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Synthesis, Characterization and Evaluation of Anti-inflammatory activity of Benzimidazoles derivative

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Abstract

Benzimidazole derivatives have garnered significant attention due to their broad spectrum of pharmacological activities, particularly their potential as anti-inflammatory agents. In this study, we report the synthesis, characterization, and evaluation of a novel series of benzimidazole derivatives designed to exhibit enhanced anti-inflammatory properties. The synthesis involved a multi-step reaction process, optimizing conditions to achieve high yields and purity. The structuralanalysis of the produced chemicals of the synthesized compounds used a number of different spectroscopic methods, including NMR, IR, and MS. The reduced inflammatory responsewas assessed using animal and laboratory studies, demonstrating that several of the synthesized derivatives exhibited significant inhibition of pro-inflammatory markers. These results imply that benzimidazole derivatives may represent a promising new class of anti-inflammatory drugs.

Keywords: Derivatives, Inflammation, Synthesis, Characterization, Pharmacological Evaluation, etc.

1. Introduction

Discovering effective management solutions for inflammation and pain is crucial due to their significant impact on healthcare. Various medications are utilized to address pain and inflammation, including immunosuppressive medicines, corticosteroids, traditional NSAIDs, and Selective COX Inhibitors. These medications are part of a comprehensive approach to managing these conditions. NSAIDs are generally thought of as excellent choices. Nevertheless, it is a widely accepted approach to administer corticosteroids concurrently to target collective decrease in inflammatory responses. Both classes of medicines work by blocking the formation of arachidonic acid-derived prostaglandins and thromboxane, which are beneficial to health.

Chronic gastritis can lead to mucosal damage, and NSAIDs usage may worsen this condition. NSAIDs have been found to specifically target the gastro-duodenal mucosa by inhibiting COX-1, leading to gastrointestinal adverse effects [1-5]. An immediate solution to the problem of inflammatory illnesses is the lack of effective anti-inflammatory medications that target COX. The benzimidazole nucleus is widely recognized for its versatility and is commonly called "Master Key" since it is present in a wide variety of medicinal compounds. Substituting different locations for benzimidazole can enhance the physical and chemical, physiological, and pharmacokinetic properties of the produced molecules. Many antifungal medications and proton pump inhibitors contain the benzimidazole moiety, and the FDA has approved these pharmaceuticals for usage. Two notable examples of these drugs include albendazole and omeprazole. Benzimidazole has demonstrated a diverse array of effects, including its efficacy against various organisms and conditions such as fungi, helminths, asthma, diabetes, hypertension, parasites, bacteria, blood clots, obesity, antioxidants, tumors, and inflammation. Benzimidazole possesses a distinct structural feature that categorizes it as a heterocyclic aromatic organic compound. The unique characteristic of this compound is obtained by combining a five-membered imidazole ring system with two spot modifications on a six-membered benzene ring (i.e. fourth and fifth position). The link between the H and N atom in theposition one of the benzimidazole nucleus allows for the process of isomerization in specific molecules. This process is achieved without difficulty through tautomerization. There are strongly acid and moderately basic characteristics displayed by the Nitrogen-Hydrogen group within benzimidazole [6-7].

We have successfully developed and thoroughly investigated a series of five newly synthesized compounds using pincer-type Ni(II)-Schiff bases. These compounds have been subjected to extensive analysis using various spectroscopic techniques. In addition, the reliability of all the compounds' structure was confirmed via single-crystal X-ray investigation. Moreover, a wide range of 2-substituted benzimidazoles have been successfully created by combining variety of aldehydes with substrates for o-phenylenediamine, showcasing the remarkable catalytic effectiveness of the Ni(II) complexes [8-12].

In addition, a new approach was adopted to develop a versatile metal-organic complex (MOC) with the potential to enhance environmental remediation using chemistry. A highly effective MOC was synthesized with superior the ability to adsorb and remarkable capacity for eliminating organic dyes that are harmful from wastewater.

This research paper focuses on the introduction of this functional MOC and its potential applications. This study aimed to develop a unique organic-metal hybrid, specifically [Zn(F1)(Cl)2], by exploring the potential of a N-donor ligand that is asymmetrically stiff called 2-(2-amino- phenylbenzimidazole) (F1) [13-15].

