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One pot high yield synthesis of some novel isoxazolidine derivatives using *N*-methyl-α-chloro nitrone in water and their antibacterial activity

Bhaskar Chakraborty[1*], Amalesh Samanta[2], Prawin Kumar Sharma[1], Manjit Singh Chhetri[1], Saurav Kafley[1], Annanya Banerjee[2] and Chandrima Sinha[2]

[1]Organic Chemistry laboratory, Sikkim Government College, Gangtok, Sikkim, India
[2]Division of Microbiology, Deptt.of Pharmaceutical Technology, Jadavpur University, Kolkata, India

ABSTRACT

A simple one pot and high yield efficient method is described in water for the diastereo and regioselective synthesis of some novel isoxazolidine derivatives at room temperature in a short reaction time. All the novel isoxazolidines have been screened for antimicrobial activity and found to be very active.

Keywords: *N*-methyl-α-chloro nitrone, novel isoxazolidine derivatives, stereo & regio-selectivity, aqueous phase, antibacterial activity.

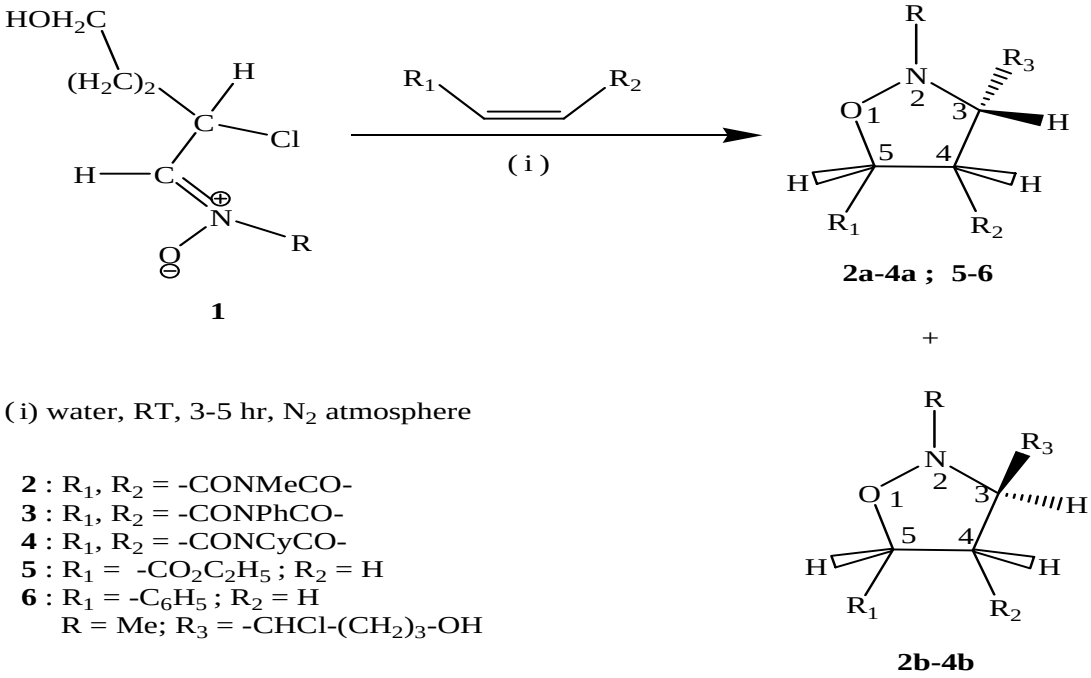
INTRODUCTION

Organic reactions in water have received increased attention primarily because of their environmental acceptability, abundance and low cost [1-3]. However, water also exhibits unique reactivity and selectivity that cannot be attained in conventional organic solvents [4-6]. Thus, the development of efficient procedures for useful chemical transformations in water without any catalyst is highly appreciated. Keeping in touch with green chemical pathway of synthesis our group has already reported 1,3-Dipolar cycloaddition reactions with *N*-phenyl-α-chloro nitrone [7] and *N*-cyclohexyl-α-amino nitrone [8] in water. Among a plethora of functional groups, the nitrone functionality has etched a place of distinction in organic synthesis. Remarkable regio, stereo, face and chemoselectivity along with efficient incorporation of multiple stereocenters have made nitrone cycloaddition reactions an attractive and efficient key step in the synthesis of a great many natural products of biological interest [9]. In recent years, focus has been shifted toward asymmetric nitrone cycloaddition reactions, enantioselective, catalytic enantioselective, and diastereoselective synthetic methodologies in aqueous phase [10,11].

Herein, we would like to report high yield diastereo and regioselective synthesis of some novel isoxazolidine derivatives in water using 1,3-Dipolar cycloaddition reaction with *N*-methyl-α-chloro nitrone [12,13] in a short reaction time and their antibacterial activities (Scheme 1). The present study has been carried out with three different maleimides (*N*-methyl/phenyl/cyclohexyl) and ethyl acrylate, styrene respectively in water. Simultaneously the reactions have been also studied in organic solvent. Although reactions are found to be diastereo and regioselective in organic solvent but reaction rate and yields are not impressive (Table 1). We classified dipolarophiles into water-super and water-normal on the basis of the magnitude of their rate response to water. A ketone (C=O) conjugated to an alkene or alkyne is a water-super dipolarophile. Esters, ethers and aryl rings conjugated to an alkene are water-normal dipolarophiles.

EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded with a Bruker Avance DPX 300 spectrometer (300 MHz, FT NMR) using TMS as internal standard. ^{13}C NMR spectra were recorded on the same instrument at 75 MHz. The coupling constants (*J*) are given in Hz. IR spectra were obtained with a Perkin-Elmer RX 1-881 machine as film or as KBr pellets for all the products. MS spectra were recorded with a Jeol SX-102 (FAB) instrument. The HRMS spectra were recorded on a DART-HRMS, JMS–T100LC, Accu-TOF instrument. Elemental analyses (CHN) were performed with a Perkin-Elmer 2400 series CHN Analyzer. TLC's were run on Fluka silica gel precoated TLC plates. All other reagents and solvents were purified after receiving from commercial suppliers. *N*-methylhydroxylamine was purchased from Aldrich Chemical Company and was used as received. *N*-phenylhydroxylamine was prepared following standard methods available in literature.



