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Integrative *in silico* and Petra/Osiris/Molinspiration (POM) analysis of baicalein: identification of therapeutically relevant pharmacophores against keloid pathology

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ABSTRACT

Introduction: Keloid scars are a kind of skin disorder in which the scar grows beyond the boundaries of the original wound. Baicalein, a flavonoid, may treat keloids by targeting fibrosis, inflammation, and possible viral factors.
Methods: In silico studies were conducted to evaluate the potential anti-keloid effects of baicalein by predicting its interactions with three key proteins of the transforming growth factor-β (TGF-β) family (PDB IDs: 1VJY, 3TZM, and 7DV6). POM analysis was also used to understand the conditions that could enhance baicalein's efficacy.
Results: The results indicated that baicalein binds effectively to TGF-β family proteins via hydrogen bonds, showing strong affinities (1VJY: -9.9 kcal/mol, 3TZM and 7DV6: -9.3 kcal/mol), indicating its potential as a TGF-β receptor ligand. Osiris analysis gave a drug score of 75% for baicalein, while Molinspiration indicated good bioavailability with a cLogP of 2.84. Atomic charge distribution and pharmacophore site mapping through POM analysis indicate that baicalein exhibits an antiviral pharmacophoric moiety akin to known antiviral agents. This indicates that baicalein may act as a pro-drug, undergoing metabolic transformation to form a bis-bidentate ligand. Such ligands are crucial for forming bimetallic complexes that can function as efficient biocatalysts against various biological targets.
Conclusion: In-silico analysis suggests that baicalein may influence TGF-β receptors and exhibit anti-keloid activity. Additionally, POM analysis recommends that baicalein may serve as a lead compound with the potential to modulate TGF-β signalling and exhibit antiviral properties, indicating it as a dual-action agent against keloids and viral infections.

Keywords: *O. indicum*, baicalein, keloid, transforming growth factor-β, anti-inflammatory, anti-viral activity

Introduction

A keloid is an abnormal scar that forms in the dermis following skin injury. This condition, known as Keloid Disease (KD), is a benign but locally aggressive fibroproliferative disorder characterized by scar tissue that extends beyond the original wound boundaries into the surrounding healthy skin (1). KD typically manifests between the ages of 10 and 30 and is less common in older individuals. It is more prevalent among people with darker skin tones, particularly those with a family history of keloid formation (2). Keloid scarring does not follow the typical pattern of evolution, stabilization, and involution observed in hypertrophic scars. It may develop soon after an injury or appear years later, sometimes arising from a previously stable and mature scar (2).

Transforming growth factor-β (TGF-β) family of cytokines significantly promotes fibrosis by stimulating the production and remodelling of extracellular matrix (ECM) in fibroblasts and connective tissues. Additionally, TGF-β is crucial for enforcing immune tolerance and suppressing inflammation, as it restricts the functioning of the immune systems (adaptive and innate) (3). According to a study, TGF-β1 and TGF-β2 cause skin scarring; however, TGF-β3 appears to have the opposite effect (4). On the other hand, elevated TGF-β1 and TGF-β2 expression may negatively impact the healing process by promoting the development of scars (5). According to

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another study, proper modulation of TGF-β and TGF-β receptor expression is crucial for optimal wound healing (5).

It is commonly recognised that tissue repair involves TGF-β1, TGF-β receptor type I (TGF-βRI), and TGF-β receptor type II (TGF-βRII) (6,7). Chin et al. reported that keloid fibroblasts exhibit altered responses to TGF-β due to differences in receptor expression. Specifically, the study found that keloid fibroblasts express higher levels of TGF-βRI and TGF-βRII compared to normal human fibroblasts (8). In addition, Bock et al. investigated the expression levels of TGF-β1, TGF-β2, and TGF-β3, along with the mRNAs of TGF-βRI and TGF-βRII receptors. Their findings revealed that hypertrophic scars exhibited significantly lower TGF-βRI mRNA expression, whereas keloid scars showed reduced TGF-βRII mRNA expression. Consequently, the TGF-βRI/TGF-βRII ratio was elevated in keloid scars compared to hypertrophic scars (9).

In contrast, when TGF-βRI/TGF-βRII receptor expression levels were compared between keloid and normal human dermal fibroblasts, the findings indicated that keloid tissues were found to exhibit significantly elevated levels of these receptors (8,10). Several treatment approaches have been put forth to lessen the severity of keloid scarring. Various signalling pathways, such as mitogen-activated protein kinase (MAPK), sphingosine-1-phosphate (S1P), nuclear factor kappa B (NF-κB), insulin-like growth factor-I (IGF-I), TGF-β1, wingless-related integration site (Wnt), janus kinase (JAK)/signal transducer and activator of transcription (STAT), and integrin pathways have been investigated as potential mechanisms involved in keloid formation. However, despite extensive research, the underlying molecular mechanisms of this skin disorder remain poorly understood and inconclusive (11,12).

It has been suggested that natural compounds with diverse bioactive properties such as asiaticoside, quercetin, madecassoside, oxymatrine, and curcuminoids extracted from plants including *Salvia miltiorrhiza* Bunge, *Astragalus membranaceus* Bunge, and *Aneilema keisak* Hassk, may have therapeutic potential in the treatment of keloid disorder (11).

