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Laboratory note

Synthesis and evaluation of some new benzimidazole derivatives as potential antimicrobial agents

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Abstract

The efficient synthesis of novel azetidin-2-ones **6** has been established. Thus, condensation of 5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine **4** with various aromatic aldehydes afforded 5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-*N*-[(substituted) phenylmethylidene]-1,3,4-thiadiazol-2-amine **5** which on cycloaddition with chloroacetyl chloride in the presence of triethylamine catalyst yielded 3-chloro-1-{5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}-4-(substituted) phenylazetidin-2-one **6**. Structures of the synthesized compounds have been elucidated on the basis of their elemental analyses and spectral data. All the synthesized compounds were screened for their antimicrobial activity.

Keywords: 2-Benzimidazoles; Azetidin-2-ones; Thiadiazole; Antibacterial activity; Antifungal activity

1. Introduction

Despite a numerous attempts to develop new structural prototype in the search for more effective antimicrobials, the benzimidazoles still remain as one of the most versatile class of compounds against microbes [1–8] and, therefore, are useful substructures for further molecular exploration. Recently, the chemistry and biological profiles of various pharmacophores of 1*N*- substituted and 2-substituted benzimidazoles derivatives have been worked out in detail [9–20]. On the other side, literature survey revealed that 1,3,4-thiadiazole and 2-azetidinones are also associated with pharmacological activities like antimicrobial, antiviral, anesthetic, anticonvulsant [21–23], etc. These findings prompted us to synthesize 1,3,4-thiadiazole and 2-azetidinones derivatives of 2-methyl-1*H*-benzimidazoles. Each of the benzimidazoles analogues prepared has been tested for their antimicrobial activities and the results are reported in this paper.

2. Results and discussion

The reaction sequence for different title compounds is outlined in Scheme 1. 2-Methyl-1*H*-benzimidazole **1** required as the starting material was prepared according to the literature procedure [24]. 2-Methyl-1*H*-benzimidazole **1** on *N*-ethoxylation with ethylchloroacetate in the presence of anhyd. potassium carbonate in dry acetone gave ethyl (2-methyl-1*H*-benzimidazol-1-yl)acetate **2** which on treatment with thiasemicarbazide resulted in the formation of 2-[(2-methyl-1*H*-benzimidazol-1-yl)acetyl]-hydrazinecarbothioamide **3**. Dehydrated annulation of compound **3** with conc. H_2SO_4 followed by NH_3 treatment yielded 5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine **4** which on condensation with various selected aromatic aldehydes furnished Schiff's bases, 5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-*N*-[(substituted) phenylmethylidene]-1,3,4-thiadiazol-2-amine **5a–l**. The four membered β-lactam ring in compound **5** was introduced by the cycloaddition of (compounds **5a–l**) chloroacetyl chloride in the presence of triethylamine catalyst to give 3-chloro-1-{5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}-4-(substituted) phenylazetidin-2-one **6a–l**. The purity of the compounds was monitored

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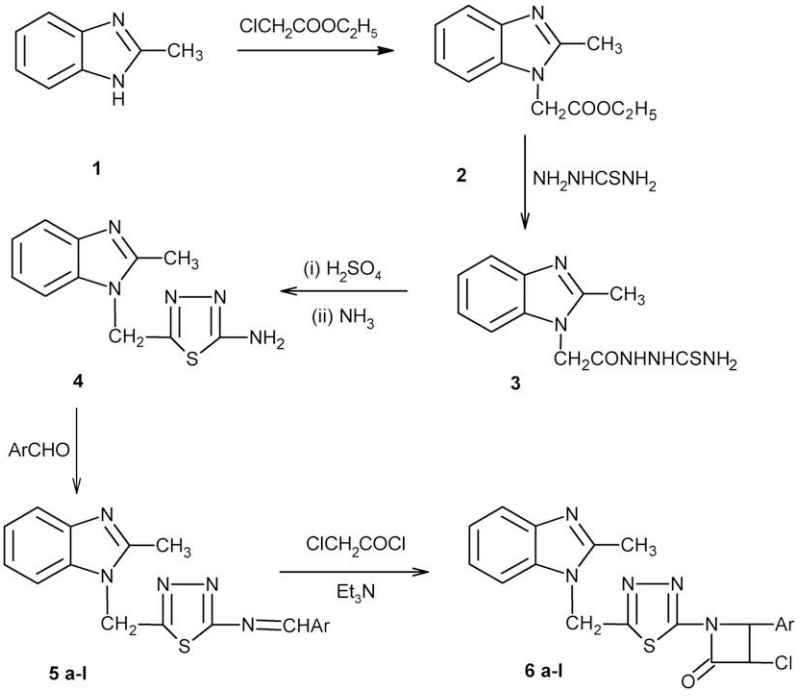

