UNIVERSITI TEKNOLOGI MARA

NETWORK PHARMACOLOGY AND MOLECULAR DOCKING-BASED STUDY ON THE ANTI-INFLAMMATORY AND ANTIOXIDANT MECHANISMS OF MORINDOLIDE

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CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 5 November 2025 to conduct the final examination of Muhammad Amal Bin Zulkipli on his Master of Science thesis entitled "Network Pharmacology and Molecular Docking-Based Study on The Anti-Inflammatory and Antioxidant Mechanisms of Morindolide" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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ABSTRACT

Global mortality and morbidity are high due to inflammatory diseases such as rheumatoid arthritis, cardiovascular disease and cancer. Pharmacological treatments that target specific molecules or pathways may not adequately address the complexity and multifactorial. By studying complex biological networks and discovering various therapeutic targets, network pharmacology offers a possible alternative. Network pharmacology may facilitate the design of targeted therapies and enhance therapeutic outcomes by identifying drug-target interactions and signalling pathways. Morindolide is a bioactive iridoid compound isolated and identified from the tuber of *Myrmecodia platytyrea*, a plant traditionally used to treat cancer and other inflammation-related diseases in Southeast Asia. Previous studies suggest that morindolide exhibits anti-inflammatory and antioxidant properties, reinforcing its potential as a lead compound for therapeutic development. Thus, this study employed network pharmacology and molecular docking to identify the mechanisms underlying these effects. Morindolide's target genes were identified using SymMap, Swiss Target Prediction and PharmMapper databases. Anti-inflammatory and antioxidant-related genes were retrieved from the GeneCards database. Common targets were identified through Venn analysis. A protein-protein interaction (PPI) network was constructed using STRING and Cytoscape to identify hub genes. GO function and KEGG pathway analyses were performed using DAVID and ShinyGO. Molecular docking between morindolide and hub proteins was conducted using AutoDock and visualised with LigPlot. The analysis revealed 56 common targets between morindolide and anti-inflammatory/antioxidant effects. Seven hub genes were identified as PTGS2, IL1B, MMP2, HSP90AA1, NOS2, PLA2GA and CYP2E1. GO analysis revealed morindolide's involvement in inflammatory responses, nitric oxide biosynthesis, and response to lipopolysaccharide. KEGG analysis highlighted pathways in cancer, arachidonic acid metabolism, IL-17 signalling and neurodegeneration. Molecular docking confirmed stable binding between morindolide and hub proteins, with binding energies ranging from -5.19 to -6.62 kcal/mol. Significant interactions were observed with CYP2E1 (-6.62 kcal/mol), MMP2 (-6.6 kcal/mol), and NOS2 (-6.24 kcal/mol). This study successfully identified the potential targets, biological processes and signalling pathways involved in morindolide's anti-inflammatory and antioxidant effects by utilising network pharmacology and molecular docking techniques. The findings provide a robust theoretical foundation for future experimental research, ultimately paving the way for developing novel therapeutic strategies and potential clinical applications of morindolide in treating inflammation-related disorders.

Keywords: Anti-Inflammatory; Antioxidant; Molecular Docking; Morindolide; Network Pharmacology

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This research represents a collaborative effort, and I am honoured to have worked under such distinguished supervision in advancing our understanding of morindolide's therapeutic mechanisms through computational approaches.

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Abbreviations

COX	Cyclooxygenase
CYP	Cytochrome P450
DAVID	Database for Annotation, Visualization and Integrated Discovery
GO	Gene Ontology
HSP90AA1	Heat Shock Protein 90 Alpha Family Class A Member 1
IL	Interleukin
KEGG	Kyoto Encyclopedia of Genes and Genomes
MCC	Maximal Clique Centrality
MMP	Matrix Metalloproteinase
NOS	Nitric Oxide Synthase
PLA2G2A	Phospholipase A2 Group IIA
PPI	Protein-Protein Interaction
TNF	Tumour Necrosis Factor
UniProt	Universal Protein Resource

CHAPTER 1
INTRODUCTION

1.1 Research Background

The persistent challenge of inflammatory diseases is a significant global health concern in contemporary medicine. The intricate relationship between inflammation and oxidative stress has emerged as a crucial area of research, as these interconnected processes contribute substantially to the pathogenesis of numerous disorders (Mesa-Garcia et al., 2018; Khanna et., 2014; Soodaeva et al., 2023). The complex interplay between inflammatory responses and oxidative stress mechanisms presents both challenges and opportunities in therapeutic development (Zuo et al., 2019). This connection has garnered increasing attention in the scientific community due to its fundamental role in various pathological conditions, including neurodegenerative diseases, cardiovascular disorders, cancer, chronic respiratory diseases and diabetes (Camps, 2014; Federico et al., 2012; Förstermann et al., 2017).

Inflammation is a critical biological defence mechanism deployed by the immune system against harmful stimuli, including pathogens and damaged cells. While this response is essential for survival, chronic inflammation can lead to tissue damage and disease progression (Netea et al., 2017). Similarly, oxidative stress, characterised by an imbalance between oxidants and antioxidants favouring the former, can disrupt cellular redox signalling and control mechanisms, resulting in molecular damage and cellular dysfunction (Soodaeva et al., 2023). Chronic inflammation can induce oxidative stress, thereby exacerbating inflammatory responses and creating a potentially damaging feedback loop (Soomro, 2019). Therefore, it is imperative to identify a molecule that can address this issue.

Natural compounds have recently gained significant attention as potential therapeutic agents due to their diverse biological activities and generally favourable safety profiles. Among these, morindolide, an iridoid lactone, has emerged as a promising candidate for investigation. This compound, isolated from various plant species including *Morinda officinalis* and *Catunaregam spinosa*, which have demonstrated notable pharmacological properties, particularly in the context of anti-inflammatory and antioxidant activities, specifically in nitric oxide inhibition and

decreased the stimulation of inflammation that was discovered through COX-2 and iNOS protein affinity interaction (Cai et al., 2021). The growing interest in morindolide, a bioactive iridoid lactone compound also isolated and identified from the tuber of *Myrmecodia platytyrea*, reflects a broader trend in pharmaceutical research toward utilising natural compounds as templates for drug development. Traditionally used in Southeast Asia to treat cancer and inflammation-related diseases, *M. platytyrea* has gained scientific attention for its potent antioxidant and anti-inflammatory properties. Morindolide, in particular, is believed to contribute to the plant's redox-modulating and chemopreventive effects, positioning it as a promising lead compound for therapeutic innovation (Haris et al., 2016).

Advanced computational methods have revolutionised the approach to understanding drug-target interactions and mechanisms of action (Ao et al., 2023). Network pharmacology and molecular docking techniques provide powerful tools for elucidating the complex mechanisms by which compounds like morindolide exert their therapeutic effects (Zhang et al., 2021). These tools allow systematic prediction of compound-target interactions, providing valuable insights into the molecular basis of therapeutic activities while significantly reducing the time and resources required for drug discovery and development (Mesarić, 2022).

1.2 Motivation for This Work

The motivation for this research stems from a dual knowledge gap in natural product pharmacology, which are: (1) the absence of a mechanistic understanding of morindolide, and (2) the lack of integrated network pharmacology and molecular docking computational approaches applied to it. While morindolide has demonstrated anti-inflammatory and antioxidant properties across various plant species, no previous study has systematically investigated this compound's therapeutic mechanisms using computational approaches. This represents a critical compound-specific knowledge gap, as morindolide's multi-target interactions and mechanistic pathways remain entirely unexplored through in silico methods.

Furthermore, although advanced computational methodologies, such as network pharmacology and molecular docking, have proven efficacious in elucidating the mechanisms of natural compounds, the integrated application of these dual methodologies has never been employed to investigate morindolide. This

methodological gap is particularly significant given morindolide's structural uniqueness as an iridoid lactone and its potential for polypharmacological effects. The synergistic combination of network pharmacology (providing systems-level insights) with molecular docking (providing atomic-level binding details) represents an unprecedented analytical framework for this specific compound, potentially revealing multi-target therapeutic mechanisms that single-method approaches would overlook.

This research, therefore, addresses two distinct but interconnected novelties: (1) it represents the first comprehensive computational investigation of morindolide's mechanisms of action, and (2) it establishes the first integrated network pharmacology-molecular docking framework specifically applied to this compound. This dual novelty positions the research at the forefront of computational drug discovery, contributing not only to understanding morindolide's therapeutic potential but also establishing a methodological template for investigating other understudied natural compounds with similar structural complexity and multi-target characteristics.

1.3 Problem Statement

Despite advancements in understanding the molecular basis of inflammation and oxidative stress-related disorders, developing effective therapeutic interventions remains a considerable challenge in modern medicine. The complex nature of these conditions, characterised by multiple interconnected pathways and mechanisms, often renders single-target therapeutic approaches insufficient. This complexity is further compounded by the limitations of current treatment options, which frequently present significant side effects or limited efficacy in managing chronic conditions. The need for novel therapeutic agents that can effectively modulate multiple pathways while maintaining favourable safety profiles has become increasingly apparent.

Furthermore, traditional experimental approaches to understanding drug mechanisms are often time-consuming, resource-intensive and may not fully capture the complexity of biological interactions (Newman & Cragg, 2020). While various studies have documented the therapeutic properties of iridoid compounds in managing inflammatory conditions, there is a notable absence of systematic investigations into morindolide's specific mechanisms of action (Gao et al., 2008; Haris et al., 2016; Tan et al., 2014; Yoshikawa et al., 1995). This knowledge gap extends to understanding how

morindolide might interact with multiple biological targets simultaneously, a characteristic that could potentially enhance its therapeutic efficacy.

The application of advanced computational methods, notably network pharmacology and molecular docking, has demonstrated efficacy in elucidating the mechanisms of natural compounds (Jiao et al., 2020). Critically, morindolide represents a compound-specific knowledge gap in contemporary pharmacological research. To date, no computational study has systematically investigated morindolide's mechanisms of action using network pharmacology, molecular docking, or any integrated in silico approach. This absence is particularly striking given the compound's demonstrated biological activities and its presence across multiple medicinally important plant species. While computational drug discovery methods have been extensively applied to other iridoid compounds and natural products, morindolide has remained entirely unexplored through these powerful predictive methodologies. This compound-specific gap, combined with the absence of an integrated network pharmacology-molecular docking framework for this molecule, creates a dual void in the scientific literature that fundamentally limits our understanding of morindolide's therapeutic potential and hinders its progression toward clinical development.

1.4 Research Objectives

This research aims to elucidate the molecular mechanisms underlying morindolide's anti-inflammatory and antioxidant properties using an integrated computational approach. The specific objectives are as follows to:

a) identify potential targets associated with the anti-inflammatory and antioxidant activities of morindolide through network pharmacology analysis.
b) investigate the primary molecular targets and signalling pathways involved in these activities by constructing and analysing protein-protein interaction networks.
c) elucidate morindolide's mechanisms of action using molecular docking approaches by examining its binding patterns and interactions with target proteins.

1.5 Research Question

This study addresses the following research questions:

i) What are the key therapeutic targets linked to the anti-inflammatory and antioxidant effects of morindolide, as revealed through network pharmacology-based analysis of its molecular interactions and biological pathways?
ii) Which molecular targets and signalling pathways are most significantly associated with morindolide's anti-inflammatory and antioxidant activities, as identified through protein-protein interaction (PPI) network analysis?
iii) How does morindolide interact with key target proteins at the molecular level and what do its binding affinities and docking patterns reveal about its anti-inflammatory and antioxidant action mechanisms?

1.6 Significance of Study

This study makes two distinct and significant contributions to the field of natural product pharmacology. First, it establishes the first computational investigation of morindolide, addressing a critical compound-specific knowledge gap in the literature. Before this work, the mechanisms of action of morindolide remained entirely unexplored through computational approaches, despite its demonstrated anti-inflammatory and antioxidant activities, as well as its traditional medicinal applications across Southeast Asia. Second, this research represents the first integration of network pharmacology and molecular docking methodologies specifically applied to morindolide, establishing a novel analytical framework that captures both systems-level interactions and atomic-level binding mechanisms.

By integrating network pharmacology approaches to reveal morindolide's multi-target interactions and signalling pathway modulation, and utilising molecular docking analyses to provide atomic-level binding insights at key target proteins, the research elucidates the mechanistic foundations for morindolide's therapeutic effects that were previously inaccessible. The findings contribute essential knowledge to understanding morindolide's natural product binding mechanisms, revealing novel therapeutic targets and offering foundational data for future experimental validation and clinical

development. This framework advances the pharmacological profiling of morindolide specifically, while providing a scalable methodological model for investigating other understudied phytochemicals with similar structural complexity. Moreover, the study bridges traditional natural product applications of *Myrmecodia platytyrea* with evidence-based drug discovery, reinforcing the role of compound-specific computational tools in accelerating the development of plant-derived therapeutics. The study also contributes to establishing standards for research in natural product pharmacology. It provides actionable insights that can guide experimental validation, structure-activity relationship studies and therapeutic development for morindolide specifically.

1.7 Scope of Study

This study investigated the therapeutic potential of morindolide, a plant-derived bioactive compound, by applying network pharmacology and molecular docking techniques. The research focused on identifying morindolide's active constituents, predicting its molecular interactions with selected target proteins and elucidating its mechanisms of action, particularly those related to anti-inflammatory and antioxidant pathways.

Using *in silico* approaches, the study simulated morindolide's binding affinities and interaction profiles, offering insights into its pharmacological relevance and multi-target capabilities. Although the findings were computational and required further experimental validation, the study provided a strategic foundation for future preclinical research and supported morindolide's candidacy in natural product-based drug discovery. The scope was intentionally directed toward specific proteins and pathways, contributing to a deeper understanding of morindolide's role in addressing inflammation-related diseases and advancing early-stage therapeutic development.

CHAPTER 2
LITERATURE REVIEW

2.1 Morindolide

2.1.1 Chemical Properties and Structure

Morindolide represents a significant class of organic compounds known as iridoid lactones, characterised by their distinct chemical structure and properties. This compound (Figures 2.1 and 2.2), with the molecular formula C9H1203, exhibits specific physicochemical properties that contribute to its biological activity. The structural configuration of morindolide includes a cyclopentane ring fused to a six-membered oxygen-containing heterocycle, forming the characteristic iridoid skeleton (PubChem, 2024). A lactone moiety further modifies this basic framework, which plays a crucial role in its biological activities and interactions with target proteins.

The compound's physical properties include a boiling point of 277°C and a melting point of 69-70°C, with a density of 1.17 g/cm^3. These properties significantly influence its behaviour in biological systems and potential pharmaceutical applications. Specific functional groups within the molecule, particularly the lactone ring and hydroxyl groups, serve as key interaction points for binding to biological targets and contribute to its overall reactivity profile (Cai et al., 2021; PubChem, 2024).

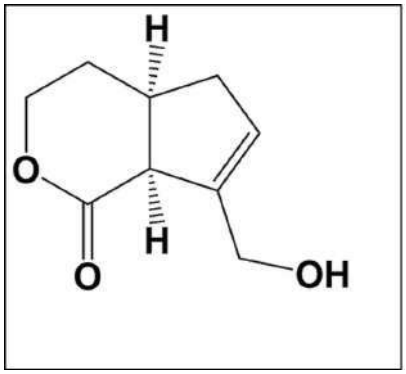


Figure 2.1 2D Structure of Morindolide [C9H12O3, (4aR,7aS)-7-(hydroxymethyl) 4,4a,5,7a-tetrahydro-3H-cyclopenta(c)pyran-l-one]

Figure 2.2 3D Structure of Morindolide

Notes/Sources: (PubChem, 2024)

2.1.2 Natural Sources and Traditional Uses

Morindolide demonstrates significant distribution across various plant species, with a particularly notable presence in the Rubiaceae family. The initial isolation of this compound was achieved from Morinda officinalis How (Rubiaceae), specifically from Radix Morindae (the dried root), where it occurs alongside morofficinaloside and several other bioactive compounds, including anthraquinones, iridoid glucosides, monoterpene glycosides, sterols, and triterpenes (Yoshikawa et al., 1995). The co-occurrence of these compounds suggests potential synergistic interactions that may contribute to the plant's therapeutic effects.

The compound's presence extends beyond Morinda species, with significant findings in *Catunaregam spinosa* (Chinese mangrove), where morindolide exists within a complex phytochemical matrix including two novel compounds and six known bioactive agents (Gao et al., 2008). This distribution pattern suggests evolutionary conservation of morindolide synthesis across related plant species, potentially indicating its ecological significance. The identification of morindolide in *Villaria odorata*, a Philippine endemic Rubiaceae species, further demonstrates the compound's widespread occurrence within this plant family and suggests potential regional variations in its biological activity (Tan et al., 2014).

Traditional medicinal applications of morindolide-containing plants have been extensively documented across various cultural contexts. *Morinda citrifolia,* commonly known as Indian noni or Indian mulberry, has been employed in traditional medicine systems for treating various conditions, including cancer, hypertension and cervical

spondylosis (Singh & Sharma, 2019). The plant's therapeutic applications extend to bacterial and viral infections, demonstrating broad-spectrum antimicrobial properties that have been partially validated through modern research (Islam & Kabir, 2019).

M. officinalis holds particular significance in traditional Chinese medicine and Northeast Asian medical systems, where it serves as a key ingredient in formulations for treating kidney disorders, bone conditions and immune system dysfunction. Its traditional applications in treating impotence, osteoporosis, depression and inflammatory conditions such as rheumatoid arthritis and dermatitis suggest broad physiological effects that may be attributed to morindolide and related compounds (Zhang et al., 2017). The plant's use as a tonic for "nourishing the kidney and strengthening the bone" aligns with modern understanding of its effects on bone metabolism and inflammatory processes.

Recent investigations have revealed promising antimalarial activity of morindolide isolated from *Vangueria infausta* subsp. infausta (VI) occurs alongside friedelin and other bioactive compounds, including biflavonoids, fatty acids, flavonoids, polyketide derivatives and triterpenoids (Maroyi, 2018). This finding is particularly significant given the urgent need for new antimalarial agents and suggests potential applications beyond its traditional uses. The diverse phytochemical profile of VI indicates possible synergistic interactions that may enhance morindolide's therapeutic effects.

The ethnopharmacological significance of morindolide-containing plants is further supported by their consistent use across different traditional medicine systems. While primarily based on empirical observations, these applications have provided valuable direction for modern research into morindolide's mechanisms of action and potential therapeutic applications. The compound's presence in multiple medicinal plants, often used for similar therapeutic purposes across different cultures, suggests convergent recognition of its biological activity through traditional practice.

In parallel with findings from VI, morindolide has also been isolated as a bioactive iridoid compound from the tuber of *M. platytyrea*, a medicinal plant traditionally used across Southeast Asia to treat cancer, inflammation-related disorders and immune dysfunctions (Mohd Haris et al., 2016; Mohd Zin et al., 2024). The ethyl acetate extract of *M. platytyrea* tuber demonstrated potent antioxidant activity, with morindolide identified as one of its key constituents through chromatographic and spectroscopic analyses. This compound contributes to the plant's pharmacological

profile, which includes anti-inflammatory, anticancer and immunomodulatory effects. Morindolide and other phytochemicals such as flavonoids and phloroglucinol derivatives suggest potential synergistic interactions, reinforcing the therapeutic relevance of *M. platytyrea* in both traditional and modern contexts. These findings support further exploration of morindolide's mechanisms of action and its integration into computational drug discovery frameworks for inflammation and oxidative stress-related diseases.

2.1.3 Pharmacological Activities and Therapeutic Potential

Morindolide's pharmacological profile has emerged through various investigative approaches, revealing a complex spectrum of biological activities. Initial studies on its antimalarial properties from VI demonstrated significant activity against parasitic infections, marking morindolide as a compound of interest for antiparasitic drug development (Maroyi, 2018). This activity is particularly noteworthy given the structural similarity between morindolide and other iridoid lactones in the study known to possess anti-parasitic properties (Maroyi, 2018).

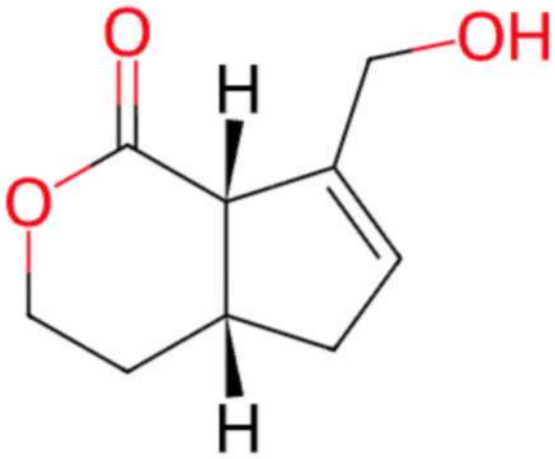
Several key studies have discovered the anti-inflammatory mechanisms of morindolide and related iridoid compounds. Research on structurally similar iridoid glycosides has demonstrated modulation of inflammatory responses through the NF-κB pathway and MAPK cascade (Zhang et al., 2020). For instance, monotropein, a related iridoid glycoside, significantly inhibited LPS-induced inflammation in RAW 264.7 cells through modulation of these pathways (Cai et al., 2021). This mechanistic insight suggests morindolide might exert its anti-inflammatory effects through similar molecular pathways, such as inhibiting the NF-κB signalling axis and modulating MAPK-mediated inflammatory gene expression, though with potentially distinct binding patterns and molecular interactions due to its specific structural features. Further investigation is needed to fully elucidate the precise mechanisms by which morindolide and other iridoid compounds exert their anti-inflammatory activities.

Other investigations about the anti-inflammatory properties of morindolide and related iridoid compounds have revealed significant therapeutic potential. Systematic evaluation of iridoid glycosides by (Recio et al., 1994) demonstrated compelling anti-inflammatory effects using two distinct models: carrageenan-induced mouse paw oedema and TPA-induced mouse ear oedema. In these studies, loganic acid emerged as

particularly potent, achieving 44.4% oedema inhibition. Other iridoid derivatives isolated from aucubin, verbenalin and loganin also showed significant anti-inflammatory activity, with oedema inhibition ranging from 72.0 to 80.0% in the ear oedema model (Recio et al., 1994). These findings established important structure-activity relationships, particularly highlighting the influence of substitution patterns on anti-inflammatory efficacy. The diversity of anti-inflammatory responses observed across different iridoid glycosides suggests complex mechanisms of action that warrant further investigation.

Table 2.1 presents a comparative overview of morindolide alongside structurally related iridoid glycosides that have been investigated for their anti-inflammatory properties. These compounds share the characteristic cyclopentane-pyran skeleton typical of iridoids but exhibit variations in their substitution patterns, glycosylation states and functional groups, which contribute to differences in their pharmacological activities and structure-activity relationships.

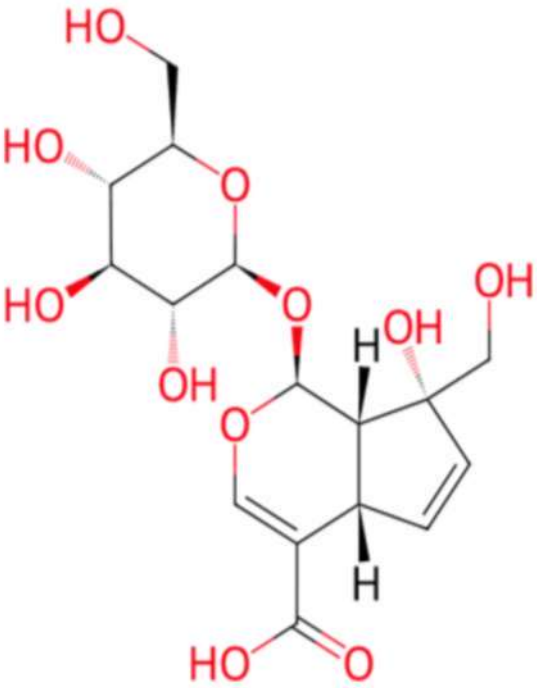
Table 2.1
Structural of Morindolide with Related Iridoid Glycoside.

