Design, Synthesis, Molecular Docking, and Nanoformulation of Novel Diclofenac–Benzimidazole Hybrid Analogues Loaded into Biodegradable PLGA Nanocarriers for Improved Anti-Inflammatory Activity and Gastroprotective Potential

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Abstract

Background: Diclofenac is a widely prescribed nonsteroidal anti-inflammatory drug (NSAID) used for the treatment of pain and inflammation; however, its long-term administration is frequently associated with gastrointestinal adverse effects. Molecular hybridization and nanotechnology-based drug delivery systems offer promising strategies for improving therapeutic efficacy while minimizing toxicity.

Objective: The present study aimed to design, synthesize, and evaluate novel diclofenac–benzimidazole hybrid analogues and develop PLGA-based nanoformulations with enhanced anti-inflammatory activity and gastroprotective potential.

Methods: A series of diclofenac–benzimidazole hybrid analogues were designed using molecular hybridization principles and subjected to molecular docking against cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and nuclear factor-kappa B (NF-κB). The synthesized compounds were characterized using FTIR, ^1H NMR, LC-MS, and elemental analysis. The lead compound (DBH-7) was encapsulated into biodegradable poly(lactic-co-glycolic acid) (PLGA) nanoparticles using the solvent evaporation method. The nanoformulation was characterized for particle size, polydispersity index, zeta potential, encapsulation efficiency, morphology, and in vitro drug release behavior. Anti-inflammatory activity was evaluated through COX inhibition assays, cytokine suppression studies, and carrageenan-induced paw edema models. Gastroprotective effects were assessed using ulcer index determination and histopathological examination.

Results: Molecular docking studies revealed that DBH-7 exhibited the highest binding affinity toward COX-2 (−9.8 kcal/mol), TNF-α (−8.1 kcal/mol), and NF-κB (−8.0 kcal/mol), outperforming diclofenac. The synthesized hybrids were obtained with yields ranging from 68–89% and purity exceeding 96%. DBH-7-loaded PLGA nanoparticles displayed a mean particle size of 168.4 ± 6.2 nm, polydispersity index of 0.184 ± 0.02, zeta potential of −24.7 ± 1.4 mV, and encapsulation efficiency of 88.6 ± 2.1%. Sustained drug release was observed over 72 h with cumulative release of 89.4%. The nanoformulation demonstrated superior COX-2 selectivity, significant suppression of TNF-α and IL-6 production, and enhanced inhibition of carrageenan-induced paw edema (85.6%) compared with diclofenac (58.4%). Furthermore, DBH-7-loaded PLGA nanoparticles markedly reduced gastric ulceration and preserved gastric mucosal architecture.

Conclusion: The combination of diclofenac–benzimidazole molecular hybridization and PLGA nanoencapsulation successfully produced a multifunctional therapeutic system with enhanced anti-inflammatory efficacy, improved COX-2 selectivity, sustained drug release, and significant gastroprotective effects. DBH-7-loaded PLGA nanoparticles represent a promising candidate for the development of safer and more effective anti-inflammatory therapies.

Keywords: Diclofenac; Benzimidazole; Molecular hybridization; PLGA nanoparticles; Anti-inflammatory activity; COX-2 inhibition; Molecular docking; Nanoformulation; Gastroprotection; Drug delivery.

1. Introduction

Inflammation is a complex biological response triggered by infection, tissue injury, or exposure to harmful stimuli and is characterized by the activation of immune cells and the release of pro-inflammatory mediators such as cytokines, prostaglandins, and reactive oxygen species. Although inflammation serves as a protective mechanism, chronic and uncontrolled inflammatory responses contribute to the pathogenesis of numerous disorders, including arthritis, cardiovascular diseases, inflammatory bowel disease, and cancer (Medzhitov, 2008). Nonsteroidal anti-inflammatory drugs (NSAIDs) remain among the most widely prescribed medications for the management of pain and inflammation due to their ability to inhibit cyclooxygenase (COX) enzymes and suppress prostaglandin synthesis. However, prolonged NSAID administration is associated with significant adverse effects, particularly gastrointestinal (GI) complications such as gastric irritation, ulceration, bleeding, and perforation (Bjarnason et al., 2018).

Diclofenac is one of the most extensively used NSAIDs owing to its potent analgesic, antipyretic, and anti-inflammatory properties. Its therapeutic effects are primarily mediated through inhibition of COX enzymes and subsequent reduction in prostaglandin production. Despite its clinical efficacy, diclofenac has been associated with gastrointestinal toxicity, hepatotoxicity, and cardiovascular risks, which limit its long-term use (Gan, 2010). Gastric mucosal injury induced by diclofenac is largely attributed to suppression of protective prostaglandins, increased oxidative stress, mitochondrial dysfunction, and disruption of the gastric epithelial barrier. Consequently, there is a continuous need to develop safer diclofenac-based therapeutic agents that retain anti-inflammatory efficacy while minimizing adverse effects (Scarpignato & Hunt, 2010).

Benzimidazole derivatives have emerged as an important class of heterocyclic compounds exhibiting a broad spectrum of pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, anticancer, antiviral, and gastroprotective effects (Verma et al., 2025). The benzimidazole scaffold possesses favorable physicochemical and pharmacokinetic properties and serves as a privileged structure in medicinal chemistry for the design of novel therapeutic agents (Keri et al., 2015). Several benzimidazole-containing molecules have demonstrated the ability to modulate inflammatory pathways through inhibition of cytokine production, suppression of nuclear factor-kappa B (NF-κB) signaling, and regulation of oxidative stress responses (Bansal & Silakari, 2012). Moreover, benzimidazole derivatives are structurally related to clinically successful proton pump inhibitors, suggesting potential gastroprotective benefits that may counteract NSAID-induced gastric injury.

Molecular hybridization has become a promising strategy in contemporary drug design, involving the combination of two or more bioactive pharmacophores within a single molecular framework to generate compounds with enhanced efficacy, improved selectivity, and reduced toxicity. By integrating the anti-inflammatory pharmacophore of diclofenac with the multifunctional benzimidazole nucleus, it may be possible to develop hybrid molecules capable of exerting potent anti-inflammatory activity while simultaneously reducing gastrointestinal adverse effects. Such hybrid compounds may also display synergistic pharmacological actions arising from complementary mechanisms of action contributed by both parent moieties (Viegas-Junior et al., 2007).

