CHAPTER III

Results and Discussion

The present study reports about the synthesis, 1,3-dipolar cycloaddition reaction and applications of *N*-phenyl and *N*-methyl-α-amino nitrones (**1a & 1b**)[1-4] following green chemistry methodologies. In the first segment of this chapter of the dissertation, we have reported the results based on spectroscopic studies in the high yield diastereoselective synthesis of some new amino isoxazolidines in aqueous phase. In the second segment, we have reported microwave assisted regioselective synthesis of amino isoxazoline derivatives while in the third segment we have reported synthesis of spiro isoxazolidine derivatives and peptide synthesis (a new concept of functionalization reactions of amino isoxazolidine derivatives). In the fourth segment of this chapter we have introduced a new methodology of atom efficient aldehyde synthesis and finally we have reported the results obtained for the biological screening of the new cycloadducts.

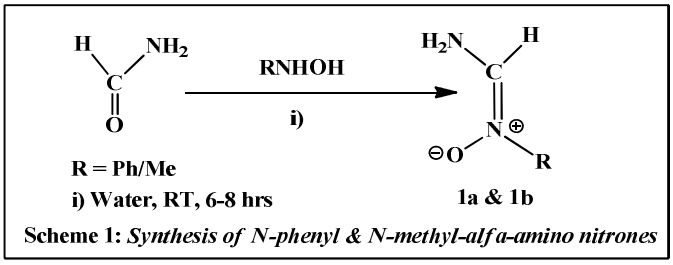
The 1,3-dipolar cycloaddition reactions are among the most important synthetic routes for the construction of five-membered heterocycles, important frameworks of various natural products[5]. 1,3-dipolar cycloaddition reactions are an integral and weighty part of organic chemistry in pedagogy and research as well. In particular the 1,3-dipolar cycloaddition reactions of nitrones with alkenes and alkynes afford isoxazolidines, isoxazolines which are interesting intermediates for the synthesis of *β*-amino alcohols and alkaloids[6,7]. Isoxazolines posses' medicinal activities such as antibacterial, anticonvulsant, antibiotic, antituberculer and antifungal activity[8,9]. Despite their potential utility, many of these procedures require high temperature and prolonged reaction times (drastic experimental conditions) and also suffer from poor regioselectivity, and lack of simplicity. In few cases, the yields and selectivities reported are far from satisfactory due to the occurrence of several side reactions[10].

The wealthy literature on cycloaddition reactions from their birth up to now, unequivocally witnesses to their leading chemistry. The so-called ''conventional solvents'' are organic solvents that have undoubtedly promoted their success. Yet, the toxicity aspect of these solvents impedes their use freely and with no fear. Not only is the operating chemist uncomfortable while experimenting, but also the environment is equally threatened. Working out the cycloaddition reactions and other organic ones in aqueous system would certainly bring some relief to the chemist and to the environment as well. Unusual outcomes in terms of yield, reactivity and selectivity compared to those performed in organic solvents were commonly observed, and have overwhelmed the chemists with surprise indeed[11,12]. The 1,3-dipolar cycloaddition methodology applied to aqueous media has brought forth a number of heterocyclic compounds, usually with a regio and stereoselectivity pecularity. These heterocycles include isoxazoles, isoxazolidines and pyrrolidines.

This rate of acceleration of organic reactions in aqueous media was ascribed to one or a combination of the following factors and phenomena[13], the high cohesive energy density of water, the high internal pressure within the medium, the hydrogen-bonding ability[14], the hydrophobic packing of diene and dienophile in cycloaddition reactions, the hydrophobic vs. antihydrophobic effects, the micellar catalysis, the solvophobicity, and the solvent polarity[15]. Today's status, the insolubility of organic reactants in water, once considered a drawback, turns out to be advantageously a leading factor for the success of organic reactions in pure water. In 2005, Sharpless coined these heterogeneous reactions as ''*on-water*'' reactions[16,17]. The ''*on water*'' method consists simply of stirring the reactant (s) with water to generate an aqueous suspension and it has been observed that both kinetics and yields are extremely enhanced in most cases, compared with those in organic solvents.

In continuation of our efforts to establish green methodologies in nitrone cycloaddition reactions[18-25], herein, we wish to report a new route to the synthesis and 1,3-dipolar cycloaddition reaction of formamide derived α-amino nitrones **1a & 1b**[1-4] having vast synthetic potentials (**Scheme 1**) with a variety of alkenes and alkynes to produce new isoxazolidine and isoxazoline derivatives (**2-14**) in water and under microwave irradiation (**Scheme 2 & 3**).

General procedure of synthesis of N-Phenyl & N-Methyl-α-amino nitrones (1a & 1b) in aqueous phase[1-4]



Scheme 1: ***Synthesis of N-phenyl & N-methyl-alfa-amino nitrones***

N-phenylhydroxylamine (250mg, 2.30 mmole) has been added to dry; distilled formamide (1 equivalent) in water (15 mL) under N_2 atmosphere and the reaction mixture was kept at RT with constant stirring with a magnetic stirrer for 10 hr. The formation of nitrone was monitored by TLC (R_f = 0.33). After completion of reaction, the nitrone 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 nitrone was isolated under reduced pressure vaccum pump as white needle shape crystals (92%; m.p:42^{0}C, uncorrected, $UV_{\lambda max}$: 238 nm) which decomposes if kept for 6-8 hrs at RT. Therefore, the nitrone has been trapped in situ for the cycloaddition reactions.

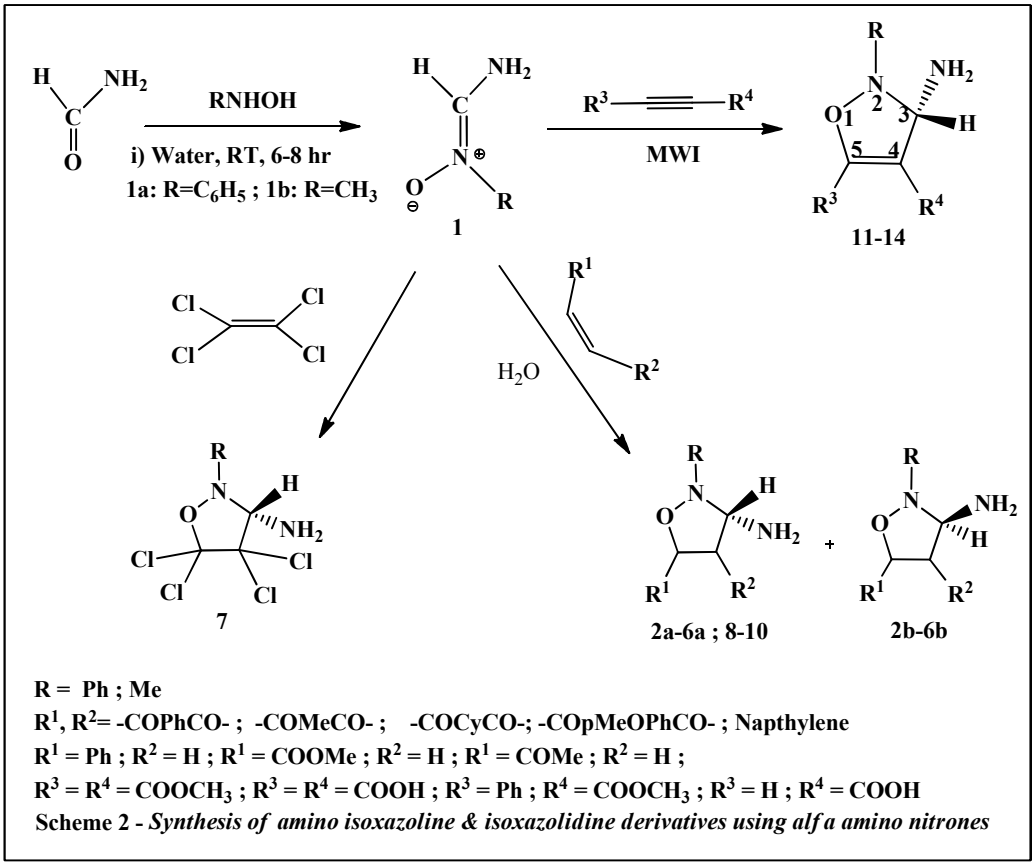
Same methodology was adopted for the synthesis of *N*-methyl-α-amino nitrone (**1b**). Both the α-amino nitrones (**1a & 1b**) decomposes on keeping at room temperature, therefore in majority of the cases *in situ* cycloaddition reactions were performed with various activated alkene and alkynes.

Spectral data of N-Phenyl-α-amino nitrone (1a):

White crystalline solid. Yield: 92%; R_f = 0.36, m.p: 42^0C (uncorrected); $UV_{\lambda max}$: 238 nm; IR (KBr): 3310 (br), 3020 (m), 1610 (s), 1180 (s), 785 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.49-7.13 (m, 5H, C_6H_5), 6.84 (s, 1H, CH=N+), 1.62 (s, 2H, $-NH_2$).

Spectral data of N-Methyl-α-amino nitrone (1b):

Pale yellow crystalline solid. Yield: 87%; R_f = 0.36, m.p: 43^0C (uncorrected); $UV_{\lambda max}$: 237 nm; IR (KBr): 3312 (br), 3021 (m), 1610 (s), 1180 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.40 (br,s, 2H, NH_2, exchanged in D_2O), 6.72 (s, 1H, CH=N+), 3.36 (s, 3H, N^+- CH_3).

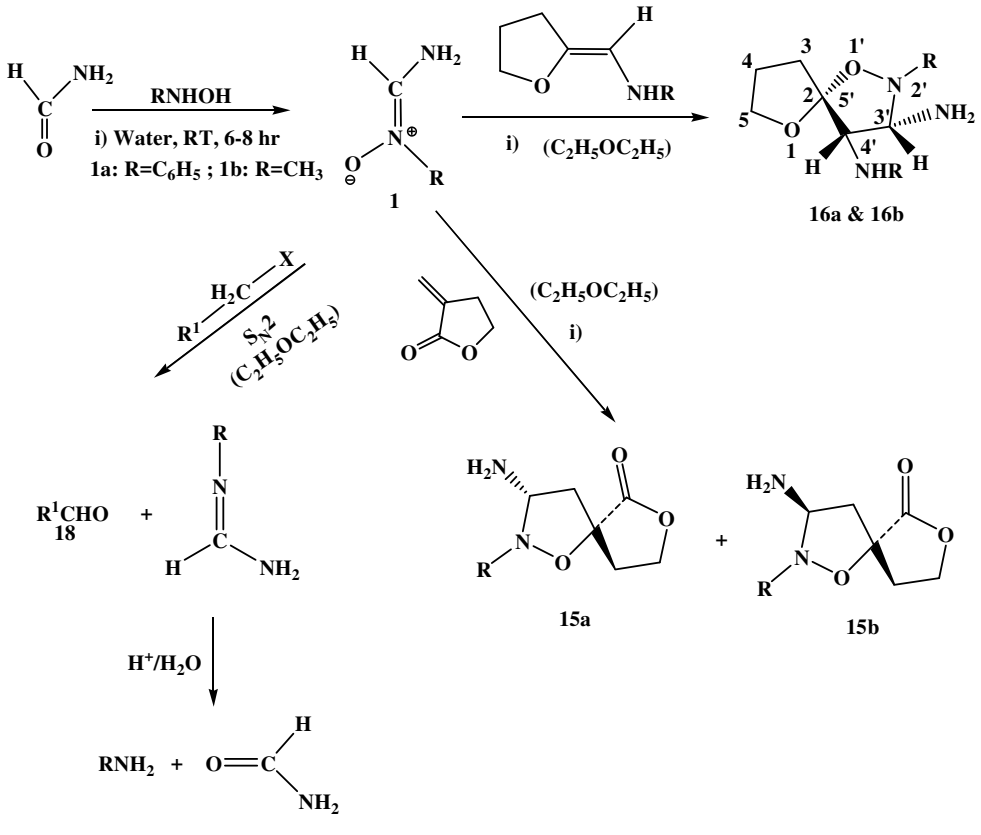


R = Ph ; Me
R^1, R^2= -COPhCO- ; -COMeCO- ; -COCyCO-; -COpMeOPhCO- ; Napthylene
R^1 = Ph ; R^2 = H ; R^1 = COOMe ; R^2 = H ; R^1 = COMe ; R^2 = H ;
R^3 = R^4 = $COOCH_3$; R^3 = R^4 = COOH ; R^3 = Ph ; R^4 = $COOCH_3$; R^3 = H ; R^4 = COOH

Scheme 2 - ***Synthesis of amino isoxazoline & isoxazolidine derivatives using alfa amino nitrones***

This is quite a new approach of the synthesis of nitrone from formamide. The present study has been carried out with four different maleimides (*N*-methyl/phenyl/cyclohexyl/P-methoxy-*N*-phenyl) and methyl acrylate, methyl vinyl ketone and styrene respectively in water for the synthesis of isoxazolidine derivatives while electron deficient alkynes like phenyl methyl propiolare, dimethyl acetylene carboxylate, acetylene dicarboxylic acid etc were employed for the synthesis of new isoxazoline derivatives using microwave irradiation. Simultaneously the reactions have been also studied in organic solvent (CH_2Cl_2) as well.

