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Research Article
Molecular Docking Study of 2, 3-Dimethylmaleic Anhydride (3, 4-Dimethyl-2, 5-Furandione) as Anti-inflammatory Agent

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Abstract

Background and Objective: Extracts from *Colocasia esculenta* plant have been found to possess anti-inflammatory properties. 2, 3-Dimethylmaleic anhydride (3, 4-Dimethyl-2, 5-furandione) is a recent root stock extract of *Colocasia esculenta*. This study aimed to look for the possible anti-inflammatory activity of the 2, 3-Dimethylmaleic anhydride by docking study with the target protein, COX-2. **Materials and Methods:** Crystal structure of COX-2 was retrieved from RCSB protein data bank. The structure of 2, 3-Dimethylmaleic anhydride was retrieved from PubChem compound database in NCBI. Docking study was performed with the help of AutoDock 4.2.6. **Results:** Docking study showed that 2, 3-Dimethylmaleic anhydride fits into the cyclooxygenase active site of COX-2 with favourable binding energy. **Conclusion:** NSAIDs best known for their anti-inflammatory properties act by blocking COX enzymes suggest the compound 2, 3-Dimethylmaleic anhydride may possess anti-inflammatory potential bearing a role in alleviating the signs and symptoms of inflammation.

Key words: 2, 3-Dimethylmaleic anhydride, NSAIDs, anti-inflammatory activity, COX enzymes, molecular docking and *Colocasia esculenta*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cyclooxygenase (COX) enzymes, also known as Prostaglandin-endoperoxide synthase (PTGS), catalyze the metabolic conversion of arachidonic acid (AA) to prostaglandins (PGs) that play an important role in the process of inflammation[1]. COX exists in three isoforms: Cyclooxygenase-1, 2 and 3 (COX-1, COX-2 and COX-3)[2,3]. COX-2 plays more of a role in prostaglandin mediated inflammation, while COX-1 plays some housekeeping roles mediating homoeostatic functions in the gastrointestinal and cardiovascular system but exact functions of COX-3 (a splice variant of COX-1) are still unclear[4,5].

COX-2 converts AA to prostaglandin H2 (PGH2). COX-2 is a sequence homodimer. Each monomer of the enzyme has a peroxidase and a Cyclooxygenase active site. The COX enzymes catalyze the conversion of arachidonic acid to prostaglandins in two steps. First, hydrogen is abstracted from carbon 13 of arachidonic acid and then two molecules of oxygen are added by the COX-2, giving prostaglandin G2 (PGG2). Second, PGG2 is reduced to PGH2 in the peroxidase active site[6].

For decades nonsteroidal anti-inflammatory drugs (NSAIDs) have been used as anti-inflammatory agents[7]. They act by blocking the COX enzymes. Compared with non-selective NSAIDs that inhibit both COX-1 and COX-2, COX-2 selective inhibitors inhibit only COX-2 enzymes. These drugs are highly effective but nevertheless have lesser or more side effects[8,9]. Use of naturally occurring anti-inflammatory agents are yet a rare sight. Research on such agents is very much awaited approach that could subsidize inflammation to a greater extent and at the same time possessing lesser toxicity in comparison to the classically used NSAIDs. Attempts have been made at regular intervals to find out such a naturally occurring potent alternative.

Colocasia esculenta (L.) commonly called taro, is a tropical plant grown primarily for its edible corms, the root vegetables that has been known since ancient times for its medicinal properties[10] and has been utilized for treatment of various ailments including inflammation[11]. 2, 3-Dimethylmaleic anhydride (3, 4-Dimethyl-2, 5-furandione) (Fig. 1) has been isolated from the root stock of *Colocasia esculenta*[12]. This compound has been reported as insecticidal molecule[12] but no study has been conducted yet to establish its role as a remedy of inflammation. This compound could additionally have anti-inflammatory potential. If its role in inflammation could be established then this finding would be a great procurement as a replacement of NSAIDs by a natural extract. No *in silico* study of this compound has been conducted so far to prove its anti-inflammatory role. For the first time the compound 2, 3-Dimethylmaleic anhydride has been reported to have anti-inflammatory property and this study had been analyzed using *in silico* approach. The use of *in silico* method has provided as the opportunity to study the interaction at molecular level. This study aimed to explore the possible anti-inflammatory activity of the compound 2, 3-Dimethylmaleic anhydride by docking study with the target protein, COX-2.

