Original Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

E-PHARMACOPHORE SCREENING AND INDUCED FIT DOCKING OF PHYTOCOMPOUNDS AGAINST HUMAN DIHYDROOROTATE DEHYDROGENASE

PRIYA SWAMINATHAN*

Assistant Professor, Department of Biotechnology,SRM Univesrity, Kattankulathur, Chennai 603203, India

ABSTRACT

Human Dihydroorotate dehydrogenase (DHODH) is the fourth enzyme in the de novo pyrimidine biosynthetic pathway. Leflunomide is rapidly metabolized to its active form, A77 1726. Two mechanisms of action have been identified for A77 1726: inhibition of dihydroorotate dehydrogenase (DHODH) and inhibition of tyrosine kinases. Leflunomide is a novel drug with proven efficacy in rheumatoid arthritis but it is a synthetic inhibitor for DHODH which can cause side effects in the form of hepatotoxicity. Leflunomide is known to have two properties which from the literature as immunomodulatory and anti-rheumatic drug. Based on this activity of the leflunomide, the natural compounds in the Dr. Duke's database was searched and 62 compounds which are both, immunomodulatory or anti-rheumatic are collected from PubChem. An already validated E-pharmacophore model for leflunomide was searched against these compounds. The search resulted in three lignin based phytocompounds that was found to be most similar to leflunomide. Further, binding efficiency studies using induced fit docking was performed to explore the possibility that they could be inhibitors of human DHODH. This study helps to shortlist novel compounds from Duke's database as inhibitors of human DHODH.

KEYWORDS: *Immunomodulatory, leflunomide, rheumatoid arthritis, induced fit docking*

PRIYA SWAMINATHAN*

Assistant Professor, Department of Biotechnology,SRM Univesrity, Kattankulathur, Chennai 603203, India

Received on : 24-11-2016
Revised and Accepted on : 23-01-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b641-647

INTRODUCTION

Dihydroorotate dehydrogenase [DHODH] is an enzyme present in the mitochondrial inter-membrane of eukaryotes. It catalyses the fourth step of the *denovo* pyrimidine synthesis that takes place in the cytosol[1]:

$$\text{(S)-dihydroorotate} + O_2 \rightleftharpoons \text{orotate} + H_2O_2$$

The pathway provides pyrimidine for nucleotide replication, thus is active during growth and proliferation phases like in inflammation, cancer and tissue regeneration[2]. Human dihydroorotate dehydrogenase is a ubiquitous FMN flavoprotein[3]. Gene pyrD, in bacteria is located on the inner side of the cytosolic membrane while in some yeasts, such as in *Saccharomyces cerevisiae* (gene URA1), it is a cytosolic protein, whereas in other eukaryotes, it is found in the mitochondrial inter-membrane[4]. DHODH enzyme is very important target for inflammatory and immunohyperactive disorders[5].The drug named Leflunomide is usually used as a potent inhibitor of DHODH[6]. It binds to an alpha helix tunnel that leads to the alpha/beta-barrel domain based active site for dihydroorotate to form orotate[7]. Leflunomide prevents orotate formation thus decreasing inflammatory effects. Thus patients suffering from Rheumatoid arthritis show relief from pain during the action of leflunomide. However, longer course of taking leflunomide may cause hepatotoxicity related side effects[8]. Studies to find substitutes to leflunomide that might be efficient are happening. Among many methods used for finding new inhibitors for DHODH, one is using already known compounds for a QSAR model and analysis[9-11]. Various side chain substitution based compounds for leflunomide have been generated and analyzed for better activity[12-14]. Such studies are ligand focused and the novel compounds obtained are active against human DHODH.In an earlier study done by this author, an effective feature based model was created and validated for finding novel inhibitors of DHODH[15]. This E-harmacophore which was based on leflunomide and its binding mode in DHODH promised to shortlist new inhibitors with same activity and same binding mode. In this study, the validated E-pharmacophore is searched against a database of natural compounds with known activity to find similar hits. The hits obtained can be repurposed as an anti-rheumatic phytocompound. The study uses more accurate docking methodology to find new lead for human DHODH. Molecular docking is a good simulation that helps to provide an insight in to the type of molecules that might be good inhibitors of a target. Computational tools have been used to simulate and to analyze the binding pose of a novel ligand with the target. Induced fit docking is an advanced docking method where the receptor and ligand are kept flexible contrary to the conventional docking methods.