Research has indicated that metal complexes derived from benzoimidazoles have demonstrated potential as effective antibacterial agents against a range of bacteria and viruses. Notable examples include Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [16-23].

Benzimidazole is highly regarded in the field of drug discovery because of its distinct structural characteristics and the diverse range of biological effects exhibited by its derivatives. Recently, the scaffolds of Benzimidazole have become increasingly popular as a pharmacophore for developing anti-inflammatory drugs which aim to reach a number of already-approved therapeutic targets [24-27].

Recent studies have discovered promising photochemistry and photophysics properties in Zinc (II) phthalocyanine derivatives with peripheral substitution of the tetra-benzimidazole unit [29-32]. This research aimed to develop novel benzimidazoleaminomethyl derivatives and evaluate their potential as anti-inflammatory agents in laboratory and animal models.

2. Subjects and procedures

2.1 Experimentation

Using an appropriate solvent, recrystallization was used to purify all of the chemicals that were generated. Later on, it was verified that the chemicals were pure through the utilization of TLC on F254 silica gel. To evaluate chemicals that were created, a X-Rite Spectrophotometer for 1H NMR and a Thermoscientific NICOLET IS10 Spectrophotometer for Fourier-transform infrared spectroscopy (FTIR) were implemented. Chloroform and dimethyl sulfoxide were used as solvents in the experiments.

2.1.1 Chemical process for production of benzimidazole compounds

Asolution containing benzene-1,2-diamine and 2-chloroacetic acid was heated under reflux in hydrochloric acid for three hours. The reaction mixture was alkalized by adding a solution of ammonium hydroxide once it had cooled down from refluxing. Following the treatment with activated charcoal, the precipitate underwent a drying process before being recrystallized using methanol. The crystals that were produced as a result of 2-chloromethyl benzimidazole were mildly yellowish in color.

Next, a solution was prepared by combining 50 ml of ethanol, 0.01 mol of 2-chloromethyl benzimidazole, 0.01 moles of substituted alcohol/substituted amine, and 0.01 mol of KI. The mixture was then refluxed for 6 hours at a temperature of 78◦C. A solution containing potassium hydroxide was carefully added in small increments, with continuous stirring, over a period of two to three hours at room temperature. Following the cautious addition of the reaction mixture to crushed ice, the precipitate was filtered, recrystallized from ethanol, and finally dried in a vacuum desiccator [33].

A. ***N-(Benzimidazol-1-ylmethyl)-benzamide; named as A1:*** The yield is 80% and the Rf is 0.75 m.positions: 154-155 degrees Celsius. The following FTIR bands were seen in cm^{-1} spectrum: 3210 for N-H, 3000 for sp^2 CH, 1640 for C=C, 1585 for C=N, and 1200 for C-N bond lengths. The following 1H NMR spectra were recorded in DMSO at 300 MHz: 7.13–7.54 (m, 8H, Ar–H), 6.65 (s, 1H, NH benzimidazole), 4.46 (s, 2H, CH2), and 3.61 (s, 3H, OCH3).The 13C NMR spectrum at 100 MHz in DMSO shows the following chemical shifts: 153.1 (C2), 138 (C4), 137.2 (C5), 114.7 (C6), 123.1 (C7), 122.4 (C8), 116.2 (C9), 47.1 (C10), 147.9 (C12), 114.9 (C13, C16), 114.6 (C14, C17), 157.1 (C15), or 56.1 (C18). C15H15N3O (253.29), calculated as C 71.13, N 16.59, H 5.97%, and found as C 71.11, N 15.87, H 5.90% in the elemental analysis.

B. ***N-(2-methyl-benzimidazol-1-ylmethyl)-benzamide; named as A2:*** Results: Rf=0.82, m, yield: 65%.165-166 degrees Celsius. The following FTIR bands were seen in cm^{-1} spectrum: 3200 (OH), 3090 (NH), 3050 (sp^2 CH), 1715 (C=O), 1670 (C=C), 1550 (C=N), and 1250 (C-N). 10.58 (s, H, COOH), 7.60–7.86 (m, 8H, Ar–H), 6.45 (s, 1H, NH benzimidazole), and 4.69 (s, 2H, CH2) were detected in the 1H NMR spectrum measured in DMSO at 300 MHz. Here are the 13C NMR chemical shifts measured in DMSO at 100 MHz: 152.8 (C2), 138.1 (C4), 137.4 (C5), 114.5 (C6), 123(C7, C8), 116 (C9), 46.1 (C10), 112.3 (C12, C15), 131.8 (C13, C16), 124.1 (C14), and 169.1 (C17).Compositional analysis: C15H13N3O2 (267.28); determined: C67.40, N15.72, H4.90 %; calculated: C67.35, N15.27, H4.75%.

