

Synthesis and biological activity of some heterocyclic compounds containing benzimidazole and beta-lactam moiety

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MS received 22 January 2009; revised 7 August 2009; accepted 7 September 2009

Abstract. A number of 1*N*-substituted-2-methyl benzimidazole derivatives have been synthesized and tested for their antibacterial activities. The chemical structures of the newly synthesized compounds were verified on the basis of spectral and elemental methods of analyses. Investigation of antimicrobial activity of the compounds was done by disc diffusion method using Gram-positive (*S. aureus*, *S. mutans* and *B. subtilis*), Gram-negative (*E. coli*, *S. typhi* and *P. aeruginosa*) bacteria and fungi (*C. albicans*, *A. flavus* and *A. niger*). Among the compounds tested **5a**, **5b**, **5d**, **5i**, **5j** and **5k** exhibited good antibacterial activities against Gram positive bacteria, while **5d** and **5i** also showed notable antifungal activity. Specially compounds **5a** and **5b** exhibited appreciable activity against *S. aureus* and *B. subtilis* comparable to reference drugs.

Keywords. 2-Methyl benzimidazole; azetidin-2-one; antimicrobial activity; ampicillin; nalidixic acid; amphotericin B.

1. Introduction

The benzimidazole ring is an important pharmacophore in modern drug discovery. A variety of benzimidazole are in use, like thiabendazole and flubendazole (anthelmintic), omeprazole and lansoprazole (antiulcerative) and astemizole (antihistaminic) (figure 1). The chemistry and pharmacology of benzimidazoles have been of great interest to medicinal chemistry,^{1,2} because its derivatives possessed various biological activities such as antioxidant,^{3,4} antimicrobial,^{5–10} anthelmintic,^{11–13} anticancer,¹⁴ antihypertensive,¹⁵ antineoplastic,¹⁶ anti-inflammatory,^{17,18} analgesic,¹⁹ antiprotozoal^{20,21} and anti-hepatitis B virus activity.²² In addition, a large number of antibiotics contain the 2-azetidinone (commonly known as β -lactam) moiety²³ such as penicillin, cephalosporin and carbapenem (figure 2). It is also associated with a variety of therapeutic activities.^{24–28} In continuation of our work to develop potential antimicrobial molecules,^{29,30} we report here the synthesis of some derivatives of the title structure type containing the above mentioned moieties for evaluation of their antimicrobial activity.

2. Experimental

All melting points were determined in an open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on Perkin-Elmer RX1 spectrophotometer. The ¹H-NMR and ¹³C-NMR spectra were measured in CDCl₃ solutions on a Bruker DRX-300 MHz spectrometer using TMS as an internal reference (chemical shift in δ ppm). The mass spectra were recorded on a Jeol SX-102 instrument. Elemental analyses were carried out with Elementar Vario EL III elemental analyzer. Thin layer chromatography was performed on silica plates pre-coated with Merck Kiesegel 60 F254 and column chromatography with silica gel.

2.1 Synthesis of 2-methyl-1*H*-benzimidazole-1-carbohydrazide (**3**)

A mixture of compound **2** (20 mmol) and hydrazine hydrate (100%; 20 mmol) in dry methanol (40 ml) was refluxed on a steam bath for 4 h the excess solvent was removed under reduced pressure and the product crystallized from methanol to obtain compound **3**. Yield 78%; mp 173–175°C; IR (KBr): 1632 (–C=N), 1673 (–C=O amido), 3157 (–NHNH₂) cm⁻¹; ¹H-NMR (DMSO-*d*₆, δ ppm): 2.58 (*s*, 3H,

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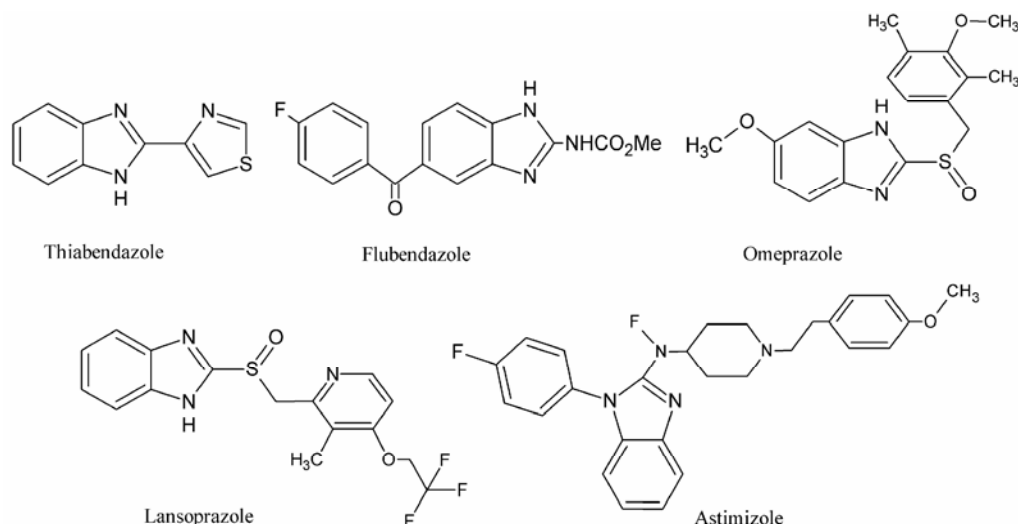


