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Research Article

# CHEMINFORMATICS OF POLYPRENOL, QUERCETIN, ELLAGIC ACID AND IT'S INTERACTION WITH ARTHRITIC PATHWAY

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**ABSTRACT**

Cheminformatics of polyprenol, quercetin, ellagic acid are done to find out druggability of compounds. The interaction studies of drug and target are done and compared with standard drug available in market. The *in silico* techniques employed in pharmaceutical companies for the process of drug discovery are used. The compounds structures are taken and docking is done against protein responsible for arthritis. Automated docking process is followed to know the binding properties of the compounds with target protein. Based on binding energies and bonds formed in docking process, it can be hypothesized that ellagic acid, polyprenol and quercetin are the potent activators of protein hypoxia inducible transcription factors (HIFs). We discuss here the synergistic application of *in silico* and cheminformatic study of the HIF-2α, the target protein in arthritic pathway. By *in silico* analysis, it seems that ellagic acid is promoting the notable anti arthritic activity through the inhibition of HIF-2α protein, with the formation of 4 hydrogen bonds and binding energy -9.38. Hence, the proposed study can be used in the development of novel lead compound for future advancement in drug designing.

**Keywords:** arthritis, cheminformatics, docking, HIF-2α

## INTRODUCTION

Cheminformatics is the use of computer and informational techniques, applied to a range of problems in the field of chemistry. Drug Designing is the inventive process of finding new medications based on the knowledge of the biological target. One way of achieving binding of small molecules to targets of biological interest is the *in silico* or virtual screening of large compound collections to identify a subset of compounds that contains relatively many hits against the target, compared to a random selection from the collection. "Docking program" is used to place computer generated representations of a small molecule into a target structure in a variety of positions, conformations and orientations. Each such docking mode is called as 'Pose'[1].

*Kirganelia reticulata* (Poir.), is a medicinal herb traditionally used to treat rheumatism. Polyprenol, quercetin and ellagic acid are isolated compounds of above mentioned plant. Ellagic acid is a dietry supplement, is purported to have anti-inflammatory, anticarcinogenic, antifibrosis, antioxidative and antinociceptive properties via cyclooxygenase inhibition. A polyphenol compound found in pomegranates, raspberries, and other foods, ellagic acid represents one of hundreds of phenolic compounds found in plant foods. Quercetin is a polyphenolic compound and a major bioflavonoid in the human diet, has anti-inflammatory properties because of direct inhibition of several initial processes of inflammation. Quercetin is present in foods as quercetin glycosides, represents 60–75% of the total dietary flavonols plus flavones intake. High concentrations are found in apples, tea, onions and red wine[2]. Polyprenols (α-unsaturated isoprenoid alcohols) occur in green tissues of many plants and have also been found in bacteria[3]. Polyprenols are low molecular natural bioregulators (physiologically active), playing a significant modulating role in the cellular process in plants referred to as biosynthesis. Polyprenols stimulate the immune system, cellular reparation and spermatogenesis, and have antistress, adaptogenic, antiulcerogenic and wound-healing activity.

Osteoarthritis (OA) is characterized by metabolic, biochemical and structural changes in the articular cartilage and surrounding tissues. Pathologic changes in the osteoarthritic joint are detected with conventional radiography, arthroscopy and magnetic resonance imaging.

Articular cartilage allows friction free movement and acts as a shock absorber in the joint. For this reason the tissue cannot afford a delicate blood supply or innervation. At least in larger animals a consequence of this lack of vasculature is that articular cartilage is maintained and functions throughout life in a hypoxic environment. This has naturally led to the study of hypoxia and the hypoxia inducible transcription factors (HIFs) in cartilage biology and pathology. HIFs mediate the response of all cells to hypoxia and the protein is continuously produced, but in the presence of sufficient oxygen it is rapidly degraded due to hydroxylation of specific proline residues that target the HIF-α subunit for von Hippel-Lindau tumor suppressor protein (pVHL)-mediated proteosomal degradation. However, when oxygen levels drop below a certain level (typically < 5%), hydroxylation becomes progressively inhibited since molecular oxygen is consumed in this reaction; HIF-α is therefore not degraded and dimerizes with the constitutively expressed HIF-β subunit. The HIF-α/β heterodimer translocates to the nucleus, binding specific hypoxia response element (HRE) consensus sequences (RCGTG) in target genes thus activating their transcription[5]. In human articular chondrocytes, HIF-2α is strongly regulated by prolyl hydroxylation, and can be stabilized even in the presence of atmospheric oxygen by specific inhibition of the HIF-targeting prolyl hydroxylases[6]. HIF-2α is expressed more in well-differentiated chrondocytes than in nascent cells, and many HIF-2α functions, including the promotion of chondrocyte hypertrophy, are not subject to loss by oxygen-dependent prolyl hydroxylation[7,8].

