Journal of Pharmaceutics and Drug Research
JPDR, 1(1): 16-22
www.scitcentral.com

Original Research Article: Open Access

In Silico Docking Studies of Alkannin and Shikonin with Cyclooxygenase-2 (COX-2)

Noboru Motohashi[1], Robert Gallagher[2], Vanam Anuradha[3] and Rao Gollapudi[2*]

[1]*Meiji Pharmaceutical University, Tokyo, Japan*

[2]*University of Kansas, Lawrence, KS 66045, USA*

[3]*Sri Venkateswara University, Tirupathi, AP, India.*

Received July 27, 2017; Accepted September 10, 2017; Published September 28, 2018

ABSTRACT

Cyclooxygenase (COX) enzymes play an important function in the biotransformation of arachidonic acid (1) into prostaglandins. They are the leading mediators of inflammation, pain and increased body temperature. Cyclooxygenase-2 (COX-2) is responsible for the inflammatory action in cancer, arthritis, thrombosis and arteriosclerosis. In cancerous tissues, an inflammatory response can encourage angiogenesis and subsequent metastasis. Hence, chronic inflammation can be reduced by COX-2 inhibition. In traditional Chinese medicine (TCM), herbs containing alkannin (2) and shikonin (3) are employed to treat ulcers, measles, smallpox, wounds, sores and skin eruptions. Furthermore, these two compounds (2,3) have been shown to reduce inflammation. Hence, the present molecular docking study suggests that the observed anti-inflammatory activity of these compounds (2,3) is due to their binding affinity to the COX-2 active site, which is comparable to the anti-inflammatory drug, celecoxib (4). Further detailed experimental investigations on alkannin (2) and shikonin (3) are necessary to support the observations of this docking study.

Keywords: Cyclooxygenase-1, Cyclooxygenase-2, arachidonic acid, Alkannin, Shikonin, Celecoxib, Lipinski's rule of five, Docking studies

Abbreviations: Ala: Alanine; Arg: Arginine; Asn: Asparagine; COX-1: Cyclooxiganese-1; COX-2: Cyclooxygenase-2; Cys: Cystine; DNA: Deoxyribose Nucelic Acid; EGF: Epithelial Growth Factor; FGF: Fibroblast Growth Factor; Glu: Glutamic Acid; Gln: Glutamine; Gly: Glycine; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; Pro: Proline; ROS: Reactive Oxygen Species; RO5: Lipinski's Rule of Five; Ser: Serine; TCM: Traditional Chinese Medicine; Tyr: Tyrosine; Val: Valine

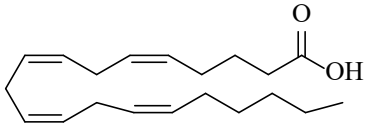
INTRODUCTION

Cyclooxygenase (COX) enzymes primarily functions to catalyze the conversion of arachidonic acid (1) to the intermediate prostaglandin H2 (PGH2), which plays an important role in the biosynthesis of prostanoids that are responsible for robust biological reactions [1]. Cyclooxygenase (COX) exists in two isomeric forms, namely cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) [2]. The majority of non-steroidal anti-inflammatory drugs (NSAIDs) target these isoforms, which suggests that COX enzymes induce pain, fever, inflammation, tumorogenesis, angiogenesis and metastasis [3-5]. Both COX-1 and COX-2 are linked with a wide-range of diseases, which includes chronic inflammation, cancer, arthritis, thrombosis and arteriosclerosis [6]. Furthermore, severe inflammation is associated with colon, breast, hematological malignancies and prostate cancers [7-9]. Tumor progression is linked to the secretion of epidermal (EGF) and fibroblast (FGF) growth factors [10]. Inflammation triggers cancer progression by inducing

Corresponding author: Rao Gollapudi, University of Kansas, Lawrence, KS-66045, USA; E-mail: gollapudirao@ku.edu

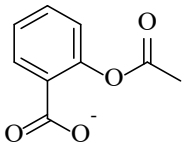
Citation: Motohashi N, Gallagher R, Anuradha V & Gollapudi R. (2018) Uptake of Hydroxy Derivatives of Benzoic Acid and Cinnamic Acid by Caco-2 cells via Monocarboxylic Acid Transporters. J Pharm Drug Res, 1(1): 16-22.