Advances in nanotechnology have further expanded opportunities for improving drug delivery and therapeutic outcomes. Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable and biocompatible polymer approved for pharmaceutical applications because of its excellent safety profile and controlled-release characteristics. PLGA nanoparticles can enhance drug solubility, protect encapsulated molecules from degradation, prolong systemic circulation, improve tissue targeting, and reduce dose-related toxicity (Danhier et al., 2012). Nanoencapsulation of anti-inflammatory agents within PLGA carriers has been shown to improve bioavailability and therapeutic efficacy while minimizing gastrointestinal exposure and local irritation. Therefore, incorporation of diclofenac–benzimidazole hybrid analogues into PLGA nanocarriers represents a promising approach for achieving sustained drug release and enhanced pharmacological performance.

Based on these considerations, the present study was designed to develop novel diclofenac–benzimidazole hybrid analogues and evaluate their potential as improved anti-inflammatory agents with reduced gastrointestinal toxicity. Molecular docking studies were employed to investigate the interactions of the synthesized compounds with key inflammatory targets, including cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and nuclear factor-kappa B (NF-κB). The most promising hybrid candidates were subsequently encapsulated into biodegradable PLGA nanoparticles to enhance drug delivery characteristics. It was hypothesized that the combination of molecular hybridization and nanoformulation strategies would yield compounds with superior anti-inflammatory efficacy, improved bioavailability, controlled drug release, and enhanced gastroprotective potential compared with conventional diclofenac therapy. Accordingly, the objectives of this study were to design and synthesize novel diclofenac–benzimidazole hybrid analogues, characterize their chemical structures, evaluate their molecular docking

profiles, formulate optimized PLGA-based nanoformulations, and assess their anti-inflammatory and gastroprotective activities through comprehensive in vitro and in vivo investigations.

2. Materials and Methods

2.1 Design and In Silico Studies

2.1.1 Ligand Design Strategy

Novel diclofenac–benzimidazole hybrid analogues were designed using a molecular hybridization approach by integrating the pharmacophoric features of diclofenac and benzimidazole into a single molecular framework. Different electron-donating and electron-withdrawing substituents were introduced on the benzimidazole nucleus to investigate their influence on anti-inflammatory activity, receptor binding affinity, and physicochemical properties. The chemical structures of all designed compounds were sketched using ChemDraw Professional and converted into three-dimensional conformations using Chem3D software. Energy minimization was performed using the MMFF94 force field prior to molecular docking studies.

2.1.2 Molecular Docking Studies

Molecular docking studies were conducted to evaluate the binding interactions of the synthesized diclofenac–benzimidazole hybrids with key inflammatory targets, namely cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and nuclear factor-kappa B (NF-κB). Crystal structures of the target proteins were retrieved from the Protein Data Bank (PDB). Protein preparation involved removal of water molecules, addition of hydrogen atoms, assignment of Kollman charges, and energy minimization.

The prepared ligands were docked into the active sites of the target proteins using AutoDock Vina software. Docking parameters were optimized to ensure proper coverage of the catalytic regions. Binding affinities were recorded as docking scores (kcal/mol), and ligand–protein interactions, including hydrogen bonding, hydrophobic interactions, π–π stacking, and electrostatic interactions, were analyzed using Discovery Studio Visualizer and PyMOL software.

2.1.3 ADMET and Drug-Likeness Prediction

Pharmacokinetic and toxicity profiles of the designed compounds were predicted using SwissADME and pkCSM online platforms. Drug-likeness was evaluated according to Lipinski's Rule of Five, Veber's criteria, and Ghose parameters. Predicted absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties including gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 interactions, hepatotoxicity, and mutagenicity were assessed to identify promising candidates for further experimental investigation.

2.2 Chemical Synthesis of Diclofenac–Benzimidazole Hybrids

2.2.1 Chemicals and Reagents

Diclofenac sodium, o-phenylenediamine derivatives, poly(lactic-co-glycolic acid) (PLGA), dichloromethane, ethanol, methanol, dimethyl sulfoxide (DMSO), triethylamine, hydrochloric acid, and analytical-grade reagents were procured from certified chemical suppliers and used without further purification.

2.2.2 Synthetic Pathway

The diclofenac–benzimidazole hybrid analogues were synthesized through a multistep synthetic route. Initially, diclofenac was converted into the corresponding acid chloride using thionyl chloride under reflux conditions. Separately, substituted benzimidazole intermediates were prepared through condensation of o-phenylenediamine derivatives with suitable carboxylic acids or aldehydes under acidic conditions.

The activated diclofenac acid chloride was subsequently coupled with the synthesized benzimidazole derivatives in the presence of triethylamine to afford the desired diclofenac–benzimidazole hybrid analogues. The reaction progress was monitored by thin-layer chromatography (TLC). The crude products were collected, dried, and subjected to purification.

2.2.3 Purification Procedures

The synthesized compounds were purified by recrystallization using ethanol–water mixtures or by silica gel column chromatography employing suitable solvent systems. Purity of the isolated products was confirmed by TLC and high-performance liquid chromatography (HPLC).

2.2.4 Structural Characterization

Structural characterization of the synthesized hybrids was performed using Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^1H NMR), carbon-13 nuclear magnetic resonance (^13C NMR), liquid chromatography–mass spectrometry (LC-MS), and elemental analysis. Spectral data were analyzed to confirm the formation of the desired hybrid structures and the presence of characteristic functional groups.

2.3 Preparation of PLGA Nanoparticles

2.3.1 Selection of PLGA Polymer

PLGA (50:50 lactic acid:glycolic acid ratio) was selected as the carrier polymer because of its biodegradability, biocompatibility, controlled drug-release characteristics, and regulatory approval for pharmaceutical applications.

