Synthesis and Microbial Activities of 2, 5, 6-Substituted Benzimidazole Derivatives

Dhondiba Vishwanath[1], Devaraju[2], Vijayanand Vithalrao[3]

[1]GWFGC Kalaburagi, Karnataka, India

[2]Yuvaraja college Mysore, Karnataka, India

[3]GC(Autonomous) kalaburagi, Karnataka, India

DOI: https://dx.doi.org/10.51584/IJRIAS.2025.101100038

Received: 24 November 2025; Accepted: 30 November 2025; Published: 09 December 2025

ABSTRACT

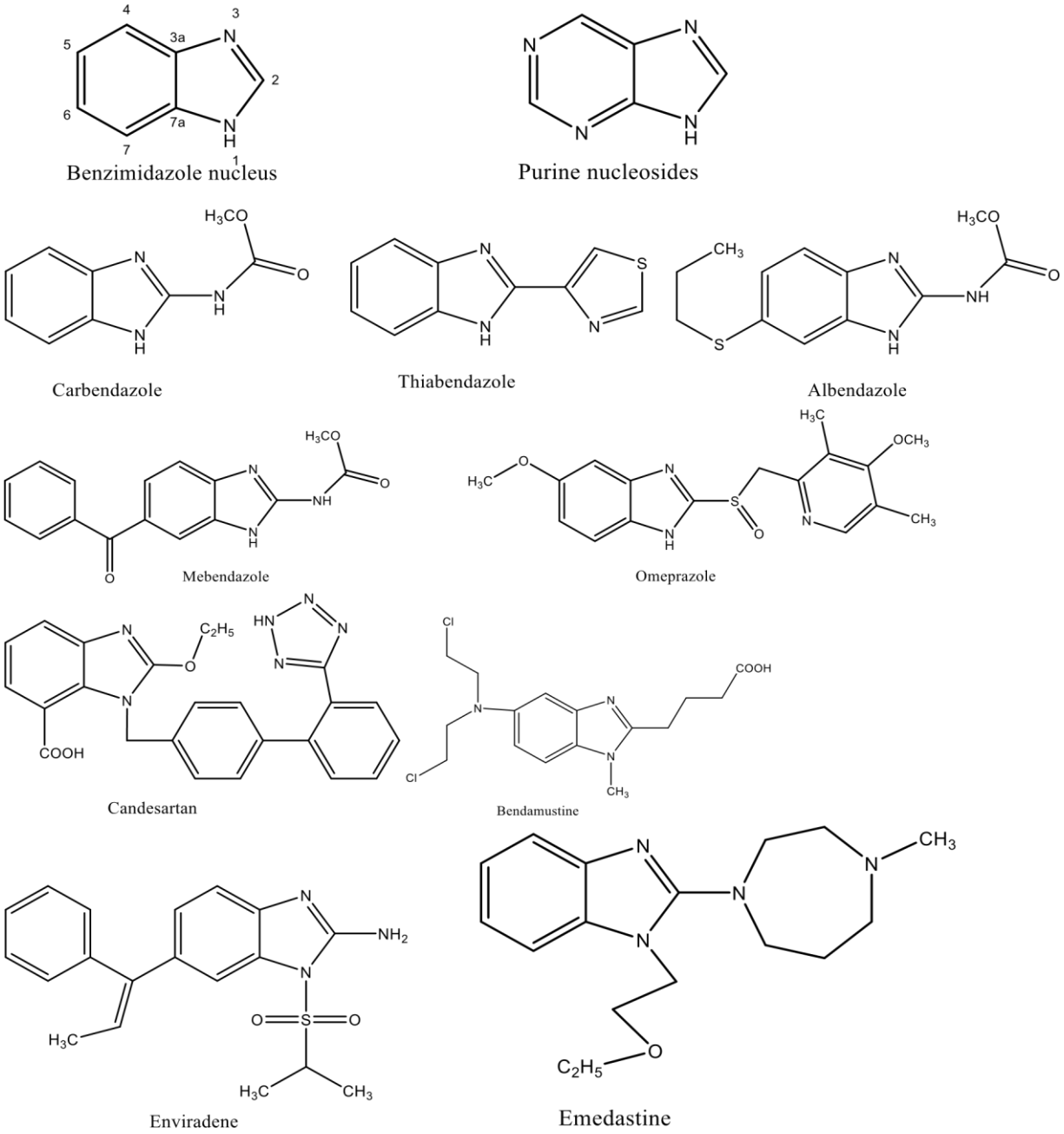
Benzimidazole derivatives are the analogues of purine nucleosides which are found in human body. These are an important heterocyclic organic compounds containing phenyl ring (six membered ring) fused with imidazole (five membered ring) which possesses wide range of starting material for various compounds and exhibit clinical applications such as anti-inflammatory, antibacterial, antifungal, antiviral, analgesic, proton pump inhibitors, antihistamines, anticancer, etc. The presence of electron-donating groups (OH, $-CH_3$, $-OCH_3$) causes significant increase in the biological activity, while the electron-withdrawing groups (-Cl, -Br,$–NO_2$) decreases the biological activity of synthesized of benzimidazole derivatives.

