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Green route for the heterocyclization of 2-mercaptobenzimidazole into β-lactum segment derivatives containing –CONH– bridge with benzimidazole: Screening in vitro antimicrobial activity with various microorganisms

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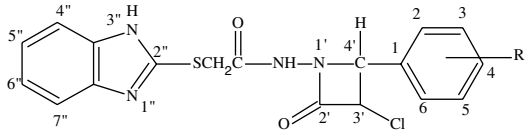
Abstract—The efficient and rapid synthesis of novel azetidin-2-ones **4a**–**j** has been established. Thus, both microwave and conventional condensation 2-{(1*H*-benzimidazol)-ylthio}-*N*′-2-(substituted phenyl) hydrazide with chloroacetylchloride were carried out in DMF-benzene solvent in the presence of Et_3N catalyst. The microwave synthesis route afforded better yield with short time. The novel heterocycles were characterized by elemental analysis and spectral features. Some of the produced compounds were screened for their antimicrobial activity.

1. Introduction

2-Mercaptobenzimidazole derivatives are known to possess varied biological activities.[1] 2-Azetidinone derivatives have been reported to possess anti-inflammatory,[2] anticonvulsant,[3] fungicidal,[4] antibiotic,[5] anticancer,[6] antielastase,[7] antiviral,[8] antimicrobial,[9] antitumor,[10] anti-HCMV,[11] antibacterial[12] activities and pharmalogical interest.[13] The incorporation of a 2-oxoazetidine moiety in to 2-mercaptobenzimidazole scaffold enhances its activity.

In the last few years, microwave-induced organic reaction enhancement (MORE) has gained popularity as a non-conventional technique for rapid organic synthesis[14] and many researchers have described accelerated organic reactions, with a large number of papers that have appeared proving the synthesis utility of MORE chemistry in routine organic synthesis.[15,16] It can be termed as 'e-chemistry' because it is easy, effective, economical, and eco-friendly, and is believed to be a step toward achieving green chemistry objectives. Within the framework of 'Green Chemistry' we have now developed an environmentally benign and novel approach for the synthesis of azetidine-2-ones. In view of the above, and in continuation to our earlier work on the application of MORE[17–19] chemistry to organic synthesis and the biological importance of 2-azetidinones, we now report a simple, novel and environmentally benign approach using facile, microwave synthesis of 2-azetidinones **4a**–**j** from precursors **3a**–**j** and $ClCH_2COCl$ using triethylamine (TEA) as a catalyst (Fig. 1).



4	R	**4**	R
a	4-NO_2	**f**	2-OCH_3
b	3, 4, 5-$(OCH_3)_3$	**g**	4-OCH_3
c	2-OH	**h**	2-Cl
d	3-OH	**i**	3-Cl
e	4-OH	**j**	4-Cl

Figure 1. General structure of synthesized 2-azetidinones **4a**–**j**.

Keywords: 2-Azetidinones; Heterocyclization; Microwave effect; Antimicrobial interest.
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2. Results and discussion

Conventional methodology sometimes has lower yields than microwave protocols. Microwave irradiation facilitates the polarization of the molecule under irradiation causing rapid reaction to occur. A comparative study in terms of yield and reaction period is shown in Table 1. The MS spectral fragmentation pattern is presented (Scheme 2) as an additional evidence for the proposed structure **4a**. The synthetic route of abovementioned compounds is shown in Scheme 1) . All the compounds synthesised were adequately characterized by their elemental analysis and IR, ^{1}H NMR, ^{13}C NMR spectroscopies and by mass spectrometry.

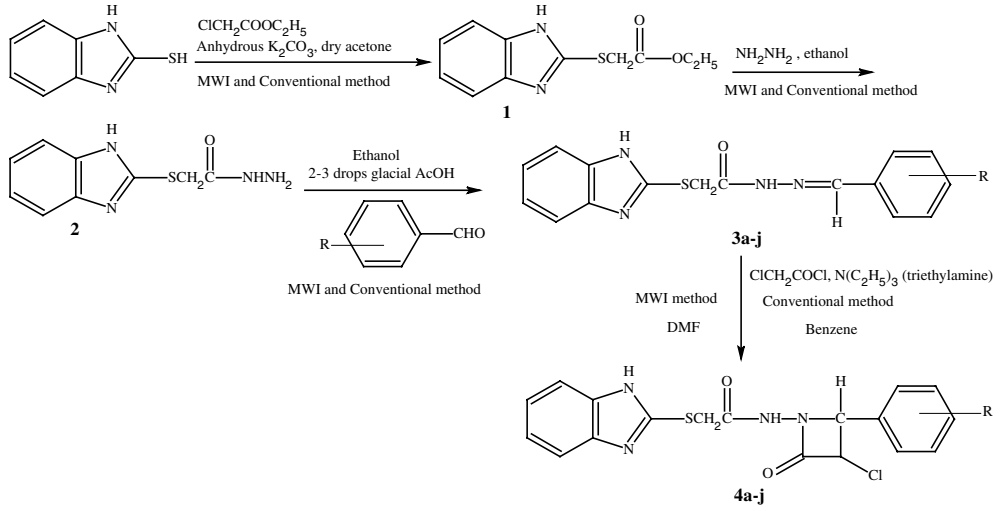
2.1. Chemistry

Condensation of 2-mercaptobenzimidazole and ethyl chloroacetate in presence of anhydrous K_2CO_3 in dry acetone as a reaction mediator afforded ethyl-2-(benzimidazolylthio)-acetate[20,21] **1**. The formation of compound **1** was evidenced by appearance of a signal at δ 1.23 and 4.13 ppm due to CH_3 and CH_2, respectively (J = 7 Hz), in –COOCH_2CH_3 of compound **1** in the ^{1}H NMR spectra and IR spectral bands at 1723 cm^{-1} due to the ester (>C=O) and 2915, 2871, 1423, 713 cm^{-1} (CH_2 and CH_3) also confirmed the formation of compound **1**. Compound **1** on ammonolysis with hydrazinehydrate in ethanol as a reaction media afforded [2-(benzimidazolylthio)-acetyl]-hydrazine[20,21] **2**. In the ^{1}H NMR spectra of compound **2**, the peak at δ 7.88 ppm were observed due to –CONH– and a peak at δ 4.40 ppm was due to the –NH_2 group of compound **2**. Furthermore, in the IR spectra, the bands at 1665 cm^{-1} (>C=O of amide) and 3352, 3378 cm^{-1} (–NHNH_2) also confirm the formation of compound **2**. Compound **2**, an aromatic aldehyde and 2–3 drops of glacial acetic acid in ethanol as a reaction mediator afforded 2-{(1*H*-benzimidazol)-ylthio}-*N*′-2-(substituted phenyl)hydrazide[20,21] **3**. The formation of compound **3** was evidenced by appearance of signal at δ 4.40 ppm due to the –N=CH– of in ^{1}H NMR spectra, the appearance of a signal at δ 60 ppm due to the >CH–N< of in ^{13}C NMR spectra and IR spectra bands, due to