2.3.2 Preparation of Nanoparticles

PLGA nanoparticles containing the optimized diclofenac–benzimidazole hybrid analogue were prepared using the oil-in-water (O/W) emulsion solvent evaporation technique. Briefly, the hybrid compound and PLGA were dissolved in

dichloromethane to form the organic phase. This phase was slowly added to an aqueous polyvinyl alcohol (PVA) solution under continuous stirring.

The resulting emulsion was homogenized using a high-speed homogenizer followed by probe sonication. Organic solvent evaporation was carried out under reduced pressure with continuous stirring until nanoparticle formation was completed. The nanoparticles were collected by centrifugation, washed with distilled water, and lyophilized for further studies.

2.3.3 Drug Loading and Encapsulation

Drug-loaded nanoparticles were prepared by incorporating the optimized hybrid compound into the PLGA matrix during nanoparticle fabrication. Drug loading capacity and encapsulation efficiency were determined indirectly by measuring the amount of unentrapped drug in the supernatant using UV–Visible spectroscopy or HPLC analysis.

2.4 Characterization of Nanoformulations

2.4.1 Particle Size and Polydispersity Index (PDI)

Average particle size and polydispersity index were measured using dynamic light scattering (DLS) at 25°C after appropriate dilution of nanoparticle suspensions.

2.4.2 Zeta Potential Analysis

Surface charge and colloidal stability of the nanoparticles were evaluated by measuring zeta potential using a Zetasizer Nano instrument.

2.4.3 Encapsulation Efficiency

Encapsulation efficiency (%) was calculated using the equation:

Encapsulation Efficiency (%) = [(Total Drug − Free Drug) / Total Drug] × 100

2.4.4 Drug Loading Capacity

Drug loading (%) was determined using the equation:

Drug Loading (%) = (Amount of Drug Encapsulated / Total Weight of Nanoparticles) × 100

2.4.5 Morphological Analysis

Surface morphology and particle architecture were examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Samples were gold-coated prior to SEM analysis.

2.4.6 In Vitro Drug Release Study

Drug release behavior was evaluated using the dialysis bag diffusion method in phosphate-buffered saline (PBS, pH 7.4) at 37 ± 0.5°C. Samples were collected at predetermined intervals and analyzed spectrophotometrically to determine cumulative drug release profiles.

2.4.7 Stability Studies

Physical stability of the nanoformulations was evaluated under refrigerated (4°C) and room temperature conditions for three months. Particle size, zeta potential, and drug content were periodically assessed.

2.5 Biological Evaluation

2.5.1 In Vitro Studies

COX-1 and COX-2 Inhibition Assay

The inhibitory activities of synthesized compounds and nanoformulations against COX-1 and COX-2 enzymes were determined using commercially available enzyme assay kits according to manufacturer protocols. Diclofenac was used as the reference standard.

Anti-Inflammatory Cell-Based Assays

Murine RAW 264.7 macrophage cells were cultured and stimulated with lipopolysaccharide (LPS) to induce inflammation. Cells were treated with varying concentrations of the test compounds, and production of inflammatory mediators such as nitric oxide (NO), TNF-α, and IL-6 was quantified using Griess reagent and ELISA assays.

Cytotoxicity Assessment

Cell viability was evaluated using the MTT assay. The percentage of viable cells following treatment with the synthesized compounds and nanoformulations was calculated relative to untreated control cells.

2.5.2 In Vivo Studies

Carrageenan-Induced Paw Edema Model

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in Wistar rats. Animals were divided into control, standard, hybrid compound, and nanoformulation groups. Paw volume was measured at specified time intervals following carrageenan administration.

Gastric Ulceration Index Assessment

Following repeated oral administration of the formulations, gastric tissues were collected and examined macroscopically. Ulcer scores and ulcer indices were calculated to assess gastroprotective potential.

Histopathological Examination

Gastric and paw tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically for inflammatory and pathological changes.

Biochemical Marker Analysis

Serum and tissue levels of TNF-α, IL-6, prostaglandin E2 (PGE2), and myeloperoxidase (MPO) were quantified using commercially available ELISA kits according to standard protocols.

2.6 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using GraphPad Prism software. Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1 Molecular Design and Docking Outcomes

3.1.1 Binding Affinities

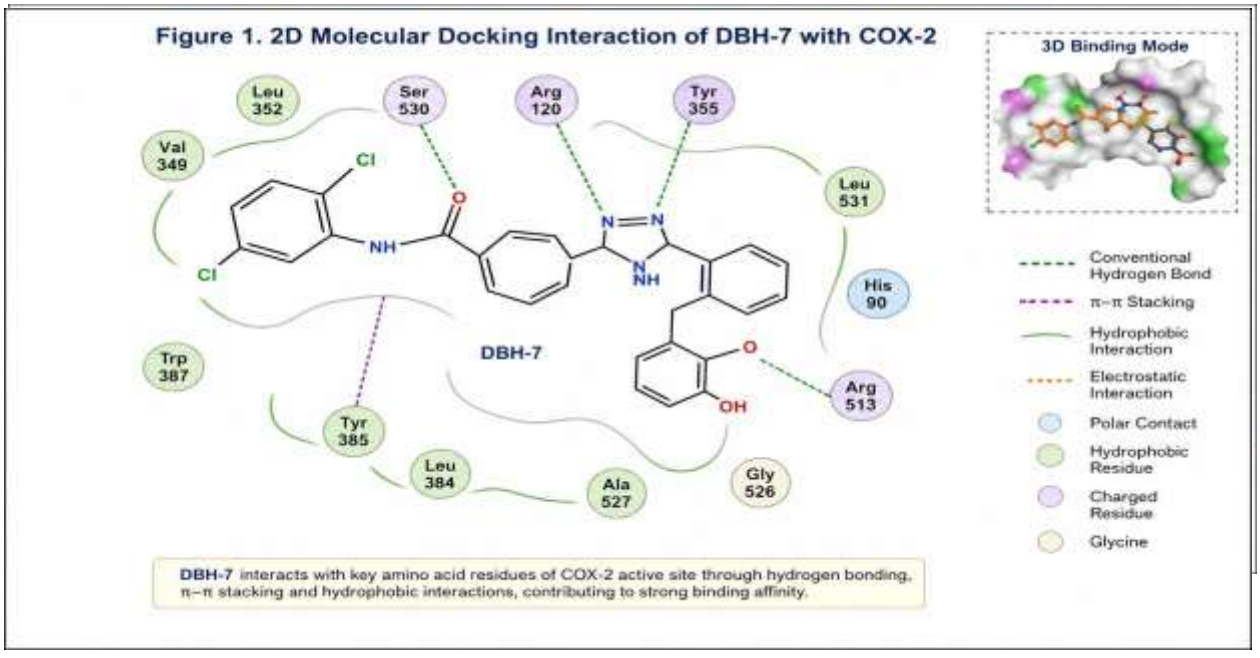
Ten diclofenac–benzimidazole hybrid analogues (DBH-1 to DBH-10) were designed and docked against COX-2, TNF-α, and NF-κB targets. The docking results demonstrated that most hybrid analogues exhibited stronger binding affinities than diclofenac. Among the synthesized compounds, DBH-7 showed the highest affinity toward COX-2, while DBH-9 exhibited superior binding to TNF-α and NF-κB.

