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RESEARCH ARTICLE

Microwave assisted, one-pot synthesis of 5-nitro-2-aryl substituted-1H-benzimidazole libraries: Screening *in vitro* for antimicrobial activity

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Abstract

The efficient and rapid synthesis of 5-nitro-2-aryl substituted-1H-benzimidazole libraries **(1a-1j)** has been established. Thus, both microwave and conventional cyclo-condensation of 4-nitro *ortho*-phenylenediamine with various phenoxyacetic acids were carried out in the presence of HCl catalyst. The microwave synthesis route afforded advantages, such as good to excellent yields, shorter reaction time (2.5-3.5 min), readily available starting material, and simple purification procedure, which distinguish the present protocol from other existing methods used for the synthesis of benzimidazole libraries. Bioassay indicated that all the compounds showed *in vitro* antimicrobial activity against *Vancomycin resistant enteroccoccus, Staphylococcus aureus, Micrococcus, Bacillus subtilis* (gram-positive bacteria) and *Shigella dysentery, Escherichia coli* (gram-negative bacteria) and *Candida albicans, Aspergillus niger, Penicillium* (fungi). The minimum inhibitory concentration **(MIC)** was determined for test compounds as well as for reference standards.

Keywords: *Benzimidazole libraries; one-pot synthesis; microwave irradiation; antimicrobial activity*

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Introduction

Benzimidazole has been an important pharmacophore and privileged structure in medicinal chemistry [1] *e.g.*, as antifungal, antimicrobial, anticancer, anaesthetic, HIV-Rt inhibitor, antihistaminic [2–6], Mean while 5-nitrobenzimidazole with varying substituents at 2-position used as angiotensin II receptor antagonists [7]. Many approaches for the synthesis of benzimidazoles continue to utilize the condensation reactions of *o*-phenylenediamine with carboxylic acids, carboxylic acid esters, lactones, anhydrides and aldehydes [8].

Modern drug discovery uses techniques such as combinatorial and parallel synthesis, as well as automated library production, to accelerate the lead identification process [9]. There is, however, an acute need to implement more sustainable methods, not only for large-scale production but also for lab-scale medicinal chemistry research [10]. A set of environmentally friendly approaches and technologies are available that can help to make progress in this somewhat forgotten research area [11]. Beyond the development of a environmentally friendlier synthetic methods, there is a need for decreased reaction times. Thus, microwave irradiation has become a helpful processing tool because it allows rapid and convenient super heating to high temperatures in combination with excellent reaction control and low-energy consumption [12,13]. Hence the decomposition of reactants and/or products is diminished in these reactions leading to enhanced yields. Often, microwave assisted organic reactions proceed rapidly in the absence of solvent also [14]. This expeditious and solvent-free approach involves the exposure of neat reactants to microwave irradiation in conjunction with the use of supported reagents or catalysts, which are primarily of mineral origin. The salient features of these high yield protocols are the enhanced reaction rates, greater selectivity and the experimental ease of manipulation, which is in many ways superior to traditional heating [15,16]. This novel synthetic route did not lead to formation of some carcinogenic by-products as reported with the literature methods [17–20]. But in our preliminary *in vitro*

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bioassay against six strains of bacteria [*Vancomycin resistant enteroccoccus* (ATCC-51299), *Staphylococcus aureus* (ATCC-29213), *Micrococcus* (natural isolates), *Bacillus subtilis* (natural isolates), *Shigella dysentery* (natural isolates) *and Escheria coli* (ATCC-25922)] and three strains of fungi [*Candida albicans, Aspergillus niger and Penicillium*] indicated that these benzimidazole libraries displayed significant antimicrobial activities.

As part of our ongoing work devoted toward the development of a rapid synthesis of heterocyclic molecules of biological interest, we explored the possibility of synthesizing 5-nitro-2-aryl substituted phenoxymethyl-1H-benzimidazole libraries from 4-nitro-*o*-phenylenediamine and substituted phenoxyacetic acids using mineral acid as a catalyst in solvent-free conditions under microwave irradiation to facilitate the rapid synthesis of drugs from commercially available building blocks.

