



ISSN: 2231-3354  
Received on: 26-05-2012  
Revised on: 31-05-2012  
Accepted on: 09-06-2012  
DOI: 10.7324/JAPS.2012.2619

## ***In Silico* analysis of compounds characterized from *Pseudarthria viscida* root with TNF- $\alpha$**

**Thinakaran Rajan**

**Thinakaran Rajan**  
Department of Biochemistry,  
Muthayammal College of Arts and  
Science, Rasipuram,  
Tamilnadu, India

### **ABSTRACT**

Tumour necrosis factor is a polypeptide cytokine involved in inflammation and the acute phase response. TNF-alpha is present in larger quantities in persons with rheumatoid arthritis. Direct inhibition of TNF-alpha by the commercial biological agents has produced significant advances in rheumatoid arthritis treatment and validated the extra-cellular inhibition of this proinflammatory cytokine as an effective therapy. However, viable leads molecule that inhibits TNF-alpha have not been reported. Bioinformatics is seen as an emerging field with the potential to significantly improve how drugs are found brought to the clinical trials and eventually released to the marketplace. Computer-Aided Drug Design (CADD) is a specialized discipline that uses computational methods to stimulate drug-protein interaction. Discovery studio 2.1 provides a set of protocols for predicting and analyzing the interaction between protein and ligands. Molecular Docking experiments were carried out for the compounds identified from *Pseudarthria viscida* root extract with TNF-alpha using Accelry's DISCOVERY STUDIO 2.1. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Out of 13 compounds characterized from *Pseudarthria viscida*, only one of them docked with Tumour necrosis factor  $\alpha$ .

**Keywords:** Inflammation, TNF  $\alpha$ , *Pseudarthria viscida*, Rutin.

### **INTRODUCTION**

Tumour necrosis factor alpha (TNF  $\alpha$ ) is a pleotropic inflammatory cytokine produced by the immune system that suppresses tumour cell proliferation. Subsequent studies established that TNF  $\alpha$  is a key mediator of inflammation (Warren *et al.*, 1988; Vasanthi *et al.*, 2007). It is a proinflammatory cytokine capable of inducing multiple signaling cascades that can serve in host defense and paradoxically contribute to inflammatory tissue injury (Mu Culloch *et al.*, 2006). The elevated level of TNF  $\alpha$  has been reported in arthritic patients and in experimentally induced arthritis (Philippe *et al.*, 1997).

**For Correspondence**  
Email: [rajanpgbc@yahoo.co.in](mailto:rajanpgbc@yahoo.co.in)

Molecular Docking continues to hold great promise in the field of Computer Aided Drug Design (CADD) which screens small molecule by orienting and scoring them in the binding site of a protein. It is a study of how two (or) more molecular structure, fit together. In other words, the problem is like solving a 3 dimensional puzzles. The action of a harmful protein in human body may be prohibited by finding an inhibitor, which binds to that particular protein. Novel ligands for enzymes of known structure were designed and their interaction energies were calculated using the scoring function (Irwin *et al.*, 2002).

Secondary metabolites are the substances, which are produced by plants as defense chemicals. It includes alkaloids, flavonoids, essential oils, phenols, terpenes etc. These metabolites are sought after because they are known to exhibit number of biological activities that promotes health effects (Saxena, 2001). Plants have played a remarkable role in healthcare since the ancient times. Traditional plant based medicines still exert a great deal of importance to people living in developing countries and also leads to the discovery of new drug candidates (Orhan *et al.*, 2007).

The plants were initially used in unmodified form, later as extracts, and in the 19<sup>th</sup> century, advances in chemistry made it possible to isolate the active compounds of some medicinal plants. A large number of the pharmaceutical agents used today contain natural compounds, including those with various modification of the original molecule (Kingham, 2001). In addition, bioactive plant compounds have served as templates for several synthetic drugs, and as precursors used in the production of semi-synthetic drugs (Newman *et al.*, 2003; Verdine, 1996; Wessjohann, 2000). Historically, Willow bark (*Salix*) was used for its anti-inflammatory properties, which later resulted in isolation and synthesis of pure compound salicylic acid, and its acetylated derivative acetyl salicylic acid, commonly known as Aspirin (Vane and Botting, 1996).