Figure 1. Clinically used benzimidazoles.

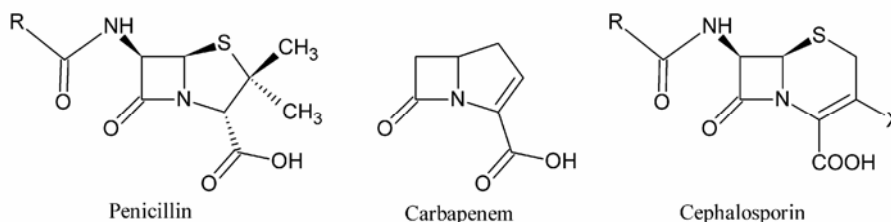


Figure 2. Antibiotics containing β -lactam ring.

–CH₃ at C-2 of benzimidazole), 2.60 (*s*, 2H, –NH₂), 7.79–7.92 (*m*, 4H, aromatic protons); EI–MS: 191 ($M^+ + 1$).

2.2 2-Methyl-*N'*-(substituted) alkyl/aryl methylidene]-1-carbohydrazido-1*H*-benzimidazoles (4a–4m)

General procedure: To a stirred solution of compound 3 (30 mmol) containing 4–5 drops of glacial acetic acid in methanol (30 ml) was added appropriate aldehyde (30 mmol) and the mixture refluxed for 4–8 h on a steam bath. Methanol was removed under vacuum and the resultant semisolid treated with distilled water (3 × 10 ml). The separated solid was filtered, dried and recrystallized from ethanol to give title compounds.

2.2a *N'*-Ethylidene-1-carbohydrazido-2-methyl-1*H*-benzimidazole (4a): IR (KBr): 1624 (–C=N), 1588 (–N=CH), 1680 (–C=O amido), 3300 (–C–NH) cm^{-1} ; ¹H-NMR (DMSO-*d*₆, δ ppm): 1.34 (*d*, 3H,

–CH₂CH₃), 2.48 (*s*, 3H, –CH₃ at C-2 of benzimidazole), 3.95 (*q*, 1H, –CH₂CH₃), 7.94–7.75 (*m*, 4H, aromatic protons); EI–MS: 217 ($M^+ + 1$).

2.2b 2-Methyl-*N'*-propylidene-1-carbohydrazido-1*H*-benzimidazole (4b): IR (KBr): 1657 (–C=N), 1605 (–N=CH), 1681 (–C=O amido), 3336 (–C–NH) cm^{-1} ; ¹H-NMR (DMSO-*d*₆, δ ppm): 1.00 (*t*, 3H, –CH₂CH₃), 1.48 (*p*, 2H, –CH₂CH₃), 2.35 (*s*, 3H, –CH₃ at C-2 of benzimidazole), 7.77–7.56 (*m*, 4H, aromatic protons), 8.49 (*t*, 1H, –N=CH); EI–MS: 231 ($M^+ + 1$).

2.2c *N'*-Butylidene-1-carbohydrazido-2-methyl-1*H*-benzimidazole (4c): IR (KBr): 1610 (–C=N), 1590 (–N=CH), 1677 (–C=O amido), 3323 (–C–NH) cm^{-1} ; ¹H-NMR (DMSO-*d*₆, δ ppm): 0.99 (*t*, 3H, –CH₂CH₂CH₃), 1.39 (*m*, 2H, –CH₂CH₂CH₃), 1.54 (*m*, 2H, –CH₂CH₂CH₃), 2.46 (*s*, 3H, –CH₃ at C-2 of benzimidazole), 3.96 (*m*, 1H, –CH₂CH₂CH₃), 7.82–7.60 (*m*, 4H, aromatic protons); EI–MS: 245 ($M^+ + 1$).

2.2d 2-Methyl-*N'*-(phenylmethylidene)-1-carbohydrazido-1*H*-benzimidazole (**4d**): IR (KBr): 1610 (–C=N), 1590 (–N=CH), 1677 (–C=O amido), 3323 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.39 (s, 3H, –CH₃ at C-2 of benzimidazole), 7.96–7.70 (m, 9H, aromatic protons) 8.51 (s, 1H, –N=CH); EI-MS: 279 ($\text{M}^+ + 1$).