(i) water, RT, 3-5 hr, N_2 atmosphere

2 : R_1, R_2 = -CONMeCO-
3 : R_1, R_2 = -CONPhCO-
4 : R_1, R_2 = -CONCyCO-
5 : R_1 = $-CO_2C_2H_5$; R_2 = H
6 : R_1 = $-C_6H_5$; R_2 = H
R = Me; R_3 = $-CHCl-(CH_2)_3-OH$

Scheme 1

General procedure for cycloaddition (for diastereomers)

To a stirred solution of nitrone **1** (1 mmole) in 15 mL water dipolarophiles were added (1 equivalent) at RT under nitrogen atmosphere and the reaction mixture was stirred for 3-4 hr. The progress of the reaction was monitored by TLC. After completion of the reaction, the products were extracted with ether (2 X 25 mL), the organic layer was washed with saturated brine (2 X 15 mL), dried over anhydrous Na_2SO_4 and concentrated. The mixture of diastereomers were purified and separated by column chromatography using ethyl acetate - hexane to afford cycloadducts **2-4** (Scheme 1). This procedure was followed for the substrates **1**–**3** listed in Table 1.

Table 1. **Physicochemical data of synthesized compounds**

Entry	Nitrone	Dipolarophile[a]	Time (hr)	Cycloadduct[b] & m.p (^{o}c) *Cis/trans ratio*(%) **2a-4a : *cis* ; 2b–4b: *trans***	Yield[c] (%)
1	*N*-methyl-α-chloro nitrone	*N*-methyl maleimide	3 (27)	**2a**: White crystals, 137 **2a**: 66 **2b**: White crystals, 106 **2b**: 31	97 (78)
2	*N*-methyl-α-chloro nitrone	*N*-phenyl maleimide	3 (29)	**3a**: White solid, 145 **3a**: 63 **3b**: White solid, 122 **3b**: 32	95 (76)
3	*N*-methyl-α-chloro nitrone	*N*-cyclohexyl maleimide	4 (32)	**4a**: Yellow crystals, 140 **4a**: 68 **4b**: Yellow crystals, 113 **4b**: 26	94 (76)
4	*N*-methyl-α-chloro nitrone	Ethyl acrylate	4 (34)	**5**: Colourless gummy liquid	92 (69)
5	*N*-methyl-α-chloro nitrone	Styrene	5 (38)	**6**: Colourless viscous liquid	91 (67)

[a] *Reaction condition: α-chloro nitrone (1 mmol), dipolarophile (1 equivalent), water, N_2 atmosphere, RT*
[b] *All the reactions were carried out at RT*
[c] *Isolated yields after purification*
Figures in parentheses indicate reactions performed in CH_2Cl_2

(*3S*)–3-(1-chloro-4-hydroxybutyl)-5-methyl-2-methyldihydro-2H-pyrrolo[3,4-d] isoxazole-4,6(5H,6 a-H)-dione, 2a

White crystals. Yield 66%; R_f = 0.46; IR (KBr): 3486 - 3430 (br), 2915 (m), 2832 (m), 1762 (s), 1660 (s), 1474 (m), 1190 (m), 814 (s), 778 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.83–4.72 (br, s, 1H, OH, exchanged in D_2O), 4.60 (dd, 1H, *J* = 6.06, 6.20 Hz, C_4H), 3.30 (s, 2x3H, 2-CH_3 protons), 3.00 (d, 1H, *J* = 6.60 Hz, C_5H), 2.70 (dd, 1H, *J* = 6.42, 6.20 Hz, C_3H), 2.34 (dt, 1H, *J* = 6.04, 6.00 Hz, CHCl), 1.82 - 1.50 (m, 6H, CH_2 protons); ^{13}C NMR ($CDCl_3$**)**: δ 178.12, 176.80 (carbonyl carbons), 87.15 (C_5), 76.00 (C_3), 67.10 (CH_2OH), 53.54 (C_4), 50.70 (CHCl), 38.00, 37.14 (2xCH_3), 22.32, 21.45 (2 CH_2 carbons); MS: *m/z* 278 (M^++2), 276 (M^+), 261, 255, 226, 169, 154 (B.P), 107; HRMS–EI: Calcd for $C_{11}H_{17}O_4N_2Cl$ (M) *m/z* 276.1240. Found: M^+ 276.1228. Anal. Found: C, 47.69; H, 6.10; N, 10.07. $C_{11}H_{17}O_4N_2Cl$ requires C, 47.80; H, 6.19; N, 10.14%.

(*3R*)–3-(1-chloro-4-hydroxy butyl)-5-methyl-2-methyl dihydro-2H-pyrrolo[3,4-d]isoxazole-4,6(5H,6 a-H)-dione, 2b

White crystals. Yield 31%; R_f = 0.52; IR (KBr): 3510 - 3454 (br), 2920 (m), 2826 (m), 1750 (s), 1664 (s), 1470 (m), 1205 (m), 810 (s), 780 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 5.03–4.90 (br, s, 1H, OH, exchanged in D_2O), 4.54 (dd, 1H, *J* = 2.52, 2.36 Hz, C_4H), 3.14 (s, 2x3H, 2-CH_3 protons), 3.04 (d, 1H, *J* = 4.54 Hz, C_5H), 2.62 (dd, 1H, *J* = 3.60, 3.12 Hz, C_3H), 2.27 (dt, 1H, *J* = 1.80, 1.64 Hz, CHCl), 1.90 - 1.54 (m, 6H, CH_2 protons); ^{13}C NMR ($CDCl_3$): δ 179.00, 178.30 (carbonyl carbons), 86.92 (C_5), 75.46 (C_3), 64.77 (CH_2OH), 54.32 (C_4), 51.20 (CHCl), 41.10, 39.00 (2xCH_3), 24.00, 23.22 (2 CH_2 carbons); MS: *m/z* 278 (M^++2), 276 (M^+), 261, 255, 246, 226, 169, 154 (B.P), 107; HRMS–EI: Calcd for $C_{11}H_{17}O_4N_2Cl$ (M) *m/z* 276.1240. Found: M^+

276.1231. Anal. Found: C, 47.72; H, 6.11; N, 10.10. $C_{11}H_{17}O_4N_2Cl$ requires C, 47.80; H, 6.19; N, 10.14%.

(*3S*)-3-(1-chloro-4 hydroxy butyl)-5-phenyl-2-methyl dihydro-2H-pyrrolo[3,4-d]isoxazole-4,6(5H,6 a-H)-dione, 3a

