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DOCKING STUDIES ON XANTHONES OF MANGOSTEEN AS COX-2 INHIBITORS

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ABSTRACT : The prostaglandins found in most of the tissues and organs are synthesized by sequential oxidation of cyclooxygenases (COX-1 and COX-2). Prostaglandins synthesized by COX-1 are responsible for the protection of gastrointestinal tract and by COX-2 are responsible for inflammation and pain. The objective of this investigation was to characterize and determine the effect of α-mangostin, β-mangostin and γ-mangostin on COX-1 and COX-2. We have carried out the docking of α, β and γ-mangostin inhibitors into the three dimensional structure of COX-1 and COX-2 enzymes using GOLD software. The inhibitor binding positions and affinity were evaluated using GOLD scoring fitness functions. We identified that amino acid residues Leu52, Arg49, Val33 in COX-1 and Ala18, Ser23, Asp38, Cys22 in COX-2 are important for inhibitor recognition via hydrogen bonding interactions. These hydrogen bonding interactions play an important role for stability of the complex. This information can be exploited to design Mangostin based inhibitors. Our results may be helpful for further experimental investigations.
Key words: Cyclooxygenases, Xanthones, Mangosteen, GOLD software and COX-2 inhibitors.

INTRODUCTION

Prostaglandins (PGs) are the arachidonic acid (AA) metabolites of cyclooxygenase (COX) pathway and are major mediators in the regulation of inflammation and immune function (Smith et al., 2000). Cyclooxygenase (COX), also known as Prostaglandin endoperoxide H synthase (PGHS, EC.1.14.99.1), catalyzes the conversion of arachidonic acid to prostaglandins. This enzyme exists in two isoforms; PGHS-1 (COX-1) and PGHS-2 (COX-2), which has same enzymatic activities (Smith et al., 2000), (Smith et al., 1996), (Marnett et al., 1999). COX-1 and COX-2 enzymes are homodimers that are widely distributed heme proteins (Alex et al., 2011). Both enzymes are associated primarily with cell membrane structures; COX-1 primarily associated with the endoplasmic reticulum where as COX-2 on the nuclear envelope (Morita et al., 1995). In terms of amino acid composition, these enzymes are approximately 60% identical, and their catalytic regions are widely conserved (Picot et al., 1994), (Luong et al., 1996), (Kurumbail et al., 1996). Moreover, the two active sites of these isoforms differ only by two amino acids, at positions 513 (His for COX-1 and Arg for COX-2) and 523 (Ile for COX-1 and Val for COX-2) (Zhang et al., 1996).

COX-1 is expressed constitutively in most mammalian tissues and is thought to be responsible for housekeeping functions of prostaglandins such as regulation of gastric response (Seibert et al., 1995), (Masferrer et al, 1994). COX-2 is an inducible enzyme that is thought to give rise to the increased prostaglandin levels produced during inflammation (Seibert et al., 1994). COX-2 gene is an early inducible gene in response to many inflammatory cytokines, including IL-1, TNF-α and Lipopolysaccharide (LPS). COX-2 gene expression is controlled at the transcriptional and post-transcriptional levels (Dixon et al., 2000). Because COX-2 isozyme was found to be over expressed during inflammation, drug investigation was focused on selective COX-2 inhibition, hoping to prevent inflammation.

Nonsteroidal anti-inflammatory drugs (NSAIDs) block the production of prostaglandins by inhibiting both COX-1 and COX-2. Most of these drugs are associated with well-known side effects at the gastrointestrial level and less frequently at the renal level. NSAIDs appear to produce at least some of their beneficial effects by inhibiting COX-2 and with lethal side effects by inhibiting COX-1 (Singh et al., 2009). Thus, there is a need to design new compounds with optimum COX-1 and COX-2 inhibition by docking. With our long standing interest in the transcriptional regulation based control of inflammation, we are particularly interested in xanthones derived from mangosteen of *Garcinia mangostana*, Mangosteen has been used as traditional medicine for the treatment of skin infection, wounds and diarrhea in south East Asia. (Nakatani et al., 2002). The main objective of the present study is to perform the docking analysis of xanthones of mangosteen.

METHODOLOGY

The molecular structures of α-mangostin (Figure-1), β-mangostin (Figure-2) and γ-mangostin (Figure-3) were generated and optimized using chemsketch software. The ligands were docked into Cyclooxygenase-1 (COX-1, PDB_ID: 3N8V) and Cycloxygenase-2 (COX-2, PDB_ID: 3NTG) using docking program GOLD 3.0.1. Hetero atoms were removed from the binding site and the chain A was selected for docking studies. Hydrogen atoms were added to COX-1 and COX-2 enzymes. The binding sites of the target enzymes were identified using CASTp server (Joe Dundas et al., 2006) based on precise computational geometry methods, including alpha shape and discrete flow theory. The chain A was selected for docking studies, hetero atoms were removed and hydrogen atoms were added to the binding site of COX-1 and COX-2 enzymes CASTp automatically locates and measures the volume and area of protein pockets and cavities. In addition CASTp provides information about the atoms lining pockets, pocket openings, and buried cavities; circumference of mouth openings.