2.2e 2-Methyl-*N'*-[(2-methylphenyl)methylidene]-1-carbohydrazido-1*H*-benzimidazole (**4e**): IR (KBr): 1617 (–C=N), 1594 (–N=CH), 1680 (–C=O amido), 3310 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.29 (s, 3H, –CH₃ at C-2 of benzimidazole), 2.46 (s, 3H, –CH₃ at benzene ring), 7.87–7.61 (m, 8H, aromatic protons), 8.42 (s, 1H, –N=CH); EI-MS: 293 ($\text{M}^+ + 1$).

2.2f 2-Methyl-*N'*-[(3-methylphenyl)methylidene]-1-carbohydrazido-1*H*-benzimidazole (**4f**): IR (KBr): 1612 (–C=N), 1580 (–N=CH), 1689 (–C=O amido), 3313 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.12 (s, 3H, –CH₃ at benzene ring), 2.32 (s, 3H, –CH₃ at C-2 of benzimidazole), 7.79–7.51 (m, 8H, aromatic protons), 8.71 (s, 1H, –N=CH); EI-MS: 293 ($\text{M}^+ + 1$).

2.2g *N'*-[(2-Chlorophenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4g**): IR (KBr): 1624 (–C=N), 1587 (–N=CH), 1682 (–C=O amido), 3315 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.40 (s, 3H, –CH₃ at C-2 of benzimidazole), 7.84–7.50 (m, 8H, aromatic protons), 8.60 (s, 1H, –N=CH); EI-MS: 313 ($\text{M}^+ + 1$).

2.2h *N'*-[(4-Chlorophenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4h**): IR (KBr): 1623 (–C=N), 1595 (–N=CH), 1698 (–C=O amido), 3324 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.38 (s, 3H, –CH₃ at C-2 of benzimidazole), 7.98–7.72 (m, 8H, aromatic protons), 8.45 (s, 1H, –N=CH); EI-MS: 313 ($\text{M}^+ + 1$).

2.2i *N'*-[(2-Hydroxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4i**): IR (KBr): 1627 (–C=N), 1574 (–N=CH), 1679 (–C=O amido), 3328 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, δ ppm): 2.42 (s, 3H, –CH₃ at C-2 of benzimidazole), 5.82 (s, 1H, Ar-OH), 7.73–7.59 (m, 8H, aromatic protons), 8.52 (s, 1H, –N=CH); EI-MS: 295 ($\text{M}^+ + 1$).

2.2j *N'*-[(3-Hydroxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4j**): IR (KBr): 1628 (–C=N), 1580 (–N=CH), 1677 (–C=O amido), 3323 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, δ ppm): 2.48 (s, 3H, –CH₃ at C-2 of benzimidazole), 5.55 (s, 1H, Ar-OH), 7.80–7.62 (m, 8H, aromatic protons), 8.49 (s, 1H, –N=CH); EI-MS: 295 ($\text{M}^+ + 1$).

2.2k *N'*-[(4-Hydroxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4k**): IR (KBr): 1625 (–C=N), 1576 (–N=CH), 1682 (–C=O amido), 3327 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ ($\text{DMSO-}d_6$, δ ppm): 2.40 (s, 3H, –CH₃ at C-2 of benzimidazole), 5.75 (s, 1H, Ar-OH), 7.83–7.62 (m, 8H, aromatic protons), 8.42 (s, 1H, –N=CH); EI-MS: 295 ($\text{M}^+ + 1$).

2.2l *N'*-[(2-Methoxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4l**): IR (KBr): 1618 (–C=N), 1592 (–N=CH), 1673 (–C=O amido), 3320 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.39 (s, 3H, –CH₃ at C-2 of benzimidazole), 3.75 (s, 3H, –OCH₃), 7.97–7.70 (m, 8H, aromatic protons), 8.60 (s, 1H, –N=CH); EI-MS: 309 ($\text{M}^+ + 1$).

2.2m *N'*-[(4-Methoxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1*H*-benzimidazole (**4m**): IR (KBr): 1617 (–C=N), 1595 (–N=CH), 1680 (–C=O amido), 3322 (–C–NH) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , δ ppm): 2.35 (s, 3H, –CH₃ at C-2 of benzimidazole), 3.64 (s, 3H, –OCH₃), 7.90–7.65 (m, 8H, aromatic protons), 8.57 (s, 1H, –N=CH); EI-MS: 309 ($\text{M}^+ + 1$).