Materials and methods

Melting points were determined on a open capillary apparatus and are uncorrected. Elemental analyses were recorded as both experimental as well as theoretical. Infrared spectra were determined in KBr on Nicolet 5700 FTIR instrument. The ^{1}H and ^{13}C NMR spectras were recorded on a 300MHz Bruker-Avanace NMR instrument in $CDCl_3$ and the chemical shifts were expressed in parts per million (ppm) with tetramethylsilane (TMS) as an internal standard. All compounds were routinely checked by thin-layer chromatography (TLC) on aluminium-backed silica gel plates. Microwave synthesis was carried out on a Ken Star synthesizer (Model: OM-25DGQ).

Chemistry

The synthesis of the target compounds was carried out as outlined in scheme 1. Cyclo-condensation of 4-nitro *ortho*-phenylenediamine with various phenoxyacetic acids were carried out in the presence of HCl catalyst, afforded 5-nitro-2-aryl substituted-1H-benzimidazole libraries under microwave conditions.

The new benzimidazole libraries were characterized by their spectral data. IR spectra of the target substituted benzimidazoles showed NH stretching bands at 3350- 3500 cm^{-1}, NO_2 asymmetric stretching bands at 1510- 1550 cm^{-1} & symmetric stretching bands at 1335- 1365 cm^{-1}, respectively. The absorption bands associated with other functional groups appeared in the expected regions. In the ^{1}H NMR spectra of the target substituted benzimidazoles, the NH proton appeared at 3.5 ppm as a broad D_2O exchangeable singlet, whereas sharp singlet at 4.5 ppm was characterstic of the CH_2-O group. The other protons appeared at the expected chemical shifts.

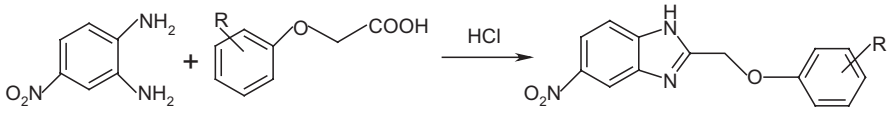
General method for the synthesis of 5-nitro-2-aryl substituted-1H-benzimidazole libraries

Microwave synthesis of 5-nitro-2-aryl substituted-1H-benzimidazole libraries. The mixture of 4-nitro-*o*-phenylenediamine (0.01 mole) and substituted phenoxyacetic acids (0.01 mole) in presence of 1 or 2 mL of HCl 6N, was irradiated in a sealed vessel under microwave oven (Model: Ken Star OM-25DGQ) for 2.5–3.5 min at 400W output power and the reaction progress examined under thin layer chromatography (TLC). After the reaction was completed the mixture was cooled to room temperature and then poured into ice cold water. Stirring was continued for few minutes and the mixture was neutralized with aqueous ammonia, then pure product is obtained in most of the cases as evident from ^{1}H NMR. Otherwise the products were recrystalized from water-ethanol system. The yield and m.p. of compounds are given in Table 1.

Conventional synthesis of 5-nitro-2-aryl substituted-1-H-benzimidazole libraries. A mixture of 4-nitro-*o*-phenylenediamine (0.01 mole) and substituted phenoxyacetic acids (0.01 mole) was heated at 100°C for 3-4 h in 15mL of 6N HCl and the reaction progress examined under thin layer chromatography (TLC). After the reaction was completed the mixture was cooled to room temperature and then poured into ice cold water. Stirring was continued for a few minutes and the mixture was neutralized with aqueous ammonia and the products were recrystalized from water-ethanol system. The yield and m.p. of compounds are given in Table 1.

