https://doi.org/10.48048/tis.2023.6110

In vitro Studies of Antibacterial and Antifungal Activity of Novel Substituted Benzimidazole Derivatives

Periyasamy Parthiban[1,*], Arthanari Saravanakumar[2], Subramanian Mohanraj[3], Chellamuthu Kavinkumar[1], Sangeetha Saravanan[1], Palani Bharat[1], Mohan Santhosh[1] and Murugesan Abbinanthan[1]

[1]*Department of Pharmaceutical Chemistry, Vellalar College of Pharmacy, Tamilnadu 638012, India*
[2]*Department of Pharmaceutical Biotechnology, Vellalar College of Pharmacy, Tamilnadu 638012, India*
[3]*Department of Pharmacology, Vellalar College of Pharmacy, Tamilnadu 638012, India*

([*]Corresponding author's e-mail: drparthiban82@gmail.com)

Received: 27 September 2022, Revised: 9 November 2022, Accepted: 28 November 2022, Published: 15 August 2023

Abstract

A series of 2-substituted amino-1-(1*H*-benzo[d]imidazol-1-yl)ethanone was prepared by reacting with benzimidazole and chloroacetyl chloride by stirring method. The resultant compound was dissolved in ethanol and refluxed with aryl/aralkyl amine in the presence of anhydrous potassium carbonate. The tested compounds were evaluated against bacterial agents such as *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. typhi*, and *S. aureus*, amoxicillin has been used as a control drug, and also screened for antifungal activity against *C. albicans* and *A. niger* at 50 and 100 µg/mL level, griseofulvin used as control drug. The structure-activity relationship led to the conclusion compound PS3 showed equipotent potent, PS4 showed moderate, and PS2 showed less moderate activity. The aromatic/aliphatic chain containing compounds PS1 and PS5 showed the least activity against all bacteria. Compound PS3, PS4 and PS1 showed better activity against *C. albicans* at 100 µg/mL concentration. The tested compounds PS1, PS3, PS2 and PS4 showed good activity at 100 µg/mL concentration. The compound PS3 can serve as a lead molecule for further development as a new class of antimicrobial agent.

Keywords: Benzimidazole, Chloroacetyl chloride, Amines, Antibacterial and antifungal activity