Inflammation triggers cancer progression by inducing moderate reactive oxygen species (ROS) levels resulting in DNA damage and subsequent genetic mutations [11]. COX-1 is responsible for the synthesis of prostaglandin (PG) and thromboxane in numerous tissues, while COX-2 plays a major role in PG biosynthesis in inflammatory cells and within the central nervous system (CNS). Prostaglandin biosynthesis is an important factor in the inflammatory response and in the development of hyperalgesia.COX-2 inhibitors selectively display analgesic and anti-inflammatory activities by blocking the arachidonic acid (1) to prostaglandin H_2 transformation [12].



Arichidonic acid **1**

The cyclooxygenase (COX) active site is composed of a long hydrophobic channel, where NSAIDs intercalate with the enzyme to induce a therapeutic response. The arachidonate-binding site (Arg-120 to Tyr-385) is located at the end of a narrow channel withinCOX-2, while the primary site for acetylsalicylate (aspirin, acetylsalicylic acid) (5) binding is found on a single residue (Ser-530) in the middle of the channel.

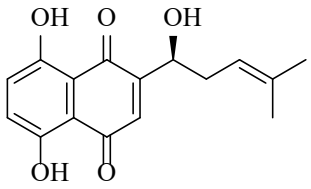


Acetylsalicylate **5**

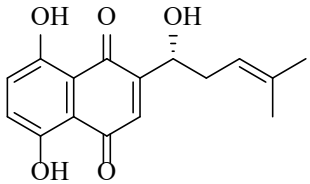
Acetylation of the Ser-530 hydroxyl-group by acetylsalicylate (5) blocks the channel, which prevents arachidonic acid (1) reaching the active site, thereby inactivating COX functionality and reducing inflammation. Compared to COX-1, COX-2 contains a larger and more accessible channel due to three residue substitutions (I434V, H513R, and I523V). Rather than the relatively bulky Ile-523 in COX-1, the presence of Val-523 generates access to an additional side pocket that allows for COX-2 drug selectivity. Similarly, the presence of Val-434 (Ile-434 in COX-1) allows the adjoining Phe-518 residue to swing out of the way to reveal further access to the side cavity of COX-2. Specifically, the Arg-513 (His-513 in COX-1) residue within the side pocket of COX-2 is open to interact with polar moieties. Therefore the tertiary structural characteristics of cyclooxygenases encourage the development of target oriented drug candidates with selective inhibition activity [13,14].

Several last resort synthetic drugs are generally known to produce numerous negative side effects, making them non-applicable as terminal disease treatments. Since natural products are naturally present in living cells, it has been suggested that secondary metabolites derived from natural sources may avoid the side effects associated with these synthetic drugs. Lead candidate drugs derived from natural sources are suitable for optimization through semisynthetic methods to achieve greater activity with reduced side effects. The ability of natural products to interact with other biological molecules is a prerequisite in the discovery of drugs with increased efficacy [5].

Alkannin (2) and shikonin (3) are the major enantiomeric constituents of the *Alkannatinctoria* and *Lithospermumerythrorhizon* (Family: Boraginaceae) plant species. Traditionally, these natural products are utilized as a natural dye, a food additive, in cosmetic preparations, and used to treat ulcers, measles, smallpox, wounds, inflammation, sores as well as skin eruptions in traditional Chinese medicine (TCM) [15,16]. Furthermore, the absolute configuration difference between alkannin (2) and shikonin (3) has relatively no effect on their anti-inflammatory activities [17].

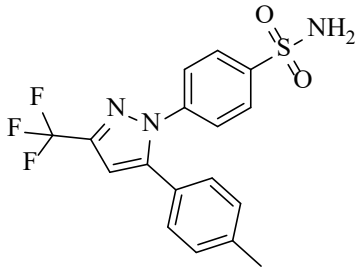


Alkannin **2**



Shikonin **3**

Rational drug design employs a variety of computational methods to explore novel compounds for biological activity potential. A molecular docking study allows the prediction and analysis of the interactions between a protein receptor and the associated ligand [18-24]. In the present study, the enantiomers (2 and 3) are compared against celecoxib (4) through molecular docking simulations to evaluate their interactions and binding efficacy with the COX-2 enzyme [15].



