CHAPTER- 1

*Synthesis of novel 5-substituted- 3-arylisoaxazolines through organometallic reactions and their immunomodulatory activity.*
Section-A: Introduction
1.1.0. Introduction:

Breakthrough and advancement in science rest always on the foundations laid by earlier workers. The history of nitrile oxide goes back to 1800, when E. Howard prepared the explosive mercury fulminate. The correct structure of fulminic acid and its salts remained unknown for a long time and was the subject of much speculation. It had to wait for about 100 years for its elucidation. In 1899 H. Ley suggested that fulminic acid was nitrile oxide of formic acid, i.e., the parent compound of nitrile oxides.

\[ \text{HC} \equiv \text{N} - \text{O}^- \]

Fulminic acid

The present status of chemistry of dipolar nitrile oxide acknowledges particularly the contributions by H. Wieland in early 1900’s, A. Quilico and associates in the 1940’s, and R. Huisgen, who, in the 1960’s, synthesized comprehensively the 1,3- dipolar reactions and arrived at a better understanding of their mechanism based on molecular orbital theory.

1.1.1. Isoxazolines, reactions and physiochemical properties; the masked functionality and the aldol concept:

The isoxazolines occur in three isomeric forms: 2-, 3-, and 4-isoaxazolines, of which the 2-isoaxazolines are the most common, most stable, as well as synthetically the most versatile. 2-isoaxazolines are readily available by 1,3- dipolar addition of nitrile oxide or silyl nitronates to olefins.

\[
\begin{align*}
\text{2- isoaxazoline} & & \text{3- isoaxazoline} & & \text{4- isoaxazoline} \\
\end{align*}
\]

The aromatization of 2-isoaxazolines to isoxazoles is important because 1,3- dipolar addition is easier with olefins than with the corresponding acetylenes, and olefins are generally more available. Isoxazolines are stable towards peracids. It is thus possible to epoxidize a vinyl substituent without oxidizing the heterocycle.¹
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Peracids

Scheme 1

Isoxazolines with hydrogen at C-3 are cleaved by the bases to α,β-cyanohydroxy derivatives.2

Scheme 2

Thermal decarboxylation of 3-carboxy-2-isoxazolines causes a similar fragmentation.3

Scheme 3

The reductive cleavage of the N—O bond, in isoxazolines, can be accomplished readily and selectively by various methods (scheme 4), and each of these reactions lead to the synthesis of very important intermediates (like β-ketoalcohols, β-aminoalcohols etc.), which are otherwise prepared through difficult and expensive routes.4
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2-isoxazolines contain a masked aldol moiety. This class of compounds is formed from inexpensive accessible starting material by simple procedures. They are reasonably stable, can be functionalized, allow steric operations and consequently, are suitable as versatile structural entities in organic synthesis.

1.1.2. Biological importance of 2-isoxazolines:

Amongst five-membered heterocycles, isoxazolines represent a class of compounds with great importance in heterocyclic chemistry. These compounds have intrinsic biological activities and constitute the structural feature of many bioactive compounds. Substituted 2-isoxazolines are small nitrogen heterocycles with broad spectrum of biological activities and application in pharmacological and synthetic intermediates as they represent masked aldol functionality, unmasking of which leads to β-amino alcohols or β-keto alcohols. Substituted 2-isoxazolines are well documented in the literature to possess significant biological activities. Isoxazolines have gained importance due to their various chemotherapeutic properties. The 3,5-disubstituted and 3,4,5-trisubstituted 2-isoxazolines have been reported to exhibit broad range of biological activities such as antimicrobial, anti-inflammatory, factor Xa inhibitory, anticancer, anti-HIV, caspase inhibitory, antidepressant and also act as fibrinogen receptor & glycoprotein IIb/IIIa antagonistic.\(^5,6\) Hence, it is considered worthwhile to prepare molecules having
isoxazoline rings with various substitution. Isoxazolines have been synthesized by 1,3-
dipolar cycloaddition of nitrile oxides to various alkenes.

1.1.3. Applications of 2-isoxazolines in synthesis:
As already discussed 2-isoxazolines are important class of synthetic compounds, which
have wide application in organic synthesis. 2-isoxazolines are masked aldols and this
important fact has been emphasized by several research groups exploring their synthetic
potentialities. The usefulness of the isoxazoline route becomes evident when the starting
material and the simple experimental conditions of the 1,3-dipolar cycloaddition are
considered. The isoxazoline route has been developed into a standard procedure for
forming carbon-carbon bonds, intermolecularly as well as intramolecularly. The
reductive cleavage, as carried out by the catalytic hydrogenation or by the metals (e.g.
Ti^{3+}), has been known for a fairly, long time, but isoxazoline methodology has been fully
recognized and appreciated only recently (scheme 5).\textsuperscript{4} We can say that reduction of
isoxazoline (route B) leads to the synthesis of familiar aldol moiety, as is obtained by the
Claisen route (route A) and complete reduction of 2-isoxazolines gives 1,3-
aminoalcohols (route C).

So a wide variety of functionalized alkenes can be prepared \textit{via} the isoxazoline route and
many of the products are useful as building blocks in organic synthesis. In conclusion
isoxazoline are considered to be very important class of compounds with wide variety of
applications in organic synthesis and can be used directly or indirectly in the synthesis of
various important class of compounds as furans, pyrones, aziridines, pyrazoles and many
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7 These compounds are not only synthetically important but are having biological importance also.

1.1.4 1, 3-Dipolar-cycloaddition reaction:
The 1,3-dipolar cycloaddition is a reaction between a 1,3-dipole and an alkene / alkyne known as a dipolarophile. Like the Diels-Alder, it is also a concerted process. The 1,3-dipolar cycloaddition is also referred to as a [3+2] cycloaddition. The [3+2] nomenclature refers to the number of atoms in the two reacting molecules, i.e., a three-atom unit and a two-atom unit (scheme 6). By analogy, the Diels-Alder reaction could also be called a [4+2] cycloaddition. The 1,3-dipolar cycloaddition is also amenable to Lewis acid catalysis.

\[
\begin{align*}
\text{1,3-dipole} & \quad \text{dipolarophile} \\
\end{align*}
\]

Scheme 6

1,3-dipoles are important class of compounds with resonating structure as shown below:

\[
\begin{align*}
X=Y^+Z^- & \quad \text{X=Y=Z} \\
\end{align*}
\]

Scheme 7

1,3-Dipoles (dipoles) vary in stability greatly. Some can be isolated and stored, others are relatively stable, but are usually freshly prepared. Others are so unstable that are generated and reacted in situ.

Reactivity Profile of 1,3-Dipoles: The reaction between dipoles and dipolarophiles fits into the following general profile:

It is currently accepted that cycloadditions are concerted processes - i.e., they have no distinct intermediates, but the bond formation may be asynchronous. (b) The reaction rates are not influenced much by solvent polarity indicating little change in polarity between reactants and transition state. (c) Rates of reaction between dipoles and
dipolarophiles vary considerably. This can be explained by Frontier Molecular Orbital Theory (fig 1), which considers the interaction between molecular orbital of the dipole and dipolarophile.

The most important interactions are those between the Lowest Occupied Molecular Orbital (LUMO) of one reactant and the Highest Occupied Molecular Orbital (HOMO) of the other reactant. Possible combinations are with $\text{HOMO}_{\text{dipole}} - \text{LUMO}_{\text{dipolarophile}}$ (Type I, fig 2) and $\text{LUMO}_{\text{dipole}} - \text{HOMO}_{\text{dipolarophile}}$ (Type III, fig 2). Which interaction is dominant depends on the difference in energies between the relevant pairs of orbital. The closer in energy the two overlapping orbitals are then the more important the interaction is and a faster reaction takes place. If the energy gap between the two combinations is similar, both are important and the interaction is referred to as Type II.
1.1.5. Mechanism of 1,3-dipolar cycloaddition:

There are certain characteristic features of the 1,3-dipolar cycloadditions irrespective of the reactants. The reactants are oriented in a two-plane complex and interact via their π-orbitals in a πς-πς process (scheme 8). Much attention has been given to the problem about the timing of two-bond formation process (i.e. c-d/ a-e bond formation).

Is it synchronized or are the bonds formed successively? Calculations on transition state (TS) geometry give ambiguous results. They depend on the method chosen. *Ab initio* calculation favors a symmetrical or close to symmetrical transition state with synchronized bond formation, whereas parameterized MINDO calculations result in a
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highly unsymmetrical and late transition state of zwitter ionic or biradical character. Apparently calculations are not refined enough to allow reliable quantitative predictions. Hence some times these are also included among the no-mechanism reactions.

1.1.6. Regioselectivity of dipolar cycloaddition reactions:

In many cases the reactions are highly regioselective if not regiospecific. An unsymmetrical dipolarophile may give two possible regioisomers depending on which way the dipole adds to it. Below are mentioned some of the parameters which decides the regiochemistry of dipolar cycloaddition reactions:

- Monosubstituted olefins and acetylenes show high regioselectivity and give 5-substituted derivatives for both electron donating and electron withdrawing groups. Very strong electron withdrawing groups (e.g. –SO₂R) gives predominantly 4-substituted derivatives.
- 1,1-Disubstituted olefins show high regioselectivity and give 5,5-disubstituted products. Strong electron withdrawing groups give 4, 4-disubstituted products.
- 1,2-Disubstituted olefins and acetylenes give mixture of regioisomers.
- Both electron withdrawing and electron donating groups and strain in the dipolarophiles increases the reactivity of the dipolarophiles.
- The addition is a concerted cis-addition (suprafacial).
- The regioselectivity of the acetylenes are less pronounced than that of olefins.

