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Phytochemical screening and *In-silico* Investigation of 2-Benzoxazolinone from *Acanthus Ilicifolius* Linn. as dual inhibitors of Cyclooxygenase-2 and 5-lipooxygenase enzymes

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Abstract

2-Benzoxazolinone (BOA) is a phytoconstituent of a mangrove plant *Acanthus ilicifolius* Linn used to treat inflammatory diseases. Anti-inflammatory agents with dual inhibiting properties of COX-2/5-LOX enzymes activities are said to possess anti-inflammatory effect and are devoid from side effects but not yet available on the market. Therefore, the dual inhibiting property of BOA and its derivatives [6-Bromo-BOA (6-BrBOA), 6-Chloro-BOA (6-ClBOA), Hydroxy-BOA (HBOA) and 6-methoxy BOA (6-MBOA)] is worth investigating. Initially, BOA content in supercritical CO_2 fluid extract (SCFE) of *A.ilicifolius* leaves was estimated using ultra high performance liquid chromatography (UHPLC). Later, genetic algorithm based GOLD docking simulation was employed to dock BOA and its derivatives into the binding pocket of COX-2 and allosteric binding site of 5-LOX. Probable hit ligand was ranked using an evaluation criterion based on the GOLD docking score and the ligand binding mode. The UHPLC chromatogram revealed the presence of BOA in SCFE. High gold docking scores ranging from 40.89 to 43.13 were observed in all the tested ligands with COX-2 and three ligands namely; 6-BrBOA (42.67), 6-ClBOA (41.79) and BOA (38.90) showed high docking scores with 5-LOX allosteric binding site. In conclusion, UHPLC method could be used for the determination of BOA content in *A.ilicifolius*. BOA and its analogues could be a promising lead for developing dual inhibitors of COX-2/5-LOX enzymes as verified by computational studies.

Keywords: Supercritical CO_2 fluid extract, *Acanthus ilicifolius* Linn, UHPLC, Mangroves, COX-2/5-LOX, Genetic Optimization for Ligand Docking.

Introduction

Naturally occurring bioactive compounds are gaining importance as potential drug candidates for a number of pathological conditions including chronic inflammatory diseases [1]. Identification of anti-inflammatory compounds from natural products is of great interest and could provide an opportunity to replace synthetic drugs in future. Cyclooxygenases (COXs) and lipoxygenase (LOXs) are key enzymes involved in the inflammation, pain and arachidonic acid metabolism. Emerging clinical evidences indicate that inhibitors of COX and LOX, particularly their respective isoforms (COX-2 and 5-LOX) are effective in ameliorating inflammatory diseases. However, most of non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the COX enzymes and are ineffective in inhibiting 5-LOX mediated inflammatory mediators such as leukotriene. The adverse effects such as gastric damage and bronchoconstriction with use of conventional NSAIDs are due to COX inhibition and leukotriene formation [2]. Recently, dual COX-2/5-LOX enzyme inhibition is considered significant in managing the molecular mechanisms during chronic inflammation and possess minimal adverse effects compared to NSAIDs [3]. The market withdrawal of selective COX-2 inhibitors namely rofecoxib in 2004 and valdecoxib in 2005 due to their cardiovascular toxicity [4] have led to identify compounds with dual COX/LOX inhibitory effect. Computer stimulated experiments could provide theoretical support to identify pharmacophore, later synthesis and test compounds for dual COX-2/5-LOX inhibitory activity [5]. 2-Benzoxazolinone (BOA) pharmacophore is a naturally occurring bioactive compound having a wide range of biological activities [6] including analgesic and anti-inflammatory effect [7]. BOA is an alkaloid isolated from *Acanthus ilicifolius*, a mangrove plant used for the treatment of inflammatory diseases. A wide variety of BOA derivatives were reported in this species [8-10]. Therefore, the impact of plant-derived BOA and/or its derivatives on COX-2/5-LOX enzyme inhibition is worthy investigating. Supercritical fluid CO_2 extraction is more effective than solvent based extraction since these extracts are free from toxic solvent residues and heat labile substances could be extracted by employing low temperatures [11]. The BOA content was estimated using high performance liquid chromatography [12]; however ultra high performance liquid chromatography (UHPLC) method is advantageous in speed as it could offer a threefold decrease in retention time without sacrificing efficiency [13]. In the first study, supercritical fluid CO_2 extract from *A.ilicifolius* leaves were

prepared under optimized conditions and UHPLC method was employed to determine the presence of BOA. In the second study, the binding modes of selected BOA and selected derivatives of BOA [6-Bromo-BOA (6-BrBOA), 6-Chloro-BOA (6-ClBOA), Hydroxy-BOA (HBOA) and 6-methoxy BOA (6-MBOA)] were evaluated by *in silico* molecular docking studies on COX-2 and 5-LOX as inflammatory therapeutic targets.

Materials and Methods

Chemicals and Reagents

2-Benzoxazolinone (BOA; Purity >98%) was obtained from Tokyo Chemical Industry (TCI), Japan. 99.9 % pure methanol (Chromasolv) were purchased from Sigma Aldrich, U.S.A. Purified water obtained from Milli-Q gradient system (Millipore, USA) with the resistance value of 18M measured at the time of sampling was used in this study.

