Analgesic and antiinflammatory activities of taxoids from Taxus wallichiana Zucc

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Anti-inflammatory activities of Taxusabietane A isolated from *Taxus wallichiana* Zucc.

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ABSTRACT

Current study was conducted to identify constituents of *Taxus wallichiana* Zucc. that might be responsible for its folk use in anti-inflammatory conditions. Taxusabietane A was isolated from the bark extract of *Taxus wallichiana* Zucc. Taxusabietane A was analyzed for *in-vitro* and *in-vivo* anti-inflammatory activities using Lipoxygenase (LOX) inhibition assay and carrageenan-induced paw edema model. Taxusabietane A revealed considerable LOX inhibitory activity with the IC_{50} value being 57 ± 0.31. Standard compound Baicalein showed the IC_{50} value being 22.1 ± 0.03 μM. Taxusabietane A also showed significant (5 and 10 mg/kg) anti-inflammatory activity induced by carrageenan. However, this study highlighted the potential of Taxusabietane A to be further explored as a new lead compound for management of conditions associated with inflammation.

1. Introduction

Taxus wallichiana Zucc. (Himalayan Yew) is a small to medium sized evergreen tree, growing 10–20 m tall, exceptionally up to 28 m. Its leaves are flat, dark green, arranged spirally on the stem. It is one of the oldest native plants known in the northern area of Pakistan. Literature survey revealed that this plant is used traditionally for treatment of high fever and painful and inflammatory conditions [1]. Leaves of the plant are used to make herbal tea for indigestion and epilepsy [2]. Some of these folk medicinal uses of *T. wallichiana* Zucc. have been validated through pharmacological studies According to previously published literature of *T. wallichiana* Zucc., immunomodulatory activities [3], antibacterial and antifungal activities [4], analgesic, antipyretic and anticonvulsant activities [5], have been reported so far. In the current study we have made an effort to explore anti-inflammatory activities of isolated abietane diterpenes from *T. wallichiana* Zucc. based on its traditional usage in pathological conditions associated with inflammation.

2. Materials and methods

2.1. Plant material

Plant material was collected from Hazara division of the North-western Frontier Province, Pakistan, in March 2005, authenticated by Prof. Dr. Jahandar Shah. Aerial parts of the

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plant were air-dried under shade for 2 weeks at room temperature. The dried plant material was later on chopped, pulverized and stored in a polyethylene bag under refrigeration for further experimentation.

2.2. Extraction and isolation

Dried and powdered bark (4 kg) was macerated in methanol for a period of 48 h [6–9]. After filtration, the process was repeated three times using 2.5 L methanol each time. The combined filtrates were concentrated in vacuo at 40 °C to afford crude methanol extract (512 g, 12.8% (w/w)). Crude methanolic extract of bark was redissolved in distilled water and successively extracted with hexane, chloroform; ethyl acetate and finally water give the respective fractions. Chloroform fraction (211 g) was subjected to column chromatography and was eluted using chloroform:n-hexane (1:9) gradient on Si gel with gradual increase of polarity to chloroform (100%). This process afforded 13 fractions (fr. 1–13). Further elution with 13% methanol–chloroform gradient has afforded 14 fractions (fr. 14–27). Sub-fraction (C-6c, 228 mg) was loaded to flash column chromatography (Silica gel, 5 g) and eluted with *n*-hexane-chloroform; (30:70) to yield a uv-active compound. (39 mg) Structure of the compound was identified as Taxusabietane A (Fig. 1) via comparison of spectral data with the spectral data cited in literature [6].

2.3. Animals

Adult Wistar rats of either sex were used in various *in vivo* studies. Animals were housed 10 per cage under standard environmental condition with 12 h light–dark cycle and free access to food and water. All the ethical principles established in 1979 for laboratory animals were followed as described previously [7–9,14].

2.4. In vitro lipoxygenase inhibition assay

Enzyme inhibition assays were performed by using different concentrations of the isolated compound Taxusabietane A. Lipoxygenase inhibitory activity was measured by slightly modifying the spectrometric method as developed by Tappel (1962). 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 addition of 10 μL linoleic acid substrate solution and absorption change with 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 [7]. The IC_{50} values were calculated using the EZFit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA).

