Journal of Saudi Chemical Society (2016) **20**, S448–S450

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

Molecular docking of Diospyrin as a LOX inhibitory compound

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Received 24 October 2012; accepted 30 January 2013
Available online 17 March 2013

KEYWORDS

Diospyros lotus;
Diospyrin;
Lipoxygenase;
Inflammation;
Docking

Abstract *Diospyros lotus* is traditionally used in various diseases including inflammation. In the current study an effort has been made to identify a bioactive constituent from *D. lotus* in order to scientifically validate its use in inflammatory disorders. Diospyrin was isolated from *D. lotus* and exhibited significant lipoxygenase (LOX) inhibitory activity (IC_{50} value: 31.89 ± 0.14 μmol). Molecular docking revealed significant molecular interactions between Diospyrin and LOX showing promising potential for further optimization as a potential anti-inflammatory lead compound.

1. Introduction

Genus Diospyros belongs to the family Ebenaceae which, consists of woody shrubs and trees distributed in the tropical and subtropical regions of the world. Around 500 species are known worldwide of which, 24 species are native to India (Uddin et al., 2011a). *Diospyros lotus* tree grows up to 9 m in height and around 6 m in width. This plant is rarely available in Britain and it is native to the Himalayan region. In folk medicine, *Diospyros* species are known for their multiple medicinal uses in many traditional and folk medicinal systems all over the world. All parts of these plants have been used for medicinal purposes, which include its use for treatment of lumbago; pain, fever, inflammation, and microbial infections. *Diospyros* species are carminative, astringent, sedative and ferifuge (Pant and Samant, 2010; Uddin et al., 2011a). Triterpenoids belonging to lupane, oleanane and ursane series have been isolated and showed anti-inflammatory activity (Uddin et al., 2011b). Phytochemical studies have been previously carried out on many Diospyros species and have revealed the widespread presence of naphthoquinones and naphthalene derivatives, dimeric naphthoquinones and lupane triterpenes (Ahmad et al., 2010; Uddin et al., 2011a). In the present study, a successful attempt has been made regarding ethnopharmacological validation, computational and experimental identification of a potential

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Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2013.01.009
1319-6103

anti-inflammatory lead compound from *D*. lotus as a lipoxygenase (LOX) inhibitor. LOX is one of the major therapeutic targets employed for the discovery of new anti-inflammatory agents (Khan et al., 2011).

2. Experimental

2.1. Plant material

Roots of *D*. *lotus* were collected from Toormang Razagram, Dir, KPK, Pakistan, in May 2009. The sample was authenticated by Dr. Abdur Rashid, Taxonomist, Department of Botany, University of Peshawar, Pakistan. A voucher specimen (RF/01) has been deposited at the Herbarium, Department of Botany, University of Peshawar, Pakistan.

2.2. Extraction and isolation

Shade-dried roots of *D*. *lotus* (14 kg) were powdered and then kept at room temperature in MeOH for 6 days with continuous stirring by simple percolation. After this period the extracts were concentrated by evaporating solvents using a rotary vacuum evaporator under reduced pressure at temperature below 55 °C. This process was repeated four times until the extraction was completed and finally 202 g of dark green residue was obtained. The MeOH extract (202 g) was suspended in water and successively partitioned with hexane, $CHCl_3$, EtOAc and BuOH. The chloroform fraction (16 g) was subjected to Column chromatography on silica gel (Merck Silica gel 60(0.063–0.200 mm), 5×60 cm). The column was first eluted with hexane–ethyl acetate (100:0 → 0:100) as solvent system. A total of 105 fractions, RF-1–RF-105 were obtained based on TLC profiles. Elution of the chromatogram with Hexane–Ethyl acetate (100:0 → 10:100) gave a reddish oil of fatty acid residues which is followed by 1.25 g of colorless needles that were identified as lupeol (m.p. 210–212 °C. Eluting the column with hexane–ethyl acetate (100:0 → 20:80) results in the isolation of a violet red fraction and revealed two compounds on TLC. This fraction was purified by preparative chromatography which resulted in the isolation of a violet compound (8-Hydroxyisodiospyrin) and ^{1}H and $^{13}CNMR$ data were found to be identical with those published by Baker et al. (1998). Fraction RF-20 obtained at (100:0 → 15:100) contained red crystals of various sizes, shape and was separated from the solution by washing with *n*-hexane. To obtain pure and larger crystals, these crystals were re-grown from a mixture of hexane–chloroform and thus obtained a polar orange–red crystalline compound Diospyrin (m.p. 252–255 °C) (1.4 g), ^{1}H and $^{13}CNMR$ data were found to be identical to the data reported previously for Diospyrin (Yoshida and Mori, 2000).

