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Research Article

In-Silico Design, Synthesis and Biological Evaluation of Some Noval Benzimidazole Derivatives

Akshara Vinayakrishnan*, Aneesha Thomas, Malavika K., Aswagosh K.

College Of Pharmacy, Kannur Medical College Anjarakandy.

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ABSTRACT

Benzimidazole, a heterocyclic compound with versatile biological activity, serves as a scaffold for medicinal chemistry. We aimed to develop new derivatives with anti-inflammatory and anti-bacterial effects. The design was done using software tools, followed by synthesis, isolation, purification and characterization of two derivatives. The two novel derivatives are synthesized by the reaction of benzimidazole with 5-sulphosalicylic acid (BM 1) and 3,5-dinitro salicylic acid(BM 2). Biological activity was evaluated using protein denaturation and agar well diffusion method. The results showed these derivatives have effects comparable to standard drugs, making them promising candidates for further development.

INTRODUCTION

Drug discovery is the process of researching and developing new medications through various stages, such as target identification and validation, hit identification, lead generation and optimization and selecting a candidate for further development. Drug development focuses on optimizing chemical synthesis and formulation, conducting animal safety tests, performing clinical trials and securing regulatory approval. Both processes are costly and time consuming and the industry is currently facing significant challenges due to regulatory requirements environmental issues and reduced profits from patent expiration.

Benzimidazole

Benzimidazole is a heterocyclic compound consist of a fused benzene and imidazole ring in its structure. It is a biologically significant structure, playing a key role in various drugs due to its biological activities such as anti-bacterial, anti-inflammatory, anti-fungal, anti-cancer, etc. and is actively studied for its potential in drug discovery.

***Corresponding Author:** Akshara Vinayakrishnan
Address: College Of Pharmacy, Kannur Medical College Anjarakandy.
Email ✉: aksharvin@gmail.com
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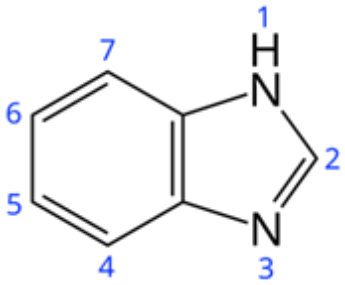


Figure 1: structure of benzimidazole

Plan Of Work

- **Overview**

To design, synthesize, characterize and evaluate the biological activity of benzimidazole derivatives.

- **Criteria for selection**

The selection of benzimidazole derivatives is based on their structural diversity, established pharmacological properties and accessible chemical information with their molecular structures retrieved from data bases like Pub Chem, ChEMBL and others.

- **Preparation of molecular structures**

Using ACD/Labs Chemsketch, KingDraw chemistry station software the molecular structures of benzimidazole derivatives can be generated.

- **Prediction of properties and activity**

Pharmacological properties such as permeability, solubility, lipophilicity, toxicity and metabolic stability can be predicted using software like Molinspiration, Swiss ADME and PASS online.

- **Molecular docking**

Using AutoDock Vina and PyMOL software docking simulation can be carried out to evaluate the binding affinity and activity of the synthesized analogues towards their protein targets.

- **Synthetic strategy**

To synthesize two benzimidazole derivatives using o-phenylenediamine as starting material.

- **Characterization**

Vibrational spectroscopy (IR) can be used for characterization of the synthesized compounds.

- **Evaluation of analogues for activity**

Biological evaluation of the synthesized compounds can be carried out by,

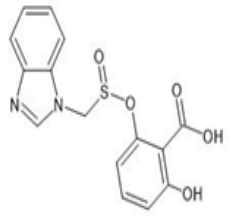
Protein denaturation method for anti-inflammatory activity and agar well diffusion method for anti-bacterial activity.

EXPERIMENTATION

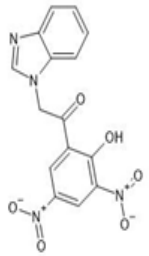
In-silico studies

The software used for in-silico studies includes; Kingdraw, ACD/Labs Chemsketch, molinspiration, PASS online, Swiss ADME, AutoDock Vina and PyMOL.

Chemsketch software were used for constricting and visualizing the molecular structures of the compounds.



