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Evolution of selective COX-2 inhibitor from *Alangium salvifolium*: an in silico approach

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ABSTRACT

Pain and inflammation are linked with a number of pathological conditions. Several studies are in progress worldwide to find natural healing agents with better safety profile. Our current study was aimed to evaluate *Alangium salvifolium* (family: Alangeaceae) derived analgesic compounds for therapeutic drug discovery by computational approach. Literature based studies were used to explore the compounds of *A. salvifolium*. Ligands were prepared by following the appropriate procedures and finally *in silico* molecular docking analysis performed by GOLD 4.2. After post docking analysis, salviifosides A of *Alangium salvifolium* was found to have interaction on COX-2 protein by obtaining highest fitness score 50.64 and molecular interaction suggests that it could be a potent anti-inflammatory compound and it may be worth for further clinical trials.

INTRODUCTION

Pain as a form of Inflammation, is suffered by almost everybody during their lifetime (Kennedy, 2007). Inflammation is the defense response of body, characterized by redness, swelling, heat, pain, and loss of function to eliminate or limit the spread of an injurious agent (Riedel *et al.*, 2014). It involves a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, thermal and physical injury, and antigen-antibody interaction (Khan et al. 2014). Cyclooxygenases (COX) or prostaglandin endoperoxide synthase (PGHS) exists in two isoforms COX-1 and COX-2, are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain and increased body temperature (hyperpyrexia) (Watson *et al.*, 2000). While both isoform catalyse the same reactions, COX-1 is a constitutive enzyme in most cells-its activity is not changed once the cell is fully grown. Conversely, COX-2 normally present in insignificant amounts is inducible by cytokines, growth factor and other stimuli during the inflammation response.

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It is believed that eicosanoids produced by COX-1 participate in physiologic functions such as secretion of mucosa, haemostasis and maintenance of renal function, while those produced by COX-2 leads to inflammatory other pathological changes (Kulkarni *et al.*, 2000; Hinz and Brune, 2002). Nonsteroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic effects through inhibition of COX, the enzyme that makes prostaglandins. Non selective inhibition of COX iso-enzyme leads to not only beneficial therapeutic effects but also a number of detrimental effects. Beneficial effects are due to inhibition of COX-2 and detrimental effects are due to inhibition of physiological COX-1 (Hinz and Brune, 2000; Urban, 2000).

Recently, a class of anti-inflammatory medications has been developed that primarily inhibits COX-2 while sparing the enzymatic activity of COX-1 at therapeutic dosages. Two medications that predominantly inhibit only COX-2, rofecoxib and celecoxib, are currently available by prescription in the United State, India and Bangladesh (Hinz and Brune, 1999; Everts *et al.*, 2000).

The use of coxib drugs such as rofecoxib and valdecoxib were withdrawn from the market in 2004 and 2005, respectively, because of increased risk of heart attacks and strokes with long term use (Mason *et al.*, 2006; Mason *et al.*, 2007). Given the need for more effective and/or less toxic pain therapies, a great deal of

emphasis has been placed on identifying novel molecular targets that could form the basis for new analgesics. Natural products, including medicinal plants, have been the primary source for obtaining new drugs with therapeutic potential throughout history. It is estimated that approximately half of the drugs in use are derived from natural products. According to the World Health Organization (WHO), poverty and lack of access to modern medicine leads from 65% to 80% of the world population in developing countries to critically depend on plants for primary health care (Parker *et al.,* 2007; Muccillo-Baisch *et al.,* 2010). In this context, at first, we designed our study to screen out a potent analgesic compounds of *Alangium salvifolium*. *A. salvifolium* Wang (syn. *Alangium lamarckii)* family: Alangeaceae, also called as Ankola, is extensively cultivated in India and Bangladesh. It is a popular folk medicine and has been studied for its anti-microbial, anti-fertility, cardiotonic, anti-diabetic, anticancer, diuretic, laxative, and antiepileptic activity (Sharma *et al.,* 2011; Ahad *et al.,* 2012). Evidence revealed that it showed a wide range activity against nociception and inflammation (Sharma *et al.,* 2011; Ahad *et al.,* 2012; Zahan *et al.,* 2013). Earlier studies reported that its leaves contain compound like salicin, kaempferol, salviifosides A-C (Tran *et al.,* 2009), analgimarckine and ankorine (Battersby *et al.,* 1966). Interaction of these compounds against the active site of COX-2 enzyme and find out a potent selective COX-2 blocker was our main approach the study which is done by *in silico* molecular docking analysis (Ghosh *et al.,* 2006; Dash *et al.,* 2014).