White solid. Yield 63%; R_f = 0.38; IR (KBr): 3585 - 3453 (br), 2920 (m), 2835 (m), 1758 (s), 1660 (s), 1480 (m), 1346 (m), 805 (s), 770 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.10 – 6.95 (m, 5H, C_6H_5), 5.80 (d, 1H, *J* = 6.74 Hz, C_5H), 5.04 – 4.93 (br, s, 1H, OH, exchanged in D_2O), 3.44 (dd, 1H, *J* = 6.04, 6.16 Hz, C_4H), 3.26 (s, 3H, CH_3), 2.70 (dt~m, 1H, CHCl), 1.84 (dd, 1H, *J* = 6.22, 6.28 Hz, C_3H), 1.55 – 1.14 (m, 6H, CH_2 protons); ^{13}C NMR ($CDCl_3$**)**: δ 174.50, 173.00 (carbonyl carbons), 135.10, 134.34, 132.00, 131.20 (aromatic carbons), 85.00 (C_5), 77.86 (C_3), 62.73 (CH_2OH), 57.40 (C_4), 54.00 (CHCl), 39.55 (CH_3), 26.40, 25.00 (2 CH_2 carbons); MS: *m/z* 340 (M^+ +2), 338 (M^+), 323, 307, 261, 247, 231, 216 (B.P), 107, 77; HRMS–EI: Calcd for $C_{16}H_{19}O_4N_2Cl$ (M) *m/z* 338.1360. Found: M^+ 338.1347. Anal. Found: C, 56.77; H, 5.53; N, 8.22. $C_{16}H_{19}O_4N_2Cl$ requires C, 56.82; H, 5.65; N, 8.28%.

(*3R*)-3-(1-chloro-4-hydroxybutyl)-5-phenyl-2-methyl-dihydro-2H-pyrrolo[3,4-d]isoxazole-4,6 (5H,6a-H)-dione, 3b

White solid. Yield: 32%, R_f = 0.44; IR (KBr): 3580 - 3448 (br), 2935 (m), 2830 (m), 1762 (s), 1664 (s), 1485 (m), 1280 (m), 800 (s), 776 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.98 – 6.93 (m, 5H, C_6H_5), 5.84 (d, 1H, *J* = 4.50 Hz, C_5H), 4.96 – 4.82 (br, s, 1H, OH, exchanged in D_2O), 3.51 (dd, 1H, *J* = 1.20, 1.35 Hz, C_4H), 3.30 (s, 3H, CH_3), 2.61 (dt~m, 1H, CHCl), 1.90 (dd, 1H, *J* = 1.60, 1.54 Hz, C_3H), 1.50 – 1.10 (m, 6H, CH_2 protons); ^{13}C NMR ($CDCl_3$**)**: δ 176.00, 175.12 (carbonyl carbons), 136.25, 135.28, 133.60, 132.45 (aromatic carbons), 84.90 (C_5), 75.65 (C_3), 63.55 (CH_2OH), 56.60 (C_4), 55.20 (CHCl), 40.15 (CH_3), 21.87, 20.46 (2 CH_2 carbons); MS: *m/z* 340 (M^+ +2), 338 (M^+), 323, 307, 288, 261, 255, 247, 231, 216 (B.P), 107, 77; HRMS – EI: Calcd for $C_{16}H_{19}O_4N_2Cl$ (M) *m/z* 338.1360. Found: M^+ 338.1344. Anal. Found: C, 56.75; H, 5.54; N, 8.17. $C_{16}H_{19}O_4N_2Cl$ requires C, 56.82; H, 5.65; N, 8.28%.

(*3S*)-3-(1-chloro-4 hydroxy butyl)-5-cyclohexyl–2-methyl dihydro-2H-pyrrolo[3,4-d]isoxazole-4,6(5H, 6a-H)-dione, 4a

Yellow crystals. Yield 68%, R_f = 0.44; IR (KBr): 3530 – 3465 (br), 2870 (s), 1770 (s), 1683 (s), 1446 (m), 1380 (m), 1265 (m), 815 (s), 780 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.30 (d, 1H, *J* = 6.64 Hz, C_5H), 5.02 - 4.92 (br, s, 1H, OH, exchanged in D_2O), 4.70 (dd, 1H, *J* = 7.26, 7.18 Hz, C_3H), 4.20 (dd, 1H, *J* = 6.26, 6.08 Hz, C_4H), 2.92 (dt~m, 1H, CHCl), 2.34 (s, 3H, CH_3), 1.43 – 1.14 (m, 17H, cyclohexyl and CH_2 protons); ^{13}C NMR ($CDCl_3$): δ 177.58, 176.00 (carbonyl carbons), 86.80 (C_5), 77.08 (C_3), 63.50 (CH_2OH), 55.00 (C_4), 50.66 (CHCl), 38.80 (CH_3), 31.10, 29.52, 27.70, 26.30, 25.00, 23.28, 22.00, 18.27 (cyclohexyl and CH_2 carbons); MS: *m/z* 346 (M^+ +2), 344 (M^+), 329, 294, 255, 237, 222 (B.P), 107, 83; HRMS – EI: Calcd for $C_{16}H_{25}O_4N_2Cl$ (M) *m/z* 344.1720. Found: M^+ 344.1707. Anal. Found: C, 55.69; H, 7.25; N, 8.05. $C_{16}H_{25}O_4N_2Cl$ requires C, 55.78; H, 7.31; N, 8.13%.

(*3R*)-3-(1-chloro-4-hydroxybutyl)-5-cyclohexyl–2-methyl-dihydro-2H-pyrrolo[3,4-d] isoxazole- 4,6 (5H, 6a-H)-dione, 4b

Yellow crystals. Yield 26%, R_f = 0.56; IR (KBr): 3523 – 3474 (br), 2880 (s), 1770 (s), 1680 (s), 1440 (m), 1385 (m), 1260 (m), 810 (s), 774 (s) cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.20 (d, 1H, *J* = 3.70 Hz, C_5H), 4.97 - 4.86 (br, s, 1H, OH, exchanged in D_2O), 4.66 (dd, 1H, *J* = 2.80, 2.04 Hz, C_3H), 4.30 (dd, 1H, *J* = 3.42, 3.60 Hz, C_4H), 2.90 (dt~m, 1H, CHCl), 2.30 (s, 3H, CH_3), 1.48 – 1.08 (m, 17H, cyclohexyl and CH_2 protons); ^{13}C NMR ($CDCl_3$): δ 179.00, 178.10 (carbonyl carbons), 86.00 (C_5), 76.40 (C_3), 64.25 (CH_2OH), 56.36 (C_4), 51.90 (CHCl), 37.23 (CH_3), 29.57, 28.00, 27.20, 25.34, 23.00, 20.54, 19.20, 18.28 (cyclohexyl and CH_2 carbons); MS: *m/z* 346 (M^+ +2),

344 (M^+), 329, 294, 261, 237, 236, 222 (B.P), 107, 83; HRMS–EI: Calcd for $C_{16}H_{25}O_4N_2Cl$ (M) *m/z* 344.1720. Found: M^+ 344.1704. Anal. Found: C, 55.64; H, 7.22; N, 8.06. $C_{16}H_{25}O_4N_2Cl$ requires C, 55.78; H, 7.31; N, 8.13%.

