SHORT COMMUNICATION

Discovery and molecular docking simulation of 7-hydroxy-6-methoxy-2H-chromen-2-one as a LOX Inhibitor

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Abstract: *Millettia ovalifolia* is traditionally used in variety of diseases including inflammation. In our investigation in to the phytochemical constituents of *Millettia ovalifolia* an effort was made to find out bioactive constituent from medicinal Plant *M. ovalifolia* to scientifically validate its use in inflammatory disorders. The compound 7-hydroxy-6-methoxy-2H-chromen-2-one was isolated from the bark of *M. ovalifolia* and was found to exhibited significant lipoxygenase (LOX) inhibitory activity with (IC_{50} value: 116.83±0.02µM). The Standard compounds Baicalein and Tenidap sodium revealed IC_{50} value being 22.1±0.03µM and 41.6±0.02µM. Molecular docking study further displayed significant molecular interactions between 7-hydroxy-6-methoxy-2H-chromen-2-one and LOX showed potential for further optimization as a possible anti-inflammatory lead compound.

Keywords: *Millettia ovalifolia* bark, 7-hydroxy-6-methoxy-2H-chromen-2-one, lipoxygenase, inflammation, docking.

INTRODUCTION

Lipoxygenase (EC 1.13.11.12) constitutes a family of non-heme iron containing enzymes, as versatile biocatalysts and are capable of catalyzing many reactions involved in xenobiotic metabolism and inflammation signaling (Khan *et al.*, 2011). 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) (Nisar *et al*., 2011). Catalytic or active site of LOX is composed of three important amino acid residues the so-called catalytic triad (His523, His518, Ile875) along with iron atom (Khan *et al.*, 2013). Any compound, which is capable to show strong molecular interaction with the catalytic triad, can be developed as new and therapeutically effective LOX inhibitor for better management of inflammatory disorders (Khan *et al.*, 2011). So keeping in view the above background, a study was designed to identify a new LOX inhibitor from *M. ovalifolia*.

MATERIALS AND METHODS

Plant material

The bark of *M. ovalifolia* was collected during the month of June 2008 from Pakistan Forest Institute (PFI), Peshawar. The plant was identified and authenticated by Dr. Samin Jan, Associate Professor, Department of Botany, Islamia College University, Peshawar, Pakistan. The voucher specimen (SJ-33) was deposited in the herbarium of Botany Department, Islamia College University, Peshawar, Pakistan.

Extraction and isolation

The stem bark was shade dried and powdered weighed (70kg) and were soaked in 5% aqueous methanol for one week (x3). The combined brownish extract was concentrated under reduce pressure by a vacuum rotary evaporator to obtained brownish residue F1 (5kg) which was suspended in water and partitioned with *n*-hexane to get *n*-hexane fraction FX (1.2kg). The *n*-hexane insoluble portion was acidified with HCl (pH 2) and subjected to further fractionation with ethyl acetate (x3), afforded ethyl acetate fraction FX1 (1.0kg), while remaining insoluble fraction was basified with ammonia (pH 8) and fractionated with chloroform; to obtained chloroform fraction FX2 (1.6kg) and insoluble fraction FX4 (0.8kg). The chloroform fraction (500g) was subjected to column chromatography using silica gel with *n*-hexane: chloroform increasing polarity to obtain several fractions, which were combined on the basis of TLC profile yielded six sub fractions (1-6). Out of these fractions the fraction 3 (14.3 g), 4 (25g) and 5 (19.7g) were combined on the basis of TLC profile which afforded a major fraction which was subjected to chromatography using silica gel and *n*-hexane: chloroform in increasing polarity orde furnished 56 fractions. Fractions 20-40 were combined based on TLC profile and purified by using Prep TLC

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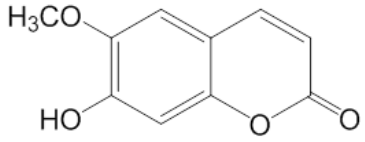
which afforded a compound known as 7-hydroxy-6-methoxy-2H-chromen-2-one (Arcos *et al*., 2006).



