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RESEARCH PAPER

Network pharmacology and molecular docking simulation uncovered the potential of hexacyclinic acid as anti-osteoarthritis by regulating IL-17 signaling pathway

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Abstract: Hexacyclinic acid has shown promising pharmacological activities, yet its molecular mechanisms and therapeutic potential remain largely unexplored. This study aimed to identify potential disease targets and elucidate the mechanism of action of hexacyclinic acid using an integrated computational approach. We employed network pharmacology analysis to predict potential targets and pathways of hexacyclinic acid using SuperPred and Swiss Target server, followed by protein-protein interaction network construction via STRING database. Pathway enrichment analysis was performed using ShyniGO and DAVID databases. Molecular docking studies were conducted using AutoDock Vina to evaluate binding affinities between hexacyclinic acid and identified target proteins. Binding poses and interactions were visualized using Biovia Discovery Studio Visualizer. Disease prediction analysis identified osteoarthritis as the most promising target, with the IL-17 signaling pathway emerging as the most significant KEGG pathway. TNF-α and IL-1β were identified as key molecular targets within this pathway. Molecular docking simulations corroborated these findings, revealing favorable binding energies between hexacyclinic acid and TNF-α (-8.62 kcal/mol) and IL-1β (-8.76 kcal/mol). These results suggest that hexacyclinic acid may exert its anti-osteoarthritis effects by modulating the IL-17 signaling pathway, particularly through interactions with TNF-α and IL-1β. The strong binding affinities observed indicate a potentially high efficacy of hexacyclinic acid in targeting these inflammatory mediators. These results have significant clinical implications, potentially leading to the development of new therapeutic strategies for osteoarthritis management with reduced side effects compared to current treatments. Future research should focus on experimental validation through in vitro and in vivo models to confirm these computational predictions and establish hexacyclinic acid as a viable candidate for clinical development.

Keywords: Hexacyclinic acid, Network pharmacology, Molecular docking, Osteoarthritis, IL-17 signaling pathway

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INTRODUCTION

In the realm of natural product research, marine-derived compounds have emerged as a rich source of novel bioactive molecules with diverse pharmacological properties [1,2]. Among these, hexacyclinic acid (**Figure 1**), isolated from the marine actinomycete *Streptomyces cellulosae* subsp. *griseorubiginosus* (strain S1013), has garnered significant attention due to its unique

Acta. Chim. Asiana., 2025, 8(1), 584 – 598

structural features and promising biological activities [3]. This complex polyketide compound, characterized by its distinctive hexacyclic structure, represents a fascinating subject for pharmacological investigation [4]. Hexacyclinic acid's structural complexity, featuring a highly oxygenated hexacyclic carbon skeleton, has intrigued organic chemists and pharmacologists alike [5]. Its unique molecular architecture suggests potential for diverse biological interactions, making it an attractive candidate for drug discovery efforts [6]. The elucidation of its structure through various spectroscopic methods has paved the way for further studies into its pharmacological properties [7,8].

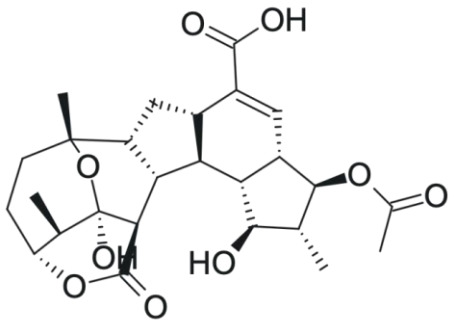

Figure 1. Chemical structure of hexacyclinic acid

To date, the primary focus of research on hexacyclinic acid has been its cytotoxic activity. Investigations have revealed promising antitumor properties against various cancer cell lines, opening new avenues for potential oncological applications. In vitro experiments have demonstrated its ability to inhibit the growth of several human cancer cell lines, including those derived from gastric (HM02), hepatocellular (HEPG2), and breast cancers (MCF-7), with GI50 values up to 14.0 μmol/L [3,4]. These findings suggest a broad spectrum of anticancer activity that warrants further investigation. While the cytotoxic properties of hexacyclinic acid have been the primary focus of research thus far, it is important to note that its full pharmacological profile remains largely unexplored. The compound's complex structure and unique origin suggest potential for other biological activities that have yet to be discovered or thoroughly investigated.

Despite the growing interest in hexacyclinic acid, there remains a significant gap in our understanding of its mechanisms of action, potential targets, and full spectrum of biological activities. While initial studies have provided valuable insights into its cytotoxic properties, the compound's effects on other physiological systems, particularly inflammatory pathways implicated in degenerative joint diseases, remain largely unexplored. Osteoarthritis, a debilitating condition affecting millions worldwide, involves complex inflammatory cascades where IL-17 signaling has emerged as a critical mediator of cartilage degradation and disease progression. Recent evidence suggests that IL-17 promotes chondrocyte catabolism, synovial inflammation, and subchondral bone remodeling, key pathological features of osteoarthritis. Given hexacyclinic acid's structural complexity and potential for diverse biological interactions, investigating its effects on IL-17 signaling pathways could reveal novel therapeutic applications for osteoarthritis management. Therefore, this study was employed to comprehensively investigate the broader pharmacological potential of hexacyclinic acid, with particular focus on its anti-osteoarthritic properties. By integrating network pharmacology and molecular docking simulation, we seek to identify novel therapeutic targets and elucidate the molecular mechanisms underlying its potential inhibition of IL-17-mediated inflammatory processes in osteoarthritis.

MATERIALS AND METHODS

Tools

In silico studies were employed using a unit of personal computer equipped with a 12th Gen Intel® Core™ i9-12900KF (24 CPUs) 3.2 GHz, coupled with 32 GB of RAM and 16 GB of NVIDIA GeForce RTX 4060 VGA, run in Windows 11 Pro operating system. The study used several software including Autodock 4.2.6 assisted by ADT interface provided by The Scripp Research Institute (https://autodock.scripps.edu), Biovia Discover Studio Visualizer 2021 version provided by Dassault Systemes (https://discover.3ds.com), Notepad++ 8.7 version provided by Mr. Don HO (https://notepad-plus-plus.org), Cytoscape 3.10.1 version provided by Cytoscape Consortium (https://cytoscape.org), and ChemDraw Ultra 12.0 version provided by Cambridge Soft.