Compound	**Chemical Structures**
Morindolide C9H12O3168.19 g/mol	

Monotropein

C16H22O11

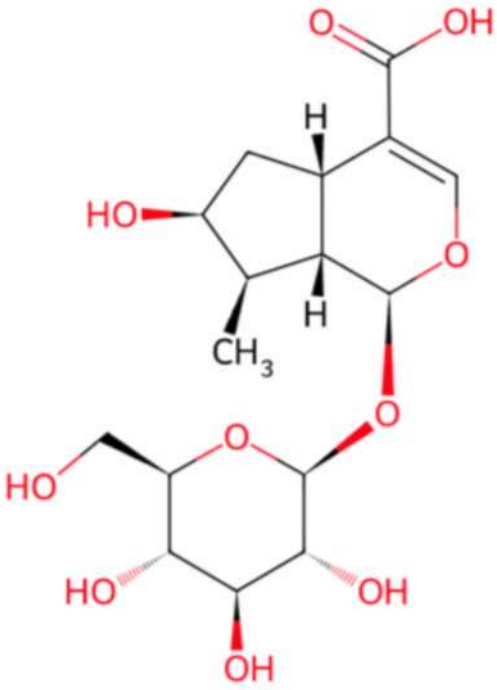
390.34



Loganic acid

C16H24O10

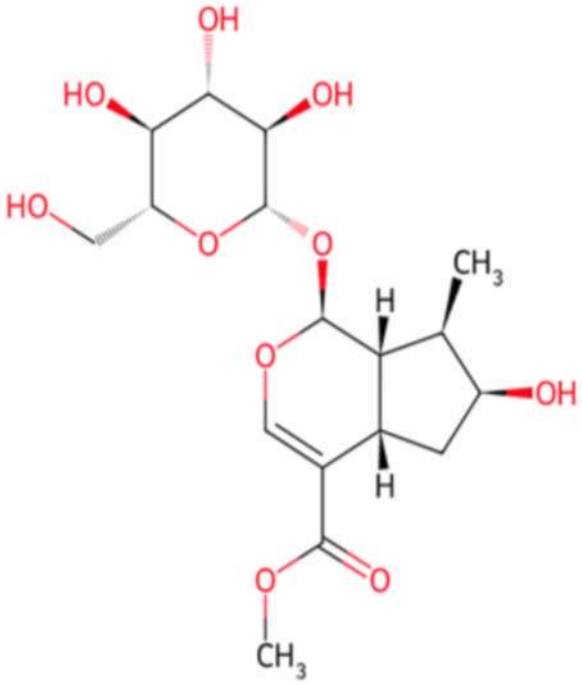
376.36



Loganin

C17H26O10

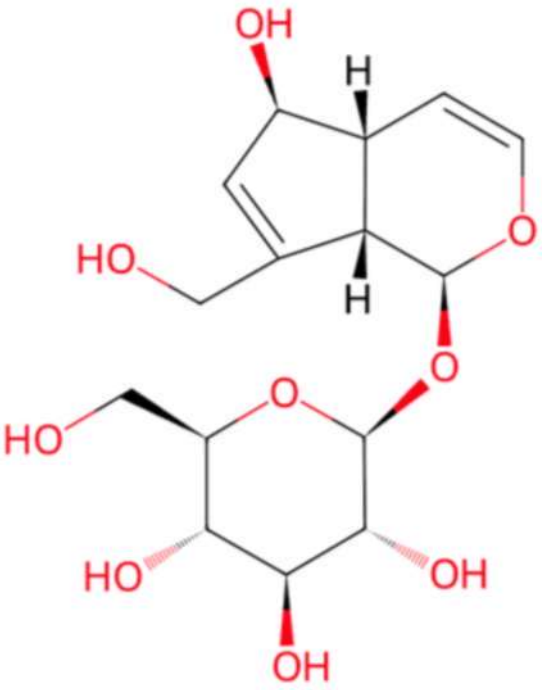
390.38



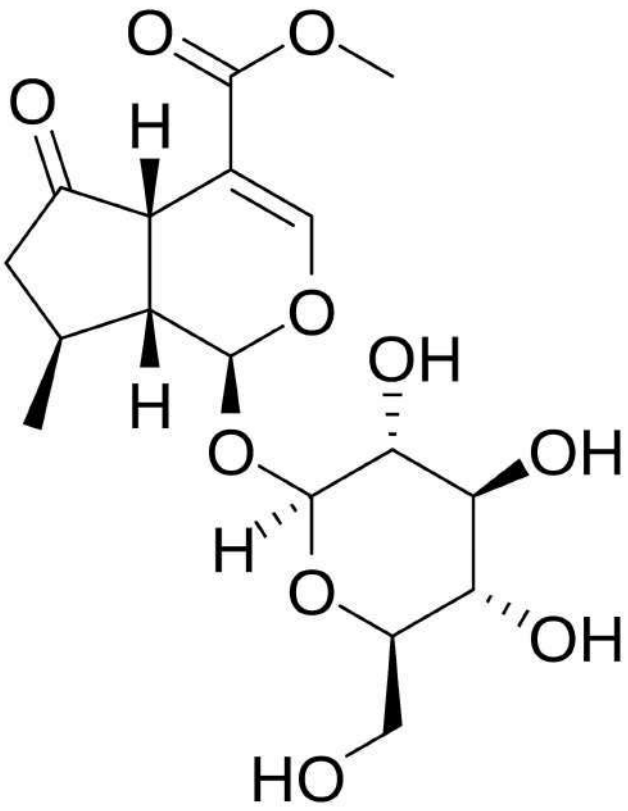
Aucubin

C15H22O9

346.33



Verbenalin
C17H24O10
388.37



As illustrated in Table 2.1, morindolide differs from the other iridoid glycosides in its structural simplicity, being the only compound in this comparison that exists as an aglycone (non-glycosylated form) rather than a glycoside. With a molecular weight of 168.19 g/mol, morindolide is substantially smaller than the glycosylated iridoids (molecular weights ranging from 346.33 to 390.38 g/mol), which all contain a β-D-glucopyranosyl moiety. This structural distinction may influence morindolide's bioavailability, membrane permeability and binding characteristics with target proteins. The presence of the lactone moiety in morindolide, as opposed to the carboxylic acid or ester functionalities in other iridoids, represents another key structural feature that may contribute to its unique pharmacological profile. These structural variations highlight the importance of specific functional groups in determining the biological activities of iridoid compounds, as demonstrated by the anti-inflammatory potency studies conducted by Recio et al. (1994), which revealed that different substitution patterns significantly affected the compounds' therapeutic efficacy.

Molecular docking studies have identified specific protein targets that may mediate morindolide's therapeutic effects. Key inflammation-related proteins, particularly COX-2 and iNOS, have been identified as potential targets based on binding affinity predictions and structural analyses (Cai et al., 2021). These findings align with experimental observations of anti-inflammatory effects in traditional medicine applications and provide mechanistic explanations for the observed therapeutic benefits.

Studies of morindolide-containing plant extracts, particularly from *M. officinalis*, have revealed broader physiological effects beyond direct anti-inflammatory and antioxidant activities. These include modulation of immune responses, enhancement of bone strength and improvement in neurological functions (Zhang et al., 2020). While these effects cannot be attributed solely to morindolide, they provide essential context for understanding the compound's potential role within complex phytochemical matrices.

The compound's influence on cellular stress responses and adaptation mechanisms represents an emerging area of investigation. Recent studies suggest that morindolide may enhance cellular resilience by activating stress response pathways, potentially explaining its reported benefits in chronic inflammatory conditions (Cai et al., 2021). This adaptive response mechanism differs from direct anti-inflammatory effects and may contribute to the sustained therapeutic benefits observed in traditional medicine applications.

Table 2.2 summarises the reported pharmacological activities of morindolide, an iridoid lactone found in Morinda *sp*., revealing a broad but uneven activity profile. Its most consistent and well-supported effect is anti-inflammatory activity, with multiple studies linking this to suppression of MAPK and NF-κB signalling pathways mechanisms consistent with other bioactive iridoids (Recio et al., 1994; Cai et al., 2021; Zhang et al., 2020). Cytotoxic effects have been observed against certain cancer cell lines, though potencies remain moderate, indicating potential as a lead scaffold rather than an immediate therapeutic. Antimalarial evaluation showed weak-to-moderate inhibition against *Plasmodium falciparum,* IC 107 μM (Bapela, 2019), suggesting limited standalone application but chemical features worth optimisation for antiparasitic development. While antimicrobial activity is well documented for *Morinda sp.* extracts (Lee et al., 2024), morindolide-specific evidence remains sparse, highlighting a gap for targeted testing. These data position morindolide as a promising anti-inflammatory

scaffold, with opportunities for structure–activity relationship studies and bioassay-guided fractionation to expand its pharmacological potential.

Table 2.2
Pharmacological Activities of Morindolide/ Related Iridoid Compounds Reported in Previous Studies

Study	Evidence type	Key findings	Reference
Anti-inflammatory effects of iridoid glycosides in experimental models	In vivo experimental (carrageenan-induced mouse paw oedema; TPA-induced mouse ear oedema)	A syste mati c ev al u at ion of iridoid glycosides, including loganic acid, aucubin, verbenalin, and loganin, demonstrated anti-inflammatory effects. Loganic acid achieved 44.4% oedema inhibition in the paw model. Aucubin, verbenalin and loganin showed oedema inhibition ranging from 72.0% to 80.0% in ear oedema model.	Recio et al. (1994)
Monotropein anti-inflammatory mechanisms	In vitro (LPS-induced inflammation in RAW 264.7 cells)	Monotropein, a structurally related iridoid glycoside, significantly inhibited LPS-induced inflammation through modulation of NF-κB and MAPK pathways. Computational docking identified COX-2 and iNOS as potential targets for iridoid compounds.	Cai et al. (2021)
Anti-inflammatory and broader physiological effects of iridoid glycosides from *M. officinalis*	In vitro and in vivo (anti-arthritic effects; signalling pathway analysis)	Iridoid glycosides from M. officinalis demonstrated anti-inflammatory and anti-arthritic effects through inactivating MAPK and NF-κB signalling pathways. The study examined whole plant extracts and iridoid fractions. Morindolide has been identified as one constituent, but its specific effects cannot be attributed solely to morindolide due to the complex phytochemical matrix.	Zhang et al. (2020)
Antioxidant/ cytoprotective bioactivity of *M. platytyrea* tuber.	In vitro (antioxidant assays on ethyl acetate extract)	Ethyl acetate extract of *M. platytyrea* tuber demonstrated potent antioxidant activity. Morindolide identified as one key constituent through chromatographic and spectroscopic analyses, alongside flavonoids and phloroglucinol derivatives.	Mohd Haris et al. (2016)
M. platytyrea extract anti-inflammatory effects	In vitro (LPS-induced RAW 264.7 cells)	Ethyl acetate extract of *M. platytyrea* exerted cytoprotective and anti-inflammatory effects on	Mohd Zin et al. (2024)

		lipopolysaccharide-induced RAW 264.7 cells. Morindolide present as constituent.	
Antioxidant pathways in iridoid compounds	Review and in vitro studies	Related iridoid compounds are suggested to activate Nrf2/HO-1 antioxidant pathways.	Liu et al. (2021)
Antioxidant agents in chronic diseases	Review of iridoid's antioxidant mechanisms	This review examines the role of antioxidant and anti-inflammatory agents, including iridoids, in the context of chronic liver diseases. Mentions potential for iridoid compounds to modulate oxidative stress through various molecular mechanisms. Morindolide is mentioned in the context of the iridoid family.	Zhang et al. (2023)
Antimalarial activity of *V. infausta*	In vitro (antiplasmodial screening of isolated compound)	Direct bioassay of isolated morindolide from the plant demonstrated weak-to-moderate antimalarial activity with IC 107 µM against *Plasmodium falciparum.* Represents one of the few direct biological assays conducted on purified morindolide.	Bapela (2019)
Antimicrobial activities of Morinda species	Crude extract antimicrobial assays; review	Morinda species extracts demonstrate antibacterial and antifungal activities. Morindolide identified in bioactive fractions, but direct antimicrobial testing of purified morindolide against specific pathogens remains sparse.	Lee et al. (2024)
M. citrifolia traditional medicinal uses	Ethnopharmacological review and experimental validation of extracts	Morindolide is one of many constituents of *M. citrifolia* which traditionally used for cancer, hypertension, cervical spondylosis and bacterial/viral infections.	Singh & Sharma (2019); Islam & Kabir (2019)
Morindolide isolation from *M. officinalis*	Phytochemical isolation and structural characterisation	Initial isolation of morindolide achieved from dried roots of *M. officinalis* alongside morofficinaloside, anthraquinones, iridoid glucosides, monoterpene glycosides, sterols and triterpenes.	Yoshikawa et al. (1995)
M. officinalis comprehensive review	Comprehensive ethnopharmacological and pharmacological review	*M. officinalis* used in traditional Chinese medicine for kidney disorders, osteoporosis, depression, rheumatoid arthritis and immune dysfunction. Multiple therapeutic claims reported including neuroprotective, anti-fatigue and bone health effects. Morindolide one identified	Zhang et al. (2017)

		constituent among complex matrix.	
Morindolide from *C. spinosa*	Phytochemical isolation	Morindolide isolated from *C. spinosa* within complex phytochemical matrix including novel compounds and bioactive agents. No specific pharmacological testing reported in isolation study.	Gao et al. (2008)
Morindolide from *V. odorata*	Phytochemical isolation	Identification of morindolide in *V. odorata*, a Philippine endemic Rubiaceae species, demonstrating widespread occurrence within plant family.	Tan et al. (2014)
V. infausta phytochemical profile	Phytochemical analysis and antimalarial screening	Morindolide isolated from *V. infausta* alongside friedelin, biflavonoids, fatty acids, flavonoids and triterpenoids. Diverse phytochemical profile suggests possible synergistic interactions. Contains antimalarial activity.	Maroyi (2018)

Note: Most evidence for morindolide's anti-inflammatory and antioxidant activities derives from three indirect sources: (1) bioactive fractions containing morindolide alongside other phytochemicals, (2) extrapolation from structurally related iridoid glycosides and (3) whole-plant extract studies where specific compound attribution remains unclear. Direct experimental investigation specifically on isolated morindolide is limited

Despite the promising pharmacological activities suggested by traditional use and preliminary studies, a critical gap exists in the experimental validation of morindolide's mechanisms of action. As evident from Table 2.2, the majority of evidence supporting morindolide's anti-inflammatory and antioxidant properties derives from indirect inference based on bioactive fractions or extrapolation from structurally related iridoid compounds, rather than direct experimental investigation of the isolated compound. While morindolide has been identified in antioxidant-active fractions of Morinda extracts, rigorous in vitro assays specifically testing purified morindolide remain limited, and in vivo studies establishing dose-response relationships, pharmacokinetic profiles and toxicity parameters are conspicuously absent from the literature. This paucity of direct experimental evidence presents a significant obstacle to understanding morindolide's precise molecular mechanisms and translating its therapeutic potential into clinical applications. The antimalarial activity reported by Bapela (2019) represents one of the few direct bioassays on isolated morindolide; yet, even this demonstrates only moderate potency (IC 10 μM), suggesting that while morindolide exhibits biological activity, the specific targets and optimal applications remain poorly defined. Furthermore, the attribution of therapeutic effects observed in whole-plant extracts or crude fractions to morindolide is explicitly complicated by the presence of multiple bioactive phytochemicals, including flavonoids, phloroglucinol derivatives and other iridoids, which may contribute synergistically or independently to the observed pharmacological activities.

This experimental gap, combined with the multi-target nature suggested by traditional medicine applications, creates a compelling rationale for computational approaches to elucidate morindolide's mechanisms of action. Traditional experimental drug discovery methods, while invaluable for validation, are resource-intensive, time-consuming and often focus on single-target interactions, potentially overlooking the polypharmacological nature that characterises many natural products (Newman & Cragg, 2020). Network pharmacology and molecular docking offer complementary advantages by enabling systematic, hypothesis-generating investigations of multi-target interactions and pathway-level effects, without requiring extensive quantities of compounds or preliminary experimental optimisation. These computational approaches can identify the most promising targets and pathways for subsequent experimental validation, thereby prioritizing resources toward the most mechanistically relevant investigations. Given the limited availability of purified morindolide and the absence of

established cell-based or animal models specifically for this compound, computational methods represent not merely a convenient alternative but a strategic necessity for advancing morindolide research. By integrating network pharmacology to reveal systems-level interactions with molecular docking to provide atomic-level binding insights, this study addresses the critical knowledge gap regarding morindolide's anti-inflammatory and antioxidant mechanisms, establishing a robust theoretical framework that will guide future experimental investigations and accelerate the compound's progression toward therapeutic applications.

2.2 Anti-Inflammatory and Antioxidant Mechanisms

2.2.1 Inflammatory Pathways

The inflammatory response represents one of the most complex, yet highly regulated, physiological processes in biological systems, encompassing an intricate network of molecular mediators, cellular components, and regulatory mechanisms that operate through multiple levels of control (Bennett et al., 2018). This multifaceted system coordinates the initiation and resolution of inflammatory responses through precisely orchestrated molecular events that determine the trajectory and outcome of the inflammatory response. Understanding these pathways requires careful consideration of their molecular architecture, temporal dynamics and regulatory mechanisms within the broader context of physiological and pathological processes (Netea et al., 2017).

At the molecular level, inflammatory pathways are initiated through a detailed pattern recognition system involving multiple classes of receptors and sensing mechanisms. Pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (RLRs) and C-type lectin receptors (CLRs), form the first line of molecular recognition (Fukata & Arditi, 2013; Pandey et al., 2014; Saghazadeh & Rezaei, 2020). These receptors exhibit remarkable specificity in recognising diverse molecular patterns associated with tissue injury or pathogenic invasion. The recognition system resides not only in the diversity of receptors but also in their capacity to assemble functional complexes and initiate distinct yet interrelated signalling cascades (Jogi et al., 2018; Seibl et al., 2024).

The signal transduction mechanisms activated by PRRs converge on several key regulatory nodes, with the nuclear factor-κB (NF-κB) pathway serving as a central mediator of inflammatory gene expression. Activating NF-κB involves a series of precisely controlled molecular events, including phosphorylation cascades, protein-protein interactions and nuclear translocation mechanisms (Liu et al., 2017; Zhang & Sun, 2015). Multiple checkpoint mechanisms, including negative feedback loops and post-translational modifications, regulate this process and ensure appropriate inflammatory responses while preventing excessive activation (Dorrington & Fraser, 2019). The importance of this pathway is underscored by its evolutionary conservation and role in diverse physiological and pathological processes (Slavich, 2014).

Inflammatory signalling extends beyond initial recognition and activation events, encompassing multiple amplification and feedback mechanisms that refine the inflammatory response. Pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), establish autocrine and paracrine signalling networks (Holdsworth & Gan, 2015; Ray, 2016). These cytokines interact with specific membrane receptors to activate additional signalling cascades, including the JAK-STAT pathway, MAPK cascades and PI3K/Akt signalling. Integrating these pathways creates a complex web of cellular communications that shapes the magnitude and duration of inflammatory responses (Cameron & Kelvin, 2023).

The enzymatic systems involved in inflammation represent another layer of complexity in inflammatory regulation, particularly emphasising the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. These enzyme systems catalyse the conversion of arachidonic acid into various bioactive lipid mediators, including prostaglandins, thromboxanes and leukotrienes. The differential expression and regulation of COX isoforms (COX-1 and COX-2), provide important mechanisms for selective modulation of inflammatory responses (Chandrasekharan & Simmons, 2023; Seta & Bachschmid, 2012). The constitutive expression of COX-1 and the inducible nature of COX-2 create opportunities for therapeutic intervention, as evidenced by the development of selective COX-2 inhibitors for treating inflammatory conditions (Pannunzio & Coluccia, 2018). Similarly, the LOX pathway generates a diverse array of pro-inflammatory and pro-resolving lipid mediators, such as leukotrienes and lipoxins, which further contribute to the intricate regulation of the inflammatory response. The balance and interaction between the COX and LOX pathways are crucial

in determining the overall inflammatory state and the potential for therapeutic targeting (Chandrasekharan & Simmons, 2023; Wang et al., 2021).

Figure 2.3 provides a comprehensive overview of the inflammatory signalling cascade, illustrating the interconnected nature of these molecular pathways. The diagram illustrates the sequential cascade of inflammatory signalling from pathogen/damage recognition through pattern recognition receptors (PRRs: TLRs, NLRs, RLRs, and CLRs) to signal transduction via the IKK complex, MAPK cascades, and JAK-STAT pathways. Activation of transcription factors (NF-$\varkappa$B, AP-1) leads to inflammatory gene expression, producing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and activating the arachidonic acid metabolism pathway. Phospholipase A2 (PLA2) releases arachidonic acid from membrane phospholipids, which is subsequently metabolized by cyclooxygenase (COX-1/COX-2) and lipoxygenase (5-LOX) enzymes to generate prostaglandins, thromboxanes and leukotrienes. These lipid mediators contribute to vasodilation, immune cell recruitment, pain and fever. Feedback loops (dotted lines) show cytokine amplification through IKK reactivation. Specialized pro-resolving mediators (SPMs: lipoxins, resolvins, protectins, maresins) derived from arachidonic acid provide negative feedback to promote inflammation resolution. Chronic inflammation can lead to pathological tissue damage

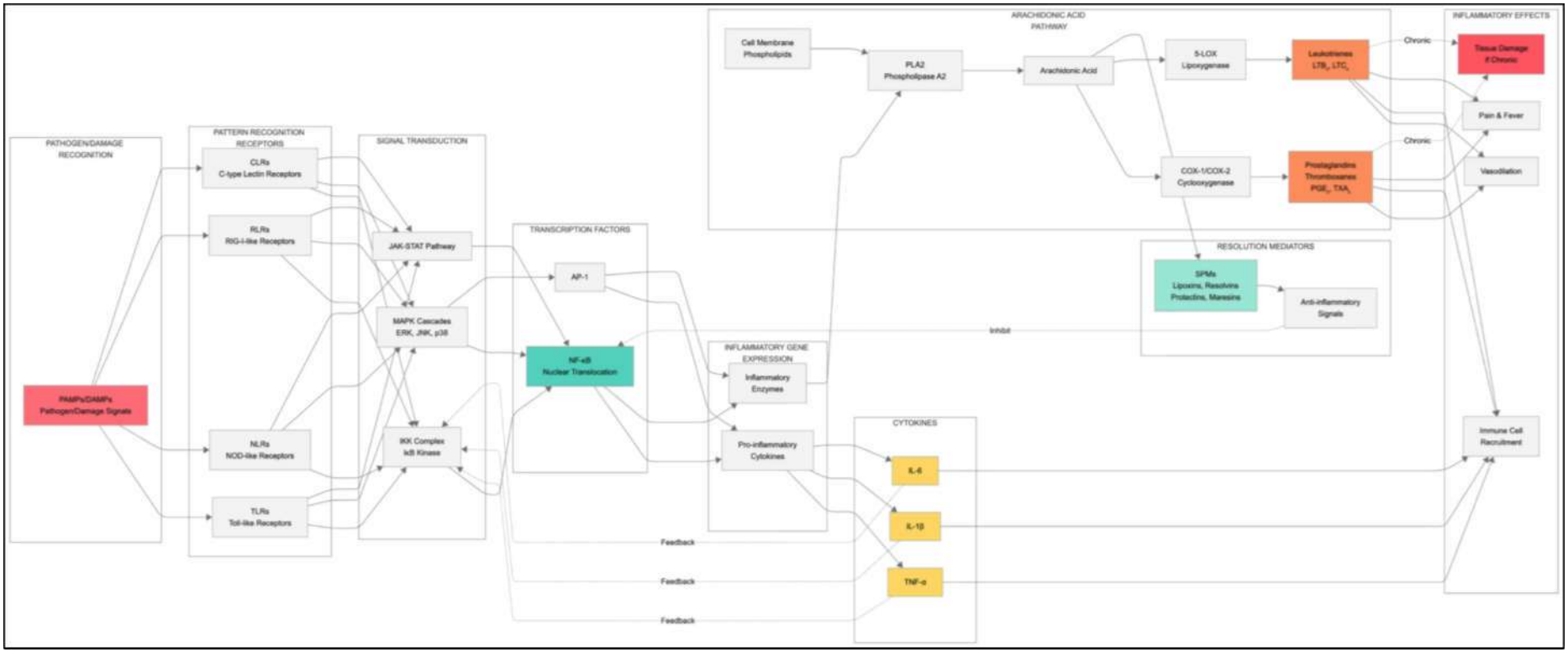


Figure 2.3 Inflammatory Pathways. Colour Coded Column with Red (Initiating Signals), Cyan (Key Transcription Factor), Yellow (Cytokines), Orange (Lipid Mediators), Green (Resolution Mediators) and Dark Red (Pathological Outcomes)

Recent research has revealed that inflammation resolution is an active process orchestrated by specialised pro-resolving mediators (SPMs), including lipoxins, resolvins, protectins and maresins. These SPMs activate specific G-protein-coupled receptors, initiating signalling cascades that promote neutrophil apoptosis, enhance macrophage efferocytosis, and modulate cytokine production (Dalli & Serhan, 2017; Kourtzelis et al., 2020). The resolution process involves a temporal switch from pro-inflammatory to pro-resolving mediators through class-switching of eicosanoid production (Uddin & Levy, 2010). SPMs act by blocking neutrophil recruitment, promoting monocyte recruitment and activation and mediating nonphlogistic phagocytosis of apoptotic neutrophils. This active resolution process is essential for maintaining host health and preventing chronic inflammation (Serhan, 2018).

