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RESEARCH ARTICLE

BIOINFORMATICS

IN SILICO DRUG DESIGNING APPROACHES FOR LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA)

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ABSTRACT

Autoantibody positivity together with subsequent development of insulin deficiency led to the introduction of latent autoimmune diabetes in adults (LADA) or type 1.5 diabetes for this subgroup of diabetic patients who clinically resemble type 2 diabetes at diagnosis. β- cell dysfunction in type 1.5 diabetes is caused mainly by Glutamic acid decarboxylase antibody (GADA). β- cell stress results in an induction of Heat shock protein 90(Hsp 90) expression, where Hsp 90 is a regulator of Class II antigen processing and presentation. *In silico* docking studies and drug likeliness analysis have shown that docking target protein Hsp 90 α with the ligands geldanamycin ,radicicol and chimeric ligand radanamycin had a protective role against autoimmune destruction. This study paves way for treating the autoimmune diabetes at the immunity level .

KEY WORDS

Glutamic acid decarboxylase antibody, Heat shock protein 90, ligands.

INTRODUCTION

Diabetes management remains a challenge for developed and developing countries alike[1]. Latent autoimmune diabetes in adults (LADA) also known as type 1.5 diabetes is a common form of diabetes initially presents as non-insulin dependent and easily misdiagnosed as type 2 diabetes[2] .

There is no established therapeutic intervention for patients with LADA so far and they are currently treated as patients with type 2 diabetes[3]. Although there is a good proportion of patients with LADA , surprisingly there are only a few studies that have evaluated interventions for this group and several others are ongoing[4] . Identification of novel drug targets and their inhibitors is a major challenge in the field of drug designing and development for diseases [5].

Any potential therapeutic approach for LADA should not only aim at obtaining good metabolic control, but also allow better preservation of the residual β- cell function. GADA is recognized as the marker with highest sensitivity for LADA[6] . β- cell function deteriorates in LADA faster than in type 2 diabetes but slower than in type 1 diabetes[7] .In auto immune diabetes, β- cell stress results in an induction of Heat shock protein 90(HSP 90) expression[8] . Recent studies have implicated HSP 90 as a regulator of Class II antigen processing and presentation[9] . *In vitro* studies have shown that the inhibition of HSP 90 by pharmacological agents such as geldanamycin or radicicol decrease presentation of both exogenous and endogenous GAD by Class II molecules. Radanamycin , a macrocyclic chimera of geldanamycin and radicicol was found to have biological activity [10] .

In the present study, an *in silico* approach has been carried out to study the inhibitory effect of geldanamycin , radicicol and chimeric ligand radanamycin on HSP 90 α protein and to study the drug likeliness of these ligands.

MATERIALS AND METHODS

The structure of the target protein HSP 90 α protein complexed with 1-4 - [(2r) -1- (5-chloro-2,4 – dihydroxybenzoyl) pyrrolidin – 2 -yl] benzyl } -3,3 -difluoropyrrolidinium complex with the protein databank identification number (PDB ID : 3HEK) was retrieved from PDB(www.pdb.org) . The structure of the drug geldanamycin and radicicol were retrieved from the drug bank (www.drugbank.ca).The structure of the chimeric drug radanamycin was retrieved from pubchem (http://www.ncbi.nlm.nih.gov/pccompound).
Docking was done using GLIDE module of Schrodinger version 7.5 and drug likeliness was analysed by using Lipinski drug filter of the Supercomputing Facility for Bioinformatics and Computational Biology ,

(i) Protein Preparation

OPLS-AA forcefield (Optimized Potential for Liquid Simulations for All Atoms) aids to perform Glide (Schrodinger) calculations. Energy minimization using OPLS-AA forcefield in the protein preparation wizard and refinement was carried out until the average root mean square deviation of the non-hydrogen atoms reached 0.3Aº using default

settings, Site points were generated followed by generation of the grid displaying the active site with an enclosing box at the centroid of the workspace.

(ii) Ligand Preparation

Each structure was assigned an appropriate bond order using ligPrep module of Schrodinger. Since the crystal structure contains only one ligand structure but there is a chance that one of the tautomeric forms interacts more strongly with the binding site relative to the other forms, ligprep module generates tautomers all of the other possible tautomeric states of one inhibitor. All the three ligands were prepared one by one for docking.

