

Green synthesis and antiviral activity screening of some benzimidazole derivatives

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Abstract : A series of benzimidazole derivatives having substitutions at position 2 (BZ1-BZ7) and one of the two imidazole nitrogens (BZ8-BZ14) has been synthesized. Antiviral activities of these derivatives have also been tested against two of the viruses namely Tobacco mosaic and Sunhemp rosette.

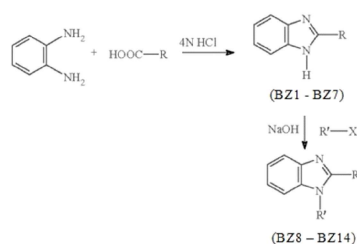
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Introduction

Interest in imidazole and pyrazole condensed heterocyclic/aromatic 1, 2 systems is due to their great therapeutic index³⁻⁵. A series of analogous and derivatives of benzimidazole have been reported to establish the effect of structure on antiviral activity⁶⁻¹⁰.

Benzimidazole derivatives have been demonstrated to inhibit Picornaviruses¹¹, Polioviruses¹², Enteroviruses¹³⁻¹⁹ etc. Broad antiviral applications of benzimidazole derivatives prompted us to synthesize various N-substituted and 2-substituted benzimidazoles and to evaluate their antiviral activities against Tobacco mosaic virus and Sunhemp rosette virus. The compounds were synthesized using Phillips condensation, by condensing the o-phenylene diamine and carboxylic acid derivatives in 4 N HCl, whereas N-substituted derivatives have been synthesized by reaction with alkyl/aryl halide in the presence of base, sodium hydride (Scheme-I). This intermediate was then used to synthesize benzimidazole derivatives. The general fragmentation pattern of these compounds has started from the substituent at positions 1 and 2. The molecular ion peak has

been observed in all the compounds. The base peak in all the spectra has been observed at m/z 118, which is assigned to the unsubstituted benzimidazole nucleus.



Scheme I

Antiviral activity

Source of inoculum

Cultures of Tobacco mosaic virus¹⁴ (TMV, common strain) and Sunhemp rosette virus (SRV) were maintained by regular passage in their systemic hosts *Nicotiana tabacum* L. "NP 31" and *Gotobaria juncea*, respectively.

Compd.	R	R'
BZ1	-CH ₂ CH ₂ COOH	H
BZ 2	-C ₆ H ₄ OH(o)	H
BZ 3	-CH=CH.C ₆ H ₅	H
BZ 4	-CH ₂ CH ₂ CH ₂ CH ₂ COOH	H
BZ 5	-CHOH.CHOH.COOH	H
BZ 6	-C ₆ H ₄ COOH(o)	H
BZ 7	-C ₆ H ₄ N=N-C ₁₀ H ₆ -OH(2)	H
BZ 8	-CH ₂ CH ₂ COOH	-CH ₂ C ₆ H ₅
BZ 9	-C ₆ H ₄ OH(o)	-CH ₂ C ₆ H ₅
BZ 10	-CH=CH.C ₆ H ₅	-CH ₂ C ₆ H ₅
BZ 11	-CH ₂ CH ₂ CH ₂ CH ₂ COOH	-CH ₂ C ₆ H ₅
BZ 12	-CHOH.CHOH.COOH	-CH ₂ C ₆ H ₅
BZ13	-C ₆ H ₄ COOH(o)	-CH ₂ C ₆ H ₅
BZ14	-C ₆ H ₄ N=N-C ₁₀ H ₆ -OH(2)	-CH ₂ C ₆ H ₅

Preparation of virus inoculum

Virus inoculums was prepared by grinding 3 to 4 of fresh diseased leaves in a mortax with distilled water (DW, 1 g/mL). The pulp was squeezed through two layers of muslin cloth, and the filtrate was centrifuged and diluted with distilled water to obtain 200-600 lesions on leaves after inoculation with a virus inoculum.

