Scientific Knowledge Group

Research Article

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Evaluation of Anti-Inflammatory Activity of Some Synthetic 3-Acetylpyridine Chalcone Derivatives

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Abstract

This study investigates the anti-inflammatory effects of different compounds using a rat model of foot swelling induced by carrageenan. This study was conducted at the Libyan Center for Medical Research-Al-Zawiya. The aim of the study was to evaluate the efficacy of compound 1 and compound 2 at varying concentrations, along with diclofenac as a reference standard.

Rats were divided into six groups. Each group received different treatments: compound 1 at 3mg/kg concentration and 6mg/kg concentration, compound 2 at 3mg/kg concentration and 6mg/kg concentration, diclofenac with carrageenan, and carrageenan only. Foot swelling measurements were taken hourly for four hours post-treatment.

The researchers used 2 compounds of 3-acetylpyridine as anti-inflammatory at 3mg/kg and 6 mg/kg concentration and comparison with diclofenac by control group. Found that the edema inhibition % of compound 1 at 3mg/kg concentration is ranging from -20.18% to 5.23%, while at 6 mg/kg concentration ranging from -6.14% to 17.73%. And the edema inhibition %of co mpound 2 at 3 mg/kg concentration ranging from 2.63% to 14.55%, while at 6 mg/kg concentration ranging from 14.91% to 38.18%. While diclofenac rangingfrom-32.46% to - 4.24%.

The researchers concluded that the compound 2 at 6mg/kg have higher activity as anti-inflammatory because have the best edema inhibition % of 38.18% at third hour.

Keywords: Inflammation, 3-acetylpyridine, Activity, Chalcones.

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Abbreviations

5-HT: 5-hydroxytryptamine
COX: Cyclooxygenase
COX-2: Cyclooxygenase-2
CRP: C-reactive protein
DAMPS: Damage-associated molecular patterns
DHCs: Dihydrochalcones
DMSO: dimethyl sulfoxide
EU: European union
GSH: Glutathione
HO-1: Heme oxygenase-1
IκB: Inhibitor of kappa B
IFN-γ: Interferon-gamma
IL-1β: Interleukin-1 beta
IL-4: Interleukin-4
IL-6: Interleukin-6
IL-10: Interleukin-10
IL-17: Interleukin-17
IKK-beta: I-kappa-B kinase beta
IUPAC: International union of pure and applied chemistry
MDA: Malondialdehyde
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
NLRs: Nucleotide-binding oligomerization

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domain-like Receptors
NO: Nitric oxide
NSAIDs: Nonsteroidal anti-inflammatory drugs
PAF: Platelet activating factor
PAMPS: Pathogen-associated molecular patterns
ROS: Reactive oxygen species
RNS: Reactive nitrogen species
SAIDs: Steroidal anti-inflammatory drugs
SAR: Structure activity relationship
TGF-β: Transforming growth factor-beta
Th1: T helper cell 1
Th2: T helper cell 2
Th17: T helper cell 17
TLRs: Toll-like Receptors
TNF-α: Tumor necrosis factor-alpha
Treg: Regulatory T cells
US FDA: United states food and drug administration

Introduction

Inflammation is a pervasive form of defense that is broadly defined as a nonspecific response to tissue malfunction and is employed by both innate and adaptive immune systems to combat pathogenic intruders [1]. A distinctive feature of inflammatory responses in relation to other facets of antiparasite defenses is that damage to the self is unavoidable. Importantly, collateral damage from inflammation is not the same as immunopathology, which involves a specific immune-mediated attack on target tissue that is no longer recognized by the immune system itself [2]. Autoimmune pathology reflects dysregulation of adaptive immune components, such as antibody and cell mediated functions, and has both genetic and environmental influences. Although inflammation-induced collateral damage can certainly contribute to immunopathology e.g. rheumatoid arthritis, multiple sclerosis, diabetes, the damage invoked by inflammation represents a basic biological trade-off between damage control and self-maintenance and does not require the presence of self-antigens to become activated [3].

Inflammation

Inflammation is a biological reaction to disrupted tissue homeostasis. It involves the recruitment of blood-derived products, such as plasma proteins, fluid, and leukocytes, into affected tissue, facilitated by vasodilation, increased vascular permeability, and increased blood flow.[1] While microbial infections are a major trigger, injury, trauma, and exposure to foreign particles or pollutants also activate inflammation [4]. This response likely evolved as an adaptation for coping with tissue damage. Both infection and trauma cause cellular and tissue damage, which trigger similar inflammatory responses [5]. The primary functions of inflammation are to rapidly destroy or isolate the disturbance's source, remove damaged tissue, and restore tissue homeostasis. Properly regulated inflammation is adaptive, as seen in the increased risk of infections in individuals with deficiencies in inflammatory components, such as neutropenia [2]. However, excessive inflammation can cause significant damage and pathology. Evolutionarily, inflammation is a conserved defense mechanism across invertebrates and vertebrates. Components like chemotaxis and phagocytosis are used by unicellular organisms and have been adapted for complex multicellular organisms. Innate immunity, including phagocytosis and antimicrobial peptides, is present in early invertebrates, while adaptive immunity, unique to jawed vertebrates, evolved to manage complex microbial communities in the vertebrate digestive tract [6].

Causes of Inflammation

Infections

The most common cause of inflammation is infections. Different microbial organisms and toxins elicit varying inflammatory responses from mild to severe or acute to chronic reactions. Outcome depends largely on type of pathogen, host response and host characteristics [7].

Tissue Necrosis

Ischemia, trauma, thermal or chemical injury are the common cause of cell death and tissue necrosis leading to inflammation [8].

Foreign Bodies

Different endogenous foreign bodies e.g. urate crystal deposits in gout and exogenous foreign bodies as microbes are the main causes of inflammation [9].

Immune Reactions

Immune reactions are also called hypersensitivity. When the normally protective immune system is inappropriately directed against self-antigens or environmental substances, damaging the individuals own tissues e.g. autoimmune diseases and allergies. As these stimuli cannot be eliminated, the inflammation tends to be persistent and difficult to cure, causing significant morbidity and mortality [10].

Signs and Symptoms of Inflammation

The five cardinal signs of acute inflammation are:

Redness

This is due to dilation of small blood vessels within damaged tissue as it occurs in cellulitis [11].

Heat

Heat results from increased blood flow due to regional vascular dilation [12].

Swelling

Swelling is due to accumulation of fluid in the extravascular space which, in turn, is due to increased vascular permeability [13].

Pain

Pain partly results from the stretching and destruction of tissues due to inflammatory edema and in part from pus under pressure in, as abscess cavity [16]. Some chemicals of acute inflammation, including bradykinins, prostaglandins and serotonin are also known to induce pain [14].

