Research Article

Identification of Novel Inhibitors for Systemic Lupus Erythematosus by Targeting IFN-α and FCγR2A: A Structure Based pharmacophore Approach

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ABSTRACT

Systemic Lupus Erythematosus (SLE), a multisystem autoimmune disease. Exact pathology of SLE not known. Radiations, Environmental causes and genetic polymorphism lead to develop SLE. Epstein-Barr virus (EBV) might be involved in disease pathology. Clinical evidence put forward that interferon is the main root for the progress of SLE. FcγR2A low responder polymorphism is responsible to produce interferon from Plasmacytoid Dendritic cells (pDCs). Structure based virtual screening carried out to find new inhibitors by targeting serum interferons and low responder polymorphism of FcγR2A. ZINC94773831 shows good binding with low responder polymorphism of FcγR2A, which cause for the production of interferons.

Keywords: Systemic lupus erythematosus, FcgammaR2A, CD32a antigen, interferon alpha, Drug Design.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease mostly affects women in pregnancy. Malfunction in the immune system, mainly in Cellular and humoral immunity, result the development of auto antibody, and SLE.[1] Multisystem disease affects Kidney, Skin, joints, brain and other organs. Lupus nephritis[2] and Neuropsychiatric lupus (NPSLE) manifestations[3] displays high mortality rate. Common in the race of African, Afro-Caribbean, Native Americans, Asians and East Asians.[4] Research shows that SLE affect male and female in the ratio of 1:9.

Even though SLE caused by the failure of the cellular and humoral immunity. In normal human it plays a major part in the defense mechanism and responsible for immunity. B cells and T cells are responsible for attacking the pathogens and foreign particles which enter our body. Lymphokines a protein mediator prominently responsible for regulating the immune system, secreted by the helper T cell, a group of T cell.[5] Lacking of lymphokines results in total immune system paralyzed.

It is well known that acquired immuno deficiency syndrome demolish the helper T cell which leads to vulnerable against infectious disease. Interleukin-2 a lymphokines has a prominent role to develop cytotoxic and suppressor T cell. Interleukin-4, 5, 6 are involved in the stimulation of B cells.[6]

SLE is a heterogeneous autoimmune disease and the complete pathogenesis remains mystery. Environmental exposure and genetic polymorphism are high risk for developing systemic lupus erythematosus.[7] Delayed apoptotic cell clearance in SLE patient's results in secondary necrosis, a cause for development of autoantibody.[8] B cell malfunction, mainly overexpression of BLyS, results in forming auto antibody. T cell abnormality, inflammatory T cell supports in production

of auto reactive B cells and auto antibody.[9] Increase in production of proinflammatory cytokines and chemokine's a major cause for SLE. It is well-known that lupus nephritis patient's serum shows elevation in the interferon alpha, a proinflammatory cytokine.

Interferon alpha (IFN-α) plays a key role in SLE. Clinical evidence suggests that 99% of neonatal lupus is caused because of increase in expression interferon alpha. Type I interferon is involved in the defense mechanism during viral attach, helps in B cell activation and antibody production.[10] Clinical report reveals that prolong use of IFN-α results in lupus like symptom.[11] Thus interferon act both in defense mechanism as well as in Autoantibody. pDCs play a prominent role in the production of interferon alpha.

Furthermore, failure in the apoptosis cell clearance leads to necrosis results in activation of immune complex(IC). Toll like receptor (TLR), TLR7 or TLR9, expression was activated by IC in binding with low affinity FcγR2A present on the surface of pDCs.[12] TLR increases the production of IFN α by pDCs result in inflammation and the development of SLE.[13-16] It is believe that Epstein-Barr virus (EBV) may be involved in the development of systemic lupus erythematosus.[17-18]

Our present study is to identify new drugs which inhibit the interferon-α production. We inhibit low responder polymorphism of FcγR2A, which is responsible for the production of IFN-α from pDCs. We also try to inhibit the sera IFN-α of SLE patients. Certain therapies include Immunosuppressive agents, Corticosteroids, Antimalarial and NSAID are used which increase the life of patients. B cell depilation therapy and monoclonal antibody are used, but prolong use leads to the self-tolerance.[19-20] Therefore, it is necessary to find the potent drug which inhibit the IFN-α production in pDCs, which is responsible for the formation of SLE. In our research Structure based

pharmacophore was generated with the help of Ligand Scout. Drug like compounds were screened using Zinc pharmer and virtual screening was carried out.[21]

MATERIALS AND METHODS

Target Identification

Target identification was handled by collecting information from different databases and literature review. Notably, while identifying target; protein 3D structure, their sequence and protein function, motifs, folds and domain analysis are vital. To validate the target we used various databases like NCBI, Therapeutic Target Database, Gene card and UniProtKB. Table 1 shows the detail information concerning access number of the database and the protein details. X-Ray crystallography structure of interferon α and low respondent polymorphism of FcγR2A was used for the docking studies.