5-{[(1*H*-1,3-benzimidazol-1-yl)methanesulfinyl]oxy}-2-hydroxybenzoic acid



2-(1*H*-1,3-benzimidazol-1-yl)-1-(2-hydroxy-3,5-dinitrophenyl)ethan-1-one

Figure 2: structure of synthesized benzimidazole derivatives (BM 1 & BM 2)

- **Molinspiration**

Molinspiration is a software used for prediction and analysis of molecular properties. It calculates a range of molecular descriptors which are essential for evaluating a compounds behavior in biological systems, chemical reactivity or its pharmacokinetic properties. Using this molecular properties and bioactivity of benzimidazole derivatives can be calculated.

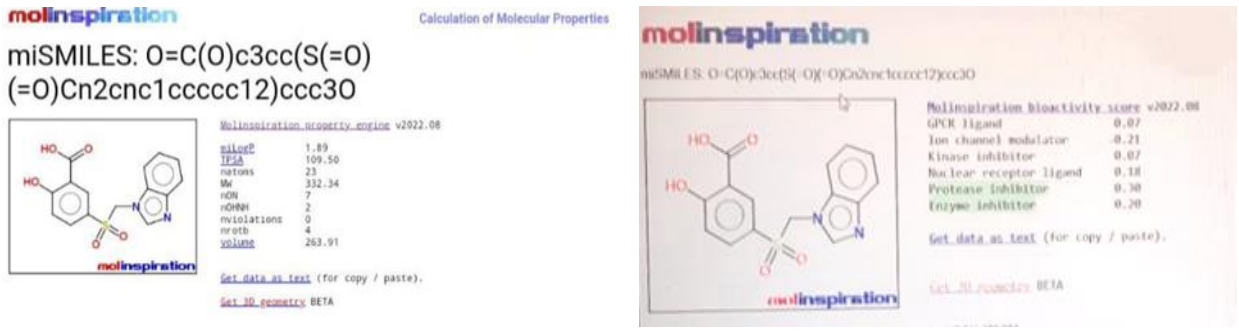


Figure 3: calculation of molecular properties and bioactivity of BM 1

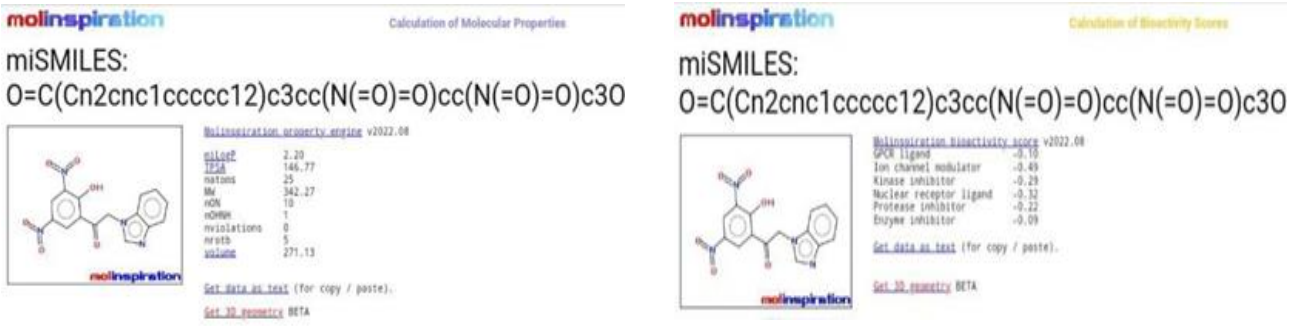


Figure 4: calculation of molecular properties and bioactivity of BM 2

- **Pass Online**

PASS online (Prediction of Activity Spectra for Substances) is a tool used to predict the biological activity of chemical compounds by analyzing their molecular structure. It helps researchers in fields like drug discovery and medicinal chemistry to identify potential therapeutic agents.

- **Swiss ADME**

Swiss ADME provides free access to fast, reliable predictive models for evaluating physicochemical properties, pharmacokinetics, drug-likeness and compatibility with medicinal chemistry, all with easy input and interpretation. Accurate ADME predictions can help prevent unnecessary testing on compounds that are unlikely to succeed and guide the optimization of compounds to enhance their desired characteristics before conducting costly experimental work.