5-nitro-2-phenoxymethyl-1H-benzimidazole(1a): IR(KBr): γ (cm^{-1}) 3422 br (ArNH), 1533 and 1344 (NO_2 asymmetric and symmetrical stretching), 1629 and 1455 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.74 (br, s, 1H, NH-benzimidazole), 3.5 (s, 1H, OCH_3), 7.27 (s, 1H, Ar-H), 7.28 (s, 2H, Ar-H), 7.63 (s, 1H, Ar-H), 8.22 (s, 1H, Ar-H), 8.51 (s, 1H, Ar-H); CIMS: *m/z* 269.24: Anal. Calcd. For $C_{14}H_{11}N_3O_3$: C 62.45, H 4.12, N 15.61. Found: C 62.43, H 4.11, N 15.60%.

5-nitro-2-*o*-toloxymethyl-1H-benzimidazole(1b): IR(KBr): γ (cm^{-1}) 3423 br (ArNH), 1540 and 1344 (NO_2 asymmetric and



1a–1j

R = H, 2-CH_3, 3-CH_3, 4-CH_3, 2-Cl, 4-Cl, 2-NO_2, 4-NO_2, 2-NH_2, 2,4-*di*-Cl

Scheme 1. Synthesis of 5-nitro-2-aryl substituted phenoxymethyl-1H-benzimidazoles.

Table 1. Comparison between microwave-assisted and thermal method synthesis of 5-nitro-2-aryl substituted phenoxymethyl-1H-benzimidazole libraries

Compound	Phenoxy acetic Acid used	Mol. Formula	Conventional method			Microwave method		
			Time (h)[a]	Yield	M.P.	Time W/(min)[a]	Yield	M. P.
1a	Unsubstituted	$C_{14}H_{11}N_3O_3$	3.35	58.40	219–221	400/2.5	87.86	218–220
1b	2-Methyl-	$C_{15}H_{13}N_3O_3$	3.20	67.59	89–91	400/3.0	88.55	88– 90
1c	3-Methyl-	$C_{15}H_{13}N_3O_3$	3.25	70.57	215–217	400/3.0	85.61	213–215
1d	4-Methyl-	$C_{15}H_{13}N_3O_3$	3.20	65.01	77–79	400/3.0	91.32	79–81
1e	2-Chloro-	$C_{14}H_{10}N_3O_3Cl$	3.50	74.70	187–189	400/3.5	85.24	185–187
1f	4-Chloro-	$C_{14}H_{10}N_3O_3Cl$	3.40	58.21	265–267	400/3.5	87.81	266–268
1g	2-Nitro-	$C_{14}H_{10}N_4O_5$	3.50	64.14	230–232	400/2.5	82.03	227–229
1h	4-Nitro-	$C_{14}H_{10}N_4O_5$	3.45	60.95	98–100	400/3.5	90.15	98–100
1i	2-Amino-	$C_{14}H_{12}N_4O_3$	3.15	63.97	215–217	400/3.0	83.08	216–218
1j	2,4-Dichloro-	$C_{14}H_9N_3O_3Cl_2$	3.40	61.86	86–88	400/2.5	86.46	85–68

[a]Time to finish the reaction monitored by TLC.

symmetrical stretching), 1630 and 1388 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 1.70 (s, 1H, CH_3), 2.78 (br, s, 1H, NH-benzimidazole), 3.87 (s, 1H, OCH_3), 7.41 (s, 5H, Ar-H), 8.20 (s, 1H, Ar-H), 8.41 (s, 1H, Ar-H); CIMS: *m*/*z* 283.27: Anal. Calcd. For $C_{15}H_{13}N_3O_3$: C 63.60, H 4.63, N 14.83. Found: C 63.59, H 4.62, N 14.81%.

5-nitro-2-*m*-toloxymethyl-1H-benzimidazole (1c): IR (KBr): γ (cm^{-1}) 3420 br (ArNH), 1533 and 1344 (NO_2 asymmetric and symmetrical stretching), 1630 and 1388 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.19 (s, 1H, CH_3), 2.70 (br, s, 1H, NH-benzimidazole), 3.87 (s, 1H, OCH_3), 7.27 (s, 4H, Ar-H), 8.21 (d, 2H, *J*=9.0, Ar-H), 8.50 (s, 1H, Ar-H); CIMS: *m*/*z* 283.27: Anal. Calcd. For $C_{15}H_{13}N_3O_3$: C 63.60, H 4.63, N 14.83. Found: C 63.59, H 4.62, N 14.81%.