Introduction

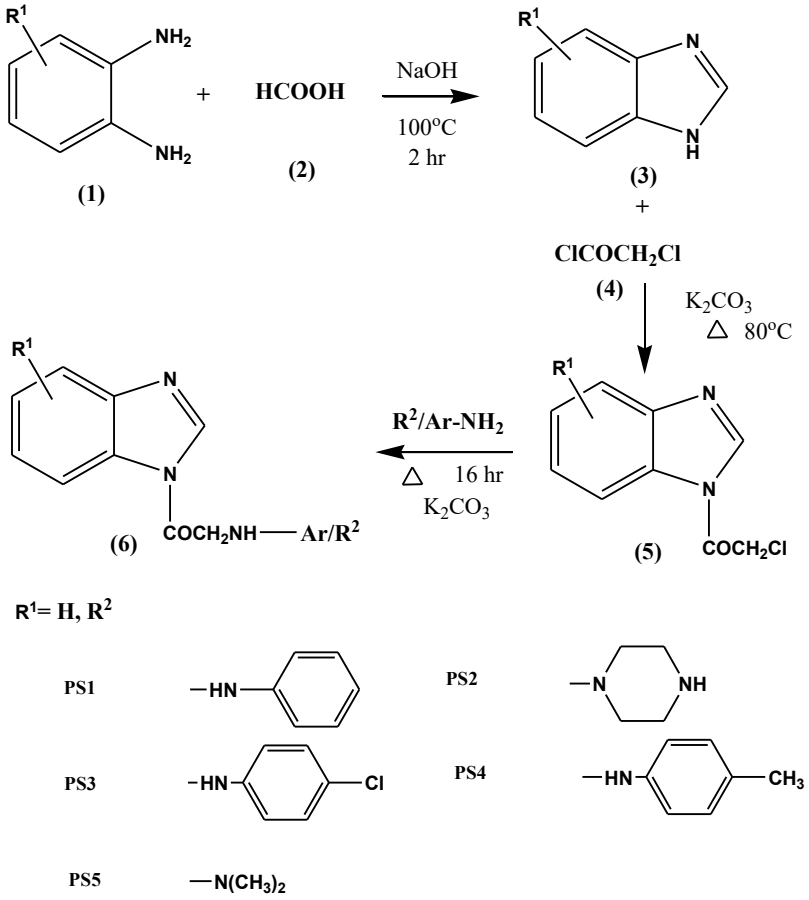
Nowadays, a severe issue is urbanized due to microbial drug resistance. Several strains are incredibly resistant to multiple antimicrobial agents, with some bacteria now immune to all available antibiotics. The heterocyclic system of benzimidazole and their derivatives awakened great interest in the past and recent years due to nitrogen-containing moiety exhibiting a diverse range of biological activities like antiviral [1], anti-inflammatory [2], antimicrobial activity [3], anticancer and antitubercular [5,6], and anticonvulsant activities [7]. Heterocyclic Nitrogen-containing systems can be incorporated to produce molecules with enhanced biological properties. Past reports reveled that benzimidazoles bearing the 1,3,4-oxadiazole moiety, which have broad spectrum antimicrobial properties [8], and molecules containing both the benzimidazole and indole heterocycles, which exhibit selective antibacterial activity [9]. According to research results, the antibacterial mechanism of benzimidazoles is due to their structural similarity to purine. It is well known that purine plays an essential role in the biosynthesis of nucleic acids and proteins in the bacterial cell wall. As competitive inhibitors, benzimidazoles can replace purine, thereby blocking the biosynthesis of crucial components, and killing or inhibiting the growth of bacteria [10]. Three representative compound classes containing active benzimidazole groups have been produced commercially as antimicrobial drugs. There is an urgent need to determine new benzimidazoles endowed with antimicrobial activity that possibly act through novel mechanisms of action distinct from those of effective antibacterial drugs in current use. Recently, much attention has been paid to developing potent benzimidazole-containing antibacterial agents, which has led to the appearance of many novel molecules that share desirable properties.

Materials and methods

Chemicals

Melting point (MP) was performed in open capillary tubes by using Thomas Hoover apparatus. It may be uncorrected. The Infrared (IR), Proton Nuclear magnetic resonance (NMR) and Mass spectra were performed using

a Perkin-Elmer 398 spectrometer, DPX-500 MHz Bruker FT-NMR spectrometer and JEOL-SX-102 instrument respectively. The chemical shifts were reported in parts per million (δ ppm) relative to TMS as an internal reference. Elemental analysis of C, H and N was performed by using Perkin-Elmer 2400 analyzer, and the obtained values were within the acceptable limits of the calculated values. The reactions can be monitored by silica gel plates and chloroform-methanol (9:1) used as a solvent system. The spots were developed in an iodine chamber. Spectral data (IR, NMR and Mass spectra) confirmed the structures of the synthesized compounds, and the purity of these compounds was ascertained by microanalysis. All chemicals and reagents were procured from Aldrich (USA), Lancaster (UK), or Spectrochem Pvt.Ltd (India) and were used without further purification. The title compound is 2-Substituted amino-1-(1*H*-benzo[d]imidazol-1-yl) ethanone (PS1 - PS5) depicted in scheme.



Scheme

Synthesis of benzimidazole or 1H-benzo[d]imidazole (3)

A 0.5 mole of O-Phenylenediamine (1) was treated with 0.75 moles of 90 % formic acid (2). The mixture was heated in a water bath at 100 ºC for 2 h. After cooling, 10 % sodium hydroxide solution was added slowly with thorough mixing by rotating of the flask, until the mixture was just alkaline to litmus [11]. The crude benzimidazole was collected with suction in a 75 mm. Ice cold water was used to rinse all solids out of the reaction flask. The simple product was pressed thoroughly on the filter, washed with about 50 mL of cold water, and then purified without previous drying.