- **Docking**

Docking predicts how a ligand will bind to a target, helping to determine the binding strength. This process involves positioning molecules in the enzyme's active site and uses scoring functions to estimate biological activity. The interaction between the ligand and protein at the binding site is crucial for drug design and understanding biochemical processes. The software tools used for docking are;

*Auto Dock Vina : It is a program used for molecular docking, which predicts how ligands bind to a target protein. It helps in drug discovery and design.

*PyMOL : PyMOL is a software used to visualize and analyze molecular structures in 3D, helping researchers to understand the shape and interactions of molecule.

*PDB : The Protein Data Bank (PDB) is a data base that stores 3D structures of proteins, nucleic acids and other biological molecules, helping researchers study their shapes and functions.

S/No.	Target	PDB ID
1	Cyclooxygenase- 1	6Y3C

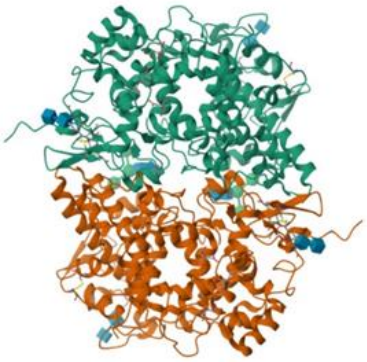


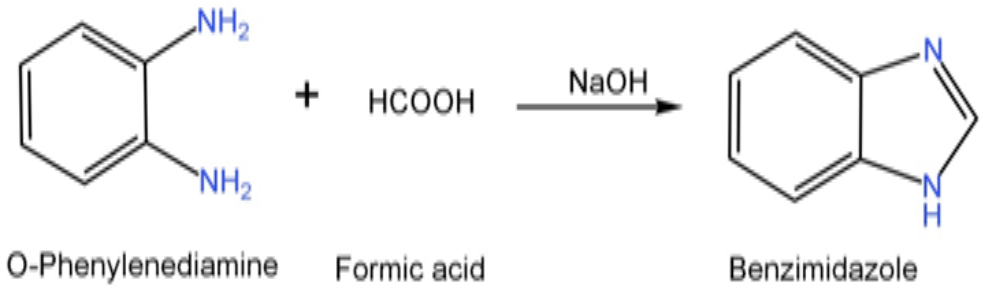
Figure 5: structure of binding protein

Synthesis

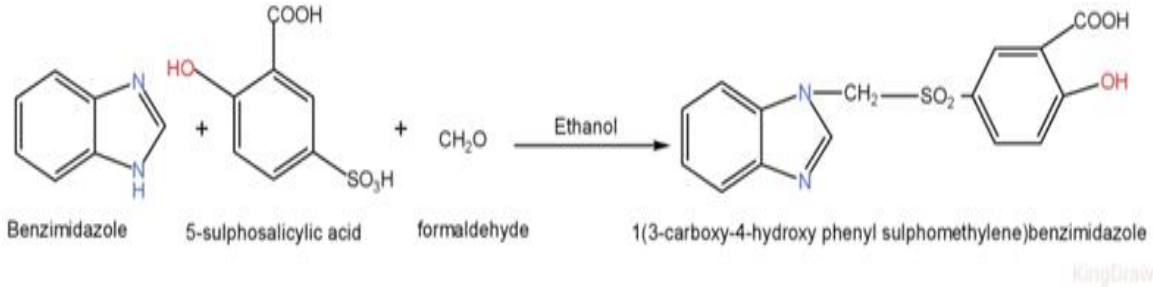
Synthetic scheme

Scheme-1

Step-1: Synthesis of benzimidazole from o-phenylenediamine.

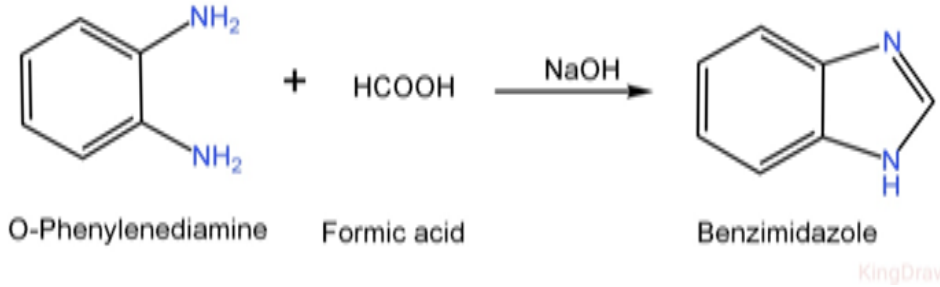


Step-2: Synthesis of benzimidazole derivative-1(BM 1) from 5-sulphosalicylic acid and formaldehyde.