Keywords: Benzimidazole; 4,5-substituted phenylene-1,2-diamine, trimethoxy benzaldehyde; magnetic stir; $Na_2S_2O_5$; microbial activity.

INTRODUCTION:

Benzimidazole derivatives are an important benzfused heterocyclic organic compounds which contains benzene ring and imidazole ring [1-2]. They are analogue of nucleosides which are found in living organism. They possess an extensive range of clinical applications such as anti-inflammatory, antibacterial, antifungal, antiviral, analgesic, and anticancer [3-6]. Benzimidazole compounds can be further improved by changing substituent groups on the core structure. This method is the most familiar to improve their biological activities. Many commercially available drugs based on the benzimidazole skeleton such as antifungal (carbendazole), anthelmintics (albendazole, mebendazole, fenbendazole, and oxibendazole), proton pump inhibitors (omeprazole), antihypertensives (candesartan and telmisartan), anticancer (bendamustine, nocodazole, and abemaciclib), antiviral(enviradene) and antihistamines(emedastine and clemizole). In this article we planned to modify the benzimidazole nucleus at 2, 4 and 5 –positions to prepare better biological active compounds. The synthesis of benzimidazole derivatives typically involved in the reaction between 4,5-substituted phenylene 1,2 dimine and 4,5,6-trimethoxy benzaldehyde using oxidizing agent sodium metabisuphite in ethanol[7-10].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue XI November 2025



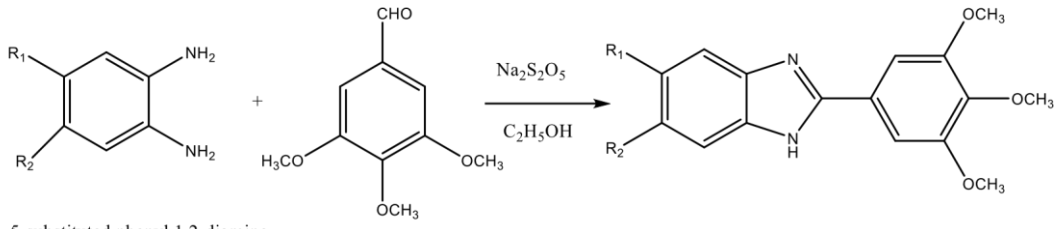
Experimental: Material and Methods

All the reagents and chemicals were purchased from Sigma-Aldrich and used without further purification. Melting points were found using thiel's tube and paraffin oil with open capillary tubes and are uncorrected. TLC is performed with E. Merck pre-coated silica gel plates (60F-254) with ninhydrin as a spot developing agent. Acme, India silica gel, 60–120 mesh is used for column chromatography. IR spectra in KBr were recorded on Perkin-Elmer model 683 spectrometers. ^{1}H NMR (300 MHz) and ^{13}C NMR (100 MHz) spectra were recorded $CDCl_3$ solvent containing tetra methyl silane (TMS) as internal references were recorded on Bruker spectrometer; Elemental analyses were performed on a PerkinElmer 2400. Mass spectra were obtained by Water-QTOF ultima spectrometer. Micro analytical data were obtained by elemental-Vario EL-III [10-13].

