ONE POT FACILE STEREO AND REGIOSELECTIVE SYNTHESIS OF SPIRO ISOXAZOLIDINE DERIVATIVES USING α-METHYLENE-γ-BUTYROLACTONE AND NOVEL α-N-METHYL/PHENYL FURAN DERIVATIVES WITH α-AMINO NITRONES AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT
Cycloaddition of α-amino nitrones to α-methylene-γ-butyrolactone afforded two diastereomeric 5-spiro substituted isoxazolidines with high selectivity while the same reaction to novel α-N-methyl and α-N-phenyl furan derivative afforded single regioselective 5-spiro cycloadducts. All the compounds have been screened for their antibacterial activity and found to be active.

Keywords: α-amino nitrone, cycloaddition reaction, spirocycloadducts, stereo & regioselectivity, antibacterial activity

INTRODUCTION
Nitrones are versatile synthetic intermediates and excellent spin trapping reagents\(^1\) and can be used also as a stable potential oxidizing reagents in the synthesis of aldehyde & ketones\(^2\). Nitrone cycloaddition to olefins are one of the most versatile methods for the construction of isoxazolidine derivatives\(^3\). In continuation of our earlier work for the isoxazolidine and isoxazoline synthesis using α-amino & α-chloro nitrones in solid phase and in hydrated media\(^4,5\), we report herein for the first time novel α-N-methyl/phenyl furan derivatives (obtained as side product during aldehyde & ketone synthesis)\(^2\) as dipolarophile in the regioselective synthesis of 5-spiro isoxazolidines using α-amino nitrones at RT with an excellent yield (Scheme - 1, Table-1) along with their antibacterial activity. The present paper also reports synthesis of diastereoselective 5-spiro isoxazolidines using α-methylene-γ-butyrolactone as dipolarophile with α-amino nitrones. Following the reported synthesis methodology of N-phenyl-α-amino nitrone\(^6\)(1, R=Ph), novel N-methyl-α-amino nitrone (1, R=Me) has been synthesized as pale yellow crystalline solid, m.p 43°C (uncorrected) which decomposes on standing.

EXPERIMENTAL
General remarks: \(^1\)H NMR spectra were recorded with a Bruker Avance DPX 400 spectrometer (400 MHz, FT NMR) using TMS as internal standard. \(^13\)C NMR spectra were recorded on the same instrument at 100 MHz. The coupling constants (\(J\)) are given in Hz. IR spectra were obtained with a Perkin-Elmer RX 1-881 machine as film or KBr pellets for all the products. MS spectra were recorded with a Jeol SX-102 (FAB) instrument. The HRMS spectra were recorded on a Q–ToF micro instrument (YA–105). TLC was carried out on Fluka silica gel TLC cards while column chromatography was performed with silica gel (E.Merck India) 60–200 mesh. 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 and has been used in synthesis\(^4,5\)
Reagents and conditions: i) RT, N2 atmosphere, 4 - 5 hr

**General procedure for the synthesis of nitrone 1 (R = Me)**

N-methylhydroxylamine (250mg, 5.3127 mmole) was added to dry, distilled formamide (9 mL) and anhydrous MgSO4. The reaction mixture was kept at RT with constant stirring with a magnetic stirrer under N2 atmosphere for 12 hr. The formation of nitrone was monitored by TLC (Rf = 0.36). The nitrone was isolated under reduced pressure vacuum pump as pale yellow crystalline solid, m.p 43°C (uncorrected) which decomposes on standing.

**Spectroscopic data for nitrone 1 (R = Me)**

1: Pale yellow crystalline solid. Yield: 87%; Rf = 0.36, m.p: 43°C (uncorrected); IR (KBr): 3312 (br), 3021 (m), 1610 (s), 1180 (s) cm⁻¹; ¹H NMR (CDCl₃): δ 7.40 (br,s, 2H, NH₂, exchanged in D₂O), 6.72 (s, 1H, CH=N⁺); 13C NMR(CDCl₃): δ 143.23 (CH=N⁺), 33.16 (N⁺- CH₃); HRMS – EI: Calcd. for C₂H₆ON₂, (M), 74.0632, Found: M+, 74.0617.

**General procedure of cycloaddition for spiro diastereomers**

To a well stirred solution of nitrone 1 (R = Me; 1 mmole) in diethyl ether (20 mL) taken in a 50 mL conical flask, was added α-methylene-γ-butyrolactone (1 equivalent) and was stirred at RT with a magnetic stirrer under N₂ atmosphere for 4 hr. The progress of the reaction was monitored by TLC (Rf = 0.38;0.44). After completion of the reaction, the crude spiro diastereomers were concentrated in a rotary evaporator and finally the mixture of diastereomers were purified and separated by column chromatography using ethyl acetate - hexane to afford pure spiro cycloadducts (entry 1, Table 1, Scheme - 1). This procedure was followed for the reaction of nitrone 1 (R=Ph) with α-methylene-γ-butyrolactone listed in Table 1.

