Inhibition of Leishmania Arginase PDB 1T5F by using Pharmacophore

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Abstract: **Leishmaniasesare a group of parasitic diseases caused by several species of the genus Leishmania. Like Docking, Pharmacophore searching is an In-silico technique which is widely used for drug discovery. In pharmacophore searching the main focus is on the hydrogen bond interactions between the ligand and the target protein. Pharmacophore models are hypotheses on the 3D arrangement of structural properties, such as hydrogen bond donor and acceptor properties, hydrophobic groups and aromatic rings of compounds that bind to a biological target. In the presence of the 3D structure of this target of by comparison with inactive analogs, further geometric or steric constraints. The pharmacophore models were generated by using the already known actives as templates and by utilizing the significant chemical features of the active site. In this research the pharmacophore searching has been used to find potential ligands or inhibitors for arginase1T5F. The May bridge portfolio offers a comprehensive range of chemistry products and services tailored to the drug discovery and biotechnology sector. By searching from these data base through software's called catalyst that measure them through various parameter i.e. bond angle, bond length, hydrophobicity, and molecular weight it searched the hit for each specific pharmacophore. Pharmacophore models were generated by using DSV (Discovery studio visualizer, Accelrys. models were generated through vector method and were modified and improved on the basis of hits obtained and by considering the chemistry of the active site.The pharmacophores were improved by adding hydrogen bond acceptor and donor vectors to the previous pharmacophores.The top hits were selected on the basis of fitting well in the active site, forming maximum hydrogen bonds, offering minimum steric clash and in no violation of the exclusion spheres.Through this study it appears that the selected ligands having hydroxyl group at C1 position and directed towards Aspartic acid 128, 183, Glu 186, 277 of the active site can be a good inhibitor.**

Key words: Leishmania, Arginase PDB 1T5F, inhibitor, Pharmacophore

I. INTRODUCTION

Leishmaniases is a group of parasitic diseases caused by several species of the genus Leishmania. On epidemiological grounds, classically cutaneous leishmaniasis (CL) of the Old World can be broadly separated into two categories: anthroponotic cutaneous leishmaniasis (ACL) caused mainly by Leishmania (L) tropica, found in urban,towns and cities, clinically characterized by dry-type lesions. Zoonotic cutaneous leishmaniasis (ZCL) caused mainly by Leishmania (Leishmania) major, found in rural or semi-urban areas and clinically characterized by wet-type lesions. Pakistan has five provinces namely; Khyber Pakhtunkhwa, Punjab, Sindh,Balochistan and GilgitBaldistan. Various epidemiological studies have shown that the presence of L.tropica in each province of anthroponotic cutaneous leishmaniasisamong Afghan refugee camps has been reported in northwest of country [1]. In Pakistan, CL has been differentiated on the basis of clinical presentations (dry- and wet-type lesions) .The carrier of this parasite is the fly which is female phlebotomine sand fly Leishmaniasisis caused by protozoan leishmania parasiteas an infection, spread to people through the bite of the female phlebotomine sand fly. This parasite exists in many tropical and temperate countries the organisms are microscopic in size. There are about 21 species of Leishmaniawhich affect humans, including the L. donovani complex and the L. Mexicana complex, among others. The life cycle is relatively simple. When the sand fly bites a human, it injects small numbers of parasites which are rapidly taken up by mononuclear blood cells this stage is called the promastigotestage. Once the parasite enters at the amastigote stage inside the human

mononuclear cells,it begins to multiply and infect other cells and tissues.

Leishmaniasisis a vector-borne disease. Itshows different clinical symptoms including cutaneous, mucosal, and visceral forms, both the cutaneous and mucosal forms can cause severe deformities to patients, including ulcerative skin lesions and the destruction of mucous membranes and in some cases leading to permanent disfigurement. Visceral leishmaniasis due to Leishmaniadonovani is the most severe form of Leishmania infections. Its annual incidence is estimated to be about 500,000 cases [2]. Uninfected sand flies acquire the parasite by feeding on infected people or animals such as dogs, foxes, or rodents. The implication is that the privileged structures provide the scaffold and the substitutions on it; provide the specificity for a particular receptor. Two monographs deals with the privileged structure concept [3-4]. Among the most popular privileged structures, historical representatives are arylethylamines(including indolylethylamines), diphenylmethane derivatives, tricyclic psychotropics and sulfonamides Dihydropyridines[5].