General methods of preparation of 2, 5, 6-substituted derivatives benzimidazoles: A mixture of substituted phenyl 1,2-dimine 1(a-f) 0.02 mole, 3, 4, 5-trimethoxybenzaldehyde 0.02 mole and 0.04 mole sodium metabisulphite was added in 30cm^3 of ethanol. The resulting reaction mixture was stirred using magnetic stirrer at room temperature for about 2-3 hours. The progress of reaction was checked by TLC. After complete completion of reaction, the product was concentrated with vacuum distillation. The solid product 2(a-f) was washed with water and then n-hexane and dried in anhydrous P_2O_5 for overnight. Product was again dried at room temperature and determined the physical constant.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue XI November 2025

Scheme

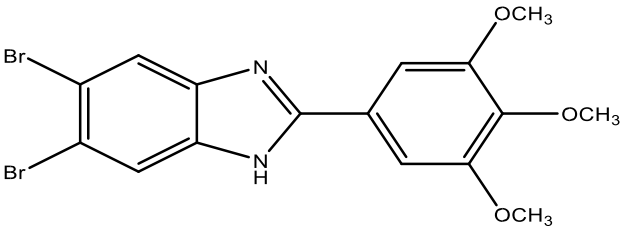


4, 5-substituted phenyl 1,2-diamine 1(a-f) + 3,4,5-trimethoxy benzaldehyde → Benzimidazole derivative 2(a-f)

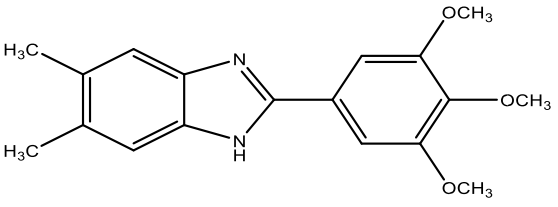
Compound	R_1	R_2
1-2(a)	Cl	Cl
1-2(b)	Br	Br
1-2(c)	CH_3	CH_3
1-2(d)	OCH_3	OCH_3
1-2(e)	OH	H
1-2(f)	NO_2	H

5, 6-dichloro-2-(3, 4, 5-trimethoxyphenyl)-1H-benzimidazole 2(a): Colour: Light yellow solid. M. P. 145149^{0}C, Yield 75.5%. IR (KBr): 3250cm^{-1}(N-H), 1500cm^{-1}(C=N), 750cm^{-1}(Ar-Cl) 1387cm^{-1}(Ar-OCH_3).^{1}H-NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 9H, OCH_3), 6.97 – 8.30 (m, 4H, Ar.-H), 12.5(s, 1H, NH). ^{13}C-NMR ($CDCl_3$); δ (ppm):56.1, 60.1, 60.8, 139.2, 153.1, 104.6, 124.9, 152.9, 130.9, 117.2, 128.6. Mass (*m/z*): 354.20. Elemental Analysis (%): For $C_{16}H_{14}O_3Cl_2N_2$, Calculated: C, 54.41; H, 4.00; O, 13.59; Cl, 20.07; N, 7.93. Found: C, 54.45; H, 3.91; O, 20.00; Cl, 13.52; N, 7.89.

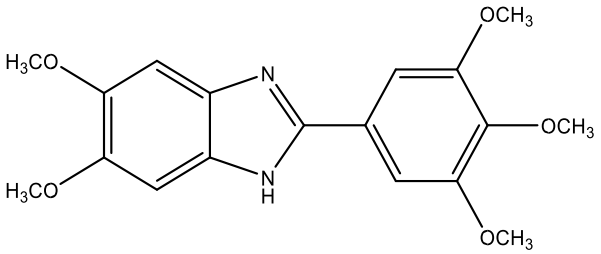
5, 6-dibromo-2-(3, 4, 5-trimethoxyphenyl)-1H-benzimidazole 2(b): Colour: Yellow solid. M. P. 155-159^{0}C, Yield 83.7%. IR (KBr): 3250cm^{-1}(N-H), 1500cm^{-1}(C=N), 650cm^{-1}(Ar-Br) 1387cm^{-1}(Ar-OCH_3).^{1}H- NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 9H, OCH_3), 6.97 – 7.76 (m, 4H, Ar.-H), 12.56(s, 1H, NH). ^{13}C-NMR ($CDCl_3$); δ (ppm):56.1, 60.1, 139.2, 153.1, 104.6, 124.9, 152.9, 140.1, 120.9, 120.8. Mass (*m/z*): 442.11. Elemental Analysis (%): For $C_{16}H_{14}O_3Br_2N_2$, Calculated: C, 43.47; H, 3.19; O, 10.85; Br, 36.15; N, 6.34. Found: C, 43.35; H, 3.25; O, 10.91; Br, 36.10; N, 6.39.



