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Synthesis and microbial inhibition study of novel 5-imidazolyl substituted isoxazolidines

M. P. Sadashiva,[a] H. Mallesha,[a] N. A. Hitesh[b] and K. S. Rangappa[a,*]

[a]*Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India*
[b]*Department of Studies in Biochemistry, University of Mysore, Manasagangotri, Mysore 570 006, India*

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Abstract—Cycloaddition of *C*-imidazolyl-*N*-phenylnitrones with monosubstituted alkenes afforded 5-imidazolyl substituted isoxazolidines with high regioselectivity. Novel isoxazolidines were screened for their antibacterial activities against *S. aureus*, *E. coli* and *B. subtilis* by using streptomycin as a positive control. They were also tested for their antifungal activities against *F. moniliforme*, *A. niger* and *C. acremonium* by using nystatin as a positive control. Isoxazolidines, **4a** and **4f** exhibited more potent inhibition towards antifungal activity than the other isoxazolidines prepared.

1. Introduction

The frequency of microbial infection in man has increased dramatically because of multi-drug resistant microbial isolates (e.g., fungi and bacteria).[1–4] Hence, the search of novel and active antimicrobial agents to treat serious microbial infection has increased significantly over the last two decades. Currently, physicians use three major classes of prescription therapies to treat serious fungal infections:[3,5,6] (a) amphotericin B, a polyene; (b) azoles, including ketoconazole, fluconazole and itraconazole; and (c) the allylamine terbinafine. Azoles are safer and more water-soluble than amphotericin B. Hence, the chemistry of imidazoles occupies an extremely important niche possessing diverse pharmacological activity[7–12] within the family of five-membered heterocyclic compounds.

In our previous studies,[13–17] we have described the synthesis of biologically active isoxazolidines via 1,3-dipolar cycloaddition reactions of nitrones[18–20] with olefins. Literature survey reveals that imidazolyl moieties are rarely included in the preparation of isoxazolidines.[21,22] Recently, we have identified 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-substituted δ^2-isoxazoline as a new structural alternative for strong antifungal activity in the substituted imidazolyl isoxazoline class.[11] In our continuing interest, we recognized an opportunity to apply our knowledge of 1,3-dipolar species to the synthesis of a series of novel 5-imidazolyl substituted isoxazolidines through the 1,3-dipolar cycloaddition reactions of *C*-imidazolyl-*N*-phenylnitrones with monosubstituted alkenes. The resulting 5-imidazolyl substituted isoxazolidines were expected to differ in their chemical and antimicrobial properties from the known azole derivatives, thus providing valuable information of a new generation of antimicrobial agents.

2. Chemistry

2-*n*-Butyl-4-chloro-1*H*-imidazole-5-carboxaldehyde, **1** was synthesized according to the procedure reported earlier.[23] *C*-Imidazolyl-*N*-phenylnitrones, **3**/**3′** were synthesized by the reduction of a mixture of nitro compounds, **2** and imidazoaldehyde, **1** with zinc dust using histidine as a catalyst.[18] The novel 5-imidazolyl substituted isoxazolidines, **4**/**4′** were prepared by 1,3-dipolar cycloaddition of nitrones, **3**/**3′** with monosubstituted olefins as briefed in Scheme 1.

3. Results and discussion

Arylhydroxylamines of corresponding nitro compounds were generated in situ and were condensed with **1** to

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* Corresponding author. Tel.: +91 821 2412191; fax: +91 821 2518835/2421263; e-mail: rangappaks@chemistry.uni-mysore.ac.in

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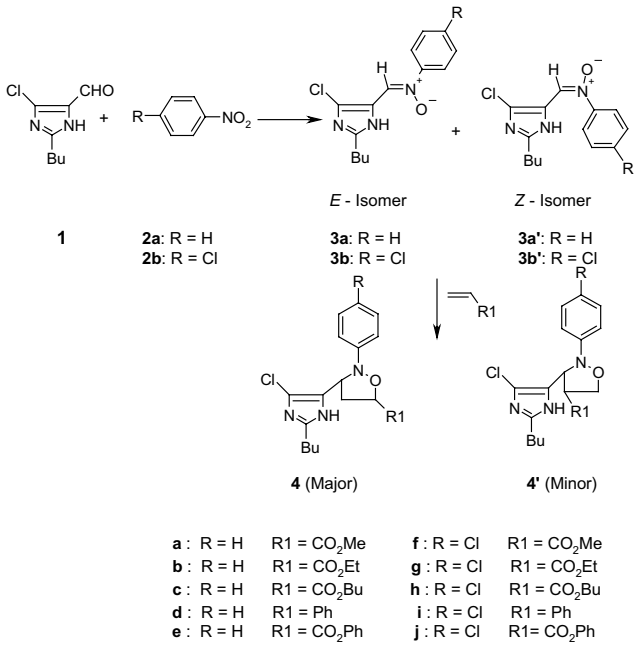

