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Original Article

Design, Synthesis, Molecular Docking, and *in vitro* Anti-Inflammatory Evaluation of Novel Benzimidazole Derivatives as Potential COX-2 Inhibitors

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ABSTRACT

Background: Cyclooxygenase-2 (COX-2) is a key enzyme involved in inflammation, making it an important target for anti-inflammatory drug discovery. Derivatives of benzoimidazole are well recognized for diverse pharmacological activities, including COX inhibition. **Materials and Methods:** Novel benzimidazole derivatives (NB1-NB6) were synthesized and structurally confirmed through spectral characterization. The COX-2 enzyme (PDB: 4COX) was created with Discovery StudioVisualizer, and Glide was used for docking (Schrödinger suite). Binding interactions were analyzed in 2D and 3D, and results were validated against Celecoxib's crystal structure. *In vitro* anti-inflammatory activity was evaluated using the protein denaturation method, with diclofenac sodium as the reference drug. **Results:** Docking studies revealed that all compounds exhibited favorable binding affinities (-6.902 to -8.94 kcal/mol). NB6 showed the strongest binding (-8.94 kcal/mol), interacting with key residues ARG120, TYR355, TYR385, TRP387, GLY526, and ALA527. *In vitro* assays demonstrated concentration-dependent inhibition of protein denaturation, with NB6 and NB4 showing activity comparable to diclofenac sodium. **Conclusion:** The study identified NB6 as the most promising COX-2 inhibitor candidate. According to these results, benzimidazole derivatives possess strong potential for development as selective anti-inflammatory agents.

Keywords: Benzimidazole Derivatives, COX-2 Inhibition, Molecular Docking, Anti-Inflammatory Activity.

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INTRODUCTION

Inflammation is a fundamental defense mechanism of the human body against infections, injuries, and various harmful stimulus. It involves a cascade of biochemical events leading to redness, swelling, heat, and pain at the affected site (Harvanov and Duranková, 2025). Although acute inflammation is beneficial in restoring homeostasis, prolonged or chronic inflammation contributes to the onset and progression of several pathological conditions such as rheumatoid arthritis (Alivernini *et al.*, 2022), cardiovascular diseases (Henein *et al.*, 2022), diabetes, neurodegenerative disorders (Missiroli *et al.*, 2020), and cancer (Khandia and Munjal, 2020). Therefore, the development of safe and effective anti-inflammatory agents remains an important area of pharmaceutical research.

Nonsteroidal Anti-inflammatory medications (NSAIDs) are frequently recommended to relieve fever and pain (Arfeen *et al.*, 2024). These drugs exert their effect primarily by inhibiting Cyclooxygenase (COX) enzymes, which catalyze the conversion of arachidonic acid to prostaglandins (Rouzer and Marnett, 2009). Two isoforms of COX exist: COX-1, constitutively expressed in most tissues, plays a vital role in gastric mucosal protection, platelet aggregation, and renal function, whereas COX-2 is an inducible isoform, overexpressed at sites of inflammation and responsible for the synthesis of pro-inflammatory mediators(Rodrigues *et al.*, 2024). Conventional NSAIDs inhibit both COX-1 and COX-2, often leading to adverse effects such as gastrointestinal ulceration, renal impairment, and bleeding tendencies (El-Malah *et al.*, 2022). Consequently, selective COX-2 inhibitors (coxibs) such as celecoxib, etoricoxib, and rofecoxib

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were developed to minimize COX-1-mediated side effects while maintaining anti-inflammatory efficacy (Cui and Jia, 2021). However, several coxibs have been withdrawn from the market due to cardiovascular toxicity, underscoring the urgent need for new chemical scaffolds with improved safety and therapeutic profiles (Arora *et al*., 2020).

Heterocyclic compounds have played a crucial role in drug discovery, and among them, Derivatives of benzimidazoles have garnered a lot of interestdue to their structural resemblance to naturally occurring nucleotides and their ability to interact with diverse biological targets (Hernández-López *et al*., 2022). Benzimidazole derivatives exhibit a wide spectrum of pharmacological activities including antimicrobial, antiviral, anticancer, antihypertensive, and anti-inflammatory effects (Brishty *et al*., 2021; Veerasamy *et al*., 2021). Prior research has documentedthat substitution at specific positions of the benzimidazole nucleus enhances binding affinity toward COX-2, thereby improving anti-inflammatory potential (Kaur and Silakari, 2018). These findings suggest that benzimidazole-based scaffolds represent promising candidates for the creation of new COX-2 inhibitors.