Scheme 1.

by TLC and the structures of all the derivatives (**5a–l** and **6a–l**) were supported by spectral data. The IR, ^{1}H NMR and mass spectra are in agreement with the proposed structures. Physical and analytical data of the synthesized compounds are reported in Table 1.

3. Biological activities

The compounds **5a–l** and **6a–l** were screened for their antibacterial activity against *Bacillus subtilis* MTCC 121 (Gram-positive) and *Escherichia coli* ATCC 25922 (Gram-negative)

Table 1
Physical and analytical data of the compounds **2**, **3**, **4**, **5a–l** and **6a–l**

Compounds	Ar	Yield (%)	M.P. (°C)	Mol. formula	Analysis % found (calculated)		
					C	H	N
2	–	88	186–188	$C_{12}H_{14}N_2O_2$	66.04 (66.02)	6.47 (6.45)	12.84 (12.81)
3	–	83	195–197	$C_{11}H_{13}N_5OS$	50.17 (50.15)	4.98 (4.96)	26.60 (26.57)
4	–	79	182–184	$C_{11}H_{11}N_5S$	53.86 (53.83)	4.52 (4.50)	28.55 (28.52)
5a	$-C_6H_5$	82	198–200	$C_{18}H_{15}N_5S$	64.81 (64.84)	4.52 (4.53)	21.00 (21.01)
5b	4-BrC_6H_4	67	188–190	$C_{18}H_{14}N_5SBr$	52.41 (52.43)	3.40 (3.42)	16.97 (16.99)
5c	2-ClC_6H_4	79	176–178	$C_{18}H_{14}N_5SCl$	58.75 (58.77)	3.81 (3.84)	9.62 (9.64)
5d	4-ClC_6H_4	80	181–183	$C_{18}H_{14}N_5SCl$	58.76 (58.77)	3.83 (3.84)	9.62 (9.64)
5e	2-$OCH_3C_6H_4$	84	166–168	$C_{19}H_{17}N_5OS$	62.77 (62.79)	4.69 (4.71)	19.26 (19.27)
5f	4-$OCH_3C_6H_4$	85	165–167	$C_{19}H_{17}N_5OS$	62.76 (62.79)	4.68 (4.71)	19.24 (19.27)
5g	2-$CH_3C_6H_4$	89	182–184	$C_{19}H_{17}N_5S$	65.65 (65.68)	4.93 (4.93)	20.12 (20.16)
5h	3-$CH_3C_6H_4$	80	161–162	$C_{19}H_{17}N_5S$	65.67 (65.68)	4.91 (4.93)	20.13 (20.16)
5i	2-OHC_6H_4	75	154–156	$C_{18}H_{15}N_5OS$	61.85 (61.87)	4.30 (4.33)	20.00 (20.04)
5j	3-OHC_6H_4	73	150–151	$C_{18}H_{15}N_5OS$	61.84 (61.87)	4.32 (4.33)	20.01 (20.04)
5k	4-OHC_6H_4	84	157–159	$C_{18}H_{15}N_5OS$	61.85 (61.87)	4.30 (4.33)	20.00 (20.04)
5l	4-$NH_2C_6H_4$	81	166–167	$C_{18}H_{16}N_6S$	62.02 (62.05)	4.62 (4.63)	24.11 (24.12)
6a	$-C_6H_5$	81	212–214	$C_{20}H_{16}N_5OSCl$	58.58 (58.60)	3.91 (3.93)	17.05 (17.09)
6b	2-ClC_6H_4	89	200–202	$C_{20}H_{15}N_5OSCl_2$	54.04 (54.06)	3.38 (3.40)	15.72 (15.76)
6c	4-ClC_6H_4	85	203–205	$C_{20}H_{15}N_5OSCl_2$	58.04 (54.06)	3.39 (3.40)	17.75 (15.76)
6d	2-$OCH_3C_6H_4$	82	187–189	$C_{21}H_{18}N_5O_2SCl$	57.30 (57.33)	4.10 (4.12)	15.89 (15.92)
6e	4-$OCH_3C_6H_4$	78	181–183	$C_{21}H_{18}N_5O_2SCl$	57.31 (57.33)	4.10 (4.12)	15.90 (15.92)
6f	2-$CH_3C_6H_4$	86	178–180	$C_{21}H_{18}N_5OSCl$	59.47 (59.50)	4.27 (4.28)	16.50 (16.52)
6g	3-$CH_3C_6H_4$	77	166–168	$C_{21}H_{18}N_5OSCl$	59.48 (59.50)	4.25 (4.28)	16.48 (16.52)
6h	4-$CH_3C_6H_4$	85	175–177	$C_{21}H_{18}N_5OSCl$	59.48 (59.50)	4.25 (4.28)	16.50 (16.52)
6i	2-OHC_6H_4	84	170–172	$C_{20}H_{16}N_5O_2SCl$	56.38 (56.40)	3.75 (3.79)	16.41 (16.44)
6j	3-OHC_6H_4	88	161–163	$C_{20}H_{16}N_5O_2SCl$	56.37 (56.40)	3.76 (3.79)	16.43 (16.44)
6k	4-OHC_6H_4	82	173–175	$C_{20}H_{16}N_5O_2SCl$	56.38 (56.40)	3.77 (3.79)	16.42 (16.44)
6l	4-$NH_2C_6H_4$	87	189–190	$C_{20}H_{17}N_6OSCl$	56.30 (56.53)	4.00 (4.03)	19.77 (19.78)