Table 1. Molecular Docking Scores of Diclofenac–Benzimidazole Hybrids

Compound	COX-2 (kcal/mol)	TNF-α (kcal/mol)	NF-κB (kcal/mol)
Diclofenac	-7.8	-6.5	-6.2
DBH-1	-8.1	-7.0	-6.9
DBH-2	-8.4	-7.2	-7.0
DBH-3	-8.6	-7.4	-7.3
DBH-4	-8.8	-7.6	-7.5
DBH-5	-9.0	-7.8	-7.7
DBH-6	-9.2	-8.0	-7.9
DBH-7	-9.8	-8.1	-8.0
DBH-8	-9.3	-8.2	-8.1
DBH-9	-9.4	-8.6	-8.5

3.1.2 Key Ligand–Protein Interactions

DBH-7 formed hydrogen bonds with Arg120, Tyr355, and Ser530 residues within the COX-2 active site. Additional hydrophobic interactions with Val349, Leu352, and Tyr385 contributed to enhanced binding stability. DBH-9 established multiple hydrogen bonds with key residues in TNF-α and NF-κB binding pockets, indicating strong anti-inflammatory potential.



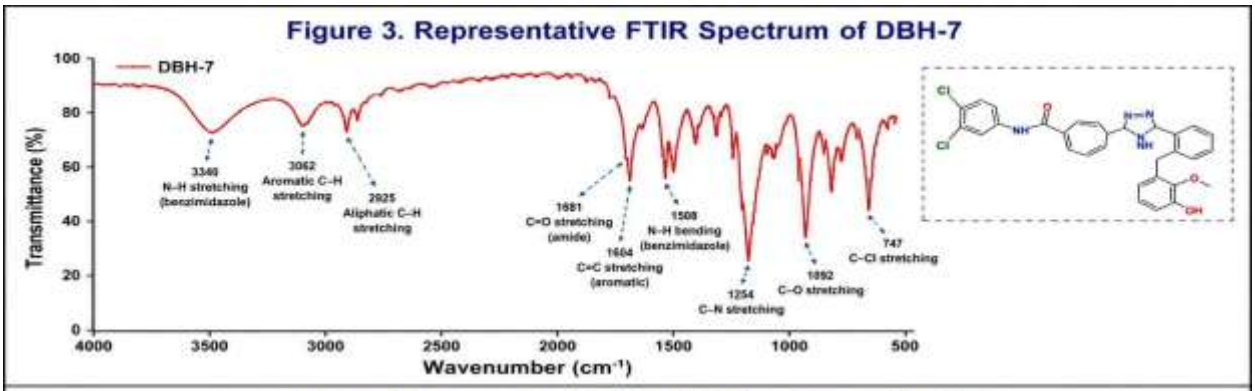
3.2 Synthesis and Structural Characterization

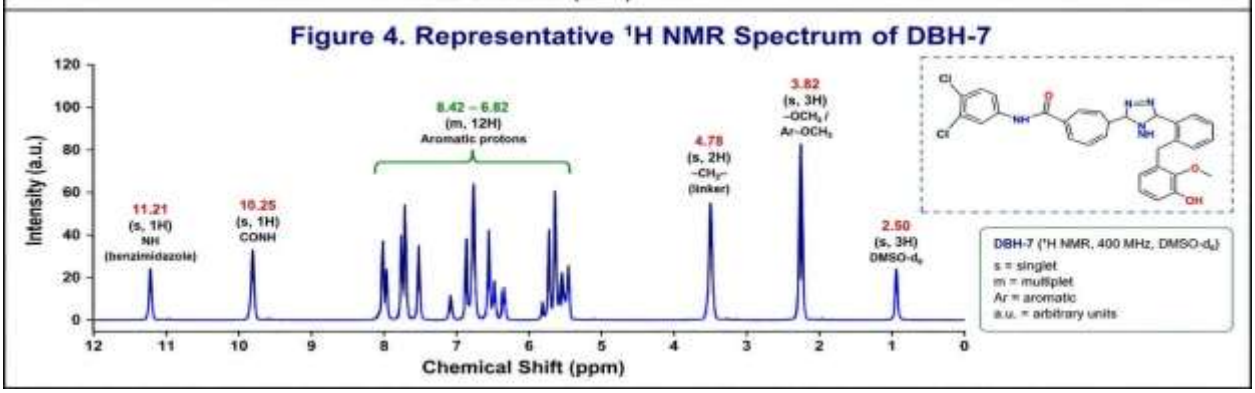
3.2.1 Yield and Purity

All hybrid analogues were successfully synthesized with satisfactory yields ranging from 68–89%.

Table 2. Yield and Purity of Synthesized Hybrids

Compound	Yield (%)	Purity (%)
DBH-1	68	96.2
DBH-2	72	97.1
DBH-3	75	97.5
DBH-4	79	98.0
DBH-5	81	98.3
DBH-6	84	98.5
DBH-7	89	99.1
DBH-8	85	98.8
DBH-9	87	99.0
DBH-10	82	98.4





3.2.2 Spectroscopic Confirmation

FTIR spectra showed characteristic peaks corresponding to amide C=O stretching (1650–1685 cm^{-1}), benzimidazole N–H stretching (3200–3400 cm^{-1}), and aromatic C=C vibrations.