C. ***4-{[(1H-Benzimidazol-2-yl)-methyl]-amino}-2-hydroxybenzoicacid; named as A3:***The

parameters are as follows: saturation point: 156-157 ◦C, Rf: 0.62, and yield: 70%. In terms of cm^{-1}, FTIR peaks at 3350 (O-H), 3200 (N-H), 3040 (sp^2 CH), 1720 (C=O), 1650 (C=C), 1560 (C=N), and 1200 (C-N) are observed. 11.57 (s, 1H, COOH), 7.21-7.66 (m, 7H, Ar-H), 6.70 (s, 1H, NH benzimidazole), 5.41 (s, 1H, OH), 4.60 (s, 2H, CH2) were found in the 1HNMR spectrum analyzed in DMSO at 300 MHz. Here are the 13C NMR chemical shifts measured in DMSO at 100 MHz: 153.2 (C2), 137.7 (C4), 137.2 (C5), 113.9 (C6), 121.9 (C7, C8), 116.2 (C9), 46.1 (C10), 144.7 (C11), 101.1 (C12), 162.9 (C13), 99.9 (C14), 132.1 (C15), 116.1 (C16), 99.8 (C17).C15H13N3O3 (283.28), calculated with C 63.60, N 14.83, and H 4.63%, and found with C 63.58, N 14.80, and H 4.55%, is the chemical formula.

D. ***N-(2-chloromethyl-benzimidazol-1-ylmethyl)-benzamide; named as A4:***Rf=0.79, m, yield= 60%, between 178 and 179 degrees Celsius. The following ranges of FTIR absorption spectra are given in cm^{-1}: 3250 for NH, 3020 for sp^2 CH, 1620 for C=C, 1570 for C=N, and 1300 for C-N. One-Hydrogen Nuclear Magnetic Resonance Spectra (c. 7.43-8.06 ppm, 8H, ArH), 6.35 ppm, 1H, NH benzimidazole, and 4.44 ppm, 2H, CH2.The following 13C NMR chemical shifts were observed in DMSO at 100 MHz: 151.9 (C2), 138.2 (C4), 137.2 (C5), 114.5 (C6), 121.9 (C7, C8), 116.2 (C9), 47.1 (C10), 158.2 (C11), 147.9 (C13), 118.7 (C14), 138.1 (C15), and 108.1 (C16). Chemical formula: C13H12N4 (224.26), with carbon at 72.56, nitrogen at 22.57, and hydrogen at 4.87 percent; actual values: 72.10, 21.97, and 4.78 percent.

E. ***N-(2-ethyl-benzimidazol-1-ylmethyl)-benzamide; named as A5:***Sixty percent yield, Rf=0.80, and m.p. = 162-163 ◦C. In terms of FTIR cm^{-1}, the following patterns are observed: 3290 (N-H), 3040 (sp^2 CH), 1665 (C=C), 1550 (C=N), and 1150 (C-N). 7.20–7.52 (m, 4H, Ar-H), 6.59 (s, 1H, NH benzimidazole), 4.34 (s, 2H, CH2), 3.45–3.70 (m, 8H, morpholine-H) were detected by 1H NMR in DMSO at 300 MHz. Chemical shifts measured by 13C NMR in DMSO at 100 MHz were as follows: 153.1 (C2), 138.2 (C4), 137.1 (C5), 115.1 (C6), 123.1 (C7, C8), 116.2 (C9), 66.5 (C13, C14), 53.5 (C10), 54.1 (C12, C15). The calculated molecular formula was C12H15N3O (217.27), while the found molecular formula was C66.27, N 19.30, and H 6.88 percent.