Synthesis of 1-(1H-benzo[d]imidazol-1-yl)-2-chloroethanone (5)

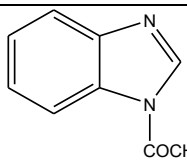
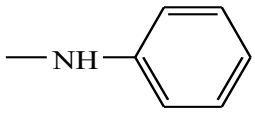
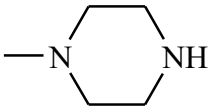
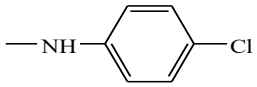
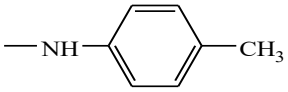
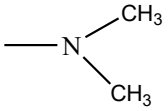
To the solution of compound (3) (1.71 g; 0.01 mol) in dioxan, chloroacetyl chloride (1.12 g; 0.01 mol) [4] and potassium carbonate was added as a catalyst and heated (with stirring) at 80 ºC for 6 h [12]. The reaction mixture was cooled to room temperature and filtered off to remove the inorganic materials. The filtrate was poured into

crushed ice, and extracted with ether. The ether was removed under reduced pressure to yield the compound (5). Yield: 95 %, MP 110 - 112 ºC; IR (KBr) cm^{-1}: 1660 cm^{-1} (C=O), 757 (C-Cl), ^{1}H-NMR ($CDCl_3$) δ: 7.26 (m, CH), 8.08 (m, CH), 4.49 (m, CH_2 methylene); MS (m/z): 194 (M + 1), 196 (M + 2): Anal. Calcd for $C_9H_7ClN_2O$: C, 55.54; H, 3.63; Cl, 18.22; N, 14.39; O, 8.22. Found: C, 55.54; H, 3.65; Cl, 18.19; N, 14.35; O, 8.41 %.

General procedure for the synthesis of 2-Substituted amino-1-(1H-benzo[d]imidazol-1-yl) ethanone (PS1 - PS5)

A mixture of 1-(1*H*-benzo[d]imidazol-1-yl)-2-chloroethanone (5) (2.48 g; 0.01 mol), anhydrous potassium carbonate (100 mg) and Aryl/aralkyl amine (0.01 mol) in dioxan (15 mL) was refluxed for 16 h [13]. The reaction mixture was then poured into crushed ice. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol. The title compounds physical data were given in **Table 1**.

Table 1 Physical data for synthesized derivatives.

Compound Code	R^2/Ar	Mol. Formula	Mol.Weight	Rf Value	Melting Point (MP)	Percentage of Yield (%)
Intermediate		$C_9H_7ON_2Cl$	194 [M + 1] +, 196 [M + 2] +	0.76	110	95
PS1		$C_{15}H_{13}ON_3$	251+	0.92	136	82
PS2		$C_{13}H_{16}ON_4$	244+	0.54	126	86
PS3		$C_{15}H_{12}ON_3Cl$	285 [M + 1] + 287 [M + 2] +	0.78	138	90
PS4		$C_{16}H_{15}ON_3$	265+	0.86	142	89
PS5		$C_{11}H_{13}ON_3$	203+	0.62	114	60

2-Phenylamino-1-(1H-benzo[d]imidazol-1-yl)ethanone (PS1)

Yield: 82 %; MP 136 ºC; IR (KBr) cm^{-1}: 1666 cm^{-1} (C=O), 3280 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$) δ: 7.26 (m, CH), 8.08 (m, CH), 4.17 (m, CH_2 methylene), 7.04 (m, Ar ring), 4.0 (s, Aromatic NH); MS (m/z): 251 (M^+); Anal. Calcd for $C_{15}H_{13}ON_3$: C, 71.70; H, 5.21; N, 16.72; O, 6.37. Found: C, 71.59; H, 5.31; N, 16.51; O, 6.07 %.

2-Piperazinyl-1-(1H-benzo[d]imidazol-1-yl)ethanone (PS2)

Yield: 86 %; MP 126 ºC; IR (KBr) cm^{-1}: 1670 cm^{-1} (C=O), 3284 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$) δ: 7.24 - 8.08 (m, CH, Ar), 8.08 (m, CH), 2.48 (m, CH_2 methylene), 2.65 - 3.47 (m, CH_2 Piperazinyl), 2.0 (NH, Amine); MS (m/z): 254 (M^+); Anal. Calcd for $C_{13}H_{16}ON_4$: C, 63.91; H, 6.60; N, 22.93; O, 6.55. Found: C, 63.81; H, 6.66; N, 22.87; O, 6.75 %.