Network Pharmacology Study

Target identification of hexacyclinic acid

The chemical structure of hexacyclinic acid was represented using its Simplified Molecular-Input

Line-Entry System (SMILES) notation, which was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). This SMILES representation was then used as input for two different target prediction tools: Super-PRED (https://prediction.charite.de/) [9] and SWISS Target Prediction (http://www.swisstargetprediction.ch/) [10]. These tools were employed to forecast potential molecular targets of hexacyclinic acid. In all cases, Homo sapiens was specified as the target organism for data collection. To ensure consistency and remove any redundant entries, the protein identifiers were standardized and aligned using UniProt IDs (https://www.uniprot.org/).

Protein-Protein Interaction (PPI) Network Construction

The previously identified proteins were subjected to further analysis using the STRING database (https://string-db.org) [11], employing its multiple protein feature capability. The analysis was configured with the following parameters: the organism was set to Homo sapiens, a full STRING network was selected, a high confidence score threshold of 0.400 was applied, and a medium false discovery rate (FDR) stringency of 5% was used. The resulting PPI data was then exported in TSV format using the "explore" option. Subsequently, this data was imported into Cytoscape 3.10.1 software to visualize and construct the PPI network, allowing for a comprehensive graphical representation of the protein interactions.

Network Construction and Topological Analysis

Cytoscape 3.10.1 software was utilized to conduct a topological analysis of the protein-protein interaction (PPI) data, which was imported in TSV format. The software's network analyzer tool was employed to examine the network topology parameters. In the resulting visualization, nodes represent targets, pathways, and compounds, while edges illustrate their interactions. Node significance is quantified by its degree centrality - the number of direct connections it has to other nodes. This metric serves as a proxy for the node's influence within the network, with highly connected nodes presumed to play more crucial roles in the biological system under study. Th 587 ach allows for a comprehensive understan the network's structure and the identification o /ers in the molecular interactions surrounding hexacyclinic acid's potential effects.

Disease Analysis, Gene Ontology Biological Process, and KEGG pathway

Disease predictions were generated by inputting the core targets into the DAVID database (https://david.ncifcrf.gov) [12]. Subsequently, a comprehensive analysis of the identified targets' functional roles was conducted using the ShinyGO 0.80 tool (http://bioinformatics.sdstate.edu/go/) [13]. This analysis was extensive, encompassing various Gene Ontology (GO) categories – including biological processes and molecular functions – as well as KEGG pathways. To ensure statistical rigor, a false discovery rate (FDR) threshold of 0.05 was applied throughout the analysis. The resulting data were then transformed into visually compelling bar plot maps.

Molecular Docking Study

Protein and Ligand Selection

Crystallographic data for the macromolecules were sourced from the Protein Data Bank (https://www.rcsb.org). PDB IDs were chosen based on specific criteria, including a resolution lower than 2.5 Å, absence of mutations, and the presence of a native ligand. The PubChem database (https://pubchem.ncbi.nlm.nih.gov) provided the 3D structures of hexacyclinic acid and various protein inhibitors. These protein inhibitors served as benchmarks for performing and evaluating the results of the molecular docking simulation.

Protein and Ligand Preparation

The macromolecule structures retrieved previously from Protein Data Bank underwent preparation in Biovia Discovery Studio Visualizer to eliminate water, ligands, and unnecessary components and chains. Additionally, the hydrogen atoms were added to the macromolecule structures. On the other hand, ligands (hexacyclinic acid and protein inhibitors) structure underwent structure optimization in Chem3D Pro through MM2 energy minimization. The Ligands in PDB format from Chem3D Pro were further processed in Autodock to set the center nodes and rotatable bonds and saved into PDBQT format.

Molecular Docking Simulation

A molecular docking study was performed using hexacyclinic acid as the ligand and the main targets from the network pharmacology analysis as macromolecules. The study utilized Autodock

4.2.6, which is integrated into ADT interface software. Prior to simulation, validation of the docking protocol was conducted by re-docking the native ligand with adjusting the grid box to encompass binding site residues. The docking process focused on the active site of macromolecules following the binding site of their native ligand, with the grid-box size adjusted to a spacing of 0.375 Å. A flexible-rigid docking approach was used, employing the Genetic Algorithm with 100 runs and default crossover and mutation rates. The Lamarckian GA was chosen as the output method. The best conformation of the re-docking native ligand was selected and compared to the original native ligand. The similarity was analysis based on the RMSD value, with value of <2.0 Å was set as a threshold of validity. The validated protocols were then applied to docking simulation of hexacyclinic acid in the similar steps. To ensure reliability, the molecular docking process was repeated ten times. The best pose was selected for further analysis and visualization using Biovia Discovery Studio Visualizer.

Drug-likeness Properties Assessment

The drug-likeness properties of hexacyclinic acid were assessed based on the Lipinski's rule of five by observing its physicochemical properties including number of H donor, H acceptor, LogP and molecular weight [14]. Molsoft: molecules in silico (https://molsoft.com) was used to assess drug-likeness of hexacyclinic acid.

Pharmacokinetics and Toxicological Assessment

ADMET evaluation was conducted using the pkCSM prediction server (https://biosig.lab.uq.edu.au/pkcsm). The process involved entering the canonical SMILES of hexacyclinic acid, which were obtained from the PubChem database, into the SMILES string column on the pkCSM web server. The result was interpreted based on the theory described by Pires et al. (2015) [16].