3. Molecular docking simulations

FRED 2.1 (McGann et al., 2003; Khan et al., 2009) was used in this study to dock the OMEGA pre-generated multiconformer library mentioned above. FRED 2.1 strategy is to exhaustively dock/score all possible positions of each ligand in the binding site. The exhaustive search is based on rigid rotations and translations of each conformer within the binding site defined by a box. FRED filtered the poses ensemble by rejecting the ones that clash with the protein (LOX) or that does not have enough contacts with the protein. The final poses can then be scored or re-scored using one or more scoring functions. In this study, the smooth shape-based Gaussian scoring function (shapegauss) was selected to evaluate the shape complementarily between each ligand and the binding pocket. Default FRED protocol was used except for the size of the box defining the binding sites. In an attempt to optimize the docking-scoring performance we performed exhaustive docking with shapegauss applying the "Optimization" mode. The "Optimization" mode involves a systematic solid body optimization of the top ranked poses from the exhaustive docking. Three different boxes were explored for LOX (PDB ID: 1JNQ). Three different simulations were carried out with an added value of 8 Å around the reference ligand.

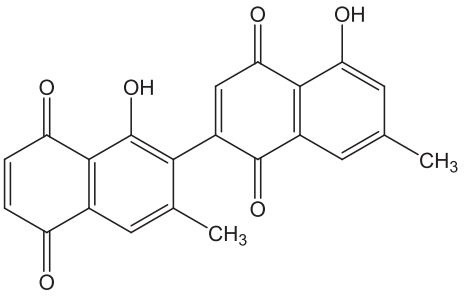

Figure 1 Chemical structure of Diospyrin.

4. In vitro lipoxygenase inhibition assay

Enzyme inhibition assays were performed by using different concentrations of the isolated compound Diospyrin (Nisar et al., 2011). Lipoxygenase inhibitory activity was measured by slightly modifying the spectrometric method as developed by Lipoxygenase (EC 1.13.11.12) type I-B (Soybean) and linoleic acid were purchased from Sigma (St. Louis, MO) and were used without further purification. All other chemicals were of analytical grade and purchased from the same vendor i.e. Sigma (St. Louis, MO). 160 μL of sodium phosphate buffer, 0.1 mM (pH 7.0), 10 mL of the sample solutions (test compound and standards) and 20 μL of lipoxygenase solution were mixed and incubated for 5 min at 258 °C. Reaction was initiated by the addition of 10 μL linoleic acid substrate solution and absorption change with the formation of (9Z,11E)-13S)-13-hydroperoxyo-ctadeca-9,11-dienoate was followed for 10 min. Test sample and control were dissolved in 50% ethanol. All the reactions were performed in triplicate. Baicalein and Tenidap sodium were used as positive controls for lipoxygenase inhibition (Khan et al., 2009). The IC_{50} values were calculated using the EZFit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

5. Results and discussion

5.1. In vitro lipoxygenase inhibition assay

All compounds showed promising inhibitory activity against lipoxygenase. IC_{50} value of Diospyrin (Fig. 1) was 62.7 ± 0.12. Standard compounds Baicalein and Tenidap sodium showed the IC_{50} value being 22.1 ± 0.03 μM and 41.6 ± 0.02 μM respectively.