bacterial strains. Ampicillin was used as a reference standard. The results of the antibacterial activity screening of the tested compounds are summarized in Tables 2 and 3. The compounds not shown in tables have no antibacterial activity. Some of the compounds tested were found to have good antibacterial activity against *B. subtilis*, however, they were found to have less or poor activity against *E. coli*. The compounds having *o*-chloro, *o*-methyl, *p*-methoxy, *o*-hydroxy and *p*-amino group in phenyl ring showed good antibacterial activity.

The antifungal activity of the compounds **5a–l** and **6a–l** was assayed by using the cup-plate agar diffusion method [25] against *Candida albicans* MTCC 1637, *Aspergillus flavus* AIIMS and *Aspergillus niger* AIIMS fungal strains using amphotericin B as standard. Antifungal results indicated that some of the derivatives possessed a broad spectrum of activity against tested fungi, however, none of the derivatives showed a better spectrum of activity than the reference drug (Table 4). The compounds which have no antifungal activity are not included in the table.

4. Experimental protocol

4.1. Chemistry

Melting points were determined in open capillary tubes and are uncorrected. FTIR spectra were recorded on Perkin–Elmer RX1 spectrophotometer and ^{1}H NMR spectra in $CDCl_3$ on a Brucker 400 MHz spectrometer using TMS as an internal reference (chemical shift in δ ppm). Mass spectra were taken on a Jeol SX-102 instrument. Elemental analyses were carried out with Elementar-Vario EL III elemental analyzer. The purity of the compounds was checked on a silica gel-G plates and visualization was done using iodine/UV lamp.

Table 2
Antibacterial activity of compounds **5a–l** and **6a–l** against *Bacillus subtilis*

Compounds	Concentrations					
	1 μg/ml	10 μg/ml	100 μg/ml	200 μg/ml	500 μg/ml	App. MIC μg/ml
5a	++	+	−	−	−	100
5b	++	++	+	P	−	500
5c	++	+	−	−	−	100
5f	++	+	−	−	−	100
5g	++	++	P	−	−	200
5i	++	+	−	−	−	100
5k	++	P	P	−	−	200
5l	++	P	−	−	−	100
6a	++	++	+	−	−	200
6b	++	++	P	−	−	200
6e	++	+	P	−	−	200
6f	++	+	−	−	−	100
6h	++	+	P	−	−	200
6i	++	+	P	−	−	200
6j	++	+	+	P	−	500
6k	++	+	P	−	−	200
6l	++	P	−	−	−	100
Ampicillin	+	−	−	−	−	10

