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

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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] antihelmintic,[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) moiety[23] 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.

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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 ^{1}H-NMR and ^{13}C-NMR spectra were measured in $CDCl_3$ solutions on a Brucker 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-1H-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 (–$NHNH_2$) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm): 2·58 (*s*, 3H,

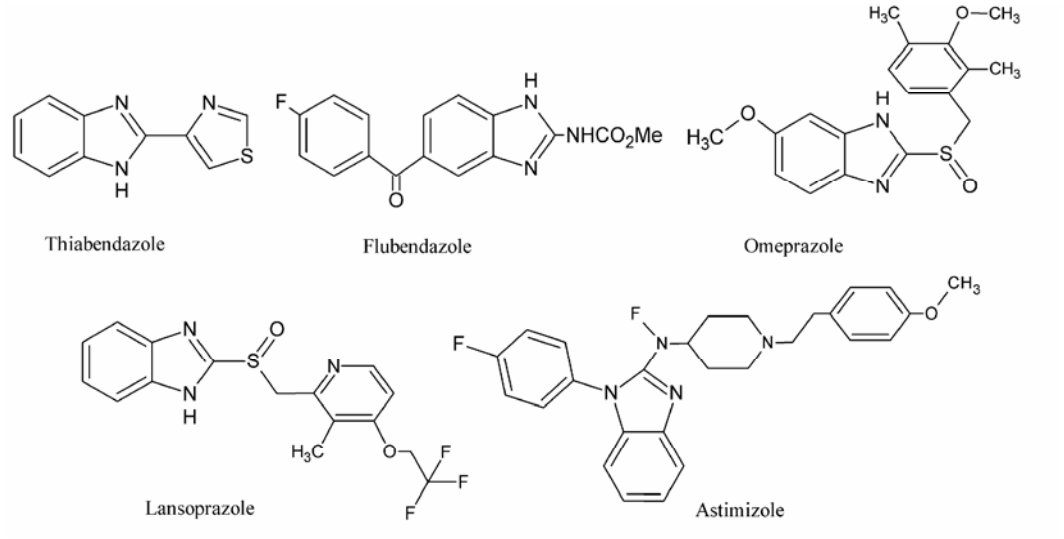


Figure 1. Clinically used benzimidazoles.

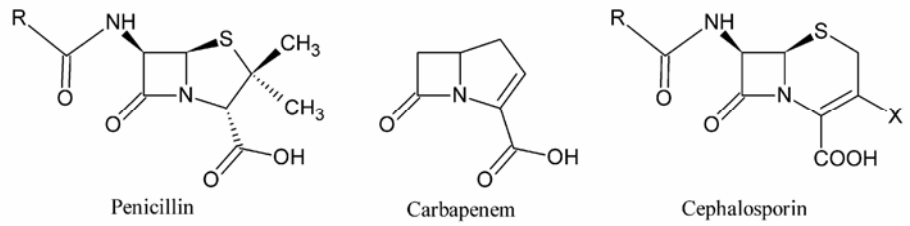


Figure 2. Antibiotics containing β-lactam ring.

$-CH_3$ at C-2 of benzimidazole), 2·60 (*s*, 2H, $-NH_2$), 7·79–7·92 (*m*, 4H, aromatic protons); EI–MS: 191 ($M^+ + 1$).

2.2 *2-Methyl-N′-[(substituted) alkyl/aryl methylidene]-1-carbohydrazido-1H-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-1H-benzimidazole* **(4a)**: IR (KBr): 1624 (–C=N), 1588 (–N=CH), 1680 (–C=O amido), 3300 (–C–NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm): 1·34 (*d*, 3H, $-CH\underline{CH_3}$), 2·48 (*s*, 3H, $-CH_3$ at C-2 of benzimidazole), 3·95 (*q*, 1H, $-\underline{CH}CH_3$), 7·94–7·75 (*m*, 4H, aromatic protons); EI–MS: 217 ($M^+ + 1$).

