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*International Journal of Current Research*
*Vol. 6, Issue, 11, pp.10067-10070, November, 2014*

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

**ISSN: 0975-833X**

**RESEARCH ARTICLE**

# IDENTIFICATION OF NOVEL LEAD COMPOUNDS ACTIVE AGAINST INTERLEUKIN-17 PATHWAY INVOLVED IN RHEUMATOID ARTHRITIS USING IN SILICO APPROACH

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**ARTICLE INFO**

***Article History:***
Received 15th August, 2014
Received in revised form
26th September, 2014
Accepted 15th October, 2014
Published online 30th November, 2014

***Key words:***
Rheumatoid Arthritis,
Interleukin-17,
Docking; natural sources,
Probable drug.

**ABSTRACT**

**Objective**: The objective of the study was to identify novel lead compounds active against Interleukin-17 pathway that could reduce the effect of Rheumatoid Arthritis.
**Methods**: Work was done on the IL17 (PDB ID: 4HR9) protein. Protein minimization and active site prediction was carried out using CHARMm algorithm on Discovery Studio. Compounds from plant and animal sources having immunosuppressant, anti-TNF and anti-inflammatory properties as well as standard drugs active against Rheumatoid Arthritis were identified. ADMET studies were done. Protein-Ligand Docking was done using Lead IT. The best results were chosen based on their e-values.
**Results**: Probable drugs effective against Rheumatoid Arthritis were identified from various plant and animal sources based on lowest e-value and compared with the FDA approved standard drugs.
**Conclusion**: The use of various natural compounds as probable drugs could reduce the effects of Rheumatoid Arthritis. QSAR and molecular dynamics can be done on the best compounds chosen as probably drugs and further experimental analysis can be carried out.



## INTRODUCTION

Rheumatoid Arthritis is a progressive inflammatory autoimmune disease with articular and systemic effects (Choy, 2012). Modern advances in the medical treatment of Rheumatoid Arthritis have greatly alleviated patients' symptoms. Development of even more effective remedies could be spurred by discovery of the disease's etiology, but the cause of Rheumatoid Arthritis remains poorly understood and may involve a combination of genetic, environmental and stochastic factors (Choy, 2012). It is of unknown etiology affecting approximately 1% of the world (Gibofsky, 2012). Recent reports have provided convincing evidence that IL-17-producing T cells play a key role in the pathogenesis of organ-specific autoimmune diseases, a function previously attributed exclusively to IFN-c-secreting Th1 cells. Furthermore, it appears that IL-17-producing T cells can also function with Th1 cells to mediate protective immunity to pathogens. Although much of the focus has been on IL-17-secreting CD41 T cells, termed Th17 cells, CD81 T cells, cd T cells and NKT cells are also capable of secreting IL-17. The differentiation of Th17 cells from naive T cells appears to involve signals from TGF-b, IL-6, IL-21, IL-1b and IL-23. Furthermore, IL-1a or IL-1b in synergy with IL-23 can promote IL-17 secretion from memory T cells. The induction or function of Th17 cells is regulated by cytokines secreted by the other major subtypes of T cells, including IFN-c, IL-4, IL-10 and at high concentrations, TGF-b. The main function of IL-17-secreting T cells is to mediate inflammation, by stimulating production of inflammatory cytokines, such as TNF-a, IL-1b and IL-6, and inflammatory chemokines that promote the recruitment of neutrophils and macrophages (Mills, 2008). Interleukin 17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma. IL-17 is produced by T-helper cells and is induced by IL–23 which results in destructive tissue damage in delayed-type reactions. Interleukin 17 as a family functions as a proinflammatory cytokine that responds to the invasion of the immune system by extracellular pathogens and induces destruction of the pathogen's cellular matrix. Interleukin 17 acts synergistically with tumor necrosis factor and interleukin-1 (Pappu *et al*., 2011).