Loss of Function

The inflamed area is inhibited by pain while severe swelling may also physically immobilize the tissue [5].

Mechanisms of Inflammation

Inflammation is a tightly regulated cascade of immunological, physiological and behavioral processes orchestrated by soluble immune signaling molecules called cytokines. It begins with

the recognition of infection or damage through the detection of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which activate the innate immune [1].

Immune Receptors

The main receptors in the immune system are:

Toll-Like Receptors (TLRs)

These are transmembrane receptors that recognize ligands and activate signaling pathways leading to the release of the transcription factor (nuclear factor kappa- light-chain-enhancer of activated B cells NF-κB), which is found in virtually all cell types and remains in an inactivated state bound to an inhibitor protein, inhipitor of kappa B (IκB). Upon signal transduction, NF-κB is released from inhipitor of kappa B (IκB) and translocated to the nucleus, where it binds to target genes and upregulates proinflammatory gene expression [15].

Nucleotide-Binding Oligomerization Domain-Like Receptors (NLRs)

These are intracellular receptors that respond to damage-associated molecular patterns (DAMPs). They activate the inflammasomes, which convert cytokines into active forms that promote inflammation system while minimizing harm to host cells [16].

Stages of Inflammation

1- Recognition of Infection or Damage

Pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) recognized by toll-like receptors (TLRs) and nod- like receptors (NLRs). This leads to the activation of intracellular signaling pathways [17].

2- Signal Transduction

- Upon ligand recognition, nod-like receptors (TLRs) activate signaling pathways that release nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κB) from its inhibitor (inhibitor of kappa B) (IκB).
- NF-κB then translocates to the nucleus and activates the expression of proinflammatory genes.
- NLRs respond to DAMPs signals and activate the inflammasome [18].

3- Cytokine Production

- Proinflammatory cytokines like interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and Tumor necrosis factor-alpha (TNF-α) are produced.
- These cytokines, along with chemokines, recruit effector cells to the site of disturbance [19].

4- Effector Cell Recruitment

- Monocytes and neutrophils migrate to the site of inflammation.
- Neutrophils release reactive oxygen species (ROS), reactive nitrogen species (RNS), and proteases, creating a toxic environment for both pathogens and host cells [20].

Effector Mechanisms

- Inflammation is characterized by the cardinal signs: heat, swelling, redness, pain, and loss of function [21].
- Neutrophils release toxic compounds including (ROS), (RNS), and proteases, leading to the destruction of both pathogens and host tissues [20].
- Macrophages and dendritic cells participate in the phagocytosis of antigens and present them to T cells [22].

Adaptive Immune Regulation T Helper Cells (Th)

T helper cells differentiate into various subsets T helper cell 1 (Th1), T helper cell 2 (Th2), regulatory T cells (Treg), T helper cell 17 (Th17). Th1 and Th17 cells promote inflammation, whereas Th2 and Treg cells dampen it. These cells secrete cytokines that can either enhance or inhibit the inflammatory response [23].

- **T helper cell 1(Th1)**: secretes interferon-gamma (IFN-γ) and interleukin-2 (IL-2) and promotes a proinflammatory response [24].
- **T helper cell 2(Th2)**: secretes interleukin-4 (IL-4) and interleukin-5 (IL-5) and interleukin-10 (IL-10), helping regulate the immune response and reduce inflammation [25].
- **Regulatory T cell (Treg)**: secretes transforming growth factor-beta (tgf-β) and interleukin-10 (IL-10) and works to regulate and decrease the inflammatory response [26].
- **T helper cell 17(Th17)**: secretes interleukin-17 (IL-17) and interleukin-6(IL-6) tumor necrosis factor-alpha (TNF-α) and (contributes to the inflammatory response [27].

Resolution of Inflammation

- The resolution of inflammation is crucial to limit collateral damage.
- This involves switching from proinflammatory mediators to anti-inflammatory lipoxins.
- Lipoxins promote the apoptosis of neutrophils and their phagocytosis by macrophages, leading to tissue repair and healing [28]. The mechanism of inflammation is shown in figure 1.

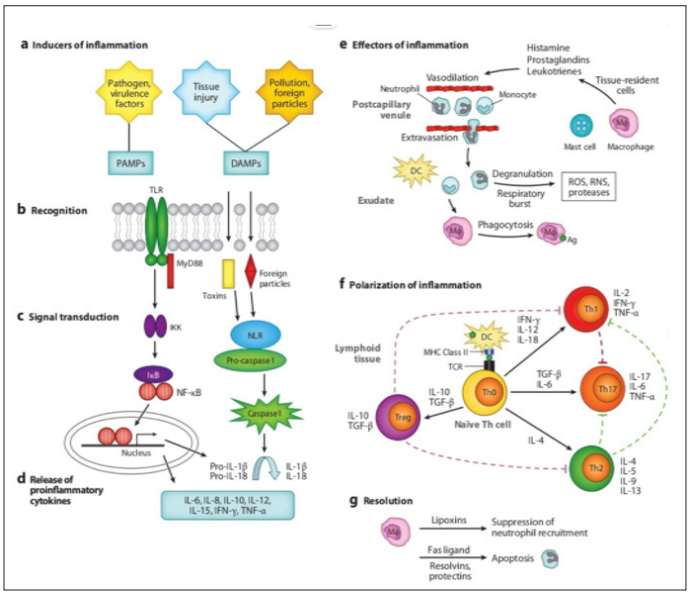

Figure 1: Mechanism of Inflammation [28]

Inflammatory Markers

Inflammatory markers are crucial in clinical diagnostics for

distinguishing between normal and pathological processes and evaluating therapeutic responses [29]. They predict and correlate with inflammatory diseases such as cardiovascular conditions and infections. Key markers include inflammatory cytokines (e.g., interleukins (ILs), tumor necrosis factors (TNFs), interferons (IFNs)), which regulate immune responses, and proteins/ enzymes (e.g., C- reactive protein (CRP), cyclooxygenase-2 (COX-2)), which help restore homeostasis during trauma or infection. Additionally, oxidative stress markers (e.g., reactive oxygen species (ROS), malondialdehyde (MDA)) indicate inflammation and are linked to various diseases [30]. The inflammatory response involves multiple cell types, including neutrophils, monocytes, macrophages, and mast cells, which coordinate to manage tissue damage and infection [31].

Types of Inflammation

Inflammation is a biological response to harmful stimuli, such as pathogens, damaged cells, or irritants, and it serves as a protective mechanism to initiate healing.

There are two main types of inflammation:

Acute Inflammation

Acute inflammation is the body's initial response to harmful stimuli. It is characterized by redness, heat, swelling, pain, and loss of function. This type of inflammation is typically short-lived, lasting from a few minutes to a few days. It involves the rapid influx of immune cells, particularly neutrophils, to the site of injury or infection. Example: a common example of acute inflammation is the redness and swelling that occurs around a cut or scrape [32].