Plant Material

Fresh *A. ilicifolius* leaves were collected from Pichavaram Mangrove forest, TamilNadu, India. The shade dried leaves were made into a coarse powder using a pulveriser and used for supercritical CO_2 fluid extraction.

Supercritical CO_2 Fluid Extract of *A. ilicifolius*

A. ilicifolius leaves powder (5 kg) was packed in the pilot supercritical fluid extraction vessel (10 L). Four parameters such as pressure, temperature, CO_2 flow rate and extraction time were optimized for better yield. The boiling point of BOA was considered while optimizing the experimental parameters.

UHPLC Analysis of SCFE for BOA Content

The content of BOA in SCFE of *A.ilicifoius* was analysed using Accela 1250 UHPLC system (Thermo Fisher Scientific Inc., USA) equipped with Accela 1250 photodiode array (PDA) detector and Accela 1250 binary pump. 10.72 mg of SCFE of *A.ilicifoius* was dissolved in 10 mL of methanol, filtered using 0.22 μm nylon syringe filter (Millipore) and 1μL (1.072 μg of SCFE) was injected into the UHPLC system. The separation was employed by isocratic elution mode with 100% methanol as mobile phase at a flow rate of 1mL/min using Thermo Hypersil C18 column (240 4.6 mm, i.d., 5 μ) maintained at 27 C. Column effluent was monitored at 265nm using PDA detector. 1 μL of BOA (1 μg) was used as a internal standard under above experimental conditions. The retention time and percentage area was calculated using ChromQuest chromatography data system software (Version 5.0, Thermo Fisher Scientific Inc., USA). The percent of BOA in SCFE of *A.ilicifoius* was calculated *via* external standard method using ChromQuest software.

In-Silico Molecular Docking Analysis

Protein preparation and optimization

The X-ray crystallographic three dimensional (3D) structure of mouse cyclooxygenase-2 (COX-2) (PDB id: 1CX2) is a homotetramer enzyme with EC Number: 1.14.99.1 classified under Oxidoreductase class of enzymes, complexed with a selective inhibitor S58 with 3.0 A resolution [14]. While 5-LOX enzyme with EC Number: 1.13.11.34 with 2.39A resolution (PDB id: 3O8Y) were used as inflammatory drug target respectively [15]. For both COX-2 and 5-LOX PDB protein structures water molecules were removed and hydrogen atoms were added and energy minimization was done using UCSF Chimera [16].

Ligand preparation and optimization

The canonical SMILES (Simplified molecular-input line-entry system) of antagonists Benzoxazolinone (BOA), 6-Bromo-2-Benzoxazolinone (6-BrBOA), 6-Chloro-2- Benzoxazolinone (6-ClBOA), Hydroxy-2-Benzoxazolinone (HBOA) and 6-Methoxy benzoxazolinone (6-MBOA) were obtained from NCBI PubChem compound [17]. The 3D structures for the ligands were generated and optimized using VIDA version 4.2.1, OpenEye Scientific Software (Figure 1).

Docking studies

In the docking study, the optimized COX-2 crystal structure A-chain was used for the initially molecular docking studies targeting the SC-558 binding site as reference with a cut off of 12 Å radius around with 100% genetic algorithm (GA) parameter setting using GoldScore as fitness function to dock BOA using Genetic Optimization for Ligand Docking (GOLD V 5.1) [18]. From initial docking study, it's known that SC-558 binding site was not a promising binding site of BOA. So, in the later part of the study prediction of possible allosteric binding-site of BOA and its derivatives on COX-2 protein was determined used Pocket-Finder, a Ligsite based algorithm [19].

Blind Docking: BOA and its derivatives binding site identification

To determine the preferred allosteric binding site of BOA and its derivatives among the pockets predicted by Pocket-Finder, a blind docking study was carried out using Autodock [20] by forcing the entire COX-2 protein into grid in order to dock BOA by parameterizing the docking with GA run of 100 and population size 150.