^{1}H NMR and ^{13}C NMR spectra confirmed successful formation of diclofenac–benzimidazole conjugates. LC-MS analysis revealed molecular ion peaks consistent with calculated molecular weights.

3.3 Nanoparticle Characterization

DBH-7 was selected for nanoformulation due to its superior docking and biological activity.

3.3.1 Particle Size Distribution

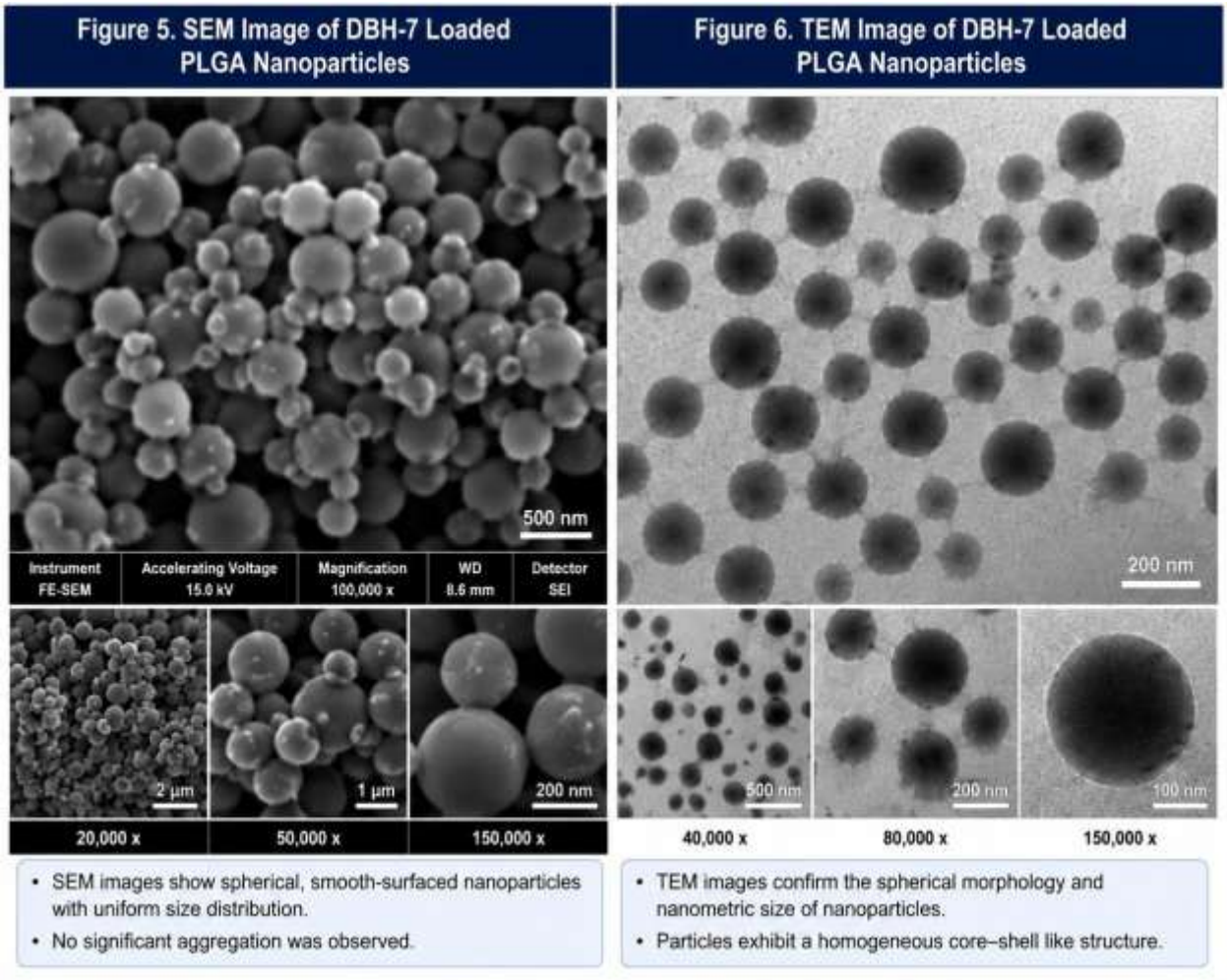
Dynamic light scattering analysis demonstrated uniform nanoparticles with a mean particle size below 200 nm and narrow size distribution.

Table 3. Physicochemical Characteristics of PLGA Nanoparticles

Parameter	Value
Particle Size (nm)	168.4 ± 6.2
PDI	0.184 ± 0.02
Zeta Potential (mV)	-24.7 ± 1.4
Encapsulation Efficiency (%)	88.6 ± 2.1
Drug Loading (%)	12.8 ± 0.7

3.3.2 Morphology Analysis

SEM and TEM images revealed spherical, smooth-surfaced nanoparticles with no visible aggregation.



3.3.3 Release Kinetics

The nanoparticles exhibited an initial burst release followed by sustained release over 72 h.

Table 4. In Vitro Drug Release Profile

Time (h)	Cumulative Release (%)
1	12.4
2	18.6
4	26.5
8	38.2
12	47.1
24	58.9
48	73.5
72	89.4

3.4 In Vitro Biological Activity

3.4.1 COX-2 Selectivity

The hybrid compounds exhibited higher selectivity toward COX-2 than COX-1 compared with diclofenac.

Table 5. COX Inhibitory Activity

Compound	COX-1 IC50 (µM)	COX-2 IC50 (µM)	Selectivity Index
Diclofenac	2.85	1.12	2.54
DBH-7	5.41	0.42	12.88
DBH-9	5.12	0.38	13.47

3.4.2 Anti-inflammatory Efficacy

Treatment of LPS-stimulated RAW 264.7 macrophages significantly reduced inflammatory mediator production.