Table 3
Antibacterial activity of compounds **5a–l** and **6a–l** against *Escherichia coli*

Compounds	Concentrations					
	1 μg/ml	10 μg/ml	100 μg/ml	200 μg/ml	500 μg/ml	App. MIC μg/ml
5a	++	+	P	−	−	200
5b	++	++	+	P	−	500
5c	++	+	−	−	−	100
5f	++	++	+	−	−	200
5g	++	+	−	−	−	100
5i	++	++	P	−	−	200
5k	++	+	P	−	−	200
5l	++	+	P	−	−	200
6a	++	++	+	P	−	500
6b	++	+	−	−	−	100
6e	++	+	P	−	−	200
6f	++	+	−	−	−	100
6h	++	+	+	+	−	500
6i	++	+	P	−	−	200
6j	++	+	+	P	−	500
6k	++	++	+	P	−	500
6l	++	+	P	−	−	200
Ampicillin	+	−	−	−	−	10

Symbols: total inhibition (no growth of organism) = (−); poor growth compared to control = (P); medium growth compared to control = (+); confluent growth (no inhibition) = (++).

4.2. Synthetic methods

4.2.1. Synthesis of ethyl (2-methyl-1H-benzimidazol-1-yl)acetate **2**

Ethylchloroacetate (0.028 mol, 3 ml) was added to a solution of 2-methyl-1*H*-benzimidazoles (0.028 mol, 3.7 g) in dry acetone (40 ml). To this mixture, anhyd. K_2CO_3 (3 g)

Table 4
Antifungal activity of compounds **5a–l** and **6a–l**

Compounds	Zone of inhibition (30 μg/ml)		
	Candida albicans	*Aspergillus niger*	*Aspergillus flavus*
5a	++	+++	+++
5d	+	−	+
5e	+	++	++
5f	+	++	++
5g	+	−	+
5j	+++	+	+
5k	++	++	++
5l	++	+	+++
6a	++	++	+
6d	−	−	−
6e	+	−	−
6f	+	−	++
6h	−	++	+
6i	+	+	++
6k	++	++	++
6l	++	+	+
Amphotericin B	+++	+++	+++

Symbols: zone diameter of growth inhibition: (−) = Inactive (<10 mm); (+) = weakly active (10–15 mm); (++) moderately active (16–21 mm); (+++) = highly active (22–28 mm); (+++) = amphotericin B.

was added and the reaction mixture refluxed for 10 h. Acetone was removed *in vacuo* and the residue crystallized from ethanol to give **2**, yield 88%, mp 186–188 °C, IR (KBr): 1613 (−C=N), 1720 (−C=O ester), 1460 (−N−CH_2) cm^{-1}; ^1H NMR ($CDCl_3$): 3.67 (s, 2H, −N−CH_2), 4.21 (q, 2H, −$COOCH_2CH_3$), 1.32 (t, 3H, −$COOCH_2CH_3$), 2.92 (s, 3H, −CH_3), 6.69–7.71 (m, 4H, Ar−H); EI-MS: 219 ($M^+ + 1$).

4.2.2. Synthesis of 2-[(2-methyl-1H-benzimidazol-1-yl) acetyl]hydrazinecarbothioamide **3**

A mixture of compound **2** (0.03 mol, 6.5 g) and thiosemicarbazide (0.03 mol) in 1,4-dioxan (40 ml) was refluxed on a water bath for about 7 h. The excess solvent was removed under reduced pressure and the product crystallized from acetone to give compound **3**, yield 83%, mp 195–197 °C, IR (KBr): 1605 (−C=N), 1652 (−CONH), 3189 (−NH, −NH_2), 1138 (−C=S) cm^{-1}; ^1H NMR ($CDCl_3$): 3.15 (s, 2H, −N−CH_2), 8.33 (m, 4H, −NHNHCSNH_2), 2.85 (s, 3H, −CH_3), 6.62–7.80 (m, 4H, Ar−H); EI-MS: 264 ($M^+ + 1$).