(iii) Docking using Glide

The Glide SP (Standard Precision), a ligand docking program of the software Schrodinger version 7.5 used in the present study for predicting protein-ligand binding modes and ranking ligands via high-throughput virtual screening utilizes scoring functions SP GlideScore, to rank-order compounds. The docking process involves a conformational search for a compound which complements a target binding site, with the aim of identifying the best matching binding pose. The Glide docking algorithm performs a series of hierarchical searches for locations of possible ligand affinity within the binding site of a receptor .

The stability of the docked ligand–protein complex is due to hydrogen bonding and van der Waals Interactions. The glide score, glide energy value, H-bonds and vander Waals contacts (good, bad and ugly) to the receptor were visualized in the Glide pose viewer using default settings to analyze the binding modes of the ligands to receptor .

(iv) Finding drug likeliness using Lipinski drug filter

The ligands namely geldanamycin, radicicol and radanamycin used in the present study were subjected to Lipinski rule screening using the tool Lipinski drug filter of the Supercomputing Facility for Bioinformatics and Computational Biology (http://www.scfbio-iitd.res.in/utility/LipinskiFilters.jsp) according to which prediction of high probability of success or failure is based on drug likeness for molecules complying with 2 or more of the rules namely- molecular mass less than 500 dalton, high lipophilicity (expressed as LogP less than 5),less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors and molar refractivity should be between 40-130.

RESULTS

(i) Protein Preparation

The complex 1-4 - [(2r) -1- (5-chloro-2,4 – dihydroxybenzoyl) pyrrolidin – 2 - yl] benzyl } - 3,3 –difluoropyrrolidinium complex was removed from the receptor protein Hsp 90 α , water molecules were removed , site points were generated and grid was generated displaying the active site with an enclosing box at the centroid of the workspace.

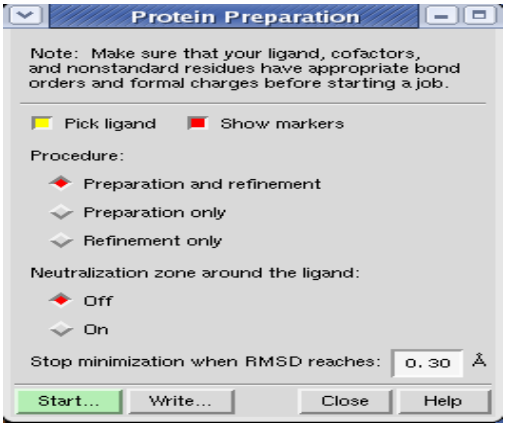


Figure 1
Protein preparation wizard used for protein preparation and refinement

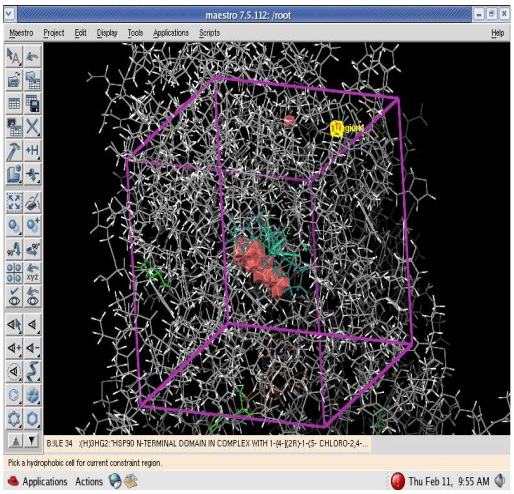


Figure 2
Grid generation in the Hsp 90 α

(ii) Ligand Preparation

Ligands were prepared using ligprep module. For each ligands geldanamycin,radicicol and radanamycin the best tautomeric form was generated.