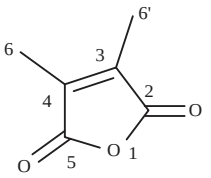


Fig. 1: Molecular structure of 2, 3-Dimethylmaleic anhydride (3, 4-dimethyl, 2, 5-Furandione)[12]

MATERIALS AND METHODS

Preparation of protein molecule: Cyclooxygenase-2 (Prostaglandin Synthase-2) complexed with a selective inhibitor, SC-558 in I222 space group (PDB ID: 6COX)[13] was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/) as a PDB file. The co-crystal selective inhibitor SC-558 was removed to get native target protein. The target protein COX-2 is a homodimer comprising of two identical chains A and B with similar active sites. Protein was prepared using the Auto Dock Tools (ADT)[14]. In protein preparation, B chain, all water molecules and hetero-molecules of 6COX were deleted. Polar hydrogen atoms and Kollman charges were added.

Preparation of ligand: The structure of 2, 3-Dimethylmaleic anhydride (PubChem CID: 13010) was retrieved from PubChem compound database[15] in NCBI[16] as a SDF file. Using Open Babel (http://openbabel.org)[16] SDF file was converted into PDF format. The ligand 2, 3-Dimethylmaleic anhydride was prepared using Auto Dock Tools (ADT). In ligand preparation Gasteiger partial charges were assigned and non-polar hydrogen atoms were merged.

Docking: Docking study was performed in order to check the binding affinity of 2, 3-Dimethylmaleic anhydride with the Cyclooxygenase active site residues of COX-2 enzyme (PDB ID: 6COX) using the software, Auto Dock 4.2.6[14]. A grid box covering the cyclooxygenase active site residues of the target

protein was generated to get the best conformational state of docking. Docking grid box size was set to 60×56×68 Å dimension, spacing of 0.514 Å and centered at 25.19, 25.805, 46.378 of X, Y and Z coordinate. Docking was performed using Lamarckian Genetic Algorithm (LGA).

RESULTS AND DISCUSSION

Before performing the docking study the docking protocol was validated. The co-crystal selective inhibitor SC-558 was removed from the protein (PDB ID: 6COX) and again docked back into the active site of the protein. The two dimensional graphical depiction of binding interaction was accessed by LIGPLOT tool[17] (Fig. 2a and b). Root mean square deviation of the protein in co-crystal complex conformation and the best docked conformation was zero. Ligand showed deviation which was negligible (Fig. 2c). This indicated the ability of the docking protocol to reproduce the binding mode of the co-crystal inhibitor. In the docking study the compound 2, 3-Dimethylmaleic anhydride showed hydrogen bonding interactions with Tyr 385(A) and Ser 530(A) and hydrophobic interactions with Val 349(A), Leu 352(A), Phe 381(A), Leu 384(A), Met 522(A) and Gly 526(A) (Fig. 3) with a binding energy (G) of -4.59 kcal mol^{-1}.