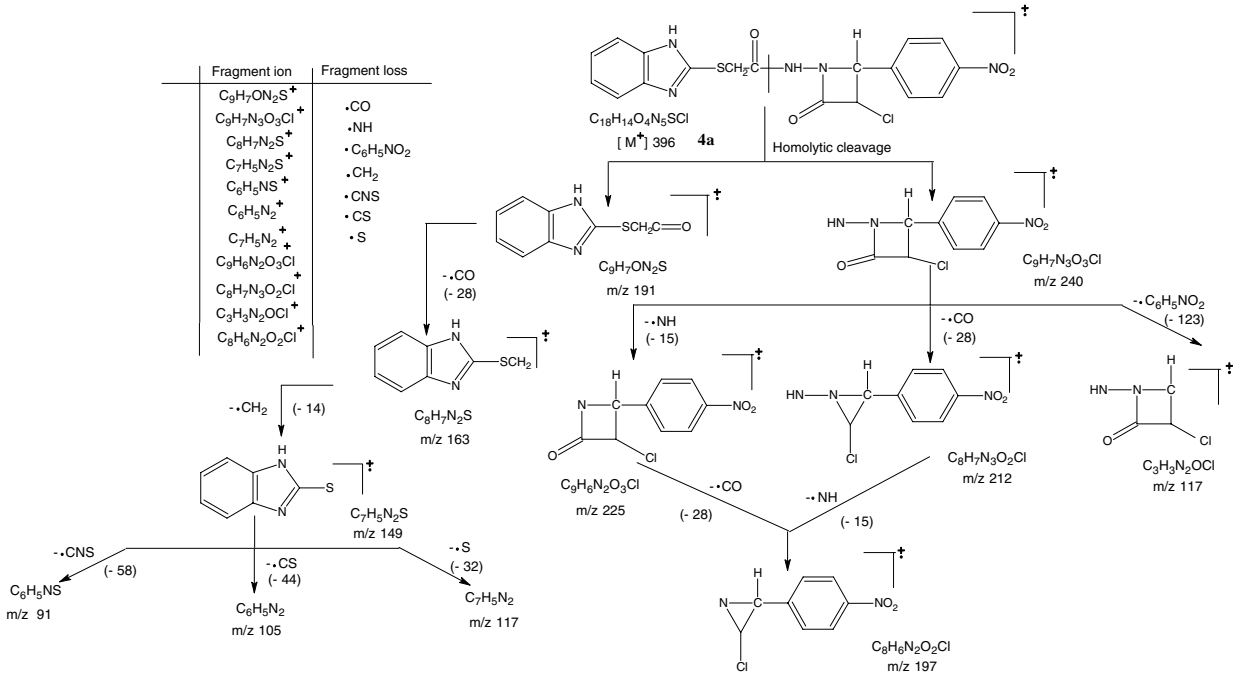
Table 1. Comparative study in terms of yield and reaction period in presence of different power watts and constant temperature for microwave and conventional techniques **4a–j**

Products	[M]Microwave irradiation technique (MWI)					[C]Conventional method	
	Irradiation condition		[A]Yield (%)	[B]Yield (%)	Constant temperature (°C)	Time (h)	[C]Yield (%)
	[A,M]Power P_1 (W)/Time T_1 (min)	[B,M]Power P_2 (W)/Time T_2 (min)					
4a	250/5.0	400/3.0	78	89	145	5.0	70
4b	300/4.0	400/3.0	79	88	149	6.0	69
4c	200/5.5	450/2.5	75	90	150	6.5	65
4d	250/5.0	350/3.5	78	85	148	6.5	75
4e	200/5.0	350/3.0	75	84	148	6.0	70
4f	300/4.0	400/3.0	80	86	146	5.0	58
4g	250/5.0	450/2.5	76	90	147	6.5	61
4h	200/5.5	450/3.0	75	88	146	5.0	72
4i	250/5.0	400/2.5	78	87	144	5.5	73
4j	300/4.0	450/2.5	80	90	145	5.0	69

[A,M]Yield of isolated products (P_1-200-3-0 W, T_1-4.0-5.5 min); [B,M]Yield of isolated products (P_2-350-450 W, T_2-2.5-3.5 min);[C] Yield of isolated products.



Scheme 1.



Scheme 2. Mass spectral fragmentation pattern of 1′-[(benzimidazol-2-yl)thioacetamidyl]- 3′-chloro-4′-(4-nitrophenyl-azetidin- 2′-one) **4a**.

(–N=CH–) at 1626 cm^{-1}, also confirmed the formation of compound **3**. This compound **3** on cycloaddition with chloroacetylchloride in presence of triethylamine [Et_3N] in DMF/benzene as a reaction mediator afforded 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(substituted phenyl-azetidin-2′-one) **4**. In the ^1H NMR spectra of compound **4**, the peak was observed at δ 4.0 ppm due to the >CH–Cl in the β-lactam ring; in the ^{13}C NMR spectra of compound **4**, peaks at δ 50 ppm was observed, due to >CH–Cl, at 172.5 ppm (cyclic, >C=O) and 157.5 ppm (heteroaromatics) in the β-lactam moiety. In the IR spectra of compound **4**, the bands at 1717 cm^{-1} (>C=O, cyclic) also confirmed the formation of azetidinones **4**. In the mass spectra of compound **4a**, the molecular ion peak 396 [M^+] (24%) also confirmed the formation of the azetidinone. The fragment ion (m^+) peak was observed at 240 *m/z* (78%), 225 *m/z* (37%), 212 *m/z* (31%), 197 *m/z* (29%), 191 *m/z* (34%), 163 *m/z* (24%), 149 *m/z* (35%), 117 *m/z* (30%), 105 *m/z* (38%) and 91 *m/z* (29%) by the loss of fragment radicals and neutrals ·CO (−28), ·NH (−15), $\cdot C_6H_5NO_2$ (−123), $\cdot CH_2$ (−14), ·CNS (−58), ·S (−32) and ·CS (−44).

