Pergamon

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Bioorganic & Medicinal Chemistry 11 (2003) 4539–4544

BIOORGANIC &
MEDICINAL
CHEMISTRY

# Solution-Phase Synthesis of Novel $\Delta^2$-Isoxazoline Libraries via 1,3-Dipolar Cycloaddition and Their Antifungal Properties

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Received 21 June 2003; revised 9 August 2003; accepted 11 August 2003

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**Abstract**—The synthesis of novel imidazolyl substituted $\delta^2$-isoxazoline libraries are currently of high interest. We report here in the full details of a study leading to the synthesis and antifungal activities of 3-(-2-butyl-4-chloro-1*H*-imidazolyl)-substituted $\delta^2$-isoxazolines. The solution phase synthesis of the title compounds was accomplished via 1,3-dipolar cycloaddition of in situ generated nitryl oxides from aldoximes with mono substituted alkenes to obtain the compound libraries contain an imidazole functionality in addition to the isoxazoline rings. The newly synthesized compounds when tested in vitro in solid agar culture exerted a potent antifungal activity against *Aspergillus flavus*, *Fusarium moniliforme* and *Botrydiplodia theobromae* also MIC values were determined. The title 5-substituted-3-imidazolyl-$\delta^2$-isoxazoline compounds represent a novel class of potent antifungal agents.

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## Introduction

The rising prevalence of multi-drug resistant fungi continues to provide impetus for the search and discovery of novel antifungal agents active against these pathogens.[1,2] Ketoconazole was the first orally active antifungal agent that was effective against a broad array of systematic and superficial fungal infections,[3] inhibiting fungal cytochrome-P 450 activity, which has many ramifications.

Previous studies show several isoxazoline moieties, novel isoxazolidines[4–6] showed possessing a wide spectrum of activity like antifungal, anticancer, antiviral, insecticidal, antibiotic activities and precursors for different natural products.[7] The chemistry of the imidazole ring occupies an extremely important niche possessing diverse pharmacological activity within the family of five-membered ring heteroatom.

DuP 753 (losartan) is a nonpeptide angiotensin II antagonist, which is an orally active antihypertensive agent.[8] 2-Butyl-4-chloro-1*H*-imidazole-5-carboxaldehyde is a major active metabolite, which is one of the key intermediate of DuP 753 (losartan). 1, 3-dipolar cycloaddition of nitryl oxides are well documented and provide efficient entries to the synthesis of isoxazolines. Nitryl oxides as typical 1,3-dipoles undergo a wide range of cycloaddition with alkynes (alkenes) leading to five membered isoxazol (in) es.[10,11] We setout to develop a protocol for the polyfunctionalisation of 3-substituted imidazolyl-5-substituted-$\delta^2$-isoxazolines (4,5-dihydro isoxazoles), thus providing valuable information of a new generation of antifungal agents. In this paper, we describe the synthesis and antifungal activity of series of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-substituted $\Delta^2$-isoxazoline derivatives bearing the two five membered ring nitrogen heterocycles (isoxazoline and imidazolyl moieties).

## Chemistry

Microwave-assisted synthesis of 2-*n*-butyl-4-chloro-5-imidazolaldehyde **1** and its X-ray crystallographic studies have been reported.[9] The 50% probability of the molecule is as shown in Figure 1. The key intermediate, imidazolyl aldoxime **2** was prepared from substituted imidazolaldehyde **1** with hydroxylamine sulfate in the presence of sodium acetate in methanol at 60 °C for 5–6 h. Recent investigation from our laboratory has been concerned with the use of imidazolyl aldoxime **2** as a suitable synthon for the synthesis of $\delta^2$-isoxazolines. The synthetic scheme involves the generation of isoxazolines by trapping in situ generated nitryl oxide with appropriate olefins in a practical and efficient one-pot