5-nitro-2-*p*-toloxymethyl-1H-benzimidazole (1d): IR (KBr): γ (cm^{-1}) 3422 br (ArNH), 1533 and 1345 (NO_2 asymmetric and symmetrical stretching), 1631 and 1387 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.18 (s, 1H, CH_3), 2.79 (br, s, 1H, NH-benzimidazole), 3.67 (s, 1H, OCH_3), 7.27 (s, 3H, Ar-H), 7.30 (d, 2H, *J*=6.1, Ar-H), 8.41 (s, 2H, Ar-H); CIMS: *m*/*z* 283.27: Anal. Calcd. For $C_{15}H_{13}N_3O_3$: C 63.60, H 4.63, N 14.83. Found: C 63.59, H 4.62, N 14.81%.

2-(2-chloro-phenoxymethyl)-5-nitro-1H-benzimidazole (1e): IR (KBr): γ (cm^{-1}) 3425 br (ArNH), 1517 and 1343 (NO_2 asymmetric and symmetrical stretching), 1627 and 1388 (C=C and C=N ring stretching), 1284 and 733 (ArCl); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.72 (br, s, 1H, NH-benzimidazole), 3.41 (s, 1H, OCH_3), 7.27 (s, 4H, Ar-H), 7.58 (s, 1H, Ar-H), 8.18 (s, 1H, Ar-H), 8.49 (s, 1H, Ar-H); CIMS: *m*/*z* 303.68: Anal. Calcd. For $C_{14}H_{10}N_3O_3Cl$: C 55.37, H 3.32, N 13.84. Found: C 55.38, H 3.34, N 13.85%.

2-(4-chloro-phenoxymethyl)-5-nitro-1H-benzimidazole (1f): IR (KBr): γ (cm^{-1}) 3425 br (ArNH), 1517and 1345 (NO_2 asymmetric and symmetrical stretching), 1631 and 1387 (C=C and C=N ring stretching), 1283 and 734 (ArCl); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.72 (br, s, 1H, NH-benzimidazole), 3.67 (s, 1H, OCH_3), 7.28 (s, 4H, Ar-H), 8.23 (s, 2H, Ar-H), 8.50 (s, 1H, Ar-H); CIMS: *m*/*z* 303.68: Anal. Calcd. For $C_{14}H_{10}N_3O_3Cl$: C 55.37, H 3.32, N 13.84. Found: C 55.38, H 3.34, N 13.85%.

5-Nitro-2-(2-nitro-phenoxymethyl)-1H-benzimidazole (1g): IR (KBr): γ (cm^{-1}) 3425 br (ArNH), 1517 and 1344 (NO_2 asymmetric and symmetrical stretching), 1629 and 1387 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.71 (br, s, 1H, NH-benzimidazole), 4.81 (s, 1H, OCH_3), 7.27 (s, 3H, Ar-H), 7.60 (s, 1H, Ar-H), 8.19 (d, 2H, *J*=8.8, Ar-H), 8.49 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz,): δ (ppm) 41.21 (s, C, OCH_3), 110.05 (s, C, Ar-C), 118.07 (s, C, Ar-C) and 143.0 (s, C, Ar-C); CIMS: *m*/*z* 314.23: Anal. Calcd. For $C_{14}H_{10}N_4O_5$: C 53.51, H 3.21, N 17.83. Found: C 53.49, H 3.19, N 17.82%.