1.1.7. Nucleophilic addition to nitrile oxide:

Nitrile oxide reacts with nucleophiles to form an array of hydroxamic acid derivatives (scheme 9). Most of these additions have limited synthetic interest. On the other hand, the reverse reaction, e.g. the elimination of hydrogen chloride from hydroximoyl chloride, is, as we have seen the best choice for synthesizing nitrile oxides, since the hydroximoyl chlorides are readily accessible by the chlorination of aldoximes.

\[
R-C≡N=O^- + \text{Nu}^- + H^+ \rightleftharpoons R-\overset{\text{Nu}}{\text{N-OH}}
\]

\[\text{Nu} = \text{nucleophile}\]

Scheme 9
Reactivity of organometallic reagents to C=N bonds:
As in the case for the addition reaction of carbanions to the carbonyl group of aldehydes and ketones, the addition of organometallic reagents to the C=N bonds of imine derivatives is an old and well known reaction. However, the development of these additions has been severely limited both by the poor electrophilicity of the azomethine carbon and the tendency of enolizable imines and imine derivatives to undergo deprotonation rather than addition. To circumvent these two problems, a variety of methods have been developed and have greatly improved the scope of organometallic additions to the imines or imine derivatives. The electrophilicity of the carbon atom of the C=N bond can increased by the N alkylation, N-oxidation, N- acetylation, or N-sulphonation to give reactive iminium salts, reactive nitrones/ nitrile oxide, acylimines, and sulphonamines. Organometallic reagents are highly reactive towards the C=N bond, but resonance-stabilized allyl organometallic reagents are much more reactive to C=N bond as compared to the ordinary organometallic reagents. Various reactions are already reported on the same, such as reaction of allyl organometallics to the free C=N bond of imines, hydrazones, oximes, and nitrones. Our continued interest in organometallic additions to various C=N compounds, prompted us to present in this chapter, the reaction of nitrile oxides to various resonance stabilized organometallic reagents. In this chapter the novel methods for the synthesis of 5-substituted isoxazoline (i.e. 5-butenyl isoxazoline, 5-methyl isoxazoline and 5-vinyl isoxazoline) is presented. It involves the reaction of nitrile oxide (both in situ generated and stable ones) with resonance stabilized organometallic species (i.e. organometallic reagents of allyl bromide and 1,4-dibromo-trans-2-butene). The product formed in this reaction is novel and are of great biological importance. Some of the earlier approaches for the synthesis of these novel isoxazoles are mentioned below.

1.1.8. Earlier approach for the synthesis of 5-substituted isoxazolines:
1.1.8a. Synthesis of 5-butenyl isoxazoline:
Synthesis of 5-butenyl isoxazoline has been reported long back in 1987 by Mark J. Kurth and Michael J. Rodriguez by simple cycloaddition reaction of nitrile oxide and hexadiene
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(scheme 10). These compounds were prepared because they were having great synthetic importance and can be used for the stereo-controlled synthesis of tetrahydrofurans through a simple procedure involving bis-addition of oxygen nucleophile across the 1,5-diene moiety (scheme 11).

Further in 2004, Hyoung Rae Kim and coworkers reported the synthesis of 2-Cyanomethyl-3-hydroxy-5-iodomethyltetrahydrofuran from various substituted 5-butenyl isoxazolines as depicted in scheme 12.10

1.1.8b. Synthesis of 5-methyl isoxazoline:
Synthesis of 5-methyl isoxazoline has been reported in 1981 by T. Kumagai et al. by cycloaddition reaction of nitrile oxide and propylene gas (scheme 13), which might be relatively inconvenient, as the reaction has to be carried out under adiabatic conditions.9
1.1.8c. Synthesis of 5-vinyl isoxazoline:

5-vinyl isoxazoline are important heterocycles compounds with great synthetic potential. These isoxazolines are used as intermediates for synthesizing a variety of useful compounds like tetrahydrofurans, D, L-lividosamine etc.\textsuperscript{12} The reported procedure for the synthesis of 5-vinyl isoxazoline involves the simple cycloaddition reaction between nitrile oxide and butadiene (scheme-14). However the resulting reaction affords mixture of mono and \textit{bis} adduct.
Section B: -Domino addition of allylzinc bromide to nitrile oxides: synthesis of 5-butenylisoxazolines.
1.2.0. Introduction:
Chemists are constantly working to discover new and improved reactions. One of the primary motivating goals of this research is the development of cleaner, more efficient transformations to shorten syntheses and save money on chemicals. The strategy of using reactions in tandem is also aimed at shortening syntheses. Tandem reactions are commonly referred to under the nebulous phrase “multistep one-pot reactions.” However, more rigorous definitions have been suggested. Ho’s definition is probably the most descriptive: tandem reactions are “combinations of two or more reactions whose occurrence is in a specific order, and if they involve sequential addition of reagents the secondary reagents must be integrated into the products.” Tandem reactions have several advantages over a series of individual reactions. First, they allow construction of complex structures in as few steps as possible. In theory, they also eliminate the need for a purification step (or steps). Since the intermediates are not isolated, it becomes easier to work with sensitive or unstable intermediates. Finally, employing reactions in tandem will save on cost and amounts of reagents, solvents, and reduce the amount of waste that is generated. Chemists have grouped tandem reactions into three categories. The first is “cascade or domino” reactions in which both or all reactions take place without the need for additional reagents or a change in reaction conditions. Everything that is necessary for both reactions is incorporated into the starting materials. The second class, “consecutive” reactions, is where the intermediate formed in the first reaction has the necessary functionality, but additional energy must be added in order to overcome an activation barrier. The last class is “sequential,” where the functionality for the second reaction has been created but additional reagents must be added in order for the second reaction to occur. We are here interested in domino reactions. Among the numerous types of known organic reactions available to use in tandem, cycloadditions are particularly attractive. While there are many types of cycloadditions known, our focus is on the 1,3-dipolar cycloaddition reactions (also known as [3+2] cycloadditions).
As discussed before 1,3-dipolar cycloaddition reactions are important tools for the synthesis of a variety of 5-membered heterocyclic compounds that are difficult to access
through other routes. In the past two decades, much work has been carried out to examine and optimize these reactions with various dipoles. Keeping in view the synthetic & biological importance of isoxazolines derived dipolar cycloaddition; a novel domino addition of allyl zinc reagent to nitrile oxide has been developed and presented.

1.2.1. Present work:
Continued interest in organometallic additions to various C=N compounds, prompted us to present in this section, 1,3-dipolar cycloadditions of nitrile oxides to allylzinc bromide, the intermediate products undergoing addition in a domino fashion to generate 5-butenylisoxazolines in good yields. Several stable benzonitrile oxides and some generated in situ, were reacted with excess (>2 mole eq.) allylzinc bromide in THF under an inert atmosphere. In most cases, 5-butenylisoxazolines were isolated in moderate to good yields (52-82%) after 12-14 h. reaction at ambient temperature. The reaction was found to be general with regard to various substituted nitrile oxides bearing electron-donating or electron-withdrawing groups on the aromatic ring (scheme 15).

![Scheme 15](image)

In some cases, the crude product mixture also contained minor quantities (10-15%) of oximes (5) derived from adduct (7), as well as 5-methyl isoxazolines (6, Scheme 16). The 5-methylisoxazolines 6 presumably arise by protonation of intermediate (3) on quenching with water.
In the case of a sterically hindered dipole viz, 2,6-dichlorobenzonitrile oxide, only the oxime (5) could be isolated (64%) with traces of cyclized product, 5-methyl isoxazoline (<3%) and no 5-butenyl isoxazoline. No products derived from nucleophilic addition of allylzinc bromide to the C=\text{N} i.e. 5-butenylisoxazolines (4) could be detected. The formation of 5-butenylisoxazolines (4) can be visualized as involving two reactions happening in domino fashion: 1,3-dipolar cycloaddition of the nitrile oxide to allylzinc bromide generating intermediate (3) then reaction with a second mol. of allylzinc bromide to generate the final product (4), Wurtz type of coupling of (3) with unreacted allyl bromide or via a mechanism involving Schlenk complex formation (Scheme 17). We exclude the former possibility on the grounds that no product from reaction of (unchanged) allyl bromide and the nitrile oxide was observed and so no allyl bromide was available to participate in the Wurtz-type coupling.
1.2.2. Conclusion:
An unprecedented and direct synthesis of 5-butenylisoxazolines through a domino 1,3-dipolar cycloaddition of allylzinc bromide to nitrile oxides is presented in this section. Apparently reasonable mechanism involving Schlenk complex explaining the formation of 5-butenyl isoxazolines in high yields is also proposed. Overall, the utility & the reproducibility of the method as a preparative protocol for the synthesis of 5-butenyl isoxazolines is presented for the first time in this section.
1.2.3. Experimental section:

Synthesis of 5-but-3-en-1-yl-3-(4-chlorophenyl)-4,5-dihydroisoxazole:

General procedure:

In a typical procedure, a suspension of freshly activated zinc dust (0.65 g, 10 mmol) and allyl bromide (0.6 g, 5 mmol) in dry THF (20 ml) was stirred under nitrogen until the metal dissolved completely to form a clear solution. The allylzinc bromide solution generated as above was cooled to 0-5 °C and added dropwise to a solution of p-chlorobenzonitrile oxide (equivalent to 0.15 g, 1 mmol) in THF (15 ml) over a period of 10 minutes while maintaining the temperature between 0-5 °C. The reaction mixture was allowed to attain rt. and stirring was continued at ambient temperature for 13 h followed by quenching with aqueous ammonium chloride solution (10 ml) and diluting with dichloromethane (50 ml). The organic layer was separated and the aqueous layer extracted with dichloromethane (2x20 ml). The combined organic layers were dried (anhydrous Na₂SO₄) and evaporated under reduced pressure to afford crude product, which was subjected to chromatography (silica gel, 100-200 mesh, elution; n-hexane/EtOAc gradient) to afford pure allyl 5-butenyl isoxazoline (0.18 g, 75%) as a colorless solid and 5-methyl isoxazoline (7%).

Compound 4a:

\[
\text{Cl} \quad \text{N} \quad \text{O} \quad \text{C} \quad \text{C} \quad \text{C} \\
\text{N} \quad \text{N} \quad \text{N} \quad \text{N} \quad \text{N}
\]

\( ^1H \text{NMR (CDCl}_3, 200 \text{ MHz}): \) 81.63-1.99 (m, 2H), 2.18-2.25 (m, 2H), 2.89-3.01 (q, 1H, J =8.1Hz.), 3.32-3.45 (dd, 1H, J=10.0 Hz., 6.2Hz.), 4.69-4.85 (m, 1H), 4.98-5.12 (m, 2H), 5.85-5.95 (m, 1H), 7.37 (d, 2H, J = 8.6 Hz), 7.6 (2H,d, J= 8.6 Hz).

\( ^{13}C \text{NMR (CDCl}_3, 200\text{MHz}): \) 829.3, 34.5, 39.8, 81.0, 115.4, 127.8, 128.3, 128.9, 135.9, 137.4, 155.5.

IR (KBr, cm⁻¹):

3446, 3087, 2983, 1603, 1596, 1492, 1439, 1402, 1349, 1092, 908, 825, 538, 511, 473.
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EIMS: 237 (M<sup>+</sup> +1, 20), 235 (66), 206 (17), 192 (51), 180 (100), 164 (21), 152 (100), 138 (70), 125 (49), 111 (97), 102 (25), 84 (35), 75 (62), 57 (88).

