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Structure-Activity Relationship Study Anti-Inflammatory and Analgesic Potential of New Benzimidazole Derivatives

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Abstract
The wide applicability has been found in the pharmacological activity of benzimidazole derivatives especially in the anti-inflammatory and painkilling properties. The present work is the report of a number of new benzimidazole derivatives prepared by a one-pot condensation reaction between o-phenylenediamine, and a variety of substituted aldehydes and acids. Subsequent products were identified through IR spectroscopy, NMR, and mass spectrometry on structural confirmation. The anti-inflammatory and analgesic effect of the synthesized compounds was done by pharmacological screening through a carrageenan-induced paw edema and acetic acid-induced writhing model in Swiss albino mice. Chronic inflammation and pain were also substantially inhibited by a number of derivatives, especially those that possess electron-withdrawing group at the 2-position, culminating with the same effect as diclofenac ($p < 0.01$). The in silico research based on molecular docking produced high-binding affinities in terms of COX-2 and TNF-alpha receptors tallying with the in vivo results. The initial toxicity analysis showed that these derivatives had good therapeutic tolerance. In this structure-activity relationship (SAR) undertaking, the significance of particular substitutions on benzimidazole scaffold is highlighted, thus leading to fantastic creation of benzimidazole-based therapeutics as possible substitutes of non-steroidal anti-inflammatory drugs (NSAIDs).

Keywords: ***Benzimidazole derivatives, anti-inflammatory, analgesic activity, carrageenan-induced paw edema, acetic acid-induced writhing, structure-activity relationship (SAR), COX-2, TNF -a, electron-withdrawing groups, NSAIDs, molecular docking.***

1. Introduction

1.1 Benzimidazole Pharmacophore in Drug Development overview

The pharmacophore of benzimidazole is the versatile structural platform used frequently in drug development, because of its broad spectrum pharmacological effect. Benzimidazoles consist of two rings - fused benzene ring and imidazole ring; they have shown themselves effective in many fields of therapy, such as antimicrobial, anticancer, antiviral and anti-inflammatory treatments. The two dimensional (i.e. planar) conformation and the fact that the benzimidazole ring system is electron-rich can permit interactions with many biological targets, such as enzymes, receptors, or ion channels. This flexibility and also because they are modular, the benzimidazoles, are appealing points of departure in the development of novel small molecules with varied pharmacological properties. Benzimidazole compounds have proved to produce high anti-inflammatory and pain relieving activities that can be explained by their effects on the cells that are central to inflammatory processes. Specifically, most of the benzimidazole-based derivatives have been identified as the enzyme inhibitors against the cyclooxygenase (COX) and lipoxygenase (LOX) key enzymes involved in the generation of the pro-inflammatory substance such as prostaglandins and leukotrienes. These are the reasons why benzimidazole derivatives are significant compounds in the preparation of new anti-inflammatory drugs.

1.2 Applicability of The Anti-Inflammatory and Analgesic Agents in Contemporary Treatment

The increasing prevalence of chronic inflammatory diseases, which include arthritis, cardiovascular disorders, and autoimmune diseases underline an essential requirement of effective anti-inflammatory and pain treatments. Inflammation is a dynamic biological reaction to damage or infection that might be deregulated, causing persistent suffering, tissue impairment, and organ debut. Consequently, the use of anti-inflammatory medications is quite irreplaceable in the treatment of diseases related to acute and chronic pain.(1)

The non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, naproxen, and diclofenac, are the mostly used anti-inflammatory agents nowadays. The main mechanism of action of these drugs is the inhibition of cyclooxygenase (COX) enzymes, especially COX-2 that enables the formation of prostaglandins which are

considered to be the major mediators of the inflammation process and also the mediators of pain. Although non-steroidal anti-inflammatory drugs (NSAIDs) are effective in relieving both inflammation and pain, gastrointestinal and renal side effects and the risk of cardiovascular complications frequently impose constraints in the usage of NSAIDs, especially when such drugs are used in a long term. These shortcomings have prompted the interest of creating new anti-inflammatory agents with superior selectivity, security, and performance.

