Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 4 pp. 629–636, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND ANTIMICROBIAL SCREENING OF N-[2-(2/4-SUBSTITUTED PHENYL)-1-(5/6 SUBSTITUTED 1*H*- BENZIMIDAZOL-2-YL)VINYL]BENZAMIDES

DARPAN KAUSHIK[1]*, SUROOR A. KHAN[1], GITA CHAWLA[1] and BIBHU P. PANDA[2]

[1]Medicinal Chemistry Division, [2]Microbiology and Biotechnology Division,
F/O Pharmacy, Jamia Hamdard, New Delhi-11062, India

Abstract: A series of (benzamidostyryl)benzimidazole derivatives were synthesized by hydrolyzing 2-phenyl-4-(substituted)benzylidene-5-oxazolones, the azlactone precursors in an acidic medium and treating the product with substituted *o*-phenylenediamine (OPDA) *in situ*. The structures of the synthesized compounds were confirmed by spectral and elemental analyses. All synthesized compounds were screened for their *in vitro* antimicrobial activities against some identifiable strains. Thereby, it was found that only nitro substituted benzimidazoles exhibited good to moderate antibacterial activity, while other derivatives were devoid of any antimicrobial effect.

Keywords: benzimidazole, Erlenmeyer-Plochl azlactone synthesis, identifiable strains, minimal inhibitory concentration, two fold dilution technique

Infectious microbial diseases remained pressing problems worldwide, because of resistance to a number of antimicrobial agents among variety of clinically significant species of microorganisms and has become an important global health problem (1). One way to battle with this challenge is the conscious usage of the currently marketed antibiotics; the other is the development of novel antimicrobial agents (2). Hence, there will always be a vital need to discover new chemotherapeutic agents to avert the emergence of resistance and ideally shorten the duration of therapy.

Benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications (3–6). They have is an important pharmacophore and privileged structure in medicinal chemistry both with respect to their inhibitory activity and their favorable selectivity ratio (7). Literature survey revealed that amongst the benzimidazole derivatives, 2-substituted are found to be pharmacologically more potent and hence the design and synthesis of 2-substituted benzimidazoles are the potential area of research (8). Extensive biochemical and pharmacological studies have confirmed that these derivatives are effective against various strains of microorganisms (9–14). Thus, benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibited the immense potential and varied bioactivities; therefore, efforts have been made from time to time to generate libraries of these compounds and screened them for potential biological activities. Therefore, seeing the importance of benzimidazole nucleus, it was thought that it would be worthwhile to design and synthesize some new 2-substituted benzimidazole derivatives and screen them for potential biological activities.

EXPERIMENTAL

All chemicals were of reagent grade. The melting points of synthesized derivatives were determined in an open end capillary tube on Hicon digital melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on FT-IR Rez Bio Rad Win-IR (KBr) and Brucker DRX-300 instruments, respectively. Chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard. The elemental analyses were performed on Vario EL III CHNS analyzer using sulfanilic acid as a standard,

* Corresponding author: e-mail: darpkaush@yahoo.com, mobile: +91-8059110384

and all the values were within ± 0.4% of the theoretical compositions. The fast atom bombardment (FAB) spectra were recorded on Jeol SX 102/DA-600 mass spectrometer using Argon/Xenon (6 KV 10 mA) as the FAB gas. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) accomplished with silica gel G chromatoplates of 15 cm length and 0.2 cm thick (Merck). Solvent system: toluene : ethyl acetate : formic acid (5:4:1, v/v/v), vizualization with iodine vapor.

Synthesis of hippuric acid (1)

Hippuric acid was prepared according to reported method (15). The compound was recrystallized from boiling water. The practical yield of product was 80% and its m.p. 186°C.

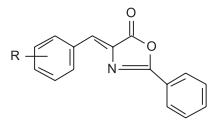
General procedure for the synthesis of 4-benzylidene-2-phenyl-4*H*-oxazol-5-one derivatives (2a–f)

The azlactones (**2a–f**) were prepared according to previously reported methods (15). The ^{1}H NMR spectral data of all the synthesized compounds are presented in Table 1.

General procedure for the synthesis of substituted (benzamidostyryl)benzimidazoles (3a-r)

To 2-phenyl-4-(substituted) benzylidene-5-oxazolone **(2a–f)**, an equimolar quantity of *o*-phenylendiamine (OPDA, substituted) was added, then 20 mL of 50% acetic acid, followed by 30 mL of dioxane and the mixture was stirred until all the solids get dissolved. The solution was then refluxed at 135–140°C for 20 h with stirring. The reaction mixture was then evaporated to near dryness to

Table 1. Physicochemical data of 4-(substituted benzylidene)-2-phenyl-4*H*-oxazol-5-ones.