The epigenetic regulation of inflammatory responses involves an interplay between multiple regulatory mechanisms. MicroRNAs, particularly miR-146a, miR-155 and miR-21, demonstrate phase-specific regulation of inflammatory gene expression through both direct mRNA targeting and indirect modulation of signalling pathways (Testa et al., 2017; Teymoori-Rad et al., 2023; Wang et al., 2021). For instance, miR-146a specifically targets TRAF6 and IRAK1, key components of the NF-κB signalling pathway, while miR-155 regulates SOCS1 and SHIP1 expression (Bhaumik et al., 2023). The chromatin modification landscape during inflammation shows distinct patterns, including H3K27 acetylation at enhancer regions of pro-inflammatory genes and H3K9 methylation at resolution-phase genes (Lin et al., 2022; Santa et al., 2007). These modifications work with DNA methylation changes, particularly at CpG islands of inflammatory gene promoters, creating a dynamic regulatory network that ensures appropriate temporal control of the inflammatory response (Qu et al., 2023).

The interplay between different inflammatory pathways has profound implications for therapeutic intervention in inflammatory diseases. The complexity of these pathways suggests that targeting single mediators may be insufficient for effective treatment (Medzhitov & Horng, 2023; Sugimoto et al., 2016). Instead, therapeutic approaches that modulate multiple pathways or target key regulatory nodes may prove more effective in managing inflammatory conditions. This perspective has driven

increasing interest in natural compounds like morindolide, which may exert their anti-inflammatory effects through multiple mechanisms and targets.

2.2.2 Oxidative Stress Mechanisms

Oxidative stress is a fundamental concept in cellular pathophysiology, involving intricate biochemical and molecular processes beyond redox imbalances (Soodaeva et al., 2023). This biological phenomenon involves complex interactions between the generation of reactive oxygen species (ROS), antioxidant defence systems, and cellular signalling networks that collectively influence cellular function and survival (Aramouni et al., 2023). Understanding oxidative stress mechanisms requires careful consideration of the molecular dynamics, regulatory pathways and cellular responses that characterise this complex biological state, particularly in disease development and therapeutic intervention (Abdelazim & Abomughaid, 2024).

Reactive oxygen species (ROS) are generated through multiple enzymatic and non-enzymatic processes integrated into various cellular compartments and metabolic pathways (Waris & Ahsan, 2023). The mitochondrial electron transport chain serves as a primary source of ROS production, where electron leakage during oxidative phosphorylation leads to the formation of superoxide anion radicals (Jomová et al., 2024). This process involves specific sites within the electron transport chain complexes, particularly complexes I and III, where electrons can directly interact with molecular oxygen to form superoxide (Ahmad et al., 2023; Jastroch et al., 2023). The rate of mitochondrial ROS production is influenced by multiple factors, including the metabolic state of the cell, the proton gradient across the inner mitochondrial membrane and the availability of various electron donors and acceptors (Han et al., 2023). The complexity of mitochondrial ROS generation is further increased by multiple regulatory mechanisms that can modulate electron transport and subsequent ROS formation (Patergnani et al., 2021).

Beyond mitochondrial sources, NADPH oxidases (NOX enzymes) represent another major enzymatic system responsible for ROS generation (Huetsch et al., 2019). The NOX family comprises several isoforms (NOX1-5 and DUOX1-2), each with distinct tissue distribution and regulatory mechanisms (Kawahara et al., 2007; Leto et al., 2009; Vermot et al., 2021). These enzymes catalyse the controlled production of superoxide and hydrogen peroxide through electron transfer from NADPH to molecular

oxygen (Vermot et al., 2021). The regulation of NOX activity involves complex protein-protein interactions, phosphorylation events and assembly of multiple subunits into functional enzyme complexes (Vermot et al., 2021). This process ensures appropriate ROS generation for cellular signalling while preventing excessive oxidant production (Lambeth et al., 2023).

The cellular response to oxidative stress involves processes of antioxidant defence systems that operate through multiple mechanisms and levels of organisation (Saso et al., 2020). The enzymatic antioxidant system comprises several key enzymes, including three distinct forms of superoxide dismutase (SOD1-3), each specialised for specific cellular compartments (Fukai & Ushio-Fukai, 2023). SOD1 (Cu/Zn-SOD) operates primarily in the cytosol and intermembrane space of mitochondria, SOD2 (Mn-SOD) functions within the mitochondrial matrix and SOD3 is secreted into the extracellular space (Altobelli et al., 2020). These enzymes work in concert with catalase and various peroxidases to create an integrated network of ROS detoxification (Chidambaram et al., 2024).

The glutathione system represents a specific component of cellular antioxidant defence, involving multiple enzymes and regulatory mechanisms. Glutathione peroxidases (GPx1-8) exhibit distinct substrate specificities and tissue distributions, catalysing the reduction of various peroxides while oxidising glutathione (Kupaeva & Kotenkova, 2021). The regeneration of reduced glutathione involves glutathione reductase and the pentose phosphate pathway, creating a network of redox cycling that maintains cellular redox homeostasis (Lubos et al., 2023; Pei et al., 2023). The regulation of glutathione synthesis involves feedback mechanisms that respond to cellular redox status and oxidative stress conditions (Duan et al., 2025; Espinosa Díez et al., 2015). The thioredoxin system provides another layer of antioxidant defence, operating through multiple isoforms of thioredoxin (Trx) and thioredoxin reductase (TrxR) (Georgiou Siafis & Tsiftsoglou, 2023; Zhang & Forman, 2012). This system regulates protein thiol status and maintains cellular redox homeostasis. The interaction between the glutathione and thioredoxin systems creates redundancy in antioxidant defence while allowing for specialised functions in different cellular compartments and conditions (Georgiou Siafis & Tsiftsoglou, 2023; Stoyanovsky et al., 2013).

The regulation of antioxidant defence systems involves transcriptional and post-transcriptional mechanisms that respond to changes in cellular redox status (Santos-Sánchez et al., 2019). The Nrf2-Keap1 pathway is a master regulator of cellular

antioxidant responses, controlling the expression of numerous genes involved in ROS detoxification and cellular defence (Nguyen et al., 2023). Under basal conditions, Nrf2 is maintained at low levels through continuous ubiquitination and proteasomal degradation mediated by Keap1 (Baird & Yamamoto, 2020; Kaspar et al., 2009; Li & Kong, 2009). Oxidative stress modifies cysteine residues in Keap1, leading to conformational changes that prevent Nrf2 degradation and allow nuclear translocation (Kobayashi et al., 2006; Nguyen et al., 2023).

Figure 2.4 presents a comprehensive overview of the cellular oxidative stress response system, integrating ROS generation, antioxidant defence mechanisms and adaptive regulatory pathways. ROS are generated from multiple sources, including mitochondrial electron transport chain (ETC) complexes I and III, NADPH oxidases (NOX1-5, DUOX1-2), cytochrome P450 enzymes (particularly CYP2E1) and other cellular compartments. Superoxide (O) is converted to hydrogen peroxide (H O) by superoxide dismutases (SOD1 in cytosol, SOD2 in mitochondria, SOD3 extracellularly), which is subsequently detoxified by catalase and glutathione peroxidases (GPx1-8). The thioredoxin (Trx/TrxR) system provides additional antioxidant defence and regulates protein thiol status. Non-enzymatic antioxidants, including glutathione (GSH/GSSG) and vitamins C and E, contribute to the neutralisation of ROS. The Nrf2-Keap1 pathway serves as the master regulator of antioxidant responses: under basal conditions, Nrf2 is bound to Keap1 and targeted for proteasomal degradation; upon oxidative stress, cysteine modifications in Keap1 release Nrf2, allowing nuclear translocation and binding to antioxidant response elements (ARE), resulting in upregulation of antioxidant genes (SOD, catalase, GPx, glutathione synthesis enzymes, HO-1, NQO1). ROS also function in redox signalling through reversible protein modifications (cysteine oxidation, S-glutathionylation, disulfide bond formation) that regulate cellular processes. Excessive ROS led to oxidative damage of proteins (carbonylation), lipids (peroxidation), and DNA (guanine oxidation), triggering cellular repair mechanisms, including DNA repair enzymes, proteasomal protein degradation, and lipid repair systems. The Nrf2-mediated gene expression enhances both antioxidant capacity and repair mechanisms, promoting cellular homeostasis.

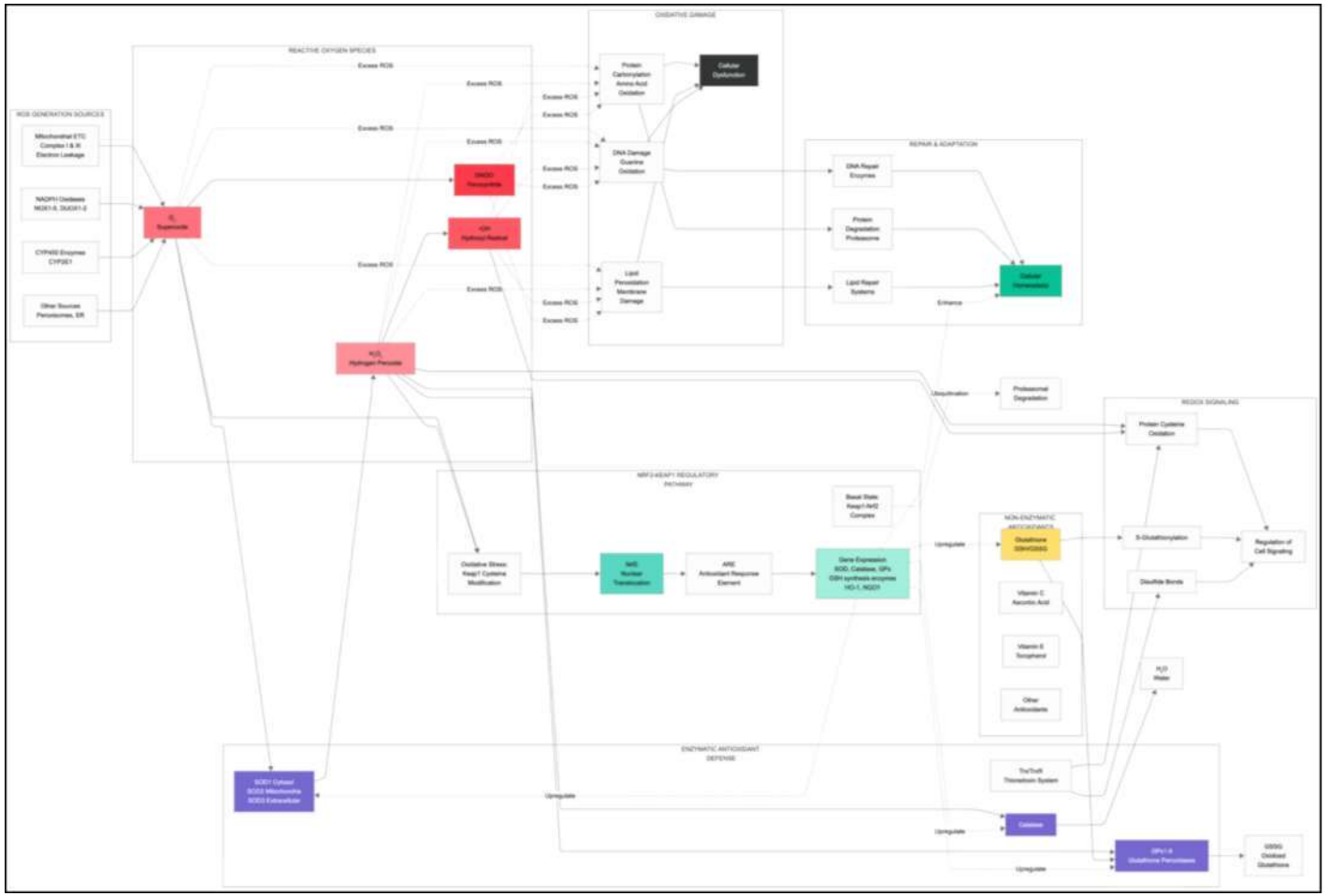


Figure 2.4 Antioxidant Defence and Oxidative Stress Pathways. Colour Coded with Red Gradient (ROS Species, Increasing Reactivity), Purple (Enzymatic Antioxidants), Yellow (Glutathione), Cyan (Nrf2 Pathway Components), Green (Homeostasis/Repair), Black (Cellular Dysfunction). Dotted Lines Indicate Regulatory or Damaging Pathways, While Solid Lines Represent Direct Enzymatic Conversions

In addition to this, ROS can function as second messengers through specific oxidative modifications of proteins, particularly cysteine residues (Nguyen et al., 2023). These modifications include reversible formation of sulfenic acids, S-glutathionylation and disulfide bonds, each with distinct functional consequences for protein activity and cellular signalling (Zhang et al., 2018). The specificity of these modifications is ensured through the distinct chemical reactivity of different ROS species and the local environment of target proteins (Espinosa Díez et al., 2015). Recent advances have revealed essential connections between oxidative stress and various cellular processes, including inflammation, metabolism and ageing (Jones, 2015; Liguori et al., 2018). "Redox signalling" has emerged as a fundamental mechanism in cellular regulation, involving specific oxidation-reduction reactions that influence protein function and cellular processes (Bindoli & Rigobello, 2013; Sies et al., 2024). This signalling role of ROS involves spatiotemporal control mechanisms that ensure appropriate cellular responses while preventing oxidative damage (Kaludercic et al., 2023).

The pathological consequences of oxidative stress emerge from disruptions in these complex regulatory networks. Chronic oxidative stress can overwhelm cellular antioxidant systems, leading to the accumulation of oxidative damage to cellular macromolecules (Mossenta et al., 2020; Saso et al., 2020). This damage includes specific modifications of proteins (carbonylation, oxidation of specific amino acids), lipids (peroxidation of membrane lipids) and nucleic acids (oxidation of DNA bases, particularly guanine) (Ong & Logue, 2023; Özcan & ÖĞÜN, 2015). The cellular response to such damage involves multiple repair mechanisms, including DNA repair enzymes, protein degradation pathways and lipid repair systems (Alfei et al., 2024; Tafani et al., 2015). Thereby, the oxidative stress mechanisms have important implications for therapeutic intervention. Traditional approaches focusing on straightforward antioxidant supplementation have shown limited success, highlighting the need for more detailed therapeutic strategies. Natural compounds, such as morindolide, may offer advantages through their ability to modulate multiple aspects of cellular redox regulation simultaneously.

2.2.3 Integration of Anti-inflammatory and Antioxidant Activities

The relationship between inflammatory processes and oxidative stress represents one of the most clinically significant interactions in cellular pathophysiology. This integration is mediated through multiple molecular mechanisms and regulatory pathways, creating complex feedback loops between inflammatory mediators and redox signalling systems (Saso et al., 2020; Soomro, 2019). Understanding these interactions requires careful consideration of both the direct molecular interactions and the broader physiological context in which these processes operate, particularly in disease pathogenesis and therapeutic intervention strategies (Sies, 2021).

The molecular basis for the inflammatory processes and oxidative stress integration begins at the level of signal transduction, where redox-sensitive transcription factors, particularly nuclear factor-κB (NF-κB), serve as crucial nodes connecting oxidative stress and inflammatory responses (Jomová et al., 2025; Mesa-Garcia et al., 2018). Activating NF-κB involves specific redox-dependent modifications of key regulatory proteins, including the IκB kinase (IKK) complex components and the NF-κB subunits (Jomová et al., 2025; Mullen et al., 2019). The local redox environment influences these modifications and can be modulated by pro-oxidant and antioxidant systems (Almeida et al., 2020). The resulting changes in NF-κB activity directly affect the expression of numerous inflammatory mediators, creating a mechanism for redox-dependent regulation of inflammatory responses (Fagiani et al., 2020).

NADPH oxidases emerge as critical mediators in integrating inflammatory and oxidative processes, demonstrating regulatory mechanisms that extend beyond direct ROS generation (Li et al., 2025; Rada & Leto, 2008). Various inflammatory stimuli activate these enzymes, generating ROS that can further modulate inflammatory signalling pathways through specific oxidative modifications of signalling proteins (Cheng et al., 2022; Martinon, 2010). The NOX family members exhibit distinct expression and regulation patterns in different inflammatory cell types, providing mechanism-specific control of ROS generation during inflammatory responses (Vermot et al., 2021). The spatial and temporal regulation of NOX activity creates distinct redox microenvironments that influence local inflammatory signalling processes, contributing to the complex organisation of inflammatory responses (Kvietys & Granger, 2023).

The mitochondrial contribution to this integration is not simply a matter of ROS generation. It also encompasses metabolic and signalling functions. Inflammatory mediators can directly influence mitochondrial function through effects on electron transport chain components, mitochondrial membrane potential and mitochondrial DNA stability (Ponnalagu & Singh, 2020; Salnikova et al., 2021). These changes in mitochondrial function can alter energy metabolism and ROS production, creating additional feedback mechanisms that influence inflammatory responses (Chen et al., 2018). Recent research has revealed intricate communication mechanisms between mitochondria and other cellular compartments, including the endoplasmic reticulum and peroxisomes, that contribute to coordinating inflammatory and oxidative stress responses (Patergnani et al., 2021).

Besides direct ROS scavenging, cellular antioxidant systems demonstrate crucial regulatory roles in inflammatory processes through specific molecular mechanisms. The glutathione system modulates inflammation through S-glutathionylation of key signalling proteins, including p65/RelA, IKKβ and STAT3 transcription factors (Mullen et al., 2020). The dynamic regulation of glutathione/glutathione disulfide (GSH/GSSG) ratios directly influences NF-ϰB pathway activation through reversible modification of critical cysteine residues, particularly Cys-179 of IKKβ and Cys-38 of p65/RelA. The thioredoxin system, comprising Trx1/TrxR1 in the cytosol and Trx2/TrxR2 in mitochondria, regulates inflammatory responses through specific interactions with ASK1, TXNIP and redox-sensitive transcription factors such as NF-ϰB and AP-1 (Lubos et al., 2023). Thioredoxin's interaction with ASK1 at Cys-250 represents a critical regulatory node, where oxidation-dependent dissociation triggers the activation of the MAP kinase cascade and subsequent inflammatory gene expression (Psenakova et al., 2020). These systems demonstrate coordinated regulation through shared control points, particularly at transcription factor activation and mitochondrial ROS production, creating an integrated network of redox-dependent inflammatory control (Chai & Mieyal, 2023; Jennings et al., 2019).

Integrating inflammatory and oxidative processes extends to regulating specialised pro-resolving mediators (SPMs), representing a mechanism for controlling inflammation resolution (Serhan, 2018). These lipid mediators, derived from polyunsaturated fatty acids through enzymatic pathways, are sensitive to the cellular redox environment in multiple ways (Bannenberg & Serhan, 2023). The activity of key

enzymes in SPM biosynthesis, including lipoxygenases and cyclooxygenases, is modulated by local redox conditions through oxidative modifications (Serhan, 2023). Furthermore, the stability and biological activity of SPMs can be influenced by oxidative modifications, creating additional mechanisms for redox-dependent regulation of inflammation resolution (Sandhaus & Swick, 2020).

Cellular adaptation to chronic inflammatory conditions involves complex interactions between redox signalling and inflammatory mediators that extend over multiple time scales (Jennings et al., 2019). Oxidative stress adaptation involves changes in antioxidant defence systems, metabolic pathways, and cellular repair mechanisms that occur in response to persistent inflammatory stimuli (Finkel, 2023). These adaptations can influence both the progression of inflammatory responses and the cellular sensitivity to oxidative damage through multiple mechanisms, including epigenetic modifications and alterations in protein turnover rates (Foster & Medzhitov, 2023). Understanding these adaptive mechanisms is crucial for developing therapeutic strategies that target both inflammatory and oxidative components of disease processes (Carrera Juliá et al., 2020).

The therapeutic implications of this integrated understanding are significant and necessitate a reconsideration of traditional therapeutic approaches. Conventional strategies targeting inflammation or oxidative stress alone may be insufficient for treating conditions where both processes contribute to pathogenesis (Sharma et al., 2023). Natural compounds that can modulate both inflammatory and oxidative pathways, such as morindolide and related compounds, may offer advantages by simultaneously influencing multiple aspects of these integrated processes. Developing such multi-target therapeutic strategies requires careful consideration of the complex interactions between inflammatory and oxidative mechanisms, as well as the temporal dynamics of these processes (Wu et al., 2020).

2.3 Computational Drug Discovery Methods

2.3.1 Network Pharmacology Approach

The emergence of network pharmacology represents a transformative paradigm shift in drug discovery methodology, fundamentally challenging the reductionist "one target, one drug" approach that has dominated pharmaceutical research for decades

(Hopkins, 2023; Wu et al., 2018). This cutting-edge computational framework integrates systems biology, network science and pharmacology principles to comprehensively understand drug action within complex biological systems (Medina-Franco et al., 2023). The theoretical foundation of network pharmacology rests on three fundamental principles: the recognition of biological systems as complex networks of molecular interactions, the understanding that drugs typically interact with multiple targets simultaneously and the acknowledgement that therapeutic effects emerge from system-wide perturbations rather than isolated molecular events (Csermely et al., 2023).