The residues comprising the active site pocket of COX as reported in previous reports consists of His 90, Leu 117, Arg 120, Phe 205, Phe 209, Val 344, Ile 345, Tyr 348, Val 349, Leu 352, Ser 353, Tyr 355, Leu 359, Phe 381, Leu 384, Tyr 385, Trp 387, Val 434, Arg 513, Phe 518, Met 522(A), Val 523, Gly 526, Ala 527, Ser 530, Leu 531, Gly 533 and Leu 534[18-21]. Present docking study revealed that 2, 3-Dimethylmaleic anhydride fits favorably into the cyclooxygenase active site of COX-2

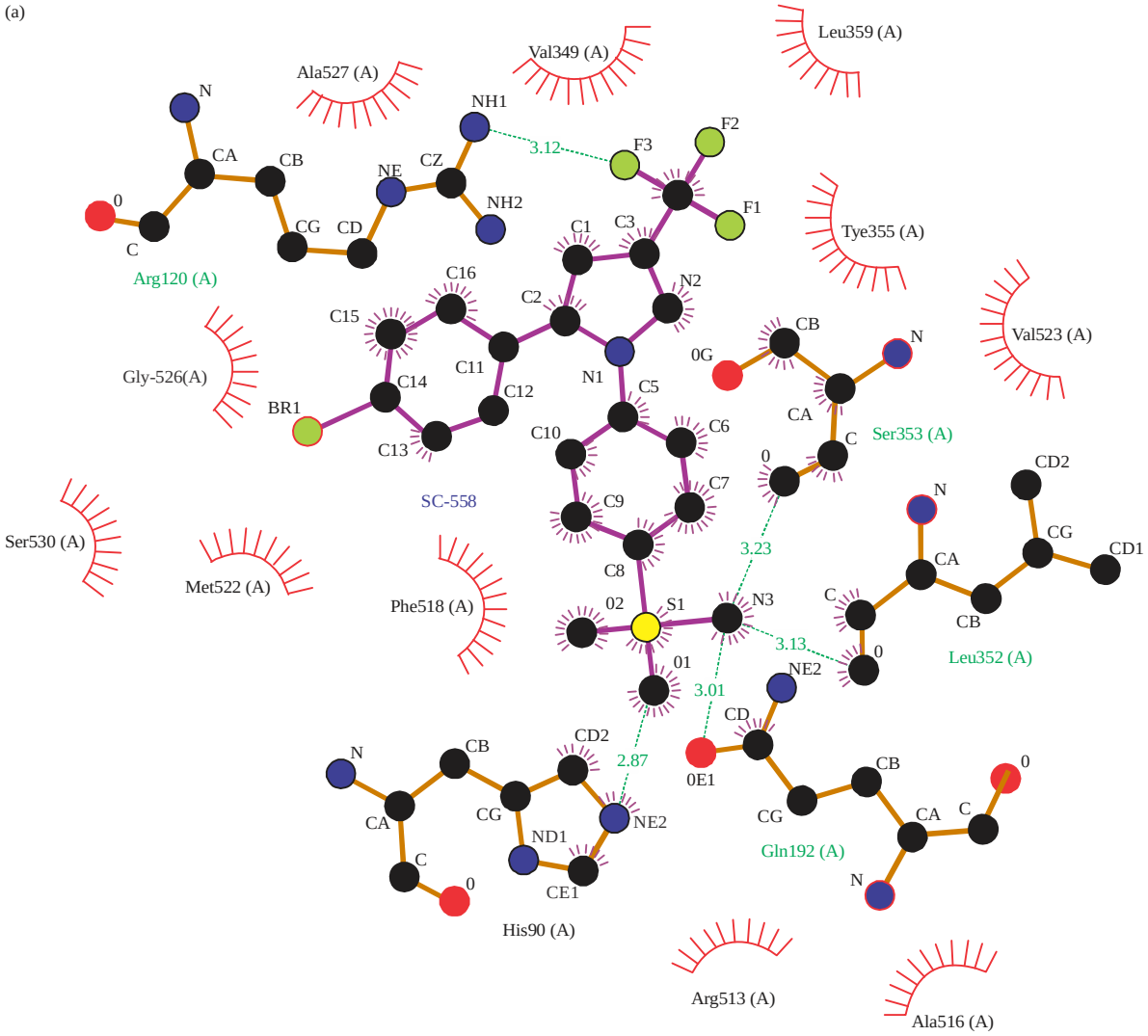
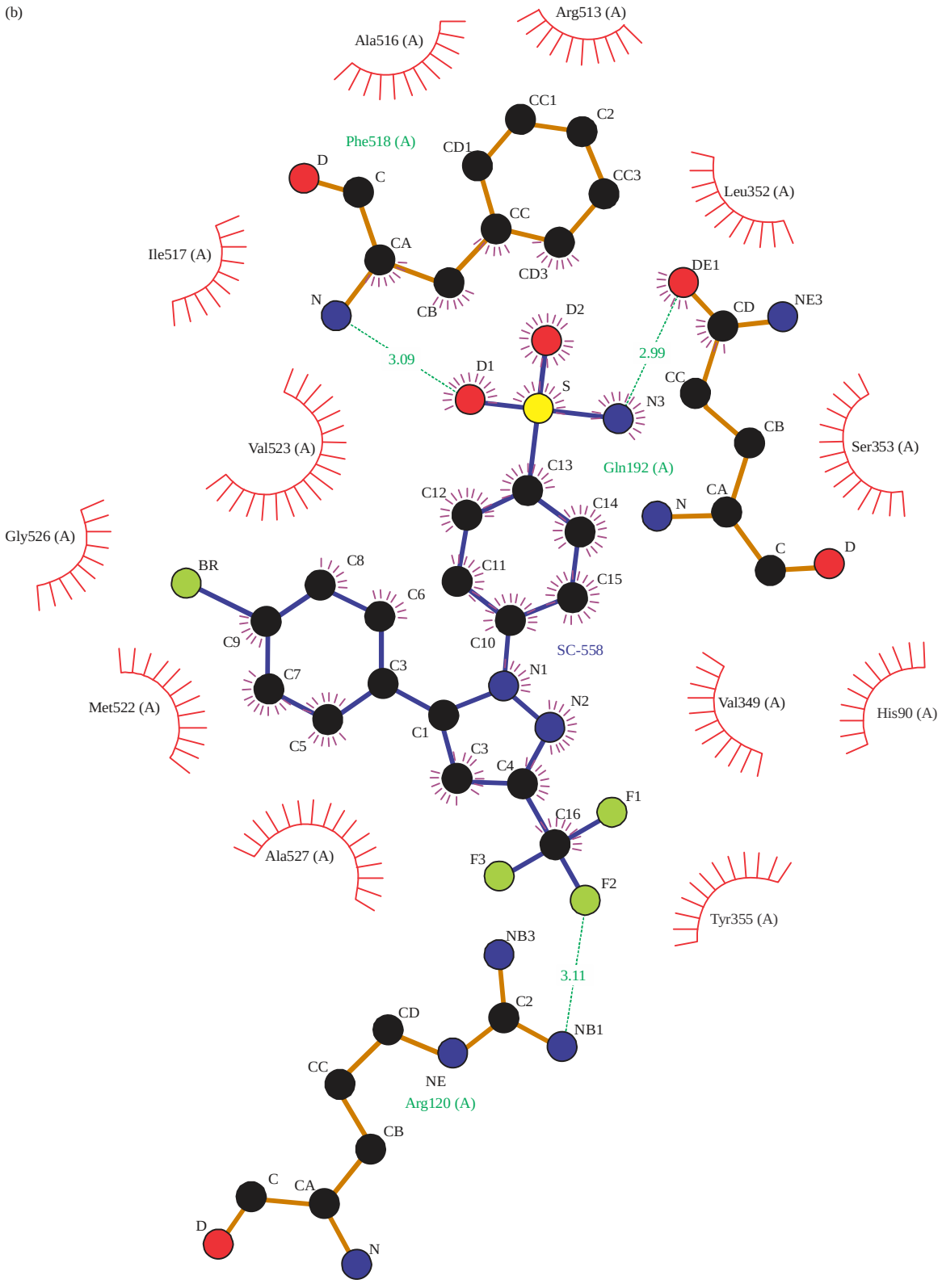