R	M.p.[a] (°C)	Yield (%)	Color	Mol. formula[b] (M.w.)	^{1}H-NMR (δ ppm, DMSO-d$_6$)
-H	158	60	Yellow needle	$C_{16}H_{11}NO_2$ (249.27)	8.31–8.29 (d, 2H, 2',6' Ar-*H*), 8.14–8.12 (d, 2H, 2,6 Ar-*H*), 7.74–7.52 (m, 6H, Ar-*H*), 7.35 (s, 1H, C*H*=C)
p-CH_3	134	58	Parrot green	$C_{17}H_{13}NO_2$ (263.20)	8.38–8.35 (d, 2H, 2',6' Ar-*H*), 8.14–8.12 (d, 2H, 2,6 Ar-*H*), 7.74–7.49 (m, 3H, Ar-*H*), 7.45 (s, 1H, C*H*=C), 7.28–7.26 (d, 2H, 3,5 Ar-*H*), 2.37(s, 3H, CH_3)
p-OH	172–173	62	Yellow	$C_{16}H_{11}NO_3$ (265.27)	10.62 (s, 1H, O*H*), 8.38–8.35 (d, 2H, 2',6' Ar-*H*), 8.19–8.16 (d, 2H, 2,6 Ar-*H*), 7.70–7.45 (m, 3H, Ar-*H*), 7.39 (s, 1H, C*H*=C), 6.94–6.91 (d, 2H, 3,5 Ar-*H*)
p-OCH_3	156	52	Yellow fluffy	$C_{17}H_{13}NO_3$ (279.30)	8.20–8.17 (d, 2H, 2',6' Ar-*H*), 8.14–8.12 (d, 2H, 2,6 Ar-*H*), 7.63-7.43 (m, 3H, Ar-*H*), 7.39 (s, 1H, C*H*=C), 6.85–6.83 (d, 2H, 3,5 Ar-*H*), 3.82 (s, 3H, OCH_3)
o-Cl	156–158	61	Yellow	$C_{16}H_{10}ClNO_2$ (283.72)	8.17–8.15 (d, 2H, 2',6' Ar-*H*), 7.92–7.90 (d, 1H, 6 Ar-*H*), 7.66–7.56 (m, 5H, Ar-*H*), 7.48 (s, 1H, C*H*=C), 7.18–7.13 (d, 1H, Ar-*H*)
p-$N(CH_3)_2$	217–219	55	Dark brown	$C_{18}H_{16}N_2O_2$ (292.34)	8.20–8.17 (d, 2H, 2',6' Ar-*H*), 8.08–8.06 (d, 2H, 2,6 Ar-*H*), 7.67–7.61 (t, 3H, 3',4',5' Ar-*H*), 7.24 (C*H*=C), 6.85–6.83 (d, 2H, 3,5 Ar-*H*), 3.08 (s, 6H, N(CH_3)$_2$)

[a]Melting point of the compounds at their decomposition. [b]Elemental analyses for C, N were within ± 0.4 % of the theoretical values.

yield brownish material, which was further redissolved in methanol, to which activated charcoal was added, and the mixture was again refluxed for 20 min, followed by filteration while hot and the filtrate was then allowed to cool and the solid thus precipitated out was recrystallized from methanol, to give colored crystals (16). The physicochemical data of all the synthesized compounds are given in Table 2.

The spectral data of all newly synthesized derivatives are mentioned below:

N-[-1-(1 *H*-benzimidazol-2-yl)-2-(phenyl)vinyl] benzamide (**3a**)

IR: ν_{max} (cm^{-1}): 3278 (NH), 1656 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.01 (bs, 1H, CON*H*, D_2O exchangeable), 9.6 (s, 1H, N*H*, D_2O exchangeable), 8.14–8.11 (d, 2H, 2',6' Ar-*H*), 7.76–7.42 (m, 12H, Ar-*H*), 7.35 (s, 1H, C*H*=C). MS (m/z): M^+ (339), M–77 (262), M–105 (234).

N-[-1-(1*H*-benzimidazol-2-yl)-2-(4-methylphenyl)vinyl]benzamide (**3b**)

Table 2. Physicochemical data of *N*-[- 2 -(2/4-substituted phenyl)-1-(6 substituted 1*H*-benzimidazol-2-yl)vinyl] benzamides.