Recent advances in computational chemistry have enabled the use of molecular docking to predict and optimize drug-target interactions. Docking not only offers information on binding affinity and interaction patterns but also guides rational drug design by identifying favorable binding modes (Kairys *et al*., 2019). Glide (Schrödinger suite) is a widely employed docking tool that offers accurate predictions of ligand-protein interactions through advanced scoring functions and flexible binding site analysis (Reddy *et al*., 2020). In combination with experimental assays, docking studies provide a robust platform for the evaluation of novel therapeutic agents.

In this work, we created and synthesized a brand-new class of benzimidazole derivatives and assessed their capacity to reduce inflammation. The synthesized compounds were Glide was used in molecular docking investigations against COX-2 (PDB: 4COX) to predict binding affinities and interaction profiles (Araújo *et al*., 2020). Furthermore, *In vitro* testing was done to evaluate the synthetic compoundsanti-inflammatory propertiesusing the protein denaturation method, with diclofenac sodium as a standard. The combined computational and experimental findings aim to Determine possible lead compounds usingwith strong COX-2 inhibitory activity and reduced toxicity, thereby contributing to the development of safer anti-inflammatory agents.

MATERIALS and METHODS

Materials

The synthesis involved several key chemicals, ortho-phenylenediamine, chloroacetic acid, 4-chloro-3-methyl phenol, 3-hydroxy benzoic acid, formic acid, acetic acid, acetyl chloride, phenyl acetyl chloride, 4-chloroaniline, benzyl chloride, formyl chloride and chloroacetic acid. Reagents used were analytical quality, sourced from, Loba chemie. Infrared spectra were checked by Bruker FTIR, for ^{1}H-NMR, recorded on Bruker AVANCE III 500 MHz (AV 500), with TMS serving internal standard in DMSO-D_6- d6 solvent. Additionally, mass spectra obtained by JEOL GCMATE II, with the mass spectrum depicted as m/z values.

Methods

Molecular Docking Studies

The enzymeCOX-2 (PDB: 4COX) from the Protein Data Bank (Araújo *et al*., 2020). By using Discovery Visual studio (v.24) protein was cleaned. Chemdraw was used to draw the ligand structure (Ultra 12.0). Glide (Schrödinger suite) was used for investigations involving molecular docking. After docking to study Analysis of 2D interactions was done using Glide and for 3D interaction's Discovery Visual studio was used. Celecoxib crystal structures were compared to the anticipated conformations of docking data to optimize the docking method (Dhamak and Amrutkar, 2025).

Synthesis of 2-(chloro-methyl)-1*H*-benzimidazole

A mixture of OPD (0.01 mol) and chloro-acetic (0.01 mol) acid was added in 0.3 N HCl in a RBF and heatedfor3hrs.at 70-90°C. After heatingroom temperature was used to cool the reaction mixture and then precipitate by NaOH. After filtering, the precipitate was dried under UV lamp. Product was recrystallized by ethanol (Figure 1) (Choudhary *et al*., 2023).

Synthesis of 2-[(4-chloro-3-methylphenoxy) methyl]-1*H*-1,3-benzimidazole

A mixture of 2-(chloromethyl)-1H -benzimidazole (0.01 mol) and 4-chloro-3-methyl phenol (0.01mol) with strong base was reflux for 3 hr at 60-70°C in DMF as solvent. The reaction mixture was cooled, and water was added to form precipitate. After filtering, the precipitate was dried under UV lamp. Product was recrystallized by ethanol (Figure 1) (Sunil, 2020).

Synthesis of 4-[(1*H*-1,3-benzimidazol-2-yl) methoxy]-2-methylphenyl 3-hydroxybenzoate

A mixture of 2-[(4-chloro-3-methylphenoxy) methyl]-1*H*-1,3-benzimidazole (0.01 mol) and 3-hydroxy benzoic acid (0.01 mol) with (K_2CO_3) For 3 hr, potassium carbonate refluxed at 60-70°C in DMF as solvent. The reaction mixture was cooled, and water was added to form precipitate. The precipitate was filtered and dried under UV lamp. Product was recrystallized by ethanol. All compounds completion of reaction was verified by TLC (Figure 1) (Gupta Atyam *et al*., 2010).