Oroxylum indicum (L.) Kurz (Bignoniaceae), also called the Indian trumpet flower, is widely found across various Asian countries, including Thailand, Vietnam, Malaysia, Indonesia, the Philippines, Japan, China, Taiwan, and India (13). The plant is renowned for its therapeutic properties (14). For millennia, it has been utilized in Asian ethnomedical systems as a traditional medicine to prevent and treat a wide range of illnesses, including jaundice, rheumatoid arthritis, peptic ulcers, cancers, respiratory conditions, diabetes, diarrhea, and dysentery, among others (15). Flavonoids, alkaloids, tannins, glycosides, saponins, phenols, and quinones are only a few of the many secondary metabolites that contribute to the biological activity of the *O. indicum* plant. Chrysin, baicalein, oroxylin-A, baicalein-7-O-diglucoside, and baicalein-7-O-glucoside are all derived from this group. Among all the flavonoids found in *O. indicum*, baicalein is the most common and the most significantly active component (13). Baicalein strongly inhibits endoproteases and proprotein convertases, contributing to its broad spectrum of biological effects, including anti-inflammatory, anticancer, antihyperglycemic, neurogenic, anti-adipogenic, cardioprotective, antibacterial, and wound healing activities (13-16). It also inhibits collagen synthesis and cell proliferation (16) and exhibits anti-inflammatory properties (17). Moreover, it is believed that using baicalein can alleviate keloid scarring.

Therefore, the current study is aimed at performing *in silico* studies to predict baicalein as an anti-keloid scar molecule by targeting TGF-βRI and II receptors.

Methodology

Structure preparation

Protein Data Bank (PDB) IDs 1VJY (TGF-βRI), 3TZM (TGF-βRI), and 7DV6 (TGF-βRII) were used to download the crystalline structures and sequences of TGF-βRI and II receptors through the Research Collaboratory for Structural Bioinformatics (RCSB) protein repository (Online). Using BIOVIA Discover Studio 2021, hetero-atoms and water, as well as inhibitors, were carefully eliminated from the target receptor. The three-dimensional (3D) molecular structures of the ligands, baicalein and conventional medications (1,5-Naphthyridine;4-(5-benzo(1,3)dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl)benzamide and 5-[(3S)-5,5-dimethyloxolan-3-yl]-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-a] pyrimidine), in the structured data format (SDF) (Fig. SI A-D) were obtained using PubChem databank (Table SI). BIOVIA Discover Studio 2021 was used to convert the ligands' SDF to PDB file format. Open Babel 2.4.1 software, which was in line with PyRx, was used to convert all PDB file types into PDBQT file formats to generate atomic coordinates. The Open Babel was also utilized to minimize the energy of ligands following the insertion of H-atoms.

Ramachandran plot and hydropathy analysis

Ramachandran plot and hydropathy analysis were carried out to assess the structural and physicochemical properties of the proteins (18-20). In brief, the Procheck server (Online) was used to verify the stereochemical character of the proteins obtained via the Protein Data Bank (PDB). The relevance and positioning of secondary structural elements, such as α-helices and β-sheets, were tested for relevance using Ramachandran plot analysis, which examines the φ (phi) and Ψ (psi) dihedral angles to determine the conformational stability of the polypeptide backbone. Protein modeling and structural evaluations were guided by these angular measurements. The statistical distribution of amino acids within the secondary structures was also analyzed. Additionally, the amphiphilic nature of the receptor molecule was confirmed using a hydropathy plot generated by BIOVIA Discovery Studio Software 2021.

Molecular docking

PyRx, written in Python, is a virtual molecular screening tool that docks ligands to macromolecules to locate the compounds of interest with the necessary biological significance. The application of this in silico technique in computer-aided drug design is well established (21).

The ligand and macromolecule (target proteins 1VJY, 3TZM, and 7DV6) were uploaded to the PyRx tool to closely monitor and assess any potential interaction between the ligand

(a chemical compound) and target protein. The output included a list of models arranged in accordance with the anticipated binding energies in kilocalorie per mole (kcal/mol), whereas the input included a ligand, docking box, flexible receptor, and rigid receptor (21). All targeted TGF-β receptors and docking simulations were started with active sites as the CASTp server suggested (22). Discovery Studio was used to view several interactions posed to assess theresidual interaction of ligands with the target enzyme.The Discovery Studio Visualizer was used to extract and align the docking model containing the lowest possible energy related to the receptor.

Absorption, distribution, metabolism, excretion properties (ADME) and drug-likeness prediction

Swiss ADME (http://www.swissadme.ch/), a free online tool, examines ligands' drug-likeness prediction and ADME properties (23). Drug-likeness was evaluated based on Lipinski's rule of five, which states that a compound is more likely to be orally active if it meets the following criteria: no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, a molecular weight below 500 Da, a calculated LogP value (lipophilicity) ≤ 5, and a polar surface area (PSA) ≤ 140 Å². Compounds that violate two or more of these criteria are generally considered to have poor absorption and bioavailability (24).

Target prediction

Finding potential crossover reactions or physiological adverse effects from the intercommunication of tiny biomolecules requires pharmacological research (25). Canonical SMILES of baicalein were entered into the Swiss Target Prediction website (Online) for target prediction research, and the results were examined.

Toxicity prediction

Animal and human models are essential for predicting the toxicological profile of a molecule, which is essential for determining its acceptability before administration. The Protox II server (Online) was used to assess the molecule's toxicity. To predict toxicity, the canonical SMILES of baicalein that were obtained from PubChem were utilised as input (26).

