BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, September 2024. Vol. **21**(3), p. 1241-1253

Insilico and Biological Evaluation of Anti-Inflammatory Activity of synthesized Benzimidazoles Derivatives

Khemchand R. Surana[1]*, Pallavi S. Jadhav[1], Harshada S. Shewale[1], Dipa B. Wagh[1], Sunil K. Mahajan[1], Jayesh V. Musale[2], Jubershaha S. Fakir[3] and Yogesh P. Sharma[4]

[1]Department of Pharmaceutical Chemistry, Shreeshakti Shaikshanik Sanstha, Divine College of Pharmacy, Satana, Nashik, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra 423301, India.
[2]Department of Pharmaceutics, Mahatma Gandhi Vidyamandir's SPH College of Pharmacy, Malegaon, Nashik, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India-423203.
[3]Department of Pharmacology, Shreeshakti Shaikshanik Sanstha, Divine College of Pharmacy, Satana, Nashik, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra 423301, India.
[4]Department of Pharmaceutics, SSS's Divine College of Pharmacy, Nampur Road, Satana, Nashik, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India – 423301.

http://dx.doi.org/10.13005/bbra/3300

(Received: 03 July 2024; accepted: 05 October 2024)

We have developed a mild, easy, and highly efficient green catalyst for the synthesis of 2-substituted benzimidazole. In this study, Ace-dock and DockThore performed molecular docking of the designed benzimidazole molecules with the selected protein FAAH (PDB ID: 3LJ7). We assessed the drug's likeliness (Lipinski's rule of 5) and potential toxicity using the Protox-II software. We can confidently state that the synthesized molecules adhere to Lipinski's rule of five, given that the design molecules' properties are within acceptable limits. In comparison to the reference Ibuprofen, the proposed compounds exhibited favorable pharmacokinetic properties and achieved docking scores ranging from -10.88 to -27.31 (Acedock) and -6.045 to 9.122 (DockThore). We synthesized the benzimidazole derivatives 3a to 3g. Based on an in silico study, we synthesized the molecules, chose the best ones, and then tested their anti-inflammatory action in a lab setting. We employed the albumin denaturation assay test to determine the extent of heat-induced protein denaturation inhibition. Both of the synthesized compounds and the standard drug, diclofenac sodium, inhibit denaturation of proteins at concentrations between 10 and 50 ppm. At a dose of 10 ppm, compound 3f showed the highest level of inhibition, at 70%. Diclofenac sodium exhibited the highest suppression, measuring 97.20% at a concentration of 40 ppm. We could further investigate 3F to determine its anti-inflammatory characteristics.

Keyword: AceDock, Anti-inflammatory activity, Benzimidazoles, DockThor, Protox-II software.

Imidazole heterocycle is found in benzimidazoles, which are the benzo derivatives of imidazole [1]. The word used to refer to the parent compound in the series is widely accepted, and the numbering scheme follows the standardized pattern for heterocyclic compounds. Heteroaromatic compounds have garnered significant attention due to their role in the development of intricate

*Corresponding author E-mail: khemchandsurana772@gmail.com

organic materials and physiologically active molecules [2]. Organic molecules containing nitrogen atoms in their ring structures, known as nitrogen-containing heterocyclic compounds, have attracted considerable attention due to their relevance in pharmacology and biology [3]. Scientists are continuously seeking novel applications and purposes for this heterocycle, which has been widely utilized in medicinal chemistry and drug development because of its benzimidazole structural motif [4]. Compounds containing benzomidazole display a wide range of biological and therapeutic properties, such as anti-inflammatory, analgesic, antibacterial, antifungal, antiviral, antidepressant, anticonvulsant, anticancer, and anti- diabetic effects [5]. Hoebrecker synthesized the inaugural benzimidazole compound in 1872 through the reduction of 2-nitro-4-methyl acetanilide. In recent times, a multitude of researchers have reported a range of methods for synthesizing benzimidazole derivatives with 1-substituted or 1-disubstituted structures [6]. These methods involve the use of different chemical groups and are carried out under different atmospheric reaction circumstances [7]. Fused imidazole derivatives have become prominent in the field of medicinal chemistry due to their significant features as clinically useful medications [8]. Consequently, the pharmaceutical sector is researching benzimidazole, and its derivatives have been found in several medical uses. Benzimidazole derivatives demonstrate a diverse array of pharmacological effects as a result of their adaptable core, which is found in multiple molecules [9, 10].

EXPERIMENTAL METHODS

Molecular Docking

Selection of Ligand: There are a lot of written descriptions of synthetic benzimidazole molecules and derivatives, which is why we chose to focus on this moiety for our in silico study. In addition, we kept making new substances while we were doing our study [11, 12].

Selection of Protein

The enzyme fatty acid amide hydrolase hydrolyzes anandamide, an endocannabinoid. The enzyme fatty-acid amide hydrolase breaks down the endocannabinoid anandamide inside cells. Only in some cells does epirazole successfully halt this process [13]. The fact that epirazole (Figure 2) prevents anandamide oxidation while maintaining carrier-mediated absorption is interesting. This prevents the breakdown of anandamide, leading to its accumulation in neurons and eventual release from the cells [14, 15].