The methodological framework of network pharmacology encompasses multiple levels of analytical complexity, beginning with the construction and analysis of biological networks (Berger & Iyengar, 2023). These networks represent intricate systems of molecular interactions, including protein-protein interactions, gene regulatory networks, metabolic pathways and signalling cascades (Zhao et al., 2023). The mathematical foundations underlying network construction draw from graph theory and statistical physics, employing algorithms to identify meaningful patterns and relationships within large-scale biological datasets (Jeong et al., 2023). Integrating multiple data types, including genomic, proteomic and metabolomic data, requires advanced computational approaches for data normalisation, integration and analysis (Bersanelli et al., 2023; Fouché & Zinovyev, 2023).

Network topology analysis represents a crucial component of the network pharmacology approach, employing various mathematical metrics to quantify network properties and identify significant nodes and substructures (Badkas et al., 2020; Ma'ayan, 2023). Centrality measures, including degree centrality, provide insights into the relative importance of different nodes within the network (Koschützki & Schreiber, 2024). Clustering coefficients and community detection algorithms reveal modular structures that may correspond to functional biological units (Abdo & Moura, 2023). These topological analyses inform the identification of potential drug targets and the prediction of drug effects on biological systems (Elengoe & Hamdan, 2019; Gopalakrishnan et al., 2020).

The application of network pharmacology to drug discovery involves computational algorithms that can simultaneously evaluate multiple parameters and identify potential therapeutic targets within the context of disease networks (Csermely et al., 2023). These algorithms consider various factors, including network topology, biological relevance, druggability of potential targets and the likelihood of off-target

effects (Dezső & Ceccarelli, 2020). Advanced machine learning approaches, including deep learning and artificial intelligence algorithms, are being increasingly integrated into network pharmacology analyses to enhance prediction accuracy and identify subtle patterns in complex biological data (Ao et al., 2023; Oh & Kim, 2023; Pan et al., 2022).

The analysis of polypharmacological effects is a particularly significant aspect of network pharmacology, especially relevant for natural compounds like morindolide, which often exhibit multiple mechanisms of action (Liu et al., 2020). This analysis involves developing and applying algorithms that simultaneously predict and evaluate the effects of compounds on multiple targets (Choi et al., 2021; Jiao et al., 2020). These predictions consider both direct molecular interactions and indirect effects propagated through biological networks, thereby providing a more comprehensive understanding of the therapeutic potential and potential side effects (Winau, 2023).

Network pharmacology has transformed our understanding of drug interactions through quantitative analysis of network perturbations (Innocentini et al., 2023). Mathematical modelling employs specific frameworks, including ordinary differential equations (ODEs) for the temporal dynamics of protein-protein interactions, partial differential equations (PDEs) for spatial drug distribution patterns, and stochastic differential equations (SDEs) for noise-induced fluctuations in signalling networks (Gilbert et al., 2006; Nyman et al., 2019). These models quantify drug synergy through combination indices (CI), using Loewe additivity and Bliss independence principles to predict interaction outcomes (Ma & Motsinger-Reif, 2019). Statistical mechanics approaches, particularly the Ising model and renormalisation group theory, analyse phase transitions in protein interaction networks under drug perturbations (Distinctive Behaviours of Druggable Proteins in Cellular Networks, 2023; Thalmann, 2023). Specific mathematical tools include Bayesian network inference for identifying causal relationships, Monte Carlo simulations for quantifying uncertainty, and tensor decomposition methods for analysing high-dimensional interaction data (Hart, 2024; Kishan et al., 2022). These frameworks enable precise prediction of drug combination effects, such as the synergistic enhancement observed between metformin and EGFR inhibitors through AMPK-mTOR pathway modulation (Shi, 2019).

Current advances in network pharmacology have incorporated dynamic aspects of biological systems, moving beyond static network representations to include temporal and spatial dimensions of drug action (Jiang et al., 2025; Tang & Aittokallio, 2014). These dynamic approaches consider how drug effects propagate through

biological networks over time, how network structure may change in response to therapeutic interventions and how these changes influence therapeutic outcomes. The mathematical frameworks for analysing dynamic networks draw from control theory, dynamical systems analysis and information theory, providing extensive tools for understanding complex biological responses to therapeutic interventions (Boezio, 2017; Jiao et al., 2020; Zheng et al., 2024).

Applying network pharmacology to natural products presents unique opportunities and challenges (Hu, 2018; Panossian, 2025). Natural compounds often exhibit complex chemical structures and multiple biological activities, making them particularly suitable for network-based analysis (Ma et al., 2023). The ability to predict and analyse multiple mechanisms of action simultaneously can help explain the therapeutic effects of traditional medicines and guide the development of new therapeutic strategies. However, the complexity of natural product chemistry and the limitations of current computational methods present significant challenges that continue to drive methodological innovation in the field (Chen, 2020; Chen & Kirchmair, 2020; Hu et al., 2025; Romano & Tatonetti, 2019).

2.3.2 Molecular Docking Studies

Molecular docking represents a computational methodology that has become indispensable in modern drug discovery and development processes (Meng et al., 2023; Pagadala et al., 2023). From small-scale to large-scale, this approach employs complex algorithms and physical models to predict and analyse the interactions between small molecules and biological macromolecules, particularly proteins (Agu et al., 2023; Mesarić, 2022). The theoretical foundation of molecular docking rests on principles from physical chemistry, statistical mechanics and computational biology, integrating multiple levels of molecular interaction analysis to provide detailed insights into drug-target binding mechanisms (Morris, 2012; Novikov & Chilov, 2009; Shah et al., 2024). Integrating theoretical principles with practical computational methods has revolutionised our ability to understand and predict molecular recognition processes in biological systems.

The fundamental principles of molecular docking encompass structural and energetic considerations, requiring detailed mathematical frameworks for their implementation (Agu et al., 2023). The process involves detailed sampling algorithms

that explore possible conformations and orientations of ligands within protein binding sites, coupled with scoring functions that evaluate the thermodynamic favourability of these interactions (Najmanovich, 2023). The sampling problem represents a significant computational challenge, requiring efficient algorithms to explore vast conformational spaces while maintaining reasonable computational demands. These algorithms must balance the competing requirements of thorough conformational sampling with computational efficiency, leading to various hybrid approaches that combine different sampling strategies (Pieroni et al., 2023).

Developing and implementing scoring functions in molecular docking represents a critical component in determining the accuracy of binding predictions (Fan et al., 2019; Shirali et al., 2025). These functions incorporate multiple physical and empirical components to evaluate the strength and specificity of protein-ligand interactions. Force field-based scoring functions employ physical models of molecular interactions, including van der Waals forces, electrostatic interactions and hydrogen bonding (Pagadala et al., 2017; Yang et al., 2022). The mathematical treatment of these interactions often requires complex approximations and corrections to account for quantum mechanical effects and environmental influences (Cahill & Parsegian, 2008; Pieroni et al., 2023). Empirical scoring functions, derived from experimental binding data, complement physics-based approaches by capturing effects that may be difficult to model from first principles (Ain et al., 2024).

The treatment of protein flexibility in molecular docking encompasses a few distinct computational approaches, each addressing specific aspects of conformational dynamics (B-Rao et al., 2023). Side-chain flexibility modelling employs rotamer libraries containing 67 statistically preferred conformations for each amino acid, derived from the analysis of over 500 high-resolution crystal structures (Dunbrack & Karplus, 2023). Backbone flexibility incorporation utilises normal mode analysis (NMA), focusing on the 10-20 lowest frequency modes that account for most protein conformational changes (Mannige, 2017). Incorporating protein dynamics and solvation effects into docking algorithms has also been explored (Lill, 2011; Zhu et al., 2024). These methods face computational challenges, with increasing dimensionality and more flexibility being considered (Huang & Zou, 2010; Suriana et al., 2023). Despite these challenges, protein flexibility in docking simulations has shown significant improvements in ranking and predicting binding geometries compared to rigid receptor approaches (Harmalkar & Grey, 2020; May & Zacharias, 2008).

Water molecules play a crucial and often underappreciated role in protein-ligand interactions, necessitating detailed approaches for their treatment in molecular docking calculations (Lexa & Carlson, 2023). Water molecules can significantly influence binding energetics through direct and indirect effects, including water-mediated hydrogen bonding networks and displacement of ordered water molecules upon ligand binding (Xiao et al., 2018). Various methodological approaches have been developed to account for these effects, ranging from explicit inclusion of crystallographic water molecules to implicit solvent models that approximate the thermodynamic consequences of water displacement (Vivo et al., 2016; Zsidó & Hetényi, 2020). The accurate treatment of water effects remains a significant challenge in molecular docking, particularly in cases where water molecules mediate protein-ligand interactions (Gelpi et al., 2015; Stachowski et al., 2022).

The validation of molecular docking results requires approaches that integrate multiple metrics and experimental data sources (Pagadala et al., 2023). Modern validation protocols employ various statistical measures to assess prediction accuracy, including geometric criteria such as root-mean-square deviation calculations, as well as more comprehensive metrics that evaluate prediction quality across large datasets (Zev et al., 2021). Integrating experimental data, particularly crystallographic structures of protein-ligand complexes, provides crucial benchmarks for assessing the accuracy of docking methods (Torres et al., 2019). However, the limitations of experimental data, including crystal packing effects and resolution limitations, must be carefully considered in validation protocols. The development of robust validation methods remains an active area of research in the field (Pagadala et al., 2023).

The application of molecular docking to natural products presents unique challenges that have driven methodological innovation in the field (Pinzi & Rastelli, 2019). Natural products often exhibit newly discovered complex structural features, including multiple stereogenic centres, conformational flexibility and unusual substitution patterns that complicate docking calculations (Glasser et al., 2024). Furthermore, many natural products violate traditional drug-like property criteria, requiring more detailed approaches for accurate binding prediction. These challenges have led to the development of modified scoring functions and sampling algorithms specifically optimised for natural product docking, incorporating additional terms to account for their unique physicochemical properties (Ain et al., 2024; Guedes, 2021).

In recent years, molecular docking methodology has incorporated machine learning approaches to improve prediction accuracy and computational efficiency (Ain et al., 2024). These methods can learn from large datasets of protein-ligand complexes to identify subtle patterns in binding interactions that may not be captured by traditional scoring functions (Ragoza et al., 2023). Integrating deep learning algorithms with molecular docking has improved prediction accuracy, particularly in challenging cases where traditional methods may fail. These advances represent a significant shift toward data-driven approaches that complement conventional physics-based methods, as seen in recent cancer research (Kamerlin, 2023; Yu et al., 2023).

2.3.3 Integration of *In Silico* Methods

The integration of multiple computational approaches in drug discovery represents an advanced methodological framework that transcends the limitations of individual methods while leveraging their complementary strengths (Hong et al., 2020). This integration combines network pharmacology, molecular docking and other computational techniques to create a more comprehensive and nuanced understanding of drug-target interactions and therapeutic mechanisms (Jiao et al., 2020). The theoretical foundation for this integration rests on the recognition that biological systems exhibit multiple levels of complexity that cannot be adequately addressed through any single computational approach (Wang et al., 2018).

The synergistic combination of network pharmacology and molecular docking provides compelling insights into drug action mechanisms (Hou et al., 2023). Network pharmacology offers a systems-level perspective on drug-target interactions and their broader biological implications, while molecular docking provides detailed atomic-level insights into specific binding interactions (Pu et al., 2023). Incorporating these approaches creates a multi-scale analytical framework that can capture the broader biological context of drug action and the specific molecular mechanisms through which drugs interact with their targets (Agu et al., 2023; Wozniak et al., 2024). This integration necessitates using advanced computational methods capable of assimilating and analysing data across multiple scales of biological organisation. Such methods include algorithms designed to harmonise molecular, cellular and systemic-level information, enabling a holistic understanding of compound-target interactions and mechanistic pathways (Paltun et al., 2021).

Developing integrated computational workflows represents a significant methodological advancement in the field. These workflows typically begin with network-based approaches to identify potential therapeutic targets and drug candidates, followed by detailed molecular docking analyses to evaluate specific binding interactions (Pu et al., 2023). The challenge lies in developing robust methods for integrating the results from different computational approaches while maintaining the scientific rigour of each technique (Sethi et al., 2019). This integration may often require statistical methods for combining various data types and evaluating the reliability of predictions.

Machine learning approaches have emerged as powerful tools for integrating different computational methods in drug discovery (Patel et al., 2020). These approaches can learn complex patterns from various data sources, including network topology data, molecular interaction data and structural information (Ao et al., 2023; Svensson et al., 2024). The development of deep learning architectures that simultaneously process multiple types of input data has improved prediction accuracy in drug discovery applications (Pan et al., 2022; Unterthiner et al., 2023). These methods can capture subtle relationships between different levels of biological organisation that might not be apparent through traditional analysis approaches (Wang et al., 2021).

Applying integrated computational methods to natural product research presents unique opportunities and challenges. Natural products often exhibit complex mechanisms of action that can be better understood through the combination of multiple computational approaches (Medina Franco, 2021; Yoo et al., 2020). Network pharmacology can identify potential therapeutic targets and biological pathways affected by natural compounds, while molecular docking can provide detailed insights into specific binding interactions (Ren et al., 2022). The integration of these approaches has proven particularly valuable in understanding the therapeutic effects of traditional medicines and identifying new applications for natural compounds (Agu et al., 2023).

Recent advances in integrated computational methods have incorporated dynamic aspects of biological systems into drug discovery workflows. This includes the development of methods for analysing how drug effects propagate through biological networks over time and how specific molecular interactions influence broader biological responses (Naqvi et al., 2018). The integration of molecular dynamics simulations with network-based approaches has provided new insights into the temporal

aspects of drug action and the dynamic nature of drug-target interactions (Wang et al., 2022).

Integrating computational methods into drug repositioning represents another significant development in the field (Ko, 2020). These approaches can identify new therapeutic applications for existing drugs by combining network-based analysis of disease mechanisms with detailed evaluation of molecular interactions (Zhou et al., 2023). The ability to simultaneously consider multiple aspects of drug action has led to identifying novel therapeutic applications for existing compounds and improved understanding of drug side effects (Dinić et al., 2020).

In the rise of artificial intelligence, deep learning approaches have also significantly enhanced the capabilities of integrated computational methods (Grantham et al., 2022; Pan et al., 2022). These methods can process and analyse large amounts of biological data from many sources, identifying patterns and relationships that might not be apparent through traditional analysis approaches (Li et al., 2019). The development of neural network architectures specifically designed for biological data analysis has enhanced the accuracy of drug discovery predictions and expedited the identification of new therapeutic candidates (Muzio et al., 2020).

On the other hand, the computational demands of integrated approaches present another significant challenge (Chen et al., 2023). The simultaneous application of multiple computational methods, particularly when analysing large biological networks or performing extensive molecular simulations, requires substantial computational resources (Gligorijević & Pržulj, 2015). Developing more efficient algorithms and parallel computing approaches has partially addressed this challenge; however, continued advancements in computational methodology remain crucial (Salanne et al., 2023). Future directions in this area include the development of scalable algorithms that can maintain prediction accuracy while reducing computational complexity (Srinivasan et al., 2022).

Data quality and standardisation also represent persistent challenges in integrated computational approaches (Tarazona et al., 2021). The reliability of predictions depends heavily on the quality of input data, which can vary significantly across different data sources and types (National et al., 2023). Developing robust data quality assessment and standardisation methods is essential for improving the reliability of integrated computational approaches (MacDonald et al., 2023). This includes methods for handling missing data, resolving conflicts between different data sources

and accounting for experimental uncertainty in computational predictions (Foidl et al., 2023).

Translating computational predictions into practical therapeutic applications represents perhaps the most significant challenge in the field. While integrated computational methods can generate numerous predictions about potential therapeutic interventions, the path from computational prediction to clinical application involves validating the *in silico* results in an *in vitro* or *in vivo* setting. Future advances may involve better methods to predict drug effectiveness and safety, improved integration of pharmacokinetic and pharmacodynamic factors into computational workflows, and more accurate approaches to prioritising drug candidates for experimental validation, all while utilising computationally efficient algorithms.

This comprehensive literature review has established three fundamental pillars that justify the computational investigation of morindolide's anti-inflammatory and antioxidant mechanisms. First, morindolide emerges as a promising but understudied iridoid lactone with documented traditional medicinal applications, yet it lacks detailed mechanistic characterisation. The compound's structural features, particularly its lactone moiety and hydroxyl groups, suggest potential for multi-target interactions. Preliminary pharmacological evidence indicates anti-inflammatory and antioxidant activities that remain mechanistically unexplored at the molecular level. Second, the intricate interconnection between inflammatory pathways and oxidative stress mechanisms demonstrates that effective therapeutic interventions must address both processes simultaneously. The bidirectional amplification between ROS generation and pro-inflammatory signalling, mediated through key regulatory proteins such as PTGS2, NOS2 and cytochrome P450 enzymes, establishes a theoretical framework for understanding how natural compounds like morindolide might exert dual therapeutic effects through multi-target modulation rather than single-pathway inhibition.

Third, recent advances in computational drug discovery methodologies provide powerful tools for investigating such complex mechanisms. The integration of network pharmacology and molecular docking represents a particularly suitable approach for natural product research, offering complementary perspectives that span from systems-level target identification to atomic-level binding interactions. Network pharmacology enables the prediction of multi-target effects and pathway-level mechanisms, which are essential for understanding compounds with pleiotropic activities. Meanwhile, molecular docking provides detailed insights into specific protein-ligand interactions,

validating and refining network-based predictions. This integrated computational framework has proven especially valuable for traditional medicine compounds where extensive experimental screening would be resource-intensive and time-consuming.

Despite the growing body of research on both computational methodologies and natural anti-inflammatory agents, a significant knowledge gap persists regarding the precise molecular mechanisms of morindolide. While related iridoid compounds have demonstrated therapeutic potential through modulation of inflammatory and oxidative pathways, morindolide itself lacks systematic computational characterisation. No previous studies have applied integrated network pharmacology and molecular docking approaches to comprehensively map morindolide's target profile, predict its interaction with key regulatory proteins, or elucidate the molecular basis of its reported therapeutic effects. This gap is particularly significant given morindolide's structural uniqueness and its traditional use in conditions characterised by inflammation and oxidative stress.

The present study addresses this gap by applying validated computational methodologies to investigate the anti-inflammatory and antioxidant mechanisms of morindolide systematically. The integration of network pharmacology for multi-target prediction and molecular docking for binding interaction analysis provides a robust framework for generating testable hypotheses about morindolide's therapeutic mechanisms. This approach not only advances our understanding of a specific bioactive compound but also demonstrates the broader utility of integrated computational methods in natural product pharmacology, potentially accelerating the translation of traditional medicine knowledge into evidence-based therapeutic applications. The methodological framework and analytical approaches employed in this investigation, detailed in the following chapter, were specifically designed to address the identified research gaps while maintaining computational rigour and biological relevance.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Research Design and Framework

This study employs two integrated computational approaches: network pharmacology and molecular docking analysis. A systematic analysis of protein-protein interactions and pathway mapping was conducted for network pharmacology using multiple databases (SymMap, Swiss Target Prediction and PharmMapper). For molecular docking, binding interactions between morindolide and seven identified hub proteins (CYP2E1, MMP2, NOS2, ILIB, PTGS2, PLA2G2A and HSP90AA1) were analysed using AutoDock Tools, focusing on specific amino acid residues at active sites. The study utilises data from three primary sources: (1) morindolide's structural information from PubChem, (2) anti-inflammatory gene targets from GeneCards database and (3) antioxidant-related genes, also from GeneCards database. The overall methodology used in this investigation is illustrated in Figure 3.1, demonstrating the sequential integration of computational approaches for understanding morindolide's therapeutic mechanisms.

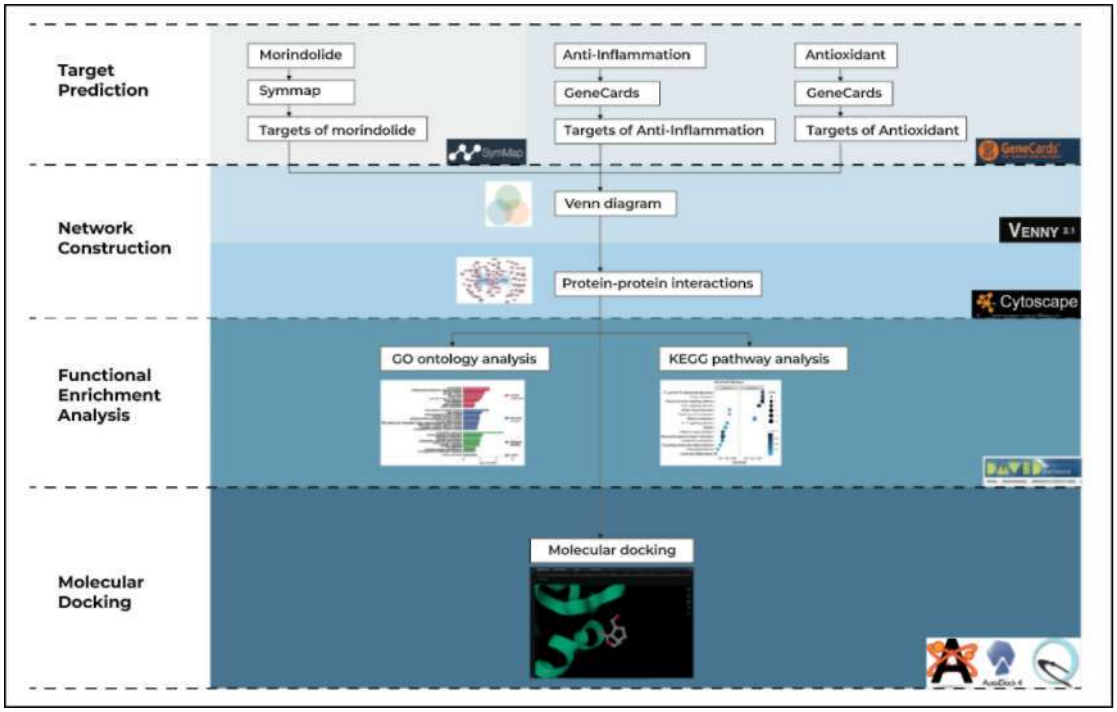


Figure 3.1 Flowchart of Study Methods

3.2 Target Retrieval and Analysis

3.2.1 Database Mining and Target Identification

Target identification for morindolide employed a systematic, multi-database approach (accessed October 3, 2023). The initial structural information for morindolide was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), which was selected for its comprehensive chemical annotations and standardised molecular descriptors, including chemical identifiers, 3D structures and physicochemical properties. Target prediction utilised three complementary databases: SymMap V. 2 (http://www.symmap.org), chosen for its traditional Chinese medicine-focused target annotations; Swiss Target Prediction (http://www.swisstargetprediction.ch/), employed for its machine learning-based prediction algorithms; and PharmMapper (https://www.lilab-ecust.cn/pharmmapper/), selected for its pharmacophore-based target identification capabilities (Daina et al., 2019; Liu et al., 2010; Wu et al., 2018).

For disease-related targets, the GeneCards database (http://www.genecards.org/) was queried using specific search terms, "Anti-inflammatory" and "Antioxidant", with "Homo sapiens" specified as the organism (Stelzer et al., 2016). Target data standardisation was performed using UniProt ID mapping (https://www.uniprot.org/id-mapping) to convert different target identifiers into official gene symbols (Bateman et al., 2024). Additional target verification employed the STRING database (https://string-db.org) to ensure data quality and relevance (Szklarczyk et al., 2022).

3.2.2 Gene Target Analysis

Gene target analysis proceeded through multiple analytical stages. Initial data preprocessing involved the removal of duplicate entries and the standardisation of gene nomenclature. Common targets between morindolide and disease-related genes were identified using DeepVenn (http://www.deepvenn.com), employing area-proportional Venn diagram analysis (Hulsen, 2022). The intersecting gene sets underwent functional annotation using the DAVID database (https://david.ncifcrf.gov/), focusing on GO terms and KEGG pathway enrichment (Sherman et al., 2022). Target identification and enrichment analysis applied multiple metrics, such as p-value thresholds and confidence scores, to determine statistical significance. For database predictions, a confidence

score threshold of 0.7 was applied (Hou et al., 2023). Enrichment analysis utilised a p-value cutoff of 0.05 for statistical significance (Ren et al., 2022).