MATERIALS AND METHODS

Searching for natural compounds similar to Leflunomide

In the Duke's database of phytochemicals[16] the chemical activity of the compounds that are immunomodulatory and anti-rheumatic just like the action of Leflunomide was shortlisted. 62 compounds were searched and listed as having immunomodulatory and anti rheumatic activity. Those compounds that were duplicated in the list were removed. These compounds' structures were obtained from Pubchem- the chemical structure repository.

E-Pharmacophore structure

The E-pharmacophore from a previous study was used as a scaffold to search for new compounds with the same profile features as the pharmacophore. The pharmacophore used, is based on Leflunomide and has 4 featurs namely ADHR which was validated in the aforementioned paper using known inhibitors of DHODH[15]. In this study, the pharmacophore can be used to pick new inhibitors which might be leflunomide like inhibitors using the advanced screening option of Schrodinger's Pharmacophore modeling. The number of sites to match were set at 4 and 3 respectively.

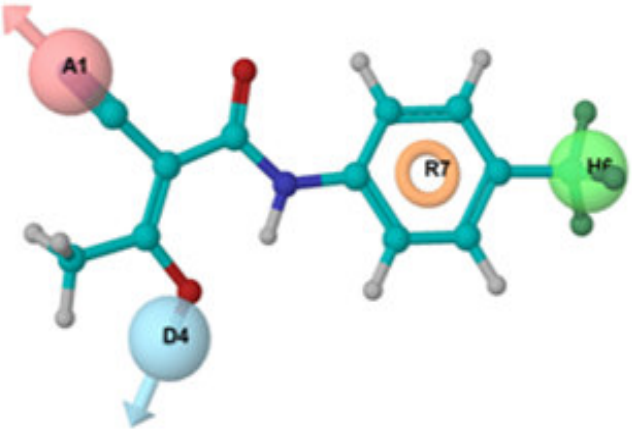

Figure 1
Four featured pharnmacophore (ADHR) which is capable of screening out novel inhibitors of human DHODH

Finding matches

Taking 62 natural compounds as unknown, and E-pharmacophore as known, a pharmocophore search was performed where the hits obtained were considered to have features similar to the known and hits with a high fitness value were shortlisted.

Protein preparation

Human DHODH [1D3H] was prepared for induced fit docking by removing hetero atoms, optimizing hydrogen bonds and minimizing the energy of the structure. This was done using the Protein Preparation wizard of Schrodinger Maestro.

Induced fit docking

In induced fit docking when the ligand structure is not exactly complementary to protein active site structure, the protein flexibly induces to accommodate the ligand of interest[17]. This is advantageous than glide docking using Schrödinger's Maestro Induced fit docking. This helps to evaluate false negatives and find a more accurate binding energy for ligand protein complex.

RESULTS AND DISCUSSION

Table 1
Phytochemicals with immunomodulatory and anti-rheumatic effects from Duke's Database