2.2. Microwave irradiation technique

All the reactions that used microwave irradiation (MWI) were completed with in 2–5.5 min, whereas similar reactions under conventional heating (steam bath) at similar temperatures (80–100 °C) gave poor yields with comparatively longer reaction time period (Table 1), demonstrating that the effect of microwave irradiation is not purely thermal. Microwave irradiation facilitates the polarization of the molecules under irradiation causing rapid reaction to occur. This is consistent with the reaction mechanism, which involves a polar transition state.[22] The effectiveness of microwave irradiation and conventional heating for the synthesis of compounds **4a**–**j** has been compared (Table 1). Under microwave irradiation conditions, the yields of **4a**–**j** are high (90–84%). Whereas using conventional heating the yields are only 58–75%. The effects of irradiation power and time on the reaction were also studied and the results summarized in Tables 2 and 3. High yields of compounds **4a**–**j** can be obtained in 450 W for 2.5 min under microwave irradiation.

2.3. Antibacterial activity

The antibacterial screening results (the zone of inhibition), presented in Table 4, the results revealed that all newly synthesized compounds were exhibited potent antibacterial activity. In general, compounds **4a**, **4i** and **4j** exhibited more pronounced antibacterial activities than the compounds **4b**–**h**, with better activity against both Gram positive and Gram negative bacteria. Among all the compounds tested, **4i** and **4j** exhibited remarkable antibacterial activity against the Gram neg-

Table 2. The effect of microwave irradiation power[K]

Irradiation power (W)	200	250	300	350	400	450
Yield (%)	75	78	80	85	88	90

[K] Irradiation time is 2.5 min.

Table 3. The effect of microwave irradiation time[G]

Irradiation time (min)	5.5	5.0	4.0	3.5	3.0	2.5
Yield (%)	73	75	79	81	87	90

[G] Irradiation power is 450 W.

Table 4. The zone of inhibition values (mm) of compounds **4a–j**

Compounds	Antibacterial activity (mm)		
	Gram positive (+ve)		Gram negative (−ve)
	B.s [k] (ATCC 6633)	*S.a* [r] (ATCC 6538)	*E.c* [u] (ATCC 6538)
4a	+ + +	+ +	+ +
4b	+ +	+ +	+ +
4c	−	−	−
4d	+ +	+ +	+ +
4e	+ +	+ +	+ +
4f	−	−	−
4g	+ +	+ +	+ +
4h	+ +	+ +	+ +
4i	+ +	+ + +	+ + +
4j	+	+	+ + +
Zone of inhibition of standard drugs (mm)			
Streptomycin	+ + + +	+ + + +	+ + + +

Diameter of the zone of inhibition: (−) 6 mm; (+) 6–15 mm; (+ +) 15–20 mm; (+ + +) 20–25 mm; (+ + + +) 25–30 mm.

[k] *B.s*, *Bacills substilis*; [r] *S.a*, *Staphylococcus aureus*; [u] *E.c*, *Escherchia coli*.

Table 5. The MIC values (μg/mL) of compounds **4a–j**

Compounds	Antifungal activity (μg/mL)		
	C.a [n] (ATCC 64550)	*C.k* [a] (ATCC 14243)	*A.n* [l]
4a	−	−	−
4b	+ +	+ +	+ +
4c	+ +	+ + +	+ +
4d	+ +	+ +	+ +
4e	+	+ +	+ +
4f	+ +	+ +	+ + +
4g	+ +	+ +	+ +
4h	+ +	+ +	+ +
4i	−	−	−
4j	+ +	+ +	+
Minimum inhibitory concentration of standard drugs (μg/mL)			
Flucanozole	+ + + +	+ + + +	+ + + +

50 μg/mL = + + + +, 100 μg/mL = + + +, 150 μg/mL = + +, 200 μg/mL = +, Not active upto 200 μg/m = Flucanozole[23] is (+ + + +) at ⩽50 μg/mL.

Diameter of the MIC: (−) 6 μg/mL; (+) 6–15 μg/mL; (+ +) 15–20 μg/mL; (+ + +) 20–25 μg/mL; (+ + + +) 25-30 μg/mL.

[n] *C.a*, *Candida albicans*; [a] *C.k*, *Candida krusei*; [l] *A.n*, *Aspergillus niger*.

ative *Escherichia coli* (ATCC 6538) as compared with antibiotic streptomycin.

On the other hand, compounds **4a** and **4i** exhibited potent activity against the Gram positive *Bacills substilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538) as compared with Streptomycin. The antibacterial activities of compounds **4c**, **4f** and **4j** were 70% lower than, the standard against the Gram positive bacteria species and **4c** and **4f** against Gram negative bacteria species. Moreover, compounds **4b**, **4d**, **4e**, **4g** and **4h** were moderately active against the same organisms.

2.4. Antifungal activity

For all drugs, the minimum inhibitory concentration (MIC) of the compounds was defined as the lowest concentration at which there was 100% inhibition of growth compared with the growth for a drug free control.[23] Sensitivity of the selected fungal pathogens to some synthesized compounds **4a–j** was determined in vitro at four concentrations (50, 100, 150 and 200 μg/mL). Standard minimum inhibitory concentration for Flucanozole (FLU)[23] is (+ + + +) at ⩽50 μg/mL against all microbes.

The antifungal screening results (MIC), presented in Table 5, it is evident from the screening data compounds **4c** and **4f** were more effective against *Candida krusei* (100 μg/mL) and *Aspergillus niger* (100 μg/mL), respectively, compared with the other derivatives. Compounds **4e** and **4j** showed weak antifungal activity against *Candida albicans* and *Aspergillus niger*, respectively, with a MIC of 200 μg/mL, but compounds **4b**, **4d**, **4g** and **4h** showed moderate antifungal activity against all fungal species with a MIC of 150 μg/mL, compounds **4c**, **4f** and **4j** showed moderate antifungal activity against *Candida albicans* with a MIC of 150 μg/mL, compounds **4e**, **4f** and **4j** showed moderate antifungal activity against *Candida krusei* with a MIC of 150 μg/mL and compounds **4c** and **4e** showed moderate antifungal activity against *Aspergillus niger* with a MIC of 150 μg/mL. By visualizing the antifungal data it could be observed that some of the compounds possess significant activity. However, none of compounds were was superior to standards used against any fungi. Generally, the compounds **4e** and **4j** (both have R = –OH and –Cl, respectively) were less active their methoxy counterparts, with exception of compound **4c** against *Candida krusei*. The rest of the tested compounds **4a** and **4i** were not active against all organisms with MIC values upto ⩾200 μg/mL.

