Insilico Analysis of Binding of Boeravinone E with GMCSFR and Tnfαr

Praveen Kumar P.K, Sivanandham M, Karthick J, and Sivagami U

***Abstract*—** *Boerhavia diffusa* is a medicinal plant used in treatment of autoimmune diseases like Rheumatic arthritis and, Alzheimer's disease. The present study investigates the application of bioinformatics tools showing binding affinity of BoeravinoneE, a rotenoid with the human immunomodulatory receptor molecules GMCSFR and TNFαR, etc. The binding pocket sites, the internal energy, the hydrogen bond interactions and the interacting amino acid residues of the human immunomodulatory protein molecules with BoeravinoneE were analyzed through molecular docking method. Among the human immunomodulatory molecules docked, which are responsible for DCs maturation and activation, BoeravinoneE was binding with receptors of GMCSF, and TNFα exhibiting a maximum Dock score of 47.43 and, 59.31 and internal binding energy of 5.5 J/mol, 0.10 J/mol, respectively. BoeravinoneE was docked with binding site aminoacids, LEU246 of GMCSFR and SER74 and ASN110 of TNF-αR as predicted from UNIPROT and PDBSum. This present study results could be verified by doing studies in *invitro* and *invivo.*

***Keywords*—** Boeravinone E, *Boerhavia diffusa*, Granulocyte Macrophage Colony Stimulating Factor, Tumor Necrosis Factor - α

I. Introduction

IMMUNOREGULATION is a complex balance between regulatory and effectors cells and any imbalance in the immunological mechanism may lead to pathogenesis of several diseases [1]. Dendritic cells are crucial to the presentation of peptides and proteins to T & B lymphocytes and are widely recognized as the key antigen presenting cells (APCs) promoting immunomodulation [2]. The T cell receptors (TCRs) on T lymphocytes recognize fragments of antigens (Ags) bound to molecules of the Major Histocompatibility Complex (MHC) on the surfaces of APCs [3]. They can regulate the cytokine production such as Tumor Necrosis Factor (TNF), Interleukins (ILs) and Interferon's (IFNs) and these cytokines may, in turn, activate different cells of immune system such as T-cells or natural killer (NK) cells [4]. In most tissues, DCs are present in a so-called 'immature' state and are unable to stimulate T cells [5]. Once immature DCs have acquired and processed the foreign Ags, they migrate to the T cell areas of lymph nodes (LNs) and the spleen, undergo maturation and stimulate on immune response [6].

Modulation of the immune system is an emerging trend in chemotherapeutic research. Immunomodulators are materials derived from plant or microbial sources which can modify the body's defense mechanism either by enhancing or controlling immune responses [7]. In the present study, the plant taken is *Boerhaavia diffusa*, belonging to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (known also under its traditional name as Punarnava) is used for the treatment of various auto-immune diseases like Rheumatic arthritis, Alzheimer's disease and Colon cancer. The other 5 species of *Boerhaavia* present in India are *B.chinensis, B.erecta, B.repens, B.rependa*, and *B.rubicunda* [8]. The root of *B.diffusa* contains alkaloids (punarnavine), rotenoids (Boeravinones A-J), flavanoids, aminoacids, lignans (liriodendrons), β-sitosterols and tetracosanoic, esacosanoic, stearic and urosolic acids [8]. Rheumatoid arthritis; an auto-immune disease can reduce the number of RBCs (Anemia) and WBCs in humans. The usage of BoeravinoneE extracted from *Boerhaavia diffusa* as an adjuvant for Th4 cell Immunomodulation in humans through cytokines like TNF-α and, GMCSF expressed on Dendritic cells can reduce the Rheumatoid Inflammation of the lung lining (pleuritis), which causes chest pain with deep breathing or coughing [9]. Antagonism of GM-CSF can markedly reduce established disease in mouse models of RA [10]. Tumor necrosis factor alpha (TNFα) is known to exert pleiotropic effects on the host defense which fundamentally differ depending on an acute or chronic release, the occurrence of disequilibrium between pro-and anti-inflammatory mediators, and the concomitant regulation of other cytokines sharing certain biological properties. Trimeric TNFα acts by binding to one of the TNFα receptors (TNFRs): TNFR1 or TNFR2 [10, 11].