2.3 *N*-[3-Chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1*H*-benzimidazoles (**5a–5m**)

General procedure: To a stirred solution of compound (**4a–4m**) (20 mmol) and triethylamine (10 mmol) in dry dioxane (50 ml) was added chloroacetyl chloride (10 mmol) dropwise at room temperature. The reaction mixture was stirred for 1h and then refluxed for 5–10 h. The solid obtained after removal of dioxane was crystallized from aq. ethanol to give the title compounds.

2.3a *N*-(3-Chloro-2-methyl-4-oxoazetidin-1-yl)-1-carboxamido-2-methyl-1*H*-benzimidazole (**5a**): IR (KBr): 1618 (–C=N), 1670 (–C=O amido), 1726 (>C=O monocyclic β -lactam), 3318 (–C–NH), 772

(-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , δ ppm): 1.30 (*d*, 3H, -CH₃ of side chain at β -lactam), 2.45 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 4.11 (*p*, 1H, -CHCH₃, $J = 7.01$, 7.01, 7.03 and 7.05 Hz), 5.44 (*d*, 1H, -CHCl, $J = 6.96$ Hz), 7.82–7.71 (*m*, 4H, aromatic protons); EI-MS: 293 ($M^+ + 1$).

2.3b *N*-(3-Chloro-2-ethyl-4-oxoazetidin-1-yl)-1-carboxamido-2-methyl-1H-benzimidazole (**5b**): IR (KBr): 1620 (-C=N), 1676 (-C=O amido), 1720 (>C=O monocyclic β -lactam), 3319 (-C-NH), 763 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , δ ppm): δ 1.00 (*t*, 3H, -CH₂CH₃), 1.45 (*p*, 2H, -CH₂CH₃), 2.43 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 3.98 (*q*, 1H, -CH of β -lactam, $J = 7.16$, 7.16 and 7.20 Hz), 5.41 (*d*, 1H, -CHCl, $J = 6.96$ Hz), 7.80–7.62 (*m*, 4H, aromatic protons); EI-MS: 307 ($M^+ + 1$).

2.3c *N*-(3-Chloro-2-oxo-4-propylazetidin-1-yl)-1-carboxamido-2-methyl-1H-benzimidazole (**5c**): IR (KBr): 1616 (-C=N), 1673 (-C=O amido), 1723 (>C=O monocyclic β -lactam), 3317 (-C-NH), 780 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , δ ppm): 0.98 (*t*, 3H, -CH₂CH₂CH₃), 1.38 (*m*, 2H, -CH₂CH₂CH₃), 1.56 (*q*, 2H, CH₂CH₂CH₃), 2.49 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 3.90 (*q*, 1H, -CH of β -lactam, $J = 7.12$, 7.12 and 7.15 Hz), 5.48 (*d*, 1H, -CHCl, $J = 7.00$ Hz), 7.76–7.61 (*m*, 4H, aromatic protons); EI-MS: 321 ($M^+ + 1$).

2.3d *N*-(3-Chloro-2-oxo-4-phenylazetidin-1-yl)-1-carboxamido-2-methyl-1H-benzimidazole (**5d**): IR (KBr): 1625 (-C=N), 1687 (-C=O amido), 1730 (>C=O monocyclic β -lactam), 3324 (-C-NH), 772 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): 2.37 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 5.02 (*d*, 1H, -CH of β -lactam, $J = 8.6$ Hz), 5.25 (*d*, 1H, -CHCl, $J = 7.9$ Hz), 7.94–7.65 (*m*, 9H, aromatic protons); EI-MS: 355 ($M^+ + 1$).

2.3e *N*-[3-chloro-2-(2-methylphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazole (**5e**): IR (KBr): 1630 (-C=N), 1680 (-C=O amido), 1716 (>C=O monocyclic β -lactam), 3340 (-C-NH), 760 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): δ 2.34 (*s*, 3H, -CH₃ at benzene), 2.40 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 5.05 (*d*, 1H, -CH of β -lactam, $J = 8.7$ Hz), 5.45 (*d*, 1H, -CHCl, $J = 7.9$ Hz), 7.90–7.74 (*m*, 8H, aromatic protons); EI-MS: 369 ($M^+ + 1$).

2.3f *N*-[3-Chloro-2-(3-methylphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazole (**5f**): IR (KBr): 1611 (-C=N), 1682 (-C=O amido), 1727 (>C=O monocyclic β -lactam), 3318 (-C-NH), 758 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): 2.38 (*s*, 3H, -CH₃ at benzene), 2.44 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 4.99 (*d*, 1H, -CH of β -lactam, $J = 8.4$ Hz), 5.42 (*d*, 1H, -CHCl, $J = 7.2$ Hz), 7.87–7.61 (*m*, 8H, aromatic protons); EI-MS: 369 ($M^+ + 1$).