Benzodiazepines,N-arylpiperazines, biphenyls and pyridazines are more recent contributions [6]. A statistical analysis of NMR-derived binding data on 11 protein targets indicates that the biphenyl motif is a preferred substructure for protein binding [7].Liposomal amphoteric β is now available therapeutic failure lead to focus on drug development. There is need to develop new vaccines for leishmania.

II. MATERIAL AND METHOD

Leishmaniaprotein ITF5 was obtained from **Protein DataBank ID**research collaboratory for structural Bioinformatics (**RCSB PDB**). After screening and applying filter to database parameters,we selected particular protein for detail analysis. Thebest fit pharamacophoremodels were docked against the ligand. The legend*(s)-2amin 7,7dihydroxyheptanoic acid* was used and the information of protein IT5Fis shown in Table 1.

Protein	***Arginase***
Classification	*Hydrolase*
Molecule	*Arginase*
Polymer	*1*
Length	*314bp*
Pdb	*1T5F*
Chain	*3(a,b,c)*
Organism	*Rattus Norvegius*
Gene name	*Arg1*
Ligand name	*(s)-2amin7,7dihydroxy heptanoic acid*
Molecular formula	$C_7H_{15}NO_4$
Protein weight	*102401.92g/mol*
Ligand formula weight	*177.2g/mol*
Prosthetic group	Mn

Table 1: Information about Arginase

Discovery studio was used for the preparation ofpharmacophore models of ITF5. Molecule construction and 2D to 3D conversion was performed by using the LigPrep application in the Maestro modeling environment (8-9). Ionization of a Pharmacophore Model Generation Software Tools 32;atgiven pH or neutralization, tautomer enumeration and stereoisomer enumeration was also supported. Stereoisomers can be treated either as being separate or identical molecules .The molecule preparation steps also include conformational expansion using a torsional search or a combined Monte Carlo Multiple Minimum or Low Mode search. During the search, the intra-molecular hydrogen bonds were excluded. Molecules can be minimized; OPLS-2005 or MMFF force fields [10] and two continuum solvation models (distance-dependent dielectric or GB/SA) were available. A double criterionmethod was used to eliminate redundant conformations; it uses distances between pairs of corresponding atoms within 1 kcal/mol–1energy window. Around the center of the active site the exclusion spheres in a radius of about 10Å were generated. This generates a large number of football-like spheres for the pharmacophore.Among

pharmacophoremodel; best models were selected on the basis of ligand inthe active site.

In the MOE environment, a scheme is a collection of functions that defines how each ligand is annotated this is accessed via SVL function. This default scheme is called PCH (Polarity-Charged-Hydrophobicity).New schemes can be created to better represent certain molecules, e.g. PlanarPolar-Charged-Hydrophobicity.For structural information of a receptor, molecule alignments can also be performed using an all-atom flexible alignment procedure that combines a force field and 3D similarity function based on Gaussian descriptions of shape and pharmacophore features to produce an ensemble of possible alignments of collection of small molecules. Pharmacophore queries can be derived from the resulting set of aligned conformations of known actives. The properties of prepared pharmacophore models are shown in Table 2.

Pharamacophore Model	Donor	Acceptor	Model Interaction
1st	Protein H→O H_2O:837	Ligand O →H Glu:277	N→H ASP: 183
2nd	Protein H→O H_2O:837	Ligand N→H H_2O:832	o→H Glu: 277
3rd	Protein N→H	LigandO→H Ala141	H →O H_2O:837
4th	N→H ASP: 183	Protein H→O H_2O:837	O →H Glu:277

Table 2: Properties of pharmacophore models

The 1st created pharmacophore consists of the following constraints:

Hydrogen bond accepter, here ‘’O” of ligand is accepting “H” from Glu277,Hydrogen bond donor, Water molecule (837) act as an ‘H’ donor and ‘O’ of ligand as ‘H’acceptor. Hydrogen bond donor ASP(183)act as ‘H’ accepter and nitrogen of ligand is a donor. The first pharmacophore is shown in Fig 1.