Compound	**R**	**R^1**	Yield (%)	M.p[a] (^oC)	Mol. formula[b] (M.w.)
3a	-H	-H	43	235–238	$C_{22}H_{17}N_3O$ (339.40)
3b	*p*-CH_3	-H	32	266–268	$C_{23}H_{19}N_3O$ (353.43)
3c	*p*-OH	-H	26	187–190	$C_{22}H_{17}N_3O_2$ (355.40)
3d	*p*-OCH_3	-H	23	278	$C_{23}H_{19}N_3O_2$ (369.43)
3e	*o*-Cl	-H	36	236–238	$C_{22}H_{16}ClN_3O$ (373.85)
3f	*p*-$N(CH_3)_2$	-H	23	234–235	$C_{24}H_{22}N_4O$ (382.47)
3g	-H	-NO_2	24	255–259	$C_{22}H_{16}N_4O_3$ (384.40)
3h	*p*-CH_3	-NO_2	33	220–221	$C_{23}H_{18}N_4O_3$ (398.42)
3i	*p*-OH	-NO_2	32	187–189	$C_{22}H_{16}N_4O_4$ (400.40)
3j	*p*-OCH_3	-NO_2	28	198–200	$C_{23}H_{18}N_4O_4$ (414.42)
3k	*o*-Cl	-NO_2	29	280	$C_{22}H_{15}ClN_4O_3$ (418.84)
3l	*p*-$N(CH_3)_2$	-NO_2	27	248–251	$C_{24}H_{21}N_5O_3$ (427.47)
3m	-H	-CH_3	39	281	$C_{23}H_{19}N_3O$ (353.43)
3n	*p*-CH_3	-CH_3	33	206–209	$C_{24}H_{21}N_3O$ (367.45)
3o	*p*-OH	-CH_3	43	165–168	$C_{23}H_{19}N_3O_2$ (369.43)
3p	*p*-OCH_3	-CH_3	28	233–235	$C_{24}H_{21}N_3O_2$ (383.45)
3q	*o*-Cl	-CH_3	37	279	$C_{23}H_{18}ClN_3O$ (387.87)
3r	*p*-$N(CH_3)_2$	-CH_3	23	236–238	$C_{25}H_{24}N_4O$ (396.50)

[a]Melting point of the compounds at their decomposition. [b]Elemental analyses for C, N were within ± 0.4 % of the theoretical values

IR: ν_{max} (cm^{-1}): 3269 (NH), 1640 (C=O), 1597 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.08 (bs, 1H, CON*H*), 9.54 (s, 1H, N*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.70–7.34 (m, 9H, Ar-*H*), 7.33 (C*H*=C), 7.12–7.10 (d, 2H, Ar-*H*), 2.37 (s, 3H, CH_3).

N-[-1-(1*H*-benzimidazol-2-yl)-2-(4-hydroxyphenyl)vinyl]benzamide (3c)

IR: ν_{max} (cm^{-1}): 3274 (NH), 1648 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.03 (bs, 1H, CON*H*), 10.41 (s, 1H, O*H*), 9.40 (s, 1H, N*H*), 8.06–8.03 (d, 2H, Ar-*H*), 7.77–7.38 (m, 9H, Ar-*H*), 7.33 (s, 1H, C*H*=C), 6.94–6.91 (d, 2H, Ar-*H*).

N-[-1-(1*H*-benzimidazol-2-yl)-2-(4-methoxyphenyl)vinyl]benzamide (3d)

IR: ν_{max} (cm^{-1}): 3265 (NH), 1643 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.01 (bs, 1H, CON*H*), 9.54 (s, 1H, N*H*), 812–8.10 (d, 2H, 2', 6' Ar-*H*), 7.66–7.41 (m, 9H, Ar-*H*), 7.19 (s, 1H, C*H*=C), 6.85–6.82 (d, 2H, Ar-*H*), 3.79 (s, 3H, OCH_3). MS (m/z): M^+ (369), M–77 (292), M–105 (264).

***N*-[-1-(1*H*-benzimidazol-2-yl)-2-(2-chlorophenyl)vinyl]benzamide (3e)**

IR: ν_{max} (cm^{-1}): 3271 (NH), 1654 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.04 (bs, 1H, CON*H*), 9.41 (s, 1H, N*H*), 8.05–8.03 (d, 2H, Ar-*H*), 7.76–7.43 (m, 7H, Ar-*H*), 7.42 (s, 1H, C*H*=C), 7.22–7.04 (m, 4H, Ar-*H*).