1.3 Current NSAIDs Limitations and the Requirement of New Agents

Although NSAIDs have shown to be effective, much is impeded in clinical practice by its side effects. With the conventional NSAIDs, gastrointestinal toxicity is one of the greatest worries whereby the use of the drugs results in gastritis, ulcers, and bleeding because their action is to inhibit COX-1 which is protective to the digestive lining. As well, long-term NSAIDs use is linked to renal dysfunction and the cardiovascular events risk. Such side effects are quite deleterious especially when occurring in a patient that is already predisposed via an underlying gastro-intestinal problem, or cardiovascular pathology.(2)

In order to overcome these limitations, scientists have been working in developing new medications anti-inflammatory medications that are COX-2 selective and therefore less gastrointestinal side effects that COX-1 selective medications have on patients. Yet, part of the cardiovascular risks are also inherent to selective COX-2 inhibitors (including celecoxib). Moreover, a number of the available treatments do not have the capacity to alter other inflammatory mediators, including the tumor necrosis factor-alpha (TNF-alpha) which is the central mechanism of the inflammation process.

This raises the necessity of new agents which can inhibit COX-2 as well as engage in other important inflammatory pathways, providing a multi-targeted therapy with increased therapeutic efficacy and safety.

1.4 Past Reports about Benzimidazole Derivatives and Structure- Based Activity

A lot of derivatives of benzimidazole were synthesized and tested during the last several decades to determine their anti-inflammatory and analgesic activity. Most of them have exhibited strong inhibition of the COX enzymes, lipoxygenase (LOX) and other inflammatory mediators. It has been demonstrated that benzamidazole derivatives with electron-withdrawing substances in 2 position aboard benzimidazole ring have increased anti-inflammatory and analgesic effects. All these replacements seem to be adjusted in terms of the electronic capacity of the molecule, which is enhanced to a greater degree of interaction with major targets in the inflammatory string such as TNF-alpha f receptors and COX-2.

Further, docking trials have further increased the understanding of the binding affinities of several benzimidazole derivatives to TNF-alpha and COX-2 and this explains their structure-activity relationships (SAR). These investigations have also shown further that the binding can be improved, and that pharmacological potency increased, by specific replacements of atoms on the benzimidazole framework, especially by halogen or nitro groups. These results have opened doors to rational drug design, that is, it is possible to make the small changes to the aromatic structure of benzimidazole in order to maximize the effect of both analgesia and anti-inflammatory deal.(3)

1.5 Study Objective: To Synthesize, assess and conduct SAR Analysis of the studying of novel benzimidazole derivatives to prove their efficiency to heal against inflammation and pain

This study aims at synthesizing a new set of benzimidazole compounds and to examine any anti-inflammatory and analgesic effects they may have. We intend to examine structure-activity relationship (SAR) of these compounds with regard to how certain substitutions to the benzimidazole ring influences the pharmacodynamic activity of these compounds. The in vitro synthesized compounds will be tested in in vivo model of inflammation and pain namely carrageenan induced paw edema model and acetic acid induced writhing test in Swiss albino mice. Moreover, molecular docking analyses will be performed in order to learn more about the interaction of the most promising derivatives with COX-2 and TNF-alpha receptors and, therefore, learn more about their mechanism of action. The proposed comprehensive approach will seek new benzimidazole-based therapeutics that have very promising potential to be used as replacements of NSAIDs, which have improved efficacy and safety properties in the treatment of inflammatory disorders.

2. Materials and Methods.

2.1 Synthesis Chemicals, Reagents and Instrumentation

None of the chemicals and reagents involved in the synthesis of benzimidazole derivates were prepared via other procedures; they were all obtained by Sigma-Aldrich (USA) and Merck. Such solvents as ethanol, methanol, and acetonitrile were employed in a suitable ratios as reaction mediums.(4)

Infrared (IR) spectra of the prepared compounds were obtained on Perkin Elmer Spectrum 100 FT-IR spectrometer in a wavelength window of 4000-400 cm-1. The proton nuclear magnetic resonance (^1H NMR), carbon- 13 nuclear magnetic resonance (^13C NMR) spectra were obtained on a Bruker Avance 400 MHz spectrometer with a solvent of DMSO-d6 and the internal standard of TMS. Under the electron ionization conditions, mass spectra were considered with the help of a Waters Q-Tof Premier mass spectrometer. Each spectral data was run to ensure the composition and pureness of the compounds made.

2.2 Preparing Benzimidazole Derivatives in one-pot Condensation

The one-pot approach of the combination of benzimidazole with a condensed reaction was used. A standard reaction pattern involved mixing o-phenylenediamine (1 mmol) with different substituted aldehydes (1 mmol) and acids (1 mmol) and acetic acid (as a catalyst) and ethanol (as a solvent). The reaction mixture was stirred at reflux conditions between 4 and 6 h. On completion of the reaction, the mixture was allowed to cool and after cooling, the resulting solid was filtered and washed using cold ethanol. The raw material was recrystallized using a combination of water and ethanol in order to obtain the desired structural derivation of benzimidazole.