2.3g *N*-[3-Chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazole (**5g**): IR (KBr): 1634 (-C=N), 1690 (-C=O amido), 1733 (>C=O monocyclic β -lactam), 3327 (-C-NH), 765 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , δ ppm): 2.42 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 5.02 (*d*, 1H, -CH of β -lactam, $J = 8.7$ Hz), 5.44 (*d*, 1H, -CHCl, $J = 7.7$ Hz), 7.69–7.41 (*m*, 8H, aromatic protons); EI-MS: 389 ($M^+ + 1$).

2.3h *N*-[3-Chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazole (**5h**): IR (KBr): 1617 (-C=N), 1679 (-C=O amido), 1729 (>C=O monocyclic β -lactam), 3312 (-C-NH), 769 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): δ 2.39 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 5.02 (*d*, 1H, -CH of β -lactam, $J = 8.6$ Hz), 5.40 (*d*, 1H, -CHCl, $J = 7.5$ Hz), 7.79–7.54 (*m*, 8H, aromatic protons); EI-MS: 389 ($M^+ + 1$).

2.3i *N*-[3-Chloro-2-(2-hydroxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazole (**5i**): IR (KBr): 1613 (-C=N), 1677 (-C=O amido), 1728 (>C=O monocyclic β -lactam), 3313 (-C-NH), 770 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): 2.46 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 4.97 (*d*, 1H, -CH of β -lactam, $J = 8.0$ Hz), 5.42 (*d*, 1H, -CHCl, $J = 6.8$ Hz), 5.58 (*s*, 1H, Ar-OH), 7.84–7.61 (*m*, 8H, aromatic protons); EI-MS: 371 ($M^+ + 1$).

2.3j *N*-[3-Chloro-2-(3-hydroxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-benzimidazole (**5j**): IR (KBr): 1618 (-C=N), 1668 (-C=O amido), 1729 (>C=O monocyclic β -lactam), 3318 (-C-NH), 768 (-C-Cl) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , δ ppm): 2.44 (*s*, 3H, -CH₃ at C-2 of benzimidazole), 4.88 (*d*, 1H, -CH of β -lactam, $J = 7.9$ Hz), 5.39 (*d*, 1H, -CHCl, $J = 7.1$ Hz), 5.73 (*s*, 1H, Ar-OH), 7.87–7.62 (*m*, 8H, aromatic protons); EI-MS: 371 ($M^+ + 1$).

2.3k *N*-[3-Chloro-2-(4-hydroxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1*H*-benzimidazole (**5k**): IR (KBr): 1617 (–C=N), 1674 (–C=O amido), 1730 (>C=O monocyclic β -lactam), 3323 (–C–NH), 761 (–C–Cl) cm^{-1} ; $^1\text{H-NMR}$ (DMSO- d_6 , δ ppm): 2.49 (s, 3H, –CH₃ at C-2 of benzimidazole), 4.96 (d, 1H, –CH of β -lactam, $J = 8.2$ Hz), 5.45 (d, 1H, –CHCl, $J = 7.1$ Hz), 5.65 (s, 1H, Ar–OH), 7.82–7.61 (m, 8H, aromatic protons); EI–MS: 371 ($\text{M}^+ + 1$).

2.3l *N*-[3-Chloro-2-(2-methoxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1*H*-benzimidazole (**5l**): IR (KBr): 1624 (–C=N), 1687 (–C=O amido), 1734 (>C=O monocyclic β -lactam), 3299 (–C–NH), 759 (–C–Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): 2.41 (s, 3H, –CH₃ at C-2 of benzimidazole), 3.73 (s, 3H, –OCH₃), 4.96 (d, 1H, –CH of β -lactam, $J = 8.0$ Hz), 5.42 (d, 1H, –CHCl, $J = 6.95$ Hz), 7.71–7.52 (m, 8H, aromatic protons); EI–MS: 385 ($\text{M}^+ + 1$).

2.3m *N*-[3-Chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1*H*-benzimidazole (**5m**): IR (KBr): 1629 (–C=N), 1693 (–C=O amido), 1731 (>C=O monocyclic β -lactam), 3305 (–C–NH), 763 (–C–Cl) cm^{-1} ; $^1\text{H-NMR}$ (CDCl₃, δ ppm): 2.45 (s, 3H, –CH₃ at C-2 of benzimidazole), 3.78 (s, 3H, –OCH₃), 4.92 (d, 1H, –CH of β -lactam, $J = 8.0$ Hz), 5.46 (d, 1H, –CHCl, $J = 6.8$ Hz), 7.75–7.58 (m, 8H, aromatic protons); EI–MS: 385 ($\text{M}^+ + 1$).