RESULTS AND DISCUSSION

Network Pharmacology Analysis

The network pharmacology study of hexacyclinic acid yielded significant insights into its potential therapeutic mechanisms and targets. Gene mining from super-PRED and Swiss Target databases identified 183 unique target genes, which were subsequently subjected to protein-protein interaction (PPI) network construction and topological analysis. This analysis revealed ten core targets with high degrees of connectivity: TNF, IL1B, ESR1, JUN, PTGS2, NFKB1, MMP9, GSK3B, and MAPK1, as depicted in **Figure 2**. Disease identification based on these core targets highlighted osteoarthritis (OA) as the most probable therapeutic target for hexacyclinic acid, demonstrating the highest fold enrichment score (**Figure 3a**). KEGG pathway analysis implicated the IL-17 signaling pathway as a potential mechanism through which hexacyclinic acid may modulate OA pathogenesis (**Figure 3b**). Gene Ontology (GO) analysis further elucidated the compound's multifaceted effects, indicating its involvement in regulating responses to exogenous and endogenous stimuli, including oxygen-containing, organonitrogen, and nitrogen compounds. Additionally, GO analysis revealed hexacyclinic acid's influence on catalytic activity, inflammatory response, and anatomical structure morphogenesis. Complete GO enrichment result can be seen in **Figure 3c** – **d**.

To elucidate the interconnections between hexacyclinic acid, its primary molecular targets, and the related medical conditions, a network visualization was generated based on the identified core targets and associated diseases (see **Figure 4**). This visual representation serves to illustrate and clarify the complex relationships uncovered in the study. The IL-17 signaling pathway mechanism, implicated in the study, was extrapolated and refined based on the canonical pathway map available in the KEGG database (https://www.kegg.jp/pathway/hsa04657).

The implication of the IL-17 signaling pathway as a potential mechanism of action for hexacyclinic acid in OA is particularly noteworthy. IL-17 has been increasingly recognized as a key pro-inflammatory cytokine in OA progression [17,18]. Recent studies have shown that IL-17 promotes cartilage degradation and synovial inflammation in experimental OA models [19]. Several core targets, including TNF, IL1B, PTGS2 (COX-2), MMP9, and MAPK1, involved in IL-17 signaling pathway, are well-known to play crucial roles in OA pathogenesis [20,21]. This multi-target profile offers intriguing insights into the compound's potential therapeutic mechanisms and implications for OA treatment. TNF-α and IL-1β, pro-inflammatory cytokines central to OA progression, stimulate the production of matrix-degrading enzymes, inhibit matrix synthesis, and promote chondrocyte apoptosis [22–24].

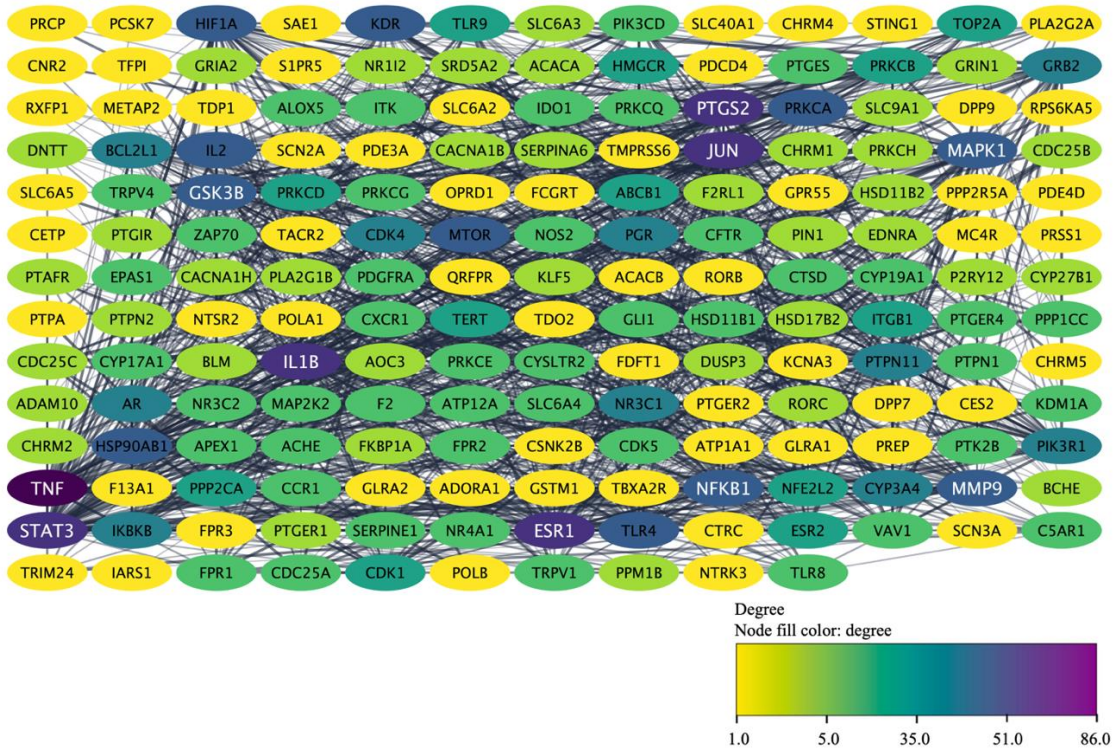

Figure 2. Protein-protein interaction network of all target genes.

