
Synthesis and Antimicrobial Activity of Some Benzimidazoles

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Reaction between 4-nitro-1,2-diaminobenzene, phthalic anhydride and p-amino acetophenone yielded the starting compound N-(4-acetylphenyl)-4-(5-nitro-1H-benzimidazol-2-yl)-benzamide 1. The compound 1 on condensation with different aldehydes yielded the chalcones 2. Chalcone being a good synthon, reacted with variety of reagents like malononitrile and ammonium acetate, thiourea and hydroxylamine hydrochloride furnished the corresponding compounds i.e. cyanopyridines 3, thienopyrimidines 4 and isoxazoles 5. All the compounds have been characterized using elemental analyses, IR and NMR spectral data. All the compounds have been evaluated for their antimicrobial and antitubercular activity against different strains of Gram positive and Gram negative bacteria and *Mycobacterium smegmatis* species and MIC values have also been determined.

Compounds bearing benzimidazole nucleus have been of great interest to synthetic and medicinal chemists for a long time due to their unique chemical and biological properties mainly related to traditional anthelmintics, albendazole and oxibendazole. Benzimidazole derivatives have also been found to possess biological activities such as antiviral¹, antibacterial² and anticancer³. Continuous increase in bacterial resistance to existing drugs has been resulted due to wide spread use of antibiotics leading to research on new substances possessing antimicrobial activity⁴. On the basis of above considerations and in order to contribute to the definition of antimicrobial properties of structurally related analogs, some new derivatives like chalcones^{5,6}, cyanopyridines^{7,8}, thienopyrimidines^{9,10} and isoxazoles^{11,12} bearing benzimidazole nucleus has been investigated in order to study their pharmacological profile.

The starting compound N-(4-acetylphenyl)-4-(5-nitro-1 H-benzimidazol-2-yl)-benzamide 1 was synthesised by the reaction of 4-nitro-1,2-diaminobenzene, phthalic anhydride and 4-amino acetophenone. The compound 1 on reaction with different aldehydes yielded the chalcones i.e. N-[4-(3-