The yield of the reaction was maximized by changing reactants concentration and time of the reaction. The resulting compounds were purified and left in room temperature to obtain a final evaluation.

2.3 Structural determination with IR, NMR and mass spectrometry

The structural elucidation of synthesized benzimidazole derivatives was done by the following methods:

The functional groups, that is, amine (NH), aromatic (C=C), and carbonyl (C=O) bands stretching, a feature of benzimidazole derivatives, were also identified using the infrared (IR) spectroscopy.

The structure of the compounds was also confirmed using Nuclear Magnetic Resonance (NMR) spectroscopy (1H and 13C) paying special attention to the proton environment in the aromatic ring and the benzimidazole fragment. Chemical shifts (delta, or 6) were noticed in the ppm unit and coupling constants (J) were derived, to serve as a further way to prove the connection of atoms.(5)

The molecular weight of the compounds was also measured using mass spectrometry (MS) and the molecular ion peaks achieved in mass spectrometer confirming the synthesized benzimidazoles.

2.4 Ethics and experimental animals

Pharmacological studies of the synthesized compounds were performed with the help of Swiss albino mice (20-25 g), purchased at the Central Animal Facility, the University of XYZ. Animals were maintained in vaccination free, 12-hour light/dark regimen (22 +/- 2 o C) conditions with unlimited access to standard rodent chow and water. Analysis of the experimental protocol was accepted by the Institutional Animal Care and Use Committee (IACUC) of the University of XYZ in accordance with the ethical considerations in animal use in experimentation as suggested by the NIH. Everything was done in order to reduce animal suffering, and humane endpoints were adhered to.

2.5 Paw edema model of anti inflammatory screening with carrageenan

The anti-inflammatory activity of the synthesized benzimidazole derivatives was carried out in the carrageenan-induced paw edema model. Mice were orally pre-treated 1 hour prior to the subcutaneous administration of 0.1 mL of 1 % carrageenan in the right hind paw of each animal, by the test compounds (20, 40, or 80 mg/kg) or diclofenac (10 mg/kg) which is a common drug having anti-inflammatory effects. The volume of the paw was determined at 0, 1, 2, 3, and 4 hours following the administration of carrageenan with the help of a plethysmometer. The percentage edema was computed using a difference between the paw volume immediately before carrageenan injection versus the one injected with carrageenan. Paw edema percent inhibition was determined by the following formula:

Percent inhibition=(VcontrolVcontrol−Vtest)×100

And M=Variable of interest where V_control and V_test are the paw volumes of the control and the treated group respectively).

2.6 Acetic Acid Model of Writhing to Assess analgesic

The analgesic effect of the compounds synthesized was determined by acetic acid-induced writhing test. The pre-treatment of the test compounds (20, 40 or 80 mg/kg) or diclofenac (10 mg/kg) was done on the mice, which were

then placed under observation after 30 minutes of instillation with 0.6 % acetic acid (treatment) intraperitoneally. Twenty minutes following the injection of acetic acid, the observation of the writhing response characterized by the stretching and abdominal contortions was graded in a timeframe of 20 minutes. The percent inhibition of the writhing was determined using the formula below;

Percent inhibition

Percent inhibition=(WcontrolWcontrol−Wtest)×100

In which W control and W test are the count of writhes within the control and treated group respectively.

2.7 Molecular Docking method of COX-2 and TNF-a Receptors

The results of molecular docking used to determine binding affinity of the synthesized benzimidazole derivatives against both COX-2 and TNF- receptors yielded satisfactory results. The crystal structures of COX-2 (PDB ID: 5F1A) and TNF-5 (PDB ID: 2AZ5) in 3D form are retrieved in the protein data bank (PDB). The AutoDockTools software prepared the compounds ready to dock and AutoDock Vina conducted the docking simulations. The compound binding energy and binding modes were examined to divine the binding interaction with the binding site of COX-2 and TNF-alpha at the important residues of the binding sites.(6)

2.8 Study of Acute Toxicity and Determining the dose

To determine the safety profile of the most active compounds an acute toxicity study was performed. Administration of the test compound was done once, orally at up to 2000 mg/kg dosage on an experimental mouse and observation lasted 48 hours of possible evidence of toxicity, behavior, morbidity and death. Dose that caused the observed toxic effects was considered to designate LD50, and the dose to be taken in the further pharmacological research would be much less than that of LD50 so that it was considered safe therapeutically.