## MATERIALS AND METHODS

### Compounds identified from *Pseudaerthria viscida* root:

The presence of compounds like 3-O-Methyl-d-glucose, Butane-1,1 Diethoxy-3-methyl, d-Mannitol-1-decyl sulfonyl, n-Hexadecanoic acid, Oleic acid, Oxirane tetra decyl, Tetradecanoic acid, Undecanoic acid was identified by GC-MS study. By HPLC analysis, the existence of phenolic compounds such as Rutin, Quercetin, Gallic acid, Ferulic acid and Caffeic acid was characterized. So in total 13 compounds identified in the root of *Pseudaerthria viscida* was taken for binding analysis with TNF-  $\alpha$ .

### Ligand preparation

The three dimensional structures of compounds taken for binding analysis were downloaded in .sdf format from PubChem database. Hydrogen bonds were added and the energy was minimized using CHARMM force field. Lipinski properties such as Molecular weight, XLog P, number of hydrogen bond donors and acceptors for the compounds were obtained from PubChem (shown in Table: 1)

## Protein preparation

The PDB is a key resource in areas of structural biology, is a key repository for 3D structure data of large molecules. The molecule which taken is TNF  $\alpha$  for our consideration. The PDB ID 2TNF and a resolution factor is 1.40 Å and the method of incorporation is X-ray diffraction method. The ligand 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 connected using alternate conformations and valence monitor options. Following the above steps of preparation, the protein was subjected to energy minimization using the CHARMM Force field.

## Docking studies

The docking method used in this study is LigandFit. To perform docking process the modeled protein, a protocol called "Dockligands" (LigandFit) is selected among those listed under receptor-ligand interaction protocol cluster. Each ligand compound is given as input in the parameter meant for "input ligands" and the protocol was run for each of the inhibitors selected for the study. The various conformations for ligand in this docking procedure were generated by Monte Carlo trials. The final energy refinement of the ligand pose (or) pose optimization in ligandfit occurs by Broyden-Fletcher Gold Farbshanno (BFGS) method. The Dock score of the best poses docked in to the enzyme for all the 13 compounds is calculated.

## RESULTS AND DISCUSSION

The crystal structure of Tumour Necrosis Factor  $\alpha$  with PDB ID 2TNF having structural weight 52000.04 is retrieved from PDB. The resolution factor is 1.40 Å and the method of incorporation is X-ray diffraction method. The structure of 2TNF has in total 3 chains viz. A, B and C. All the chains are sequence unique. The structure of TNF has one helices and twelve strands. 1% of the structure comprising 3 amino acid residues belongs to helical part and 48% of structure comprising 75 amino acid residues belongs to strands.

**Table. 1:** Physicochemical Properties of Compounds Identified In *Pseudaerthria Viscida*.

S.No	Compound name	Mol. wt	X logp	H- Bond donor	H- Bond acceptor
1	3-O-Methyl-d-glucose	194.18246	-2.9	4	6
2	Butane -1,1-diethoxy-3-methyl	1602539	2.5	0	2
3	d-Mannitol-1-decyl sulfonyl	370.50	0.9	5	7
4	n-Hexadecanoic acid	256.42	6.4	1	2
5	Oleic acid	282.46	6.5	1	2
6	Oxirane tetra decyl	240.42	7.3	0	1
7	Tetradecanoic acid	228.37	5.3	1	2