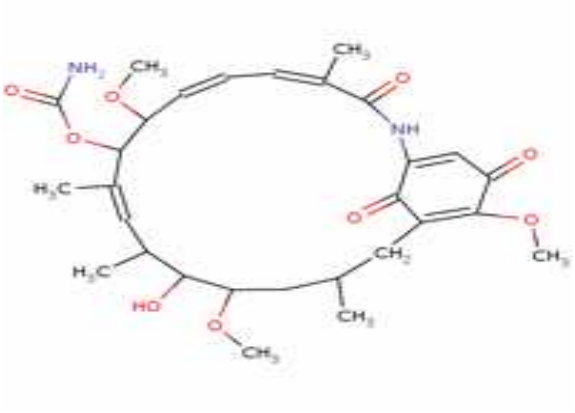


Figure 4
Chemical structure of geldanamycin

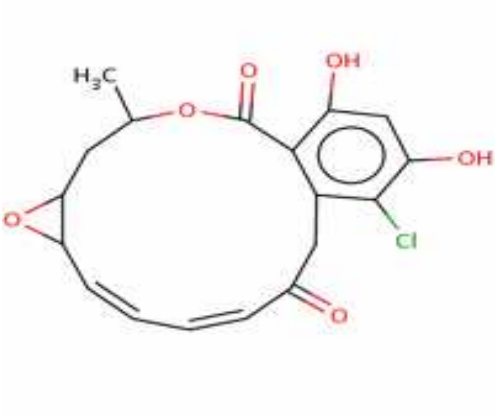


Figure 5
Chemical structure of radicicol

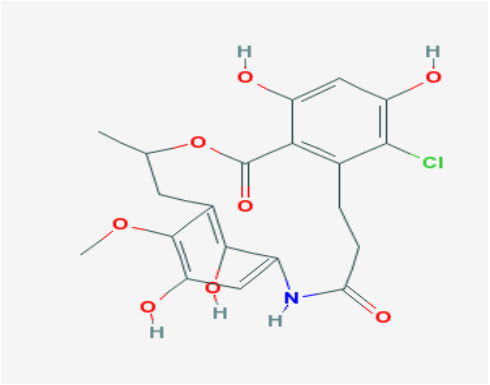


Figure 6
Chemical structure of radanamycin

iii) Receptor – ligand docking using Glide

Docking procedures basically aim to identify the correct conformation of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. It is a process by which two molecules fit together in a 3-dimensional space .Docking algorithm based on the tetrahedral grid model of proteins allows a more precise description of shape complementarity .

The Glide SP (Standard Precision), a ligand docking program of the software Schrodinger version 7.5 used in the present study predicts protein-ligand binding modes and ranks ligands via high-throughput virtual screening utilizing scoring functions SP GlideScore, to rank-order compounds.

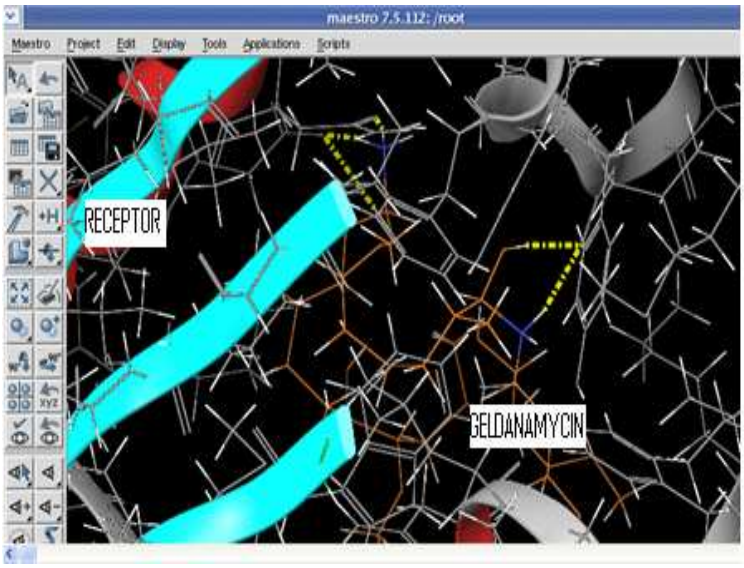
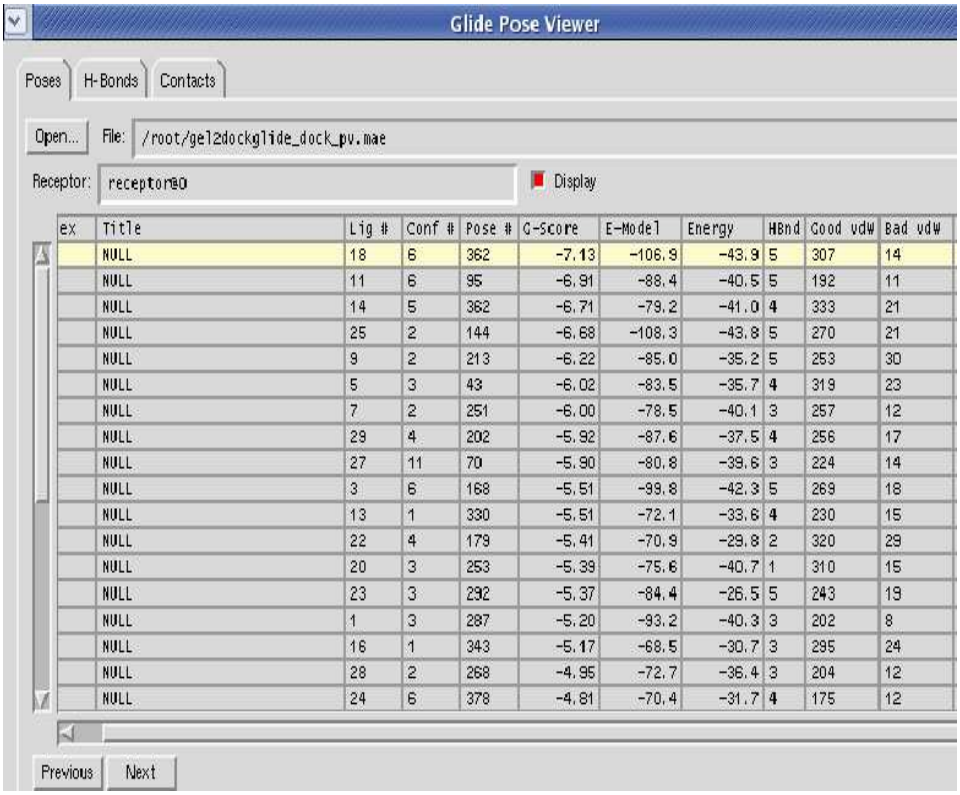