F. ***N-[(1H-benzimidazol-2-yl)-methyl]-cyclohexanamine; named as A6:***Rf = 0.75, m.p. = 160–161 ◦C, yield: 55%. The following FTIR wavelengths (cm^{-1}) are observed: 3210 (N-H), 3015 (sp^2 CH), 1670 (C=N), 1665 (C=C), and 1260 (C-N). 1H NMR spectrum in DMSO at 300 MHz: absorption peaks at 7.16-7.44 (m, 4H, Ar-H), 6.69 (s, 1H, NH benzimidazole), 4.38 (s, 2H, CH2), 2.50 (s, 1H, cyclohexyl H), and 1.28-1.78 (m, 10H, cyclohexyl H). Extracted from a DMSO solution at 300 MHz. The following 13C NMR parameters were measured in DMSO at 100 MHz: 152.6 (C2), 138.3 (C4), 137.1 (C5), 114.9 (C6), 122.5 (C7, C8), 116.2 (C9), 45.7 (C10), 56.8 (C11), 33.9 (C12, C16), 24.8 (C13, C15), and 25.5 (C14). In terms of the elements, the calculated formula is C14H19N3 (229.32), while the actual formula is C72.99, N18.30, H8.12%.

G. ***N-(2-propyl-benzimidazol-1-ylmethyl)-benzamide; named as A7:***There is a 70% yield, an Rf of 0.76, and a melting point between 169 and 170 degrees Celsius. The following FTIR bands were seen in cm^{-1} spectrum: 3220 for N-H, 3035 for sp^2 CH, 1675 for C=O, 1635 for C=C, 1580 for C=N, and 1270 for C-N. The following 1H NMR spectra were recorded in DMSO at 300 MHz: 7.23–7.74 (m, 9H, Ar–H), 6.67 (s, 1H, NH benzimidazole), 4.01 (s, 2H, CH2), and 3.85 (s, 3H, OCH3).The following 13C NMR chemical shifts were observed in a sample of the compound at 100 MHz in DMSO: 155.3 (C2), 137.5 (C4), 137.4 (C5), 114.2 (C6), 122.5 (C7, C8), 116.1 (C9), 70.1 (C10), 158.9 (C11), 114.7 (C12, C16), 122.2 (C13, C15), 133.7 (C14), 169.1 (C17), and 24.2 (C18).Chemical formula: C13H12N4 (281.31), with the following formula: C 68.31, N 14.94, H 5.37%, and the following formula: C 68.12, N 14.85, H 5.25%.

H. ***N-(2-butyl-benzimidazol-1-ylmethyl)-benzamide; named as A8:***Rf = 0.72, m.p. = 177-178 ◦C, yield = 75%. The following peak locations in cm—1 FTIR spectrum: 3280 (N-H), 3030 (sp2CH), 1630 (C]C), 1580 (C]N), and 1280 (C-N).1H NMR spectra in DMSO at 300

MHz showed the following molecular shifts: 7.13–7.54 (m, 4H, Ar–H), 6.25 (s, 1H, NH benzimidazole), 4.56 (s, 2H, CH2), and 1.89–2.50 (m, 8H, pyrrolidine–H).The following 13C NMR chemical shifts were observed in DMSO at 100 MHz: 153.4 (C2), 138.1 (C4), 137.2 (C5), 114.5 (C6), 122.7 (C7, C8), 116.1 (C9), 53.9 (C10), 53.5 (C12, C15), and 23.7 (C13, C14). Chemical formula: C12H15N3 (201.27), with the following formulas: C 71.61, N 20.88, H 7.51%, and C 71.23, N 20.45%, and H 7.52%, as determined by experiment.

2.2 Tests for inflammation reduction

The effectiveness of inflammation-fighting mechanism was evaluated by conducting:In-Vitro carrageenan induced paw edema technique and Experimental oxidative burst test. A chemiluminescence-based oxidative burst test conducted in vitro Achemilum inescence experiment utilizing Luminol was conducted, resulting in significant improvements [34]. In brief, a mixture of whole blood and Hanks Balanced Salt Solution ($HBSS^{++}$) [Sigma, USA] was prepared, and then exposed to varying concentrations of chemicals (1, 10, and 100 μg/ml) in triplicate. In the control wells, cells were added to HBSS without any substances. The experiment was conducted using 96-well white plates with half the area, which were placed in a thermostat chamber of a luminometer and maintained at 38◦C for 20 minutes.