Table 1: Target protein information's on different databases

Target	TTD:ID	UniProtKB ID	PDB ID	Chain used
Interferon α	Nil	P01562	3UX9	Chain A
FcγR2A	TTDS00515	P12318	3RY4	Chain A

Active Site Prediction

Subsequently with the uprising in the human genomic sequencing, Insilico drug discovery is emergent. Over lakhs of available protein only few has protein-ligand complex and active sites.[22] To find an active site various binding site predictions like protein-ligand binding site, Protein-DNA binging site, Protein-Protein binding site predictors are in practice. Based on our work we used Protein-Ligand binding site for predicting the active site. Protein ligand binding site uses two methods, geometric methods and energy based methods.[23] We used energy based methods for the prediction of protein ligand binding site. In our study, receiving information from protein 3D structure, binding site predicted by using different algorithms. We used potent servers like CASTP, W Pool, FT Site, Meta Pockets, BS Pred, Residue Depth, LIGSITE, Co Factor, TM Site and COACH. Binding residues from the different servers were compared. Top three pockets were further preceded for pharmacophore generation and virtual screening.

Pharmacophore Design

IUPAC outlines Pharmacophore as "an ensemble of steric and electronic features that is necessary to ensure optimal supramolecular interactions with a specific biological target structure and to trigger (or block) its biological response".[24-25] Pharmacophore is the characterization of the steric futures necessary for biological activity in 3D or 2D representation. Oddly features like Hydrogen bond donor, Hydrogen bond acceptor, Aromatic, Positive charge, Negative charge, Negative ionisable, Ring aromatic are ideal for

pharmacophore generation. Structure based Pharmacophore, an emerging technique in insilico drug design, was handled in our study. Besides, 3D protein with ligand information certain protein lacks information regarding their active sites that is characterized as macromolecular approach.[26] Macromolecular approach was to create pharmacophore based on active site. Our present work was based on Macromolecular approach. Pharmacophore was generated based on active site pockets by using ligand scout as shown in Figure 1.
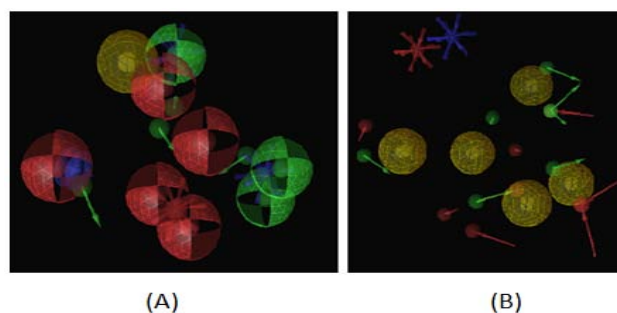

Figure 1: pharmacophore generated from Ligand scout. (A) is the pharmacophore of interferon α 3UX9 chain A and (B) is the pharmacophore of FcγR2A 3RY4.

Chemical Library

Generally speaking, chemical library is a database that encloses collection of drug like compounds in SDF and MOL2 format. Library encompasses information regarding molecular weight, molecular formula, smiles, LogP, Hydrogen bond donor, hydrogen bond acceptor, rotatable bonds etc. Zinc Pharmer, a free database comprise millions of drug like compounds available for virtual screening. http://zincpharmer.csb.pitt.edu/Zincpharmer generates conformations based on fingerprint as well as an alignment based conformer. We screened the drugs based on Lipinski rule.

Based on different pharmacophore parameters several drug like files were generated for virtual screening with Interferon α and FcγR2A. Discovery studio and Marvin view software were used to visualise the structure and properties.[27-28]

Data Mining

In our study with interferon α, best two active site pocket selected for further virtual screening study. Based on six different sets of pharmacophore Zincpharmer generated 30,251 drug like compound gained from first pocket. Successively, in second pocket a total of 25,831 drug like files were generated from three sets of pharmacophore. KNIME a java based data mining software used, to remove duplicates and bring together the compounds repeated in all the pharmacophores.

Workspace was generated as shown in Figure 2A. We got over-all 687 drugs from the first pocket and 340 drugs from the second pocket. Files were splitted and handled for protein ligand docking with interferon α (3UX9).