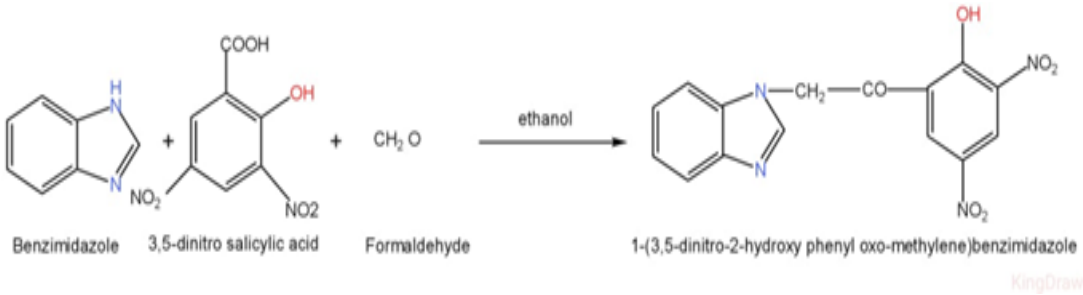


Scheme-2

Step-1: Synthesis of benzimidazole from o-phenylenediamine.



Step-2: Synthesis of benzimidazole derivative-2(BM 2) from 3,5-dinitrosalicylic acid and formaldehyde.



Synthesis of benzimidazole derivative-1(BM 1)

Benzimidazole was synthesized using previously reported method. A mixture of benzimidazole (1g), formaldehyde (1.5ml) and 5-sulphosalicylic acid (1g) in ethanol (20ml) was heated under reflux for 3 hour. Subsequently ethanol was distilled off and the reaction mixture is cooled to obtain the product. The separated product is filtered, dried and recrystallized using suitable solvent.

Synthesis of benzimidazole derivative-2(BM 2)

Benzimidazole was synthesized using previously reported method. A mixture of benzimidazole (1g), formaldehyde (1.5ml) and 3,5-dinitrosalicylic acid (1g) in ethanol (20ml) was heated under reflux for 3 hour. Subsequently ethanol was distilled off and the reaction mixture is cooled to obtain the product. The separated product is filtered, dried and recrystallized using suitable solvent.

Evaluation Of Biological Activity.

Evaluation of anti-inflammatory activity-Protein denaturation method

The method of protein denaturation is used for evaluating the efficacy of anti-inflammatory agents. This method involves, the reaction mixture is 0.2 ml of egg albumin, 2.8ml of normal saline and 2ml of varying concentration of sample (25,50,75,100µg/ml). 2ml of egg albumin and 4.8ml of double distilled water taken as control. Incubate the mixture (37ºC) for 30 minutes and heated at 70ºC in a water bath for 15 minutes. After cooling the absorbance was measured by UV spectrophotometer (280nm) using triple-distilled water as blank. The percentage inhibition was calculated by using the formula:

$$\% \ Inhibition = \frac{absorbance \ of \ test \ sample}{absorbance \ of \ control} \times 100$$

Evaluation of anti-bacterial activity-Agar well diffusion method

The agar well diffusion technique is commonly employed to assess the anti-microbial properties of a test sample.

Mueller-Hinton agar (15-20ml) was poured into sterile glass petri dishes of equal size and allowed to solidify. A standardized inoculum of test organism was evenly spread over the surface of the agar using a sterile cotton swab. Four wells, each 8mm in diameter and spaced 20mm apart, where aseptically created in each plate using a sterile cork borer. The test sample was added to wells T1 and T2 from a 10mg/ml stock solution. Gentamycin and methanol were used as the positive and negative controls respectively. The plates were incubated for 24 hours at 37ºC under aerobic conditions. Following incubation, the plates were examined and zone of inhibition of bacterial growth surrounding the wells was measured in millimeters.