5-nitro-2-(4-nitro-phenoxymethyl)-1H-benzimidazole (1h): IR (KBr): γ (cm^{-1}) 3422 br (ArNH), 1516 and 1343 (NO_2 asymmetric and symmetrical stretching), 1627 and 1386 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.72 (br, s, 1H, NH-benzimidazole), 3.61 (s, 1H, OCH_3), 7.60 (s, 1H, Ar-H), 7.97 (s, 2H, Ar-H), 8.30 (d, 2H, *J*=8.7, Ar-H), 8.50 (s, 2H, Ar-H); CIMS: *m*/*z* 314.23: Anal. Calcd. For $C_{14}H_{10}N_4O_5$: C 53.51, H 3.21, N 17.83. Found: C 53.49, H 3.19, N 17.82%.

2-(5-nitro-1H-benzimidazol-2-ylmethoxy)-phenyl amine (1i): IR (KBr): γ (cm^{-1}) 3562 s ($ArNH_2$); 3327br (ArNH), 1518 and 1332 (NO_2 asymmetric and symmetrical stretching), 1627 and 1390 (C=C and C=N ring stretching); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.72 (s, 1H, NH_2), 3.5 (br, 1H, NH-benzimidazole), 7.27 (s, 4H, Ar-H), 8.06 (d, 2H, *J*=8.1, Ar-H), 8.50 (s, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz,): δ (ppm) 41.19 (s, C, OCH_3), 112.106 (s, C, Ar-C), 114.66 (s, C, Ar-C), 118.09 (s, C, Ar-C) and 143.017 (s, C, Ar-C); CIMS: *m*/*z* 284.28: Anal. Calcd. For $C_{14}H_{12}N_4O_3$: C 59.15, H 4.25, N 19.71. Found: C 59.15, H 4.23, N 19.70%.

2-(2,4-Dichloro-phenoxymethyl)-5-nitro-1H-benzimidazole (1j): IR (KBr): γ (cm^{-1}) 3416 br (ArNH), 1517 and 1344 (NO_2 asymmetric and symmetrical stretching), 1629 and 1388 (C=C and C=N ring stretching), 1285 and 734s (ArCl); ^{1}H NMR ($CDCl_3$, 300 MHz,): δ (ppm) 2.69 (br, 1H, NH-benzimidazole), 4.82 (s, 1H, CH_3), 7.77 (d, 2H, *J*= 5.5, Ar-H), 7.80 (s, 1H, Ar-H), 8.18 (d, 2H, *J*= 8.4, Ar-H), 8.47 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 75 MHz,): δ (ppm) 41.20 (s, C, OCH_3), 111.66 (s, C, Ar-C), 115.10 (s, C, Ar-C), 119.56 (s, C, Ar-C) and 144.113 (s, C, Ar-C); CIMS: *m*/*z* 338.14: Anal. Calcd. For $C_{14}H_9N_3O_3Cl_2$: C 49.73, H 2.68, N 12.43. Found: C 49.72, H 2.67, N 12.41%.

Biology

Antimicrobial activity

All the test compounds were assayed *in vitro* for antibacterial activity against *Vancomycin resistant enteroccus, S. aureus, micrococcus* and *B. subtilis* representative for gram-positive bacterias. *S. dysentery* and *E. coli* representative for gram-negative bacterias, and the antifungal activities were evaluated against *C. albicans*, *A. niger* and *Penicillium.* The strains used in this study were maintained at the Department of Botany, Karnatak University, Dharwad. The antimicrobial activity was determined by using disk diffusion method [21–23] and MIC by twofold serial dilution method. Streptomycin and Nystatin were used as reference standards to compare antibacterial and antifungal activities, respectively. For determining the antimicrobial activity, the synthesized compounds were dissolved in dimethyl sulphoxide (the stock solution 1 mg/mL). Further dilutions were prepared at the required quantities of 100, 50 and 25 μg/mL concentrations. In order to ensure that the solvent had no effect on bacterial growth, a control test was also performed containing disc loaded with only DMSO at the same dilution used in our experiment. For determining the MIC of the synthesized compounds, they were diluted at 100, 50, 25 and 12 μg/mL concentrations and expressed in μM/mL (0.318, 0.159, 0.0795 and 0.0381 respectively for compound 1 h). In order to ensure that the solvent had no effect on fungal growth, a control test was also performed containing broath supplemented with only DMSO at the same dilution used in our experiment. The MIC of the compounds was defined, as the lowest concentration at which there was 100% inhibition of growth compared with the growth for a drug free control. Every experiment in the antibacterial assay and MIC was replicated thrice.