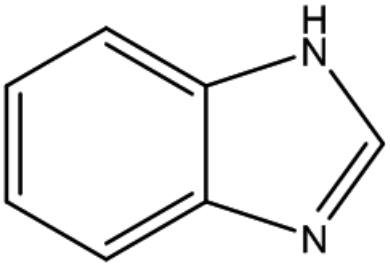


Fig. 1. benzimidazole Structure (Self prepared with the help of ChemBio Draw Ultra 14.0)

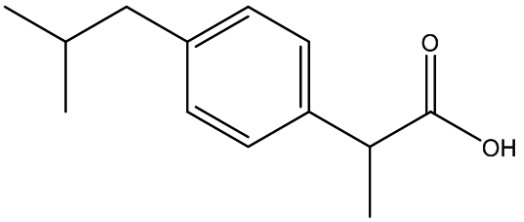


Fig. 2. Ibuprofen Structure (Self-prepared with the help of ChemBio Draw Ultra 14.0)

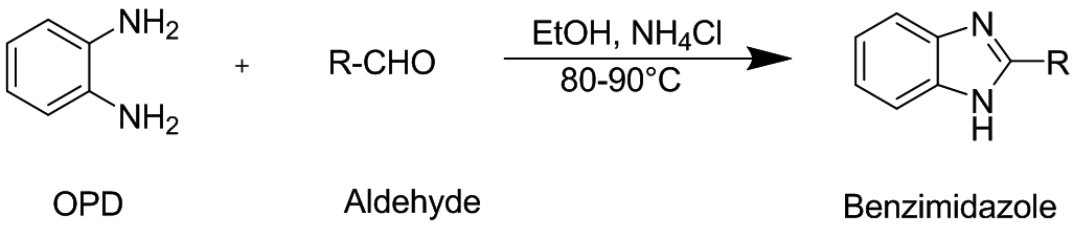


Fig. 3. Synthetic Scheme for benzimidazole derivatives

Molecular Docking

For molecular docking of benzimidazole derivatives, one can use various computational tools like Ace-dock and DockThore. We used the Protein Data Bank (PDB ID: 3LJ7) to obtain the protein's structure. Next, we extracted the heteroatoms and water molecules from the structure to deprotonate it. The protein's structure determines the binding sites. We used Chem-Draw software to display the molecules' structures. After changing its shape, we labeled the molecule as a ligand. We created a receptor grid for ligand docking and recorded the found docking number [16-18].

Toxicity studies

Toxicity studies involve the application of predictive toxicology in the development of ecologically friendly products and chemicals that are less harmful. This approach helps reduce exposure and waste [19]. The Protox-II software was utilized to forecast the organ

Molecular docking: Following are the design molecules for molecular docking

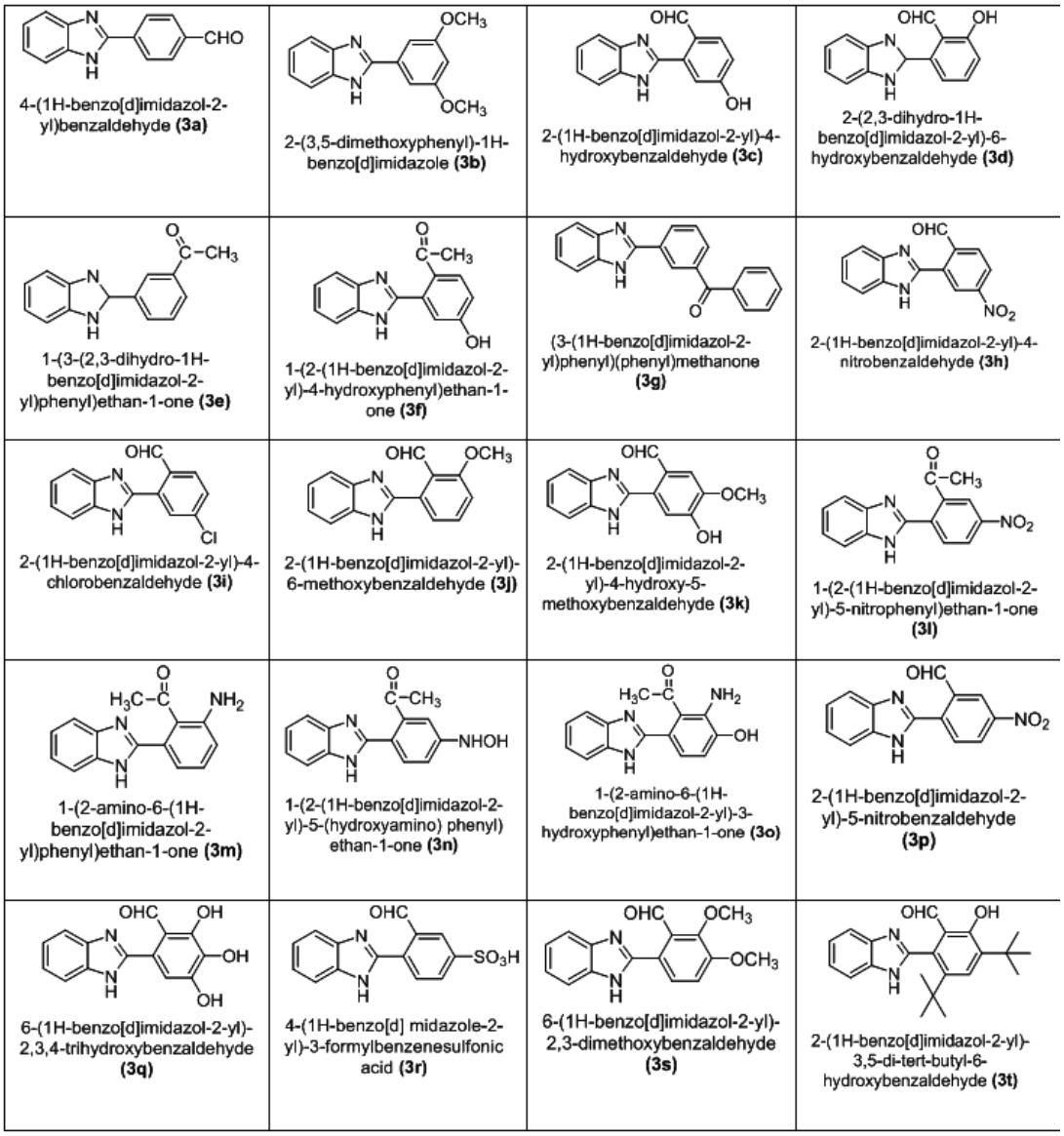


Fig. 4. Benzimidazole design molecules for molecular docking

toxicities and toxicological endpoints of the ligands, encompassing their LD50 values. The Protox-II software can be used to determine the hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity of the substances. Illustrate the molecular configuration, select the predetermined degree of toxicity, and record the resulting consequence [20, 21].