Synthesis of 4-((1H-benzo[d]imidazol-2-yl)methoxy) phenyl 4 hydroxybenzoate derivates (Figure 2)

NB1:4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(formyloxy)benzoate

Take 4-((1H-benzo[d]imidazol-2-yl) methoxy) phenyl 4 hydroxybenzoate (0.01 mol) and formyl chloride (0.01 mol) was heated for a duration of 4 hr at 60-70°C under continuous stirring using a magnetic stirrer in 10 mL of ethanol as the reaction A basic, the solvent bicarbonate, was added to the reaction mixture in order to facilitate the nucleophilic substitution process. Following the reaction's mixture allowed to cool to room temperature. Then, a small volume of water was added to the mixture to induce precipitation. The precipitate that resulted was separated using filtration, thoroughly dried, and recrystallized from ethanol to obtain the purified compound.

NB2 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-acetoxybenzoate

Take 4-((1H-benzo[d]imidazol-2-yl) methoxy) phenyl 4 hydroxybenzoate (0.01 mol) and acetyl chloride (0.01 mol) was heated for a duration of 4 hr at 60-70°C under continuous stirring using a magnetic stirrer in 10 mL of ethanol as the reaction solvent. Add 2-3 drops of sulphuric acid as a catalyst. Sodium bicarbonate was then added. as a base to the reaction mixture. Following the reaction's conclusion, the mixture was allowed to cool to room temperature. Then, a small volume of ice-cold water was added to the mixture to induce precipitation. The precipitate that resulted was separated using filtration, thoroughly dried, and recrystallized from ethanol to obtain the purified compound.

NB3 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(2-phenylacetoxy) benzoate

Take 4-((1H-benzo[d]imidazol-2-yl) methoxy) phenyl 4 hydroxybenzoate (0.01 mol) and Phenyl Acetyl chloride (0.01 mol) was heated for a duration of 4 hr at 60-70°C under continuous stirring using a magnetic stirrer in 10 mL of ethanol as the reaction solvent. Add 2-3 drops of sulphuric acid as a catalyst. Sodium bicarbonate was then added. as a base to the reaction mixture. Following the reaction's conclusion, the mixture was allowed to cool to room temperature. Then, a small volume of ice-cold water was added to the mixture to induce precipitation. The precipitate that resulted was separated using filtration, thoroughly dried, and recrystallized from ethanol to obtain the purified compound.

NB4 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-((chlorocarbonyl)oxy) benzoate

Take 4-((1H-benzo[d]imidazol-2-yl) methoxy) phenyl 4 hydroxybenzoate (0.01 mol) and chloroacetic acid (0.01 mol) was subjected to reflux for a duration of 3 hr within the temperature range of 60-70°C using a conventional heating mantle, with ethanol (10 mL) as the reaction solvent. Upon completion of the reflux, the reaction mixture was allowed to cool to room temperature. Subsequently, an aqueous solution of sodium bicarbonate was added to the cooled mixture to facilitate the neutralization of acidic by-products and to promote the formation of the desired precipitate. The resulting precipitate was isolated via filtration, thoroughly dried, and recrystallized from ethanol to yield the purified compound.

Synthesis of 4-[(1H-benzo[d]imidazole-2-yl) methoxy)-2-methylphenyl-3-(benzyloxy) benzoate NB5 scheme 1B

NB5:4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(benzoyloxy)benzoate.

Take 4-((1H-benzo[d]imidazol-2-yl) methoxy) phenyl 4 hydroxybenzoate (0.01 mol) and benzyl chloride (0.01 mol) was heated for a duration of 4 hr at 60-70°C under continuous stirring using a magnetic stirrer in 10 mL of ethanol as the reaction solvent. Subsequently, triethylamine was added as a base to the reaction mixture to facilitate the nucleophilic substitution process. Following the reaction's conclusion, the mixture was allowed to cool to room temperature. Then, a small volume of water was added to the mixture to induce precipitation. The precipitate that resulted was separated using filtration, thoroughly dried, and recrystallized from ethanol to obtain the purified compound.