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. Almost all the reactions in water are very fast (3-4 hrs in case of maleimides, methyl acrylate and 5 hrs for styrene) compared with the normal cycloaddition reactions in organic solvents which are reported to take longer periods (26 - 48 hrs)[6,14,24].

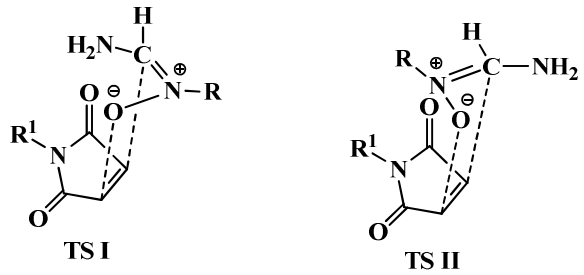


Scheme - 3: ***Synthesis of spiro isoxazolidine derivatives and atom efficient aldehyde synthesis***

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, methyl acrylate and thereby greatly facilitates the reaction.

The 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[26,27]. 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 is weak hydrogen bond acceptor, which means that their FMO's are only slightly affected by hydrogen bond interactions and leads 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. α-amino nitrones (**1a** & **1b**) reacted with *N*-substituted maleimides, acenapthylene and tetrachloroethylene giving isoxazolidine derivatives (**2-7 ; Scheme 2**).

Excellent diastereofacial selectivity is observed in nitrone cycloadditions in water. The addition of nitrone **1** to maleimides result in a mixture of diastereomer **2a–6a** and **2b–6b** (almost 65:35 ratio in all cases) and generation of as many as three asymmetric centers in a single step. Studies 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[13,14]. These results can be rationalized by an *exo* approach of nitrone **1** which has Z configuration for the formation of major cycloadducts **2a–6a** (transition state **I,** Figure **1**). The minor cycloadducts **2b–6b** are formed by the *endo* approach of Z nitrone (transition state **II,** Figure **1**).

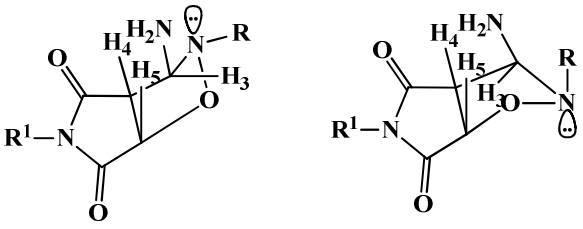


$R^1 = CH_3/C_6H_5/C_6H_{11}/P\text{-}MeoC_6H_4$

Figure 1 - ***Exo/endo approach of the nitrone 1 to the maleimides in cycloaddition reactions***

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[28,29]. The most significant differences in the ^{1}H NMR data for the diastereomers are the position and multiplicity of the 3-H signal.

In the minor adducts **2b–6b**, 3-H resonates upfield around δ_H 2.00 while for the same proton in major adducts **2a–6a** around δ_H 3.30 and the coupling constant has been found to be $J_{3,4}$ ~ 6.20 Hz for major adducts whilst for minor adducts $J_{3,4}$ is ~ 2.00 Hz. These differences can be explained by considering the available isoxazolidine ring conformations. Due to the 4,5-fued 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 (Figure **2**). 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 **2**).



Minor conformation 1 **Major conformation 2**

R = Ph; Me ; R^1 = Me; Ph ; Cy ; P-MeoC$_6$H$_4$

Figure 2 : ***Major & minor conformations of amino isoxazolidine derivatives***

The diastereomeric isoxazolidines **2a–6a** and **2b–6b** were separated by column chromatography and obtained in analytically pure form. The *endo/exo* stereochemistry mentioned above is based on extensive NMR investigations. Most relevant are the coupling constants ($J_{H3,\ H4}$) of the diastereomers. For **2a-6a**, this coupling constant is almost 6.50–7.00 Hz, implying a *cis* relationship between H-3 and H-4, whereas for **2b–6b**, the coupling constant is almost 1.70–2.50 Hz which implies a *trans* relationship between H-3 and H-4[28,29]. In all the diastereomers, the configurations of H-5 and H-4 are *cis* as evidenced from their coupling constant values. In case of tetrachloro ethylene cycloadducts (**7**), H-5 and H-4 protons are absent and the presence of sole H-3 proton cannot determine the stereochemistry of the molecule.

For methyl acrylate, styrene and methyl vinyl ketone the regioselectivity was rationalized by using frontier orbital theory[30] (FMO approach) and ^{1}H NMR experiments. Cycloadditions to α,β-unsaturated carboxylic acid derivatives, e.g. methyl acrylate are particularly useful because high regioselectivity is often observed in water[31]. The reactions were found to be highly regioselective to form solely **5**-substituted isoxazolidines. This is due to the fact that, nitrone (LUMO)-dipolarophile (HOMO) interactions completely dominate the reaction and lead to the formation of only 5-substituted adducts[32].

From the ^{1}H NMR spectrum of cycloadducts **8–10**, it was found that clear double doublet signal for H-4 protons and triplet signals for both H-3 & H-5 protons were obtained in these cycloadducts and hence confirms in favour of **5**-substituted adducts. From the detailed investigations on the nature of these cycloaddition reactions using TLC and ^{1}H NMR spectrum studies for the cycloadducts **8–10**, it is also confirmed that no diastereomers are formed. The relative configurations of H-3, H-4 and H-5 protons in these cycloadducts are *syn* and the cycloadducts are in favour of *exo* transition state geometry as evidenced from their coupling constant values ($J_{H4,H5}$ ~ 6.06–7.40 Hz; $J_{H4,H3}$ ~ 6.20-6.80 Hz).

Table 1 — *Physicochemical data of synthesized compounds* **2a-6a ; 2b-6b; 7 & 8-10**

Entry	Nitrone	Dipolarophile[a]	Time (hr)	Cycloadduct, m.p.(°C), **2a-6a: *cis*** ; **2b–6b: *trans***	*Cis/trans ratio (%)*	Yield[b] (%)
1	*N*-phenyl-α-amino nitrone	*N*-methyl maleimide	3 (12h)	**2a**: White crystals, 121 **2b**: White crystals, 107	**2a**: 68 **2b**: 28	96 (68)
2	*N*-phenyl-α-amino nitrone	*N*-phenyl maleimide	3 (13h)	**3a**: Brown solid, 116 **3b**: Yellow solid, 97	**3a**: 70 **3b**: 24	94 (66)
3	*N*-phenyl-α-amino nitrone	*N*-cyclohexyl Maleimide	4 (13h)	**4a**: White crystals, 132 **4b**: White crystals, 120	**4a**: 63 **4b**: 31	94 (66)
4	*N*-phenyl-α-amino nitrone	Acenapthylene	4 (12h)	**5a:** Yellow crystals, 130 **5b**: Yellow crystals, 112	**5a**: 68 **5b**: 26	94 (62)
5	*N*-phenyl-α-amino nitrone	P-methoxy-*N*-phenyl maleimide	4 (13h)	**6a**: Yellow crystals, 104 **6b**: Yellow crystals, 126	**6a**: 70 **6**b: 21	91 (65)
6	*N*-phenyl-α-amino nitrone	Tetrachloro ethylene	2 (10h)	**7:** Colourless liquid	**7** : 90	90 (66)
7	*N*-phenyl-α-amino nitrone	Methyl acrylate	4 (14h)	**8**: Red viscous liquid	**8**: 85	86 (66)
8	*N*-phenyl-α-amino nitrone	Styrene	4 (14h)	**9**: Colourless thick liquid	**9**: 85	85 (63)
9	*N*-phenyl-α-amino nitrone	Methyl vinyl ketone	5 (16h)	**10**: Colourless thick liquid	**10**: 83	83 (61)

[a] Reaction conditions: nitrone (1 mmol), dipolarophile (1 equivalent), water, N_2 atmosphere, RT
[b] All products were characterized by IR, ^{1}H NMR, ^{13}C NMR and MS spectral data.
[c] Isolated yield after purification. Figures in parentheses indicate reactions performed in conventional methods.

The cycloadducts **2–10** have been confirmed on the basis of ^{1}H and ^{13}C NMR spectroscopy in $CDCl_3$ solution along with MS and IR spectra. ^{1}H NMR spectra and TLC studies of **2–6** indicate that these isoxazolidine derivatives are formed as a mixture of diastereomers in almost 65:35 ratio with *cis* and *trans* configurations relative to the spatial orientation of the NH_2 group at C_3 with respect to the H atom at C_4 position. These diastereomers have been separated by column chromatography and recrystallized from heptane-ethyl acetate. In this cycloaddition reaction the C-C & C-O bond formation in the transition state may not happen in a synchronous manner. The C-C bond of isoxazolidine ring is more developed in the transition state than C-O bond. This process would afford products having *syn* configuration at C_5 & C_4 respectively[28]. In the ^{13}C NMR spectrum, four signals were obtained in case of phenyl ring carbon atoms due to the equivalent nature of C-2 and C-6 and, C-3 and C-5 carbons. IR spectral studies of the maleimide cycloadducts also support the structural correlation as far as the carbonyl group and aromatic C-H absorptions are concerned. In general, the reactions are very clean and high yielding compared to usual cycloaddition reactions of nitrones. No catalyst or co-organic solvent are required. All the new cycloadducts (**2-10**) are stable and prominent molecular ion peak, base peaks are obtained in the mass spectrum as expected. All the maleimide cycloadducts have shown a common mass fragmentation pattern leading to the development of a base peak (B.P) due to the fragmentation of phenyl and substituted phenyl ring from these molecules and thereby showing a correlation in their structures.

The use of microwaves in organic synthesis has increased dramatically within the past decade, receiving widespread acceptance and becoming an indespensible tool[33,34,35]. In continuation of our green methodological synthesis of spiro isoxazolidine, isoxazolidine, aldehyde, ketone synthesis using α-amino & α-chloro nitrones in solid phase and in hydrated media[18-25], in this dissertation, we have reported microwave assisted green synthesis of some new isoxazoline derivatives (**11-14**) having high synthetic potential using *N*-phenyl-α-amino nitrone (**1a**) with different activated alkynes with excellent yield (**Scheme 2 ;Table 2**)[1b].

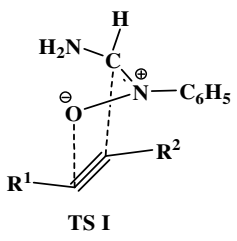
For the present study, we have chosen activated alkynes like dimethyl acetylene dicarboxylate, phenyl methyl propiolate and acetylene dicarboxylic acid (but-2-ynedioic acid), propiolic acid respectively as dipolarophiles in 1,3-Dipolar cycloaddition reaction with *N*-phenyl-α-amino nitrone (**1a**)[1b] for the synthesis of new isoxazoline derivatives (**11-14; Scheme 2; Table 2**). These results can be rationalized by an *exo* approach of the nitrone (**1a**) in Z configuration (transition state **1;** Figure **3**)[28,29] for the dipolarophiles (alkynes) in the development of new cycloadducts (**11–14; Scheme 2; Table 2**).