Scheme 1.

yield a mixture of nitrones *E* (**3**) and *Z* (**3′**) isomers. The *E*- and *Z*-isomers were formed in the ratio of 65:35 (a: R = H) and 60:40 (b: R = Cl). The mixtures, after evaluating by 300 MHz ^{1}H NMR, were directly used for cycloaddition with olefins. The 1,3-dipolar-cycloaddition reaction of nitrones, **3/3′** with monosubstituted olefins was carried out in refluxing toluene as solvent and obtained two regioisomeric 5-imidazolyl substituted isoxazolidines **4** and **4′** with high regioselectivity. Ratio of regioisomers, **4** and **4′** was evaluated by integration from 300 MHz ^{1}H NMR spectra of crude reaction mass after passing through a short silica gel column using H/EA (5:1) as eluent (Table 1). The major isomers, **4** were separated either by recrystallization or using appropriate combination of solvents as eluent as shown in the Table 1. Isoxazolidines, **4** showed a set of ^{1}H NMR signals at δ 2.43–2.74 (ddd), 2.69–3.17 (ddd), 4.32–5.50 (dd) and 4.56–5.93 (dd) for C-4H$_\alpha$, C-4H$_\beta$, C-3H and C-4H, respectively. The yields of **4** were in the range of 59–76% with high purity, it is found to be absent of minor isomers, **4′** supported by ^{1}H NMR signals. All novel isoxazolidines **4a–j** were structurally characterized by ^{1}H NMR, IR and elemental analysis.

4. Antibacterial activity

All novel isoxazolidines, **4a–j** were screened for their antibacterial activity in blotter disc method by using streptomycin as a positive control. The minimum inhibitory concentration (MIC) values of **4a–j** against *Staphylococcus aureus*, *E. coli* and *B. subtilis* are summarized in Table 2. Antibacterial activity of **4c**,**d**,**h** and **4i** showed about 50% inhibition when compared with streptomycin and remaining were not effective against any of the three strains.

Table 1. Reaction conditions and physical data of 5-imidazolyl substituted isoxazolidines (**4a–j**)

Isoxazolidine	Solvent[a]	Time (h)	R^f value[b]	Ratio[c] of **4/4′**	Eluent[d]	Yield[f] of **4** in %	Mp (°C)
4a	Toluene	26	0.33	77/23	H:EA (7:1)	60	Oil
4b	Toluene	26	0.38	81/19	H:EA (9:1)	62	Oil
4c	Toluene	28	0.48	87/13	H:EA (9:1)	59	Oil
4d	Xylene	34	0.32	89/11	H:EA (8:1)	76	Oil
4e	Xylene	36	0.35	90/10	H:EA (4:1)[e]	72	128
4f	Toluene	24	0.38	78/22	H:EA (9:1)	61	Oil
4g	Toluene	24	0.41	84/16	H:EA (9:1)	60	Oil
4h	Toluene	26	0.48	90/10	H:EA (9:1)	60	Oil
4i	Xylene	27	0.38	91/9	H:EA (8:1)	72	Oil
4j	Xylene	29	0.31	93/7	H:EA (4:1)[e]	63	134

[a] Used for cycloaddition reaction.
[b] Used 7:1 ratio of H (hexane)/EA (ethylacetate).
[c] Regioisomeric ratio of crude ascertained by ^{1}H NMR (300 MHz) after passing through a short column using H/EA (4:1).
[d] Used for purification of isoxazolidines.
[e] Recrystallized in alcohol after passing through a short column.
[f] Calculated with respect to nitrone.