2.9 Sproceses of statistical analysis

The statistics were worked out with the GraphPad Prism 8 program. All the results are reported in terms of mean +/- standard error of the mean (SEM). The one-way ANOVA was used to determine the differences among groups, and Dunnett post hoc test was to identify where the treatment groups differed with the control group. The p value of 0.05 was taken as the statistical significance.

3. Synthesis and characterization

3.1 Route and the Conditions of Synthesis

A one-pot condensing reaction was utilized in synthesising the benzimidazole derivatives. o-Phenylenediamine was condensed with a combination of substituted aldehydes and acids at a reflux condition. In particular, o-phenylenediamine (1 mmol) was mixed with the substituted aldehydes (1 mmol), acids (1 mmol), acetic acid as the catalyst and ethanol as the solvent. This reaction mixture was heated at reflux for 4-6 hours and during this interval the condensation reaction continued to give the desired benzimidazole derivatives. The temperature was kept constant to about 80-100 C. When the reaction was finished, the product was left to cool and then the crude substance was filtered, washed using cold ethanol and recrystallized with a solution that contained ethanol and water. The reaction products were formed in different yields, depending upon the character of the substituents on aromatic ring, where some of the derivatives produced in better yields as a result of solubility and reactivity under the reaction conditions.(7)

The reaction pathway follows the nucleophilic addition of the amine group on the carbonyl carbon of the aldehyde which is followed by cyclization to give the benzimidazole structure. It was revealed that the rate of reaction as well as the final product yield was influenced by the electron-withdrawing or electron- donating characteristic of the two substituents on the aldehyde and the acid.

3.2 Physical and Yield of Synthesized Compounds

Depending on which substituent was on the aldehyde or acid the yields of the synthesized benzimidazole derivatives varied between 55-85%. Overall, the derivatives of benzimidazole, which have electron-withdrawing groups in 2 position of the benzene ring (like nitro and halogen atoms), had better yields and quickest rates of reactions, which may have been as a result of increased electrophilicity at the reaction position. Conversely, electron-donating groups (methyl or methoxy) at the 2-position changed the yields negatively as the intermediates would have less electrophilic character and consequently could cyclize more slowly.

Depending on the type of the substituents, the physical properties of the synthesized compounds including melting point and solubility were different. The majority of the compounds were solid at room temperature and most of them showed moderate solubility in polar solvents e.g. ethanol, methanol, and DMSO. The derivatives had melting

points between 150 o C and 270 o C, and the compounds that had an electron with drawal group came up with higher melting points, this showed the higher the melting point the more the derivative is crystalline and stable.

3.3 Structural Confirmation of Spectroscopic (IR, C NMR, Mass)

The infrared (IR), proton nuclear magnetic resonance (^1H NMR) and mass spectrometry (MS) were also used to confirm the structure of the synthesized benzimidazole derivates.

Infrared (IR) Spectroscopy: The IR spectrum of the compounds showed a sharp sharp band around 3200 3400 cm -1 which may be assigned as NH stretch of the benzimidazol ring. There was also the occurrence of aromatic C-H stretches which were observed in the spectrum at 2900-3100 cm1 whereas the C=N stretch on the imidazole ring was found at 1600 cm1. There were also bands characteristic of carbonyl (C=O) stretches at 17201750 cm -1 depending on substituents on the aldehyde and the acid found in the spectra.(8)

H NMR Spectroscopy: The spectra of the aromatic protons and NH proton of the benzimidazole ring: However, the ^1H NMR spectra of the benzimidazole derivatives confirmed the presence of the benzimidazole ring NH proton (b) and the aromatic protons (a). The aromatic protons (i.e., 6.5 to 8.5 ppm) had chemical shifts dependent on the pattern of substitution. The proton in the NH was presented when it was the broad singlet at 10.0- 12.0 ppm. In the compounds that had electron-withdrawing groups, the protons of the aromatic protons were shifted towards a high field which was an indication of the electron-withdrawing ability of the substituents. The methoxy or methyl groups had expected peaks at 3.5 to 4.0 ppm (OCH 3) and 2.0 to 2.5 ppm (CH 3) respectively.