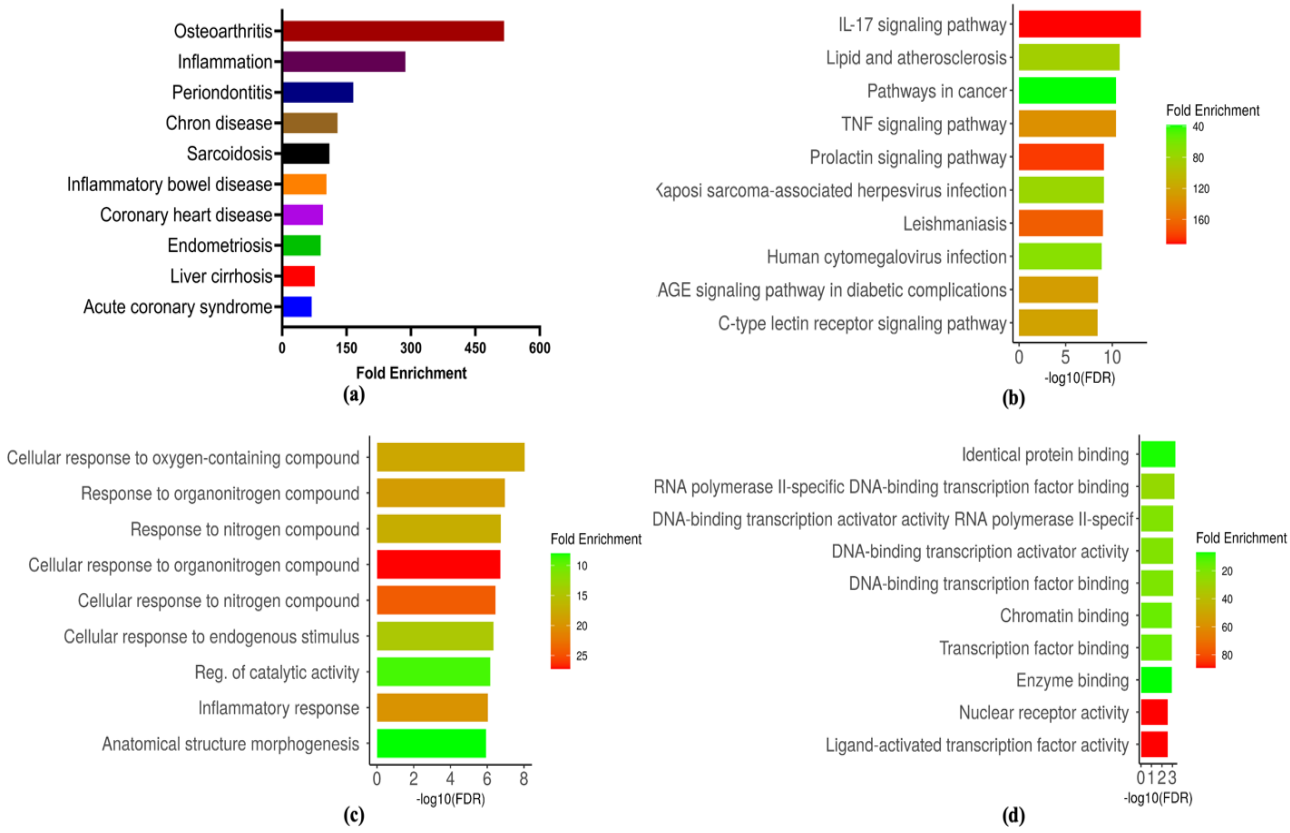

Figure 3. Enrichment analysis. (a) Disease prediction, (b) KEGG pathway, (c) GO biological process, and (d) GO molecular function related to 10 core targets.

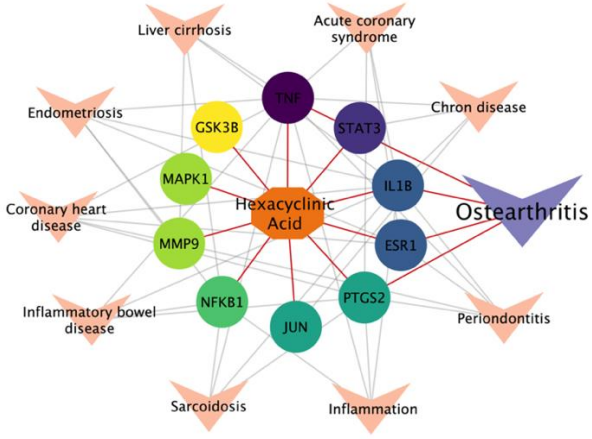

Figure 4. Network diagram of hexacyclinic acid-core targets-diseases. The color of the core target nodes transitions from yellow to purple, indicating a shift from low to high degree values in the network.

The identification of these targets suggests that hexacyclinic acid may exert significant anti-inflammatory effects. However, it's worth noting that while TNF-α inhibitors have shown efficacy in rheumatoid arthritis, their effectiveness in OA has been limited [25], highlighting the complex nature of OA and the potential advantage of hexacyclinic acid's multi-pathway approach. The targeting of PTGS2 (COX-2), a key enzyme in prostaglandin biosynthesis and the target of NSAIDs, indicates potential analgesic and anti-inflammatory properties similar to current OA treatments but possibly with a broader mechanism of action [26–28]. MMP9, involved in the degradation of cartilage extracellular matrix components, represents another critical target. Its modulation by hexacyclinic acid suggests a potential chondroprotective effect that could slow disease progression [29,30]. The identification of MAPK1 (ERK2) as a core target further emphasizes the compound's multi-faceted approach, as the MAPK/ERK pathway regulates various cellular processes relevant to OA, including inflammation, apoptosis, and matrix degradation [31,32]. This multi-target profile aligns with the complex pathophysiology of OA and may offer advantages over single-target therapies by simultaneously modulating inflammatory cytokines, matrix-degrading enzymes, and key signaling pathways [33,34].

Molecular Docking Simulation

The docking results of hexacyclinic acid with various targets using a validated protocol (see **Figure 5**) reveal interesting interactions. TNF-a and IL-1B demonstrate the strongest binding affinity, both with free binding energies of -8.62 kcal/mol and 8.76 kcal/mol respectively, while PTGS2 shows a weaker interaction at -5 kcal/mol (**Figure 6**). Hexacyclinic acid demonstrates quite distinct type of molecular interaction with essential amino acid residues to each target macromolecule.