POM analysis

The identification of the pharmacophore sites of drugs is performed with great precision, based on the geometric and electronic properties of the functionalised groups. The organigram of POM theory is shown in Fig. S1. This model was developed using a combination of three computational tools: 1) Petra: Calculation of atomic charges (27), 2) Osiris: for the prediction of potential side effects, bioavailability, and Drug-score (Online), 3) Molinspiration: for the evaluation of Lipinski's five rules and 3D structure optimisation (Online).

Results and discussion

Baicalein chemistry

Baicalein ($C_{15}H_{10}O_5$) belongs to the flavone subclass of flavonoids (Fig. S2 A). It is naturally found in the leaves of *Thymus vulgaris* L. and *O. indicum* as well as in the roots of *Scutellaria baicalensis* Georgi (28,29). Baicalein is a potent anti-inflammatory that primarily works by binding to several chemokines, such as IL-8, monocyte chemotactic protein-2, and macrophage inflammatory protein-1β, thereby inhibiting their biological activity. Furthermore, baicalein has been shown to modulate the immune response by promoting the polarization of macrophages from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype (30). These properties highlight baicalein's potential in mitigating inflammatory processes (31,32). Given that, inflammation plays a crucial role in keloid pathogenesis, baicalein's anti-inflammatory activity may contribute to its therapeutic potential in managing keloid disease.

Interestingly, baicalein's therapeutic use was constrained due to its low bioavailability and solubility. Therefore, it is essential to modify baicalein using biological and chemical approaches to generate analogues with improved solubility and absorption. Baicalein contains three hydroxyl groups (5-OH, 6-OH, and 7-OH) on ring A (Fig. S2A), with the C-6 and C-8 positions serving as active sites for nucleophilic substitution reactions. The 5-OH group is a key structural feature linked to the cytotoxic activity of baicalein and its derivatives. Furthermore, nitrogenous and water-soluble amino acids can be used as substituents in structural modifications to enhance solubility (33). Researchers have successfully replicated the biosynthetic conversion of chrysin to baicalein in laboratory testing by employing a multistep synthetic strategy (34-36). After conducting a groundbreaking study, Chen et al. developed a novel and efficient method for synthesizing baicalein from 3,4,5-trimethoxyphenol. This approach offers a promising and more effective strategy for the large-scale production of baicalein (14,34). In addition, Huang et al. synthesized baicalein, oroxylin A, and wogonin very effectively by employing inexpensive reagents and working in mild conditions (37).

Our results emphasized the significance of baicalein as the key active compound, highlighting its strong binding affinity to the active sites of TGF-βRI and TGF-βRI. Additionally, baicalein exhibited notable anti-inflammatory properties, thereby revealing potential mechanisms for its therapeutic application in keloid disease. It also served as an essential tool for assessing the structural stability of the molecule and validating the amphiphilic nature of the receptor, respectively (18).

Ramachandran plot and hydropathy analysis

The Ramachandran plot illustrates the stable conformations of amino acid residues by identifying favourable and unfavourable regions, particularly highlighting low-energy configurations for the torsion angles φ and Ψ. Each point on the plot represents the φ and Ψ angles of an amino acid residue in a three-dimensional protein structure (Fig. S3). Upon analysing the sequences of 1VJY, 3TZM, and 7DV6, it was found that 268, 265, and 275 non-glycine and non-proline residues were present, respectively, out of a total of 299, 295, and 304 residues in the corresponding proteins.

The stereochemical characteristics are displayed in Table S-II for all likely values of φ and Ψ for the dihedral angle. This includes hydrophilic and hydrophobic regions in the amino acid chain, starting at the N-terminal and progressing

towards the C-terminal, further supporting protein architectures (Fig. S4).

Molecular docking assessment for targeting TGF-β receptors

Binding energies measured with PyRx software powered by Autodock Vina were **-9.9** (1VJY-Baicalein), -**9.3** (3TZM-Baicalein) and -**9.3** kcal/mol (7DV6-Baicalein) with favourable root mean square deviation (RMSD) values compared with reference standards, 1,5-Naphthyridine (-10.3 kcal/mol), 4-(5-benzo(1,3) dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl)benzamide (-10.8 kcal/mol) and 5-[(3S)-5,5-dimethyloxolan-3-yl]-6-methoxy-3-(2-methoxypyridin-4-yl)pyrazolo[1,5-a]pyrimidine (-9.3 kcal/mol), respectively (Table 1). The ASP 351 and GLU 245 residues are targeted by the baicalein interaction with 1VJY through hydrogen bonding at the protein's active site. Additionally, they used several pi bonds to engage with LYS 232, VAL 219, ILE 211, ALA 350, 230, and LEU 260 at the enzyme's catalytic pocket. Van der Waals interactions were observed with more amino acids, including LEU 278, 352, 340, TYR 249, PHE 262, LEU 278, VAL 231, 279, SER 280, and GLY 286 (Fig. 1A).