II. Databases And Softwares Used

A.Prodrg Server

Boeravinone E, a rotenoid from *Boerhaavia diffusa* [12] is screened from the 10 Boeravinone compounds by Lipinski's rule from MolInspiration. Boeravinone E is drawn in Prodrg Server [13] and the resultant 3-D coordinates in PDB format is

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retrieved for docking analysis with human GM-CSF and TNF-α were shown in Figure1.

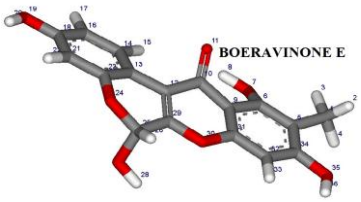


Fig. 1 Structure of Boeravinone E[13]

B. RCSB

Research **C**ollaboratory for **S**tructural **B**ioinformatics is an information portal and a resource house for many biological macromolecular structures. Often structures of proteins, enzymes, vitamins, polysaccharides, polypeptides etc are available as .pdb files.

C. UNIPROT

The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data. The Uniprot Knowledgebase (UniProtKB) is the central access point for extensive curated protein information, including function, classification, and cross-reference. The active sites are predicted from the PDBSum and UniProtKB data from the databases.

D. Accelrys discovery studio

In the present study, a new shape-based method called LigandFit [14] is used for accurately docking ligands into protein active sites. The method employs a cavity detection algorithm for detecting invaginations in the protein as candidate active site regions. A shape comparison filter is combined with a Monte Carlo conformational search for generating ligand poses consistent with the active site shape. Candidate poses are minimized in the context of the active site using a grid-based method for evaluating protein-ligand interaction energies. The method appears quite promising, reproducing the X-ray structure ligand pose within an RMSD of 2A°. A high-throughput screening study applied to the Human GMCSF and TNF-α receptors are also presented in which LigandFit, when combined with LigScore [15], an internally developed scoring function, yields very good hit rates for a ligand pool seeded with known actives.

III. Methodology

A. Immunomodulatory proteins preparation

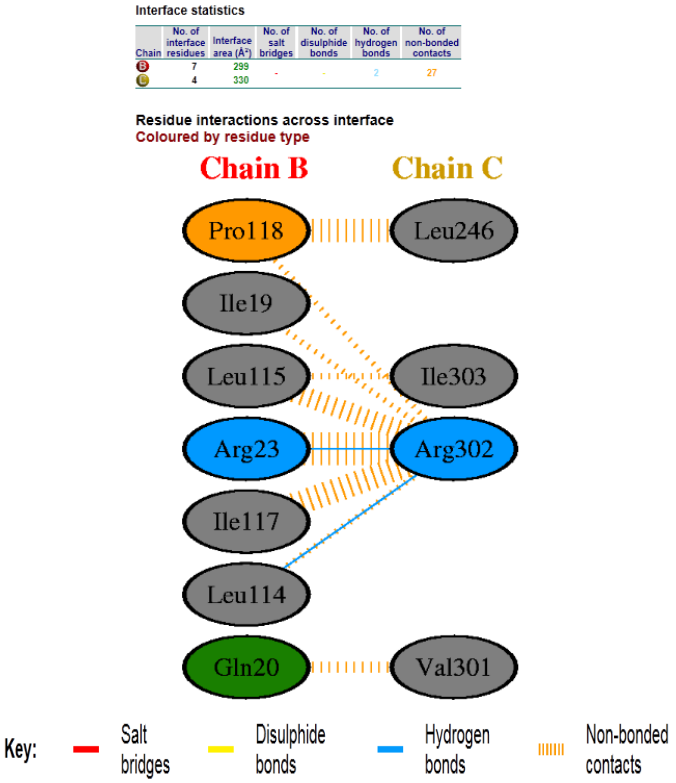
Protein-protein complexes remain enticing, but extremely challenging, targets for small-molecule drug delivery. Although antibody therapeutics have been developed that block protein-protein interactions, no approved small-molecule drugs have yet been produced for this important target class. GMCSF, TNFα receptor and ligand protein sequences were retrieved for Human in FASTA format from UniprotKB databases represented in Table 1.