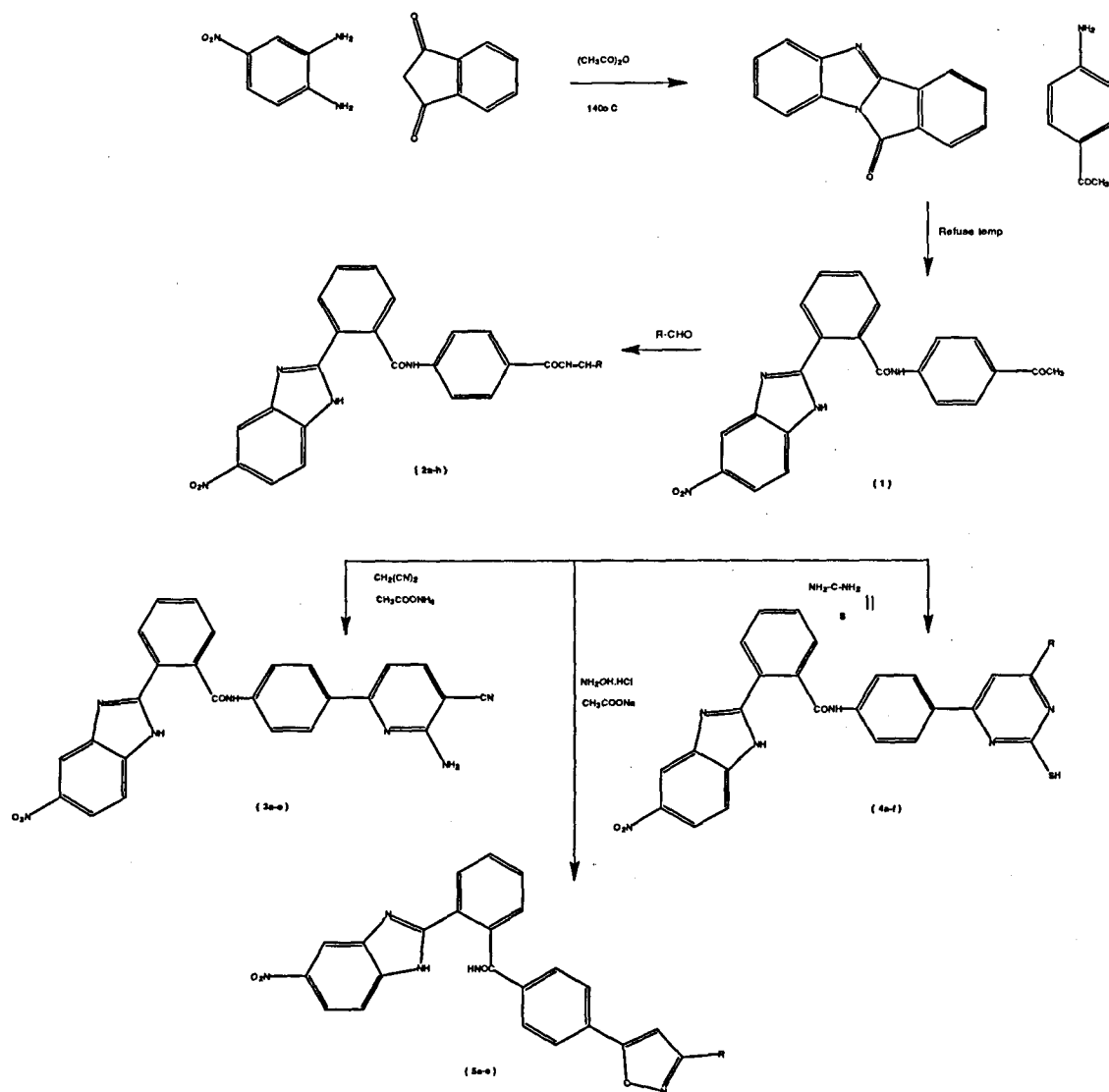
aryl-acryloyl)-phenyl]-2-(5-nitro-1 H-benzimidazol-2-yl)-benzamide 2. The compound 2 on reaction with malononitrile and ammonium acetate, thiourea and hydroxylamine hydrochloride separately yielded the corresponding N-[4-(2-amino)-6-aryl-3-cyano-pyridin-4-yl]-phenyl]-2-[5-nitro-1 H-benzimidazol-2-yl)-benzamide 3, N-[4-(2-mercapto-6-aryl-pyrimidin-4-yl)-phenyl]-2-(5-nitro-1 H-benzimidazol-2-yl)-benzamide 4 and N-[4-(5-aryl-isoxazol-3-yl)-phenyl]-2-[5-nitro-1 H-benzimidazol-2-yl)-benzamide 5. The synthetic sequence leading to the formation of targeted compound is depicted in scheme 1.

The constitution of the products has been supported by elemental analyses, IR and NMR spectral data. All the compounds have been evaluated for antibacterial and antitubercular activity against five different strains of *Staphylococcus aureus*, three different strains of *Mycobacterium smegmatis*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* at different concentrations of 100, 200, 300 and 500 µg/ml and the MIC values have also been determined for all the compounds.

MATERIALS AND METHODS

All the melting points are uncorrected. IR spectra (KBr)

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Scheme 1: Reaction Scheme

were recorded on a Shimadzu spectrophotometer, FT IR-8400. ¹H NMR spectra were recorded on a 300 MHz spectrophotometer using TMS as an internal standard.

N-(4-Acetylphenyl)-4-(5-nitro-1H-benzimidazol-2-yl)-benzamide 1:

A mixture of 4-nitro-1,2-diaminobenzene (0.01 mol) and phthalic anhydride (0.01 mol) was fused for 45 min and then refluxed for 6 h with acetic anhydride. The reaction mixture was poured onto ice and isolated product further reacted with 4-aminoacetophenone (0.01 mol) in DMF. The reaction mixture was refluxed for 6 h. The product was isolated by pouring onto ice and crystallised from DMF. Yield 70%, m.p.

245°. IR (KBr) ν_{max} : 3346 (N-H), 1679 (-COCH₃), 1662 (-CONH), 1595 (-C=N), 1178 (C-O-C), 829 cm⁻¹ (-NO). ¹H NMR: δ : 2.7 (s, 3H, -CH₃), 6.57-8.21 (m, 11H, Ar-H), 8.35 (s, 1H, NH).

