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Construction and Spectral Analysis of Fused Benzimidazole Heterocycles as Putative Anti-Inflammatory Agents

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INTRODUCTION

Global Burden of Inflammation stands as a fundamental physiological response to injury, infection, or tissue damage, orchestrating immune cell recruitment and cytokine release to restore homeostasis. Yet, when dysregulated, it evolves into chronic pathology, underpinning disorders like rheumatoid arthritis, inflammatory bowel disease, and cardiovascular complications. Globally, inflammatory conditions afflict over 350 million people, imposing a staggering economic burden exceeding $1 trillion annually in healthcare costs and lost productivity. Conventional therapies, including non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and selective COX-2 inhibitors like celecoxib, alleviate symptoms by blocking prostaglandin synthesis. However, their prolonged use triggers severe adverse effects gastrointestinal ulcers, renal toxicity, and heightened cardiovascular risks stemming from off target inhibition of protective prostanoids. This therapeutic shortfall underscores the urgent need for novel anti-inflammatory agents that target multifaceted pathways with enhanced selectivity and safety profiles Heterocyclic scaffolds dominate medicinal chemistry, comprising over 60% of FDA-approved drugs due to their ability to mimic biomolecular interactions and modulate protein**.**

Keywords: Construction and Spectral Analysis, Fused Benzimidazole, Heterocycles, Putative Anti-Inflammatory Agents

ABSTRACT

Inflammatory processes are closely associated with the pathogenesis of several chronic diseases, creating a substantial need for the identification of novel therapeutic candidates that may offer alternatives to conventional anti-inflammatory medications. The present research was undertaken to develop and investigate a series of fused benzimidazole-based heterocyclic compounds for their potential anti-inflammatory properties. A total of seven derivatives, designated PB-1 to PB-7, were synthesized using a one-pot synthetic approach involving condensation and subsequent cyclization of substituted benzaldehydes with *o*-phenylenediamine. The synthesized compounds were subjected to molecular docking investigations against cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) to examine their predicted binding behaviour and interactions within the respective enzyme binding sites. The docking results indicated favourable molecular interactions and promising binding affinities, suggesting the potential relevance of these derivatives as candidates for further investigation. The synthesized molecules were characterized using complementary spectroscopic and analytical methods, namely Fourier-transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance (^1H NMR), carbon-13 nuclear magnetic resonance (^13C NMR), and high-resolution mass spectrometry (HRMS), to establish their structural characteristics. Biological evaluation revealed appreciable in vitro COX-2 inhibitory activity among the synthesized derivatives. Comparative assessment of their anti-inflammatory potential identified PB-7 as the most active compound in the investigated series, demonstrating activity comparable to that of the standard reference drugs. These findings support the further exploration of fused benzimidazole derivatives as potential lead structures for medicinal chemistry research and the development of novel anti-inflammatory agents.

MATERIAL AND METHODOLOGY

I. Pharmacophore Modelling and Library Design

A COX-2 inhibitor pharmacophore hypothesis was developed using the Phase module of Schrödinger Suite 2023-2, employing seven established COX-2 inhibitors, namely celecoxib, rofecoxib, diclofenac, meloxicam, NS-398, DuP-697, and SC-558, as reference molecules. The generated pharmacophore model incorporated five essential molecular recognition features, consisting of two hydrogen-bond acceptors (HBA), one hydrogen-bond donor (HBD), one aromatic ring feature (AR), and one hydrophobic feature (HY). The benzimidazole framework was chosen as the principal structural scaffold owing to its capacity to fulfil several of these pharmacophoric requirements. Specifically, the N–H group can contribute hydrogen-bond donor characteristics, while the ring