2.2b *2-Methyl-N′-propylidene-1-carbohydrazido-1H-benzimidazole* **(4b)**: IR (KBr): 1657 (–C=N), 1605 (–N=CH), 1681 (–C=O amido), 3336 (–C-NH) cm^{-1}; ^{1}H- NMR (DMSO-d_6, δ ppm): 1·00 (*t*, 3H, $-CH_2\underline{CH_3}$), 1·48 (*p*, 2H, $-\underline{CH_2}CH_3$), 2·35 (*s*, 3H, $-CH_3$ 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-1H-benzimidazole* **(4c)**: IR (KBr): 1610 (–C=N), 1590 (–N=CH), 1677 (–C=O amido), 3323 (–C–NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm): 0·99 (*t*, 3H, $-CHCH_2CH_2\underline{CH_3}$), 1·39 (*m*, 2H, $-CHCH_2\underline{CH_2}CH_3$), 1·54 (*m*, 2H, $-CH\underline{CH_2}CH_2CH_3$), 2·46 (*s*, 3H, $-CH_3$ at C-2 of benzimidazole), 3·96 (*m*, 1H, $-\underline{CH}CH_2CH_2CH_3$), 7·82–7·60 (*m*, 4H, aromatic protons); EI–MS: 245 ($M^+ + 1$).

2.2d *2-Methyl-N′-(phenylmethylidene)-1-carbohydrazido-1H-benzimidazole* (**4d**): IR (KBr): 1610 (–C=N), 1590 (–N=CH), 1677 (–C=O amido), 3323 (–C–NH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ ppm): 2·39 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 7·96–7·70 (*m*, 9H, aromatic protons) 8·51 (*s*, 1H, –N=CH); EI–MS: 279 ($M^+ + 1$).

2.2e *2-Methyl-N′-[(2-methylphenyl)methylidene]-1-carbohydrazido-1H-benzimidazole* (**4e**): IR (KBr): 1617 (–C=N), 1594 (–N=CH), 1680 (–C=O amido), 3310 (–C–NH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ ppm): 2·29 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 2·46 (*s*, 3H, $–CH_3$ at benzene ring), 7·87–7·61 (*m*, 8H, aromatic protons), 8·42 (*s*, 1H, –N=CH); EI–MS: 293 ($M^+ + 1$).

2.2f *2-Methyl-N′-[(3-methylphenyl)methylidene]-1-carbohydrazido-1H-benzimidazole* (**4f**): IR (KBr): 1612 (–C=N), 1580 (–N=CH), 1689 (–C=O amido), 3313 (–C–NH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ ppm): 2·12 (*s*, 3H, $–CH_3$ at benzene ring), 2·32 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 7·79–7·51 (*m*, 8H, aromatic protons), 8·71 (*s*, 1H, –N=CH); EI–MS: 293 ($M^+ + 1$).

2.2g *N′-[(2-Chlorophenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4g**): IR (KBr): 1624 (–C=N), 1587 (–N=CH), 1682 (–C=O amido), 3315 (–C–NH) cm^{-1}; ^{1}H- NMR ($CDCl_3$, δ ppm): 2·40 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 7·84–7·50 (*m*, 8H, aromatic protons), 8·60 (*s*, 1H, –N=CH); EI–MS: 313 ($M^+ + 1$).

2.2h *N′-[(4-Chlorophenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4h**): IR (KBr): 1623 (–C=N), 1595 (–N=CH), 1698 (–C=O amido), 3324 (–C–NH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ ppm): 2·38 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 7·98–7·72 (*m*, 8H, aromatic protons), 8·45 (*s*, 1H, –N=CH); EI–MS: 313 ($M^+ + 1$).

2.2i *N′-[(2-Hydroxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4i**): IR (KBr): 1627 (–C=N), 1574 (–N=CH), 1679 (–C=O amido), 3328 (–C–NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm): 2·42 (*s*, 3H, $–CH_3$ 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 ($M^+ + 1$).

2.2j *N′-[(3-Hydroxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4j**): IR (KBr): 1628 (–C=N), 1580 (–N=CH), 1677 (–C=O amido), 3323 (–C–NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm): 2·48 (*s*, 3H, $–CH_3$ 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 ($M^+ + 1$).

2.2k *N′-[(4-Hydroxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4k**): IR (KBr): 1625 (–C=N), 1576 (–N=CH), 1682 (–C=O amido), 3327 (–C-NH) cm^{-1}; ^{1}H-NMR (DMSO-d_6, δ ppm): 2·40 (*s*, 3H, $–CH_3$ 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 ($M^+ + 1$).