Chemical structure of 7-hydroxy-6-methoxy-2H-chromen-2-one

In-vitro lipoxygenase inhibition assay

Enzyme inhibition assays were performed by using different concentrations of the isolated compound. Lipoxygenase inhibitory activity was measured by slightly modifying the spectrometric method as reported earlier (Khan *et al*., 2009; Khan *et al*., 2011). Lipoxygenase (EC 1.13.11.12) type I-B 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). About 160µL of sodium phosphate buffer, 0.1mM (pH 7.0), 10mL of the sample solutions (test compound and standards) and 20µL of lipoxygenase solution were mixed and incubated for 5 min at 258^{o}C. Reaction was initiated by addition of 10µL linoleic acid substrate solution and absorption change with formation of (9Z, 11E)-13S)-13-hydroperoxyo-ctadeca-9,11-dienoate being followed for 10min. 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). Results of the study were expressed as mean ±S.E.M.

Molecular docking simulations

FRED 2.1 (Khan *et al*., 2009) was used in this study to dock the OMEGA pre-generated multi-conformer library mentioned above. FRED 2.1 strategy is to exhaustively dock/score all possible positions of each ligand in the binding site. Epigallocatechin-bound (co-crystallized) protein structure (PDB ID: 1JNQ) was used for docking study. 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 (α-glucosidase) 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 (shape gauss) 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 shape gauss 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 α-glucosidase. Three different simulations were carried out with an added value of 8 Å around the reference ligand.

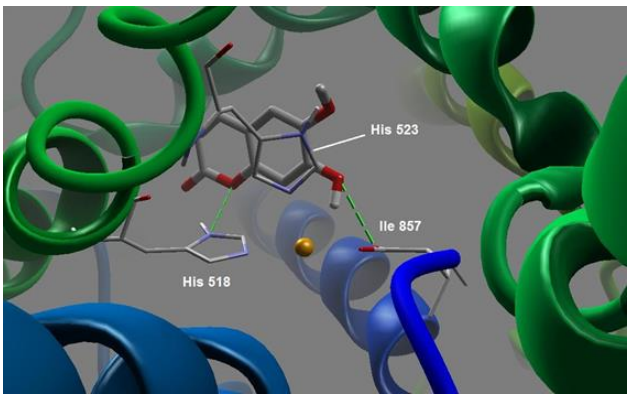


Fig. 1: Binding mode of compound 1 inside catalytic site of lipoxygenase. Orange colored round object is Fe atom. Hydrogen bonding (green dotted lines) are selectively (only for His523 and His519, for details see fig. 3) shown for clarity. Hydrogen atoms (except polar ones) were omitted for clarity.

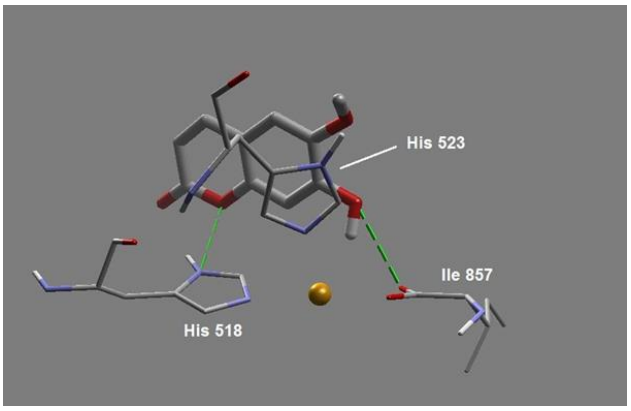


Fig. 2: Closer view of molecular interactions of Viscosine inside catalytic site of lipoxygenase. Orange colored round object is Fe atom. Hydrogen atoms (except polar ones) were omitted for clarity. (For interpretation of the references to color in this fig. legend, the reader is referred to the web version of the article).