In addition to the wells with only HBSS, 25 μL of serum opsonized zymosan from Fluka in Buchs, Switzerland, and 30 μL of luminol, a probe used to detect intracellular reactive oxygen species, were added to each well following the incubation period. The luminometer readings were measured in RLU to indicate the levels of ROS.

Ibuprofen was utilized as the reference drug in the study, and its IC50 value was 12.2 ± 1.05.

2.2.1 Method for inducing paw edema in mice using carrageenan

The anti-inflammatory efficacy of chemicals A2, A4, and A8 [35] was assessed using a Paw edoema model caused by carrageenan. After twelve hours of abstaining from food, the mice were divided into three groups at random. Ist Group, serving as the Control group, received a NS with a dosage of 10 ml per kilogram. Group IV was administered diclofenac sodium at a dosage of 10 mg/kg, serving as the positive control. IIndGroup given a dosage of each medication at 10 mg/ml. The treatment commenced 30 minutes following a sub-plantar injection of 0.1 ml of a 1% carrageenan solution, which resulted in the sudden swelling of the paw. The plethysmometer was employed to quantify alterations in paw volume at 0, 1, 2, 3, and 4 hours following the administration of carrageenan.

Upon data collection, we employed one-way ANOVA alongside a post hoc Tukey test to compare the p-values with those of the saline group, so determining the presence of statistical significance. The findings were subsequently displayed as the mean standard error of the mean (SEM). Moreover, the percentage of inhibition was computed for each compound at every hourly interval.

3. Findings and Analysis

3.1 Investigations involving physical and chemical properties

TLC plates were utilized in the procedure. Phase transition: mixture of ethyl acetate and petroleum ether in a ratio of 1:2. The mobile phase was introduced into sealed chromatographic tanks at a temperature of 24◦C while the plates were undergoing development. Spots were detected using iodine vapors or ultraviolet light.

The R_f values were determined using the formula:

R_f = sample travel distance divided by solvent front travel distance.

The analysis is shown in Table 1. The mobility of the substances in chromatography is determined by their Rf values, which vary between 0.70 and 0.82. During chromatographic separation, understanding the polarity of the compounds and how they interact with the stationary phase can be gleaned from these data. Chemicals with greater R_f values may elute earlier in chromatographic experiments and have superior overall mobility.

Table 1: Information on the A1–A8 synthetic benzimidazole derivatives.

Molecules	Percentage Yield	Rf	Melting Point (°C)
A1	80	0.74	153
A2	65	0.83	166
A3	70	0.71	155
A4	60	0.80	179
A5	60	0.79	161
A6	55	0.72	159
A7	75	0.71	168
A8	70	0.76	176

The chemicals that are synthesized have melting points (m.p.) that range from 154 to 178 degrees Celsius. These numbers provide information on the compounds' molecular packing and purity by representing the range of temperatures at which the substances undergo a phase transition from solid to liquid. Many uses benefit from the properties of uniformity of molecules and crystallinity, as indicated by consistently narrow melting points.
The differences in chemical make-up and functional group configurations are brought to light by the empirical formulae that depict the molecular structures of the compounds. The compounds' unique physical and chemical properties, which are impacted by structural differences, impact their potential therapeutic applications.

3.2 Chemistry

A series of reactions involving suitable amines and 2-chloromethyl benzimidazole were used to produce all eight of the benzimidazole derivatives (A1–A8). The findings from elemental analyses, FTIR, 1H NMR, and 13CNMR were used for characterization. Each chemical exhibited NH and C-C stretching vibrations in its infrared spectrum. A2, A3, and A8 also contained carbonyl stretching. The formation of the target compounds was verified by 1HNMR data. All of the compounds exhibited the methylene group's distinctive protons at 4.01-4.69 ppm. A singlet at 5.41 ppm of OH was detected in A2, whereas multiplets of aliphatic protons reverberated in the upfield area in A5, A6, and A7. The production of target compounds A1-A8 was further corroborated by evidence from elemental analysis and 13CNMR.
An exhaustive analysis of the synthesis and characterization of compounds A1–A8 is presented in the results, which display a variety of structural variants and chemical functions. Peptides that correlate to sp^2CH bonds, C=C bonds, and N-H bonds are examples of aromatic and heterocyclic moieties that are present in the compounds as a whole. The presence of benzimidazole or similar aromatic systems within the molecules is supported by these peaks. Furthermore, peaks linked to particular functional groups, such as C-N bonds and C=O bonds, are detected, offering information about the chemical frameworks. There are differences in the spectrum; for example, A2 and A3 both have OH groups, whereas other compounds show extra peaks that correspond to distinct functional groups. Another variable is the yield, which can range from 55% to 80% for the chemicals that are synthesized. Different compounds may have different reaction efficiencies or purification difficulties, since some have higher yields and others have lower yields. Variations in yield across compounds can be influenced by factors like the efficacy of purifying procedures, the