2.2l *N′-[(2-Methoxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4l**): IR (KBr): 1618 (–C=N), 1592 (–N=CH), 1673 (–C=O amido), 3320 (–C–NH) cm^{-1}; ^{1}H-NMR ($CDCl_3$, δ ppm): 2·39 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 3·75 (*s*, 3H, $–OCH_3$), 7·97–7·70 (*m*, 8H, aromatic protons), 8·60 (*s*, 1H, –N=CH); EI–MS: 309 ($M^+ + 1$).

2.2m *N′-[(4-Methoxyphenyl)methylidene]-1-carbohydrazido-2-methyl-1H-benzimidazole* (**4m**): IR (KBr): 1617 (–C=N), 1595 (–N=CH), 1680 (–C=O amido), 3322 (–C–NH) cm^{-1}; ^{1}H- NMR ($CDCl_3$, δ ppm): 2·35 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 3·64 (*s*, 3H, $–OCH_3$), 7·90–7·65 (*m*, 8H, aromatic protons), 8·57 (*s*, 1H, –N=CH); EI–MS: 309 ($M^+ + 1$).

2.3 *N-[3-Chloro-2-(substituted) alkyl/aryl-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-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-1H-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}H-NMR (DMSO-d_6, δ ppm): 1·30 (*d*, 3H, $–CH_3$ of side chain at β-lactam), 2·45 (*s*, 3H, $–CH_3$ at C-2 of benzimidazole), 4·11 (*p*, 1H, $–\underline{CH}CH_3$, 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}H-NMR (DMSO-d_6, δ ppm): δ 1·00 (*t*, 3H, $–CH_2\underline{CH_3}$), 1·45 (*p*, 2H, $–\underline{CH_2}CH_3$), 2·43 (*s*, 3H, $–CH_3$ 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}H- NMR (DMSO-d_6, δ ppm): 0·98 (*t*, 3H, $–CH_2CH_2\underline{CH_3}$), 1·38 (*m*, 2H, $–CH_2\underline{CH_2}CH_3$), 1·56 (*q*, 2H, $\underline{CH_2}CH_2CH_3$), 2·49 (*s*, 3H, $–CH_3$ 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}H-NMR ($CDCl_3$, δ ppm): 2·37 (*s*, 3H, $–CH_3$ 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}H-NMR ($CDCl_3$, δ ppm): δ 2·34 (*s*, 3H, $–CH_3$ at benzene), 2·40 (*s*, 3H, $–CH_3$ 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}H-NMR ($CDCl_3$, δ ppm): 2·38 (*s*, 3H, $–CH_3$ at benzene), 2·44 (*s*, 3H, $–CH_3$ 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}H-NMR (DMSO-d_6, δ ppm: 2·42 (*s*, 3H, $–CH_3$ 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}H-NMR ($CDCl_3$, δ ppm): δ 2·39 (*s*, 3H, $–CH_3$ 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}H-NMR ($CDCl_3$, δ ppm): 2·46 (*s*, 3H, $–CH_3$ 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}H-NMR (DMSO-d_6, δ ppm): 2·44 (*s*, 3H, $–CH_3$ 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-1H-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}H-NMR (DMSO-d_6, δ ppm): 2·49 (*s*, 3H, $-CH_3$ 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 (M^+ + 1).

2.3l *N-[3-Chloro-2-(2-methoxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-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}H-NMR ($CDCl_3$, δ ppm): 2·41 (*s*, 3H, $-CH_3$ at C-2 of benzimidazole), 3·73 (*s*, 3H, $-OCH_3$), 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 (M^+ + 1).