Celecoxib **4**

EXPERIMENTAL

Lipinski's rule of five

During the initial stages of drug discovery, it is crucial to assess the drug-likeness of each potential candidate to

minimize cost by removing hits presenting false positive results. An ideal drug candidate should not violate more than one of the criteria, as defined by the "Lipinski's rule of five" (RO5). These parameters include molecular weight (no greater than 500 Da or g/mol), octanol-water partition coefficient (Log_p of 5 or less), as well as hydrogen bond donors (5 or less) and acceptors (10 or less). In the current study, these characteristics were determined with the Drug Likeness Tool (DruLiTo), which revealed that 2 and 3 presented lower Log_p values compared to 4 **(Table 1)**. This suggests that 2 and 3 should exhibit better bioavailability compared to 4 [25].

Table 1. Examination of the "Lipinski's rule of five" parameters in alkannin (2), shikonin (3) and celecoxib (4).

	"Lipinski's Rule of Five" Parameters (Ideal Drug Value)			
Ligand	Mol. Wt (≤ 500)	Log_p (≤ 5)	H-Donors (≤ 5)	H-Acceptors (≤ 10)
Alkannin (2)	288.1	1.154	3	5
Shikonin (3)	288.1	1.08	3	5
Celecoxib (4)	381.08	2.266	1	5

Docking simulations between ligands (2-4) and the target (COX-2) were conducted in Auto Dock Vina (Molecular Graphics Lab, La Jolla, CA, USA) [26,27]. The Auto Dock Vina software sets the target in a rigid conformation, while the ligands were allowed to be flexible and adaptable towards the target. The software determines the lowest binding affinity by utilizing different confirmations of each ligand. The resulting lowest binding energy docking poses of each ligand are selected for further evaluation.

Preparation of the target

The three-dimensional (3D) crystal structure of human COX-2 enzyme in complex with celecoxib (4) (PDBID: 3LN1), retrieved from Protein Data Bank (PDB) (http://www.rcsb.org), was selected as the protein target model for this virtual screening study. Water molecules, ligands and the B-D chains were removed from the PBD file using Discovery Studio4.5 (Dassault Systemes BIOVIA, Discovery Studio Modelling Environment, Release 2017, San Diego, USA). Hydrogen atoms were added to the protein model using the virtual screening tool, PyRxv0.8 (http://pyrx.sourceforge.net/). Energy minimization of the protein chain was implemented using Chimera (UCSF, San Francisco, CA, USA). The docking grid was prepared using PyRx software interlinked with Auto Dock Vina [15]. Docking simulations were performed by defining the grid (Box size: 59.37 × 78.58 × 64.62 Å and box center: 34.9329×-28.9785×-9.5134 for x, y and z, respectively) and exhaustiveness (8.2.1) values with PyMol v1.3 (Schrodinger, New York, NY, USA). Chimera software was used for visual inspection and graphical representations of the docking results.

Preparation of the ligands

The structures of 2 (alkannin, CID: 72521) and 3 (shikonin, CID:5208) were initially retrieved from the PubChem Compound Database (National Center for Biotechnology Information, U.S. National Library of Medicine). Molecular geometry optimization of the ligands was optimization of the ligands was performed using Avogadro (an open-source molecular builder and visualization tool, Version 1.90.0, http://avogadro.cc/) [28].The protein and ligand structures were optimized, saved in Protein Data Bank (PDB) file format, and used for the docking study.

Protein and ligand docking

Docking of COX-2 with 2 and 3 were accomplished with the help of PyRx virtual screening software interlinked with Auto Dock Vina. A population of possible conformations was generated by docking different orientations of each ligand within the COX-2 binding site. Throughout the docking process, the protein was kept rigid, while the ligands were left flexible. After docking completion, ligand conformations displaying greatest binding affinity and lowest docked energies were chosen. The hydrogen bonds, bond lengths and hydrophobic interactions between protein (COX-2) and ligand (2 and 3) were determined by using Lig Plot (http://www.ebi.ac.uk/thornton-srv/software/LIGPLOT/) and PyMol programs.