MATERIALS AND METHODS

Ligands Preparation

From the literature review, all compounds like salicin, kaempferol, salviifosides A-C, analgimarckine, ankorine along with celecoxib were drawn in Symyx Draw 4.0 and then prepared for docking using the Sybyl 7.3 Molecular Modeling Suite of Tripos, Inc. 3D conformations were generated using Concord 4.0 (Hevener *et al.,* 2009), hydrogen atoms were added and charges were loaded using the Gasteiger and Marsili charge calculation method (Hristozov *et al.,* 2007). The individual ligand was minimized with the Tripos Force Field prior to docking using the Powell method with an initial Simplex (Osolodkin *et al.,* 2011) optimization and 1000 iterations or gradient termination at 0.01 kcal/(mol*A). Input ligand file format was mol2 for all docking programs investigated.

Protein preparation and Active Site Determination

The crystal structure COX-2 (pdb id : 6 COX) enzyme was collected protein data bank (Berman *et al.,* 2000). Enzymes was prepared according to the docking protocol of Gold.

The active site of these enzymes was identified according to the giving information Kurumbail *et al.* (1996).

Docking using GOLD (Genetic Optimization for Ligand Docking)

GOLD utilizes genetic algorithm to explore the rotational flexibility of receptor hydrogens and ligand conformational flexibility (Jones *et al.,* 1997). In GOLD docking was carried out using the wizard with default parameters population size (100); selection pressure (1.1); number of operations (10,000); number of islands (1); niche size (2); and operator weights for migrate (0), mutate (100) , and crossover (100) were applied.

The active site with a 10 Å radius sphere was defined by selecting an active site residue of protein. Default Genetic Algorithm settings were used for all calculations and a set of 10 solutions were saved for each ligand. GOLD was used by a GoldScore fitness function. GoldScore is a molecular mechanism like function and has been optimized for the calculation of binding positions of ligand. It takes into account four terms:

$$\text{Fitness} = S_{(hb\_ext)} + 1.3750*S_{(vdw\_ext)} + S_{(hb\_int)} + 1.0000*S_{(int)}$$

$$S_{(int)} = S_{(vdw\_int)} + S_{(tors)}$$

Where, S_{hb_ext} is the protein-ligand hydrogen bonding and S_{vdw_ext} are the vanderwaals interactions between protein and ligand. S_{hb_int} are the intramolecular hydrophobic interactions whereas S_{vdw_int} is the contribution due to intra molecular strain in the ligand.

RESULT

Structure-functional relationship of the compounds of *A. salvifolium*'s leaves along with celecoxib was evaluated to know their biological activity against the COX-2 using the 3D structure of the receptor retrieved from protein data bank site of COX-2 enzyme (pdb code: 6COX).

For this the docked binding mode was established to link the docking score function with these selected compounds and protein. Analysis of the binding pattern between COX-2 protein and ligands suggested that the binding pattern also varied with the ligand nature (Fig. 1 and 2).

The results of all compounds were established (Table 1) by GOLD scoring parameter. The compound obtained the highest score was further subjected to analyse. The binding mode was compared with standard selective drugs including celecoxib which was done by Accelrys discovery studio visualize 3.5 version shown in table 2. The highest fitness score was obtained by salviifosides A which was 50.64.

Table 1: Fitness score values as well as hydrogen bonding interaction values between COX-2 protein and ligand molecules.

Compounds Name	Gold Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
Salicin	41.41	6.65	33.25	0.00	-10.95
Analgimarckine	43.30	1.17	43.25	0.00	-17.34
Kaempferol	45.61	3.45	39.44	0.00	-12.08
Salviifosides B	49.36	1.27	51.26	0.00	-22.39
Salviifosides C	50.37	2.18	50.71	0.00	-21.55
Salviifosides A	50.64	2.00	50.76	0.00	-21.16
Ccelecoxib	62.85	1.93	49.61	0.00	-7.29

