Mizoribin as a inhibitor for leukocyte immunoglobulin receptor subfamily A member3

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INTRODUCTION

LILR S family:

✓The leukocyte Ig-like receptor (LILR/ILT/LIR) family comprises 13 members regulating a broad range of cells in the immune responses. They can recognize MHC (major histocompatibility complex) class I molecules

✓The leukocyte immunoglobulin-like receptors contains receptors possessing extra cellular immunoglobulin domains .

✓They are also known as CD85,ILTs and LIR, and can exert immuno modulatory effects on a wide range of immune cells .

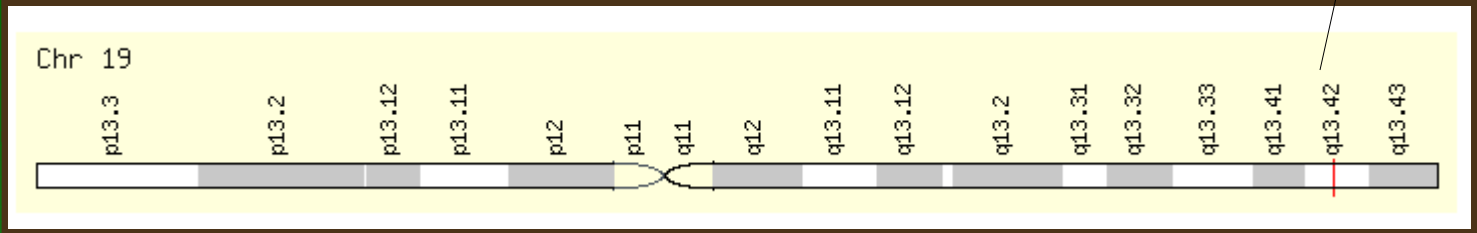
✓Leukocyte IG like receptors are also expressed predominantly on monocytes and B cells .

Leukocyte immunoglobulin receptor sub family A member 3(LILRA3):

- LILRA3 is highly homologous to other LILR genes and bind with the human leukocyte antigen (HLA) class I and acts as a soluble receptor for class -1 molecules.

- LILRA3 is the only secretary LILR and control the inhibitory immune response induced by LILRB1, LILRB2, and other HLA-binding LILR molecules like LILRA1.

- LILRA3 gene located receptor complex on chromosome 19q13.4.



Disease related to LILRA3

- LILRA3 impair interactions on membrane-bound LILRs with their HLA ligands, thus modulating immune responses and leads to HLA class-1 associated diseases
- The increased levels of LILRA3 in serum of patients with RA(Rheumatoid Arthritis), monocytes and B cells from patients with RA show increased expression of LILRA3 mRNA. These suggest that lymphocyte and/or monocyte-derived LILRA3 play a role of inflammation in RA.
- RA is an autoimmune disease leading to chronic inflammation of the joints affecting the synovial membrane, cartilage and bone.
- The Elevated levels of LILRA3 in RA patients leads to stroke.

To identify a novel inhibitor for leukocyte immunoglobulin receptor subfamily A member3 against stroke in RA patients

OBJECTIVES

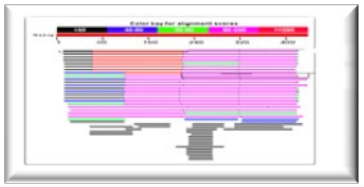
- Sequential and functional analysis of the protein LILRA3
- Building a 3D model for LILRA3 using Modeller 9V9.
- Docking Analysis of LILRA3 using SCHRODINGER software