Table 2. The minimum antibacterial inhibition concentrations of **4a–j** against *S. aureus*, *E. coli* and *B. subtilis* by Bolter disc method; concentration: 10 mg/mL; vol. of sample taken from the stock soln −10 μL

Micro-organisms	Zone of inhibition (1 mm)										
	Compounds										
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	Streptomycin
S. aureus	—	6	21	17	2	—	5	18	16	—	35
E. coli	—	5	19	19	3	2	4	16	18	2	36
B. subtilis	2	9	20	19	7	—	8	21	17	—	35

5. Antifungal activity

All novel isoxazolidines **4a**–**j** were screened for their antifungal activity by two methods, cup borer and turbidometric, using nystatin as a positive control. The MIC values of **4a**–**j** against *Fusarium moniliforme*, *Aspergillus niger* and *Cephalosporium acremonium* are summarized in Tables 3–5.

Compounds **4a** and **4f** exhibited better antifungal activity than *nystatin*. The MIC values of **4a** were 17, 15 and 23 while **4f** were 13, 15 and 17 against *F. moniliforme*, *A. niger* and *C. acremonium*, respectively. The MIC values of these compounds were less than 50% when compared with the corresponding values of nystatin (Table 5). The antifungal potential of **4a** and **4f** are found to be dose dependant (Tables 3 and 4). Compounds **4e** and **4g** exhibited similar inhibition to that of nystatin against *A. niger*. Compound **4j** showed similar activity against *F. moniliforme* but not effective against the other two fungal strains. Therefore, compounds **4e**,**g** and **4j** are strain dependant.

6. Conclusion

Isoxazolidines, **4a**–**j** were synthesized and screened for their antimicrobial activities (antifungal and antibacterial). Amongst those tested, **4a** and **4f** exhibited more potent antifungal activity against *F. moniliforme*, *A. niger* and *C. acremonium*.

7. Experiment

7.1. General

Melting points were determined on SELACO-650 instrument and are uncorrected. The IR was recorded on a Perkin–Elmer model RX1 FT-IR spectrophotometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-300 spectrometer at 300 and 75 MHz, respectively, using TMS as internal standard. The chemical shift values are on the δ scale and the coupling constants (J) are in Hertz. The elemental analyses were obtained on a Vario-EL instrument. The analytical TLCs were performed on a coated Merck silica gel $60F_{254}$ plates; the spots were detected either under UV light (or) by charring with 4% alcoholic H_2SO_4. All extracted solvents were dried over Na_2SO_4, followed by evaporation in vacuum.

Table 5. The minimum antifungal inhibitory concentrations (MIC) μM of (**4a**–**j**) by Turbidometric method

Isoxazolidine	*F. moniliforme*	*A. niger*	*C. acremonium*
4a	17 ± 1 ± 0.6	15 ± 0.3	23 ± 1.3
4b	135 ± 2.1	112 ± 1.7	93 ± 2.7
4c	160 ± 3.2	83 ± 2.7	97 ± 1.9
4d	156 ± 2.1	121 ± 1.7	98 ± 1.5
4e	85 ± 1.3	27 ± 0.9	95 ± 2.5
4f	13 ± 0.7	15 ± 1.1	17 ± 0.5
4g	95 ± 1.4	31 ± 1.2	127 ± 3.0
4h	83 ± 2.3	107 ± 2.4	79 ± 3.0
4i	115 ± 3.0	68 ± 2.7	97 ± 2.7
4j	85 ± 3.0	76 ± 1.7	31 ± 2.1
Nystatin	35	32	39

7.2. Synthesis

Nitrones, **3a**,**a′** and **3b**,**b′** were prepared according to the procedure reported earlier.[18]

A mixture of nitrones *E* (**3a**) and *Z* (**3a′**) isomers (yellow oil, 0.42 mg, 51%) was obtained from nitrobenzene **2a** (0.33 mL, 2.68 mmol) and 2-butyl-4-chloro-1*H*-imidazole formaldehyde **1** (0.5 g, 2.68 mmol).

7.2.1. *E*-*C*-(2-Butyl-4-chloro-1*H*-imidazolyl)-*N*-phenylnitrone 3a. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.93 (t, 3H, CH_3); 1.38 (m, 2H, CH_2); 1.75 (m, 2H, CH_2); 2.75 (t, 2H, CH_2); 7.32–7.48 (m, 3H, Ar–H, merged with *Z*

Table 3. Determination of MIC of (**4a**–**j**) against *F. moniliforme*, *A. niger C. acremonium* by Cup borer method; concentration: 1 mg/mL; vol. of sample taken from the stock soln −50 μL