Chronic Inflammation

Chronic inflammation is a prolonged inflammatory response that can last for weeks, months, or even years. It occurs when the body fails to eliminate the cause of an acute inflammation or when an inappropriate immune response persists [1]. Chronic inflammation is characterized by the presence of macrophages, lymphocytes, and fibroblasts. It can lead to tissue damage and is associated with various diseases, such as rheumatoid arthritis, atherosclerosis, and chronic infections. Example: rheumatoid arthritis is an example of chronic inflammation where the immune system mistakenly attacks the joints, causing pain and swelling over long periods [33].

Inflammatory Disease

Inflammatory diseases are characterized by chronic, abnormal inflammatory responses that complicate distinguishing cause from effect [34]. Chronic inflammation is central to diseases like obesity-related diabetes and rheumatoid arthritis, where inflammation both contributes to and results from disease processes. In rheumatoid arthritis, chronic synovial inflammation involves immune cell infiltration and activation, leading to autoantibody production against citrullinated proteins [35]. Asthma and allergic inflammations involve dysregulated immune interactions and are influenced by genetic and environmental factors. Inflammatory disorders affect various body systems, contributing to neurodegenerative diseases, inflammatory bowel disease, and glomerulonephritis [36].

Oedema

Oedema is the abnormal accumulation of fluid in the body, resulting from an imbalance in fluid distribution. It can be categorized into two types: intracellular oedema, where fluid accumulates within cells, and interstitial oedema, where fluid gathers in the spaces between cells [37].

Treatment of Inflammation by Anti-Inflammatory Drugs

Anti-inflammatory drugs are essential for treating both acute and chronic inflammatory conditions, such as osteoarthritis and rheumatoid arthritis [38]. These drugs are broadly categorized into steroidal and nonsteroidal types [39]. Steroidal anti-inflammatory drugs (SAIDs), like betamethasone, prednisolone, and dexamethasone, and nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, diclofenac, ibuprofen, indomethacin, naproxen, nimesulide, and celecoxib, are commonly used [40]. However, prolonged use of these drugs is associated with significant side effects. Steroidal drugs can cause adrenal atrophy, osteoporosis, suppressed immune response, euphoria, cataracts, and glaucoma [41]. NSAIDs can lead to peptic ulcers and bronchospasm due to the blockade of both physiological and inflammatory prostaglandins and the concurrent production of leukotrienes [42]. Due to the adverse effects and high costs associated with synthetic steroidal and nonsteroidal drugs, there is an increasing interest in finding new anti- inflammatory agents from herbal sources, aiming for greater safety, better efficacy, and more economical treatment options [43]. chalcones, a class of plant-derived flavonoids, have emerged as promising natural agents for combating inflammation. Recent studies have highlighted their anti-inflammatory properties, along with antioxidant and antimicrobial activities, making chalcones an intriguing subject for developing new treatments targeting various types of inflammation [44].

Chalcones

Chalcone is the trivial name given to the α, β-unsaturated ketones obtained by condensing an aromatic aldehyde with an aryl methyl ketone in the presence of a base. They are designated structurally as Ar CH=CHC(O) Ar′ and their IUPAC name is 1,3-Diphenyl-2-propene-1-one [45]. The substituents attached to the benzene rings of chalcone are numbered as shown in the figure below figure 2.

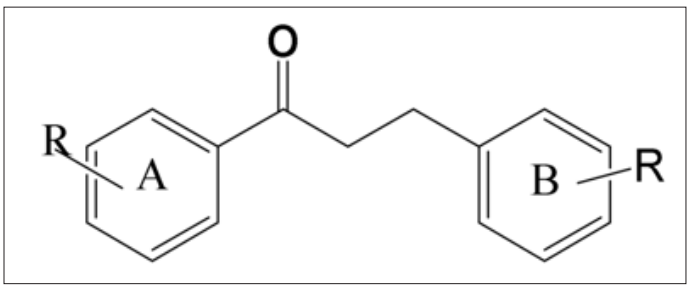

Figure 2: 1,3-Diphenyl-2-propene-1-one or Chalcone [45]

In this structure, the group ─CH=CHC(=O) ─ is known as the chalcone functionality or chalcone moiety or ketoethylenic group.[46] Due to this functionality, chalcones are also called α, β-unsaturated carbonyl systems or α, β- unsaturated

ketones. The parent member of the chalcone series is benzylideneacetophenone. Other names given to chalcone are phenyl styryl ketone, β-phenylacrylophenone, γ-oxo-α, γ-diphenyl-α-propylene, and α-phenyl- β-benzoylethylene [47]. The term "chalcone" was first used by kostanecki [48]. All the α,β-unsaturated ketones are not necessarily be chalcones but all the chalcones are α,β-unsaturated ketones e.g. mesityl oxide figure 3 is an α,β-unsaturated ketone (but not a chalcone) with the formula CH3C(O)CH=C(CH3). This compound is a colorless, volatile liquid with a strong peppermint odor [49].

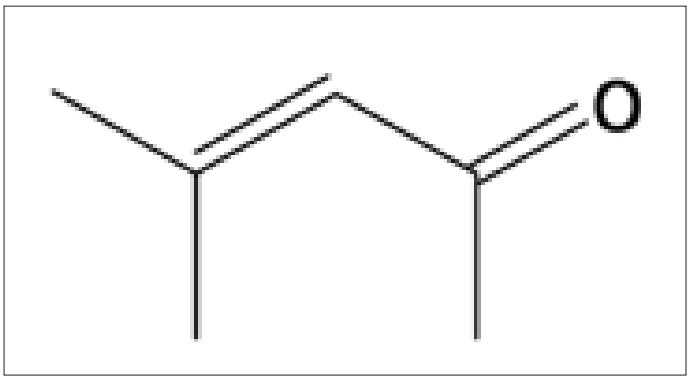

Figure 3: Mesityl Oxide [49]

The difference is the "aromaticity" on position 1 and 3 of the α, β-unsaturated carbonyl system. If the groups attached to ─CH=CHC(=O) ─ moiety do not possess aromaticity, then the resulting compound is just α, β-unsaturated ketone and not the chalcone. Chalcones have a diverse array of groups on the two aromatic rings of 1,3-Diaryl-2-propene-1-one, as shown in the figure 2 above, where the substituents R1 and R2 may be same or different and they may be present anywhere on the two rings. Moreover, R1 or R2 may not necessarily be a single substituent i.e. more than one substituent may be present on any of the two rings. Also, the two aromatic rings may be homocyclic or heterocyclic [50].