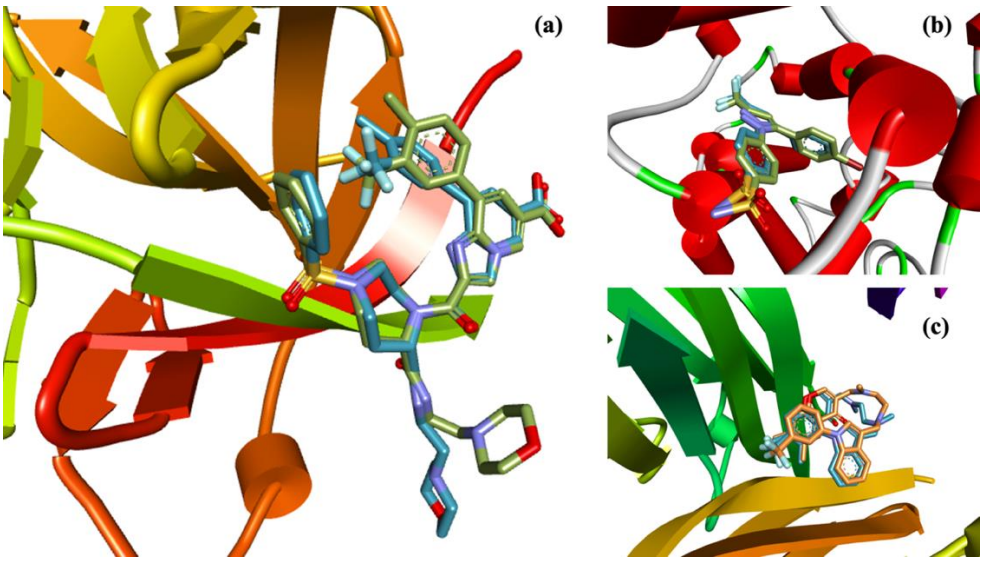

Figure 5. Superimposed of re-docking (blue) and original native ligand from protocol validation. (**a**) IL-1β (RMSD 1.539 Å), (**b**) COX-2 (RMSD 0.831 Å), and (**c**) TNF-α (RMSD 0.980 Å).

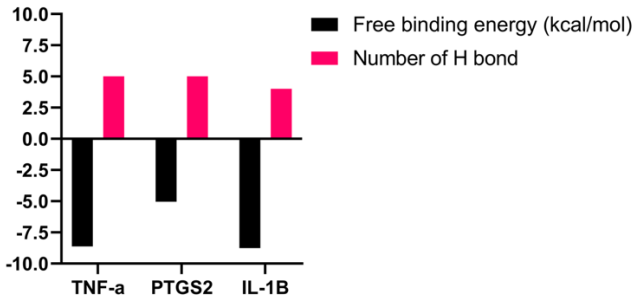

Figure 6. Docking score of Hexacyclinic acid on several core targets.

As illustrated in **Figure 7 – 9**, hexacyclinic acid tend to form more hydrogen bond and has less hydrophobic interaction in TNF-α and IL-1 β. This suggests that polar interactions dominate these complexes, potentially leading to more specific and stable binding. The prevalence of hydrogen bonding in these interactions aligns with research indicating the importance of such bonds in stabilizing protein-ligand complexes and enhancing binding specificity [35]. These strong, directional interactions may contribute to the compound's potential anti-inflammatory effects by modulating TNF-α and IL-1β activity, key mediators in inflammatory processes [36]. Furthermore, hexacyclinic acid demonstrates significant binding interactions with key inflammatory cytokines. In the case of TNF-α, this compound forms interactions with several critical amino acid residues, specifically Tyr 151, Gly 121, and Tyr 119, all located within the TNF-α binding site [37]. Similarly, hexacyclinic acid exhibits binding activity with IL-1β through interactions with essential amino acids at its active site. Of particular significance is Lys 103, which has been identified by Vulpetti et al. [38] as a strategic target residue for developing covalent, low-molecular-weight IL-1β antagonists.

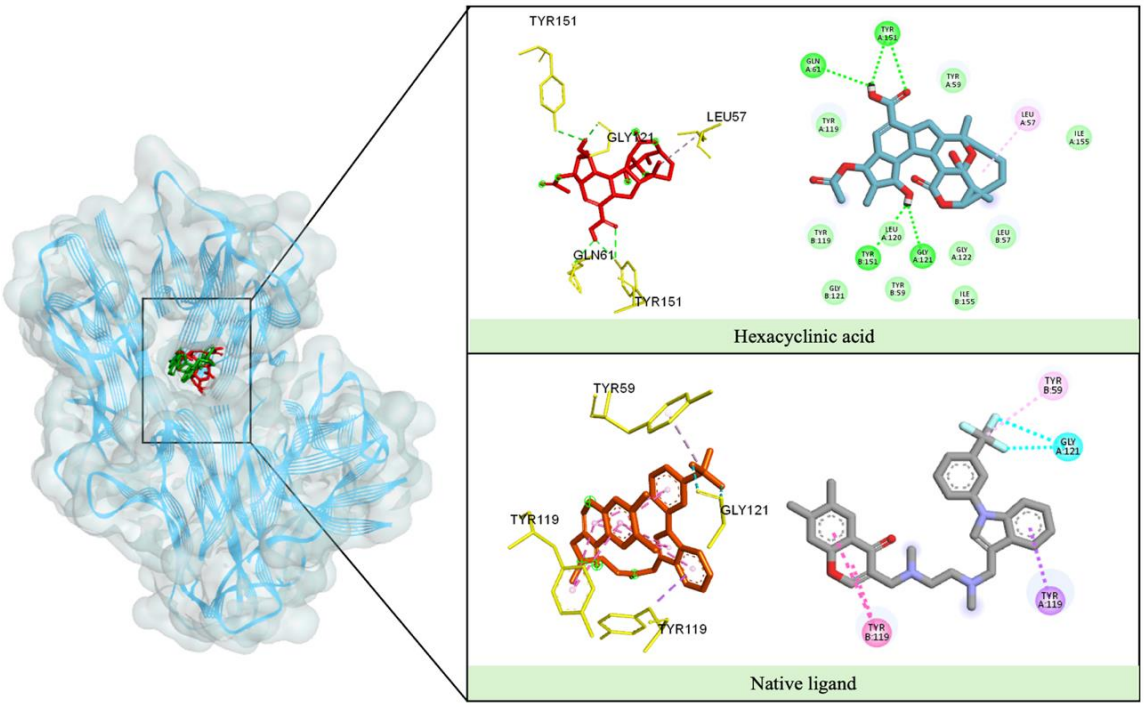

Figure 7. Molecular interaction of Hexacyclinic acid and native ligand with essential amino acid residues of TNF-α.