3. Experimental

3.1. General

Regents, instrumentation and measurements: all reagents, 2-mercaptobenzimidazole, solvents and catalyst were of analytical grade and used directly. All the melting points were determined in PMP–DM scientific melting point apparatus and are uncorrected. The completion of reaction was monitored by thin-layer chromatography (TLC) using silica gel-G coated Al-plates (0.5 mm thickness, Merck) and spots were visualized by exposing the dry plates to iodine vapour. The product was purified by silica gel column chromatography (60–120 mesh) eluted by benzene and ethyl acetate (8:2 v/v) mixture.[24] IR spectra (υ_{max} in cm^{-1}) were recorded on a Shimadzu FT-IR 8300 spectrophotometer using KBr or the Nujol technique; ^1H NMR spectra were acquired on a Bruker WM 400FT 400 MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as the solvent and TMS as the internal reference (chemical shifts in δ, ppm); ^{13}C NMR was performed on a Varian AMX

400 (100 MHz) spectrometer using solutions in $CDCl_3$ and mass spectra were acquired on a Jeol JMS D-300 spectrometer operating at 75 eV. The elemental analysis (C, H, N) of compounds was performed on Carlo Erba-1108 elemental analyzer. Their results were found to be in good agreement with the calculated values. The microwave assisted reactions are carried out using QPro-M Microwave Sample Preparation System (Questron Technologies Corporation, Mississauga, Ontario L4Z 2E9, Canada), wherein this unit, microwaves are generated by magnetron at a frequency of 2450 MHz having an output energy range of 100–500 W and a fibre optic sensor for temperature control with an attached reflux condenser with constant stirring (to avoid the risk of high pressure development) and permitting synthesis on preparative scales.

The QPro-M apparatus used was especially well suited for stringent reaction conditions, including anhydrous atmosphere.

3.2. Synthesis

3.2.1. Microwave assisted synthesis of ethyl-2-(benzimidazolylthio)-acetate (1). 2-Mercaptobenzimidazol (0.01 mol, 1.50 g) and ethyl chloroacetate (0.01 mol, 1.22 mL) in dry acetone (4 mL) in the presence of anhydrous K_2CO_3 (1 g) were placed in a round-bottomed flask inside a microwave oven and irradiated (350 W, 61–62 °C) for 3.5 min.[20,21] Upon completion of the reaction (monitored by TLC), the reaction mixture was allowed to attain room temperature and treated with cold water. The separated solid was filtered, washed with water and recrystallized from chloroform to furnish compound **1**, yield 84%, as a white crystal. Mp 60 °C, Anal. Calcd for $C_{11}H_{12}N_2O_2S$: C, 55.93; H, 5.08; N, 11.86. Found: C, 55.95; H, 5.10; N, 11.90%; IR (KBr): υ (cm^{-1}) 3023 (aromatic ring), 1070 (aliphatic ether), 638 (C–S), 1723 (>C═O of ester), 1614 (–C═N–), 1223 and 1041 (C–O–C), 721 (C–S–C) and 2915, 2871, 1423, 713 (–CH_2 and –CH_3); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 1.23 (t, 3H, J = 7 Hz, –$COOCH_2CH_3$), 4.13 (q, 2H, J = Hz, –$COOCH_2CH_3$), 4.46 (s, 2H, S–CH_2–), 6.73–7.87 (m, 4H, Ar–H), 8.6 (s, 1H, –NH–benzimidazole).

3.2.2. Conventional synthesis of ethyl 2-(benzimidazolylthio)acetate (1). An equimolar solution of 2-mercaptobenzimidazol (0.01 mol, 1.50 g) and ethyl chloroacetate (0.01 mol, 1.22 mL) in dry acetone (4 mL) in the presence of anhydrous K_2CO_3 (1 g) was refluxed on a water bath for 6 h. The solvent was removed by vacuum distillation and the residue was recrystallized from chloroform to furnish compound **1**, yield 67%, as a white solid. Mp 60 °C.

3.2.3. Microwave assisted synthesis of [(2-benzimidazolylthio)-acetyl]-hydrazine (2). Compound **1** (0.01 mol, 2.36 g) and hydrazine hydrate (0.01 mol, 0.9 mL) in ethanol (20 mL) was placed in a round-bottomed flask inside a microwave oven and irradiated (300 W, 76–78 °C) for 4.0 min.[20,21] After completion of reaction (monitored by TLC), the mixture was cooled and the resulting solid was filtered, dried, and recrystallized from ethanol to give compound **2**, yield 80%, as a pinkish white powder. Mp 195 °C. Anal. Calcd for $C_9H_{10}N_4OS$: C, 48.64; H, 4.5; N, 25.22. Found: C, 48.66; H, 4.8; N, 25.25%; IR (KBr): υ (cm^{-1}) 3352, 3378 (–$NHNH_2$), 1665 (>C═O of amide); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 6.80–7.90 (m, 4H, Ar–H), 4.40 (s, 2H, –NH_2), 4.81 (s, 2H, S–CH_2–), 7.88 (s, 1H, –CONH–), 8.4 (s, 1H, –NH–benzimidazole).

3.2.4. Conventional synthesis of 2. Compound **1** (0.01 mol, 2.36 g) and hydrazine hydrate (0.01 mol, 0.9 mL) in ethanol (20 mL) were refluxed for about 5 h on a steam bath. After cooling the resulting solid was filtered, dried, and recrystallized from ethanol to obtain compound **2**, yield 63%, as a pinkish white solid. Mp 195 °C.

3.2.5. Microwave assisted synthesis of 2-{(1*H*-benzimidazol)-ylthio}-*N*′-2-(4-nitrophenyl) hydrazide (3a). A mixture of compound **2** (0.01 mol, 2.22 g), 4-nitrobenzaldehyde (0.01 mol, 1.51 g) and 2–3 drops glacial acetic acid in ethanol (20 mL) were placed in a round-bottomed flask inside a microwave oven and irradiated (450 W, 76–78 °C) for 2–3 min.[20,21] After completion of reaction (monitored by TLC). The solvent was removed and residue recrystallized from chloroform–methanol mixture to afford compound **3**, yield 87%, as a dark yellow solid. Mp 161 °C. Anal. Calcd for $C_{16}H_{13}N_5O_3S$: C, 54.08; H, 3.66; N, 19.71. Found: C, 54.05; H, 3.65; N, 19.73%; IR (KBr): υ (cm^{-1}) 3340, 1335 (–NH–), 1668 (>C═O), 1626 (–N═CH–); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 4.40 (s, 1H, –N═CH–), 8.13 (s, 1H, –CONH–), 6.93–7.73 (m, 4H, Ar–H), 8.8 (s, 1H, –NH–benzimidazole).

3.2.6. Conventional synthesis of 3a. A mixture of compound **2** (0.01 mol, 2.22 g) and 4-nitrobenzaldehyde (0.01 mol, 1.51 g) and 2–3 drops of glacial acetic acid in ethanol (20 mL) was refluxed on a water bath for about 6 h. The solvent was removed and residue recrystallized from chloroform methanol mixture to yield compound **3a**, (61%) as a pale yellow powder. Mp 161 °C. Compounds **3b–j** were prepared similarly by treating **2** with various aromatic aldehydes.