Microorganism	Zone of inhibition (1 mm)										
	Compounds										
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	Nystatin
F. moniliforme	21 ± 1	—	1	—	8	22 ± 1	5	—	1	7	12
A. niger	21 ± 2	2	—	2	7	23 ± 2	6	—	—	3	13
C. acremonium	23 ± 1	1	—	—	3	23 ± 2	5	—	—	2	13

Table 4. Determination of MIC of (**4a**–**j**) against *F. moniliforme*, *A. niger*, *C. acremonium* by Cup borer method; concentration: 1 mg/mL; vol. of sample taken from the stock soln −100 μL

Microorganism	Zone of inhibition (1 mm)										
	Compounds										
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	Nystatin
F. moniliforme	31 ± 1	4	2	2	5	37 ± 1	7	1	2	15 ± 1	12
A. niger	37 ± 1	7	—	3	15 ± 1	39 ± 1	11	—	—	5	13
C. acremonium	35 ± 1	6	—	1	7	43 ± 1	8	—	1	5	13

isomer); 7.76–7.78 (m, 2H, Ar–H, merged with *Z* isomer); 7.97 (s, 1H, CH=N); 12.13 (s, 1H, NH). IR (KBr): 3230, 3045, 2863, 2857, 1548, 1176, 893, 740, 660 cm^{-1}. Anal. Calcd CHN: 60.54, 5.81, 15.13. Found 60.63, 5.84, 15.18.

7.2.2. *Z*-*C*-(2-Butyl-4-chloro-1*H*-imidazolyl)-*N*-phenyl-nitrone 3a′. δ 0.76 (t, 3H, CH_3); 1.11 (m, 2H, CH_2); 1.49 (m, 2H, CH_2); 2.36 (t, 2H, CH_2); 7.32 (s, 1H, CH=N); 7.32–7.48 (m, 3H, Ar–H, merged with *E* isomer); 7.76–7.78; (m, 2H, Ar–H, merged with *E* isomer); 12.72 (br s, 1H, NH). IR (KBr): 3230, 3045, 2863, 2857, 1548, 1176, 893, 740, 660 cm^{-1}. Anal. Calcd CHN: 60.54, 5.81, 15.13. Found 60.63, 5.84, 15.18.

A mixture of nitrones *E* (**3b**) and *Z* (**3b′**) isomers (yellow oil, 0.44 mg, 48%) was obtained from 4-chloronitrobenzene **2b** (0.42 g, 2.68 mmol) and 2-butyl-4-chloro-1*H*-imidazole formaldehyde **1** (0.5 g, 2.68 mmol).

7.2.3. *E*-*C*-(2-Butyl-4-chloro-1*H*-imidazolyl)-*N*-(4-chloro-phenyl) nitrone 3b. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 3H, CH_3); 1.35 (m, 2H, CH_2); 1.73 (m, 2H, CH_2); 2.68 (t, 2H, CH_2); 7.71(d, 2H, Ar–H); 7.93 (s, 1H, CH=N); 8.46 (d, 2H, Ar–H); 12.41 (s, 1H, NH); IR (KBr): 3220, 3015, 2854, 2863, 1566, 1166, 903, 790, 688 cm^{-1}. Anal. Calcd CHN: 53.86, 4.84, 13.46. Found 53.91, 4.89, 13.42.

7.2.4. *Z*-*C*-(2-Butyl-4-chloro-1*H*-imidazolyl)-*N*-(4-chloro-phenyl) nitrone 3b′. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.76 (t, 3H, CH_3); 1.12 (m, 2H, CH_2); 1.51 (m, 2H, CH_2); 2.37 (t, 2H, CH_2); 7.30 (s, 1H, CH=N); 7.74 (d, 2H, Ar–H); 8.53 (d, 2H, Ar–H); 12.71 (br s, 1H, NH). IR (KBr): 3220, 3015, 2854, 2863, 1566, 1166, 903, 790, 688 cm^{-1}. Anal. Calcd CHN: 53.86, 4.84, 13.46. Found 53.91, 4.89, 13.42.

7.3. General procedure for the synthesis of novel isoxazolidines 4a–j

To a solution of the corresponding nitrone (1 mmol) in toluene/xylene was added monosubstituted alkene (50 mmol) and the resulting solution was refluxed until reaction completes, monitored by TLC. Reaction mixture was cooled to room temperature and concentrated under reduced pressure. The regioisomeric ratio (rr%) of the residue was determined by ^{1}H NMR analysis. The crude material was purified by silica gel column using appropriate eluent to give major product **4** with 96% purity analysed by ^{1}H NMR.