Stereochemistry of the new isoxazoline derivatives (**11-14**) could not be determined in detail since two most important protons are absent at C_4 and C_5 positions and the lone singlet signal due to C_3 proton unable to predict it. Expected broad signal for amino groups at δ 3.05-2.80 ppm and sharp singlet for ester methyl protons at δ 3.30-3.20 ppm were obtained while carboxylic protons showed sharp singlet signals around δ 10.15 ppm in the ^{1}H NMR spectrum. The major and minor conformers of the new isoxazoline ring systems (**11–14**) may be represented in Figure **3**.

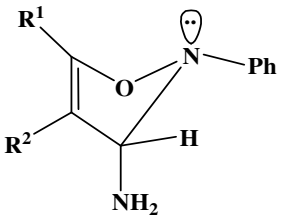
Table 2 - Physicochemical data of synthesized novel isoxazoline derivatives (**11-14**)

Entry	Nitrone	Dipolarophile[a]	Time (min)	Cycloadduct[b]	Yield[c] (%)
1	*N*-phenyl-α-amino nitrone (**1**)	Phenyl methyl propiolate	4	**11**: Red viscous liquid	93
2	*N*-phenyl-α-amino nitrone (**1**)	Dimethyl acetylene dicarboxylate	4	**12**: Dark yellow liquid	91
3	*N*-phenyl-α-amino nitrone (**1**)	Acetylene dicar -boxylic acid	5	**13**: Colourless gummy liquid	90
4	*N*-phenyl-α-amino nitrone (**1**)	Propiolic acid	5	**14:** Colourless liquid	90

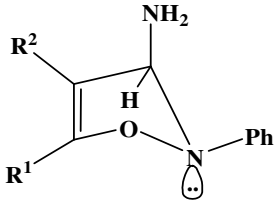
[a] Reaction condition : Nitrone **1** (1 mmol), dipolarophile (1 equivalent), dry DMF, MWI
[b] All the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, HRMS spectral data.
[c]Isolated yields after purification



TS I



Conformation 1



Conformation 2

Figure 3

R^1 = Ph, R^2 = $COOCH_3$; R^1 = R^2 = $COOCH_3$; R^1 = R^2 = COOH ; R^1 = H, R^2 = COOH

In all the new isoxazoline derivatives (**11-14**), we have obtained expected fragmentation peaks due to the development of different aziridine derivatives. Base peaks are obtained due to loss of PhCO for phenyl methyl propiolate, $COOCH_3$ for dimethyl acetylene dicarboxylate and COOH for acetylene dicarboxylic acid cycloadducts respectively.

Hence it is confirmed that during mass fragmentation, the isoxazoline cycloadducts underwent rearrangement to aziridine derivatives (Details are explained in analysis of mass spectra section of this chapter). Structures of all the new isoxazolidine and isoxazoline derivatives (**2-14**) have been confirmed on the basis of expected signals obtained in ^{1}H NMR, ^{13}C NMR, MS and FT-IR spectrum. Satisfactory elemental analysis values were also obtained for all the new cycloadducts.

In the third segment of this dissertation, we have reported synthesis of few new spiro isoxazolidine derivatives (**Scheme 3**; **15a & 15b ; 16a & 16b**)[36]. In this synthesis for the first time we have introduced novel α-*N*-methyl/phenyl furan derivatives (obtained as side product during aldehyde synthesis)[25] as dipolarophile in the regioselective synthesis of *5-spiro isoxazolidines* using α-amino nitrones at RT with an excellent yield (**Scheme - 3, Table 3**). This methodology also includes diastereoselective synthesis of *5-spiro isoxazolidines* using α-methylene-γ-butyrolactone as dipolarophile with α-amino nitrones.

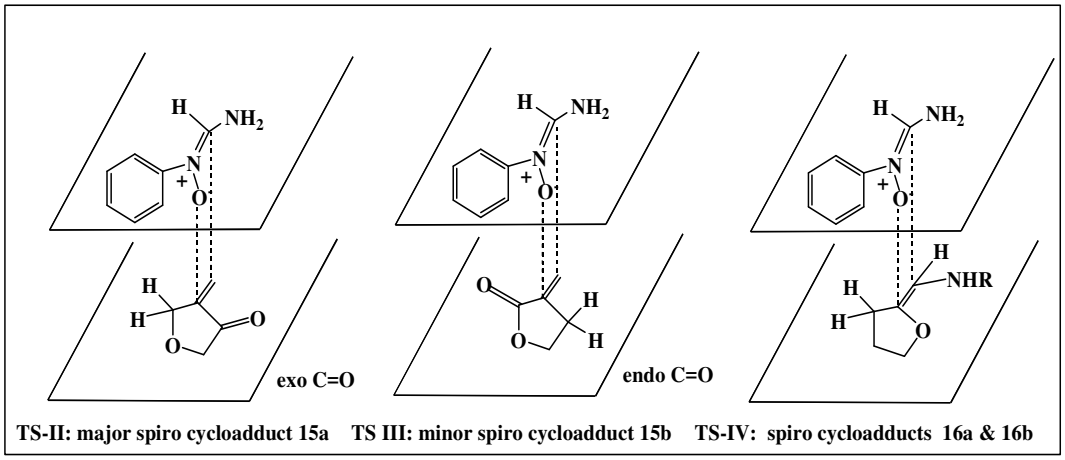
Table 3 : Physical characteristics of the spiro cycloadducts

Entry	Nitrone	Dipolarophile[a]	Spiro cycloadduct[b]	*Cis*/*trans* ratio	Time (hr)	Yield[c] (%)
1	*N*-methyl-α-amino nitrone	α-methylene-γ--butyrolactone	**15a**: Pale yellow liquid **15b**: Yellow liquid	**15a**:68 **15b**:24	4	92
2	*N*-methyl-α-amino nitrone	α-*N*-methyl furan--derivative	**16a**: Yellow viscous liquid		7	93
3	*N*-phenyl-α-amino nitrone	α-*N*-phenyl furan--derivative	**16b**: Yellow gummy liquid		8	92

[a] Reaction condition : α-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

The cycloaddition reaction of *N*-methyl-α-amino nitrone (**1b**) to α-methylene-γ-butyrolactone is faster (4-5 hrs) compared to reaction with α-*N*-methyl/phenyl furan derivatives (7-8 hrs). Good diastereoselectivity is observed in nitrone cycloadditions described here with α-methylene-γ-butyrolactone. The addition of *N*-methyl-α-amino nitrone (**1b**; Scheme **3**) to α-methylene-γ-butyrolactone results in a mixture of diastereoisomer **15a** & **15b** (almost 70:30 ratio). These results can be rationalized by an *exo* approach nitrone **1b** (in *Z* configuration) with respect to dipolarophile (α-methylene-γ-butyrolactone) for the major spiro cycloadduct **15a** (transition state **II**).

The minor spiro cycloadduct **15b** is formed by an *endo* approach of dipolarophile to *Z* nitrone **1b** (transition state **III**).



TS-II: major spiro cycloadduct 15a TS III: minor spiro cycloadduct 15b TS-IV: spiro cycloadducts 16a & 16b

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 **15a**, 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 in **15b** coupling constant ($J_{H3',H4'}$) is 2.5 to 3.4 Hz which implies a *trans* relationship between 3'-H and 4'-H (Ref 28,29).

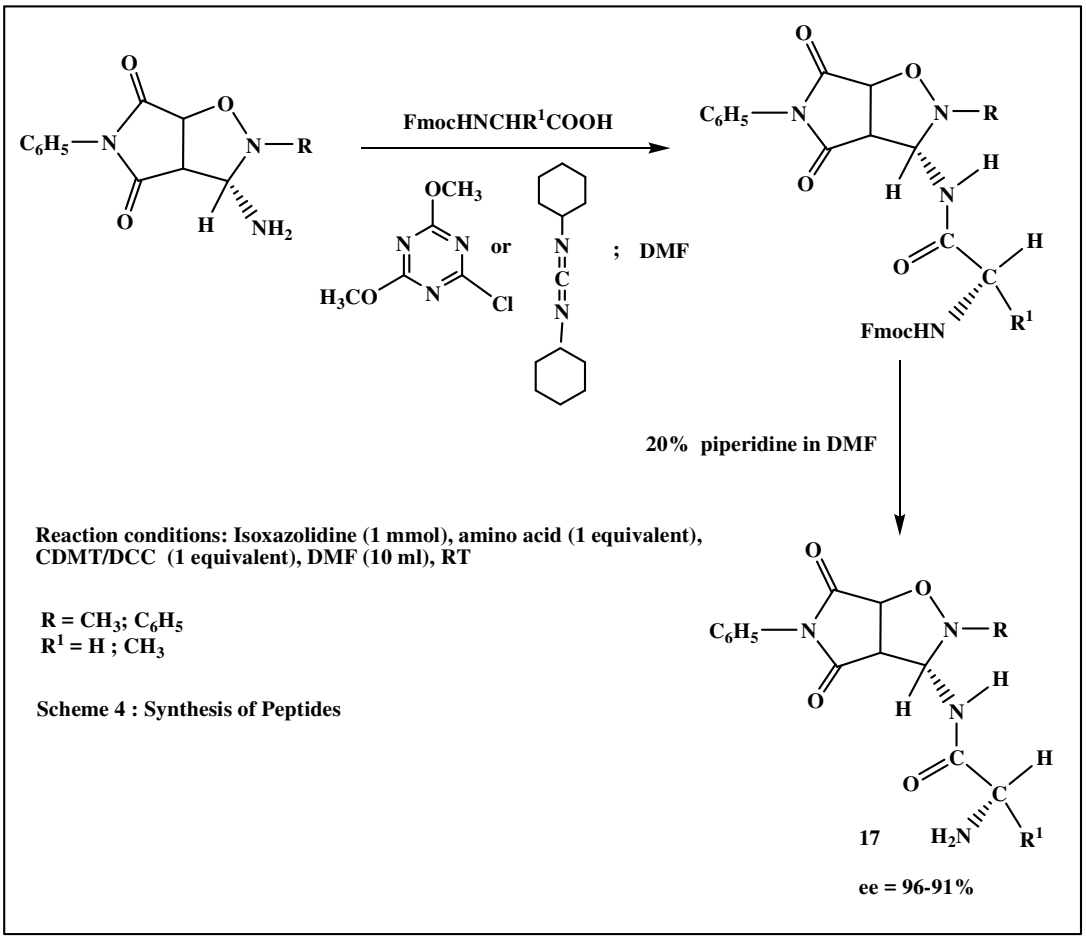
On the other hand, reactions of nitrone **1a & 1b** (**R=Ph, Me**) with newly reported α-*N*-methyl/phenyl furan derivatives[25] as dipolarophile are found to be highly regioselective to form solely *5-spiro isoxazolidine derivatives* (**16a & 16b**). It could be due to the fact that nitrone (LUMO) – dipolarophile (HOMO) interactions are strong enough to dominate the reaction and leads to the formation of soley regioselective *5-spiro isoxazolidines*[30,31] via an *exo* approach of nitrone **1** (in *Z* configuration) to the furan derivatives (α-*N*-methyl/phenyl furan derivatives ; transition state **IV**). 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 spiro cycloadducts and their coupling constants ($J_{H3,H4}$ = 6 – 7.4 Hzs) are also indicative of this stereochemical relationship[29].

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 **15a, 15b** and **16a, 16b** shows significant long range coupling between H_4 with $H_{4'}$ and vice versa in **15a** and **15b** while H_4 with $H_{3'}$ and vice-versa in **16a & 16b**. Mass fragmentation peaks of different value are also obtained for diastereomers of a particular spiro cycloadduct.

The diastereomeric spiro isoxazolidines **15a** and **15b** were separated by column chromatography and obtained in analytically pure form. The ^{1}H NMR spectrum of **15a** and **15b** displayed different spectrum (position of signals) for the diastereomers. In contrast, the ^{1}H NMR spectrum of **16a & 16b** 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[5,6]. The products were characterized from their spectroscopic (IR, ^{1}H NMR, HRMS, ^{13}C NMR) data. No catalyst or co-organic solvent was required.