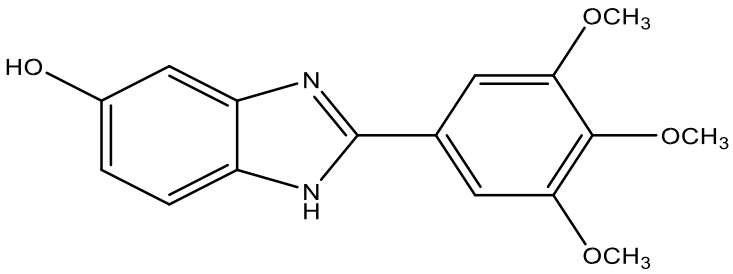
5, 6-dimethyl-2-(3, 4, 5-trimethoxyphenyl)-1H-benzimidazole 2(c): Colour: Colourless solid. M. P. 107109^{0}C, Yield 71.8%. IR (KBr): 3250cm^{-1}(N-H), 1500cm^{-1}(C=N), 1387cm^{-1} (Ar-OCH_3).^{1}H- NMR ($CDCl_3$); δ (ppm): 2.47(s, 6H, CH_3), 3.71-3.83 (bs, 9H, OCH_3), 6.97 – 7.35 (m, 4H, Ar.-H), 12.56(s, 1H, NH). ^{13}C-NMR ($CDCl_3$); δ (ppm):18.8, 56.1, 60.8, 139.2, 153.1, 104.6, 124.9, 132.6, 115.2, 130.3. Mass (*m/z*): 312.37. Elemental Analysis (%): For $C_{18}H_{20}O_3N_2$, Calculated: C, 69.21; H, 6.45; O, 15.37; N, 8.97. Found: C, 69.25; H, 6.41; O, 15.42; N, 8.92.



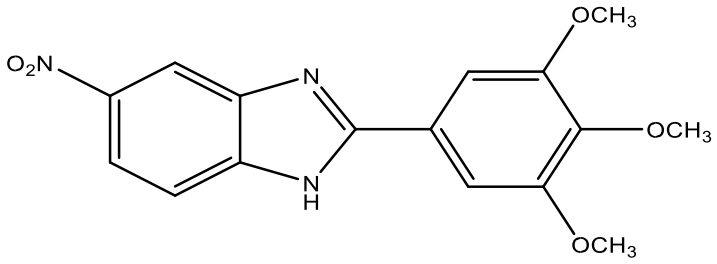
5, 6-dimethoxy-2-(3, 4, 5-trimethoxyphenyl)-1H-benzimidazole 2(d): Colour: Colourless solid. M. P. 127129^{0}C, Yield 79.4%. IR (KBr): 3250cm^{-1}(N-H), 1500cm^{-1}(C=N), 1387cm^{-1} (Ar-OCH_3).^{1}H- NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 15H, OCH_3), 6.97 – 7.14 (m, 4H, Ar.-H), 12.56(s, 1H, NH). ^{13}C-NMR ($CDCl_3$); δ (ppm): 56.1, 60.8, 139.2, 153.1, 104.6, 152.9, 132.2, 101.8, 142.0. Mass (*m/z*): 344.37. Elemental Analysis (%): For $C_{18}H_{20}O_5N_2$, Calculated: C, 62.78; H, 5.85; O, 23.23; N, 8.13. Found: C, 62.70; H, 5.88; O, 23.28; N, 8.14.