Host Plant

Seeds of *N-tabacum* 'samsun NN' and *Cyamopsis tetragonoloba* (L) Taub were sown in clay pots. The seedlings were transplanted to 12 cm diameter clay pots filled with compost and transferred to an insect free green house. For experimental work, somsun NN and N Glutinosa L plants were used at the 5 to 6 leaf stage and cyamopsis plants were used at the 4-leaf stage (3 unifoliate leaves and 1 trifoliate leaf). All the experiments were performed with a minimum of three replicates (minimum three plants with three or four leaves) per treatment.

Assay for virus inhibition

Cyamopsis tetragonoloba/SRV and *N Talacum samsun NN*/TMV were the host/virus system employed in the assay for virus infectivity. The two basal leaves of the host plants were treated with buffer extracted and centrifuged sap from *C. aculeatum* leaves or purified protein solution. Post-treatment done 24 hr after the treatment unless otherwise specified. The basal leaves of the control set of plants were treated with distilled water before virus inoculation. Percent inhibition of virus infectively was calculated by the formula:

$$\text{Percent inhibition} = (C - T/C) \times 100$$

C = average number of lesions on control leaves

T = average number of lesions on tested leaves

Minimum time for induction of systemic resistance

The two lower leaves of the test plants, *N. glutinosa* and *Cyomopsis tetragonoloba* were tested with various N-substituted- and 2-

substitutedbenzimidazoles. After varying treatment intervals, these leaves were removed and the virus SRV was inoculated on the upper untreated leaves 24 hr later. The lesions were counted and percent reduction in number of lesions were calculated using the formula:

$$\% \text{ inhibition} = \frac{R-L}{L} \times 100$$

Almost all the compounds have shown average % inhibition. Antiviral activities of compounds are summarised in (Table-I).

Experimental Section

Melting points were taken in an electrically heated instrument and are uncorrected. Compounds were routinely checked for their purity on silica gel G TLC plates and the spots were visualized by iodine vapours. IR spectra were recorded on a Shimadzu 8201 PC FTIR spectrometer; ¹H NMR spectra on a Bruker DRX 300 MHz FT NMR spectrometer using TMS as internal reference (chemical shifts in , ppm), and mass spectra on a Jeol SX-102 spectrometer.

Preparation of 2-substituted-benzimidazole BZ1-BZ7.

General procedure o-Phenylenediamine (4 g, 0.04 mole) was condensed with carboxylic acids (0.03 mole) in 50 mL 4 N. HCl.

The reaction mixture was stirred for about 4 hr with magnetic stirrer at 80°C. The compounds were precipitated by adding concentrated ammonia solution, filtered through suction and washed with cold water. Compounds were recrystallized from water and ethanol.

Compound BZ 1: Yield 4.6 g (61%), m.p. 256°C; ¹H NMR (CD₃OD): δ 7.2 (m, 4H, ArH), 3.4 (t, 2H, H-2), 3.2 (s, 1H, NH), 2.9 (t, 2H, H-1); MS: m/z 190 (M⁺), 146 (C₉H₁₀N₂), 119 (C₄H₁₁N₂O₂), 77 (C₆H₅), 74 (C₃H₁₀N₂), 55 (C₃H₅N). Anal. Calcd for C₁₀H₁₀N₂O₂ C, 63.16; H, 5.26; N, 14.73. Found: C, 62.76; H, 5.32; N, 15.42%.

Table-I
Antiviral activity of 1-benzyl-2-substituted- benzimidazoles

Sl.No. Control	Average number of lesions		% inhibition	
	Site leaf	Remote leaf	Site leaf	Remote leaf
	133	130		
BZ1	124	90	12.5	32.3
BZ 2	158	85	-17.5	34.6
BZ 3	124	88	15.6	28.6
BZ 4	119	99	10.9	23.4
BZ 5	125	92	20.4	38.1
BZ 6	111	98	36.3	15.6
BZ 7	120	110	17.9	25.0
BZ 8	85	89	36.5	31.9
BZ 9	86	90	32.9	23.6
BZ 10	125	119	6.7	8.4
BZ 11	112	102	24.6	16.3
BZ 12	130	127	2.9	2.3
BZ 13	100	94	10.5	9.5
BZ 14	110	98	17.9	25.0