In contrast, the interaction with PTGS2 (also known as COX-2) shows a different pattern. The weaker binding energy coupled with a similar number of hydrogen bonds (similar to TNF-a) suggests that other factors are at play. **Figure 9** illustrated an increase in hydrophobic interactions, pi-sigma, pi-alkyl, and pi-lone pair interactions with essential amino acids, including Tyr 355 and Ala 527, which should be in hydrogen interaction for PTGS2 provides insight into this discrepancy [39]. While these interactions contribute to binding, they are generally weaker and less directional than hydrogen bonds [40]. The prevalence of these interactions in the PTGS2 complex might explain the reduced binding energy despite the similar number of hydrogen bonds. The distinct interaction profile with PTGS2 could have implications for the compound's activity. COX-2 inhibitors often rely on a balance of polar and non-polar interactions to achieve selectivity and potency [41]. The increased hydrophobic and pi interactions observed with hexacyclinic acid might not be optimal for COX-2 inhibition, potentially explaining its decreased activity compared to its effects on TNF-α and IL-1β.

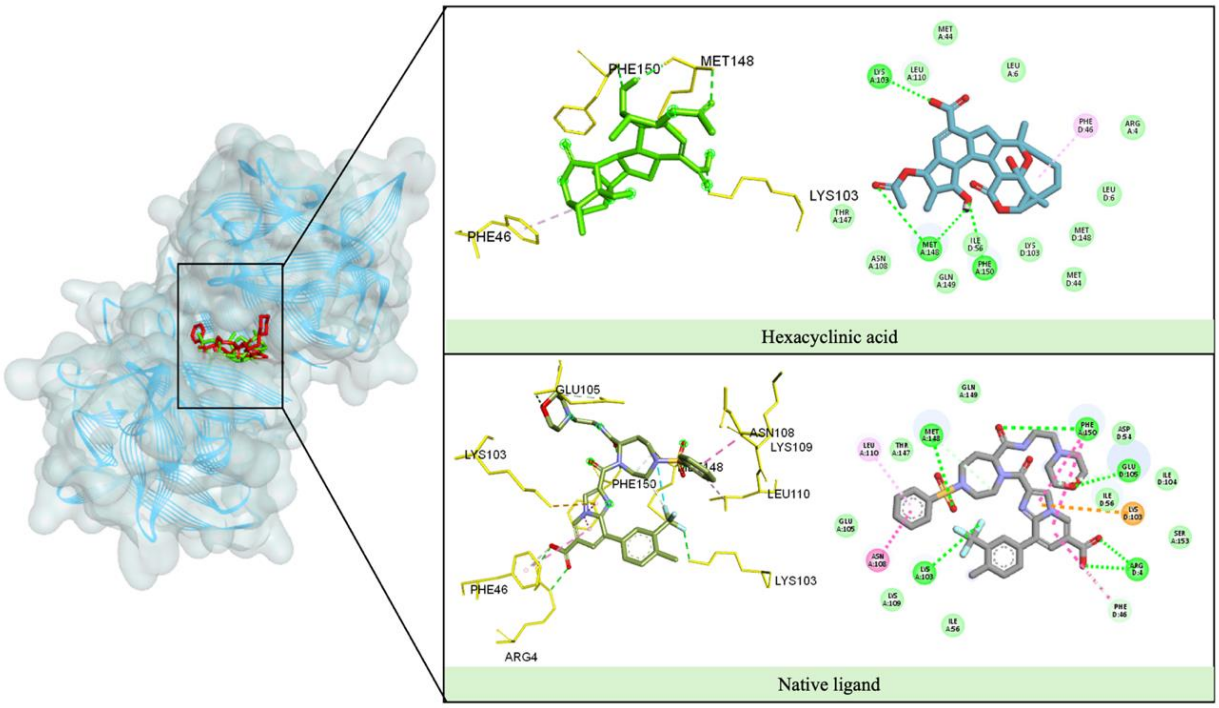

Figure 8. Molecular interaction of Hexacyclinic acid and native ligand with essential amino acid residues of ILB-1β.

