

Synthesis and biological evaluation of some novel isoxazoles and cyanopyridines, a new class of potential anti-tubercular agents

Rajeev Doshi, Preeti Kagthara & Hansa Parekh*

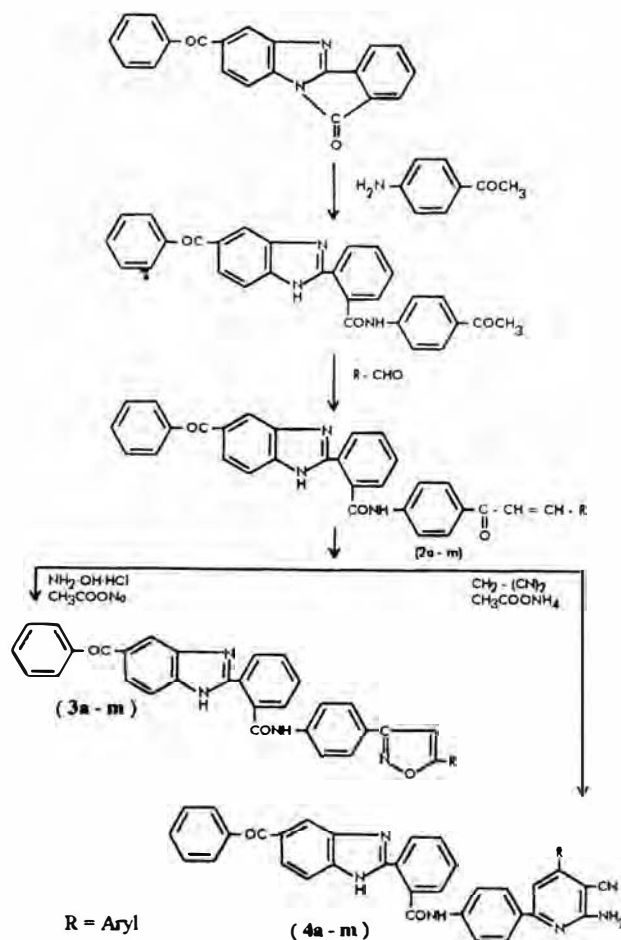
Department of Chemistry, Saurashtra University, Rajkot 360 005, India

Received 23 February 1998; accepted (revised)

Substituted chalcones **2a-m** prepared by the treatment of 5-benzoyl-benzimidazol-2-yl-*o*-benzoyl(4'-aminoacetophenone) **1** with araldehydes, on cyclisation with hydroxylamine hydrochloride in ethanol furnish isoxazoles **3a-m**. The same chalcones on condensation with malononitrile yield cyanopyridines **4a-m**. The structure of the compounds synthesised have been assigned on the basis of elemental analyses, IR and NMR spectral studies. Most of the compounds exhibit significant activity against *Mycobacterium tuberculosis* H37 Rv and MIC value is reported. Compounds **2c**, **3c** and **4d** have been selected by Merck Incorporation USA for further pharmacological screening.

As a part of the surge of interest in heterocycles that have been explored for developing pharmaceutically important molecules, isoxazoles¹⁻³ and cyanopyridines⁴⁻⁶ have played an important role in medicinal chemistry. Some of them received considerable attention as potential antimicrobial⁷ and antitubercular agents⁸. Benzimidazole derivatives show a wide variety of biological activity which is reflected by their use as antitubercular, antitumor and antimicrobial agents⁹⁻¹⁴. In view of the high incidence of desirable pharmacological properties, it appeared of interest to synthesis some novel isoxazole and cyanopyridine derivatives for evaluating their pharmacological profile.

p-Amino acetophenone was condensed with 5-benzoyl-*o*-benzoylene-2, 1-benzimidazole to get 5-benzoyl-benzimidazole-2-yl-*o*-benzoyl (4'-amino acetophenone) **1**, which was further treated with various araldehydes to get (*p*-5'-benzoylbenzimidazol-2'-yl-*o*-benzoyl amino chalcones) **2a-m**. The chalcones **2a-m** on cyclisation with hydroxylamine hydrochloride in ethanol in presence of sodium acetate afforded corresponding 3-(5'-benzoyl-benzimidazol-2'-yl-benzamido-phenyl)-5-arylisoxazoles **3a-m**. Moreover compounds **2a-m** also reacted with malononitrile in ethanol in presence of ammonium acetate which afforded 2-amino-3-cyano-4-aryl-6-(5'-benzoyl benzimidazol-2'-yl-*o*-benzamido phenyl) pyridines **4a-m** (Scheme I; Table I).