(3S,3′S)-spiro[tetrahydrofuran-2-one-3,5′-(2′-methyl,3′-amino)tetrahydroisoxazole] 2a

2a: Pale yellow liquid. Yield. 68%; Rf = 0.38; IR (KBr): 3390 (br), 3024 (m), 2910 (m), 1755 (s), 1470 (m), 1290 (m), 1178 (s) cm⁻¹; ¹H NMR (CDCl₃): δ 6.80 Hz, C₃′ H), 4.02 (dt, C₄ 2H, J = 6.08, 7.42 Hz), 3.33 (s, 3H, N-CH₃), 2.60 (t, 2H, J = 6.18 Hz, C₅ 2H), 2.00 (ddd, C₄ 2H, J = 6.56, 6.20 Hz); ¹³C NMR (CDCl₃): δ 178.22 (carbonyl carbon), 88.43 (C₅′/C₃′), 73.70 (C₁), 65.20 (C₄′), 56.13 (C₄′), 38.30 (N-CH₃); MS: m/z 172 (M⁺), 157, 156, 141 (B.P); HRMS – EI: Calcd for C₃H₁₂O₃N₂ (M) m/z 172.1140. Found: M⁺ 172.1112.

(3S,3′R)-spiro[tetrahydrofuran-2-one-3,5′-(2′-methyl,3′-amino)tetrahydroisoxazole] 2b
2b: Yellow liquid. Yield 24%; $R_f = 0.44$; IR(KBr): 3378 (br), 3012 (m), 2904 (m), 1762 (s), 1655 (s), 1478 (m), 1286 (s), 1182 (s) cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 7.27 (br,s, 2H, NH$_2$, exchanged in D$_2$O), 4.40 (t, 1H, $J = 3.42$ Hz, C$_{3''}$ H), 4.12 (dt, C$_{4''}$ 2H, $J = 2.24, 3.40$ Hz), 3.26 (s, 3H, N-CH$_3$), 2.54 (t, 2H, $J = 3.08$ Hz, C$_5$ 2H), 1.96 (ddd, C$_4$ 2H, $J = 4.24, 2.68$ Hz); $^{13}$C NMR (CDCl$_3$): δ 176.58 (carbonyl carbon), 86.90 (C$_5'/C_3$), 75.25 (C$_{3''}$), 64.32 (C$_{4''}$), 54.30 (C$_4$), 51.76 (C$_5$), 37.14 (N-CH$_3$); MS: m/z 172 (M$^+$), 156, 155, 141 (B.P); HRMS – EI: Calcd for C$_7$H$_{12}$O$_3$N$_2$ (M) m/z 172.1140. Found: M$^+$ 172.1117.

(3S,3'S)-spiro[tetrahydrofuran-2-one-3,5'-(2'-phenyl,3'-amino)tetrahydroisoxazole] 3a
3a: Dark red liquid. Yield 70%, $R_f = 0.46$; IR (KBr): 3385 (br ), 3036 (m), 2916 (m), 1778 (s), 1660 (s), 1480 (m), 1282 (m), 1190 (s), 782 (s) cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 7.30 (br,s, 2H, NH$_2$, exchanged in D$_2$O), 6.86 – 6.75 (m, 5H, C$_6$H$_5$), 4.46 (t, 1H, $J = 7.14$ Hz, C$_3'$ H), 4.00 (dt, C$_{4''}$ 2H, $J = 6.16, 6.70$ Hz), 2.54 (t, 2H, $J = 7.06$ Hz, C$_5$ 2H), 2.05 (ddd, C$_4$ 2H, $J = 8.32, 7.10$ Hz); $^{13}$C NMR (CDCl$_3$): δ 181.20 (carbonyl carbon), 133.43, 132.12, 130.86, 128.73 (aromatic carbons), 85.42 (C$_5'/C_3$), 72.67 (C$_{3''}$), 62.90 (C$_{4''}$), 57.84 (C$_4$), 53.68 (C$_5$); MS: m/z 222 (M$^+$), 206, 145, 129 (B.P), 77; HRMS – EI: Calcd for C$_{11}$H$_{14}$O$_3$N$_2$ (M) m/z 222.1344. Found: M$^+$ 222.1327.