5-hydroxy-2-(3, 4, 5-trimethoxyphenyl)-1H-benzimidazole 2(e): Colour: Orange solid. M. P. 162-165^{0}C, Yield 84.1%. IR (KBr): 3250cm^{-1}(N-H), 1500cm^{-1}(C=N), 3450cm^{-1}(Ar-OH) 1387cm^{-1}(Ar-OCH_3).^{1}H- NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 9H, OCH_3), 6.97 – 7.39 (m, 5H, Ar.-H), 9.45(s, 1H, OH), 12.56(s, 1H, NH). ^{13}C-NMR ($CDCl_3$); δ (ppm): 56.1, 60.8, 139.2, 153.1, 104.6, 124.9, 152.9, 134.3, 140.3, 102.4, 116.6, 111.4, 151.7. Mass (*m/z*): 300.31. Elemental Analysis (%): For $C_{16}H_{16}O_4N_2$, Calculated: C, 63.99; H, 5.37; O, 21.31; N, 9.33. Found: C, 63.91; H, 5.35; O, 21.35; N, 9.39.



5-nitro-2-(3, 4, 5-trimethoxyphenyl)-1H-benzimidazole 2(f): Colour: Red solid. M. P. 187-199^{0}C, Yield 85.0%. IR (KBr): 3250cm^{-1}(N-H), 1500cm^{-1}(C=N), 1450cm^{-1}(Ar-NO_2) 1387cm^{-1}(Ar-OCH_3).^{1}H- NMR ($CDCl_3$); δ (ppm): 3.71-3.83 (bs, 9H, OCH_3), 6.97 – 8.09 (m, 5H, Ar.-H), 12.56(s, 1H, NH). ^{13}C-NMR ($CDCl_3$); δ (ppm): 56.1, 60.8, 139.2, 153.1, 104.6, 124.9, 152.9, 139.8, 147.8, 112.9, 116.1, 118.6, 144.3. Mass (*m/z*): 329.31, Elemental Analysis (%): For $C_{16}H_{15}O_5N_3$, Calculated: C, 58.36; H, 4.59; O, 24.29; N, 12.76. Found: C, 58.31; H, 4.62; O, 24.33; N, 12.74.



Antimicrobial Activity:

1. Antibacterial Activity

The pure compounds 2(a-f) were carried out for their antibacterial activity by using disc diffusion method. The nutrient agar broth prepared by the usual method, was inoculated aseptically with 0.5 cm^3 of 24 hours old subculture of Staphylococcus aureus(SA) and Escherichia coli(EC) in separate conical flask at 35ºC-45ºC and mixed well by shaking. About 30 cm^3 of the contents of the flask were poured and evenly spread in Petridis (90

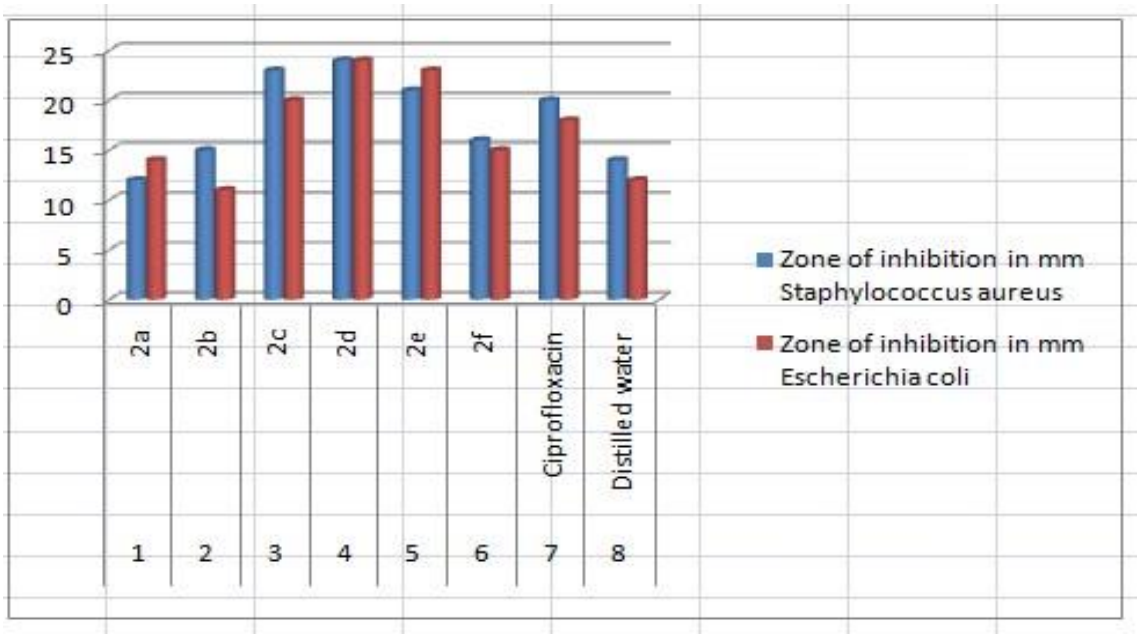
ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue XI November 2025

