

Molecular docking studies of withanolides against Cox-2 enzyme

Yogeswaran Prabhakaran¹, Sathis Kumar Dinakaran*¹, Sravan Prasad Macharala²,
Somsubhra Ghosh¹, Sridevi Ranjitha Karanam³, Naveena Kanthasamy⁴ and
Harani Avasarala¹

¹Nalanda College of Pharmacy, Nalgonda, Andhra Pradesh, India

²Sri Indhu College of Pharmacy, Ibrahimpatan, India

³Sri Sai Aditya Institute of Pharmaceutical Sciences and Research, Surampalem, India

⁴SASTRA University, Thanjavur, Tamilnadu, India

Abstract: Withaniasomnifera (Ashwaganda) belonging to the family solanaceae is the subject of our present study. Withanoloides which are the major chemical constituents have been proved of interest because of their structural variations in the hybrids of different races. Docking is the process which brings the two structures together. In the present study we focus the extensive use of tool and graphical software for the identification of the binding energy of selected Withanolides like Withaferin -A, Withanolide-D from Withaniasomnifera and to screen the phytoconstituents that will dock/bind to the active sites of COX-2 enzyme. The relief from the symptoms of inflammation and pain can be by the Pharmacological inhibition of COX which involves the prediction of potential ligand for the treatment of inflammation. The energy value of docking between the target and the phytoconstituents under investigation and comparison with Diclofenac sodium was taken into consideration for coming into conclusion regarding the best pose and the binding ability.

Keywords: Withaferin a, withanolide d, diclofenac sodium, COX-2 enzyme, docking.

INTRODUCTION

Inflammation is a complex biological response given by the vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (Suredra, 2008). Inflammation either acute or chronic is an attempt by the organism not only to remove the injurious stimuli but also initiate the healing process (Ferrero *et al.*, 2007). Acute inflammation that is a short-term process appears within a few minutes or hours and ceases upon the removal of the injurious stimulus and considered as the initial response of the body to harmful stimuli, occurring due to the increased movement of plasma and leukocytes from the blood into the injured tissues is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and mastocytes. This is characterized by five cardinal signs: rubor (redness), calor (increased heat), tumor (swelling), dolor (pain), functiolaesa (loss of function).

Chronic inflammation which is a prolonged inflammation is characterized by simultaneous destruction and healing of the tissue from the inflammatory process leads to a progressive shift in the type of cells which are present at the site of inflammation (www.humpath.com).

Rheumatoid arthritis (RA), a chronic autoimmune disease resulting in inflammation and deformity of the joints

(The John Hopkins Arthritis Center), further may lead to various Systemic problems including vasculitis, rheumatoid nodules in various parts of the body, lung disease, blood disorders, and osteoporosis.

The formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane involves Cyclooxygenase (COX) enzyme (EC 1.14.99.1) (Rang *et al.*, 2003). At present, three COX isoenzymes COX-1, COX-2, and COX-3 are known of which COX-3 is splice variant of COX-1, a constitutive enzyme, found in most mammalian cells and COX-2 is undetectable in most normal tissues. COX an inducible enzyme abundant in activated macrophages and other cells at sites of inflammation catalyzes the formation of prostaglandins, the messenger molecules in the process of inflammation and thromboxane from arachidonic acid derived from the cellular phospholipid bilayer by phospholipase A₂. The suppression of inflammation may be by the inhibition of COX or prostaglandins. The non-steroidal anti-inflammatory drugs become main COX inhibitors (Koeberle *et al.* 2009). The classical COX inhibitors which are not selective inhibit all types of COX, causing peptic ulceration and dyspepsia. It is believed that such lack of selectivity is caused by the "dual-insult" of NSAIDs - direct irritation of the gastric mucosa and inhibition of prostaglandin synthesis by COX-1. Selectivity for COX-2

*Corresponding author: e-mail: satmpdina@yahoo.co.in

is the main feature of newer NSAIDs like celecoxib, rofecoxib, and other members of this drug class (Wilson *et al.*, 2003).