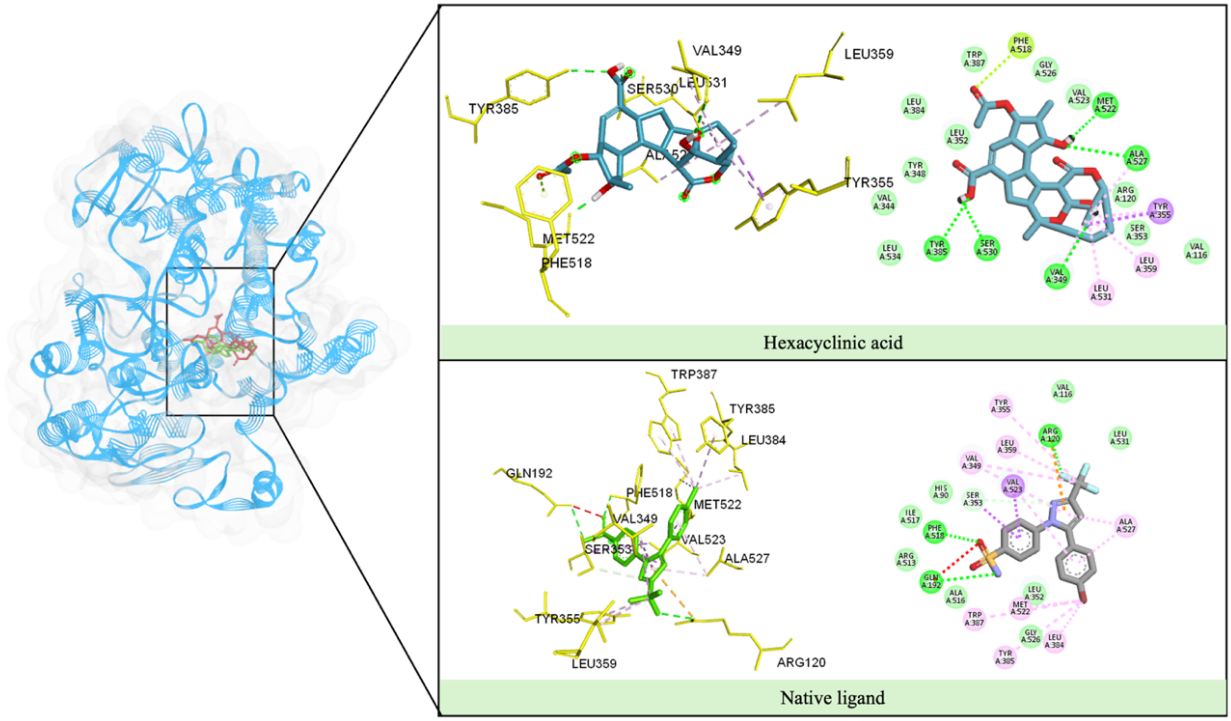

Figure 9. Molecular interaction of Hexacyclinic acid and native ligand with essential amino acid residues of COX-2.

Our computational analysis of hexacyclinic acid as a potential therapeutic agent for osteoarthritis provides valuable preliminary insights. However, several inherent limitations of computational approaches warrant acknowledgment and consideration. The target prediction methodology relies heavily on existing databases, which may contain knowledge gaps or biases toward well-studied proteins and pathways. This potentially limits our ability to identify novel or less-characterized targets. Additionally, the protein structures utilized for molecular docking simulations represent static conformations that may not fully capture the

dynamic nature of protein-ligand interactions occurring in biological systems.

The docking simulations, while informative, employ scoring functions that approximate binding energies and do not account for all physicochemical factors influencing ligand binding in vivo, such as solvent effects, entropy changes, and protein flexibility. Furthermore, our pathway analysis identifies potential mechanisms but cannot definitively establish causal relationships between hexacyclinic acid and observed therapeutic effects. Another significant limitation is that computational models cannot adequately reproduce the complex multicellular environment of osteoarthritic joints, where various cell types interact through multiple signaling pathways. The inflammatory processes in osteoarthritis involve intricate feedback loops and compensatory mechanisms that are difficult to model computationally.

To address these limitations and validate our computational findings, we propose several targeted experimental approaches. Cell-based assays using primary human chondrocytes and synoviocytes would provide direct evidence of hexacyclinic acid's effects on relevant cell types. These experiments should measure inflammatory cytokine production, cartilage matrix degradation, and activation of the IL-17 signaling pathway components.

Direct binding studies using biophysical techniques such as surface plasmon resonance or isothermal titration calorimetry would confirm the predicted interactions with TNF-α and IL-1β targets and provide quantitative binding parameters. These studies would validate the molecular docking results and establish structure-activity relationships. Functional assays measuring PTGS2 enzymatic activity would determine whether hexacyclinic acid directly inhibits this inflammatory mediator, as predicted by our analysis. Additionally, gene expression profiling in relevant cell types would comprehensively assess the compound's impact on inflammatory pathways beyond our computational predictions. Ex vivo models using cartilage explants or 3D tissue constructs could bridge the gap between computational predictions and in vivo efficacy by providing a more physiologically relevant environment while maintaining experimental control. These systems would allow evaluation of hexacyclinic acid's effects on cartilage degradation markers and tissue integrity.

Through this systematic validation approach, we can address the limitations of our computational methodology and establish a robust foundation for further development of hexacyclinic acid as a potential therapeutic agent for osteoarthritis.

Drug-likeness Analysis

The drug-likeness properties of hexacyclinic acid were evaluated using Lipinski's rule of five (RO5). Compounds adhering to Lipinski's RO5 criteria are considered to possess physicochemical properties similar to those of established oral medications [14]. As indicated in **Table 1**, hexacyclinic acid met all of these criteria, with the drug-likeness score was 0.30, suggesting that hexacyclinic acid exhibits drug-likeness properties comparable to conventional oral drugs. Applying the RO5 is crucial during the initial stages of identifying pharmacologically active lead compounds to ensure their drug-like properties [42]. Drug candidates that comply with RO5 criteria typically experience lower attrition rates in clinical trials, thus increasing their chances of reaching the market [43].

Table 1. Physicochemical and drug-likeness properties of Hexacyclinic acid

Molecular weight (g/mol)	490.22
H bond donor	3
H bond acceptor	9
Log P	1.84
Drug-likeness model score	**0.30**

Pharmacokinetics and Toxicological Assessment

An ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) evaluation was conducted to examine the pharmacokinetic and toxicological profiles of hexacyclinic acid. The interpretation of all data in this section was based on the pkCSM theory as described by Pires et al. (2015) [15]. The pharmacokinetic screening of hexacyclinic acid revealed generally excellent absorption profiles (**Table 2**). Hexacyclinic acid demonstrated fair intestinal absorption, as indicated by its Caco2 and Human Intestinal Absorption (HIA) values. Even though its Caco2 value was 0.472 (< 0.9), its HIA value was 65.452% (> 30%), which still

suggests good absorption. HIA is categorized into three levels based on molecular absorbance: high absorption (HIA > 80%), moderate absorption (HIA 30%-79%), and poor absorption (HIA <30%) [44]. Additionally, hexacyclinic acid showed high skin permeability, as evidenced by log Kp values < -2.5. Regarding P-glycoprotein (Pgp) interactions, hexacyclinic acid acts as Pgp substrates. These Pgp-related properties are important to consider, as they can influence the pharmacokinetics of other drugs through Pgp-mediated interactions [45].