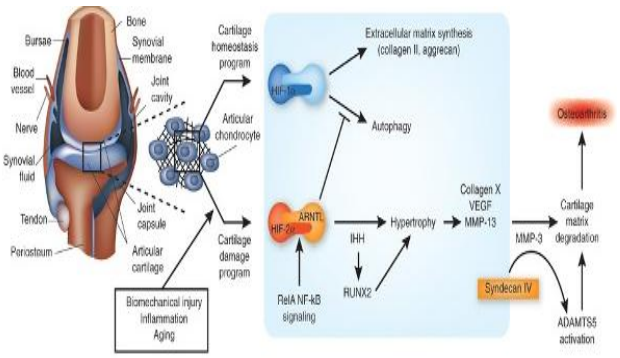


**Fig 1: HIF-1α and HIF-2α have different functions in the cartilage[4].**

The chemical and molecular properties of drugs- polyprenol, ellagic acid, quercetin and acetyl salicylic acid have been studied and the interaction between drugs and target protein hypoxia inducible transcription factors (HIFs) in arthritis is done using automated docking process.

**MATERIALS AND METHODS**

PUBCHEM (http://pubchem.ncbi.nlm.nih.gov) – PubChem is organized as three linked databases within the NCBI's Entrez information retrieval system. The drug structures are downloaded from this database.

CHEM DRAW ULTRA 6.0 - ChemDraw Ultra is a chemical structure drawing software designed for drawing stereochemically correct structures from chemical names, to get accurate IUPAC names for structures and to estimate NMR spectra from a ChemDraw structure with direct atom to spectral correlation.

KEGG (http://www.genome.jp/kegg/) - It is a collection of online databases dealing with genomes, enzymatic pathways, and biological chemicals. The PATHWAY database records networks of molecular interactions in the cells, and variants of them specific to particular organisms. Arthritis pathway and interacting proteins are studied using this.

PDB (www.rcsb.org) - The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. The 3D structure of HIF-2α having PDB ID: 3H7W is retrieved.

Swiss PDB Viewer - Swiss-PdbViewer is tightly linked to SWISS-MODEL, an automated homology modeling server and an application that provides a user friendly interface allowing analyzing several proteins at the same time. The PDB file is edited by removing the heteroatoms, adding C terminal oxygen.

Cast P (http://cast.engr.uic.edu.) - Computed Atlas of Surface Topography of proteins provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins.

Ligplot (http://www.ebi.ac.uk ) - The LIGPLOT program automatically generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input. Interacting amino acids with ligands on the surface of protein are predicted.

PRODRG (http://davapc1.bioch.dundee.ac.uk/prodrg/) - PRODRG will take a description of a small molecule and from it generate a variety of topologies for use with GROMACS, PRODRG takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein-ligand complexes.

Autodock - AutoDock is a suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoGrid calculates the energy of the non-covalent interactions between the protein and probe atoms that are located in the different grid points of a lattice that defines the area of interest.

As a result of these calculations the output file of the protein-ligand complex with flexible residues and the ligand located within the binding pocket is obtained. Each structure was scored and ranked by the program by the calculated interaction energy.

**RESULTS AND DISCUSSION**

The chemical and molecular properties of drugs- polyprenol, ellagic acid, quercetin and acetyl salicylic acid have been studied using Chem Ultra Draw 6.0. The study was conducted for the isolated compounds keeping in view of parameters such as exact mass, molecular weight, elemental analysis, boiling point, melting point, critical temperature, critical pressure, critical volume, Gibb's energy, Log p, Henry's law etc., and the results of which are presented in Table 1.

According to the designed protocol we retrieved the crystal structures of the hypoxia inducible transcription factors (HIFs) from PDB- 3H7W. The selected drug molecules; polyprenol, ellagic acid, quercetin and acetyl salicylic acid which underwent 2D screening were docked with respective receptor using ADT i.e Python and MGL Tools, an interactive protein docking and molecular super position program. The model depicted here opens the door to additional, compelling strategies for potential disease modification in human osteoarthritis, such as suppressing the activity of HIF-2α, inhibiting the development of articular chondrocyte hypertrophy in Arthritis.