Chalcones belong to flavonoid family. Structurally, chalcones are open-chain flavonoids, which were derived by the cleavage of the C ring in the flavonoids as shown below figure 4 [51].

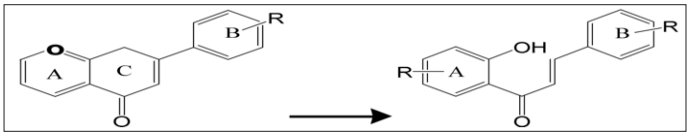

Figure 4: conversion of flavone to chalcone [51]

Natural Sources of Chalcones

Chalcones are abundantly present in nature, from ferns to higher plants. During 1960"s and 70"s many chalcones have been reported to be isolated from the various parts of plants: buds, leaves, blossoms, heart wood, roots, seeds, flowers, and inflorescence. These compounds exist both in free and combined states either in the form of chalcones or glycosides respectively [52].

Dietary Chalcones

Chalcones is one of the major classes of natural products which are widely distributed in spices, tea, beer, fruits and vegetables and have been recently a subject of great interest for their pharmacological activities [53]. |The major dietary source of dihydrochalcones is apples [51]. The US FDA (united state food and drug administration) and EU (european union) have approved the neohesperidin dihydrochalcone to be used as sweetener in various foods like non-alcoholic soft drinks, desserts and confectionery etc. At concentrations in the range 10-400 mg kg-1 (or mg l-1) [55].

or as a flavor modifier at concentrations of up to 5 mg kg-1 [56]. Native chalcone glycosides tend to convert to flavanone glycosides during extraction. Due to this characteristic, chalcones by themselves have limited occurrence in foods. Naringenin chalcone is found in tomato skin but it is present in traces in its juice, paste and ketchup [57]. Mixtures of retrochalcones (e.g. echinatin, licochalcones A and B) are present in licorice (liquorice) root (glycyrrhiza spp) and some medicines based on licorice. Also, the confectionery containing licorice root extracts, might contain these chalcones [58]. Similarly, prenyl-chalcones (e.g. xanthohumol, desmethylxanthohumol) occur in hops and beer. Mixture of flavanone-chalcone (e.g. cerasinone, cerasin) have also been shown to exist in prunus spp [56]. Dihydrochalcones (DHCs) are characteristic of apples and derived products (apple juice, cider, pomace, etc). Phloretin 2'-glucoside (phloridzin) content of apples varies widely depending upon the cultivar. Some english cider apples contain phloridzin as much as 190 mgkg-1, while cultivar verde doncella contains less than 0.1 mgkg-1. They are present in the skin, pulp and especially the seeds, but the skin is 5-10 times richer than that of flesh [57,58].

Pharmacological Profile of Chalcones

Chalcones, either natural or synthetic, are known to exhibit a broad spectrum of various biological activities. The presence of α, β-unsaturated carbonyl moiety as well as of substituted aromatic rings render the chalcones biologically active. Some substituted chalcones and their derivatives, including some of their heterocyclic analogues have been reported to possess strong biological properties which have been proved detrimental to the growth of microbes, tubercle bacilli, malarial parasites and intestinal worms [59-62]. Many chalcones have been claimed to be toxic to various animals and insects and have also shown inhibitory effects on several enzymes and herbaceous plants [63,34]. A few major biological activities which have been reported to be associated with chalcones include:
anti-inflammatory, antifungal, antioxidant, antimalarial, antituberculosis, analgesic, anti-HIV and antitumor activities [65-69]. Some of them act as anticancer, antiviral, and anti-AIDS agents. Quinoline-based chalcones have been reported to possess antimalarial activity [70].

Anti-Inflammatory Activity of Chalcone

Chalcone derivatives have been extensively studied for their anti-inflammatory properties. These compounds exhibit significant potential in reducing inflammation through various mechanisms, including the inhibition of pro-inflammatory cytokines, suppression of NF-κB signaling, and reduction of reactive oxygen species (ROS) production

[71,72]. Their effectiveness is attributed to the presence of α, β-unsaturated carbonyl system, which is crucial for their biological activity [73].

Structure-Activity Relationship (SAR) of Chalcones

The structure-activity relationship (SAR) of chalcones is crucial for predicting the bioactivity of newly identified derivatives [74]. Chalcones with anti-inflammatory properties typically feature an alpha-beta unsaturated bond, allowing them to act as michael acceptors for nucleophilic species such as glutathione (GSH) or cysteine residues on proteins like I-kappa-B kinase beta (IKK-beta) [75]. These modifications often result in reduced or lost anti-inflammatory activity [76]. Additionally, the presence of hydroxyl groups on the chalcone scaffold enhances antiproliferative effects against cancer cells, with hydroxyl derivatives being more potent than others [77]. The presence of a 2'-hydroxy group in chalcone molecules significantly enhances their anti-inflammatory properties by increasing the electrophilic nature of the alpha-beta unsaturated ketone through hydrogen bonding with the ketone moiety. This effect is due to the hydrogen bond between the 2'-hydroxy group and the ketone moiety (also referred to as the keto, carbonyl, or enone moiety) [77]. Furthermore, electron-donating groups on the A ring can stabilize the glutathione adduct by reducing the acidity of the alpha hydrogen. Modifying chalcone structures with prenyl side chains also affects their biological activities, as prenylation increases protein lipophilicity and targets the modified protein to cell membranes. This modification influences solubility, cellular uptake, and subcellular localization. Chalcones with various glycosidic substitutions on the aromatic rings exhibited reduced ability to suppress proliferation compared to their aglycones. This reduction is likely due to decreased lipophilicity, complicating passive diffusion through cell membranes. For radical scavenging activity, the ability to form intramolecular hydrogen bonds is essential. Chalcone derivatives that can adopt conformations where the A ring and carbonyl group are orthogonal are more successful radical scavengers [78]. The inhibitory activity of chalcones on nitric oxide (NO) production is linked to the presence of an alpha- beta double bond. Molecules with a single bond exhibited a weaker effect on nitric oxide production than corresponding molecules with an unsaturated bond. Similar mechanisms apply to chalcones potential to inhibit the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappa-B) pathway, as reduction of the alkene to a single bond attenuates their inhibition potential. Chalcones with 3,4,5-trimethoxyphenyl groups on the A ring and an alpha-methyl group within the enone moiety fit better into the colchicine-binding site of tubulin than other chalcones derivatives. Thus, alpha-methyl chalcones are believed to exhibit greater cytotoxic activity than unsubstituted analogues. Additionally, studies on the induction of heme oxygenase-1 (HO-1) by methoxychalcones revealed that an increasing number of methoxy groups on the aromatic rings at the 3, 4, 5 and 3', 4', 5' positions correlated with progressive induction of heme oxygenase-1, while methoxy substituents at the 2, 4, 6 positions or alone at the 4 and 4' positions were ineffective.[78]

Significance of the Study

1. This research could contribute to the development of new anti-inflammatory drugs for the treatment of inflammatory disease.
2. New 3-acetylpyridine chalcone derivatives may provide better treatment options with fewer side effects than current NSAIDs drugs.