Inoculum details: Inoculums were obtained from The Microbial Type Culture Collection (MTTC) Chandigarh.

Name of Microorganism	MTCC No.	Incubation conditions
Escherichia coli	443	37°C for 24 hours

RESULTS AND DISCUSSION

This work entitled “In-silico design, synthesis and biological evaluation of some novel

benzimidazole derivatives" reveals the significance of rational insilico design and development of benzimidazole derivatives as anti-inflammatory and anti-bacterial agents.

In-silico studies

- ACD/Lab Chemsketch

The derivatives where designed and the molecular descriptors were analyzed.

Table 1: molecular descriptors for designed derivatives

Name	Molecular weight	Molar volume(cm^3)	Parachor (cm^3)	Surface Tension(dyne/cm)	Polarizability (cm^3)	Molar refractivity(cm^3)
BM 1	332.33	211.5±7.0	624.8±8.0	76.1±7.0	$33.20±0.5×10^{-24}$	83.76±0.5
BM 2	342.26	209.2±7.0	625.2±8.0	79.6±7.0	$33.32±0.5×10^{-24}$	84.06±0.5

Table 2: analysis of drug likeness score for standard drug

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand
Diclofenac	0.14	0.20	0.17	0.09
Ibuprofen	-0.17	-0.01	-0.72	0.05

Table 3: analysis of drug likeness score for derivatives

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand
BM 1	0.07	-0.21	0.07	0.18
BM 2	-0.10	-0.49	-0.29	-0.32

Table 4: analysis of Lipinski's rule of five for standard drug

Compound	Log P	Molecular weight	nON	nOHNH	No. of rotable bonds	Violations
Diclofenac	4.5	296.15	3	2	4	0
Ibuprofen	3.46	206.28	2	1	4	0

Table 5: analysis of Lipinski's rule of five for derivative

Compound	Log P	Molecular weight	nON	nOHNH	No. of rotable bonds	Violations
BM 1	1.89	332.34	7	2	4	0
BM 2	2.20	342.27	10	1	5	0

All the compounds obeyed the Lipinski's rule of five and showed good likeness score.

- **Pass Online**

Benzimidazole exhibits a broad spectrum of pharmacological effects, and the synthesized derivatives were assessed for their activities. The biological activity of the derivatives was predicted using the PASS online tool, with the results summarized in the table below:

Table 6: prediction of biological activity of derivatives using PASS software

Compound	Effect of anti-inflammatory		Effect of anti-bacterial	
	Pa	Pi	Pa	Pi
BM 1	0,344	0,043	0,345	0,045
BM 2	0,158	0,100	0,226	0,097

- **ADME**

Using Swiss ADME the pharmacokinetic properties of derivatives were analyzed.

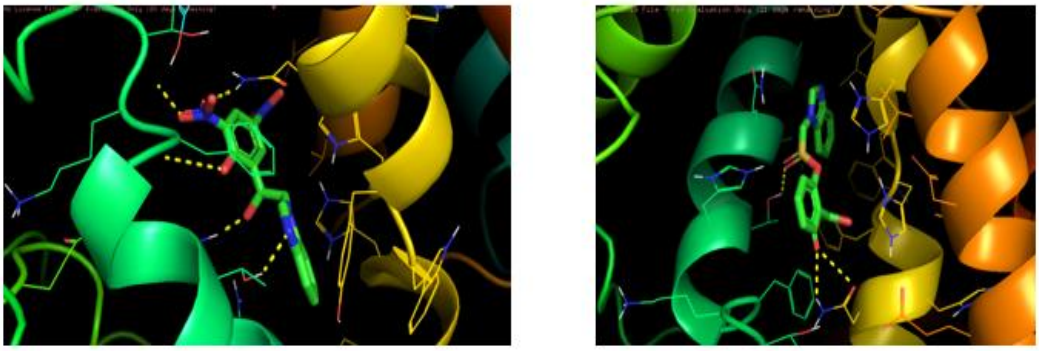
Table 7: prediction of pharmacokinetic properties by Swiss ADME

Compound	Log P	Log S	GI absorption	BBB permeation	Log K_p (cm/s)	Bioavailability
BM 1	1.54	Soluble	High	No	-6.81	0.56
BM 2	0.70	Soluble	Low	No	-6.40	0.55

The designed derivatives show greater solubility. Compound BM 1 shows greater gastro-intestinal absorption. The compounds are not able to cross the Blood Brain Barrier. Both compounds show good bioavailability score.