ADME Properties

For the ADME, it is very important to get the factors of absorption, distribution, metabolism, and excretion just right. It is well known that people with these traits are good candidates for medicines. Along with how well and selectively it works, a medicine's success depends on how badly it is supposed to be broken down by the body [22]. In the early stages of ADME predictions, a few basic ADME or ADME-related factors are often used. These include intrinsic solubility (logS), partition coefficient (logP), and apparent partition coefficient (logD). We used Protox-II software to guess the ADME properties of a certain molecule based on these qualities. Lipinsky's rule of five is used to figure out factors like molecular weight, logP, hydrogen bond acceptors and hydrogen bond donors [23, 24].

Table 1. Comparative study using different docking software

Sr. No.	Molecule	DockThor	ACE DOCK
	Standard		
1	Ibuprofen	-5.656	-11.88
	Benzimidazole derivatives		
1	3a	-6.444	-25.93
2	3b	-9.122	-10.88
3	3c	-6.226	-19.71
4	3d	-8.318	-15.89
5	3e	-6.535	-21.65
6	3f	-6.231	-21.85
7	3g	-6.621	-17.72
8	3h	-6.045	-23.61
9	3i	-6.458	-17.27
10	3j	-6.432	-17.72
11	3k	-7.432	-18.82
12	3l	-6.407	-18.73
13	3m	-8.874	-18.38
14	3n	-8.140	-25.71
15	3o	-6.483	-14.69
16	3p	-6.424	-18.71
17	3q	-6.424	-27.31
18	3r	-5.565	-30.00
19	3s	-7.633	-18.64
20	3t	-6.172	111.67

Synthesis of benzimidazole derivatives

We combined 0.15 grams (30%) of NH4Cl with 0.100 grams (0.92 mmol) of OPD and 0.125 grams (0.92 mmol) of anisaldehyde in 4 mL of ethanol. We stirred the mixture for two hours at a temperature of 80 °C. We determined the conclusion of the TLC reaction using a solvent mixture of ethylacetate and hexane at a ratio of 1:2 v/v. When the reaction mixture was added to ice-cold water, a pale yellow solid formed as a precipitate. After filtering the contents, we subjected the product to two water washes, dried it, and then recrystallized it with ethanol to produce a pure product [25, 26].

Evaluation of Anti-inflammatory activity

For the purpose of determining the anti-inflammatory efficacy in vitro, a protein denaturation experiment was conducted utilizing egg albumin as the experimental protein.

Procedure

Preparation of Control Solution: In order to prepare the control solution, 2 ml of freshly prepared egg albumin were combined with 20 ml of distilled water to get 50 ml [27].

Preparation of Standard Solution: 2 ml of freshly prepared egg albumin (pH 6.4) were combined with 28 ml of phosphate buffer saline. Subsequently, a 20 ml solution of diclofenac sodium varying in concentration from 10 to 50 ppm was added to make the 50 ml standard solution.

Preparation of Test Solution: Test Solution was prepared by adding 20 milliliters of the synthesized drug solution, which ranged in concentration from 10 to 50 ppm, to 2 milliliters of freshly made egg albumin, further it was mixed with the 28 milliliters of phosphate buffered saline (pH 6.4).

All prepared solutions was subjected to heat for 5 minutes at 70 °C in a water bath and incubated for 15 minutes at 37 ± 2 °C. We allowed room-temperature cooling for the solutions. Next, we used a vehicle and a UV-visible spectrophotometer to determine the absorbance at 660 nm [28-30].

RESULTS

A comprehensive analysis was carried out on the molecular structure of benzimidazole derivatives, including their docking scores and interactions with certain receptor proteins (Table 1). The compounds 3r (DockThore) and 3r (Ace dock) exhibited the highest affinity (docking score)

Interactions of Ibuprofen with FAAH Protein (PDB ID: 3LJ7):

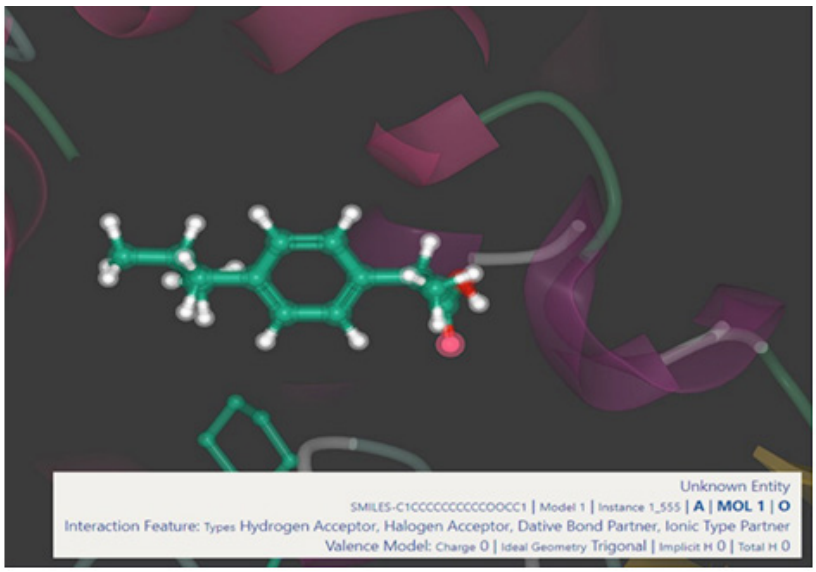


Fig. 5a. The fatty acid amide hydrolase (PDB ID: 3LJ7) protein and Ibuprofen drug receptor interaction were represented in 3D using AceDock software. (3D representation of the drug receptor interaction diagram available after molecular docking study by using AceDock software)