M.p 60.2 °C.

3-(4-Chlorophenyl)-5-methyl-4, 5-dihydroisoxazole (compound no-6a):

\[
\text{\textsuperscript{1}H NMR (CDCl}_3, 200 MHz): \delta 1.43 (d, 3H, J= 6.2 Hz), 2.85-2.95 (q, 1H, J=8.0 Hz), 3.33-3.46 (dd, 1H, J=10.1Hz. and 6.3 Hz), 4.88 (m, 1H), 7.36 (d, 2H, J=9.0 Hz), 7.59 (d, 2H, J=9.0 Hz).
\]

\[
\text{\textsuperscript{13}C NMR (CDCl}_3, 200MHz): \delta 20.9, 41.4, 77.8, 127.8, 128.4, 128.9, 135.8, 155.5.
\]

IR (KBr, cm<sup>-1</sup>): 3443, 2956, 2924, 2852, 1596, 1459, 1379.

EIMS: 195 (M+), 180 (28), 152 (51), 135 (18), 111 (35).

M.p: 53-54 °C.

5-But-3-en-1-yl-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (compound no-4b):

\[
\text{\textsuperscript{1}H NMR (CDCl}_3, 200 MHz): \delta 1.63-1.99 (m, 2H), 2.18-2.25 (m, 1H), 2.89-3.01 (q, 2H, J=8.1 Hz), 3.32-3.45 (dd, 1H, J=10.3 Hz, 6.12 Hz), 3.81 (s, 3H), 4.69-4.85 (m, 1H), 4.98-5.12 (m, 2H), 5.85-5.95 (m, 1H), 6.85 (d, 2H, J =8.6 Hz), 7.72 (d, 2H, J =8.6 Hz).
\]

\[
\text{\textsuperscript{13}C NMR (CDCl}_3, 200 MHz): \delta 24.56, 29.28, 34.99, 50.12, 75.18, 108.86, 110.06, 117.20, 122.87, 132.31, 150.76, 155.72.
\]

IR (KBr, cm<sup>-1</sup>): 3441, 3082, 2968, 2946, 2839, 1643, 1610, 1596, 1518, 1469, 1439, 1421, 1358, 1308, 1252, 1177, 1109, 1044, 830.

EIMS: 231 (M<sup>+</sup>, 70), 186 (30), 176 (100), 147 (56), 134 (47), 121 (99), 92 (35), 77 (73), 41 (57).

M.p 59.5 °C.

3-(4-Methoxyphenyl)-5-methyl-4, 5-dihydroisoxazole (compound no-6b):
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$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.43 (d, 3H, $J$ = 6.2 Hz), 2.85-2.95 (q, 1H, $J$ = 8.0 Hz.), 3.33-3.46 (dd, 1H, $J$ = 10 Hz and 6.3 Hz), 3.80 (s, 3H), 4.88 (m, 1H), 6.82 (d, 2H, $J$ = 8.8), 7.65 (d, 2H, $J$ = 8.8).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 21.2, 42.1, 55.6, 79.7, 114.4, 122.8, 128.4, 130.0, 156.3.

IR (KBr, cm$^{-1}$): 3453, 2948, 2918, 2857, 1600, 1465, 1375.

ESI-MS: $M_p$ 201 °C.

5-But-3-en-1-yl-3-phenyl-4, 5-dihydroisoxazole (pale yellow liquid, compound no-c):

\[
\begin{align*}
\text{N} & \text{O} \\
\text{N} & \text{O}
\end{align*}
\]

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.63-1.99 (m, 2H), 2.18-2.25 (m, 2H), 2.89-3.01 (q, 1H, $J$ = 8.1 Hz.), 3.32-3.45 (dd, 1H, $J$ = 10 Hz, 6.2 Hz.), 4.69-4.85 (m, 1H), 4.98-5.12 (m, 2H), 5.85-5.95 (m, 1H), 7.37 (d, 2H, $J$ = 8.6 Hz), 7.7 (d, 2H, $J$ = 8.6 Hz).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 29.3, 34.5, 39.8, 81.0, 119.4, 127.8, 128.3, 128.9, 135.9, 137.4, 155.5.

IR (KBr, cm$^{-1}$): 3424, 3073, 2930, 2361, 1640, 1596, 1498, 1402, 1357, 1076, 1019, 911, 760, 692, 545, 474.

ESI-MS: $M_p$ 201 (M$^+$ + Na).

3-(Phenyl)-5-methyl-4, 5-dihydroisoxazole (compound no-6c):

\[
\begin{align*}
\text{N} & \text{O} \\
\text{N} & \text{O}
\end{align*}
\]

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.43 (d, 3H, $J$ = 6.2 Hz), 2.85-2.95 (q, 1H, $J$ = 8.0 Hz.), 3.33-3.46 (dd, 1H, $J$ = 10 Hz and 6.3 Hz), 4.88 (m, 1H), 7.35 (d, 3H, $J$ = 3.4), 7.67 (q, 2H, $J$ = 3.3).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 20.9, 41.4, 77.8, 127.8, 128.4, 128.9, 135.8, 156.4.

IR (KBr, cm$^{-1}$): 3444, 2957, 2924, 2855, 1596, 1466, 1360.

ESI-MS: $M_p$ 162 (M$^+$ +1).

M.p 72 °C.
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5-But-3-en-1-yl-3-(2-nitrophenyl)-4,5-dihydroisoxazole (yellowish brown liquid, compound no-4d):

\[
\begin{align*}
\text{N—O} & \\
& \end{align*}
\]

\[\text{IR (KBr, cm}^{-1}\text{): 3445, 3073, 2973, 1607, 1595, 1495, 1440, 1405, 1359, 1093, 908, 835, 539, 517, 474.}\]

\[\text{EIMS: 245 (M}+ - 1, 39), 217 (21), 204 (31), 191 (78), 132 (18), 104 (64), 91 (43), 71 (100), 56 (61), 45 (71).]\]

3-(2-Nitrophenyl)-5-methyl-4,5-dihydroisoxazole (yellow colored liquid, compound no-6d):

\[
\begin{align*}
\text{N—O} & \\
& \end{align*}
\]

\[\text{IR (KBr, cm}^{-1}\text{): 3448, 2950, 2852, 1594, 1458, 1370.}\]

\[\text{ESI-MS: 229 (M}^+ + \text{Na).}\]

5-But-3-en-1-yl-3-(2-chloro-5-nitrophenyl)-4,5-dihydroisoxazole: (Compound no-4e)

\[
\begin{align*}
\text{N—O} & \\
& \end{align*}
\]

\[\text{IR (KBr, cm}^{-1}\text{): 3448, 2950, 2852, 1594, 1458, 1370.}\]

\[\text{ESI-MS: 229 (M}^+ + \text{Na).}\]
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$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 29.3, 34.5, 42.8, 81.0, 117.4, 125, 125.8, 130, 130.8, 138, 139, 157.5.

IR (KBr, cm$^{-1}$): 3442, 3081, 2934, 2913, 1610, 1579, 1464, 1433, 1413, 1351, 1083, 946, 911, 841, 635, 562, 529, 497.

EIMS: 280 (M$^+$, 21), 251 (22), 238 (70), 225 (19), 196 (43), 151 (37), 110 (27), 75 (32), 56 (49), 43 (100).

M.p: 50.3 °C.

3-(2-Chloro-5-nitrophenyl)-5-methyl-4, 5-dihydroisoxazole (compound no-6e):

![Image](image.png)

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.41 (d, 3H, J=6.2 Hz), 3.01-3.13 (q, 1H, J=8.1 Hz.), 3.46-3.59 (dd, 1H, J=10 Hz. and 6.9 Hz), 4.80 (m, 1H), 6.63 (dd, 1H, J=2.82 Hz and 5.75 Hz), 6.93 (d, 1H, J=2.8 Hz) 7.14 (d, 1H, J=8.6).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 20.6, 44.1, 78.2, 116.2, 117.5, 121.5, 129.6, 131.1, 145.3, 157.

IR (KBr, cm$^{-1}$): 3435, 2945, 2920, 2845, 1605, 1450, 1370.

ESI-MS: 241 (M$^+$ +1).

M.P: 50.3 °C.

5-But-3-en-1-yl-3-(4-N,N-dimethylphenyl)-4,5-dihydroisoxazole (light yellow liquid, compound no. 4f):

![Image](image.png)

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.63-1.99 (m, 2H), 2.18-2.25 (m, 2H), 2.89-3.01 (m, 7H), 3.32-3.45 (dd, 1H, J=10.0 Hz., 6.2Hz.), 4.69-4.85 (m, 1H), 4.98-5.12 (m, 2H), 5.85-5.95 (m, 1H), 7 (d, 2H, J=8.6 Hz), 7.5 (2H,d, J=8.6 Hz).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 29.3, 34.5, 39.8, 44, 81.0, 115.4, 127.8, 128.3, 128.9, 135.9, 137.4, 155.5.

IR (KBr, cm$^{-1}$): 3422, 2938, 1602, 1506, 1439, 1409, 1343, 1080, 910, 822, 535, 478.

EIMS: 278 (M$^+$ + HCl, 100%), 223 (26), 196 (37), 181 (25), 161 (12), 118 (13), 77 (11), 56 (28), 44 (28).
5-But-3-en-1-yl-3-(3-nitrophenyl)-4,5-dihydroisoxazole (yellow liquid, compound no-4g):

![Chemical structure](image)

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.63-1.99 (m, 2H), 2.18-2.25 (m, 2H), 2.89-3.01 (q, 1H, $J=8.1$ Hz.), 3.32-3.45 (dd, 1H, $J=10.0$, 6.2 Hz.), 4.69-4.85 (m, 1H), 4.98-5.12 (m, 2H), 5.85-5.95 (m, 1H), 7.5 (m, 3H), 8.1 (d, 1H).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 29.3, 34.5, 39.8, 81.0, 115.4, 127.8, 128.3, 128.9, 135.9, 137.4, 155.5.

IR (KBr, cm$^{-1}$): 3442, 3086, 2985, 1605, 1599, 1496, 1445, 1407, 1344, 1096, 918, 826, 535, 513, 472.

ESI-MS: 269(M$^+$ + Na).

