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Molecular Docking Studies of *Rhizophora mucronata* Alkaloids Against Neuroinflammatory Marker Cyclooxygenase 2

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ABSTRACT

Computer aided drug design is playing an important role in identifying the drug targets. The contribution of cyclooxygenase-2 (COX-2) to peripheral inflammation and brain inflammation is well documented. Therefore, the present study aimed to evaluate inhibitory effect of *Rhizophora mucronata* derived alkaloids such as ajmalicine, vindoline, catharanthine, serpentine and tabersonin on COX 2. Based on Lamarckian genetic algorithm, the alkaloids were docked with target protein using Auto Dock 4.0. The results indicated serpentine and ajmalicine expresses higher binding energy (-9.16 and -8.12 kcal mol^{-1}), length of a hydrogen bond (2.211 and 2.079), amino acid residues (HIS 388) on cyclooxygenase 2 receptor than compared to other derivatives. This study concludes that serpentine and ajmalicine acts as a potent source for anti-neuro-inflammatory agents. Further preclinical studies will be carried out to find out the exact molecular level mechanism and drug development for neuro inflammation disorders.

Key words: Ajmalicine, amino acid, auto dock, binding energy, COX2, neuro-inflammation

INTRODUCTION

Neuro inflammation is the serious reaction of endogenous central nervous system which leads to cause several neurodegenerative diseases (NDD) such as Alzheimer's disease, stroke, sclerosis, Parkinson's disease and Huntington's disease. Neuro inflammation is characterized by glial cell activation, class II antigens, acute phase proteins and cell surface adhesion molecule. Earlier research has reported on the contribution of Cyclooxygenase (COX) in neuro inflammation. COX-2 immuno reactivity has been localized primarily in meningeal macrophages, endothelial cells, microglia and astrocytes (Breder, 1997). Prostaglandins are found elevated in brain following injury and COX-2 appears to play a significant role in neuro prostaglandin production. Ischemic cerebral injury is caused by COX-2 mRNA induction accompanied by prostaglandin production that is blocked by COX-2 inhibitor (O'Banion *et al*., 1996). COX-2 played a vital role in synaptic transmission, neurotransmitter release, blood flow regulation and cerebro vascular coupling (Stefanovic *et al*., 2006). Irregular action of central nervous system leads to the presence of abnormal level of COX-2 which direct to neuro inflammation with high mortality rate, economic burden and increase the diseased persons from 22 million to triple the amount by 2050 around the worldwide (Scatena *et al*., 2007).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of various types of inflammatory conditions. The target for these drugs is COX, a rate limiting enzyme involved in the conversion of arachidonic acid into inflammatory prostaglandins (Van Ryn *et al*., 2000). COX-1 is constitutively expressed in all tissues, while COX-2 is constitutively expressed only in kidney, brain and ovaries. COX-2 is increasingly expressed during inflammatory conditions by pro-inflammatory molecules such as interlukins-1, tumour necrosis factor-α, lipo-polysaccharide and agents such as carrageenan (Carter, 2000). The traditional NSAIDs are reported to be associated with an increased risk of gastrointestinal ulcers, including gastrointestinal hemorrhage, perforation and obstruction, due to COX-2 as well as COX-1 inhibition. As for selective COX-2 inhibitors, they are reported to have serious cardiovascular side effects such as an increase in blood pressure, stroke and myocardial infarction. These unpalatable situations indicate that it is still a challenge for the drug companies to develop more effective and less toxic NSAIDs. The reason for the failure of these agents in the treatment of neuro-inflammatory disorders can be attributed to their inability to target the key component involved in neuro-inflammation. Therefore, recent study has focussed their interest towards COX 2 inhibitors developments from plant origin (Phillis *et al*., 2006). Phenolic compounds have believed to be one of the most widely occurring groups of phytochemicals throughout the plant kingdom and it will be for novel drug development (Jimeno *et al*., 2004). Traditional Chinese medicine dendrobium alkaloids have reports as potential protective effects against neuronal damages induced by LPS, oxygen-glucose deprivation and reperfusion, resulting in decreases in neuron apoptosis and Aβ deposition in rat hippocampus (Chen *et al*., 2008). *Rhizophora mucronata* (Tamil: Kandal; Family: Rhizophoraceae) is a mangrove found along the tropical and subtropical coastal regions. Traditionally, it is used to treat pain, diarrhea, diabetes, inflammation and healing of wounds caused by toxic marine organisms to the fishermen community (Ramanathan, 2000). The leaves, flowers, aerial parts and barks of *R. mucronata* were scientifically validated on their antimicrobial, anti-inflammatory, anti-diabetic, cytotoxicity and dipeptidyl peptidase IV inhibitory potential (Gurudeeban *et al*., 2012). Our previous study indicated that alkaloids of *R. mucronata* posses radical scavenging and antioxidant (Gurudeeban *et al*., 2013). But there was no scientific evidence of alkaloids in neuro-inflammation. Therefore, cost effective molecular docking is used to determine the binding affinity of ajmalicine, vindoline, catharanthine, serpentine and tabersonin on the inhibitory action of cyclooxygenase 2.