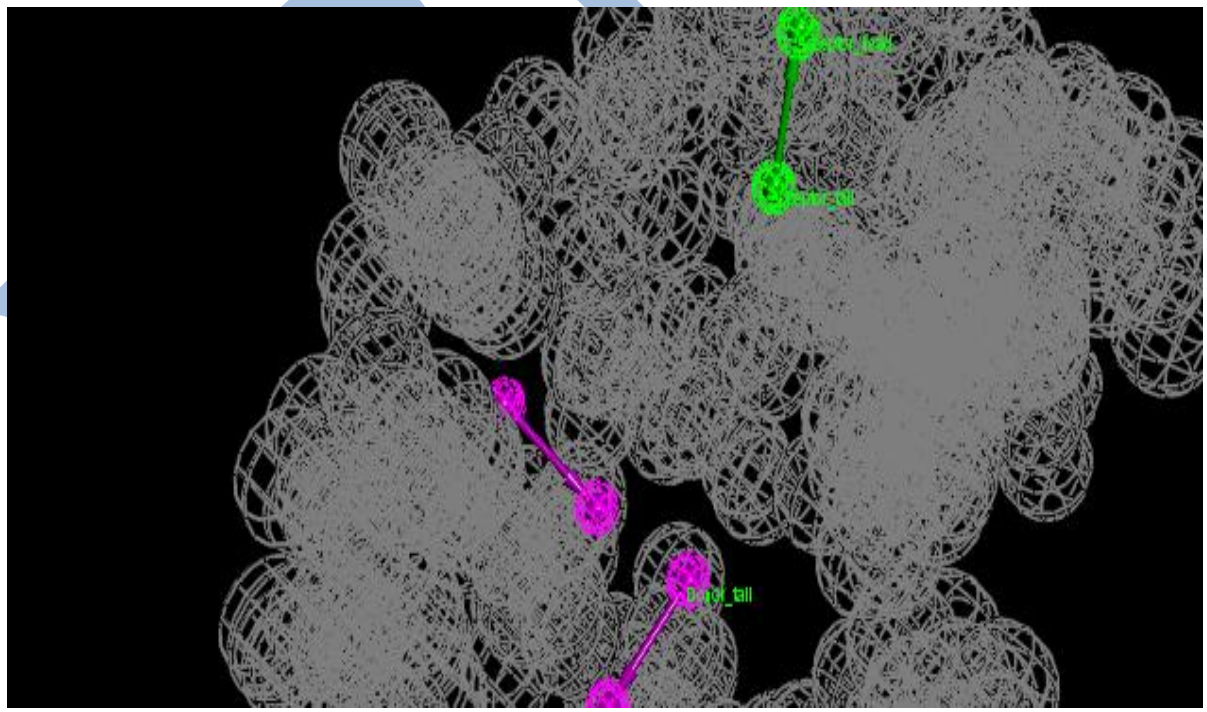

Figure 1: 1st Pharmacophore with grey ex-spheres 2 H-b donor &1accepter

The 2nd pharmacophore which contain the following constraints:

Hydrogen bond acceptor,H_2O (832) act as a donor to nitrogen of the ligand,Hydrogen bond accepter. H_2O 837 act as a donor of proton while oxygen of ligand Act as accepter,Hydrogen bond donor, oxygen of ligand act as donor for Glu277 of the enzyme active site. The secondpharmacophore is shown in Fig 2