3-(2-Bromophenyl)-5-methyl-4,5-dihydroisoxazole (brownish colored liquid, compound 6f):

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.44 (d, 3H, $J=6.2$ Hz), 3.07-3.12 (q, 1H, $J=8.0$ Hz.), 3.15-3.56 (dd, 1H, $J=10.1$, 6.3 Hz), 4.88 (m, 1H), 7.36 (d, 2H, $J=9.0$ Hz), 7.59 (d, 2H, $J=9.0$ Hz).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 20.9, 44.2, 78.1, 121.9, 127.5, 130.8, 131.8, 157.5.

IR (KBr, cm$^{-1}$): 3445, 2952, 2925, 2853, 1604, 1460, 1340.

ESI-MS: 241 (M$^+$ +1).

By following the same general procedure (as mentioned above) for the sterically hindered dipole viz., 2,6-dichlorobenzonitrile oxide, only the oxime 5 (scheme 8) could be isolated (64%) with traces of cyclized product, 5-methyl isoxazoline (<3%) and no 5-butenyl isoxazoline. No products derived from nucleophilic addition of allylzinc bromide to the C=N i.e. 5-butenylisoxazolines could be detected. So here I am reporting the spectra of oxime and 5-methyl isoxazolines of the above-mentioned compound.

---

Chapter I
(1E)-1-(2,6-dichlorophenyl) but-3-en-1-one oxime: (compound 5)

![Chemical structure of (1E)-1-(2,6-dichlorophenyl) but-3-en-1-one oxime]

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.65 (br. s, 1H), 3.53 (d, 2H, $J$=7.3 Hz), 4.98-5.16 (m, 2H), 5.63-5.83 (m, 1H), 5.74-7.36 (m, 3H).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 33.6, 118.7, 128.0, 130.2, 130.9, 134.1, 135.0, 155.9.

IR (KBr, cm$^{-1}$): 3241, 732, 1427, 940, 778.

EIMS: 229 (M$^+$, 23), 187 (100), 170 (47), 136 (27), 124 (34), 74 (32).

M.P 94-96 °C.

3-(2,6-Dichlorophenyl)-5-methyl-4, 5-dihydroisoxazole: (compound 6g)

![Chemical structure of 3-(2,6-Dichlorophenyl)-5-methyl-4, 5-dihydroisoxazole]

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 1.44 (d, 3H, $J$=6.2 Hz), 2.81-2.95 (q, 1H, $J$=8.9 Hz), 3.17-3.50 (dd, 1H, $J$=10.1 Hz and 10.0 Hz), 4.98 (m, 1H), 7.36 (m, 3H).

$^{13}$C NMR (CDCl$_3$, 200 MHz): $\delta$ 21.2, 44.2, 78.8, 128.3, 129.4, 131.4, 135.2, 154.1.

IR (KBr, cm$^{-1}$): 3436, 2950, 2915, 2842, 1598, 1465, 1375.

ESI-MS: 253 (M$^+$ +Na).

M.P 157 °C.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Nitrile oxide 1</th>
<th>Isoxazoline 4</th>
<th>Reaction time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cl—CNO</td>
<td>Cl—N—O</td>
<td>13</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>MeO—CNO</td>
<td>MeO—N—O</td>
<td>12</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>CNO</td>
<td>N—O</td>
<td>12</td>
<td>78</td>
</tr>
<tr>
<td>4</td>
<td>O2N—CNO</td>
<td>O2N—N—O</td>
<td>12</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>NO2—CNO</td>
<td>NO2—N—O</td>
<td>12</td>
<td>80</td>
</tr>
<tr>
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<td>Me2N—CNO</td>
<td>Me2N—N—O</td>
<td>14</td>
<td>60</td>
</tr>
<tr>
<td>7</td>
<td>O2N—Cl—CNO</td>
<td>O2N—Cl—N—O</td>
<td>12</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>Cl—CNO</td>
<td>—</td>
<td>24</td>
<td>—</td>
</tr>
</tbody>
</table>

a) All products were characterized by IR, $^1$H and $^{13}$C NMR, DEPT and mass spectroscopy.
b) Yields obtained after column chromatography.
c) The corresponding oxime 5j was isolated in 64% yield.
Section C: Anionic domino C-O-heterocyclization approach for the synthesis of 5-vinyl isoxazolines.
1.3.0. Introduction:

1.3.1. Trans-1, 4-dibromo-2-butene and metal in organic synthesis:

This belongs to very important class of reactants. It is mostly used to impart vinyl substitution. It has also been used for a number of anionic domino transformations, developed mainly by Rodriguez et al. using that resulted in the synthesis of very important class of compounds. Some of these novel reactions reported by Rodriguez et al. are described below:

1.3.1a. Preparation of chromane derivatives via indium mediated intramolecular allylation reactions:

Intramolecular indium mediated allylation has been utilized to prepare chromanes that are important structural features of various natural products. The substrate, a precursor for the cyclization, was prepared by the reaction of salicylaldehyde with 1,4-dibromo-2-butene in the presence of potassium carbonate as a base and potassium iodide (catalytic amount) in acetone at room temperature (Scheme 18).

Subsequently, one pot synthesis of chromane was also reported using EtOH as solvent (scheme 19).
1.3.1b. Synthesis of vinyl dihydrofurans:
These are another important class of compounds, which was synthesized by Rodriguez et al using dibromobutene and 1,3-acetonedicarboxylate. This is also an example of one pot synthesis exhibiting high yields as well. The general scheme for this reaction is shown below (scheme 20):

![Scheme 20](image)

1.3.1c. Synthesis of fused functionalised tetrahydrofurans:
These were synthesized by the reaction of disubstituted cyclopentanone with trans-dibromobutene. The schematic representation of the same is represented below (scheme 21):

![Scheme 21](image)

Many such important organometallic reactions reported in literature prompted us to choose dibromobutene mediated nucleophilic addition/C-O heterocyclization approach for the facile synthesis of vinyl isoxazolines.

1.3.2. Present work:
5-Vinyl isoxazolines are valuable intermediates for the generation of a variety of biologically active molecules including D, L-deoxy sugars and isoazolidinyl N-alkylethanolamines. The sole method for the synthesis of 5-vinyl isoxazolines employs the addition of nitrile-oxide to trans-1,3-butadiene (scheme 22), which is taken as limiting reactant. The derived product mixture generally contains both mono and bis adducts.
Chapter I

In order to improvise the synthetic procedure silyl nitronates were employed for the generation of 5-vinyl isoxazolines in good yields. Thus to my knowledge, there has not been any simple and alternative route for the generation of isoxazolines in general and 5-vinyl isoxazolines in particular. Nucleophilic additions of organometallic reagents to various C=N compounds such as imines, hydrazones, oxime-ethers are well established.\(^{21a}\) Nitrones have been successfully employed as substrates for such additions, even though the same has not been studied in depth with regard to nitrile oxides despite the fact that nitrile oxides are known to add to a number of nucleophiles to generate hydroximic acid derivatives.\(^{21b,c}\) The nucleophilic addition of allyl organometallic species (generated by the reaction of 1, 4-dihalobutene with metals) to nitrile oxides resulting in the formation of 5-vinyl isoxazolines via concomitant anionic domino C-O-heterocyclization (Scheme 23) is presented in this section. Thus, 1,4-dibromo-2-butene was stirred with magnesium metal in dry THF at ambient temperature followed by the reaction with a variety of stable and \textit{in situ} generated nitrile oxides. 5-Vinyl isoxazolines were isolated in good yields within a short reaction time (2-5 h) and high yields (65-85%).

Scheme 22

\[ \text{butadiene} \quad \xrightarrow{R\text{=}N\text{=O}^-} \quad \text{mono aduct} + \text{bis aduct} \]

The reaction was found to be general with regard to various metals \textit{viz}, zinc, magnesium, indium and also dihalocompounds (1, 4-dichlorobutene also gave analogues results). In case of indium metal, the reaction proceeds well in aqueous media (THF/water, 1:1) even
though it takes longer reaction time as compared to magnesium to achieve comparable yields (30-45 h, 62-83%). When zinc/THF was employed, the yields were comparatively low (45-63%) even at the end of 48h of reaction time. The formation of 5-vinyl isoxazolines may be visualized through a mechanism involving the nucleophilic attack by the allylic organometallic species 9 (Scheme 24) on iminium carbon of benzonitrile oxide followed by the ring closure with concomitant loss of bromine through repositioning of double bond.

Even though similar anionic domino C-O heterocyclization reactions involving 1,4-dihalo olefins and acetylenes has been pioneered by Rodriguez et. al,\textsuperscript{22} the initial reaction in their case largely involved base catalyzed condensation prior to domino cyclization, whereas, our approach involves cascade of C and O-nucleophilic reactions, occurring in domino fashion.

1.3.3. Conclusion:
A facile and high yielding alternate preparative protocol for 5-vinyl isoxazolines has been developed. The reaction in the presence of indium offers an ecofriendly approach for the synthesis of these compounds in aqueous media. 1, 4-Dihalobutene offers a safe and convenient raw material for the generation of 5-vinyl isoxazolines as compared to butadiene in terms of convenience in handling. A plausible mechanism to explain the product formation is presented. Overall the methodology presented here being novel, high yielding & ecofriendly, may become an alternate preparative protocol for the synthesis of 5-vinyl isoxazolines.
1.3.4. Experimental section:

Procedure for synthesis of 5-vinyl isoxazoline:

Magnesium mediated synthesis of 3-(4-fluorophenyl)-5-vinyl 4,5-dihydroisoxazole, compound 8a):

In a typical procedure, to a suspension of magnesium turnings (0.015 g, 5 mmol.) in dry tetrahydrofuran (distilled over benzophenone ketyl-Na) was added trans-1, 4-dibromobutene (0.214 g, 1 mmol.) in small portions while stirring the reaction mixture at room temperature (note: A small grain of iodine is generally required to promote formation of the Grignard reagent.). The mixture was stirred at room temperature for 1-2 hours. The Grignard reagent generated as above was cooled to 0-5 °C and added drop wise to a solution of p-fluorobenzonitrile oxide (equivalent to 0.137 g, 1 mmol, generated in situ by the treatment of triethyl amine with the corresponding chlorooxime (0.173g, 1 mmol) in THF (15 ml) over a period of 10 minutes while maintaining the temperature between 0-5 °C. The reaction mass was allowed to attain rt. and stirring was continued at ambient temperature for 2-3 h followed by quenching with aqueous ammonium chloride solution (10 ml) and diluting with dichloromethane (50 ml). The organic layer was separated and the aqueous layer extracted with dichloromethane (2x20 ml). The Combined organic layers were dried (anhydrous Na₂SO₄) and evaporated under reduced pressure to afford crude product, which was subjected to chromatography (silica gel, 60-120 mesh, elution; n-hexane/EtOAc gradient) to afford pure 3-(4-fluorophenyl)-5-vinyl 4,5-dihydroisoxazole (0.16 g, 83 %) as a colorless amorphous solid.