3.2.7. Microwave assisted synthesis of 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-nitrophenyl- azetidin-2′-one) (4a). A mixture of **3a** (0.01 mol, 3.55 g) in DMF and chloroacetyl chloride (0.01 mol, 1.12 mL) with a catalytic amount of triethylamine (1 mL) was placed in a round-bottomed flask inside a microwave oven and irradiated (400 W, 143–145 °C) for 3 min.[20,21] After completion of the reaction (monitored by TLC), it was then diluted with ice cold water. The solid product formed was filtered, dried, and recrystallized from ethanol, yield 89%, as a brown solid. Mp 221 °C. Anal. Calcd for $C_{18}H_{14}N_4O_5S$: C, 54.54%; H, 3.53%; N, 17.67%. Found: C, 54.52%; H, 3.55%; N, 17.70%; IR (KBr): υ (cm^{-1}) 3340 and 1330 (–NH–), 1665 (>C═O, amidyl), 1717 (>C═O, cyclic), 1340 (Ar–NO_2). ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.53 (s, 1H, –CONH–), 7.00–7.95 (m, 8H, Ar–H), 3.15 (d, 1H,

>CH–Ar), 4.48 (s, 2H, S–CH_2–), 4.0 (s, 1H, >CH–Cl), 8.2 (s, 1H, –NH–benzimidazole). ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 127 (C_1), 128.9 (C_2, C_6),126.2 (C_3, C_5), 150 (C_4), 60 (> $C_{4'}$H–N <), 30 (–S–CH_2–), 172.5 (cyclic, > $C_{2'}$=O), 169.2 (amide, >C=O), 51 (> $C_{3'}$H–Cl), 157.5 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 396 [M^+] (24%), 240 (78%), 240 (78%), 225 (37%), 212 (31%), 197 (29%), 191 (34%), 163 (24%), 149 (35%), 117 (30%), 105 (38%), 91 (29%).

3.2.8. Conventional synthesis of 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-nitrophenyl-azetidin-2′-one) (4a). A mixture of **3a** (0.01 mol, 3.55 g) in benzene and chloroacetyl chloride (0.01 mol, 1.12 mL) with a catalytic amount of triethylamine (1 mL) was placed in a round-bottomed flask. It was refluxed for 5 h on a steam bath. After completion of the reaction (monitored by TLC), the benzene was distilled off to obtain product **4a**. The solid product was filtered, dried, and recrystallized from ethanol, yield 70%, as a brownish crystal. Mp 221 °C. Other compounds **4b–j** were prepared in the similar way using **3b–j**, respectively.

4. Spectroscopic data of compounds 4b–j

4.1. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(3,4,5-trimethoxyphenyl-azetidin-2′-one) (4b)

Mp 250 °C; Anal. Calcd for $C_{21}H_{21}N_4O_5S$: C, 57.16; H, 4.05; N, 15.27. Found: C, 57.14; H, 4.08; N, 15.25%; IR (KBr): υ (cm^{-1}) 3335, 1335 (–NH–), 1660 (>C=O, amidyl), 1720 (>C=O, cyclic), 2825 (Ar–OCH_3), 779 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.50 (s, 1H, –CONH–), 6.98–7.93 (m, 8H, Ar–H), 3.18 (d, 1H, >CH–Ar), 4.46 (s, 2H, S–CH_2–), 4.1 (s, 1H, >CH–Cl), 8.4 (s, 1H, –NH–benzimidazole), 3.91(s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 127.1 (C_1), 129.1 (C_2, C_6),126.4 (C_3, C_5), 153 (C_4), 52.3 (> $C_{4'}$H–N <), 30.1 (–S–CH_2–), 172 (cyclic, > $C_{2'}$=O), 168.2 (amide, >C=O), 58 (> $C_{3'}$H–Cl), 156.5 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics), 35.4 ($CH_3OC_6H_4$–); MS (*m*/*z*): 441[M^+] (69%), 250 (33%), 235 (17%), 222 (70%), 207 (83%), 191 (47%), 163 (27%), 149 (40%), 117 (32%), 105 (19%), 91 (49%), 82 (55%).

4.2. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(2-hydroxyphenyl-azetidin-2′-one) (4c)

Mp 141 °C; Anal. Calcd for $C_{18}H_{16}N_4O_3S$: C, 58.81; H, 4.09; N, 15.38. Found: C, 58.85; H, 4.07; N, 15.36%; IR (KBr): υ (cm^{-1}) 3290, 1338 (–NH–), 1670 (>C=O, amidyl), 1725 (>C=O, cyclic), 3590 (Ar–OH), 811 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.49 (s, 1H, –CONH–), 7.20–7.90 (m, 8H, Ar–H), 3.17 (d, 1H, >CH–Ar), 4.31 (s, 2H, S–CH_2–), 3.9 (s, 1H, >CH–Cl), 8.1 (s, 1H, –NH–benzimidazole), 3.65(s, 1H, –OH); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 126.9 (C_1), 128.8 (C_2, C_6), 127 (C_3, C_5), 155 (C_4), 51.9 (> $C_{4'}$H–N <), 53.3 (> $C_{3'}$H–Cl), 31.5 (–S–CH_2–), 175 (cyclic, > $C_{2'}$=O), 167.2 (amide, >C=O), 154.5 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 367[M^+] (70%), 191 (32%), 176 (45%), 163 (25%), 161 (47%), 149 (40%), 148 (25%), 133 (17%), 117 (15%), 105 (33%), 91 (23%), 82 (57%).

4.3. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(3-hydroxyphenyl-azetidin-2′-one) (4d)

Mp 153 °C; Anal. Calcd for $C_{18}H_{16}N_4O_3S$: C, 58.71; H, 4.10; N, 15.23. Found: C, 58.69; H, 4.07; N, 15.21%; IR (KBr): υ (cm^{-1}) 3333, 1341 (–NH–), 1680 (>C=O, amidyl), 1734 (>C=O, cyclic), 3571 (Ar–OH), 810 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.55 (s, 1H, –CONH–), 6.85–7.65 (m, 8H, Ar–H), 3.11 (d, 1H, >CH–Ar), 4.36 (s, 2H, S–CH_2–), 4.3 (s, 1H, >CH–Cl), 8.5 (s, 1H, –NH–benzimidazole), 3.64 (s, 1H, –OH); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 127.1 (C_1), 129.3 (C_2, C_6), 126.8 (C_3, C_5), 153.5 (C_4), 52.5 (> $C_{4'}$H–N <), 54.3 (> $C_{3'}$H–Cl), 32.5 (–S–CH_2–), 175.2 (cyclic, > $C_{2'}$–O), 166.7 (amide, >C=O), 153.8 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 366[M^+] (75%), 191 (40%), 175 (85%), 163 (48%), 160 (35%), 149 (50%), 147 (28%), 133 (42%), 117 (39%), 105 (22%), 91 (20%), 82 (52%).