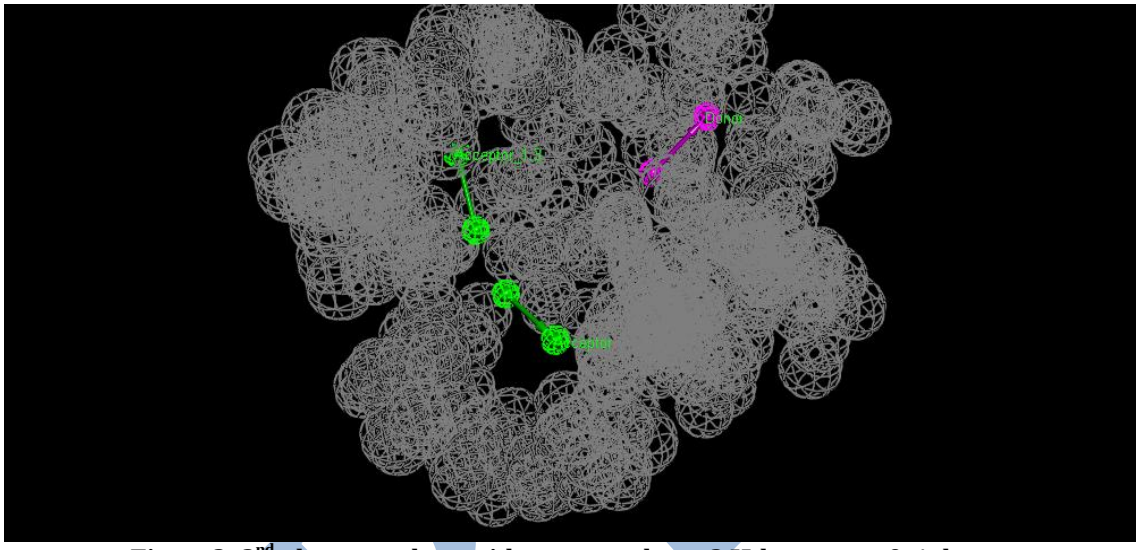

Figure 2: 2nd pharmacophore with grey ex-spheres 2 H-b acceptor & 1 donor

The3rd pharmacophore which we create contain the following constraints:

Hydrogen bond acceptors, Oxygen of ligand act as acceptor for Ala141, Hydrogen bond acceptor, Nitrogen of ligand act as acceptor, Hydrogen bond donor.H_2O act as donor for oxygen of the ligand. Third pharmacophore is shown in Fig 3.

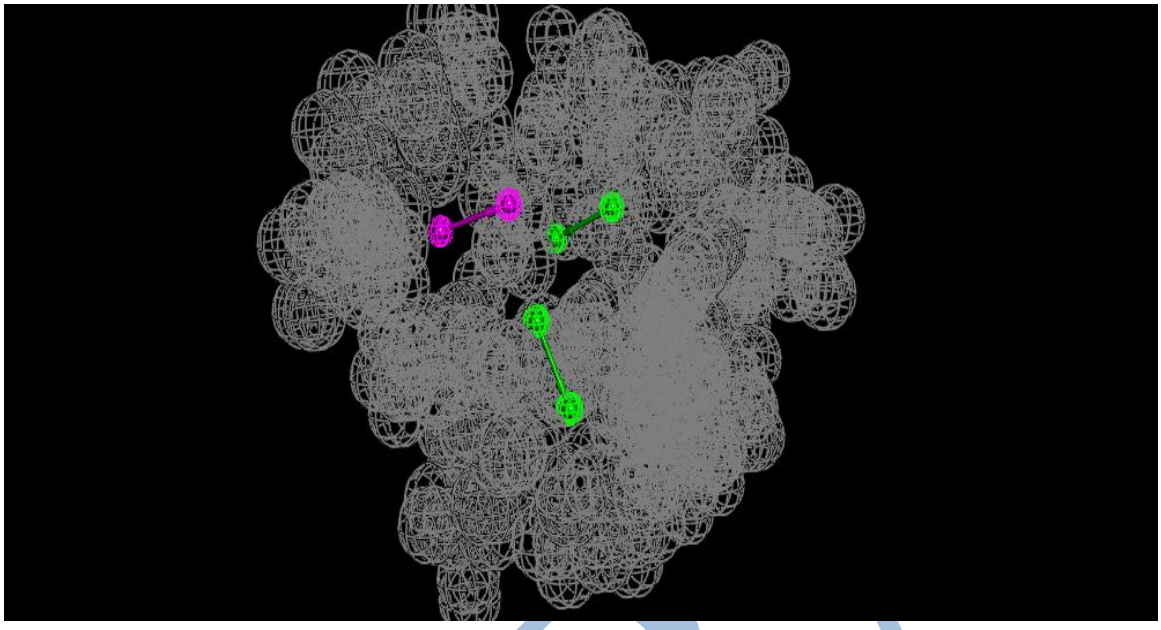

Figure 3: 3rd pharmacophore with grey ex-spheres 2 H-b acceptor & 1 donor

The 4thpharmacophore fourth consists of the constraints:

Hydrogen bond donor, H_2O donate its proton to oxygen of ligand, Hydrogen bond acceptor. Nitrogen of ligand acts as an acceptor for ASP183. Fourth pharmacophore is shown in Fig 4.