Indium mediated synthesis of 3-(4-fluorophenyl)-5-vinyl 4,5-dihydroisoxazole: (compound 8a):

In a typical procedure, a suspension of indium powder (1.15 g, 10 mmol) and trans-1,4-dibromobutene (1.2 g, 10 mmol) taken in 15 ml of THF/water (1:1) was stirred at ambient temperature for 5 h until the metal dissolved completely to form allylindium reagent. The above reagent was cooled to 0-5 °C and added drop wise over a period of 5 minutes to a stirred solution of p-fluorobenzonitrile oxide generated in situ by treating
triethyl amine with the corresponding chloroxime (equiv. 1.53 g, 10 mmol) in THF (15 ml), while maintaining the temperature between 0-5 °C. The reaction mass was allowed to attain room temperature and stirring was continued at ambient temperature for 45 h followed by quenching with aqueous ammonium chloride solution (10 ml). Reaction mass was diluted with DCM (50 ml) and extracted (2x20 ml). Combined organic layers dried (anhydrous Na$_2$SO$_4$) and evaporated under reduced pressure to afford crude product that was subjected to column chromatography (silica gel, finer than 200 mesh, elution; $n$-hexane/ EtOAc gradient) to afford pure 3-(4-fluorophenyl)-5-vinyl-4,5-dihydroisoxazole (3d, 1.53 g, 80 %) as a colorless amorphous solid.

$$\text{N—O}$$

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 2.85-2.95 (q, 1H, $J=8.0$ Hz.), 3.33-3.46 (dd, 1H, $J=10.1$Hz and 6.3 Hz), 5.1 (m, 1H), 5.12-5.55 (m, 2H), 5.85-5.95 (m, 1H 7.36), 7.2 (d, 2H, $J=9.0$ Hz), 7.59 (d, 2H, $J=9.0$ Hz);

$^{13}$C NMR (CDCl$_3$, 200 MHz): 40.4, 82.5, 118.2, 128.4, 128.5, 137.3, 157.5, 161.1, 163.5.

IR (KBr, cm$^{-1}$): 3444, 2959, 2919, 2851, 1602, 1430, 1383.

ESI-MS: 192 (M$^+$ +1).

M.p 52.8 °C.

3-(4-Chlorophenyl)-5-vinyl-4,5-dihydroisoxazole (compound 8b):

$$\text{N—O}$$

$^1$H NMR (CDCl$_3$, 200 MHz): $\delta$ 2.85-2.95 (q, 1H, $J=8.0$ Hz.), 3.33-3.46 (dd, 1H, $J=10.1$Hz and 6.3 Hz), 5.1 (m, 1H), 5.12-5.55 (m, 2H), 5.85-5.95 (m, 1H), 7.36 (d, 2H, $J=9.0$ Hz), 7.59 (d, 2H, $J=9.0$ Hz).

$^{13}$C NMR (CDCl$_3$, 200 MHz): 40.4, 82.5, 118.2, 128.4, 128.5, 137.3, 157.5, 161.1, 163.5.

IR (KBr, cm$^{-1}$): 3452, 2964, 2925, 2861, 1612, 1439, 1387.

ESI-MS: 230 (M$^+$ + Na).

M.p 77 °C.
3-(4-methoxyphenyl)-5-vinyl-4, 5-dihydroisoxazole (compound 8c):

\[
\text{MeO-} \quad \begin{array}{c}
\mid \\
\text{N} \quad \text{O} \\
\end{array} \quad \begin{array}{c}
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\text{C} \\
\end{array} \quad \begin{array}{c}
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\text{H} \\
\end{array}
\]

\(^1\)H NMR (CDCl\(_3\), 200 MHz): \(\delta\) 2.85-2.95 (q, 1H, \(J=8.1\) Hz.), 3.33-3.46 (dd, 1H, \(J=10.0\) Hz. and 6.4 Hz), 3.87 (s, 3H), 5.1 (m, 1H), 5.12-5.54 (m, 2H), 5.84-5.95 (m, 1H), 6.82 (d, 2H, \(J=8.6\)), 7.65 (d, 2H, \(J=8.6\)).

\(^13\)C NMR (CDCl\(_3\), 200 MHz): \(\delta\) 40.1, 55.4, 81.3, 115.4, 122.4, 127.4, 130.3, 157.3, 160.8, 163.2.

IR (KBr, cm\(^{-1}\)):

ESI-MS:

M.p 67-70 °C.

3-(4-N, N-dimethylphenyl)-5-vinyl-4, 5-dihydroisoxazole (semi solid, compound 8d):

\[^1\)H NMR (CDCl\(_3\), 200 MHz): \(\delta\) 2.89-3.01 (m, 7H), 3.33-3.46 (dd, 1H, \(J=10.1\) Hz. and 6.3 Hz), 5.1 (m, 1H), 5.12-5.55 (m, 2H), 5.85-5.95 (m, 1H), 7.0 (d, 2H, \(J=8.6\) Hz), 7.5 (2H,d, \(J=8.6\) Hz).

\(^13\)C NMR (CDCl\(_3\), 200 MHz): 40.2, 44.0, 82.1, 115.2, 127.4, 128.3, 128.9, 157.2, 160.3, 163.5.

IR (KBr, cm\(^{-1}\)):

ESI-MS:

M.p 67-70 °C.

3-Phenyl-5-vinyl-4, 5-dihydroisoxazole (compound 8e):

\[^1\)H NMR (CDCl\(_3\), 200 MHz): \(\delta\) 2.82-2.93 (q, 1H, \(J=8.1\) Hz.), 3.33-3.46 (dd, 1H, \(J=10.2\) Hz. and 6.1 Hz), 5.10 (m, 1H), 5.11-5.55 (m, 2H), 5.85-5.95 (m, 1H), 7.62 (m, 3H), 7.96 (d, 2H, \(J=7.6\) Hz).

\(^13\)C NMR (CDCl\(_3\), 200 MHz): 40.2, 82.1, 119.2, 127.4, 128.1, 128.7, 157.2, 161.3, 163.5.

IR (KBr, cm\(^{-1}\)):

ESI-MS:

M.p 67-70 °C.
Chapter I

ESI-MS: 174 (M$^+$ + 1).

M.p 44-45 °C.

3-(4-methylphenyl)-Phenyl-5-vinyl-4, 5-dihydroisoxazole (semi solid, Compound 8f):

\[
\begin{align*}
\text{N—O} \\
\end{align*}
\]

$^1$H NMR (CDCl$_3$, 200 MHz): \( \delta \) 2.40(s, 3H), 2.98-3.23 (q, 1H, \( J = 8.3 \) Hz.), 3.33-3.45 (dd, 1H, \( J = 10.1 \) Hz. and 6.5 Hz), 5.10 (m, 1H), 5.11-5.55 (m, 2H), 5.85-5.95 (m, 1H), 7.12 (d, 2H, \( J = 7.8 \)), 7.57 (d, 2H, \( J = 7.7 \) Hz).

IR (KBr, cm$^{-1}$): 3444, 2959, 2919, 2887, 1602, 1432, 1383, 1352.

ESI-MS: 210 (M$^+$ + Na).

3-(4-methylphenyl)-Phenyl-5-vinyl-4, 5-dihydroisoxazole (semi solid, compound 8g):

\[
\begin{align*}
\text{N—O} \\
\end{align*}
\]

$^1$H NMR (CDCl$_3$, 200 MHz): \( \delta \) 2.40(s, 3H), 3.18-3.29 (q, 1H, \( J = 8.5 \) Hz.), 3.52-3.62 (dd, 1H, \( J = 10.3 \) Hz. and 6.6 Hz), 5.30 (m, 1H), 5.34-5.55 (m, 2H), 6.01-6.29 (m, 1H), 7.32 (m, 3H), 7.72 (d, 1H, \( J = 7.3 \) Hz).

IR (KBr, cm$^{-1}$): 3450, 2961, 2931, 2883, 1609, 1435, 1381, 1356.

ESI-MS: 210 (M$^+$ + Na).
Table 2: - Synthesis of 5-vinyl isoxazolines through metal mediated addition of 1,4-dibromo butene to nitrile oxides.

<table>
<thead>
<tr>
<th>Entr</th>
<th>Nitrile oxide(1)</th>
<th>5-Vinyl isoxazoline(4)</th>
<th>Indium mediated addition</th>
<th>Grignard addition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Reaction time, (Yield, %)</td>
<td>Reaction time, (Yield, %)</td>
</tr>
<tr>
<td>1</td>
<td>F-CNO</td>
<td>F-CNO</td>
<td>45(80)</td>
<td>3(83)</td>
</tr>
<tr>
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<td>Cl-CNO</td>
<td>Cl-CNO</td>
<td>30(81)</td>
<td>2(85)</td>
</tr>
<tr>
<td>3</td>
<td>MeO-CNO</td>
<td>MeO-CNO</td>
<td>45(73)</td>
<td>3(78)</td>
</tr>
<tr>
<td>4</td>
<td>N-CNO</td>
<td>N-CNO</td>
<td>45(71)</td>
<td>4(75)</td>
</tr>
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<tr>
<td>7</td>
<td>Me-CNO</td>
<td>Me-CNO</td>
<td>45(68)</td>
<td>3(73)</td>
</tr>
</tbody>
</table>

a) All products were characterized by IR, $^1$H and $^{13}$C NMR, DEPT and mass spectroscopy.
b) Yields obtained after column chromatography.
Section D: - Immunomodulatory activity of selected compounds.
1.4.0. Introduction:

Immunomodulation\textsuperscript{23} is a developing segment of immunopharmacology. Immunomodulatory compounds and compositions, as the name implies, are useful for modulating or regulating immunological functions in warm-blooded animals. Immunomodulators\textsuperscript{24} may be immunostimulants for building up immunities to initiate healing of certain diseases and disorders. Conversely, they may be immunoinhibitors\textsuperscript{24} or immunosuppressors for preventing undesirable immune reactions of the body, e.g., to foreign materials and autoimmune diseases. Immunomodulators have been found to be useful for treating systemic autoimmune diseases, such as lupus erythematosus, as well as immunodeficiency diseases. Further, immunomodulators may be useful for immunotherapy of cancer or to prevent rejections of foreign organs or other tissues in transplants, e.g., kidney, heart or bone marrow. Various immunomodulator compounds have been discovered including muramyl dipeptide derivatives, levamisole, niridazole, oxysuran, flagyl and others from the groups of interferons, interleukins, leukotrienes, corticosteroids and cyclosporine. However, many of these compounds have been found to have undesirable side effects and have high toxicity. Thus, doses are typically kept low (and may include delivery in a time release formulation) and one or more compounds may be administered to patients in need of such treatment. One such class of additive agent is anti-proliferative agents. Such agents include azathioprine, brequinar sodium, deoxyspergualin, mizoribine, mycophenolic acid morpholino ester, cyclosporin, and FK-506.\textsuperscript{24c, 24e, 25} New immunomodulators are therefore needed to provide a wider range of immunomodulator function for specific areas with a minimum of undesirable side effects. The discovery of low molecular weight immunomodulator compounds would be yet another advance in this area.

