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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF CHALCONE-BEARING BENZIMIDAZOLE DERIVATIVES AS ANTI-INFLAMMATORY AGENTS

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ABSTRACT

Inflammation is a fundamental biological response of living tissues to harmful stimuli, including physical injury, infection, toxins, and immune reactions. It is an essential component of the innate immune system, acting as the body's first line of defense against infections and injuries. The main goal of this study was to come up with, make, and test biologically chalcone-containing benzimidazole derivatives that could be used as anti-inflammatory drugs. The study successfully showed a methodical way to create new molecules by combining two pharmacologically important scaffolds: chalcone, an α,β-unsaturated carbonyl system known for its anti-inflammatory properties, and benzimidazole, a privileged heterocyclic structure known for its wide range of biological activities. A new group of chalcone-containing benzimidazole derivatives was made using the well-known Claisen–Schmidt condensation process, which is often used to make α,β-unsaturated carbonyl systems. The chalcone moiety is made when a ketone and an aldehyde come together in this process, which takes place in basic conditions. This trend strongly supports further development of EWG-substituted derivatives as promising anti-inflammatory agents.

KEYWORDS: chalcone-containing benzimidazole, scaffolds, Claisen–Schmidt condensation, α,β-unsaturated carbonyl systems.

1. INTRODUCTION

1.1 Background of Inflammation and Its Biological Role

Inflammation is a fundamental biological response of living tissues to harmful stimuli, including physical injury, infection, toxins, and immune reactions. It is an essential

component of the innate immune system, acting as the body's first line of defense against infections and injuries. The primary goal of inflammation is to eliminate the initial cause of cell injury, remove damaged tissue, and initiate tissue repair processes. Although inflammation is a protective mechanism, an exaggerated or chronic inflammatory response can lead to various pathological conditions.

Biologically, inflammation is characterized by a series of well-coordinated molecular and cellular events. These events are mediated by immune cells, blood vessels, and numerous molecular mediators such as cytokines, chemokines, prostaglandins, and leukotrienes. The classical clinical signs of inflammation include redness (rubor), heat (calor), swelling (tumor), pain (dolor), and loss of function (functio laesa). These manifestations result from vasodilation, increased vascular permeability, and the migration of immune cells to the site of injury or infection.

- **Pathophysiology of Inflammation**

Inflammation refers to a complicated biological reaction of the body tissues to some adverse agent like organisms, deteriorated cells or irritants. It is a vital body defense mechanism that is supposed to eradicate the source of the injury, destroy the dead cells and activate the healing process. Acute inflammation is protective and desirable, whereas chronic inflammation played a role in the pathogenesis of many diseases, such as rheumatoid arthritis, cardiovascular diseases, neurodegenerative diseases and cancer as well as the metabolic syndromes.

- **Therapeutic Targets in Inflammation**

Understanding the molecular mechanisms of inflammation has led to the identification of multiple therapeutic targets.

1. Enzymatic Targets

Cyclooxygenase (COX-1 and COX-2) enzymes catalyze the synthesis of prostaglandins, key mediators of pain and inflammation. NSAIDs like ibuprofen and diclofenac inhibit these enzymes.5-Lipoxygenase (5-LOX) produces leukotrienes involved in allergic and inflammatory responses. LOX inhibitors target this pathway. Phospholipase A2 (PLA2) releases arachidonic acid, a precursor for both prostaglandins and leukotrienes.

2. Cytokines and Their Receptors

Cytokines like TNF-α, IL-1β, IL-6, and IL-17 are crucial drivers of chronic inflammation. Biological agents such as infliximab (anti-TNF-α) and tocilizumab (anti-IL-6 receptor) have been developed to neutralize these cytokines.

3. Transcription Factors

NF-κB and AP-1 regulate the expression of numerous inflammatory genes. Inhibiting these transcription factors can broadly suppress inflammatory responses.

4. Inflammasomes

The NLRP3 inflammasome, which controls the activation of IL-1β and IL-18, has emerged as a key therapeutic target in diseases like gout and metabolic syndromes.

5. Other Targets

Janus Kinase (JAK) inhibitors (e.g., tofacitinib) block cytokine signaling pathways. Cell adhesion molecules like ICAM-1 facilitate leukocyte migration and are potential targets. Antioxidants aim to neutralize ROS and mitigate oxidative stress-induced inflammation.