Fig. 2(a-c): Continue



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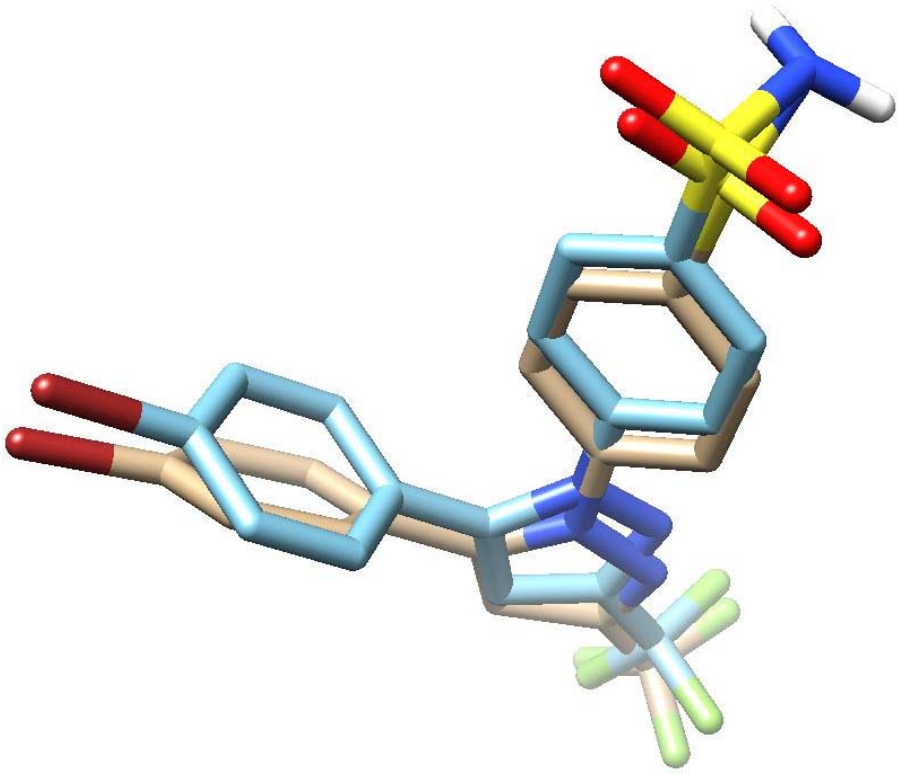

Fig. 2(a-c): (a) Binding interaction of the co-crystal selective inhibitor SC-558 with the active site residues of COX-2 (PDB ID-6COX) Chain A found in crystallographic structure shown using Ligplot, Dashed lines between atoms indicate Hydrogen bond, arc with spokes radiating toward SC-558 represents hydrophobic contacts, spokes on SC-558 show the contacted atoms (b) Binding interaction of the co-crystal selective inhibitor SC-558 with the active site residues of COX-2 (PDB ID-6COX) chain A obtained by docking with Auto Dock 4.2.6 shown using Ligplot. Dashed lines between atoms indicate Hydrogen bond, arc with spokes radiating toward SC-558 represents hydrophobic contacts, spokes on SC-558 show the contacted atoms and (c) Overlay of co-crystal selective inhibitor SC-558 conformation extracted from 6COX (shown in grey) with the best docked conformation (shown in cyan) in COX-2

displaying hydrogen bonding interactions with Tyr 385(A) and Ser 530(A) and hydrophobic interactions with Val 349(A), Leu 352(A), Phe 381(A), Leu 384(A), Met 522(A) and Gly 526(A). This docking study formed the base for the proposed compound to be used as NSAIDs analogue as all the amino acid residues that interacted with the compound form a part of the active site. Previous studies explained about an important region in the COX-2 active site, the hydrophobic pocket (lined by Trp 387, Tyr 385, Phe 518, Phe 381, Leu 352) and also described the involvement of this region in the proper positioning of fatty acid substrates for oxygenation[21]. Tyr 385(A), Phe 381(A) and Leu 352(A) residues, lied within the hydrophobic pocket of the COX-2 showed interaction with 2, 3-Dimethylmaleic anhydride suggest the possibility of the compound in blocking the AA from binding with the COX-2 for oxygenation.