2.3m *N-[3-Chloro-2-(4-methoxyphenyl)-4-oxoazetidin-1-yl]-1-carboxamido-2-methyl-1H-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}H-NMR ($CDCl_3$, δ ppm): 2·45 (*s*, 3H, $-CH_3$ at C-2 of benzimidazole), 3·78 (*s*, 3H, $-OCH_3$), 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 (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 *Ecsherichia 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 suspension 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}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	$C_{11}H_{12}N_2O_2$ (204·23)	64·67 (64·69	5·90 5·92	13·70 13·72)
3	–	78	173–75	$C_9H_{10}N_4O$ (190·20)	56·80 (56·83	5·27 5·30	29·42 29·46)
4a	$-CH_3$	82	185–87	$C_{11}H_{12}N_4O$ (216·24)	61·07 (61·10	5·57 5·59	25·88 25·91)
4b	$-CH_2CH_3$	80	184–86	$C_{12}H_{14}N_4O$ (230·27)	62·56 (62·59	6·11 6·13	24·30 24·33)
4c	$-CH_2CH_2CH_3$	76	190–92	$C_{13}H_{16}N_4O$ (244·29)	63·88 (63·91	6·57 6·60	22·90 22·93)
4d	$-C_6H_5$	80	180–82	$C_{16}H_{14}N_4O$ (278·31)	69·03 (69·05	5·04 5·07	20·11 20·13)
4e	2–$CH_3C_6H_4$	83	183–85	$C_{17}H_{14}N_4O$ (292·34)	69·81 (69·85	5·50 5·52	19·15 19·17)
4f	3–$CH_3C_6H_4$	75	175–77	$C_{14}H_{16}ClN_4O$ (292·34)	69·82 (69·85	5·48 5·52	19·15 19·17)
4g	2–ClC_6H_4	71	169–71	$C_{16}H_{13}ClN_4O$ (312·75)	61·41 (61·44	4·17 4·19	17·89 17·91)
4h	4–ClC_6H_4	69	168–70	$C_{16}H_{13}ClN_4O$ (312·75)	61·43 (61·44	4·17 4·19	17·89 17·91)
4i	2–OHC_6H_4	85	181–83	$C_{16}H_{14}N_4O_2$ (294·31)	65·28 (65·30	4·75 4·79	19·01 19·04)
4j	3–OHC_6H_4	82	175–77	$C_{16}H_{14}N_4O_2$ (294·31)	65·27 (65·30	4·74 4·79	19·02 19·04)
4k	4–OHC_6H_4	81	166–68	$C_{16}H_{14}N_4O_2$ (294·31)	65·27 (65·30	4·74 4·79	19·01 19·04)
4l	2–$OCH_3C_6H_4$	74	160–62	$C_{17}H_{16}N_4O_2$ (308·33)	66·20 (66·22	5·21 5·23	18·16 18·17)
4m	4–$OCH_3C_6H_4$	75	155–57	$C_{17}H_{16}N_4O_2$ (308·33)	66·19 (66·22	5·20 5·23	18·14 18·17)
5a	$-CH_3$	86	188–90	$C_{13}H_{13}ClN_4O_2$ (292·72)	53·31 (53·34	4·46 4·48	19·11 19·14)
5b	– CH_2CH_3	80	185–87	$C_{14}H_{15}ClN_4O_2$ (306·75)	54·79 (54·82	4·92 4·93	18·24 18·26)
5c	$-CH_2CH_2CH_3$	82	193–95	$C_{15}H_{17}ClN_4O_2$ (320·77)	56·12 (56·16	5·31 5·34	17·44 17·47)
5d	$-C_6H_5$	86	150–52	$C_{18}H_{15}ClN_4O_2$ (354·79)	60·92 (60·94	4·24 4·26	15·76 15·79)
5e	2–$CH_3C_6H_4$	64	147–49	$C_{19}H_{17}ClN_4O_2$ (368·82)	61·84 (61·87	4·63 4·65	15·17 15·19)
5f	3–$CH_3C_6H_4$	79	149–51	$C_{19}H_{17}ClN_4O_2$ (368·82)	61·85 (61·87	4·62 4·65	15·16 15·19)
5g	2–ClC_6H_4	75	141–43	$C_{18}H_{14}Cl_2N_4O_2$ (389·24)	55·51 (55·54	3·61 3·63	14·35 14·39)
5h	4–ClC_6H_4	84	160–62	$C_{18}H_{14}Cl_2N_4O_2$ (389·24)	55·52 (55·54	3·60 3·63	14·37 14·39)
5i	2–OHC_6H_4	85	120–22	$C_{18}H_{15}ClN_4O_3$ (370·79)	58·29 (58·31	4·07 4·08	15·09 15·11)
5j	3–OHC_6H_4	87	110–112	$C_{18}H_{15}ClN_4O_3$ (370·79)	58·28 (58·31	4·05 4·08	15·07 15·11)
5k	4–OHC_6H_4	84	126–28	$C_{18}H_{15}ClN_4O_3$ (370·79)	58·26 (58·31	4·05 4·08	15·08 15·11)
5l	2–$OCH_3C_6H_4$	81	135–37	$C_{19}H_{17}ClN_4O_3$ (384·82)	59·27 (59·30	4·43 4·45	14·53 14·56)
5m	4–$OCH_3C_6H_4$	84	151–53	$C_{19}H_{17}ClN_4O_3$ (384·82)	59·27 (59·30	4·41 4·45	14·53 14·56)