***N*-[-1-(1*H*-benzimidazol-2-yl)-2-(4-dimethylamino-phenyl)vinyl]benzamide (3f)**

IR: ν_{max} (cm^{-1}): 3273 (NH), 1656 (C=O), 1603 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.01 (bs, 1H, CON*H*), 9.42 (s, 1H, N*H*), 8.05–8.03 (d, 2H, 2',6' Ar-*H*), 7.78–7.41 (m, 9H, Ar-*H*), 7.28 (s, 1H, C*H*=C), 6.85–6.83 (d, 2H, Ar-*H*), 3.04 (s, 6H, CH_3).

***N*-[-1-(6-nitro-1*H*-benzimidazol-2-yl)-2-phenylvinyl]benzamide (3g)**

IR: ν_{max} (cm^{-1}): 3298 (NH), 1644 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 13.21 (bs, 1H, CON*H*), 10.38 (s, 1H, N*H*), 8.49 (s, 1H, Ar-*H*), 8.18–8.16 (d, 1H, Ar-*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.73–7.70 (d, 2H, Ar-*H*), 7.64–7.58 (m, 5H, Ar-*H*), 7.42 (s, 1H, C*H*=C), 7.37–7.32 (t, 2H, Ar-*H*). MS (m/z): M^+ (384), M–77 (307), M–105 (279).

***N*-[-2-(4-methylphenyl)-1-(6-nitro-1*H*-benzimidazol-2-yl)vinyl]benzamide (3h)**

IR: ν_{max} (cm^{-1}): 3312 (NH), 1658 (C=O), 1607 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 13.37 (bs, 1H, CON*H*), 10.37 (s, 1H, N*H*), 8.51 (s, 1H, Ar-*H*), 8.14–8.12 (d, 1H, Ar-*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.66–7.38 (m, 6H, Ar-*H*), 7.34 (s, 1H, C*H*=C), 7.12–7.10 (d, 2H, Ar-*H*), 2.38 (s, 3H, CH_3).

***N*-[-2-(4-hydroxyphenyl)-1-(6-nitro-1*H*-benzimidazol-2-yl)vinyl]benzamide (3i)**

IR: ν_{max} (cm^{-1}): 3296 (NH), 1647 (C=O), 1606 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 13.32 (bs, 1H, CON*H*), 10.49 (s, 1H, O*H*), 10.38 (s, 1H, N*H*), 8.49 (s, 1H, Ar-*H*), 8.14–8.12 (d, 1H, Ar-*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.76–7.32 (m, 6H, Ar-*H*), 7.29 (s, 1H, C*H*=C), 6.91–6.89 (d, 2H, Ar-*H*).

***N*-[-2-(4-methoxyphenyl)-1-(6-nitro-1*H*-benzimidazol-2-yl)vinyl]benzamide (3j)**

IR: ν_{max} (cm^{-1}): 3308 (NH), 1650 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 13.34 (bs, 1H, CON*H*), 10.38 (s, 1H, N*H*), 8.49 (s, 1H, Ar-*H*), 8.14–8.12 (d, 1H, Ar-*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.76–7.41 (m, 6H, Ar-*H*), 7.34 (s, 1H, C*H*=C), 6.86–6.84 (d, 2H, Ar-*H*), 3.84 (s, 3H, OCH_3).

***N*-[-2-(2-chlorophenyl)-1-(6-nitro-1*H*-benzimidazol-2-yl)vinyl]benzamide (3k)**

IR: ν_{max} (cm^{-1}): 3306 (NH), 1654 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 13.30 (bs, 1H, CON*H*), 10.33 (s, 1H, N*H*), 8.52 (s, 1H, Ar-*H*), 8.14–8.11 (d, 1H, Ar-*H*), 7.99–7.97 (d, 2H, Ar-*H*), 7.77 (s, 1H, C*H*=C), 7.73–7.70 (d, 2H, Ar-*H*), 7.64–7.58 (m, 4H, Ar-*H*), 7.37–7.32 (t, 2H, Ar-*H*).