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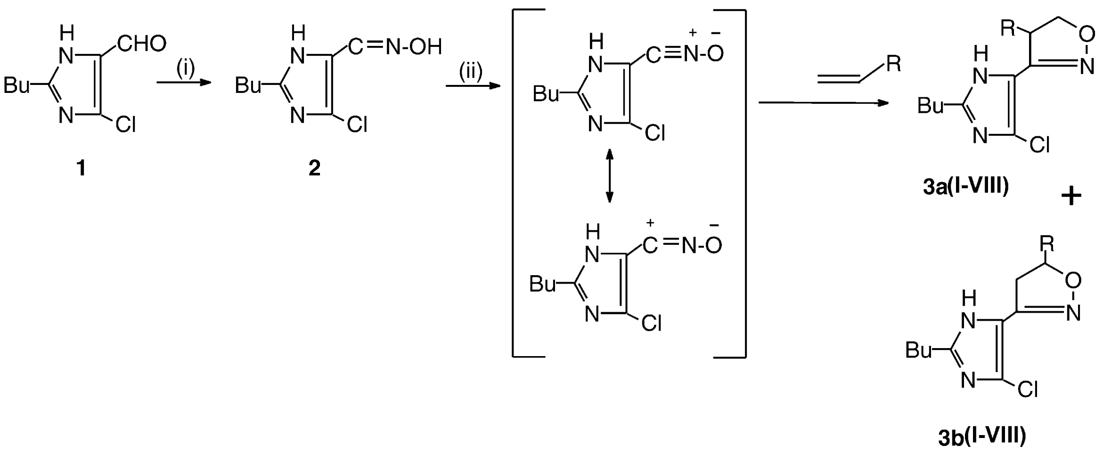
doi:10.1016/j.bmc.2003.08.007



Reaction condition:

(i) $(NH_2OH).H_2SO_4$ $NaOCOCH_3$ Methanol

(ii) $NaOCl/Et_3N$ THF/MDC

Where: **R**= (I) -CN
(II) $-C_6H_5$
(III) $-COOC_6H_5$
(IV) $-COOC_2H_5$
(V) $-CH_2COOCH_3$
(VI) $-CH_2OH$
(VII) $-COOCH_3$

**Scheme 1.**

operation. The solution-phase synthesis of the title compounds was accomplished via 1,3-dipolar cycloaddition reactions of aldoximes with mono substituted alkenes.[12] Nitryl oxides, which are having bulky group, do not tend to dimerise rapidly in solution or as solids and exhibit a characteristic high regioselectivity towards different types of dipolarophiles, which can be interpreted in terms of frontier orbital interactions.[13] Solid-phase synthesis of isoxazol (in) es via nitryl oxides has not been successful due to low yields and low purity of the products. Solution-phase synthesis of novel isoxazoline derivatives was obtained by treating with the imidazolyl aldoxime **2** solution in tetrahydrofuran/dichloromethane with monosubstituted alkenes in the presence of sodium hypochlorite and triethylamine (Scheme 1).

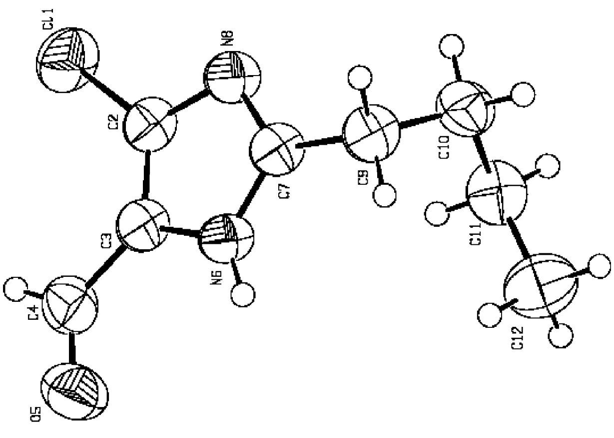


**Figure 1.** Crystal structure of the molecule **1**.

## Result and Discussion

The cycloaddition of all monosubstituted olefins with the generated nitryl oxides showed high regioisomeric products (approximately 85:15, which shown by TLC) and gave 5-substituted regioisomer predominantly as major products as shown in Library 1. The major products of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-substituted $\delta^2$-isoxazoline derivatives were separated on silica gel column using appropriate combination of chloroform, n-hexane, methanol and ethyl acetate as eluent. The reaction condition and the physical data of cycloadducts are given in Table 1.