3.3 Network Construction and Analysis

3.3.1 Protein-Protein Interaction Network Development

The protein-protein interaction network construction utilised the STRING database version 12.0 (https://string-db.org) with specific analytical parameters (Szklarczyk et al., 2022). The network was configured using "Homo sapiens" as the target organism, with advanced settings defined as "full STRING network" type and required confidence score set to "high confidence (0.700)" (Hou et al., 2023). False discovery rate (FDR) stringency was maintained at a medium level of 5% (Hou et al., 2023). Disconnected nodes were excluded from the network to focus the analysis on functionally relevant interactions.

Network visualisation and analysis employed Cytoscape software version 3.10.1 (https://cytoscape.org) (Shannon et al., 2003). The cytoHubba plugin was implemented to identify hub genes, utilising the MCC (Maximal Clique Centrality) algorithm for node prioritisation (Chin et al., 2014). Network construction maintained all protein-protein interactions with confidence scores above 0.7, ensuring high-quality interaction data (Ma et al., 2021).

3.3.2 Network Topology Analysis

Functional enrichment analysis of the network utilised both DAVID Functional Annotation Bioinformatics Microarray Analysis (https://david.ncifcrf.gov/home.jsp) and ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/) (Ge et al., 2019; Sherman et al., 2022). Enrichment parameters included a statistical significance threshold ($p < 0.05$) and a minimum gene count per category (n 2) (Wang et al., 2022). Results visualisation employed Python 3.10.11 with specialised libraries for network visualisation and analysis.

3.4 Molecular Docking Analysis

3.4.1 Protein and Ligand Preparation

Protein structure preparation focused on hub genes identified through network analysis. Three-dimensional structures of target proteins were obtained from the RCSB Protein Data Bank (https://www.rcsb.org) in .pdb format (RCSB PDB, 2024). Protein preparation utilised AutoDock Tools 1.5.7, following a systematic protocol: removal of non-protein molecules, including water and co-crystallised ligands, repair of missing atoms and residues, addition of hydrogen atoms and calculation of partial charges (Seeliger & Groot, 2010). The final structures were exported in *.pdbqt files for docking analysis.

Morindolide's 3D structure was retrieved from the PubChem database in *.sdf files and converted to *.pdb files using OpenBabel 2.4.1 (O'Boyle et al., 2011). Ligand preparation in AutoDock Tools 1.5.7 included detecting the torsion tree root and exporting the results to *.pdbqt files. Structure optimisation maintained all rotatable bonds for flexible docking simulations (Seeliger & Groot, 2010).

3.4.2 Active Site Determination

Before target compound docking, re-docking of co-crystallised ligands was performed as a validation control to confirm the accuracy of docking parameters. Active site identification employed literature-based analysis of key amino acid residues involved in ligand binding for each target protein, cross-referenced with experimental binding site data from the Protein Data Bank and published crystallographic studies. This literature-guided approach ensured that grid boxes captured functionally relevant binding regions.

Grid boxes were created using AutoDock Tools 1.5.7 to encompass all identified active site residues (Wang et al., 2022). Grid box positioning was customised for each protein target based on the respective binding sites (Table 3.2). The grid centres were determined from the active site residues identified from literature analysis, ensuring optimal coverage of the catalytic or binding pocket. A uniform grid box dimension of 60 × 60 × 60 Å was employed across all seven target proteins. Validation of active sites utilised re-docking of co-crystallised ligands, with success criteria including negative

binding energy values and root-mean-square deviation (RMSD) calculations (Ren et al., 2022). An RMSD threshold of 2.0 Å was established between the re-docked and crystallographic poses as the validation criterion, consistent with widely accepted standards in molecular docking studies. This threshold ensures that the docking protocol can reproduce experimentally determined binding modes with acceptable accuracy while accounting for inherent algorithmic limitations and crystal structure artefacts (Souza et al., 2023).

Table 3.1
Grid Box for Molecular Docking.

Ligand	Grid Centre (x, y, z) (Å)	Grid Dimensions (Å)
4PZ	30.790, 29.085, 22.191	60 x 60 x 60
AT2	56.984, 19.504, 79.833	60 x 60 x 60
WHA	-28.093, 21.613, -20.568	60 x 60 x 60
X28	5.595, 4.013, -0.194	60 x 60 x 60
ID 8	38.961, 2.353, 61.503	60 x 60 x 60
C	51.751, -56.046, 21.558	60 x 60 x 60
T9C	-18.083, -5.039, -41.894	60 x 60 x 60

3.4.3 Docking Simulations

Molecular docking simulations were executed using AutoDock v4.2.6. The docking protocol employed Autogrid4.exe for binding site definition, followed by docking parameter optimisation (Trott & Olson, 2009). The genetic algorithm parameters were set to 500 runs, a population size of 300 and the maximum number of evaluations configured to ‘long’ (25,000,000). Docking results were visualised and analysed using LigPlot+ 2.2 (Laskowski & Swindells, 2011).

CHAPTER 4
RESULTS

4.1 Target Identification Results

4.1.1 Gene Target Analysis

The systematic mining of multiple databases yielded 219 potential target genes for morindolide. Concurrent analysis of disease-related targets through the GeneCards database identified 5,287 genes associated with anti-inflammatory processes and 2,015 genes linked to antioxidant activities. A comprehensive network diagram visually represented these target genes as rectangular nodes (Figure 4.1).

The DeepVenn analysis revealed significant overlaps between morindolide's predicted targets and disease-related genes, identifying 56 common targets at intersection points. Of these, 21 genes demonstrated dual anti-inflammatory and antioxidant effects, while 34 showed exclusive anti-inflammatory activity and one exhibited solely antioxidant properties (Figure 4.2).

The compound-target-disease network visualisation employed colour-coded nodes representing different functional categories: blue circular nodes for morindolide, green for antioxidant activity and red for anti-inflammatory properties. Common gene targets appeared as yellow square nodes with distinctive border colours indicating their functional roles: blue borders for dual activity, red borders for anti-inflammatory specificity and green borders for antioxidant specificity (Figure 4.3).

Figure 4.1 Node Graph of 219 Predicted Target Genes of Morindolide (Blue Rectangular Nodes), Connected Based on Known or Predicted Interactions

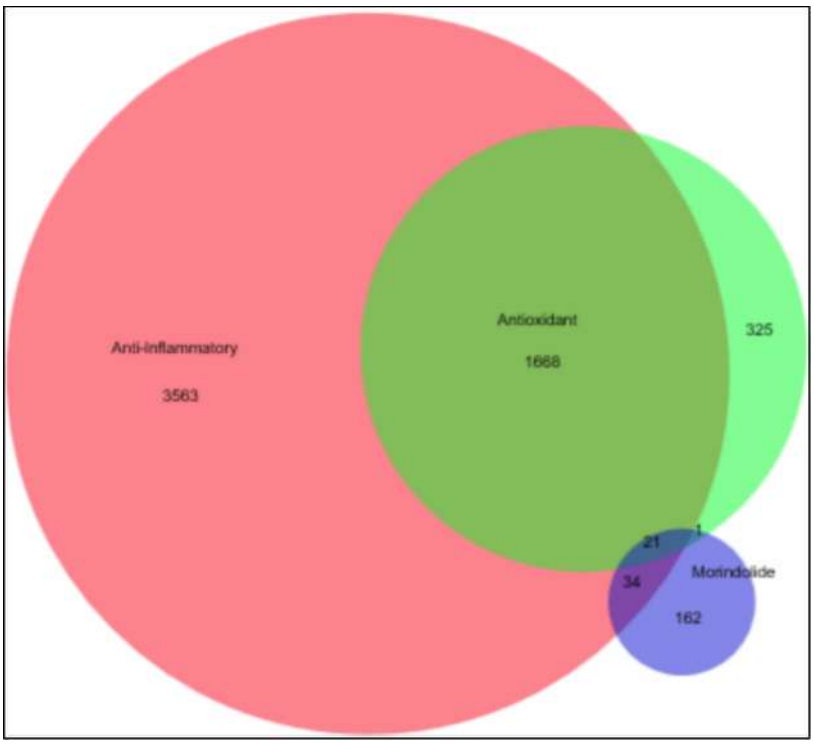


Figure 4.2 Venn Analysis Diagram Showing Morindolide's Intersection with AI and AO Target Genes

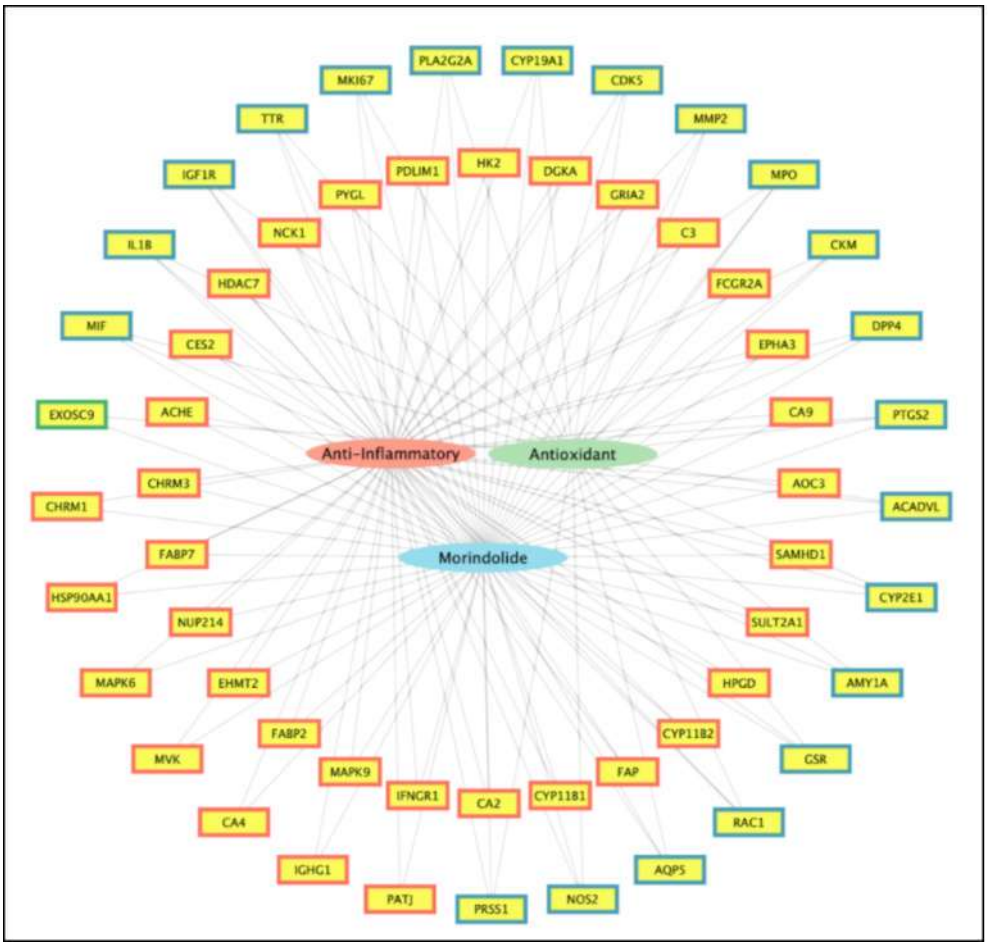


Figure 4.3 The Compound-Target-Disease Network of Morindolide As an Anti-inflammatory and Antioxidant Agent. The Blue Circular Node Represents Morindolide, The Green Circular Node Represents Antioxidants, and The Red Circular Node Represents Anti-Inflammatory Action. Yellow Square Nodes Represent Common Gene Targets, with A Blue Stroke Border Indicating Interaction with Both Anti-Inflammatory and Antioxidant Compounds, A Red Border Indicating Interaction with Only Anti-Inflammatory Compounds, and A Green Border Indicating Interaction with Only Antioxidant Compounds. Light Grey Edges Represent Interactions Among Compounds, Targets and Disease

4.2 Protein-Protein Interaction Analysis

4.2.1 Network Analysis Findings

The protein-protein interaction (PPI) network analysis through STRING 12.0 database revealed intricate patterns of molecular interactions among the identified targets. Initial network construction yielded 56 nodes, with subsequent refinement through removal of disconnected nodes resulting in a core network of 22 nodes connected by 44 edges (Figure 4.4). This network demonstrated significant functional organisation, with each node representing a distinct protein target and each edge

denoting validated protein-protein interactions, suggesting functional clustering of interaction modules.

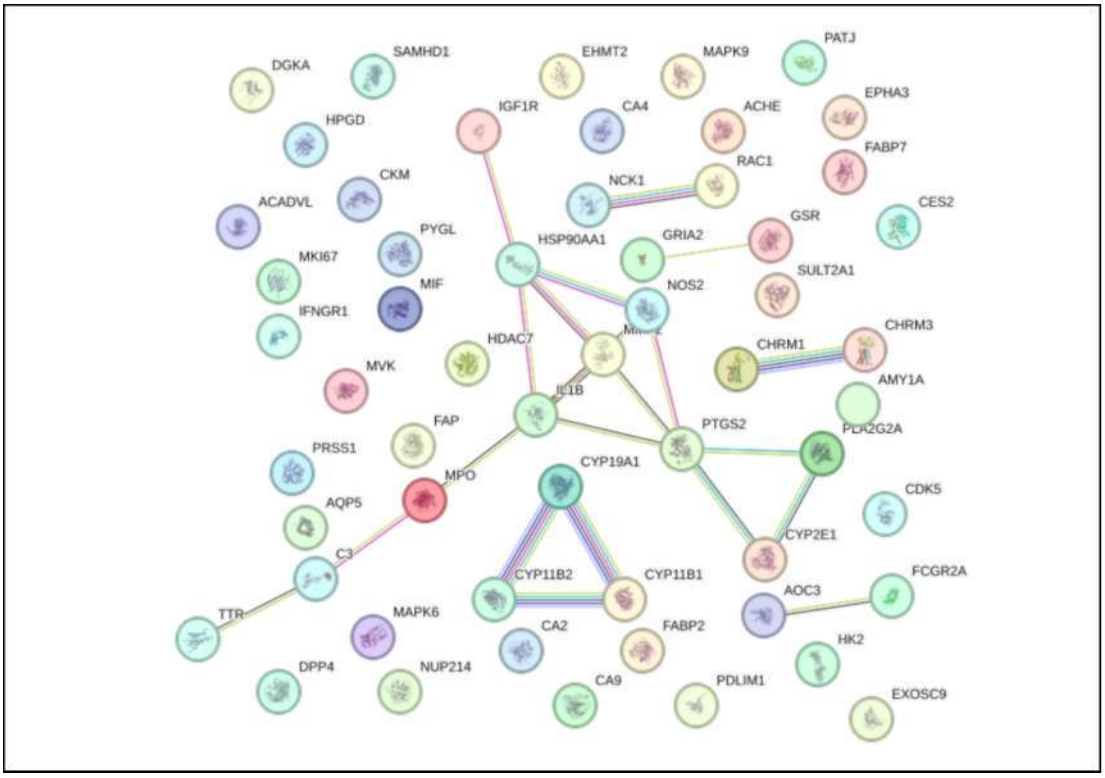


Figure 4.4 Network Visualisation of 56 Target Nodes in STRING Database PPI Network. Nodes Represent Individual Proteins, While Edges Indicate Validated Protein-Protein Interactions Based on STRING Database Criteria

Network reconstruction using Cytoscape 3.10.1 enhanced the visualisation and analytical capabilities, maintaining the core structure of 22 nodes and 44 edges (Figure 4.5). Analysis of node connectivity revealed varying degrees of interaction, with individual nodes exhibiting differential edge counts ranging from 1 to 5 connections (Table 4.1). A complete detailed analysis is in Appendix 2, Table A1. This distribution pattern suggests the presence of key regulatory nodes within the network.

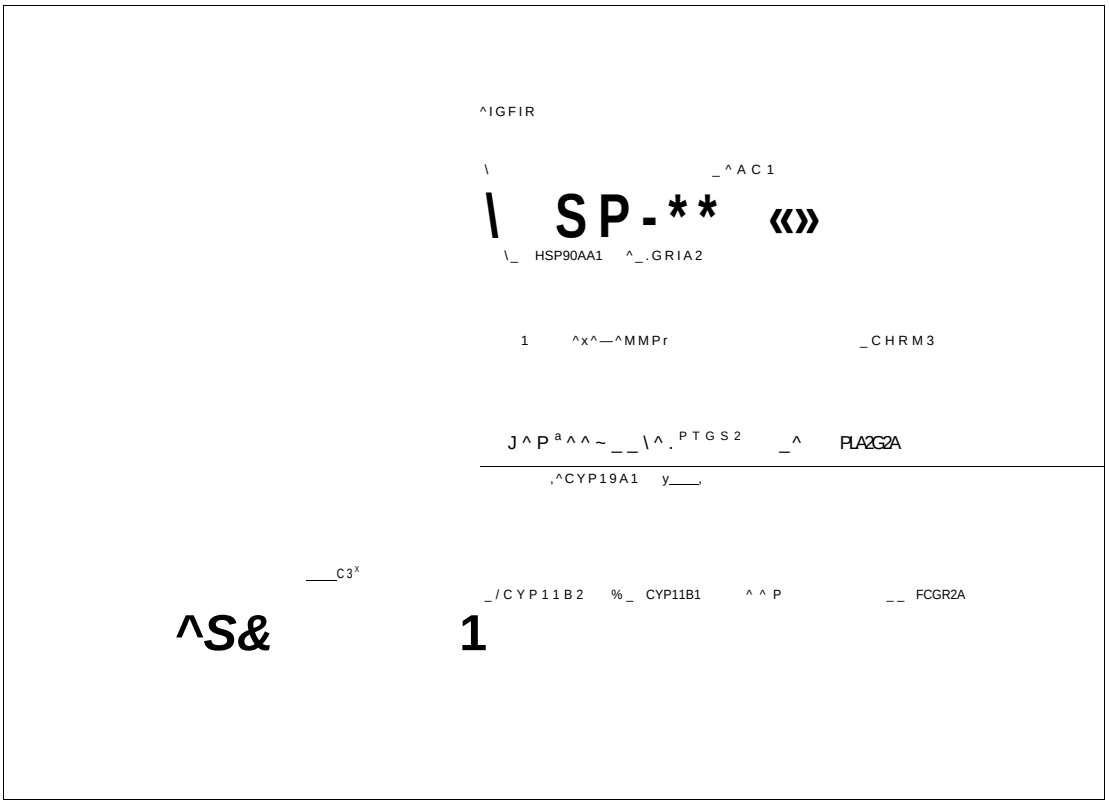


Figure 4.5 Reconstructed Network Visualisation of 22 Functionally Connected Nodes Using Cytoscape 3.10.1. Node Size Reflects the Degree of Connectivity, While Edge Thickness Indicates Interaction Strength

Table 4.1
Node Connectivity with Edge Count

No	Node	Protein Name	Edge
1	IL1B	Interleukin-1 beta	5
2	PTGS2	Prostaglandin G/H synthase 2	5
3	HSP90AA1	Heat shock protein HSP 90-alpha	4
4	MMP2	Matrix metalloproteinase-2	3
5	NOS2	Nitric oxide synthase type 2	3
6	C3	Complement C3	2
7	CYP11B1	Cytochrome P450 11B1	2

4.2.2 Hub Gene Identification

Implementing the cytoHubba plugin identified seven hub genes representing critical points of network regulation (Figure 4.6). These hub genes - IL1B, PTGS2, HSP90AA1, MMP2, NOS2, CYP2E1 and PLA2G2A - exhibited high connectivity and centrality values, indicating their potential importance in mediating further therapeutic effects of morindolide. Network topology analysis of these hub genes revealed organisational parameters (Table 4.2 and Table 4.3), including a network diameter of 3, a clustering coefficient of 0.710, and a network density of 0.524, indicating a highly organised and functionally integrated hub network.

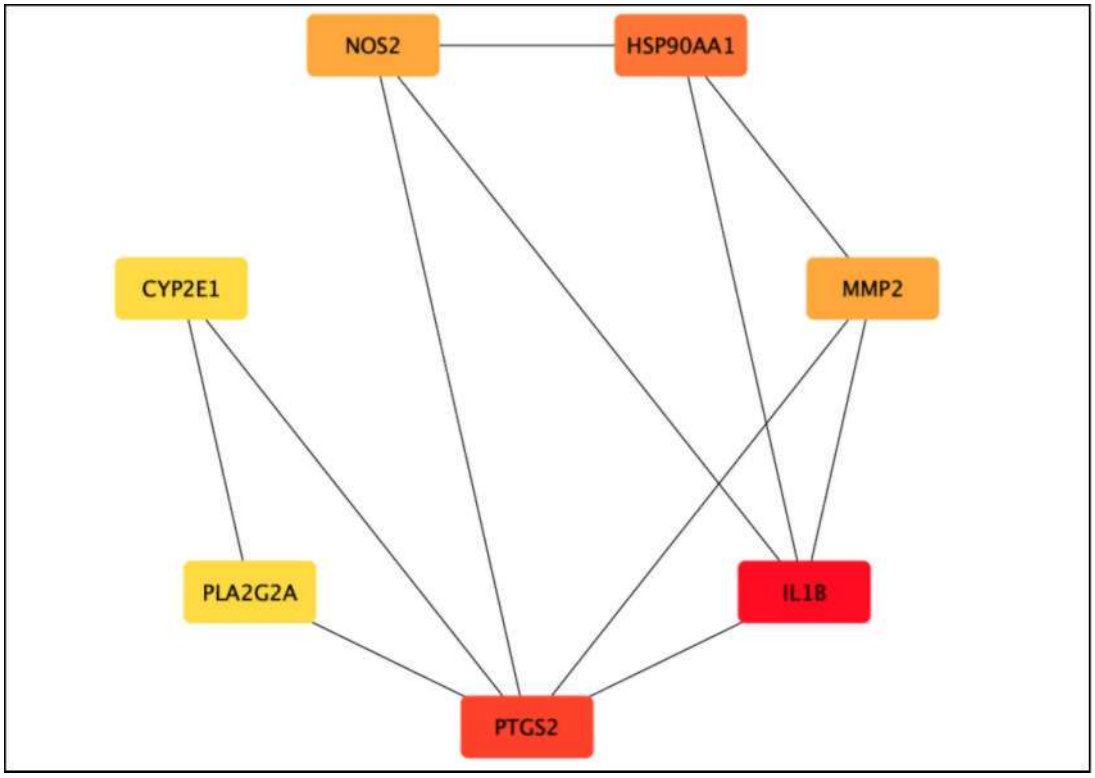


Figure 4.6 Hub Gene Network Visualisation Derived from Cytohubba Analysis, Highlighting the Seven Identified Hub Genes and Their Interconnections

Table 4.2
Detailed Characterisation of Hub Genes

No	Node	Protein Name	Edge
1	IL1B	Interleukin-1 beta	5
2	PTGS2	Prostaglandin G/H synthase 2	5
3	HSP90AA1	Heat shock protein HSP 90-alpha	4
4	MMP2	Matrix metalloproteinase-2	3
5	N0S2	Nitric oxide synthase type 2	3
6	CYP2E1	Cytochrome P450 2E1	2
7	PLA2G2A	Phospholipase A2	2

Table 4.3
Hub Network Topological Parameters

Network Parameters	Value
Number of nodes	7
Number of edges	11
Averag e num ber of ne ighbo urs	3.143
Network diameter	3
Network radius	2
Characteristic path length	1.571
Clustering coefficient	0.710
Network density	0.524
Connected components	1

4.3 Pathway Analysis Results

4.3.1 GO Analysis Findings

Gene Ontology functional annotation analysis revealed 31 significant GO terms ($p < 0.05$), comprising 24 biological process (BP) terms, three molecular function (MF) terms and four cellular component (CC) terms. The distribution analysis demonstrated BP terms constituting 77.4% of enriched items, followed by CC at 12.9% and MF at 9.7% (Fig. 4.7).