Aim of the Study

The aim of this study is evaluating the anti-inflammatory activity of some synthetic 3-acetylpyridine chalcone derivatives aim to assess the potential of these novel compounds as anti-inflammatory drugs.

Literature Reviews

The Study 1

In June 1998, Dhanaji Jadhav and C. Ramaa. Synthesized twelve 2',4'- difluorinated chalcones using claisen-schmidt condensation and evaluated their anti-inflammatory activities. Four compounds showed anti-inflammatory activity comparable to indomethacin at 20 mg/kg, demonstrating the potential of fluorinated derivatives in inflammation treatment [79].

The Study 2

In 2006, Lucky Okunrobo, Cyril Usifoh, and John Uwaya. Synthesized 1,3-diaryl propen-1-ones (chalcones) and evaluated their anti-inflammatory and gastroprotective activities. One compound showed significant anti-ulcer effects comparable to cimetidine, indicating the dual therapeutic potential of chalcones [80].

The Study 3

In 2010, Zhang et al. Synthesized a series of substituted chalcone derivatives and evaluated their anti-inflammatory activity. Compounds [E-1-(2,4- dihydroxyphenyl)-3-(4-dimethylamino) phenyl) prop-2-en-1-one and [E-3-(4- chlorophenyl)-1-(2,4-dihydroxyphenyl) prop-2-en-1-one] showed the highest anti-inflammatory activity, comparable to or slightly more potent than ibuprofen [81].

The Study 4

In 2011, Jianzhang et al. Synthesized and screened a series of chalcone derivatives for anti-inflammatory activities. Two compounds exhibited significant inhibition of TNF-α and IL-6 release and interfered with JNK/NF-κB signaling. These compounds showed promising anti-inflammatory activities, highlighting their potential in treating inflammatory diseases [82].

The Study 5

In India in 2012, Selvakumar S. et al. Synthesized a series of 2-substituted benzimidazolyl chalcones and evaluated their biological activities. They found that 5 chalcones showed good anti-inflammatory activities. The ortho-substituted derivatives, especially with electron-donating hydroxy and electron-withdrawing chloro groups, exhibited better analgesic and anti-inflammatory activities [83].

The Study 6

In 2012, Shyam Sunder and Sandeep Reddy, Ch. Synthesized four novel chalcone derivatives two of them using a solvent-free

method and confirmed their structures using 1H NMR and FTIR spectra. two compounds exhibited significant anti-inflammatory activity, one of them showing activity equal to diclofenac, highlighting the efficiency of the solvent-free synthesis method [84].

The Study 7

In 2012, Rajendra Prasad, Venkatesh, and Subas Chandra Dinda. Evaluated the anti-inflammatory activity of novel chalcones, pyrimidines, and pyrazolines. Three chalcones were notably potent. The study used a carrageenan-induced rat paw oedema model and found that the synthesized compounds exhibited significant anti-inflammatory activity, comparable to the standard drug aceclofenac [85].

The Study 8

In 2013, Jahirul Islam Talukdar, Monica Kachroo, and Rema Razdan. Synthesized, purified, and characterized a series of new chalcone derivatives. Compounds with methoxy and ethoxy substitutions showed enhanced anti-inflammatory activities [86].

The Study 9

In India in 2022, Amit N. Panaskar, Ashish Jain, Pradeep Kumar Mohanty. synthesize chalcone hydrazide derivatives and evaluate their anti-inflammatory activity using a carrageenan-induced edema model in rats. The synthesized chalcone hydrazide derivatives showed significant anti-inflammatory activity [87].

The Study 10

In turkey in 2024, Iman Baramati, Mehlika Dilek. et al. Discovery New indole- chalcone hybrids were synthesized, showing significant pain relief and anti- inflammatory effects in mice, indicating therapeutic potential.

Compound 4 demonstrated the highest efficacy and good predicted oral bioavailability, making it a strong candidate for further pain and inflammation treatment research [88].

Materials and Method

Materials

Collection of Study Samples

The study samples were collected for 24 rats provided from the Animal House of Libyan Medical Research Center-Alzaweia, from 8 January to 22 April 2024. Their weights ranges from 145 to 355 gm and the age vary from 2 to 3 months.

Synthetic Derivatives

Two synthetic derivatives of 3-acetylpyridine chalcone were utilized in this study. These derivatives were synthesized by reacting 3-acetylpyridine with specific ketonic compounds under laboratory conditions. 20 mg of each derivative was diluted with 10 mg of dimethyl sulfoxide (DMSO) compound. Chemicals were synthesized by Dr. Mohammed El-Shambari.

Carrageenan

Type alpha carrageenan was used in this study, obtained from the Libyan Medical Research Center-Alzaweia. A 50 mg of 1% carrageenan solution was prepared by dissolving it in 10 ml of hot distelled water.

Diclofenac

Diclofenac sodium 75 mg / 3 ml was used in this study, obtained from the pharmacy. 0.2 ml of diclofenac diluted with 5 mg of DMSO compound. Used as solution injectable.

Animals

Male sprague-dawley rats weighing between 145 and 355 grams were used in the study. Rats were obtainid from the Libyan Medical Research Center-Alzaweia. A total of 24 rats were randomly divided into six experimental groups.

Methods

Experiment Animal Preparation

Rats were acclimatized under controlled laboratory conditions for three months prior to the experiment, with ad libitum access to water and standard rodent diet.

Experimental Procedure

Paw edema was induced by subcutaneously injecting 0.1 ml of 1% carrageenan solution into the rats' paw. Synthetic derivatives were administered via intraperitoneal injection at varying doses.

1- Division of Rats into Groups

The rats were divided into the following groups:

- Group 1: 3-(4-methylphenyl)-1-(pyridine-3-yl) prop-2-en-1-one compound group at 3mg/kg concentration.
- Group 2: 3-(4-methylphenyl)-1-(pyridine-3-yl) prop-2-en-1-one compound group at 6mg/kg concentration.
- Group 3: 3-(4-dimethylaminophenyl)-1-(pyridine-3-yl) prop-2-en-1-one compound group at 3mg/kg concentration.
- Group 4: 3-(4-dimethylaminophenyl)-1-(pyridine-3-yl) prop-2-en-1-one compound group at 6mg/kg concentration.
- Group 5: positive control Group (edema induction with diclofenac and carrageenan).
- Group 6: Control Group (standard group): carrageenan only.