Scheme I

Table I—Physical data of compounds 2a-m, 3a-m and 4a-m

Compd	R	Mol. Formula	m.p. °C	Yield (%)	N (%)	
					Calcd	Found
2a	C ₆ H ₅ –	C ₃₆ H ₂₅ O ₃ N ₃	115	79	7.67	7.65
2b	2-Cl-C ₆ H ₄ –	C ₃₆ H ₂₄ O ₃ N ₃ Cl	120	82	7.22	7.21
2c	4-Cl-C ₆ H ₄ –	C ₃₆ H ₂₄ O ₃ N ₃ Cl	125	82	7.22	7.10
2d	2,4-Cl ₂ -C ₆ H ₃ –	C ₃₆ H ₂₃ O ₃ N ₃ Cl ₂	130	88	6.82	6.90
2e	3,4-(OCH ₃) ₂ -C ₆ H ₃ –	C ₃₈ H ₂₉ O ₅ N ₃	120	71	6.92	6.99
2f	4-N,N-(CH ₃) ₂ -C ₆ H ₄ –	C ₃₈ H ₃₀ O ₃ N ₄	130	82	9.49	9.57
2g	C ₄ H ₃ O–	C ₃₄ H ₂₃ O ₄ N ₃	115	80	7.82	7.73
2h	4-OCH ₃ -C ₆ H ₄ –	C ₃₇ H ₂₇ O ₄ N ₃	110	85	7.27	7.39
2i	3-(OCH ₃)-4-(OH)C ₆ H ₃ –	C ₃₇ H ₂₇ O ₅ N ₃	130	80	7.07	7.19
2j	2-NO ₂ -C ₆ H ₄ –	C ₃₆ H ₂₄ O ₅ N ₄	120	79	9.45	9.59
2k	3-NO ₂ -C ₆ H ₄ –	C ₃₆ H ₂₄ O ₅ N ₄	110	83	9.45	9.53
2l	–CH=CH–C ₆ H ₅ –	C ₃₈ H ₂₇ O ₃ N ₃	115	75	7.33	7.39
2m	3,4,5-(OCH ₃) ₃ -C ₆ H ₂ –	C ₃₉ H ₃₁ O ₆ N ₃	120	78	6.59	6.67
3a	C ₆ H ₅ –	C ₃₆ H ₂₄ O ₃ N ₄	125	78	9.99	10.10
3b	2-Cl-C ₆ H ₄ –	C ₃₆ H ₂₃ O ₃ N ₄ Cl	120	62	9.42	9.59
3c	4-Cl-C ₆ H ₄ –	C ₃₆ H ₂₃ O ₃ N ₄ Cl	130	68	9.42	9.60
3d	2,4-Cl ₂ -C ₆ H ₃ –	C ₃₆ H ₂₂ O ₃ N ₄ Cl ₂	90	72	8.90	8.99
3e	3,4-(OCH ₃) ₂ -C ₆ H ₃ –	C ₃₈ H ₂₈ O ₅ N ₄	150	58	9.03	9.13
3f	4-N,N-(CH ₃) ₂ -C ₆ H ₄ –	C ₃₈ H ₂₉ O ₃ N ₅	120	62	11.60	11.79
3g	C ₄ H ₃ O–	C ₃₄ H ₂₂ O ₄ N ₄	130	64	10.18	10.09
3h	4-OCH ₃ -C ₆ H ₄ –	C ₃₇ H ₂₆ O ₄ N ₄	150	69	9.49	9.54
3i	3-(OCH ₃)-4-(OH)C ₆ H ₃ –	C ₃₇ H ₂₆ O ₅ N ₄	140	72	9.24	9.19
3j	2-NO ₂ -C ₆ H ₄ –	C ₃₆ H ₂₃ O ₅ N ₅	175	75	11.56	11.49
3k	3-NO ₂ -C ₆ H ₄ –	C ₃₆ H ₂₃ O ₅ N ₅	140	79	11.56	11.60
3l	–CH=CH–C ₆ H ₅ –	C ₃₈ H ₂₆ O ₃ N ₄	90	53	9.55	9.43
3m	3,4,5-(OCH ₃) ₃ -C ₆ H ₂ –	C ₃₉ H ₃₀ O ₆ N ₄	130	59	8.61	8.69
4a	C ₆ H ₅ –	C ₃₉ H ₂₆ O ₂ N ₆	160	55	13.76	13.89
4b	2-Cl-C ₆ H ₄ –	C ₃₉ H ₂₅ O ₂ N ₆ Cl	120	60	13.03	13.01
4c	4-Cl-C ₆ H ₄ –	C ₃₉ H ₂₅ O ₂ N ₆ Cl	130	62	13.03	13.19
4d	2,4-Cl ₂ -C ₆ H ₃ –	C ₃₉ H ₂₄ O ₂ N ₆ Cl ₂	150	69	12.37	12.44
4e	3,4-(OCH ₃) ₂ -C ₆ H ₃ –	C ₄₁ H ₃₀ O ₄ N ₆	130	54	12.53	12.57
4f	4-N,N-(CH ₃) ₂ -C ₆ H ₄ –	C ₄₁ H ₃₁ O ₂ N ₇	140	63	15.00	15.05
4g	C ₄ H ₃ O–	C ₃₇ H ₂₄ O ₃ N ₆	155	69	13.99	14.09
4h	4-OCH ₃ -C ₆ H ₄ –	C ₄₀ H ₂₈ O ₃ N ₆	155	73	13.12	13.20
4i	3-(OCH ₃)-4-(OH)C ₆ H ₃ –	C ₄₀ H ₂₈ O ₄ N ₆	150	79	12.80	12.69
4j	2-NO ₂ -C ₆ H ₄ –	C ₃₉ H ₂₅ O ₄ N ₇	140	75	14.95	15.05
4k	3-NO ₂ -C ₆ H ₄ –	C ₃₉ H ₂₅ O ₄ N ₇	145	79	14.95	15.10
4l	–CH=CH–C ₆ H ₅ –	C ₄₁ H ₂₈ O ₂ N ₆	155	82	13.20	13.30
4m	3,4,5-(OCH ₃) ₃ -C ₆ H ₂ –	C ₄₂ H ₃₂ O ₅ N ₆	150	81	11.99	12.05

