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In silico Screening of Cyclooxygenase Inhibitory Molecules from Margroves

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ABSTRACT

Cyclooxygenase (COX) plays a vital role in physiological process of inflammation. Therefore, we evaluated the inhibitory effect of phytocompounds derived from mangrove plants against COX protein using computational method. The 3D structure of avicenol, betulinic acid, heritonin, halprogin, lupeol, pyretrin, quercetin, rubrolide and triterpenoid obtained from PubChem database and docked against COX receptor by using Auto dock 4.0. The results indicated all the compounds have significant interaction and formation of hydrogen bonds. Lupeol acts as a potential inhibitor among the nine compounds, with binding energy -8.17 kcal mol^{-1} has formed two hydrogen bonds interaction at the residue THR212 and THR212. This study concludes number of effective bioactive compounds from mangrove will have great interest in pharmaceutical industry.

Key words: Avicenol, mangrove, binding energy, inflammation

INTRODUCTION

Inflammation is a protective response of body cells to various intrinsic or extrinsic stimuli such as irradiation, extreme temperature and toxic pathogens (Anderson and Borlak, 2008). Although, deregulated chronic inflammation can promote heart attack, paralysis and might contribute with atherosclerosis, hepatitis and rheumatoid arthritis (Chung *et al*., 2007). Non Steroidal Anti Inflammatory Drugs (NSAIDs) have therapeutic potential to rheumatoid arthritis and various types of inflammation. The target for NSAIDs is cyclooxygenase, a rate limiting receptor protein involved in the production and generation of inflammatory mediator such as prostaglandins from arachidonic acid and lipooxygenase. Particularly, COX-1 commonly known as housekeeping enzymes and constitutively expressed isoform in all tissues and associated with the production of prostaglandins (Selvam *et al*., 2005).

The mangrove ecosystem is one of the important coastal biodiversity. Generally, mangroves are woody plant or shrubs that grow in tropical, subtropical, estuaries, back waters, rivers. Commonly found in the Asian countries, islands of Indian Ocean, Arabian Sea, Bay of Bengal, South China and the Pacific (Qasim, l998). It contains array of primary and secondary metabolites such as alkaloids, flavonoids, terpenes, pheromones, tannins have toxicological, pharmacological and ecological importance (Bandaranayake, 2002). Specifically, phenolic compounds including flavonoid inhibit the expression of the inducible forms of COX, interleukins, tumor necrosis factor-α and adhesion molecules in inflammatory cells and tissues (Kim *et al*., 2004). Number of compounds was reported from mangroves which have therapeutic potential on diabetes, ulcer, inflammation, wound healing, antibiotics, cancer and cardiovascular disease by *in vitro* and *in vivo* methods (Silambarasan, 2012; Gurudeeban *et al*., 2013; Satyavani, 2013). Recently, Gurudeeban *et al*.

(2012a, b) evidently proved the DPPIV and alpha ketoglutarate inhibitory effect of mangrove based compounds using computational approaches, this might be minimize the usage of cost and experimental animals while entered in to *in vivo* studies. However, compounds with COX inhibitory effect are not validated by *in silico* approaches. Therefore, the present study aimed to evaluate the COX inhibitory effect of nine phyto compounds from mangrove plants using computational methods.

MATERIALS AND METHODS

Preparation of coordinate file: Three dimensional structure of cyclooxygenase was obtained from Protein Data Bank. The active sites of COX 1 protein was analysed by PDB sum. The 2D structure of compounds viz., avicenol A, betulinic acid, halprogin, heritonin, lupeol, pyretrin II, quercetin, rubrolide N and triterpenoid were retrieved from PubChem database (Table 1). The optimized ligand molecules were docked into distinguished model using Lig and Fit theory.

***In silico* analysis:** Auto-dock Tools 4.0 was used to prepare, run and analyze the docking simulations. The pre calculated grid maps, one for each atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. The Lamarckian Genetic Algorithm (LGA) 23 was chosen search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 250000, maximum number of generations 50000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, step sizes were 0.2 Å for translations, 5.0 Å for quaternions and 5.0 Å for torsions. Cluster tolerance 0.5 Å, external grid energy 1000, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed. Auto Dock was compiled and run under Windows XP operating system. Autodock results were analyzed to study the interactions and the binding energy of the docked structure. All the Auto Dock docking runs were performed in Intel CORE™ i5, 64 bit operating system and 4GB RAM in Lenovo Win 7 PC.

