIANT

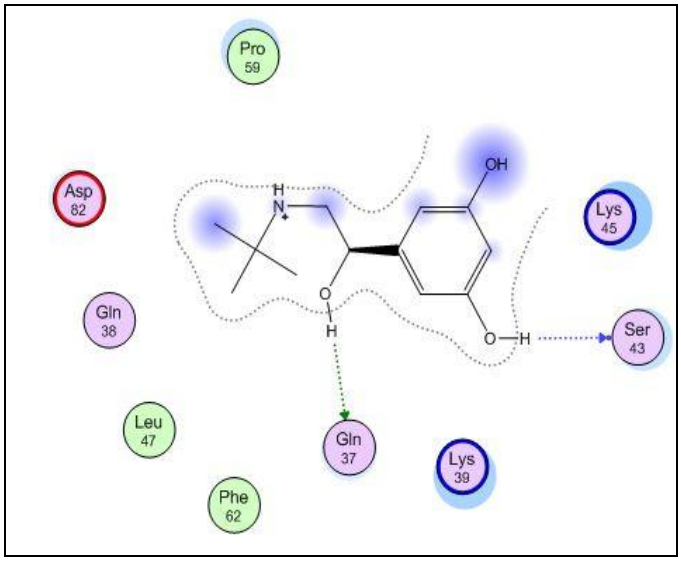

FIG. 4g: TERBUTALINE WITH Gly16 VARIANT

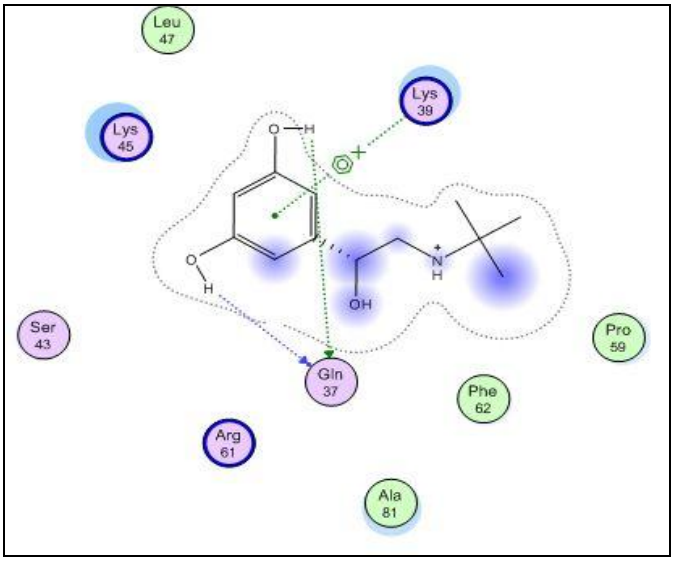

FIG. 4h: TERBUTALINE WITH Arg16 VARIANT

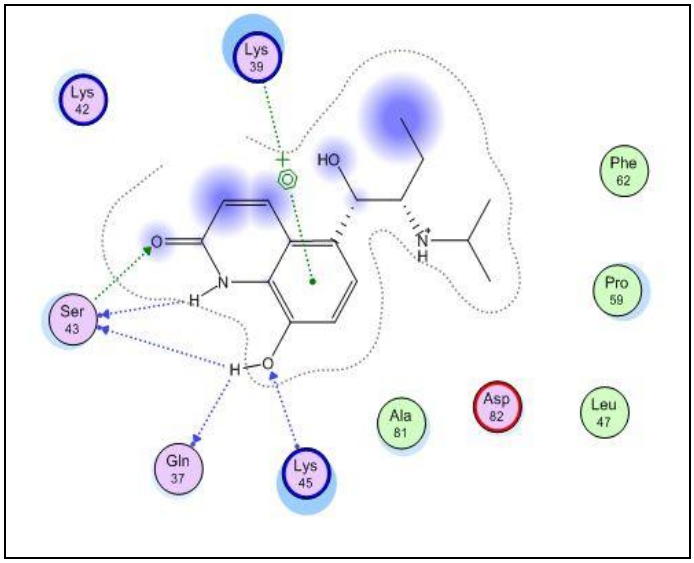

FIG. 4 i: PROCATEROL WITH GLY16 VARIANT

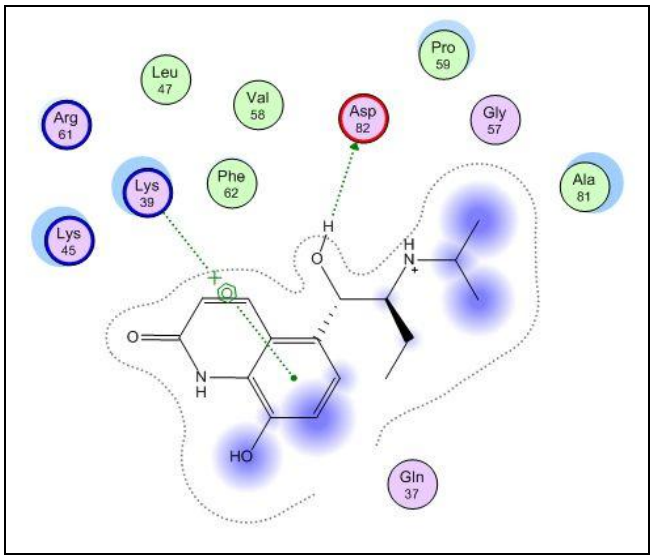

FIG. 4j: PROCATEROL WITH Arg16 VARIANT

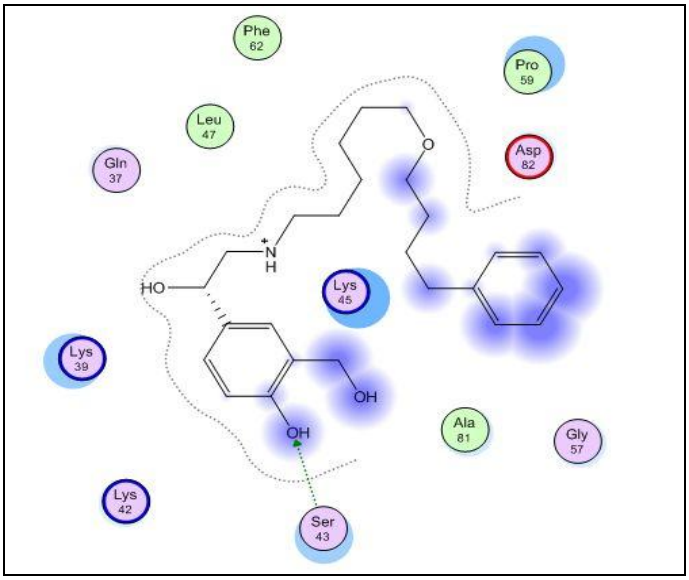