TABLE I
IMMUNOMODULATORY PROTEINS INFORMATION OF *HOMOSAPIENS* FROM UNIPROT

S. No	Immunomodulatory Protein Name	Uniprot Accession No	Sequence Length (AA)	PDB Id	PDB Resolution (A°)
1	GMCSFR	P15509	400 AA	3CXE	3.3
2	GMCSFL	P04141	144 AA	1CSG	2.7
3	TNF-αR	P19438	461 AA	1EXT	1.85
4	TNF-αL	P01375	233 AA	1A8M	2.30

The active sites of Immunomodulatory cytokines responsible for activation and maturation of dendritic cells were predicted from PDBSum. The bonding contacts of Immunomodulatory ligand and receptor molecules like GMCSF [16] and TNFα [17] is shown in Fig 2.

GMCSF-L (Chain B) vs GMCSF-R (Chain C)



TNF-αL (Chain A) vs TNF-αR (Chain

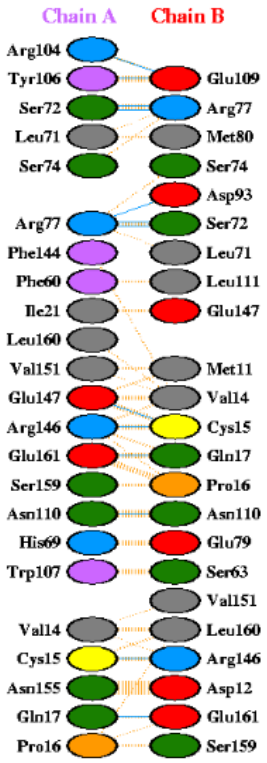


Fig 2. Bonding contact AAs of GMCSF-L (PDB Id: 1CSG) vs GMCSF-R (PDB Id: 3CXE) and TNF-αL (PDB Id: 1A8M) vs TNF-αR (PDB Id: 1EXT) from PDBSum

The ligands and crystallographic water molecules were removed from the protein; and the chemistry of the protein was corrected for missing hydrogen. Crystallographic disorders and unfilled valence atoms were corrected using alternate conformations and valence monitor options. Following the above steps of preparation, the proteins were subjected to energy minimization using the CHARMm force field.

B.DOCKING

Understanding the interactions between proteins and ligands is crucial for the pharmaceutical industries. The experimental structures of these protein/ligand complexes are usually obtained, under highly expert control, by time-consuming techniques such as X-ray crystallography or NMR. These techniques are therefore not suitable for routinely screening the possible interaction between Human GMCSF and TNFα receptors molecule with Boeravinone E. To overcome this limitation, computational algorithms (i.e. docking algorithm) have been developed that uses the individual structures of the receptor and ligand to predict the structure of this complex.

Thus docking analysis of Boeravinone E with Human GMCSF and TNFα receptors were carried out by LigandFit of Discovery studio. It explores the ways in which these Boeravinone E molecule and the Human Immunomodulatory receptor molecules like GMCSF and TNF-α fit together and docks to each other well, like pieces of a three-dimensional jigsaw puzzle. The Boeravinone E compound and the 2 human immunomodulatory receptor molecules necessary of immunomodulation expressed on Dendritic Cells were identified via docking and their relative stabilities were evaluated using their binding affinities. Thus binding sites were designed based on the ligands already present in the PDB file which were followed by site sphere definition for determining probe site radius. Here top ranked ligands were taken for binding affinity studies. The validation process consisted of two parts: (i) Hydrogen bond details of the top-ranked docked pose and (ii) Prediction of binding energy between the docked ligand and the three immunomodulatory molecules using various score calculated using Discovery studio (LigScore1, LigScore2, -PLP1, -PLP2, Jain, -PMF, Dock scores and Internal Energy scores) were taken for analysis.