- **Docking**

The docking is carried out by using AutoDock Vina and PyMOL and tabulated below:



(a)BM 1 **(b) BM 2**

Figure 6: docking images of benzimidazole derivatives on binding protein 6Y3C

Table 8 : docking scores of derivatives with target protein by AutoDock Vina

Compound	Binding affinity
BM 1	-8.6
BM 2	-9.1

Proposed derivatives were subjected to flexible docking on to the binding site of COX-1 using AutoDock Vina and PyMOL. The compound BM 2 shows more binding affinity compared to BM 1.

Synthetic Methodology

The synthetic scheme involved a two step reaction process. The starting material for the synthesis was o-phenylenediamine, from which benzimidazole was synthesized. The second step involves reaction of benzimidazole with 5-sulphosalicylic acid and 3,5- dinitro salicylic acid to produce different derivatives.

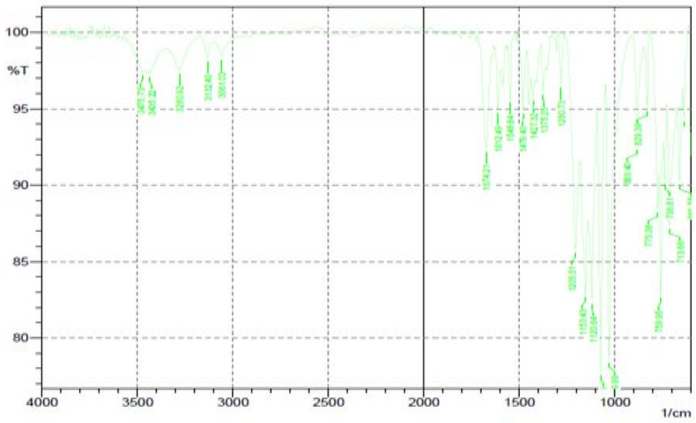
Table 9: description of synthesized compounds

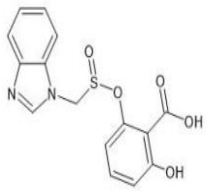
Compound	Molecular formula	Molecular weight	Density (g/cm^3)	Practical yield (g)
BM 1	$C_{15}H_{12}N_2O_5S$	332.33	1.57±0.1	3.2
BM 2	$C_{15}H_{10}N_4O_6$	342.26	1.63±0.1	1.5

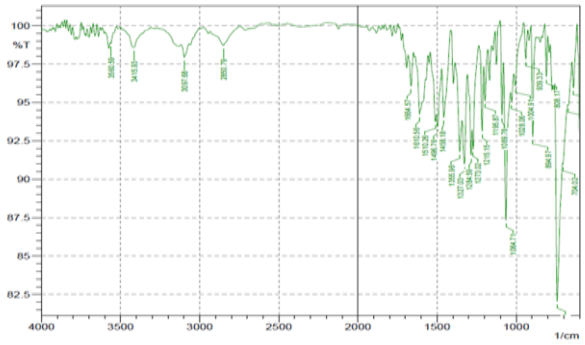
Figure 7: synthesized derivatives (BM 1 & BM 2)

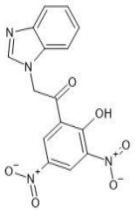
Characterization

The synthesized compounds were characterized using analytical and spectral techniques, which are especially effective for identifying the functional groups in a molecule.



Compound	IR characterization(cm^{-1})
	C=C-1612.49 stretching, C-N-1029.99 SO_2-1153.43, C=O stretching, OH-3475.73 Ar-OH-3435.22



Compound	IR characterization(cm^{-1})
	C=C-1612.49 stretching, C-N-1029.99, SO_2-1153.43, COOH-1674.21,C=O stretching OH-3475.73, Ar-OH-3435.22

FT-IR spectra and spectral analysis of benzimidazole derivatives BM 1 and BM 2

Evaluation of anti-inflammatory activity by protein denaturation method

Biological activity

Table 10: comparative evaluation of anti-inflammatory effect of BM 1, BM 2 with Diclofenac sodium