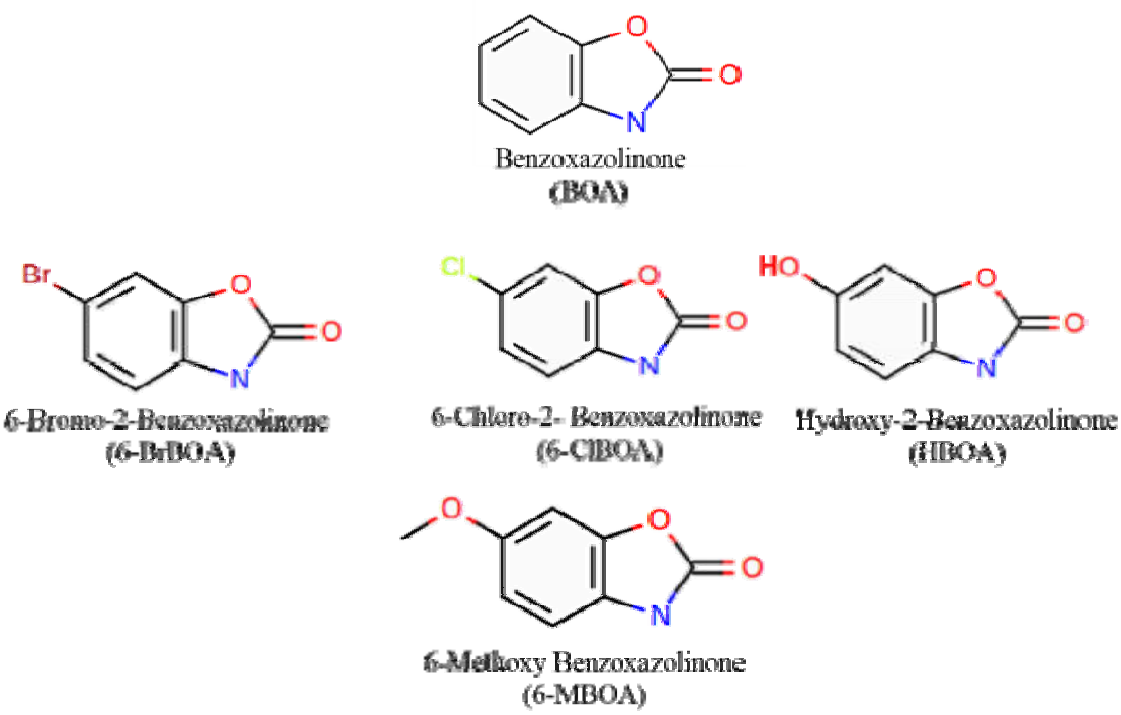


Figure 1: List of BOA and its derivatives.

Docking studies on COX-2 allosteric site

The predicted COX-2 allosteric binding site through Pocket-Finder followed by blind docking study using Autodock was used as the possible allosteric target site for docking BOA and its derivatives. Later docking studies were performed by GOLD V 5.1 [18] using validated allosteric binding site with a cut off of 10Å radius around residue Arg469 with 100% GA, using GoldScore as fitness function with ligands all atom RMSD differing at 1.5 Å were clustered together. BOA and its derivatives first ranked solution, as well the lowest energy conformation of the most populated cluster were analyzed by visual inspection using the software PyMol (The PyMOL Molecular Graphics System. DeLano Scientific LLC, NY, USA. http://www.pymol.org). BOA and its derivatives with the highest affinity for allosteric binding site with high goldscore were selected and ranked. Here, we examined the performance of these docking softwares to select best compound without any bias. Binding mode of the docked molecules were analysed based on the docking score and visual inspection of the binding mode and interaction pattern of each compound.

Docking studies on 5-LOX

Similar, docking studies with BOA and its derivatives were performed targeting enzyme 5-lipoxygenase with PDB ID: 3O8Y, using GOLD V 5.1 [18] binding site with a cut off of 12 Å radius around residue Arg246 and with rest of parameters as mentioned in later part of COX-2 docking studies.

Results and Discussion

SCFE and UHPLC analysis of BOA

The dried leaves of *A. ilicifolius* yielded 0.5% w/w of SCFE under the optimized experimental conditions, 350g bar pressure, 45°C, CO_2 at a flow rate of 3 L/h for 3h and the total CO_2 consumed was 7 kg. The amount of BOA in SCFE of *A. ilicifolius* was identified by comparing the UHPLC-PDA spectral data comparison obtained with SCFE and internal standard BOA based on their retention time (Figure 2). The retention time for SCFE and BOA was 3.1 and 3.09 min respectively, and the total run time was 22.5min. The total content of BOA in SCFE was 48.79 % w/w. Quantification of BOA was earlier reported using HPLC [12, 21] and this is the first study to report the quantification of BOA in *A. ilicifolius* using UHPLC.