NB6 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(4-aminophenoxy) benzoate

Take 4-((1H-benzo[d]imidazol-2-yl) methoxy) phenyl 4 hydroxybenzoate (0.01 mol) and 2-Chloroaniline (0.01 mol) was heated for a duration of 4 hr at 60-70°C under continuous stirring using a magnetic stirrer in 10 mL of ethanol as the reaction solvent. Subsequently, triethylamine was added as a base to the reaction mixture to facilitate the nucleophilic substitution process. Following the reaction's conclusion, the mixture was allowed to cool to room temperature. Then, a small volume of water was added to the mixture to induce precipitation. The precipitate that resulted was separated using filtration, thoroughly dried, and recrystallized from ethanol to obtain the purified compound.

In vitro Anti-Inflammatory Activity: Protein Denaturation Methods

A sample concentration of 50,100,200,300 µg/mL were taken. After adding 0.5 mL of 1.5 mg/mL egg albumin serum to each sample, they were incubated for 20 min at 37°C. After heating all of the prepared solutions for 3 min at 57°C, 250 µg/mL of 0.5M phosphate buffer pH 6.3 was added. Then same proportion of sample solution and fol-in-ciocateu reagent was added followed by make up to 10 mL. After 10 min incubate at 55°C; Then

test tube were allowing to cool. Absorbance was determined at wavelength 650nm using UV-Visible Spectrophotometer. Diclofenac sodium 100 µg/mL was used to evaluate recorded measurement as a reference drug and measure the inhibition % of protein denaturation was calculated by using following formula(Lalit *et al.*, 2025):

$$\% \ denaturation \ inhibition = \frac{Abs \ control - Abs \ Std}{Abs \ Control} \ X \ 100$$

RESULTS

Physical, analytical and spectral data of synthesized compounds (NB1- NB6)

NB1 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(formyloxy)benzoate

Yield: 64%, m.p.: 185°C and Rf: 0.63 (ethyl acetate: methanol, 4:1). IR KBR Pellet: 3329.45 N-H stretch(imidazole), 1728.73 C=O stretch (ester), 1251.77 -O- stretch (ether), 1491.60 C=C stretch (aromatic). H^1NMR (400 MHz, DMSO-d_6): δ 11.06 Aldehydic C-H (s, 1H), δ 8.138 -7.126 Ar C-H (m, 11H), δ 4.686 CH_2 Group (s, 2H), δ 3.991 CH_3 Group (s, 3H). Mol wt. m/z402.41.

NB2 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-acetoxybenzoate

Yield: 61%, m.p.: 172°C and Rf: 0.73 (ethyl acetate: methanol, 4:1). IR KBR Pellet: 3329.74 N-H stretch (imidazole), 1726.93 C=O stretch (ester), 1491.22 C=C stretch (aromatic), 1121.68 -O- stretch (ether). H^1NMR (400 MHz, DMSO-d_6): δ 12.75 Ar N-H (s, 1H), δ 8.009 -7.136 Ar C-H (m, 11H), δ 5.733 N-H (s, 1H), δ 4.670 CH_2 Group (s, 2H), δ 3.995 CH_3 Group (s, 3H), δ 2.951 CH_3 Group (s, 3H). Mol wt. m/z 416.50.

NB3 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(2-phenylacetoxy)

Yield: 66%, m.p.: 193°C and Rf: 0.68 (ethyl acetate: methanol, 4:1). IR KBR Pellet: 3445.05 O-H stretch(aromatic), 3329.69 N-H stretch (Acid), 1725.95 C=O stretch (ester), 1495.82 C=C stretch (aromatic), 1124.56 -O- stretch (ether). H^1NMR (400 MHz, DMSO-d_6): δ 11.55 Ar N-H (s, 1H), δ 8.008 -7.049 Ar C-H (m, 16H), δ 4.521 benzylic CH_2 Group (s, 2H), δ 3.652 CH_2 Group (s, 2H), Mol wt. m/z 492.67.