Substituted-2-isoxazolines are small nitrogen heterocycles with broad spectrum of biological activities and applications in pharmacological and synthetic intermediates as they represent masked aldol functionality unmasking of which leads to $\beta$-amino alcohols or $\beta$-keto alcohols. Recently we reported the synthesis of 5-butenylisoxazolines that involves domino addition of allylzinc bromide to various nitrile oxides.\textsuperscript{7} We synthesized
a variety of 2-isoxazolines following this method and classical 1,3-dipolar cycloaddition\textsuperscript{4,5,6} approach and screened their effects on different aspects of immune response. Compounds were tested for T and B-cell proliferation through cell mediated immune response, CD4+/CD8+ T-cell counts through flowcytometry and cytotoxicity.

1.4.1. Chemistry:

1.4.1a. Synthesis of 5-substituted isoxazolines:

5-substituted isoxazoline (3) (Scheme-25/table-3) were prepared through 1,3-dipolar cycloaddition of nitrile oxides (1) to various monosubstituted dipolarophiles (2) according to literature procedure\textsuperscript{15} and were characterized by IR, \textsuperscript{1}H, \textsuperscript{13}C-NMR, mass and also by the comparison on their physical characteristics with literature data for authentic compounds.

\[
\text{Ar} \xrightarrow{\text{N-O}} + \text{R} \xrightarrow{\text{THF, RT}} \text{3}
\]

\[ R = \text{-Ph, -CH}_2\text{OH, -CO}_2\text{Me, -CO}_2\text{allyl, -Ph, -CH}_2\text{Br, -CHClCH}_2\text{-OAc, -OBu.} \]

Scheme 25

5-substituted isoxazolines are formed in most of the cases as sole products as such additions are observed to be highly regioselective. However, 5-butenyl isoxazolines, 5-methyl isoxazolines and 5-vinyl isoxazolines were prepared via novel methods developed by us recently,\textsuperscript{26} employing resonance stabilized organometallics as dipolarophiles. The detail of synthesis is already explained in the earlier two sections of this chapter.
Table 3. Synthesis of various isoxazolines.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Isoxazoline a)</th>
<th>Entry</th>
<th>Isoxazoline a)</th>
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<td>33</td>
<td><img src="image33" alt="Isoxazoline 33" /></td>
</tr>
</tbody>
</table>

a) All products were characterized by IR, $^1$H and $^{13}$C NMR, DEPT and mass spectroscopy.
1.4.2. Biological activity:
In order to evaluate the immunomodulatory activity of test compounds, a number of assays in a variety of immune responsive cells were employed. Humoral incompetence, cell mediated immunity (delayed type hyper sensitivity reaction to SRBC), T and B-cell surface markers and cytokine release by lymphocytes were investigated. Levamisole a known immunostimulant drug as positive control has been used in these studies. In all 33 different derivatives of isoxazolines for their possible immunomodulator activity were tested. Out of these, 9 compounds belonging to 5-butenyl and 5-methyl series exhibited varying degrees of immunomodulatory activities, whereas rest of the compounds have shown either moderate or no activity. Therefore these compounds were used for further evaluation and the results are summarized below.

1.4.2a. Results pertaining to evaluation of immunomodulatory activities of representative 3-aryl-5-substituted isoxazoline:

1.4.2b. Effect of test compounds on SRBC induced antibody synthesis in mice: -
Group of 10 mice was treated intraperitonially with SRBC followed by concomitant treatment of isoxazolines (0.001-10 mg/kg p.o) daily for fifteen days followed by haemagglutination antibody titre and out of 33 different isoxazoline derivatives studied, 5-butenylisoxazolines (1-7, Table-3), and 5-methylisoxazoline (8-14, Table-3) series exhibited potential immunomodulatory activities (Fig 1, 2, 3 & 4). Effect of these compounds on SRBC induced antibody titre (both IgM and IgG) in Balb/C mice clearly shows that 5-butenylisoxazolines exhibit dose dependent immunomodulatory effect and among all the compounds in 5-butenyl series, isoxazoline-5 showed highest activity at 1 mg/kg dose and hence this has been taken up for detailed evaluation. Similarly, in 5-methylisoxazoline series, isoxazoline-10 exhibited stronger immunostimulatory activity at lower doses in comparison with other test compounds. Isoxazoline-10 shows maximum activity at 0.01mg/kg body weight. This compound shows significant rise in secondary antibody production in comparison with primary antibody production. Thus isoxazoline-
10 exhibited highest immunostimulator effect and therefore, it has been chosen for detailed investigation.

1.4.2c. Effect of test compounds on SRBC induced delayed type hypersensitivity reaction (DTH) in Balb/C mice:

Delayed type Hypersensitivity reaction (DTH) to SRBC was induced in mice following a standard procedure by treatment of animals with a single dose of betamethasone. Mice were immunized on day 0 and challenged on day 7 with the same concentration of SRBC. Test compounds (0.001-10 mg/kg p.o) were administered following the same schedule as above. Out of 5 test compounds that were studied under 5-butenyl series, isoxazoline-5 shown promising immunostimulatory activity at 1mg/Kg dose and hence this has been taken up for detailed evaluation under different doses. Dose dependent effect of isoxazoline-5 at doses ranging from 0.001 to 10 mg/Kg (table 6) has clearly shown highest activity at 1mg/Kg (90.34%) as compared to betamethasone. Similarly in 5-methylisoxazoline series, isoxazoline-10 shows significant rise in delayed type hypersensitivity reaction at dose range of 0.01mg/kg (91.72 %, table 10).

1.4.2d. Effect of different doses of test compounds on surface markers (CD4+ & CD8+ and CD3 & CD19) by flow cytometry analysis:

Isoxazoline-5 and isoxazoline-10 were administered orally at dose ranges (0.01-1mg/kg) and (0.0001-0.01 mg/kg) respectively for 15 days. Among all the compounds, isoxazoline-5 and isoxazoline-10 showed significant rise in CD4+ and CD8+ population.

1.4.2e. Effect of isoxazoline-5 and isoxazoline-10 on spleen T cells subtypes CD4+ and CD8+:

Isoxazoline-5 showed effect of 35.61 ± 0.2 % (1mg/kg p.o); 44.25 ± 0.1% (0.01mg/kg p.o) of CD4+ and 10.96 ± 0.2% (1mg/kg p.o); 22.56 ± 0.3 (dose 0.01mg/kg p.o) CD8+ T cells whereas isoxazoline-10 shown effect of 31.95 ±0.1 % (dose 0.1mg/kg p.o); 20.84 ± 0.2 % (0.001mg/kg p.o) of CD4+; and 11.22 ± 0.3 (dose 0.1mg/kg p.o); 11.85 ± 0.4 (dose 0.001mg/kg p.o) of CD8+ T cells respectively. This shows a significant increase in CD4+ and CD8+ T cell count (Table 7, 8). Levamisole, a standard T cell stimulator at 2.5 mg/kg oral dose stimulated both CD4+ and CD8+ T cells showed 31.8±1.30% of CD4+ and
19.29±0.53% of CD8+ T cells. Out of two compounds, isoxazoline-10 showed more prominent activity at lower doses (0.01mg/kg). So, isoxazoline-10 was subjected for detailed investigation.

1.4.2f. Effect of isoxazoline-10 on spleen T cells subtypes CD3 and CD19:
Isoxazoline-10 at 0.01 mg/kg dose showed drastic increase in CD3 (27.84%) and CD19 (38.63%, Table-9) in comparison with control. This shows isoxazoline-10 to have sustained immunostimulatory effect on both CD4+ and CD8+ T cells at higher doses.

1.4.2g. Effect of different doses of Isoxazoline-10 on cytokine release (IL-10, IL-4 & IFN-γ) by flow cytometry analysis:
In order to understand the specific effects of isoxazoline-10 on cytokine profiles, a characteristic TH1 cytokine, IFN-γ and TH2 cytokine IL-4, IL-10 were analysed by flowcytometry. Isoxazoline-10 showed a drastic increase in levels of cytokines like IFN-γ, IL-4 and IL-10 at dose range of 0.01mg/kg in comparison with control (Table 11).
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Fig: 1. Effect of test compounds (Isoxazoline-2, 3, 4, 5 & 6) on SRBC induced antibody synthesis in Balb/C mice.

![Graph showing HA titre (Mean ± SE) for different doses mg/kg of test compounds.](image)

Fig: 2. Effect of test compounds (Isoxazoline-2, 3, 4, 5 & 6) on SRBC induced delayed type hypersensitivity (DTH) reaction in Balb/C mice.

![Graph showing DTH response Mean ± S.E. for different doses mg/kg of test compounds.](image)
Chapter I

Fig: 3. Effect of test compounds (Isoxazoline-9, 10, 11 & 12) on SRBC induced antibody synthesis in Balb/C mice

Fig: 4. Effect of test compounds (Isoxazoline-9, 10, 11 & 12) on SRBC induced delayed type hypersensitivity (DTH) reaction in Balb/C mice.

Results are expressed as Mean ± S.E. Significant difference from control by student t-test *P< 0.05; ** P< 0.01; *** P<0.001
Out of 9 test compounds under butenyl and methyl-isoxazoline series, isoxazoline-5 and isoxazoline-10 exhibited promising immunostimulatory activity at 1mg/kg and 0.01mg/kg respectively.