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

	Mean zone inhibition (in mm)[a]					
	Gram-positive bacteria			Gram-negative bacteria		
Compounds	*S. aureus*	*S. mutans*	*B. subtilis*	*E. coli*	*S. typhi*	*P. aeruginosa*
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

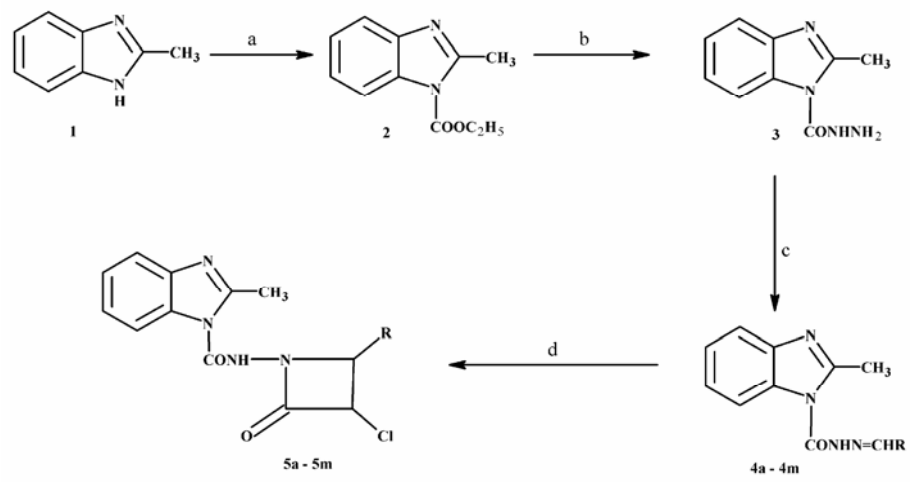
[a]Values are mean (n = 3)
[b]Ampicillin (10 μg/disc) and Nalidixic acid (30 μg/disc) used as positive reference; synthesized compounds (300 μg/disc)
'–' indicates no sensitivity or mean inhibition zone diameter lower than 7 mm

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

	Mean zone inhibition (in mm)[a]		
Compounds	*Candida albicans*	*Aspergillus niger*	*Aspergillus flavus*
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

[a]Values are mean (n = 3)
[b]Amphotericin B (30 μg/disc) used as positive reference; synthesized compounds (300 μg/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) $ClCOOC_2H_5/K_2CO_3$, acetone; (b) $NH_2NH_2{\cdot}H_2O$/ ethanol; (c) corresponding aldehyde/ethanol (d) $ClCOCH_2Cl/Et_3N$).

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.