*All compounds gave satisfactory C, H and N analyses.

The structure of the compounds synthesised were assigned on the basis of elemental analyses, IR and H NMR spectral data. The compounds were

evaluated for antimicrobial screening against Gram +ve, Gram –ve bacteria and fungal strain. Most of the compounds were evaluated for the *in vitro* anti-

Table II—Biological evaluation of the compounds exhibiting the highest activity against the microbes investigated

Standard Antibiotic	<i>B. megaterium</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>Ps. Fluorescens</i>	<i>A. awamori</i>	<i>Mycobacterium Tuberculosis H37 Rv</i>
	2f, 3e, 3i, 4f, 4b	2h, 3g, 3k, 4f	2e, 2g, 2j, 3f, 3a, 3m, 4e, 4g, 4k	2b, 3d, 3i, 4i, 4a, 4f	2a, 2e, 2j, 3d, 3a, 3k, 4i, 4a, 4e, 4g, 4h	2i, 2j, 3c*, 3h, 3b, 3a, 3k, 4b*, 4f, 4c, 4i, 4g, 4h
Chloramphenicol	20-30 mm					
Ampicillin	16-25 mm					
Norfloxacin	15-30 mm					
Griseofulvin	20 mm					
Rifampin	0.25-0.031 µg/ml					

*see Table III level-II assay summary for MIC *in vitro* evaluation of anti-tuberculosis activity

Table III—Level-II assay summary for MIC *in vitro* evaluation of anti-tuberculosis activity

Sample ID	Where, R=	MIC vs H37 Rv (µg/mL)	IC 50 VERO Cells (µg/mL)	SI (IC50/MIC)	Comment
3c	4-Cl-C ₆ H ₄ -	12.5	31.4	2.51	IC50 (INH>1000; RMP=137.8 DMSO=3%)
4b	2-Cl-C ₆ H ₄ -	12.5	25.3	2	IC50 (INH>1000; RMP=137.8 DMSO=3%)

mycobacterial activity towards a strain of *Mycobacterium tuberculosis H37 Rv*.

In vitro evaluation of pharmacological studies

The antimycobacterial activity of the compounds was carried out at Tuberculosis Antimicrobial Acquisition and Co-ordinating Facility (TAACF), USA.

Primary screening of the compounds for anti-tubercular activity have been conducted at 12.5 µg/mL against *Mycobacterium tuberculosis H37 Rv* in BACTEC 12B medium using the BACTEC 460 radiometric system. Compounds demonstrating at least 90% inhibition in the primary screen have been retested at lower concentration against *M. tuberculosis H37 Rv* to determine the actual minimum inhibitory concentration in the CABTEC 460. The antimycobacterial activity data were compared with standard drug Rifampin at a 0.25 to 0.031 µg/mL concentration.