Figure 7
Docked structure of geldanamycin with Hsp 90 α



ex	Title	Lig #	Conf #	Pose #	G-Score	E-Model	Energy	HBnd	Good vdW	Bad vdW
	NULL	18	6	362	-7.13	-106.9	-43.9	5	307	14
	NULL	11	6	95	-6.91	-88.4	-40.5	5	192	11
	NULL	14	5	362	-6.71	-79.2	-41.0	4	333	21
	NULL	25	2	144	-6.68	-108.3	-43.8	5	270	21
	NULL	9	2	213	-6.22	-85.0	-35.2	5	253	30
	NULL	5	3	43	-6.02	-83.5	-35.7	4	319	23
	NULL	7	2	251	-6.00	-78.5	-40.1	3	257	12
	NULL	29	4	202	-5.92	-87.6	-37.5	4	256	17
	NULL	27	11	70	-5.90	-80.8	-39.6	3	224	14
	NULL	3	6	168	-5.51	-99.8	-42.3	5	269	18
	NULL	13	1	330	-5.51	-72.1	-33.6	4	230	15
	NULL	22	4	179	-5.41	-70.9	-29.8	2	320	29
	NULL	20	3	253	-5.39	-75.6	-40.7	1	310	15
	NULL	23	3	292	-5.37	-84.4	-26.5	5	243	19
	NULL	1	3	287	-5.20	-93.2	-40.3	3	202	8
	NULL	16	1	343	-5.17	-68.5	-30.7	3	295	24
	NULL	28	2	268	-4.95	-72.7	-36.4	3	204	12
	NULL	24	6	378	-4.81	-70.4	-31.7	4	175	12