***N*-[-2-(4-dimethylamino-phenyl)-1-(6-nitro-1*H*-benzimidazol-2-yl)vinyl]benzamide (3l)**

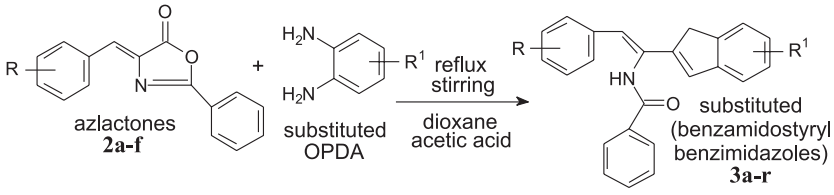
IR: ν_{max} (cm^{-1}): 3310 (NH), 1645 (C=O), 1611 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 13.38 (bs, 1H, CON*H*), 10.24 (s, 1H, N*H*), 8.50 (s, 1H, Ar-*H*), 8.24–8.22 (d, 1H, Ar-*H*), 8.13–8.11 (d, 2H, Ar-*H*), 7.71–7.68 (d, 2H, Ar-*H*), 7.64–7.58 (m, 4H, Ar-*H*), 7.26 (s, 1H, C*H*=C), 6.86–6.83 (d, 2H, Ar-*H*), 3.07 (s, 6H, N(CH_3)$_2$).

***N*-[-1-(6-methyl-1*H*-benzimidazol-2-yl)-2-phenylvinyl]benzamide (3m)**

IR: ν_{max} (cm^{-1}): 3305 (NH), 1648 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.41 (bs, 1H, CON*H*), 10.15 (s, 1H, N*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.81 (s, 1H, Ar-*H*), 7.68–7.44 (m, 8H, Ar-*H*), 7.34 (s, 1H, C*H*=C), 7.14–7.12 (d, 2H, Ar-*H*), 2.37 (s, 3H, CH_3).

***N*-[-2-(4-methylphenyl)-1-(6-methyl-1*H*-benzimidazol-2-yl)vinyl]benzamide (3n)**

IR: ν_{max} (cm^{-1}): 3310 (NH), 1647 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.33 (bs, 1H,



R = -H, -CH_3, -OH, -OCH_3, *o*-Cl, *p*-$(CH_3)_2$N-

R^1 = -H, -NO_2, -CH_3

Scheme 1. Synthetic pathway of compounds **3a–r**. OPDA = *o*-phenylenediamines

CON*H*), 10.13 (s, 1H, N*H*), 8.06–8.04 (d, 2H, Ar-*H*), 7.82 (s, 1H, Ar-*H*), 7.76–7.37 (m, 6H, Ar-*H*), 7.36 (s, 1H, C*H*=C), 7.20–7.13 (m, 3H, Ar-*H*), 2.39 (s, 3H, CH_3), 2.26 (s, 3H, CH_3).

***N*-[-2-(4-hydroxyphenyl)-1-(6-methyl-1*H*-benzimidazol-2-yl)vinyl]benzamide (3o)**

IR: ν_{max} (cm^{-1}): 3296 (NH), 1656 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.30 (bs, 1H, CON*H*, D_2O exchangeable), 11.05 (s, 1H, O*H*, D_2O exchangeable), 10.11 (s, 1H, N*H*, D_2O exchangeable), 8.03–8.01 (d, 2H, Ar-*H*), 7.79 (s, 1H, Ar-*H*), 7.68–7.42 (m, 6H, Ar-*H*), 7.34 (s, 1H, C*H*=C), 7.14–7.12 (d, 1H, Ar-*H*), 7.03–7.01 (d, 2H, Ar-*H*), 2.37 (s, 3H, CH_3).

***N*-[-2-(4-methoxyphenyl)-1-(6-methyl-1*H*-benzimidazol-2-yl)vinyl] benzamide (3p)**

IR: ν_{max} (cm^{-1}): 3312 (NH), 1648 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.44 (bs, 1H, CON*H*), 10.12 (s, 1H, N*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.80 (s, 1H, Ar-*H*), 7.62–7.37 (m, 6H, Ar-*H*), 7.34 (s, 1H, C*H*=C), 7.14–7.12 (d, 1H, Ar-*H*), 6.85–6.82 (d, 2H, Ar-*H*), 3.79 (s, 3H, OCH_3), 2.38 (s, 3H, CH_3).

***N*-[-2-(2-chlorophenyl)-1-(6-methyl-1*H*-benzimidazol-2-yl)vinyl]benzamide (3q)**

IR: ν_{max} (cm^{-1}): 3308 (NH), 1654 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 12.54 (bs, 1H, CON*H*), 10.14 (s, 1H, N*H*), 7.98–7.95 (d, 2H, Ar-*H*), 7.78 (s, 1H, Ar-*H*), 7.67–7.48 (m, 6H, Ar-*H*), 7.41 (s, 1H, C*H*=C), 7.34–7.23 (m, 2H, Ar-*H*), 7.04–6.90 (t, 1H, Ar-*H*), 2.40 (s, 3H, CH_3).