(3S,3'R)-spiro[tetrahydrofuran-2-one-3,5'-(2'-phenyl,3'-amino)tetrahydroisoxazole] 3b
3b: Red liquid. Yield 20%, $R_f = 0.50$; IR(KBr): 3298 (br ), 2908 (m), 1780 (s), 1656 (s), 1485 (m), 1277 (m), 1198 (s), 780 (s) cm$^{-1}$; $^1$H NMR (CDCl$_3$): δ 7.24 (br,s, 2H, NH$_2$, exchanged in D$_2$O), 6.80 – 6.67 (m, 5H, C$_6$H$_5$), 4.46 (t, 1H, $J = 3.42$ Hz, C$_{3''}$ H), 4.13 (dt, C$_{4''}$ 2H, $J = 2.84, 2.30$ Hz), 2.50 (t, 2H, $J = 3.10$ Hz, C$_5$ 2H), 2.14 (ddd, C$_4$ 2H, $J = 4.32, 2.36$ Hz); $^{13}$C NMR (CDCl$_3$): δ 178.18 (carbonyl carbon), 134.12, 132.95, 131.70, 129.82 (aromatic carbons), 87.50 (C$_5'/C_3$), 74.54 (C$_{3''}$), 63.87 (C$_{4''}$), 58.43 (C$_4$), 52.41 (C$_5$); MS: m/z 222 (M$^+$), 206, 205, 129 (B.P), 77; HRMS – EI: Calcd for C$_{11}$H$_{14}$O$_3$N$_2$ (M) m/z 222.1344. Found: M$^+$ 222.1321.

Reagents and conditions: i) RT, N$_2$ atmosphere, 7 - 8 hr

**General procedure of cycloaddition for regioselective spiro cycloadducts**

To a well stirred solution of nitronone 1 (R=Me; 1 mmole) in diethyl ether (20 mL) taken in a 50 mL conical flask, was added a-N-methyl furan derivative [(E)-1-(dihydrofuran-2-(3H)-ylidene)-N-methyl methanamine] (1 equivalent) and was stirred at RT with a magnetic stirrer under N$_2$ atmosphere for 7 hr. The progress of the reaction was monitored by TLC ($R_f = 0.52$). After completion of the reaction, the crude spiro cycloadduct was concentrated in a rotary evaporator and finally purified by column chromatography using ethyl acetate - hexane to afford pure spiro cycloadduct 5a (entry 3, Table 1, Scheme - 2). This procedure was followed for the reaction of nitronone 1 (R=Ph) with a-N-phenyl furan derivatives [(E)-1-(dihydrofuran-2-(3H)-ylidene)-N-phenyl methanamine] listed in Table-1.
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(S)-3-amino-(3S,4S,5S)-2-methyl-4-(methylamino)-1,6-dioxa-2-azaspiroisoxazole 5a
5a: Yellow viscous liquid. Yield 93%, Rf = 0.52; IR (KBr): 3396 (br ), 3033 (m), 2917 (m), 1773 (s), 1662 (s), 1470 (m), 1282 (m), 1178 (s) cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 4.90 (br,s, 2H, NH\(_2\), exchanged in D\(_2\)O), 4.60 (br, 1H, NHCH\(_3\)), 3.36 (s, 6H, 2 x N-CH\(_3\)), 3.00 (d, 1H, \(J = 7.54\) Hz, C\(_3\)H), 2.70 (dt, 2H, \(J = 6.24, 6.28\) Hz, C\(_{3'}\)H\(_2\)), 2.38 (dt, 1H, \(J = 7.12, 6.70\) Hz, C\(_4\)H), 1.70 – 1.48 (m, 4H); 13C NMR (CDCl\(_3\)): \(\delta\) 88.50 (C5/C2'), 77.12 (C3), 56.26 (C4), 40.94 (N-CH\(_3\)), 38.13 (NH-CH\(_3\)), 32.07, 31.22, 29.34 (3',4',5' CH\(_2\) carbons); MS (m/z): 187 (M+), 172, 157, 156 (B.P), 141. HRMS-EI: Calcd. for C\(_8\)H\(_{17}\)O\(_2\)N\(_3\) (M), 187.1633, Found; M+, 187.1613.