Figure 8
Glide pose viewer displaying the glide score for geldanamycin

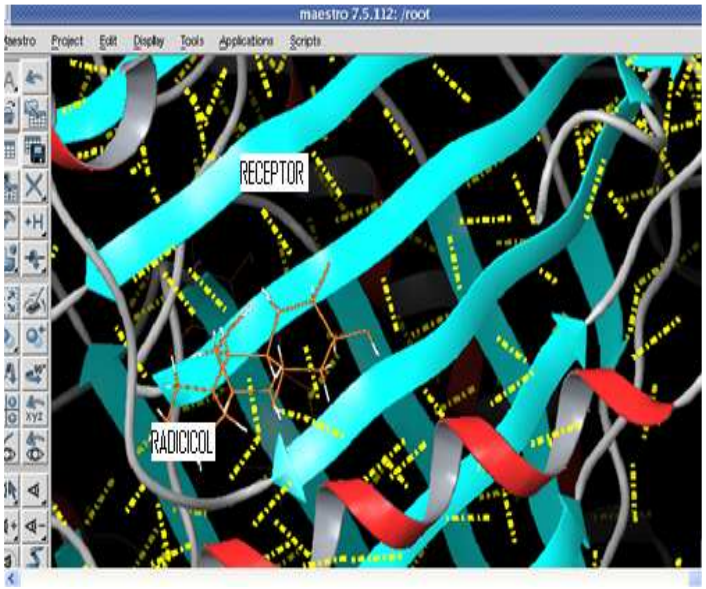
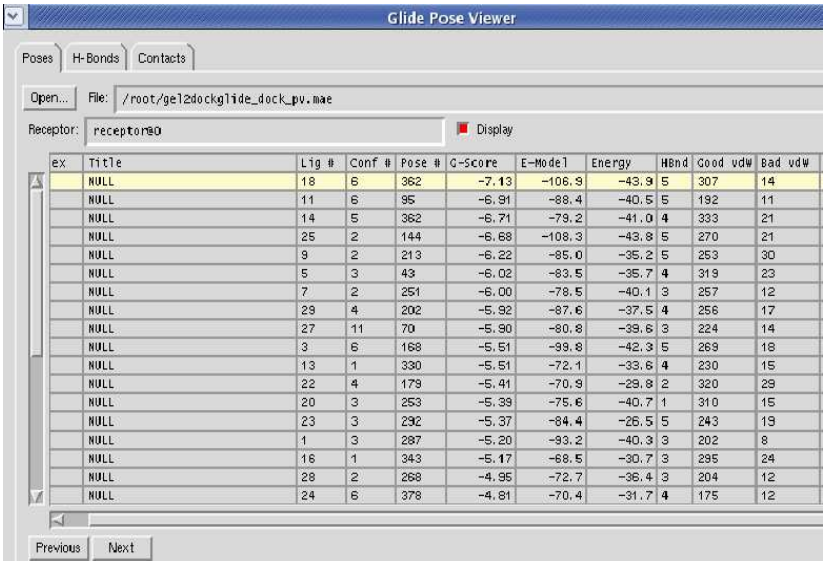


Figure 9
Docked structure of radicicol with Hsp 90 α



ex	Title	Lig #	Conf #	Pose #	G-Score	E-Model	Energy	HBnd	Good vdW	Bad vdW
	NULL	18	6	362	-7.13	-106.9	-43.9	5	307	14
	NULL	11	6	95	-6.91	-88.4	-40.5	5	192	11
	NULL	14	5	362	-6.71	-79.2	-41.0	4	333	21
	NULL	25	2	144	-6.68	-108.3	-43.8	5	270	21
	NULL	9	2	213	-6.22	-85.0	-35.2	5	253	30
	NULL	5	3	43	-6.02	-83.5	-35.7	4	319	23
	NULL	7	2	251	-6.00	-78.5	-40.1	3	257	12
	NULL	29	4	202	-5.92	-87.6	-37.5	4	256	17
	NULL	27	11	70	-5.90	-80.8	-39.6	3	224	14
	NULL	3	6	168	-5.51	-99.8	-42.3	5	269	18
	NULL	13	1	330	-5.51	-72.1	-33.6	4	230	15
	NULL	22	4	179	-5.41	-70.9	-29.8	2	320	29
	NULL	20	3	253	-5.39	-75.6	-40.7	1	310	15
	NULL	23	3	292	-5.37	-84.4	-26.5	5	243	19
	NULL	1	3	287	-5.20	-93.2	-40.3	3	202	8
	NULL	16	1	343	-5.17	-68.5	-30.7	3	295	24
	NULL	28	2	268	-4.95	-72.7	-36.4	3	204	12
	NULL	24	6	378	-4.81	-70.4	-31.7	4	175	12