1.2 Need for Novel Anti-Inflammatory Agents

Inflammation is a vital defense mechanism of the human body that plays a crucial role in combating infections, healing wounds, and repairing tissue damage. While inflammation is an essential part of immune function, uncontrolled or chronic inflammation is a significant contributor to the pathogenesis of various diseases, including rheumatoid arthritis, asthma, psoriasis, inflammatory bowel disease, cardiovascular diseases, diabetes, neurodegenerative disorders, and certain cancers.

Limitations of Existing Anti-Inflammatory Drugs

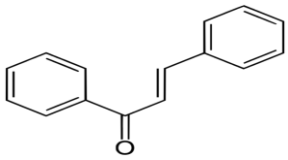
Existing anti-inflammatory medication is separately divided into non-steroidal anti-inflammatory medication (NSAIDs), glucocorticoids (steroidal anti-inflammatory medication), and biological agents that give specific consideration to cytokines and receptor targeting.

The main mechanism of action of the NSAIDs is to suppress cyclooxygenase (COX) enzymes which produce prostaglandins and control inflammations, pain, and fevers. Although its usage is effective in cases of acute inflammation, NSAIDs have extreme

gastrointestinal anomalies such as gastric ulcers, haemorrhaging, renal unit function, and cardiovascular hazards especially when repeatedly used.

1.3 Chalcone Scaffold: Biological Importance and Pharmacological Applications

Chalcones are an important class of natural and synthetic compounds characterized by the presence of an α, β-unsaturated carbonyl system linking two aromatic rings (commonly referred to as ring A and ring B). Chemically, they are open-chain flavonoids with the core structure of 1,3-diaryl-2-propen-1-one. Chalcones are biosynthetically derived as intermediates in the flavonoid biosynthetic pathway in plants. Their simple structure allows easy derivatization, leading to diverse biological activities. The structural flexibility of chalcones has made them a privileged scaffold in medicinal chemistry.

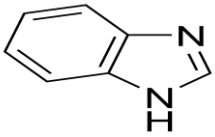


- **Biological Importance of Chalcone Scaffold**

The biological significance of the chalcone moiety stems from its α, β-unsaturated carbonyl system, which acts as a Michael acceptor, enabling covalent interaction with biological nucleophiles such as cysteine residues in enzymes and proteins. This reactivity underlies many of the pharmacological activities observed in chalcone derivatives. Furthermore, the presence of two aromatic rings provides the opportunity to introduce a variety of substituents, influencing the electronic, steric, and lipophilic properties, thereby affecting biological activity.

In nature, chalcone derivatives are widely distributed in various plant families, including Leguminosae, Compositae, and Moraceae, where they contribute to plant defense mechanisms against pathogens and environmental stress. Their occurrence in dietary sources has been associated with numerous health benefits, including antioxidant and anti-inflammatory effects.

1.4 Benzimidazole as a Privileged Structure in Drug Design



Benzimidazole is a fused heterocyclic compound consisting of a benzene ring fused with an imidazole ring. It is a significant pharmacophore in medicinal chemistry owing to its remarkable ability to interact with a wide variety of biological targets. The benzimidazole core has been widely recognized as a privileged structure in drug design due to its structural versatility, ease of modification, and the broad spectrum of pharmacological activities it imparts to drug candidates.

3. Objectives of Research

- To design novel chalcone-bearing benzimidazole derivatives based on rational drug design principles targeting inflammatory pathways.
- To synthesize the designed compounds using efficient, eco-friendly, and cost-effective synthetic routes.
- To characterize the synthesized compounds using spectroscopic techniques such as IR, NMR, and Mass Spectrometry.
- To evaluate the in vitro and in vivo anti-inflammatory activity of the synthesized compounds.
- To perform structure-activity relationship (SAR) analysis to understand the influence of different functional groups on anti-inflammatory activity.
- To compare the anti-inflammatory efficacy of synthesized compounds with standard drugs (e.g., Diclofenac or Ibuprofen).
- To perform molecular docking studies to predict binding affinities with key inflammatory targets such as COX-2, NF-κB, or iNOS.
- To assess preliminary toxicity or safety profiles of the most active compounds.
- To explore the potential of chalcone–benzimidazole hybrids as safer alternatives to current nonsteroidal anti-inflammatory drugs (NSAIDs).