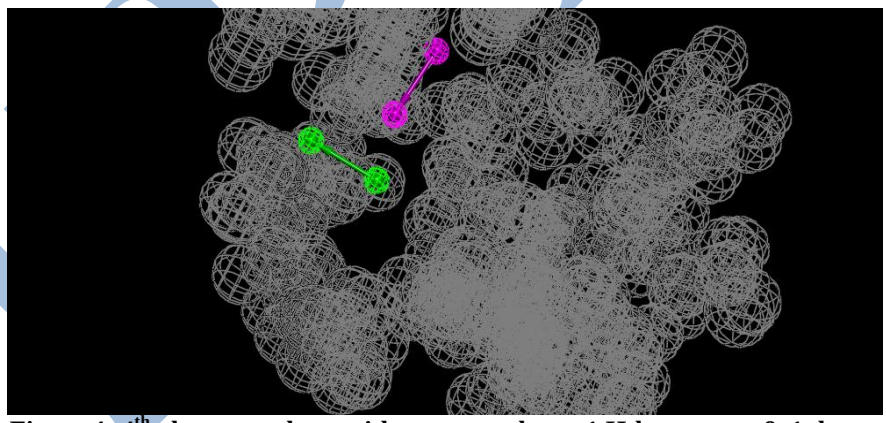

Figure 4: 4th pharmacophore with grey ex-spheres 1 H-b acceptor & 1 donor

III. RESULTS AND DISCUSSIONS

It is the most useful data base that is available online. That has large number of the hits in it. It contains approximately 14,400 entries. After that another online data base is EBI. ChEBI database was developed in 2006, standsfor(The database and ontology of Chemical Entities of Biological Interest).Chemical Entities of Biological Interest (**ChEBI**) is a freely available dictionary of molecular entities focused on 'small' chemical compounds. The term 'molecular entity' refers to any constitutionally or isotopically distinct atom, molecule, ion, ion pair, radical, radical ion, complex and conformer etc. identifiable as a separately distinguishable entity. The molecular entities in question are either the product of nature or synthetic products used to intervene in the processes of living organisms. ChEBI incorporates an ontological classification, whereby the relationships between molecular entities or classes of entities and their parents and/or children are specified.ChEBI uses nomenclature, symbolism and terminology endorsed by the following international scientific bodies.

The hits obtained in the results of first, second, third and fourth pharmacophore were respectively 112, 8, 5 and 22 hits. These hits (ligand) were searched in the may-bridge data base by means of the software named as catalyst. This software searches all the related ligand that has a bit of correlation with the samples we made. The software searchessuch types of molecules which have some interaction with the made pharmacophore models.As mentioned above,may-bridge searched at least 421 such ligand or molecules which haveat least only one parameter match. The parameters on which the related hits were searched in the any databases are:

Bond angles, *Bond length* and *Functional groups*

On these bases; the related molecules then searched in the database.Unauthorized person may sometime find it difficult to search them in the may-bridge99 data base as may-bridges association put charges on them.The generated pharmacophore modelswere subjected to May-bridge 99(53,000 compounds) database search through the use of Catalyst.

The software probed out those moleculeswhich have some resemblance with the ligand of arginase. The next most important step was the self-visualization of all those molecules which were searched out by the software catalyst. As a result many molecules which were searched, come out of the active site had longer length, their molecular weight were not same as that of the molecular weight of original ligand of the enzyme arginase. After visualizing all those hits one by one,through different parameter we have rejected numerous molecules from the molecules searched out by the software as it searched only through bond angle and bond length etc.Among them some of the molecules were also fulfilling the required criteria in terms of molecular weight; functional group and the residue were approximately same as that of the original so those molecules were selected as the potential inhibitor for the arginase.

No. of pharmacophore	Alcohol (OH)	Benzene (C_6H_6)	Amine (NH_2)	Carbonyl (CO)	Carboxyl (COO)
1	77	53	47	11	27
2	8	6	2	1	1
3	5		1		1
4	19	9	13	2	1

Table 3: table of functional groups in Ligands

This table gives information about the functional groups that were present in the structure of the searched molecules. We have sum up our knowledge from the amount of functional groups present in all the molecules as which and how much amount of functional groups were present in these all searched out molecules. The common residues which surround ligand are shown in Table 4.