RESULTS

The structure of protein viewed by PyMol and PDB sum predicted active sites are NAG 622, NAG 661 and ASN 68 (Fig. 1). The docking poses were ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses (Zhang *et al.*, 2008). 10 docking runs were performed. Grid parameters were set as mentioned earlier and spacing between grid points was 0.375 Å. After the simulations were complete, the docked structures were

Table 1: List of ligand used for docking

Name of the compound	PubMed ID	Hydrogen donor/acceptor	Molecular weight (g mol^{-1})
Avicenol A	11208912	2/5	-8.06
Betulinic acid	64971	2/3	456.70
Haloprogin	3561	0/1	361.39
Heritonin	130118	0/3	258.31
Lupeol	259846	1/1	426.71
Pyretrin II	6433155	0/5	372.45
Quercetin	5280343	5/7	302.23
Rubrolide N	5472704	2/4	472.52
Triterpenoid	9804218	2/3	458.60

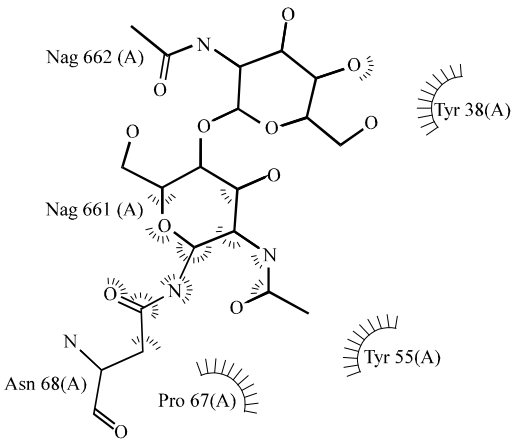

Fig. 1: Active site of target receptor protein cyclooxygenase

Table 2: Molecular interactions of phyto compounds on cyclooxygenase receptor

Name of the ligand	No. of H-bonds	Binding energy	Length of hydrogen bond (Å)	Cluster RMSD	Reference RMSD
Avicenol A	2	-7.15	1.951	0.0	211.12
			2.091		
Betulinic acid	2	-4.34	1.884	1.67	197.28
			1.916		
Haloprogin	1	-7.04	2.817	0.0	203.69
Heritonin	1	-6.84	1.805	0.0	203.04
Lupeol	2	-8.17	1.962	0.3	197.30
			2.208		
Pyretrin II	4	-6.28	1.936	0.0	198.29
			1.991		
			1.734		
			1.791		
Quercetin	4	-5.57	2.161	0.0	197.71
			1.756		
			2.084		
			1.649		
Rubrolide A	2	-7.66	1.842	0.0	198.06
			2.738		
Triterpenoid	3	-6.79	2.204	0.3	196.61
			2.073		
			1.998		

analyzed and the interactions were observed. Hydrogen bond interactions and the binding distance between the donors and acceptors were measured for the best conformers. Distinct conformational clusters RMSD-tolerance and Van der Waals scaling factor was found to be 2.0 and 1.0 Å, respectively.

***In silico* studies:** Molecular docking of mangrove derived bioactive molecules such as avicenol A, betulinic acid, heritonin, halprogin, lupeol, pyretrin, quercetin, rubrolide and triterpenoid into COX produced six clusters of conformers (Fig. 2). The binding energy, hydrogen bond interactions distance between hydrogen donor and acceptor atoms of each ligand represented in Table 2. Among