4.4. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-hydroxyphenyl-azetidin-2′-one) (4e)

Mp 169 °C; Anal. Calcd for $C_{18}H_{16}N_4O_3S$: C, 59.80; H, 4.48; N, 14.71. Found: C, 59.84; H, 4.46; N, 14.69%; IR (KBr): υ (cm^{-1}) 3390, 1337 (-NH-), 1679 (>C=O, amidyl), 1730 (>C=O, cyclic), 3583 (Ar–OH), 818 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.46 (s, 1H, –CONH–), 6.88–7.90 (m, 8H, Ar–H), 3.12 (d, 1H, >CH–Ar), 4.45 (s, 2H, S–CH_2–), 4.6 (s, 1H, >CH–Cl), 8.7 (s, 1H, –NH–benzimidazole), 3.58(s, 1H, –OH); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 126.8 (C_1), 128.5 (C_2, C_6), 127.1 (C_3, C_5), 154 (C_4), 52.1 (> $C_{4'}$H–N <), 56 (> $C_{3'}$H–Cl), 32 (–S–CH_2–), 176.2 (cyclic, > $C_{2'}$=O), 168.7 (amide, >C=O), 155.8 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 368[M^+] (78%), 191 (80%), 177 (43%), 163 (51%), 162 (20%), 149 (54%), 134 (48%), 117 (15%), 105 (24%), 91 (28%), 82 (57%).

4.5. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(2-methoxyphenyl-azetidin-2′-one) (4f)

Mp 198 °C; Anal. Calcd for $C_{19}H_{17}N_4O_3S$: C, 59.99; H, 4.49; N, 14.71. Found: C, 60.00; H, 4.46; N, 14.69%; IR (KBr): υ (cm^{-1}) 3375, 1337 (–NH–), 1671 (>C=O, amidyl), 1726 (>C=O, cyclic), 2828 (Ar–OCH_3), 820 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.47 (s, 1H, –CONH–), 6.88–7.95 (m, 8H, Ar–H), 3.13 (d, 1H, >CH–Ar), 4.49 (s, 2H, S–CH_2–), 4.3 (s, 1H, >CH–Cl), 8.5 (s, 1H, –NH–benzimidazole), 3.96 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 126.8 (C_1), 128.7 (C_2, C_6),125.9 (C_3, C_5), 153.2 (C_4), 52.0 (> $C_{4'}$H–N <), 53.1 (> $C_{3'}$H–Cl), 32 (–S–CH_2–), 172.5 (cyclic, > $C_{2'}$=O), 168 (amide, >C=O), 157.5 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics), 35.7 ($CH_3OC_6H_4$-); MS (*m*/*z*): 381[M^+] (72%), 191 (44%), 190 (88%), 175 (43%), 163 (50%), 162 (54%), 149 (36%), 147 (29%), 117 (13%), 105 (27%), 91 (24%), 82 (52%).

4.6. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-methoxyphenyl-azetidin-2′-one) (4g)

Mp 218 °C; Anal. Calcd for $C_{19}H_{17}N_4O_3S$: C, 56.05; H, 3.65; N, 14.55. Found: C, 56.03; H, 3.63; N, 14.53%; IR (KBr): υ (cm^{-1}) 3378, 1339 (–NH–), 1675 (>C=O, amidyl), 1721 (>C=O, cyclic), 2830 (Ar–OCH_3), 816 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.53 (s, 1H, –CONH–), 6.65–7.77 (m, 8H, Ar–H), 3.16 (d, 1H, >CH–Ar), 4.29 (s, 2H, S–CH_2–), 4.2 (s, 1H, >CH–Cl), 8.2 (s, 1H, –NH–benzimidazole), 3.89 (s, 3H, –OCH_3); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 127.8 (C_1), 129.7 (C_2, C_6),124.9 (C_3, C_5), 152.2 (C_4), 52.3 (> $C_{4'}$H–N <), 54.1 (> $C_{3'}$H–Cl), 33 (–S–CH_2–), 171.5 (cyclic, > $C_{2'}$=O), 167 (amide, >C=O), 156.5 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics), 34.7 ($CH_3OC_6H_4$-); MS (*m*/*z*): 380 [M^+] (69%), 191 (47%), 189 (83%), 174 (40%), 163 (48%), 161 (50%), 149 (31%), 146 (30%), 117 (10%), 105 (30%), 91 (22%), 82 (50%).

4.7. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(2-chlorophenyl-azetidin-2′-one) (4h)

Mp 201 °C; Anal. Calcd for $_{18}H_{14}N_4O_2SCl$: C, 55.98; H, 3.63; N, 14.58. Found: C, 55.95; H, 3.62; N, 14.56%; IR (KBr): υ (cm^{-1}) 3385, 1340 (–NH–), 1678 (>C=O, amidyl), 1722 (>C=O, cyclic), 835 (Ar–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.50 (s, 1H, –CONH–), 6.72–7.82 (m, 8H, Ar–H), 3.17 (d, 1H, >CH–Ar), 4.38 (s, 2H, S–CH_2–), 4.1 (s, 1H, >CH–Cl), 8.1 (s, 1H, –NH–benzimidazole); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 128.8 (C_1), 128.7 (C_2, C_6),125.9 (C_3, C_5), 151.2 (C_4), 54.3 (> $C_{4'}$H–N <), 33.2 (–S–CH_2–), 171.7 (cyclic, > $C_{2'}$=O), 167.2 (amide, >C=O), 57.4 (> $C_{3'}$H–Cl), 156.7 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 385.5[M^+] (77%), 194 (51%), 191 (80%), 179 (45%), 166 (47%), 163 (51%), 151 (30%), 149 (29%), 117 (13%), 105 (29%), 91 (21%), 82 (54%).

4.8. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(3-chlorophenyl-azetidin-2′-one) (4i)

Mp 216 °C; Anal. Calcd for $C_{18}H_{14}N_4O_2SCl$: C, 56.24; H, 3.64; N, 14.60. Found: C, 56.25; H, 3.62; N, 14.58%; IR (KBr): υ (cm^{-1}) 3380, 1341 (–NH–), 1673 (>C=O, amidyl), 1719 (>C=O, cyclic), 825 (C–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.48 (s, 1H, –CONH–), 7.15–7.92 (m, 8H, Ar–H), 3.18 (d, 1H, >CH–Ar), 4.41 (s, 2H, S–CH_2–), 4.4 (s, 1H, >CH–Cl), 8.3 (s, 1H, –NH–benzimidazole); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 127.7 (C_1), 129.8 (C_2, C_6),124.8 (C_3, C_5), 152.3 (C_4), 53.2 (> $C_{4'}$H–N <), 34.3 (–S–CH_2–), 170.6 (cyclic, > $C_{2'}$=O), 168.3 (amide, >C=O), 58.3 (> $C_{3'}$H–Cl), 157.8 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 386[M^+] (76%), 195 (50%), 191 (79%), 180 (43%), 167 (46%), 163 (49%), 152 (28%), 149 (32%), 117 (17%), 105 (31%), 91 (24%), 82 (56%).