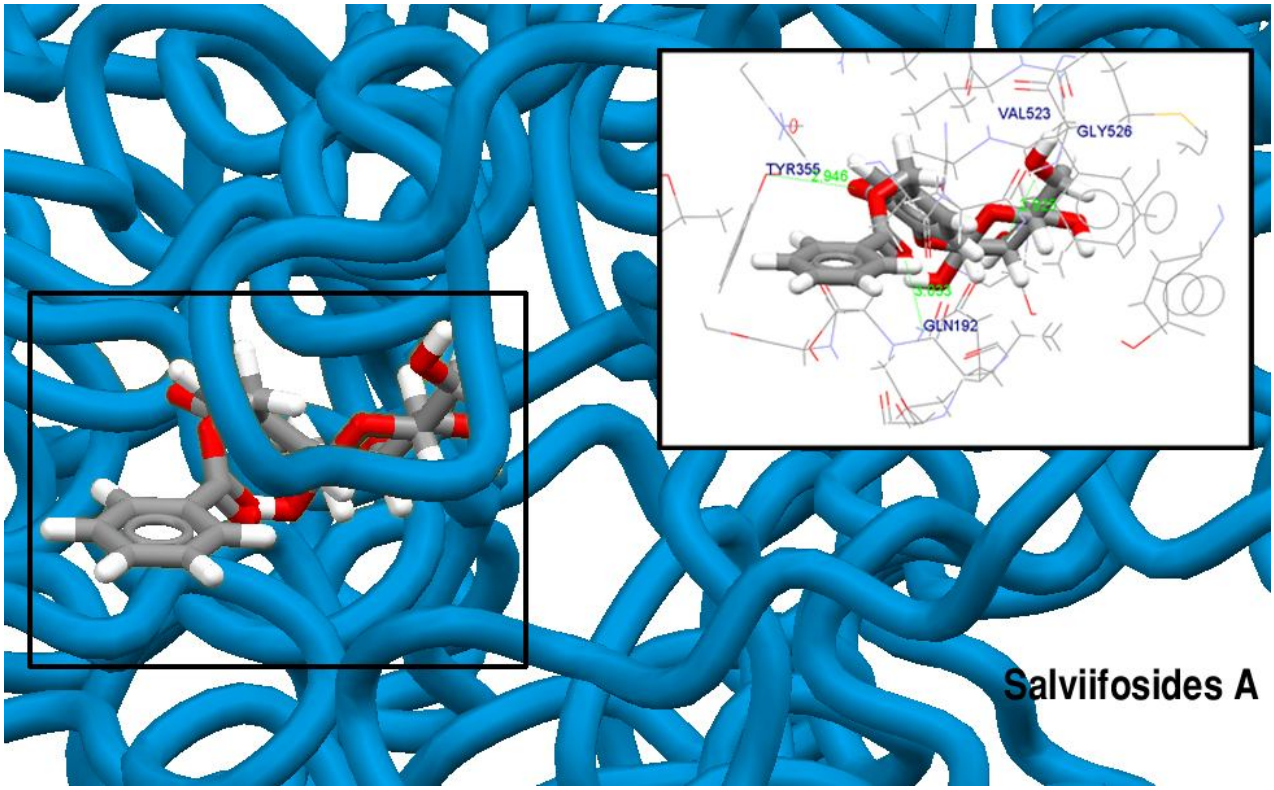

Fig. 1: Interaction of COX-2 protein with salviifosides A.