UniProt **sequence**

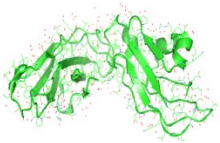
MTPILTVLICLGLSLDPRTHVQA
GPLPKPTLWAEPGSVITQGSPVT
LRCQGSLETQEYHL

BLASTp

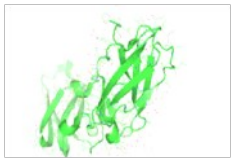
Template selection



1GOX



3P2T



Multiple templates

Ligand database

In house library

Docking

SCHRÖDINGER

Target and template alignment

Identity 39 and 45%

Query coverage 93 and 92%

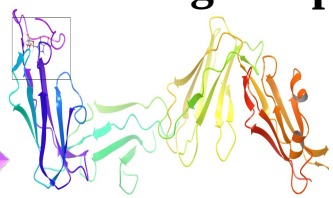
Model building

Modeller

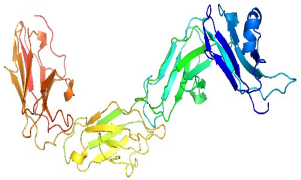
Model evaluation

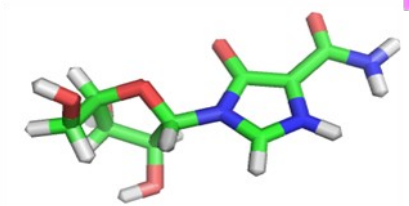
AD IT! Validation Server

Docking complex



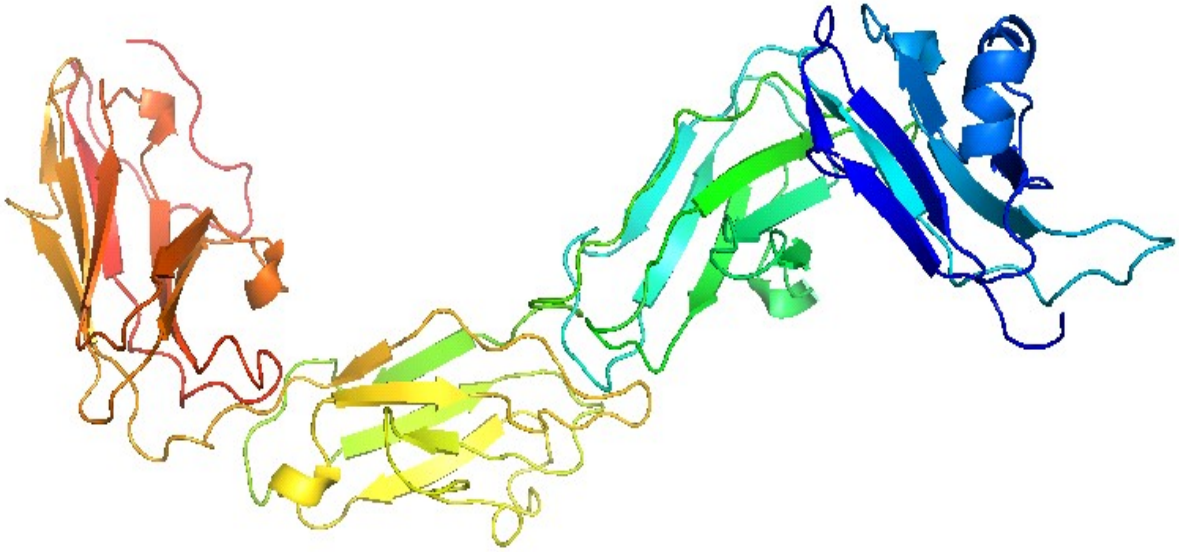
Model visualization



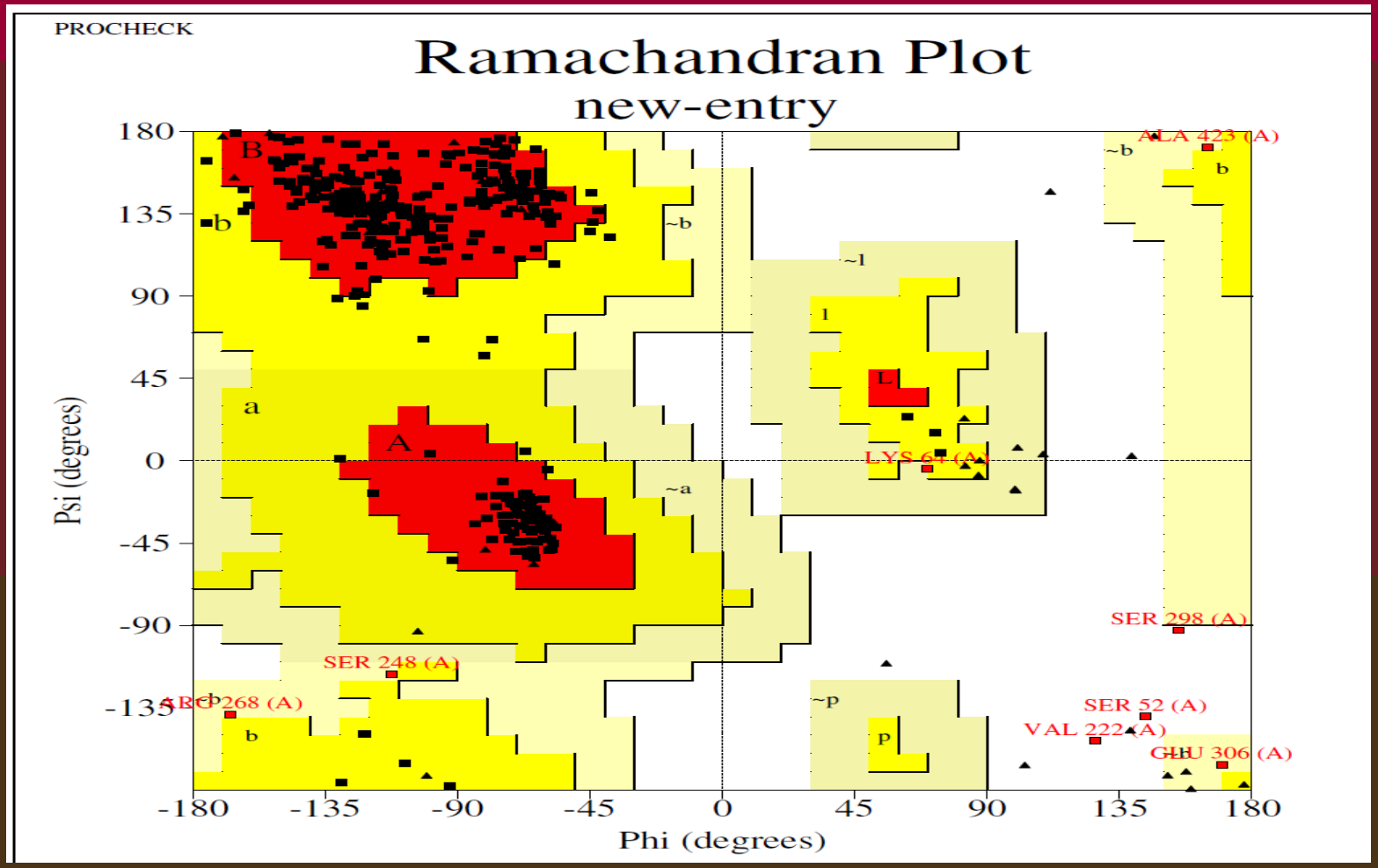


MATERIALS AND METHODS

Visualization of Modeled Structure



VALIDATION RESULTS



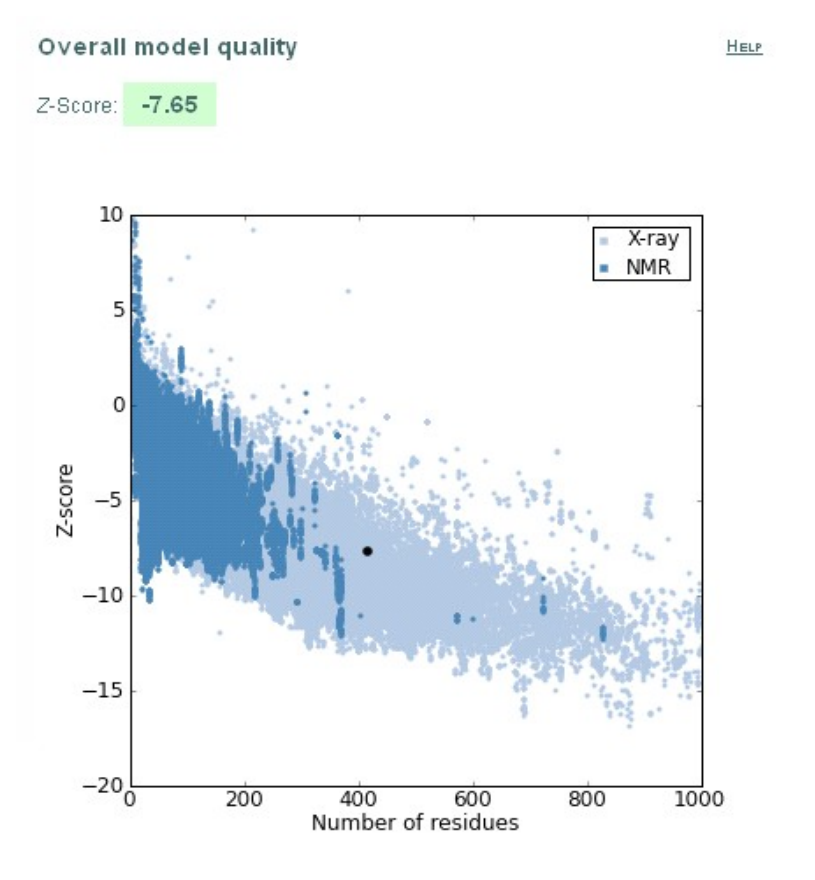
Plot statistics

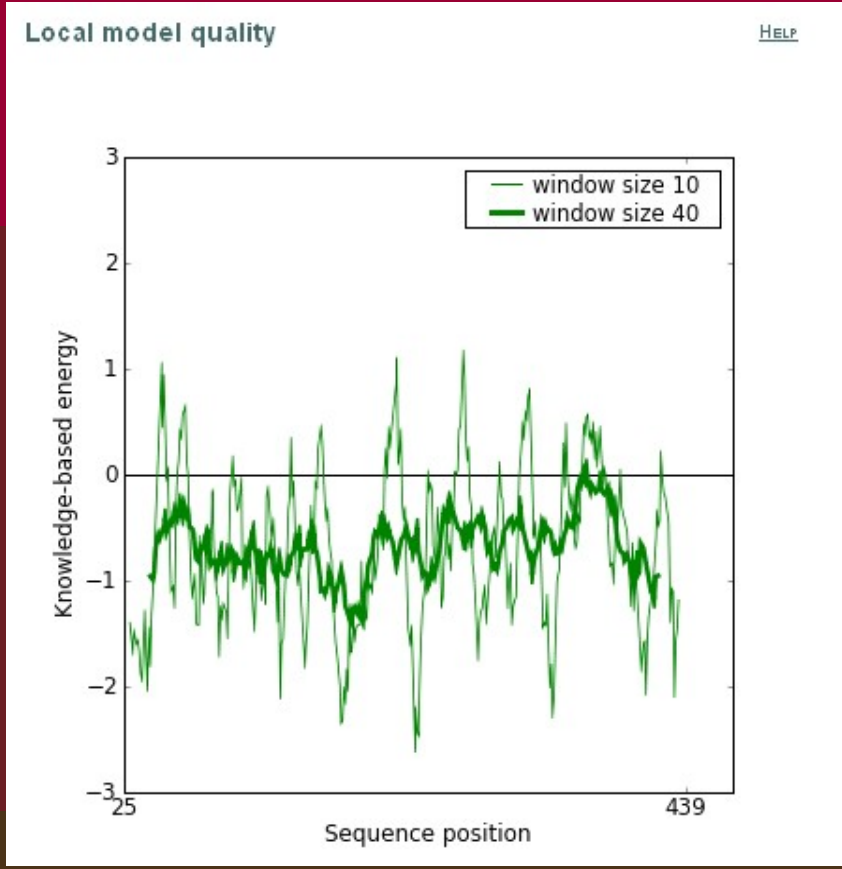
Residues in most favoured regions [A,B,L]	307	90.6%
Residues in additional allowed regions [a,b,l,p]	24	7.1%
Residues in generously allowed regions [~a,~b,~l,~p]	5	1.5%
Residues in disallowed regions	3	0.9%
	---	------
Number of non-glycine and non-proline residues	339	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	38	
Number of proline residues	37	

Total number of residues	415	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