General procedure for cycloaddition (for regioselective cycloadducts)

To a stirred solution of nitrone **1** (1 mmole) in 15 mL water dipolarophiles were added (1 equivalent) at RT under nitrogen atmosphere and the reaction mixture was stirred for 4-5 hr. The progress of the reaction was monitored by TLC. After completion of the reaction, the product was extracted with ether (2 X 25 mL), the organic layer was washed with saturated brine (2 X 15 mL), dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified by column chromatography using ethyl acetate - hexane to afford pure cycloadducts **5-6** (Scheme 1). This procedure was followed for the substrates **4** and **5** listed in Table 1.

***(3S)*-ethyl-3-(1-chloro-4 hydroxy butyl)-2-methyl isoxazolidine-5-carboxylate, 5**

Colourless gummy liquid. Yield 92%, R_f = 0.40; IR (KBr): 3514 – 3440 (br), 2925 (s), 2842 (m), 1755 (s), 1444 (s), 790 (s) cm^{-1}; ^{1}HNMR ($CDCl_3$): δ 4.90 - 4.78 (br, s, 1H, -OH, exchanged in D_2O), 4.18 (q, 2H, *J* = 4.64, 4.34 Hz, $-OCH_2CH_3$), 4.02 (t, 1H, *J* = 7.46 Hz, C_5H), 3.40 (dt, 1H, *J* = 6.54, 6.70 Hz, C_3H), 3.18 (dd, 2H, *J* = 7.12, 7.44 Hz, C_4 2H), 2.84 (dt~m, 1H, CHCl), 2.15 (s, 3H, CH_3), 1.26 (t, 3H, *J* = 5.40 Hz, $-OCH_2CH_3$), 1.18 – 0.86 (m, 6H, CH_2 protons); ^{13}C NMR ($CDCl_3$): δ 169.32 (carbonyl carbon), 84.70 (C_5), 79.12 (C_3), 67.42 (CH_2OH), 61.00 (CH_2 carbon of $-OCH_2CH_3$), 56.90 (C_4), 53.64 (CHCl), 37.20 (CH_3), 22.40, 21.35 (2 CH_2 carbons), 15.45 (CH_3 carbon of OCH_2CH_3); MS: *m/z* 267 (M^+ +2), 265 (M^+), 220, 192, 191, 158, 143 (B.P), 108, 107, 73, 45; HRMS - EI: Calcd for $C_{11}H_{20}O_4NCl$ (M) *m/z* 265.1320. Found: M^+ 265.1303. Anal. Found: C, 49.69; H, 7.48; N, 5.24. $C_{11}H_{20}O_4NCl$ requires C, 49.78; H, 7.59; N, 5.28%.

4-chloro-4-((*3S*)-2-methyl-5-phenyl-isoxazolidin-3-yl)butan-1-ol, 6

Colourless viscous liquid. Yield 91%, R_f = 0.50; IR (KBr): 3520 - 3380 (br), 2925 (s), 2844 (m), 1710 (s), 1440 (m), 1324 (s), 804 (m), 776 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.80 - 6.73 (m, 5H, C_6H_5), 4.94 (t,1H, *J* = 6.08 Hz, C_5H), 4.85 – 4.77 (br, s, 1H, exchanged in D_2O), 4.28 (dt, 1H, *J* = 6.52 Hz, C_3H), 3.90 (dd, 2H, *J* = 6.48, 6.12 Hz, C_4 2H), 3.66 (dt~m, 1H, CHCl), 2.18 (s, 3H, CH_3), 1.47 - 1.08 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 136.75, 135.22, 133.00, 132.14 (aromatic carbons), 85.95 (C_5), 77.32 (C_3), 60.64 (CH_2OH), 56.40 (C_4), 52.38 (CHCl), 38.66 (CH_3), 20.27, 19.20 (2 CH_2 carbons); MS: *m/z* 271 (M^+ +2), 269 (M^+), 192, 191, 162, 147 (B.P), 107, 77; HRMS-EI: Calcd for $C_{14}H_{20}O_2NCl$ (M) *m/z* 269.1370. Found: M^+ 269.1357. Anal. Found: C, 62.32; H, 7.40; N, 5.13. $C_{14}H_{20}O_2NCl$ requires C, 62.42; H, 7.48; N, 5.20%.

Antibacterial screening

All the isoxazolidine derivatives **2-6** were screened for antimicrobial activity and were found soluble in dimethyl sulphoxide (DMSO) upto 4%, which was found to be completely free from any type of antimicrobial activity. A stock solution of concentration 1mg/mL was prepared which was further diluted as per requirement. All the cycloadducts (**2-6**) were subjected to *in vitro* screening against the 14 bacterial strains. Sensitivity test was performed by Agar dilution method and then minimum inhibitory concentration (MIC) of the drugs were determined by Disc Diffusion Method and Broth Dilution Method [14]. Previously prepared drug dilutions (4µg/mL, 8µg/mL, 16µg/mL, 32µg/mL, 64µg/mL, 128µg/mL, 256µg/mL and 512µg/mL) of the isoxazolidines with appropriate antibiotic control (Streptomycin and Gentamycin) were prepared with Mueller Hinton Agar [15]. For agar dilution assay those cycloadduct plates were spot inoculated (2×10^6 cfu per spot). A plate without isoxazolidines was taken as control (blank) in order to compare the results. The results were then recorded after incubation for 72 hrs at 37ºC [16]. The minimum drug concentration for which no visible growth was observed was considered as the MIC. MIC was determined by Kirby-Bauer disc diffusion method [17] and

Table 2. Determination of Minimum Inhibitory Concentration (MIC)