Interestingly, it is noteworthy that the target macromolecule 3TZM active binding pocket contains the amino acid GLU 245 via a traditional hydrogen bond with baicalein. Furthermore, GLY 286, LEU 352, VAL 231, 279, PHE 262, and TYR 249 interacted through van der Waals at the protein catalytic site, while VAL 219, LEU 260, and ALA 350 interacted through various pi-bonds (Fig. 2A). The binding site of 7DV6-baicalein, another receptor interaction, was made up of hydrogen-bonded HIS 328 and VAL 250 residues. Through various pi-bonds, baicalein targets VAL 258, LEU 305, 386, LYS 277, ALA 275, and CYS 396. Via van der Waals, it targets ALA 326, 329, ASN 332, GLY 251, THR 325, LEU 323, PHE 294, 327, and ASP 397. The sole amino acid interacting with the ligand through a carbon-hydrogen bond is GLY 331 (Fig. 3A).

TABLE 1 - Baicalein's protein-ligand binding energy values with TGF-βRI and RII receptors

S.N.	Target molecule	Binding Energy (kcal/mol)			
		Baicalein	**1,5-Naphthyridine**	**4-(5-benzo(1,3)dioxol-5-yl-4-pyridin-2-yl-1H-imidazol-2-yl) benzamide**	**5-[(3S)-5,5-dimethyloxolan-3-yl]-6-methoxy-3-(2-methoxypyridin-4-yl) pyrazolo[1,5-a]pyrimidine**
1.	1VJY (TGF-βRI)	−9.9	−10.3	---	---
2.	3TZM (TGF-βRI)	−9.3	---	−10.8	---
3.	7DV6 (TGF-βRII)	−9.3	---	---	−9.3

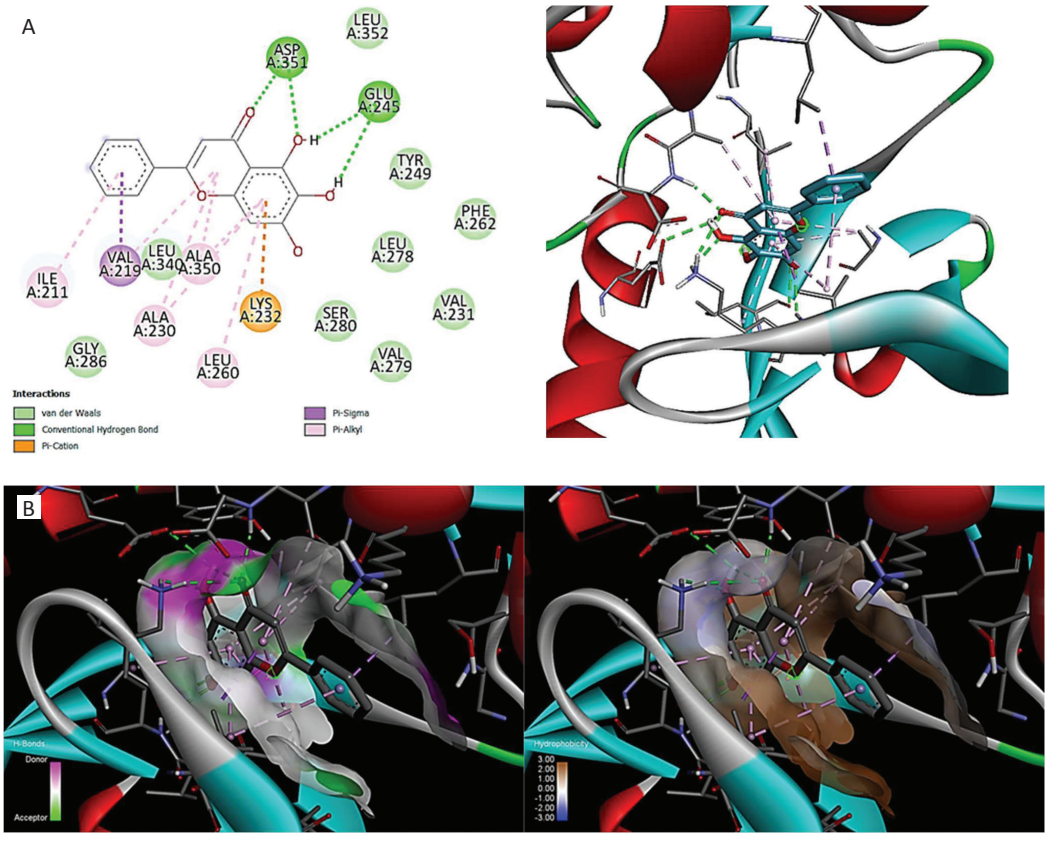

FIGURE 1 - A. 2D (left) and 3D (right) view of protein ligand interaction (1VJY-Baicalein). B. Hydrogen (Left) and Hydrophobicity (Right) surface view of receptor-ligand interaction (1VJY-Baicalein).

The strength of the interaction between a ligand and its target protein is predicted by the hydrogen interactions between the two, which is why our study closely examined the donor and recipient regions of the hydrogen bonds between the two, as well as the distance between the interacting atoms (Figs 1B-3B).

Swiss ADME and drug-likeness prediction

Table S-III displays the baicalein's drug-likeness and ADME data. The scores showed that baicalein met the requirements of Lipinski's rule of five, indicating that it's a reliable medication that works well when taken orally. In summary, this molecule is said to have good permeability, oral bioavailability, low toxicity, and favorable absorption characteristics. The bioavailability radar map summarized the drug-likeness of baicalein (Fig. S5). The pink area represents the molecule's (baicalein) effective range for each attribute.