(S)-3-amino-(3S,4S,5S)-2-phenyl-4-(phenylamino)-1,6-dioxa-2-azaspiroisoxazole 5b
5b: Yellow gummy liquid. Yield 92%, Rf = 0.48; IR (KBr): 3387 (br ), 3030 (m), 2920 (m), 1780 (s), 1674 (s), 1276 (m), 785 (s) cm\(^{-1}\); 1H NMR (CDCl\(_3\)): \(\delta\) 7.02 - 6.90 (m, 10H, 2 x C\(_6\)H\(_5\)), 5.86 (d, 1H, \(J = 6.30\) Hz, C3H), 5.00 (br,s, 2H, NH\(_2\), exchanged in D\(_2\)O), 3.50 (dt, 2H, \(J = 6.74, 6.06\) Hz, C\(_3\)H\(_2\)), 3.38 (br,s,1H, NHC\(_6\)H\(_5\)), 2.70 (dt, 1H, \(J = 7.20, 6.18\) Hz,C\(_4\)H), 1.52 – 1.28 (m, 4H); 13C NMR (CDCl\(_3\)): \(\delta\) 137.21, 135.44, 134.00, 133.10, 130.66, 129.40, 128.32, 127.84 (aromatic carbons), 86.94 (C 5/C2'), 74.24 (C3), 55.70 (C 4), 27.87, 25.63, 24.00 (3',4',5' CH\(_2\) carbons); MS (m/z): 311 (M +), 295, 218, 203 (B.P), 202, 92, 77; HRMS-EI: Calcd. for C\(_{18}\)H\(_{21}\)O\(_2\)N\(_3\) (M), 311.2054, Found; M+, 311.2037.

Table-1: Physical characteristics of the spiro cycloadducts

<table>
<thead>
<tr>
<th>Entry</th>
<th>Nitron (R = )</th>
<th>Dipolarophile (a)</th>
<th>Nature of spiro cycloadduct (b)</th>
<th>Cis/trans ratio</th>
<th>Time (hr)</th>
<th>Yield (c) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Me</td>
<td>(\alpha)-methylene-(\gamma)-butyrolactone</td>
<td>2a: Pale yellow liquid</td>
<td>2a: 68</td>
<td>4</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Ph</td>
<td>(\alpha)-methylene-(\gamma)-butyrolactone</td>
<td>2b: Yellow liquid</td>
<td>2b: 24</td>
<td>5</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>Me</td>
<td>(\alpha)-N-methyl butyrolactone</td>
<td>furan</td>
<td>5a: Dark red liquid</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>Ph</td>
<td>(\alpha)-N-phenyl butyrolactone</td>
<td>furan</td>
<td>5b: Yellow viscous liquid</td>
<td>8</td>
<td>92</td>
</tr>
</tbody>
</table>

\(a\) Reaction condition: \(\alpha\)-amino nitrone (1 mmol), dipolarophile (1 equivalent), dry ether, N\(_2\) atmosphere, RT

\(b\) All the compounds were characterized by IR, \(^1\)H NMR, \(^13\)C NMR, MS, HRMS spectral data.

\(c\) Isolated yields after purification

RESULTS AND DISCUSSION

The cycloaddition reaction of \(\alpha\)-amino nitrones (I) to \(\alpha\)-methylene-\(\gamma\)-butyrolactone are faster (4-5 hrs) compared with reaction to \(\alpha\)-N-methyl/phenyl furan derivatives (7-8 hrs). Excellent diastereofacial selectivity is observed in nitrone additions described here with \(\alpha\)-methylene-\(\gamma\)-butyrolactone. The addition of \(\alpha\)-amino nitroate 1 (Scheme - 1, R=Me, Ph) to \(\alpha\)-methylene-\(\gamma\)-butyrolactone results in a mixture of diastereoisomer 2a-3a & 2b-3b (almost 70:30 ratio in both cases). These results can be rationalized by an exo approach of both nitroate 1 (in Z configuration) and dipolarophile 2 for the major cycloadducts 2a-3a (transition state 1).

The minor cycloadducts 2b-3b are formed by an endo approach of dipolarophile 2 to Z nitroate 1 (transition state 2). The mixture of diastereomers are identified by considering the multiplicity of the proton signals at 3'-H along with their coupling constant values and significant differences with respect to position and multiplicity of the 3'-H signal in the \(^1\)H NMR spectrum. For 2a-3a (R=Ph,Me), this coupling constant (\(J_{H3',H4'}\)) is almost 6.8 to 7.4 Hz, implying a cis relationship between 3'-H and 4'-H whereas 2b-3b (R=Ph,Me) has a coupling constant (\(J_{H3',H4'}\)) of 2.5 to 3.4 Hz which implies a trans relationship between 3'-H and 4'-H (Ref 6).
On the other hand, reaction of nitrone $1 (R=\text{Ph,Me})$ with newly reported $\alpha$-$N$-methyl/phenyl furan derivatives as dipolarophile are found to be highly regioselective to form solely 5-spiro isoxazolidine derivatives ($5a$-$5b$). It could be due to the fact that nitrone (LUMO) – dipolarophile (HOMO) interactions are strong enough to dominate the reaction and leads to the formation of solely regioselective 5-spiro isoxazolidines via an exo approach of nitrone $1$ (in $Z$ configuration) to the furan derivatives $4$ (transition state $3$). The relative configurations of $H_3$ & $H_4$ protons in the regioselective spiro cycloadducts are in favour of exo transition state geometry. The $H_3$ & $H_4$ protons are $syn$ in these cycloadducts and their coupling constants ($J_{H_3,H_4} = 6 – 7.4$ Hzs) are also indicative of this stereochemical relationship.