2.4 Antimicrobial activity test

The compounds (**5a–5m**) were tested for their *in vitro* growth inhibitory activity against different microbes. The bacterial strains used were *Staphylococcus aureus* ATCC 29213, *Streptococcus mutans* MTCC 890 and *Bacillus subtilis* MTCC 741 (all Gram positive) and *Escherichia coli* ATCC 25922, *Salmonella typhi* MTCC 733 and *Pseudomonas aeruginosa* MTCC 741 (all Gram negative). For testing the antifungal activity of the synthesized compounds the fungal strains *Candida albicans* MTCC 1637, *Aspergillus flavus* AIIMS and *Aspergillus niger* AIIMS were used.

The inhibition zones of synthesized compounds were determined using disc diffusion method.³¹ In this method, paper disks (6 mm) containing specific amounts of an antimicrobial agent (300 μg for the synthesized compounds) were placed on the surface of an agar plate inoculated with a standardized sus-

pension of the microorganisms tested. The plates were incubated at 35°C for 24 and 48 h, respectively for bacteria and fungi. Ampicillin (10 μg) for Gram positive bacteria, Nalidixic acid (30 μg) for Gram negative bacteria and Amphotericin B (30 μg) for fungi, were used as standard drugs. Paper disks with only DMSO were utilized as negative control. All experiments were carried out three times. The inhibition zones produced by the various synthesized compounds on the microbial growth were measured (diameter in mm). Physical and analytical data of the synthesized compounds are reported in table 1. The data on antimicrobial activity of compounds (**5a–5m**) are shown in tables 2 and 3.

3. Results and discussion

3.1 Chemistry

The reaction sequenced for different title compounds is outlined in scheme 1. 2-Methyl-1*H*-benzimidazole **1** and ethyl 2-methyl-1*H*-benzimidazole-1-carboxylate **2** were prepared according to the literature procedure.³² Compound **2** on treatment with hydrazine hydrate in ethanol yielded 2-methyl-1*H*-benzimidazole-1-carbohydrazide **3**. Compound **3** on condensation with various aldehydes furnished 2-methyl-*N'*-[(substituted) alkyl/aryl methylidene]-1-carbohydrazido-1*H*-benzimidazoles (**4a–4m**). The four-membered β -lactam ring was introduced in compounds (**4a–4m**) at the azomethine group by the cycloaddition of chloroacetyl chloride in the presence of triethylamine, according to literature,⁷ to yield *N*-[3-chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1*H*-benzimidazoles (**5a–m**). The purity of the compounds was monitored by TLC. The structural evaluation of the newly synthesized derivatives and of the intermediates synthesized in this study were performed using IR, $^1\text{H-NMR}$ and mass spectroscopic techniques which are in agreement with the proposed structures.

3.2 Antimicrobial evaluation

The *in vitro* antimicrobial activity was performed using the disc diffusion method with different strains of bacteria and fungi. Ampicillin and nalidixic acid were used as positive control for bacteria and amphotericin B was used against fungi.

Table 1. Physical and analytical data of the compounds **2**, **3**, (**4a–4m**) and (**5a–5m**).