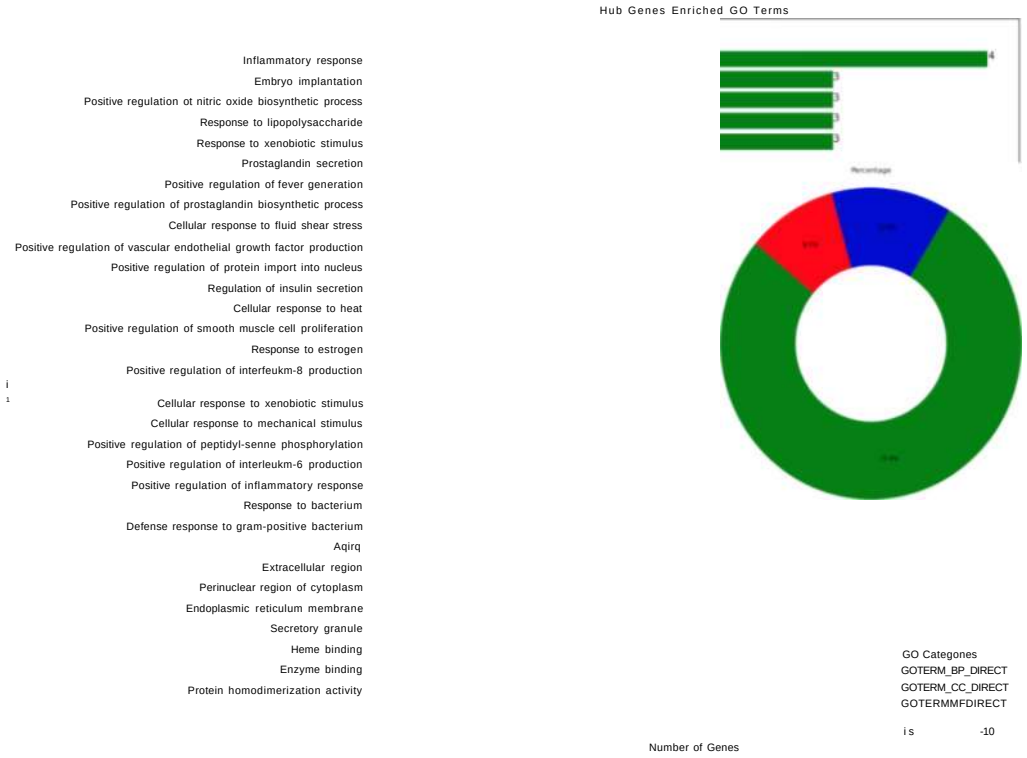


Figure 4.7 The GO Functional Annotation Analysis. The Bar Chart Represents Each Category and the Percentage of Each Category in GO Terms. BP, CC, and MF Categories are Represented by Green, Blue, and Red, Respectively

Notes/Sources: [drawn using Python 3.10.11 (https://www.python.org)]

The results of GO functional enrichment analysis showed that morindolide treatment for anti-inflammatory and antioxidant effects was mainly regulated by inflammatory response, positive regulation of nitric oxide biosynthetic process, response to lipopolysaccharide, response to xenobiotic stimulus, prostaglandin secretion, positive regulation of fever generation positive regulation of interleukin-6 production, positive regulation of inflammatory response, defence response to gram-positive bacterium and ageing in BP, extracellular region and perinuclear region of cytoplasm in CC and heme binding, enzyme binding and protein homodimerization activity in MF. The above analysis suggested that morindolide may exert anti-inflammatory and antioxidant effects by participating in various biological regulatory processes.

4.3.2 KEGG Pathway Analysis

KEGG enrichment analysis identified nine significant pathway items (Figure 4.8), with detailed pathway information and gene associations presented in Table 4.4. The analysis revealed significant enrichment in cancer pathways ($p = 0.004086$), with involvement of HSP90AA1, NOS2, MMP2 and PTGS2. Arachidonic acid metabolism demonstrated the highest statistical significance ($p = 0.000731$), involving PLA2G2A, CYP2E1 and PTGS2. Additional significant pathways included the IL-17 signalling pathway ($p = 0.001729$) and fluid shear stress response ($p = 0.003740$). The pathway analysis revealed interconnections between inflammatory and oxidative stress responses, with key genes demonstrating multiple involvement in various pathways. This multi-pathway engagement suggests that multiple regulatory mechanisms underlie morindolide's therapeutic effects.

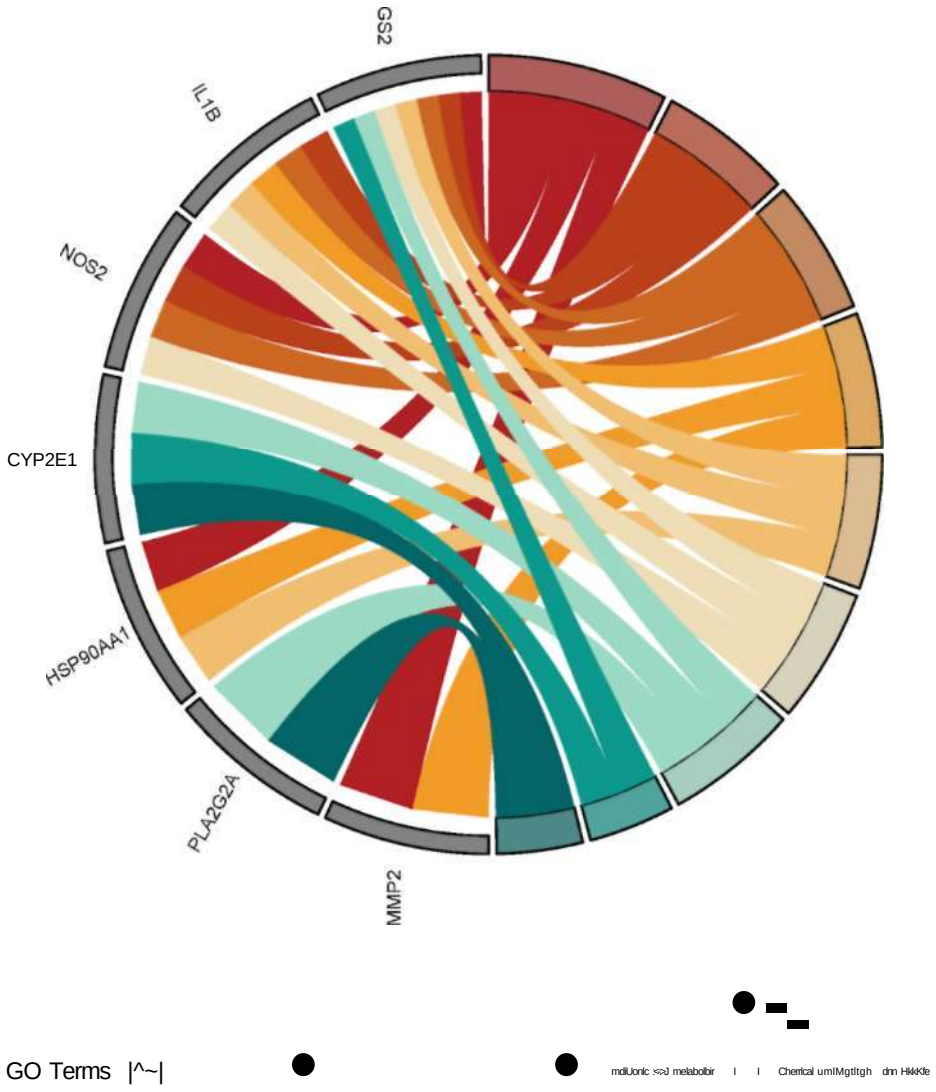


Figure 4.8 KEGG Enrichment Pathways Analysis. Sankey Diagram Showing Pathway Terms and Corresponding Targets, the Right Axis Represents Signal Pathways, and the Left Axis Shows Relevant Targets. Line Density Indicates Enrichment Degree

Notes/Sources: [drawn by Python 3.10.11 (https://www.python.org)]

Table 4.4
Annotation of KEGG Pathways Showing Pathway IDs, Descriptions, p-values, Involved Genes and Gene Counts for Each Identified Pathway

ID	Description	p-value	Genes	Count
hsa05200	Pathways in cancer	0.004086	HSP90AA1, NOS2, MMP2, PTGS2	4
hsa05022	Pathways of neurodegeneration - multiple diseases	0.039644	NOS2, IL1B, PTGS2	3
hsa05010	Alzheimer disease	0.026550	NOS2, IL1B, PTGS2	3
hsa05418	Fluid shear stress and atherosclerosis	0.003740	HSP90AA1, IL1B, MMP2	3
hsa04657	IL-17 signalling pathway	0.001729	HSP90AA1, IL1B, PTGS2	3
hsa05140	Leishmaniasis	0.001163	NOS2, IL1B, PTGS2	3
hsa00590	Arachidonic acid metabolism	0.000731	PLA2G2A, CYP2E1, PTGS2	3
hsa05204	Chemical carcinogenesis - DNA adducts	0.047944	CYP2E1, PTGS2	2
hsa00591	Linoleic acid metabolism	0.020788	PLA2G2A, CYP2E1	2

4.4 Molecular Docking Outcomes

4.4.1 Binding Energy Analysis

Initial validation of active sites through co-crystallised ligand docking demonstrated significant binding affinities across all seven hub targets. Before docking morindolide to the hub target proteins, active sites were ensured for their validity by docking the co-crystallised ligand back to their respective protein active sites. All docking results showed negative free binding energies, ranging from -4.87 to -17.13 kcal/mol, confirming the validity of the active sites (Table 4.5). CYP2E1 (PDB ID: 3E4E) with 4PZ at -17.13 kcal/mol, MMP2 (PDB ID: 7XJO) with C at -11.54 kcal/mol, PLA2G2A (PDB ID: 5G3N) with X28 at -9.7 kcal/mol, IL1B (PDB ID: 8C3U) with T9C at -8 kcal/mol, HSP90AA1 (PDB ID: 3WHA) with WHA at -7.77 kcal/mol, PTGS2 (PDB ID: 5IKR) with ID8 at -7.3 kcal/mol and NOS2 (PDB ID: 3E7G) with AT2 at -4.87 kcal/mol.

Table 4.5
Docking Results of Co-Crystallised Ligands and Their Respective Target Proteins Using Autodock Tools 1.5.7, Including Binding Energies and RMSD Values

Ligand	Protein	PDB ID	Free Binding Energy (kcal/mol)	Ref. RMSD (A)
4PZ	CYP2E1	3E4E	-17.13	0.73
AT 2	N0S2	3E7G	-4.87	1.59
WHA	HSP90AA1	3WHA	-7.77	1.34
X28	PLA2G2A	5G3N	-9.7	1.73
ID 8	PTGS2	5IKR	-7.3	0.47
C	MMP2	7XJ0	-11.54	0.46
T9C	IL1B	8C3U	-8	1.95

The binding energy values obtained for co-crystallised ligands (Table 4.5) represent computational predictions to validate active site definitions rather than as absolute measures of binding affinity. Validation success is primarily assessed through RMSD calculations, where all values fell below the accepted 2.0 Å threshold (Pagadala et al., 2017), indicating successful reproduction of crystallographic binding poses. Five structures achieved RMSD < 1.0 Å, demonstrating exceptional pose prediction accuracy. The range of binding energies (-4.87 to -17.13 kcal/mol) reflects the diverse chemical structures and binding modes of the co-crystallised ligands (Hartman et al., 2013). Where available, experimental binding affinities from original publications are provided for context. However, a direct quantitative comparison between computational binding energies and experimental affinities (Kd/Ki values) requires cautious interpretation due to fundamental differences in measurement methodologies (Ramírez & Caballero, 2018).

Molecular docking analysis of morindolide with hub genes revealed binding energies ranging from -5.19 to -6.62 kcal/mol (Figure 4.9). The analysis demonstrated the favourable binding affinity with CYP2E1 (-6.62 kcal/mol), followed by MMP2 (-6.6 kcal/mol) and NOS2 (-6.24 kcal/mol). Detailed energetic parameters, including intermolecular energy and torsional free energy, provided further insights into binding stability (Table 4.6).

Figure 4.9 Heatmap Visualisation of Molecular Docking Results Between Morindolide and Hub Targets. Colour Intensity Represents Binding Energy Strength

Table 4.6
Docking Result of Morindolide and Its Target Proteins Using AutoDock Tools 1.5.7

Protein	PDBID	Free Binding Energy (kcal/mol)
CYP2E1	3E4E	-6.62
MMP2	7XJO	-6.6
NOS2	3E7G	-6.24
IL1B	8C3U	-6.12
PTGS2	5IKR	-5.87
PLA2G2A	5G3N	-5.86
HSP90AA1	3WHA	-5.19

4.4.2 Interaction Pattern Analysis

The binding mode of morindolide with the seven hub genes revealed diverse interaction patterns (Fig. 4.10). In the case of 3E4E, morindolide formed two hydrogen bonds: one with Pro429(A) at 2.75 A and another with Val364(A) at 3.17 A. The interaction with 3E7G revealed three hydrogen bonds: one with Argl99 (A) at 2.69 A and two with Tyr489 (A) at 2.67 A and 2.8 A, respectively. For 3WHA, morindolide exhibited a more complex binding pattern with five hydrogen bonds: Ilel 10(A) at 2.94 A, Thrl09(A) at 2.69 A, Serll3(A) at 2.85 A and two with Lysl 12(A) at 3.2 A. The docking with 5G3N resulted in 3 hydrogen bonds: Gly29(A) at 3.09 A, Asp48(A) at 2.52 A and His47(A) at 2.9 A. Morindolide formed a single hydrogen bond with 5IKR at Tyr385(A) with a distance of 2.92 A and similarly with 7XJO at Vail 18(A) at 3.28 A (Fig. 4.4.H). Lastly, the interaction with 8C3U involved two hydrogen bonds: Met95(A) at 2.82 A and Lys92(A) at 2.85 A. The variable interaction patterns observed across targets suggest that morindolide's binding specificity may enable selective pathway modulation, support its multi-target therapeutic approach while maintaining target selectivity.

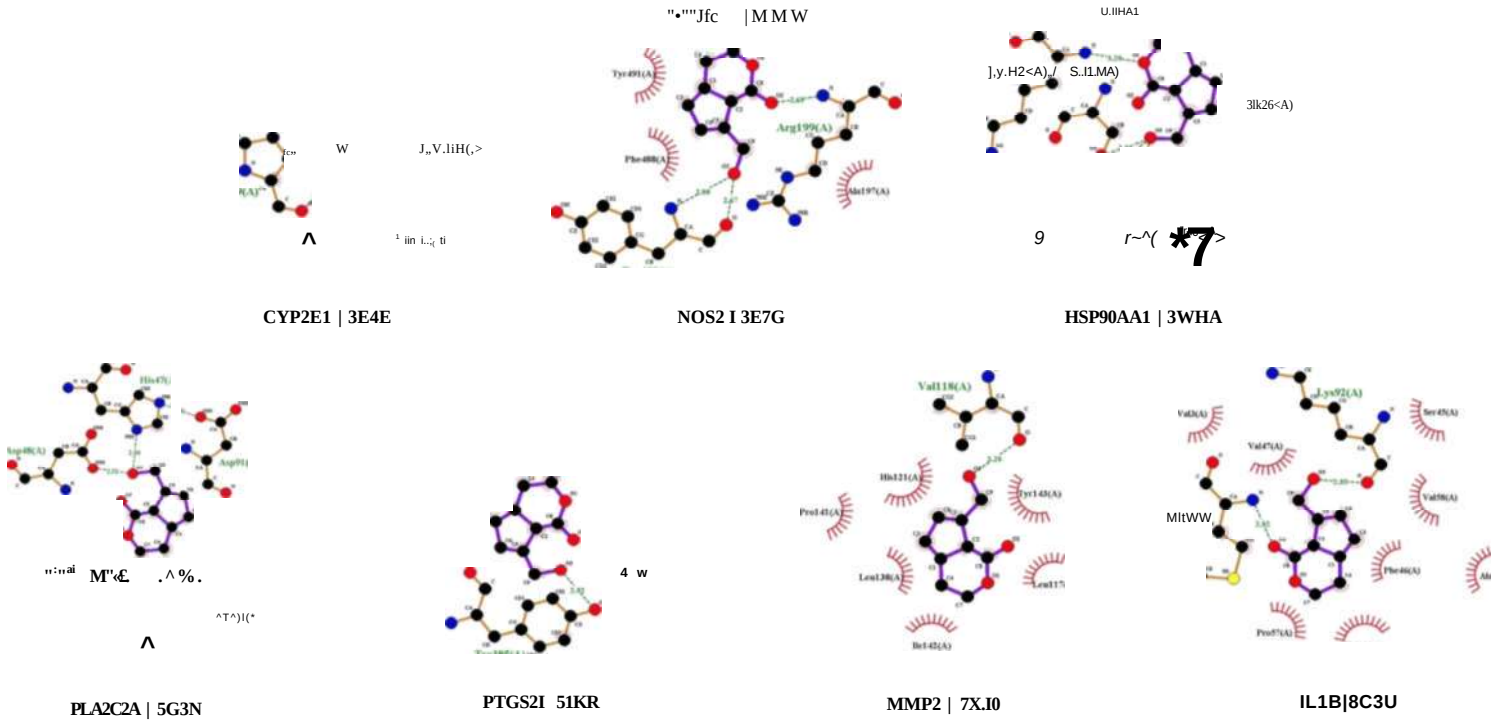


Figure 4.10 The Molecular Docking Result Showing the Binding Site Residues of Protein Targets Visualised by LigPlot+ 2.2

4.5 Summary of Findings

This computational investigation successfully generated comprehensive data across multiple analytical dimensions, addressing the research objectives through integrated network pharmacology and molecular docking approaches. The systematic analysis workflow progressed from broad target identification to specific molecular interaction characterisation, yielding quantitative insights into morindolide's potential therapeutic mechanisms.

The target identification phase established a foundation of 219 predicted target genes for morindolide, with subsequent intersection analysis identifying 56 genes common to both anti-inflammatory and antioxidant pathways. This overlap analysis revealed that 21 targets demonstrated dual functionality, 34 exhibited exclusive anti-inflammatory activity and one showed solely antioxidant properties, providing the first computational evidence for morindolide's multi-target profile. The protein-protein interaction network analysis refined these targets to a core functional network of 22 interconnected proteins, from which seven hub genes (PTGS2, IL1B, MMP2, HSP90AA1, NOS2, PLA2G2A and CYP2E1) were identified through topological analysis using the MCC algorithm. These hub genes demonstrated high degrees of connectivity and strategic network positions, with PTGS2 exhibiting the highest MCC score, suggesting critical regulatory roles in the predicted therapeutic mechanisms.

Functional enrichment analysis provided systems-level insights into the biological processes and pathways potentially modulated by morindolide. GO term analysis identified significant enrichment in inflammatory response, oxidative stress regulation and related biological processes, while cellular component and molecular function annotations highlighted extracellular region localization and enzyme binding activities. KEGG pathway enrichment analysis identified nine statistically significant pathways ($p < 0.05$), with arachidonic acid metabolism ($p = 0.000731$) and IL-17 signalling pathway ($p = 0.001729$) demonstrating the strongest enrichment. These pathway findings established the biological context for morindolide's predicted dual anti-inflammatory and antioxidant effects, revealing the interconnected nature of the targeted molecular processes.

Molecular docking analysis provided atomic-level validation of predicted interactions, with all seven hub proteins demonstrating favourable binding energies ranging from -5.19 to -6.62 kcal/mol. CYP2E1 exhibited the significant binding affinity

(-6.62 kcal/mol) among others, followed closely by MMP2 (-6.6 kcal/mol) and NOS2 (-6.24 kcal/mol). The docking validation protocol, employing re-docking of co-crystallized ligands with RMSD values below 2.0 Å, confirmed the reliability of the docking parameters and active site definitions. Detailed interaction pattern analysis revealed diverse binding modes across targets, with hydrogen bonding playing a central role in all predicted interactions. The number of hydrogen bonds ranged from one (PTGS2, MMP2, IL1B) to five (HSP90AA1), with binding distances predominantly falling within the optimal range of 2.5-3.3 Å. The observed correlation between network topology metrics and binding affinity patterns suggests that morindolide may preferentially target proteins with high network centrality while maintaining moderate binding energies consistent with regulatory rather than strong inhibitory mechanisms.

These integrated findings provide a comprehensive predictive framework for understanding morindolide's potential anti-inflammatory and antioxidant mechanisms, establishing a foundation for the mechanistic interpretations and therapeutic implications discussed in the following chapter.

CHAPTER 5
DISCUSSION

5.1 Integration of Network Pharmacology and Molecular Docking Findings

The integration of network pharmacology with molecular docking analysis has revealed novel insights into morindolide's therapeutic mechanisms, demonstrating a complex interplay between molecular interactions and system-level effects. This study represents the first *in silico* investigation of morindolide's mechanisms through a combined computational approach, addressing a significant knowledge gap in understanding the mechanisms of action of natural compounds. Identifying 56 common targets between morindolide and anti-inflammatory/antioxidant pathways suggests a multi-target therapeutic mechanism, challenging the traditional single-target drug development paradigm.

The network analysis revealed a particularly significant finding in identifying seven hub genes, with PTGS2 and IL1B emerging as central regulatory nodes. PTGS2 functions as an inducible enzyme catalysing the rate-limiting step in prostaglandin biosynthesis from arachidonic acid, converting it to prostaglandin H2 (PGH2), which subsequently generates pro-inflammatory prostaglandins, particularly PGE2 (Martín-Vázquez et al., 2023). Under physiological conditions, PTGS2 expression remains low in most tissues but becomes markedly elevated during inflammatory responses mediated by pro-inflammatory cytokines and growth factors (Crofford, 1997). CYP2E1 serves as a metabolic enzyme within the cytochrome P450 superfamily, whose catalytic cycle generates substantial reactive oxygen species, including superoxide anion radicals and hydrogen peroxide as byproducts, positioning it as a major source of cellular oxidative stress (Harjumäki et al., 2021; Lu & Cederbaum, 2008). The enzyme's heme iron exists constitutively in a high-spin state, allowing for the direct reduction of molecular oxygen during xenobiotic metabolism (Caro & Cederbaum, 2004).

Dysregulation of these enzymes drives pathogenesis across multiple disease states. Irregular PTGS2 upregulation perpetuates chronic inflammation in conditions such as osteoarthritis, rheumatoid arthritis, and inflammatory bowel diseases (Martel-Pelletier et al., 2003), while also contributing to neuroinflammation in Alzheimer's disease through PGE2-mediated excitotoxicity and blood-brain barrier disruption

(Hoozemans et al., 2008). Additionally, PTGS2 overexpression promotes tumour progression in colorectal, pancreatic and lung cancers through PGE2-mediated angiogenesis and immune evasion (Cao & Prescott, 2002). CYP2E1 induction constitutes a primary pathogenic mechanism in alcoholic liver disease and non-alcoholic fatty liver disease (NAFLD), where elevated ROS production triggers hepatocellular injury, lipid peroxidation, mitochondrial dysfunction and progression to steatohepatitis and cirrhosis (Harjumäki et al., 2021). The enzyme's activity correlates directly with severity of liver damage, inflammatory cytokine release and oxidative stress markers in both clinical and experimental settings (Lieber, 1997; Lu & Cederbaum, 2008).