Figure 10
Glide pose viewer displaying the glide score for radicicol

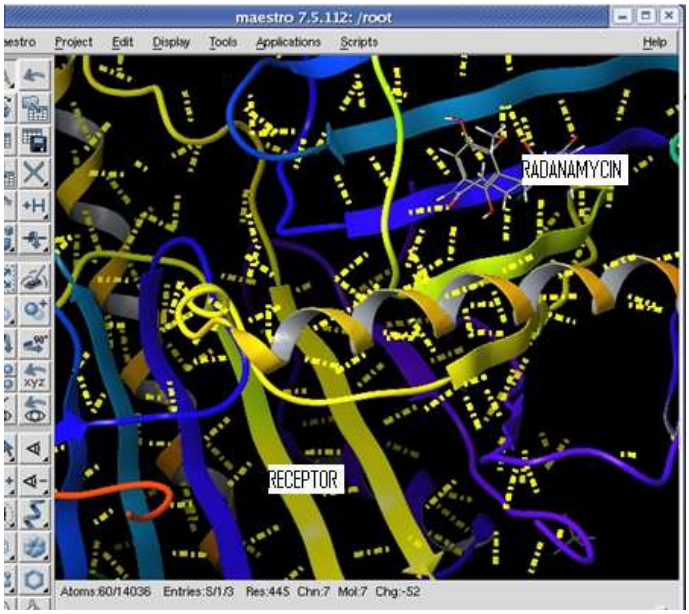


Figure 11
Docking of radanamycin with Hsp 90 α

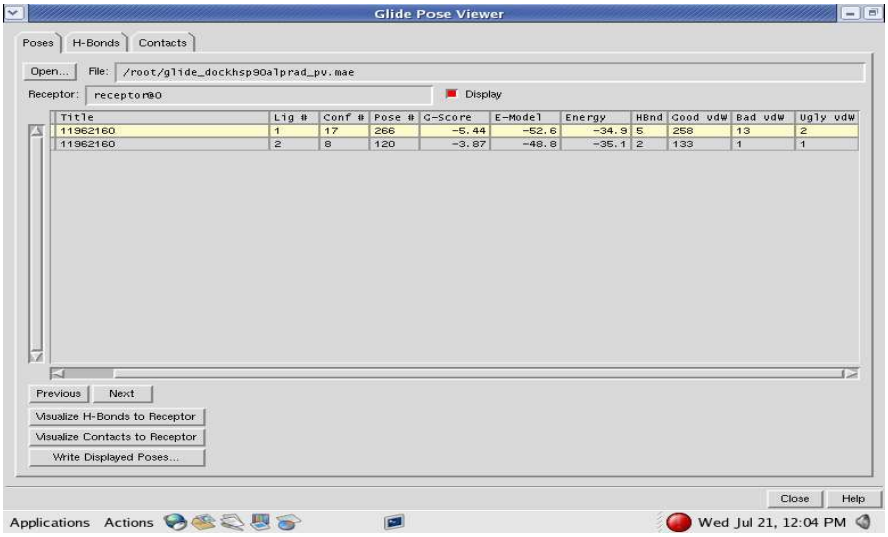


Figure 12
Glide pose viewer displaying the glide score for radanamycin

TABLE 1
Docking results of ligands for HSP 90 α

Ligands	Glide score	Glide energy	No. Of hydrogen bonds
Geldanamycin	-7.13	-43.9	2
Radicicol	-5.93	-33.3	5
Radanamycin	-5.43	-34.9	5