Compds	R	Yield (%)	m.p. (°C)	Mol-formula (formula wt.)	Analysis % found (calculated)		
					C	H	N
2	–	79	180–82	C ₁₁ H ₁₂ N ₂ O ₂ (204.23)	64.67 (64.69)	5.90 (5.92)	13.70 (13.72)
3	–	78	173–75	C ₉ H ₁₀ N ₄ O (190.20)	56.80 (56.83)	5.27 (5.30)	29.42 (29.46)
4a	–CH ₃	82	185–87	C ₁₁ H ₁₂ N ₄ O (216.24)	61.07 (61.10)	5.57 (5.59)	25.88 (25.91)
4b	–CH ₂ CH ₃	80	184–86	C ₁₂ H ₁₄ N ₄ O (230.27)	62.56 (62.59)	6.11 (6.13)	24.30 (24.33)
4c	–CH ₂ CH ₂ CH ₃	76	190–92	C ₁₃ H ₁₆ N ₄ O (244.29)	63.88 (63.91)	6.57 (6.60)	22.90 (22.93)
4d	–C ₆ H ₅	80	180–82	C ₁₆ H ₁₄ N ₄ O (278.31)	69.03 (69.05)	5.04 (5.07)	20.11 (20.13)
4e	2–CH ₃ C ₆ H ₄	83	183–85	C ₁₇ H ₁₄ N ₄ O (292.34)	69.81 (69.85)	5.50 (5.52)	19.15 (19.17)
4f	3–CH ₃ C ₆ H ₄	75	175–77	C ₁₄ H ₁₆ ClN ₄ O (292.34)	69.82 (69.85)	5.48 (5.52)	19.15 (19.17)
4g	2–ClC ₆ H ₄	71	169–71	C ₁₆ H ₁₃ ClN ₄ O (312.75)	61.41 (61.44)	4.17 (4.19)	17.89 (17.91)
4h	4–ClC ₆ H ₄	69	168–70	C ₁₆ H ₁₃ ClN ₄ O (312.75)	61.43 (61.44)	4.17 (4.19)	17.89 (17.91)
4i	2–OHC ₆ H ₄	85	181–83	C ₁₆ H ₁₄ N ₄ O ₂ (294.31)	65.28 (65.30)	4.75 (4.79)	19.01 (19.04)
4j	3–OHC ₆ H ₄	82	175–77	C ₁₆ H ₁₄ N ₄ O ₂ (294.31)	65.27 (65.30)	4.74 (4.79)	19.02 (19.04)
4k	4–OHC ₆ H ₄	81	166–68	C ₁₆ H ₁₄ N ₄ O ₂ (294.31)	65.27 (65.30)	4.74 (4.79)	19.01 (19.04)
4l	2–OCH ₃ C ₆ H ₄	74	160–62	C ₁₇ H ₁₆ N ₄ O ₂ (308.33)	66.20 (66.22)	5.21 (5.23)	18.16 (18.17)
4m	4–OCH ₃ C ₆ H ₄	75	155–57	C ₁₇ H ₁₆ N ₄ O ₂ (308.33)	66.19 (66.22)	5.20 (5.23)	18.14 (18.17)
5a	–CH ₃	86	188–90	C ₁₃ H ₁₃ ClN ₄ O ₂ (292.72)	53.31 (53.34)	4.46 (4.48)	19.11 (19.14)
5b	–CH ₂ CH ₃	80	185–87	C ₁₄ H ₁₅ ClN ₄ O ₂ (306.75)	54.79 (54.82)	4.92 (4.93)	18.24 (18.26)
5c	–CH ₂ CH ₂ CH ₃	82	193–95	C ₁₅ H ₁₇ ClN ₄ O ₂ (320.77)	56.12 (56.16)	5.31 (5.34)	17.44 (17.47)
5d	–C ₆ H ₅	86	150–52	C ₁₈ H ₁₅ ClN ₄ O ₂ (354.79)	60.92 (60.94)	4.24 (4.26)	15.76 (15.79)
5e	2–CH ₃ C ₆ H ₄	64	147–49	C ₁₉ H ₁₇ ClN ₄ O ₂ (368.82)	61.84 (61.87)	4.63 (4.65)	15.17 (15.19)
5f	3–CH ₃ C ₆ H ₄	79	149–51	C ₁₉ H ₁₇ ClN ₄ O ₂ (368.82)	61.85 (61.87)	4.62 (4.65)	15.16 (15.19)
5g	2–ClC ₆ H ₄	75	141–43	C ₁₈ H ₁₄ Cl ₂ N ₄ O ₂ (389.24)	55.51 (55.54)	3.61 (3.63)	14.35 (14.39)
5h	4–ClC ₆ H ₄	84	160–62	C ₁₈ H ₁₄ Cl ₂ N ₄ O ₂ (389.24)	55.52 (55.54)	3.60 (3.63)	14.37 (14.39)
5i	2–OHC ₆ H ₄	85	120–22	C ₁₈ H ₁₅ ClN ₄ O ₃ (370.79)	58.29 (58.31)	4.07 (4.08)	15.09 (15.11)
5j	3–OHC ₆ H ₄	87	110–112	C ₁₈ H ₁₅ ClN ₄ O ₃ (370.79)	58.28 (58.31)	4.05 (4.08)	15.07 (15.11)
5k	4–OHC ₆ H ₄	84	126–28	C ₁₈ H ₁₅ ClN ₄ O ₃ (370.79)	58.26 (58.31)	4.05 (4.08)	15.08 (15.11)
5l	2–OCH ₃ C ₆ H ₄	81	135–37	C ₁₉ H ₁₇ ClN ₄ O ₃ (384.82)	59.27 (59.30)	4.43 (4.45)	14.53 (14.56)
5m	4–OCH ₃ C ₆ H ₄	84	151–53	C ₁₉ H ₁₇ ClN ₄ O ₃ (384.82)	59.27 (59.30)	4.41 (4.45)	14.53 (14.56)

Table 2. Antibacterial activity of compounds (5a–5m).