In the fourth segment of this dissertation, we have reported a methodology of *peptide synthesis* using amino isoxazolidine derivatives[1a] (**Scheme 4**). In the realm of rapidly growing protein chemistry, synthesis of new peptides is expected to play key roles in modeling structure and function[37,38,39]. The newly reported methodology is very simple and is highly attractive as it has basic fundamental applications in the organic synthesis. Moreover, potential biological activity of the newly synthesized peptides has made this protocol more attractive.

To execute proposed study, various isoxazolidine derivatives were synthesized from α-amino nitrones[1] by employing reported protocol[3,5,6]. Initially, newly synthesized isoxazolidine derivatives were treated with various amino acids in presence of DCC as coupling reagent for the synthesis of a series of new peptides. Although DCC has provided good yield of the product, however, due to the formation of insoluble by-product (*N,N*-dicyclohexylurea) purification becomes tedious. We have also tested carbonyldiimidazole (CDI) as coupling reagent. Although yields were satisfactory but long reaction time does not benefit for the general applicability. Finally, we employed CDMT (2-chloro-4.6-dimethoxy-1,3,5-triazine) as coupling reagent for the peptide synthesis. CDMT was developed few years ago and has been widely employed for amide bond formation and peptide synthesis[40,41]. Moreover, the resulting side products can be completely removed by washing with dilute acids which circumvent the need for chromatographic purification of the product. These results encouraged us to use CDMT for the formation of new peptides. Protection of the α-amino functionality of amino acids is one of the most important issues in peptide chemistry and is mandatory to prevent polymerization of the amino acid once it is activated. The use of Fmoc chemistry for protection of the alpha amino group has become the preferred method for most contemporary solid and solution phase peptide synthetic processes[42,43]. Fmoc has also been shown to be more reliable and produce higher quality peptides than Boc chemistry.



Reaction conditions: Isoxazolidine (1 mmol), amino acid (1 equivalent), CDMT/DCC (1 equivalent), DMF (10 ml), RT

R = CH_3; C_6H_5
R^1 = H ; CH_3

Scheme 4 : Synthesis of Peptides

The advantage of Fmoc is that it is cleaved under very mild basic conditions (usually 20% piperidine in DMF), but stable under acidic conditions. Fmoc amino acids demonstrate superior solubility in DMF. It was found that the reaction between *N*-methyl isoxazolidine (entry **1**, peptide **17a, Table 4, Scheme 4**) and Fmoc-glycine in presence of CDMT was completed in 3 hr at RT with 88% yield while the same reaction in presence DCC produced 81% yield at RT in 6 hr. Simple work-up of the crude product using acid affords **17a** (R = CH_3; R^1 = H; entry **1**, peptide **17**) in 88% yield. Thus, the optimized reaction conditions were used i.e., 1 equivalent of CDMT and Fmoc-glycine at RT in DMF for 3 hr to obtain a series of new peptides with good to excellent yields (Table **3**). Each diastereomeric isoxazolidine derivative was coupled with amino acids in presence of CDMT to produce a particular targeted peptide. The structures of the newly synthesized peptides (**17a-17c**) were established on the basis of ^{1}H NMR, ^{13}C NMR, MS and IR spectroscopy[44,45,46,47].

Table 4 : Physical characteristics of the peptides (**17a-17c**)

Entry	Isoxazolidine	Amino acid[a]	Peptide[b]	Time (hr)	Yield[c] (%)
1	*N*-methyl-amino isoxazolidine	Glycine	**17a**: White crystals, M.P: 94^0C *ee* = 96%	3	88
2	*N*-phenyl-amino isoxazolidine	Glycine	**17b**: Pale yellow crystals, M.P: 121^0C *ee* = 93%	3	87
3	*N*-methyl-amino isoxazolidine	Alanine	**17c**: Gray paste *ee* = 91%	3	84

[a] Reaction condition : α-amino nitrone (1 mmol), dipolarophile (1 equivalent), dry ether, N_2 atmosphere, RT

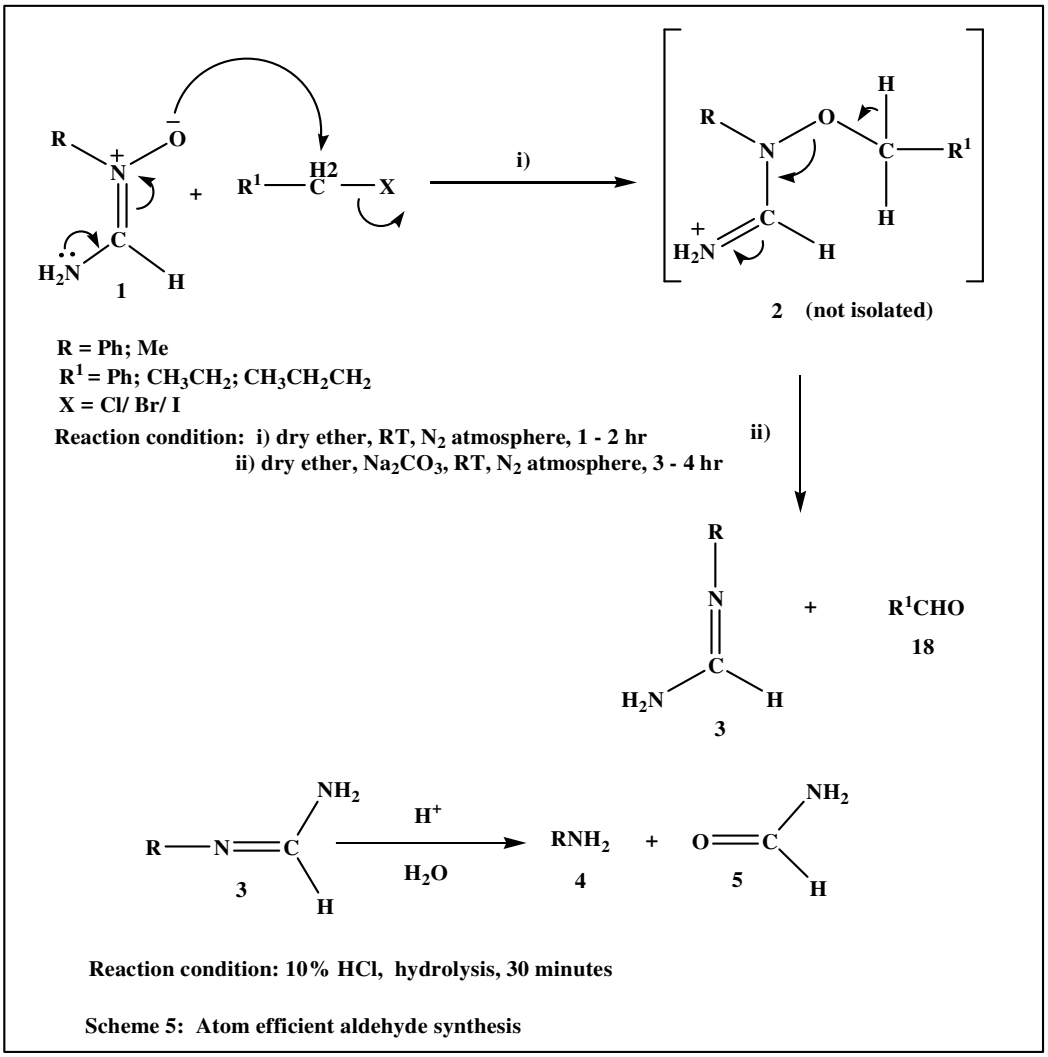
[b] All the compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MS, HRMS spectral data.

[c]Isolated yields after purification

All the new peptides are classified as *cis cis* and *cis trans* peptides as evidenced from the configurations of C_5, C_4 and C_3 protons (isoxazolidine ring numbering) as well as their coupling constant values obtained in the ^{1}H NMR spectrum. Basically, diastereomeric isoxazolidine derivatives were used in these coupling reactions for the synthesis of new peptides. At the same time we have also tested successfully coupling reactions between isoxazoline derivatives and amino acids. Our study shows that former reactions were found to have good yield in a faster reaction process while the reactions in latter were much slower with moderate yield. Purity of the new peptides was considered on the basis of enantimeric excess (*ee*). We have also tested the present protocol for the synthesis of peptides from isoxazolidine and isoxazoline derivatives that do not contain amino substituents. In such derivatives, ester substituents are initially hydrolysed to corresponding carboxylic acids followed by coupling reaction for the synthesis of peptides. All the new peptides are found to be stable and have prominent molecular ion peak and base peaks in the mass spectrum as expected.

In the fifth segment of this dissertation we have introduced a new methodology of aldehyde synthesis using α-amino nitrones as potential oxidizing reagents. Conversion of alkyl halides to aldehydes using *N*-oxide with moderate yield has been reported long time back (Krohnke reaction). In addition to the existing methods available for the synthesis of aldehydes from alkyl halides[48-52] we would like to incorporate an efficient methodology of synthesis of aldehydes from alkyl halides along with imines using for the first time α-amino nitrones[1-4] as an oxidizing reagent with an excellent yield (**Scheme 5, Table 5**). In addition, the side product (imines) obtained during aldehyde synthesis results starting material amide and amines upon simple hydrolysis.

The duly obtained amides can be successfully reused for the synthesis of nitrones while the amines can be used for further general reaction purposes. Literature survey reveals that aldehyde synthesis using α-amino nitrone as an oxidizing reagent has not yet reported and can be incorporated as an important application in nitrone chemistry.



Scheme 5: Atom efficient aldehyde synthesis

The present study has been carried out using a *N*-phenyl & *N*-methyl-α-amino nitrones and alkyl halides like benzyl chloride, 1-chloro propane etc (**Table 5**) in order to generalize the methodology for the aldehyde synthesis. The yield of the isolated aldehydes are extremely high (almost 80 - 88%) in a much lesser time and are much better in case of active alkyl halides compared to inactive alkyl halides while imines are obtained in almost 11 – 20% yields as side products. The results are summarized in **Table 5**.

The products especially aldehyde, amide and amines are known compounds and spectral data of these synthesized compounds are almost identical to the values found in literature. For example, sharp singlet signals at δ 9.80 & 198 in the ^{1}H NMR and ^{13}C NMR spectrum along with molecular ion peak at 106, base peak at 105 and 77, 51 in the MS spectrum give strong evidence in favour of benzaldehyde formation. Spectral data of the imine derivatives (**3**) also agreed well with the assigned structures. For example, prominent molecular ion peak at 196 and base peak at 103 (due to the formation of PhCN) clearly indicates in favour of imine derivative **3**. The proposed mechanism for the aldehyde synthesis using α-amino nitrone is very interesting. Nitrone **1** undergoes S_N2 reaction readily with alkyl halides and develops an intermediate compound (**2**) which was not isolated. The reaction rate is much more faster compared to the S_N2 reactions of α-chloro nitrones[24,25,53,54,55,56] due to the involvement of available electron pairs of amino group. The N-O bond of the intermediate compound (**2**) breaks[57] when the reaction mixture is stirred with solid sodium carbonate which plays an important role for the development of aldehyde and imines in a Kornblum type reaction with a very good yield (**Scheme 5; Table 5**). The imine derivative **3** on hydrolysis results starting amide (**5:** 55– 60%) and amines (**4:** 35-40%) where amides are the starting material for α-amino nitrone synthesis. In the course of the study, major difficulties were faced during isolation and identification of formaldehyde because of its volatility. The products were characterized from their spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, HRMS) data. No catalyst or co organic solvent was required.

Table 5: *Synthesis of aldehydes using α-amino nitrone*

Entry	Nitrone	Alkyl halide[a]	Product[b]	Time (hr)	Yield[c] (%)
1	*N*-phenyl-α-amino nitrone	Benzyl chloride	Benzaldehyde	4	88
2	*N*-phenyl-α-amino nitrone	1-chloro propane	Propionaldehyde	6	78
3	*N*-methyl-α-amino nitrone	Benzyl chloride	Benzaldehyde	6	77
4	*N*-methyl-α-amino nitrone	1-chloro propane	Propionaldehyde	5	75

[a] Reaction condition : α-amino nitrone & alkyl halide (1 equivalent each), 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 yield after purification.