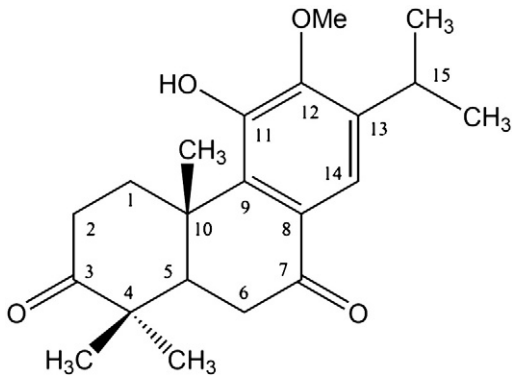

Fig. 1. Chemical structure of Taxusabietane A.

2.5. Molecular docking simulations

FRED 2.1 (McGann et al., 2003) 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. 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. 3 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.

2.6. Carrageenan-induced oedema

In-vivo model was utilized to assess the anti-inflammatory potential of test sample via testing its ability to inhibit the carrageenan-induced hind paw oedema, as reported earlier [7]. Test samples and the control samples were administered orally in groups ($n = 5$) to rats. After 1 h, acute inflammation at desired site was induced by subplantar injection of 1% suspension of carrageenan (0.1 mL) using 2% gum acacia as a suspending agent in normal saline, in the right hind paw of the rats. The paw volume was measured plethysmometrically at '0' and 3 h after the carrageenan injection. Indomethacin 5 mg/kg, p.o. suspended in 2% gum acacia was used as positive control. Percent inhibition of the inflammation was determined by applying statistics on raw data followed by the calculation of percent inhibition for each group by comparing with control group. The formula used for comparison was: $\%\ I = 1 - (dt/dc) \times 100$, where "d*t*" is the difference in paw volume in the drug-treated group and "d*c*" is the difference in

paw volume in control group. However, "*I*" stands for inhibition of inflammation.

2.7. Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and mortality was recorded.

2.8. Data analysis

Results of the study were expressed as mean ± SEM. Student's *t*-test was used to analyze data between the groups and analysis of variance (ANOVA) among groups followed by Dunnet's test for multiple comparisons. Values of $p<0.05$ were considered significant in all cases.

3. Results

3.1. In vitro lipoxygenase inhibition assay

All compounds showed promising inhibitory activity against lipoxygenase. IC_{50} values of Taxusabietane A, Taxusabietane C and Taxamairin F were 57 ± 0.31. Standard compound Baicalein and Tenidap sodium showed the IC_{50} value being 22.1 ± 0.03 μM and 41.6 ± 0.02 μM.

3.2. Molecular docking simulations

Taxusabietane A showed considerable molecular interactions with important subsites of LOX catalytic site (Fig. 2). Keto group of Taxusabietane A at position C-7 seems to play a vital role in its inhibitory activity through hydrogen bonding interactions with His523 at distance of 3.06 Å (Fig. 3). This macromolecular complex was further stabilized by the favorable hydrophobic interactions (Fig. 4) between the enzyme and Val769, Leu773, and Ile572. On other side, methoxy group at position C-12 was held strongly through hydrogen bonding (2.98 Å) with His518. Phenolic group at position was found to be interacting with Trp519 via hydrogen bonding (3.11 Å). Molecular shape of Taxusabietane A fits well into the binding pocket, thus preventing access of substrate to catalytic site of LOX. As a whole, Taxusabietane A seems to be supported by both hydrophilic and hydrophobic interactions. No electrostatic clash was observed between LOX and Taxusabietane A.

3.3. Carrageenan-induced oedema

Taxusabietane A significantly ($p<0.05$) reduced oedema induced by carrageenan at a dose 5 and 10 mg/kg (Fig. 5). However standard compound indomethacin (5 mg/kg) showed comparatively better activity than test compounds.

3.4. Acute toxicity

All the compounds were found safe after 48 h of administration. Statistically, no considerable difference was observed between the negative control and other treatment groups both in terms of mortality and morbidity.