Compound BZ 2: Yield 5.7 g (68%), m.p. 145°C; ¹H NMR (CD₃OD): δ 7.9 (m, 8H, ArH), 4.8 (s, 1H, OH), 3.2 (s, 1H, NH); MS: m/z 210 (M⁺), 194 (C₁₃H₁₀N₂), 122 (C₇H₁₀N₂), 103 (C₇H₅N), 77 (C₆H₅). Anal. Calcd for C₁₃H₁₀N₂O : C, 74.28; H, 4.75; N, 13.33. Found: C, 73.19; H, 5.31; N, 13.76%.

Compound BZ 3: Yield 6.5 g (74%), m.p. 120°C; ¹H NMR (CD₃OD): δ 7.7 (m, 5H, ArH), 7.2 (m, 4H, ArH), 3.9 (d, 1H, H-2'), 3.4 (s, 1H, NH), 3.1 (d, 1H, H-1); MS: m/z 220 (M⁺), 148 (C₉H₁₂N₂), 129 (C₉H₇N), 104 (C₈H₈), 93 (C₆H₄N), 77 (C₆H₅). Anal. Calcd for C₁₅H₁₂N₂: C, 81.81; H, 5.45; N, 12.72. Found: C, 80.93; H, 6.12; N, 12.93%.

Compound BZ 4: Yield 6.9 g (79%), m.p. 155°C; ¹H NMR (CD₃OD): δ 7.2 (m, 4H, ArH), 3.3 (s, 1H, NH), 2.8 (t, 2H, H-4'), 2.4 (m, 4H, H-2, H-3'), 2.1 (t, 2H, H-1); MS: m/z 218 (M⁺), 174 (C₁₁H₁₄N₂), 146 (C₆H₁₄N₂O₂), 102 (C₅H₁₄N₂), 93 (C₆H₇N), 83 (C₅H₉N), 77 (C₆H₅), 56 (C₄H₈). Anal. Calcd for C₁₂H₁₄N₂O₂: C, 66.05; H, 6.42; N, 12.84. Found: C, 65.87; H, 7.15; N, 12.14%.

Compound BZ 5: Yield 7.5 g (85%), m.p. 270°C; ¹H NMR (CD₃OD): δ 7.3 (m, 4H, ArH), 4.4 (s, 2H, OH), 3.9 (d, 1H, H-2), 3.4 (d, 1H, H-1), 3.2 (s, 1H, NH); MS: m/z 222 (M⁺), 178 (C₉H₁₀N₂O₂), 150 (C₄H₁₀N₂O₄), 148 (C₈H₈N₂O), 131 (C₄H₅NO₄), 106 (C₃H₆O₄), 93 (C₆H₇N), 77 (C₆H₅). Anal. Calcd for C₁₀H₁₀N₂O₄: C, 54.05; H, 4.50; N, 12.61. Found: C, 54.97; H, 5.01; N, 12.07%.

Compound BZ 6: Yield 7.5 g (79%), m.p. 190°C; ¹H NMR (CD₃OD): δ 7.8 (m, 5H, ArH), 7.3 (m, 4H, ArH), 3.1 (s, 1H, NH); MS: m/z 238 (M⁺), 194 (C₁₃H₁₀N₂), 122 (C₇H₁₀N₂), 103 (C₇H₅N), 93 (C₆H₇N), 77 (C₆H₅). Anal. Calcd for C₁₄H₁₀N₂O₂: C, 70.59; H, 4.20; N, 11.79. Found: C, 71.14; H, 5.42; N, 12.07%.