Antibacterial assay. The antibacterial activity of the benzimidazole derivatives was tested by the agar disc-diffusion method against Gram-positive and Gram-negative bacteria. Test compound solutions prepared in DMSO were serially diluted and loaded (10 μL) to Sterile filter paper discs (6 mm diameter), which finally contained (25, 50 and 100 μg/mL) of the compound per disc respectively. Impregnated disks were then dried for 1 h and placed on inoculated plates. The seeded plates were incubated at 37 °C for 16 h. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are given in Figures 1 & 2, for the compound 1h. The MIC of the compounds was defined, as the lowest concentration at which there was 100% inhibition of growth compared with the growth for a drug free control. The standard MIC for Streptomycin is *Vancomycin resistant enteroccoccus (0.85-1.71), S. aureus (≤0.85), Micrococcus (≤0.85), B. subtilis (0.85-1.71), S. dysentery (1.71-3.43) and E. coli (≤0.85)* μmoles/mL. Every experiment in the antibacterial assay was replicated thrice in order to define the MIC values, as shown in Table 2.

Antifungal assay. The synthesized Benzimidazole derivatives were tested for their antifungal activity *in vitro*, in comparison with Nystatin (Nystatin Ns 100, HIMEDIA, 100 units/disc) as a reference drug using the standard agar disk

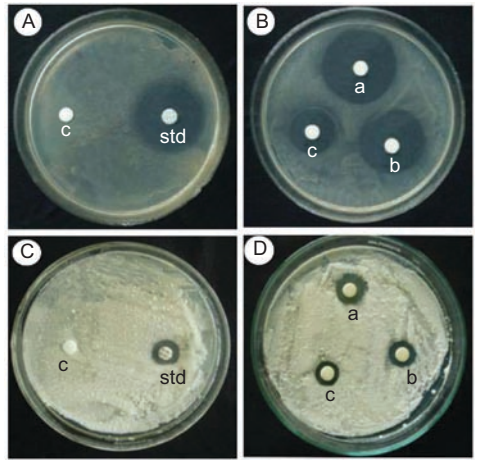


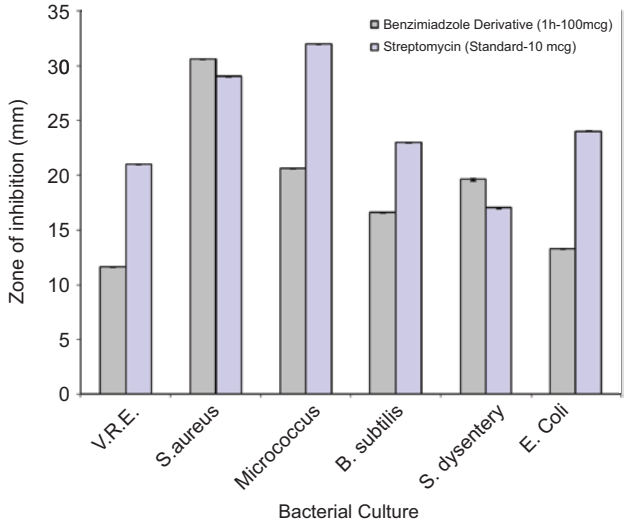
Figure 1. Photographic comparison of compound 1h with standard for antibacterial activity in *Staphylococcus aureus* and antifungal activity in *Penicillium*.