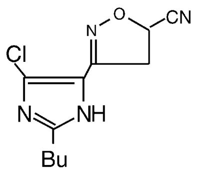
The yields were in the range of 60–80% and greater than 95% purity. All synthesized isoxazolines were structurally characterized by using IR, $^1$H NMR and C, H, N analysis.

### Antifungal activity

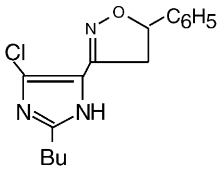
All newly synthesized isoxazoline derivatives were evaluated for antifungal activity. Nystatin, a known antifungal compound, was used for comparison.

The minimum inhibitory concentration (MIC) nM of compounds **3b** (**I**–**VII**) and nystatin against the fungal strains *Aspergillus flavus*, *Fusarium moniliforme* and *Botrydiplodia theobromae* are summarized in Table 2.

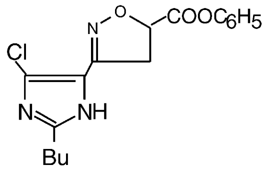
Compound **3bVI** exhibited more potent antifungal activity than nystatin. The MIC values of **3bVI** were 11 nM and 15 nM against *A. flavus* and *F. moniliforme*, respectively, which is less than 50% compared with the



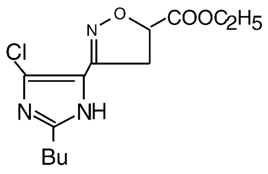
**3bI**



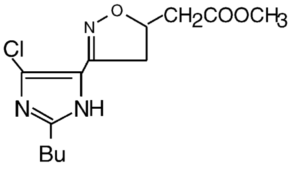
**3bII**



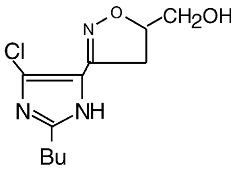
**3bIII**



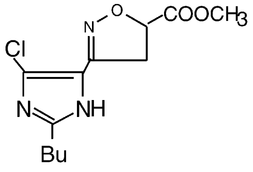
**3bIV**



**3bV**



**3bVI**



**3bVII**

**Library 1.**

**Table 1.** Reaction condition and physical data of $\delta^2$-isoxazoline derivatives

| $\delta^2$-Isoxazolines | Solvent used for refluxing | Time taken to complete the reaction (min) | $R_f$ value | Eluent used in separation | Yield in % | Mp °C |
|---|---|---|---|---|---|---|
| **3bI** | Dichloromethane | 1.30 | 0.59 | $CHCl_3$ + methanol (8:2) | 78 | Oily |
| **3bII** | Dichloromethane | 2.10 | 0.75 | $CHCl_3$ + methanol (7:3) | 69 | 160–162 |
| **3bIII** | THF | 2.45 | 0.49 | *n*-Hexane + ethyl acetate (7.5: 2.5) | 81 | Oily |
| **3bIV** | THF | 1.20 | 0.63 | *n*-Hexane + ethyl acetate (7.5: 2.5) | 71 | Oily |
| **3bV** | Dichloromethane | 1.45 | 0.52 | *n*-Hexane + ethyl acetate (8: 2) | 59 | 102 |
| **3bVI** | THF | 2.35 | 0.45 | $CHCl_3$ + methanol (9:1) | 84 | Oily |
| **3bVII** | Dichloromethane | 1.50 | 0.67 | *n*-Hexane + ethyl acetate (6.5: 3.5) | 73 | 183–185 |