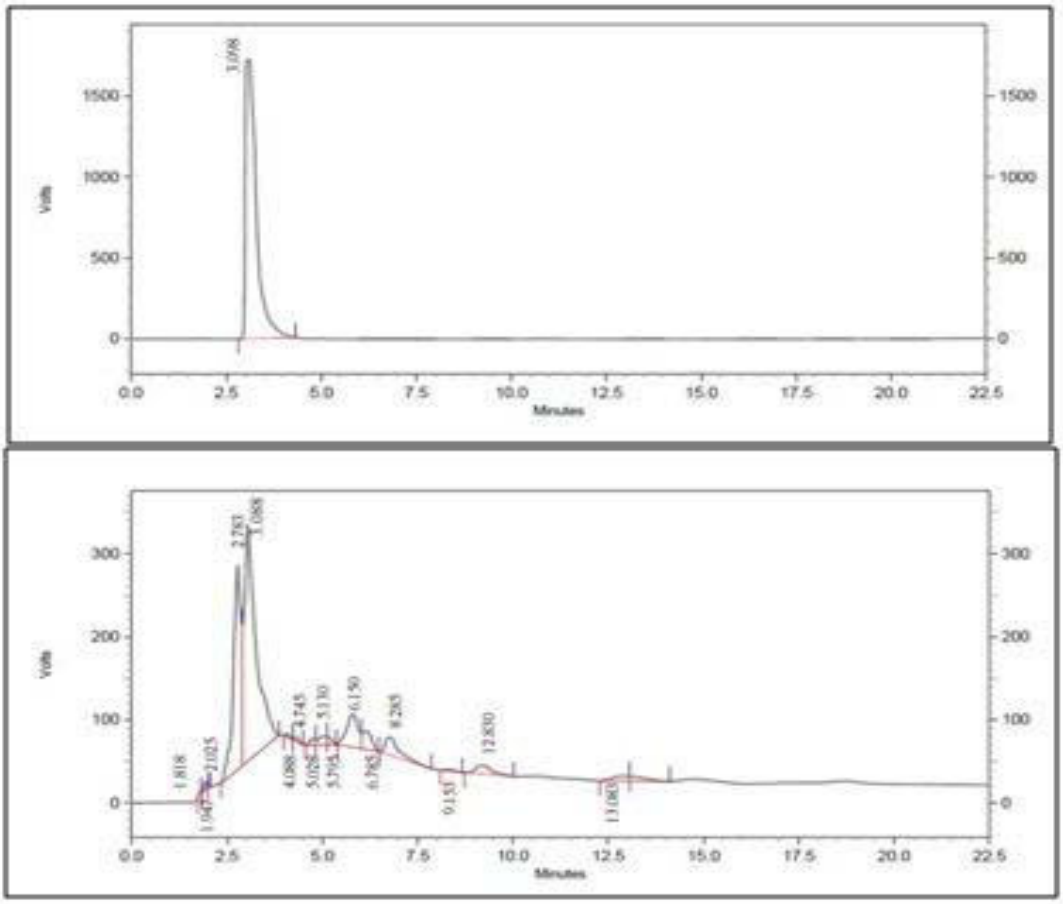


Figure 2: UHPLC Chromatographic fingerprint of BOA (top) and SCFE of A.ilicifolius (bottom). Right axis = retention time (minutes); Left axis = intensity of the peak (Volts). The chromatograms were detected at 265 nm.

Docking analysis with COX-2 enzyme

Dual inhibition of COX-2 and 5-LOX enzymes is safe and effective than targeting COX or LOX alone [3], this is first report for examining BOA and its derivatives for dual COX/LOX inhibition using *in silico* approach. Commercially available flavonoids were used as ligands in identifying potential COX [22] and LOX [23] inhibitors using *in silico* approach. A similar approach was adopted in selecting the ligands in our study. Initial GOLD docking studies with BOA, targeting SC-558 binding site of COX-2 didn't provide desirable binding affinity and this could be due to the fact that the SC-558 binding site residue's electronic features and geomantic orientation was not feasible to accommodate a small molecule like BOA with molecular weight: 135.1201 g/mol which couldn't produce stable binding at the binding site (Data not shown). So, it's proposed that there could be a possible alternative binding site for BOA and its derivatives. In order to determine the possible allosteric binding site on COX-2, we performed pocket site prediction using Pocket-Finder, which predicted totally 9 pockets for COX-2 (Figure 3).

Therefore, to determine the potentially feasible allosteric binding site for BOA among the 9 predicted pocket blinding docking studies were performed on COX-2 using AutoDock considering the entire protein under grid. From the blind docking study analysis it's known that there were 3 potential region i.e. SC-558 binding-site, hemoglobin binding site and an allosteric site to which BOA tend to bind with binding energies -5.05 ,-4.6 and -4.5 respectively. Further interaction analysis show that the allosteric binding site was more promising over HEM and S58 binding site. Therefore, it proposes a new allosteric binding site for the binding of BOA and its derivatives though our blind docking studies.

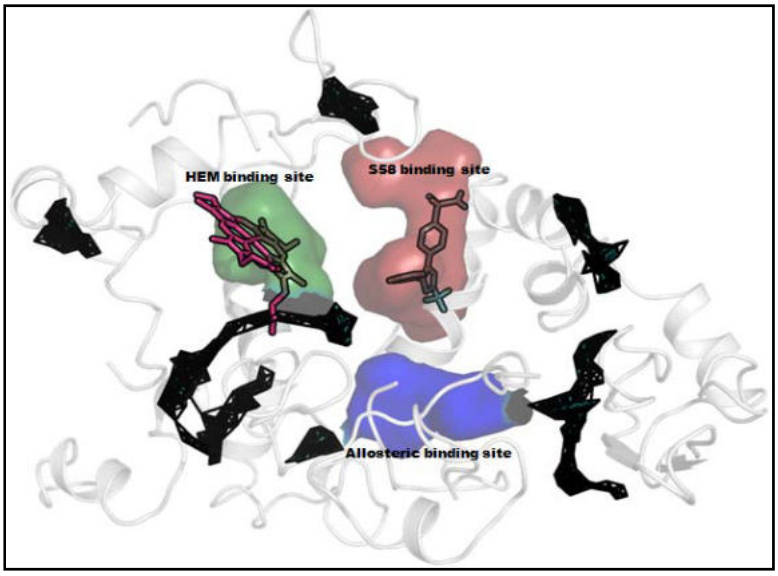


Figure 3: show the all the pocket predicted by pocket-finder, where the light green pocket is the hemoglobin (HEM) binding site, red color pocket is the S58 binding site and blue color pocket is the Allosteric binding site, while the black color are other pocket sites.