The isolated amide (starting material for α-amino nitrones) and amines are equally good in quality as obtained from commercial suppliers and thereby offering greater scope for the present methodology. The notable advantages offered by this method are simple operation, easy workup, mild and faster reaction conditions with high yield of products. Therefore, the present methodology may be incorporated as a general method of aldehyde synthesis from alkyl halides for extremely good yield and also as an important application of nitrones.

Interpretation of mass spectra

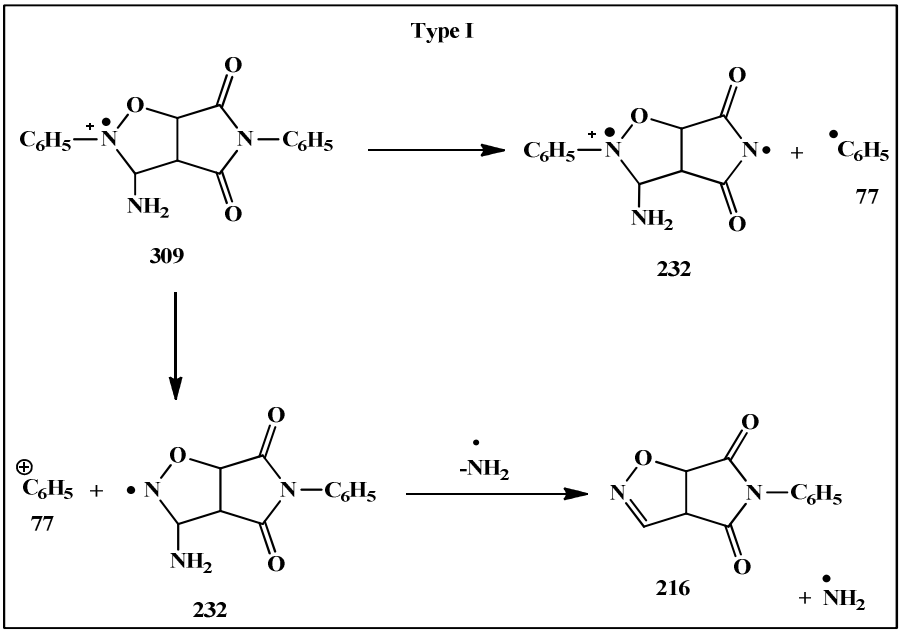
In the case of *N*-phenyl-α-amino nitrone, all the cycloadducts formed possess 2-phenyl-3-amino-1,2-isoxazolidine moiety in common. On electron impact, mass fragmentation of a molecule would generate generally a radical ion and expectedly one of the non bonding electrons of the nitrogen atom of 1,2-isoxazolidine ring would be removed as the nitrogen atom is tertiary in nature.

Taking *N-phenyl maleimide* cycloadduct as an example in 1,3-dipolar cycloaddition reaction, a general scheme was formulated (**Type I**). The fragmentation pattern of all the maleimide cycloadducts were discussed in the light of this fission pattern.

Mass fragmentation pattern of amino isoxazolidins derived from N-phenyl-α-amino nitrone

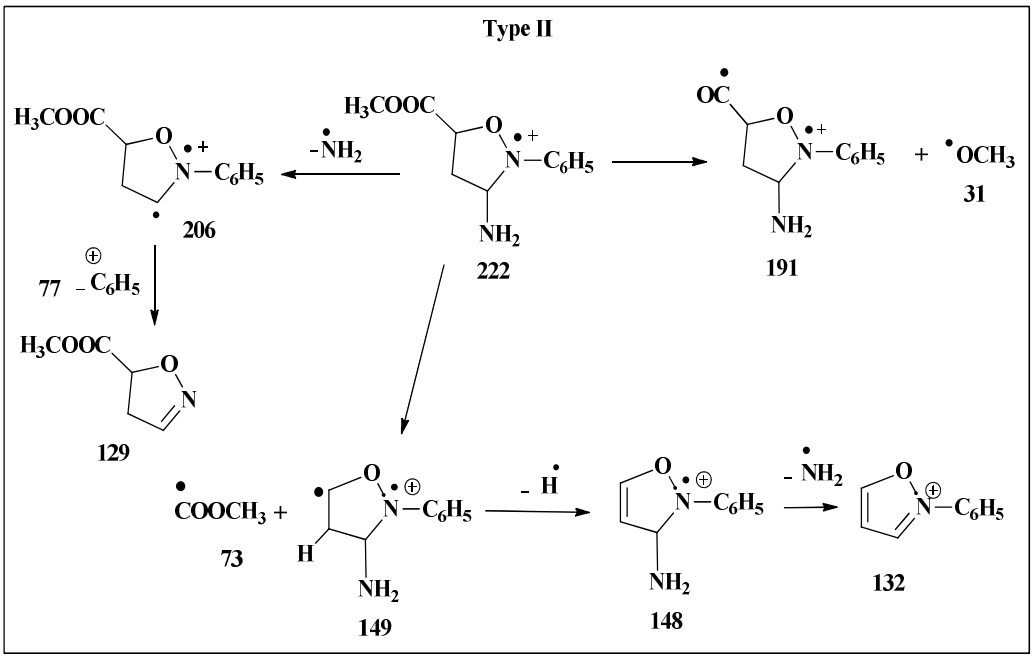
Type I: ***N-substituted maleimide isoxazolidines***

In the mass fragmentation pattern of maleimide cycloadducts (*N*-phenyl, *N*-cyclohexyl, *N*-methyl etc.) in addition to the common expected fragments, other prominent peaks at *m/e* = 83, 77, 15 for cyclohexyl, phenyl, methyl were also obtained. Example pattern of *N*-phenyl maleimide adduct.



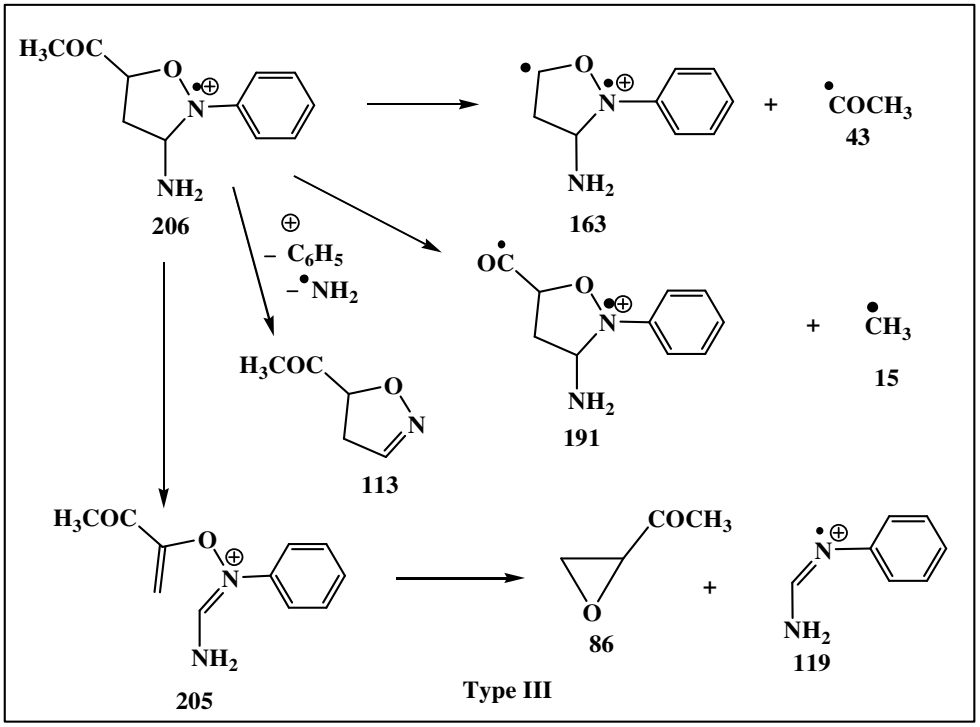
Type II: ***Regioselective (Methyl acrylate/styrene etc) cycloadducts***

The fragmentation pattern of regioselective cycloadducts (e.g, methyl acrylate cycloadduct) followed the general pattern with some typical peaks *i.e.* CH_3-O (31), CH_3-COO (59) shown in **Type II**.



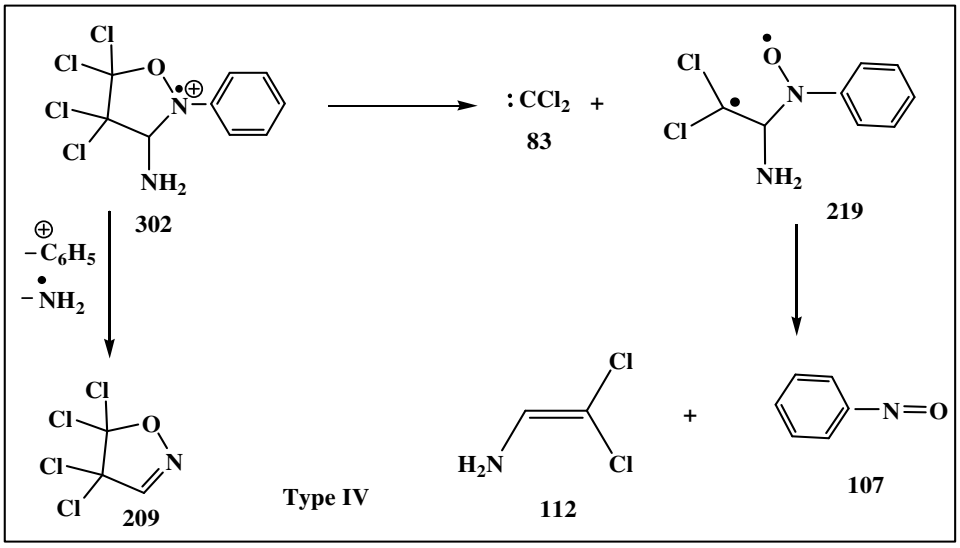
Type III: ***Methyl vinyl ketone cycloadduct***

The major fragmentation pattern of methyl vinyl ketone cycloadduct has been explained in **Type III.**