PROSA RESULT

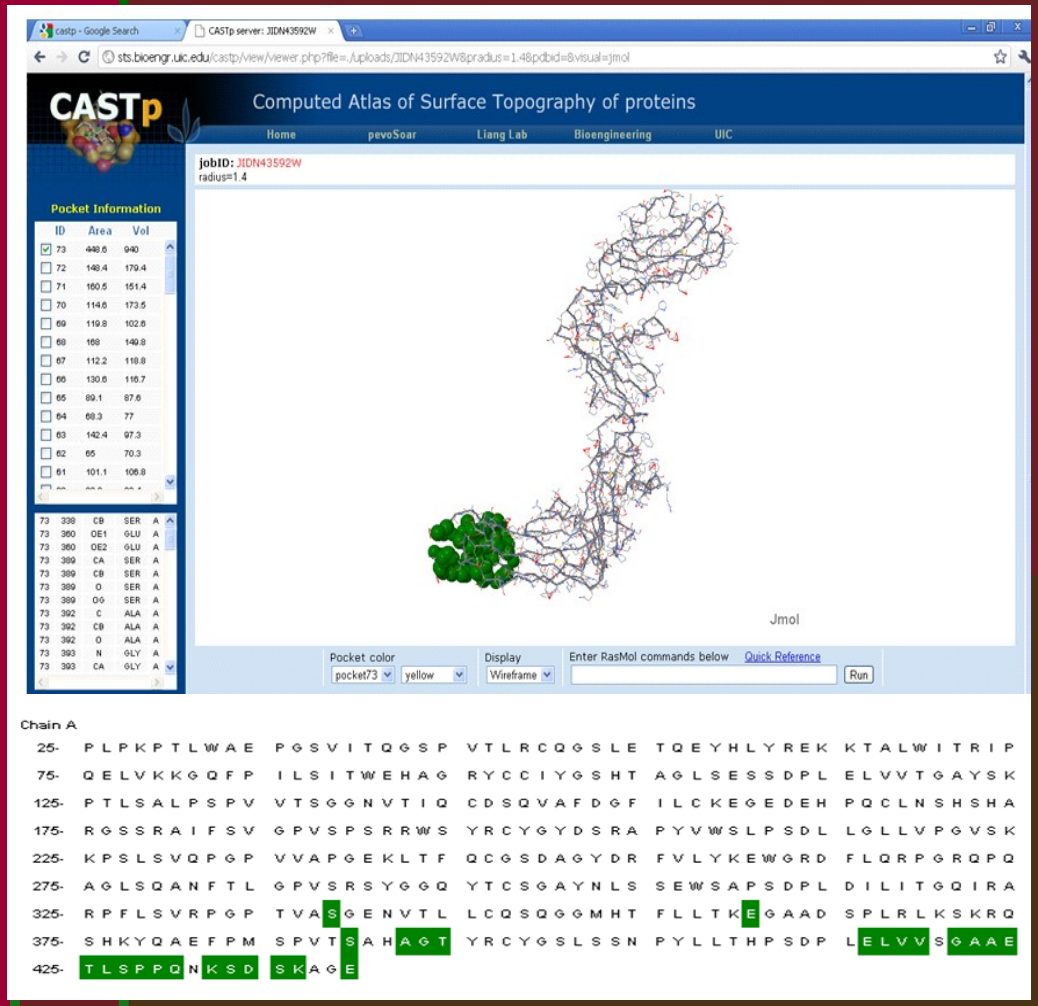




- ✓ PROSA tool graph showed all over model quality of the structure and the location of the Z-score for the structure
- ✓ The Z-score of LILRA3 was present in the range represented in black dot

CAST p Results

✓ The ligand binding sites were estimated through CASTp analysis. Binding sites from CASTp analysis are



Ser -338,Glu-360,Ser-389, Ala-392,Gly-393,Thr-394, Glu-416,Leu-417,Val-418, Val-419,421-Gly,422-Ala, 423-Ala,424-Glu,425-Thr, 426-Leu,427-Ser,428-Pro, 429-Pro,430-Gln,432-Lys, 433-Ser,434-Asp,435-Ser, 436-Leu,439-Glu.

Nature Precedings : doi:10.1038/npre.2011.6532.1 : Posted 14 Oct 2011

Docking

- Docking is frequently used to predict the binding orientation of small molecule / drug candidates to their protein targets in order to predict the affinity of the small molecule.