***N*-[-2-(4-dimethylamino-phenyl)-1-(6-methyl-1*H*-benzimidazol-2-yl)vinyl]benzamide (3r)**

IR: ν_{max} (cm^{-1}): 3310 (NH), 1650 (C=O), 1603 (C=N), ^{1}H NMR (DMSO-d_6, δ, ppm): 12.21 (bs, 1H, CON*H*), 10.14 (s, 1H, N*H*), 8.03–8.01 (d, 2H, Ar-*H*), 7.79 (s, 1H, Ar-*H*), 7.62–7.37 (m, 6H, Ar-*H*), 7.33 (s, 1H, C*H*=C), 7.14–7.12 (d, 1H, Ar-*H*), 6.85–6.83 (d, 2H, Ar-*H*), 3.08 (s, 6H, N(CH_3)$_2$), 2.38 (s, 3H, CH_3).

Antimicrobial activity test

All the synthesized compounds were screened for their *in vitro* antimicrobial activity against the standard strains of: *Staphylococcus aureus* (MTCCB, 96), *Bacillus subtilis* (MTCC 121), *Streptococcus mutans* (MTCC 890), *Escherichia coli* (MTCCB, 1610), *Pseudomonas aeruginosa* (MTCCB, 741) and various fungi: *Aspergillus niger* (MTCC, 281), *Aspergillus flavus* (MTCC, 277), *Monascus purpureos* (MTCC, 369), *Penicillium citrinum* (NCIM, 768) using the cup diffusion technique (17). Compounds showing inhibition zones of at least 18 mm were considered active and were further evaluated for their minimal inhibitory concentration (MIC) and minimal bacteriostatic concentration (MBC) values using the two-fold serial dilution method (18). Also as control, antimicrobial effects of the DMSO were determined. The results were evaluated according to the values of the controls.

Methodology for *in vitro* antimicrobial screening
Inhibition zone measurement

The antibacterial and antifungal testing was studied using the cup diffusion technique. The test compounds at 200 µg/mL solutions in dimethyl sulfoxide (DMSO), were evaluated *in vitro* for antibacterial activity against Gram positive bacterial strains i.e., *S. aureus* (MTCCB, 96), *B. subtilis* (MTCC 121), *S. mutans* (MTCC 890) and Gram negative bacteria including *E. coli* (MTCCB, 1610), *P. aeruginosa* (MTCCB, 741) and antifungal activity against *A. niger* (MTCC, 281), *A. flavus* (MTCC, 277), *M. purpureos* (MTCC, 369), *P. citrinum* (NCIM, 768). Sterile nutrient agar and potato dextrose agar (Himedia) was respectively inoculated with the test

organisms (each 100 mL of the medium received 1 mL of 24 h broth culture) for the determination of antibacterial and antifungal activity, and then seeded media were poured into sterile Petri dishes. Cups (8 mm in diameter) were cut in the agar, and each cup received 0.1 mL of the test compound solution in triplicate. The plates were then incubated at 37°C for 24 h and 5 days for the respective activity. The activities were estimated as range of zones of inhibition in mm diameter as depicted in Table 3. A 50 µg/mL solution of ciprofloxacin and fluconazole were used as reference standards. DMSO was used as a negative control and did not show any inhibition zones.

Minimal inhibitory concentration measurement

The two fold dilution technique (18) was followed to determine the minimal inhibitory concen-

Table 3. Preliminary screening of zone of inhibition against identifiable strains.

Compound	Microorganism				
	S. aureus	*B. subtilis*	*S. mutans*	*E. coli*	*P. aeruginosa*
Control	–	–	–	–	–
3a	+	+	+	+	–
3b	++	+	+	+	–
3c	+	+	+	++	–
3d	++	+	+	+	–
3e	++	+	+	+	–
3f	++	+	++	++	–
3g	++++	+++	++++	++++	++
3h	++	+	++	++	–
3i	+++	++	++	+++	–
3j	+++	+++	+++	++++	+++
3k	+++	+++	++++	+++	+++
3l	+	+	++	++	++
3m	+	+	+	++	+
3n	++	++	+	+	+
3o	+	++	+	++	+
3p	++	+	++	+	+
3q	+	+	+	+	+
3r	++	+	++	++	+
Ciprofloxacin	++++	++++	++++	++++	++++

Range of zone of inhibition (as studied in triplicate): + = 5–10 mm, ++ = 11–15 mm, +++ = 16–20 mm, ++++ = More than 20 mm, – = No inhibition.