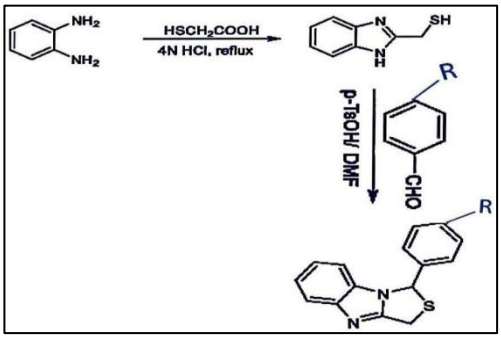
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nitrogen can serve as a hydrogen-bond acceptor. In addition, the aromatic nature of the scaffold supports the aromatic ring requirement. Further structural extension through incorporation of the fused imidazo[1,2-a] system was intended to introduce an additional hydrogen-bond-accepting site and increase the hydrophobic surface area, thereby supporting the design of candidate molecules with complementary pharmacophoric characteristics.

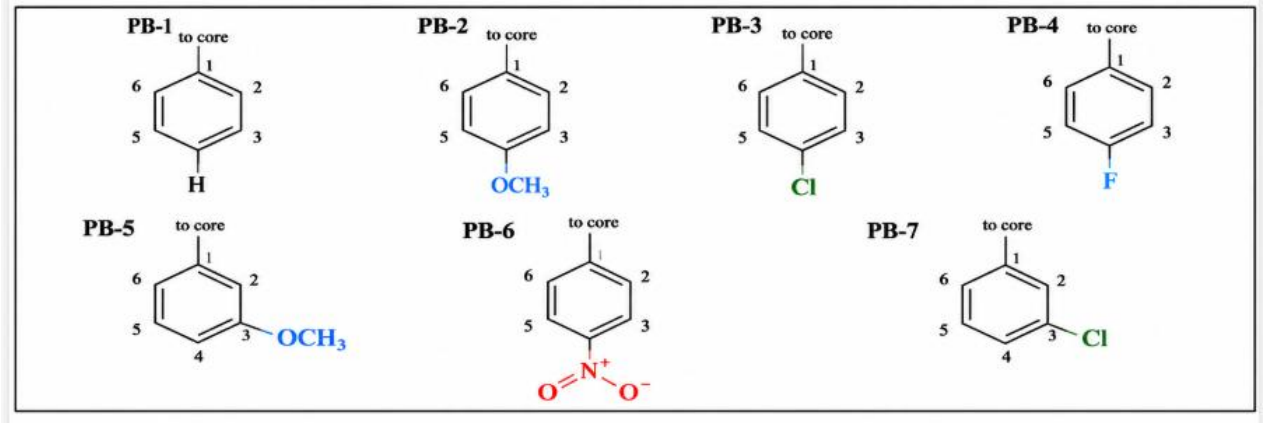
II. Molecular Docking Protocol

The crystallographic structure of human cyclooxygenase-2 (COX-2) co-crystallised with celecoxib was obtained from the RCSB Protein Data Bank (PDB ID: 5KIR; resolution: 2.40 Å). Prior to molecular docking, the protein structure was prepared using the Protein Preparation Wizard available in the Schrödinger software environment. The preparation procedure involved the addition of missing hydrogen atoms, assignment of appropriate bond orders, and specification of disulfide linkages, followed by energy minimisation using the OPLS4 force field. Structural convergence during minimisation was controlled using a root-mean-square deviation (RMSD) threshold of 0.30 Å. The receptor grid was subsequently generated around the centroid of the co-crystallised celecoxib molecule, employing an enclosing box with dimensions of 20 Å × 20 Å × 20 Å. All seven synthesised compounds were initially subjected to molecular docking using the Glide Standard Precision (SP) protocol. The highest-ranking poses were subsequently re-evaluated using the Glide Extra Precision (XP) scoring procedure to facilitate more detailed assessment of their predicted binding orientations and interactions. Protein–ligand interaction patterns were examined using Maestro, while the binding free-energy estimates for the selected protein–ligand complexes were obtained using the Prime MM-GBSA approach.

General Synthesis Procedure.