MATERIALS AND METHODS

Preparation of receptor molecules: Cyclooxygenase 2 (COX-2) of 3D crystal structure was downloaded from the PDB structural database site. PDB ID of the receptor proteins was 6COX used as a docking target. The active site of receptor protein was predicted by using PDB Sum. The function of COX-2 in the neuro-inflammation is presented in Fig. 1.

Ligand preparation: The 2D structure of mangrove derived ligands viz., ajmalicine, catharanthine, serpentine, tabersonin, vindolin (Gurudeeban *et al*., 2013) and standard drug ibuprofen were retrieved from the PubChem database (Table 1). Optimized ligand molecules were docked into distinguished model using Ligand Fit theory.

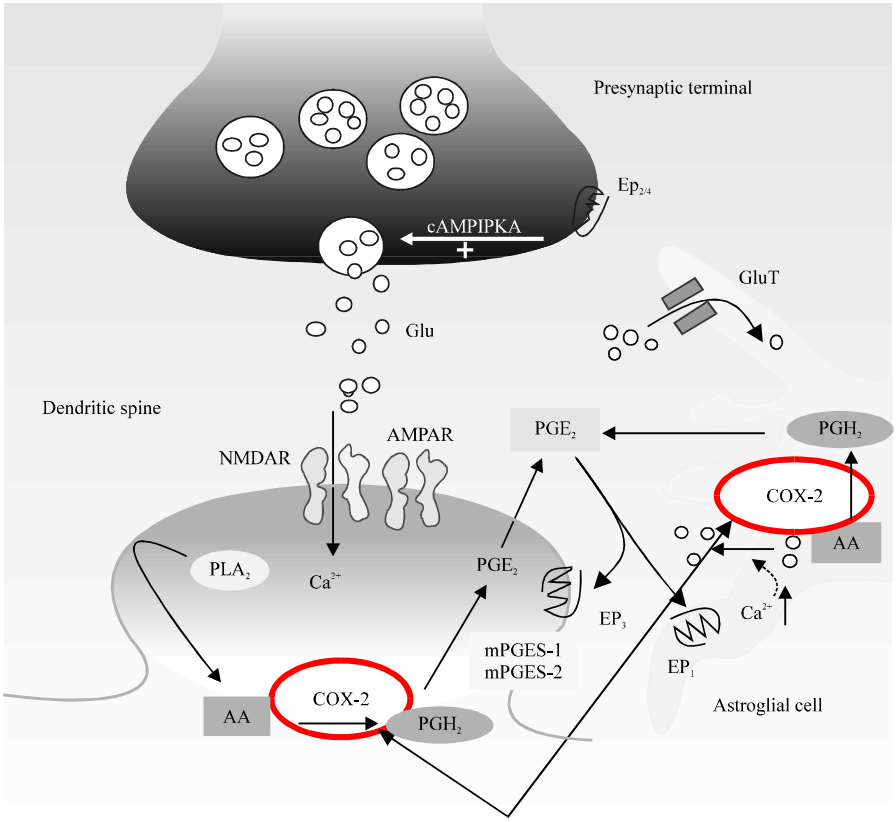


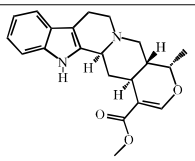
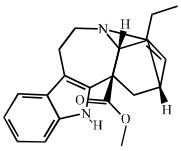
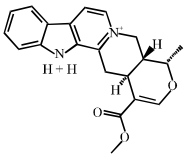
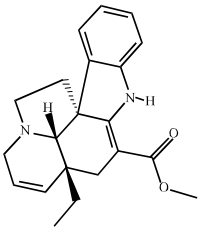
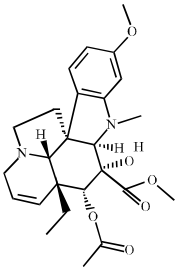
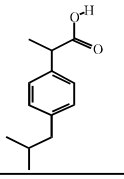
Fig. 1: Schematic representation of COX-2 on neuro-inflammation

Docking methodology: Auto Dock Tools 4.0 was used to prepare, run and analyze the docking simulations. The pre calculated grid maps, one for COX-2 atom type present in the flexible molecules being docked and its stores the potential energy arising from the interaction with rigid macromolecules. Lamarckian Genetic Algorithm (LGA) 23 was chosen search for the best conformers. Auto Dock 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 Centrino, 32 bit operating system and 1GB RAM in HP Pavilion dv6000.