RESULTS AND DISCUSSION

In previous studies, shikonin (3) displayed *in vitro* and *in vivo* antitumor and anti-metastatic activities [29-31]. In addition, 3 demonstrated anti-inflammatory activity by inhibiting leukotriene biosynthesis [32]. The anti-inflammatory activity exhibited by 3led us to further explore the mechanism of COX-2 inhibition, using docking studies. The interactions between ligand (2 and 3) and target (COX-2) were determined from molecular docking studies using PyRx software interlinked with Auto Dock Vina. These docking results were used to determine the best binding modes between each ligand to target protein, and to evaluate intermolecular interactions present in each complex (COX-2-2 and COX-2-3). As a result, both 2 and 3 satisfied the "Lipinski's rule of five" criteria (as evaluated by the DruLiTo tool) to qualify as suitable drug candidates. Compared to the control (celecoxib, 4, -8.2 kcal/mol), both enantiomers (2, -9.0 kcal/mol; 3, -8.7 kcal/mol) exhibited greater binding affinity to the target. The docking positions for each complex (COX-2-2and COX-2-3) were created in

Auto Dock Vina, transferred to Pymol, and subsequently ranked according to the docking scores of each ligand (2 and 3). Graphical representations of the binding poses of each docked ligand [alkannin (2) shikonin (3) and celebex(4)] to COX-2 were generated **(Figure 1)**.