2- Experimental Protocol in One Day

Induction of edema with carrageenan. All rats in groups from 1 to 6 were injected with carrageenan in the paw of the left foot to induce edema. Then injected by diclofenac and compound 1 and 2 in left foot subcutaneously.

Measurement of Paw Edema

Paw thickness was measured using a plethysmometer device after 1-, 2-, 3-, and 4-hours post-carrageenan injection. The device was used to determine average paw thickness and estimate edema volume.

Limitations

1. The study was limited to acute inflammation induced by carrageenan. Future studies should explore the derivatives' efficacy in chronic inflammation models.
2. The sample size of animals used in the study could affect generalizability, warranting larger-scale studies.
3. The study focused on paw edema measurements; additional biomarkers of inflammation could provide a comprehensive understanding of the derivatives' anti-inflammatory mechanisms.

Figure 5: The 6 Groups of Rats in our Experience [89]

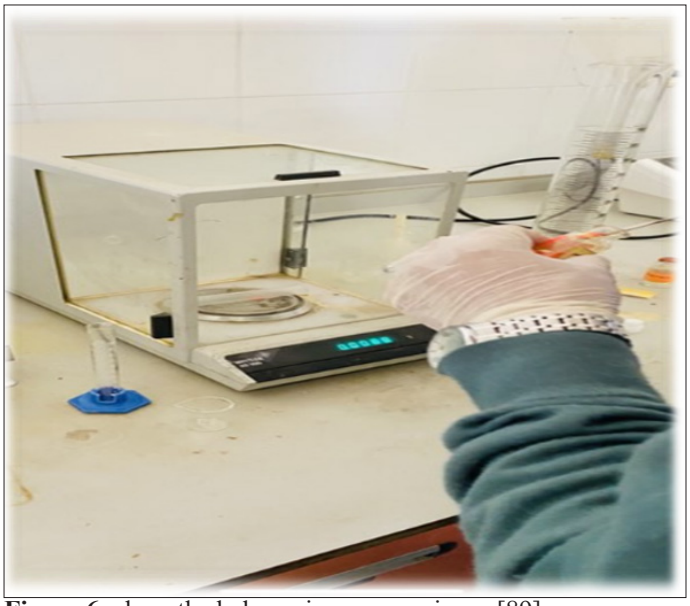

Figure 6: show the balance in our experience [89]

Figure 7: Plethysmometer device in our experience [89]

Figure 8: measured the edema by the device by the researchers [89]

Results and Discussion

The two compounds used in this research are: compound 1 is called 3-(4- methylphenyl)-1-(pyridine-3-yl) prop-2-en-1-one at 3 and 6 mg/kg concentration and the compound 2 is called 3-(4-dimethylaminophenyl)-1-(pyridine-3-yl) prop- 2-en-1-one at 3 and 6 mg/kg concentration.

The inflammation studied in this project is a chemical inflammation induced by carrageenan, rather than being caused by a viral or bacterial infection. Carrageenan is a chemical compound commonly used in animal studies to produce acute inflammation, such as foot swelling in rats, in order to evaluate the effectiveness of anti-inflammatory compounds. In this model, carrageenan triggers an inflammatory response by stimulating the body's immune system to release substances like cytokines and prostaglandins, which contribute to the inflammatory process. Typically, the inflammation caused by carrageenan is acute and lasts for a short duration, with observations extending to four hours after the administration of carrageenan. This allows researchers to study the anti- inflammatory effects of specific compounds without the presence of infection- causing agents such as bacteria or viruses.

1- Models Used in Inflammation Studies

- **Carrageenan Induced Paw Edema**

Carrageenan is a sulphated polysaccharide obtained from red sea weeds [90]. Carrageenan induces paw edema in 2 phases, the early phase and the late phase [90]. The early phase is commonly a nonphagocytic inflammation and occurs within one hour of carrageenan injection. The early phase is attributed to the release of mediators such as serotonin, histamine particularly 5-hydroxytryptamine (5-HT), cytoplasmic enzymes and platelet activating factor (PAF) from the mast cell. The late phase that

occurs after one hour is associated with the increased production of inducible cyclooxygenase (COX) and thereby increased prostaglandins synthesis which is mediated by leukotrienes, prostaglandins, bradykinin and polymorphonuclear cells in the inflammatory site [91]. The second phase edema is more sensitive to NSAIDs and steroidal anti-inflammatory agents. Carrageenan induced paw edema may involve L-arginine nitric oxide (NO) pathway whereby carrageenan injection results in release of nitric oxide (NO), this is attributed to elevated expression of inducible isoform of NO synthase. NO is a key mediator of inflammation. Tumor necrosis factor alfa (TNF-α), a key mediator of carrageenan induced edema, causes inflammatory incapacitation and stimulates production of kinins and leukotrienes which are responsible for long lasting nociceptive response [92].

2- The Results and Discussion of Each Group as in Following Tables

1- As shown in table 1 the mean paw edema volume is ranging from 1.37 to
1.63cm at four hours.

The anti-inflammatory effect of compound 1 at four hours calculated by inhibition percentage (edema inhibition %) at 3 mg/kg concentration and comparison with control group.

The edema inhibition % of group 1 = control mean edema - test group mean edema / control mean edema ×100%
The edema inhibition % at first hour = 1.14 - 1.37 / 1.14 ×100% = -20.18%

The edema inhibition % at second hour = 1.41 - 1.41 / 1.41 ×100% = 0% The edema inhibition % at third hour = 1.65 - 1.55 /1.65 ×100% = 6.06% The edema inhibition % at fourth hour = 1.72 - 1.63 / 1.72 ×100% = 5.23%.

Table 1: The volume of paw edema in group 1 using compound 1 at 3mg/kg concentration

Rats	1hour	2hour	3 hours	4 hours
R1	1.48	1.50	1.60	**1.64**
R2	1.29	1.54	1.69	**1.56**
R3	1.45	1.36	1.49	**1.68**
R4	1.24	1.25	1.42	**1.65**
Mean	1.37	1.41	1.55	**1.40**

2- And as shown in table 2 the mean paw edema volume is ranging from
1.16 to 1.43 cm at four hours. The anti-inflammatory effect of compound at four 1 hours calculated by inhibition percentage (edema inhibition %) at 6 mg/kg concentration and comparison with control group.