4.2.3. Synthesis of 5-[(2-methyl-1H-benzimidazol-1-yl) methyl]-1,3,4-thiadiazol-2-amine **4**

The compound **3** (0.02 mol, 5.3 g) with conc. H_2SO_4 (15 ml) was kept overnight at room temperature, then poured into ice cold water, neutralized with ammonia and extracted with ether. The ethereal extract was distilled off and the product obtained was crystallized from methanol to afford **4**, yield 79%, mp 182–184 °C, IR (KBr): 1610 (−C=N), 1469 (−N−CH_2), 3341 (−NH_2), 714 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.29 (s, 2H, −N−CH_2), 4.14 (s, 2H, −C−NH_2), 2.83 (s, 3H, −CH_3), 6.90–7.89 (m, 4H, Ar−H); EI-MS: 246 ($M^+ + 1$).

4.2.4. General procedure for the synthesis of compounds **5a–l**

To a stirred solution of compound **4** (0.02 mol, 5 g) in methanol (50 ml) containing a glacial acetic acid (2 ml) was added appropriate aromatic aldehyde (0.02 mol) and the mixture refluxed for 6–8 h on a water bath. The separated solvent was distilled off at reduced pressure and the resulting solid was collected, dried and crystallized from chloroform–benzene mixture to give the title compounds.

4.2.4.1. 5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-N-(phenyl methylidene)-1,3,4-thiadiazol-2-amine (**5a**).

The following spectral data were recorded for compound **5a**: IR (KBr): 1615 (−C=N), 1475 (−N−CH_2), 1580 (−N=CH), 703 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.82 (s, 2H, −N−CH_2), 8.20 (s, 1H, −N=CH), 2.25 (s, 3H, −CH_3), 7.12–7.79 (m, 9H, Ar−H); EI-MS: 334 ($M^+ + 1$).

4.2.4.2. N-[(4-Bromophenyl)methylidene]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (**5b**).

The following spectral data were recorded for compound **5b**: IR (KBr): 1608 (−C=N), 1462 (−N−CH_2), 1583 (−N=CH), 682 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.80 (s, 2H, −N−CH_2), 8.15 (s, 1H, −N=CH), 2.28 (s, 3H, −CH_3), 7.29–7.70 (m, 8H, Ar−H); EI-MS: 413 ($M^+ + 1$).

4.2.4.3. N-[(2-Chlorophenyl)methylidene]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (**5c**).

The following spectral data were recorded for compound **5c**: IR (KBr): 1598 (−C=N), 1475 (−N−CH_2), 1580 (−N=CH), 701 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.85 (s, 2H, −N−CH_2), 8.12 (s, 1H, −N=CH), 2.25 (s, 3H, −CH_3), 7.28–7.73 (m, 8H, Ar−H); EI-MS: 368 ($M^+ + 1$).

4.2.4.4. N-[(4-Chlorophenyl)methylidene]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (**5d**).

The following spectral data were recorded for compound **5d**: IR (KBr): 1618 (−C=N), 1469 (−N−CH_2), 1585 (−N=CH), 705 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.80 (s, 2H, −N−CH_2), 8.11 (s, 1H, −N=CH), 2.32 (s, 3H, −CH_3), 7.15–7.72 (m, 8H, Ar−H); EI-MS: 368 ($M^+ + 1$).

4.2.4.5. N-[(2-Methoxyphenyl)methylidene]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (**5e**).

The following spectral data were recorded for compound **5e**: IR (KBr): 1620 (−C=N), 1460 (−N−CH_2), 1582 (−N=CH), 712 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.92 (s, 2H, −N−CH_2), 8.13 (s, 1H, −N=CH), 3.15 (s, 3H, −OCH_3), 2.10 (s, 3H, −CH_3), 7.82–7.96 (m, 8H, Ar−H); EI-MS: 364 ($M^+ + 1$).

4.2.4.6. N-[(4-Methoxyphenyl)methylidene]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (**5f**).

The following spectral data were recorded for compound **5f**: IR (KBr): 1609 (−C=N), 1466 (−N−CH_2), 1570 (−N=CH), 720 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.99 (s, 2H, −N−CH_2), 8.05 (s, 1H, −N=CH), 3.52 (s, 3H, −OCH_3), 2.22 (s, 3H, −CH_3), 7.80–7.95 (m, 8H, Ar−H); EI-MS: 364 ($M^+ + 1$).