The gastrointestinal absorbency and brain penetrative effectiveness of baicalein are predicted by the boiled-egg plot between the water partition coefficient (WLOGP) and topological polar surface area (TPSA), as illustrated in Fig. S6. Baicalein is quickly absorbed into the gastrointestinal tract and cannot cross the blood-brain barrier (BBB) by interacting with the innermost yellow nucleus/yolk, as the plot (Fig. S6) demonstrates. The red dot indicates that baicalein is a non-substrate of permeability glycoprotein.

Swiss target prediction

A pie chart was created to illustrate the leading 15 plausible targets (Fig. S7). These predicted targets include 26.7% enzymes, 20% lyases and kinases, 13.3% cytochrome P450 enzymes, and 6.7% each for oxidoreductases, primary active transporters, and erasers.

The prediction algorithms estimate potential target interactions for the molecule, with likelihood scores ranging from 1.0000 to 0.3299. These values suggest a high probability of the molecule binding to its intended targets. The target prediction results displayed on the server page contributed to several significant findings (Table S-IV).

ProTox 3.0 toxicity prediction

The organ toxicity (hepatotoxicity), Tox21 Stress response pathways, Tox21 Nuclear receptor signalling pathways, and toxicity endpoints (immunotoxicity, carcinogenicity, mutagenicity, and cytotoxicity) served as the foundation for the toxicity prediction. Table S-V provides a summary of the toxicity prediction results. According to class 5 projected toxicity, baicalein's LD50 was 3919 mg/kg, with an average similarity of 81.74% and a prediction accuracy of 70.97% (Fig. S8).

One of the main causes of keloid scar development has been identified as aberrant regulation of the apoptotic pathway. Keloid fibroblasts' inability to halt proliferation

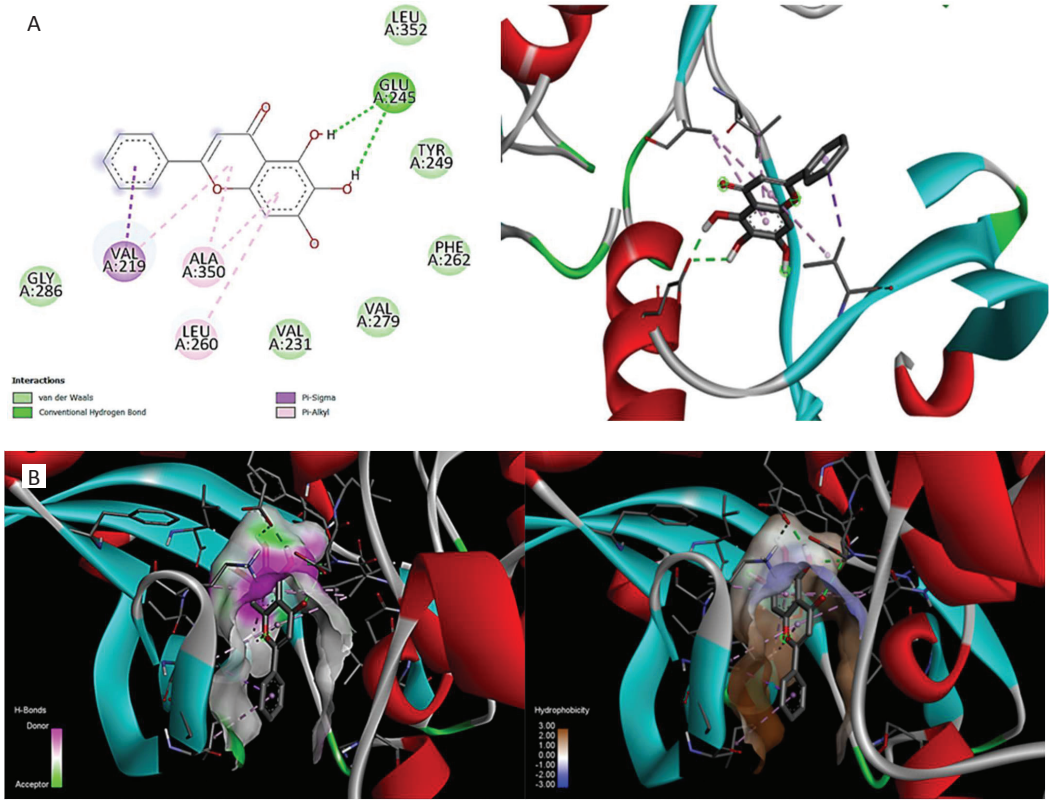

FIGURE 2 - A. 2D (left) and 3D (right) view of 3tzm-Baicalein interaction. B. Hydrogen (Left) and Hydrophobicity (Right) surface view of receptor-ligand interaction (3tzm-Baicalein).