Virtual screening work flow

Modeled LILRA3

11,693 Analogues

Protein and ligand preparation

Post Ligprep 1741 Compounds

Lipinski filter

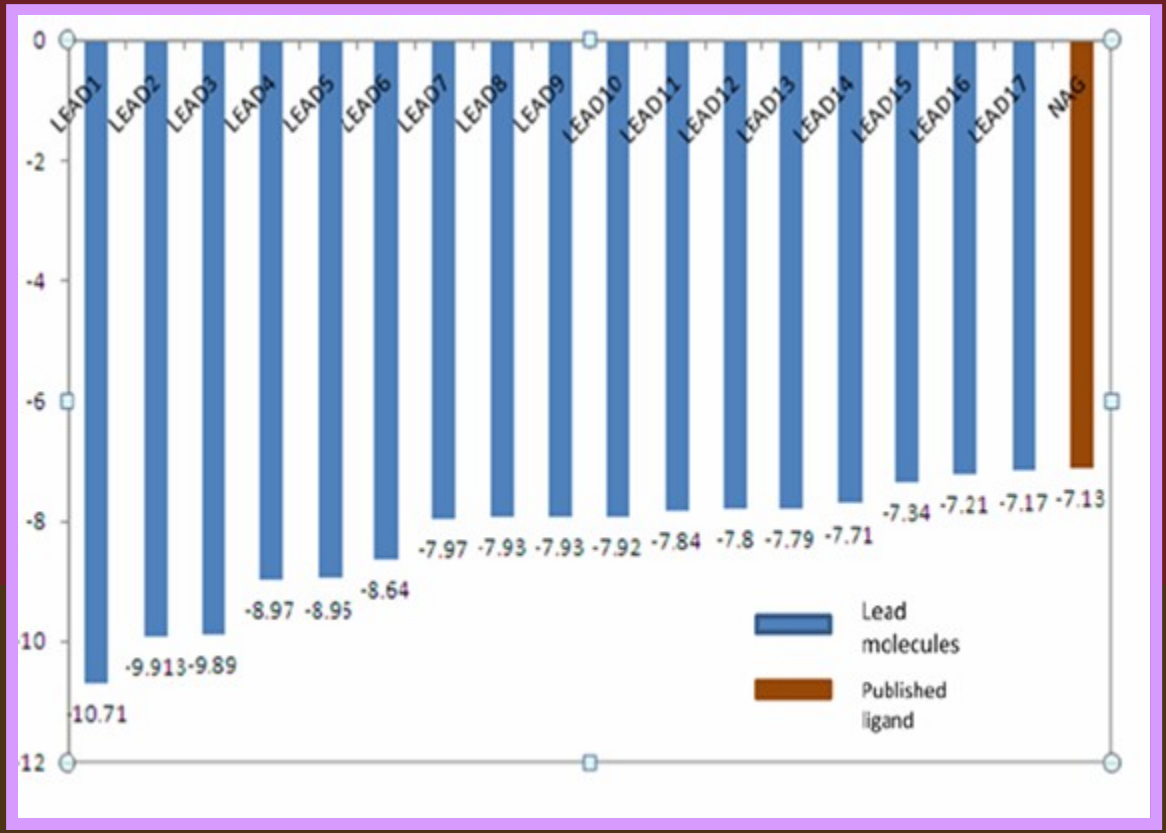
Reactive filter

676 and 558 compounds

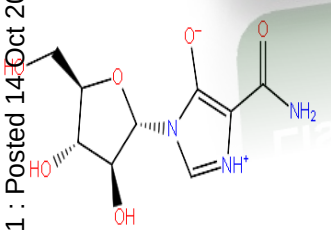
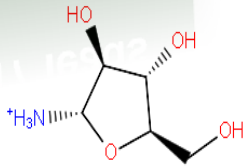
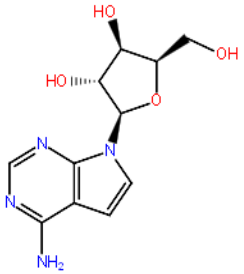
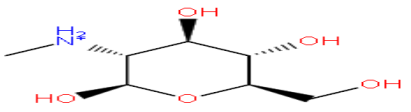
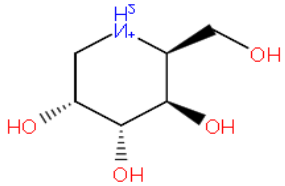
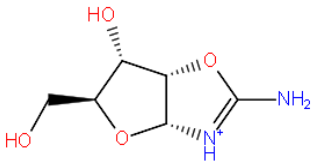
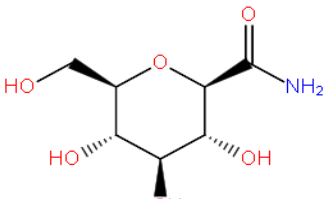
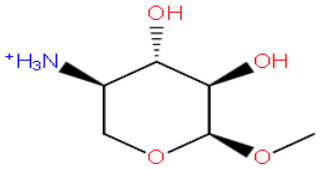
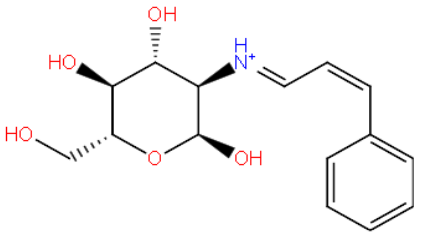
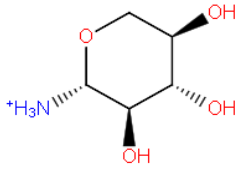
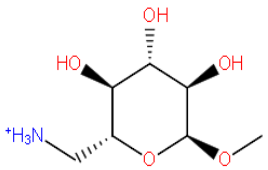
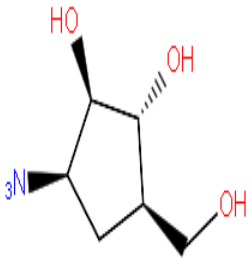
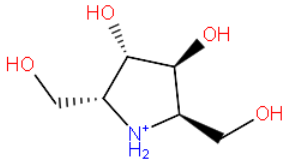
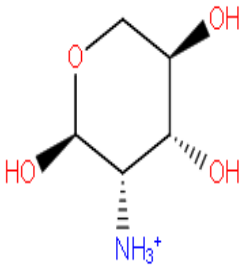
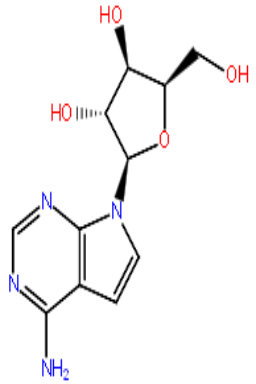
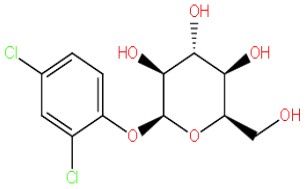
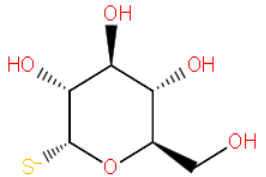
Virtual screening through HTVS ,sp and XP dock(315, 125 and 32)