2-(4-Chlorophenylamino-1-(1H-benzo[d]imidazol-1-yl)ethanone (PS3)

Yield: 90 %; MP 138 ºC; IR (KBr) cm^{-1}: 1674 cm^{-1} (C=O), 3280 cm^{-1} (NH), 752 (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 7.26 - 8.10 (m, CH, Ar), 6.37 - 7.05 (m, CH, Ar), 4.17 (m, CH_2 methylene), 4.0 (NH, Amine); MS (m/z): 285[M^+], 287[M + 2]; Anal. Calcd for $C_{15}H_{12}ON_3Cl$: C, 63.05; H, 4.23; Cl, 12.41; N, 14.71; O, 5.60. Found: C, 63.17; H, 4.28; Cl, 12.04; N, 14.84; O, 5.75 %.

2-(4-Methylphenylamino-1-(1H-benzo[d]imidazol-1-yl)ethanone (PS4)

Yield: 89 %; MP 142 ºC; IR (KBr) cm^{-1}: 1670 cm^{-1} (C=O), 3284 cm^{-1} (NH); ^{1}H-NMR ($CDCl_3$) δ: 7.26 - 8.08 (m, CH, Ar), 6.31 - 6.84 (m, CH, Ar), 4.17 (m, CH_2 methylene), 2.35 (m, CH_3 (methyl)), 4.2 (NH, Amine); MS (m/z): 265[M^+]: Anal. Calcd for $C_{16}H_{15}ON_3$: C, 72.43; H, 5.70; N, 15.84; O, 6.03. Found: C, 72.43; H, 5.70; N, 15.84; O, 6.03 %.

2-(Dimethylamino-1-(1H-benzo[d]imidazol-1-yl)ethanone (PS5)

Yield: 60 %; MP 114 ºC; IR (KBr) cm^{-1}: 1676 cm^{-1} (C=O), 2607 cm^{-1} (Dimethylamine); ^{1}H-NMR ($CDCl_3$) δ: 7.24 - 8.06 (m, CH, Ar), 6.30 - 6.81 (m, CH, Ar), 3.47 (m, CH_2 methylene), 2.27 (m, 2-CH_3 (methyl)); MS (m/z): 203 [M^+]: Anal. Calcd for $C_{11}H_{13}ON_3$: C, 65.01; H, 6.45; N, 20.68; O, 7.87. Found: C, 65.22; H, 6.49; N, 20.76; O, 7.67 %.

Biological

The novel title compounds were screened for their antibacterial and antifungal activity by ditch dilution method [3]. Antibacterial activity was screened against gram negative viz *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. typhii* and 1 g positive bacteria, i.e *S. aureus* at 1 concentration. The test organism was a 2 h culture of *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. typhii* and *S. typhii* incubated and grown in a peptone-water medium (37 ºC). Dimethyl formamide (DMF) [4] was used as solvent control which did not show any zone of inhibition. Muller-Hilton agar medium was used as a culture medium. The culture plates were incubated at 37 ºC for 24 h. Antibacterial activity was determined by measuring the diameter (mm) of the inhibition zone. Amoxicillin had been used as the control drug for antibacterial activity and griseofulvin had been used as the control drug for antifungal activity. The antifungal activity was performed against *C. albicans* and *A. niger* at 50 and 100 µg/mL levels. The results are given in the results and discussion part.