IMMUNOMODULATORY	ANTI-RHEUMATIC
(+)-EPIPINORESINOL	1,8-CINEOLE
3-ACETYLACONITINE	24-METHYLENE-CYCLOARTANOL
ACONITINE	ALPHA-TOCOPHEROL
ALKANNIN	ANISIC-ACID
ARABINO-3,6-GALACTAN-PROTEIN	APOCYNIN
ARCTIGENIN	ASCORBIC-ACID
ARGLABRIN	BETA-CAROTENE
ARTEMISININ	CITROSTADIENOL
BOLDINE	COLCHICINE
CHIMAPHYLIN	CYCLOARTENOL
EUCOMMIN-A	DIHYDROHELENALIN
EUPALINE	DIHYDROHELENALIN-ESTERS
GALLIC-ACID	FRAXETIN
GAMMA-LINOLENIC-ACID	GENTIANIDINE
GINKGOLIDE	GENTIANINE
GINSENOSIDES	GENTISIC-ACID
GLYCYRRHIZAN-UC	GLYCYRRHETIC-ACID
INOSINE	GLYCYRRHETINICACID
IRILONE	GLYCYRRHIZIC-ACID
LIMONENE	GLYCYRRHIZIN
LINOLEIC-ACID	HELENALIN
OLEANOLIC-ACID	HELENALIN-ESTERS
PAEONOL	LUPEOL
ROSMARINIC-ACID	MENTHOL
RUTIN	METHYL-SALICYLIC-ACID-ESTER
SAIKOSAPONIN	SALICIN
SANCHINAN-A	SALICYLIC-ACID
SWAINSONINE	THYMOL
SYRINGIN	THYMOQUINONE
TYLOPHORINE	TOCOPHEROL
URSOLIC-ACID	
WITHANOLIDE	

Table 1 lists the different compounds used for screening from duke's database. Among the types of compounds obtained, the compounds that are common aminoacids, vitamins and metals were disregarded as they cannot be used as a targeted ligand towards the target especially as they have other physiological roles in a body. So among the collected compounds, 62 compounds were used as the ligand list for pharmacophore search. Recurring entries were also deleted.

Pharmacophore searching

The E-Pharmacophore ADHR was searched against the 62 compounds. The search was conducted by varying the "must match" option as 4 or 3. This means that all four features of the pharmacophore or atleast 3 features of the pharmacophore must match to be shortlisted as a hit. When match by all 4 features was run, it gave Arctigenin as an only hit. The Search to match Atleast three features gave 4 hits listed in Table 2.

Table 2
Fitness score of compounds screened by the ADHR pharmacophore.(3 match criterion)

Compounds	A	D	H	R	Fitness score
CID 6021- Inosine	✓	✓		✓	1.89
CID 5281779-Irilone	✓	✓		✓	1.57
CID 64981 -Arctigenin	✓	✓	✓	✓	1.39
CID 637584-Epipinoresinol	✓		✓	✓	1.35

The fitness score ranged from 1.35 to 1.89. All the hits were above 1. A fitness score is a measure of similarity between a chemical structure and a pharmacophore with features. A tetra or a hexa hydroxyfuran fused ring was the key cohesion factor between the compounds in the hits list. Among the 62 compounds obtained, only four compounds including Arctigenin were found to match the E-pharmacophore. Table 2 gives the features that were matched also. In the case of epipinoresinol, it matched the Acceptor, Ring and Hydrophobic features of the Epharmacophore. Inosine and Irilone both had matched Acceptor, Donor and ring features while Arctigenin matched all the four features.

Induced fit docking

Induced fit docking [IFD] was performed to obtain flexible docking results for the Pharmacophore screened hits. The prepared protein PDB Structure of Human DiHydroOrotate Dehydrogenase (1D3H) was added as the receptor for induced fit docking. The program would simulate the induced conformations in the receptor using Prime Modules , such that the ligand flexibilitycan be accomodated accommodated in a superior manner than rigid docking .Alternative poses of the ligand can be explored.Also false negatives can be minimized by this methodology.All the four compounds obtained from Pharmacophore screening and Leflunomide (CID 3088)was used for induced fit docking to Human DHODH