RESULTS

The structure of COX-2 protein is viewed by PyMol and predicted active sites of having delH-7.410e-04. The docking poses were ranked according to their docking scores, list of docked ligands and their corresponding binding poses (Zhang *et al.*, 2008). Ten docking runs were performed. Grid parameters were set as mentioned earlier and spacing between grid points were 0.375 Å. After the simulations were complete, the docked structures were analyzed and the interactions were observed. Hydrogen bond interactions and binding distance between the donors and acceptors were measured for the best conformers. Distinct conformation clusters RMSD (Root Mean Square Deviation) tolerance and Van der Waals scaling factor were found to be 2.0, 1.0 Å, respectively. The active sites of the receptor protein have been presented in Fig. 2.

Table 1: *Rhizophora mucronata* derived ligands

Name of the ligand	Molecular weight (g mol^{-1})	H donor/acceptor	Molecular structure of the ligand
Ajmalicine	352.42	1/4	
Catharanthin	456.53	1/8	
Serpentine	336.43	1/3	
Tabersonin	336.42	1/4	
Vindolin	350.42	1/3	
Ibuprofen (Standard drug)	206.28	1/2	

Interaction ligands with COX-2: Docking simulation of all the ligands into COX-2 produced single cluster of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. The binding energy of ligands ajmalicine, catharanthine, serpentine, tabersonin and vindolin into COX-2 are represented in Table 2. Among the five ligands serpentine has highest binding energy -9.16 kcal mol^{-1}. The hydrogen bond interactions are at residue HIS388, VAL523, HIS207 and ARG 120, respectively (Fig. 3).

Interaction with ibuprofen: Docking simulation of ibuprofen into COX-2 produced single cluster of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs. Cluster rank 1 at 2nd run