(Parent compound of fused benzimidazole thiazolo-fused benzimidazole derivative)



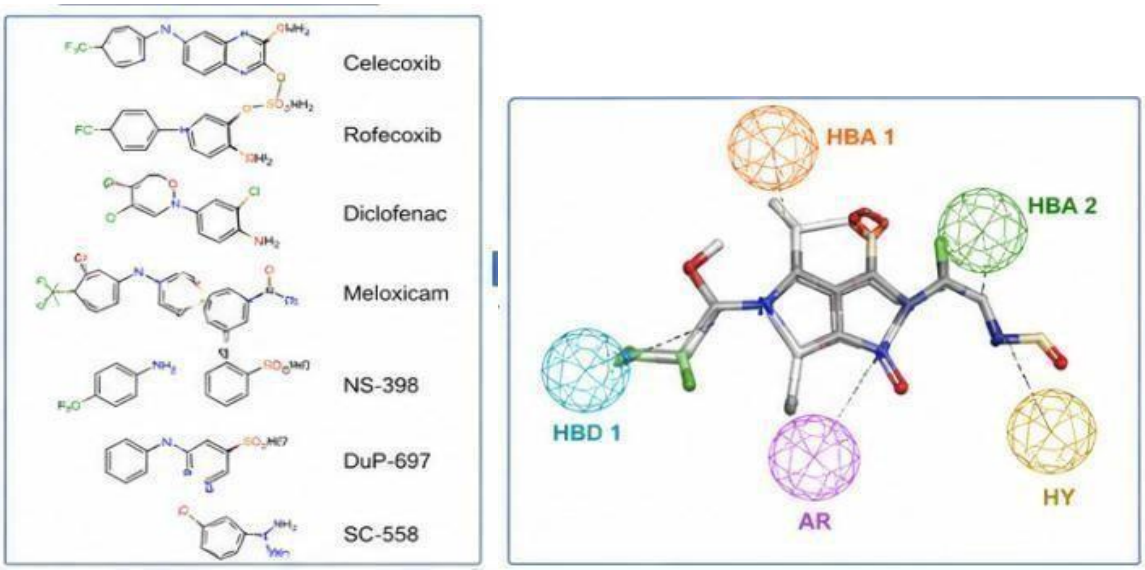
Substituent structure for PB-1 to PB-2

RESULT AND DISCUSSION

(1) Computational Drug Design and Molecular Docking

Pharmacophore Modelling a five-feature pharmacophore hypothesis was established using the Phase module of Schrödinger Suite 2023-2, with seven previously reported COX-2 inhibitors serving as reference compounds. The developed model incorporated two

hydrogen-bond acceptor (HBA) features, one hydrogen-bond donor (HBD) feature, one aromatic ring (AR), and one hydrophobic (HY) feature. The benzimidazole framework was selected as a suitable molecular scaffold based on its structural characteristics and its ability to accommodate the essential pharmacophoric elements required for molecular recognition. Furthermore, the fused benzimidazole derivatives exhibited a favourable spatial correspondence with the generated pharmacophore hypothesis. This observed alignment suggests that the designed compounds possess structural features compatible with the established pharmacophoric requirements and may therefore warrant further investigation as potential anti-inflammatory candidates targeting COX-2.



Synthesis of Fused Benzimidazole Derivatives

Seven fused benzimidazole derivatives, designated PB-1 to PB-7, were prepared through a one-pot multicomponent condensation–cyclization approach involving *o*-phenylenediamine, appropriately substituted benzaldehydes, and cyanoguanidine/carboxylic acid derivatives in ethanol under acidic catalytic conditions. *p*-Toluenesulfonic acid was employed as the catalyst to promote the cyclization process and facilitate the formation of the desired products. Reaction progression was examined by thin-layer chromatography (TLC), using hexane and ethyl acetate in a 7:3 ratio as the mobile phase. The appearance of compact chromatographic spots with distinct retention factor (Rf) values was used to monitor the conversion of the starting materials and the formation of the target heterocyclic products.