Name of Organism	MIC values in µg/mL	Name of Organism	MIC values in µg/mL	Name of Organism	MIC values in µg/mL	Name of Organism	MIC values in µg/mL	Name of Organism	MIC values in µg/mL
	2a 4% DMSO	**2b 0.17% DMSO**	**3a 0.17% DMSO**	**3b 0.17% DMSO**	**4a 0.17% DMSO**	**5 4% DMSO**	**6 4% DMSO**	**Strepto-mycin**	**Genta-mycin**
1 *Escherichia coli* ATCC 25938	32	128	512	>512	>512	128	16	2	0.25
***Klebsiella pneumonia* J/1/4**	64	64	>512	>512	>512	128	32	4	2
***Staphylococcus aureus* ATCC 27853**	32	64	>512	>512	>512	128	64	2	1
***Pseudomonas aeruginosa* ATCC 27853**	64	256	>512	>512	512	64	8	64	2
***Vibrio cholerae* 14035**	32	64	>512	>512	256	128	64	64	0.5
***Bacillus subtilis* UC 564**	64	64	>512	>512	64	32	8	8	4
***Shigella dysenteriae* 3**	64	64	>512	>512	>512	128	16	64	1
***Streptococcus faecalis* 29212**	64	128	>512	>512	>512	128	64	64	0.50
***Shigella flexneri* DN 13**	8	16	16	16	32	64	32	32	1
1 *Salmonella typhi* DIRW	8	64	16	16	64	256	128	4	1
1 *Vibrio parahaemolyticus* 72016	256	256	>512	>512	>512	256	128	16	1
1 *Micrococcus luteus* AGD 1	128	64	>512	>512	>512	512	128	4	8
1 *Salmonella typhimurium* 11	32	64	>512	>512	>512	128	32	8	1
1 *Enterococcus faecalis*	64	256	>512	>512	>512	128	32	4	2

Broth Dilution Method [14]. The antifungal activity of the isoxazolidine derivatives (**2-6**) has been assayed *in vitro* at a concentration of 100µg/mL, 200µg/mL, 400µg/mL, 600µg/mL, 800µg/mL and 1000µg/mL by Agar dilution and Broth dilution method against *Aspergillus niger, Candida albicans, Candida tropicalis, Cryptpcoccus neoformans*, which were maintained on sabouraud dextrose agar slants stored at 4°C. Test drugs (**2-6**) exhibited considerable antibacterial and antifungal activity, were diluted double fold with Mueller Hinton broth for bacterial strains and sabouraud dextrose broth for fungi in a series of test tubes. An aliquot of 1mL of the bacterial suspension (2×10^6 cfu/mL) and fungal spores (2×10^5 spores/mL) were inoculated into each tube. The control tubes were inoculated with same quantity of broth culture only.

Table 3. Determination of Minimum Inhibitory Concentration (MIC)

fungal strains	MIC values in µg/mL							
	2a	**2b**	**3a**	**3b**	**4a**	**5**	**6**	**Fluconazole**
Aspergillus niger	100	200	600	800	400	100	200	10
Candida albicans	200	100	>1000	600	600	200	200	4
Candida tropicalis	400	200	800	>1000	400	400	200	8
Cryptococcus neoformans	400	400	>1000	>1000	1000	400	100	8
Saccharomyces cerevisiae	200	100	600	800	800	200	100	16

Determination of Minimal Bacteriocidal Concentration (MBC)