4.2.4.7. 5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-N-[(2-methylphenyl)methylidene]-1,3,4-thiadiazol-2-amine (**5g**).

The following spectral data were recorded for compound **5g**: IR (KBr): 1621 (−C=N), 1458 (−N−CH_2), 1581 (−N=CH), 695 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.70 (s, 2H, −N−CH_2), 8.51 (s, 1H, −N=CH), 2.10 (s, 3H, −CH_3), 6.60–6.82 (m, 8H, Ar−H); EI-MS: 348 ($M^+ + 1$).

4.2.4.8. 5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-N-[(3-methylphenyl)methylidene]-1,3,4-thiadiazol-2-amine (**5h**).

The following spectral data were recorded for compound **5h**: IR (KBr): 1616 (−C=N), 1470 (−N−CH_2), 1578 (−N=CH), 702 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.72 (s, 2H, −N−CH_2), 8.58 (s, 1H, −N=CH), 2.55 (s, 3H, −CH_3), 6.62–6.80 (m, 8H, Ar−H); EI-MS: 348 ($M^+ + 1$).

4.2.4.9. 2-[({5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}imino)methyl]phenol (**5i**).

The following spectral data were recorded for compound **5i**: IR (KBr): 1618 (−C=N), 1468 (−N−CH_2), 1574 (−N=CH), 680 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.70 (s, 2H, −N−CH_2),

8.72 (s, 1H, −N=CH), 5.60 (s, 1H, Ar−OH), 2.65 (s, 3H, $-CH_3$), 7.25−7.80 (m, 8H, Ar−H); EI-MS: 350 ($M^+ + 1$).

*4.2.4.10. 3-[({5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}imino)methyl]phenol (**5j**).* The following spectral data were recorded for compound **5j**: IR (KBr): 1604 (−C=N), 1470 ($-N-CH_2$), 1580 (−N=CH), 708 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.75 (s, 2H, $-N-CH_2$), 8.71 (s, 1H, −N=CH), 5.55 (s, 1H, Ar−OH), 2.60 (s, 3H, $-CH_3$), 7.28−7.93 (m, 8H, Ar−H); EI-MS: 350 ($M^+ + 1$).

*4.2.4.11. 4-[({5-[(2-Methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}imino)methyl]phenol (**5k**).* The following spectral data were recorded for compound **5k:** IR (KBr): 1625 (−C=N), 1480 ($-N-CH_2$), 1585 (−N=CH), 710 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.84 (s, 2H, $-N-CH_2$), 8.67 (s, 1H, −N=CH), 5.46 (s, 1H, Ar−OH), 2.67 (s, 3H, $-CH_3$), 7.25−7.90 (m, 8H, Ar−H); EI-MS: 350 ($M^+ + 1$).

*4.2.4.12. N-[(4-Aminophenyl)methylidene]-5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-amine (**5l**).* The following spectral data were recorded for compound **5l**: IR (KBr): 1618 (−C=N), 1473 ($-N-CH_2$), 1580 (−N=CH), 712 (C−S−C) cm^{-1}; ^1H NMR ($CDCl_3$): 3.82 (s, 2H, $-N-CH_2$), 8.65 (s, 1H, −N=CH), 4.08 (s, 2H, $-C-NH_2$), 2.62 (s, 3H, $-CH_3$), 7.72−7.95 (m, 8H, Ar−H); EI-MS: 349 ($M^+ + 1$).

*4.2.5. General procedure for the synthesis of compounds **6a–l***

To a stirred solution of the particular 5-[(2-methyl-1*H*-benzimidazol-1-yl)methyl]-*N*-[(substituted) phenylmethylidene]-1,3,4-thiadiazol-2-amine **5a–l** (0.02 mol) and triethylamine (0.01 mol) in dioxan (50 ml), chloroacetyl chloride (0.01 mol) was added dropwise at 0−5 °C. The reaction mixture stirred for 3 h and the separated solid was crystallized from methanol to give the title compounds.

*4.2.5.1. Synthesis of 3-chloro-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}-4-phenylazetidin-2-one (**6a**).* The following spectral data were recorded for compound **6a**: IR (KBr): 1600 (−C=N), 1748 (−C=O), 702 (C−S−C), 766 (−C−Cl) cm^{-1}; ^1H NMR ($CDCl_3$): 3.90 (s, 2H, $-N-CH_2$), 4.11 (s, 1H, −N−CH), 5.15 (s, 1H, −CHCl), 2.23 (s, 3H, $-CH_3$), 7.00−7.82 (m, 9H, Ar−H); EI-MS: 410 ($M^+ + 1$).