**Table 2.** The minimum antifungal inhibitory concentrations (MIC)[a] (nM) of $\delta^2$-isoxazolines **3b(I-VII)**

| $\delta^2$-Isoxazolines | *Aspergilus flavus* | *Fusarium moniliform* | *Botrydiplodia theobrome* |
|---|---|---|---|
| Nystatin | 27 ± 1.1 | 32 ± 1.4 | 30 ± 1.1 |
| **3bI** | 152 ± 5.1 | 128 ± 4.6 | 197 ± 1.3 |
| **3bII** | 29 ± 1.3 | 40 ± 1.7 | 29 ± 3.2 |
| **3bIII** | 160 ± 6.8 | 84 ± 3.6 | 93 ± 1.5 |
| **3bIV** | 24 ± 1.1 | 19 ± 0.7 | 40 ± 3.6 |
| **3bV** | 93 ± 3.2 | 166 ± 6.8 | 91 ± 1.5 |
| **3bVI** | 11 ± 0.3 | 15 ± 0.4 | 90 ± 2.1 |
| **3bVII** | 89 ± 3.4 | 68 ± 2.4 | 118 ± 4.6 |

[a] Values are means of three determinations, the ranges of which were less than 5% of the mean in all cases.

corresponding MIC value for standard drug nystatin, which showed 27 and 32 nM, respectively. The reduction in MIC is significant in both the cases ($P < 0.005$ and $P < 0.0005$ respectively, one tailed *t*-test).[14,15]

Compounds **3bII** and **3bIV** were comparable to nystatin with MIC of 29 and 24 nM against *A. flavus*. **3bII** was less potent than nystatin against *F. moniliforme* and infact the MIC of **3bII** was more than that of nystatin. However **3bIV** had significantly less MIC compared to nystatin against *F. moniliforme*. The reduction in MIC is significant ($P < 0.005$, one tailed *t*-test).

**3bII**, **3bIV** and **3bVI** were not much effective compared to nystatin against *B. theobromae*.

The antifungal activities of **3bI**, **3bIII**, **3bV**, **3bVII** were not effective against any of the three strains.

## Conclusion

In conclusion, 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-substituted $\delta^2$-isoxazoline derivatives were synthesized and their antifungal activities against the antifungal susceptible standard and clinically isolated resistant fungi including *A. flavus*, *F. moniliforme* and *B. theobromae* were evaluated. **3bVI** demonstrated strong in vitro activity, comparable to or better than Nystatin.

We have identified the 3-(2-butyl-4-chloro-1H-imidazolyl)-5-methanolyl-$\delta^2$-isoxazoline as a new structural alternative for strong antifungal activity in the substituted imidazolyl isoxazolines class.

## Experimental

The melting points were determined on SELACO-650 hot stage apparatus and are uncorrected. IR (nujol) spectra were measured on Shimadzu 8300 IR spectrophotometer, $^1$H NMR were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer by using $CDCl_3$ as solvent and TMS as an internal standard (chemical shift in δ ppm). Elemental analyses were obtained on a Vario-EL instrument. TLC was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck) and Column by silica gel BDH 60–120 mesh. All extracted solvents were dried over $Na_2SO_4$, followed by evaporation in vacuo.

### Syntheses

**Synthesis of 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2.** A solution of 2-butyl-4-chloro-1*H*-imidazolyl)-5-carboxaldehyde (2 g, 9.918 mmol) in methanol (10 mL) was added to a mixture of hydroxylamine sulfate (0.975 g, 7.438 mmol) and sodium acetate (4.067 g, 0.495 mol). The reaction mass was refluxed for 4–5 h till the reaction completes. Evaporated the solvent under vacuo and added 40 mL of demineralised water. Cooled to 5–8 °C and filtered at the same temperature to obtained the crystalline solid of 1.76 g (88%). Mp: 179–181 °C. $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.9 (q, 3H $CH_3$); 1.4 (qt or multiplet, 2H $CH_2$); 2.4 (m, 2H $CH_2$); 2.7 (t, 2H $CH_2$); 2.9 (s, 1H CH); 6.75 (s, 1H NH); 7.1 (s, 1H, OH) IR (KBr pellet, cm$^{-1}$): v 3461.3, 3256.6, 1620.4,1255.6, 1101.3. Anal. CHN: calcd 47.629, 5.995, 20.847, found 47.612, 6.004, 20.835.