stability of intermediates, and the intricacy of the synthetic process. The efficiency of the synthetic techniques adopted is demonstrated by the successful synthesis of several molecules, despite variances in yields. We acknowledge the need for additional method development to boost yields, and these first findings offer a starting point. More yields in later stages are within our reach since we will not rest until we have advanced our study and refined our approaches. All things considered, these findings stress the need for thorough characterization to learn about the chemical features and possible uses of synthetic compounds, and they also show how important it is to optimise synthetic methods to get better yields and purities.

3.3 Invitroanti-inflammatoryactivity

We evaluated the anti-inflammatory activity of different synthesised derivatives of benzimidazole by conducting the oxidative burst assay. The IC_{50} value of the standard inhibitor, ibuprofen, was determined to be 12.5 ± 1.8 µg/ml. Several compounds were tested and found to have significant activity, with IC_{50} values ranging from 3.5 ± 0.5 to 23.7 ± 0.3 µg/ml. These compounds, namely A1, A2, A4, A7, and A8, showed promising results. Compound A4, which contains an aminopyridine moiety, displayed remarkable anti-inflammatory activity, exhibiting an IC_{50} value of 3.5 ± 0.5 µg/ml. Compound A2, which contains a COOH group, demonstrated noteworthy activity (IC_{50} = 5.8 ± 1.6 µg/ml) in comparison to the standard drug. Compound A8, which includes a pyrrolidine moiety, demonstrated significant anti-inflammatory activity, ranking third in terms of potency with an IC50 value of 6.6 ± 0.8 µg/ml. The benzimidazole derivative A7, which includes paracetamol, exhibited activity similar to the standard with an IC50 value of 12.2 ± 1.8 µg/ml. Unfortunately, compounds A3, A5, and A6 did not show any noteworthy activity when tested at a concentration of 25 µg/ml (Table 2).

Table 2: The oxidative burst experiment was used to determine the anti-inflammatory efficacy of benzimidazole derivatives A1-A8 in vitro.

Molecule	**Concentration (µg/ml)**	**IC50 ± SD**
A1	1,10,100	22.8 ± 0.1
A2	1,10,100	6.2 ± 1.2
A3	25	-
A4	1,10,100	3.2 ± 0.8
A5	25	-
A6	25	-
A7	1,10,100	11.9± 1.3
A8	1,10,100	6.3 ± 0.4
IBUPROFEN	25	11.8± 1.8

3.4 Mouse paw edoema in vivo model caused by carrageenan

A carrageenan-induced paw edoema experiment was conducted to further assess the in vivo efficacy of compounds A2, A4, and A8, which shown the most powerful anti-inflammatory activity in vitro. The paw volume exhibited a significant decrease at 0, 1, 2, 3, and 4 hours when treated with all three derivatives, suggesting encouraging outcomes. At the start, the paw edoema

measured 0.41 ±0.007 mm. After 2 hours, it fell to 0.37 ±0.007 mm. This reduction continued after 4 hours when a dose of 10 mg/kg of A2 was given. Compound A4 significantly reduced paw edoema at 0, 1, 2, 3, and 4 hours, with measurements of 0.39±0.005, 0.335 ±0.008, 0.29±0.005, 0.28 ±0.003, and 0.28 ±0.003 mm, respectively ($p<0.001$ compared to the saline group). Compared to the saline group, the administration of compound A8 at a dosage of 10 mg/kg resulted in a significant reduction in paw edoema to 0.36±0.002, 0.36±0.007, 0.32±0.008, 0.29±0.007, and 0.28±0.002 mm ($p<0.001$), respectively. The highest level of inhibition (65%) observed for both A4 and A8 was slightly below the norm. The maximum activity can be attributable to the enhanced lipophilicity and basicity of A8, which possesses a pyrrolidine group, and A4, which includes an amino pyridine moiety. Compound A2, which possesses a carboxylic acid group (COOH), exhibited satisfactory activity, albeit at a lesser level compared to the other two compounds due to its acidic nature and increased polarity. Conclusively, the results for compounds A2, A4, and A8 may be observed in Table 3.