### Th17 Pathway

Many chronic inflammatory diseases are characterized by inappropriate or dysregulated CD4+ T cell responses. The understanding of the immunopathology of many chronic inflammatory diseases has been transformed by advances in basic studies of CD4+ T cell biology, especially the proposition, put forward more than two decades ago, that CD4+ T cells undergo sub specialization for coordinating "help" for distinct types of immunity on the basis of their

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expression of alternative patterns of cytokines: the T helper (Th) 1–Th2 hypothesis (Weaser *et al*., 2013). The idea instigated by the original Th1– Th2 hypothesis, that CD4+ T cells emerge from their primary thymic education poised for a secondary education that diverts them down divergent programs of differentiation contingent upon cytokine cues from innate immune cells, has provided a strong mechanistic foundation on which new advances and discoveries have been integrated. Among the most important of these was the discovery of a third major subset of effect or CD4+ T cells: Th17 cells (Weaser *et al*., 2013). Th17 cells are named for their production of the IL-17 family cytokines, IL-17A and IL-17F, that target innate immune cells and epithelial cells, among others, to produce granulocyte colony stimulating factor (G-CSF) and IL-8 (CXCL8), which induce increased neutrophil production and recruitment, respectively (Weaser *et al*., 2013).

The development of chronic immune disease mediated by dysregulated Th17 responses appear to be particularly extensive. In fact, it was the discovery that the cytokine IL-23, an important factor for the development of Th17 cells, was essential for several key models of autoimmune disease previously attributed to dysregulated (Weaser *et al*., 2013).

Th1 immunity that led to the discovery of Th17 cells as a distinct effector T cell subset. Since then, the Th17 pathway has been linked to a growing number of chronic inflammatory diseases in humans through genome-wide association studies (GWAS) and Th17 cell–specific biomarkers. Notable among these are inflammatory diseases centred on tissues with broad epithelial interfaces with the external environment, specifically mucosal tissues and the skin—sites where cells of the Th17 pathway are normally present in greatest abundance (Weaser *et al*., 2013). The differentiation of Th17 cells from naive T cells appears to involve signals from TGF-b, IL-6, IL-21, IL-1b and IL-23. Furthermore, IL-1a or IL-1b in synergy with IL-23 can promote IL-17 secretion from memory T cells. The induction or function of Th17 cells is regulated by cytokines secreted by the other major subtypes of T cells, including IFN-c, IL-4, IL-10 and at high concentrations, TGF-β. The main function of IL-17-secreting T cells is to mediate inflammation, by stimulating production of inflammatory cytokines, such as TNF-α, IL-1b and IL-6, and inflammatory chemokines that promote the recruitment of neutrophils and macrophages (Choy, 2012).

**Bone resorption and joint destruction**

The pathobiology of RA is multifaceted and involves T cells, B cells and the complex interaction of many pro-inflammatory cytokines, including TNF-a and IL-6. These cytokines are messengers that activate and differentiate effect or cells that cause local and systemic symptoms associated with this disease (Choy, 2012). Due to the association between ICAM1on the synovial macrophage and LFA1 on the self-reactive Th 1 cell, cytokines such as, IL-1, IL-6, IL-11, IL-18, TNF-a are released. TGF-b, IL-6, IL-21, IL-1b and IL-23 appear to be involved in Th cell differentiation, thereby are involved in the release of IL-17. The action of all these pro-inflammatory cytokines results in the conversion of RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) present on the Osteoblasts, mononuclear cells that synthesize the bone, to RANK (Receptor Activator of Nuclear Factor Kappa-B) on the Osteoclast. Osteoclasts are multinucleated cells formed by the fusion of mononuclear progenitors of the monocyte/ macrophage family. The primary mediators of bone resorption, these cells populate the synovial membranes of patients with RA and are polarized on bone. Macrophage-driven osteoclastogenesis requires the presence of macrophage colony-stimulating factor (MCSF) and results from the interaction of the RANK and the RANK ligand (RANKL). RANKL expression is regulated by pro-inflammatory cytokines such as TNF-a, IL-1, IL-6 and IL-17. MCSF, IL-6 and IL-11 can also support human osteoclast formation from peripheral blood mononuclear cells by a RANKL-independent mechanism (Choy, 2012).

There are certain other cells that enhance bone resorption such as,

- V-ATPase: V-ATPase stands for Vacuolar type H+ ATPase. They are ATP- dependent, multi-subunit proton pumps. For bone resorption, V-ATPases on the plasma membranes of Osteoclasts acidifies the extracellular milieu adjacent to the bone surface, by secreting protons from the Osteoclasts (Kartner N *et al*., 2012).
- TRAP: TRAP stands for Tartrate Resistant Acid Phosphatase. They are multinuclear cells released by the Osteoclasts that enhance bone resorption.

Agents such as parathyroid hormone, 1,25-dihydroxyvitamin D3 and interleukin (IL)-3 are known to increase the number of TRAP-positive cells in the synovial fluid (Fujikawa Y *et al*., 1996). This study aims to generate natural compounds that might have the potential to be candidate drugs thereby relieving the symptoms of Rheumatoid Arthritis.