The antimicrobial activity was assayed by using the Cup-plate agar diffusion method¹⁵ by measuring the inhibition zones in mm. All the compounds were screened *in vitro* for their antimicrobial activity against a variety of bacterial strains such as *Bacillus megaterium*, *escherichia coli*, *Bacillus subtilis*, *Pseudomonas flourescens* and fungi such as

aspergillus awamori at a concentration of 50 µg. Known antibiotics like chloramphenicol, ampicillin, norfloxacin and griseofulvin were used for comparison purpose.

The antimicrobacterial activity of the tested compounds 2a-m, 3a-m, 4a-m against *Mycobacterium tuberculosis H37 Rv* is shown in Table II. The obtained data show that the most potent derivatives in the series were compounds 3c and 4b (Table III).

The chalcone derivatives 2a-m exhibited various degree of activity except 2i which showed 82% inhibition. Isoxazole analogues were generally more active than the corresponding chalcones which showed a significant activity. Cyanopyridine analogs 4b, 4c, 4f and 4i displayed best activity than the corresponding isoxazoles. These preliminary results confirm the importance of benzimidazole moiety with respect to the antimycobacterial activity.

From Table II it can be concluded that all the compounds have displayed maximum activity against *E. coli*. The compounds 2f, 3e, 3i, 4b, 4f were highly active against *B. megaterium*. The compounds 2h, 3g, 3k, 4f showed significant activity against *B. subtilis*. Compounds 2a, 2e, 3a, 3d, 4e, 4f displayed moderate activity against *A. Awamori*. Compounds 3a, 3d, 3f, 3i were found to be less active against *Ps. Fluore-scens*.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Shimadzu 435-IR spectrophotometer and ^1H NMR spectra on Bruker AC 300 MHz using TMS as an internal standard (chemical shifts in δ , ppm). All the compounds showed satisfactory C, H and N analyses.

***p*-5'-Benzoyl-benzimidazol-2'-yl-*o*-benzoyl amino chalcones 2f.** To a well stirred solution of 5-benzimidazol-2-yl-*o*-benzoyl(4'-amino acetophenone) (0.01 mole, 3.48 g) and *N,N*-dimethyl benzaldehyde (0.01 mole, 1.49 g) in ethanol (25 mL), 40% KOH (3 mL) was added. The reaction mixture was heated for 10 min and left overnight. The contents were poured into ice water acidified, filtered and crystallised from ethanol to give **2f**, yield 82%, m.p. 130°C. Anal. Found: C, 77.25; H, 5.15; N, 9.57. Calc. for: $\text{C}_{38}\text{H}_{30}\text{O}_3\text{N}_4$ C, 77.2; H, 5.1; N, 9.49%; IR (KBr): 3300 (N-H), 1705 (C=O chalcone), 1670 (C=O benzoyl), 1635 (C=O amide) 1610 (C=N) cm^{-1} ; ^1H NMR (CDCl_3 +TFA): δ 3.40-3.34 (s, 6H, $-\text{N}(\text{CH}_3)_2$), 6.90 (d, 2H, =CH), 7.5-8.0 (m, 2OH, $-\text{Ar}-\text{H}$), 7.78 (d, CH=CH-R), 8.2 (s, 1H, $-\text{NH}$), 8.25 (s, 1H, $-\text{CONH}$).

Similarly, other members of **2a-m** were prepared and their physical data are given in **Table I**.

3-(5'-Benzoyl-benzimidazol-2'-yl-benzamido-phenyl)-5-arylisoxazole 3h. Anhydrous sodium acetate (0.001 mole, 0.82 g) dissolved in a minimum amount of hot acetic acid was added to a solution of hydroxylamine hydrochloride (0.01 mole, 0.79 g) in ethanol (10 mL). This was added to a solution of **2h** (0.01 mole, 5.77 g) in ethanol (15 mL). The mixture was refluxed on a sand-bath for 2-3 hr. The contents were poured into crushed ice, filtered and crystallised from ethanol to give **3h**, yield 69%, m.p. 150°C. Anal. Found: C, 75.2; H, 4.40; N, 9.54. Calc. for: $\text{C}_{37}\text{H}_{26}\text{O}_4\text{N}_4$: C, 75.12; H, 4.40; N, 9.49%; IR (KBr): 3247 (N-H), 1694 (C=O benzoyl), 1652 (C=O amide), 1603 (C=N) cm^{-1} ; ^1H NMR (CDCl_3 +TFA): δ 3.9 (s, 3H, $-\text{OCH}_3$), 7.0-8.0 (m, 20H, $-\text{Ar}-\text{H}$), 8.2 (s, 1H, $-\text{NH}$), 8.4 (s, 1H, $-\text{CONH}$).