Concurrently for the subsequent protein ligands docking with FcγR2A best three pockets were selected.

Overall, we got 50,653 from first pocket 80,096 from second pocket and 95,011 drugs from the succeeding pocket. The workspace was generated for cluster analysis, based on Lipinski rule and molecular weight shown in Figure 2B. Best two clusters were further processed for protein ligand docking with FcγR2A (3RY4). A total of 2173 drug was extracted from KNIME. The first pocket contains 635 drugs, second pocket contains 827 drugs and third contain 711 drugs respectively.
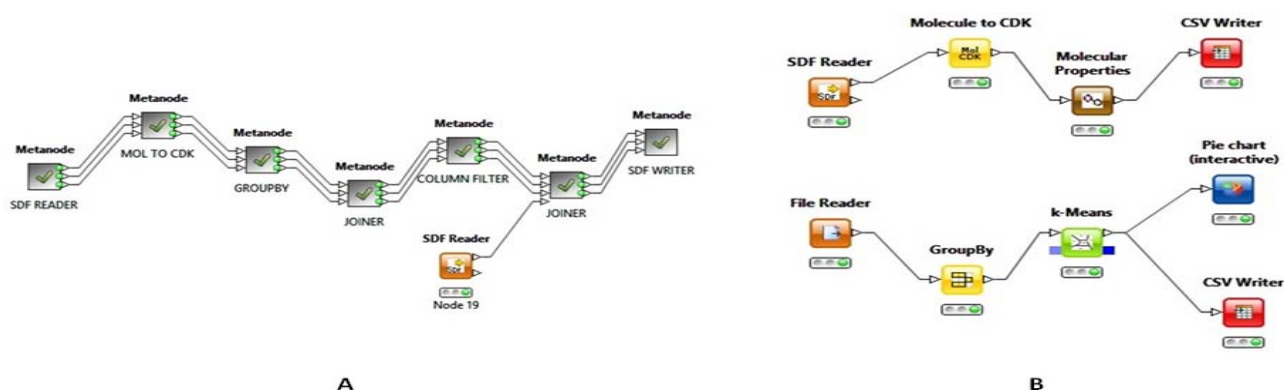

Figure 2: (A) KNIME workflow for combine drugs repeated in all the pharmacophore. (B) KNIME workflow for cluster analysis based on molecular weight.
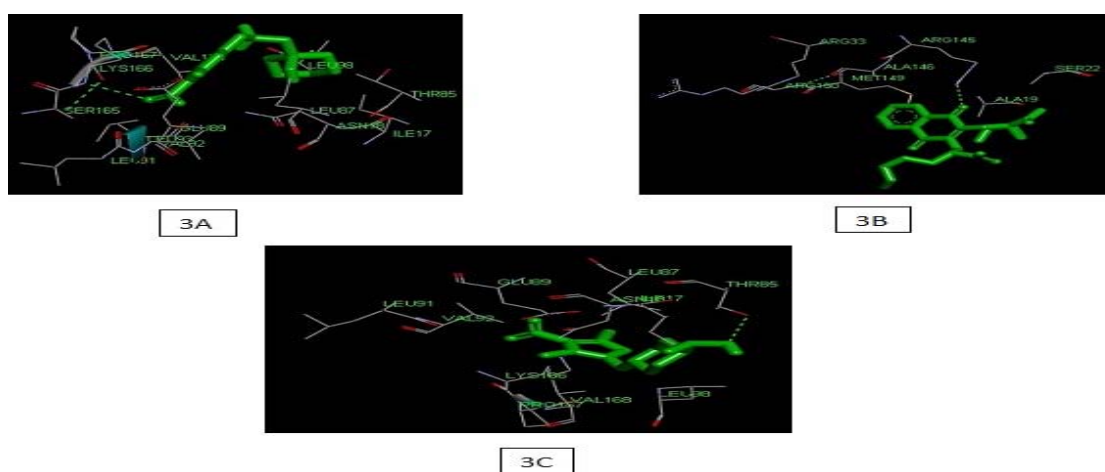

Figure 3: (3A): Protein ligand interaction of FcγR2A (3RY4) with ZINC94773831 shows interaction in LYS166. (3B): Protein ligand interaction of IFNα (3UX9) with Zinc32603325 shows interaction in ARG145. (3C): Protein ligand interaction of FcγR2A (3RY4) with ZINC77209292 shows interaction in LYS166.