### General procedure for the synthesis of novel $\delta^2$-isoxazolines 3b(I–VII)

A solution of aldoxime (2, 2.0 equiv) in dichloromethane/tetrahydrofuran was added to a solution of a dipolaraphiles (1 equiv), sodium hypochlorite (Aldrich, 2 equiv) and triethylamine (1 equiv) stirred for 1–2.5 h to complete the reaction, which was monitored by TLC. The solvent was evaporated under vacuo. Eight volumes of demineralised water was added to the residue and extracted the crude with 4 volumes of dichloromethane thrice. The organic layer was washed with 5% $NaHCO_3$ solution. Using appropriate mixture of chloroform, *n*-hexane, methanol and ethyl acetate as eluent in silica gel column separated the pure products.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-cyano-$\delta^2$-isoxazoline 3bI.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), acrylonitrile (0.1344 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.87 (q, 3H $CH_3$); 1.24 (m, 2H $CH_2$); 2.2 (q, 2H $CH_2$), 2.4 (t, 2H $CH_2$); 3.35 (d, $J=18$, 7.4 Hz $H_4$); 5.05 (dt or multiplet, 1H, $H_5$); 6.68 (s, 1H, NH) IR (KBr pellet, cm$^{-1}$): v 3429.6, 2231.4, 1440.4,1253.2, 1116.8. Anal. CHN: calcd 52.283, 5.183, 22.170, found 52.275, 5.179, 22.179.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-phenyl-$\delta^2$-isoxazoline 3bII.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), styrene (0.1344 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.91 (q, 3H $CH_3$); 1.34 (m, 2H $CH_2$); 2.18 (q, 2H $CH_2$); 2.6 (t, 2H $CH_2$); 3.55 (d, $J=17.0$, 10 Hz, $H_4$); 5.7 (dd, $J=11.0$, 8 Hz, $H_5$); 6.75 (s, 1H, NH); 6.95 (s, 1H, Ar-H); 7.15 (t, 2H, $J=6.1$ Hz, Ar-H); 7.45 (t, 2H, Ar-H) IR (KBr pellet, cm$^{-1}$): v 3428.1, 1451.4,1249.1, 1107.2. Anal. CHN: calcd 63.25, 5.971, 13.831, found 63.241, 5.94, 13.853.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-benzoyl-oxy-$\delta^2$-isoxazoline 3bIII.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), phenyl acetate (0.367 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.71 (q, 3H $CH_3$); 1.36 (m, 2H $CH_2$); 2.29 (q, 2H $CH_2$), 2.6 (t, 2H $CH_2$); 3.32 (d, $J=17.0$, 7 Hz, $H_4$); 5.05 (m, IH, $H_5$); 6.96 (d, 2H, $J=7.0$ Hz, Ar-H); 7.25 (m, 3H, Ar-H); 6.75 (s, 1H, NH) IR (KBr pellet, cm$^{-1}$): v 3445.7, 1710.6, 1468.4,1255.6, 1101.3. Anal. CHN: calcd 58.708, 5.216, 12.08, found 58.694, 5.25, 12.126.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-ethoxycarbonyl-$\delta^2$-isoxazoline 3bIV.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), ethyl acrylate (0.2481 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.81 (q, 3H $CH_3$); 0.95 (t, 2H–$CH_2$–$CH_3$); 1.02 (t, 3H $CH_3$); 1.25 (m, 2H $CH_2$); 2.3 (q, 2H $CH_2$); 2.6 (t, 2H $CH_2$); 4.98 (dd, $J=14.0$, 6 Hz, $H_5$); 3.45 (d, 2H $H_4$); 6.75 (s, 1H, NH) IR (KBr pellet, cm$^{-1}$): v 3433.1, 2231.4, 1721.4, 1440.4, 1255.6, 1101.3. Anal. CHN: calcd 52.09, 6.052, 14.018, found 52.182, 5.986, 14.174.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-acetoxy-methyl-$\delta^2$-isoxazoline 3bV.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), methyl-3-butenoate (0.248 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1$H NMR ($CDCl_3$, 400 MHz): δ 0.94 (q, 3H $CH_3$); 1.19 (m, 2H $CH_2$); 2.43 (q, 2H $CH_2$); 2.36 (d, 2H $CH_2$); 2.6 (t, 2H $CH_2$); 2.84 (m, 2H $CH_2$); 3.75 (s, 3H $OCH_3$); 4.45 (d, 1H, $H_4$); 5.24 (m, $J=12.0$, 8 Hz, 1H $H_5$); 6.75 (s, 1H, NH). IR (KBr pellet, cm$^{-1}$): $\nu$ 3421.4, 1745.2, 1436.2, 1219.4, 1124.6.