Compound BZ 7: Yield 9.8 g (67%), m.p. 210°C; ¹H NMR (CD₃OD): δ 5.4 (1H, s), 3.9 (1H, s), 7.9-9.1 (14H, m); MS: m/z 364 (M⁺), 194 (C₁₃H₁₀N₂), 144 (C₁₀H₈O), 128 (C₁₀H₈), 122 (C₄H₁₀N₂), 103 (C₇H₅N), 93 (C₆H₇N), 77 (C₆H₅). Anal. Calcd for C₂₃H₁₆N₄O: C, 75.82; H, 4.39; N, 15.38. Found: C, 75.14; H, 3.99; N, 15.87%.

Preparation of 1-benzyl 2-substituted-benzimidazoles BZ8-BZ14.

General procedure. 2-Substitutedbenzimidazoles 1-7 (0.02 mole) were treated with benzyl chloride (2.5 g, 0.02 mole) in the presence of a little quantity of sodium hydride (2 g) in THF. The reaction mixture was stirred for 8-12 hr at 40 °C. Excess solvent was removed by distillation and crude product was washed with water, extracted with ethyl acetate and finally recrystallized from water and ethanol.

Compound BZ 8: Yield 4.16 g (79%), m.p. 102 °C; IR (KBr): 3540 (O-H stretching), 1722 (C=O stretching), 1214 cm^{-1} (C-N stretching); ^1H NMR (CDCl_3): δ 7.4 (m, 9H, ArH), 2.6 (t, 2H, 2 -CH₂), 2.1 (t, 2H, 1 -CH₂), 2.4 (s, 1H, 1 -CH₂); MS: m/z 280 (M^+), 236 ($\text{C}_{16}\text{H}_{16}\text{N}_2$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 143 ($\text{C}_9\text{H}_8\text{N}_2$), 140 ($\text{C}_6\text{H}_8\text{N}_2\text{O}_2$), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 91 (C_7H_7), 72 ($\text{C}_3\text{H}_8\text{N}_2$), 58 ($\text{C}_3\text{H}_8\text{N}$). Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_2$: C, 77.27; H, 6.06; N, 10.60. Found: C, 76.03; H, 6.12; N 10.52%.

Compound BZ 9: Yield 4.10 g (68%), m.p. 238 °C; IR (KBr): 3540 (OH stretching), 1216 cm^{-1} (C-N stretching); ^1H NMR (CDCl_3): δ 8.2 (m, 4H, ArH), 7.7 (m, 5H, ArH), 7.3 (m, 4H, ArH), 4.3 (s, 1H, OH), 2.5 (s, 2H, 1 -CH₂); MS: m/z 300 (M^+), 284 ($\text{C}_{20}\text{H}_{16}\text{N}_2$), 234 ($\text{C}_{16}\text{H}_{14}\text{N}_2$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 194 ($\text{C}_{13}\text{H}_{10}\text{N}_2$), 144 ($\text{C}_9\text{H}_8\text{N}_2$), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 103 ($\text{C}_7\text{H}_5\text{N}$), 91 (C_7H_7). Anal. Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_2\text{O}$: C, 80.0; H, 5.33; N, 9.33. Found: C, 80.33; H, 6.12; N 9.14%.

Compound BZ 10: Yield 4.12 g (67%), m.p. 120 °C; ^1H NMR (CDCl_3): δ 8.1 (m, 5H, ArH), 7.5 (m, 5H, ArH), 7.2 (m, 4H, ArH), 4.3 (d, 1H, -CH), 3.9 (d, 1H, -CH), 2.5 (s, 2H, 1 -CH₂); MS: m/z 310 (M^+), 234 ($\text{C}_{16}\text{H}_{14}\text{N}_2$), 220 ($\text{C}_{15}\text{H}_{12}\text{N}_2$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 148 ($\text{C}_9\text{H}_{12}\text{N}_2$), 144 ($\text{C}_9\text{H}_8\text{N}_2$), 132 ($\text{C}_9\text{H}_{10}\text{N}$), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 91 (C_7H_7), 77 (C_6H_5). Anal. Calcd for $\text{C}_{22}\text{H}_{16}\text{N}_2$: C,

85.16; H, 5.80; N, 9.03. Found: C, 85.63; H, 5.92; N 9.79%.