Table 2: Protein ligand docking of best drugs based on binding affinity by targeting interferon-α and FcγR2A

S. No	Molecule: ID	Receptor	Affinity (kcal/mol)	Interaction
1	ZINC04244476	3ux9	-7.3	MET149:SD,ARG145:NH2
2	ZINC32603325	3ux9	-6.8	ARG145:NH1
3	ZINC34104693	3ux9	-6.9	SER25:OG
4	ZINC38200551	3ux9	-8	ARG145:NH2
5	ZINC06941198	3RY4	-5	LYS166:N
6	ZINC09498678	3RY4	-5.4	LYS166:N
7	ZINC11565632	3RY4	-4.8	THR85:OG1
8	ZINC77209292	3RY4	-5	THR85:OG1
9	ZINC89513211	3RY4	-5.1	LYS166:N
10	ZINC94773831	3RY4	-6.2	LYS166:N
11	ZINC94774380	3RY4	-5.5	THR85:OG1
12	ZINC94774418	3RY4	-5.3	LYS166:N
13	ZINC00502855	3RY4	-6.3	LYS166:N
14	ZINC02186707	3RY4	-4.9	THR85:OG1

Table 3: Re-Docking result of selected three drugs by targeting IFNα (3UX9) and FcγR2A (3RY4)

Zinc ID	Docking	Receptor	Affinity (kcal/mol)	Interaction
ZINC94773831	MOL:1	3ry4	-6.1	LYS166:N
	MOL:2	3ry4	-6	LYS166:N
	MOL:3	3ry4	-6.1	LYS166:N
	MOL:4	3ry4	-6	LYS166:N6:NIIIII
	MOL:5	3ry4	-6.1	LYS166:N
	MOL:6	3ry4	-6	LYS166:N 66:NII
	MOL:7	3ry4	-6	LYS166:N
	MOL:8	3ry4	-6	TRP85:OG1
	MOL:9	3ry4	-6	LYS166:N
	MOL:10	3ry4	-6	LYS166:N
ZINC32603325	DOCK:1	3ux9	-6.7	ARG145:NH1
	DOCK:2	3ux9	-6	ARG145:NH2
	DOCK:3	3ux9	-6.6	ARG145:N2
	DOCK:4	3ux9	-6.4	ARG145:NH1
	DOCK:5	3ux9	-6.8	ARG145:NH1
	DOCK:6	3ux9	-6.3	ARG145:NH1
	DOCK:7	3ux9	-6.7	ARG145:NH1
	DOCK:8	3ux9	-6.5	ARG145:NH
	DOCK:9	3ux9	-6.6	ARG145:NH2
	DOCK:10	3ux9	-6.8	ARG145:NH2
ZINC77209292	DOCK:1	3ry4	-4.9	THR85:OG1
	DOCK:2	3ry4	-5	THR85:OG1
	DOCK:3	3ry4	-4.9	thr85:og1
	DOCK:4	3ry4	-5.3	THR85:OG1
	DOCK:5	3ry4	-4.9	THR85:OG1
	DOCK:6	3ry4	-4.9	THR85:OG1
	DOCK:7	3ry4	-4.8	THR85:OG1
	DOCK:8	3ry4	-4.9	THR85:OG1
	DOCK:9	3ry4	-5	THR85:OG1
	DOCK:10	3ry4	-5.6	THR85:OG1

Table 4: Drug likeness and ADMET property of the selected top three drugs

ADMET Property	ZINC32603325	ZINC77209292	ZINC94773831
Bioavailability	YES	YES	Yes
Carcinogenic	Non Carcinogen	Non Carcinogen	Non Carcinogen
Mutagenic	Not mutagenic	Not mutagenic	Not mutagenic
Irritant	No irritation	No irritation	No irritation
Drug score	0.37	0.75	0.44
Molecular Wt	446.22	282.17	283.15
Log P	0.964	1.71	2.65
Lipinski	Yes	Yes	Yes
Hydrogen bond donor	8	4	1
Hydrogen bond acceptor	4	1	2

Protein Ligand Docking

AutoDock Vina is one of the finest, fast and accurate free software for virtual screening. In our study docking was handled by using Vina. It requires protein preparation as rigid and fixed. Confirmation files contain information regarding XYZ coordinates and the ligand which has to be docked with receptor. Vina does not require GRID file as it creates its own file. Cygwin, a UNIX based software was used to perform vina scripts. Vina scores nine conformation files. All the drugs from KNIME is docked with protein in their binding site and further analyzed for best score and energy.[29-31]

Docking Analysis

Docking analysis was performed by MGL tools where the binding energy and the repeated confirmations and the variance between the confirmations was noted, the best result was further re-docked.