NB4 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-((chlorocarbonyl)oxy) benzoate

Yield: 67%, m.p.: 162°C and Rf: 0.61 (ethyl acetate: methanol, 4:1). IR KBR Pellet: 3329.45 N-H stretch (Imidazole), 1726.73 C=O stretch (ester), 1491.60 C=C stretch (aromatic), 1123.99 -O- stretch (ether). H^1NMR (400 MHz, DMSO-d_6): δ 12.5 Ar N-H (s, 1H), δ 8.26-7.43 Ar C-H (m, 11H), δ 4.755 CH2 (s, 2H), δ 3.367 CH_3 Group (s, 3H). Mol wt. m/z 436.85.

Table 1: Represents interaction of amino acids; Types of Bonds and Docking Score (Kcal/mol) of NB1 to NB6 with reference Diclofenac Sodium.

Sl. No	Compound Code	Interacting with amino acids	Types of Bonds	Docking Score (Kcal/mol)
1	Diclofenac Sodium	TYR385, SER530, VAL349, VAL523 ALA527	Hydrogen Bond, Hydrophobic Bond and others.	-5.781
2	NB1	ARG120, TYR355, TYR385, TRP387, GLY526, ALA527, VAL116, LEU359, LEU531, LEU352, VAL89, and LEU93.	Hydrogen Bond, Hydrophobic Bond and others.	-8.006
3	NB2	TYR355, ARG120, MET522, PHE518, TYR385, TRP387, GLY526, ALA527, VAL116, LEU359, LEU531, LEU352, and LEU93	Hydrogen Bond, Hydrophobic Bond and others.	-6.902
4	NB3	TYR355, TYR115, SER353, GLU524, TYR385, TRP387, LEU93, VAL116, PRO86, VAL89, and ALA527.	Hydrogen Bond, Hydrophobic Bond and others.	-7.11
5	NB4	ARG120, SER530, TYR385, TRP387, GLY526, ALA527, VAL523, MET522, VAL349, LEU531, LEU93, VAL116, and TYR355.	Hydrogen Bond, Hydrophobic Bond and others.	-7.326
6	NB5	TYR355, SER119, VAL523, TYR115, ARG120, LEU93, VAL116, VAL89, VAL349, and ALA527.	Hydrogen Bond, Hydrophobic Bond and others.	-7.446
7	NB6	ARG120, TYR355, PHE381, TYR385, TRP387, GLY526, ALA527, VAL116, LEU359, LEU531, LEU352, VAL89, and LEU93	Hydrogen Bond, Hydrophobic Bond and others.	-8.94

In vitro anti-inflammatory activity: Protein Denaturation Methods by UV Spectroscopy

NB5 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(benzoyloxy)benzoate

Yield: 58%, m.p.: 150°C and Rf: 0.58 (ethyl acetate: methanol, 4:1). IR KBR Pellet: 3329.45 N-H stretch (imidazole), 1726.73 C=O stretch (ester), 1491.60 C=C stretch (aromatic), 1123.99 -O- stretch (ether). H^1NMR (400 MHz, DMSO-d_6): δ 11.87 Ar N-H (s, 1H), δ 8.009-7.083 Ar C-H (m, 16H), δ 4.762 CH2 (s, 2H), δ 3.34 CH3 (s, 3H). Mol wt. m/z 478.60.

NB6 4-((1H-benzo[d]imidazol-2-yl) methoxy)-2-methylphenyl 4-(4-aminophenoxy) benzoate

Yield: 71%, m.p.: 132°C and Rf: 0.60 (ethyl acetate: methanol, 4:1). IR KBR Pellet: 3320.35 N-H stretch (imidazole), 1726.70 C=O stretch (ester), 1484.70 C=C stretch (aromatic), 1123.99 -O- stretch (ether). H^1NMR (400 MHz, DMSO-d_6): δ 11.57 Ar N-H (s, 1H), δ 8.010-7.082 Ar C-H (m, 15H), δ 4.762 CH2 (s, 2H), δ 3.34 CH3 (s, 3H).Mol wt. m/z 465.61.