In the $^{13}$C NMR spectrum, four signals were obtained in case of phenyl ring carbons due to equivalent nature of C-2 & C-6 and C-3 & C-5 carbons. $^1$H NMR spectrum of $2a$-$3a$, $2b$-$3b$ and $5a$-$5b$ shows
significant long range coupling between H₄ with H₄' and vice versa in 2a-3a and 2b-3b while H₄ with H₃' and vice-versa in 5a-5b. Mass fragmentation peaks of different value are also obtained for diastereomers of a particular spiro cycloadduct. Studies of HRMS spectra shows almost exact masses for the majority of the compounds. The diastereomeric spiro isoxazolidines 2a-3a and 2b-3b were separated by column chromatography and obtained in analytically pure form. The ¹H NMR spectrum of 2a-3a and 2b-3b displayed different spectrum (position of signals) for the diastereomers. In contrast, the ¹H NMR spectrum of 5a-5b displayed only one set of signals indicating that they are formed as unique spiro cycloadducts. The experimental procedure is very simple. α-methylene-γ-butyrolactone and novel α-N-methyl/phenyl furan derivatives are added to nitrone 1 in diethyl ether. Smooth reaction ends with the production of diastereomeric spiro cycloadducts and regioselective spiro cycloadducts with extremely good yield. In general the reactions are very clean and high yielding compared to usual cycloaddition reactions of α-amino nitrones. The products were characterized from their spectroscopic (IR, ¹H NMR, HRMS, ¹³C NMR) data. No catalyst or co-organic solvent was required.

Antibacterial screening test
All the spiro isoxazolidine derivatives 2a-3a, 2b-3b & 5a-5b (R=Ph,Me) were subjected to in vitro screening against Vibrio parahaemolyticus, Klebsiella pneumoniae, Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus, Shigella flexneri, Eschericia coli, Salmonella typhi and Vibrio cholerae. The minimum inhibitory concentration (MIC) was determined using cup plate assay method according to the standard procedure. Nutrient agar was used as a culture medium. Initially strains of desired bacteria were isolated and were suspended in normal saline. From each bacterial suspension 0.1 mL was taken with the help of pipette and was spread on preprepared nutrient agar plate, with the help of spreader. Then cups were scooped out from each plate with the help of a cork borer and then to the respective cups different derivatives of the isoxazolidine (2a-3a, 2b-3b & 5a-5d) of concentrations (1000 µg/mL, 600, 400, 200, 100, 50, 25, 10 µg/mL) were added. The plates were incubated at 37°C for 24 hr and then results were recorded. The lowest concentration, which showed no visible growth, was taken as an end point minimum inhibitory concentration (MIC). All the compounds showed MIC 10 µg/mL except 2a and 5b (R=Me). They showed MIC 50 µg/mL against Bacillus subtilis and Proteus vulgaris. It has been observed that the derivatives of spiro isoxazolidine 2a (R=Me), 3b & 5b (R=Ph) have antibacterial activity against both gram positive (S.aureus, B.subtilis) and gram negative (E.coli, S.flexneri) bacteria, hence it can be concluded that the derivatives used were broad spectrum antibiotics.

CONCLUSION
In summary, the present procedure introduces novel α-N-methyl/phenyl furan derivatives as highly efficient dipolarophile in 1,3-dipolar cycloaddition reaction for the synthesis of regioselective spiro isoxazolidines and may be included as effective dipolarophile like other conventional dipolarophiles for regioselective synthesis in nitrone cycloaddition reactions. Studies on diastereoselective synthesis of isoxazolidine derivatives using novel α-N-methyl/phenyl furan derivatives with simple nitrones are in progress at present in our laboratory. Almost all the synthesized spiro cycloadducts are having significant antibacterial activity. The notable factors of this methodology are: (a) high yields (b) faster reaction (c) and mild reaction conditions. Therefore, it is believed that procedure described here will find important applications in the synthesis of spiro isoxazolidine derivatives using newly reported dipolarophiles and thereby offering greater scope in cycloaddition reactions.

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