The edema inhibition % of group 2 = control mean edema - test group mean edema/control mean edema ×100%

The edema inhibition % at first hour = 1.14 - 1.21 / 1.14 ×100% = - 6.14% The edema inhibition % at second hour = 1.41 - 1.16 / 1.41 ×100% = 17.73% The edema inhibition % at third hour = 1.65 - 1.36 /1.65 ×100% = 17.57% The edema inhibition % at fourth hour = 1.72 - 1.43 / 1.72 ×100% = 16.86%

Table 2: The volume of paw edema in group 2 using compound 1 at 6mg/kg concentration

Rats	1hour	2hour	3 hours	4 hours
R1	1.56	1.4	1.73	**1.81**
R2	1.36	1.34	1.48	**1.58**
R3	0.8	0.78	0.85	**0.96**
R4	1.12	1.12	1.37	**1.39**
Mean	1.21	1.16	1.36	**1.43**

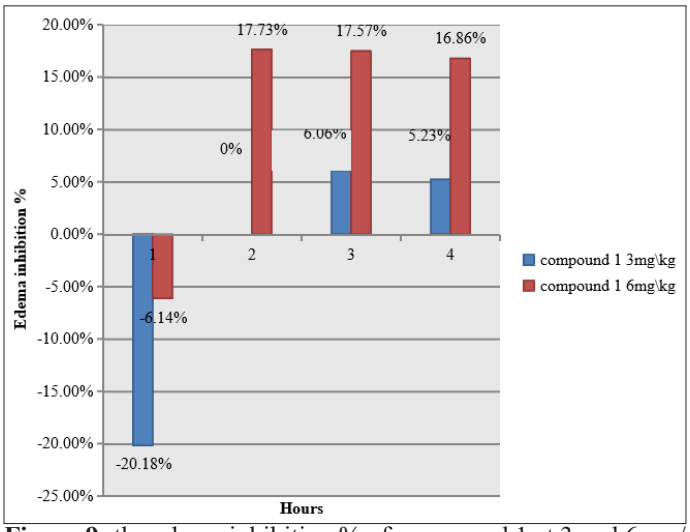

Figure 9: the edema inhibition % of compound 1 at 3 and 6 mg/kg concentration

3- As shown in table 3 the mean paw edema volume is ranging from 1.11 to 1.51 cm at four hours. The anti-inflammatory effect of compound 2 at four hours calculated by inhibition percentage (edema inhibition %) at 3 mg/kg concentration and comparison with control group.

The edema inhibition % of group 3 = control mean edema - test group mean edema / control mean edema ×100%.

The edema inhibition % at first hour = 1.14 - 1.11 / 1.14 × 100% = 2.63%. The edema inhibition % at second hour = 1.41 - 1.28 / 1.41 ×100% = 9.22%.
The edema inhibition % at third hour= 1.65 - 1.41 / 1.65 ×100% = 14.55%.

The edema inhibition % at fourth hour = 1.72 - 1.51 / 1.72 ×100% = 12.21%.

Table 3: The volume of paw edema in group 3 using compound 2 at 3mg/kg concentration

Rats	1hour	2hour	3 hours	4 hours
R1	1.14	1.30	1.52	**1.48**
R2	1.55	1.89	2.02	**2.04**
R3	0.88	1.02	1.10	**1.22**

R4	0.88	0.89	1.00	**1.28**
Mean	1.11	1.28	1.41	**1.51**

4- As Shown in table 4 the mean paw edema volume is ranging from 0.92 to 1.10 cm at four hours. The anti-inflammatory effect of compound 2 at four hours calculated by inhibition percentage (edema inhibition %) at 6mg/kg concentration and comparison with control group.

The edema inhibition % of group 4 = control mean edema - test group mean edema / control mean edema ×100%

The edema inhibition % at first hour = 1.14 - 0.97 / 1.14 ×100% = 14.91%.

The edema inhibition % at second hour =1.41 - 0.92 / 1.41 ×100%= 34.75%.

The edema inhibition % at third hour = 1.65 - 1.02 / 1.65 × 100%= 38.18%.

The edema inhibition % at fourth hour = 1.72 -1.10 / 1.72 ×100% = 36.05%.

Table 4: The volume of paw edema in group 4 using compound 2 at 6mg/kg concentration

Rats	1hour	2hour	3 hours	4 hours
R1	0.97	0.78	0.94	**1.01**
R2	1.00	0.9	0.89	**1.11**
R3	0.93	0.9	1.07	**1.00**
R4	0.96	1.1	1.18	**1.26**
Mean	0.97	0.92	1.02	**1.10**

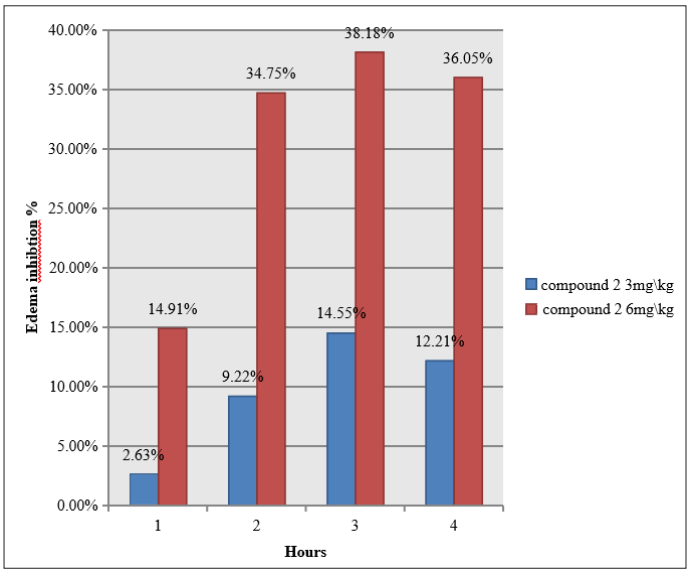

Figure 10: The Edema Inhibition % of Compound 2 at 3 and 6 mg/kg Concentration

5- As shown in table 5 (positive control) the mean paw edema volume is ranging from 1.51 to 1.80 cm at four hours. The anti-inflammatory effect of diclofenac after 3 hours by calculated inhibition percentage inhibition (edema inhibition %) at 2 ml/kg concentration and comparison with control group.

The edema inhibition % of group 5 = control mean edema - test group mean edema / control mean edema ×100%
The edema inhibition % at first hour = 1.14 - 1.51 / 1.14 ×100% = -32.46%

The edema inhibition % at second hour = 1.41 - 1.59 / 1.41 ×100% = - 12.77%

The edema inhibition % at third hour = 1.65 -1.72 / 1.65 ×100% = -4.24%

The edema inhibition % at fourth hour = 1.72 - 1.80 / 1.72 ×100% = -4.65%

Diclofenac with cargeenan: did not show a significant difference although it is a reference standard for reducing inflammation; other factor might have influenced results.