Results and discussion

Chemistry

Five benzimidazole derivatives were synthesized in 2 steps. In the 1st step, condensation of HCOOH (2), NaOH (10 %), and OPDA (1) yield benzimidazole (3). The compound 5 (2-chloro-1-benzimidazole ethanone) was prepared by reflexive a solution of benzimidazole (3) and chloro acetyl chloride (4) in dry dioxane in the presence of a base. The stirring was continued further for 6 h with heating. The IR spectrum of compound (3) indicates that the NH peaks usually appear around 3200 - 3300 cm^{-1}. The IR spectrum of compound (5) shows intense peaks at 757 cm^{-1} for Cl and 1660 cm^{-1} for Carbonyl (C=O). The NH peak of benzimidazole disappeared in the IR spectrum 3200 - 3400 cm^{-1}. The title compounds 2-(substituted amino)-1-benzimidazole ethanone (PS1 - PS5) were obtained in good yields through the nucleophilic displacement of –Cl group of 2-chloro-1-benzimidazole ethanone (5) with a variety of amines using dioxan as solvent. All the title compounds showed in the IR spectrum the disappearance of –Cl peaks at 757 cm^{-1} and the appearance of NH peaks around 3280 cm^{-1}. The disappearance of peaks indicates the formation of title compounds due to –Cl, the starting materials, and the appearance of NH at 3280 cm^{-1} in the IR spectrum of the PS1. The compound (3) mass spectrum indicates that the peaks are obtained clearly, and the most intense peak was observed. The compound (5) mass spectrum demonstrates that the most intense peak was honored along with Halogen (Cl) peak. The Halogen peak was marked as one part of the intense peak. So, it can be named as daughter peak or M + 2 peak. The –Cl peak was disappearing in all the title compounds.

Biological

Biological studies indicated that different substituents over the 1st position of the benzimidazole ring exerted varied biological activity. The compounds containing 2-(substituted amino)-1-benzimidazole ethanone (PS1 - PS5) were evaluated for their activity against bacteria and fungi to determine the inhibition of the growth of bacteria and fungus. All the tested compounds exhibited good and moderate exercise (**Table 2**). Percentage protection data showed that all tested series combinations showed significant protection in the range of 6 - 19 mm. Biological studies indicated that different substituents over the 1st position of the benzimidazole ring exerted varied physical

activity. The compound PS3 showed equipotent (19 mm) against most bacteria due to the Presence of an electron-withdrawing group (–Cl) than compounds PS4 containing electron-donating (–CH_3) groups. i.e. The inhibitory activity increases when position 4 is chlorinated as in compound PS3. Methyl substitution at 4th position of the phenyl ring shows moderate the inhibitory activity (18 mm) of compound PS4 against all bacteria. Placement of additional heteroatoms, such as nitrogen-containing the cyclic aliphatic chain PS2 showed less moderate (17 mm) exercise, and placement of the aromatic/aliphatic chain containing compounds PS1 (8 mm) and the presence of dimethyl group PS5 (6 mm) showed minor activity against all bacteria. Amoxicillin has been used as a controlled drug. The antimicrobial activity data is shown in **Table 2**, and the bar diagram is shown in **Figures 1** and **2**. All the title compounds were evaluated against *C.albicans* and *A.nigar* at 50 and 100 µg/mL concentration using griseofulvin as control. Most of the tested compounds, such as PS3, PS4 and PS1 showed better activity against *C.albicans* at 100 µg/mL concentration. The tested compounds PS1, PS3, PS2 and PS4 showed good activity at 100 µg/mL concentration. All the tested compounds were not produced activity against all fungi at 50 µg/mL concentration. The compound PS5 showed less activity. Compound 2-(4-Chlorophenylamino-1-(1*H*-benzo[d]imidazol-1-yl)ethanone (PS3) exhibited equipotent of the series.

Table 2 The antimicrobial data for synthesized compounds PS1 - PS5.

Compounds	Antibacterial Activity					Antifungal Activity			
	E. coli (mm)	*P. aeruginosa* (mm)	*K. pneumoniae* (mm)	*S. typhii* (mm)	*S. aureus* (mm)	*C. albicans*		*A. nigar*	
						50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
PS1	11	10	12	11	8	+	+++	+	++++
PS2	16	12	17	14	11	+	++	+	+++
PS3	19	18	18	16	11	+	++++	+	++++
PS4	18	16	17	15	12	+	+++	+	+++
PS5	11	10	11	8	6	+	++	+	++
Amoxicillin	20	19	18	18	15	–	–	–	–
Griseofulvin	–	–	–	–	–	++++	++++	++++	++++

Note: ++++ = (13 - 17 mm) Strong active range, +++ = (8 - 12 mm) Moderately active range, ++ = (Less than 8 mm (or) ≤ 8 mm) Weekly active range, + = Inactive range.

