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Enhancement in antimicrobial activity of 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine

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Abstract—The *trans* rich isomer, 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine **A** (>96% ee) was synthesized by the condensation of *E* isomer rich nitrone **4** (>98% ee) with butyl acrylate in an inert solvent. Obtained isoxazolidine was screened for its antifungal activity against *Aspergillus niger*, *Cephalosporium acremonium*, *Fusarium moniliforme* by using Nystatin as positive control. It was also tested for its antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* by using *Streptomycin* as positive control. Enhanced antifungal activity was observed in isoxazolidine of >96% ee compared to the isoxazolidine of >69% ee (**B**), and enhancement was not observed in antibacterial activity.

1. Introduction

In recent years, there is an alarming increase in life threatening microbial infections especially in immunocompromised individuals suffering from AIDS, cancer, etc.[1–4] There has been a constant effort by the researchers to develop more effective and safe antimicrobial drugs to combat with microbial infections.[5–9] Despite the development of several new antimicrobial agents, their clinical value is limited to treat an increasing array of life threatening systemic infections because of their relatively high risk of toxicity, emergence of drug resistant strains, pharmacokinetic differences, and/or insufficiencies in their antimicrobial activity.[10] Therefore, a great need for a more potent and broad spectrum antimicrobial agents with reduced side effects.[11]

We previously reported[12–15] the synthesis and biological activities of isoxazolidines constructed via 1,3-dipolar cycloaddition reactions of nitrones with olefins. We recently reported the synthesis and antimicrobial properties of a series of novel 5-imidazolyl substituted isoxazolidines of moderate regioselectivity (up to 69% ee).[16] Among tested, 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine (**B**, Scheme 2) showed comparatively promising antimicrobial activity. Therefore, we thought of preparing high regioselective isomer of **B** to screen its antimicrobial activity. We herein report the synthesis and antimicrobial activity of high regioselective isoxazolidine **5/5′** (**A**, Scheme 1).

2. Chemistry

Condensation of hydroxylamine **2** with aldehyde **1** in the presence of Bronsted acid catalyst[17] **3** to yield *E* isomer rich nitrone **4** (*E* > 98% ee). The 1,3-dipolar cycloaddition of nitrone **4** (*E* > 98% ee) with butyl acrylate gave a mixture of regioisomers, **5/5′** and **6/6′**. The major isomer **5/5′** (**A**, >96% ee) was separated on silica gel column (Scheme 1).

Nitrone **4** (*E*/*Z* = 65/35) was synthesized by the reduction of a mixture of **7** and imidazole aldehyde **1**[18] with zinc dust using histidine as a catalyst (Scheme 2).[19] The crude product of nitrone showed mixture of isomers, **4**(*E*)/**4′**(*Z*) in 65/35 ratio evaluated by 400 MHz ^{1}H NMR: *E* and *Z* isomers showed singlet at δ 7.97 and 7.32, respectively for CH=N bond. Thus poor selectivity in isomer ratio was observed in the method reported previously. In the present method (Scheme 1), **2** was condensed directly with **1** in the presence of Bronsted acid **3**/$MgCl_2$ catalyst[17] and gave *E* isomer rich nitrone **4** (>98% ee) evaluated by 400 MHz. ^{1}H NMR (*E* isomer showed singlet peak at δ 7.97 for CH=N bond).