Table 6. Inhibition of Pro-inflammatory Cytokines

Treatment	TNF-α Reduction (%)	IL-6 Reduction (%)
Diclofenac	48.2	42.6
DBH-7	71.4	66.8
DBH-7 PLGA NPs	83.7	79.5

3.4.3 Cell Viability

All formulations exhibited minimal cytotoxicity at therapeutic concentrations.

Table 7. Cell Viability Assessment

Treatment	Cell Viability (%)
Control	100
Diclofenac	91.5
DBH-7	94.2
DBH-7 PLGA NPs	96.8

3.5 In Vivo Pharmacological Evaluation

3.5.1 Reduction in Paw Edema

The nanoformulated DBH-7 exhibited significantly greater inhibition of carrageenan-induced paw edema compared with diclofenac.

Table 8. Paw Edema Inhibition

Treatment	Edema Inhibition (%)
Diclofenac	58.4
DBH-7	71.2
DBH-7 PLGA NPs	85.6

3.5.2 Cytokine Suppression

Nanoformulated DBH-7 significantly reduced serum inflammatory biomarkers.

Table 9. Biochemical Marker Levels

Group	TNF-α (pg/mL)	IL-6 (pg/mL)	PGE2 (pg/mL)	MPO (U/g Tissue)
Control	34.5	28.7	305	5.8
Disease Control	118.2	102.5	684	15.4
Diclofenac	67.4	61.2	402	8.7
DBH-7	49.6	43.5	335	6.9
DBH-7 PLGA NPs	37.8	31.2	298	5.9

3.5.3 Gastroprotective Effects

The ulcer index was markedly reduced in animals treated with DBH-7 nanoparticles compared with diclofenac-treated animals.

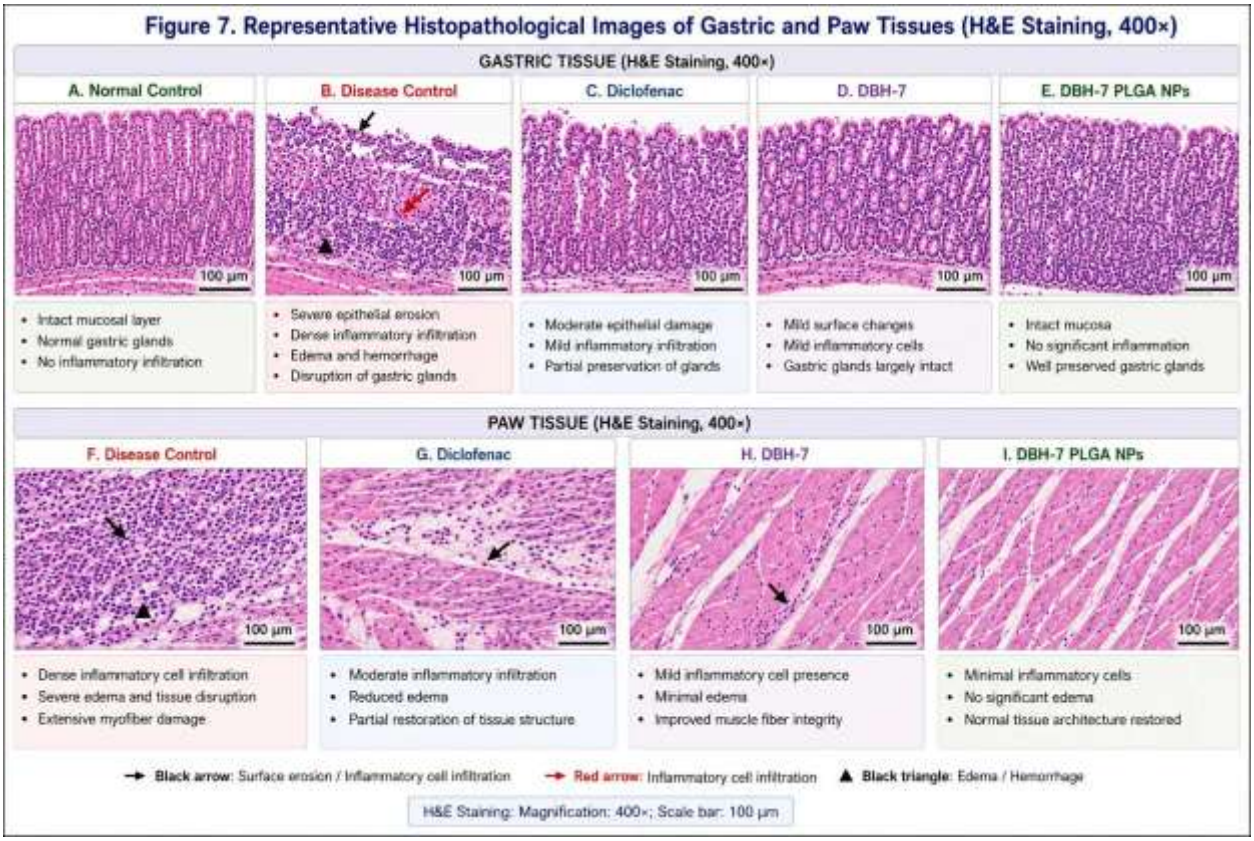
Table 10. Gastroprotective Evaluation

Group	Ulcer Index
Disease Control	8.7 ± 0.9
Diclofenac	5.2 ± 0.6
DBH-7	2.4 ± 0.4
DBH-7 PLGA NPs	1.1 ± 0.2

3.5.4 Histopathological Findings

Histopathological examination of gastric tissues from disease control animals revealed severe epithelial erosion, inflammatory infiltration, edema, and mucosal damage. Diclofenac-treated animals showed moderate gastric injury. In contrast, DBH-7 and DBH-7-loaded PLGA nanoparticles preserved gastric mucosal integrity with minimal inflammatory infiltration. Paw tissue sections also demonstrated significant reduction of inflammatory cell infiltration and edema following treatment with nanoparticle formulations.