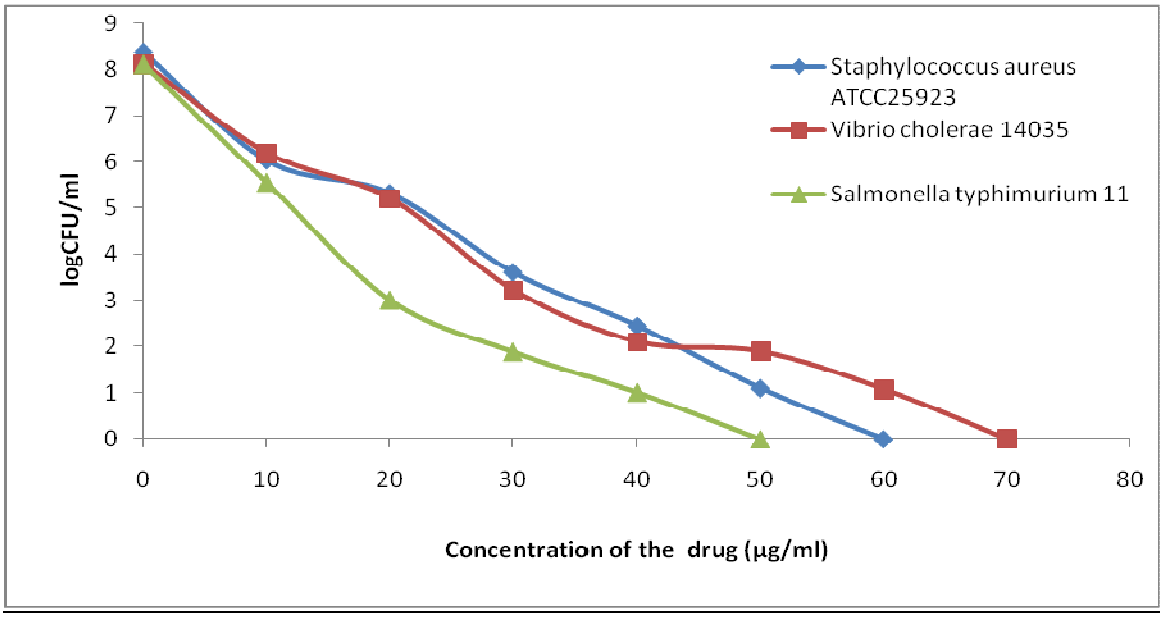


Fig.1. Effect of drug 2a on three bacteria at different concentrations

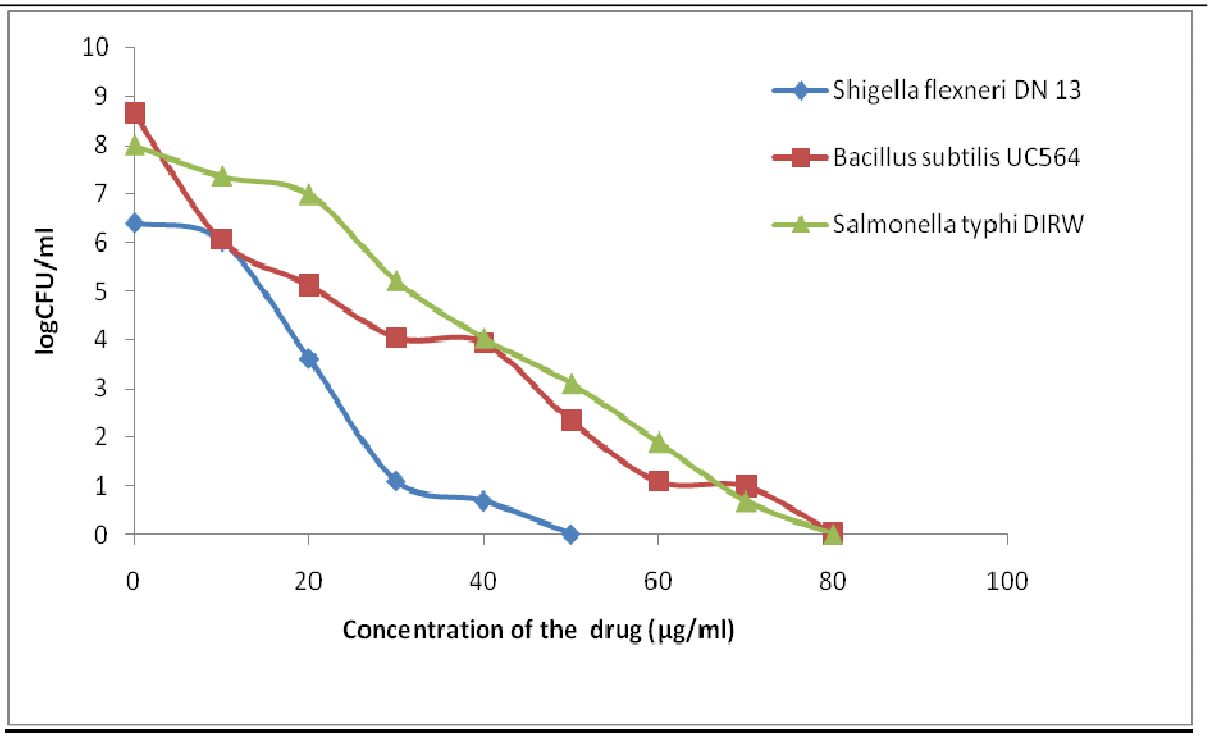


Fig.2. Effect of drug 3b on three bacteria at different concentrations

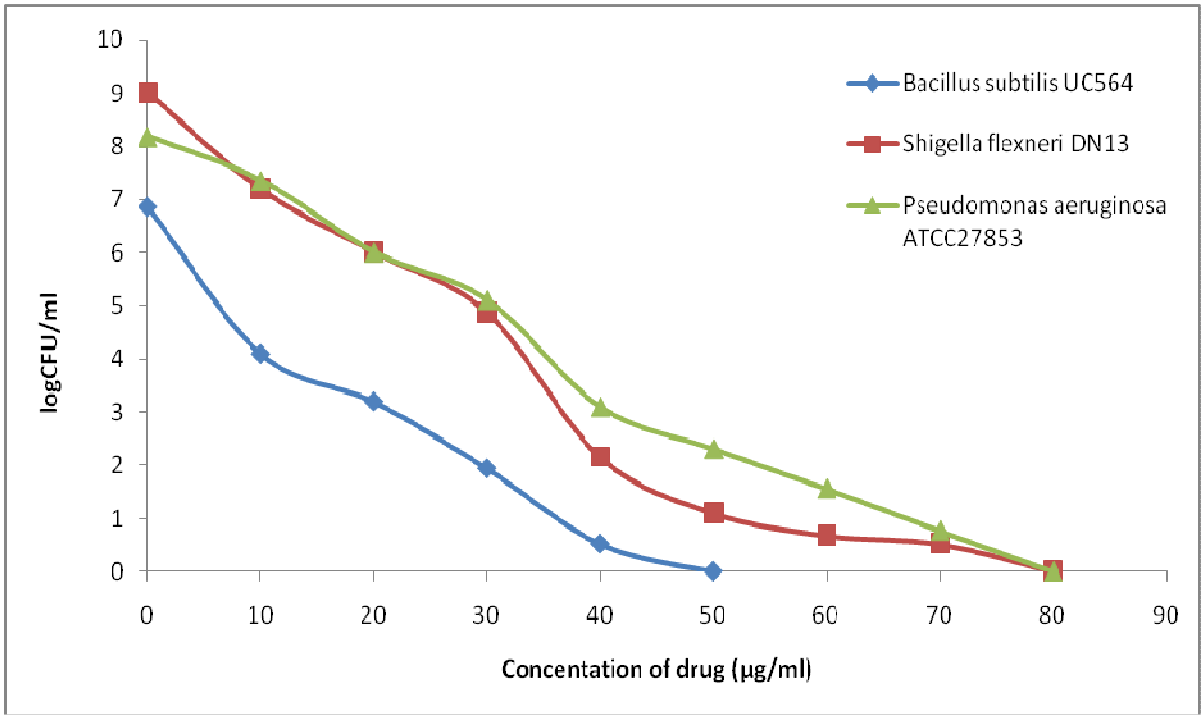


Fig.3. Effect of drug 4b on three bacteria at different concentrations

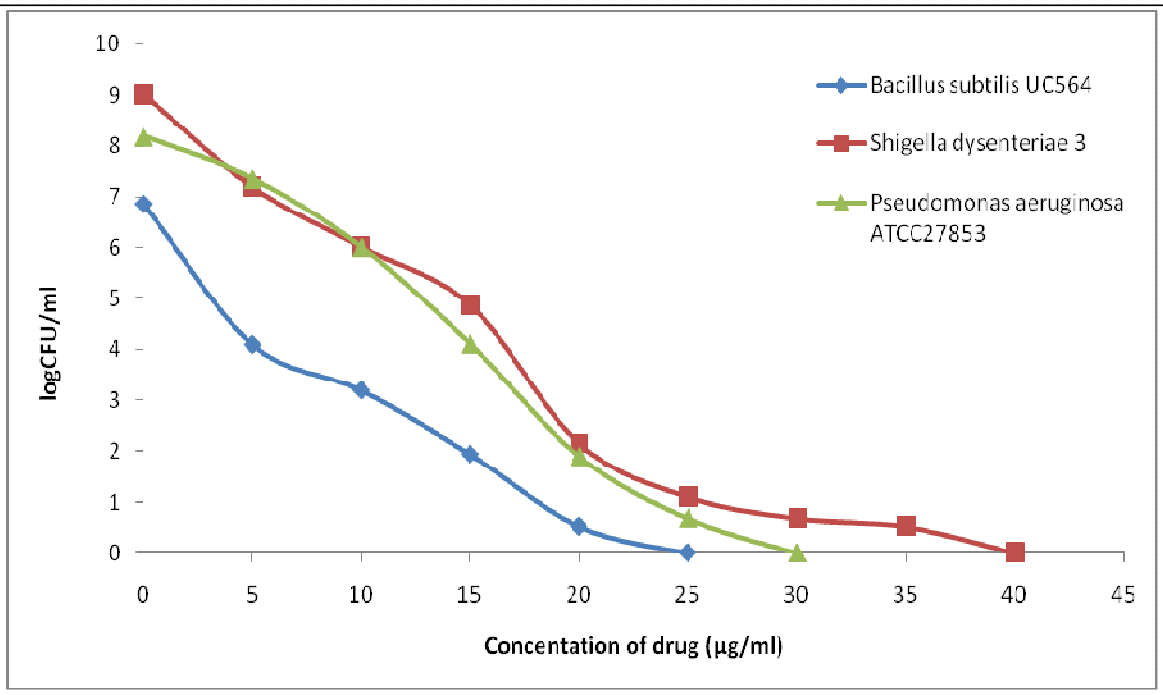


Fig.4. Effect of drug 5 on three bacteria at different concentrations

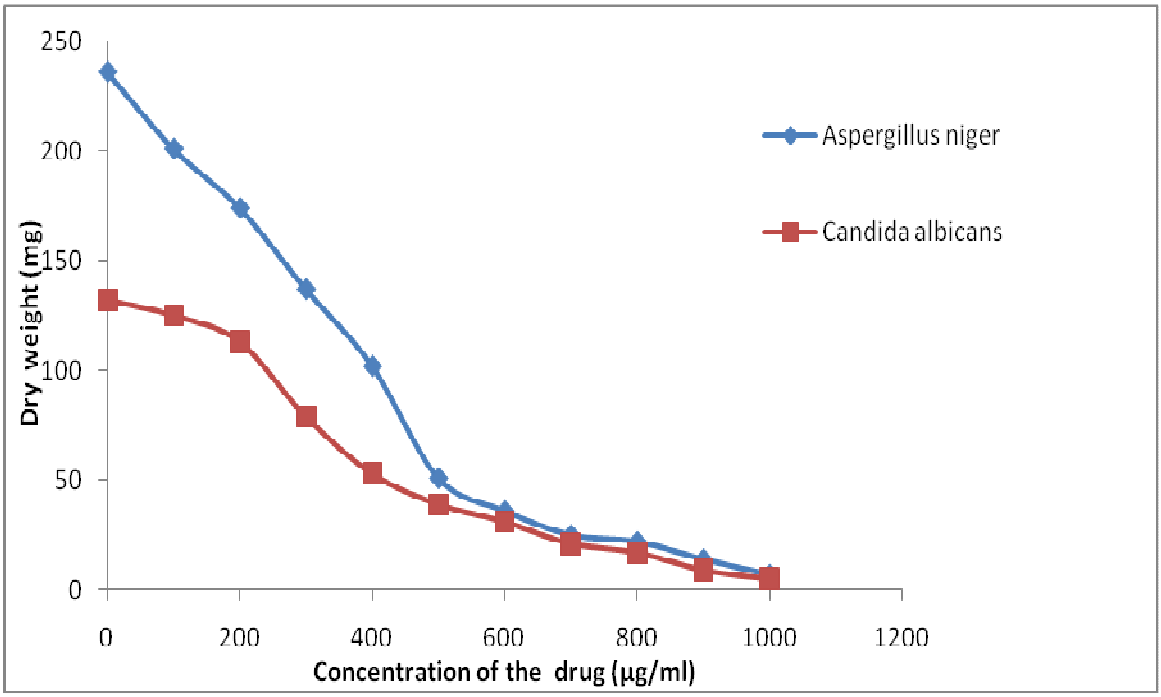