COX-2 as is specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibitors and thus the decreased risk of peptic ulceration but this very selectivity does not seem to negate other side-effects of NSAIDs, mainly the increased risk of renal failure, along with an increase in the risk for heart attack, thrombosis, and stroke through an increase in thromboxane.

Ashwaganda (*Withania somnifera*) of the family Solanaceae, cultivated for its roots which are dried immediately grows wild in all dry parts and subtropical India (Kokate *et al.*, 2008) has been traditionally used in the treatment of rheumatism, gout, hypertension, nervous and skin diseases. Ashwaganda has sedative and hypnotic effects. It is used as an immunomodulatory agent. It has hypotensive, respiratory, stimulant action along with bradycardia. This drug prevents body degenerative change in arthritic condition. The plant contains alkaloids and steroidal lactones. The alkaloids present are withanin, somniferin, somnine, tropine, pseudotropin and anafenin. The steroidal lactones include withaferin, withaferin A, withanolide-A, withanolide-D. In this current project we explain the anti-inflammatory and anti-rheumatoid arthritis activity of withanolides from the roots of *Withania somnifera* by using *insilico* methods.

MATERIALS AND METHODS

Various tools and softwares are used to analyze the protein (Cox-2) structure and to study the binding energy properties with Diclofenac sodium, Withaferin-A, Withanolide-D. Cox-2 enzyme sequence was obtained from protein data bank (www.pdb.org/pdb/). Active site of enzyme was obtained by PAR-3D online tool and CASTp server. Molecular properties of withanolides were taken from MOLSOFT tool. The prediction of ADME/T was done by using ALOGPS 2.1 online tool. SYBYL-FLEXI-X software was used for docking purpose and for visualization docking molecular structure.

RESULT

PDB is used to get the 3D structure of the target protein but this pdb file has not only the protein binding sites and active sites of proteins and DNA's but also some other molecules associated with structural pockets and cavities and hence the exact binding site is located by CASTp and PAR 3D servers Delaunay triangulation and the alpha complex for shape measurements. Active sites like Cys-907, Cys-910 Cys-962 and Cys-964 were retrieved, which were shown in the fig. 1.

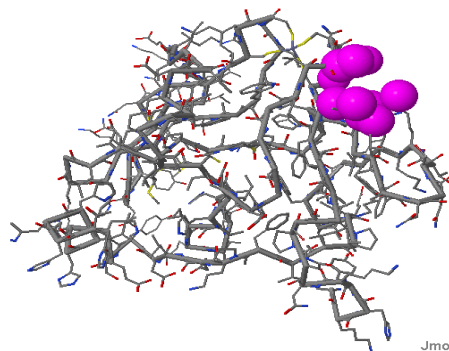


Fig. 1: Active Site Pocket of Cox-2 by CASTp server

Drug likeness determines whether particular molecule is similar to the known drugs or not defined as it is a complex balance of various molecular properties and structure features like hydrophobicity, electronic distribution, Hydrogen bonding characteristics, molecule size and flexibility. This was done by using MOLSOFT tool. The structure of withanolide D and withaferin A was drawn in MOLSOFT tool and the drug likeness data was retrieved. The hydrogen bond acceptor values, hydrogen bond donor values of withanolide-D and withaferin-A were 5, 1 and 6, 2 respectively and molecular polar surface area, the molecular weights of withanolide-D and withaferin-A were 76.132, 454.607 and 96.36, 470.606 respectively.