The score values include Ligscore1&2 (Protein-Ligand Affinity Energy)[18], PLP1, PLP2 (Steric and H-bonding intermolecular function, Higher PLP scores indicate stronger receptor ligand binding (larger PKi values)[19], JAIN (sum of five interaction terms namely Lipophilic interactions, Polar attraction interactions, Polar repulsive interactions, Solvation of the protein and ligand, An entropy term for the ligand) [20] PMF (developed based on statistical analysis of the 3D structures of protein-ligand complexes, scores were calculated by summing pairwise interaction terms over all interatomic pairs of the receptor-ligand complex, a higher score indicates a stronger receptor-ligand binding affinity) [21] and DockScore (Candidate ligand poses are evaluated and prioritized according to the DockScore function). The determination of the ligand binding affinity was calculated using the shape-based interaction energies of the ligand with the protein. The two scoring methodologies namely LigScore and PLP1 were used to estimate the ligand-binding energies. Larger score value indicated better ligand-binding affinity.

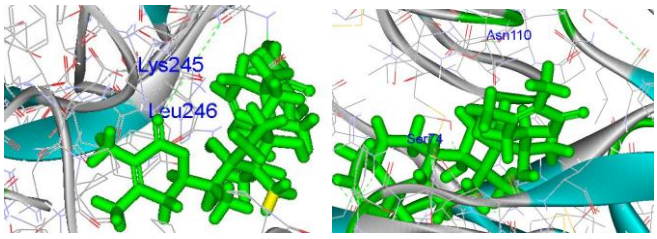
IV. Results And Discussion

Molecular Docking continues to holds great promise in the field of computer based drug design which screens small molecules by orienting and scoring them in the binding site of a protein. As a result, novel ligands for receptors of unknown structure were designed and their interaction energies were calculated using the scoring functions [22]. Number of reports citing successful application of CADD in developing specific drugs in different therapeutic areas was expanding rapidly. A very interesting example which can also serve as a proof of principle of the *insilico* approach involves in immunomodulation cytokines responsible for expression of dendritic cells. The elevation of GM-CSF in the joints of patients with Rheumatoid arthritis (RA) suggests a pathogenic role of GM-CSF in these auto-immune diseases [23]. TNFα accelerates thrombus formation in an invivo model of arteriolar thrombosis, supporting a more selective therapeutic approach in anticytokine therapy favouring TNFR2 specific antagonists [24]. The impact of TNFα in the pathogenesis of

autoimmune disorders such as systemic lupus erythematosus and RA has been widely accepted [25]. Targeting TNFα may be of therapeutic benefit in Osteoarthritis and requires further evaluation in controlled trials [26].

To ensure that the ligand orientation obtained from the docking studies were likely to represent valid and reasonable binding modes of the Boeravinone E, the LigandFit program docking parameters had to be first validated for the crystal structure's active site. UniprotKB database is used to find out the active sites in the structure and it was find out the active sites in the structure and it was found that the active site contains aminoacids such as LEU246 of GMCSFR and SER74, ASN110 of TNFαR. Results of docking showed that LigandFit determined the optimal orientation of the docked BoeravinoneE, exactly to these active sites. The low RMS deviation of between the docked and crystal ligand coordinates indicate very good alignment of the experimental and calculated positions especially considering the resolution of the crystal structure (2.00 A°) shown in Table 2.