Table 4. Antibacterial activity of compounds (MIC in µg/mL)

Compound	Microorganism									
	S. aureus		*B. subtilis*		*S. mutans*		*E. coli*		*P. aeruginosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3g	3.12	12.5	6.25	12.5	3.12	6.25	3.12	6.25	50	100
3i	12.5	25	12.5	25	25	25	12.5	25	X	X
3j	6.25	12.5	6.25	12.5	6.25	12.5	3.12	12.5	25	50
3k	6.25	12.5	6.25	12.5	3.12	12.5	6.25	12.5	12.5	50
Ciprofloxacin	0.8		< 0.8		< 0.8		< 0.8		< 0.8	

X – Not tested

tration (MIC) of the synthesized compounds. The test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium at the required final concentration with variation of 1.6–100 µg/mL. A plate containing only the culture medium and DMSO in the same dilution was used as negative control. The final amount applied was 10^4 CFU/plate. The MIC values were recorded after incubation at 37°C for a period of 24 h. The lowest concentration of the test substance that completely inhibited the growth of the microorganism was reported as MIC expressed in terms of µg/mL. Ciprofloxacin was used as reference drug. All experiments were performed three times.

Minimal bacteriostatic concentration (MBC) measurement

A loopful from the plate not showing visible growth (MIC) was spread over a quarter of a nutrient agar plate. After an overnight incubation (18 h), the plates were examined for growth. The tube containing the lowest concentration of the test compound that failed to yield growth on subculture plates was judged to contain the MBC (19) of that compound for the respective test organism.

RESULTS AND DISCUSSION

Title compounds were synthesized by hydrolyzing 2-phenyl-4-benzylidene-5-oxazolone, the azlactone precursor in an acidic medium and treating the subsequent product formed on hydrolysis with substituted *o*-phenylenediamine (OPDA) *in situ*. The hydrolysis of azlactone and condensation of acid with OPDA are both acid catalyzed reactions.

The Erlenmeyer azlactone synthesis of arylideneoxazolones involves condensation of aromatic aldehyde under the influence of base with the reactive methylene group in azlactone (2-phenyloxazol-5-one), which is formed by the dehydration of benzoylglycine, when the latter is heated with acetic anhydride in the presence of sodium acetate. The azalactone ring of arylideneoxazolones is readily cleaved hydrolytically and yielded compounds of substituted acylaminoacrylic acids (*N*-benzoyl-α-aminocinnamic acid). The hippuric acid (benzoylglycine) is prepared by benzoylation of amino group of glycine by Schotten-Baumann method. The final synthesized compounds were characterized by elemental analysis, FT-IR, ^{1}H NMR and mass spectra. FT-IR spectrum showed bands at 1656–1640 cm^{-1} for C=O stretch, 3091–3010 CH-Ar and 3312–3265 cm^{-1} for N-H stretch. The ^{1}H NMR of compounds confirms the presence of singlet at δ 13.38–12.01, 10.49–9.41, 7.77–7.19 ppm for –CONH, -NH, -CH=C, respectively, of which first two are D_2O exchangeable.

The final products **3a–r** were tested for their *in vitro* growth inhibitory activity against human pathogens. The *in vitro* activity was performed against Gram positive and negative bacterial strains, and various fungi to test the antifungal activity. All the test compounds were preliminary screened at 200 µg/mL concentration, whereas the standard was kept at 50 µg/mL. Based on initial preliminary screening of percentage inhibition of zone with reference to the positive control, the synthesized compounds were further evaluated for their MIC and MBC for bacteria. Known antibiotics like ciprofloxacin (the reference for antibacterial drugs) and fluconazole (the reference for antifungal drugs) were used as positive control.

According to the obtained results, no antifungal activity has been observed for any of compounds. The antibacterial profile of all the synthesized compounds **3a-r** revealed that all of the nitro substituted synthesized derivatives **3g-k** only exhibited good to moderate activity against the Gram-positive and negative strains, while other compounds had very weak potency.