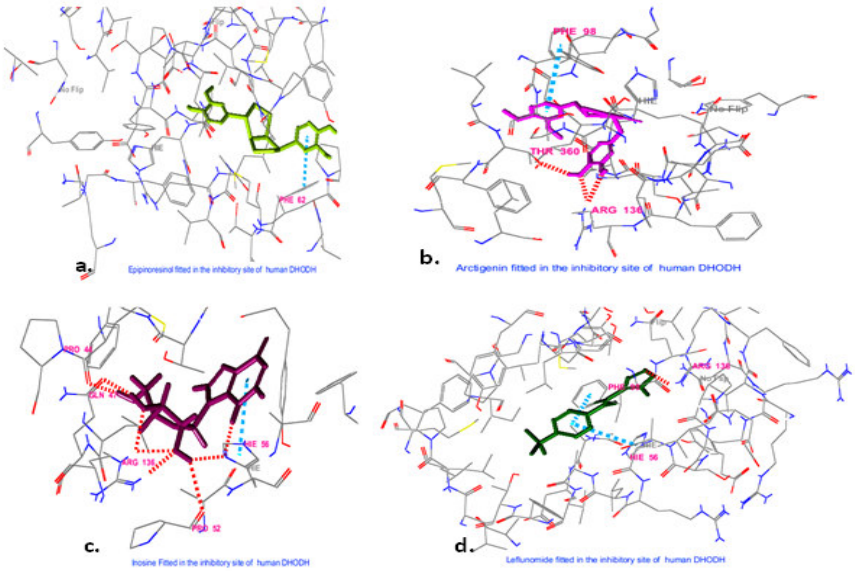

Figure 2

Induced fit docking poses for the ligands shortlisted from Duke's Database with human DHODH receptor: a. Epipinoresinol b. Actigenin c. Inosine and d. A771726(Active form of Leflunomide)

Flexible induced fit docking of the compounds screened out from E-pharmacophore search gave superior results when compared to the known inhibitor Leflunomide. Induced fit docking provides an IFD score which is the sum of glide Gscore and the prime energy. Glide score enumerates the binding efficiency of the ligand while the Prime energy finds the best conformation of the receptor for the ligand to bind.IFD score signifies that the obtained compounds have a conformational energy close to the known inhibitor of lefluomide

Table 3

IFD score for the compounds used in Induced fit docking

S.No	Compounds	IFD score Kcal/mol
1	CID 6021- Inosine	-807.39
2	CID 5281779-Irilone	-
3	CID 64981 -Arctigenin	-808.62
4	CID 637584-Epipinoresinol	-814.38
5	CID 3088-A7717269 (Active form of Leflunomide)	-808.69

The IFD score for Leflunomide was -808.69 Kcal /mol. The other compounds used for IFD showed its score to be either minimized or nearer to the IFD score of leflunomide. The IFD score ranged from -807 to-814 Kcal/mol (Table 3). Since the variations in the score are less, it is indicative that the poses of the ligands obtained are near to or conformationally stable just like that of Leflunomide. Epipinoresinol had highest IFD score of -814.38 Kcal/mol. Irilone alone did not bind with human DHODH. This could be due the bulky nature of 3 fused rings in its di-oxolo chemical structure. The structure could be too bulky to accommodate in the tunnel like inhibitory site of DHODH. Though the fitness score was high for irilone, it still did not bind to the human DHODH receptor. Thus IFD protocol helped to rule out false positives like irilone as inhibitors of human DHODH.

Binding interaction of leflunomide with Human DHODH

Leflunomide (Active form-A771726) is a known inhibitor of human DHODH. It binds to a hydrophobic pocket surrounded by alpha helices. It binds to Arg136 with a Hydrogen bond of 2.29 Å length and also has 3 stacking π bonds each with Arg136, Phe98 and His56 (Figure 2d). π stacking strengthens the interactions between ligand and receptor . The XP Glide Gscore was -8.87 Kcal/mol (Table 4)

Binding interaction of Epipinoresinol with human DHODH

Epipinoresinol had no known hydrogen bonds with the inhibitor site but interacts with a π- π stacking bond with Phe62. Epipinoresinol forms a π stacking bond between its methoxyphenyl group and the phenyl ring of phenylalanine. This bond is in the deeper hydrophobic region of the DHODH pocket. It had a good XPGscore of -11.48 Kcal/mol. IFD produced only two different poses for epipinoresinol but based on the E-model score, which is the score to choose the most stable conformation, the ligand pose in Figure 2a was chosen.