In the previous studies NSAIDs were reported to be interactive with the cyclooxygenase active site residues. Aspirin was reported as covalent modifier of both COX-1 and COX-2 enzymes through acetylation of the hydroxyl group of Ser 530[22-24]. Tyr 385 formed hydrogen-bonds with the acetyl group of aspirin, which increases its reactivity by stabilizing the negative charge of the tetrahedral intermediate of acetylation[25]. Interestingly present docking study showed hydrogen bonding interactions of 2, 3-Dimethylmaleic anhydride with Tyr 385(A) and Ser 530(A) in a much similar way to the binding mode of aspirin in the COX enzyme suggesting that it may have anti-inflammatory potential like aspirin.

Indomethacin showed deep binding within the cyclooxygenase active site through its p-chlorobenzoyl group

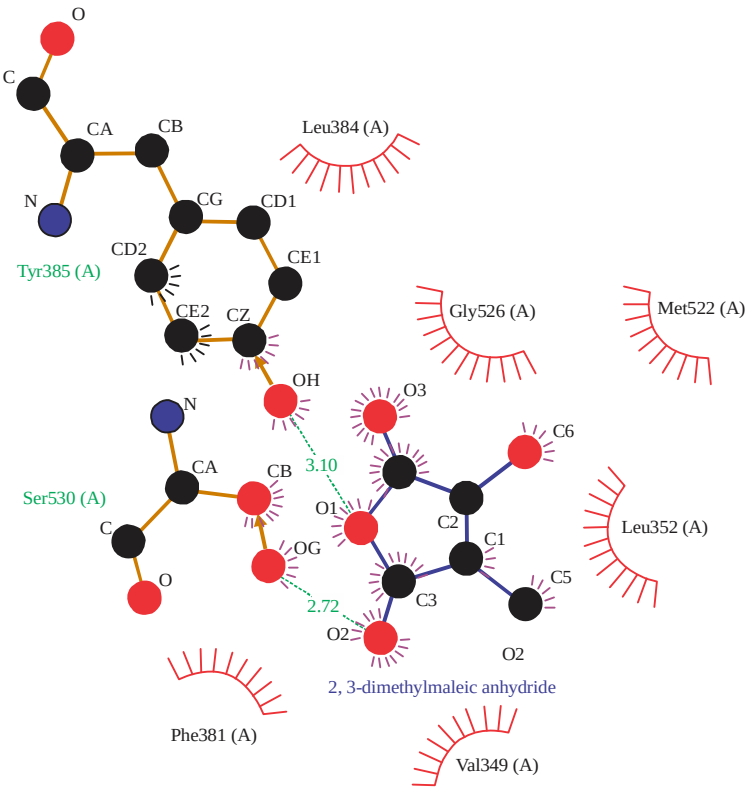

Fig. 3: Docking pose of 2, 3-Dimethylmaleic anhydride into the active site residues of COX-2 (PDB ID-6COX) chain A shown using Ligplot. Dashed lines between atoms indicate Hydrogen bond, arc with spokes radiating toward the 2, 3-Dimethylmaleic anhydride represents hydrophobic contacts, spokes on 2, 3-Dimethylmaleic anhydride show the contacted atoms

that interacts with Leu 384 and Ser 530. The benzoyl group showed hydrophobic interactions with Leu 384, Tyr 385, Phe 381 and Trp 387. The indole ring of indomethacin showed interaction[13] with Val 349. The proposed compound exhibited bonding with majority of the residues mentioned above that includes Ser 530(A), Leu 384(A), Tyr 385(A), Phe 381 and Val 349 (A). This result unveiled similar mode of binding shown by indomethacin and the present compound with the COX enzyme.