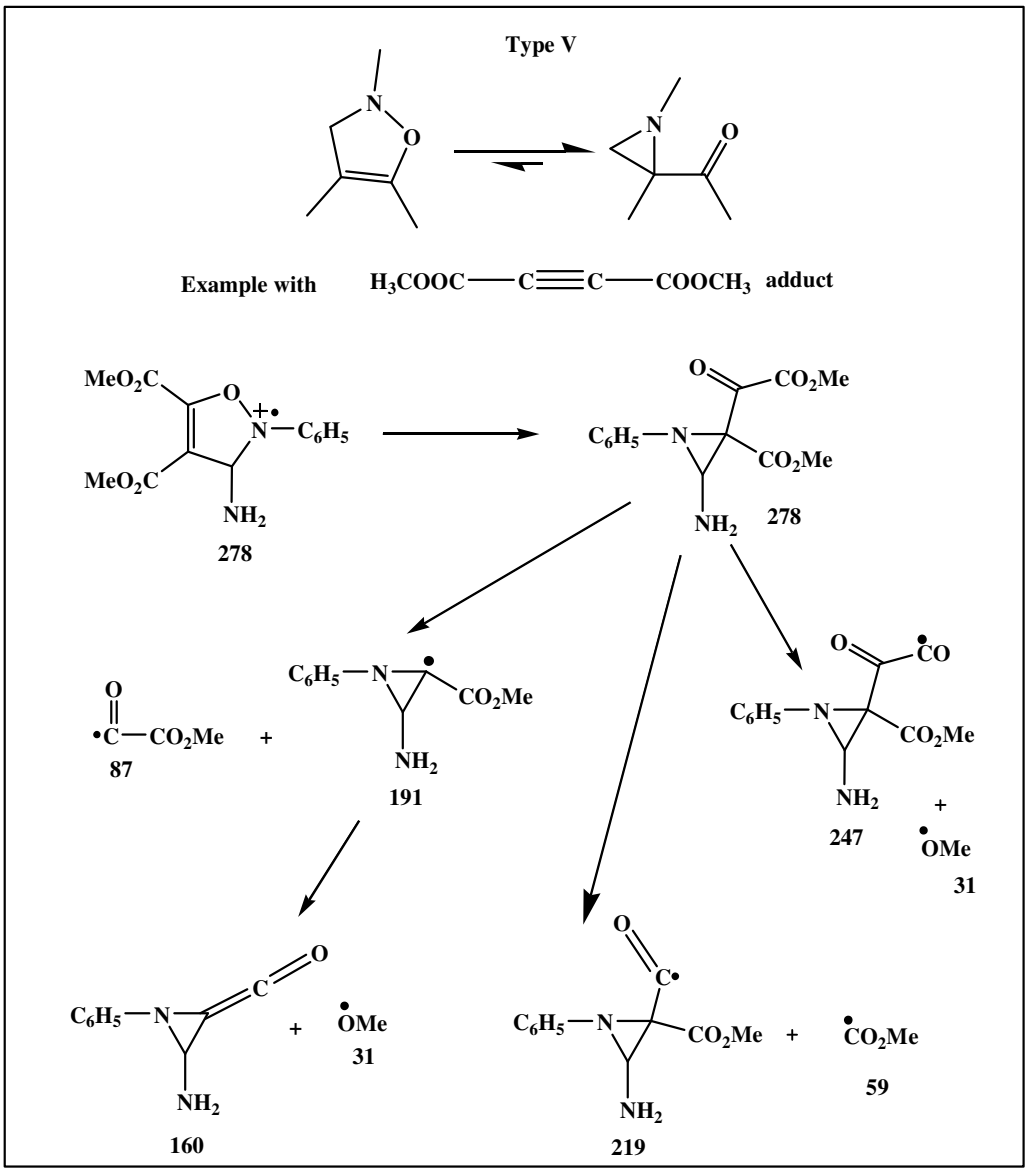
Type IV: ***Tetrachloro ethylene cycloadduct***

The major fragmentation pattern of tetrachloro ethylene cycloadduct has been explained in **Type IV.** Development of dichloro carbene and nitroso benzene is the salient features observed in this pattern of mass fragmentation.



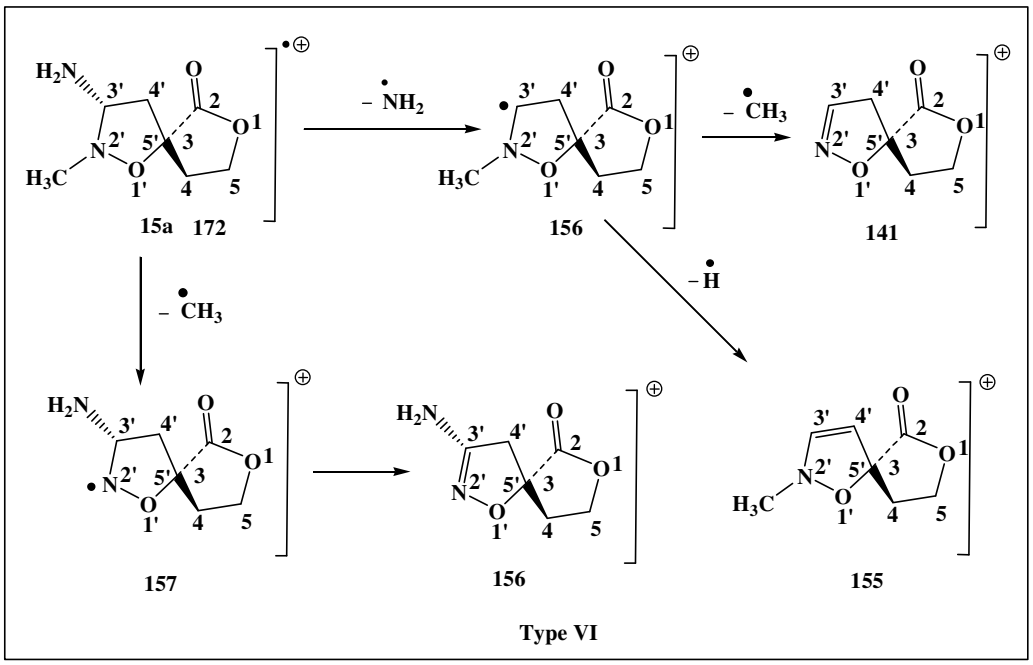
Type V: Mass fragmentation pattern of amino isoxazoline cycloadducts

The fragmentation patterns of acetylenic cycloadducts are completely different and are explained in **Type V.** It has been observed that all the isoxazoline derivatives underwent aziridine derivatives during electron impact mass fragmentation. We have obtained expected fragmentation peaks in the mass spectral studies and are due to the development of *aziridine derivatives*. Base peaks were obtained due to loss of $COOCH_3$ for dimethyl acetylene dicarboxylate, methyl phenyl propiolate while COOH for acetylene dicarboxylic acid, $COOCH_3$ for ethyl propiolate respectively. Taking dimethyl acetylene dicarboxylate cycloadduct as an example, the mass fragmentation pattern of isoxazoline derivatives has been described in **Type V** scheme.



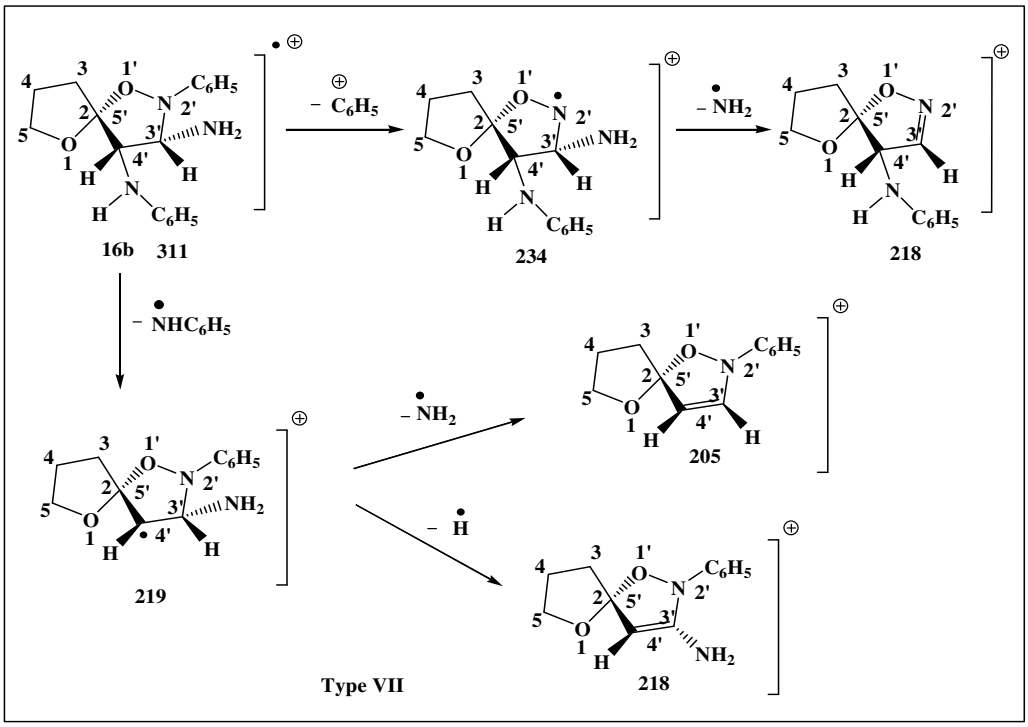
Type VI: Mass fragmentation pattern of spiro isoxazolidine cycloadducts

The major fragmentation pattern of the spiro cycloadducts (**15a & 15b**) have been described in **Type VI.**

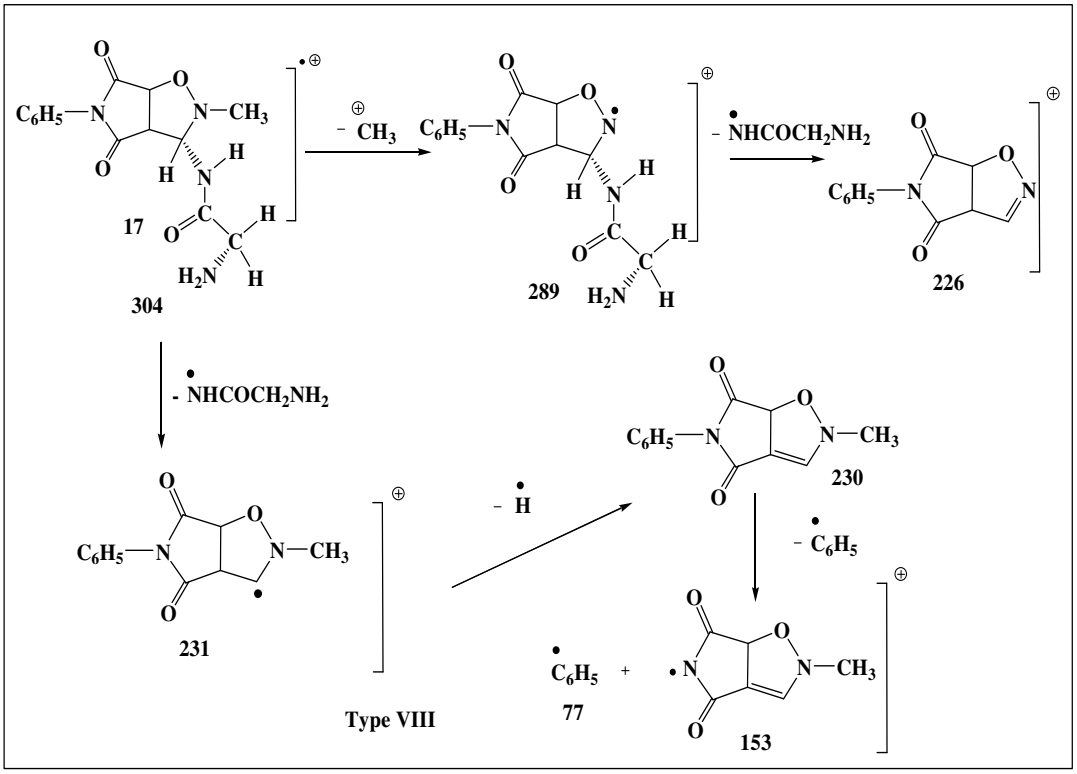


Type VII: Mass fragmentation pattern of novel spiro isoxazolidine cycloadducts

The major fragmentation pattern of the novel spiro cycloadducts (**16a & 16b**) have been described in **Type VII.**



Type VII: General scheme of mass fragmentation pattern of novel peptides 17a-c



Interpretation of ^{1}H NMR spectra

On interpretation of ^{1}H NMR spectra of the amino isoxazolidine and isoxazoline derivatives the chemical shifts and the coupling constants for C_5, C_4, C_3 were considered. The *J* value i.e. coupling constant determines the stereochemistry at these positions. In most often cases C_5, C_4, C_3 are asymmetric in nature. In case of diastereomers the products were identified considering the multiplicity of the proton signals at 3-*H* and 4-*H* along with coupling constant values. During the course of the study regarding the *J* values of the cycloadducts the following representation gives us an idea regarding the stereochemistry of the cycloadducts. Exact stereochemistry of the isoxazoline derivatives could not be assigned due to the absence of C_5 and C_4 protons and only C_3 proton cannot determine the stereochemistry.