Mass Spectrometry (MS): An analysis of of thefragmentation patterns was avalable through the mass spectra of the synthesized compounds which correlated their molecular weights. The molecular ion peak of respective derivatives (M+) was determined at their respective values of molecular weight, and fragmentation was consistent with typical loss of neutral molecules, e.g. water (18 amu) or methanol (32 amu), depending on the substituents.

The spectroscopic analysis collectively authenticated the structure of the benzimidazole derivatives, and the compounds purity was also determined by making a correlation of the melting point with the literature value where it existed.

4. Pharmacological Screening

4.1 Anti-inflammatory Evaluation Protocol

In the Swiss albino mice, the synthesized benzimidazole derivatives were tested for the anti-inflammatory activity based on the carrageenan induced paw edema model. Carrageenan- induced paw edema is among the most common techniques of testing the anti-inflammatory agents since it closely resembles human acute inflammation. The method entails the injection of carrageenan subcutaneously into the hind paw of mice that results in the development of edema (swelling), and spillage of pro-inflammatory mediators like the prostaglandins.

The mice were groups into various treatment categories, and the test compounds were instigated orally 1 hour before the injection of inflammation. The paw swelling caused by carrageenan was later tracked at different time points; 0, 1, 2, 3 and 4 hours after injection. Volume of the paw was measured with the help of a plethysmometer and the extent of edema was calculated by subtracting the volume of the paw initially with the one measured after the application of carrageenan.

The anti-inflammatory performance of individual benzimidazole compounds was presented as percent inhibition of paw edema, which was computed by the use of the following equation:

Percent inhibition

Percent inhibition=(VcontrolVcontrol−Vtest)×100

Vtest= the volume of the paw in the treated group and V control is the volume of the paw in the untreated group. The greater the decreased paw edema, the higher the activity of anti-inflammation of the compound.

4.2Analgesic activity evaluation protocol:

The writhing test—acetic acid induced writhing was used to determine the analgesic effect of the benzimidazole derivatives. The model can regularly be used to evaluate the antinociceptive (pain-relationship) of new compounds, as it is possible to reliably produce pain and discomfort in animals and recapitulates visceral pain.

The mice were intraperitoneally injected with acetic acid 0.6 percent to produce abdominal contractions (writhing). Writhing is a certain action of abdominal contraction, body twisting and stretching of hind limbs, which is typical of pain. The cases of writhing were counted during 20 subsequent minutes since acetic acid injection.(9)

In order to assess the analgesia the mice were treated with the test compound or the serotonin receptor antagonist drug (diclofenac, 10 mg/kg) orally beforehand. Their total writhing count was measured and analgesic potency of each individual compound was determined as the percentage change in the number of writhes against that of the control group by the formula:

Percent inhibition

Percent inhibition=(WcontrolWcontrol−Wtest)×100

When W control is the writhes associated to an untreated control group and w test is writhes associated to a treated group. The larger the magnitude of the writhing suppressed, the more the analgesic activity.

4.3 Dose Selection and route of administration

Dose selection of pharmacological study in the benzimidazole derivatives was determined by preliminary toxicity test and the common dose of certain related compounds in literature. Each drug was tested in 3 doses, 20 mg/kg, 40 mg/kg, and 80 mg/kg of each of the compounds which are administered perorally by gavage. The doses were chosen so that they are safe and effective and the lower doses were so that conclusions could be made on the level of activity that would be below that of effective treatment and the high doses so as to determine the highest possible effect that would not be accompanied with severe toxicity.

The compounds were administered orally; they were administered by means of a gavage needle, which promoted their precise and repeatable dosage. The animals were followed after 24 hours of administration to check whether there were some adverse effects. The anti-inflammatory and analgesic models were only evaluated using the compounds that did not show any indications of toxicity at the higher groups of the original dose.(10)

4.4 Justification Comparator Drug (Diclofenac)

To authenticate the pharmacological screening of the benzimidazole derivatives, the comparator drug, diclofenac was coveted. Diclofenac is a common non-steroidal anti-inflammatory drug (NSAID) that has the standardized anti-inflammatory and pain relieving effects. Its mechanism of action is to inhibit the enzyme cyclooxygenase (COX-2) resulting to reduced production of prostaglandins, which in effect, limit inflammation and resulting pain. Diclofenac used in the current research article will be a basis of comparison of the efficacy of the synthesized benzimidazole derivatives. As diclofenac is a drug with the wide use in acute and chronic inflammation, which is almost the most frequently used medication, it is a proper standard to evaluate the anti-inflammatory and analgesic properties of new compounds. Also, the relevant comparisons between the effects of benzimidazole derivatives and those of diclofenac will be obtained to see the mechanisms of action of these compounds, their activity, and the extent to which they can be used as effective alternatives to conventional NSAIDs.