Compound **3g** was found to be most active with MIC of 3.12 µg/mL and MBC of 12.5 µg/mL against the *S. aureus* while **3g** and **3k** were equipotent with MIC of 3.12 µg/mL while MBC were 6.25 and 12.5 µg/mL, respectively, against the *S. mutans*. Compounds **3g**, **3j-k** were equally potent with MIC of 6.25 µg/mL and MBC of 12.5 µg/mL against *B. subtilis*, while **3g** and **3j** were equally potent against *E. coli,* with the MIC of 3.12 µg/mL and MBC of 6.25 and 12.5 µg/mL, respectively. Only **3g** and **3k** were found to have activity against *P. aeruginosa* with MIC of 50 and 12.5 µg/mL as presented in Table 4. The activity from studies of *P. aeruginosa* is interesting as point of research but not have any therapeutic application. These data showed that the compounds possess varying degree of antibacterial effect compared to reference compound.

CONCLUSION

The results give clear indication that the nitro substitution on benzimidazole ring only results in very good activity against bacterial strains, but none of the synthesized derivatives showed promising result against the fungal strains. Moreover, these nitro derivatives showed both bacterostatic and bactericidal effect, but not at the same concentrations.

As the compounds tested are not numerous, it is premature to discuss the SAR of the tested series, but one point can be assured from our study that the nitro group on benzimidazole moiety played an important role in the antibacterial activity. Since this preliminary series of nitro benzimidazoles has shown good activity against the tested organisms, the possibility remains that more divergent structural modifications might improve the spectrum of antibacterial activity. Thus, our results may provide some guidance for future development of some novel nitrobenzimidazoles.

Acknowledgments

Authors are thankful to Dr Salim Javed, Hamdard University, New Delhi for generation of FT-IR and elemental analysis data. The authors are really grateful to CDRI, Lucknow for the generation of FABMS and to IIT, New Delhi and Hamdard University, New Delhi for providing the ^{1}H-NMR spectra. We are thankful to Dr Santosh Pasha, Scientist, IGIB, New Delhi for providing the bacterial strains and Biotech Dept., Jamia Hamdard for providing the fungal strains.

REFERENCES

1. He Y., Wu B., Yang J., Robinson D., Risen L. et al.: Bioorg. Med. Chem. Lett. 13, 3253 (2003).
2. Metwally K.A., Abdel-Aziz L.M., Lashine E.M., Husseiny M.I., Badawy R.H.: Bioorg. Med. Chem. 14, 8675 (2006).
3. Kazimierczuk Z., Upcroft J.A., Upcroft P., Górska A., Starościak B. et al.: Acta Biochim. Pol. 49, 185 (2002).
4. Yang Y.H., Cheng M.S., Wang Q.H., Nie H., Liao N. et al.: Eur. J. Med. Chem. 44, 1808 (2009).
5. Kaur N., Kaur A., Bansal Y., Shah D.I., Bansal G. et al.: Bioorg. Med. Chem. 16, 10210 (2008).
6. Cheng J., Xie J., Luo X.: Bioorg. Med. Chem. Lett. 15, 267 (2005).
7. Ansari K.F., Lal C.: Eur. J. Med. Chem. 44, 4028 (2009).
8. Foks H., Pancechowska-Ksepko D., Kuzmierkiewicz W., Zwolska Z., Augustynowicz-Kopec E., Janowiec M.: Chem. Heterocycl. Comp. 42, 611 (2006).
9. Sharma D., Narasimhan B., Kumar P., Jalbout A.: Eur. J. Med. Chem. 44, 1119 (2009).
10. Guven O.O., Erdogan T., Goker H., Yildiz S.: Bioorg. Med. Chem. Lett. 17, 2233 (2007).
11. Mohamed B.G., Abdel-Alim, A.M., Hussein M.A.: Acta Pharm. 56, 31 (2006).
12. Boiani M., Gonzalez M.: Mini. Rev. Med. Chem. 5, 409 (2005).
13. Tuncbilek M., Kiper T., Altanlar N.: Eur. J. Med. Chem. 44, 1024 (2009).
14. Desai K.G., Desai K.R.: Bioorg. Med. Chem. 14, 8271 (2006).
15. Kaushik D., Khan S. A., Chawla G.: Eur. J. Med. Chem. 45, 3960 (2010).
16. Kaushik D., Khan S.A., Chawla G.: Med. Chem. Res**.** (2011), DOI: 10.1007/s 00044-011-9552-1**.**
17. Jain S. R., Kar A.: Planta Med. 20, 118 (1971).
18. National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 2003. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically NCCLS Approval Standard Document M7-A6.
19. Scott A. C., Laboratory control of antimicrobial therapy, in Mackie and MacCartney Practical Medical Microbiology, 13th edn., Colle J.G., Duguid J.P., Fraser A.G., Marmion B.P. Eds., p. 161, Churchil Livingstone, Edinburgh 1989.

Received: 7. 04. 2011