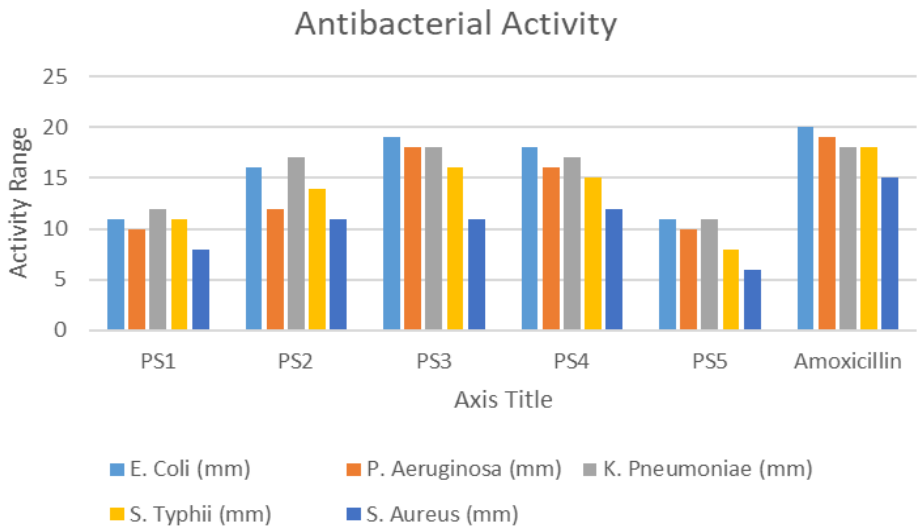


Figure 1 Antibacterial activity.

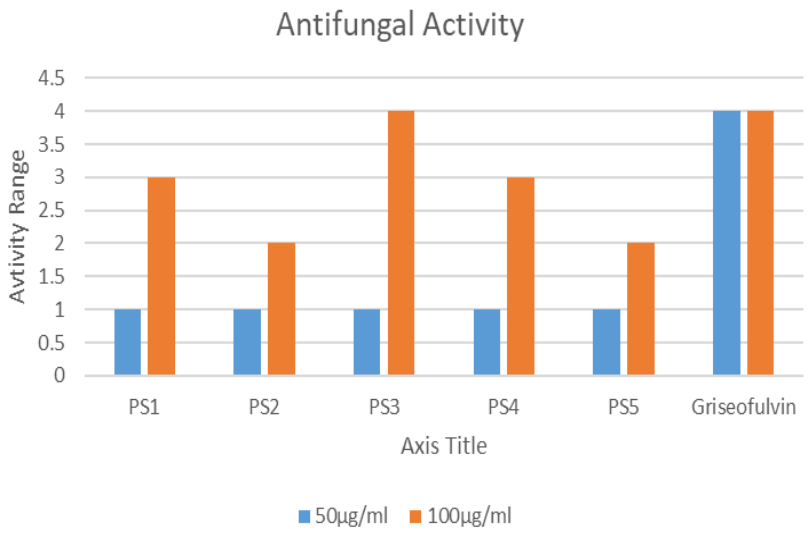


Figure 2 Antifungal activity.

Conclusions

Benzimidazole has a variety of simple chemistry, which enables a diversity of substitutions with easy synthesis and different pharmacological potentials in dependence on particular structural modifications. Within this work, 5 substituted compounds (2-Substituted amino-1-(1*H*-benzo[d]imidazol-1-yl) ethanone (PS1 - PS5) have been synthesized and characterized. All novel compounds were tested on antibacterial and antifungal agents. The title compounds have exhibited good antimicrobial activity against them. As far as SAR is concerned, the presence of an electron-withdrawing group in the 4th position on the phenyl ring of benzimidazole (2-(4-chlorophenylamino)-1-(1*H*-benzo[d]imidazole-1-yl) ethanone) compound PS3 was found to be the most active compound against all bacteria's, which is equipotent (19, 18, 18, 16 and 11 mm) to reference control amoxicillin. Placement of the aromatic/aliphatic chain containing compounds PS1 (8 mm) and the presence of dimethyl group PS5 (6 mm) showed minor activity against all bacteria. Interestingly compound PS3 also showed appreciable antifungal activity compared to griseofulvin and could therefore serve as a lead molecule for further modification to obtain a clinically helpful novel class of antimicrobial agents.