Table 6 (^{1}H NMR values of some C_5H & C_4H protons and coupling constant values in δ ppm): *Cycloadducts derived from N-phenyl-α-amino nitrone) in aqueous phase.*

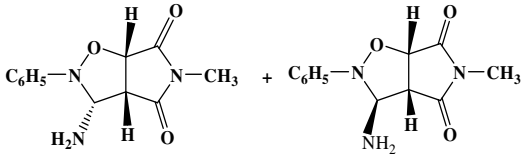
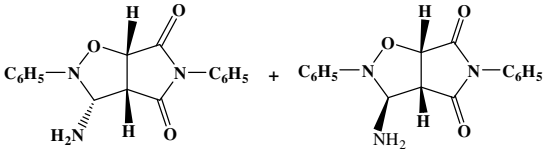
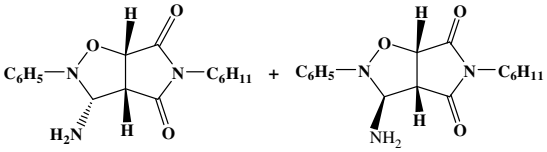
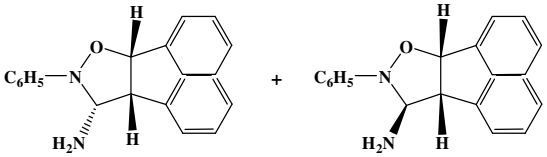
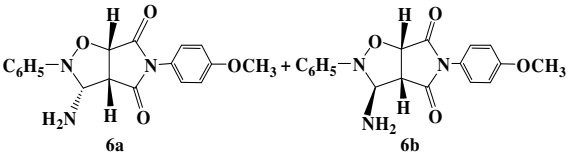
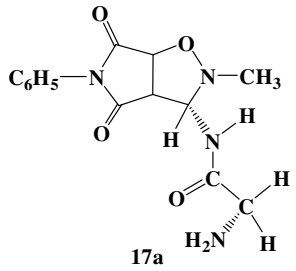
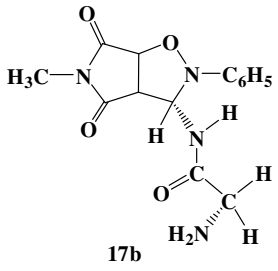
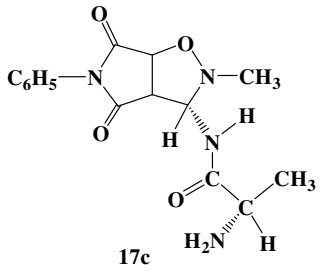
Amino isoxazolidines	C_5H [coupling constant values (Hz) in parentheses]	C_4H [coupling constant values (Hz) in parentheses]
 **2a** + **2b**	5.24 (6.06) 5.15 (2.54)	4.93 (6.60, 6.52) 4.70 (3.40, 3.10)
 **3a** + **3b**	5.16 (7.26) 4.94 (2.80)	4.15 (7.24, 7.50) 4.26 (2.08, 1.94)
 **4a** + **4b**	1.85 (6.80) 1.86 (2.74)	2.10 (6.08, 6.14) 2.42 (2.08, 2.06)
 **5a** + **5b**	4.37 (6.12) 4.16 (2.10)	2.89 (6.14, 6.14) 2.62 (2.06, 2.08)
 **6a** + **6b**	6.45 (6.06) 6.40 (3.00)	3.51 (6.12, 6.12) 3.34 (2.06, 2.06)

Table 7 (^{1}H NMR values of some C_5H & C_4H protons and coupling constant values in δ ppm): *New Peptides derived from amino isoxazolidines (N-phenyl-α-amino nitrone).*

Peptides	C_5H coupling constant values (Hz) in parentheses	C_4H coupling constant values (Hz) in parentheses	C_3H coupling constant values (Hz) in parentheses
 **17a**	6.45 (6.10)	2.36 (6.04, 6.06)	6.23 (3.22)
 **17b**	4.72 (6.64)	3.32 (6.12, 6.10)	4.35 (2.84)
 **17c**	4.75 (6.02)	3.54 (6.52, 6.56)	4.36 (3.92)

In our present study nitrone **1** (both *N-phenyl-α-amino & N-methyl-α-amino nitrone*) exists exclusively in *Z* configuration and *syn* cycloadducts are formed from *Z* nitrone through an *exo* transition state geometry. The relative configurations of C_3, C_4, C_5 protons of the cycloadducts are *syn*, as evidenced by their coupling constant (J ~ 6.06-8.18Hz, for C_4-C_5 & J ~ 6.02-7.50 Hz, for C_3-C_4) values[28,29]. It may be concluded from the J values that the dipolarophiles with *cis* configuration about the double bond gave rise to *cis* adducts and therefore the nitrone additions were stereospecifically *syn* in nature.

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[28,29]. The most significant differences in the ^{1}H NMR data for the diastereomers are the position and multiplicity of the 3-H signal. In the major adducts **2a–6a**, coupling constant between 3-H & 4-H has been measured as $J_{3,4}$ ~ 6.26 Hz implying a *cis* relationship between H-3 and H-4, whilst for minor adducts **2b–6b**, $J_{3,4}$ is ~ 2.26 Hz implies a *trans* relationship between H-3 and H-4[28,29].

From the coupling constant values for *C*-5 proton of the amino cycloadducts we have calculated the dihedral angles between *C*-5 and *C*-4 protons of *N-methyl maleimide, N-phenyl maleimide and methyl acrylate amino isoxazolidines* from the standard graph[5,6] (**Figure 4**). From these calculated values and with the assumption that 2-phenyl-3-amino isoxazolidines will prefer the envelope configuration. *N*-phenyl group at equatorial position and amino group will also be at equatorial position at *C*-3 (**Figure 5**).

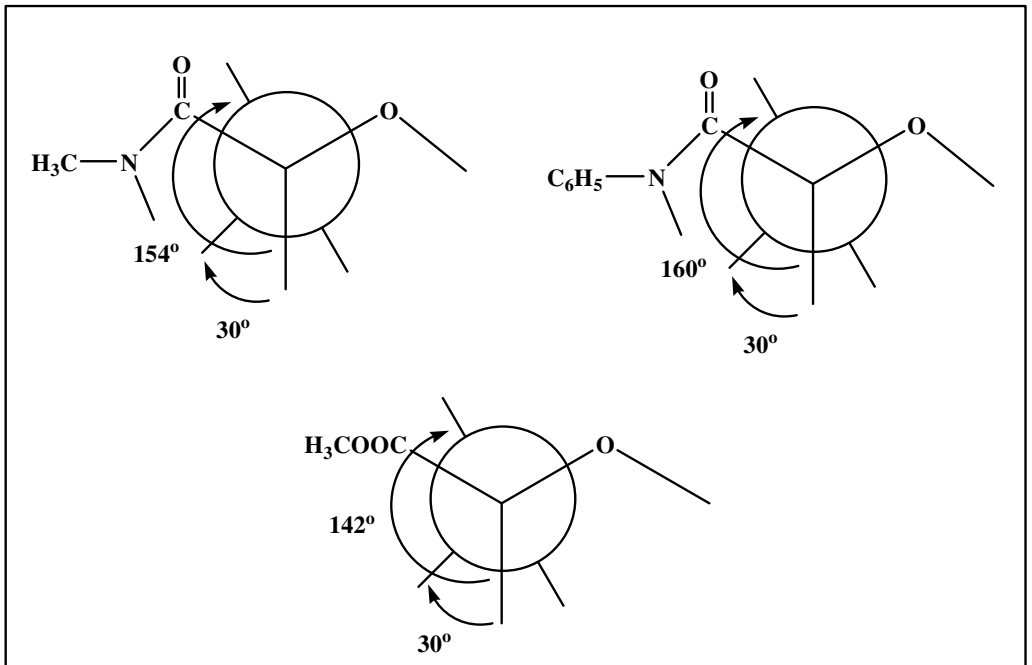


Figure 4 : ***Calculation of the values of dihedral angles between C_5 & C_4 protons in amino isoxazolidine derivatives***

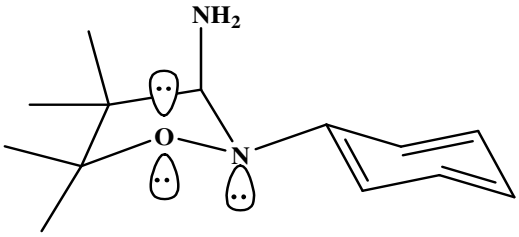


Figure 5: ***Probable conformational structure of amino isoxazolidines***

From these figures it is clear that the substituent at the *C*-3 position tries to have an equatorial position as well as the substituent at the *C*-5 position from the quasi equatorial position of the envelope form. As a result the 1,2-isoxazolidine conformation shifts from envelope to half chair form depending upon the bulkiness of the *C*-5 substituent (**Figure 5**).

This indicates that in the reported amino cycloadducts, the *C*-5 and *C*-4 protons couple in the same way and comparison with the corresponding dihedral angles suggests that the angles of the protons are nearly 30°. The normal dihedral angle has been found to be 40-30° as found from dihedral angle reported for the cycloadducts in the literature[5,6].

Significant long range coupling between C_3 & $C_{4'}$ protons were observed in the *5-spiro isoxazolidine derivatives* (**16a & 16b**) obtained by the reactions of nitrone **1a & 1b** (**R=Ph, Me**) with newly reported α-*N*-methyl/phenyl furan derivatives[25] as dipolarophile. 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 spiro cycloadducts and their coupling constants ($J_{H3,H4}$ = 6 – 7.4 Hzs) are also indicative of this stereochemical relationship[28].

In case of new peptides (**17a-17c**), coupling pattern of C_5 & C_4 protons of the isoxazolidine ring are similar to normal isoxazolidines as reported (**2a-6a**). Coupling constant values are reflected in **Table 7**. All the new peptides are classified as *cis cis* and *cis trans* peptides as evidenced from the configurations of C_5, C_4 and C_3 protons (isoxazolidine ring numbering) as well as their coupling constant values obtained in the ^{1}H NMR spectrum.

In case of amino isoxazoline derivatives (**11-14**) exact stereochemistry based on ^{1}H NMR spectroscopy (coupling constant values of adjacent protons) could not be determined as C_5 & C_4 protons were absent and only C_3 proton is unable to predict the stereochemistry. Stereochemistry of tetrachloro isoxazolidine (**7**) derivative also could not be assigned for the same reason.

Three new chiral centers are developed in the newly developed cycloadducts (amino isoxazolidines) at C_3, C_4, C_5 positions. The relative configurations of C_3, C_4, C_5 protons of the cycloadducts are *syn*, as evidenced by their coupling constant (J ~ 6.06 -8.18Hz, for C_4-C_5 & J ~ 6.02 - 7.50 Hz, for C_3-C_4) values[28,29].

Nitrone cycloaddition reactions are believed to be a process with similarity of LUMO and HOMO energies in dipole and dipolarophile. As such both HOMO (dipole) - LUMO (dipolarophile) and LUMO (dipole) - HOMO dipolarophile) interactions are important in determining reactivity and regiochemistry. In these cycloaddition, the *C-C* & *C-O* bond formation in the transition state may not happen in a synchronous manner. The *C-C* bond of isoxazolidine ring is more developed in the transition state than *C-O* bond. This process helps to afford products having *syn* configuration at C_3 & C_4 respectively. In addition to the above explanations, all expected signals are obtained and the values are in good agreement with the reported values found in literature[5,6]. Signals of amino protons are generally obtained as broad peaks around 2-5 δ ppm while ester methyl protons were obtained in the range of 3.30 δ ppm. In the atom efficient aldehyde synthesis (reaction between amino nitrones and alkyl halides), signals of aldehydic carbon of benzaldehyde were obtained around 9.80 δ ppm.

In the present text, following abbreviations are used for identifying NMR signal.

s = singlet, d = doublet, dd = double doublet, ddd = doublet of double doublet. dt = doublet of triplet, q = quartet, m = multiplet, br = broad

Interpretation of ^{13}C NMR Spectra

On exhaustive study regarding ^{13}C NMR spectra of reported amino nitrones, cycloadducts, spiro cycloadducts and peptides we have found that in almost all the cycloadducts the expected signals for the carbon atom bonded with nitrone ($CH=N^+$) are obtained in the range between δ 140 -142 in ppm while expected signals for *C*-5, *C*-4, *C*-3 carbon atoms of the amino isoxazolidine, isoxazoline derivatives, phenyl and carbonyl carbons are obtained. All the signals are in good agreement with the published research articles found in literature. Remarkably the deviated values for the carbonyl groups are obtained when the carbonyl group is either methyl ester or ethyl ester. The signals obtained for the phenyl carbons of the cycloadducts in most often cases are found to be four (4) ranging between δ 138-120 ppm.