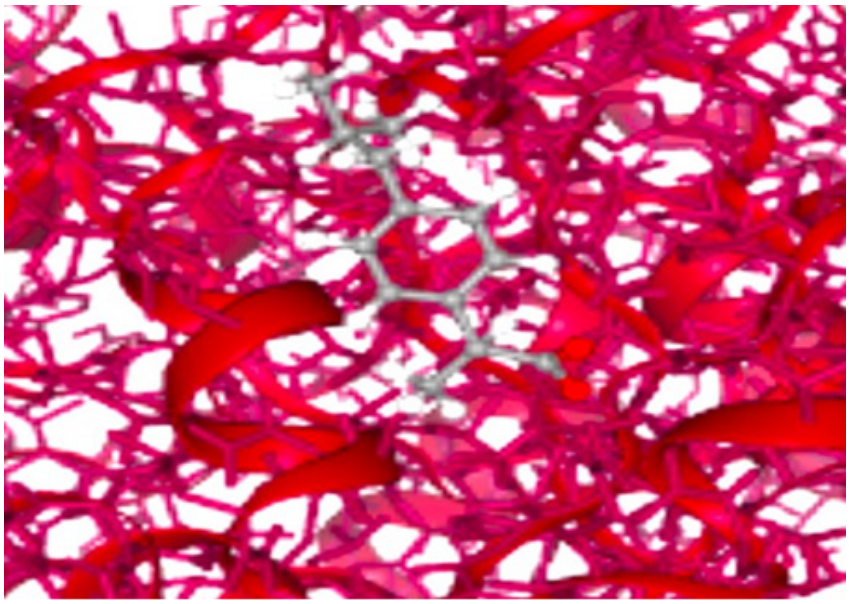


Fig. 5b. Ibuprofen drug receptor interaction with the protein fatty acid amide hydrolase (PDB ID: 3LJ7) is represented in 3D using DockThore software. (3D representation of the drug receptor interaction diagram available after molecular docking study by using DockThore software)

Interaction of benzimidazole molecule with FAAH protein (PDB ID: 3LJ7)

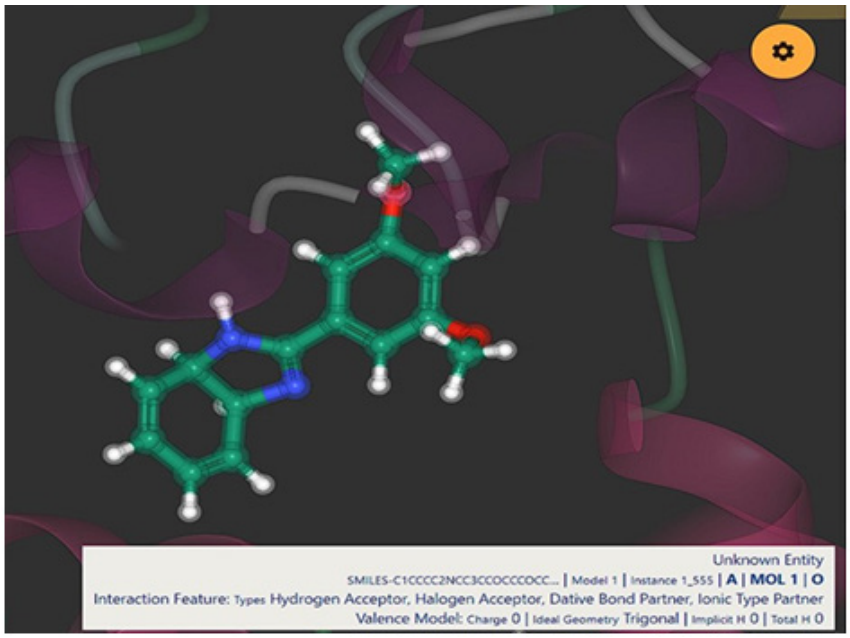


Fig. 6a. The figure illustrates the 3D representation of the drug interaction between 2-(3,5-dimethoxyphenyl)-1H-benzo[d]imidazole (3b) and the fatty acid amide hydrolase (PDB ID: 3LJ7) protein by usning Ace dock. (3D representation of the drug receptor interaction diagram available after molecular docking study by using AceDock software)

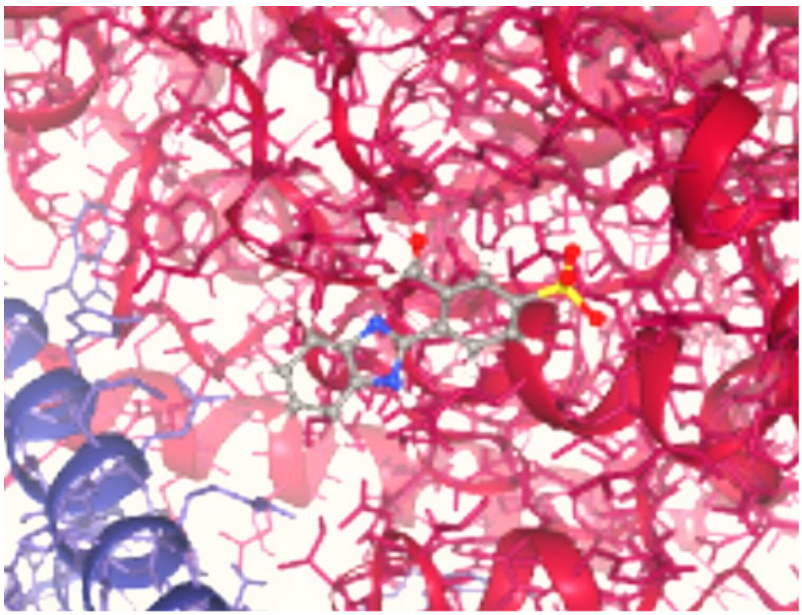


Fig. 6b. The 3D representation depicts the drug interaction between 4-(1H-benzo[d]imidazole-2-yl)-3-formylbenzenesulfonic acid (3r) and the fatty acid amide hydrolase (PDB ID: 3LJ7) protein by using DockThore) (3D representation of the drug receptor interaction diagram available after molecular docking study by using DockThore software)

of -9.122 and -30.00, respectively, towards the FAAH (PDB ID: 3LJ7) receptor. This indicates that these molecules should be further investigated in vitro. To facilitate comparisons, this molecule is also juxtaposed with the reference molecule, ibuprofen. The molecule exhibits a docking score (affinity) of -5.656 (DockThore) and -11.88 (Ace dock). In addition, both the ligand molecule for the FAAH protein and the other proposed compounds exhibited docking scores (Affinity) ranging from -6.045 to 9.122 (DockThore) and -10.88 to -27.31 (Acedock), which fall within the permitted range of docking scores for conventional Ibuprofen. These chemicals, which have been specifically developed, may possess anti-inflammatory characteristics due to their high docking score (Figure 5a, 5b, 6a and 6b).