Compound BZ 11: Yield 3.92 g (64%), m.p. 133 °C; ^1H NMR (CDCl_3): δ 7.8 (m, 5H, ArH), 7.3 (m, 4H, ArH), 3.3 (t, 2H, 4 -CH₂), 2.8 (t, 2H, 1-CH₂), 2.4 (s, 2H, 1-CH₂), 2.4 (t, 4H, 2-CH₂, 3 -CH₂); MS: m/z 308 (M^+), 236 ($\text{C}_{16}\text{H}_{16}\text{N}_2$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 172 ($\text{C}_{11}\text{H}_{12}\text{N}_2$), 168 ($\text{C}_8\text{H}_{12}\text{N}_2\text{O}_2$), 144 ($\text{C}_5\text{H}_{14}\text{N}_2$), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 102 ($\text{C}_5\text{H}_{14}\text{N}_2$), 91 (C_7H_7), 85 ($\text{C}_5\text{H}_{11}\text{N}$), 58 (C_4H_{10}). Anal. Calcd for $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2$: C, 74.02; H, 6.49; N, 9.09. Found: C, 74.92; H, 7.15; N, 8.72%.

Compound BZ 12: Yield 3.96 g (63%), m.p. 180 °C; ^1H NMR (CDCl_3): δ 7.8 (m, 5H, ArH), 7.2 (m, 4H, ArH), 3.5 (d, 1H, 2 -CH), 3.2 (d, 1H, 1 -CH), 2.6 (s, 2H, 1 -CH₂); MS: m/z 312 (M^+), 234 ($\text{C}_{16}\text{H}_{14}\text{N}_2$), 222 ($\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_4$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 178 ($\text{C}_9\text{H}_{10}\text{N}_2\text{O}_2$), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 91 (C_7H_7). Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_4$: C, 65.39; H, 5.13; N, 8.97. Found: C, 65.18; H, 6.02; N, 9.33%.

Compound BZ 13: Yield 5.68 g (87%), m.p. 196 °C; ^1H NMR (CDCl_3): δ 7.8 (m, 5H, ArH), 7.2 (m, 4H, ArH), 3.5 (d, 1H, 2 -CH), 3.2 (d, 1H, 1 -CH), 2.6 (s, 2H, 1 -CH₂); MS: m/z 312 (M^+), 234 ($\text{C}_{16}\text{H}_{14}\text{N}_2$), 222 ($\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_4$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 178 ($\text{C}_9\text{H}_{10}\text{N}_2\text{O}_2$), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 91 (C_7H_7). Anal. Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_4$: C, 65.39; H, 5.13; N, 8.97. Found: C, 65.18; H, 6.02; N, 9.33%.

Compound BZ 14: Yield 7.3 g (80%), m.p. 275 °C; ^1H NMR (CDCl_3): δ 8.1 (m, 8H, ArH), 7.6 (m, 7H, ArH), 7.2 (m, 4H, ArH), 2.4 (s, 2H, 1 -CH₂); MS: m/z 454 (M^+), 346 ($\text{C}_{23}\text{H}_{14}\text{N}_4$), 264 ($\text{C}_{20}\text{H}_{16}\text{N}_2$), 230 ($\text{C}_{16}\text{H}_{10}\text{N}_2$), 208 ($\text{C}_{14}\text{H}_{12}\text{N}_2$), 194 ($\text{C}_{13}\text{H}_{10}\text{N}_2$), 128 (C_{10}H_8), 118 ($\text{C}_7\text{H}_6\text{N}_2$), 91 (C_7H_7). Anal. Calcd for $\text{C}_{30}\text{H}_{22}\text{N}_4\text{O}$: C, 79.29; H, 4.85; N, 12.33. Found: C, 78.13; H, 5.01; N, 12.33%.

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