FIG. 4k: SALMETEROL WITH GLY16 VARIANT

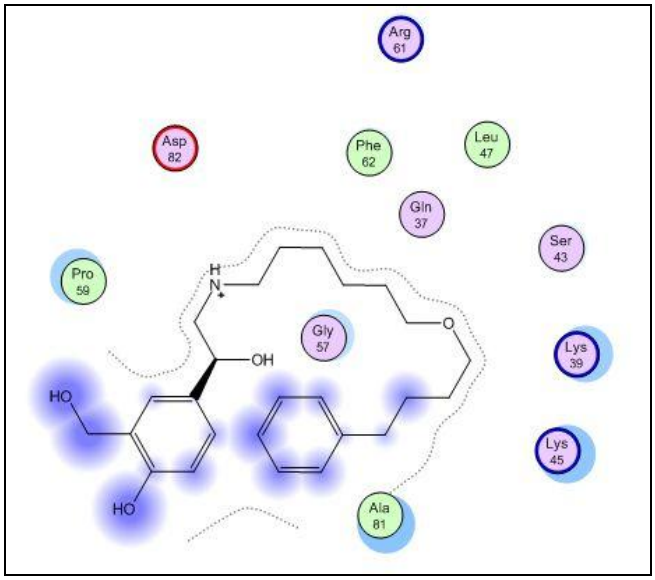

FIG. 4l: SALMETEROL WITH ARG16 VARIANT

FIG 4: LIGAND INTERACTION MAPS

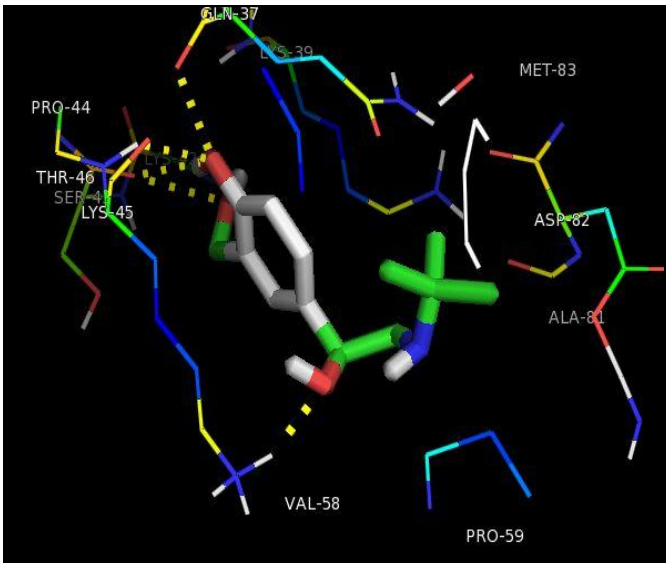

FIG. 5a: SALBUTAMOL WITH Gly16 VARIANT

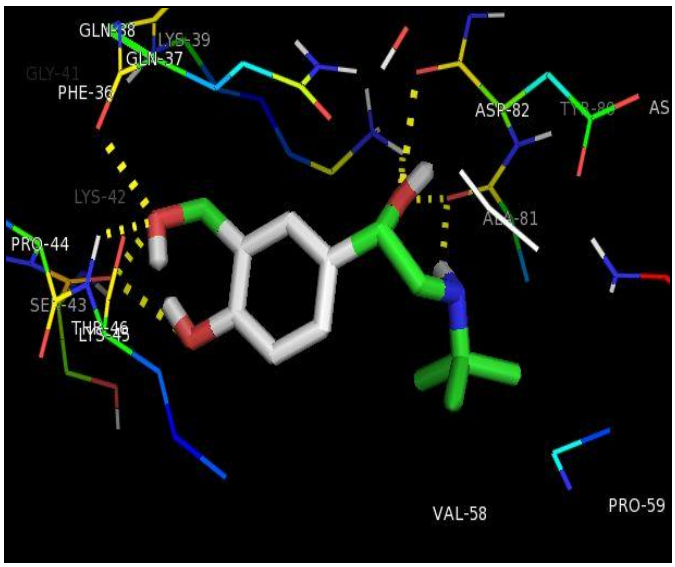

FIG. 5b: SALBUTAMOL WITH Arg16 VARIANT

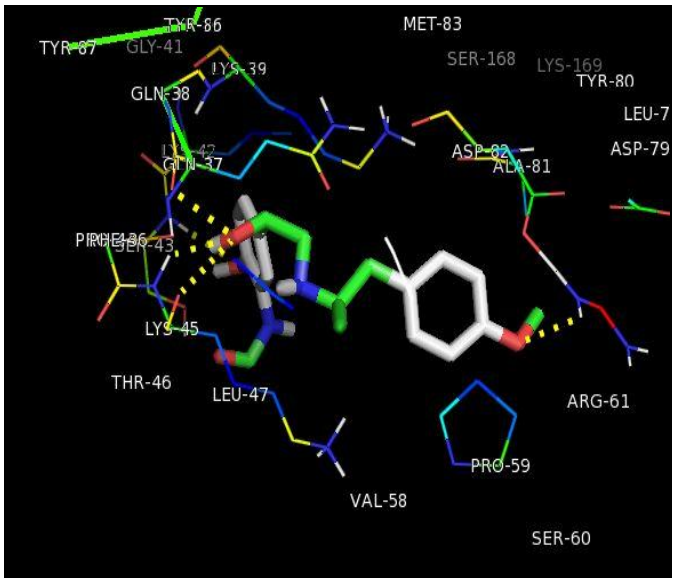

FIG. 5c: FORMETEROL WITH Gly16 VARIANT