7.3.1. 2-(Phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-methylate isoxazolidine 4a. This compound was obtained by the cycloaddition of **3a** (0.4 g, 1.37 mmol) with methyl acrylate (4.7 mL, 50 mmol). Brownish thick liquid, 0.24 g (60%). IR (KBr): 3230, 3058, 2962, 1730, 1254, 1080, 820, 754, 694 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.87 (t, 3H, CH_3); 1.21–1.38 (m, 2H, CH_2), 1.65–1.83 (m, 2H, CH_2); 2.51 (ddd, 1H, H_4, J = 12.58, 8.70 and 5.10 Hz); 2.61 (t, 2H, CH_2); 3.03 (ddd, 1H, H_4 J = 12.40, 6.3 and 4.0 Hz); 3.78 (s, 3H, OCH_3); 4.84 (dd, 1H, J = 8.40 and 5.12 Hz); 4.98 (dd, 1H, CH, J = 8.6 and 5.3 Hz); 7.25–7.44 (m, 5H, Ar–H); 10.73 (br s, 1H, NH). Anal. Calcd CHN: 59.42, 6.05, 11.55. Found 59.45, 6.11, 11.58.

7.3.2. 2-(Phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-ethylate isoxazolidine 4b. This compound was obtained by the cycloaddition of **3a** (0.42 g, 1.44 mmol) with ethyl acrylate (5.4 mL, 50 mmol). Brownish thick liquid, 0.26 g (62%). IR (KBr): 3258, 3071, 2950, 1730, 1250, 1075, 828, 750, 692 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (t, 3H, CH_3); 1.14 (t, 3H, CH_3); 1.23–1.40 (m, 2H, CH_2); 1.61–1.80 (m, 2H, CH_2); 2.45 (ddd, 1H, H_{4a}, J = 13.3, 8.2 and 3.0 Hz); 2.72 (t, 2H, CH_2); 2.98 (ddd, 1H, H_{4b}, J = 13.3, 7.6 and 5.1 Hz); 4.18 (q, 3H, OCH_2); 4.85 (dd, 1H, H_3, J = 8.6 and 5.1 Hz); 4.99 (dd, 1H, CH, J = 8.5 and 4.8 Hz); 6.96 (m, 3H, Ar–H); 7.15 (m, 2H, Ar–H); 10.55 (br s, 1H, NH). Anal. Calcd CHN: 60.40, 6.40, 11.12. Found 60.43, 6.44, 11.09.

7.3.3. 2-(Phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidines 4c. This compound was obtained by the cycloaddition of **3a** (0.44 g, 1.5 mmol) with butyl acrylate (7.2 mL, 50 mmol). Brownish thick liquid 0.26 g (59%). IR (KBr): 3247, 3062, 2959, 2873, 173, 1598, 1258, 1085, 825, 757, 694, 598 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.81–1.01 (m, 6H, CH_3); 1.22–1.42 (m, 4H, CH_2); 1.55–1.82 (m, 4H, CH_2); 2.65 (t, 2H, CH_2); 2.43 (ddd, 1H, H_{4b}, J = 13.4 5,87 and 4.7 Hz); 2.99 (ddd, 1H, H_{4b}, J = 13.28, 7.96 and 5.3 Hz); 3.08 (t, 2H, OCH_2); 4.57 (dd, 1H, CH, J = 8.8 and 5.4 Hz); 4.82 (dd, 1H, CH, J = 8.4 and 4.7 Hz); 6.95–7.15 (m, 3H, Ar–H); 7.22–7.35 (m, 2H, Ar–H); 10.5 (br s, 1H, NH). Anal. Calcd CHN: 62.14, 6.95, 10.35. Found 62.19 7.06, 10.38.