In contrast, diclofenac showed interaction with the active site of COX-2 in a unique inverted binding mode with its carboxylic acid moiety hydrogen-bonded[26] to Ser 530 and Tyr 385. It was clearly evident from above reports that Ser 530 and Tyr 385 were the two key residues involved in diclofenac mode of COX blocking. As the studied compound also showed interaction with these two key residues so it could be concluded that it might also behave like diclofenac.

Recently, a crystal structure of mCOX-2 with lumiracoxib, a COX-2 selective phenylacetic acid derivative of diclofenac, was solved and showed that lumiracoxib also bound to the COX active site in an inverted orientation. Lumiracoxib formed hydrogen-bonding interactions with Ser 530 and Tyr 385, similar to diclofenac[27]. The present compound 2, 3-Dimethylmaleic anhydride also showed hydrogen-bonding interactions with Ser 530(A) and Tyr 385(A).

Stellatin is a naturally occurring anti-inflammatory chromone[28]. Some of stellatin derivatives were reported as COX inhibitor. In docking study with COX target proteins these stellatin derivatives showed hydrogen bonding[29] with Tyr 385, Met 522 and Ser 530. These results have marked resemblance with present docked result.

Curcumin, a major pigment in the Indian spice turmeric was reported to have anti-inflammatory property. From docking analysis it was observed that curcumin analogues were involved in the hydrogen bonding with a residue Ser 530. Active site amino acid residues Ser 530, Gly 526, Met 522, Tyr 385 and Ala 526 surrounded one of the phenyl rings of curcumin[30]. Majority of these amino acids showed interaction with studied compound suggesting similar curcumin like anti-inflammatory behaviour.

Xanthone derivatives were found to be inhibitors against COX enzyme showing interaction with amino acids Arg 120,

Ser 530 and Met 522, Tyr 355, Tyr 385 and Ser 353 of COX enzyme[31]. These docking data are in line with present study, so the studied compound could act like xanthone derivatives.

In docking study nimesulide showed hydrogen[32] bonds with Ser 530 and/or Tyr 385. Whereas celecoxib bound by several amino acid residues; among them[31] are Trp 385, Trp 387, Phe 518, Val 523, His 90, Leu 352, Leu 531, Ala 527, Val 349, Gly 526 and Ser 530. These similar modes of binding further strengthened COX blocking nature of the present compound.

These similarities in interaction of present studied compound with other agents such as naturally derived substances or NSAIDs including both traditional as well as new generations indicate that the compound 2, 3-Dimethylmaleic anhydride is able to occupy the active site of both COX-1 and COX-2 enzyme and are believed to be involved in inhibition of both. As the key difference between the COX-1 and COX-2 enzyme active site is the exchange of isoleucine in COX-1 for valine in COX-2 at positions[19,33] 434 and 523 and the *in silico* study showed no interaction with these amino acids which revealed present studied compound could be effective as non selective inhibitor.

CONCLUSION

NSAIDs exhibit anti-inflammatory properties by blocking COX enzymes. Molecular docking study revealed that the compound 2, 3-Dimethylmaleic anhydride (3, 4-Dimethyl-2, 5-furandione) may behave like NSAIDs in regard to its binding with COX enzyme and may be used as an anti-inflammatory agent.

SIGNIFICANCE STATEMENT

The structure of the studied compound also can prove to be an important scaffold for designing more potent anti-inflammatory drugs. Further, it can also be used as a supplement along with the available anti-inflammatory drugs to enhance their activity. Being a natural compound the compound will have minimum side effects, which is an utmost requirement in drug research today. This study has been reported based on *in silico* approach and in order to understand the stability of the complex (ligand-protein), molecular dynamics simulation study is being carried out. To support the *in silico* findings, *in vitro* as well as *in vivo* studies have to be performed to analyze the efficacy of the compound. The NSAIDs are potent COX inhibitors but not free of adverse effects. So naturally occurring agents will be very useful that could inhibit COX and at the same time less harmful to human being. So our finding could help to procure a replacement of NSAIDs by a natural extract.

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