17 leads

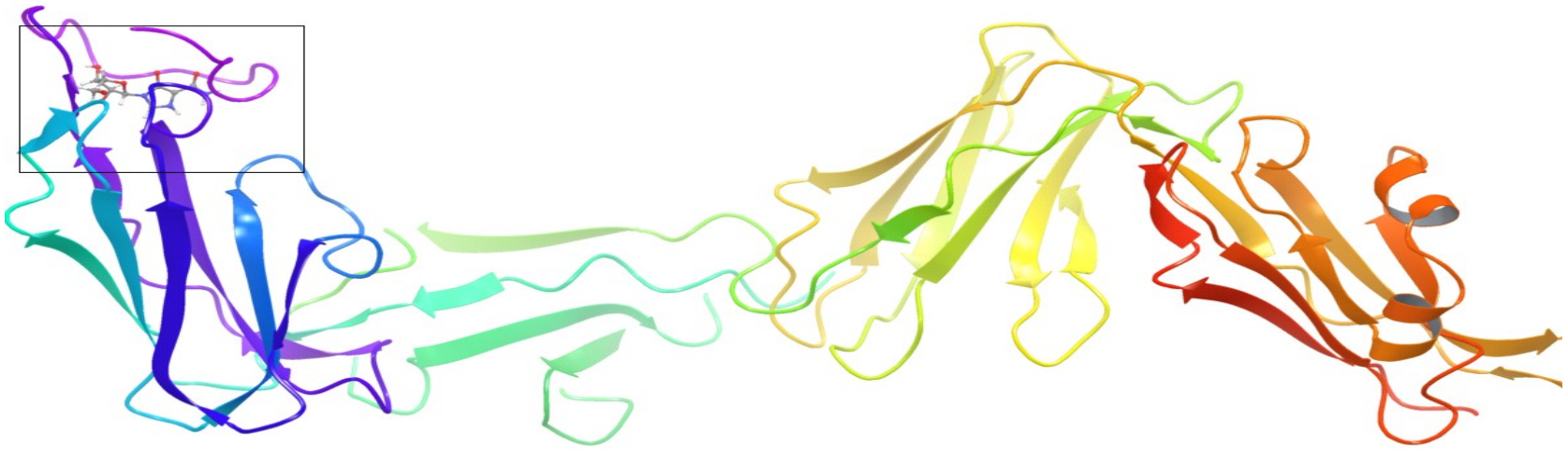
Docking score plot of ligand and leads



List of 17 leads

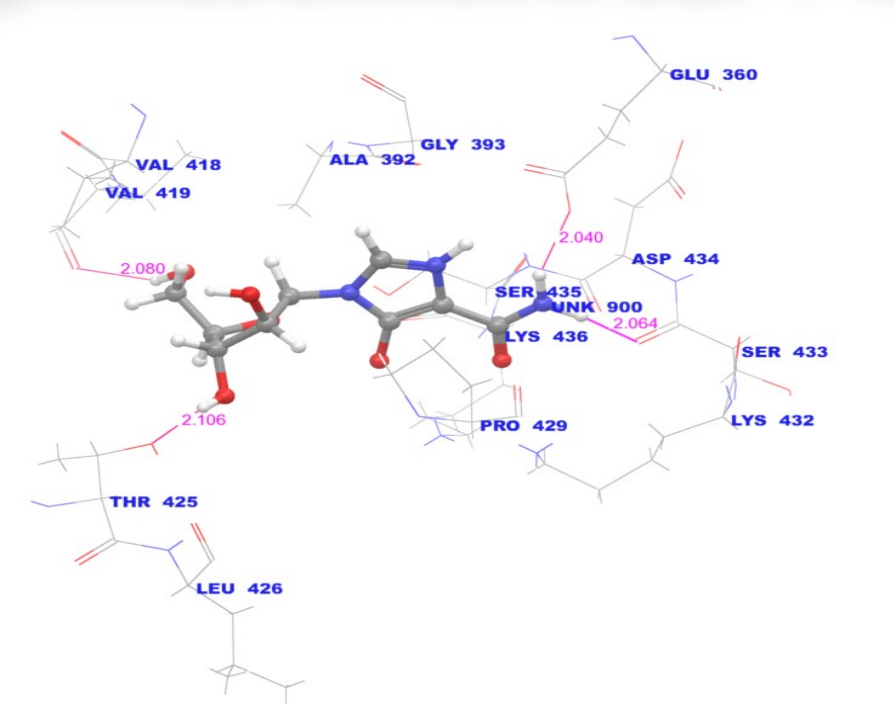


















Docking Complex



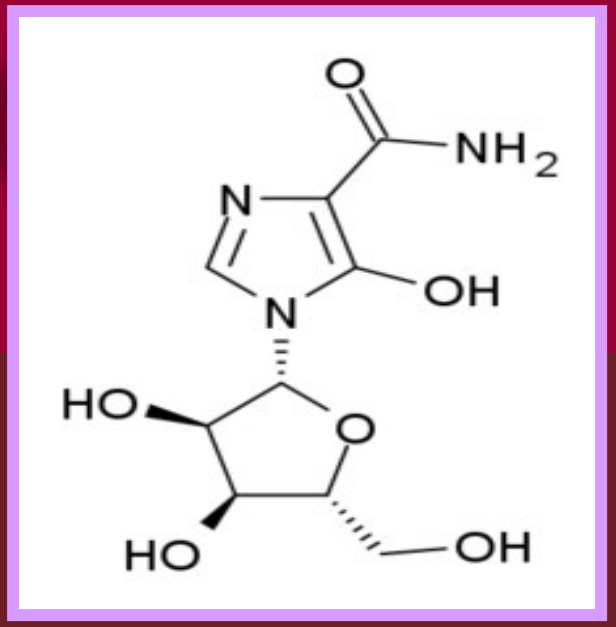
✓ Docking complex of lead 1 with LILRA3 protein with an negative XP G Score of -10.70 K cal/mol

Hydrogen bond network of lead “1” with LILRA3 protein



The lead1 forms 4 hydrogen bonds Thr 425, Glu -360, Ser-433,Val-419. The first hydrogen bond forms a bond length of 2.106. The second hydrogen has 2.040 length and third one has 2.064 and the fourth has 2.080 bond lengths respectively . The other van der Waal residues like Val-418,Gly-393,Asp-434, Ser-433,Lys -432 forms hydrogen bond network

Mizoribine as potent Lead



✓The lead “1” obtained is identified as MIZORIBINE

✓ **Mizoribine** (trade name **Bredinin**) is an immuno suppresive drug. It is a natural product, first isolated from the mould *Eupenicillium brefeldianum*.

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Eupenicillium brefeldianum, an ascomycetes harvested from the soil of Hachijo Island, Tokyo, Japan, in 1971, produces mizoribine (MZB). MZB is a nucleoside of the imidazole class, and was found to have weak antimicrobial activity against *Candida albicans*, but it proved ineffective against experimental candidiasis

Conclusion

- LILRA3 belongs to Ig family consisting of 439 amino acids
- The function of LILRA3 is to act as soluble receptor for class1 MHC antigens.
- The over expression of LILRA3 in serum of patients with RA show lymphocyte suppression leads to stroke .
- The lead ‘1’obtained (mizoribine) have better binding affinity, good docking score and orientation with LILRA3 for the suppression of lymphocyte in RA.
- Hence lead’1’(mizoribine) can be suggested as a promising lead for the treatment of RA patients causing stroke.

ACKNOWLEDGMENTS

- My deep sense of gratitude to the honorable **Dr.A.Umamaheswari,** Coordinator of BIF & Head of the Department, Bioinformatics, SVIMS, Tirupati for her guidance to carry out my project work

- I am highly thankful to DBT, ministry of science and technology, Govt. of India for providing all the necessary facilities to carryout project.