### Table 4: Effect of compounds on SRBC induced antibody synthesis in Balb/C mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses mg/kg</th>
<th>Primary antibody (IgM) titer</th>
<th>% Stimulation/suppression</th>
<th>Secondary antibody (IgG) titer</th>
<th>% Stimulation/suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SRBC</td>
<td>5.1 ± 0.33</td>
<td></td>
<td></td>
<td>5.6 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>2.50</td>
<td>7.6 ± 0.22*</td>
<td>49.01†</td>
<td>8.2 ± 0.33</td>
<td>46.42†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>0.001</td>
<td>6.3 ± 0.20</td>
<td>23.52†</td>
<td>6.7 ± 0.25**</td>
<td>19.64†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>0.01</td>
<td>6.8 ± 0.11</td>
<td>33.33†</td>
<td>7.3 ± 0.25</td>
<td>30.35†</td>
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<tr>
<td>Isoxazoline-5</td>
<td>0.1</td>
<td>7.3 ± 0.22*</td>
<td>4.13†</td>
<td>8.7 ± 0.11**</td>
<td>55.35†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>1</td>
<td>9.1 ± 0.57**</td>
<td>78.43†</td>
<td>9.3 ± 0.11</td>
<td>66.07†</td>
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<tr>
<td>Isoxazoline-5</td>
<td>3</td>
<td>8.3 ± 0.11</td>
<td>62.74†</td>
<td>8.3 ± 0.11</td>
<td>48.21†</td>
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<tr>
<td>Isoxazoline-5</td>
<td>10</td>
<td>8.0 ± 0.25**</td>
<td>56.86†</td>
<td>6.6 ± 0.20</td>
<td>17.85†</td>
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</tbody>
</table>

### Table 5: Effect of compound Isoxazoline-5 on SRBC induced delayed type hypersensitivity (DTH) reaction in Balb/C mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses mg/kg</th>
<th>DTH Response</th>
<th>% Change compared to betamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.79 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>0.01mg/kg</td>
<td>1.45 ± 0.040</td>
<td></td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>0.001</td>
<td>1.98 ± 0.02</td>
<td>36.55†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>0.01</td>
<td>2.38 ± 0.02*</td>
<td>64.13†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>0.1</td>
<td>2.53 ± 0.04***</td>
<td>74.48†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>1</td>
<td>2.76 ± 0.03**</td>
<td>90.34†</td>
</tr>
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<td>Isoxazoline-5</td>
<td>3</td>
<td>2.4 ± 0.11*</td>
<td>65.51†</td>
</tr>
<tr>
<td>Isoxazoline-5</td>
<td>10</td>
<td>2.1 ± 0.03</td>
<td>44.82†</td>
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</tbody>
</table>

### Table 6: Effect of different doses of Isoxazoline-5 on spleen T cell subtypes

<table>
<thead>
<tr>
<th>Treatment group (n)</th>
<th>CD4+ T-Cell(%)</th>
<th>CD8+ T-Cell(%)</th>
<th>CD4+/CD8+ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle)</td>
<td>28.21±0.1</td>
<td>12.02±0.2</td>
<td>2.34±0.07</td>
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<tr>
<td>Isoxazoline-5 (0.01mg/kg)</td>
<td>44.25±0.1**</td>
<td>22.56±0.3*</td>
<td>1.96±0.06</td>
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<tr>
<td>Isoxazoline-5 (0.1mg/kg)</td>
<td>35.26±0.6*</td>
<td>15.4±0.3</td>
<td>2.28±0.02</td>
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<tr>
<td>Isoxazoline-5 (1mg/kg)</td>
<td>35.61±0.2**</td>
<td>10.96±0.2</td>
<td>2.55±0.03</td>
</tr>
<tr>
<td>Levamisole</td>
<td>31.8±1.30</td>
<td>19.29±0.53**</td>
<td>1.68±0.06</td>
</tr>
</tbody>
</table>
### Table 7: Effect of different doses of Isoxazoline-10 on spleen T cell subtypes

<table>
<thead>
<tr>
<th>Treatment group (n)</th>
<th>CD4+ T-Cell (%)</th>
<th>CD8+ T-Cell (%)</th>
<th>CD4+/CD8+ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle)</td>
<td>25.1±0.3</td>
<td>16.29±0.2</td>
<td>1.54±0.02</td>
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<tr>
<td>Isoxazoline-10 (0.001 mg/kg)</td>
<td>20.84±0.2**</td>
<td>11.85±0.4</td>
<td>1.75±0.08*</td>
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<tr>
<td>Isoxazoline-10 (0.01 mg/kg)</td>
<td>34.4±0.2*</td>
<td>11.9±0.1**</td>
<td>2.89±0.04**</td>
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<tr>
<td>Isoxazoline-10 (0.1 mg/kg)</td>
<td>31.95±0.1**</td>
<td>11.22±0.3</td>
<td>2.84±0.1*</td>
</tr>
<tr>
<td>Levamisole</td>
<td>31.8±1.30</td>
<td>19.29±0.53**</td>
<td>1.68±0.06</td>
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</table>

### Table 8: Effect of Isoxazoline-10 on SRBC induced antibody synthesis in Balb/C mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (mg/kg)</th>
<th>Primary antibody (IgM) titer</th>
<th>% Stimulation/suppression</th>
<th>Secondary antibody (IgG) titer</th>
<th>% Stimulation/Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control SRBC</td>
<td>5.1±0.33</td>
<td>6.4±0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levamisole</td>
<td>2.50</td>
<td>7.6±0.21*</td>
<td>49.01↑</td>
<td>9.6±0.33</td>
<td>50.0↑</td>
</tr>
<tr>
<td>BMS</td>
<td>0.05</td>
<td>4.8±0.25**</td>
<td>18.45↓</td>
<td>3.8±0.21</td>
<td>40.62↓</td>
</tr>
<tr>
<td>Isoxazoline-10</td>
<td>0.001</td>
<td>8.1±0.11</td>
<td>58.82↑</td>
<td>9.8±0.21*</td>
<td>53.12↑</td>
</tr>
<tr>
<td>Isoxazoline-10</td>
<td>0.01</td>
<td>9.0±0.21*</td>
<td>76.47↑</td>
<td>11.2±0.33</td>
<td>75.0↑</td>
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<tr>
<td>Levamisole</td>
<td>0.1</td>
<td>8.5±0.41*</td>
<td>66.66↑</td>
<td>10.4±0.33</td>
<td>62.5↑</td>
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<td>Isoxazoline-10</td>
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<td>8.2±0.16</td>
<td>60.78↑</td>
<td>9.2±0.25**</td>
<td>43.75↑</td>
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</tbody>
</table>

### Table 9: Effect of compounds Isoxazoline-10 on SRBC induced delayed type hypersensitivty (DTH) reaction in Balb/C mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Doses (mg/kg)</th>
<th>DTH_Response %</th>
<th>% Change compared to betamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.79±0.02</td>
<td></td>
</tr>
<tr>
<td>Betamethasone</td>
<td>0.01</td>
<td>1.45±0.04*</td>
<td>37.93↑</td>
</tr>
<tr>
<td>Isoxazoline-10</td>
<td>0.001</td>
<td>2.0±0.02</td>
<td>37.93↑</td>
</tr>
<tr>
<td>Isoxazoline-10</td>
<td>0.01</td>
<td>2.78±0.02**</td>
<td>91.72↑</td>
</tr>
<tr>
<td>Isoxazoline-10</td>
<td>0.1</td>
<td>2.43±0.02***</td>
<td>67.58↑</td>
</tr>
<tr>
<td>Isoxazoline-10</td>
<td>1</td>
<td>2.14±0.03**</td>
<td>47.58↑</td>
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</table>

### Table 10: Effect of different doses of Isoxazoline-10 on cytokine release (IL-10, IL-4 & IFN-γ) by flow cytometry analysis

<table>
<thead>
<tr>
<th>Treatment group (n) (mg/kg)</th>
<th>IL-10 Population (Mean ± S.E.)</th>
<th>IL-4 Population (Mean ± S.E.)</th>
<th>IFN-γ Population (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle)</td>
<td>6.1±0.13</td>
<td>9.78±0.4</td>
<td>1.03±0.12</td>
</tr>
<tr>
<td>Isoxazoline-10 (0.0001)</td>
<td>12.17±0.3*</td>
<td>15.38±0.18</td>
<td>2.32±0.18</td>
</tr>
<tr>
<td>Isoxazoline-10 (0.001)</td>
<td>13.6±0.76</td>
<td>17.6±0.53**</td>
<td>2.37±0.22</td>
</tr>
<tr>
<td>Isoxazoline-10 (0.01)</td>
<td>16.53±0.88</td>
<td>18.67±0.33</td>
<td>2.98±0.12**</td>
</tr>
<tr>
<td>Levamisole</td>
<td>0.68±0.16**</td>
<td>12.98±0.29</td>
<td>2.54±0.3*</td>
</tr>
<tr>
<td>BMS (0.05)</td>
<td>5.13±0.36</td>
<td>3.9±0.29</td>
<td>0.9±0.02</td>
</tr>
</tbody>
</table>
Table 11. Effect on of different doses of Isoxazoline-10 on surface markers (CD 3 & CD19) by flow cytometry analysis

<table>
<thead>
<tr>
<th>Treatment group (n) (mg/kg)</th>
<th>CD3 population (Mean ± S.E.)</th>
<th>% Change compared to control</th>
<th>CD19 population (Mean ± S.E.)</th>
<th>% Change compared to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle)</td>
<td>38.86 ± 0.01</td>
<td></td>
<td>32.33 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Isoxazoline-10 (0.0001)</td>
<td>39.81± 0.98</td>
<td>2.44↑</td>
<td>35.18 ± 0.5</td>
<td>8.8↑</td>
</tr>
<tr>
<td>Isoxazoline-10 (0.001)</td>
<td>44.51± 0.3*</td>
<td>14.53↑</td>
<td>38.23± 0.53**</td>
<td>18.24↑</td>
</tr>
<tr>
<td>Isoxazoline-10 (0.01)</td>
<td>49.68 ±0.85</td>
<td>27.84↑</td>
<td>44.82± 0.16**</td>
<td>38.63↑</td>
</tr>
<tr>
<td>Levamisole</td>
<td>45.2 ±0.16**</td>
<td>16.31↑</td>
<td>41.6 ± 0.4</td>
<td>28.67↑</td>
</tr>
<tr>
<td>BMS (0.05)</td>
<td>22.81 ± 0.4</td>
<td>41.30↓</td>
<td>31.6 ± 1.42</td>
<td>2.2↓</td>
</tr>
</tbody>
</table>