*4.2.5.2. 3-Chloro-4-(2-chlorophenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6b**).* The following spectral data were recorded for compound **6b**: IR (KBr): 1616 (−C=N), 1673 (−C=O), 718 (C−S−C), 780 (−C−Cl) 1718 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.99 (s, 2H, $-N-CH_2$), 4.13 (s, 1H, −N−CH), 5.30 (s, 1H, −CH−Cl), 2.55 (s, 3H, $-CH_3$), 7.32−7.78 (m, 8H, Ar−H); EI-MS: 445 ($M^+ + 1$).

*4.2.5.3. 3-Chloro-4-(4-chlorophenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6c**).* The following spectral data were recorded for compound **6c**: IR (KBr): 1602 (−C=N), 1683 (−C=O), 714 (C−S−C), 775 (−C−Cl) 1721 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.81 (s, 2H, $-N-CH_2$), 4.11 (s, 1H, −N−CH), 5.25 (s, 1H, −CH−Cl), 2.55 (s, 3H, $-CH_3$), 7.30−7.84 (m, 8H, Ar−H); EI-MS: 445 ($M^+ + 1$).

*4.2.5.4. 3-Chloro-4-(2-methoxyphenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6d**).* The following spectral data were recorded for compound **6d:** IR (KBr): 1611 (−C=N), 1689 (−C=O), 670 (C−S−C), 778 (−C−Cl) 1728 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.75 (s, 2H, $-N-CH_2$), 4.15 (s, 1H, −N−CH), 5.37 (s, 1H, −CH−Cl), 4.08 (s, 3H, $-OCH_3$), 3.72 (s, 3H, $-CH_3$), 7.37−7.92 (m, 8H, Ar−H); EI-MS: 440 ($M^+ + 1$).

*4.2.5.5. 3-Chloro-4-(4-methoxyphenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6e**).* The following spectral data were recorded for compound **6e**: IR (KBr): 1619 (−C=N), 1690 (−C=O), 705 (C−S−C), 765 (−C−Cl) 1722 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.66 (s, 2H, $-N-CH_2$), 4.10 (s, 1H, −N−CH), 5.33 (s, 1H, −CH−Cl), 4.12 (s, 3H, $-OCH_3$), 3.83 (s, 3H, $-CH_3$), 7.62−7.98 (m, 8H, Ar−H); EI-MS: 440 ($M^+ + 1$).

*4.2.5.6. 3-Chloro-1-{5-[(2-methyl-1H-benzimidazol-1-yl)-methyl]-1,3,4-thiadiazol-2-yl}-4-(2-methylphenyl)azetidin-2-one (**6f**).* The following spectral data were recorded for compound **6f**: IR (KBr): 1615 (−C=N), 1685 (−C=O), 669 (C−S−C), 760 (−C−Cl) 1727 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.82 (s, 2H, $-N-CH_2$), 4.20 (s, 1H, −N−CH), 5.29 (s, 1H, −CH−Cl), 3.45 (s, 3H, $-CH_3$), 7.20−7.90 (m, 8H, Ar−H); EI-MS: 424 ($M^+ + 1$).

*4.2.5.7. 3-Chloro-1-{5-[(2-methyl-1H-benzimidazol-1-yl)-methyl]-1,3,4-thiadiazol-2-yl}-4-(3-methylphenyl)azetidin-2-one (**6g**).* The following spectral data were recorded for compound **6g**: IR (KBr): 1620 (−C=N), 1688 (−C=O), 690 (C−S−C), 762 (−C−Cl) 1718 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.75 (s, 2H, $-N-CH_2$), 4.26 (s, 1H, −N−CH), 5.17 (s, 1H, −CH−Cl), 3.32 (s, 3H, $-CH_3$), 7.33−7.98 (m, 8H, Ar−H); EI-MS: 424 ($M^+ + 1$).