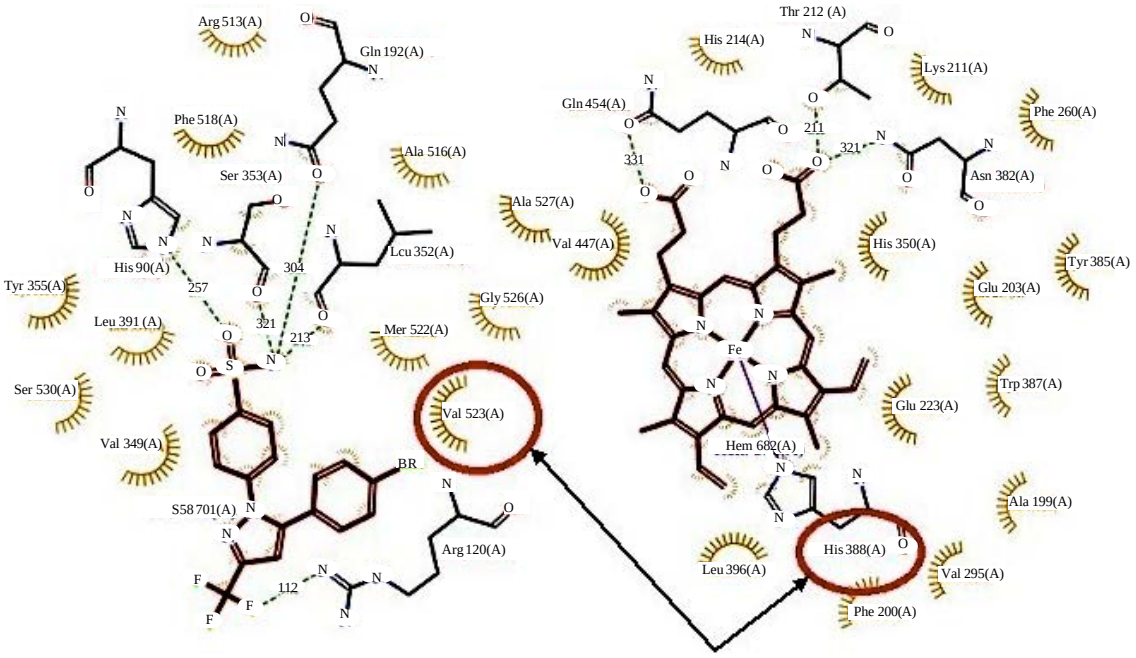


Fig. 2: Predicted active sites in the receptor protein COX-2

Table 2: Molecular interaction of alkaloids on COX2 receptor

Name of the ligand	No. of H-bonds	Binding energy	Length of hydrogen bond (Å)	Cluster RMSD	Reference RMSD
Ajmalicine	1	-8.12	2.079	1.15	52.34
Catharanthine	1	-7.97	1.888	0.52	56.19
Serpentine	1	-9.16	2.211	0.00	54.08
Tabersonin	1	-6.91	2.011	0.01	54.10
Vindoline	2	-5.64	1.936	0.83	51.29
			1.989		
Ibuprofen (Standard drug)	2	-6.36	2.094	0.00	57.31
			1.922		

with binding energy -6.36 kcal mol^{-1} has formed two hydrogen bond interactions at residue with reference RMSD 57.31 (Fig. 3). Hydrogen bond distance between the donor and acceptor was found to be 2.094 and 1.922, respectively. Compared to other four alkaloids and standard drug ibuprofen, serpentine had significant binding energy -9.16 kcal mol^{-1} and interacted with receptor protein in HIS 388 amino acid residue (Table 2).

DISCUSSION

Inflammation plays an important role in CNS disorders not currently available anti-inflammatory agent offers significant neuroprotection in such disorders. Anti-inflammatory agents used in therapy can be broadly classified as steroidal and nonsteroidal agents (SAIDs and NSAIDs, respectively). These drugs pose potential health hazards in long-term treatment. The role of steroidal agents such as estrogen in the treatment of dementia is controversial and is not recommended that age-associated dementia (Espeland *et al.*, 2004). Selective COX-2 inhibitors