(iv) Finding drug likeliness using Lipinski drug filter

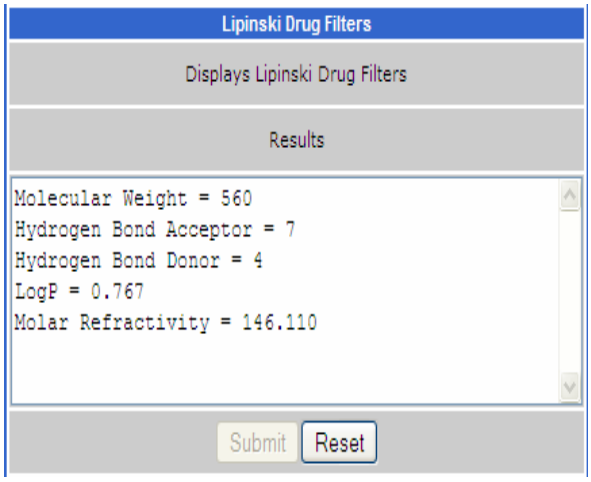


Figure 13
Results of Lipinski drug filters for geldanamycin

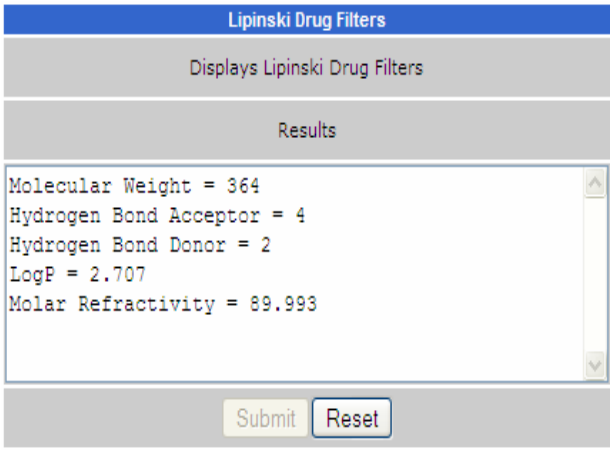


Figure 14
Results of Lipinski drug filters for radicicol

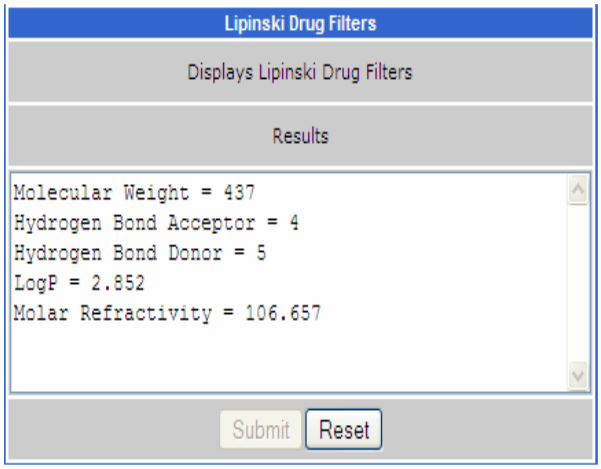


Figure 15
Results of Lipinski drug filters for radanamycin

TABLE 2
Lipinski filters of the three ligands analysed in the study

Name of the Compound	Molecular Weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log p	Molecular Refractivity
Geldanamycin	**560**	**4**	**7**	**0.767**	**146.110**
Radicicol	**364**	**2**	**4**	**2.707**	**89.993**
Radanamycin	**437**	**5**	**4**	**2.852**	**106.657**

In the present study all the three ligands satisfied more than 2 rules predicting high probability of success to show drug likeliness. Geldanamycin satisfied three rules; radicicol satisfied all the five rules and radanamycin satisfied four rules .

DISCUSSIONS

The ligand molecule geldanamycin was docked with the target protein Hsp 90 α involving five hydrogen bonds. Geldanamycin interacted with the HSP 90 α with a glide score value of -7.13. Docking of the ligand molecule radicicol with the target protein Hsp 90 α was found to involve of two hydrogen bonds. Radicicol interacted with the Hsp 90 α with a score value of -5.93. The ligand molecule radanamycin was docked with the target protein HSP 90 α and five hydrogen bonds were involved in binding the ligand and the receptor. Radanamycin interacted with the HSP 90 α with a score value of -5.44.

In the present study all the three ligands satisfied more than 2 rules of Lipinski's rule out of five predicting high probability of success to show drug likeliness. Geldanamycin satisfied three rules; radicicol satisfied all the five rules; radanamycin satisfied four rules evidencing that all the

three ligands used in the present study showed drug likeliness.

The results of the present study shows that all the three ligands displayed a good docking score and drug likeliness. However, docking was best for geldanamycin with least energy value compared to other two ligands. Drug likeliness was best for radicicol as it satisfied all the five rules of Lipinski. Geldanamycin, is found to be the best ligand compared to other two ligands as it had lowest energy value and also satified more than two rules of Lipinski.

CONCLUSION

Thus the *insilico* method adopted in the present study helped in identifying the ligands using the commercial software and online tools for the pandemic disorder diabetes. This method reduces the time and cost in designing a drug as well as in analyzing the drug likeliness before it enters the clinical trials.

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