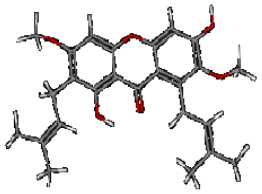

Figure-1: Structure of α-mangostin

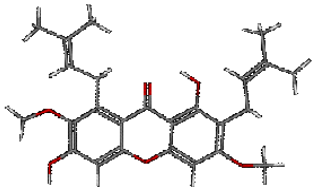

Figure-2: Structure of β-mangostin

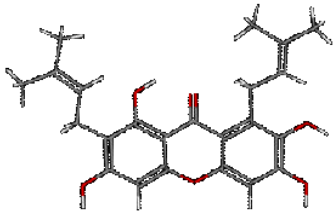

Figure-3: Structure of γ-mangostin

Docking with GOLD 3.0.1

GOLD (Genetic Optimization of Ligand Docking) a genetic algorithm (GA) based software, mainly utilizes an evolutionary strategy involving 3 genetic operators; cross overs, mutations and migrations (Jones et al., 1997). GOLD imports the partial flexibility to proteins and full flexibility to inhibitors. The compounds are docked into the active site of COX-1 and COX-2 and the interaction of these ligands with the active site residues are thoroughly studied using calculations of molecular mechanics. The parameters used for GA were population size (100), selection pressure (1.1), number of operations (10,000), number of island (1) and niche size. Operator parameters for crossover, mutation and migration were set to 100, 100 and 10 respectively. Default cutoff values of 3.0A° (dH-X) for hydrogen bonds and 6.0A° for vanderwaals were employed. The default algorithm speed was selected and the inhibitor binding site in the COX-1 and COX-2 was defined within a 10A° radius with the centroid as HH atom of PHE220 and ARG170 respectively. The number of poses for each inhibitor was set 100, and early termination was allowed if the top three bound conformations of inhibitors were within 1.5A° RMSD. After docking, the individual binding poses of each inhibitor were observed and their interactions with the protein were studied. The best and most energetically favorable conformation of each inhibitor was selected.

GOLD Score fitness function

The four components vig, Protein-ligand hydrogen bond energy (external H-bond); Protein-ligand vanderwaals energy (external vdw); Ligand internal vanderwaals energy (internal vdw); and Ligand intramolecular hydrogen bond energy (internal- H- bond) were considered for calculating the fitness function of GOLD score. The protein-ligand hydrophobic contact was encouraged by making an empirical correction by multiplying external vdw score with 1.375. The fitness function has been optimized for the prediction of ligand binding positions.

Gold Score = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

Where S (hb_ext) is the protein-ligand hydrogen bond score, S (vdw_ext) is the protein-ligand vanderwaals score, S (hb_int) is the score from intramolecular hydrogen bond in the ligand and S (vdw_int) is the score from intramolecular strain in the ligand.

RESULTS AND DISCUSSION

The concept of docking is important to determine the properties associated with protein-ligand interactions such as binding energy, electron distribution, hydrogen bond donor acceptor properties and hydrophobicity. In the present study, CASTp server was used to found the possible binding site of COX-1 (Figure-4) and COX-2 (Figure-5). From the binding site analysis it was observed that binding pockets are identical both in COX-1 and COX-2 and the largest binding pocket was selected for the docking studies. Due to similar crystal structures, 3N8V and 3 NTG were used as representative structures for COX-1 and COX-2 respectively. The xanthone ligands were docked into COX-1 and COX-2 using GOLD 3.0.1 and all docking solutions for COX-1 and COX-2 were ranked according to the GOLD fitness function. The docking results showed that all the xanthone derivatives of mangosteen are active COX inhibitors with a significant preference for COX-2.

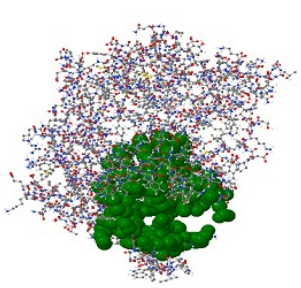

Figure-4: Active site of COX-1

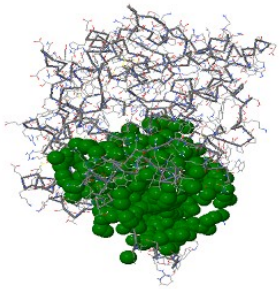

Figure-5: Active site of COX-2

Among the three xanthones, α-mangostin and β-mangostin showed common hydrogen bond interactions with Leu52, Arg49 of COX-1. α-mangostin showed a bond length of 1.817A° and 2.656 A° (Figure-6); β-mangostin showed a bond length of 1.806 A° and 2.180 A° (Figure-7), and γ-mangostin showed a different pattern of hydrogen bonding with Val33 of COX-1 (Figure-8). On the other hand α-Mangostin extended O11 and O13 of its oxygen atoms to form two hydrogen bonds with Ala18 of COX-2 with a bond length of 2.019A° and 2.556A° respectively (Figure-9). Similarly, β-mangostin exhibited two hydrogen bonds with Asp38, Ser23 of COX-2; one bond is between oxygen atom of Asp38 with H39 and another bond is seen between hydrogen atom of Ser23 with O20 (Figure-10). As shown in Figure-11 γ-mangostin showed strong hydrogen bond interactions with Ala18, Cys22 and Asp38 of COX-2 between hydroxyl group of Ala18 and O22; other bondings observed between oxygen atom of Asp38 and H37, hydrogen atom of Cys22 and oxygen atom O21.