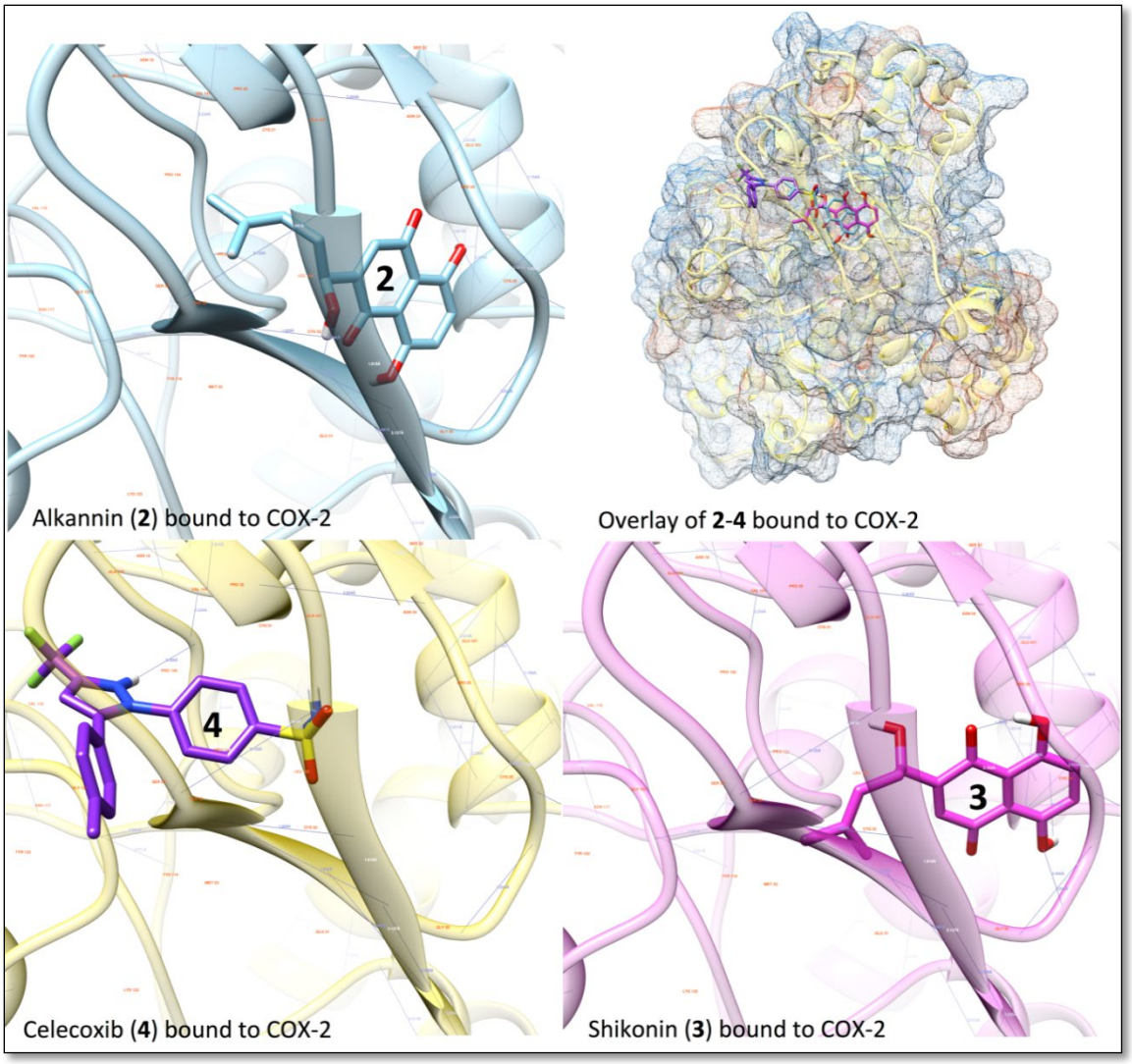


Figure 1. Docking poses of alkannin (2), shikonin (3) and celecoxib (4) to COX-2 enzyme.

Examination of the best docking conformations (lowest binding energy) in each complex revealed that alkannin (2) and shikonin (3) predominately bound to the hydrophobic pocket of COX-2. The stabilization of complexes (COX-2-2, COX-2-3) was evaluated by examining the non-covalent interactions between each ligand (2 and 3) and the target (COX-2). The LigPlot algorithm generates a postscript file containing a schematic 2-D representation from 3-D co-ordinates of protein-ligand complexes. This postscript file gives a simple and informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions and atom accessibilities. The LigPlot examination of the ligand-target complexes (COX-2-2, COX-2-3, COX-2-4) revealed that alkannin (2) produced four hydrogen bonds [Cys-21 (2.94Å) Asn-24 (2.83Å), Tyr-116 (2.83Å) and Gly-121 (2.99Å)] and ten hydrophobic interactions [Cys-26, Arg-29, Gly-30, Glu-31, Cys-32, Leu-138, Pro-139, Gln-451, Lys-454, and Arg-455]; shikonin (3) produced three hydrogen bonds [Cys-21 (2.97Å), Asn-24 (2.78Å) and Tyr-116 (2.93Å)] and eight hydrophobic interactions [Cys-26, Gly-30, Glu-31, Cys-32, Leu-138, Gln-451, Lys-454 and Arg-455]; while celecoxib (4) formed only two hydrogen bonds [Cys-32 (3.17Å) and Ser-34 (3.05Å)] and seven hydrophobic interactions [Asn-19, Cys-21, Met-33, Gly-121, Tyr-122, Pro-140 and Ala-142] with COX-2 **(Figure 2)**.