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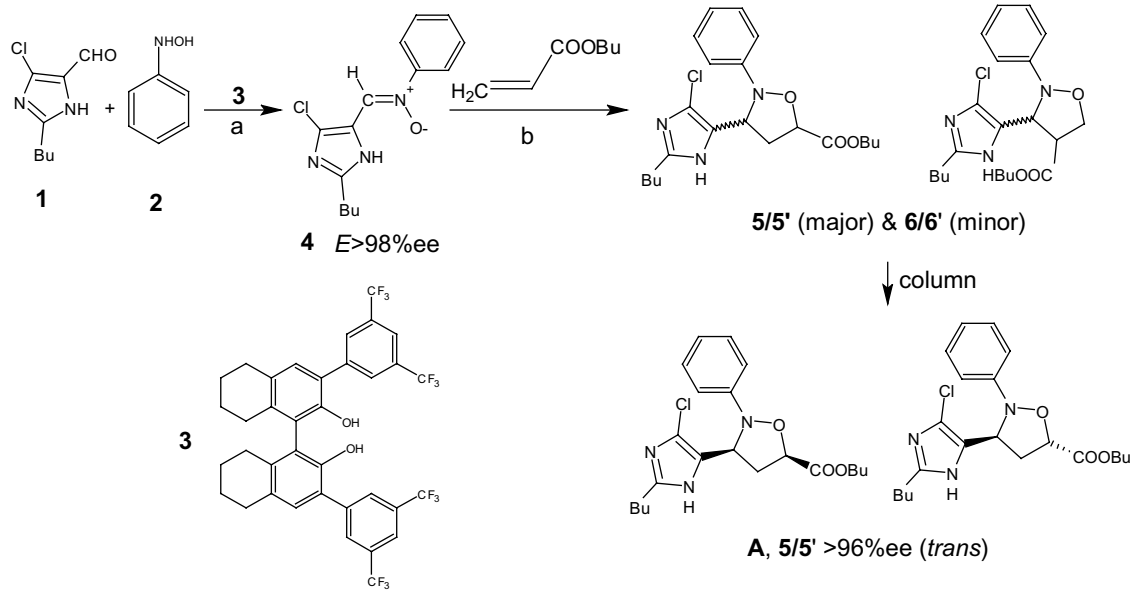
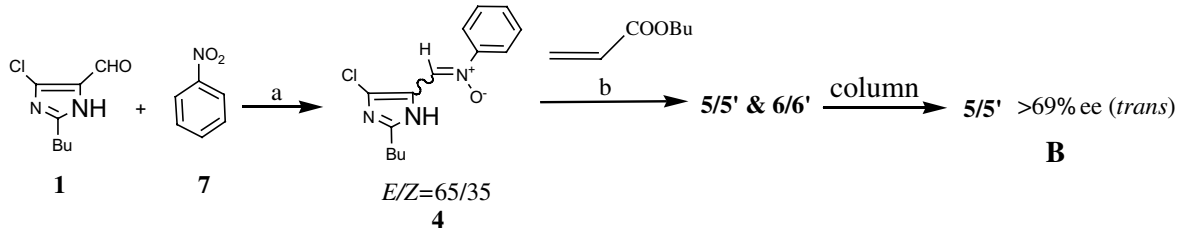


Scheme 1. Reagents and conditions: (a) $MgCl_2$, methylene dichloride; (b) toluene, reflux, 38 h.



Scheme 2. Reagents and conditions: (a) H_2O:ethanol:DMF (7:2:1), zinc dust, −5 °C, pH 7.2–7.4; (b) toluene, reflux, 38 h.

Thus, **3**/$MgCl_2$ catalytic system greatly enhanced the *E* isomeric ratio of **4** from >65% ee to >98% ee. The 1,3-cycloaddition of **4** (*E* > 98% ee) with butyl acrylate in inert solvent gave a mixture of 5- and 4-substituted regioisomers, **5/5′** and **6/6′**. After separation on silica gel column, the major stereoisomers, **5/5′** (**A**) ratio showed high stereoselectivity (>96% ee) evaluated by the integration from 400 MHz ^{1}H NMR spectra.

3. Material and methods

3.1. Microorganisms used

Bacteria: *Bacillus subtilis* (ATCC-23857D), *Escherichia coli* (ATCC-700926D), and *Staphylococcus aureus* (ATCC-10832D).

Fungi: *Aspergillus niger* (ATCC-1004), *Cephalosporium acremonium* (ATCC-10141), and *Fusarium moniliforme* (ATCC-10052).

3.2. Culture media

Media were prepared according to the manufacturer's (Hi-media) instructions. Brain heart infusion agar (BHIA) and brain heart infusion broth (BHIB) were used for the cultivation of bacteria. The composition of BHIA is—calf brain infusion (solids) 12.5 g/L, beef heart infusion (solids) 5 g/L, peptic digest of animal tissue 10.0 g/L, sodium chloride 5.0 g/L, dextrose 2.0 g/L, agar 20.0 g/L, distilled water 1 L, and pH 7.4 ± 0.2 (25 °C). Apart from agar, the composition of BHIB is similar to BHIA. The fungal species were cultured in potato dextrose agar (PDA) and potato dextrose broth (PDB) media. The composition of PDA is potato extracts 200.0 g/L, dextrose 2.0 g/L, agar 20.0 g/L and distilled water 1 L. Except agar, the composition of PDB is similar to PDA.