The three dimensional structure of HIF-2α protein has been obtained as in Fig 2A from Protein Data Bank and PDB ID 3H7W of HIF-2α was selected for the interaction with different compounds. The schematic two dimensional representations of protein-ligand complexes as generated by LIGPLOT program from standard Protein Data Bank file input is shown in Fig 2B. During Autodocking, number of hydrogen bond formation and binding energy required has been shown between HIF-2α protein and drugs polyprenol (Fig 2C), quercetin (Fig 2D), ellagic acid (Fig 2E) and acetyl salicylic acid (Fig 2F).

In Autodocking, the binding energy of polyprenol, quercetin, ellagic acid and acetyl salicylic acid are -2.48, -5.8, -9.38, -6.76 and H-bond formed between protein and ligand is 1, 3, 4 and 2 respectively. Among these compounds Ellagic acid is having more number of hydrogen bonds and less binding energy is required. The values are represented in Table 2. Hence, ellagic acid has been proved to be one of the potent anti arthritic agents.

**CONCLUSION**

Current docking methods follow the assumption that protein structures are rigid entities and that it is the ligand that during the binding process changes its three dimensional structure to find the best spatial and energetic fit to the protein's binding site. This model does reflect the actual physical process of binding and limits or in some cases even prevents the correct identification of potential drug candidates. Based on Autodock binding energies it can be hypothesized that ellagic acid, polyprenol and quercetin are the potent activators of protein hypoxia inducible transcription factors (HIFs). It is a new approach to discriminate between good binders and non-binders to a protein. Further work aiming towards isolation of compounds is in progress.

**Table 1: Cheminformatic Analysis of Compounds**

| | Polyprenol | Quercetin | Ellagic Acid | Acetyl Salicylic Acid |
|---|---|---|---|---|
| **Formula** | $C_{19}H_{34}O_2$ | $C_{15}H_{10}O_7$ | $C_{15}H_8O_7$ | $C_9H_8O_4$ |
| **Exact Mass** | 294.26 | 302.04 | 300.03 | 180.04 |
| **Molecular Weight** | 294.47 | 302.24 | 300.22 | 180.16 |
| **Elemental Analysis** | C,77.50;H,11.64;O,10.87 | C,59.61;H,3.33;O,37.06 | C,60.01;H,2.69;O,37.30 | C,60.00; H,4.48;O.35.52 |
| **Boiling Point** | 680.29K | 822.35K | 828.14K | 589.05K |
| **Melting Point** | 366.87K | 970.62K | 977.10K | 432.56K |
| **Critical Temperat-ure** | 833.81K | 1021.68K | 1039.02K | 797.61K |
| **Critical Pressure** | 13.88[Bar] | 82.20[Bar] | 70.14[Bar ] | 35.77[Bar] |
| **Critical Volume** | 1073.50$cm^3$/mol | 706.50$cm^3$/mol | 704.50$cm^3$/mol | 481.50$cm^3$/mol |

| | | | | |
|---|---|---|---|---|
| **Gibb's Energy** | -56.58KJ/mol | -606.34KJ/mol | -604.40KJ/mol | -526.60KJ/mol |
| **Log P** | 4.71 | 0.35 | 0.72 | 1.18 |
| **MR** | 95.51 cm3/mol | 75.49 cm3/mol | 72.28 $cm^3$/mol | 43.95 $cm^3$/mol |
| **Henry's Law** | 3.20 | 25.30 | 22.84 | 7.27 |
| **Heat of Form** | -413.15KJ/mol | -900.69KJ/mol | -879.05KJ/mol | -671.58KJ/mol |

**Table 2: Molecular docking results with HIF-2α protein**

| Molecule | Binding Energy | Docking Energy | Inhibitory Constant | Internal Energy | H-Bond | Bonding |
|---|---|---|---|---|---|---|
| **Polyprenol** | -2.48 | 3.35 | 0.02 | 2.86 | 1 | HIF:A:CYS257:SG:PP::DRG1:HA7 |
| **Quercetin** | -5.8 | -5.89 | 5.61e-005 | -0.09 | 3 | HIF:A:ALA277:O::QR::DRG1:HAT<br>HIF:A:ASN341:OD1::QR::DRG1:HAR<br>HIF:A:THR321:HG1::QR::DRG1:OAU |
| **Ellagic Acid** | -9.38 | -9.39 | 1.32e-007 | -0.01 | 4 | HIF:A:ASN341:OD1:EA::DRG1:HAR<br>HIF:A:SER292:O:EA::DRG1:HAS<br>HIF:A:THR321:HG1:EA::DRG1:OAM<br>HIF:A:SER246:HG:EA::DRG1:OAQ |
| **Acetyl Salicylic Acid** | -6.76 | -7.25 | 1.06e-005 | 0.47 | 2 | HIF:A:SER246:HG:ASP::DRG1:OAB<br>HIF:A:HIS248:HE2:ASP::DRG1:OAB |