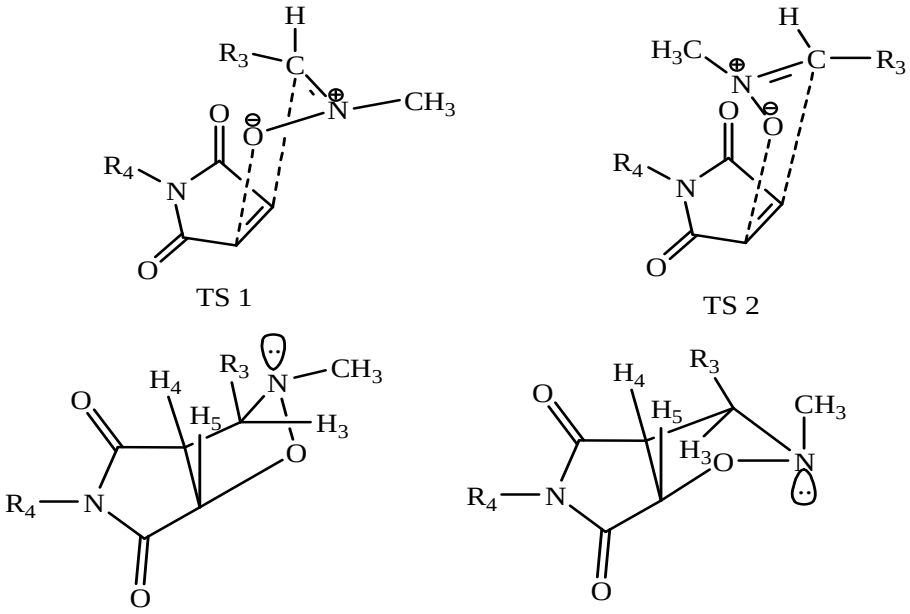
Fig.5. Effect of drug 6 on *invitro* growth of two fungi at different concentrations

All tubes were incubated at 37^{o}C for 24h and 28°C for 96 h with shaking on a platform shaker at 200 rpm. The test drugs were added to the mid-logarithmic phase of growth and aliquots of 1.0 mL were withdrawn for determination of colony count [18] while the growth of the fungi was determined by dry weight of the sample at 60^{0}c for 20 h for 3 days [19].