Binding interaction of Arctigenin with human DHODH

The compound arctigenin was bound with the residue Arg136 with two bonds like Leflunomide. Additional hydrogen bond with Thr360, both amino acids were found in the polar region of the inhibitor site of DHODH (Figure 2b.). π- π bonding with Phe98 in a deeper hydrophobic pocket. Arctigenin gave a XP Gscore of -8.87 Kcal/mol. Two Hydrogen bonds with Arg136 and Thr360 were at 2.09Å while the other bond with Arg136 was at a distance of 2.63 Å.

Binding interaction of Inosine with human DHODH

Inosine forms hydrogen bonds with the side chains of Arg136, and Gln47.It also forms Hydrogen bonds with the backbone atoms of Met43 and Pro52. It forms a π bond with His56. The Hydrogen bonds were at lengths ranging from 1.84 Å to 2.34 Å (Figure 2c). This indicates that close contacts between the ligand and the receptor. Inosine had a Glide XP Gscore of -9.70 Kcal/mol.

Table 4
Glide XP GScore from IFD docking

Title	Fitness	IFDScore Kcal/mol	Glide emodel Kcal/mol	XP Gscore Kcal/mol
CID637584 EPIPINORESINOL	1.35	-814.38	-62.07	-11.48
CID6021 INOSINE	1.89	-807.03	-69.87	-9.70
CID64981 ARCTIGENIN	1.39	-808.62	-72.62	-8.87
CID3899 LEFLUNOMIDE	-	-808.69	-66.18	-8.11

Table 4 gives the IFD docking XPGscore for the compounds shortlisted by the E-pharmacophore. The table also indicates the associated IFD score and the Emodel score for the most favorable poses. The XP score ranged from -11.48 Kcal/mol for epipinoresinol to -8.87 Kcal/mol for arctigenin. All the XP scores were higher compared to that of Leflunomide (-8.11 Kcal/mol). Though the fitness score of inosine was highest, its docking score was second best (-9.70 Kcal/mol) to that of epipinoresinol. However all the molecules screened by the E-pharmacophore, when docked , bound to the same pocket around Arg136 and other amino acids in the 3Å distance surrounding it. Figure 3 gives the exact orientation as well as the molecular surface of the molecules bound to inhibitor site.

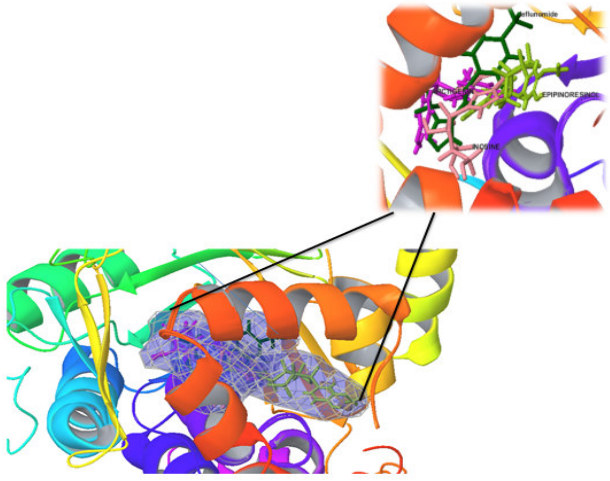

Figure 3
The phytomolecules from Duke's database binding in the same binding pocket.