4.9. 1′-[(Benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-chlorophenyl-azetidin-2′-one) (4j)

Mp 209 °C; Anal. Calcd for $C_{18}H_{14}N_4O_2SCl$: C, 49.63; H, 3.26; N, 9.68. Found: C, 49.61; H, 3.23; N, 9.66%; IR (KBr): υ (cm^{-1}) 3383, 1342 (–NH–), 1668 (>C=O, amidyl), 1718 (>C=O, cyclic), 831 (Ar–Cl); ^{1}H NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 8.51 (s, 1H, –CONH–), 6.96–7.86 (m, 8H, Ar–H), 3.11 (d, 1H, >CH–Ar), 4.40 (s, 2H, S–CH_2–), 4.5 (s, 1H, >CH–Cl), 8.1 (s, 1H, –NH–benzimidazole); ^{13}C NMR ($CDCl_3$-DMSO-d_6): δ (ppm) 126.8 (C_1), 128.9 (C_2, C_6), 123.9 (C_3, C_5), 151.4 (C_4), 52.9 (> $C_{4'}$H–N <), 52.3 (> $C_{3'}$H–Cl), 33.4 (–S–CH_2–), 169.7 (cyclic, > $C_{2'}$=O), 167.4 (amide, >C=O), 156.7 ($C_{1''}$, $C_{2''}$, $C_{4''}$, $C_{5''}$, $C_{6''}$, $C_{7''}$, heteroaromatics); MS (*m*/*z*): 384[M^+] (79%), 193 (49%), 191 (83%), 178 (42%), 165 (49%), 163 (52%), 150 (31%), 149 (38%), 117 (20%), 105 (29%), 91 (23%), 82 (59%).

5. Antibacterial activity (the zone of inhibition)

The antimicrobial susceptibility testing (AST) was accomplished by the Kirby Bauer method of disc diffusion method. The sample solution was prepared by dissolving 10 μg of each of the compound in 1.0 mL of Dimethylformamide (DMF). The sterilized Whatman filter paper (No. 1) discs of approximately 6 mm were dipped in sample solution and dried in oven. These discs were placed on the medium previously seeded with the organisms in petri dishes at suitable distances. The petri dishes were stored in an incubator at 30 ± 2 °C for 24 h. The zone of inhibition thus formed around each disc containing the test compound was measured accurately in mm.

The synthesized derivatives **4a**–**j** were screened for their in vitro antibacterial activity against *Staphylococcus aureus* (ATCC-6538), *Bacillus substilis* (ATCC-6633) and *Escherichia coli* (ATCC-6538) using disc diffusion method.[25] Mueller–Hinton agar (Hi Media, Difco, Detroit, USA) was used for bacterial strains. Streptomycin was also screened under similar conditions for comparison.

6. Antifungal activity (the minimum inhibitory concentration)

Study design. Microdilution is used according to a standard protocol described by the NCCLS.[26,27] Three stains were tested each of the following species: *Candida albicans* (ATCC-64550), *Candida krusei* (ATCC-14243) and *Aspergillus niger*.

Medium. RPMI 1640 broth with L-glutamine without sodium bicarbonate and 0.165 μ MOPS buffer (34.54 *g*/L) was used. The medium was adjusted to pH 7.0 at 25 °C. Sterility of each bottle was performed before it was used.

Antifungal agents. Terbinafine was provided by the manufacturer as a standard powder. All drugs dissolved 100% dimethylsulfoxide according to the NCCLS methods.[26,27] The final drug concentrations were 50–0.01 μg/mL for all drugs.

Preparation of inoculum. The preparation of inoculum suspensions was based mainly on the NCCLS guideline[27] and as described previously.[28–30] For dermato-

phytes the final inoculum size was adjusted from 1.2×10^4 to 6×10^4 CFU/mL and for *C. albicans* it was approximately 1×10^3 and 5×10^3 CFU/mL.[26,31,32]

Test procedure. The test procedure was applied according to the NCCLS protocols.[26,27] Microdilution plates (96 U-shaped) were prepared and frozen at −70 °C until needed. Each microdilution well containing 100 μL of the 2-fold drug concentration was inoculated 100 μL of the final inoculum suspension. Two drug-free growth controls were included for each test plate, one without any drug (growth control) and the other with media containing an equivalent amount of solvent used to dissolved the drug (solvent control).

The fungal pathogens were maintained in RPMI 1640 Broth after incubation for 48 h at 25 ± 1 °C. Testing was performed in RPMI 1640 Broth at pH 7.0 and 2-fold dilution was applied. A set of tubes containing only inoculated broth were kept as controls. After incubation for 48 h at 25 ± 1 °C, the last tube with no fungal growth was recorded to represent minimum inhibitory concentration (MIC), expressed in μg/mL.

All of the compounds **4a**–**j** were tested for their in vitro growth inhibitory antifungal activity against *Candida albicans* (ATCC-64550), *Candida krusei* (ATCC-14243) and *Aspergillus niger*. Flucanozole (FLU) was also screened under similar conditions for comparison.

7. Conclusion

In conclusion, this new method for the synthesis of 2-azetidinones using catalytic amount of Et_3N in DMF under microwave irradiation offers significant improvements over existing procedures an thus, helps facile entry into a variety of 2-azetidinones of potentially high synthetic utility. Also, this simple and reproducible technique affords various 2-azetidinones with short reaction times, excellent yields and without formation of undesirable by-products.