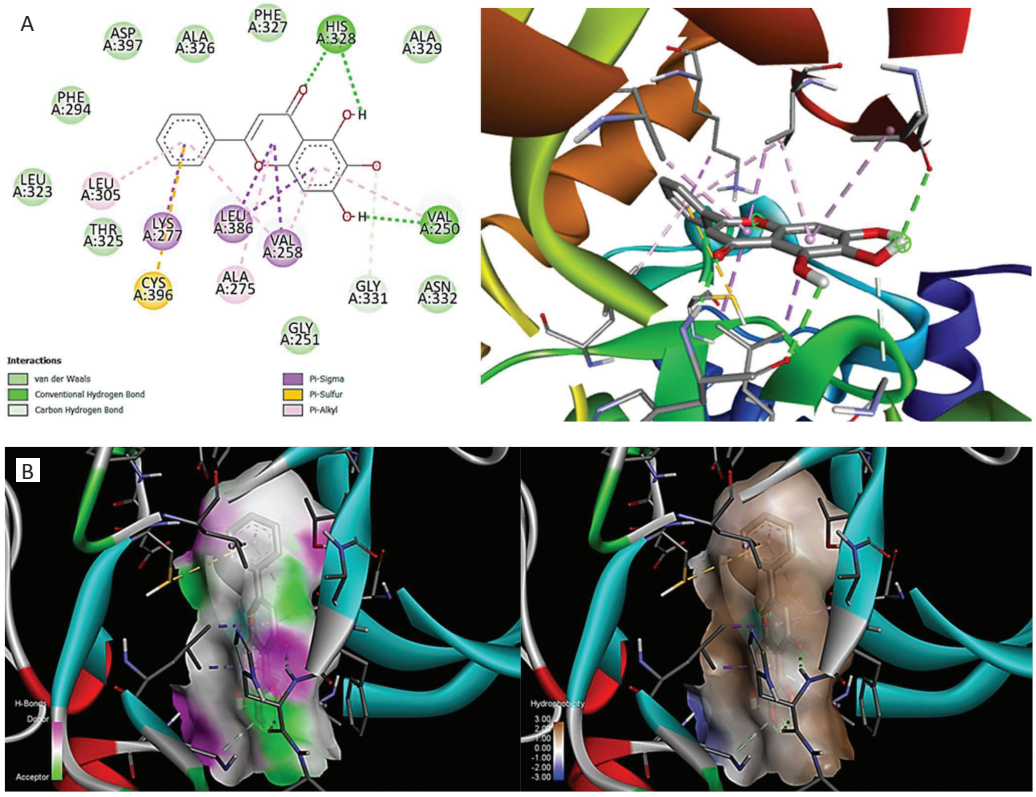

FIGURE 3 - A. 2D (left) and 3D (right) view of 7dv6-Baicalein interaction. B. Hydrogen (Left) and Hydrophobicity (Right) surface view of receptor-ligand interaction (7dv6-Baicalein).

and collagen formation due to their resistance to apoptotic behaviour causes an imbalance in the deposition and breakdown of collagen. Although the basic molecular mechanism of keloid development is still unknown, it is widely accepted that the TGF-β1 pathway plays a significant role in keloid pathogenesis (2,11,38).

Interestingly, maintaining the integrity of an organism depends heavily on the TGF-β regulatory mechanism. TGF-β signaling controls metazoan embryo development, tissue homeostasis, and damage repair through its coordinating impacts on the proliferation of cells, phenotypic variability, migration, metabolic flexibility, and immune monitoring of various cell types in communal environments (39). The pathogenesis of fibrosis and scars is caused by defects in TGF-β signalling, which disrupt immune tolerance, increase inflammation, and result in abnormal collagen synthesis and deposition, a higher proportion of collagen I/III, and the formation of abnormally cross-linked collagen fiber bundles. These defects are especially prevalent in epithelial cells, tissue fibroblasts, and immune cells (3,39,40). It is now thought that flavonoids are primarily responsible for lowering scar formation by inhibiting the production of collagen and fibroblast proliferation. The suppression of TGF-β1 and -β2 and SMAD proteins may be the mechanism underlying these inhibitory actions (41).

According to multiple pharmacological studies, baicalein is closely linked to a number of the plant's biological actions, including anti-inflammatory, antibacterial, antiviral, anti-cardiovascular, and anti-neurodegenerative illnesses (13,42-45). Interestingly, baicalein shows promise as a treatment for skin fibrogenesis. It inhibits the TGF-β/Smad2/3 signalling pathway in fibroblasts *in vitro* and *in vivo* by down-regulating the amounts of phosphorylated Smad2/3 in a dose-dependent manner. Baicalein effectively decreases the activation and multiplication of hypertrophic scar-derived fibroblast cells. It restrains the α-SMA expression throughout the proliferative stage of scarring in a mouse model triggered by mechanical strain. This results in decreased collagen accumulation and attenuated scar development by impairing the contractile and migratory capabilities of fibroblasts produced from hypertrophic scars (40). Zhang et al. (40) suggested that without affecting the expression of Smad6/7, TGF-β1, or TGF-β receptor I/II, baicalein selectively binds to the ATP-binding pocket of activin receptor-like kinase 5 (ALK5), a TGF-β receptor I. Furthermore, it is well known that the TGF-β1 pathway is a major contributor to keloid scarring (1).

Any chemical entity's intricate journey to its destination usually involves successfully navigating several obstacles and surviving a challenging biological process. Several factors and processes determine a chemical entity's bioavailability, which can significantly affect its pharmacokinetic characteristics. Historically, there was a high attrition rate in the development of new medications; ADME issues were linked to over 40% of all therapy failures. Comprehensive studies of ADME techniques are routinely carried out at the early stage of drug development to lower the attrition rate, and the combined evaluation of the biological properties and efficacy of active compounds has been standardized. Researchers are using computational methods to predict a drug's future by

identifying the early dangers of toxicity. Before doing *in vitro* tests, *in silico*-based ADMET profiling techniques are commonly employed to give a general understanding (18).

Using molecular docking, baicalein was employed as a substance (ligand) to assess the compound's possibilities as an anti-keloid molecule (Table 1). This was done by utilising the compound's capacity to attach and downregulate the TGF-βs/Activin pathway. The 3D-interaction map of the best-ranking posture in our investigation demonstrated that baicalein docked nicely inside the catalytic area of all three receptors and formed favourable interactions.