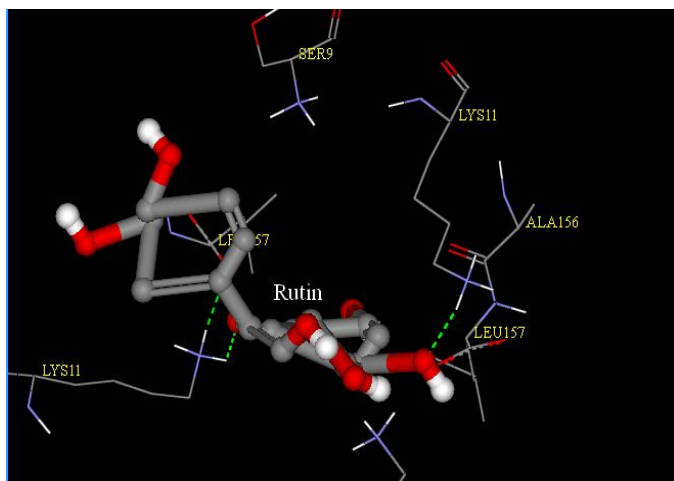
8	Undecanoic acid	186.29	3.7	1	2
9	Rutin	610.5175	-1.3	10	16
10	Quercetin	302.2357	1.5	5	7
11	Galic acid	610.5175	-1.3	10	16
12	Ferrulic acid	194.18	1.5	2	4
13	Caffeic acid	180.15	1.2	3	4

Out of different compounds (ligands) taken for docking analysis only one compound docked with the protein tumour necrosis factors alpha. Docked pose of the compound with protein (Tumour necrosis factor  $\alpha$ ) is presented in Figure 1. The dockscore values includes Ligscore 1&2 (Mayo et al., 1990); Piecewise Linear Potential (PLP)- PLP1(Krammer et al., 2005; Gehlhaar et al., 1995) and PLP 2 (Gehlhaar et al., 1995); Jain (Jain 1996); Potential of Mean Force- PMF (Mugge and Martin YC, 1999); PMF04 (Mugge, 2006), Ligand internal energy and dockscore obtained using LigandFit protocol of Discovery studio 2.1.

The ligand rutin docked with protein tumour necrosis factor with 22.035 dock score value and formed two hydrogen bonds. The atoms involved in forming hydrogen bond between rutin and protein (TNF $\alpha$ ) are presented in Table: 2

**Table. 2:** Hydrogen Bond details between the TNF $\alpha$  and the ligand rutin

Amino acid in Protein	Atom in amino acid	Position of amino acid	Atom in ligand
Lys	HZ	11	O19
Lys	HZ	11	O17



**Fig. 1 :** Docking pose of Rutin with TNF $\alpha$

To ensure that the ligand orientation obtained from the docking studies was likely to represent valid and reasonable binding modes of the inhibitors, the ligand Fit program docking parameters had to be first validated for the crystal structure's active site. Protein utilities and health protocol of Discovery's studio was used to find out the active site contains amino acids such as Ser 9, Lys 11, Ala 156, Leu 157 etc. Results of docking showed that the LigandFit determined the optimal of the docking inhibitor, exactly to these active sites.

Here top ranked ligand is taken for binding affinity studies. The validation process consisted of two parts;

1. Hydrogen bond details of the top-ranked docked pose.
2. Prediction of binding energy between the docked ligand and the enzyme using various score calculated using Discovery studio

## CONCLUSION

In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are the substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes / macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors on target cells and can increase vascular permeability and neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short-lived but cause harmful effects. One such a mediator is TNF  $\alpha$ . *In silico* molecular docking in one of the most powerful techniques to discover novel ligand for proteins of known structure and thus play key role in structure based drug. Investigators often use docking computer programs to find the binding affinity for molecules that fit a binding site on the protein. Hence in this present work we have carried out *in silico* molecules docking to analyze the binding properties of the mediator called TNF  $\alpha$  with 13 different compounds reported from root of *Pseudarthria viscida*. The wet analysis carried out by us showed very good result with regard to anti-inflammatory property of this plant extract. So the present study may act as supportive evidence that substantiate property of this plant extract may because of the inhibiting ability of Rutin identified from this plant with TNF  $\alpha$ .