A & B - Streptomycin (Standard) and Compound 1h for *Staphylococcus aureus*

C & D - Nystatin (Standard) and Compound 1h for *Penicillium*

A & C - c- control disc with DMSO, std- standard (Streptomycin-10mcg & Nystatin-100units)

B & D - a-1mcg, b-0.5mcg and c-0.25mcg



Each value represents the mean ± SEM (n = 6). 1h = 5-nitro-2-(4-nitro-phenoxymethyl)-1H-benzimidazole

Figure 2. Bar curves for comparison of antibacterial activity of compound 1h with Streptomycin standard.

Table 2. The MIC values (μmoles/mL) of compounds 1a-1j for Antibacterial activity.

Compound	[c]V. r. e.	[c]S. a.	[c]M.	[c]B. s.	[c]S. d.	[c]E. c.
1a	≥3.71	0.445–0.929	1.858–3.71	≥3.71	1.858–3.71	1.858–3.71
1b	≥3.533	0.882–1.765	≥3.533	≥3.533	1.765–3.533	≥3.533
1c	≥3.533	0.423–0.882	1.765–3.533	≥3.533	1.765–3.533	≥3.533
1d	≥3.533	1.765–3.533	0.423–0.882	1.765–3.533	0.423–0.882	1.765–3.533
1e	1.64-3.29	0.823–1.64	1.64–3.29	≥3.29	1.64–3.29	≥3.29
1f	≥3.29	0.823–1.64	0.823–1.64	≥3.29	1.64–3.29	1.64–3.29
1g	≥3.182	0.381–0.795	≥3.182	≥3.182	1.59–3.182	≥3.182
1h	≥3.182	≤0.381	0.795–1.59	≥3.182	0.795–1.59	1.59–3.182
1i	≥3.521	0.88–1.76	≥3.521	≥3.521	1.76–3.521	≥3.521
1j	1.47-2.957	0.739–1.47	0.739–1.47	1.47–2.957	1.47–2.957	≥2.957
[b]SD	0.85-1.71	≤0.85	≤0.85	0.85–1.71	1.71–3.43	≤0.85

[c]*V. r. e.- Vancomycin resistant enterococcus*; [c]*S. a.- Staphylococcus aureus*; [c]*M.- Micrococcus*; [c]*B. s.- Bacillus subtilis*; [c]*S. d.- Shigella dysentery*; [c]*E. c.- Escheria coli*
[b]SD–Streptomycin (Standard)

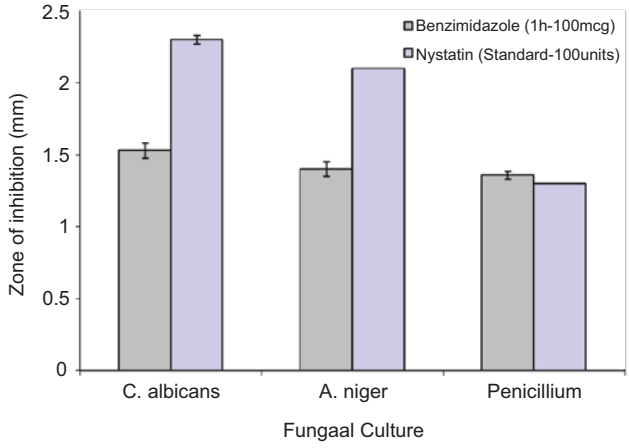


Figure 3. Bar curves for comparison of antifungal activity of compound 1h with Nystatin standard.

Table 3. The MIC values (μmoles/mL) of compounds 1a-1j Antifungal activity.

Compound	cC. a.	cA. n.	cP.
1a	≤0.4457	0.929–1.858	1.858–3.71
1b	0.8827–1.7655	1.858–3.71	>3.533
1c	≤0.4237	0.882–1.765	1.765–3.533
1d	0.423–0.882	0.423–0.882	0.882–1.765
1e	0.411–0.823	>3.29	1.64–3.29
1f	0.823–1.64	1.64–3.29	>3.29
1g	1.59–3.182	>3.182	>3.182
1h	0.381–0.795	≤0.381	1.59–3.182
1i	1.76–3.521	1.76–3.521	1.76–3.521
1j	0.739–1.47	1.47–2.957	0.739–1.47
[b]SD	≤ 1.71	≤3.43	≤3.43