Docking studies performed targeting the COX-2 validated allosteric binding site with BOA, 6-BrBOA, 6-ClBOA, HBOA and 6-MBOA are shown in Figure 4a. By comparing the binding mode of the docked BOA derivatives, it's clear that 6-MBOA, 6-BrBOA, BOA, HBOA and 6-ClBOA were well docked within COX-2 allosteric binding site with a gold score of 43.13, 41.98 and 40.89, 43.80 and 41.59 respectively. Where in all the BOA derivative's Oxazolidone O forms H-bond with guanidinium NH of Arg469, while the keto group O of oxazolidone forms H-bond with guanidinium NH of Arg44, while the benzene part of BOA form hydrophobic interaction with Phe529 benzyl group and with the side chain of Asp125 as shown in Figure 4b-f. Additionally, in the case of 6-MBOA, 6-BrBOA and 6-ClBOA their methoxy methyl, Bromo and Chloro group forms hydrophobic interaction with Phe529 respectively (Figure 4b-d), whereas this sort of interaction was lost with BOA and HBOA interaction. Thereby, we could clearly say that 6-MBOA, 6-BrBOA and 6-ClBOA have a stronger affinity to COX-2 than BOA and HBOA. Nevertheless, both BOA and its derivatives could be a potential candidate that could inhibit COX-2 by binding to the allosteric site.

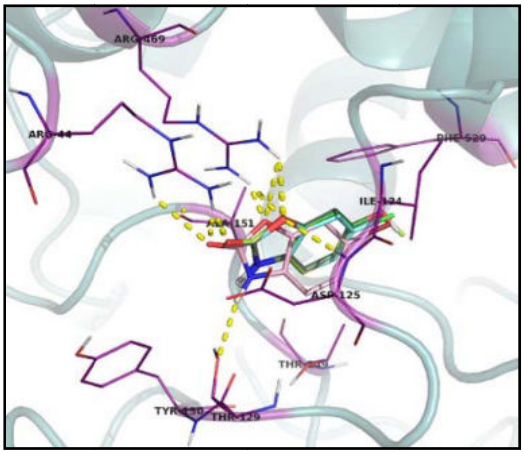
Docking analysis with 5-LOX enzyme

Gold docking study with BOA, 6-BrBOA, 6-ClBOA, HBOA and 6-MBOA on 5-LOX considering the binding site of 5-LOX shows that BOA and its derivatives are good class of 5-LOX inhibitor. Out of the 5 docked compounds, BOA, 6-BrBOA and 6-ClBOA binds well within 5-LOX binding site with a goldscore of 38.90, 42.67 and 41.79 respectively. Docking studies with well-known dual COX-2 and 5-LOX inhibitor, Darbufelone and Licofelone, with gold score of 57.72 and 62.76 respectively higher, when compared to the BOA and its derivatives and moreover, the binding mode was different among Darbufelone, Licoflone and BOA series as shown in Figure 5.

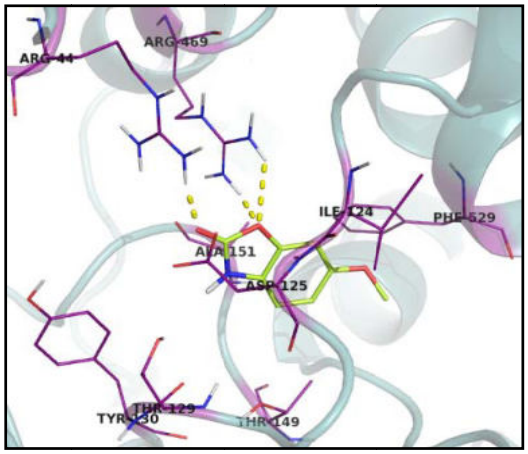
The binding mode of BOA series was very similar to one another binding deep in the 5-LOX active site, meaning that this class of compound would be a possible 5-LOX inhibitor, shown in Figure 6a**.** While, docking studies with HBOA and 6-MBOA produced poor clustering and unstable binding mode.

The binding mode of BOA is shown in Figure 6b, where BOA is in gray color, with the Arg370 guanidinium NH forms H-bond with Oxazolidone O of BOA, additionally the benzoxazolinone forms hydrophobic interaction with side chain of Phe450, Trp478 Leu448, Gl549, Val541 and Phe554. While, in the case of BrBOA colored in pink as in Fig 6c, shows that Arg370 of guanidinium NH forms H-bond with Oxazolidone O of BrBOA, Moreover the benzoxazolineone and Bromine forms hydrophobic interaction with side chain of Phe450, Trp478 Leu448, Gl549, Val541 and Phe554. In Figure 6d, ClBOA in orange colored stick with its Oxazolidone O forms H-bond with guanidinium NH of Arg370.

Thus, molecular docking simulations to screen *A.ilicifolius* for phytochemical was successful in screening BOA and its derivatives as promising drug candidates for targeting COX-2 and 5-LOX as dual target inhibitors.



(4a)



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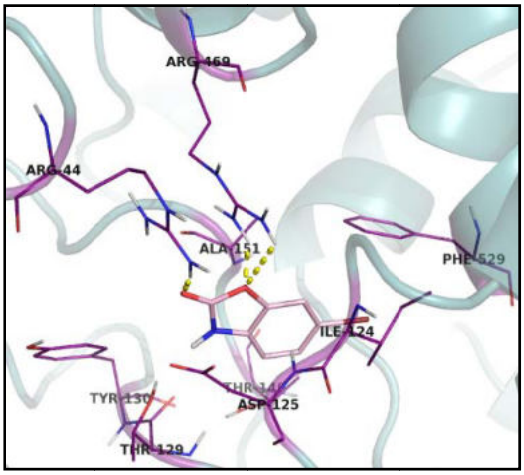