A series of 2-azetidinone (β-lactam) derivatives were prepared and tested for their in vitro antibacterial activity against the three strains of bacteria (gram +ve, gram −ve) and antifungal activities against the three strains of human pathogenic fungi. Five compounds of the obtained series showed high in vitro antimicrobial activity. 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-nitrophenyl- azetidin-2′-one) (**4a**) showed excellent activity against *B. substilis*, 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(3-chlorophenyl-azetidin-2′-one) (**4i**) showed excellent activity against *S. aureus*, (**4i**) and 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(4-chlorophenyl-azetidin-2′-one) (**4j**) showed excellent activity against *E. coli* indicated in vitro antibacterial activity comparable to or slightly lower than that of streptomycin. 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(2-hydroxyphenyl-azetidin-2′-one) (**4c**) showed excellent activity against *C. krusei* and 1′-[(benzimidazol-2-yl)thioacetamidyl]-3′-chloro-4′-(2-methoxyphenyl-azetidin-2′-one) (**4f**) showed excellent activity against *A. niger* indicated in vitro antifungal activity comparable to or slightly lower than that of flucanozole. The different substituents on the aromatic ring exert a significant influence on the biological activity. The presence of electron-withdrawing group on the aromatic ring in general decreases the antimicrobial activities of test compounds compared to compounds having electron-donating groups. Based upon the results it will also be necessary to optimize the lead compound by substituting series of electron-donating groups on aromatic ring and selectively modifying the β-lactum nucleus. The substitution in the $C_{4\prime}$ position in of the phenyl ring by methoxy, chloro and polar group (phenolic or nitro moiety) seems to be very important for antifungal effect, as well as the presence and the position of the –CONH– group in the connecting linker between the benzimidazole and phenyl ring and β-lactam derivatives seems to be very important for antibacterial effect.

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References and notes

1. Guru, N.; Srivastava, S. D. *J. Sci. Ind. Res.* **2001**, *60*, 601.
2. Muhammad, M.; Bhat, I. *Indian J. Heterocycl. Chem.* **2003**, *13*, 183.
3. Biradar, J. S.; Manjunath *Indian J. Chem. (B)* **2004**, *43B*, 141.
4. Sharma, P.; Kumar, A. *Indian J. Chem. (B)* **2004**, *43B*, 385.
5. Desai, J. M.; Shah, V. H. *Indian J. Chem. (B)* **2003**, *42B*, 631.
6. (a) Banik, B. K.; Becker, F. F.; Banik, I. *Bioorg. Med. Chem.* **2004**, *12*(10), 2523; (b) Banik, I.; Becker, F. F.; Banik, B. K. *J. Med. Chem* **2003**, *46*(1), 12.
7. Anaya, Josefa; Gero, D. S.; Grande, Manuel; Hernando, José Ignacio M.; Laso, N. M. *Bioorg. Med. Chem.* **1999**, *7*(5), 837.
8. Gerona-Navarro, G.; Perez de Vega, M. J.; Garcia-Lopez, M. T.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J.; Gonzalez-Muniz, R. *J. Med. Chem.* **2005**, *48*(7), 2612.
9. (a) Singh, G. S.; Mmolotsi, B. J. *IL Farmaco* **2005**, *60*(9), 727; (b) Güner, Vıldan; Yıldırır, Süleyman; Özçelık, Berrın; Abbaso, Lu. U. *IL Farmaco* **2000**, *55*(2), 147.
10. Veinberg, Grigory; Shestakova, Irina; Vorona, Maxim; Kanepe, Iveta; Lukevics, Edmunds *Bioorg. Med. Chem. Lett.* **2004**, *14*, 47.
11. Gerona-Navarro, Guillermo; Jesús Pérez de Vega, M.; Teresa García-López, M.; Andrei, Graciela; Snoeck, Robert; Balzarini, Jan; De Clercq, Erik; González-Muñiz, Rosario *Bioorg. Med. Chem. Lett.* **2004**, *14*(9), 2253.
12. Afonso, A.; Hon, F.; Fett, N.; Weinstein, J.; Ganguly, A. K.; Naples, L.; Hare, R. S.; Miller, G. H. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2793.
13. Aoyama, Y.; Uenaka, M.; Kii, M.; Tanaka, M.; Konoike, T.; Hayasaki-Kajiwara, Y.; Naya, N.; Nakajima, M. *Bioorg. Med. Chem.* **2001**, *9*, 3065.

14. (a) Varma, S. *Green Chem.* **1999**, *1*, 43; (b) Desai, K. G.; Desai, K. R. *J. Sulfur Chem. (Taylor and Francis Groups)* **2006**, *27*, 315.
15. Borah, R.; Kalita, D. J.; Sharma, J. C. *Indian J. Chem* **2002**, *41B*, 1032.
16. Kidwai, M.; Dave, B. *Indian J. Chem* **2002**, *41B*, 2430.
17. (a) Patel, V. M.; Desai, K. R.; *ARKIVOC (USA)* (www.arkat-usa.org). **2004**, *1*, 123; (b) Desai, K. G.; Desai, K. R.; Raval, J. P. *J. Iranian Chem. Soc.* **2006**, *3*, 233.
18. Desai, K. G.; Desai, K. R.; *Molbank* (www.mdpi.org/molbank) (*Switzerland*), 2006, M475 (MOL File: m477.mol).
19. (a) Desai, K. G.; Desai, K. R.; D. Padmanabhan.; *Molbank* (www.mdpi.org/molbank) (Switzerland), 2006, M475 (MOL File: m478.mol); (b) Desai, K. G.; Desai, K. R. *Indian J. Chem. (B)* **2005**, *B*, 2096.
20. Desai, K. G.; Desai, K. R. *Indian J. Chem. (B)* **2005**, *44B*, 2093.
21. Desai, K. G.; Desai, K. R. *J. Saudi Chem. Soc.* **2006**, *9*, 631.
22. Loupy, A.; Perreux, L.; Moneuse, M. *Pure. Appl. Chem.* **2001**, *11*, 161.
23. National committee for clinical laboratory standards, 1997, Reference method for broth dilution antifungal susceptibility testing of yeast. Approved standard NCCLS document M27 A. (ISBN 1 56238 328 0, ISSN 0273 3099). National committee for clinical laboratory standards, 940, West Valley Road, Suite 1400, Wayne, Pennsylvania, 19807.
24. Kidwai, M.; Mothsra, P. *Syn. Commun.* **2006**, *36*, 817.
25. National committee for clinical laboratory standards, 2000.
26. National committee for clinical laboratory standards, 1997.
27. National committee for clinical laboratory standards, 1998.
28. (a) Fernandez, B.; Cabanes, A. J.; Inza, I. *J. Clin. Microb.* **2002**, *40*, 4003; (b) Desai, K. G.; Desai, K. R. *J. Heterocyclic Chem.* **2006**, *43*, 1083.
29. Fernandez, B.; Carillo, A. J.; Martin, E. *Antimicrob. Agents Chemother.* **2001**, *45*, 2528.
30. Gupta, A. K.; Kohli, Y. *Brit. J. Dermatol.* **2003**, *149*, 305.
31. Kigh, C. W.; Famthing, R. A.; Rinaldi, M. G. *J. Clin. Microb.* **1992**, *30*, 3145.
32. Berenguer, J.; Sanchez, R.; Matinez, J. V. *Antimicrob. Agents Chemother.* **1996**, *40*, 2003.