[c]*C. a.- Candida albicans*; [c]*A. n.- Aspergillus niger*; [c]*P.- Penicillium*
[b]SD–Nystatin (Standard)

diffusion method against three strains of fungus (*A. niger, Penicillium and C. albicans*). A spore suspension in sterile distilled water was prepared from 3-5 days old culture of the test fungi growing on Potato Dextrose Agar (PDA) media. The final spore concentration was 5×10^{-4} spores mL^{-1}. About 15 mL of the growth medium was placed into sterilized Petri dishes of 9 cm diameter and inoculated with 100 μL of the spore suspension. Sterile 6-mm filter paper disk (HiMedia Laboratories Pvt. Limited. India) was saturated with 10 μL of the test compound solution. Impregnated disks were then dried for 1 h and placed on inoculated plates. The seeded plates were incubated at 27 °C for 4 days. The radii of inhibition zones (in mm) of triplicate sets were measured and the results are given in Figures 1. & 3. The MIC of the compounds was defined, as the lowest concentration at which there was 100% inhibition of growth compared with the growth for a drug free control. The standard MIC for Nystatin is *A. niger* (≤*3.43*), *Penicillium* (≤*3.43*) and *C. albicans* (≤*1.71*) at 100 units, as shown in Table 3.

Results and discussion

Conventional synthesis sometimes has lower yields than microwave protocols. By using microwave irradiation 4-nitro *ortho*-phenylenediamine reacted with 1:1 equivalent of substituted phenoxyacetic acids at 400W for 2.5-3.5 min. to successfully afford the desirable 5-nitro-2-aryl substituted-1-H-benzimidazole libraries in good to excellent isolate yields **(82-92%)**. Whereas using conventional heating the yields are only **58-75%**. The effects of time on the reaction were also studied and the results summarized in Table 1. All the compounds synthesized were adequately characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopies and by mass spectrometry.

The obtained results revealed that the nature of substituents and substitution pattern on the benzimidazole ring might have a considerable impact on the antimicrobial activities of the target substituted benzimidazole libraries of particular importance. A nitro group in the aromatic ring enhances the antimicrobial activity against most of the microorganisms.

In general compounds 1a, 1c, 1d and 1h exhibited more pronounced antibacterial activities than the compounds 1b, 1e, 1f, 1g, 1i and 1j, with better activity against both gram-positive and gram-negative bacteria. Among all compounds tested 1h exhibited significant antibacterial activity against the gram-positive *Staphylococcus aureus*, as compared with antibiotic Streptomycin. On the other hand 1c, 1d and 1h against gram-negative *Shigella dysentery* as compared with antibiotic Streptomycin as showed in (Figure 1. & 2.).

The antifungal screening results (MIC), showed in Table 3. It is evident from the screening data of the compounds, 1h was more effective against *penicillium*, compared with the standard Nystatin. Compounds 1a, 1b, 1c, 1d, 1e, 1f, 1g, 1i and 1j showed moderate antifungal activity against all fungal species with a MIC of 50 μg/mL (0.269, 0.176, 0.176, 0.176, 0.164, 0.164, 0.159, 0.176 and 0.147μM/mL respectively). Examination of the antifungal data it is seen that some of the compounds possess significant activity.

Conclusion

In summary we have described a simple, rapid, efficient and convenient protocol for the preparation of 5-nitro-2-aryl substituted phenoxymethyl-1H-benzimidazole libraries in one pot-synthesis. All these reactions were carried in microwave irradiation as well as conventional method under solvent free conditions. Furthermore, the procedure used commercially available reagents and equipment, and most of the reactions involved are efficient, giving the desired compounds in higher purity and yield. The versatility of this methodology is suitable for library synthesis in drug discovery efforts. Bioassay indicated that all the compounds showed *in vitro* antimicrobial activity against six strains of bacteria and three strains of fungus. Further *in vivo* biological evaluations are underway.

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