mm in diameter) and allowed to set for three hours. The cups (8 mm in diameter) were formed by the help of borer in agar medium and filled with 0.1 cm^3 (1mg/ cm^3) solution of sample in propanone. The plates were incubated at 25-30°C for 7 days. After the completion of incubation period, the zones of inhibition growth is in the form of diameter in mm was measured. Along the test solution in each Petridis one cup was filled up with ciprofloxacin as standard and another cup was filled up with distilled water (DW) which acts as negative control all over the experiment of 50 µg concentration [11-13].

Table-1: Antibacterial activity of the synthesized compounds 2(a-f)

Sl. No.	Compounds	Zone of inhibition in mm	
		Staphylococcus aureus	Escherichia coli
1	2a	12	14
2	2b	15	11
3	2c	23	20
4	2d	24	24
5	2e	21	23
6	2f	16	15
7	Ciprofloxacin	20	18
8	Distilled water	14	12

Graph-1: Inhibition zone against bacteria of the synthesized compounds 2(a-f)



Antifungal Activity

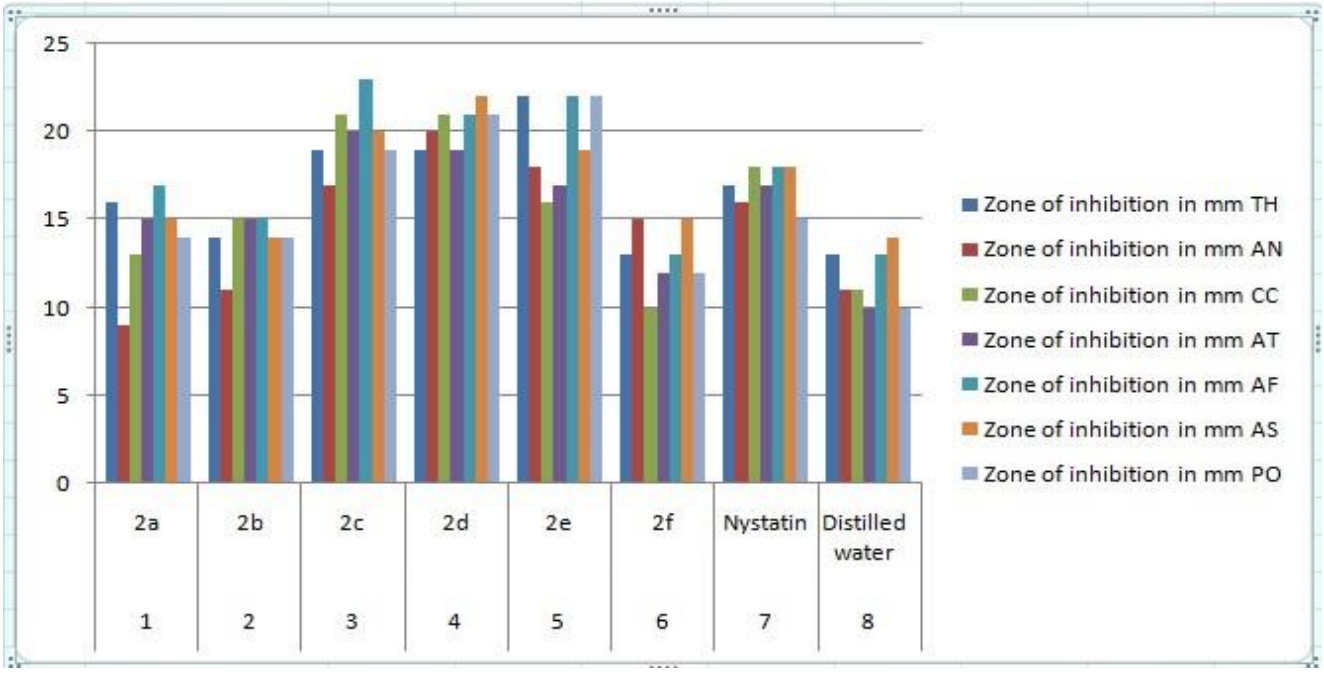
The pure compounds 2(a-f) were screened for their antifungal activity by using disc diffusion method. The fungi like Trichoderma harzianum(TH), Aspergillus niger(AN), Colletotrichum capsici(CC), Aspergillus tamari(AT), Aspergillus flavus(AF), Alternaria solani(AS), and Penicillium oxalicum(PO) were employed for testing. The culture was maintained on sabouraud dextrose agar slants. Sterilized Sabouraud dextrose agar medium was inoculated with 72 hr old 0.5 cm^3 suspension of fungal spores in a separate flask. About 25 cm^3 of the inoculated medium was evenly spreader in a sterilized Petridis and allowed to set for 5 hours. The cups (8 mm in diameter) were punched in Petridis and loaded with 0.1 cm^3 (2 mg/ cm^3) of solution of sample in propanone. The plates were incubated at 25-30°C for 7 days. After the completion of incubation period, the zones of inhibition growth is in the form of diameter in mm was measured. Along the test solution in each Petridis one cup was filled up with nystatin as standard and another cup was filled up with distilled water (DW) which acts as negative control all over the experiment of 50 µg concentration [14-16].