*4.2.5.8. 3-Chloro-1-{5-[(2-methyl-1H-benzimidazol-1-yl)-methyl]-1,3,4-thiadiazol-2-yl}-4-(4-methylphenyl)azetidin-2-one (**6h**).* The following spectral data were recorded for compound **6h**: IR (KBr): 1600 (−C=N), 1748 (−C=O), 713 (C−S−C), 767 (−C−Cl) 1728 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.70 (s, 2H, $-N-CH_2$), 4.18 (s, 1H, −N−CH), 5.25 (s, 1H, −CH−Cl), 3.30 (s, 3H, $-CH_3$), 7.40−7.92 (m, 8H, Ar−H); EI-MS: 424 ($M^+ + 1$).

*4.2.5.9. 3-Chloro-4-(2-hydroxyphenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6i**).* The following spectral data were recorded for compound **6i**: IR (KBr): 1605 (−C=N), 1750 (−C=O), 705 (C−S−C), 762 (−C−Cl) 1720 (>C=O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.78 (s, 2H, $-N-CH_2$),

4.10 (s, 1H, −N−CH), 5.10 (s, 1H, −CH−Cl), 5.79 (s, 1H, −C−OH), 2.45 (s, 3H, $-CH_3$), 7.69–8.08 (m, 8H, Ar−H); EI-MS: 426 ($M^+ + 1$).

*4.2.5.10. 3-Chloro-4-(3-hydroxyphenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6j**).* The following spectral data were recorded for compound **6j**: IR (KBr): 1601 (−C═N), 1738 (−C═O), 675 (C−S−C), 780 (−C−Cl) 1722 (>C═O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.75 (s, 2H, $-N-CH_2$), 4.17 (s, 1H, −N−CH), 5.22 (s, 1H, −CH−Cl), 5.78 (s, 1H, −C−OH), 2.44 (s, 3H, $-CH_3$), 7.60–7.92 (m, 8H, Ar−H); EI-MS: 426 ($M^+ + 1$).

*4.2.5.11. 3-Chloro-4-(4-hydroxyphenyl)-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6k**).* The following spectral data were recorded for compound **6k**: IR (KBr): 1605 (−C═N), 1738 (−C═O), 715 (C−S−C), 766 (−C−Cl) 1720 (>C═O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.73 (s, 2H, $-N-CH_2$), 4.13 (s, 1H, −N−CH), 5.25 (s, 1H, −CH−Cl), 5.80 (s, 1H, −C−OH), 2.48 (s, 3H, $-CH_3$), 7.67–7.97 (m, 8H, Ar−H); EI-MS: 426 ($M^+ + 1$).

*4.2.5.12. 4-(4-Aminophenyl)-3-chloro-1-{5-[(2-methyl-1H-benzimidazol-1-yl)methyl]-1,3,4-thiadiazol-2-yl}azetidin-2-one (**6l**).* The following spectral data were recorded for compound **6l**: IR (KBr): 1609 (−C═N), 1744 (−C═O), 702 (C−S−C), 763 (−C−Cl) 1725 (>C═O monocyclic β-lactam) cm^{-1}; ^1H NMR ($CDCl_3$): 3.70 (s, 2H, $-N-CH_2$), 4.12 (s, 1H, −N−CH), 4.85 (s, 1H, −CH−Cl), 4.32 (s, 2H, $-C-NH_2$), 2.38 (s, 3H, $-CH_3$), 7.10–7.85 (m, 8H, Ar−H); EI-MS: 425 ($M^+ + 1$).

4.3. Microbiology

4.3.1. Antibacterial activity test

For the antibacterial activity of compounds **5a–l** and **6a–l**, the test organisms were grown overnight at 37 °C in buffered glucose broth. A loopful (5mm diameter) of the test organisms was added to the buffered glucose broth containing test compounds and incubated at 37 °C for 24 h. The control experiments were run in a similar manner and contained equal amounts of buffered and test compound. The last dilution inhibition growth was taken as minimum inhibitory concentration (MIC) in μg/ml.

4.3.2. Antifungal activity test

The synthesized compounds **5a–l** and **6a–l** were tested for their antifungal activity *in vitro*, in comparison with amphotericin B as a reference drug using cup-plate technique in nutrient agar media by measuring the inhibition zone in millimeters. DMSO was used as a control and the test was performed at 30 μg/ml concentration.

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