DISCUSSION

The benzimidazole derivatives were synthesized (Figure 1 and 2) and confirmed for its structure by physical, analytical and spectral techniques. The synthesized benzimidazole derivatives (NB1-NB6) were successfully evaluated for their COX-2 inhibitory potential using molecular docking studies (Table 1) and *in vitro* anti-inflammatory assays (Table 2). Docking results obtained through Glide showed that every compound hasfavorable binding affinities towards COX-2, with docking scores between -6.902 to -8.94 kcal/mol. Among these, NB6 demonstrated the strongest binding (-8.94 kcal/mol, Table 2), followed by NB1 (-8.006 kcal/mol, Table 2), suggesting strong and stable interactions within the COX-2 active site. Key amino acid residues such as ARG120, TYR355, TYR385, TRP387, GLY526, ALA527, and VAL116 were recurrently involved in hydrogen bonding and hydrophobic interactions across the series, indicating a conserved binding mode similar to that of the reference drug celecoxib. The interaction of amino acids; Types of Bonds and Docking Score (Kcal/mol) of NB1 to NB6 with reference Diclofenac Sodium were represented in Table 1.

The 2D and 3D interaction analyses revealed that the benzimidazole scaffold facilitated multiple hydrogen bonds and hydrophobic contacts, contributing to the stabilization of ligand-enzyme complexes. Compounds NB1 and NB6 showed optimal alignment with the COX-binding pocket, which might explain their higher docking scores. Importantly, comparison with the celecoxib crystal structure validated the docking protocol, confirming that the predicted conformations closely resembled the known inhibitor.