N-[4-(3-Aryl-acryloyl)-phenyl]-2-(5-nitro-1H-benzimidazol-2-yl)-benzamide (2a-h):

A solution of 4-methoxybenzaldehyde (0.01 mol) in minimum quantity of ethanol was added to a mixture of 1 (0.01 mol) in ethanol and 40% NaOH (2-3 drops) was added to make it alkaline. The reaction mixture was then stirred for 24 h at room temperature. The reaction mixture was poured onto ice. The isolated product was crystallised from

dioxane. For **2e**: Yield 55%, m.p. 250°. (Found: C, 69.44; H, 4.20; N, 10.76; $C_{30}H_{22}N_4O_5$ requires: C, 69.49; H, 4.24; N, 10.81%). IR (KBr) ν_{max} : 3311 (N-H), 1666 (-CONH), 1650 (C=O), 1595 (-C=N), 1170 cm^{-1} (C-O-C); 1H NMR: δ : 3.81 (s, 3H, -OCH₃), 6.52-8.33 (m, 17H, Ar-H), 8.45 (s, 1H, NH). Similarly other chalcones were prepared. The physical data are recorded in Table 1.

N-{4-[2-Amino-6-aryl-3-cyano-pyridine-4-yl]-phenyl}-2-(5-nitro-1H-benzimidazol-2-yl)-benzamide (**3a-e**):

A mixture of **2e** (0.01 mol), malononitrile (0.01 mol) and ammonium acetate (0.08 mol) in ethanol was refluxed for 8 h. The reaction mixture was poured onto ice and the isolated product was crystallised from ethanol. For **3d**: Yield 65%, m.p. 195°. (Found: C, 68.10; H, 3.90; N, 16.82; $C_{33}H_{23}N_7O_4$ requires: C, 68.15; H, 3.95; N, 16.86%). IR (KBr) ν_{max} : 3310(N-H), 2185 (C=N), 1668 (-CONH), 1581 (C=N), 1182 cm^{-1} (C-O-C); 1H NMR: δ : 3.82 (s, 3H, -OCH₃), 6.62-8.36 (m, 16H, Ar-H), 8.48 (s, 1H, NH). Similarly other cyanopyridines were prepared. The physical data are re-

TABLE 1: PHYSICAL DATA OF THE COMPOUNDS

Compd. No.	R	Molecular Formula	MP° (%)	Yield Value	Rf	% of Nitrogen	
						Calcd.	Found
2a	2-Cl-C ₆ H ₄	C ₂₉ H ₁₉ N ₄ O ₄ Cl	140	62	0.46	10.72	10.68
2b	2,4-Cl ₂ -C ₆ H ₃	C ₂₉ H ₁₈ N ₄ O ₄ Cl ₂	120	50	0.46	10.05	10.00
2c	2-OH-C ₆ H ₄	C ₂₉ H ₂₀ N ₄ O ₅	266	58	0.44	11.11	11.08
2d	3-OCH ₃ -4-OH-C ₆ H ₃	C ₃₀ H ₂₂ N ₄ O ₆	252	54	0.40	10.48	10.43
2e	4-OCH ₃ -C ₆ H ₄	C ₃₀ H ₂₂ N ₄ O ₅	250	55	0.50	10.81	10.76
2f	2-NO ₂ -C ₆ H ₄	C ₂₉ H ₁₉ N ₅ O ₆	240	50	0.41	13.13	13.10
2g	3-NO ₂ -C ₆ H ₄	C ₂₉ H ₁₉ N ₅ O ₆	188	55	0.46	13.13	13.09
2h	-(CH=CH)-C ₆ H ₅	C ₃₁ H ₂₂ N ₄ O ₄	212	60	0.54	10.87	10.83
3a	2-Cl-C ₆ H ₄	C ₃₂ H ₂₀ N ₇ O ₃ Cl	188	65	0.39	16.75	16.70
3b	2,4-Cl ₂ -C ₆ H ₃	C ₃₂ H ₁₉ N ₇ O ₃ Cl ₂	208	60	0.60	15.80	15.75
3c	3,4-(OCH ₃) ₂ -C ₆ H ₃	C ₃₄ H ₂₅ N ₇ O ₅	170	62	0.49	16.03	16.00
3d	4-OCH ₃ -C ₆ H ₄	C ₃₃ H ₂₃ N ₇ O ₄	195	65	0.48	16.86	16.82
3e	-(CH=CH)-C ₆ H ₅	C ₃₄ H ₂₃ N ₇ O ₃	190	70	0.50	16.98	16.93
4a	2-Cl-C ₆ H ₄	C ₃₀ H ₁₉ N ₆ O ₃ ClS	250	58	0.42	14.53	14.50
4b	3,4-(OCH ₃) ₂ -C ₆ H ₃	C ₃₂ H ₂₄ N ₆ O ₅ S	242	57	0.60	13.90	13.85
4c	4-OH-C ₆ H ₄	C ₃₀ H ₂₀ N ₆ O ₄ S	160	55	0.42	15.00	15.05
4d	4-OCH ₃ -C ₆ H ₄	C ₃₁ H ₂₂ N ₆ O ₄ S	246	60	0.47	14.63	14.60
4e	2-NO ₂ -C ₆ H ₄	C ₃₀ H ₁₉ N ₇ O ₅ S	142	62	0.63	16.63	16.60
4f	-(CH=CH)-C ₆ H ₅ -	C ₃₂ H ₂₂ N ₆ O ₃ S	100	65	0.46	14.73	14.70
5a	C ₆ H ₅	C ₂₉ H ₁₉ N ₅ O ₄	252	55	0.50	13.97	13.93
5b	2,4-Cl ₂ -C ₆ H ₃	C ₂₉ H ₁₇ N ₅ O ₄ Cl ₂	238	50	0.42	12.28	12.23
5c	2-OH-C ₆ H ₄	C ₂₉ H ₁₉ N ₅ O ₅	213	58	0.38	13.53	13.50
5d	2-NO ₂ -C ₆ H ₄	C ₂₉ H ₁₈ N ₆ O ₆	240	52	0.45	15.38	15.33
5e	-(CH=CH)-C ₆ H ₅	C ₃₁ H ₂₁ N ₅ O ₄	170	60	0.51	13.28	13.24