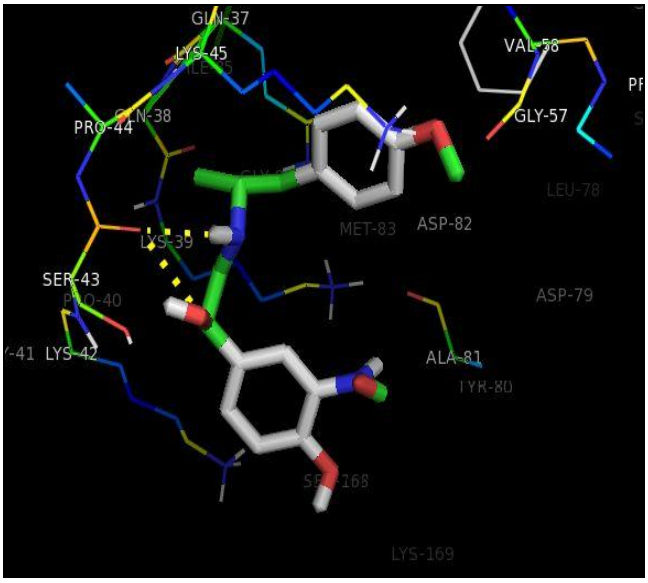

FIG. 5d: FORMETEROL WITH Arg16 VARIANT

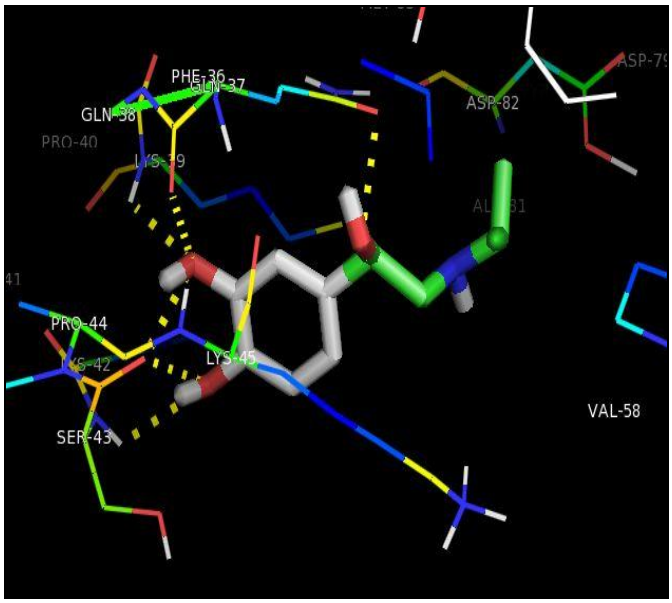

FIG. 5e: ISOPRENALINE WITH Gly16 VARIANT

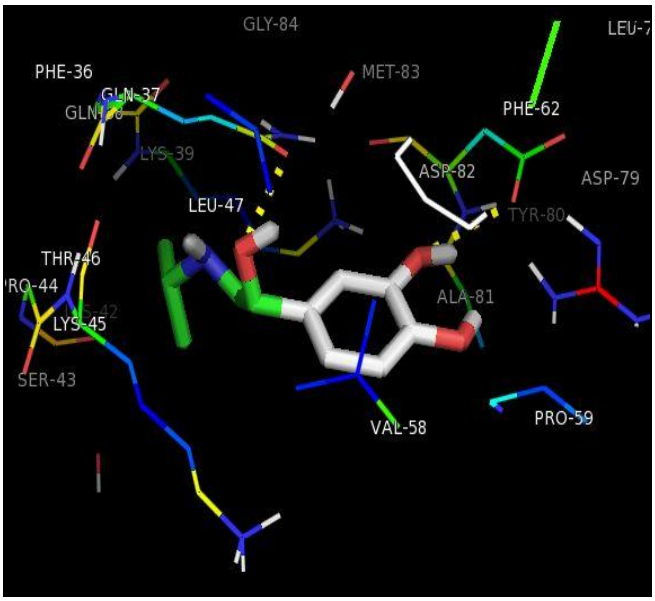

FIG. 5f: ISOPRENALINE WITH Arg16 VARIANT

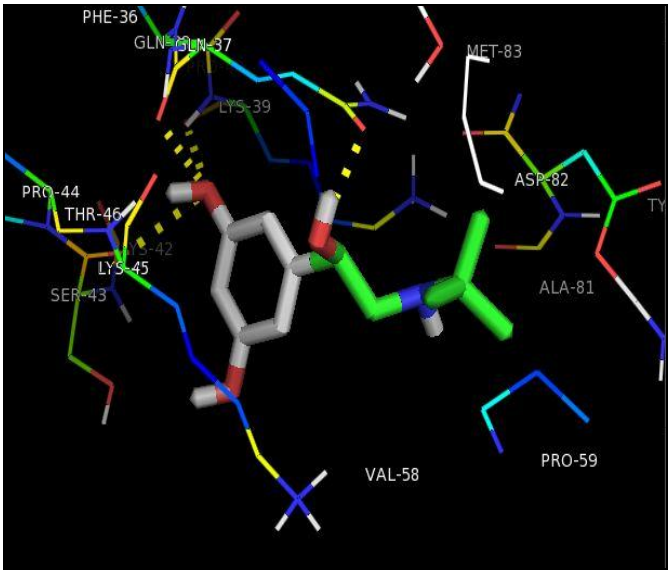

FIG. 5g: TERBUTALINE WITH Gly16 VARIANT

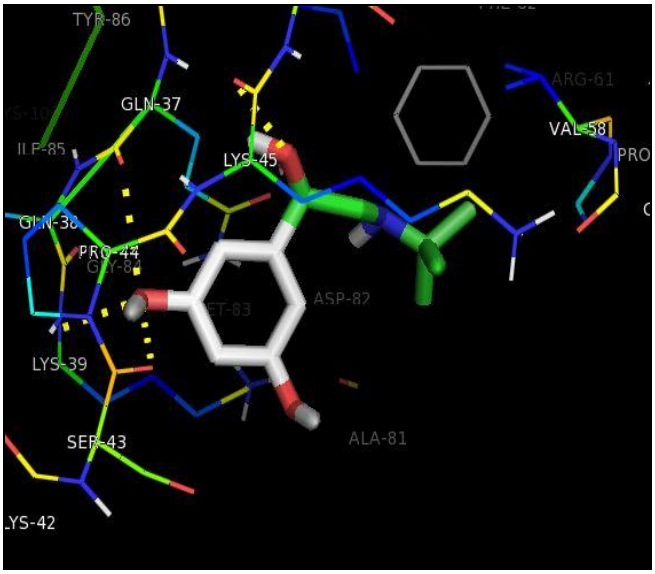