7.3.4. 3-(2-Butyl-4-chloro-1*H*-imidazolyl)-2,5-diphenylisoxazolidine 4d. This compound was obtained by the cycloaddition of **3a** (0.48 g, 1.65 mmol) with styrene (5.73 mL, 50 mmol). White solid 0.364 g (76%). IR (KBr): 3428, 3033, 2956, 2867, 2745, 2664, 1487, 1452, 1598, 1238, 1023, 760, 697 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.90 (t, 3H, CH_3); 1.34 (m, 2H, CH_3); 1.65 (m, 2H, CH_2); 2.61 (t, 2H, CH_2); 2.48–2.61 (ddd, 1H, H_{4a}, J = 13.0, 6.9 and 2.7 Hz); 3.07–3.16 (ddd, 1H, H_{4b}, J = 13.66, 6.75 and 4.2 Hz); 5.10 (dd, 1H, CH, J = 8.10 and 5.7 Hz); 5.24 (dd, 1H, CH, J = 8.4 and 6.90 Hz); 7.0 (q, 2H, Ar–H); 7.11 (d, 2H, Ar–H, J = 7.8 Hz); 7.24–7.44 (m, 6H, Ar–H); 9.68 (br s, 1H, NH). Anal. Calcd CHN: 69.20, 6.33, 11.00. Found 69.28, 6.37,11.12.

7.3.5. 2-(Phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-benzoate isoxazolidine 4e. This compound was obtained by the cycloaddition of **3a** (0.43 g, 1.47 mmol) with vinyl benzoate (4.7 mL, 50 mmol). Brownish thick liquid 0.31 g (71.9%). IR (KBr): 3019, 2963, 2875, 1732, 1597, 1260, 1026, 1489, 1454, 866, 759, 659, 668 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.02 (t, 3H, CH_3); 1.41 (m, 2H, CH_2); 1.68 (m, 2H, CH_2); 2.66 (ddd, 1H, H_{4a}, J = 13.3, 7.8 and 5.6 Hz); 2.72 (t, 2H, CH_2); 2.92 (ddd, 1H, H_{4b}, J = 13.3, 7.0 and 5.1 Hz); 5.5 (dd, 1H, CH, J = 7.3 and 5.4 Hz); 5.93 (dd, 1H, CH, J = 8.2 and 6.1 Hz); 7.05–8.0 (m, 10H, Ar–H); 10.8 (br s, 1H, NH).

Anal Calcd CHN: 64.86, 5.68, 9.87. Found 64.91, 5.70, 9.89.

7.3.6. 2-(4-Chlorophenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-methylate isoxazolidine 4f. This compound was obtained by the cycloaddition of **3b** (0.44 g, 1.42 mmol) with methyl acrylate (4.7 mL, 50 mmol). Brownish colour 0.268 g (61%). IR (KBr): 3260, 3088, 2862, 1740, 1260, 1060, 840, 754, 684 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): 0.96 (t, 3H, CH_3); 1.30 (m, 2H, CH_2); 1.83 (m, 2H, CH_2); 2.71 (t, 2H; CH_2); 2.57 (ddd, 1H, H_{4a}, $J = 13.6$, 9.4 and 2.6 Hz); 2.78 (ddd, 1H, H_{4b} $J = 13.6$, 6.7 and 4.0 Hz); 3.73 (s, 3H, OCH_3); 4.42 (dd, 1H, CH $J = 8.8$ and 5.5 Hz); 4.56 (dd, 1H, CH, $J = 8.8$ and 5.8 Hz); 7.05 (d, 2H, Ar–H, $J = 8.0$ Hz); 7.39 (d, 2H, Ar–H, $J = 8.3$ Hz); 10.6 (br s, 1H, NH). Anal. Calcd CHN: 54.28, 5.31, 10.55. Found 54.31, 5.38, 10.50.

7.3.7. 2-(4-Chlorophenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-ethylate isoxazolidine 4g. This compound was obtained by the cycloaddition of **3b** (0.4g, 1.3 mmol) with ethyl acrylate (7.2 mL, 50 mmol). Brownish colour 0.24 g (60%). IR (KBr): 3268, 3081, 2960, 1736, 1255, 1085, 838, 760, 698 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.97 (t, 3H, CH_3); 1.14 (t, 3H, CH_3); 1.38 (m, 2H, CH_2); 1.88 (m, 2H, CH_2); 2.69 (t, 2H, CH_2); 2.55 (ddd, 1H, H_{4a}, $J = 13.3$, 8.2 and 3.0 Hz); 2.69 (ddd, 1H, H_{4b}, $J = 13.3$, 7.6 and 5.1 Hz); 4.19 (s, 2H, OCH_2); 4.42 (dd, 1H, H_3, $J = 8.6$ and 5.1 Hz); 4.88 (dd, 1H, CH, $J = 8.5$ and 4.8 Hz); 7.04 (d, 2H, Ar–H, $J = 8.0$ Hz); 7.49 (d, 2H, Ar–H, $J = 8.3$ Hz); 10.53 (br s, 1H, NH). Anal Calcd CHN: 55.35, 5.62, 10.20. Found 55.41, 5.66, 10.16.