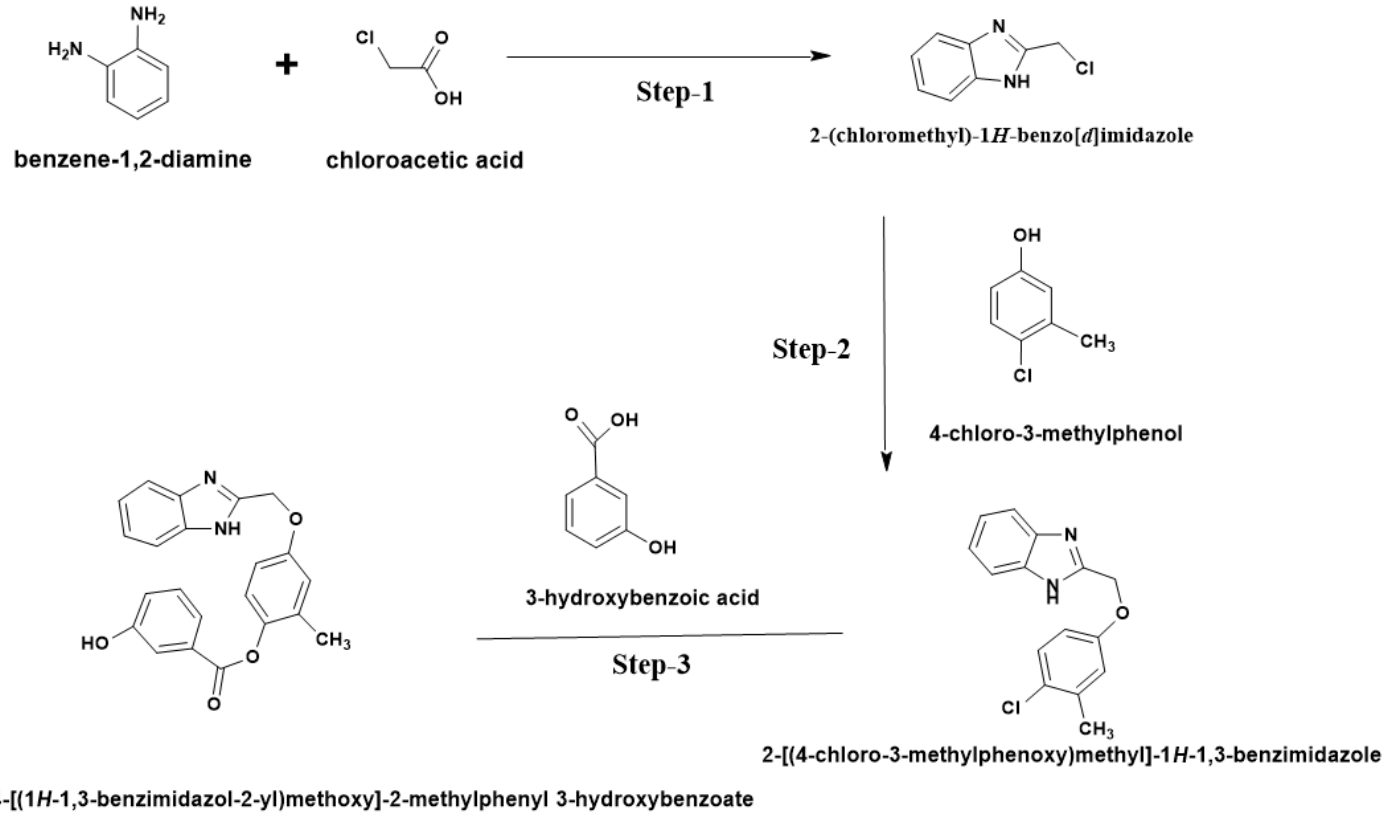

Figure 1: Synthesis of 4-[(1H-1,3-benzimidazol-2-yl) methoxy]-2-methylphenyl 3-hydroxybenzoate.

Table 2: ***In vitro*** **anti-inflammatory activity with Protein Denaturation Methods by UV Spectroscopy for NB1 to NB6 with reference Diclofenac Sodium.**

Comp. Code	50 µg/mL	100 µg/mL	200 µg/mL	300 µg/mL
Diclofenac sodium	3.750	2.834	2.577	1.452
NB1	2.092	2.031	2.003	1.807
NB2	2.375	1.925	1.604	1.388
NB3	2.410	2.052	1.811	1.656
NB4	2.118	1.652	1.484	1.389
NB5	2.054	1.902	1.767	1.567
NB6	1.968	1.712	1.624	1.493