Compounds	Mean zone inhibition (in mm) ^a					
	Gram-positive bacteria			Gram-negative bacteria		
	<i>S. aureus</i>	<i>S. mutans</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>
5a	38	20	28	18	18	14
5b	37	18	28	–	16	–
5c	32	18	22	–	–	12
5d	36	16	27	–	13	12
5e	30	15	20	–	–	–
5f	30	13	20	–	–	–
5g	31	13	18	–	–	–
5h	31	10	20	–	–	–
5i	37	16	27	–	10	10
5j	36	16	26	–	10	–
5k	36	15	26	–	–	–
5l	25	14	22	–	–	–
5m	22	16	18	–	–	–
Ampicillin ^b	38	22	28	20	–	–
Nalidixic acid ^b	–	–	–	28	20	18

^aValues are mean ($n = 3$)^bAmpicillin (10 $\mu\text{g}/\text{disc}$) and Nalidixic acid (30 $\mu\text{g}/\text{disc}$) used as positive reference; synthesized compounds (300 $\mu\text{g}/\text{disc}$)

‘–’ indicates no sensitivity or mean inhibition zone diameter lower than 7 mm

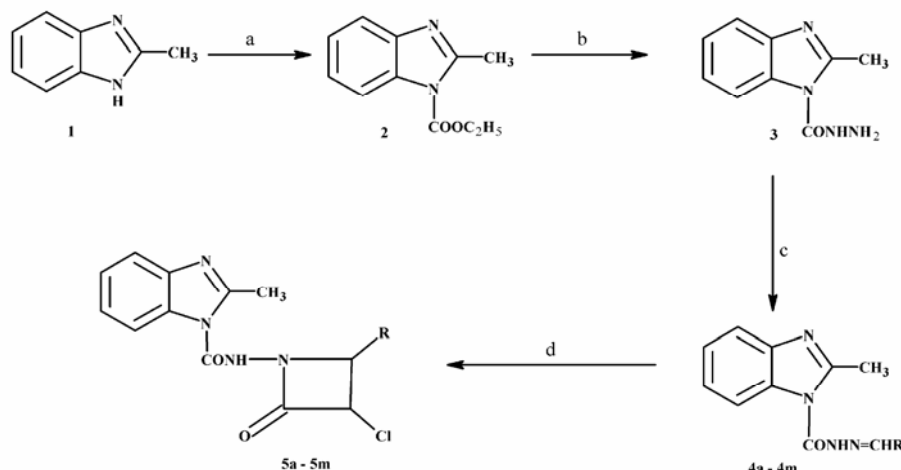
Table 3. Antifungal activity of compounds (5a–5m).

Compounds	Mean zone inhibition (in mm) ^a		
	<i>Candida albicans</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
5a	26	24	22
5b	24	25	24
5c	24	24	20
5d	27	26	27
5e	22	18	14
5f	20	18	13
5g	20	16	15
5h	18	14	14
5i	27	28	26
5j	15	20	13
5k	15	14	18
5l	16	16	16
5m	18	19	16
^b Amphotericin B	28	>28	28

^aValues are mean ($n = 3$)^bAmphotericin B (30 $\mu\text{g}/\text{disc}$) used as positive reference; synthesized compounds (300 $\mu\text{g}/\text{disc}$)

The results of the final compounds for preliminary antibacterial testing are shown in table 2. The results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms. In general, the

inhibitory activity against the Gram-positive bacteria was higher than that of the Gram-negative bacteria. The alkyl, phenyl and hydroxyphenyl substitutions at the 4-position of azetidin-2-one subunit has the best overall antibacterial profile. The methyl, chloro



Scheme 1. (Reagents: (a) $\text{ClCOOC}_2\text{H}_5/\text{K}_2\text{CO}_3$, acetone; (b) $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ /ethanol; (c) corresponding aldehyde/ethanol (d) $\text{ClCOCH}_2\text{Cl}/\text{Et}_3\text{N}$).

and methoxy substituents on phenyl ring at azetidin-2-one moiety of final compounds displayed least activity.

As can be seen in table 3, although all the compounds are not as active as standard Amphotericin B, compounds **5d** and **5i** were found to be more active against *Candida albicans* and *Aspergillus flavus*. Again in antifungal activity compounds **5e**, **5f**, **5g**, **5h**, **5l** and **5m** showed less or negligible activity than the other derivatives of the same series. Although the rest of the compounds showed varying degree of inhibition, none were as effective as Amphotericin B.

4. Conclusions

We have synthesized series of *N*-[3-chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1*H*-benzimidazoles. Among the synthesized benzimidazoles, compounds with alkyl, phenyl and hydroxyphenyl at 4-position of azetidin-2-one were found to increase the antibacterial activity. Compounds with phenyl and hydroxyphenyl substituents at azetidin-2-one sub-unit showed good antibacterial and antifungal activities. More extensive study is needed to confirm the preliminary results and mode of action studies are required to be able to optimize the effectiveness of this series of compounds.

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