Compound	Concentration (µg/m l)	Absorbance(nm)	Percentage inhibition
Control	-	0.045	0
BM 1	25	0.007	15
	50	0.019	42.2
	75	0.020	44.4
	100	0.021	46.6
BM 2	25	0.009	20
	50	0.014	31.1
	75	0.034	75.5
	100	0.039	86.6
Diclofenac sodium	25	0.011	24.4
	50	0.013	28.8
	75	0.020	44.4
	100	0.040	88.8

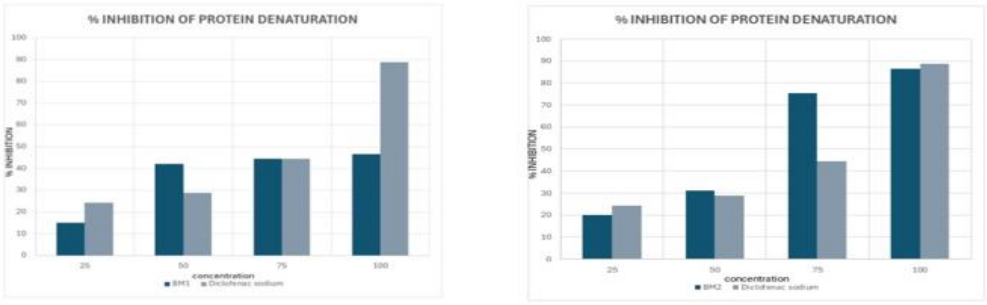


Figure 8: comparative evaluation of anti-inflammatory effect of BM 1, BM 2 with diclofenac sodium

The percentage inhibition of protein denaturation of standard and BM 1 was found to be similar at 75µg/ml and percentage inhibition of standard and BM 2 was found to be similar at 50 and 100 µg/ml concentration.

Evaluation of anti-bacterial activity

Evaluation of anti-bacterial activity by agar well diffusion method. Plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm which is tabulated below:

Figure 9: agar well diffusion method for anti-bacterial activity of benzimidazole derivatives

Table 11: comparative evaluation of anti-bacterial effect of BM 1, BM 2, and gentamycin

Name of microorganism	Sample code	Zone of inhibition (mm)			
		Standard Gentamycin (160 µg)	Negative Control	T1 (500 µg)	T2 (1000µg)
Escherichia coli	BM 1	+ve (23mm)	-ve	-ve	+ve (10mm)
	BM 2	+ve (23mm)	-ve	+ve (9mm)	+ve (10mm)

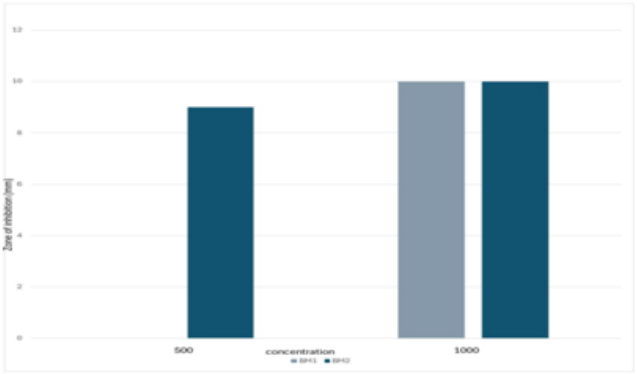


Figure 10: graphical interpretation of agar well diffusion method of BM 1 and BM 2

The agar well diffusion method for benzimidazole derivatives showed anti-bacterial activity. BM 2 showed anti-bacterial activity at both concentrations, while BM 1 showed activity only at 1000µg.

CONCLUSION

This study resulted in the creation of novel benzimidazole derivatives, focusing on structure based drug design and the development of these derivatives, followed by their biological evaluation. Two new

analogues were designed, synthesized (BM 1&BM 2) and characterized through solubility and FT-IR analysis after in-silico molecular modeling and docking studies. Anti-inflammatory and anti-bacterial activities were assessed and compared with standard drugs. The analogues exhibited comparable activity to the standards, suggesting that these benzimidazole derivatives could serve as promising candidates for the development of effective anti-inflammatory and anti-bacterial agents.

ACKNOWLEDGEMENT

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