## REFERENCES

- Gehlhaar, D.K., Bouzida, D. and Rejto, P.A. Rational drug design: Novel methodology and practical applications. American Chemical Society: Washington DC. (1999) 293-311.
- Gehlhaar, D.K., Verkhivker, G.M., Rejto, P.A., Sherman, C.J., Fogel, D.B., Fogel, L.J., Freer, S.T. Molecular recognition of the inhibitor AG-1343 by HIV-1 Protease: Conformationally Flexible docking by evolutionary programming. *Chemistry and Biology*. 1995; 2(5): 317-324.
- Irwin, J.J., Lorbes, D.M., Mchovern, S.L., Wei, B. and Shoichet, B.K. Computational nano-science and nanotechnology. (2002) ISBN0-9708275-6-3.
- Jain, A.N. Scoring non covalent protein-ligand interactions: A continuous differentiable function turned to computer binding affinities. *J. Computer-Aided Mol. Design*. 1996; 10:427-440.
- Kinghorn, A.D. Pharmacognosy in the 21<sup>st</sup> Century. *J. Pharm. Pharmacol.* 2001; 53:135-148.
- Krammer, A., Kirchhof, P.D., Jiang, X. and Venkatachalam, M. Ligscore: A novel scoring function for predicting binding affinities. *J Mol Graph Model*. 2005; 23:395-407
- Mayo, S.L., Olafson, B.D. and Goddard, W.A DREIDING: A generic force field for molecular simulations. *J Phys Chem*. 1990;94: 8897-8909
- MuCulloch, C.A., Downey, G.P. and Ei-Gabalway, H. Signalling platform that modulate inflammatory response: New targets for drug development. *Nature Rev Drug Discov*. 2006; 5:864-876.
- Mugge, I. PMF scoring revisited. *J Med Chem*. 2006; 49(20):5895-5902.
- Mugge, I. and Martin, Y.C. A general and fast scoring function for Protein-Ligand interactions: A simplified Potential approach. *J Med Chem* 1999; 42:791-804
- Newman, D.J., Cragg, G.M. and Snader, K.M. Natural product as a source of new drugs over the period 1981-2002. *J Nat Prod*. 2003; 66:1022-1037.
- Orhan, I., Kupeli, E., Sener, B. and Yesilada, E. Appraisal of Anti-inflammatory potential of the clubmoss *Lycopodium Clavatum* L. *Journal of Ethnopharmacology*. 2007;103(2):146-150.

Philippe, L., Gegout – Pottie, P., Guingamp, C., Bordji, K., Terlian, B. and Giller, P. Relations between functional inflammatory and degenerative parameters during adjuvant arthritis in rats. *Am J Physiol.* 1997; 273:R1550-R1556.

Saxena, M.J. Relevance of herbs in improving health index of livestock animals. Proceeding of 38<sup>th</sup> congress of Nigeria. *Vet. Med. Assoc.* 2001; 4-16.

Vane, J.R. and Botting, R.M. The history of anti-inflammatory drugs and their mechanism of action. In *New targets in inflammation – inhibition of COX-2 (or) adhesion molecule.* (Bazan N, Botting J and

Vane J., Eds) Klywer Academic Publishers and William Harvey Press, Dordrecht. 1996; 1-12.

Verdine, G.L. The combinatorial chemistry of nature. *Nature.* 1996; 384: 11-13.

Warren, J.S., Ward, P.A. and Johnson, K.J. Tumour necrosis factor: a plurifunctional mediator of acute inflammation. *Modern Pathol.* 1988;1:242-247

Wessjohann, L.A Synthesis of natural product based compound libraries. *Curr Opin Chem Biol.* 2000; 4: 303-309.