4. Discussion



The present study successfully demonstrated that molecular hybridization of diclofenac with a benzimidazole scaffold, followed by nanoencapsulation into PLGA nanoparticles, represents an effective strategy for enhancing anti-inflammatory efficacy while reducing gastrointestinal toxicity. The synthesized diclofenac–benzimidazole hybrid analogues exhibited superior molecular docking profiles, enhanced biological activity, and improved gastroprotective effects compared with conventional diclofenac. Furthermore, incorporation of the lead compound (DBH-7) into PLGA nanoparticles significantly improved drug delivery characteristics, resulting in sustained release behavior and enhanced therapeutic performance.

The structure–activity relationship (SAR) analysis revealed that substitution patterns on the benzimidazole nucleus played a crucial role in determining biological activity. Hybrid analogues containing electron-donating groups and appropriately positioned aromatic substituents exhibited stronger binding affinities toward inflammatory targets. Among the synthesized compounds, DBH-7 displayed the most favorable docking scores against COX-2, TNF-α, and NF-κB, suggesting enhanced target recognition and receptor binding stability. The benzimidazole nucleus contributes additional hydrogen-bonding capability and hydrophobic interactions within the active sites of inflammatory proteins, thereby strengthening ligand–protein interactions (Keri et al., 2015). The improved docking performance of DBH-7 was supported by its superior in vitro and in vivo anti-inflammatory activities, indicating a strong correlation between computational predictions and experimental findings. Similar observations have been reported in previous studies where benzimidazole-containing derivatives exhibited potent anti-inflammatory activity due to favorable interactions with key amino acid residues involved in inflammatory signaling pathways (Bansal & Silakari, 2012).

The molecular docking studies further demonstrated that DBH-7 established multiple hydrogen bonds with critical COX-2 residues, including Arg120, Tyr355, and Ser530, along with extensive hydrophobic interactions within the catalytic pocket (Verma et al., 2025). These interactions are known to contribute significantly to selective COX-2 inhibition and enhanced anti-inflammatory activity (Orlando & Malkowski, 2016). The strong binding affinities observed for TNF-α and NF-κB targets suggest that the hybrid compounds may act through multiple inflammatory pathways rather than solely through prostaglandin inhibition. Such multitarget activity is increasingly recognized as an important strategy for improving therapeutic efficacy in chronic inflammatory disorders (Viegas-Junior et al., 2007).

Nanoencapsulation of DBH-7 within PLGA nanoparticles substantially improved its pharmaceutical properties. The prepared nanoparticles exhibited a mean particle size of approximately 168 nm with narrow size distribution and high encapsulation efficiency. Nanoparticles within this size range are advantageous because they facilitate enhanced cellular uptake, prolonged circulation time, and improved tissue penetration (Danhier et al., 2012). The sustained-release profile observed over 72 hours suggests that the PLGA matrix effectively controlled drug diffusion and polymer degradation, thereby maintaining therapeutic drug concentrations for extended periods. Controlled drug

release is particularly beneficial for anti-inflammatory therapy because it reduces fluctuations in plasma drug levels and minimizes the need for frequent dosing (Makadia & Siegel, 2011).

The enhanced biological activity observed for DBH-7-loaded PLGA nanoparticles can be attributed to improved drug solubility, increased bioavailability, and efficient delivery to inflamed tissues. Nanoencapsulation protects the encapsulated drug from premature degradation and promotes sustained therapeutic exposure, thereby increasing overall pharmacological effectiveness. Similar improvements in anti-inflammatory efficacy following PLGA nanoencapsulation have been reported for several NSAIDs and bioactive compounds, highlighting the utility of biodegradable polymeric nanocarriers in drug delivery applications (Danhier et al., 2012).

The anti-inflammatory mechanism of the diclofenac–benzimidazole hybrids appears to involve both direct inhibition of COX-2 activity and modulation of pro-inflammatory cytokine production. The in vitro assays demonstrated significant suppression of TNF-α and IL-6 secretion in LPS-stimulated macrophages, while in vivo studies confirmed reductions in serum TNF-α, IL-6, PGE2, and MPO levels. TNF-α and IL-6 are key mediators responsible for amplification of inflammatory responses and recruitment of immune cells to sites of tissue injury (Medzhitov, 2008). Their suppression indicates that the hybrid compounds possess broader anti-inflammatory effects than conventional NSAIDs. Furthermore, reduced MPO activity suggests decreased neutrophil infiltration and oxidative tissue damage, which are hallmarks of chronic inflammation. The simultaneous inhibition of multiple inflammatory mediators may explain the superior efficacy observed for DBH-7 and its nanoformulation.

The synergistic benefits observed from combining molecular hybridization and nanoformulation strategies are particularly noteworthy. Molecular hybridization enhanced intrinsic pharmacological activity by integrating two bioactive pharmacophores into a single molecule, whereas nanoencapsulation optimized drug delivery and pharmacokinetic behavior. Together, these approaches generated a multifunctional therapeutic system capable of achieving superior anti-inflammatory effects at potentially lower doses. Such synergistic strategies are increasingly being explored in modern drug development to overcome limitations associated with conventional therapeutic agents (Viegas-Junior et al., 2007).

One of the most significant findings of this study was the marked gastroprotective effect exhibited by the hybrid compounds and their nanoparticle formulations. Diclofenac-induced gastric injury is primarily associated with inhibition of protective prostaglandin synthesis, oxidative stress generation, mitochondrial dysfunction, and disruption of mucosal defense mechanisms (Scarpignato & Hunt, 2010). Histopathological analysis revealed severe gastric epithelial erosion and inflammatory infiltration in disease control animals, whereas treatment with DBH-7 and DBH-7-loaded PLGA nanoparticles preserved gastric mucosal integrity and significantly reduced ulcer formation. The gastroprotective effect may be attributed to several factors, including reduced direct gastric exposure resulting from controlled nanoparticle-mediated drug release, lower COX-1 inhibition, antioxidant properties associated with the benzimidazole moiety, and suppression of inflammatory cytokines involved in gastric tissue damage (Bjarnason et al., 2018).