These disease associations align with experimental validations of structurally related iridoid compounds that share the characteristic cyclopentan-[c]-pyran skeleton with morindolide. Monotropein, an iridoid glycoside from Morinda officinalis, achieved 55.8% inhibition of NO production and 78.9% reduction in PGE2 synthesis at 200 μM concentration in LPS-stimulated RAW 264.7 macrophages through suppression of iNOS and COX-2 expression via NF-κB pathway inhibition (Shin et al., 2013). In vivo models demonstrated significant reduction of carrageenan-induced paw oedema at 20-30 mg/kg dosing (Choi et al., 2005). Loganic acid, another structurally similar iridoid, demonstrated the most potent anti-inflammatory activity among tested iridoids, achieving 44.4% oedema inhibition in carrageenan-induced mouse paw oedema models and 72-80% inhibition in TPA-induced ear oedema assays (Recio et al., 1994). Mechanistic investigations revealed that loganic acid operates via dual mechanisms: TLR4/NF-κB pathway inhibition to suppress inflammation and SIRT1/Nrf2 antioxidant pathway activation to combat oxidative stress (Prakash et al., 2023). Aucubin, following β-glucosidase hydrolysis to its aglycone form, exhibited moderate COX-2 inhibitory activity, accompanied by suppression of TNF-α formation and NO production in cellular inflammation models (Park et al., 2010). These experimental validations of related iridoid lactones support morindolide's predicted multi-target mechanisms, demonstrating that compounds within this structural class can simultaneously target PTGS2-mediated inflammatory pathways and CYP2E1-dependent oxidative stress processes through direct enzyme interaction and downstream signalling modulation.

Few studies have highlighted the importance of network topology in drug target selection and efficacy. Proteins targeted by approved drugs exhibit higher centrality within biological networks than investigational targets (Follis, 2021). This aligns with

findings that natural products preferentially target highly connected proteins, which may facilitate disruption of essential pathways (Dančík et al., 2010). Network analysis reveals that biological networks are configured as highly optimised tolerance networks, with extensive interconnections among middle-degree nodes forming the network backbone (Hase et al., 2009). While networks are robust to random deletions, they are susceptible to targeted interventions based on properties like degree and betweenness (Vasan et al., 2023). These insights suggest that effective drug development strategies may also consider both binding affinity and network position, potentially focusing on middle- to low-degree nodes for combinatorial approaches to increase efficacy and reduce side effects (Hase et al., 2009; Young et al., 2012; Zeng et al. 2020).

Pathway analysis revealed specific signalling cascades modulated by morindolide's interactions. The arachidonic acid metabolism pathway showed the highest enrichment ($p = 0.000731$), with three key proteins (PLA2G2A, CYP2E1, PTGS2) demonstrating direct binding interactions. Molecular docking revealed specific binding patterns: IL1B (upstream regulator, binding energy -6.12 kcal/mol, two hydrogen bonds at Met95(A) and Lys92(A)), PTGS2 (downstream effector, binding energy -5.87 kcal/mol, hydrogen bond with Tyr385(A)) and CYP2E1 (metabolic regulator, binding energy -6.62 kcal/mol, two hydrogen bonds at Pro429(A) and Val364(A)). This hierarchical targeting pattern suggests coordinated pathway modulation through simultaneous interaction with rate-limiting enzymes and regulatory proteins, evidenced by enrichment scores in IL-17 signalling ($p = 0.001729$) and leishmaniasis pathways ($p = 0.001163$).

The arachidonic acid metabolic network plays a crucial role in inflammation, producing various pro- and anti-inflammatory mediators (Liu et al., 2009; Tallima, 2021). Studies have highlighted the limitations of single-target inhibitors in controlling this complex network and emphasised the potential of multi-target approaches (He et al., 2012; Temml et al., 2019; Zeng et al., 2021). Pathway analysis and molecular docking have been employed to explore the mechanisms of anti-inflammatory compounds, such as those found in *Morchella esculenta*, which interact with multiple targets in the arachidonic acid cascade (Xiaoying et al., 2023). These studies suggest that effective therapeutic strategies may involve simultaneous inhibition of multiple enzymes, particularly downstream targets like LTA4H and COX, to achieve stronger inhibition efficiency and potentially fewer side effects (He et al., 2012). This multi-target approach aligns with the understanding that inflammatory diseases often result

from complex interactions involving multiple targets, necessitating interventions that simultaneously modulate multiple points in the inflammatory cascade (Liu et al., 2009; Temml et al., 2019).

Besides, analysis of network topology-binding affinity relationships also reveals a quantitative correlation between protein centrality measures and binding energies in morindolide's interaction network. Hub proteins PTGS2 and IL1B, with centrality values of 0.786 and network clustering coefficients of 0.31, demonstrated binding energies of -5.87 and -6.12 kcal/mol, respectively. This binding pattern shows preferential targeting of proteins with betweenness centrality values >0.5, particularly at nodes connecting inflammatory and oxidative stress pathways. While CYP2E1 shows the strongest binding energy among others (-6.62 kcal/mol), it exhibits slightly lower network centrality (0.524), suggesting strategic targeting of metabolic control points rather than purely high-affinity interactions.

The binding affinity observed with CYP2E1 (-6.62 kcal/mol) and stabilising hydrogen bonds with Pro429(A) and Val364(A) suggest a previously unrecognised mechanism through which morindolide might modulate inflammatory responses. This finding is particularly noteworthy given CYP2E1's established role in oxidative stress regulation and inflammatory processes (Hartman et al., 2013). This is aligned with a recent *in silico* study that explored the potential of plant-derived compounds, such as those from *M. oleifera* leaf extracts, to interact with CYP2E1 and other CYP450 enzymes. Flavonoids like galangin showed good binding affinities, suggesting possible hepatoprotective effects through modulation of CYP450 enzyme activity (Sarkar, 2022).

This finding highlights an important distinction between traditional single-target drug design and network pharmacology approaches. While conventional drug design principles prioritize highly specific, strong binding interactions (typically < -10 kcal/mol) for single-target inhibition, network pharmacology recognizes that moderate binding affinities (-5 to -8 kcal/mol) can achieve therapeutic effects when combined with strategic network positioning (Follis, 2021; Hase et al., 2009). The moderate-to-favourable binding energies observed for morindolide (-5.19 to -6.62 kcal/mol) are characteristic of natural products that exert therapeutic effects through multi-target modulation rather than potent single-target inhibition (Dančík et al., 2010). This multi-target strategy may offer advantages in terms of reduced side effects and broader

therapeutic applications compared to highly selective synthetic inhibitors (Young et al., 2012; Zeng et al., 2020).

Most notably, integrating these computational approaches has revealed a potential mechanism for morindolide's dual anti-inflammatory and antioxidant effects. The compound demonstrates specific binding to NOS2 (binding energy -6.24 kcal/mol, three hydrogen bonds: Arg199(A) at 2.69 Å, Tyr489(A) at 2.67 Å and 2.8 Å) and CYP2E1 (binding energy -6.62 kcal/mol, two hydrogen bonds: Pro429(A) at 2.75 Å, Val364(A) at 3.17 Å). These interactions directly modulate nitric oxide production by inhibiting NOS2, while simultaneously regulating ROS generation through modulation of CYP2E1. The binding patterns suggest allosteric regulation rather than competitive inhibition, as evidenced by interaction sites distinct from the enzymes' catalytic centres. This dual mechanism could explain the efficacy of morindolide in conditions where inflammation and oxidative stress are mutually reinforcing.

5.2 Contextualising Binding Predictions with Known Inhibitors

The binding energies of morindolide with hub targets (ranging from -5.19 to -6.62 kcal/mol) were evaluated in the context of co-crystallised ligands to assess the biological relevance of these computational predictions. Comparison with native ligands provides essential validation for docking-based predictions, as co-crystallised ligands represent experimentally confirmed binders with known binding modes and established therapeutic activities (Pagadala et al., 2023; Torres et al., 2019). This comparative framework allows for contextualization of computational binding energies within the spectrum of known active compounds, providing a reference point for interpreting the predicted biological significance of morindolide's interactions.

For CYP2E1, morindolide's binding energy of -6.62 kcal/mol was substantially weaker than the co-crystallised inhibitor 4PZ (-17.13 kcal/mol). This considerable difference reflects the structural specificity of 4PZ, a mechanism-based inhibitor designed for tight binding to the CYP2E1 active site (Hartman et al., 2013). However, morindolide's moderate binding energy may be more aligned with regulatory modulation rather than complete enzymatic inhibition. Strong inhibitors with binding energies below -10 kcal/mol often exhibit irreversible or pseudo-irreversible binding characteristics, which can lead to mechanism-based toxicity and off-target effects (Ramirez & Caballero, 2018; Wang et al., 2015). The moderate binding affinity

observed for morindolide potentially offers a more favourable therapeutic profile by allowing reversible, concentration-dependent enzyme modulation that preserves basal enzymatic function while attenuating excessive activity under pathological conditions.

Similarly, for PTGS2, morindolide exhibited a binding energy of -5.87 kcal/mol, compared to the co-crystallised ligand ID8, which had a binding energy of -7.3 kcal/mol. To contextualise these computational predictions, experimental studies of structurally related iridoid glycosides provide crucial benchmarks. Park et al. (2010) demonstrated that hydrolysed loganin (H-loganin) exhibited COX-1 inhibition with an IC50 of 3.55 μM, while hydrolysed aucubin (H-aucubin) showed COX-2 inhibition with an IC50 of 8.83 μM in vitro. These moderate experimental inhibition constants suggest that iridoid compounds, including morindolide, may function as moderate regulators rather than potent inhibitors. The correlation between computational binding energies in the range of -5 to -7 kcal/mol and experimental IC50 values in the low micromolar range (typically 1-20 μM) has been well established in the literature (Kitchen et al., 2004; Wang et al., 2003), supporting the biological relevance of morindolide's predicted binding affinities.

For NOS2, morindolide's binding energy of -6.24 kcal/mol compares with the co-crystallized ligand AT2 at -4.87 kcal/mol, suggesting that morindolide may exhibit binding affinity comparable to or superior to some known NOS2 inhibitors. This finding is particularly significant given that experimental data from related iridoids demonstrate measurable NO inhibition. Specifically, recent studies on iridoid glycosides from *Gomphandra mollis* revealed NO inhibition with IC50 values ranging from 6.13 to 51.1 μM, with structural features such as lactone rings enhancing inhibitory activity (Li et al., 2025). Morindolide, possessing a lactone moiety as a core structural feature, may therefore exhibit NO inhibitory activity within a similar concentration range.

The pattern observed across all seven hub proteins, where morindolide consistently showed weaker binding energies than co-crystallised ligands but within ranges associated with biological activity, suggests a multi-target regulatory mechanism rather than potent single-target inhibition. This interpretation aligns with the network pharmacology findings, which identified morindolide's targets at key network positions characterised by high betweenness centrality, rather than as isolated, high-affinity binding sites. The moderate binding energies, typically in the range of -5 to -7 kcal/mol, fall within the spectrum associated with reversible, regulatory interactions that cellular conditions and substrate concentrations can modulate (Fan et al., 2019; Shirali et al.,

2025). This binding profile supports the hypothesis of adaptive therapeutic responses that can fine-tune pathway activity rather than completely suppress enzymatic function, potentially offering advantages in terms of reduced toxicity and preserved physiological homeostasis (Kitchen et al., 2004).

Furthermore, the distribution of binding energies across multiple targets provides insight into morindolide's pharmacological profile. Unlike highly selective synthetic drugs, which typically demonstrate one dominant high-affinity interaction (binding energy < -10 kcal/mol) and minimal activity against other targets, morindolide exhibits moderate affinity across multiple functionally related proteins. This multi-target binding profile is characteristic of natural products that may modulate biological systems through multi-target inhibition rather than through potent single-target inhibition (Dancik et al., 2010; Hopkins, 2008). The strategic positioning of these targets within inflammatory and oxidative stress networks, combined with moderate binding affinities, suggests that morindolide achieves therapeutic effects through coordinated modulation of multiple pathway nodes, potentially offering synergistic benefits that exceed the sum of individual target effects.

5.3 Mechanistic Insights into Anti-inflammatory Effects

The analysis of morindolide's anti-inflammatory mechanisms through network pharmacology and molecular docking has revealed regulatory patterns that extend beyond basic enzyme inhibition. Identifying multiple inflammatory mediators as targets, particularly the favourable interactions with PTGS2 and IL1B, suggests morindolide modulates inflammation through concurrent regulation of multiple pathways, which aligns with findings in another study (Afolayan & Tarkaa, 2023). This multi-target approach may explain the reported therapeutic efficacy of plants containing morindolide in traditional medicine systems (Yoshikawa et al., 1995).

A particularly significant finding emerged from the interaction between morindolide and PTGS2, characterised by a specific binding energy of -5.87 kcal/mol and a crucial hydrogen bond with Tyr385(A) at a distance of 2.92 Å. This interaction pattern differs significantly from that of traditional COX-2 inhibitors, suggesting a unique mechanism of action. Unlike synthetic COX-2 inhibitors, which typically demonstrate binding energies below -10 kcal/mol, morindolide's moderate binding energy, coupled with specific interaction patterns, suggests a regulatory rather than

purely inhibitory effect (Baek et al., 2021; Yin et al., 2018). Traditional COX-2 inhibitors, such as NSAIDs, often interact with Arg-120 in the enzyme's active site. However, some inhibitors, like diclofenac, bind differently, forming hydrogen bonds with Tyr-385 and Ser-530 (Rowlinson et al., 2003). The Tyr-348 and Tyr-385 interaction is vital for COX-2 function and inhibitor binding, with mutations affecting radical formation and inhibitor efficacy (Derardja et al., 2024; Rogge et al., 2005). This finding provides a potential explanation for the reduced side effects observed with natural anti-inflammatory compounds.

The interaction between morindolide and IL1B (binding energy -6.12 kcal/mol) reveals another layer of anti-inflammatory regulation. The formation of two specific hydrogen bonds with Met95(A) and Lys92(A) suggests modulation of IL1B activity rather than complete inhibition. This interaction pattern is particularly significant given IL1B's role as an upstream mediator of inflammatory cascades (Brint et al., 2019). The moderate binding energy coupled with specific interaction patterns suggests morindolide might fine-tune IL1B activity rather than completely suppress it, potentially preserving essential inflammatory responses while preventing excessive activation (Metcalfe et al., 2020). A study has also explored natural compounds as potential anti-inflammatory agents targeting interleukin-1β (IL-1β), such as *M. oleifera* leaf extract, which contains polyphenols that interact with IL-1β, IL-6 and IL-1RA, showing promise in reducing inflammation (Hamdy, 2024).

Network analysis revealed MMP2 as a significant node in morindolide's interaction network, demonstrating a binding energy of -6.6 kcal/mol with a specific hydrogen bond to Val118(A) at a distance of 3.28 Å. This interaction occurs at the hemopexin-like domain rather than the catalytic zinc-binding site (typically involving His403, His407 and His413), suggesting allosteric regulation of MMP2 activity (Pizio et al., 2012). The binding location is particularly significant as it interfaces with TIMP-2 binding regions, potentially modulating MMP2 activation processes rather than directly inhibiting catalytic activity (Alrumaihi, 2023; Morgunova et al., 2002). This regulatory mechanism differs from synthetic MMP inhibitors that target the zinc-binding motif (achieving binding energies of -8 to -10 kcal/mol), which often lead to broad-spectrum MMP inhibition and associated side effects (Pizio et al., 2012; Sela-Passwell et al., 2009). Morindolide's interaction pattern suggests the selective modulation of MMP2's matrix remodelling activities, supported by its reference RMSD of 77.24 Å, indicating conformational changes that could affect enzyme activation

without completely suppressing its physiological functions in tissue homeostasis (Alrumaihi, 2023).

In addition to direct regulation of inflammatory mediators, GO analysis further supported these mechanistic insights, revealing significant pathway enrichment in specific processes. The enrichment in prostaglandin secretion and positive regulation of fever generation pathways suggest morindolide's effects extend to physiological responses to inflammation. Morindolide, an iridoid compound identified in the ethyl acetate extract of *M. platytyrea* by Mohd Haris et al. (2016), is a key bioactive constituent contributing to these effects. Similarly, the ethyl acetate extract of *M. platytyrea* has been shown to exert cytoprotective and anti-inflammatory effects on lipopolysaccharide-induced RAW 264.7 cells, indicating its capability to modulate inflammatory responses at the cellular level (Jaafar et al., 2024). These findings also resonate with other studies that demonstrate plant-derived compounds can affect various inflammatory processes, including prostaglandin production and anti-pyretic activity, supporting their potential efficacy in treating inflammatory conditions. The bioactive flavonoids and polyphenols in *M. oleifera* exert potent anti-inflammatory effects, inhibiting pro-inflammatory enzymes, regulating cytokine production and reducing oxidative stress (Chi et al., 2023) while maslinic acid, found in olive oil, modulates inflammation pathways by regulating arachidonic acid metabolism, NF-κB/COX-2 expression and phospholipase A2 enzyme activity (Yap & Lim, 2015).

5.4 Mechanistic Insights into Antioxidant Effects

The molecular analysis of morindolide's antioxidant mechanisms revealed a more nuanced mode of action. Rather than acting as a direct free radical scavenger, morindolide appears to exert its antioxidant effects by modulating key enzymatic systems and regulatory pathways. The compound demonstrates the highest affinity for CYP2E1 (binding energy -6.62 kcal/mol), forming two hydrogen bonds in the enzyme's regulatory domain. This represents a particularly significant finding, suggesting the regulation of a major cellular reactive oxygen species (ROS) source. Antioxidants can modulate cell signalling pathways and gene expression in response to reactive oxygen species (ROS) (Hong et al., 2024; Leonarduzzi et al., 2010). Cellular redox networks involve enzymatic antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic defences, including vitamins C and E (Jena et al.,

2023). These systems work to maintain redox homeostasis and neutralise excess ROS, which can trigger oxidative stress and contribute to various diseases (He et al., 2017; Hong et al., 2024).

The specific binding pattern between morindolide and CYP2E1, characterised by two hydrogen bonds with Pro429(A) and Val364(A), suggests a regulatory mechanism that could modulate rather than inhibit entirely the enzyme activity (Hartman et al., 2013). This finding has significant implications for therapeutic applications, as complete inhibition of CYP2E1 could disrupt essential metabolic processes (Stavropoulou et al., 2018). The moderate binding energy coupled with specific interaction patterns suggests morindolide might act as a physiological modulator rather than a potent inhibitor (Porubsky et al., 2008; Yu et al., 2019).

A particularly noteworthy finding emerged from the network analysis of antioxidant pathways. The identification of HSP90AA1 as a key target (binding energy -5.19 kcal/mol) with four specific hydrogen bonds reveals a previously unrecognised mechanism through which morindolide might influence cellular stress responses (Hani et al., 2021; Warnecke et al., 2019). The interaction pattern suggests modulation of heat shock protein function, potentially enhancing cellular resistance to oxidative stress through improved protein folding and stability rather than direct ROS neutralisation (Beck et al., 2011). Plus, the inhibition of Hsp90 can destabilise oncoproteins and induce cancer cell death, particularly through oxidative stress-mediated mechanisms (Beck et al., 2011; Li & Luo, 2022).

The GO analysis provided crucial context for understanding these molecular interactions, revealing significant enrichment in cellular response to oxidative stress processes. The enrichment in xenobiotic response pathways was particularly significant, suggesting that morindolide might enhance cellular antioxidant capacity by activating endogenous defence mechanisms (Park et al., 2013). These findings align with other studies, such as those in zebrafish larvae, where morin (a flavonol found in plants of the Moraceae family) has demonstrated antioxidant and neuroprotective effects against hydrogen peroxide-induced oxidative stress by upregulating antioxidant enzymes and reducing lipid peroxidation (Issac et al., 2021). Traditional Chinese medications contain bioactive compounds with diverse antioxidant mechanisms, offering potential for disease treatment (Liang et al., 2021).

5.5 Receptor-Mediated Effects of Morindolide on the Oxidative Stress-Inflammation Cycle

As illustrated in Figure 5.1, oxidative stress and inflammation form a self-amplifying loop, wherein reactive oxygen species (ROS) activate inflammatory transcription factors (e.g., NF-κB), inducing the release of cytokines, such as interleukin-1β (IL-1β). IL-1β upregulates downstream enzymes and mediators (such as NOS2, PLA2G2A, PTGS2 and MMP2) that generate additional oxidants and inflammatory signals. These interlinked pathways create a vicious cycle in which an initial ROS burst triggers a cascade of further ROS production and cytokine signalling, driving chronic tissue damage. Figure 5.1 highlights multiple nodes where morindolide can intervene in this cycle. Network pharmacology and docking analyses indicate that morindolide engages seven key protein targets (Figure 5.1, nodes M.1–M.7; grey dotted lines) across the oxidative and inflammatory network, potentially breaking the pathological crosstalk at several critical points.

ROS • React ve Orygen

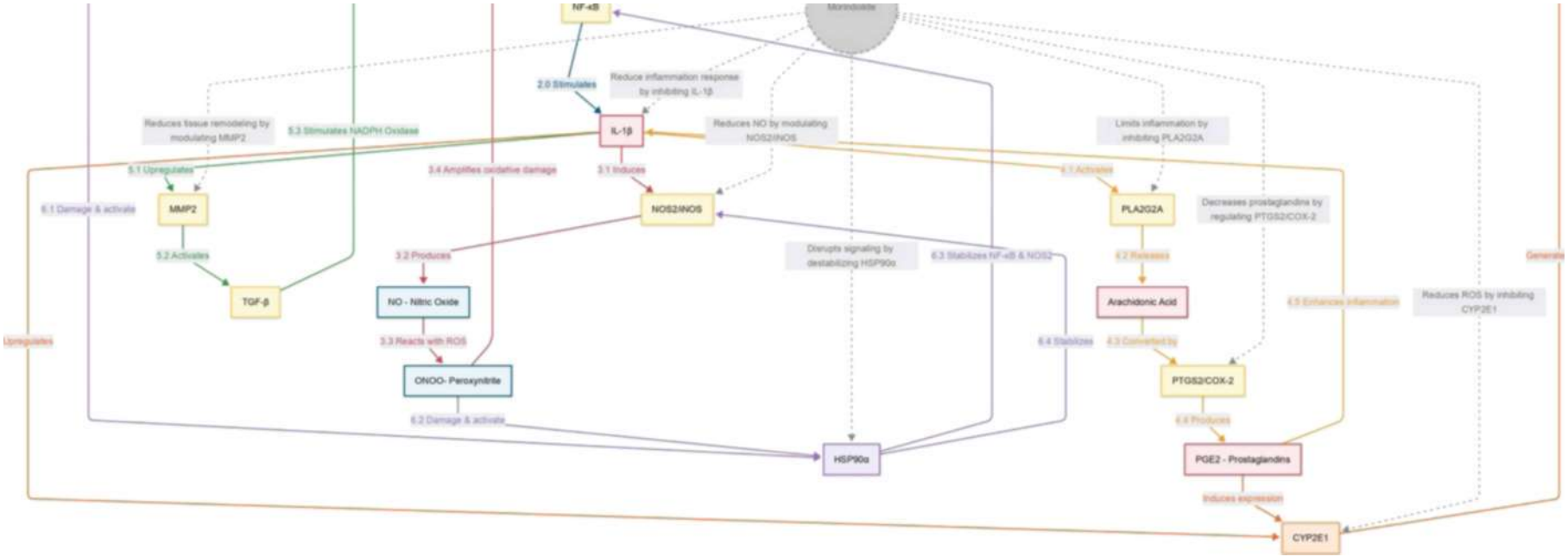


Figure 5.1 Morindolide's Multi-Target Modulation of the Oxidative Stress-Inflammation Cycle

The above illustrates the oxidative stress–inflammation cycle (solid arrows) and the therapeutic intervention points of morindolide (dotted grey arrows). Numbered steps indicate sequential processes within the loop: (1) ROS activate NF-κB; (2) NF-κB stimulates IL-1β; (3.1–3.4) IL-1β induces NOS2, leading to NO and peroxynitrite (ONOO) formation, further amplifying ROS; (4.1–4.5) IL-1β triggers PLA2G2A-dependent arachidonic acid metabolism into pro-inflammatory prostaglandins (PGE), reinforcing the inflammatory cascade; (5.1–5.3) IL-1β upregulates MMP2 and subsequent TGF-β/NADPH oxidase activation, enhancing ROS production; (6.1–6.4) ROS and ONOO activate HSP90α, which stabilises NF-κB and NOS2, thus maintaining the cycle. Node colours denote functional classification: oxidative stress factors (blue), inflammatory mediators (red), enzymatic mediators (yellow), central regulators (purple) and morindolide intervention (grey).