RESULTS AND DISCUSSION

Almost all the reactions in water are very fast (3 - 4 hrs in case of maleimides and 5 hrs for styrene) compared to the normal cycloaddition reactions in organic solvents which are reported to take longer periods (26 - 48 hrs) [9]. It is possible that water promotes the reaction through hydrogen bond formation with the carbonyl oxygen atom of the α,β-unsaturated carbonyl compounds and thereby increasing the eletrophilic character at the β-carbon which is attacked by nucleophilic oxygen atom of the nitrone. Thus water activates maleimide, ethyl acrylate and thereby greately facilitates the reaction. Reaction rate is comparatively slower in styrene because of very lesser possibility of the formation of hydrogen bonding between water and alkenes but still the rate of the reaction and the yield is higher than the cycloaddition reactions performed in solvents like THF, CH_2Cl_2 (Table 1). We suggest an explanation for these results in terms of the frontier molecular orbital (FMO) theory which has been used extensively to explain regioselectivity and to predict the yield, rate in 1,3-Dipolar cycloadditions [20,21]. This theory states that the Gibbs energy of activation is related to the energy gap between the interacting HOMO and LUMO. The dipolarophiles like styrene, cyclohexene etc. are weak hydrogen bond acceptors, which means that their FMO's are only slightly affected by hydrogen bond interactions and lead to a reduction of the energy gap between the interacting FMO's (in this case, the HOMO of the dipolarophile and LUMO of the 1,3 dipole). Consequently, the Gibbs energy of activation of the reaction is reduced and the reaction is accelerated in water with good yield.

Excellent diastereofacial selectivity is observed in nitrone cycloadditions in water. The addition of nitrone **1** to maleimides result in a mixture of diastereomer **2a–4a** and **2b–4b** (almost 65 : 35 ratio in all cases) and generation of as many as three to four chiral centers in a single step. Study of organic reactions in aqueous media shows that there is a higher probability of the formation of mixture of diastereomers when water is used as solvent rather than conventional organic solvents [5]. These results can be rationalized by an *exo* approach of nitrone **1** which has *Z* configuration for the formation of major cycloadducts **2a–4a** (transition state **1**). The minor cycloadducts **2b–4b** are formed by the *endo* approach of *Z* nitrone (transition state **2**). The mixture of diastereomers are identified by considering the multiplicity of the proton signals at 3-H and 4-H along with their coupling constant values [22,23]. The most significant differences in the ^{1}H NMR data of the diastereomers are the position and multiplicity of the 3-H signal. In the minor adducts **2b–4b**, 3-H resonates upfield around δ_H 2.50-4.60 while for the same proton in major adducts **2a–4a** around δ_H 2.60-4.70 and $J_{3,4}$ ~ 6.26 Hz for major adducts whilst for minor adducts $J_{3,4}$ is ~ 2.44 Hz. These differences can be explained by considering the available isoxazolidine ring conformations. Due to the 4,5-fused pyrrolidindione, the isoxazolidine ring adopts an envelope conformation and allowing for inversion, its nitrogen atom will either extend out from the envelope, *i.e.*, minor conformation, or point inside the envelope, *i.e.*, major conformation. The minor conformer has the N-lone pair antiperiplanar and therefore, capable of shielding 3-H proton, so this conformation is assigned to the minor conformer (Figure 6). The diastereomeric isoxazolidines **2a–4a** and **2b–4b** were separated by column chromatography and obtained in analytically pure form. The *endo/exo* stereochemistry mentioned above is based on extensive NMR investigations [22,23]. Most relevant are the coupling constants ($J_{H3,\ H4}$) of the diastereomers. For **2a-4a**, this coupling constant is almost 6.20–6.74 Hz, implying a *cis* relationship between H-3 and H-4, whereas for **2b–4b**, the coupling constant is almost 1.80–2.60 Hz which implies a *trans* relationship between H-3 and H-4 [22,23].



R_3 = $CHCl(CH_2)_3OH$; R_4 = Ph; Me; Cy

Figure 6

In all the diastereomers, the configurations of H-5 and H-4 are *cis* as evidenced from their coupling constant values. For ethyl acrylate and styrene the regioselectivity was rationalized by using frontier orbital theory [20] and ^{1}H NMR experiments. Cycloadditions to α,β-unsaturated carboxylic acid derivatives, *e.g.* ethyl acrylate are particularly useful because high regioselectivity is often observed in water [5]. The reactions were found to be highly regioselective to form solely 5-substituted isoxazolidines. Nitrone **1** has considerably higher ionization potential than normal nitrones due to the electron withdrawing effect of chlorine. Therefore, nitrone (LUMO)-dipolarophile (HOMO) interactions completely dominate the reaction and lead to the formation of only 5-substituted adducts [20,24]. From the ^{1}H NMR spectrum of cycloadducts **5–6**, it has been found that clear double doublet signal for H-4 protons and doublet of triplet signal for H-3 protons are obtained in both the cases due to further coupling from vicinal hydrogens and hence confirms in favour of 5-substituted adducts only. From the detailed investigations on the nature of these cycloaddition reactions using TLC and ^{1}H NMR spectrum studies for the cycloadducts **5–6,** it is also confirmed that no diastereomers are formed. The relative configurations of H-3, H-4 and H-5 protons in these adducts are *syn* and the cycloadducts are in favour of *exo* transition state geometry as evidenced from their coupling constant values ($J_{H4,H5}$ & $J_{H3,H4}$ ~ 6.2–7.6 Hz).

In general, the reactions are very clean and high yielding compared to usual cycloaddition reactions of nitrones. The products have been characterized from their spectroscopic (IR, ^{1}H NMR, HRMS, ^{13}C NMR) data. No catalyst or co-organic solvent are required. The exact stereochemistry at the asymmetric CHCl carbon atom of all the cycloadducts could not be determined due to multiplet signals (doublet of triplet appears almost as multiplet) obtained in the NMR spectrum and also due to freely rotating carbon centre at CHCl. In the mass spectrum, significant M^++2 ion peak signals of characteristic height are obtained in most of the diastereomers and regioselective cycloadducts as the peak of highest intensity due to the

presence of isotopic abundance of Cl^{37} atom. Studies of HRMS spectra shows almost exact mass in the majority of the compounds.

CONCLUSION

In summary, the present procedure provides an example of green chemistry methodology for the synthesis of regio and stereoselective novel isoxazolidines in aqueous phase with high yield in a short reaction time and all the synthesized novel compounds are found to have significant antibacterial activity. The notable factors of this methodology are: (a) high yields (b) faster reaction (c) mild reaction conditions and (d) green synthesis avoiding use of organic solvents. Therefore, it is believed that procedure described here will find important applications in the synthesis of isoxazolidine derivatives and thereby offering greater scope for aqueous phase cycloaddition reactions.

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