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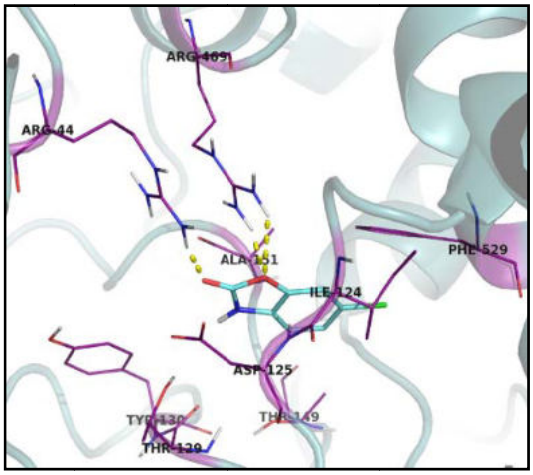

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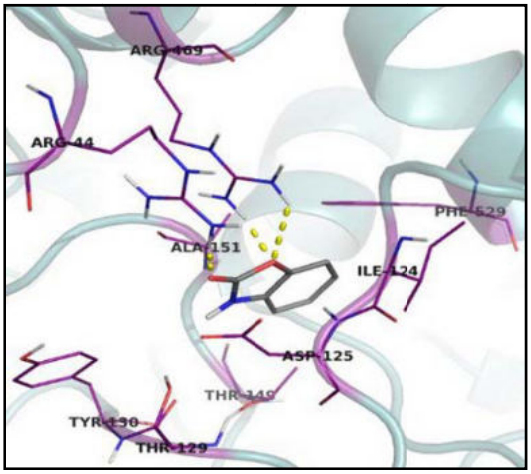

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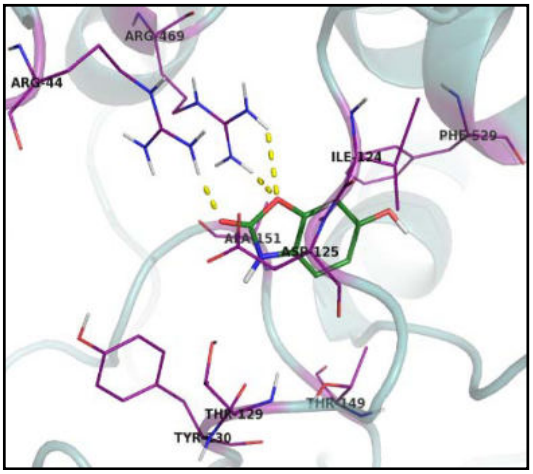


(4f)

Figure 4: (4a) show the binding modes of BOA and its derivatives in the allosteric binding site pocket predicted by pocket-finder, where the light green pocket is the HEM binding site , red color pocket is the S58 binding site and blue color pocket is the allosteric binding site, while the black color are other pockets. (4b) show the binding modes of 6-MBOA (4c) show the binding modes of 6-BrBOA (4d) show the binding modes of 6-ClBOA (4e) show the binding modes of BOA (4f) show the binding modes of HBOA.

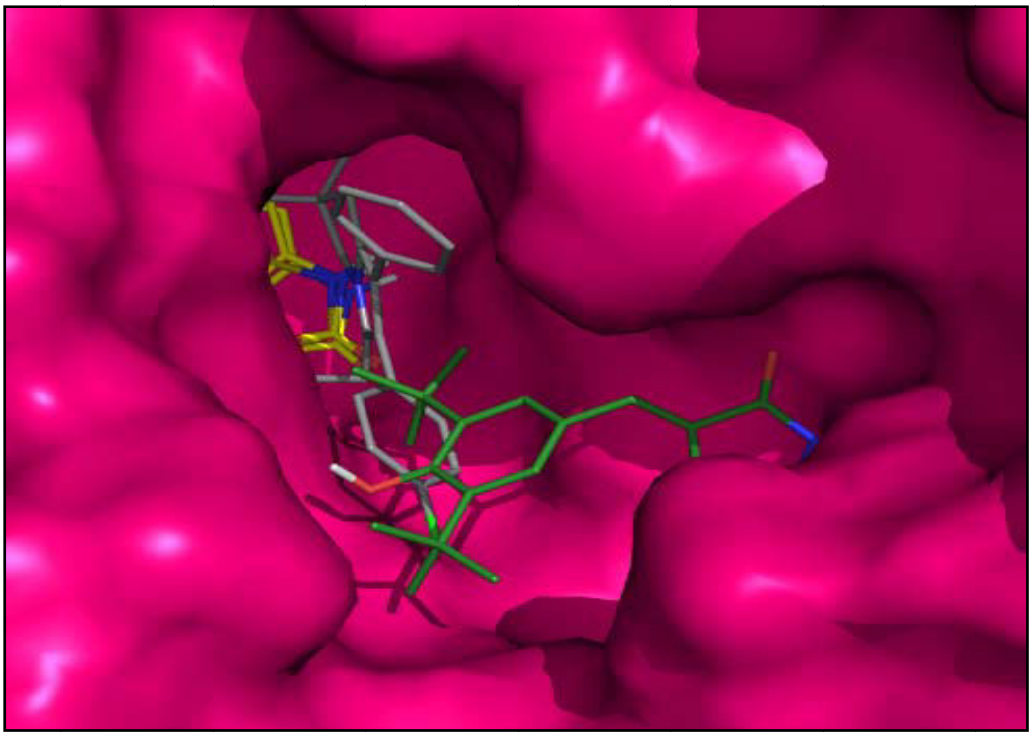
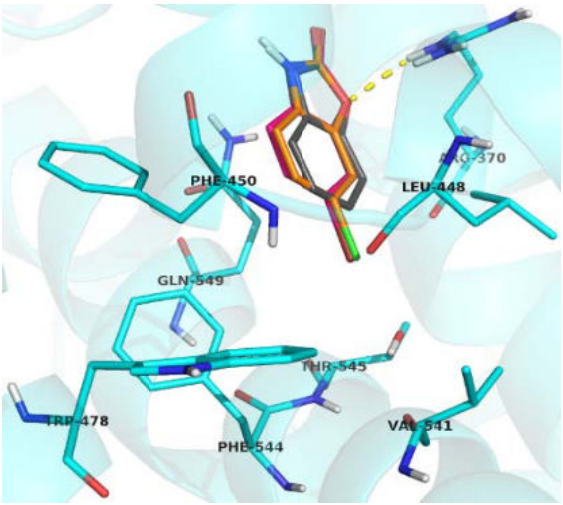