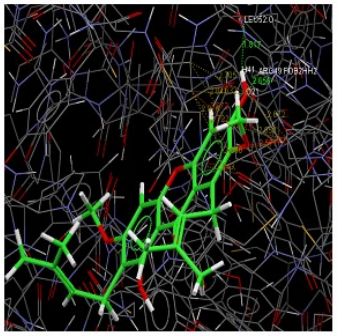

Figure-6: Docking of α-mangostin with COX-1

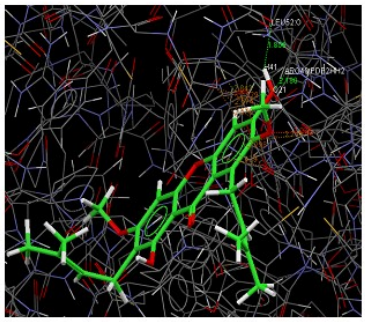

Figure-7: Docking of β-mangostin with COX-1

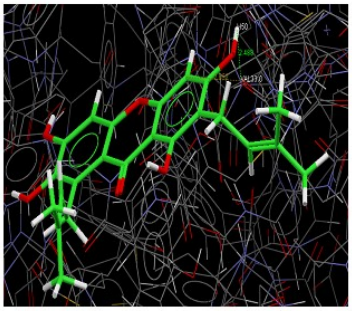

Figure-8: Docking of γ-mangostin with COX-1

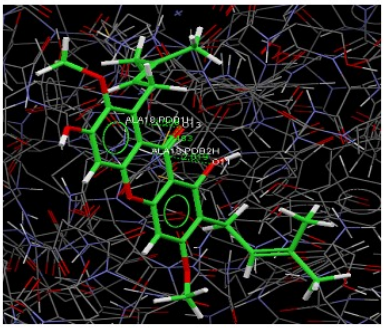

Figure-9: Docking of α-mangostin with COX-2

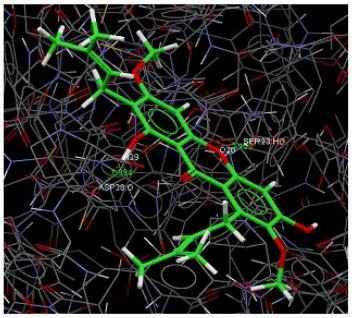

Figure-10: Docking of β-mangostin with COX-2

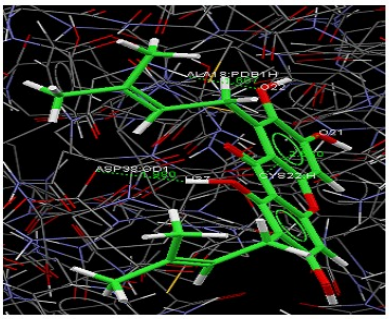

Figure-11: Docking of γ-mangostin with COX-2

The atoms involved in hydrogen bonding, their bond lengths and docking energies of all the three ligands based on GOLD fitness score were indicated for COX-1 (Table-1) and COX-2 (Table-2). The results deduce that all the three ligands were potential against COX-2 and they are ranked as γ-mangostin > α-mangostin >β-mangostin.

Table 1: Docking score and bonding of mangostins with COX-1 using GOLD 3.0.1

Molecule	No. of Hydrogen bonds	Atoms		Bond length (A°)	Docking score (kcal/mol)
		Protein	molecule		
α-mangostin	2	Arg49(HH2) Leu52(O)	O(21) H(41)	2.656 1.817	14.77
β-mangostin	2	Arg49(HH2) Leu52(O)	O(21) H(41)	2.180 1.806	10.20
γ-mangostin	1	Val33(O)	H(50)	2.488	16.20

Table 2: Docking score and bonding of mangostins with COX-2 using GOLD 3.0.1

Molecule	No. of Hydrogen bonds	Atoms		Bond length (A°)	Docking score (kcal/mol)
		Protein	molecule		
α-mangostin	2	Ala18(H2) Ala18(H1)	O(11) O(13)	2.019 2.556	22.33
β-mangostin	2	Asp38(O) Ser23(HG)	H(39) O(20)	1.894 2.122	21.34
γ-mangostin	3	Ala18(H1) Cys22(H) Asp38(OD1)	O(22) O(21) H(37)	1.667 2.630 1.980	22.91

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