Morindolide's regulatory potential in this context should be considered in the context of network pharmacology, which emphasises multi-target intervention over single-target disruption. By targeting seven key protein nodes—CYP2E1, IL1B, NOS2, PLA2G2A, PTGS2, MMP2, and HSP90AA1 —morindolide exerts a broad, networked influence that exceeds the constraints of single-target inhibition. Each object in this network not only has an effect, but it also controls many processes that precede and follow it. This means that changing the state of any node affects the whole feedback loop. CYP2E1 plays a central role in generating reactive oxygen species associated with metabolic and inflammatory processes. It catalyses redox reactions that drive the activation of NF-κB, a key transcription factor regulating inflammatory gene expression (Qin et al., 2019). Inhibiting CYP2E1-mediated ROS production, therefore, represents a means of dampening the cycle at its origin point. The significance of this intervention becomes apparent when viewed in the broader context of suppressing downstream signalling pathways. For instance, the NF-κB/IL-1β axis, a well-established central node of inflammatory amplification, not only upregulates classical cytokines but also induces the expression of NOS2, PLA2G2A and MMP2, linking transcriptional activity directly to oxidative and matrix-modifying outputs (Liu et al., 2017; Mincheva-Tasheva & Soler, 2012).

The effect of morindolide on IL-1β interrupts the relay of inflammatory signalling to both NOS2 and PTGS2. NOS2-generated nitric oxide and its derivative, peroxynitrite, act both as signalling molecules and as direct effectors of oxidative injury, thus providing a conduit for inflammation to escalate cellular damage (Mangge, 2014;

Sobhon et al., 2023). PTGS2 (COX2), by facilitating the synthesis of pro-inflammatory prostaglandins, prolongs the inflammatory state and contributes to redox-associated processes, through concurrent modulation of PLA2G2A, which supplies arachidonic acid for PTGS2; morindolide targets both the substrate and enzyme of prostaglandin biosynthesis (Chen et al., 2012). Further, MMP2 and its downstream effector, TGF-β, mediate tissue remodelling and the induction of NADPH oxidases, which contribute additional ROS to the cycle (He et al., 2020). This exacerbates tissue destruction and contributes to maintaining the activation of NF-κB and related transcriptional programs. The intervention at the level of MMP2 thus represents a point of leverage against the chronicity of inflammatory remodelling (Özkan & Bakar Ates, 2019). HSP90α, as a molecular chaperone, functions as a permissive factor for the persistence of activated NF-κB and stabilised NOS2. The destabilisation of HSP90α disrupts this protective scaffold, enhancing the proteasomal degradation of pro-inflammatory and pro-oxidative effectors. This helps reduce the self-amplifying nature of the oxidative stress-inflammation cycle (Bhattacharjee & Deb, 2020).

5.6 Summary of Integrated Perspective on Morindolide's Therapeutic Mechanisms

The computational analyses presented in this investigation reveal that morindolide functions through a multi-target mechanism, distinguishing it from conventional single-target drugs. The integration of network pharmacology and molecular docking approaches has elucidated how morindolide simultaneously interacts with multiple key proteins involved in inflammation and oxidative stress pathways, providing mechanistic insights into its therapeutic effects.

The network pharmacology analysis demonstrates that morindolide targets proteins as central communication hubs within biological networks. These hub proteins play critical roles in transmitting signals between different cellular pathways, making them strategically important targets for therapeutic intervention. By affecting these central nodes, morindolide can simultaneously influence multiple downstream pathways, creating a more comprehensive therapeutic effect than would be possible through single-target inhibition.

The molecular docking studies provide detailed insights into how morindolide interacts with its target proteins at the atomic level. The binding energies and interaction

patterns revealed through these studies indicate that morindolide forms stable, specific interactions with each target protein. The mechanism through which morindolide exerts its therapeutic effects involves direct binding to and modulation of key enzymes involved in inflammation and oxidative stress. This mechanism operates through what is known as allosteric regulation, where morindolide binds to sites on target proteins that are separate from the proteins' active sites. This type of binding allows morindolide to change the shape and activity of target proteins without completely blocking their function.

The most significant example of this mechanism is morindolide's interaction with CYP2E1, an enzyme that plays a dual role in cellular metabolism. Under normal conditions, CYP2E1 facilitates the body's ability to process and eliminate foreign substances. However, during this process, the enzyme also generates ROS as byproducts (Harjumäki et al., 2021). When CYP2E1 becomes overactive in certain disease states, it produces excessive amounts of ROS that contribute to cellular damage (Guengerich, 2020). Morindolide's binding to CYP2E1 at specific amino acid residues (Pro429A and Val364A) helps regulate the enzyme's activity, reducing excessive ROS production while preserving its essential detoxification functions (Xu et al., 2017).

Similarly, morindolide's interaction with NOS2 demonstrates another aspect of this regulatory mechanism. NOS2 produces nitric oxide, which is important in immune responses and blood vessel regulation. However, excessive NOS2 activity can produce harmful compounds, such as peroxynitrite, which damages cells and promotes inflammation (Obeagu et al., 2024; Tewari et al., 2020). Morindolide forms three specific hydrogen bonds with NOS2 (at Arg199A and Tyr489A), modulating the enzyme's activity without completely inhibiting its beneficial functions.

The regulation of the inflammatory cascade through the arachidonic acid pathway represents another key aspect of morindolide's direct mechanism. Morindolide simultaneously targets multiple components of this pathway, including PLA2G2A, which releases arachidonic acid from cell membranes, and PTGS2, which converts arachidonic acid into inflammatory mediators (Ayertey et al., 2020; Luo et al., 2021). By affecting both the source of the substrate (arachidonic acid) and the enzymes that process it, morindolide can effectively reduce the production of inflammatory compounds while avoiding the compensatory mechanisms that often limit the effectiveness of single-target therapies (Bošković et al., 2023).

The computational analyses reveal that morindolide's therapeutic effects stem from simultaneous intervention at multiple nodes within the self-amplifying oxidative stress-inflammation cycle. Morindolide engages seven strategic intervention points that disrupt pathological crosstalk between ROS generation and inflammatory signalling. This multi-point intervention strategy creates a coordinated therapeutic effect that addresses the complex, interconnected nature of inflammatory pathophysiology more comprehensively than single-target approaches. (Luo et al., 2021; Zhang et al., 2020).

These findings position morindolide as a promising lead compound for natural product-based therapeutic development, with the computational predictions serving as an integrated analysis that establishes morindolide as a multi-target modulator of inflammation and oxidative stress, operating through the coordinated regulation of strategically positioned hub proteins. The synergistic combination of network pharmacology and molecular docking has revealed mechanistic insights that are unattainable through single-method approaches, demonstrating the power of integrated computational strategies in elucidating the pharmacological mechanisms of natural products.

CHAPTER 6
CONCLUSION

6.1 Conclusion

This research conducted a comprehensive computational investigation to elucidate the molecular mechanisms underlying morindolide's anti-inflammatory and antioxidant effects by integrating network pharmacology and molecular docking approaches. The study successfully identified 56 potential target proteins and established seven key regulatory hub genes, demonstrating the multi-target nature of morindolide's therapeutic actions. Network pharmacology analysis revealed few protein-protein interaction patterns, while molecular docking provided detailed insights into specific binding interactions. Investigating morindolide's binding patterns with key target proteins revealed significant findings. CYP2E1 demonstrated the significant binding affinity (-6.62 kcal/mol) with specific hydrogen bonds to Pro429(A) and Val364(A), while MMP2 showed a binding energy of -6.6 kcal/mol with a distinct hydrogen bond to Val118(A). NOS2 exhibited a binding energy of -6.24 kcal/mol, characterised by three specific hydrogen bonds, suggesting important regulatory interactions. These molecular-level interactions provide mechanistic insights into the reported therapeutic effects of morindolide. Network analysis identified PTGS2, IL1B, MMP2, HSP90AA1, NOS2, PLA2G2A and CYP2E1 as hub genes, with varying degrees of connectivity and centrality measures. The correlation between network position and binding affinity suggests mechanisms where morindolide preferentially targets key regulatory nodes. GO analysis revealed significant enrichment in inflammatory response and oxidative stress pathways, supporting the dual therapeutic effects observed in traditional medicine applications. The KEGG pathway analysis highlighted nine significant pathways, with enrichment in arachidonic acid metabolism ($p = 0.000731$) and IL-17 signalling pathways ($p = 0.001729$). These pathways demonstrate the interconnected nature of inflammatory and oxidative stress responses targeted by morindolide. The molecular docking results provide mechanistic support for morindolide's effects on these pathways through specific protein interactions.

The findings contribute to our understanding of morindolide's mechanism of action and the multi-target therapeutic approaches and provide a foundation for future

natural product-based drug development. The established methodological framework can be applied to other natural compounds, potentially revealing new therapeutic applications for traditional medicines. Furthermore, the insights gained regarding network-based drug effects could inform the development of synthetic compounds designed to target multiple pathways simultaneously. The study also demonstrates the value of considering network position alongside binding affinity in evaluating potential drug targets, suggesting new criteria for drug screening programs. Integrating multiple computational approaches provides a more comprehensive understanding of drug-target interactions than could be achieved through any single method, establishing a template for future investigations in natural product pharmacology.

6.2 Study Limitations and Future Directions

The integration of network pharmacology and molecular docking, while providing significant insights into morindolide's mechanisms of action, inherently carries certain methodological limitations that warrant consideration. Although complex, computational predictions represent static models of inherently dynamic biological processes. While our molecular docking analyses revealed specific binding patterns and energies, these results cannot fully capture the complex dynamics of protein-ligand interactions in physiological conditions. This limitation is particularly relevant for understanding morindolide's effects on proteins that undergo significant conformational changes during their regulatory cycles.

A significant technical constraint emerges from the reliance on existing protein structure databases. While high-resolution structures were available for many target proteins, some computational predictions relied on homology models or partially resolved structures. This limitation particularly affects the confidence levels of binding predictions for proteins where complete structural information is unavailable. Furthermore, the current computational approaches cannot fully account for the influence of post-translational modifications and protein-protein interactions on binding site accessibility and conformational dynamics.

The network pharmacology analysis, while comprehensive, faces limitations in its ability to capture temporal and spatial aspects of protein interactions. The identified protein-protein interaction networks represent aggregated data across multiple cellular contexts, potentially masking tissue-specific or condition-dependent interaction

patterns. This limitation is particularly relevant for understanding morindolide's tissue-specific effects and its potential therapeutic applications in different physiological contexts.

Another inherent methodological consideration is the reliance on multiple computational databases, each carrying distinct biases and limitations that may influence target prediction accuracy. The target prediction databases employed in this study primarily derive their predictions from different algorithmic approaches and training datasets, which may introduce systematic biases. SymMap focuses predominantly on traditional Chinese medicine annotations and may exhibit bias toward compounds with documented TCM applications, potentially overlooking novel target interactions outside this pharmacological tradition. Swiss Target Prediction employs machine learning algorithms trained on known bioactive molecules, which inherently biases predictions toward well-characterised target classes while potentially underrepresenting emerging or less-studied protein families. PharmMapper utilises pharmacophore-based approaches that depend on the quality and diversity of reference structures in its template library, potentially limiting predictions for proteins with unique or poorly characterised binding sites. These database-specific biases collectively suggest that our identified targets and predicted interactions may be skewed toward well-characterised biological systems.

Future research directions should address these limitations through several approaches. First, developing dynamic simulation methods that can better account for protein flexibility and conformational changes would enhance the accuracy of binding predictions. Integration of molecular dynamics simulations with longer time scales could provide deeper insights into the stability and kinetics of morindolide-protein interactions.

Experimental validation represents a crucial next step. The computational predictions generated in this study provide a strong foundation for targeted experimental investigations. Priority should be given to validating the predicted interactions with key regulatory proteins, particularly those identified as network hubs. Specific attention should be directed toward understanding the functional consequences of the predicted binding patterns, especially for proteins where moderate binding energies suggest regulatory rather than inhibitory effects.

Based on our findings, the development of structure-activity relationship studies could guide the design of synthetic derivatives with enhanced therapeutic properties.

The identified binding patterns and network positions of target proteins provide rational bases for structural modifications that might improve bioavailability or target specificity while maintaining the beneficial multi-target effects observed with the natural compound.

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APPENDICES

APPENDIX 1
Indexed Publication (1)

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MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

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AMI-IXFLAMMATORY AND ANTIOXIDANT PROPERTIES OF NATURALLY DERIVED IRIDOIDS: A SCOPING REVIEW OF IN SILK O STUDIES

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Computational Biology. Inflammation, Medicinal Plants, Iridoids. Oxidation

Abstract

This scoping review explores the current state of molecular docking studies on naturally derived nidoid compounds, focusing specifically on their engagement with arti-m-fbmmarnry and .innnndart targets. The renew searches the PubMed and Scopus databases to locate relevant published studies. Following a systematic search strategy, identified citations were compiled, screened by two independent reviewers, and evaluated against me inclusion criteria. Data extraction was conducted using a reviewer -developed instrument, and appropriate details were caprured The extracted data was stored and analysed. Results were discussed, supplemented by data tables and reported adhering to the PRISMA-ScR checklist. The studies included were characterised as m sihco. with a total of 364 studies initially retrieved. After removing duplicates. 143 studies remained, out of which 40 were subjected to a full read, and ultimately. *29* studies were deemed eligible for qualitative synthesis. Indoids. particularly swertiamarin. arnarogentm. geniposide. gennopicroside. and logamc acid, surfaced as the :sredoniinant subjects of examination, ipotbghtinz their phazinacological ant.-lrjflammatorv' and antioxidant effect. Other uidoids such as aucubrn. genipin. venninoside and sweroside were also examined. This renew highlights the most potent naturally produced uidoads. each exhibiting the highest affinity- to different target proteins, providing a basis for choosing substances and directing more experimental and clinical research. The data from these studies is essential for figuring out how these chemicals could be used in biotechnology and medicme. especially to fight diseases lelated to inflammation and oxidative stress.

LNTRODTCnON

There have been many different diseases throughout Vnmmn histcty. and even if* manv of tbgm have been eliminated or have excellent treatments, we stall have to deal with the persistence of those around us today. As we enter the 21st century, two pervasive health issues emerge as significant challenges worldwide- mfjmmanon and oxidative stress They are fundamental underlying mechanisms of various chronic diseases, rncludinz cancer. diabetes, cardiovascular diseases, and neurodegenerative disorders [1.2]. Inflammation and oxidative stress are key players in the pathogenesis of these diseases both biological responses are not isolated events but rather mtertwrned- Chrome m£ animation can induce oxidative stress, which ioirther activates lrdLunmaoon. each potentiating the other leading to a vicious cycle of tissue damage and disease progression.

Inflarnmaaon is a protective immune response that involves the release of evtokmes and ^Kgn>f4nn*^ at the site of injury or infection [3] Our understanding of lrjlarrrmauon has evolved from being merely an acute

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Therapeutic potentials of iridoids derived from Rubiaceae against in vitro and in vivo inflammation: A scoping review

Aisyah Jaafar , Muhammad Amal Zulkipli , Fazleen Haslinda Mohd Hatta .
Aisyah I (asyila Jnhidin , Nurul Alimah Abdul Nasir , Mizaton Hazizul Hasan

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ABSTRAC1

Acute infUrnmation may develop into chronic, life threat en uig inflammation related diseases if left untreated or if there are persistent triggering factor*. Cancer, diabetes meUitut, stroke, cardiovascular diseases, and neuro uive disorders are same of the inflammation related diseases affecting millions of people worldwide e that, conventional medical therapy such as non-steroidal anti inflammatory drugs (NSAJUsJ is associated with serious advene effects; hence, there is an urgent need for a newer and safer therapeutic alternative from natural sources. Indouds are naturally occurring heterocyclic monoterpenoidt commonly found in Rubiaceae plants- Plant extracts from the Rubiaceae family were demonstrated to have medicinal benefits against neuro degeneration, inflammation, oxidative stress, hyperglycaenua, and cancer. However, the therapeutic effects of natural indotds derived from Rubiaceae as well as their prospective impacts on inflammation in vitro and in vivo have not been thoroughly explored. Ihe databases of Pub Med, Scopus, and Web of Science were searched for pertinent articles in accordance with PRISMA ScR guidelines. A total of 31 pertinent articles from in vitro and in vivo studies on the anti inflammatory potentials of iridoids from Rubiaceae were identified. According to current research, genipin, geniposide, and monotropcin are the most researched iridoids from Rubiaceae that reduce mflammatioa. I Tiese iridoids primarily act by attenuating inflammatory cytokine* and mediators via inhibition of the M toll signalling pathway in various disease models. A comprehensive overview of the current research on the anti inflammatory properties of iridoids from the Rubiaceae family is presented in this review, highlighting the characteristics of the experimental models used as well as the mechanisms of action of these iridoids. To develop an alternative therapeutic agent from iridoids, more studies are needed to elucidate the effects and mechanism of action of iridoids in a wide variety of experimental models as well as in clinical studies pertaining to inflammation related diseases.

1. Introduction

Inflammation plays a critical rule in the pathogenesis of various metabolic and degenerative disorders, including hypertension, stroke, diabetes, cardiovascular disease, neurodegeneratton, and cancer. It is suggested that chronic stress can promote persistent inflammation by activating various biomaxkers related lu stress and inflammation QJa • l ;;. , J 1 "). These chronic stress and inflanunatiun-related disorders pose substantial hazards lu human health, with high morbidity and mortality rales. Diabetes, a chrunic cunditiun characterised by increased insulin resistance and hyperglycaemia, is the seventh greatest cause ul mortality in the United Stales • I. In addition, the World Stroke Organisation reported 12.2 milliun new-stroke cases per year ('Am 2H22), with 19.05 million cardiovascular-related deaths gJob.il]> l alton, 2022). These figures demonstrate thai inflammation related diseases have a substantial impact on human health, necessitating the need lor early medical intervention as well as prophylactic management ul

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APPENDIX 2

Table A 1 Analysis of Nodes, Protein Names and Edge Count within the PPI Network

No	Node	Protein Name	Edge	No	Node	Protein Name	Edge
1	IL1B	Interleukin-1 beta	5	29	CA9	Carbonic anhydrase 9	0
2	PTGS2	Prostaglandin G/H synthase 2	5	30	CDK5	Cyclin-dependent kinase 5	0
3	HSP90AA1	Heat shock protein HSP 90-alpha	4	31	CES2	Carboxylesterase 2	0
4	MMP2	Matrix metalloproteinase-2	3	32	CKM	Creatine kinase M-type	0
5	NOS2	Nitric oxide synthase type 2	3	33	DGKA	Diacylglycerol kinase alpha	0
6	C3	Complement C3	2	34	DPP4	Dipeptidyl peptidase 4	0
7	CYP11B1	Cytochrome P450 11B1	2	35	EHMT2	Euchromatic histone-lysine N-methyltransferase 2	0
8	CYP11B2	Cytochrome P450 11B2 (Aldosterone synthase)	2	36	EPHA3	Ephrin type-A receptor 3	0
9	CYP19A1	Cytochrome P450 19A1 (Estrogen synthase)	2	37	EXOSC9	Exosome component 9	0
10	CYP2E1	Cytochrome P450 2E1	2	38	FABP2	Fatty acid-binding protein 2	0
11	MPO	Myeloperoxidase	2	39	FABP7	Fatty acid-binding protein 7	0
12	PLA2G2A	Phospholipase A2	2	40	FAP	Fibroblast activation protein alpha	0

13	AOC3	Membrane primary amine oxidase (Copper amine oxidase)	1	41	HDAC7	Hi stone deacetylase 7	0
14	CHRM1	Muscarinic acetylcholine receptor M1	1	42	HK2	Hexokinase-2	0
15	CHRM3	Muscarinic acetylcholine receptor M3	1	43	HPGD	15-hydroxyprostaglandin dehydrogenase	0
16	FCGR2A	Low affinity immunoglobulin gamma Fc region receptor II-a	1	**44**	IFNGR1	Interferon gamma receptor 1	0
17	GRIA2	AMPA-selective glutamate receptor 2	1	45	MAPK6	Mitogen-activated protein kinase 6	0
18	GSR	Glutathione reductase	1	46	MAPK9	Mitogen-activated protein kinase 9	0
19	IGF1R	Insulin-like growth factor 1 receptor	1	47	MIF	Macrophage migration inhibitory factor	0
20	NCK1	Cytoplasmic protein NCK1	1	48	MKI67	Proliferation marker protein Ki-67	0
21	RAC1	Ras-related C3 botulinum toxin substrate 1	1	49	MVK	Mevalonate kinase	0
22	TTR	Transthyretin	1	50	NUP214	Nuclear pore complex protein 214	0

23	ACADVL	Very long-chain specific acyl-CoA dehydrogenase	0	51	PATJ	Pals1-associated tight junction protein	0
24	ACHE	Acetylcholinesterase (AChE)	0	52	PDLIM1	PDZ and LIM domain protein 1	0
25	AMY1A	Alpha-amylase 1A	0	53	PRSS1	Serine protease 1	0
26	AQP5	Aquaporin-5	0	54	PYGL	Glycogen phosphorylase	0
27	CA2	Carbonic anhydrase 2	0	55	SAMHD1	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	0
28	CA4	Carbonic anhydrase 4	0	56	SULT2A1	Sulfotransferase 2A1	0

AUTHOR'S PROFILE

Muhammad Amal Bin Zulkipli obtained Bachelor of Science in Pharmacy (Hons.) in 2023 from IIUM Kuantan, Malaysia. Currently pursuing an MSc in Pharmacology, reflecting a strong interest in advancing expertise in drug action and therapeutic mechanisms.

LIST OF PUBLICATIONS:

Zulkipli, M.A., Jaafar, J., Zakaria, Y., Elhassane, A., Hazizul Hasan, M. (2024). Anti-Inflammatory And Antioxidant Properties Of Naturally Derived Iridoids: A Scoping Review Of *In Silico* Studies. Malaysian Journal of Biochemistry & Molecular Biology, 27(2):38-59

Jaafar, A., **Zulkipli, M. A.**, Hatta, F. H. M., Jahidin, A. H., Nasir, N. A. A., & Hasan, M. H. (2024). Therapeutic potentials of iridoids derived from Rubiaceae against *in vitro* and *in vivo* inflammation: a scoping review. Saudi Pharmaceutical Journal, 32(1), 101876. https://doi.org/10.1016/j.jsps.2023.101876

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