4. Discussion

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 xenobiotic metabolism. They are responsible for the metabolism of the fatty acids (FAs) and their metabolites eliciting inflammatory

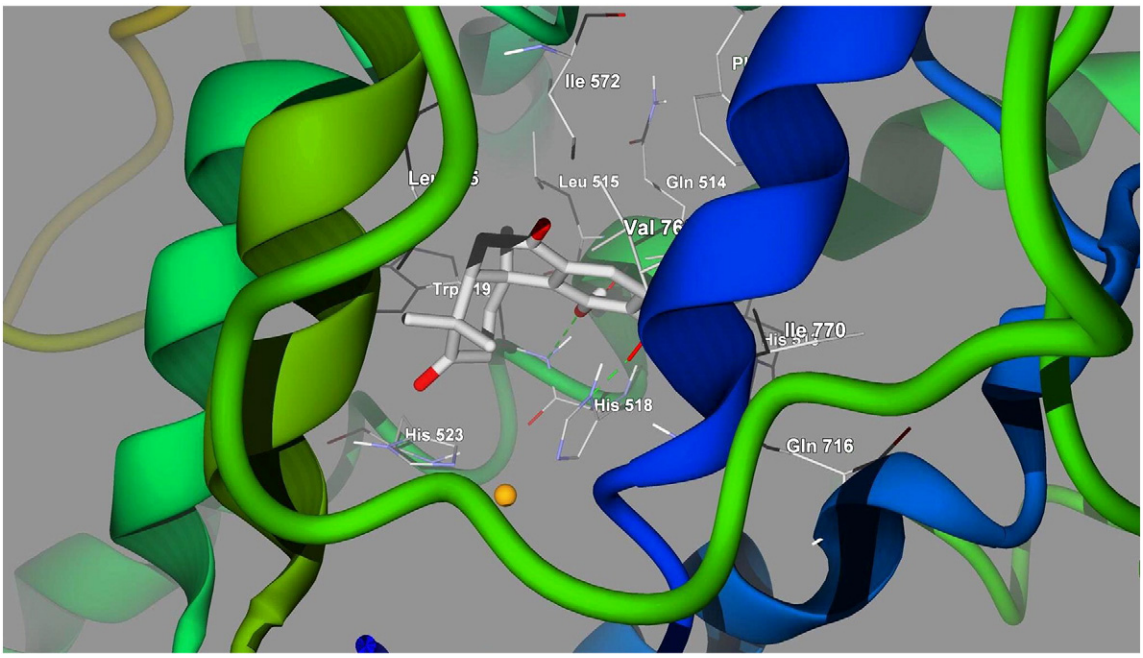

Fig. 2. Binding mode of Taxusabietane A inside catalytic site of lipoxygenase. Orange colored round object is Fe atom. Hydrogen atoms (except polar ones) were omitted for clarity.

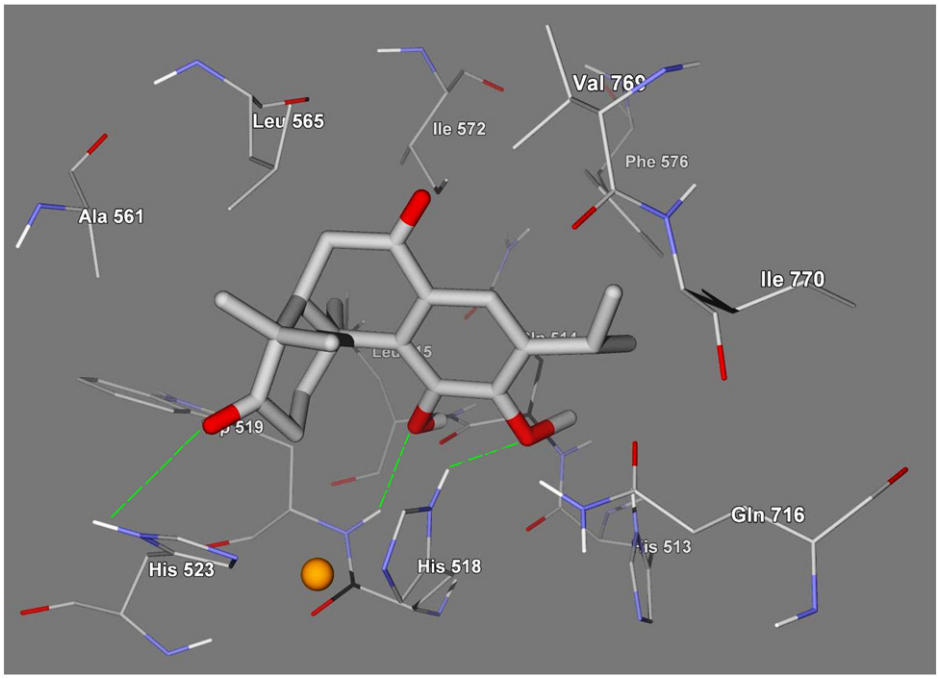

Fig. 3. Closer view of molecular interactions of Taxusabietane A inside catalytic site of lipoxygenase. Orange colored round object is Fe atom. Hydrogen atoms (except polar ones) were omitted for clarity.