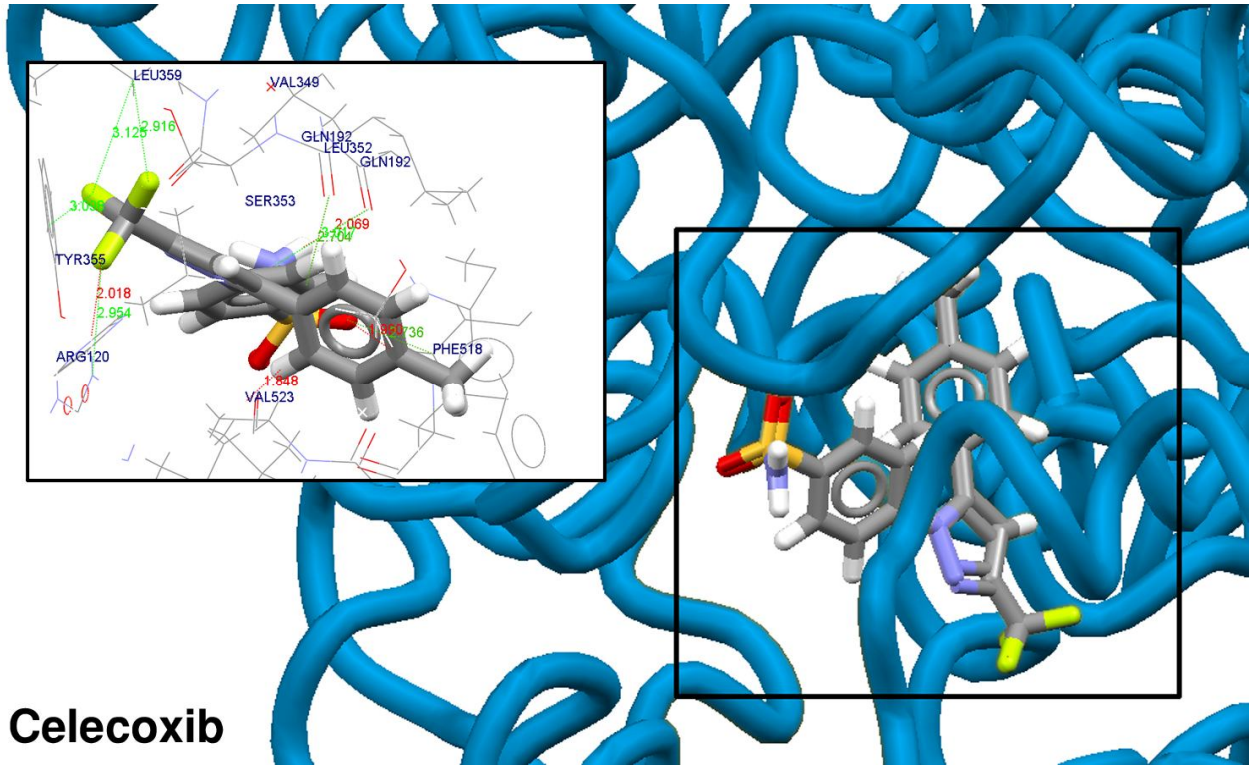

Fig. 2: Interaction of COX-2 protein with celecoxib.

DISCUSSION

Advances in computational techniques have enabled virtual screening to ave a positive impact on the drug discovery process. Virtual screening utilizes docking and scoring of each compound from a dataset and the technique used is based on predicting the binding modes and binding affinities of each compound in the dataset by means of docking to an X-ray crystallographic structure (Franca *et al.,* 2013).

In our present study, by means of gold docking, we docked all compounds from *A. salvifolium* with active site of COX-2 enzyme and salviifosides A was found to give most significant binding score with compare to other compounds. Critical evaluation of molecular docking analysis reported in (Table 2) suggested that salviifosides A has the same interaction as like as celecoxib.

The obtained docking data is in accordance with reported data on synthetic compounds where amino acid residues such as HIS^{90}, ARG^{120}, GLN^{192}, VAL^{349}, LEU^{352}, SER^{353}, TYR^{355}, LEU^{359}, TYR^{385}, TRP^{387}, ARG^{513}, ALA^{516}, PHE^{518}, VAL^{523}, GLY^{526}, ALA^{527}, LEU^{531} associated with A chain of COX-2 protein were involved for protein–ligand complementarily activity (Kurumbail *et al.,* 1996; Krishna *et al.,* 2013).

In our docking result salviifosides A was found to having three hydrogen bonding with TYR^{355}, GLN^{192}, and VAL^{523} which clearly indicates that it has a good effect in COX-2 inhibition. COX selectivity can be pursued by designing ligands targeted to interact with the non-conserved residues of the two enzymes.

Table 2: Interaction of amino acid residues of COX-2 protein with selected ligands.

Protein Ligand Interaction			
Celecoxib		Salviifosides A	
Interacted Amino acid residue	Hydrogen bond Distance (Å)	Interacted Amino acid residue	Hydrogen bond Distance (Å)
LEU359	2.916	TYR355	2.946
TYR355	3.098	VAL523	2.925
PHE518	2.736	GLN192	3.033
ARG120	2.954		
GLN192	3.017		

Both residues ARG^{513} and VAL^{523} have been demonstrated to be important for COX-2 selective inhibitor binding (Wong *et al.,* 1997). In our present study, it is revealed that salviifosides A had formed a hydrogen bond with VAL^{523} (2.925 Å). Compared to selective blocker celecoxib, salviifosides A had formed similar hydrogen bonding interaction viz. GLN^{192} and TYR^{355} with corresponding bonding distance 3.033 Å and 2.946 Å. This observation further confirms that salviifosides A may be an effective tricyclic anti-inflammatory compound especially with respect to COX-2 protein mediated inflammation compared to other traditional NSAID drugs (Llorens *et al.,* 2002).

CONCLUSION

The development of novel compounds with biological activity is an urgent need. In the present study phytocompounds of *A. salvifolium* were successfully docked into the COX-2 protein for drug interaction study to have a track in the ongoing race between drug development and new drugs especially new compounds which are more important for the discovery of new hits using molecular methods. The Fitness scores of all compounds were calculated using the GOLD software. Though the binding pattern of ligands with COX-2 differed respect to H-bonding, fitness score values substantiate the hypothesis that salviifosides A has the potentiality to selectively inhibit the COX-2 protein. Hence, it is concluded that that salviifosides A could be a potent ant inflammatory target molecule against COX-2 which may be worth for further clinical trials.

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COMPETING INTERESTS

All authors declare that they have no competing interests.

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