POM analysis

In collaboration with National Cancer Institute (NCI) and Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) of the USA, Prof. Taibi Ben Hadda (Principal inventor) group has developed a bioinformatic platform (POM theory). This POM theory is able to make pharmacological investigations and drug design easier than in the past. Its crucial role is the determination of different types of pharmacophore sites with high accuracy, on the basis of their atomic charges and 3D geometry (Fig. S1) (46,47).

For this reason, we were interested, for the first time, in looking at the role of baicalein in scar management, via a bioinformatics POM approach.

Osiris calculations of compounds

The outcomes of the pharmacokinetic profile and bioactivity score assessment of the three flavonoids (apigenin, fisetin acid, cosmosin) and baicalein are shown in Table S-VI. We chose three flavonoids (apigenin, fisetin acid, and cosmosin) for one evident reason: the flavonoids are subject to opening the central ring in acidic media. This rearrangement of flavonoids always offers two pockets, ready to chelate with two metal atoms. This structure of bimetallic complexes plays a crucial role in producing bioactive species. Hence, their structural similarities with baicalein suggest a comparable potential for pharmacological relevance.

The results show that the drug score was promising (baicalein: 75%, apigenin: 47%, cosmosiin: 44%, and fisetin acid: 40%) (Table S-VI). The toxicity risks and molecular characteristics of all flavonoids were estimated. In contrast to baicalein, all examined structures were found to be non-cancerogenic and safe.

Molinspiration calculations of compounds

The Molinspiration calculation of molecular properties of flavonoids is presented in Table S-VI. These bioavailability scores could be categorized as good (if the score cLog P < 3), moderately active (if the score was 4-5), or inactive (if the score was more than 6). Verification of every bioactivity metric revealed that most flavonoid derivatives exhibited biological activity against every enzyme (Table S-VI).

Atomic charge calculation of compounds

Based on the findings of the atomic charge calculations displayed in Fig. S9, it is evident that the most potent molecules possess an antiviral pharmacophore site (O $^{\delta-}$, O' $^{\delta-}$, O'' $^{\delta-}$). As a result, most hits were associated with antiviral drugs. Consequently, it was determined to be a pharmacophore site for the chemical baicalein, having antiviral qualities.

Identification of antiviral pharmacophore sites

Baicalein exhibits moderate to good antiviral activity ratings and can be characterized as subjects of opening/closing central rings based on the results of the theoretical and experimental POM calculations (Fig. 4).

The POM Theory is used to get more insight into the mode and mechanism of action of this (baicalein) natural product. It can be seen from Fig. 5 that baicalein is involved in the regeneration of metabolites that can coordinate with the biotargets (M = Cu, Ni, Zn, Pt, Pd, Mn, Mg, Ru, etc.) and modulate pathological processes. As a result, the regenerated metabolites may directly inhibit viral replication and the virus life cycle, and baicalein's ability to target and block TNF-β receptors would prevent abnormal scar formation. However, it's important to note that currently, there is no definitive evidence that a specific virus directly causes keloids. This is why an experimental study on anti-keloid and antiviral effect of baicalein is important because it could potentially reveal a viral factor contributing to the development of keloids, which could lead to new preventative and treatment strategies for this disfiguring skin condition (48,49).

Molecular docking is a computational technique used to predict the interaction between a small molecule (ligand) and a target macromolecule (typically a protein). In the context of baicalein, docking studies have gained attention due to its diverse pharmacological activities, including anticancer, antiviral, anti-inflammatory, and neuroprotective effects. Researchers can prioritize targets and analogues for experimental validation by using docking scores, which offer a relative assessment of the binding affinity between baicalein and certain protein targets. Docking studies reveal the preferred orientation of baicalein within the active site of the target, providing insights into key molecular interactions (e.g., hydrogen bonding, π-π stacking). These insights are crucial for structure-based drug design and rational modification of baicalein derivatives to enhance efficacy (50-52).

While molecular docking provides valuable preliminary insights into the potential interactions between baicalein and target proteins, it is imperative to recognize its limitations. Despite its utility, molecular docking has several inherent limitations that must be considered when interpreting the docking score of baicalein. Docking scores should be interpreted with caution and validated through experimental studies to ensure accurate predictions of biological activity.

Conclusion

The study demonstrates the potential of baicalein as an effective anti-keloid scar agent by targeted interaction with the TGF-βRI and TGF-βRII receptors, essential for tissue healing and scar formation. Strong binding affinities were observed between baicalein and these target proteins via in silico docking experiments, suggesting its capability to modulate critical signalling pathways implicated in keloid pathogenesis.