corded in Table 1.

N-[4-(2-Mercapto-6-aryl-pyrimidin-4-yl)-phenyl]-2-(5-nitro-1H-benzimidazol-2-yl)-benzamide (4a-f):

A mixture of **2e** (0.01 mol) and thiourea (0.02 mol) in alcoholic KOH (25 ml) was refluxed for 8 h. The reaction mixture was poured onto ice and crystallised from ethanol. For **4d**: Yield 60%, m.p. 246°. (Found: C, 64.75; H, 3.80; N, 14.60; C₃₁H₂₂N₆O₄S requires: C, 64.80; H, 3.83; N, 14.63%). IR (KBr) ν_{\max} : 3332 (N-H), 1662 (-CONH), 1595 (C=N), 1176 cm⁻¹(C-O-C); ¹H NMR: δ : 3.85 (s, 3H, -OCH₃), 7.14-8.47 (m, 16H, Ar-H), 8.48 (s, 1H, NH). Similarly, other thienopyrimidines were prepared. The physical data are recorded in Table 1.

N-[4-(5-Aryl-isoxazol-3-yl)-phenyl]-2-(5-nitro-1H-benzimidazol-2-yl)-benzamide (5a-e):

A mixture of **2b** (0.01 mol), hydroxylamine hydrochloride (0.01 mol) and sodium acetate (0.08 mol) in glacial acetic acid in ethanol was refluxed for 6 h. The reaction mixture was poured onto ice. The isolated product was crystallised from dioxane. For **5b**: Yield 50%, m.p. 238°. (Found: C, 61.00; H, 2.93; N, 12.23; C₂₉H₁₇N₅O₄Cl₂ requires: C, 61.65; H, 2.98; N, 12.28%). IR (KBr) ν_{\max} : 3369 (N-H), 1666 (-CONH), 1608 (C=N), 1091 (C-O-C), 750 cm⁻¹ (C-Cl). ¹H NMR: δ : 7.40-8.15 (m, 16H, Ar-H), 8.48 (s, 1H, NH). Similarly other isoxazoles were prepared. The physical data are recorded

in Table 1.

Antimicrobial activity:

Test microorganisms are Gram-positive bacteria (*Staphylococcus aureus* ACC131, penicillin plus streptomycin resistant strain *S. aureus* ACC132, kanamycin resistant strain of *S. aureus* ACC133, tetracycline plus erythromycin resistant strain of *S. aureus* ACC134, macrolide antibiotic resistant strain of *S. aureus* ACC135, *Mycobacterium smegmatis* ACC 136, kanamycin resistant strain of *M. smegmatis* ACC137, streptomycin resistant strain of *M. smegmatis* ACC138 and *Bacillus subtilis* ATCC-6633) and Gram negative bacteria (*Escherichia coli* ACC139 and *Pseudomonas aeruginosa* ACC140) are used in the present investigation. Except *B. subtilis* ATCC-6633, all other Gram positive and Gram negative bacteria mentioned above are taken from our own culture collection.