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) [7]. Many COX-2 or 5-LOX inhibitors have been developed as drugs to treat inflammation [10]. In this study Taxusabietane A exhibited significant inhibition of the lipoxygenase showing its strong potential to be developed as 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 adjoining sites of the enzyme thus preventing its pro-inflammatory role.

Carrageenan-induced paw edema being an *in-vivo* investigational model for acute inflammation which been extensively used to determine the anti-inflammatory effect of new investigational agents [7–9]. Taxusabietane A further established its significant ($p<0.05$) anti-inflammatory potential in *in-vivo* study by controlling biphasic inflammatory events induced by carrageenan. The early phase (90–180 min) of the inflammation is due to the release of histamine, serotonin and

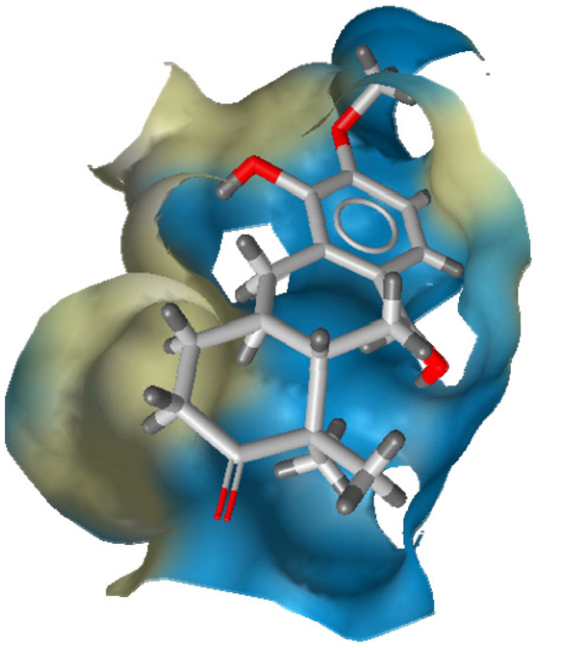

Fig. 4. Surface contacts of Taxusabietane A inside the catalytic site of lipoxygenase. Hydrophobic regions are represented as yellow colored while hydrophilic regions are represented as blue colored regions.

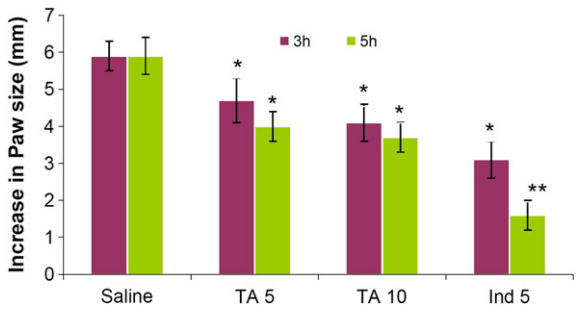

Fig. 5. Anti-inflammatory activity of Taxusabietane A based on carrageenan-induced oedema model. $^{*}p<0.05$, $^{**}p<0.01$. Ind = Indomethacin (5 mg/kg).

similar substances. The later phase (270–360 min) of edema-induced by carrageenan is characterized by the highest volume of hind limb, during which the edema reaches its highest volume and by the presence of prostaglandins and other slowly acting inflammatory mediators which include kinin-like substances, i.e. prostaglandins, proteases and lysosome [11,12]. These inflammation mediators are the main components responsible for swelling and edematous condition. Moreover, all the compounds were found safe after 48 h of test compound administration. Statistically, no considerable difference was observed between the negative control and other treatment groups both in terms of mortality and morbidity.

This significant data of Taxusabietane A might share the same anti-inflammatory mechanism as in the case of indomethacin, which involves the inhibition of inflammation process initiated through carrageenan [13]. Current investigations regarding anti-inflammatory activities reveal the fact that Taxusabietane A should be extensively studied further in order to developed as a new lead compound for treatment of inflammation.

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