All the previously mentioned features were within acceptable thresholds, and the molecules under design exhibited appropriate pharmacokinetics. The compound exhibited hydrogen bond donor and acceptor values that met the specified criteria. We determine acceptable ranges for both the molecular weight and the expected partition coefficient.

The molecular weight of ibuprofen is typically 206.28 g/mol, but the molecular weights of the design molecules range from 222.24 to 350.45 g/mol. Ibuprofen possesses a log P value of 3.07, and its designed molecule exhibits a range of 2.75 to 5.34. Ibuprofen is composed of one hydrogen bond donor and 20 hydrogen bond acceptors. The proposed molecules also have a similar number of hydrogen bond donors and acceptors, which is considered acceptable. The Lipinski rule of five is an essential criterion for developing novel molecules.

Assessing the molecules toxicity level is a pivotal step in the advancement of new pharmaceuticals. Computational methods for toxicity prediction provide a faster alternative, perhaps decreasing the necessity for animal testing. We derived the toxicity prediction from six distinct targets, including hepatotoxicity, carcinogenicity,

Table 2. ADME Properties benzimidazole molecules

Molecule Number	Compound	Mass	Log P	H Bond Acceptor	H Bond Donar
			Standard		
1	Ibuprofen	206.28	3.07	20	1
			Benzimidazole Derivatives		
1	3a	222.24	3.04	12	1
2	3b	254.28	3.25	17	1
3	3c	238.24	2.75	13	2
4	3d	252.27	3.14	15	2
5	3e	236.27	3.43	14	1
6	3f	238.24	2.75	13	2
7	3g	298.34	4.46	16	1
8	3h	251.24	3.44	13	1
9	3i	472.31	2.84	12	1
10	3j	252.27	3.05	15	1
11	3k	268.27	2.76	16	2
12	3l	265.27	3.83	15	1
13	3m	251.28	3.6	16	2
14	3n	267.28	3.31	17	3
15	3o	267.28	3.3	17	3
16	3p	251.24	3.44	13	1
17	3q	270.24	2.16	15	4
18	3r	270.31	3.21	13	2
19	3s	281.29	3.06	18	1
20	3t	350.45	5.34	29	2

and cytotoxicity, all of which are associated with unfavorable pharmaceutical effects. Each compound showed signs of hepatotoxicity 3s and 3t. Several compounds, specifically 3a, 3e, 3g, 3h, and 3r, do not exhibit any activity. A red, dark tint indicates the presence of the 3s active component in immunotoxicity. The remaining substances exhibit activity in the mutagenicity test, whereas 3a, 3g, 3i, and 3r are devoid of activity. All cytotoxicity molecules are inert, as evidenced by their dark green color. We used the Protox-II server to investigate toxicity.

In order to investigate the fundamental process behind the anti-inflammatory effects, we assessed the compound's capacity to hinder protein denaturation. The results show that both the chemical that was synthesized and Diclofenac sodium can stop protein denaturation, but the amount needed depends on the concentration, which is between 10 and 50 ppm. At a concentration of 40 ppm, the chemical 3f exhibits the greatest inhibition, reaching 70%. In comparison, diclofenac sodium demonstrates a maximum inhibition of 97.20% at the same concentration (Figure 7).

DISCUSSION

Inflammation is a widespread condition that impacts a substantial proportion of the world's population. We are currently synthesizing compounds with potential anti-inflammatory properties. We have chosen the benzimidazole

Table 3. Prediction of insilico toxicity of benzimidazole molecule

Sr. No.	Design Molecule	Hepatotoxicity with threshold value	Carcinogenicity with threshold value	Immunotoxicity with threshold value	Mutagenicity with threshold value	Cytotoxicity with threshold value
			Standard			
1	Ibuprofen	0.66	0.74	0.99	0.99	0.85
			Benzimidazole Derivatives			
1	3a	0.64	0.58	0.98	0.55	0.82
2	3b	0.50	0.50	0.94	0.73	0.70
3	3c	0.58	0.51	0.95	0.62	0.76
4	3d	0.55	0.52	0.74	0.64	0.77
5	3e	0.59	0.53	0.99	0.53	0.81
6	3f	0.58	0.51	0.95	0.62	0.76
7	3g	0.62	0.54	0.99	0.52	0.78
8	3h	0.62	0.74	0.95	0.80	0.81
9	3i	0.54	0.63	0.95	0.51	0.83
10	3j	0.50	0.56	0.74	0.67	0.75
11	3k	0.54	0.56	0.54	0.69	0.67
12	3l	0.59	0.77	0.79	0.79	0.80
13	3m	0.56	0.74	0.88	0.69	0.75
14	3n	0.67	0.70	0.86	0.78	0.79
15	3o	0.58	0.65	0.80	0.71	0.69
16	3p	0.59	0.74	0.67	0.80	0.81
17	3q	0.54	0.55	0.79	0.58	0.78
18	3r	0.59	0.57	0.81	0.54	0.67
19	3s	0.52	0.59	0.72	0.69	0.72
20	3t	0.56	0.53	0.60	0.57	0.80

Color coding represents
Active Below Threshold
Inactive Below Threshold
Active Above Threshold
Inactive Even if above Threshold