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue XI November 2025

Table-2: Antifungal activity of the synthesized compounds 2(a-f)

Sl. No.	Compounds	Zone of inhibition in mm						
		TH	AN	CC	AT	AF	AS	PO
1	2a	16	9	13	15	17	15	14
2	2b	14	11	15	15	15	14	14
3	2c	19	17	21	20	23	20	19
4	2d	19	20	21	19	21	22	21
5	2e	22	18	16	17	22	19	22
6	2f	13	15	10	12	13	15	12
7	Nystatin	17	16	18	17	18	18	15
8	Distilled water	13	11	11	10	13	14	10

Graph-2: Inhibition zone against fungi of the synthesized compounds 2(a-f)



RESULTS AND DISCUSSION

2,5,6 -substituted benzimidazole derivatives were prepared in good yields by oxidation reaction of 4,5-substituted phenyl 1,2-dimine 1(a-f) with 3, 4, 5-trimethoxybenzaldehyde using sodium metabisulphite in ethanol. The resulting reaction mixture was stirred using magnetic stirrer at room temperature for about 2-3 hours. The progress of reaction was checked by TLC. After complete completion of reaction, the product was concentrated with vacuum distillation. The solid product 2(a-f) was washed with water and then n-hexane and dried in anhydrous P_2O_5 for overnight. Product was again dried at room temperature and determined the physical constant. The products were recrystallized using hot water. The structure of 2,5,6- substituted benzimidazole derivatives of was confirmed by IR, ^{1}H- NMR, ^{13}C-NMR and mass spectral data.

The synthesized compounds were conducted for antibacterial activity and antifungal activity and the results were summarized in table-1 & graph-1 and table-2 & graph-2 respectively. From the table-1 & graph-1, the synthesized compounds **2c** and **2d** shows very active and **2e** moderately active against both bacteria due to the presence of electron releasing groups in their structures where as **2a, 2b** and **2f** compounds less active due to the presence of electron withdrawing group against the ciprofloxacin as standard. Similarly from the table-2 & graph-2, the synthesized compounds **2c, 2d** and **2e** shows very active against all the fungi due to the presence of electron releasing groups in their structures where as **2a, 2b** and **2f** compounds less or moderately active against all the fungi due to the presence of electron withdrawing group against the nystatin as standard.

CONCLUSION

We succeeded for the synthesizing of 2, 5, 6- substituted benzimidazole derivatives and their microbial activities. Some synthesized compounds were very active against fungi and bacteria, others are remains inactive compared to the standard Ciprofloxacin and nystatin which are already available in the market.