Lead Optimisation

It's noted that to confirm the lead molecule the best selected leads were Loop docked (re-docked). Re-docked, by increasing the confirmations to 60 and each lead drugs were carried out 10 times. The lead drugs with maximum conformations and minimum binding energy was selected. To optimize the potent lead drug, analysis of drug likeness, bioavailability and toxicity is needed. Inappropriate optimisation of lead drugs downfall during *in-vitro* and clinical trials. To be ideal drug must deliver active part of the drug to its proper site. Bioavailability is the vital process for the drug delivery. To obtain the good therapeutic value the drug must have bioavailable. It is also noted that the drug must not hold toxic properties like Carcinogen, Mutagenic and irritant.

RESULTS AND DISCUSSION

Active Site Validation

Active site was predicted using different servers and validated. Interferon α 3UX9 shows predicted binding residue in ARG33, HIS34, VAL143, ALA146, and GLU147. While validating FcγR2A protein 3RY4 shows residue in LEU87, GLU89, LEU91, VAL92, LEU93, SER164, SER165, LYS166, VAL168.

Validation of Docking

Molecular docking study was performed using AutoDock Vina and Cygwin plugin, the results were analysed using MGL tools of AutoDock. Crystalline structure of IFNα and FcγR2A were accessed from PDB, access ID shown in Table 1 docked with their binding site predicted by different servers. Flexible docking was handled to find best conformations with minimum binding energy.

Docking Analysis

It was observed from our docking studies that ZINC94773831 (1-[[(3-fluoro-4-nitro-phenyl)methylamino]methyl]cyclohexanol) is the top drug and is displayed 10th in the Table 2. The result is

further Re-Docked to confirm the stability of the conformations as shown in Table 3. The fluctuation between the conformations at an average of 0.3. The drug is further tested for drug likeness bioavailability and toxic property shown in Table 4. The drug displays good drug likeness bioavailability and toxic property. ZINC32603325((2S)-6-amino-2-[[3-[[(1R)-5-amino-1-carboxy-pentyl]amino]-1,4-dioxo-2naphthyl]amino]hexanoic) which is second in the Table 2 shows good binding energy but, the interaction occurs in non-pocket residue and drug likeness bioavailability and toxic property is good. Re-Docking score and the drug likeness bioavailability and toxic property is displayed in Table 3 and 4. Subsequently, ZINC77209292 ((1R)-1-[1-[(3,5-dimethyl-4-nitro-pyrazol-1-yl)methyl]-4-piperidyl]ethanol) shown 8th of the Table 2. Re-Docking shown in the Table 3 points out uniform binding affinity in most of the dockings.

Binding Interaction of Ligand and Protein

The most active drug ZINC94773831 shown 10th in the table docked with FcγR2A. The drug shows good interactions with N atom of LYS166 shown in figure 3A. Toxicity prediction shows good result and Bioavailability is astonishing. Their binding affinity in the RE-Docking results show in Table 3 shows the constancy. The second best drug ZINC32603325 displayed second of the Table 2 docked with interferon α also shows pretty good binding energy. But the drug fails to interact with any of our pocket residue. The drug interacts with ARG145, a non-pocket residue, shows optimum results in repeated docking and conformations. The interaction visualise in Figure 3b. Finally, our third drug ZINC77209292 docked with FcγR2A shows good interaction with THR 85 (as shown in Figure 3C) with good binding energy and the fluctuation between the conformations is good at an average of 0.3. The drug shows good drug likeness bioavailability and toxic property and is visualise in Table 4.

Comparatively, the docking studies perform between the two targets FcγR2A and IFNα. Low responder polymorphism of FcγR2A 3RY4 shows very good binding affinity and interacting with the pocket residues. While targeting IFNα 3UX9 it fails to interact the pocket residue, but it shows constant interaction with non-pocket residue at a very good binding affinity.

CONCLUSION

Systemic lupus erythematosus is the most lethal autoimmune disease. At present there is no potent drug to treat SLE. Molecular docking is one of the powerful technique for identifying biological significance and exploring new drugs by screening millions of compounds. Here we targeted IFNα and FcγR2A. ZINC94773831 (1-[[(3-fluoro-4-nitro-phenyl) methylamino] methyl] cyclohexanol) which shows interaction in LYS166 of protein, low responder polymorphism of FcγR2A which is responsible for the production of IFNα from pDCs. It is

concluded that the drug inhibit the IFN-α production in pDCs by blocking low responder polymorphism of FcγR2A.

The drug has better bioavailability and there is no signs of irritation, Carcinogenic property as well as non-mutagenic. However, further *in vivo* experimentation is required for the validation of the drug.

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