Table 4. Synthesized benzimidazole molecules and their percentage yields and melting point

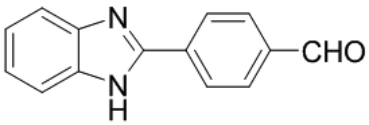
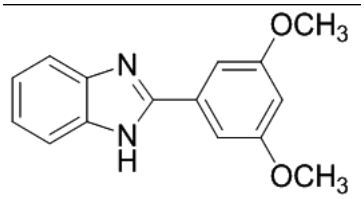
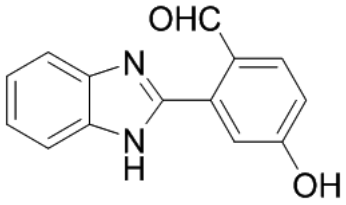
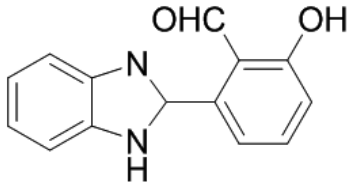
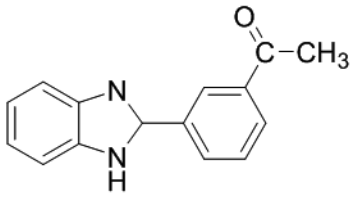
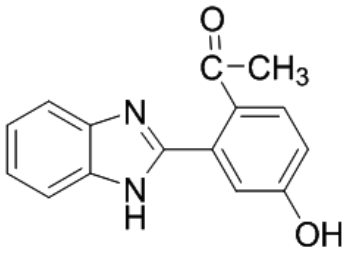
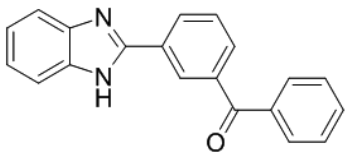
Sr.. No.	Synthesized molecules	Code of molecules	Percentage yield	M.P (°C)
1		3a	25.35	216
2		3b	23.56	230
3		3c	21.28	190
4		3d	72.56	220
5		3e	17.90	217
6		3f	39.74	195
7		3g	23.39	230

Table 5. Heat induced protein denaturation effect on synthesized compounds

Concentration (ppm)	Absorbance Control	Standard	3c	3f
10ppm	0.036	0.0021	0.034	0.058
20ppm	0.036	0.0025	0.042	0.038
30ppm	0.036	0.003	0.039	0.041
40ppm	0.036	0.001	0.051	0.061
50ppm	0.036	0.007	0.041	0.037

Table 6. Percentage inhibition activity of test compounds control and standard sample

Concentration (ppm)	Percentage inhibition Control	Standard	(3c)	(3f)
10ppm	0	94.16	5	61.6
20ppm	0	93	38.3	5.5
30ppm	0	91.6	8.6	16.1
40ppm	0	97.2	41.9	70
50ppm	0	2.9	13.8	3.3

Each value represents the mean (n=3).

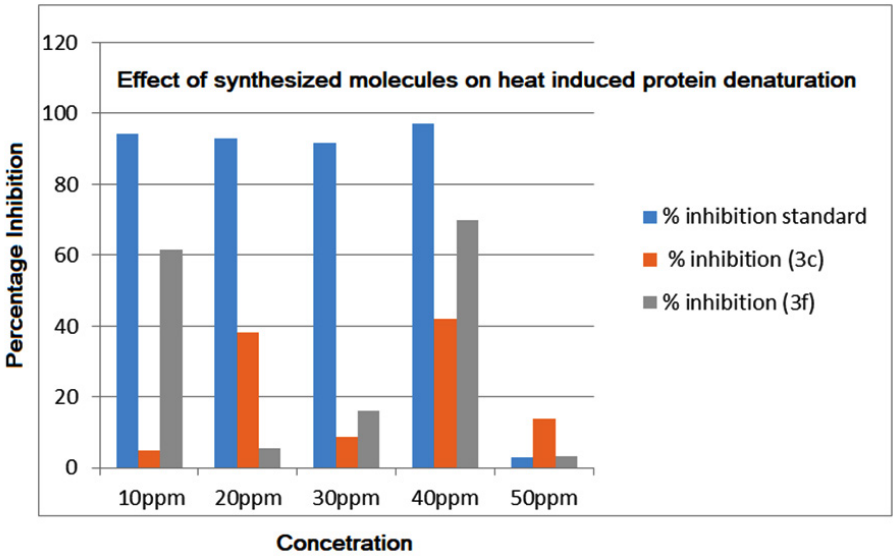


Fig. 7. Effect of synthesized molecules on heat induced protein denaturation (Self prepared by using Microsoft Excel)

moiety as the essential building block for synthesizing the desired compounds. We performed our computational analysis utilizing Ace Dock and DockThore. For this investigation, we assessed the anti-inflammatory characteristics of each synthesized molecule and compared them. Based on the docking results (Figure 2), we have selected the benzimidazole derivatives for further analysis. We will analyze their interaction with specific receptor proteins. With scores of -9.122 and -30.00, respectively, the compounds 3r (DockThore) and 3r (Ace dock) had the highest docking scores (affinities) with the FAAH receptor (PDB ID: 3LJ7). Therefore, it is necessary to conduct additional in vitro research on these compounds. Consequently, there is potential for further in vitro investigation on these compounds. In addition, we provide a baseline for comparison by juxtaposing this chemical with the reference compound, ibuprofen. Its docking scores (affinity) for the FAAH protein and the other suggested compounds were between -6.0455 and 9.122 (Dock Thore) and -10.88 to -27.31 (Acedock), which is the same range that Ibuprofen normally falls in. The compounds' elevated docking score indicates that they possess anti-inflammatory effects. Furthermore, the proposed compounds exhibit acceptor and donor hydrogen bond values that fall