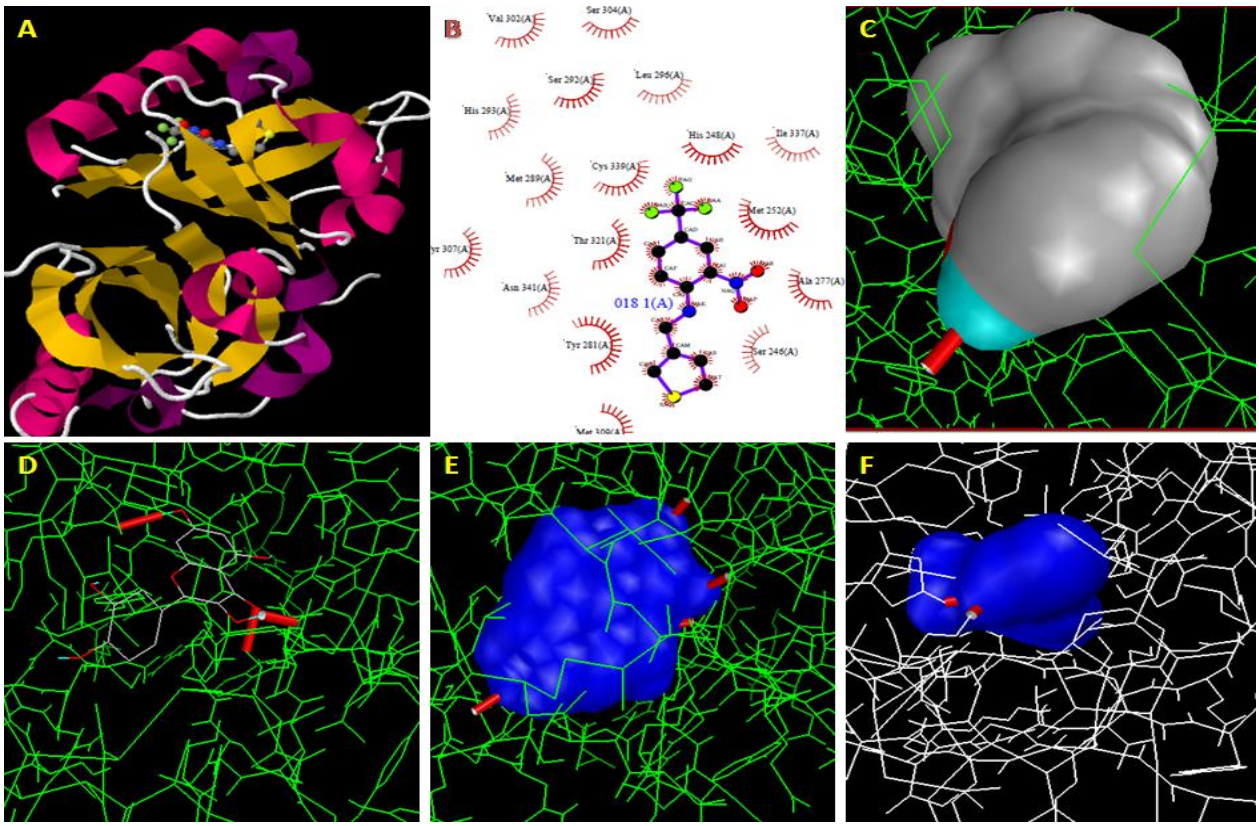


**Fig 2 :- (A) Protein structure of HIF-2α as retrieved from PDB, (B) Ligplot showing protein-ligand complexes of HIF-2α, (C) Orientation of Polyprenol in active pocket of HIF-2α, (D) Orientation of Quercetin in active pocket of HIF-2α, (E) Orientation of Ellagic acid in active pocket of HIF-2α, (F) Orientation of Acetyl salicylic acid in active pocket of HIF-2α.**

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