ALOGPS 2.1 provides interactive on-line prediction of logP, water solubility and pKa(s) of compounds for drug design (ADME/T) and environmental chemistry studies. Log p is the primary determinant of compound solubility. Higher log p values show increase in the hydrophobicity and leads to greater penetrability of the membrane. Considering the adverse effects on protein binding and drug absorption a very high lipophilicity should be avoided and the ideal drug candidates have to be decided with an idea to keep lipophilicity as low as possible. Withanolide-D and withaferin-A possess an average log p value as 5.097 and 3.856 respectively. Docking was done to identify the binding energy interaction of Diclofenac sodium, withanolide-D and withaferin-A with Cox-2 enzyme. The protein and ligand was uploaded as protein.pdb and ligand.pdb respectively. Docking energy values of Diclofenac sodium, withanolide D and withaferin A were found to be -23.979, -15.776 and -16.261 respectively and were shown in the figs. 2, 3 and 4.

DISCUSSIONS

When the binding energy of both the phyto constituents was compared to Diclofenac sodium, it was very much satisfactory and it supports its anti-inflammatory and anti-rheumatoid arthritis properties. Withanolides possess anti-inflammatory, antitumor, cytotoxic, immunomodulating

activities and for the protection against CCl₄ -induced hepatotoxicity (Ray *et al.* 1994; Anjaneyulu *et al.* 1998). Withanolides were reported to induce phase-II enzymes in animal models, which is one of the mechanisms in cancer chemoprevention (Misico *et al.*, 2002). It may be

proved that withanolides act on the COX-2 enzyme. By these studies, the selected two withanolides from *Withania somnifera* having almost optimum parameters can be used as potential ligands for the treatment for inflammation. Based on the high docking energy value,

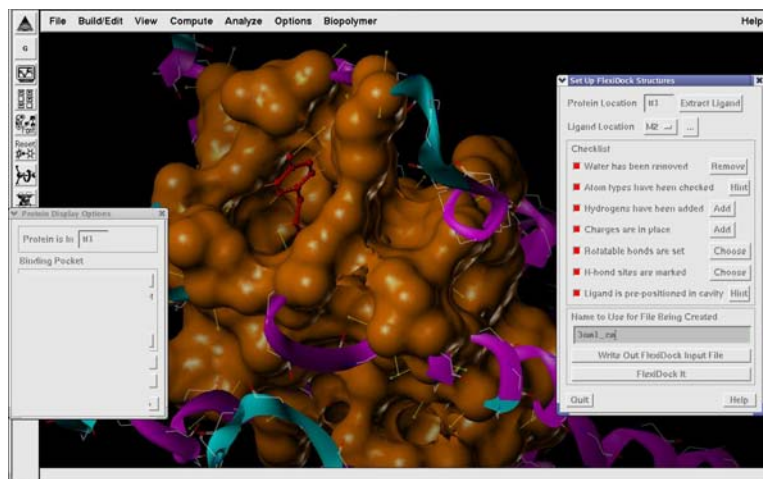


Fig. 2: Image of Diclofenac sodium in Docking study.

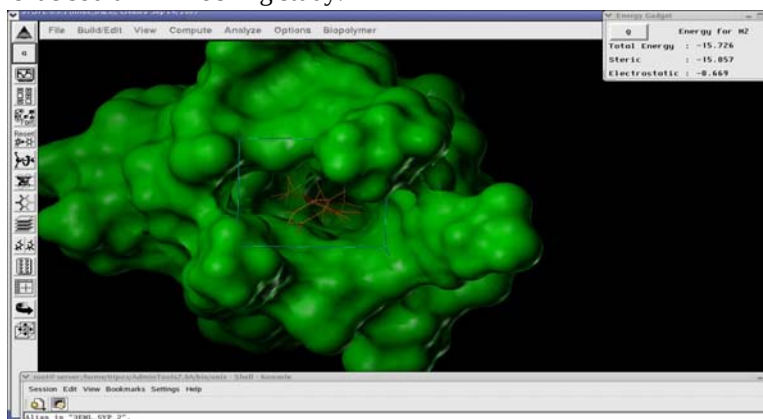


Fig. 3: Image of withanolide D in Docking study.