within a tolerable range. All molecules adhere to Lipinski's rule of five, which provides fundamental criteria for designing novel compounds based on their expected characteristics. Assessing the cumulative negative impacts is a crucial stage in the pharmaceutical development process. We have identified compounds that display cytotoxic, immunotoxic, mutagenic, carcinogenic, and hepatotoxic properties. We have identified the hepatotoxic characteristics of molecules 3s and 3t. Currently, some molecules, such as 3a, 3e, 3g, 3h, and 3r, are inactive, which contributes to the development of potential molecules. A deep crimson color indicates the presence of the 3S active component in immunotoxicity. Chemicals 3a, 3g, 3i, and 3r exhibit inactivity in the mutagenicity test, while the other chemicals demonstrate activity. All the cytotoxicity molecules are inactive, as indicated by their dark green hue. We employed the PROTOX-II server to evaluate the ligand's immediate toxicity. Additionally, we employed spectroscopic techniques to produce and examine the molecules derived from benzimidazole. After selecting these compounds based on their performance in laboratory experiments, we used computer simulations to evaluate their potential as pharmaceuticals. We examined the efficacy of the synthesized compounds in inhibiting the denaturation of egg albumin protein. This allowed us to better understand their methods for reducing inflammation. This strategy is a valuable and uncomplicated method for assessing anti-inflammatory activity. The test showed that synthetic compounds and the common medicine diclofenac sodium, when present in amounts between 10 and 50 parts per million, had different levels of success in keeping proteins from breaking down. Compound 3f, when given at a concentration of 100 parts per million (ppm), had a maximum inhibition of 70%. Diclofenac sodium at a concentration of 40 parts per million (ppm) demonstrated the highest level of inhibition, measuring 97.20%. Therefore, the compounds produced have the ability to inhibit the denaturation of the protein membrane. These compounds' benzimidazole-based structure contributes to their resilience against denaturation.

CONCLUSION

Computer-aided drug design was used to develop and evaluate benzimidazole compounds against inflammatory enzymes such as FAAH. These drugs interacted with these receptors better than ibuprofen. The Protox-II algorithm uses Lipinski's rule of 5 to verify drug similarity. All molecular features of the produced derivatives are within permissible ranges, indicating compliance with Lipinski's rule of five. Anti-inflammatory medicines with excellent pharmacokinetics and docking scores of -10.88 to -27.31 for Acedock and -6.045 to 9.122 for DockThore are likely to succeed. We tested the ligand's acute toxicity with PROTOX-II. The green synthesis method produced high-quality, pure benzimidazole derivatives. Both chemicals and diclofenac sodium reduced protein denaturation to 10–50 ppm. At 40 ppm, Compound 3f had the greatest inhibition at 70%. Diclofenac sodium gave the maximum inhibition at 40 ppm and 97.20%. This research may help design an anti-inflammatory drug by understanding the molecular process behind these molecules. In vitro and in vivo studies can confirm these findings.

ACKNOWLEDGMENT

The authors would like to thank the Principal and Secretary of Shreeshakti Shaikshanik Sanstha's Divine College of Pharmacy, Satana, Nashik, India.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Authors' Contribution

Research work carried out: Ms. Pallavi S. Jadhav, Ms. Harshada S. Shewale, Ms. Dipa B. Wagh, Dr. Khemchand R. Surana; Data collection, analysis and Interpretation of Results and Manuscript Draft Preparation: Dr. Khemchand R. Surana, Jubershah S. Shah, Yogesh P. Sharma; Reviewed the results and approved the final version of the manuscript: Dr. Khemchand R. Surana, Dr. Sunil K. Mahajan, Dr. Jayesh V. Musale