Table 5: The volume of paw edema in group 5 using diclofenac at 2mg/kg concentration

Rats	1hour	2hour	3 hours	4 hours
R1	1.80	1.93	2.01	**2.13**
R2	1.70	1.46	1.61	**1.90**
R3	1.15	1.34	1.59	**1.52**
R4	1.40	1.63	1.66	**1.67**
Mean	1.51	1.59	1.72	**1.80**

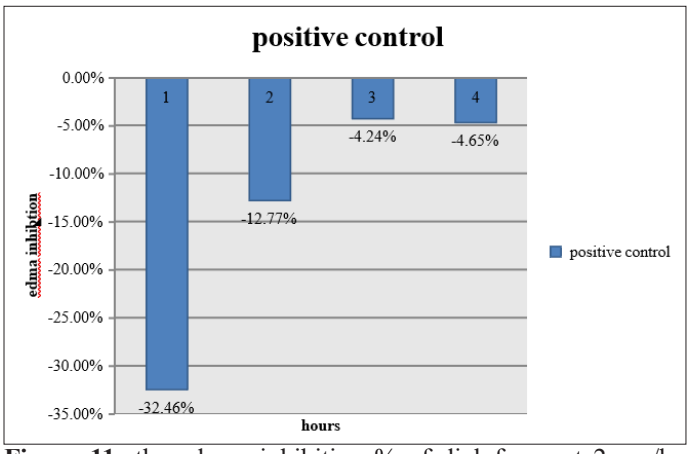

Figure 11: the edema inhibition % of diclofenac at 2 mg/kg concentration

6- And as shown in table 6 the mean paw edema volume ranging from 1.14 to 1.72 cm at four hours. This group is the control group and when carrageenan only did not show a significant effect, aligning with expectations that carrageenan is a pro-inflammatory agent.

The edema inhibition % of group 6 at all hours = 0 % because it is the control group.

Table 6: The volume of paw edema in group 6 using carrageenan only

Rats	1hour	2hour	3 hours	4 hours
R1	1.19	1.53	1.74	**1.92**
R2	1.5	1.96	2.27	**2.21**
R3	0.93	1.1	1.43	**1.51**
R4	0.93	1.03	1.14	**1.25**
Mean	1.14	1.41	1.65	**1.72**

Based on this result the researchers found the edema inhibition % of group 1 at first hour is -20.18%, while at second hour was 0%, and in third hour is 6.06%, and at fourth hour was 8.72%. While in group 2 at first hour was -6.14%, and at second hour was 17.73%, and in third hour was 17.57%, while in fourth hour was 16.86 % .and in group 3 at first hour was 2.63%, and at second hour was 9.22%, and in third hour was 14.55%. While in group 4 at first hour was 19.30%, and in second hour was 31.21%, and in third hour was 41.21%, and in fourth hour was 12.21%. while the positive control group (group 5) at first hour was - 32.46%, while in second hour was -12.77%, and at third hour was - 4.24%, and in fourth hour was -4.65%. And in group 6 at all hours was 0% becouse its control group Based on the calculations the researchers where the best effect of synthesized compounds is compound 2 at 6 mg/kg concentration in third hour. This means that the synthesized compounds have best effects when compared with diclofenac as anti-inflammatory reducer of paw edema in rats.

Conclusion

In conclusion, this study underscores the potential of synthetic compounds in medical research, particularly in the investigation of acute inflammation. The 3- acetylpyridine chalcone derivatives demonstrated promising anti-inflammatory properties in the carrageenan-induced paw edema model in rats. These compounds exhibited a dose-dependent inhibition of edema, with enhanced activity observed from the third to the fourth hour post-administration.

The findings suggest that some of these synthetic derivatives may offer greater efficacy than the reference drug, highlighting potential differences in their mechanisms of action. This underscores the importance of exploring new therapeutic agents that can provide alternative or complementary options to existing treatments.

Overall, the 3-acetylpyridine chalcone derivatives represent a promising avenue for the development of new anti-inflammatory therapies. Their significant activity in reducing inflammation, as demonstrated in this study, warrants further investigation and development, potentially leading to effective new treatments for inflammatory diseases.

Recommendations

1. **Optimization of dosage and administration**: further studies should be conducted to determine the optimal dosage and administration route for the synthetic derivatives. This includes exploring varying doses beyond the 3 mg/kg and 6 mg/kg used in this study to find the most effective concentration for reducing inflammation.
2. **Chronic inflammation models**: given that this study focused on acute inflammation, it is essential to extend research to chronic inflammation models. This would provide insight into the long-term efficacy and safety of the 3-acetylpyridine chalcone derivatives in treating chronic inflammatory conditions.
3. **Comparative analysis with indomethacin**: although the study showed that some derivatives might offer greater efficacy than indomethacin, more detailed comparative studies are necessary. This includes exploring the duration of action, side effects, and specific pathways inhibited by the synthetic derivatives versus indomethacin.
4. **Biomarker analysis**: incorporate additional biomarkers such as cytokines (e.g., TNF-α, IL-6) and histological analysis of tissue samples to provide a comprehensive understanding of the anti-inflammatory mechanisms of the synthetic derivatives. This will help in identifying specific inflammatory pathways affected by the compounds.
5. **Mechanistic studies**: detailed mechanistic studies should be conducted to elucidate the exact molecular mechanisms through which these compounds exert their anti-inflammatory effects. This could involve studying their interaction with specific enzymes, receptors, and signaling pathways involved in inflammation.
6. **Long-term safety and toxicity evaluation**: assess the long-term safety and potential toxicity of the synthetic derivatives through chronic administration studies in rats and other animal models. Monitoring for any adverse effects over an extended period will be crucial for future clinical development.
7. **Formulation development:** research should focus on developing suitable formulations for the synthetic derivatives to enhance their bioavailability and stability. This includes exploring different solvents, carriers, and delivery systems that could improve their therapeutic efficacy.
8. **Combination therapy:** investigate the potential of using the synthetic derivatives in combination with other anti-inflammatory drugs. This could result in synergistic effects, lower required doses, and reduced side effects, enhancing overall treatment efficacy.
9. **Human clinical trials:** based on promising preclinical results, plan for early-phase clinical trials to evaluate the safety, pharmacokinetics, and preliminary efficacy of the synthetic derivatives in humans. Initial studies could focus on healthy volunteers followed by trials in patients with acute and chronic inflammatory conditions.
10. **Structure-activity relationship (SAR) Studies:** conduct SAR studies to identify the key structural components responsible for the anti- inflammatory activity of the derivatives. This could lead to the design and synthesis of more potent analogues with improved efficacy and reduced side effects.
11. **Collaboration and funding:** seek collaboration with academic institutions, research organizations, and pharmaceutical companies to leverage expertise, resources, and funding opportunities. Collaborative efforts can accelerate the research and development process, leading to

faster translation of findings into clinical applications.

12. **Regulatory considerations:** engage with regulatory authorities early in the research process to understand the requirements for clinical development and eventual approval of the synthetic derivatives. This will ensure that the studies are designed to meet regulatory standards and facilitate a smoother transition to clinical trials.

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