5.2. Molecular docking simulations

Structural insights based on molecular docking revealed important subsites essentially required to be interacting with LOX inhibitors for new drug discovery of new LOX inhibitors. Among these especially between His523, Lle554 and Leo773 Diospyrin exhibited significant interactions with important subsites inside catalytic pocket of LOX. Molecular shape and electrostatic condition of diospyrin were also identified to be favorably matched with the electrostatic environment of active site inside LOX. Various molecular interactions were observed between the test ligand and LOX. Hydrogen bonding (Fig. 2) was found between His 518 (2.55 Å) and carbonyl oxygen of quinine moiety. This interaction was further reinforced by hydrogen bonding between Asp 766 (2.79 Å) and phenolic group. However molecular shape of the compound, Diospyrin was incapable to access Ile857 and His 709. Apart from hydrogen bonding, electrostatic environment (Fig. 3) of active site in LOX further supported the significant molecular interactions of Diospyrin due to its matching shape and electrostatic behavior. Other interactions like π–π interactions between ligand and LOX inside the active site monomer (not bound via hydrogen bonding) of Diospyrin was deeply penetrated inside slightly less polar cavity surrounded by aggregated positive charges. Rotatable bond between both anthraquinone dimmers provided flexibility to diospyrin for penetrating the active site with small pockets by directing in different directions.

Lipoxygenase (EC 1.13.11.12) constitutes a family of non-heme iron containing enzymes, as versatile biocatalysts are capable of catalyzing many reactions involved in the xenobiotic metabolism. They are responsible for the metabolism of the fatty acids (FAs) and their metabolites eliciting inflammatory responses in the body. They also play a significant role in cancer cell growth, metastasis, invasiveness, cell survival and induction of tumor necrosis factor (TNF) (Arfan et al., 2010). Many COX-2 or 5-LOX inhibitors have been developed as drugs to treat inflammation (Nisar et al., 2011). In this study, diospyrin exhibited a significant inhibition of the lipoxygenase showing its strong potential to be developed as an anti-inflammatory drug. Molecular docking studies revealed that its compact skeleton is the basic reason of how it holds strong contacts with the important amino acid side chains inside the active site as well as

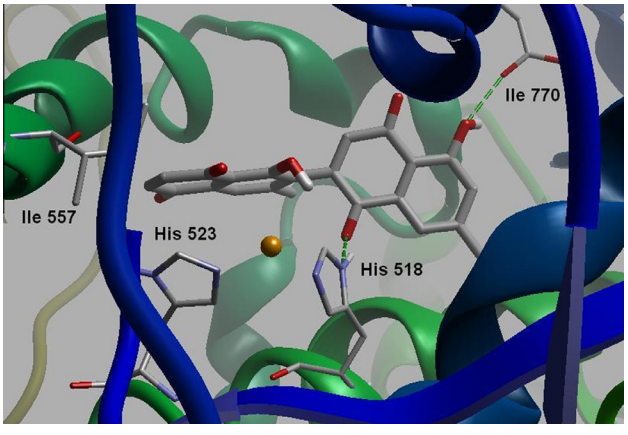

Figure 2 Binding mode of Diospyrin inside the active site of LOX. Catalytic iron atom (orange colored) is shown between the His518 and Ile 770 residues. Hydrogen atoms (except polar ones) were omitted for clarity.

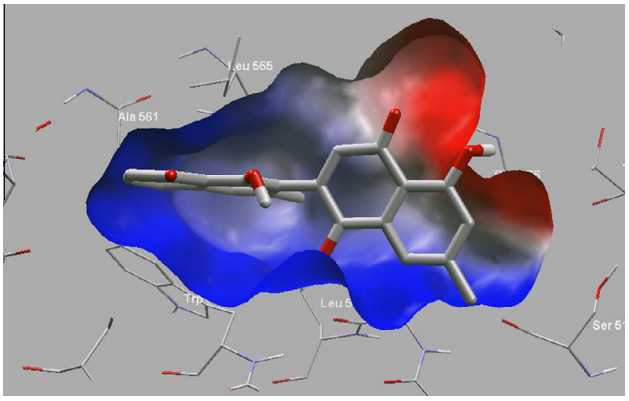

Figure 3 Electrostatic interactions of Diospyrin inside the active site of LOX. Color encoding (White area: hydrophobic region, red area: area with aggregated negative electrostatic potential, and blue area: area with aggregated positive electrostatic potential).

adjoining sites of the enzyme thus preventing its pro-inflammatory role. In the current study Diospyrin exhibited strong molecular interactions which ultimately resulted in discovery of Disoprin made a potential new lead compound targeting LOX in inflammation and related pathological conditions.

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