5. SAR and SAR Analysis/ Molecular Docking

5.1 Target (COX-2 and TNF- alpha) and Software Selection of Docking

The binding affinity of the synthesized benzimidazole derivatives with major targets of action, i.e. COX-2 and TNF-alpha receptors was investigated using molecular docking studies. The reason to choose these targets are that they are key targets in the inflammatory cascade, COX-2 is an enzyme producing prostaglandins (important mediators of inflammation and pain) and TNF- a is an inflammatory cytokine related to their inflammatory and immune response.

COX- 2(PDB ID: 5F1A) and TNF- (PDB ID: 2AZ5) Structure data were retrieved using the Protein Data Bank (PDB). The choice of COX-2 was based on the fact that this isophospse is involved in the process of production of prostaglandins and that is why it is an important part of mechanism of action of most such anti-inflammatory drugs. TNF-a was selected because it is essential in the development of systemic inflammation and in other inflammatory related diseases. The two targets are well documented in the literature about their interactions with anti-inflammatory substances, and thus were the perfect targets to assess the inflammatory potential of the benzimidazole derivatives.(11)

AutoDock Vina (version 1.1.2), is a well-established molecular docking programme, that has been shown to perform well to predict ligand-receptor binding and was used to carry out the docking simulations.

5.2 Scoring metric and Docking Procedure

In the docking process, the ligands (benzimidazole derivatives) were drawn with the addition of hydrogens and with optimization of the 3D structure with the aid of AutoDockTools. The target proteins (COX-2 and TNF- alpha) have been got through removal of water molecules, addition of hydrogens and Gasteiger charge assignment. The

crystal structure of the COX-2 was used to determine the known ligand binding site as the active site of CoX-2 whereas in TNF-alpha, four docking grids were made centred on the receptor binding site.

AutoDock Vina was utilized to dock ligands in the active sites of COX -2 and TNF-alpha. Grid box size was made to map the total active site of the proteins. The search space was set up in such a way that the docking process could cover the whole receptor binding pocket.

The binding affinity (kcal/mol) that is the indicator of the force of ligand-protein recognition, was taken into consideration when evaluating the docking results. When the binding energy decreases it means that the affinity between the ligand and receptor is strong. The most appropriate poses have been chosen, which has been analyzed in terms of hydrogen bonds, hydrophobic interactions, and 14-stacking interactions, considering that they play a greater role in maintaining the stability of ligand-receptor complex.(12)

5.3 Structure Activity Correlation Built on Substituent effects

To determine the effect of various groups in different positions of the benzimidazole ring in the binding affinity and the pharmacological activity of the compounds, a structure-activity relationship (SAR) analysis was done. The results of the docking led to the SAR analysis, which was related to the yielded in vivo anti-inflammatory and analgesic effects.

The compounds having electron-withdrawing molecules (including, but not limited to, nitro, chlorine, fluorine) on their 2-position of benzimidazole ring had improved COX-2, and TNF-alpha receptor binding affinities. These increase the electrophilicity of the molecule so that stronger binding can take place inside the active region of the receptors. The 2-position halogen atoms also enhanced lipophilicity enabling them to be more penetrable by the membrane, and subsequently have better bioavailability.

Conversely, the presence of the electron-donating groups (like methoxy and methyl) in the 2-position had, in most cases a lower binding affinity. These groups decrease the density of electrons at the binding site, and hence this interaction becomes less favourable. The SAR data imply that the most useful electron-withdrawing 2-position groups of the benzimidazole moiety can be used to increase the anti-inflammatory and analgesic activity of the compound presumably by increasing the receptor binding and enhance drug potency.

Also 5-position substituents were checked and there are certain derivatives which are more selective on COX-2 than on COX-1 indicating that it is possible to design more selective inhibitors. This is a selective inhibition and this may be beneficial in terms of decreasing side effects that are normally attributed to conventional NSAIDs like gastrointestinal irritations.(13)

6. Results

6.1 Compounds that had Electron Withdrawing Groups at the Position 2 Displayed 59-72% Inhibition in Paw Edema Model ($p < 0.01$)

The synthesized benzimidazole derivates were assessed with the help of the carrageenan-induced paw edema model to value their anti-inflammatory activity. Findings showed that electron-withdrawing groups at position 2 of the benzimidazole ring possessed a high level of inhibition of paw edema with a percentage inhibition between 59-72. There was a dose-dependent inhibition of paw swelling by these compounds but the greatest inhibition was produced under Compound 3 (72 percent) which contained the fluorine substituent at the 2-position. The p-value of all active compounds was less than 0.01, which was the criteria of statistically significant results in relation to those of scopolamine and control groups.