Similarly, others members of **3a-m** were prepared and their physical data are given in **Table I**.

2-Amino-3-cyano-4-aryl-6-(5'-benzoyl-benzimidazol-2'-yl-*o*-benzamido-phenyl)pyridines 4f: A mixture of **2h** (0.01 mole, 5.77 g), malononitrile (0.01 mole, 0.66 g) and ammonium acetate (0.08 mole, 6.61 g) dissolved in ethanol (25 mL) was heated under reflux for 6 hr. The contents were poured into crushed ice, filtered and crystallised from ethanol to give **4h**; yield 73%, m.p. 155°C. Anal. Found: C, 75.0; H, 4.40; N, 13.20. Calc. for $\text{C}_{40}\text{H}_{28}\text{N}_6\text{O}_3$: C, 75.0; H, 4.37; N, 13.12%; IR (KBr): 3336 (N-H), 2211 ($\text{C}\equiv\text{N}$), 1680 (C=O benzoyl), 1650 (C=O amide), 1594 (C=N) cm^{-1} ; ^1H NMR (CDCl_3 +TFA): δ 3.8 (s, 3H, $-\text{OCH}_3$), 7.1-8.0 (m, 21H, $-\text{Ar}-\text{H}$), 8.2 (s, 1H, $-\text{NH}$), 8.3 (s, 1H, $-\text{CONH}$).

Similarly, other members of **4a-m** were prepared and their physical data are given in **Table I**.

Acknowledgement

The authors are thankful to Dr A R Parikh, Professor and Head, Deptt. of Chemistry, Saurashtra University for providing research facilities and Dr Cecil D Kwong, Research Chemist, Tuberculosis Antimicrobial Acquisition Coordinating Facility (TAACF) USA for antimycobacterial screening facility.

References

- Murthy A K, Rao K S R & Rao N V, *Indian J Chem*, 10, 1972, 38; *Chem Abstr*, 77, 1972, 29615.
- Sharan P, Giri S & Nizamuddin, *J Indian Chem Soc*, 66, 1989, 393.
- Shah M, Patel P, Koregaokar S, Parekh H, *Indian J Chem*, 35B, 1996, 1282.
- Rigernik & Raymond H (Dow Chemical Co), US 3, 399, 205 (CI 260-294.8) 27 Aug 1968; *Chem Abstr*, 69, 1968, 96483h.
- Pavia R M, Taylor C P, Hershenson F M & Labbestael S J, *J Med Chem*, 30, 1987, 1210.
- Patel P, Koregaokar S, Shah M & Parekh H, *IL Poharmaco*, 51, 1996, 59.
- Reddy G P & Murthy A K, *Indian J Heterocycl*, 3 1994, 223; *Chem Abstr*, 122, 1995, 105724e.
- W L Hoeftling, D Elhaner & G Reckling, *VEB Leuna-werke "Water Ulbricht" Gerl* 1, 193, 506 (CI 07 D) 26 may 1965; *Chem Abst*, 63, 1965, 6979.
- H Goker, Emin Tehrizli, Ufuk Abbasoglu, *IL Pharmaco*, 51, 1996, 53.
- Gackowska Irena, Sowlewicz Josef *et al.*, *Acta pol pharm*, 44, 1987, 419; *Chem Abstr*, 110, 1989, 173148x.
- Banoglu E, Abbasogle U & Sachin M Fethi, *J Pac Pharm Gazi Uni*, 10, 1993, 1-B; *Chem Abstr*, 121, 1994, 9243f.

- 12 Andrew, Khan Adil & Mohmood Naheed, *Bio Org Med Chem lett*, 5, **1995**, 1251-4; *Chem Abstr*, 123, **1995**, 3138429.
- 13 Ru Zhou & Edward B Skib, *J Med Chem*, 39, **1996**, 4321.
- 14 Spencer C M & Faulds D, *Drugs*, 48, **1994**, 404.
- 15 Barry A L, *The antimicrobial suseptibility test, principle and practices*, edited by illus lea & Febiger, 180; *Biol Abstr*, 64, **1977**, 25183.