## MATERIALS AND METHODS

The databases and tools used for this study were Accelrys Discovery Studio, CASTp (http://sts.bioengr.uic.edu/castp/), FlexX (BioSolve IT), GenBank (www.ncbi.nlm.nih.gov/ genbank), KEGG Database (www.genome.jp/kegg/), PoPMuSiC (http://babylone.ulb.ac.be/popmusic). PubChem Project (https://pubchem.ncbi.nlm.nih.gov/), PubMed (www. ncbi.nlm.nih.gov/pubmed), RCSB PDB (www.rcsb.org/), SAVes (nihserver.mbi.ucla.edu/SAVES/), UniProt (www. uniprot.org/) and Zinc DB (http://zinc.docking.org/).

The entire work can be divided into three parts (Part A, Part B and Part C).

**Part A**

Part A dealt with identifying the target protein, active site prediction and minimization. After literature survey, the target was identified as the IL17 protein and the protein structure was downloaded from RCSB PDB having accession number 4HR9. Protein minimization for the IL17 protein was done using Discovery Studio (Refer to Table 1). The active site pockets were identified using CASTp and minimization was done using Discovery Studio. This resulted in the IL17 protein active site prediction, which is used for docking.

### Part B

Part B dealt with identifying the lead compounds and lead minimization. Through literature survey, compounds from natural sources were identified and their structures were downloaded from PubChem Project and Zinc DB. The same was done for standard drug compounds taken for comparison studies. Three separate ligand libraries were prepared using Discovery Studio for plant sources, animal sources and standard drugs. Ligand minimization was done followed by filtering using Lipinski's Rule of Five. ADMET properties were studied for plant compounds only. All animal compounds and standard drugs were considered for further studies irrespective of the filtering process because the animal compounds which were basically essential fish oils had high molecular weight, but were known to have medicinal properties. The standard drugs were already in use.

### Part C

The third part involved molecular docking using FlexX (BioSolve IT) where the IL17 protein active site was docked against the three lig and libraries of plant compounds, animal compounds and standard drugs. Best results were chosen based on e-value.

## RESULTS AND DISCUSSION

### Protein-Ligand Docking

Protein-ligand docking was done to compare effectiveness of various natural compounds obtained from plant sources and animal sources to the standard drugs used to treat Rheumatoid Arthritis. Curcumin has lowest docking score (-21.0393) amongst lead compounds from plant sources (Refer to Section Table 2) and hence shows highest binding affinity to IL17 active site. Retinol has lowest docking score (-9.9816) amongst lead compounds from animal sources (Refer to Section Table 3) and hence shows highest binding affinity to IL17 active site. Methotrexate has lowest docking score (-31.9040) amongst standard drugs (Refer to Section 1.6, Table 4) and hence shows highest binding affinity to IL17 active site. While Methotrexate has more number of interactions and lower docking score than Curcumin and Retinol (Refer to Section Table 5), it also causes various severe side effects when administered. Some side effects caused by methotrexate intake include abdominal pain, chills or fever, dizziness, hair loss, headache, light sensitivity, itching, liver problems, low blood counts. Methotrexate can cause birth defects and death in unborn babies (http://www.webmd.com/rheumatoid-arthritis/guide/rheumatoid-arthritis-medications). Women must avoid becoming pregnant while taking this medication.

**Table 1. IL17 Protein Minimization Results**

| PDB ID | Minimization stage | CHARMm energy value (kcal/mol) |
|---|---|---|
| IL17 | Before Minimization | 258.18063 |
| IL17 | After Minimization | -6011.32144 |

The above table shows energy values before and after minimization using CHARMm (Chemistry at Harvard Macromolecular Mechanics) force field was computed and results were noted. High negative CHARMm energy value indicate stable active site complex.

**Table 2. Protein-Ligand Docking of IL17 vs Plant Compounds**

| Sr. No. | Plant Compound | Docking Score (kcal/mol) |
|---|---|---|
| 1 | Curcumin | -21.0393 |
| 2 | Isoliquiritigenin | -21.0280 |
| 3 | Quercetin | -19.0907 |
| 4 | Liquiritigenin | -19.0527 |
| 5 | Cianidanol | -18.5407 |

The above table shows the top 5 Protein-Ligand Docking results of IL17 vs Plant Compounds based on lowest docking scores in kcal/mol.