7.3.8. 2-(4-Chlorophenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine 4h. This compound was obtained by the cycloaddition of **3b** (0.42 g, 1.37 mmol) with butyl acrylate (7.2 mL, 50 mmol). Brownish colour 0.25 g (60%). IR (KBr): 3239, 3042, 2966, 2822, 1738, 1608, 1260, 1088, 830, 767, 704, 618 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.00–1.06 (m, 6H, CH_3); 1.24–1.40 (m, 4H, CH_2); 1.68–1.84 (m, 4H, CH_2); 2.60 (t, 2H,CH_2); 2.53 (ddd, 1H, H_{4b}, $J = 13.45$, 8.7 and 4.7 Hz); 3.07 (t, 2H, OCH_2); 3.17 (ddd, 1H, H_{4b}, $J = 13.28$, 7.96 and 5.3 Hz); 4.32 (dd, 1H, CH, $J = 8.8$ and 5.4 Hz); 4.92 (dd, 1H, CH, $J = 8.4$ and 4.7 Hz); 7.11 (d, 2H, Ar–H, $J = 8.0$ Hz); 7.39 (d, 2H, Ar–H, $J = 8.23$ Hz); 10.66 (br s, 1H, NH). Anal. Calcd CHN: 57.28, 6.18, 9.54. Found 57.31, 6.21, 9.51.

7.3.9. 2-(4-Chlorophenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-phenyl isoxazolidine 4i. This compound was obtained by the cycloaddition of **3b** (0.44 g, 1.4 mmol) with styrene (5.7 mL, 50 mmol). Pure white solid 0.32 g (72%). IR (KBr): 3328, 3066, 2946, 2877, 2790, 2688, 1487, 1460, 1608, 1250, 1045, 770, 677 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 0.94 (t, 3H, CH_3); 1.36 (m, 2H, CH_2); 1.68 (m, 2H, CH_2); 2.64 (t, 2H, CH_2); 2.50–2.58 (ddd, 1H, H_{4a}, $J = 13.0$, 6.99 and 2.7 Hz); 3.17–3.16 (ddd, 1H, H_{4b}, $J = 13.66$, 6.75, 4.2 Hz); 5.14 (dd, 1H, CH, $J = 8.10$ and 5.74 Hz); 5.27 (dd, 1H, CH, $J = 8.4$ and 6.90 Hz); 7.0 (d, 2H, Ar–H, $J = 8.6$ Hz); 7.2 (d, 2H, Ar, $J = 7.8$ Hz); 7.24–7.44 (m, 5H, Ar–H); 10.18 (br s, 1H, NH). Anal. Calcd CHN: 63.47, 5.57, 10.10. Found 63.50, 5.61,10.07.

7.3.10. 2-(4-Chlorophenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-benzoate isoxazolidine 4j. This compound was obtained by the cycloaddition of **3b** (0.46 g, 1.5 mmol) with vinylbenzoate (6.9 mL, 50 mmol). Brownish colour 0.29 g (63%). IR (KBr): 3023, 2973, 2877, 1742, 1607, 1268, 1036, 1497, 1476, 869, 767, 688, 660 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.10 (t, 3H, CH_3); 1.46 (m, 2H, CH_2); 1.74 (m, 2H, CH_2); 2.8 (t, 2H, CH_2); 2.74 (ddd, 1H, H_{4a}, $J = 13.3$, 7.8 and 5.6 Hz); 2.99 (ddd, 1H, H_{4b}, $J = 13.3$, 7.0 and 5.1 Hz); 5.7 (dd, 1H, CH, $J = 7.3$ and 5.4 Hz); 6.01 (dd, 1H, CH, $J = 8.2$ and 6.1 Hz); 7.05 (d, 2H, Ar–H, $J = 8.2$ Hz); 7.39 (d, 2H, Ar–H); 7.44–7.51 (m, 5H, Ar–H). Anal. Calcd CHN: 60.00, 5.03, 9.13, found 60.09, 5.08, 9.12.