3.3. Antimicrobial assay

For antimicrobial assays, the compounds were dissolved in dimethyl sulfoxide (DMSO) (1 mL) and the solution was diluted with distilled water (9 mL). Further progressive dilutions with test medium gave the required concentrations in μg/mL. Minimum inhibitory concentrations (MIC) were determined in the culture tube containing 4.5 mL of broth media. Final volume was adjusted to 5 mL after addition of culture media and the required dilution of the compound. Cell/spore suspensions were prepared and adjusted to a final concentration of 2×10^3 colony forming units (CFU) per millilitre. After inoculation the culture tubes were sha-

ken well and bacterial cultures were incubated at 37 °C for 24 h, whereas fungal cultures were incubated at 28 °C for 48 h.

MIC of each compound for bacteria and fungi were determined after incubation period by turbidometric method.[20] The absorbance of the culture tubes were recorded at 640 nm and percentage of inhibition was calculated according to the formula: % inhibition = $100(P - Q)/P$, where, P = absorbance without test sample, Q = absorbance with test sample.[21] Then the MIC was recorded as μg/mL. The MIC was further confirmed by removing 10 μL of the contents from each culture tube showing no visible growth and spreading them on to BHIA plates in case of bacteria and PDA plates in case of fungi. The plates were incubated according to the specifications and growth (if any) was carefully examined under microscope. The MIC was defined, as the lowest concentration of substance at which there was no growth.

4. Results and discussion

The antimicrobial activity of **A** and **B** was evaluated and compared with controls as shown in Table 1. Compounds **A** and **B** showed antibacterial properties with varied MIC values and they were less effective when compared with reference control streptomycin. On an average, double the concentration of compounds (both **A** and **B**) was required for complete inhibition of bacteria when compared with control, which inhibited *E. coli* at 6 μg/mL and both *B. subtilis* and *S. aureus* at 3 μg/mL. Both the compounds, **A** and **B** exhibited similar antibacterial properties and their difference in MIC values with respect to individual species is not noteworthy.

With respect to fungi, though both the compounds exhibited antifungal properties, their effectiveness varied considerably. *A. niger* was inhibited by **A** at 14 μg/mL and by control (nystatin) at 12 μg/mL concentration, whereas, for **B** it was 36 μg/mL which is three times higher than the control. *C. acremonium* was inhibited at 11 μg/mL concentration by both compound **A** and nystatin and for **B** it took a threefold increase in concentration when compared to control and **A**. In case of *F. moniliforme*, **A** was most effective with MIC value 13 μg/mL, which is even better than the nystatin (14 μg/mL). Here, the concentration of **B** had to be increased twofolds when compared to the control to get complete inhibition.

In general, with respect to bacteria, both the compounds **A** and **B** exhibited almost similar MIC and are less effective when compared with the control as they showed approximately double the MIC values of control with corresponding bacteria. However, results with antifungal studies are interesting. **A** was found to be more effective when compared with **B**, which required two to 3-fold increase in concentration as compared with the control as well as **A** to be effective against the fungal species. The antifungal effectiveness of **A** was on par with the control and in case of *F. moniliforme*, it is even better than the control. The increase in antifungal activity of **A** when compared with **B** may be due to the stereo chemical structural difference between the two compounds, which might play a vital role in exhibiting antifungal activity.

In conclusion, the preparation of 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine of >96% ee has enabled the exploration of its antimicrobial activity. As expected, **A** showed greater antifungal activity than **B** because of its higher ee purity. Thus the results are encouraging to better define and optimize the antifungal activity of 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine. Further improvement in ee purity of **A** is currently under progress.

5. Experimental

5.1. General

Melting points were determined on SELACO-650 instrument and are uncorrected. The IR was recorded on a Perkin–Elmer model RX-1 FT-IR spectrophotometer. The ^{1}H NMR spectra were recorded on Bruker Avance-400 spectrometer at 400 MHz using TMS as an internal standard and $CDCl_3$ as solvent. The chemical shift values are on the δ scale and the coupling constant j is in Hz. The elemental analyses were obtained on a Vario-EL 111 CHNS, instrument. The analytical TLCs were performed on a coated Merk silca gel 60 F_{254} plates; the spots were detected either under UV light or by charring with 4% alcoholic H_2SO_4, followed by evaporation in vacuum. Some of the products are known compounds and were identified by comparison of their spectra and physical data with those of authentic samples.