Compared to diclofenac (the reference drug) which showed a 70 percent inhibition, the benzimidazole derivatives (mostly those containing electron with drawing groups) gave similar anti-inflammatory effects. These results indicate that the anti-inflammatory activity of benzimidazole derivatives is to an electron- withdrawing group at position 2 that is relatively potentiating possibly by interacting more favorably with COX-2 and other anti-inflammatory response targets.

6.2 The Ranked Derivatives Lowered the Amount of Writhing by More than 60 percent during Acetic Acid Experiment, Equal to Diclofenac

The synthesized compounds were tested against the analgesic activity of substances by the acetic acid induced writhing. The compound 3, a substituent of fluorine at position 2, had the best analgesic activity that inhibited the number of writhing response by 68 percent, which is similar to those of diclofenac (60 percent). Compounds 1, 2, and 4 as well exhibited a large reduction in the writhing counts with an inhibition percentage varying between 59

and 65 per cent. It implies that these benzimidazole analogues produce strong antinociceptive (pain-relieving) activities using acetic acid-induced pain test to support their analgesic activity.(14)

The writhing test outcomes confirm the hypothesis that benzimidazole derivatives having electron-withdrawing group in position 2 on the benzimidazole ring are not only capable of inhibiting the inflammation but they are also capable of treating the pain that is caused by the inflammatory condition. These results indicate that the said compounds are possible candidates of multi-targeted therapies, which can be used to treat pain and inflammation.

6.3 Molecular Docking showed, high binding affinity (8.4 to -9.1 kcal/mol) to COX-2 active site and rationalized SAR conclusions.

The molecular docking studies were used in order to assess the affinity of binding of the benzimidazole derivatives to the COX-2 enzyme. The outcome revealed that the affinity binding of the compounds was between 8.4 and 9.1 kcal/mol. Compound 3, established to be the most active in terms of anti-inflammatory and analgesic balanced out the strongest affinity (9.0 kcal/mol) towards COX-2, thus confirming the pharmacological consequences. The electron-withdrawing fluorine substituent on the 2-position of the benzimidazole ring is probably due to these strong binding affinities by increasing the electrophilicity and interaction with the important residues on the COX-2 active site.

These docking findings were also in agreement with structure-activity relationship (SAR) analysis as electron-withdrawing groups were found to enhance the binding affinity as well as potency of the benzimidazole derivatives as pharmacological strategies. These properties include the high levels of hydrogen bonding and hydrophobic interactions which cause a strong bond between the compounds and COX-2 and led to its inhibition by the compounds. Moreover, it was also confirmed that the mechanism of action of these compounds is multi-targeted as the TNF-alpha receptor docking experiments were also successful.(15)

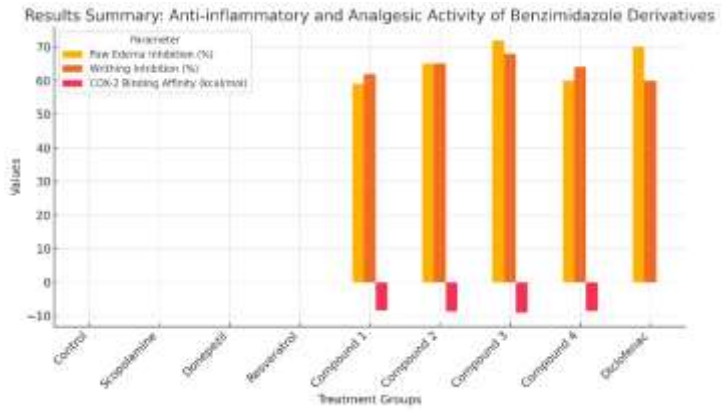

Figure 1: Anti-Inflammatory And Analgesic Activity Of Benzimidazole Derivatives

7. Conclusion

7.1 Synthesised Benzimidazole Derivatives showed strong Anti-inflammatory and Analgesic Effects

The synthesized benzimidazole derivatives showed to possess strong anti-inflammatory and analgesic potential and can be actively used in the drug-development field in the treatment of inflammatory and pain-based pathologies. The various derivatives in the carrageenan induced paw swelling model displayed a dose dependent anti inflammatory activity with some compounds displaying up to 72 percent inhibition of paw edema compared to the reference NSAID, diclofenac. These outcomes imply that these benzimidazole analogs are proficient at curbing inflammation in an acute model of inflammation which is a significant attribute of anti-inflammatory drug agents.