Four molecules were screened out by the E-pharmacophore, validated in an earlier study. Out of the four compounds, irilone didn't bind to the inhibitor site during IFD analysis probably due to its bulky nature owed to the presence of a dioxolo furan ring. The other three molecules namely epipinoresinol, arctigenin and inosine were bound to the human DHODH receptor in the same way as the known inhibitor Leflunomide (Active form A771726).Their conformational energy was almost same like the known drug , given by the IFD score. Also the binding amino acids were similar or in the 3Å vicinity of the Arg136. Others have followed similar workflow to shortlist efflux protein inhibitors in *MDR Pseudomonas*[18] and LuxP inhibitors for *Vibrio harveyi* [19]. Epipinoresinol, arctigenin and inosine gave comparatively good score and energy for their poses in the binding pocket of the inhibitor. All the three shortlisted compounds were originally categorized as immune-modulators in the Duke's Ethnobotanical database. Epipinoresinol is a lignin already well known for its antihypertensive effects from the plant *Eucommia ulmoides*[20]. Arctigenin, another lignin is a good anti hepatocellular carcinoma agent found in traditional Chinese herbs like *Forsythia intermedia*[21]. Among other studies, inosine an alternate form of adenosine, can be used as an antidepressant via the adenosine receptors in the brain[22]. Another observation that was obvious from the IFD docking was pi-pi stacking bonds played an important role. In a previous work[15], Glide XP docking did not produce any pi-pi interaction while IFD based on flexibility of the receptor produced pi stacking bonds. These bonds are important for producing structurally stable ligand- receptor interactions. Thus IFD investigations and presence of π- π bonding along with good docking score are indicative of good hits.

CONCLUSION

Active research is underway in RA for developing novel inhibitors to human DHODH. This study aims to provide novel compounds that could be possible inhibitors of human DHODH. Here, the inhibitors to DHODH are screened and shortlisted using an E-pharmacophore of a known inhibitor. It gave three compounds as possible inhibitors of DHODH. Though all the compounds are already utilized as a cure for certain diseases, our study sheds light on its usefulness in RA as inhibitors of DHODH. Another fact that was liberally noticed in this study is that, an inhibitor interacting by Pi-Pi stacking with the inhibitor site may be a good criterion to shortlist novel hits for inhibitors to DHODH. Using this study as a base, further evaluations like cytotoxicity of these compounds on cell lines can be graded and its kinetics of inhibition of DHODH enzyme can be understood.

ACKNOWLEDEMENT

I acknowledge SRM University for its infrastructure and software for writing this paper.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Munier-Lehmann H, Vidalain P-O, Tangy F, Janin YL. On Dihydroorotate Dehydrogenases and their inhibitors and uses. J Med Chem. 2013 Apr 25;56(8):3148–67.
2. Baynes JW, Dominiczak MH, Baynes SJ. Medical biochemistry: With student consult online access. 4th ed. London, United Kingdom: W.B. Saunders Company; 2014.
3. Giesmacher A, Chiba P, Muller MM. Proceedings of the joint ninth international, sixth European symposium on Purine and Pyrimidine metabolism in man, held June 1 - 7, 1997, in Gmunden, Austria. Griesmacher A, editor. New York: Kluwer Academic / Plenum Publishers; 1998.
4. Rawls J, Knecht W, Diekert K, Lill R, Löffler M. Requirements for the mitochondrial import and localization of dihydroorotate dehydrogenase. Eur J Biochem. 2000 Apr;267(7):2079–87.
5. Evans DR, Guy HI. Mammalian Pyrimidine Biosynthesis: Fresh insights into an ancient pathway. J Bio Chem. 2004 Apr 19;279(32):33035–8.
6. Herrmann ML, Schleyerbach R, Kirschbaum BJ. Leflunomide: An immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. Immunopharmacology. 2000 May;47(2-3):273–89.
7. Liu S, Neidhardt EA, Grossman TH, Ocain T, Clardy J. Structures of human dihydroorotate dehydrogenase in complex with antiproliferative agents. Structure. 2000 Jan;8(1):25–33.
8. Yuyuan L, Xuqing Z. Leflunomide-induced acute liver failure: A case report. Journal of Medical Colleges of PLA. 2010 Feb; 25(1):62–4.
9. Li S-L, He M-Y, Du H-G. 3D-QSAR studies on a series of Dihydroorotate Dehydrogenase inhibitors: Analogues of the active metabolite of Leflunomide. Int J Mol Sci. 2011 May 10;12(12):2982–93.
10. Shih K-C, Lee C-C, Tsai C-N, Lin Y-S, Tang C-Y. Development of a human Dihydroorotate Dehydrogenase (hDHODH) Pharma-Similarity index approach with scaffold-hopping strategy for the design of novel potential inhibitors. PLoS ONE. 2014 Feb 4;9(2):e87960.
11. Vyas VK, Variya B, Ghate MD. Design, synthesis and pharmacological evaluation of novel substituted quinoline-2-carboxamide derivatives as human dihydroorotate dehydrogenase (hDHODH) inhibitors and anticancer agents. Eur J Med Chem. 2014 Jul;82:385–93.
12. Li S, Luan G, Ren X, Song W, Xu L, Xu M, Zhu J, Dong D, Diao Y, Liu X, Zhu L, Wang R, Zhao Z, Xu Y, Li H. Rational design of Benzylidenehydrazinyl-Substituted Thiazole derivatives as potent inhibitors of human Dihydroorotate Dehydrogenase with in vivo anti-arthritic activity. Sci Rep. 2015 Oct 7;5:14836.