No. of Pharmacophore	Total No. of hits	Total No. of H-bond	No. of hits forming H-bond with GLU	No. of hits forming H-bond with ALA	No. of hits forming H-bond with THR	No. of hits forming H-bond with ASN	No. of hits forming H-bond with SER	No. ofhits forming H-bond with ASP	No. of hits forming H-bond with H2O
1	112	434	80	28	6	2	5	166	143
2	8	25	10	…….	3	9	…….	…….	3
3	5	16	2	…….	……	……	…….	4	10
4	22	72	6	5	……	6	…….	10	49

Table 4:Result of the residues surrounding ligand

This table gives us information about the types of residues present in the actives sites of all these ligand and the total value of each types of ligand in the active sites. Among them the most common residues were glutamic acid, thyrosine, aspartic acid, asparagines and histidine. Ligand H bonding with residues are shown in Table 5.

Pharma cophore	Total hits	Hits forming 1H-BOND	Hit with 2H-BOND	Hits with 3H-B0ND	Hits with 4H-BOND	Hits with 5H-BOND	Hits with 6H-BOND	Hits with 7H-BOND	Hits with 8H-BOND
1	112	1	13	30	29	14	10	6	3
2	8		1	3	2				
3	5	-	1	2	2				
4	22	1	6	3	7	2	6		

Table 5: frequency of the hydrogenbond making by ligands with surrounding residues:

This table gives brief information about the number of the hydrogen-bonds formation by a water molecules .

.The more the hydrogen bonds the more will be the ligand bound tightly in the active site and will catalyze the reaction more fluently.

After analyzing all ligand molecules one by one, and by collecting all possible information about them, we have selected just only the top ten molecules out of the 421 searched ligands or molecules because of the reasons given in discussion section. These top ten molecules are shown in table 6.

Hit I'd	Hits surroundingResidues	Functional group of hits	Molecular weight
(1) RJC00595	GLU,ASP,H_2O	Amine & ,imide	130.12 g
(2) SB00751	ASP,GLU,HIS	Amine, Carboxyl	155.07 g
(3) BTB14035	ASP,ALA,GLU	ASP,ALA,GLU	158.6g
(4) BTB09138	ALA,GLU,ASP	Sulfide,Amine,imide&hydoxal	167.04 g
5) JFD1639	ASP,H_2O	Amine ,imide & carbonyl	170.09 g
(6)BTB14473	GLU,ASP,ALA	Amine, carbonyl & methyl	172.1 g
(7)KM08619	ASP,GLU&H_2O	Alcohol, amine& carbonyl	172.11 g

(8) SBO1476-	ALA,ASP,GLU,H_2O	Amine & Carbonyl	174.11 g
(9)RHO1444	ALA,GLU,ASP,THR	Amine & carboxyl	174.12 g
(10) RJC00670	ASP,GLU,H_2O	Amine & Carboxyl	175.19 g

Table 6: Top 10 best fitting hits

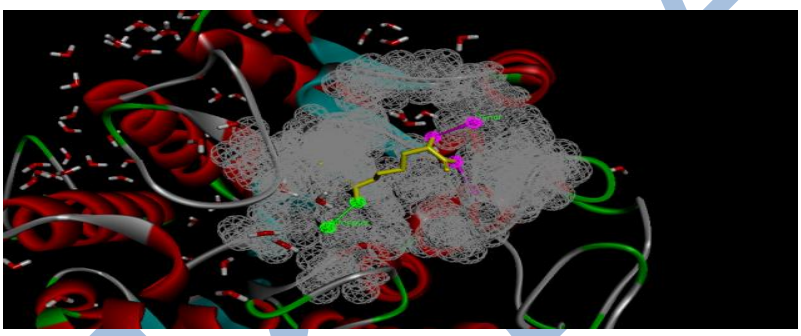

Figure 5:vector along with exclusion sphere in active site of Arginase

After doing pharmacophore searching we analyzed that residues like asportate183 and128, glutamic acid 186 and 277 are important from ligand point of view, based on formation of maximum hydrogen bonds. Both these are based on the hits obtained through pharmacophore searching. These points show that, which residues in the active sites are important for the binding of new hits.

From binding point of view the presence of hydroxyl group, carboxyl group and amino group toward asp 183,128 and glu277, 186. Residues inhits obtained through pharmacophore searching are very important.

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