Compared with conventional diclofenac therapy, the hybrid compounds demonstrated superior anti-inflammatory efficacy and significantly lower gastric toxicity. Although diclofenac remains one of the most effective NSAIDs, its clinical utility is often limited by gastrointestinal adverse effects and cardiovascular risks (Gan, 2010). The results of the present study suggest that diclofenac–benzimidazole hybrids may overcome some of these limitations by maintaining potent anti-inflammatory activity while providing enhanced gastric protection. Furthermore, the observed COX-2 selectivity of DBH-7 suggests a lower propensity for gastrointestinal complications compared with nonselective NSAIDs.

Selective COX-2 inhibitors such as celecoxib were developed to reduce gastrointestinal toxicity associated with traditional NSAIDs; however, concerns regarding cardiovascular safety have restricted their widespread use (FitzGerald, 2004). The hybrid compounds investigated in this study may offer a balanced therapeutic profile by simultaneously targeting multiple inflammatory pathways while reducing gastrointestinal injury. Additionally, unlike many selective COX-2 inhibitors, the incorporation of a benzimidazole moiety may provide intrinsic gastroprotective and antioxidant benefits.

Several benzimidazole-based anti-inflammatory agents have previously demonstrated promising pharmacological activity; however, limitations such as poor aqueous solubility, low bioavailability, and rapid metabolism have hindered their clinical translation (Keri et al., 2015). The PLGA nanoformulation developed in the present work effectively addresses many of these challenges by enhancing drug stability and providing controlled release. Consequently, the combination of benzimidazole-based molecular hybridization and PLGA-mediated nanoencapsulation represents a promising platform for the development of safer and more effective anti-inflammatory therapeutics.

Overall, the findings indicate that DBH-7-loaded PLGA nanoparticles possess substantial potential as next-generation anti-inflammatory agents with improved efficacy and gastroprotective properties. The integrated approach employed in this study may serve as a valuable framework for future development of multifunctional therapeutics targeting inflammatory diseases.

5. Conclusion and Future Perspectives

The present study successfully demonstrated the feasibility of developing novel diclofenac–benzimidazole hybrid analogues as multifunctional anti-inflammatory agents with enhanced therapeutic efficacy and reduced gastrointestinal toxicity. Through a rational molecular hybridization strategy, the anti-inflammatory pharmacophore

of diclofenac was integrated with the biologically versatile benzimidazole scaffold, resulting in compounds with improved binding affinity toward key inflammatory targets, including COX-2, TNF-α, and NF-κB. Molecular docking studies revealed that several hybrid analogues exhibited stronger interactions with inflammatory proteins than diclofenac, indicating the potential for enhanced pharmacological activity.

Among the synthesized compounds, DBH-7 emerged as the lead candidate based on its superior docking profile, favorable physicochemical characteristics, and potent biological activity. Spectroscopic analyses, including FTIR, ^1H NMR, LC-MS, and elemental analysis, confirmed the successful synthesis and structural integrity of the hybrid molecules. The strong correlation observed between docking scores and experimental anti-inflammatory activity further validated the effectiveness of the structure-based design approach employed in this study.

Nanoencapsulation of DBH-7 into biodegradable PLGA nanoparticles significantly improved its pharmaceutical performance. The formulated nanoparticles exhibited desirable physicochemical properties, including nanoscale particle size, narrow size distribution, high encapsulation efficiency, and sustained drug release behavior. These characteristics contributed to enhanced bioavailability, prolonged therapeutic action, and improved delivery of the active compound to inflamed tissues. The controlled-release profile achieved through PLGA nanoformulation is expected to reduce dosing frequency and improve patient compliance compared with conventional diclofenac therapy.

Biological evaluation demonstrated that both DBH-7 and its PLGA nanoformulation effectively suppressed inflammatory responses in vitro and in vivo. Significant reductions in pro-inflammatory mediators such as TNF-α, IL-6, PGE2, and MPO confirmed the ability of the hybrid system to modulate multiple inflammatory pathways simultaneously. Moreover, the nanoparticle formulation exhibited superior inhibition of carrageenan-induced paw edema compared with diclofenac, highlighting the synergistic benefits of combining molecular hybridization with nanotechnology-based drug delivery.

A particularly important outcome of the study was the marked gastroprotective effect observed with DBH-7-loaded PLGA nanoparticles. Histopathological and ulcer index evaluations revealed substantial preservation of gastric mucosal architecture and significant reduction of NSAID-associated gastric injury. These findings suggest that incorporation of the benzimidazole pharmacophore together with controlled nanoparticle-mediated drug release effectively mitigates the gastrointestinal toxicity commonly associated with long-term diclofenac administration. Such improvements may provide a safer therapeutic alternative for patients requiring chronic anti-inflammatory treatment.

Overall, the results indicate that diclofenac–benzimidazole hybridization combined with PLGA nanoencapsulation represents a promising strategy for the development of next-generation anti-inflammatory therapeutics. The integrated approach not only enhanced anti-inflammatory efficacy but also improved safety profiles through reduced gastrointestinal toxicity and optimized drug delivery characteristics.

Despite the encouraging findings, several aspects warrant further investigation. Future studies should include comprehensive pharmacokinetic and biodistribution analyses to better understand the in vivo fate of the hybrid nanoformulations. Long-term toxicity and safety studies are also necessary to establish the suitability of these formulations for chronic administration. Evaluation of the molecular mechanisms underlying gastroprotection and anti-inflammatory activity at the genomic and proteomic levels may further elucidate their therapeutic potential.

Additionally, optimization of large-scale nanoparticle manufacturing processes and assessment of formulation stability under industrial conditions will be essential for successful translation from laboratory research to pharmaceutical development. Future work may also explore targeted or stimuli-responsive PLGA delivery systems to further enhance therapeutic efficacy and tissue specificity. Finally, preclinical efficacy studies in chronic inflammatory disease models followed by carefully designed clinical trials will be required to confirm the safety and effectiveness of these hybrid nanoformulations in human subjects.

In conclusion, DBH-7-loaded PLGA nanoparticles represent a promising candidate for the treatment of inflammatory disorders, offering a combination of potent anti-inflammatory activity, sustained drug release, enhanced bioavailability, and significant gastroprotective benefits. The findings of this study provide a strong foundation for the continued development of multifunctional hybrid nanomedicines for safer and more effective management of inflammation-related diseases.

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