4. Experimental Work

4.1 Material

Chemicals Used: 2-Acetyl benzimidazole (analytical grade)**,** Ortho- and para-substituted benzaldehydes (e.g., o-methoxy, p-chloro, p-nitro, p-methyl benzaldehyde)**,** Potassium hydroxide (KOH, 40% aqueous solution)**,** Ethanol (absolute)**,** Methanol (analytical grade, for recrystallization)**,** Crushed ice (for precipitation)**,** Hexane and ethyl acetate (for TLC).

Instrumentation: Magnetic stirrer with stirring bar**,** Melting point apparatus**,** Thin Layer Chromatography (TLC) chamber and plates (silica gel-coated)**,** FT-IR spectrophotometer**,** NMR spectrometer (^{1}H NMR, ^{13}C NMR)**,** Mass spectrometer.

Biological Materials: Reagents for anti-inflammatory assays (e.g., egg albumin for protein denaturation, carrageenan for in vivo assay) and Laboratory animals (Wistar rats or mice) approved by the Institutional Animal Ethics Committee (IAEC)

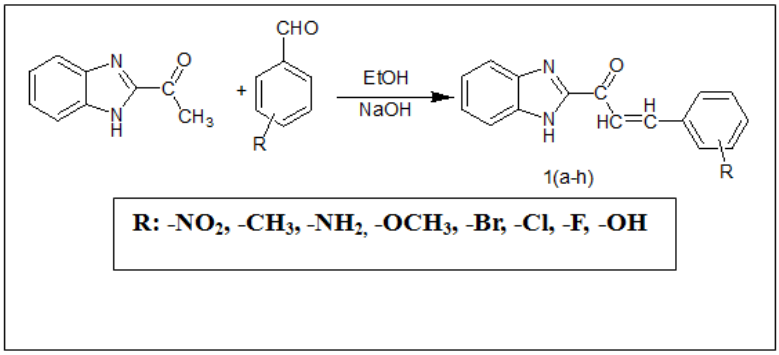
4.2. Method

Equimolar quantities of 2-acetyl benzimidazole and ortho- or para-substituted benzaldehyde were dissolved in 10 mL of ethanol and 30% KOH (7.5 mL). The reaction mixture was then allowed to stir for 18 h on a magnetic stirrer. The obtained mixture was poured into crushed ice, and the precipitate was filtered. After drying, the products were recrystallized from methanol. All reactions were monitored by performing thin-layer chromatography using hexane and ethyl acetate (1:4 v/v) as the mobile phase (Scheme 1). In this synthesis, an equimolar condensation reaction is carried out between 2-acetyl benzimidazole and an appropriately substituted benzaldehyde (either ortho- or para-substituted). This reaction was a classic example of the Claisen–Schmidt condensation, which forms a chalcone framework through the coupling of an aldehyde with a ketone in an alkaline medium. Firstly, equimolar quantities of both reactants were accurately weighed and dissolved in 10 mL of absolute ethanol, which serves as a suitable solvent due to its ability to solubilize both reactants and the potassium hydroxide. To facilitate the condensation reaction, 7.5 mL of 30% aqueous potassium hydroxide (KOH) is added to the reaction mixture. KOH acts as a strong base that abstracts an α-hydrogen from the acetyl group of 2-acetyl benzimidazole, generating an enolate ion. This nucleophilic enolate then attacks the carbonyl carbon of the benzaldehyde, leading to the formation of a β-hydroxy intermediate. The mixture is continuously stirred on a magnetic stirrer for 18 hours at room temperature, allowing sufficient time for the reaction to proceed to completion and for dehydration of the β-hydroxy intermediate to occur, yielding the desired chalcone-bearing benzimidazole derivative.

Upon completion, the reaction mixture is poured into crushed ice, which serves two purposes:

1. It neutralized excess alkali.
2. It induced the precipitation of the crude product due to the reduced solubility of the chalcone derivative in cold water.

Scheme I



4.2 Biological Evaluation

4.1.1 Animal Selection, Housing, and Ethical Considerations

Adult healthy male Wistar rats, each weighing between 170 to 190 grams, were selected for the study. The animals were procured from the Albino Research and Training Institute, Hyderabad, India, a CPCSEA-registered and approved breeder known for maintaining high standards in laboratory animal production.