References

1. (a) Wright J B 1951 *Chem. Rev.* **48** 397; (b) Preston P N 1974 *Chem. Rev.* **74** 279
2. Preston P N 1980 In *Benzimidazole and congeneric tricyclic compounds* (eds) A Weissberger and E C Taylor (New York: John Wiley and Sons), Part-2, pp. 63–147; (b) Grimmett M R 1984 In *Comprehensive heterocyclic chemistry* (ed.) K T Pots (Oxford: Pergamon) pp. 345–498; (c) Hoffman K 1953 In *Imidazole and its derivatives* (ed.) A Weissberger; *The chemistry of heterocyclic compounds* (New York: Interscience Publishers, Inc) pp. 247–317
3. Kus C, Ayhan-Kilcigil G, Can Eke B and Iscan M 2004 *Arch. Pharm. Res.* **27** 156
4. Ates-Alagoz Z, Kus C and Coban T 2005 *J. Enzyme Inhib. Med. Chem.* **20** 325
5. Goker H, Kus C, Boykin D W, Yildiz S and Altanlar N 2002 *Bioorg. Med. Chem.* **10** 2589
6. Goker H, Ozden S, Yildiz S and Boykin D W 2005 *Eur. J. Med. Chem.* **40** 1062
7. Desai K G and Desai K R 2006 *Bioorg. Med. Chem.* **14** 8271
8. Kazimierczuk Z, Upcroft J A, Upcroft P, Gorska A, Starosciak B and Laudy A 2002 *Acta Biochim. Polon.* **49** 185
9. Mohammad B G, Hussien M A, Abdel-Alim A A and Hashem M 2006 *Arch. Pharm. Res.* **29** 26
10. Pawar N S, Dalal D S, Shimpi S R and Mahulikar P P 2004 *Eur. J. Pharm. Sci.* **21** 115
11. Dubey R, Abuzar S, Sharma S, Chatterjee R K and Katiyar J C 1985 *J. Med. Chem.* **28** 1748
12. Mavrova A T, Denkova P S, Tsenov Y A, Anichina K K and Vutchev D L 2007 *Bioorg. Med. Chem.* **15** 6291
13. Ravina E, Sanchez-Alonso R, Fueyo J, Baltar M P, Bos J, Iglesias R and Sanmartin M L 1993 *Arzneim. Forsch.* **43** 684
14. Starcevic K, Kralj M, Ester K, Sabol I, Grce M, Pavelic K and Karminski-Zamola G 2007 *Bioorg. Med. Chem.* **15** 4419
15. Kubo K, Inada Y, Kohara Y, Sugiura Y, Ojima M, Itoh K, Furukawa Y, Nishikawa Y K and Naka T 1993 *J. Med. Chem.* **36** 1772

16. Ram S, Wise D S, Wotring L L, McCall J W and Townsend L B 1992 *J. Med. Chem.* **35** 539
17. Lazer E S, Matteo M R and Possanza G J 1987 *J. Med. Chem.* **30** 726
18. Lackner T E and Clissold S P 1989 *Drugs* **38** 204
19. Ito K, Kagaya H, Fukuda T, Yoshino K and Nose T 1982 *Arzneim. Forsch. Drug Res.* **32** 49
20. Navarette-Vazquez G, Cedilla R, Hernandez-Campos A, Yepez A, Hernandez-Luis F, Valdez J, Morales R, Cortes R, Hernandez M and Castillo R 2001 *Bioorg. Med. Chem.* **11** 187
21. Katiyar S K, Gordon V R, McLaughlin G L and Edlind T D 1994 *Antimicrob. Agents Chemother.* **38** 2986
22. Li Y F, Wang G F, He P L, Huang W G, Zhu F H, Gao H Y, Tang W, Luo Y, Feng C L, Shi L P, Ren Y D, Lu W and Zuo J P 2006 *J. Med. Chem.* **49** 4790
23. Kidwai M, Sapra P and Bhushan K R 1999 *Curr. Med. Chem.* **6** 195
24. Deshmukh A R, Bhawal B M, Krishnaswamy D, Govande V V, Shinkre B A and Jayanthi A 2004 *Curr. Med. Chem.* **11** 1889
25. Diurno M V, Mazzoni O, Piscopo E and Bolognese A 1992 *Il Farmaco* **47** 239
26. Alcaide B and Almendros P 2004 *Curr. Med. Chem.* **11** 1921
27. Singh S G and Boycie J M 2005 *Il Farmaco* **60** 727
28. Vaccaro W D, Sher R and Jr Davis H R 1998 *Bioorg. Med. Chem. Lett.* **8** 319
29. Ansari K F and Lal C 2009 *Eur. J. Med. Chem.* **44** 4028
30. Ansari K F and Lal C 2009 *Eur. J. Med. Chem.* **44** 2294
31. National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Disk Susceptibility tests; Approved Standard M2-A8, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2003
32. (a) Furniss B S, Hannaford A J, Smith P W G and Tatchell A R 1998 *Vogel's text book of practical organic chemistry* (England: ELBS Longman) pp. 1162–1163; (b) Registry No. 301684-45-7 (for compound 2)