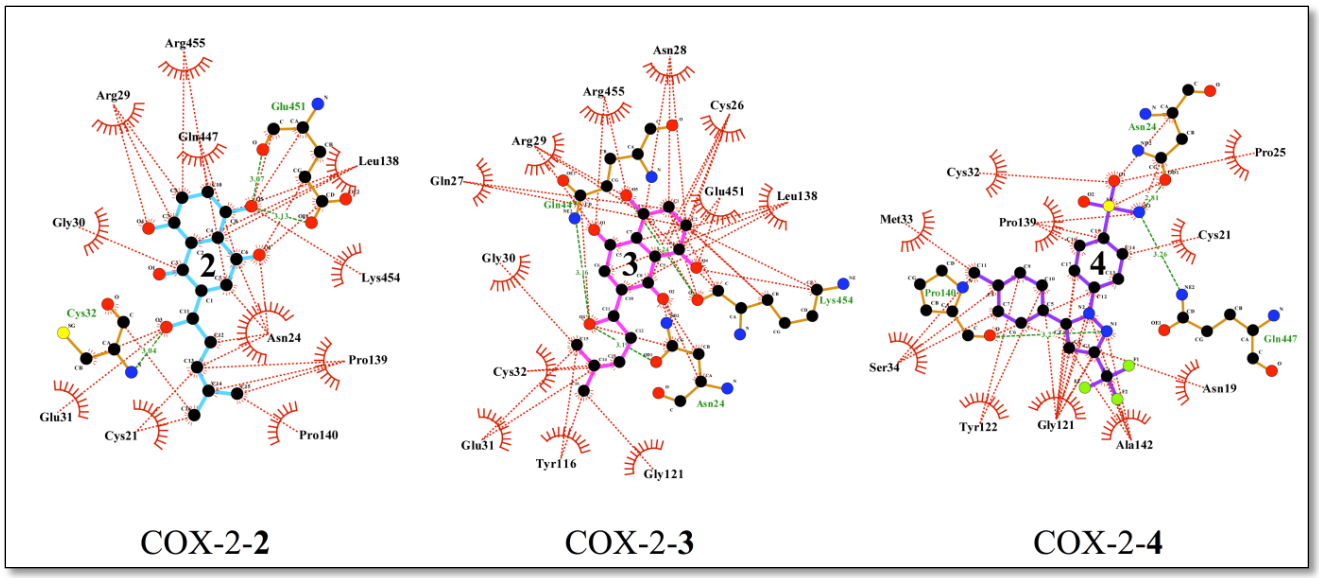


Figure 2. Interactions between ligand [alkannin (2), shikonin (3) and celecoxib (4)] and target (COX-2) in complex (COX-2-2, COX-2-3, and COX-2-4) as displayed in Lig plot.

The lowest binding energies of alkannin (2), shikonin (3) and celecoxib (4) conformers to COX-2 were arithmetically estimated as -9.0, -8.7 and -8.2 kcal/mol, respectively. The results disclosed that 2 and 3 docked with higher binding affinity to the COX-2 active cavity, when compared to the control drug celecoxib (4). The stereochemical difference between these enantiomers (2 and 3) has little impact on anti-inflammatory activity [17]. However, the current docking study on these enantiomers revealed that the orientation of the C-11-hydroxyl group produced a noticeable change in the binding affinity towards the target. Nevertheless, further evidence is still required *via* biological testing to support the hypothesis proposed based on these findings.

CONCLUSION

The wound healing properties of plant extracts containing naphthoquinone natural products - alkannin (2) and shikonin (3) - have been utilized for many centuries. Research has shown that alkannin (2) produces antioxidant, and antimicrobial activities; while shikonin (3) contains antioxidant, antimicrobial, anti-inflammatory, anticancer, wound healing and antiulcer activities. The diverse beneficial properties of these enantiomers (2 and 3) formed a sound scientific basis for the historical use of *zicao* (dried *Lithospermumerythrorhizon* root in TCM) in the treatment of inflammatory and infectious diseases. At present, there is a large demand for the discovery of novel anti-inflammatory compounds with profound biological activities with reduced side effects. However, drug discovery resulting in robust and feasible lead candidates with antitumor and antithrombotic properties coupled with lesser side-effects still remains a challenging scientific mission. Natural products are a renowned source of structural diverse therapeutic agents. Previously, molecular docking studies were applied to identify the biologically active constituents in herbal products [18-24]. The present investigation suggests that alkannin (2) and shikonin (3) have strong COX-2 inhibition activity, and may even act directly on the COX-2 enzyme. Interestingly, alkannin (2) displayed a better binding affinity to COX-2, compared to either shikonin (3) or the control drug celecoxib (4). This strongly supports further biological studies on the COX-2 inhibition activity of alkannin (2), in order to determine the potential of alkannin (2) as a novel COX-2 selective non-steroidal anti-inflammatory drug candidate.

REFERENCES

1. Rouzer CA, Marnett LJ (2009) Cyclooxygenases: structural and functional insights. J Lipid Res 50: s29-s34.
2. de Leval X, Delarge J, Soners F, de Tullio P, Henrotin Y, et al. (2000) Recent advances in inducible cyclooxygenase (COX-2) inhibition. Curr Med Chem 7: 1041-1062.
3. Costa C, Soares R, Reis-Filho S, Leitao D, Amendoeira I, et al. (2002) Cyclo-oxygenase 2 expression is associated with angiogenesis and lymph node metastasis in human breast cancer. J Clin Pathol 55: 429-424.
4. Leahy JM, Koki AT, Masferrer JL (2000) Role of cyclooxygenases in angiogenesis. Curr Med Chem 7: 1163-1170.

5. Dannhardt G, Kiefer W (2001) Cyclooxygenase inhibitors - Current status and future prospects. Eur J Med Chem 36: 109-126.

6. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, et al. (1998) Cyclooxygenase in biology and disease. FASEB 12: 1063-1073.

7. Harris RE (2009) Cyclooxigenase-2 (COX-2) blockade in the chemoprevention of cancer of the colon, breast, prostate and lung. Inflammopharmacology 17: 55-67.

8. Hussain SP, Harris CC (2007) Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 121: 2372-2380.

9. Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M (2010) The role of cyclogenase-2 in cell proliferation and cell death in human malignancies. Int J Cell Biol 2115158.