Upon arrival, the rats were acclimatized to the laboratory environment for a minimum period of 7 days to minimize stress and ensure stabilization of physiological parameters. During this acclimatization period, the animals were observed for general health, behavioral normality, and adaptation to the environmental conditions.

The rats were housed in standard polypropylene cages fitted with stainless steel wire mesh lids, with paddy husk or corn cob bedding (replaced every 48 hours to maintain hygiene). Each cage housed a maximum of 3 to 4 rats to prevent overcrowding, ensuring adequate space for free movement and reducing stress-induced variability in experimental outcomes.

The animal room was maintained under controlled environmental conditions throughout the study, which included: Temperature: 23 ± 2°C, Relative Humidity: 45–65%, Light–Dark Cycle: 12 hours light and 12 hours dark, with lights turned on at 7:00 AM.

Animals were provided with a standard pellet diet (obtained from a certified feed manufacturer) containing balanced nutrients as per CPCSEA recommendations. Filtered water was made available *ad libitum* through clean water bottles, and both feed and water were replenished daily.

Pre-Experimental Preparation

Prior to the commencement of the experiments, all rats were fasted overnight (approximately 12 hours) to ensure uniform baseline physiological conditions, especially for pharmacological, biochemical, or toxicity evaluations. During fasting, access to water was unrestricted to prevent dehydration-related physiological stress.

Throughout the experimental period, the animals were closely monitored for signs of distress, abnormal behavior, or illness, including changes in food intake, posture, coat condition, locomotion, and body weight. No mortality or adverse clinical signs were observed, indicating that the experimental conditions were well tolerated by the animals.

Ethical Approval and Compliance

All procedures involving animals were designed and conducted following the ethical principles and guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, which ensures humane treatment of laboratory animals. The experimental protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of the respective institution. The approval was granted after a thorough evaluation of the scientific rationale, ethical justification, number of animals used, and adherence to the 3Rs principle (Replacement, Reduction, Refinement) to minimize animal usage and suffering.

4.1.2 Experimental Design and Methodology

The anti-inflammatory activity of the synthesized compounds 1(a-h) was evaluated using the Carrageenan-induced rat paw edema model, as described by Winter et al. (1962), which is a widely accepted model for assessing acute inflammation mediated predominantly by prostaglandins.

Induction of Acute Inflammation

- Acute inflammation was induced by sub plantar injection of 0.05 mL of 1% (w/v) Carrageenan suspension in sterile normal saline into the left hind paw of each rat.
- The Carrageenan solution was freshly prepared before injection to maintain potency.

Drug Administration Protocol

- All test compounds (6a–6j), standard drug (Indomethacin, 10 mg/kg), and vehicle (1% Carboxymethylcellulose in water) were administered orally (p.o.) 30 minutes prior to Carrageenan injection.

- The disease control group received Carrageenan injection along with the vehicle only (no active drug).

Measurement of Paw Edema

- The paw volume of each rat was measured using a digital plethysmometer (which measures volume displacement) at the following time points:
- 0 hours (Vc) — Baseline measurement, before Carrageenan injection.
- 1 hour (Vt) — Post Carrageenan injection.
- 3 hours (Vt) — Peak of sub-acute inflammatory response.
- hours (Vt) — Late phase of inflammation, which mainly involves prostaglandin synthesis.

5. RESULT AND DISCUSSION

5.1 Spectral Analysis

➢ **1-(1H-benzo[d]imidazol-2-yl)-3-(4-nitrophenyl)prop-2-en-1-one (1a)**

"Yellow solid, 85% in yield; m.p.: 190–192°C

IR (KBr) υmax 3433, 2889, 1766, 1654, 1513, 1315, 1139, 976, 765

^{1}H NMR (DMSO-d6, 400MHz): δ= 12.62 (s, 1H, NH), 8.83 (d, 1H, J=1.22Hz, ArH), 8.41 (d, 1H, J=1.22Hz, ArH), 8.17 (d, 2H, J=7.32Hz, ArH), 7.98 (d, 1H, J=15.2Hz, CH=), 7.72 (d, 1H, J=15.2Hz, CH=), 7.58 (t, 2H), 7.64–7.67 (m, 1H, ArH)