A close view of the binding interactions of the two immunomodulatory cytokine molecules mandatory for expression of dendritic cells with the rotenoid, Boeravinone E were shown in Fig 3 & Fig 4. Table III shows the binding site radius, along with the total sites generated and total docked sites. Ligand is coloured in Green (Stick Model) whereas aminoacids involved in Hydrogen bonding were also shown in Green colour but in dotted lines. BoeravinoneE forms one hydrogen bond (shown as green dotted lines in Fig 3) and the residues involved in forming the hydrogen bonds from the chain C of GMCSFR was LEU246. BoeravinoneE forms two hydrogen bonds (shown in dotted lines in Fig 4) and here the residues involved in forming the hydrogen bonds from the chain B of TNFαR were SER74 and ASN110. Table IV showed that the detailed information of the hydrogen bond interactions of Boeravinone E with both the Immunomodulatory cytokine receptor molecules necessary for expression of dendritic cells. As a result of docking, there were 10 different conformations were generated for Boeravinone E. But only for top ranked docked complex the scores were copied from the table browser view of Discovery studio for binding affinity analysis. Table 3 shown the different score values of top ranked ligands.



(a) (b)

Fig. 3 Binding sites of 3D structure of (a) GMCSF-R (PDB Id:3CXE) and (b) TNF-αR (PDB Id:1IAR)with BoeravinoneE from DS

TABLE II
SUMMARY OF DOCKING SCORES INFORMATION OF THE TOP RANKED POSES OF BOERAVINONEE (VALUES COPIED FROM THE TABLE BROWSER WINDOW OF DS2.5.5)

Immunomodulatory Molecule	*LigScore1*	*LigScore 2*	*-PLP 1*	*-PLP 2*	*JAIN*	*-PMF*	*Dock Score*	*IE*
GMCSF-R (PDB Id: 3CXE)	4.7	3.64	56.9	62.2	1.73	67.3	47.43	5.5
TNF-αR (PDB Id: 1EXT)	4.8	3.92	80.2	84	3.2	36.7	59.31	.10

TABLE III
DOCKED SITE INFORMATION OF BOERAVINONEE WITH HUMAN IMMUNOMODULATORY RECEPTOR MOLECULES

Immunomodulatory Molecule	*Total Binding Sites*	*+ve Docked Sites*	*Probe Site Radius A°*
GMCSFR	19	8	7.9
TNF-αR	8	1	11

TABLE IV
HYDROGEN BOND INTERACTIONS OF BOERAVINONEE WITH HUMAN IMMUNOMODULATORY RECEPTOR MOLECULES

Immunomodulatory Molecule	*Chain: AA*	*Atom in AA*	*Position of AA*	*Atom in Ligand*
GMCSFR	C	LEU	246	H35
TNF-αR	B B	SER ASN	74 110	O36 OD1

V. CONCLUSION

The Protein-Ligand interaction plays a significant role in structural based drug designing for auto-immune diseases like Rheumatoid arthritis. In the present work, the human GMCSF, TNFα cytokine receptor molecules and the Boeravinone E to be as an adjuvant used for DCs Immunomodulation to explore the binding mechanism of rotenoids from *Boerhaavia diffusa*. Boeravinone E was taken for docking studies based on screening of compounds by Lipinski's rule. When the protein receptors docked to the Boeravinone E the scores obtained were shown as GMCSF (Dock Score= 47.43) and TNFα (Dock Score = 59.31). Boeravinone E binds the GMCSF and TNFα receptors stimulates the growth and differentiation of hematopoietic precursor cells from various lineages, including dendritic cells, granulocytes, macrophages, eosinophils and erthyrocytes. The TNFα intracellular domain (ICD) form induces IL12 production in dendritic cells and the cytokine produced was useful for maturation of DCs. Based on all the dock score values it was predicted that the Boeravinone E have similar and good binding affinities towards the specific

immunomodulatory cytokine molecules, expressed on dendritic cells. It was also predicted that the compound Boeravinone E can be used as an adjuvant for Rheumatoid arthritis cure by dendritic cell therapy.

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