REFERENCES

1. Keshav Anand & Sharad Wakode. "Synthesis, characterization and biological evaluation of benzimidazole derivatives." International Journal of Pharmaceutical Sciences and Research, 2018; 9(2): 617–624.
2. S. Kumari, A. Joshi, I. Borthakur, S. Kundu. Journal of Organic Chemistry, 2023, 88, 11523–11533.
3. Thi-Kim-Chi Huynh, Thi-Hong-An Nguyen, Ngoc-Hoang-Son Tran, Thanh-Danh Nguyen & Thi-Kim-Dung Hoang. "A facile and efficient synthesis of benzimidazole as potential anticancer agents." Journal of Chemical Sciences (2020) 132:84. https://doi.org/10.1007/s12039-020-01783-4
4. R.B. Vlocskó et al. "Catalyst-free synthesis of substituted benzimidazoles and benzothiazoles in a sustainable solvent." Tetrahedron Green Chemistry, 3 (2024) 100035. https://doi.org/10.1016/j.tgchem.2023.100035
5. Nannapaneni et al. "Synthesis, Characterization, and Biological Evaluation of Benzimidazole Derivatives as Potential Anxiolytics." Journal of Young Pharmacists, 2010; 2(3): 273–279. https://doi.org/10.4103/0975-1483.66809
6. Nguyen Thi Chun, Vo Cong Dung & Dau Xuan Duc. "Recent achievements in the synthesis of benzimidazole derivatives." RSC Advances, 2023, 13, 32734–32771. https://doi.org/10.1039/D3RA05960J
7. Basant Farag, Magdi E.A. Zaki, Doaa A. Elsayed & Sobhi M. Gomha. "Benzimidazole chemistry in oncology: recent developments in synthesis, activity, and SAR analysis." RSC Advances, 2025, 15, 18593–18647.
8. Shatha Ibrahim Alaqeel. "Synthetic approaches to benzimidazoles from o-phenylenediamine: A literature review." Journal of Saudi Chemical Society, 21(2), 2017, 229–237. https://doi.org/10.1016/j.jscs.2016.08.001
9. Ankit Siwach & Prabhakar Kumar Verma. "Synthesis and therapeutic potential of imidazole containing compounds." BMC Chemistry (2021) 15:12. https://doi.org/10.1186/s13065-020-00730-1
10. Ramakrishna Chintakunta & Geethavani Meka. "Synthesis, in silico studies and antibacterial activity of some novel 2-substituted benzimidazole derivatives." Future Journal of Pharmaceutical Sciences, 6:128 (2020). https://doi.org/10.1186/s43094-020-00144-9
11. Dhondiba Vishwanath, Devaraju, P. Ranjini & Y.B. Basavaraju. "Synthesis and characterisation of substituted 9-(3,4,5-trimethoxyphenyl) tetralone-triazoles by chalcone path and their biological activity." Journal of Emerging Technologies and Innovative Research, 6(2), February 2019.
12. Dhondiba Vishwanath, Devaraju Kesagodu, P. Ranjini, S. Vijayakumar & Y.B. Basavaraju. "Synthesis of Zn-catalyzed substituted 3-(3,4,5-trimethoxyphenyl)prop-2-en-1-keto-triazoles and their biological evaluation." Rasayan Journal of Chemistry, 11(3), 955–961 (2018). http://dx.doi.org/10.31788/RJC.2018.1133014
13. N.M. Goudagaon et al. "Synthesis and antimicrobial activity of N-1 substituted benzimidazoles." Indian Journal of Heterocyclic Chemistry, 13 (2004) 271.
14. Dhondiba Vishwanath, Devaraju, Vijayanand V. & Sadashivamurthy B. "Synthesis and biological activities of oxazine derivatives of 3,4-substituted chalcone." Journal of Emerging Technologies and Innovative Research, 11(12), December 2024.
15. M. Chaitramallu, Devaraju & P. Ranjini. "Synthesis of (6-hydroxy-4-phenyl-1,4-dihydronaphthalen-1-yl) amino derivatives and their antiviral activity analysis." Indian Journal of Advances in Chemical Science, 2, 61 (2014).
16. Nidhi Singh, Shamim Ahmad & M. Shamsher Alam. "Biological potentials of chalcones." International Journal of Pharmaceutical & Biological Archives, 3(6), 1298 (2012).