FIG. 5h: TERBUTALINE WITH Arg16 VARIANT

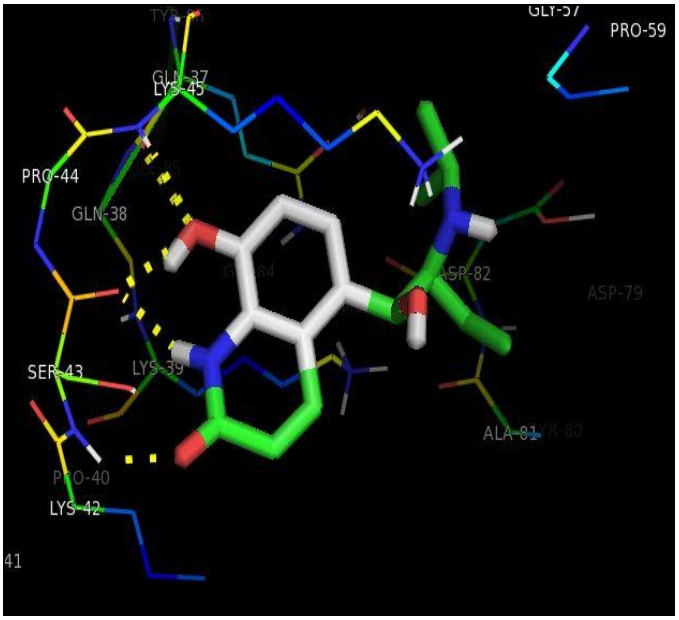

FIG. 5i: PROCATEROL WITH GLY16 VARIANT

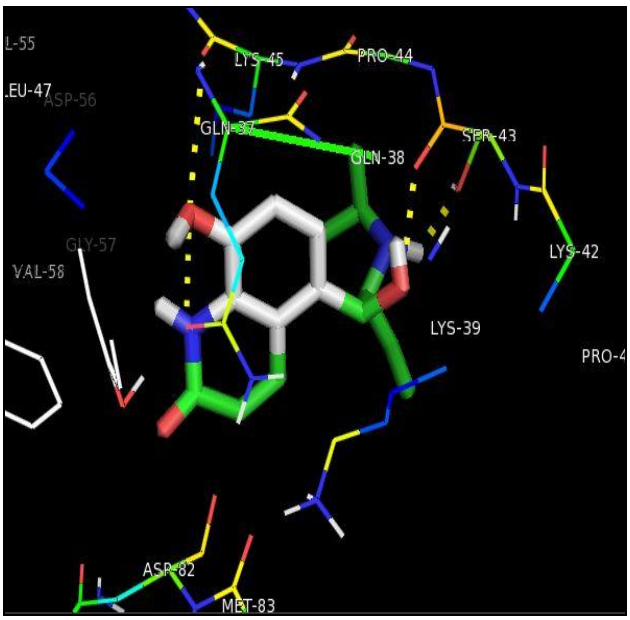

FIG. 5j: PROCATEROL WITH ARG16 VARIANT

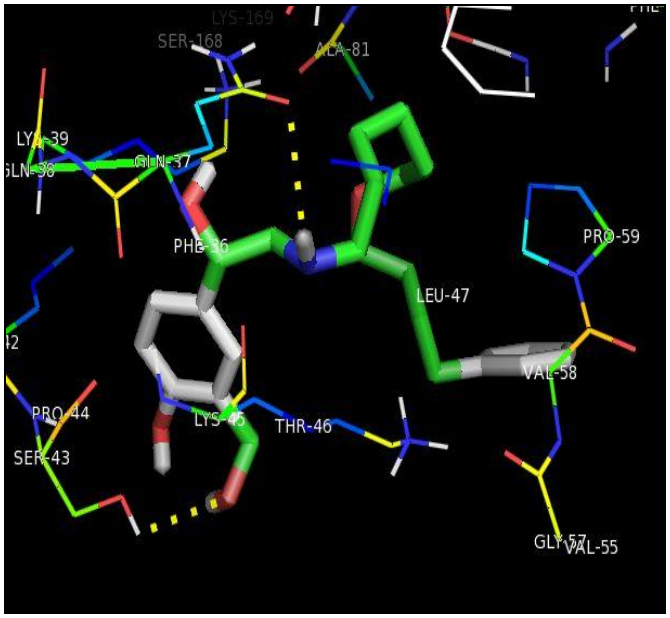

FIG 5k: SALMETEROL WITH Gly16 VARIANT

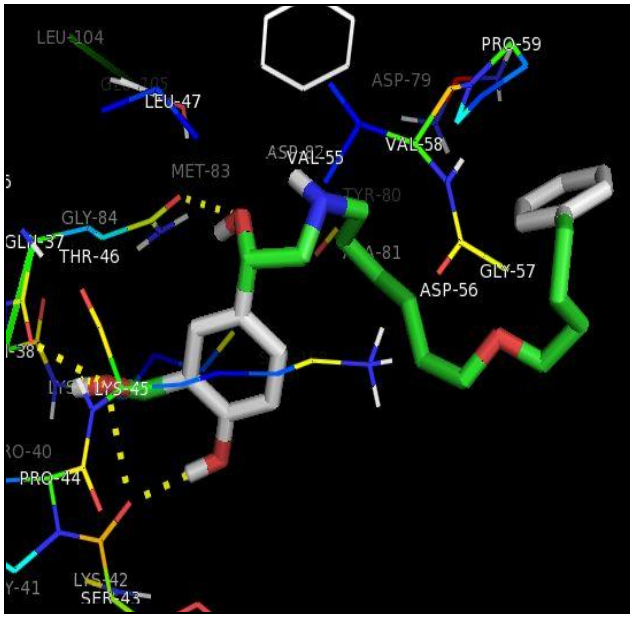

FIG 5l: SALMETEROL WITH Arg16 VARIANT

FIG 5: THREE DIMENSIONAL DOCKED CONFIRMATION OF β_2 SYMPATHOMIMETICS

CONCLUSION: Presence of functional non synonymous single nucleotide polymorphisms in the β_2 adrenoceptor significantly alters ligand binding. Binding of β_2 sympathomimetic to the Arg16 variant was found to be considerably less than that of the Gly16 variant. Hence pharmacogenetic screenings of patients who are non-responders of β_2 sympathomimetic therapy should be carried out individualize therapy regimens. Further, a constant demand for novel molecules that bind to the mutant variants of common receptors exists.

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CONFLICT OF INTEREST: The authors do not have any conflict of interest

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