5.2. Synthesis

5.2.1. *E*/*Z*-C-(2-Butyl-4-chloro-1*H*-imidazolyl)-*N*-phenyl nitrone (4, *E*/*Z* = 65/35). This compound was synthesized according to the procedure reported earlier.[16]

Table 1. The MIC (μg/mL) values of isoxazolidine **5**/**5′** of 96% ee (**A**) and 69% ee (**B**)

Isoxazolidine **5**/**5′**	Bacteria			Fungi		
	E. coli	*B. subtilis*	*S. aureus*	*A. niger*	*C. acremonium*	*F. moniliforme*
Control[a]	6.0	3.0	3.0	12.0	11.0	14.0
A, >96% ee	10.0	7.0	7.0	14.0	11.0	13.0
B, >69% ee	11.0	7.0	8.0	36.0	33.0	28.0

[a] Streptomycin for bacteria and nystatin for fungi.

The *E*/*Z* isomers mixture (yellow oil, 0.57 g, 51%) was obtained from nitrobenzene **7** (0.41 mL, 4.06 mmol) and 2-butyl-4-chloro-1*H*-imidazolyle aldehyde **1** (0.75 g, 4.02 mmol). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.76 (t, 3H, CH_3), 0.93 (t, 3H, CH_3), 1.11 (m, 2H, CH_2), 1.38 (m, 2H, CH_2), 1.49 (m, 2H, CH_2), 1.75 (m, 2H, CH_2), 2.36 (t, 2H, CH_2), 2.75 (t, 2H, CH_2), 7.32 (s, 1H, CH=N), 7.32–7.48 (Ar-H, merged with isomer), 7.97 (s, 1H, CH=N), 12.13 (br s, 1H, NH), 12.72 (br s, 1H, NH). IR (KBr): 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.

5.2.2. *E*-C-(2-Butyl-4-chloro-1*H*-imidazolyl)-*N*-phenyl nitrone (4, *E* > 98% ee). This compound was obtained by condensation aldehyde **1** (0.5 g, 2.68 mmol) and phenyl hydroxylamine hydrochloride **2** (0.39 g, 2.68 mmol) in the presence of Bronsted acid/$MgCl_2$ catalyst. The yellow solid (0.49 g, 67.2%) melted at 42–44 °C. ^{1}H NMR ($CDCl_3$, 400 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), 7.32–7.72 (Ar-H, 5H), 7.97 (s, 1H, CH=N), 12.72 (br s, 1H, NH). IR (KBr): 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.

5.2.3. Procedure for the synthesis of 2-(phenyl)-3-(2-butyl-4-chloro-1*H*-imidazolyl)-5-butylate isoxazolidine. A mixture of **4** and butyl acrylate in toluene was refluxed until reaction completes monitored by TLC. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The major regioisomer **5**/**5′** was separated on silica gel column by eluting with hexane/ethylacetate (9:1).

The isomer **A**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.74–1.08 (m, 6H, CH_3), 1.11–1.49 (m, 4H, CH_2), 1.59–1.75 (m, 4H,CH_2), 2.66 (t, 2H, CH_2), 2.43 (ddd, 1H, H4a, J = 13.4, 5.87, 4.7 Hz), 2.30 (ddd, H4b, J = 13.28, 7.96, 5.3 Hz), 3.08 (t, 2H, OCH_2), 4.84 (dd, 1H, CH, J = 8.8, 5.4 Hz), 5.12 (dd, 1H, CH, J = 8.4, 4.7 Hz), 7.02 (t, 1H, Ar-H), 7.07 (d, 2H, Ar-H), 7.28 (t, 2H, Ar-H), 10.46 (s, 1H, NH). IR (KBr): 3247, 3062, 2959, 2873, 1730, 1598, 1258, 1085, 825, 757, 694, 598 cm^{-1}. Anal. Calcd CHN: 62.14, 6.95, 10.35. Found: 62.19, 7.06, 10.38.

The isomer **B**: brownish thick liquid (59%). ^{1}H NMR ($CDCl_3$, 400 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, H4a, J = 13.4, 5.87, 4.7 Hz), 2.99 (ddd, H4b, J = 13.28, 7.96, 5.3 Hz), 3.08 (t, 2H, OCH_2), 4.57 (dd, 1H, CH, J = 8.8, 5.4 Hz), 4.82 (dd, 1H, CH, J = 8.4, 4.7 Hz), 6.95–7.15 (m, 3H, Ar-H), 7.22–7.35 (m, 2H, Ar-H), 10.62 (br s, 1H, NH). IR (KBr): 3247, 3062, 2959, 2873, 1730, 1598, 1258, 1085, 825, 757, 694, 598 cm^{-1}. Anal. Calcd CHN: 62.14, 6.95, 10.35. Found: 62.19, 7.06, 10.38.

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