Acknowledgments

The authors gratefully acknowledge the Central Instrumentation Facility, IIT Chennai, India, for the spectral analysis of the compounds used in this study. The authors also wish to thank our Management of Vellalar College of Pharmacy, Run by Vellalar Educational Trust, Maruthi Nagar, Thindal, Erode, Tamilnadu, for providing the financial and infrastructure facilities to carry out this research work.

Reference

[1] M Tonelli, G Paglietti, V Boido, F Sparatore, F Marongiu, E Marongiu, PL Colla and R Loddo. Antiviral activity of benzimidazole derivatives. Antiviral activity of 1-substituted-2-[(benzotriazol-1/2-yl)methyl]benzimidazoles. *Chem. Biodivers.* 2008; **5**, 2386-401.

[2] KCS Achar, KM Hosamani and HR Seetharamareddy. *In-vivo* analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives. *Eur. J. Med. Chem.* 2010; **45**, 2048-54.

[3] G Serban, O Stanasel, E Serban and S Bota. 2-Amino-1,3,4-thiadiazole as a potential scaffold for promising antimicrobial agents. *Drug Des. Devel. Ther.* 2018; **12**, 1545-66.

[4] ZI Kirkwood, BC Millar, DG Downey and JE Moore. Antimicrobial effect of dimethyl sulfoxide and N, N-Dimethylformamide on Mycobacterium abscessus: Implications for antimicrobial susceptibility testing. *Int. J. Mycobacteriol*. 2018; **7**, 134-6.

[5] YK Yoon, MA Ali, TS Choon, R Ismail, AC Wei, RS Kumar, H Osman and F Beevi. Antituberculosis: Synthesis and antimycobacterial activity of novel benzimidazole derivatives. *Biomed. Res. Int.* 2013; **2013**, 926309.
[6] HM Refaat. Synthesis and anticancer activity of some novel 2-substituted benzimidazole derivatives. *Eur. J. Med. Chem.* 2010; **45**, 2949-56.
[7] B Bhrigu, N Siddiqui, D Pathak, MS Alam, R Ali and B Azad. Anticonvulsant evaluation of some newer benzimidazole derivatives: Design and synthesis. *Acta Pol. Pharm.* 2012; **69**, 53-62.
[8] NC Desai and GM Kotadiya. Microwave-assisted synthesis of benzimidazole bearing 1,3,4-oxadiazole derivatives: Screening for their in vitro antimicrobial activity. *Med. Chem. Res.* 2014; **3**, 4021-33.
[9] PNK Babu, B Ramadevi, Y Poornachandra and CG Kumar. Synthesis, antimicrobial, and anticancer evaluation of novel 2-(3-methylindolyl)benzimidazole derivatives. *Med. Chem. Res.* 2014; **23**, 3970-8.
[10] D Song and S Ma. Recent development of benzimidazole-containing antibacterial agents. *Chem. Med. Chem.* 2016; **11**, 646-59.
[11] K Kamanna. *Synthesis and pharmacological profile of benzimidazoles*. *In*: M Marinescu (Ed.). Chemistry and applications of benzimidazole and its derivatives. IntechOpen, London, 2019.
[12] AN Khayyat, KO Mohamed, AM Malebari and A El-Malah. Design, synthesis, and antiproliferative activities of novel substituted imidazole-thione linked benzotriazole derivatives. *Molecules* 2021; **26**, 5983.
[13] N Vashist, SS Sambi, B Narasimhan, S Kumar, SM Lim, SAA Shah, K Ramasamy and V Mani. Synthesis and biological profile of substituted benzimidazoles. *Chem. Cent. J.* 2018; **12**, 125.