Table 2. Absorption profile of Hexacyclinic acid

Parameters	Value	Units
Caco2 permeability	-0.11	log Papp in 10-6 cm/s
Human intestinal absorption	65.452	% Absorbed
P-glycoprotein substrate	Yes	Categorical (Yes/No)
P-glycoprotein I inhibitor	No	Categorical (Yes/No)
P-glycoprotein II inhibitor	No	Categorical (Yes/No)

Hexacyclinic acid generally exhibit low volumes of distribution (VDs), with values exceeding -0.509 log L/kg, as it classified by Pires et al (2015) [15] that compounds with VDs below -0.15 log L/kg are considered as having low distribution. The VD represents the apparent volume in which a drug is distributed, either in tissues or remaining in blood plasma [46]. Lower VD values indicate greater tissue distribution of the hexacyclinic acid. Hexacyclinic acid also demonstrate high plasma protein binding, as evidenced by their low fraction unbound (Fu) values, detailed in **Table 3**. Consequently, only a small portion of the hexacyclinic acid studied is actually active, which may significantly impact their pharmacodynamics. Regarding blood-brain barrier (BBB) permeability and central nervous system (CNS) penetration, hexacyclinic acid is unable neither to cross the BBB nor enter the CNS effectively. A compound is considered to cross the BBB and penetrate the CNS if its log BB is ≥ 0.3 and log PS is ≥ -0.2. As shown in **Table 3**.

Table 3. Distribution profile of Hexacyclinic acid

Parameters	Value	Units
VDss (human)	-0.509	log L/kg
Fraction unbound (human)	0.318	Fu
BBB permeability	-1.202	log BB
CNS permeability	-3.596	Log PS

The metabolic profiles of hexacyclinic acid was comprehensively evaluated through their interactions with various cytochrome P450 (CYP450) isoforms, key enzymes in hepatic drug metabolism. These interactions, whether as substrates or inhibitors, can significantly impact a drug's therapeutic efficacy and toxicity profile [47,48]. As illustrated in **Table 4**, hexacyclinic acid exhibits diverse effects across different CYP450 isoforms. Hexacyclinic acid is expected to induce CYP3A4 yet is not predicted neither to induce nor inhibit other CYP450 isoforms. CYP3A4 plays a crucial role in several important metabolic processes: it aids in the detoxification of bile acids, terminates the effects of steroid hormones, and facilitates the elimination of both phytochemicals from food and a wide range of pharmaceutical compounds. The induction of CYP3A4 can lead to an accelerated breakdown of these substances, including hexacyclinic acid itself or any other drugs that are CYP3A4 substrates when administered concurrently. This enhanced metabolic activity can result in significant alterations to the pharmacokinetic and pharmacodynamic profiles of these compounds. Consequently, this could affect their efficacy, duration of action, and potential interactions with other substances in the body, highlighting the importance of understanding CYP3A4 interactions in drug development and administration [49,50].

Table 4. Metabolism profile of Hexacyclinic acid

Parameters	Value	Units
CYP2D6 substrate	No	Categorical (Yes/No)
CYP3A4 substrate	Yes	Categorical (Yes/No)
CYP1A2 inhibitior	No	Categorical (Yes/No)
CYP2C19 inhibitior	No	Categorical (Yes/No)
CYP2C9 inhibitior	No	Categorical (Yes/No)
CYP2D6 inhibitior	No	Categorical (Yes/No)
CYP3A4 inhibitior	No	Categorical (Yes/No)

The excretion profile of hexacyclinic acid was evaluated by examining their total clearance and potential as renal organic cation transporter 2 (OCT2) substrates. These factors are crucial in determining drug bioavailability and half-life, which in turn influence dosing strategies and therapeutic regimens [51]. As shown in **Table 5**, hexacyclinic acid exhibit a total clearance exceeding 1 mL/min/kg, categorizing it as having high clearance based

on the established classification system (high: >1 mL/min/kg, medium: 0.1-1 mL/min/kg, low: ≤0.1 mL/min/kg) [52,53]. Additionally, hexacyclinic acid is predicted not to act as OCT2 substrates, a renal transporter vital for the elimination of endogenous molecules and drugs. This OCT2 substrate status raises the potential for drug interactions when co-administered with OCT2 inhibitors [54].

Table 5. Excretion profile of Hexacyclinic acid

Parameters	Value	Units
Total Clearance	0.176	log ml/min/kg
Renal OCT2 substrate	No	Categorical (Yes/No)

The toxicity profile of hexacyclinic acid was assessed to determine their impact on cellular and tissue health. Key parameters examined included lethal dose 50 (LD50), mutagenicity (AMES toxicity), and hepatotoxicity. **Table 6** shows that the LD50 values for hexacyclinic acid is 2.107 mol/kg. Considering its molecular weights (490.549 g/mol, as per **Table 1**), the LD50 values translate to approximately 1033.587 g/kg body weight. According to Loomis and Hayes (1996) [16], compounds with LD50 between 500-5000 mg/kg body weight are classified as less toxic. Furthermore, despite these relatively high LD50 values, hexacyclinic acid is also predicted as non-hepatotoxic and non-mutagenic.

Table 6. Toxicity profile of Hexacyclinic acid

Parameters	Value	Units
AMES toxicity	No	Categorical (Yes/No)
Oral Rat Acute Toxicity (LD50)	2.107	mol/kg
Hepatotoxicity	No	Categorical (Yes/No)

CONCLUSION

Our findings identified osteoarthritis as the primary disease target, with the IL-17 signaling pathway emerging as the most prominent KEGG pathway influenced by the compound. The analysis highlighted TNF-α, IL-1β, and PTGS2 as core target genes, suggesting a multi-faceted mechanism of action. Molecular docking simulations corroborated these results, demonstrating excellent binding affinities of hexacyclinic acid to TNF-α and IL-1β, key inflammatory mediators in osteoarthritis. This multi-target approach aligns with the complex nature of osteoarthritis and current therapeutic strategies. While these computational findings provide valuable insights, they underscore the need for further experimental validation to confirm hexacyclinic acid's effects on the IL-17 signaling pathway and its interactions with identified target proteins. Future research should include in vitro validation using human chondrocyte and synoviocyte inflammation models to assess the compound's effects on inflammatory mediator expression, as well as target engagement studies to confirm direct binding to TNF-α and IL-1β through techniques such as surface plasmon resonance or isothermal titration calorimetry.

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