**Table 3. Protein-Ligand Docking of IL17 vs Animal Compounds**

| Sr. No. | Animal Compound | Docking Score (kcal/mol) |
|---|---|---|
| 1 | Retinol | -9.9816 |
| 2 | Eicosapentaenoic acid | -4.7883 |
| 3 | Docosahexaenoic acid | -3.0893 |
| 4 | Cholecalciferol | -1.0742 |
| 5 | Arachidonic acid | -0.8958 |

The above table shows the top 5 Protein-Ligand Docking results of IL17 vs Animal Compounds based on lowest docking scores in kcal/mol.

**Table 4. Protein-Ligand Docking of IL17 vs Standard Drugs**

| Sr. No. | Standard Drugs | Docking Score (kcal/mol) |
|---|---|---|
| 1 | Methotrexate | -31.9040 |
| 2 | Sulfasalazine | -27.1617 |
| 3 | Oxaprozin | -23.0278 |
| 4 | Piroxicam | -22.2226 |
| 5 | Meloxicam | -22.0046 |

The above table shows the top 5 Protein-Ligand Docking results of IL17 vs Standard Drugs based on lowest docking scores in kcal/mol.

**Table 5. Discussion of Protein-Ligand Docking of IL17 Protein**

| Compound Name | Docking Score (kcal/mol) | No. of Interactions | Amino Acid | Amino Acid Atom | Ligand Atom | Length (A°) |
|---|---|---|---|---|---|---|
| Curcumin (Plant Source) | -21.0393 | 3 | LEU97 | HN | O6 | 2.1395 |
| | | | TRP67 | HN | O2 | 2.0001 |
| | | | TRP67 | O | H41 | 1.7432 |
| Retinol (Animal Source) | -9.9816 | 1 | LEU97 | HN | O1 | 2.0267 |
| Methotrexate (Standard Drug) | -31.9040 | 8 | GLN93 | HN | O3 | 2.3294 |
| | | | GLN94 | HE21 | O3 | 1.7087 |
| | | | GLN94 | HE22 | O3 | 2.3458 |
| | | | GLU95 | HN | O4 | 2.0946 |
| | | | LEU97 | HN | N11 | 1.6908 |
| | | | GLU95 | O | H53 | 1.7530 |
| | | | LEU97 | O | H54 | 1.6524 |
| | | | LEU97 | O | H55 | 2.2204 |

The above table shows the docking scores in kcal/mol, the amino acid, and the interaction with the amino acid and ligand atom, as well as the bond length in angstrom units. The compounds being compared are Curcumin (plant source), Retinol (animal source) and Methotrexate (standard drug).

Pregnant women who have psoriasis or rheumatoid arthritis must not use methotrexate (http://www.medicinenet.com/methotrexate-oral/article.htm). Hence Curcumin can be an effective probable drug.

### Conclusion

Through literature survey, we learned that Rheumatoid Arthritis is one of the most prevalent autoimmune disorders that cause severe joint pain and inflammation in old and young alike. Probable drugs effective against Rheumatoid Arthritis were identified from various plant sources such as *Andrographis paniculata, Artemisia vestita, Berberis vulgaris,*

*Bupleurum falcatum, Camellia sinensis, Campylotropis hirtella, Clerodendron trichotomum, Curcuma longa, Dracocephalum kotschyi, Glycyrrhiza uralensis, Salvia mirzayanii, Tripterygium wilfordii, Urtica dioica* and animal sources such as fish oils. Since they are mainly derived from medicinal plants and essential fish oils, they are less likely to have side effects. While the standard drugs such as Methotrexate, Sulfasalazine, etc. showed better binding affinity, their toxic properties make them less viable as medications for chronic use. Toxicity prediction and analysis of the best natural compounds show low carcinogenicity and side effects compared to the FDA approved drugs in various animal models. According to the study, best plant compound that targeted the Interleukin-17 protein implicated in Rheumatoid Arthritis was curcumin, which could be used as a possible alternative to standard drugs such as methotrexate. Future screening of the probable drug compounds using QSAR studies will identify the best lead compounds to initiate experimental analysis and clinical trials. Molecular dynamic studies can also be carried out to garner a proper understanding of drug simulations in the body.

**Acknowledgement**

We, the authors express our sincere gratitude to the Department of Biotechnology, Siddaganga Institute of Technology, Tumkur, where the work was conducted. We, the authors also declare no conflict of interest.

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