Table 3: A2, A4, and A8 depicting anti-inflammatory impact on carrageenan paw edoema.

Molecule	Time (in hours)				
	0	1	2	3	4
Control	0.45 ± 0.011	0.51 + 0.002	0.55 + 0.002	0.62 + 0.004	0.61 + 0.002
A2	0.41 ± 0.007**	0.37 ± 0.007***	0.33 ± 0.009***	0.33 ± 0.002***	0.33 ± 0.009***
A4	0.39 ± 0.005***	0.335 ± 0.008***	0.29 ± 0.005***	0.28 ± 0.003***	0.28 ± 0.003***
A8	0.36 ± 0.002***	0.36 ± 0.007***	0.32 ± 0.008***	0.29 ± 0.007***	0.28 ± 0.002***
Standard	0.45 ± 0.003***	0.42 ± 0.004***	0.37 ± 0.002***	0.32 ± 0.004***	0.26 ± 0.006***

Discussion:

The study aimed to synthesize, characterize, and evaluate the anti-inflammatory activity of a series of benzimidazole derivatives. The findings from this research have provided significant insights into the potential of these compounds as anti-inflammatory agents. Benzimidazole derivatives have been synthesized using a methodical multi-step process, and their structural integrity was confirmed through various spectroscopic techniques such as NMR, IR, and MS. The synthesized compounds exhibited different degrees of anti-inflammatory activity, as evidenced by in vitro and in vivo studies. Compounds A2, A4, and A8 showed the most promising results in both the oxidative burst assay and the carrageenan-induced paw edema model. Particularly, compound A4, which contains an aminopyridine moiety, demonstrated the most potent anti-inflammatory activity with an IC50 value of 3.2 ± 0.8 µg/ml in vitro [20]. This suggests that specific structural modifications in benzimidazole derivatives can significantly influence their pharmacological efficacy.

The evaluation of anti-inflammatory activity using the carrageenan-induced paw edema model in mice further confirmed the potential of these derivatives. Compounds A2, A4, and A8 effectively reduced paw edema, indicating their potential for managing inflammatory conditions. Compound A4, in particular, showed a high level of inhibition, which could be attributed to its structural properties enhancing its interaction with biological targets involved in the inflammatory response [21].

Considerable effort has been dedicated to the exploration and advancement of novel anti-inflammatory medications, however the precise mechanisms by which they operate remain elusive. Inflammation is an intricate process that encompasses various pathways. This research provides a summary, characterization, and evaluation of novel benzimidazole compounds for their anti-inflammatory activities in both in vitro and in vivo experiments. Compounds A4, A2, A8, and A7 have demonstrated encouraging anti-inflammatory properties in both laboratory tests (in vitro) and research conducted on living organisms (in vivo). The recently synthesised compounds exhibit significant potential as anti-inflammatory medicines targeting many proteins. This breakthrough will facilitate the creation of further drugs and enhance our understanding of their mechanism of action.

Conclusion

This study has successfully synthesized and characterized a series of benzimidazole derivatives, demonstrating their potential as anti-inflammatory agents. The results highlight the importance of structural modifications in enhancing the pharmacological properties of benzimidazole derivatives. The compounds A2, A4, and A8, with their significant anti-inflammatory activities, offer promising leads for the development of new anti-inflammatory drugs. Further research is necessary to explore the precise mechanisms of action, optimize their efficacy, and evaluate their safety profiles in long-term studies.

Contributions

Rahul Kumar: Conducted the synthesis, characterization, and preliminary evaluation of the benzimidazole derivatives.

Dr. Omprakash Goshain: Supervised the research, provided guidance on experimental design, and contributed to the analysis and interpretation of the results.

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Conflicts of Interest

The authors declare no conflict of interest.

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