REFERENCES

1. Faheem M, Rathaur A, Pandey A, Kumar Singh V, Tiwari AK. A review on the modern synthetic approach of benzimidazole candidate. *Chemistry Select.* 2020; 5(13):3981-94. https://doi.org/10.1002/slct.201904832
2. Achar KC, Hosamani KM, Seetharamareddy HR. In-vivo analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. *Eur. J. Med. Chem.* 2010; 45(5):2048-54. https://doi.org/10.1016/j.ejmech.2010.01.029
3. Lipinski CA. Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. Adv. *Drug Deliv. Rev.* 2016; 101:34-41. https://doi.org/10.1016/j.addr.2016.04.029
4. Crawford SE, Hartung T, Hollert H, Mathes B, van Ravenzwaay B, Steger-Hartmann T, Studer C, Krug HF. Green toxicology: a strategy for sustainable chemical and material development. *Environ. Sci. Eur.* 2017; 29:1-6. https://doi.org/10.1186/s12302-017-0115-z
5. Bruce RD. An up-and-down procedure for acute toxicity testing. *Fundamental and Applied Toxicology*. 1985; 5(1):151-7. https://doi.org/10.1016/0272-0590(85)90059-4
6. Hou T, Wang J. Structure–ADME relationship: still a long way to go?. *Expert Opin Drug Metab Toxicol.* 2008; 4(6):759-70. https://doi.org/10.1517/17425255.4.6.759
7. Sajjadifar S, Mirshokraie SA, Javaherneshan N, Louie O. SBSA as a New and efficient catalyst for the one-pot green synthesis of benzimidazole derivatives at room temperature. *Am. J. Org. Chem.* 2012; 2(2):1-6. DOI: 10.5923/j.ajoc.20120202.01
8. Bharathi M, Indira S, Vinoth G, Mahalakshmi T, Induja E, Shanmuga Bharathi K. Green synthesis of benzimidazole derivatives under ultrasound irradiation using Cu-Schiff base complexes embedded over MCM-41 as efficient and reusable catalysts. *J. Coord. Chem.* 2020; 73(4):653-70. https://doi.org/10.1080/00958972.2020.1730335
9. Daraji DG, Rajani DP, Rajani SD, Pithawala EA, Jayanthi S, Patel HD. Structure based design, synthesis, and biological evaluation of imidazole derivatives targeting dihydropteroate synthase enzyme. *Bioorg Med Chem Lett.* 2021; 36:127819. https://doi.org/10.1016/j.bmcl.2021.127819
10. Opal SM, DePalo VA. Anti-inflammatory cytokines. *Chest.* 2000; 117(4):1162-72. https://doi.org/10.1378/chest.117.4.1162
11. Ley K. The role of selectins in inflammation and disease. *Trends Mol Med.* 2003; 9(6):263-8. https://doi.org/10.1016/S1471-4914(03)00071-6
12. Foroughi HO, Kargar M, Erjaee Z, Zarenezhad E. One-pot three-component reaction for facile and efficient green synthesis of chromene pyrimidine-2, 4-dione derivatives and evaluation of their anti-bacterial activity. *MonatsheftefürChemie-Chemical Monthly*. 2020; 151:1603-8. https://doi.org/10.1007/s00706-020-02692-5
13. Ibrahim DA, Ismail NS. Design, synthesis and biological study of novel pyrido [2, 3-d] pyrimidine as anti-proliferative CDK2 inhibitors. *Eur. J. Med. Chem*. 2011: 46(12): 5825-32. https://doi.org/10.1016/j.ejmech.2011.09.041
14. Kumar S, Deep A, Narasimhan B. A review on synthesis, anticancer and antiviral potentials of pyrimidine derivatives.*Curr. Bioact. Compd*. 2019; 15(3): 289-303.https://doi.org/10.2174/1573407214666180124160405
15. Abdel-Mohsen HT, Ragab FA, Ramla MM, El Diwani HI. Novel benzimidazole–pyrimidine conjugates as potent antitumor agents.*Eur. J. Med. Chem*. 2010; 45(6): 2336-44.https://doi.org/10.1016/j.ejmech.2010.02.011
16. ur Rashid H, Martines MA, Duarte AP, Jorge J, Rasool S. Muhammad R, Ahmad N, Umar MN. Research developments in the syntheses, anti-inflammatory activities and structure–activity relationships of pyrimidines.*RSC Adv.* 2021; 11(11); 6060-98.https://doi.org/10.1039/D0RA10657G
17. Ahmed K, Choudhary MI, Saleem RS. Heterocyclic pyrimidine derivatives as promising antibacterial agents.*EuropeanJ. Med. Chem*. 2023; 115701.https://doi.org/10.1016/j.ejmech.2023.115701
18. Surana KR, Mahajan SK. In silico Study of Chromane Ring Compound Rubranonoside from Plumeriarubra as Anticancer Potential. *Trends in Sciences*. 2022; 19(24): 3305. https://doi.org/10.48048/tis.2022.3305
19. Patil SJ, Surana KR, Mahajan SK. Quantification of active phytoconstituents in ethanolic extract of

Menthapiperita by modern analytical tools. *Res. Jr. Agril. Sci.* 2024; 15(3): 615-21. DOI: NA

20. Patil SJ, Surana KR, Mahajan SK. In vitro antimicrobial and antifungal activity of Menthapiperita active phytoconstituents. *Res. Jr. Agril. Sci.* 2023; 14(6): 1875-7. DOI: NA
21. Aher S. N, Sonawane S. N, Sonawane P. R, Surana K. R, Mahajan S. K, Patil D. M, Katkade P. N. Insilico Drug Design, Synthesis and Evaluation of Anti-inflammatory Activity Pyrimidine Analogue. Biotech Res Asia 2024;21(2). Available from: https://bit.ly/3XcHvg9
22. Maddila S, Gorle S, Seshadri N, Lavanya P, Jonnalagadda SB. Synthesis, antibacterial and antifungal activity of novel benzothiazole pyrimidine derivatives.*Arab. J. Chem*. 2016; 9(5):681-7.https://doi.org/10.1016/j.arabjc.2013.04.003
23. Inoyama D, Paget SD, Russo R, Kandasamy S, Kumar P, Singleton E, Occi J, Tuckman M, Zimmerman MD, Ho HP, Perryman AL. Novel pyrimidines as antitubercular agents.*Antimicrob Agents Chemother.* 2018:62(3):10-128.https://doi.org/10.1128/aac.02063-17
24. Pasunooti KK, Chai H, Jensen CN, Gorityala BK, Wang S, Liu XW. A microwave-assisted, copper-catalyzed three-component synthesis of dihydropyrimidinones under mild conditions. *Tetrahedron Lett.* 2011;52(1):80-4.https://doi.org/10.1016/j.tetlet.2010.10.150
25. Kaur R, Kaur P, Sharma S, Singh G, Mehndiratta S, MS Bedi P, Nepali K. Anti-cancer pyrimidines in diverse scaffolds: a review of patent literature. *Rec. pat. anti-can.drug dis*.2015;10(1): 23-71. https://doi.org/10.2174/1389557516666160923125801
26. Parker WB. Enzymology of purine and pyrimidine antimetabolites used in the treatment of cancer.*Chem. Rev*.2009; 109(7): 2880-93. https://doi.org/10.1021/cr900028p
27. Gyi JI, Lane AN, Conn GL, Brown T. Solution Structures of DNA™" RNA Hybrids with Purine-Rich and Pyrimidine-Rich Strands: Comparison with the Homologous DNA and RNA Duplexes.*Biochem*.1998; 37(1): 73-80. https://doi.org/10.1021/bi9719713
28. Al-Harbi NO, Bahashwan SA, Fayed AA, Aboonq MS, Amr AE. Anti-parkinsonism, hypoglycemic and anti-microbial activities of new poly fused ring heterocyclic candidates.*Int. J. Biol. Macromol*.2013: 57: 165-73.https://doi.org/10.1016/j.ijbiomac.2013.03.019
29. Lin X, Li X, Lin X. A review on applications of computational methods in drug screening and design.*Molecules*.2020; 25(6): 1375.https://doi.org/10.3390/molecules25061375
30. Shoichet BK, Leach AR, Kuntz ID. Ligand solvation in molecular docking. *Prot*.1999; 34(1): 4-16. https://doi.org/10.1002/(SICI)1097-0134(19990101)34:1%3C4::AID-PROT2%3E3.0.CO;2-6