^{13}C NMR (DMSO-d6, 100MHz): δ=189.0 (C=O), 148.0 (Ar-C), 144.0 (Ar-C), 138.1 (Ar-C), 137.5 (Ar-C), 134.4 (Ar-C), 132.6 (Ar-C), 130.8 (Ar-C), 128.6 (Ar-C), 128.4 (Ar-C), 119.1 (CH=), 117.3 (Ar-C), 112.6 (Ar-C), 111.1 (CH=)

EIMS m/z 293.8

➢ **1-(1H-benzo[d]imidazol-2-yl)-3-p-tolylprop-2-en-1-one (1b)**

Yellow solid, 79% in yield; m.p.: 271–272°C

IR (KBr) υmax 3444, 3116, 2874, 1565, 1337, 1203, 1070, 827, 670, 498

^{1}H NMR (DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 8.86 (d, 1H, J=1.83Hz, ArH), 8.41 (d, 1H, J =2.44Hz, ArH), 8.16–8.14 (m, 2H, ArH), 8.00 (d, 1H, J =15.2Hz, CH=), 7.73–7.69 (m, 2H, CH=, ArH), 7.61 (t, 1H, ArH)

^{13}C NMR (DMSO-d6, 100MHz): δ=187.7 (C=O), 148.0 (Ar-C), 144.1 (Ar-C), 140.0 (Ar-C), 138.4 (Ar-C), 134.8 (Ar-C), 133.6 (Ar-C), 132.3 (Ar-C), 130.9 (Ar-C), 130.6 (Ar-C), 127.9 (Ar-C), 127.1 (Ar-C), 119.1 (CH=), 116.8 (Ar-C), 112.8 (Ar-C), 111.2 (CH=)

EIMS m/z 262.7

3-(4-aminophenyl)-1-(1H-benzo[d]imidazol-2-yl)prop-2-en-1-one (1c)

Yellow solid, 76% in yield; m.p.: 274–276°C

IR (KBr) υmax 3428, 1748, 1486, 1334, 1143, 972, 784,2394 25:2392–2398 619

^{1}H NMR (DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 8.85 (bs, 1H, ArH), 8.41 (bs, 1H, ArH), 8.19 (d, 2H, J =7.93Hz, ArH), 7.98 (d, 1H, J=15.8Hz, CH=), 7.68 (d, 1H, J=15.2Hz, CH=), 7.63 (d, 2H, J=8.54Hz, ArH)

^{13}C NMR (DMSO-d6, 100MHz): δ=187.9 (C=O), 148.0 (ArC), 144.1 (Ar-C), 138.1 (Ar-C), 137.5 (Ar-C), 136.7 (Ar-C), 134.8 (Ar-C), 130.9 (Ar-C), 130.3 (Ar-C), 128.7 (Ar-C), 119.1 (CH=), 116.9 (Ar-C), 112.7 (Ar-C), 111.2 (CH=)

EIMS m/z 263.7

1-(1H-benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (1d)

Yellow solid, 71% in yield; m.p.: 278–280°C

IR (KBr) υmax 3429, 2611, 1652, 1459, 1326, 1139, 970, 772, 631

^{1}H NMR (DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 8.86 (bs, 1H, ArH), 8.41 (bs, 1H, ArH), 8.28 (s, 1H, ArH), 8.19 (d, 1H, J=7.32Hz, ArH), 8.00 (d, 1H, J=15.8 Hz, CH=), 7.84 (d, 1H, J=7.93Hz, ArH), 7.69 (d, 1H, J =15.8Hz, CH=), 7.55 (t, 1H, ArH)

^{13}C NMR (DMSOd6, 100MHz): δ=187.7 (C=O), 148.0 (Ar-C), 144.1 (ArC), 140.2 (Ar-C), 138.4 (Ar-C), 135.2 (Ar-C), 134.8 (Ar-C), 130.9 (Ar-C), 130.7 (Ar-C), 127.5 (Ar-C), 122.2 (Ar-C), 119.1 (CH=), 116.8 (Ar-C), 112.8 (Ar-C), 111.2 (CH=)