Anal. CHN: calcd 52.09, 6.052, 14.018, found 51.986, 6.12, 13.976.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-hydroxy-methyl-$\delta^2$-isoxazoline 3bVI.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), allyl alcohol (0.1438 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 0.75 (q, 3H $CH_3$); 1.25 (m, 2H $CH_2$); 2.3 (q, 2H $CH_2$); 2.6 (t, 2H $CH_2$); 2.89 (d, 2H); 3.35 (dd, $J = 16.0$, 7.5 Hz, 1H $H_5$); 4.45 (d, 1H, $H_4$); 5.3 (s, 1H, OH); 6.75 (s, 1H, NH) IR (KBr pellet, $cm^{-1}$): v 3433.1, 3320.5, 1440.4,1255.6, 1101.3. Anal. CHN: calcd 51.183, 6.216, 16.342, found 51.191, 6.203, 16.376.

**Synthesis of 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-acetoxy-$\delta^2$-isoxazoline 3bVII.** It was obtained from 2-butyl-4-chloro-1*H*-imidazolyl)-5-methaldoxime 2 (1 g, 4.961 mmol), methyl acrylate (0.2132 g, 2.480 mmol), triethylamine (0.250 g, 2.480 mmol) and sodium hypochlorite (0.612 mL, 9.92 mmol). $^1H$ NMR ($CDCl_3$, 400 MHz): 0.86 (q, 3H $CH_3$), 1.34 (m, 2H $J = 4.6$ Hz, $CH_2$); 2.28 (q, 2H $CH_2$); 2.45 (t, 2H $CH_2$); 3.35 (d, $J = 16.0$, 7.5 Hz, 1H $H_5$), 3.62 (s, 3H, $OCH_3$); 4.45 (d, 1H, $H_4$); $\delta$ 7.05 (s, 1H, NH); IR (KBr pellet, $cm^{-1}$): v 3429.5, 1698.5, 1465.3,1248.5, 1112.7. Anal. CHN: calcd 50.443, 5.643, 14.70, found 50.482, 5.623, 14.81.

## Determination of antifungal activity

1. **Fungus used for the antifungal activity**: *A. flavus* (ATCC76087) *F. moniliforme* (ATCC5342) *B. theobromae* (ATCC76087)
2. **Preparation of test sample**: 1 mg of $\delta^2$-isoxazolines were weighed and dissolved in $CHCl_3$ with respect to their concentration tested.
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 |

## Preparation of potato dextrose agar and broth

Potato dextrose agar and broth (HiMedia) were prepared according to the manufacturer's instruction.

Formula of the potato dextrose agar:

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

Formula of the potato dextrose broth:

| | |
|---|---|
| Potato extract | 200 g/L |
| Dextrose | 20 g/L |

The 3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-substituted $\delta^2$-isoxazoline derivatives were tested for antifungal activity by serial tube dilution technique[4,13] at different concentrations (10, 20, 30… 200 nM) against *A. flavus*, *F. moniliforme* and *B. theobromae*. Nystatin was used as reference standard and $CHCl_3$ as control. To the culture tubes containing 1.9 mL of media, 0.1 mL of test solution was added at sterile conditions. To all the tubes including standard and controls, the fresh inoculum was added using Himedia flexiloop 4 calibrated to 0.001 mL. After incubating all the tubes at 37 °C for 24 h, their absorbance was recorded at 640 nm along with Nystatin. Percentage of inhibition was calculated as follows,

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

where $P$ = absorbance without the test sample and $Q$ = absorbance with test sample. Then, the minimum inhibitory concentration (MIC) was recorded in nM.

## Acknowledgements

We are grateful to the department of University Grants commission (UGC), New Delhi for financial support under the project vides No. 12-14/2002 (SR-1).

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