8. Biological assay

8.1. Antibacterial activity test

1. *Bacterial strains used for the antibacterial activity*: *S. aureus* (ATCC10832D), *E. coli* (ATCC700926D) and *B. subtilis* (ATCC23857D).
2. *Preparation of test sample*: 10 mg of each of the isoxazolidines were dissolved in 1 mL of DMSO, which served as a stock. From the stock, 10 μL was loaded on 1 mm sterile filter discs (Sartorius-Germany) and then dried.
3. *Preparation of nutrient agar and broth*: Nutrient agar and broth (HiMedia) were prepared according to the manufacturer's instruction.

Formula of the nutrient agar:

Peptic digest of animal tissue	5.0 g/L
Sodium chloride	5.0 g/L
Beef extract	1.5 g/L
Yeast extract	1.5 g/L
Agar	20.0 g/L
pH at (25 °C)	7.4 ± 0.2

Formula of the nutrient broth:

Peptic digest of animal tissue	5.0 g/L
Sodium chloride	5.0 g/L
Beef extract	1.5 g/L
Yeast extract	1.5 g/L
pH at (25 °C)	7.4 ± 0.2

8.1.1. Blotter disc method. In order to maintain uniform growth rate of each microorganism, a fresh inoculum was prepared by sub culturing microorganisms in luria bertanii (LB) broth media and incubated until optical density (OD, turbidity at 600 nm) of the culture reaches 0.2, which indicates that, the bacterial density of 10^7–10^8 cfu/mL.

Single colony of each of the cultures were inoculated in 5 mL of LB broth and incubated under shaking condition overnight at 37 °C. Five hundred milliliters of overnight grown cultures were inoculated into 25 mL of LB and incubated at 37 °C. At 0.2 $OD_{600\,nm}$, 300 μL of each of the cultures was inoculated into seven flasks containing 30 mL each of nutrient agar medium. The contents of the flasks were poured into the petriplates and allowed to solidify. On solidifying four filter discs were appropriately labelled: first for negative control (DMSO), second for positive control (streptomycin), third and fourth for isoxazolidines **4a**–**j** (100 μL). The plates were kept at room temperature for 30 min and then maintained 37 °C for 24 h. After 24 h, the zone of inhibition caused by the compounds was recorded.[1,24]

8.2. Antifungal activity test

1. *Fungus used for the antifungal activity*: *F. moniliforme* (ATCC10052), *A. niger* (ATCC1004) and *C. acremonium* (ATCC10141).
2. *Preparation of test sample*: Isoxazolidine (1 mg) was dissolved in 1 mL of DMSO, which served as a stock.
3. *Preparation of potato dextrose agar/broth*: Potato dextrose agar and broth (HiMedia) were prepared according to the manufacturer's instruction.

Formula of the potato dextrose agar (PDA):

Potato extract	200 g/L
Dextrose	20 g/L
Agar	20 g/L

Formula of the potato dextrose broth (PDB):

Potato extract	200 g/L
Dextrose	20 g/L

8.2.1. Cup borer method. The pathogen spore suspension was prepared in 50 mL sterile distilled water. The spore concentration was adjusted to 1×10^3 spores/ml. Suspension of 2 mL was spread on PDA plates uniformly. After solidifying, four wells were bored in each plate using a cork borer (0.5 cm diameter). DMSO (negative control) in first well, nystatin (positive control) in second well, and isoxazolidine of different concentrations in the remaining two wells—third (50 μL) and fourth (100 μL) were taken in each plate. The plates were incubated at 22 ± 2 °C under alternative cycles of 12/12 h NUV and darkness. After three days, plates were evaluated based on zone of inhibition caused by the compounds.[1,24]

8.2.2. Turbidometric method. The isoxazolidines **4a**–**j** were screened for their antifungal activity at different concentrations (50 and 100 μM) against *F. moniliforme*, *A. niger* and *C. acremonium* using *nystatin* as a positive control and DMSO as a negative control. To the culture tubes containing 1.9 mL of media was added 0.1 mL of isoxazolidine at sterile conditions. To all the tubes including standard and controls, the fresh inoculum was added with a spore concentration adjusted to 1×10^2 spores/mL. After incubating all tubes at 37 °C for 24 h, their absorbance was recorded at 640 nm. Percentage of inhibition was calculated according to the formula.[24,25]

$$\%\ \text{Inhibition} = 100(P - Q)/P$$

where, P = absorbance without test sample and Q = absorbance with test sample. Then, the MIC was recorded in μM.

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