Figure 5: The 5-LOX active site is shown as pink color surface, where the Darbufelone in green color stick binds at the solvent exposed surface, while Licofelone in gray color stick at binds at the centre of pocket, while the BOA series in yellow color stick binds deep in the pocket.

6a



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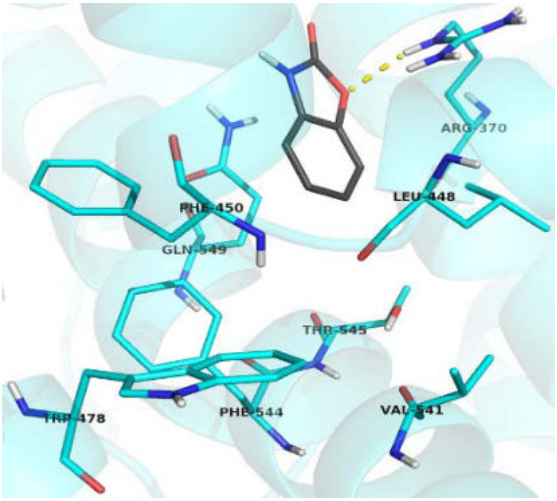

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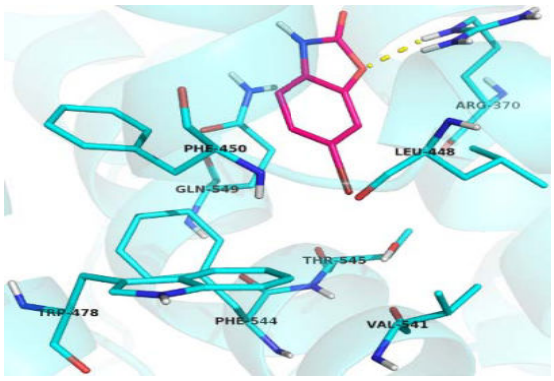

6d

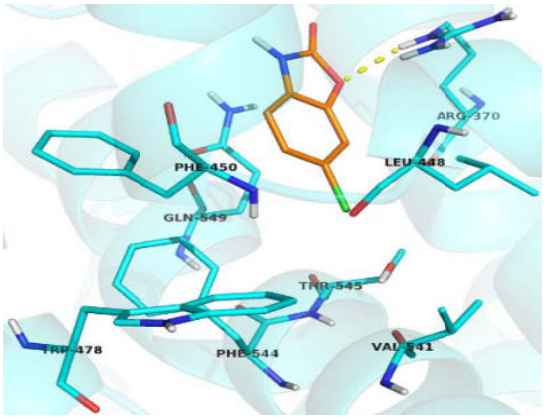

Figure 6: (6a) show the binding mode of BOA in gray color, 6-BrBOA in pink color, 6-ClBOA in orange color at the active site of 5-LOX. (6b) show the binding mode of BOA in gray color stick bound to 5-LOX binding site. (6c) show the binding mode of 6-BrBOA in pink color stick with 5-LOX interacting residues. (6d) Show the binding mode of 6-ClBOA in orange color stick at the 5-LOX binding site interacting residues.

Conclusion

Our computational results indicate that *A.ilicifolius* derived BOA and few of its derivatives possess dual inhibitory effect on COX-2/5-LOX enzyme activity. The results obtained from computational studies may trigger the interest of medicinal chemist and cell biologist for identifying novel structural analogs of BOA in future as dual COX-2/5-LOX inhibitors. The estimation of BOA using UHPLC could serve as a quality control tool to quantify BOA in standardized *A.ilicifolius* extracts.

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Conflict of Interest

The authors confirm they have has no conflict of interests.

Author's Contribution

SAR participated in plant collection, supercritical fluid extraction, phytochemical screening and UHPLC analysis. MS performed *in silico* work and analysis of *in silico* data. KKS contributed to experiment design and involved in manuscript drafting. RT contributed in UHPLC method development. TLK and SMZ provided the software analysis tools for *in silico* studies and interpretation of data. All the authors read and corrected the manuscript.