EIMS m/z 278.6

1-(1H-benzo[d]imidazol-2-yl)-3-(4-bromophenyl)prop-2-en-1-one (1e)

Yellow solid, 74% in yield; m.p.: 281–283°C

IR (KBr) υmax 3417, 2613, 1568, 1654, 1393, 1234, 1072, 902, 773, 619

^{1}H NMR (DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 8.85 (d, 1H, J=2.44Hz, ArH), 8.40 (d, 1H, J =1.83Hz, ArH), 8.11 (d, 1H, J=8.54Hz, ArH), 7.98 (d, 1H, J=15.8Hz, CH=), 7.77 (d, 2H, J=8.54Hz, ArH), 7.67 (d, 1H, J =15.2Hz, CH=)

^{13}C NMR (DMSO-d6, 100 MHz): δ=188.4 (C=O), 148.0 (Ar-C), 144.3 (Ar-C), 138.3 (Ar-C), 137.2 (Ar-C), 134.7 (Ar-C), 131.8 (Ar-C), 131.0 (Ar-C), 130.6 (Ar-C), 126.8 (Ar-C), 119.2 (CH=), 117.1 (Ar-C), 112.9 (Ar-C), 111.3 (CH=)

EIMS m/z 327.7

1-(1H-benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-one (1f)

Yellow solid, 79% in yield; m.p.: 195–197°C

IR (KBr) υmax 3434, 2912, 1588, 1445, 1268, 1158, 1019, 870, 756, 632

^{1}H NMR (DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 8.82 (d, 1H, J=2.44Hz, ArH), 8.40 (d, 1H, J =1.83Hz, ArH), 7.95 (d, 1H, J=15.9Hz, CH=), 7.79 (d, 1H, J=7.93Hz, ArH), 7.70–7.66 (m, 2H, CH=, ArH), 7.50 (t, 3H, ArH), 7.41 (t, 2H, ArH), 7.36–7.29 (m, 2H, ArH), 5.23 (s, 2H, benzy-H)

^{13}C NMR (DMSO-d6, 100MHz): δ =188.7 (C=O), 158.5 (Ar-C), 147.9 (Ar-C), 144.0 (ArC), 139.5 (Ar-C), 137.6 (Ar-C), 136.8 (Ar-C), 134.3 (Ar-C), 130.8 (Ar-C), 129.8 (ArH), 128.4 (Ar-C), 127.9 (Ar-C), 127.7 (Ar-C), 121.1 (Ar-C), 119.3 (Ar-C), 119.1 (CH=), 117.4 (Ar-C), 114.0 (Ar-C), 112.6 (Ar-C), 111.1 (CH=), 69.3 (CH2-, benzyl)

EIMS m/z 247.9

1-(1H-benzo[d]imidazol-2-yl)-3-(4-fluorophenyl)prop-2-en-1-one (1g)

Yellow solid, 90% in yield; m.p.: 263–264°C

IR (KBr) υmax 3431, 1655, 1412, 1235, 1019, 803, 637

^{1}H NMR(DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 9.37 (d, 1H, J=1.22Hz, ArH), 8.91 (d, 1H, J=1.83Hz, ArH), 8.83–8.81 (q, 1H, ArH), 8.48 (d, 1H, J=7.93Hz, ArH), 8.41 (d, 1H, J=1.83Hz, ArH), 8.01 (d, 1H, J=15.8Hz, CH=), 7.71 (d, 1H, J=15.2Hz, CH=), 7.62–7.59 (m, 1H, ArH);

^{13}CNMR (DMSO-d6, 100MHz): δ=188.1 (C=O), 152.8 (Ar-C), 149.6 (Ar-C), 148.0 (Ar-C), 144.1 (Ar-C), 138.4 (Ar-C), 135.8 (Ar-C), 135.2 (Ar-C), 133.2 (Ar-C), 131.0 (Ar-C), 123.8 (Ar-C), 119.0 (CH=), 116.9 (Ar-C), 112.8 (Ar-C), 111.1 (CH=)

EIMS m/z 266.8

1-(1H-benzo[d]imidazol-2-yl)-3-(4-hydroxyphenyl)prop-2-en-1-one (1h)