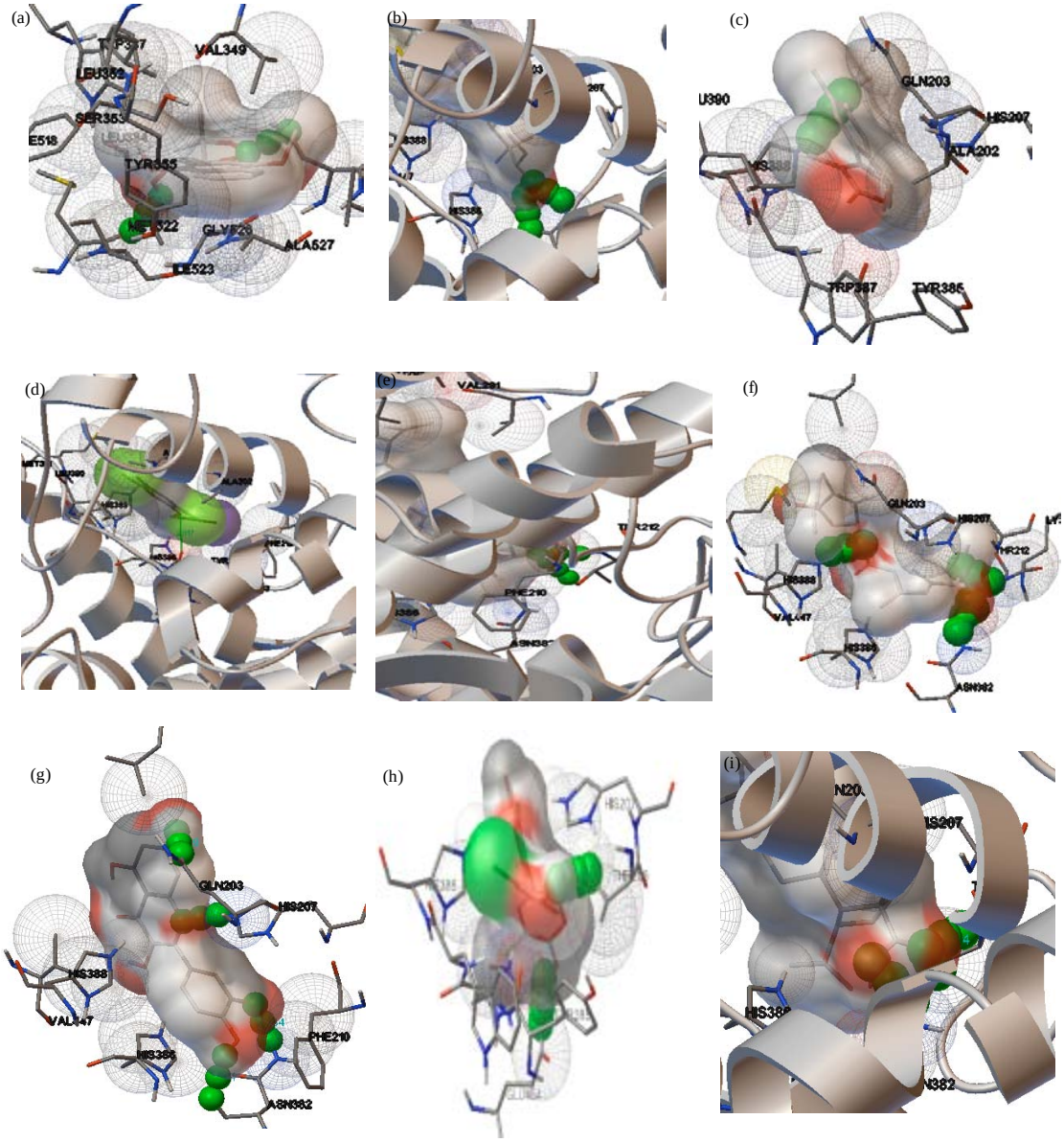

Fig. 2(a-i): Molecular interaction of cyclooxygenase receptor with mangrove derived bioactive molecules, (A) Avicenol A, (b) Betulinic acid, (c) Haloprogin, (d) Heritonin, (e) Lupeol, (f) Pyretrin II, (g) Quercetin, (h) Rubrolide N and (i) Triterpenoid

the nine bioactive molecules lupeol had significant binding energy -8.17 kcal mol^{-1}. The compounds interacted with receptor protein in the following amino acid residue LEU82, ARG388, ARG80, HIS388, TYR385, THR212, THR212, ASN 382, HIS 207, ASN 382, GLN 203, THR 206, GLU454, THR 212, ASN 382 and THR 212, respectively.

DISCUSSION

Mangrove species such as *Rhizophora apiculata*, *Rhizophora mucronata*, *Excoecaria agallocha* and *Avicennia marina* are also rich sources of phenolic, tannins, triterpenoids, alkaloids and

flavonoid. *In silico* docking depends on the scoring functions and hydrogen bonds are used to predict their binding modes, affinities and orientation on the receptor protein (Duggan *et al*., 2010). In accordance to the present findings, quercetin the member of flavonoid exhibited inhibitory activity towards the COX with binding energy -5.57. Generally the flavonoids inhibited the phospholipase A2 and cyclooxygenase (COX) involved in inflammatory responses (Bitis *et al*., 2010). The docked ligands were selected based on docking energy and good interaction with the active sites residues and hydrogen bond donors (OH-NH group), not more than ten hydrogen bond acceptors, molecular weight under 500 g/mole, partition coefficient logP of less than 5 and rotatable bonds of less than 10 is taken as drug molecules and docking procedure is carried out (Lipinski *et al*., 2001). The mangrove derived triterpenoid and rubrolide-N showed good docking energy score on dihydrofolate reductase leading to the inhibition of growth of malarial parasites (Senthilraja *et al*., 2012). In the present finding, indicates the binding energy of -6.79 and -7.66 kcal/mol with COX 1 inhibitor by triterpenoid and rubrolide. Lupeol, Taraxerol and Betulinic acid were identified from the aerial roots of *Avicennia marina* (Mahera *et al*., 2011). We found that lupeol exhibited high energy and formed two hydrogen bonds interaction at the residue THR212 and THR212 with cluster RMSD 0.00 and reference RMSD 197.3 than compared to others. In conclusion, the binding mode of the tested nine compounds from halophytes inside the active site of COX-1 enzyme was predicted using in silico docking technique.