References

[1]. Athira AP, Helen A, Saja K, Reddanna P and Sudhakaran PR. Inhibition of Angiogenesis *in vitro* by Chebulagic Acid: A COX-LOX Dual Inhibitor. Int J Vasc Med 2013; Article ID 843897, 8 pages, 2013.

[2]. Fiorucci S, Meli R, Bucci M and Cirino G. Dual inhibitors of cyclooxygenase and 5- lipoxygenase. A new avenue in anti-inflammatory therapy? Biochem Pharmacol 2001; 62(11):1433-1438.

[3]. Julemont F, Dogne JM, Pirotte B and de Leval X. Recent development in the field of dual COX / 5-LOX inhibitors. Mini Rev Med Chem 2004; 4(6):633-638.

[4]. Dogne JM, Hanson J, Supuran C and Pratico D. Coxibs and cardiovascular side-effects: from light to shadow. Curr Pharm Des 2006; 12(8):971-975.

[5]. Kaur J, Bhardwaj A, Huang Z and Knaus EE. Aspirin analogues as dual cyclooxygenase- 2/5-lipoxygenase inhibitors: synthesis, nitric oxide release, molecular modeling, and biological evaluation as anti-inflammatory agents. Chem Med Chem 2012; 7(1):144-150.

[6]. Ram RN and Soni VK. Synthesis of 3-alkylbenzoxazolones from N-alkyl-N-arylhydroxylamines by contiguous O-trichloroacetylation, trichloroacetoxy ortho-shift, and cyclization sequence. J Org Chem 2013; 78(23):11935-11947.

[7]. Gokhan-Kelekci N, Koksal M, Unuvar S, Aktay G and Erdogan H. Synthesis and characterization of some new 2(3H)-benzoxazolones with analgesic and antiinflammatory activities. J

Enzyme Inhib Med Chem 2009; 24(1):29-37.

[8]. Wöstmann R and Liebezeit G. Chemical composition of the mangrove holly *Acanthus ilicifolius* (Acanthaceae) — review and additional data. Senckenbergiana maritima 2008; 38(1):31-37.

[9]. Liu L, Fan H, Qi P, Mei Y, Zhou L, Cai L, Lin X and Lin J. Synthesis and hepatoprotective properties of alkaloid A and its derivatives. Exp Ther Med 2013; 6(3):796-802.

[10]. Ravikumar S, Raja M and Gnanadesigan M. Antibacterial potential of benzoate and phenylethanoid derivatives isolated from *Acanthus ilicifolius* L. leaf extracts. Nat Prod Res 2012; 26(23):2270-2273.

[11]. Fornari T, Vicente G, Vazquez E, Garcia-Risco MR and Reglero G. Isolation of essential oil from different plants and herbs by supercritical fluid extraction. J Chromatogr A 2012; 1250:34-48.

[12]. Chiapusio G, Pellissier F and Gallet C. Uptake and translocation of phytochemical 2- benzoxazolinone (BOA) in radish seeds and seedlings. J Exp Bot 2004; 55(402):1587- 1592.

[13]. Estella-Hermoso de Mendoza A, Campanero MA, Mollinedo F and Blanco-Prieto MJ. Comparative study of A HPLC-MS assay versus an UHPLC-MS/MS for anti-tumoral alkyl lysophospholipid edelfosine determination in both biological samples and in lipid nanoparticulate systems. J Chromatogr B Analyt Technol Biomed Life Sci 2009; 877(31):4035-4041.

[14]. Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC and Stallings WC. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature 1996; 384(6610):644-648.

[15]. Gilbert NC, Bartlett SG, Waight MT, Neau DB, Boeglin WE, Brash AR and Newcomer ME. The structure of human 5-lipoxygenase. Science 2011; 331(6014):217-219.

[16]. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC and Ferrin TE.UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004; 25(13):1605-1612.

[17]. Bolton EE, Wang Y, Thiessen PA and Bryant SH. Chapter 12 PubChem: Integrated Platform of Small Molecules and Biological Activities. In: Ralph AW, David CS, editors. Annual Reports in Computational Chemistry. Volume 4: Elsevier; 2008. p. 217-241.

[18]. Verdonk ML, Cole JC, Hartshorn MJ, Murray CW and Taylor RD. Improved protein– ligand docking using GOLD. Proteins: Structure, Function, and Bioinformatics 2003; 52(4):609-623.

[19]. An J, Totrov M and Abagyan R. Pocketome *via* comprehensive identification and classification of ligand binding envelopes. Mol Cell Proteomics 2005; 4(6):752-761.

[20]. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, and Olson, AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry 1998; 19(14):1639-1662.

[21]. Bravo HR, Copaja SV and San Martin J. Contents of 1, 4-benzoxazin-3-ones and 2- benzoxazolinone from *Stenandrium dulce* (Nees). Z Naturforsch C. 2004; 59(3-4):177-180.

[22]. Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V and Jagannath P. Discovery of potential cyclooxygenase inhibitors using *in silico* docking studies. 2012; 7(1): 21-27.

[23]. Madeswaran A, Umamaheswari M, Asokkumar K, Sivashanmugam T, Subhadradevi V and Jagannath P. Docking studies: *In silico* lipoxygenase inhibitory activity of some commercially available flavonoids. 2011; 6(2): 133-138.