In each table mentioned above the experimental parameters taken were same and are described below:
Number of observations = 10
Values are mean ± S.E of ten mice.
Significant difference from control by student t-test; *P< 0.05; **P< 0.01

1.4.3. Discussion:

A number of assays were used to investigate the immunomodulating effect of isoxazoline derivatives on different immune cells. The results of preliminary assays and structures of these compounds indicated that isoxazolines with a suitably substituted aryl group and an alkyl group such as methyl or butenyl chain on the 5th position of the ring possess immunomodulatory (potentiating) activity. Possible structural feature which primarily influences the immunomodulatory activity being the nature of alkyl group on the isoxazoline ring and thus the presence of a butenyl chain or methyl substitution together with appropriately functionalized aromatic ring at the 3rd position of isoxazoline ring seems to impart immune-enhancing activity to the molecule. Other alkyl substituents like vinyl, carboxylate, nitrile, aryl or carbinol groups resulted in complete loss of activity. Similarly, a simple substitution of one of the hydrogen of 5-methyl group with halogen atom such as bromine resulted in loss of activity. Among the butenyl series isoxazoline-5 exhibited highest activity in terms of both primary and secondary antibody titers and DTH response in Balb/C mice and hence the compound was chosen among the series for further studies. The observed enhancement in CD4+ values for this compound clearly indicate the immunogenic response through MHC-class II pathway that leads to antibody
response. Similarly among the 5-methylisoxazolines under scrutiny, which have shown immune stimulatory effect in Balb/C mice, isoxazoline-10 have shown highest immune potentiating activity and thus it has been chosen for detailed investigation. Here again the observed CD4+, CD8+ values were in conformity with the antibody response, which qualifies the isoxazoline-5 and isoxazoline-10 as the highest active molecules among all those, studied here. The varying degree of activity among the 5-butenyl or 5-methylisoxazoline series may be attributed to the further functionalization of aromatic ring and the delicate balance between the optimum substitution pattern of both 5th position of isoxazoline ring and the substitution on the aromatic ring at the 3rd position of the isoxazoline ring, decides the final activity of the molecule as such substitution patterns may facilitate or restrict the molecule to interact with cells

1.4.4. Conclusion:
The results presented in this section indicate that isoxazolines with appropriate alkyl substitution at 5th position acts as a potential immunomodulators when properly tuned with the substitution pattern on the aromatic ring at the 3rd position. 5-butenyl and 5-methylisoxazolines exhibit dose dependent immunomodulatory effect, generally acting as immune-enhancers. However, further mechanism-based studies are required for a better understanding of the mode of action of isoxazolines on immune system.
1.4.5. Experimental Section:

The typical procedure for the synthesis of 5-butenyl, 5-methyl and 5-vinyl isoxazolines is already discussed in the previous sections (section B and C) of this chapter where as rest of the isoxazolines were prepared through 1,3-dipolar cycloaddition of nitrile oxides to various monosubstituted dipolarophiles according to literature procedure\(^{15}\) and were characterized by IR, \(^1\)H, \(^13\)C-NMR, mass and also by the comparison on their physical characteristics with literature data for authentic compounds. The melting points of these simple isoxazolines are given below.

- **3-(2,6-Dichlorophenyl) isoxazoline-4-carboxylic acid methylester.** m.p 72-74°C.\(^{27}\)
- **3-(2,6-Dichlorophenyl)-5-phenyl isoxazoline.** m.p 63-65°C.\(^{27}\)
- **3,5-Diphenyl isoxazoline.** m.p 74-75°C.\(^{27}\)
- **5-Butoxy-3-phenyl isoxazoline.** Semi-solid.\(^{27}\)
- **5-(1-Chloroethyl)-3-phenyl isoxazoline.** Semi-solid.\(^{27}\)
- **5-(Bromomethyl)-3-phenyl isoxazoline.** m.p 182-185°C.\(^{27}\)
- **3-Phenyl isoxazoline -5-yl acetate.** m.p 89.5-91°C.\(^{27}\)
- **3-(4-Methoxyphenyl) isoxazoline -5-yl acetate.** m.p 140-142°C.\(^{25}\)
- **3-(4-Nitrophenyl) isoxazoline -5-yl acetate.** m.p 149.5°C.\(^{25}\)
- **Methyl 3-phenylisoxazoline -5-carboxylate.** m.p 67-68.5°C.\(^{27}\)
- **Methyl 3-(4-methylphenyl)isoxazoline -5-carboxylate.** m.p 44.5-47°C.\(^{27}\)
- **Methyl 3-(4-methoxyphenyl) isoxazoline -5-carboxylate.** m.p 74-76.5°C.\(^{27}\)
- **Methyl 3-(3-nitrophenyl) isoxazoline -5-carboxylate.** m.p 88-90°C.\(^{27}\)
- **Allyl 3-phenylisoxazoline -5-carboxylate.** Brown liquid.\(^{27}\)
- **trans-5-Hydroxymethyl-3,4-diphenylisoxazoline.** semi-solid.\(^{27}\)
- **trans-4-Hydroxymethyl-3,5-diphenylisoxazoline.** semi-solid.\(^{27}\)
- **5-Hydroxymethyl-3-phenyl-4,5-dihydroisoxazole.** colorless liquid.\(^{27}\)

**Animals:**

Study was conducted on male Balb/c mice (18-22 g). The Ethical committee of the Regional Research Laboratory (CSIR) instituted for animal handling approved all...
protocols. The animals were bred and maintained under standard laboratory conditions: temperature (25±2 °C) and photoperiod of 12 h. Commercial pellet diet and water were given ad libitum.

**Effect on antibody production:**

Groups of 10 mice each were immunized by injecting intraperitoneally with 0.2 ml of 5 x 10^9 SRBC, respectively on day 0. Blood samples were collected from individual animals by retro-orbital puncture on day 7. Antibody levels were determined by the haemagglutination technique (Nelson and Mildenhall, 1967). To two-fold dilutions of serum samples made in 25 μl volumes of normal saline containing 0.1% bovine serum albumin (BSA saline) in V bottomed Takasty microtitration plates were added 25 μl of a 0.1% suspension of SRBC in BSA saline. After thorough mixing, the erythrocytes were allowed to settle at room temperature for 60-90 min until control wells showed an equivocally negative pattern (a small button). The value of the highest serum dilution causing visible haemagglutination was taken as the antibody titre and the mean value of the titre was calculated.

**Effect on DTH reaction:**

The effect of isoxazoline-5 (0.001-10 mg/kg) and isoxazoline-10 (0.001-1 mg/kg) on development of DTH reaction to sheep erythrocytes (SRBC) was studied in Balb/C mice (18-22g) following the method of Doherty. Groups of ten mice each were immunized by injecting 20 μl of 5 x 10^9 SRBC subcutaneously (s.c.) into the right hind footpad. The day of sensitization was designated as day 0. Seven days later the animals were challenged by injecting the same amount of SRBC intradermally (i.d.) into the left hind footpad. The thickness of the left hind footpad was measured at 0 and 24 h after the challenge. The difference between two values was taken as the measure of DTH and mean percent change in foot thickness as compared to control group was determined.

**Lymphocyte immunophenotyping:**

The spleen (1/3 of the organ) was placed in PBS buffer (without Mg2+ and Ca2+) stored on ice prior to preparation of single cell suspensions. Splenic erythrocytes were lysed with red blood cell lysing buffer (BD phar ming en). Cell suspensions were refrigerated at
4 °C pending staining with antibodies. All reagents were purchased at BD pharmingen. For each sample, 2x 10^6 cells were stained with conjugated CD3, CD4+, CD8+ and CD19 antibodies. After staining with antibodies, cells were washed and resuspended in PBS for flow cytometric analysis, which was performed on a FACS Calibur flow cytometer equipped with Cell Quest software (Becton Dickinson).\textsuperscript{30}

**Study design for IFN-γ (Th1), IL-4 and IL-10 (Th2) cytokine estimation:**

Mice were immunized on day 0 by injecting 200 µl of SRBC (5x10^9 cells/ml) intraperitoneally and again challenged on day 7. Test material was administered for 15 days including the day of immunization. The blood was collected in heparinised tubes from retro orbital plexes to determine the effect of test material on IFN-γ, IL-4 and IL-10 (intracellular). In this study fluorescein isothiocyanate (FITC) labelled antimouse IFN-γ monoclonal antibody and phycoerythrin (PE) labeled IL-4 and IL-10 monoclonal antibodies were used. The fixed cells were then analyzed within 24 h in a flowcytometric analyzer using Cell Quest Pro software (BD Biosciences).\textsuperscript{31}

**Statistical analysis:**

All the data are presented as means ± SE. Statistical analysis for all the results was compared using Student’s t-test.

**Toxicity studies:**

The test compounds were tested for possible cellular toxic effects on spleen cells by MTT assay\textsuperscript{31} and the results indicate that the test compounds (as high as 100µg/ml) did not exhibit any toxic effect after 72 hrs incubation.
References
1.4.6. References:


18. All nitrile oxides were prepared as per the literature procedures and unstable nitrile oxides were used immediately without purification. Grundmann, C.; Dean, J. M.; Angew. Chem. 1964, 76, 682.


Chapter I


Chapter I


$^1$H NMR OF COMPOUND 4a
$^{13}$C NMR OF COMPOUND 4a
IR SPECTRUM OF COMPOUND 4a
MASS SPECTRUM OF COMPOUND 4a
$^1$H NMR OF COMPOUND 6a
$^{13}$C NMR OF COMPOUND 6a
The image shows an IR spectrum graph labeled "IR SPECTRUM OF COMPOUND 6a." The graph plots wavenumber cm⁻¹ on the y-axis ranging from 600 to 4000 and transmittance (%) on the x-axis ranging from 0 to 100. The spectrum includes various peaks and troughs indicating specific infrared absorption bands. The chemical structure of compound 6a is also depicted on the graph.
MASS SPECTRUM OF COMPOUND 6a
$^{13}$C NMR OF COMPOUND 5
DEPT SPECTRUM OF COMPOUND 5
IR SPECTRUM OF COMPOUND 5
1H NMR of Compound 8a
$^{13}$C NMR OF COMPOUND 8a
DEPT SPECTRUM OF COMPOUND 8a
IR SPECTRUM OF COMPOUND 8a
MASS SPECTRUM OF COMPOUND 8a