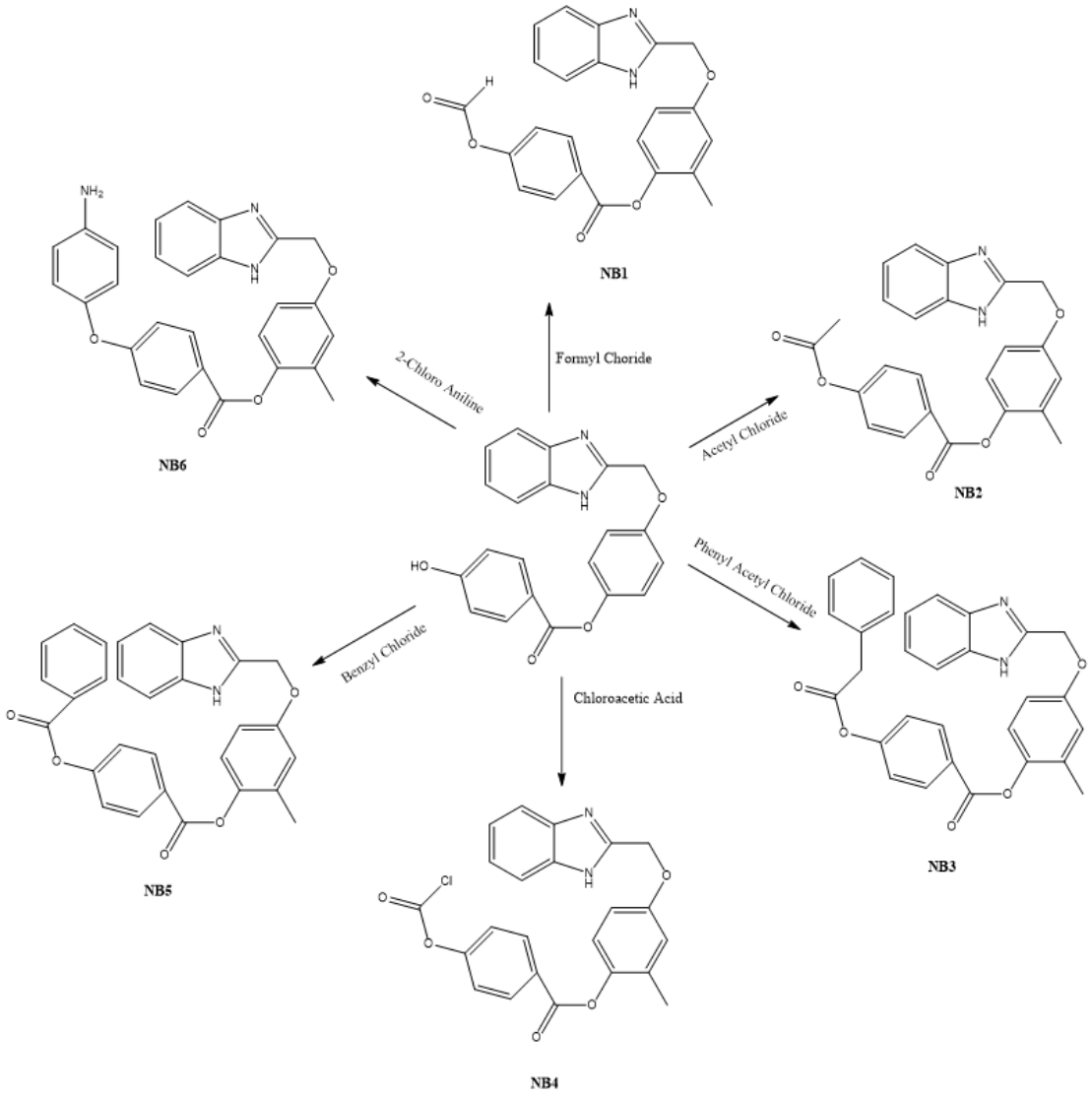

Figure 2: Scheme for synthesis of 4-((1H-benzo[d]imidazol-2-yl)methoxy)phenyl 4 hydroxybenzoate derivates.

In vitro anti-inflammatory evaluation using the protein denaturation method further supported the docking results. All synthesized compounds demonstrated concentration-dependent inhibition of protein denaturation, with NB6 and NB4 in Table 2 showing activity comparable to diclofenac sodium. These results highlight an association that is favorable between docking scores andbiological activity, indicating the potential of these derivatives as COX-2 inhibitors.

CONCLUSION

In this study, new benzimidazole-based compounds are designed, synthesized, and evaluated as possible COX-2 inhibitors. The glide-based molecular docking investigationsconfirmed favorable

binding affinities and stable interactions with critical COX-2 residues, particularly for NB6 and NB1. *In vitro* anti-inflammatory assays further validated their biological potential, with NB6 exhibiting the most promising activity. Overall, the results suggest that benzimidazole derivatives, especially NB6, may serve as lead substances for the creation of selective COX-2 inhibitors with anti-inflammatory potential. Further *in vivo* studies and pharmacokinetic evaluations are warranted to establish their therapeutic applicability.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest are disclosed by the writers.

ABBREVIATIONS

FTIR: Fourier transform infrared spectroscopy; **NMR spectroscopy:** Nuclear magnetic resonance spectroscopy; **MS:** Mass spectroscopy; **KBr:** Potassium bromide; % yield: Percentage yields; **M.P.:** Melting point; **mg/kg:** Milligram/kilograms; **sec:** Seconds; **δ:** Chemical shift; **Mol. Wt:** Molecular weight; **gm:** Gram; **COX-2:** Cyclooxygenase-2; **PDB:** Protein Data Bank; **DMF:** Dimethyl formamide; **OPD:** Ortho-phenylenediamine; **TLC:** Thin-layer chromatography; **NSAIDs:** Nonsteroidal anti-inflammatory medications; **coxibs:** Selective COX-2 inhibitors.

AUTHOR CONTRIBUTIONS

Neha Shyam Bora[1]: Synthetic work.

Mayur Shivaji Bhosale[1]: Synthetic work scheme

Vikrant Murlidhar Dhamak[2]: Interpretation of IR and NMR

Sagar Magar Dattatraya[1]: Interpretation of IR and NMR

Rohit Jaysing Bhor[1]: Molecular Docking work

Manisha Dhondiram Sonawane[1]: Molecular Docking work

Nilima Mahesh Wani[1]: Drapting the research paper

SUMMARY

The study focused on developing new anti-inflammatory agents by synthesizing six novel benzimidazole derivatives (NB1-NB6). Their structures were confirmed through spectral analysis, and molecular docking was performed against the COX-2 enzyme using Glide. All compounds showed good binding affinity, with NB6 exhibiting the strongest interactions with essential COX-2 residues. *In vitro* anti-inflammatory testing using the protein denaturation assay indicated that NB6 and NB4 had activity comparable to diclofenac. Overall, NB6 emerged as the most promising COX-2 inhibitor, highlighting benzimidazole derivatives as potential selective anti-inflammatory drug candidates.

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