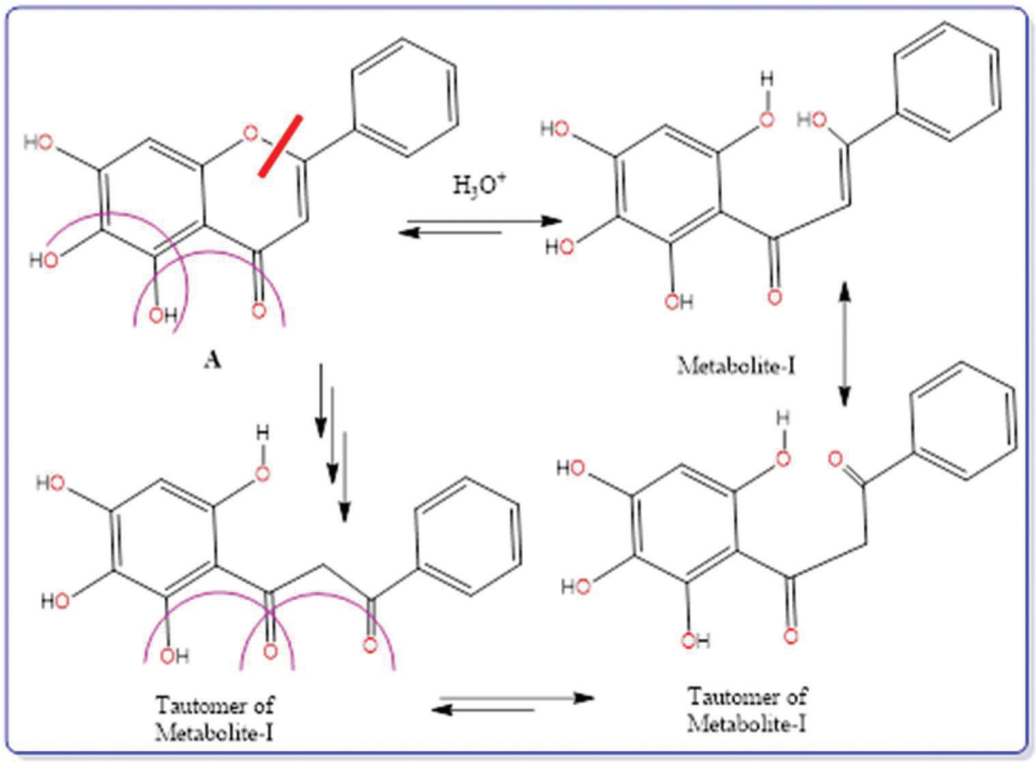

FIGURE 4 - Proposed mechanism of opening/closing the central ring leading to regeneration of the antiviral pharmacophore sites for compound baicalein.

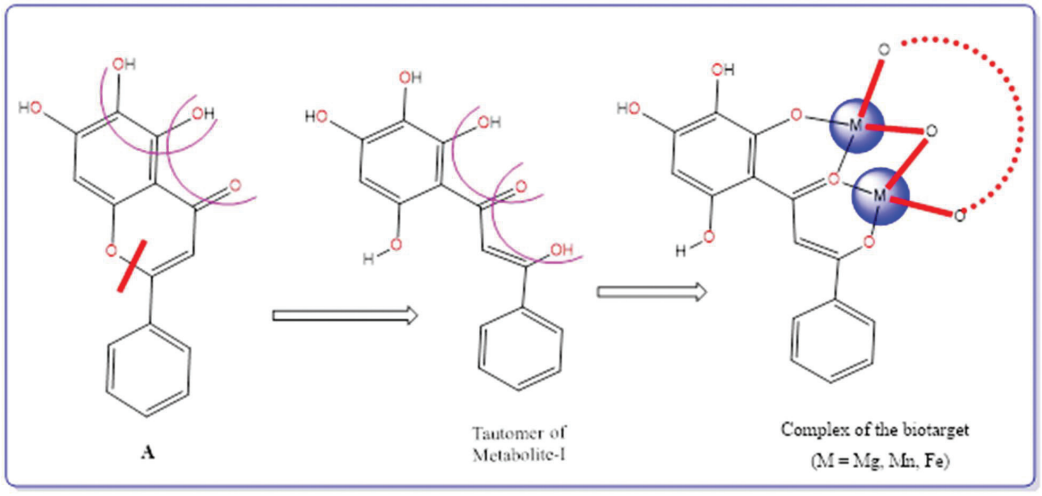

FIGURE 5 - Plausible mechanism of action of baicalein in the scar management and fibrotic disorders, showing its action as an anti-HIV drug.

Baicalein's potential as a therapeutic agent is further reinforced by its favourable ADME profile and drug-like properties. However, further *in vitro* and *in vivo* studies are necessary to elucidate its precise mechanisms of action and validate its safety and efficacy for clinical application. This study lays a promising foundation for the development of baicalein-based therapies for keloid scars and other fibrotic disorders. Moreover, the study highlights the significance of natural compounds in addressing the limitations of traditional keloid treatments, which are often associated with severe side effects and inconsistent results.

Notably, the current understanding of keloid formation remains incomplete, involving a complex interplay of genetics and the immune response. The role of viral triggers is a particularly intriguing and underexplored area, warranting further investigation to establish any causal relationship between viral infections and keloid development.

Given this context, it becomes both urgent and necessary to explore the possible link between antiviral and anti-keloid activities. This could be achieved by investigating the coordination behaviour of baicalein with various transition metals (e.g., Cu, Ni, Zn, Pt, Pd, Mn, Mg, Ru) and evaluating their catalytic bioactivity and enzyme inhibition potential. Further research is needed to clarify its mechanisms and explore its role in antiviral-based keloid prevention, particularly if a viral link is confirmed.

Furthermore, while molecular docking scores serve as valuable tools in the early stages of drug discovery, providing insights into potential targets and interaction mechanisms, their predictive value is limited by both computational and biological constraints. Therefore, docking outcomes should be interpreted with caution and always corroborated with experimental validation.

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