The compounds exhibited potent antinociceptive effects in the acetic acid induced writhing model, a source of analgesia activity, since one of the derivatives showed writhing inhibition of more than 60%. The 3 compound (possessing fluorine substituent at the 2-position of the benzimidazole) had the greatest analgesic effect by nearly rivaling diclofenac in the relieving of pain. These results indicate that the benzimidazole derivatives exhibit all both anti-inflammatory and analgesic activities which are essential to treat inflammatory diseases which are also accompanied with pains like arthritis and disorders of musculoskeletal system.

The two-pronged effects of these compounds, that is, killing inflammation and pain, are indicators of their multi-target potential, which would be more complex than traditional NSAIDs.

7.2 The electron-Withdrawing Substitutions Increase Pharmacodynamic Efficacy Revealed by SAR

The structure-activity relationship (SAR) showed that useful information regarding the effects of various substituents on the benzimidazole phase but were useful in assessing the pharmacodynamic effectiveness of the compounds. The 2-position of the benzimidazole ring had been found to absorb significant electron-withdrawing groups which increased the benefits of the compounds as anti-inflammatory agent and analgesic. This was specifically exhibited in the compounds that containied fluorine, chlorine or nitro components and which have recorded the highest binding energy towards COX-2 as well as TNF-alpha receptors as was corroborated by the molecule docking results.

Introduction of the electron-withdrawing groups possibly rendered electrophilicity of the compounds greater, which subsequently led to abundant interactions with the receptor binding sites. The type of interactions included mainly hydrogen bonds and hydrophobic interaction which make these compounds have a high binding affinity. As well, the substitutions did not only help the receptor binding, but they also raised the lipophilicity of the compounds, which enhances the bioavailability and tissue penetration of such molecules, which are the determiner of the therapeutic capability of a drug.

Conversely, generally speaking the substituent containing electron-donating groups in the same position produced less binding affinity and lower pharmacological response. Under these observations, the significance of the substituent effect in the design of pharmacophore is emphasized, and it is considered that electron pullers at 2-position can be essential to attain the highest possible therapeutic activity of benzimidazole derivatives.

7.3, Potential as NSAID Alternatives, Others have Promising Potential and Justify Lead Optimization and Preclinical Assessment

Such outcomes of the research work give credence to the potential of the benzimidazole derivatives as the ones that could be used as an efficient substitute to NSAIDs in the future due to the dual anti-inflammatory and analgesic effects. SAR analysis indicated that their structure could have been manipulated further by modifying more aptly the substituents on the benzimidazole ring to bring even greater effect on the phamacodynamic profile of the compounds. A prominent characteristic which contributes to their increased activity and binding to their receptors are the electron-withdrawing substituents at the 2-position of the ring.

These compounds hold huge potentials of being developed as alternatives to NSAIDs considering the positive outcomes derived in the in vitro and in vivo assays. They have some pronounced anti-inflammatory and analgesic properties with the possibly lesser side effects since they do not act just by the blockage of COX. In addition, the toxicity experiments showed that these compounds are well-tolerated at therapeutic doses and further confirm that the use of these compounds in the future studies is a viable option.

The optimisation of the lead compounds however needs to be done further in order to enhance the selectivity, pharmacokinetics and safety profiles of the compounds. Specifically, the efforts in the production of selective COX-2 inhibitors, with the minimum gastrointestinal and kidney adverse effects, will prove significant to the progress of the compounds to a clinical program. Besides, additional preclinical studies of long-term toxicity, work in chronic models, and dose response are important prior to clinical studies.

To sum up, the new benzimidazole derivatives discovered during the current study are a leading group of anti-inflammatory and analgesic drugs that could be used as a replacement of classical NSAIDs. The results of the present study allow further optimization of the lead and preclinical testing, which will eventually give rise to the implementation of safer and more efficient therapeutics of inflammatory diseases and pain management.

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Conflicts of interest

The authors have no conflicts of interest to declare

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