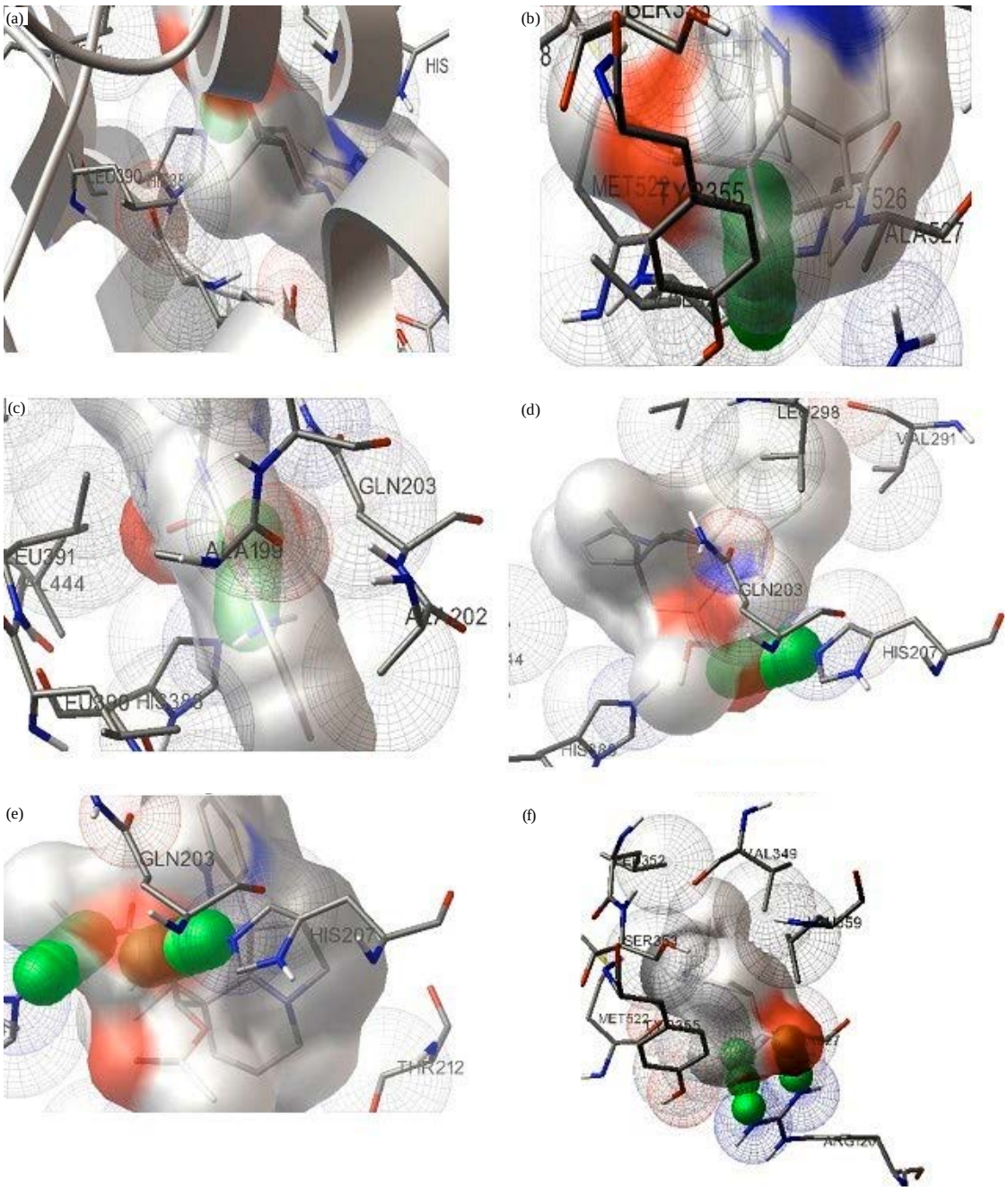


Fig. 3(a-f): Molecular interaction of five different alkaloids from *R. mucronata* on COX-2, (a) Ajmalicine, (b) Catharanthine, (c) Serpentine, (d) Tabersonin, (e) Vindoline and (f) Ibuprofen

show similar adverse drug-related events as demonstrated by nonselective NSAIDs treatment. Previously, COX-2 expression was considered inducible but recent evidence suggests that it is constitutive in the brain. It is expressed by neurons and plays a vital role in coupling synaptic activity to neocortical blood flow (Verrico *et al*., 2003). COX-2 in the brain is the primary isozyme

involved in memory consolidation and COX-1 is involved in memory formation. COX inhibitors are not only responsible for the generation of harmful prostaglandins but also involved in the generation of PGE2, which is known for its involvement in potential beneficial effects, such as membrane excitability and synaptic transmission in the hippocampus and neuroprotection against TNF-α (Lee *et al*., 2004). Recently, a selective COX-2 inhibitor Vioxx was removed from the market because of the cardio toxicity associated with its use. Previously crude extract, flavonoids and alkaloids of the following medicinal plant species, *Juglans mandshurica, Glycyrrhiza glabra, Crataeva nurvala, Ligustrum vulgare, Morinda morindoides, Osbeckia aspera, Cedrela lilloi* and *Trichilia elegans* reported with having complement inhibitory ingredients to treat neuro-inflammation (Kulkarni *et al*., 2005). Alkaloids *Isopyrum thalictroides* is used in Chinese medicine for the treatment of inflammatory disorders such as rheumatism, neuralgia and silicosis also having complement inhibitory effect.

In the previous study, *R. mucronata* alkaloids possess significant protective effect against radical scavenging and anti-oxidant properties. Oxidative stress is can reduce nerve signals in inflammatory modules and decrease symptoms neuro-inflammation. The present study, computer aided drug design, confirmed the protective effect of *R. mucronata* alkaloids, focusing on neuro-inflammation as a potential mechanism. The results clearly showed that *R. mucronata* alkaloids, particularly serpentine were effective in inhibiting over expression of COX-2. Inflammation is supposed to play a fundamental role in the progression of neuro pathological changes of Alzheimer's disease (Zilka *et al*., 2006). Diverse stimuli including LPS can activate microglia to release toxic inflammatory factors (Maccioni *et al*., 2009). Recently, COX-2 receptor protein is used to evaluate the anti-neuro-inflammatory agents. Classical targets of NSAIDs include Cyclooxygenase and peroxisome proliferators activated receptors (Townsend and Pratico, 2005). Certain NSAIDs such as ibuprofen produced an anti-inflammatory effect and rat behavioral deficits induced by LPS and this effect may be mediated through anti-inflammatory effects of ibuprofen and/or the Aβ-lowering properties of ibuprofen (Rogers *et al*., 2007). Therefore, we selected ibuprofen as the positive control in the present study. Serpentine is a type II topoisomerase inhibitor, exhibits antipsychotic properties and responsible for oxidation. This was confirmed by the inhibition of COX-2 receptor protein.

CONCLUSION

Serpentine from *R. mucronata* derived source to be an excellent lead for the development of novel neuro-inflammatory drug. However, the position of small molecules in the active site is still a challenge given the many potential poses and the shortcomings of current scoring functions. Further *in vivo* experimental studies will be determining the exact mechanism of serpentine on COX-2 inhibitors.

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