This study concludes that mangrove floral derived biomolecules will be an alternative source for inflammatory agent.

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REFERENCES

Anderson, N. and J. Borlak, 2008. Molecular mechanisms and therapeutic targets in steatosis and steatohepatitis. Pharmacol. Rev., 60: 311-357.

Bandaranayake, W.M., 2002. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol. Manage., 10: 421-452.

Bitis, L., S. Kultur, G. Melýkoglu, N. Ozsoy and A. Can, 2010. Flavonoids and antioxidant activity of *Rosa agrestis* leaves. Nat. Prod. Res., 24: 580-589.

Chung, C.P., I. Avalos, P. Raggi and C.M. Stein, 2007. Atherosclerosis and inflammation: Insights from rheumatoid arthritis. Clin. Rheumatol., 26: 1228-1233.

Duggan, K.C., M.J. Walters, J. Musee, J.M. Harp, J.R. Kiefer, J.A. Oates and L.J. Marnett, 2010. Molecular basis for cyclooxygenase inhibition by the non-steroidal anti-inflammatory drug naproxen. J. Biol. Chem., 285: 34950-34959.

Gurudeeban, S., K. Satyavani, T. Ramanathan and P. Ravikumar, 2012a. Dipeptidyl peptidase IV inhibitors derived from a mangrove flora *Rhizophora mucronata*: An in silico approach. Bangladesh J. Pharmacol., 7: 203-210.

Gurudeeban, S., K. Satyavani, T. Ramanathan and T. Balasubramanian, 2012b. An *In silico* approach of alpha-ketoglutarate dependent Dioxygenase FTO inhibitors derived from *Rhizophora mucronata*. Drug Invent. Today, 4: 594-598.

Gurudeeban, S., T. Ramanathan and K. Satyavani, 2013. Antimicrobial and radical scavenging effects of alkaloid extracts from *Rhizophora mucronata*. Pharm. Chem. J., 47: 50-53.

Kim, H.P., K.H. Son, H.W. Chang and S.S. Kang, 2004. Anti-inflammatory plant flavonoids and cellular action mechanism. J. Pharmacol. Sci., 96: 229-245.

Lipinski, C.A., F. Lombardo, B.W. Dominy and P.J. Feeney, 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev., 46: 3-26.

Mahera, S., V.U. Ahmad, S.M. Saifullah, F.V. Mohammad and K. Ambreen, 2011. Steroids and triterpenoids from grey mangrove *Avicennia marina*. Pak. J. Bot., 43: 1417-1422.

Qasim, S.Z., 1998. Mangroves. In: Glimpses of the Indian Ocean, Qasim, S.Z. (Ed.). University Press, Hyderabad, India, ISBN-13: 9788173711299, pp: 123-129.

Satyavani, K., 2013. Nanoencapsulation, characterization and ointment formulation of rutin from *Excoecaria agallocha* L. on control of type II diabetes mellitus and diabetic foot ulcer using Wistar Albino rats. Ph.D. Thesis, Annamalai University, Parangipettai, Tamil Nadu, India.

Selvam, C., S.M. Jachak, R. Thilagavathi and A.K. Chakraborti, 2005. Design, synthesis, biological evaluation and molecular docking of curcumin analogues as antioxidant, cyclooxygenase inhibitory and anti-inflammatory agents. Bioorg. Med. Chem. Lett., 15: 1793-1797.

Senthilraja, P., S.K. Sahu and K. Kathiresan, 2012. Potential of mangrove derived compounds against dihydrofolate reductase: An *in-silico* docking study. J. Comput. Biol. Bioinformat. Res., 4: 23-27.

Silambarasan, G., 2012. Studies on isolation, identification, culture and biomedical potential of cyanobacteria from parangipettai mangrove ecosystem. Ph.D. Thesis, Annamalai University, Parangipettai, Tamil Nadu, India.

Zhang, S., K. Kumar, X. Jiang, A. Wallqvist and J. Reifman, 2008. DOVIS: An implementation for high-throughput virtual screening using AutoDock. BMC Bioinform., Vol. 9. 10.1186/1471-2105-9-126