Yellow solid, 94% in yield; m.p.: 265–267°C

IR (KBr) υmax 3431, 1655, 1412, 1235, 1019, 803, 637

^{1}H NMR(DMSO-d6, 400MHz): δ=12.6 (s, 1H, NH), 9.37 (d, 1H, J=1.22Hz, ArH), 8.91 (d, 1H, J=1.83Hz, ArH), 8.83–8.81 (q, 1H, ArH), 8.48 (d, 1H, J=7.93Hz, ArH), 8.41 (d, 1H, J=1.83Hz, ArH), 8.01 (d, 1H, J=15.8Hz, CH=), 7.71 (d, 1H, J=15.2Hz, CH=), 7.62–7.59 (m, 1H, ArH)”

^{13}CNMR (DMSO-d6, 100MHz): δ=188.1 (C=O), 152.8 (Ar-C), 149.6 (Ar-C), 148.0 (Ar-C), 144.1 (Ar-C), 138.4 (Ar-C), 135.8 (Ar-C), 135.2 (Ar-C), 133.2 (ArH), 131.0 (Ar-C), 123.8 (Ar-C), 119.0 (CH=), 116.9 (Ar-C), 112.8 (Ar-C), 111.1 (CH=);

EIMS m/z 268.8

5.2 Biological Evaluation

Table 1: Result of carrageen an induced paw edema method.

Treatment	Dose	Change in paw volume ml			
		0h	1h	2h	3h
Control	Normal saline	0.93±0.005	1.52±0.04	1.85±0.03	2.51±0.04
1a	200mg/kg	0.94±0.006	1.32±0.01	1.45±0.04	1.62±0.07
1b	200mg/kg	0.93±0.004	1.4±0.01	1.43±0.04	1.63±0.07
1c	200mg/kg	0.94±0.008	1.214±0.01	1.42±0.04	1.61±0.07
1d	200mg/kg	0.92±0.007	1.24±0.01	1.45±0.04	1.60±0.07
1e	200mg/kg	0.91±0.008	1.24±0.01	1.44±0.04	1.62±0.07
1f	200mg/kg	0.93±0.006	1.26±0.01	1.42±0.04	1.65±0.07
1g	200mg/kg	0.95±0.007	1.23±0.01	1.44±0.04	1.68±0.07
1h	200mg/kg	0.94±0.006	1.24±0.01	1.40±0.04	1.66±0.07
Standard	Indomethacin	0.90±0.009	0.99±0.01	1.02±0.004	1.10±0.003

Table 2: Percentage Inhibition.

Treatment	Dose	Percentage inhibition %			
		0h	1h	2h	3h
Control	Normal saline	_	_	_	_
1a	200mg/kg	1.07	20.02	24.32	32.07
1b	200mg/kg	1.10	19.32	20.32	31.05
1c	200mg/kg	1.18	17.22	22.33	32.05
1d	200mg/kg	1.15	19.12	24.32	35.06
1e	200mg/kg	1.17	21.42	25.12	34.07
1f	200mg/kg	1.25	17.52	25.12	32.08
1g	200mg/kg	1.15	18.42	24.32	35.06
1h	200mg/kg	2.00	21.75	29.72	40.39
Standard	Indomethacine	3.22	34.86	44.86	56.17

Results are expressed as mean ± sem.*$p>0.05$, **$p<0.01$ as compared to control,

6. RESULT AND DISCUSSION

A new group of chalcone-containing benzimidazole derivatives was made using the well-known Claisen–Schmidt condensation process, which is often used to make α,β-unsaturated carbonyl systems. The chalcone moiety is made when a ketone and an aldehyde come together in this process, which takes place in basic conditions. The process started with 2-acetyl benzimidazole reacting with different substituted benzaldehydes, which have both ortho- and para-substituted methylene groups next to the carbonyl. The reaction took place with 40% potassium hydroxide (KOH) in water, which is a strong base, and ethanol, which is a polar protic liquid that made it easier for the reactants and the base to mix.

7. CONCLUSION

The main goal of this study was to come up with, make, and test biologically chalcone-containing benzimidazole derivatives that could be used as anti-inflammatory drugs. The study successfully showed a methodical way to create new molecules by combining two pharmacologically important scaffolds: chalcone, an α,β-unsaturated carbonyl system known for its anti-inflammatory properties, and benzimidazole, a privileged heterocyclic structure known for its wide range of biological activities.

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