RESULTS

In the current study, the test compound revealed lower inhibitory activity against LOX IC_{50} value: 116.83±0.02µM), as compared to standard Compounds Baicalein and Tenidap sodium (IC_{50} value being 22.1±0.03µM and 41.6±0.02µM, respectively). Molecular docking simulations revealed the reasons behind the lower activity of compound in the standard compounds. The test compound should dock score of-17.6 kcal/mol while the docking scores of Baicalein and Tanidap were -127.31 Kcal/mol and -114.79 Kcal/mol.

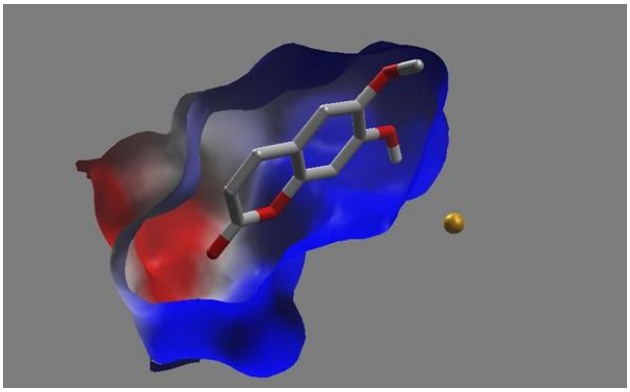


Fig. 3: Electrostatic interactions of compound 1 inside active site of LOX. Color encoding (White area: Hydrophobic region, red area: Area with aggregated negative electrostatic potential and blue area: Area with aggregated positive electrostatic potential).

DISCUSSION

The given compound on molecular docking against LOX revealed that it can effectively bind to and interact with various important sub sites of the enzyme (Fig. 1-3). The OH group at position 8 on ring A was found to be interacting with Ileu857 via hydrogen bonding at a bond distance of 2.90°A. Oxygen at position 1 of ring B also revealed a hydrogen bond interaction with His518 at a distance of 3.07°A. OH group of ring B at position 3 interacts with Gly514 and Leu515 at a bond distance of 2.23°A. Furthermore, certain π interactions were also predicted, for ring A with His 523 at a bond distance of 3.79°A, interestingly, iron atom of LOX active site was also found to be favorably interacting via charge-dipole interactions with hydroxyl moiety at position 6 of ring B. The side cyclic chain at position 3 of ring B penetrated into the active sub site showing electrostatic interaction with Gly716, His513 and Phe516, having a role in the inhibitory activity of the LOX enzyme. Replacement and extension of the alkyl side chain by a polar and bulky group could improve the activity of the compound inside the active pocket of the enzyme LOX, and hence it will further enhance the inhibitory activity of the compound. The only unfavorable interaction was the steric clash of methoxy group attached to ring B of the compound. . Molecular interactions and binding modes of the standard compounds Baicalein and Tanidap are reported previously against the same PDB file (Khan et al., 2013). Although the favorable interactions are accountable to inhibitory activity of compound against LOX but smaller size of the compound seems to be possible main reason behind lower activity as compared to the standard compounds. Lower molecular and steric size is one the factor of lower activity compounds because such compounds are loosely bound or fitted to active site (Khan *et al.,* 2011) as depicted in figure. This limiting factor can be corrected and improved by structural optimization through chemical modification followed and supported by structure-activity relationship (SAR) studies.

CONCLUSION

The compound 7-hydroxy-6-methoxy-2H-chromen-2-one isolated from the bark of *M. ovalifolia* exhibited significant lipoxygenase (LOX) inhibitory activity with (IC_{50} value: 116.83±0.02μM). Molecular docking study further displayed significant molecular interactions between 7-hydroxy-6-methoxy-2H-chromen-2-one and LOX showed potential for further optimization as a possible anti-inflammatory lead compound. Thus in overall it has been concluded that this plant is very important from medicinal point of view and it need to be further investigated phytochemically and pharmacologically for new drugs discovery.

ACKNOWLEDGMENT

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