These four signals are due to the fact that 2,6 and 3,5 positions of the phenyl ring are identical positions and give rise to only one signal. When the carbonyl carbon is methyl ester absorptions at δ 178-180 ppm are obtained while δ 168-170 ppm are obtained for normal

C=O bond absorptions. *C*-5, *C*-4, *C*-3 carbons of the cycloadducts absorb in the range of δ 85-88, δ 50-60 and δ 70-75 ppm respectively with some deviations for some certain cycloadducts. Although ^{13}C NMR spectra cannot predict the stereochemistry of the cycloadducts but plays an important role for the identification of a particular functional group, specific carbon atoms of the cycloadducts.

Interpretation of other spectra

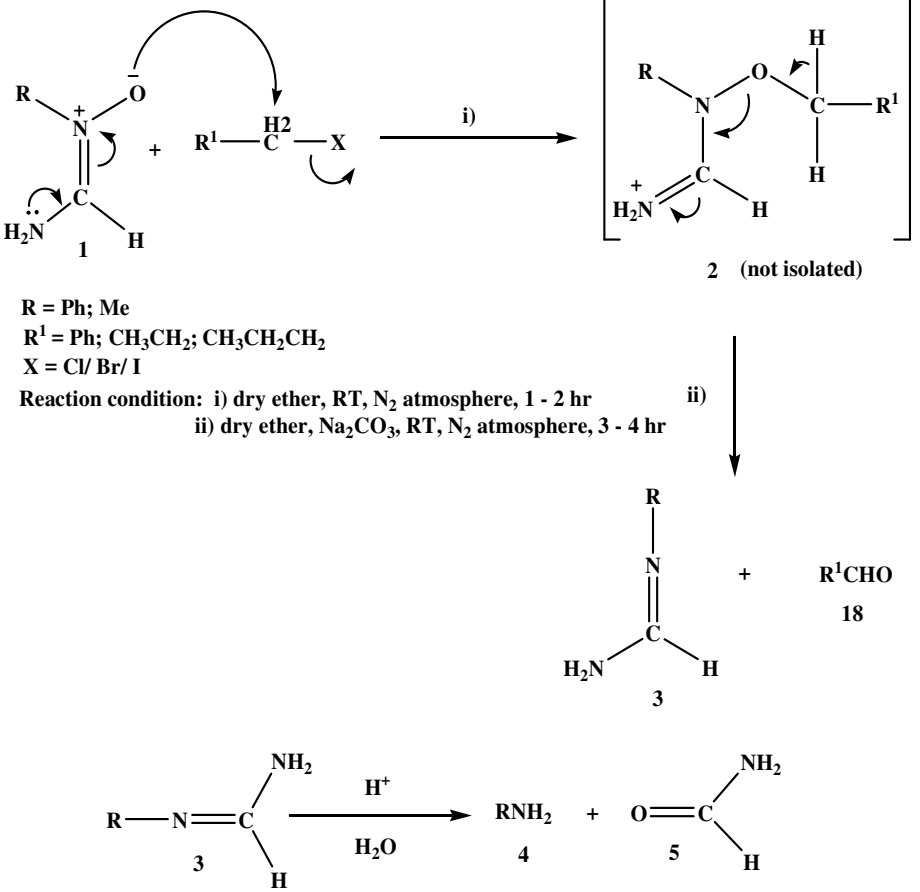
In addition to ^{1}H NMR, MS and ^{13}C NMR spectral study, IR spectral study also have been found to be an important tool for the confirmation of the various functional groups, C-C, C=C, C-N & C-H bonds of the amino cycloadducts, spiro cycloadducts and peptides reported in this dissertation. The carbonyl stretching absorption is one of the strongest IR absorptions, and is very useful in structure determination as one can determine both the number of carbonyl groups (assuming peaks do not overlap) but also an estimation of which types. The carbonyl absorptions were obtained around 1680-1740 cm^{-1} depending upon the carbonyl functionality. For example, the IR absorptions of keto group (C=O) of amino maleimide cycloadducts and aldehyde group of benzaldehyde (obtained during atom efficient synthesis of aldehydes by the reaction of amino nitrones and alkyl halides) has been found in the range of 1680-1740 cm^{-1} while IR absorptions of ester carbonyl (COOR) and carboxylic carbons (COOH) are found in the range of 1710-1750 cm^{-1}. The IR absorption bands of C-N-H stretching have been generally obtained around 1240-1320 cm^{-1}.

Sharp singlet absorptions around 750-780 cm^{-1} have been obtained due to phenyl *C-H* bending while aromatic C-H stretching absorptions are found in the range of 3010-3030 cm^{-1}. Prominent IR absorption bands of amino group ($-NH_2$) have been obtained in all the cycloadducts and peptides. The most important IR absorption band of the amino nitrones ($C=N^{+}$) have been obtained around 1610 cm^{-1}. All the reported IR absorption values in this dissertation are in good agreement with the values reported in literature.

In case of amino isoxazolidine, isoxazoline, spiro isoxazolidine and peptide derivatives, study of mass spectrum reveals that prominent molecular ion peak and the base peaks are obtained as expected. The molecular ion clearly indicates the stability of isoxazoline cycloadducts. Base peaks are obtained due to loss of PhCO for phenyl methyl propiolate and $-COOCH_3$ for dimethyl acetylene dicarboxylate cycloadducts for both *N*-phenyl-α-amino and *N*-methyl-α-amino nitrones respectively.

Elemental analysis was carried out for almost all the cycloadducts and new peptides and minimum variation was noticed in the calculated and the analyzed values which also confirms in favour of new cycloadducts and peptides.

Finally, we have reported atom efficient *aldehyde synthesis*[2] (**Scheme 5)** using the synthetic potentiality of *N*-phenyl & *N*-methyl-α-amino nitrones as a potential oxidizing reagents. The side products of the aldehyde synthesis (imines **3)** have been hydrolysed successfully and primary amines are obtained in good yield. ^{1}H NMR signals of imines and aldehydes are in good agreement with literature values.



Scheme 5: Atom efficient aldehyde synthesis

Biological study of the newly synthesized cycloadducts (amino isoxazolidine, isoxazoline derivatives, spiro isoxazolidines and peptide derivatives)

Biological study (*antibacterial, antimicrobial*) on some the newly synthesized amino isoxazolidine & isoxazoline derivatives along with spiro isoxazolidine and peptides have been successfully conducted and are found to be active. The *antifungal activity* of the amino isoxazolidine and isoxazoline derivatives (**2a, 3a, 5a, 6b, 7, 9, 12**) have 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 (**2a, 3a, 5a, 6b, 7, 9, 12**) exhibited considerable *antibacterial and antifungal activity.* Detail studies (using *SEM & TEM*) are now in progress.

Determination of Minimum Inhibitory Concentration (MIC)

All the cycloadducts (**2a, 3a, 5a, 6b, 7, 9, 12**) 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 was determined by Disc Diffusion Method and Broth Dilution Method[58]. Previously prepared drug dilutions (4µg/mL, 8µg/mL, 16µg/mL, 32µg/mL, 64µg/mL, 128µg/mL, 256µg/mL and 512µg/mL) of the fluoro isoxazolidine and isoxazoline derivatives with appropriate antibiotic control (Amoxycillin and Gentamycin) were prepared with Mueller Hinton Agar[59]. For agar dilution assay those cycloadduct plates were spot inoculated (2×106 cfu per spot). A plate without fluoro isoxazolidines or isoxazolines was taken as control (blank) in order to compare the results. The results were then recorded after incubation for 72 hrs at 37ºC. 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[25] and Broth Dilution Method[58].

Evaluation of Zone of inhibition

Zone of inhibition of test drugs were evaluated by well diffusion methods as per NCCLS protocol (NCCLS, 1993)[58]. 0.1 ml of bacterial suspension was spread on agar plates with sterile spreader to achieve uniform growth. Wells were dug by sterile borer and appropriate concentration of drugs was loaded to the wells. The plates were incubated at 37°C for 24 hrs and clear zone of inhibition around the wells were recorded**.**

Table 8. ***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	3a 0.17% DMSO	5a 0.17% DMSO	6b 0.17% DMSO	7 0.17% DMSO	9 4% DMSO	12 4% DMSO	Amoxy cillin	Genta-mycin
Escherichia coli ATCC 5938	35	126	507	>512	>512	130	17	2	0.25
Klebsiella pneumonia J/	68	64	>512	>512	>512	124	30	4	2
Staphylococcus aureus	41	60	>512	>512	>512	144	60	2	1
Pseudomonas aeruginosa ATCC 27853	60	255	>512	>512	520	64	8	60	2
Vibrio cholerae 14035	36	64	>512	>512	253	128	60	64	0.5
Bacillus subtilis UC 564	60	68	>512	>512	62	38	8	8	4
Shigella dysenteriae 3	65	64	>512	>512	>512	123	14	64	1
Streptococcus faecalis 292	62	125	>512	>512	>512	129	64	64	0.50
Shigella flexneri DN 13	7	15	16	16	36	64	32	32	1
Salmonella typhi DIRW	8	66	16	16	65	256	120	4	1
Vibrio parahaemolyticus 72016	252	256	>512	>512	>512	256	126	16	1
Micrococcus luteus AGD	120	64	>512	>512	>512	512	122	4	8
Salmonella typhimurium	30	64	>512	>512	>512	123	32	8	1
Enterococcus faecalis	60	256	>512	>512	>512	128	30	4	2

Table 9: *Minimum Inhibitory Concentration (MIC) of synthetic compounds against different bacteria*

Organism	Control	Spiroisoxazolidine & peptides (Drugs in µg/ml)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	Amoxicillin
Escherichia coli 25938	+	600	600	400	600	+	+	+	25
Salmonella typhi 62	+	600	400	+	400	+	+	600	15
Vibrio cholerae 20	+	600	600	200	600	1000	+	600	25
Klebsiella pneumoniae 1003	+	600	600	+	600	+	+	+	25
Shigella dysenteriae 1	+	800	600	400	600	1000	+	+	1
Pseudomonas AMRI 108	+	600	600	+	600	+	+	+	50
Salmonella typhimurium NTCC 74	+	600	600	600	400	+	+	+	25
Staphylococcus aureus 29737	+	600	600	400	+	1000	+	+	5
Bacillus cereus 11778	+	600	400	600	600	1000	+	+	25
Bacillus subtilis 6633	+	600	600	+	600	1000	+	+	50
Streptococcus epidermidis 12228	+	600	400	600	600	1000	+	+	5
Micrococcus luteus 10240	+	600	600	600	600	600	+	+	1
Pseudomonas aeruginosa 25619	+	600	600	600	400	+	+	+	100
Bacillus pumilus 14884	+	600	600	600	600	1000	+	+	50
Bordetella bronchiseptica 4617	+	600	600	600	600	+	+	+	50

"+" represents growth of organism.

Determination of killing rate

Antibacterial efficacy of tested compounds were determined by using viable cell count experiments (Rhim et al., 2009)[59]. From the experiment it is clear that the tested drugs did not kill the microorganisms completely but significantly reduce the bacterial growth i.e., these drugs have potential bacteriostatic effect. SEM micrographs of few spiro isoxazolidines **15 & 16** (**Table 3**) have been found to have potential antimicrobial effects on *B. cereus 11778* and *M. luteus 10240* respectively. It was clear that both the bisaziridine derivatives cleaved the cell surface leading to lyses of cell components into several fragments and thus facilitates rapid killing of cells[60].

From SEM microscopy it is expected that these drugs may act as potential antimicrobial agents. Drug **1** (**Table 9**) kills bacteria by cleaving cell membrane at different junction and leads to cell death. Drug **2** (**Table 9**) changes the cell permeability of bacteria. Due to this change fluids are accumulated within cells and eventually lead to death.

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