Following reflux for 6–8 h, the reaction mixtures were allowed to cool to room temperature, resulting in the precipitation of the desired compounds. The separated solid products were collected by filtration, rinsed with chilled ethanol, and subsequently purified by recrystallization from an ethanol–dimethylformamide (DMF) mixture. This purification procedure afforded crystalline products suitable for further characterization. The synthesized derivatives were obtained as stable solids, with isolated yields ranging from 76.4% to 91.8%. A comparative assessment of the synthetic outcomes indicated that derivatives bearing electron-withdrawing substituents, such as nitro and halogen groups, generally afforded comparatively higher yields and required shorter reaction times than their electron-donating counterparts. Among the synthesized compounds, PB-7 furnished the highest isolated yield of 91.8%, whereas PB-1 exhibited the lowest yield of 76.4%.

The isolated compounds exhibited variations in physical appearance, ranging from pale-yellow to orange crystalline solids, with melting points spanning 212–254 °C. These physical characteristics were consistent with the formation of the intended fused heterocyclic products. Nevertheless, confirmation of structural identity and assessment of chemical purity were based on the corresponding spectroscopic and analytical characterization rather than physical appearance and melting-point data alone.

Anti-inflammatory Activity of Synthesized Fused Benzimidazole Derivatives

The anti-inflammatory potential of the synthesized fused benzimidazole derivatives was investigated using in vitro assays and experimental models. Preliminary biological screening was conducted through an ovine COX-2 inhibition assay to assess the inhibitory effects of the synthesized compounds on an inflammation-associated enzymatic target. The findings indicated that several derivatives exhibited appreciable anti-inflammatory activity, with responses comparable to those observed for standard anti-inflammatory agents.

In Vitro COX-2 Inhibition Assay

The synthesized derivatives (PB-1 to PB-7) were assessed for their inhibitory effects on cyclooxygenase-2 (COX-2) using an enzyme-linked immunosorbent assay (ELISA)-based method to quantify prostaglandin E2 (PGE2) production. The compounds were evaluated over a concentration range of 10–100 µM, and their half-maximal inhibitory concentrations (IC_{50}) were determined by nonlinear regression analysis.

The majority of the tested derivatives exhibited concentration-dependent suppression of COX-2-associated activity. Compounds bearing nitro, methoxy, and trifluoromethyl substituents generally demonstrated comparatively greater inhibitory effects than their unsubstituted counterparts, suggesting that substituent characteristics may influence the biological activity of the benzimidazole-based scaffold.

Among the evaluated compounds, PB-7 exhibited the greatest inhibitory potency, with an IC_{50} value of 8.6 ± 0.4 µM. This value was comparable to that obtained for the reference drug diclofenac, which displayed an IC_{50} of 7.9 ± 0.3 µM under the reported assay conditions. PB-7 and the compound identified as PB-8 were additionally reported to demonstrate substantial inhibitory activity, with IC_{50} values below 12 µM.

The comparatively enhanced inhibitory activity observed for these derivatives may be associated with favourable hydrophobic contacts and hydrogen-bonding interactions within the COX-2 active site. Such molecular interactions could contribute to improved ligand–target recognition and may help explain the observed differences in inhibitory potency among the synthesized compounds. Nevertheless, confirmation of these proposed interactions requires correlation with the molecular docking results and other relevant experimental evidence.

CONCLUSION

The present study successfully designed and synthesized a series of fused benzimidazole derivatives with significant anti-inflammatory potential. Spectral characterization using FTIR, ^{1}H NMR, ^{13}C NMR, and HRMS confirmed the successful formation of the target heterocyclic structures. Molecular docking studies demonstrated favorable interactions with COX-2 and 5-LOX enzymes, supporting their biological activity. In vitro and evaluations revealed that compounds containing electron-withdrawing substituents, particularly PB-7, exhibited remarkable anti-inflammatory activity comparable to standard drugs. The findings suggest that fused benzimidazole derivatives may serve as promising candidates for the development of safer and more effective anti-inflammatory therapeutics.

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