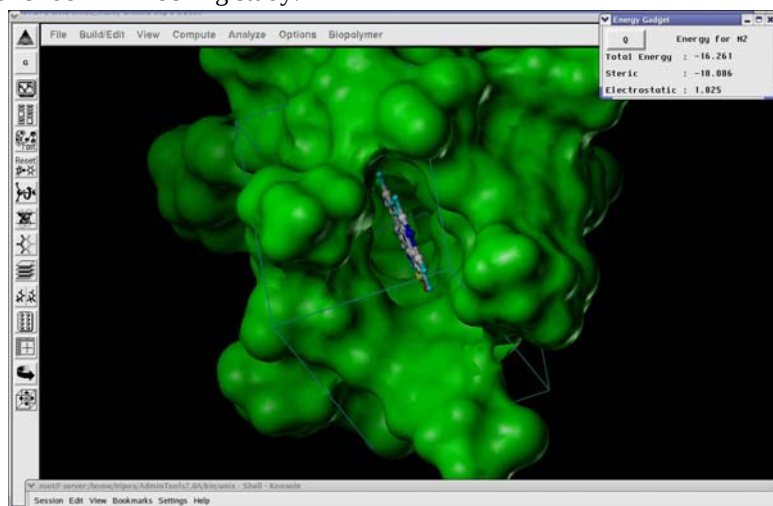


Fig. 4: Image of withaferin A in docking study.

optimum log p value, and hydrogen bond acceptor, this research study proposes that withaferin A can be treated as lead in the design of drug molecule which acts on Cox-2 enzyme which compared to withanolide-D.

CONCLUSION

By these studies it can be concluded that among the two constituents of *Withania somnifera*, withaferin A possess great activity on COX-2 followed by withanolide-D. Withanolides may have a direct action on cox-2 enzyme by binding to the Cys-907, Cys-910, Cys-962 and Cys-964 residue. In developing countries it may be useful widely because of plant availability. Different synergistically or additively acting compounds on different targets with moderate activity give larger response, than that shown by the individual compound.

REFERENCES

- Acute inflammation, <http://www.humpath.com/Acute-inflammation>. retrieved on January 2010.
- Anjaneyulu ASR, Rao DS and Lequesne PW (1998). Withanolides, biologically active natural steroidal lactones: A Review. In: Atta-ur-Rahman (Ed.). Studies in Natural products Chemistry: Structure and Chemistry (Part F), Vol.20, 1st ed., Elsevier Science BV, p.135.
- Ferrero ML, Nielsen OH, Andersen PS and Girardin SE. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1 β generation. *Clin. Exp. Immunol.*, **147**(2): 227-235.
- Koeberle A and Werz O (2009). Inhibitors of the microsomal prostaglandin E(2) synthase-1 as alternative to non steroidal anti-inflammatory drugs (NSAIDs) – A critical review. *Curr Med Chem*, **16**(32): 4274-4273.
- Kokate CK, Purohit AP and Gokhale SB (2008). Text Book of Pharmacognosy, 39th ed., Nirali Prakashan Publications, Pune, p.532.
- Misico RI, Song LL, Veleiro AS *et al.* (2002). Induction of quinine reductase by withanolides. *J. Nat. Prod.*, **65**: 677-680.
- Rang HP and Dale MM (2003). Pharmacology, 5th ed, Churchill Livingstone Publications, p.231.
- Ray AB and Gupta M (1994). Withasteroids – A growing group of naturally occurring steroidal lactones. *Prog. Chem. Org. Nat. Prod.*, **63**: 1-106.
- Suredra NP (2008). A text book of medicinal chemistry, Vol. 1, 4th ed, SG Publications. Varanshi, p.352.
- The John Hopkins Arthritis Center, Available at: http://www.hopkins-arthritis.org/arthritis-info/rheumatoid-arthritis/rheu-m_treat.html. retrieved on January 2010.
- Wilson and Gisvold (2003). Organic medicinal and organic chemistry, 11th ed., Lippincott Williams's Publications, pp.753-763.