All the Gram-positive bacteria, except *B. subtilis* and *E. coli* among gram negative bacteria were grown in nutrient broth (NB, Difco, Detroit, USA), *P. aeruginosa* was grown in heart infusion with glucose medium, all the strains of *M. smegmatis* were grown in Myco seed broth and *B. subtilis* ATCC 6633, was grown in Antibiotic medium No. 1 (Difco, Detroit, USA) for 48 to 96 h at 32-37° on shaker depending on the growth condition requirement of the different bacteria, which gave an optimum growth of the test bacteria. Each

TABLE 2: ANTIMICROBIAL SCREENING RESULTS OF THE TEST COMPOUNDS IN $\mu\text{g/ml}$.

Compd.	R	Microorganism tested					
		1	2	3	4	5	6
2b	2,4-Cl ₂ -C ₆ H ₃ -	-	200	300	-	300	-
2f	2-NO ₂ -C ₆ H ₄ -	-	-	500	-	-	-
4a	2-Cl-C ₆ H ₄ -	-	-	-	300	500	500
4c	4-OH-C ₆ H ₄ -	-	-	-	300	300	-
5a	C ₆ H ₅ -	-	500	500	500	-	500
5b	2,4-Cl ₂ -C ₆ H ₃ -	-	200	300	500	500	500
5c	2-OH-C ₆ H ₄ -	-	500	500	500	500	500
5d	2-NO ₂ -C ₆ H ₄ -	-	300	-	300	300	200
5e	-(CH=CH)-C ₆ H ₅ -	-	200	500	-	-	500

Microorganism species used are 1. *Bacillus subtilis* ATCC 6633, 2. *Staphylococcus aureus* ACC131, 3. *Staphylococcus aureus* ACC132, 4. *Staphylococcus aureus* ACC133, 5. *Staphylococcus aureus* ACC134 and 6. *Staphylococcus aureus* ACC135. (-) indicates no activity up to 500 $\mu\text{g/ml}$.

compound to be tested was dissolved in dimethyl sulfoxide (DMSO, 6 mg/ml), sterilized by filtration through sintered glass filter (G5) and added to the molten nutrient agar (NA, Difco) to achieve a final concentration of 0 (control), 100, 200, 300 and 500 ($\mu\text{g/ml}$) and poured into sterile petridishes. The inoculum consisted of freshly grown broth culture of a bacterium diluted in such a manner that 2 ml sample of the culture contained 10^5 colony forming units (CFU). These were then spot inoculated (2 ml/spot) on NA plates containing various concentrations of test compound, incubated at 37° up to 72 h for the determination of minimum inhibitory concentration (MIC)¹³.

RESULTS AND DISCUSSION

The purified products were screened for antimicrobial activity towards *B. subtilis* and different strains of *S. aureus*. MIC value of synthesised compounds was determined at a concentration range of 100, 200, 300 and 500 $\mu\text{g/ml}$. The compounds **2b**, **2f**, **4a**, **4c**, **5a**, **5b**, **5c**, **5d**, **5e**, showed MIC value in the concentration range of 100 $\mu\text{g/ml}$ to 500 $\mu\text{g/ml}$ against different microbes (Table 2). Moreover the compounds were also evaluated for their antimicrobial activity against *E. coli*, *P. aeruginosa* and different strains of *M. smegmatis*. But none of the compound showed activity towards these bacteria at concentration mentioned above.

Looking to the structure activity relationship, it can be concluded that the presence of halogen group at 2 and 2,4 position i.e. **2b**, **4a**, **5b** showed good activity towards Gram +ve bacteria. Presence of nitro group at 2 or 4 position i.e. **2f**, **5d** displayed good activity towards Gram +ve bacteria. The chalcones having phenyl, 2-hydroxyphenyl and a-cinnamyl substituents has not shown any activity but isoxazoles obtained after ring closure having same substituents showed activity towards Gram +ve bacteria, leading to

the conclusion that cyclisation of chalcones in general, enhances the antimicrobial activity.

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