13. Vyas VK, Ghate M. Development of docking-based 3D QSAR models for the design of substituted quinoline derivatives as human dihydroorotate dehydrogenase (hDHODH) inhibitors. SAR QSAR Environ Res. 2013 Aug;24(8):625–45.
14. Lolli ML, Giorgis M, Tosco P, Foti A, Fruttero R, Gasco A. New inhibitors of dihydroorotate dehydrogenase (DHODH) based on the 4-hydroxy-1, 2,5-oxadiazol-3-yl (hydroxyfurazanyl) scaffold. Euro J Med Chem. 2012 Mar;49:102–9.
15. Swaminathan P, Kalva S, Saleena L. E-Pharmacophore and molecular dynamics study of Flavonols and Dihydroflavonols as inhibitors against DiHydroOrotate DeHydrogenase. Comb Chem & High Throughput Screen. 2014 Mar 21;17(8):663–73.
16. Duke, A J[Internet]. License. 2016 Nov 16 [cited 2016 Nov 23]. Available from: http://dx.doi.org/10.15482/USDA.ADC%2F1239279 doi: 10.15482/USDA.ADC/1239279.
17. Sherman W, Day T, Jacobson MP, Friesner RA, Farid R. Novel procedure for modeling ligand/receptor induced fit effects. J Med Chem. 2006 Jan;49(2):534–53.
18. Aparna V, Dineshkumar K, Mohanalakshmi N, Velmurugan D, Hopper W. Identification of natural compound inhibitors for Multidrug Efflux pumps of Escherichia coli and Pseudomonas aeruginosa using in Silico high-throughput virtual screening and in vitro validation. PLoS ONE. 2014 Jul 15;9(7):e101840.
19. Rajamanikandan S, Srinivasan P. Pharmacophore modeling and structure-based virtual screening to identify potent inhibitors targeting LuxP of Vibrio harveyi. J Recept Sig Transd. 2016 Apr 6;36(6):617–32.
20. Hussain T, Tan B, Liu G, Oladele OA, Rahu N, Tossou MC, Yin Y. Health-promoting properties of Eucommia ulmoides: A review. Evid-Based Compl Alt. 2016;2016:1–9.
21. Lu Z, Cao S, Zhou H, Hua L, Zhang S, Cao J. Mechanism of Arctigenin-Induced specific Cytotoxicity against human Hepatocellular carcinoma cell lines: Hep G2 and SMMC7721. PLOS ONE. 2015 May 1;10(5):e0125727.
22. Muto J, Lee H, Uwaya A, Park J, Nakajima S, Nagata K, Ohno M, Ohsawa I, Mikami T. Oral administration of inosine produces antidepressant-like effects in mice. Sci Rep. 2014 Feb 26;4.