10. Motoo Y, Sawabu N, Nakanuma Y (1991) Expression of epidermal growth factor and fibroblast growth factor in human hepatocellular carcinoma: An immunohistochemical study. Liver Int 11: 272-277.

11. Salman KA, Ashraf S (2013) Reactive Oxygen Species: A link between chronic inflammation and cancer. As Pac J Mol Biol Biotechnol 21: 42-49.

12. Riccioti E, FitzGerald GA (2011) Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 31: 986-1000.

13. Marnett LJ, Kalgutkar AS (1998) Design of selective inhibitors of cyclooxygenase-2 as non-ulcerogenic anti-inflammatory agents. Cur Opin Chem Biol 2: 482-490.

14. Marnett LJ, Rowlinson SW, Goodwin BC, Kalgutkar AS, Lanzo CA (1999) Arachidonic acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. J Biol Chem 274:22903-22906.

15. Papageorgiou VP, Assimopoulou EA, Couladouros EA, Hepworth D, Nicolaou KC (1999) The chemistry and biology of alkannin, shikonin and relate naphtazarin natural products. Angew Chem 38: 270-300.

16. Chen, X, Yang L, Zhang N, Turpin JA, Buckheit RW, et al. (2003) Shikonin, a component of Chinese herbal medicine, chemokine receptor function and supresses human immunodeficiency. Antimicrob Agents Chemother 47: 2810-2816.

17. Tanaka S, Tajima M, Tsukada M, Tabata M (1986) A comparative study on anti-inflammatory activities of the enantiomers, shikonin and alkannin. J Nat Prod 49: 466-469.

18. Barlow DJ, Buriani A, Ehrman T, Bosisio E, Eberini I, et al. (2012) *In silico* studies in Chinese herbal medicines' research: Evaluation of *in silico* methodologies and phytochemical data sources and a review of research to date. Science J Ethnopharmacol 140: 526-534,

19. Sharma V, Sharma PC, Kumar V (2016) *In silico* molecular docking analysis of natural pyridoacridines as anticancer agents. Adv Chem.

20. Muhammad SA, Fatima N (2015) *In silico* analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides. Pharmacogn Mag 11: s123-s126.

21. Lagunin A, Goel RK, Gawande DY, Pahwa P, Gloriozova TA, et al. (2014) Chemo-and bioinformatics resources for *in silico* drug discovery from medicinal plants beyond their traditional use: a critical review. Nat Prod Rep.

22. Shahbazi S, Sahrawat TR, Ray M, Dash S, Kar D, et al. (2016) Drug targets for cardiovascular-safe anti-inflammatory: *In silico* rational drug studies. PLoS One.

23. Lahlou N (2013) The success of natural products in drug discovery. Pharmcol Pharm 4: 17-31.

24. Akhila S, Aleykutty NA, Manju P (2012) Docking studies on *Pepeomia pellucida* as anti-diabetic drug. Int J Pharm Pharm Sci 4: 76-77.

25. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Deliv Rev 46: 3-26.

26. Trott O, Olson AJ (2010) AutoDockvina: Improving the speed and accuracy of docking with a new drug scoring function. J Comput Chem 31: 455-461.

27. Dallakyan S, Oleson A (2015) Small-molecule library screening by docking with PyRx methods. Mol Biol 1263: 243-250.

28. Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, et al. (2012) Avogadro: An advanced semantic chemical editor, visualization and analysis platform. J Cheminform 4: 17.

29. Wada N, Kawano Y, Fujiwara S, Kikukawa Y, Okuno Y,et al. (2015) Shikonin, dually functions as a proteasome inhibitor and a necroptosis inducer in multiple myeloma cells. Int J Oncology 46:963-972.

30. Xuan Y, Hu X (2009) Naturally occurring shikonin analogues - A class of necroptotic inducers that circumvent cancer drug resistance. Cancer Lett 274: 2233-2242.

31. Wang H, Wu C, Wan S, Liu G (2013) Shikonin attenuates lung cancer by inhibiting integrin β1

expression and ERK1/2 signalling pathway. Toxicol 308: 104-112.

32. Wang WJ, Bai JY, Liu DP, Xue LM, Zhu XY (1994)The anti-inflammatory activity of shikonin and its inhibitory effect on leukotriene biosynthesis. Yao XueXueBao 29: 161-165.