CHAPTER – II

Chemistry of Isoxazolines
CHAPTER – II

CHEMISTRY OF ISOXAZOLINES

2.1.0 Introduction:

Nitrogen containing heterocycles with an oxygen atom are considered as an important class of compounds in medicinal chemistry because of their diversified biological applications. The exploitation of a simple molecule with different functionalities for the synthesis of heterocycles is a worthwhile contribution in the chemistry of heterocycles. Isoxazole (42a) is a five membered heterocyclic compound containing oxygen and nitrogen atoms in the 1,2 positions, its partially saturated analogs are called isoxazolines (42b-d) and completely saturated analog is isoxazolidine (42e).

Isoxazoles have illustrious history; their chemistry is associated with Ludwig Claisen, who first recognized the cyclic structure of 3-methyl-5-phenylisoxazole in 1888 and was shown to possess typical properties of an aromatic system under certain reaction conditions; particularly in basic media, it is very highly labile. Dunstan and Dymond were the first to synthesize the isoxazole ring; they isolated a liquid base by heating nitro ethane with aqueous alkalies to obtain 3,4,5-trimethylisoxazole. A very significant contribution to the development of isoxazole chemistry came between 1930–1946 from Quilico’s studies on the synthesis of ring system from nitrile oxides and unsaturated compounds.

Isoxazoles are an important class of heterocycles, which are largely employed in the area of pharmaceuticals and therapeutics such as insecticidal, antibacterial,
antibiotic, antitumour, antifungal, antituberculosis, anticancer and ulcerogenic. Isoxazole derivatives are used in the market as COX-2 inhibitor and anti-inflammatory drugs. Isoxazole derivatives such as sulfamethoxazole, sulfisoxazole, oxacillin, cycloserine and acivicin have been in commercial use for many years. Cycloserine is the best known antibiotic drug that possess antitubercular, antibacterial activities and in treatment of leprosy. Acivicin is an antitumour, antileishmania drug, while isoxaflutole is used as herbicidal drug.

2.1.1 Synthesis of functionalized isoxazoles using various synthetic approaches:

Diverse applications associated with isoxazole moiety led the researchers to develop various novel synthetic approaches for the synthesis of isoxazole ring systems. Oximes on treatment with PhI(OCOCF₃)₂ (hypervalent iodine) leads to rapid formation of nitrile oxides which were trapped in situ with terminal and cyclic alkynes efficiently to give 3,5-disubstituted and 3,4,5-trisubstituted isoxazoles in high yield (Scheme-48). The procedure is experimentally convenient, avoids the isolation and handling of potentially harmful and unstable hydroximoyl chlorides.

Sandeep Bhosale et al synthesized isoxazoles and isoxazolines via 1,3-dipolar cycloaddition of alkenes and alkynes with nitrile oxides generated in situ by treatment of aldoximes with magtrieve (CrO₂) in either toluene or MeCN at 80°C (Scheme-49). They observed the formation of minor amount of deoximation product.
along with isoxazoles and isoxazolines. Their methodology has been shown to be equally versatile for intramolecular nitrile oxide cycloaddition reactions.

Shravankumar et al.\textsuperscript{38} reported a facile catalytic approach to synthesize regioselectively 3,5-di- and 3,4,5-trisubstituted isoxazoles in high yields involving the nucleophilic organo-N-heterocyclic carbene-catalyzed 1,3-dipolar cycloaddition of nitrile oxide with alkynes. Triethylamine (Et$_3$N) was employed as an effective base to generate both nitrile oxide and the organo-N-heterocyclic carbene catalyst \textit{in situ}. The multi-nucleus structures like isoindole linked disubstituted isoxazoles and sterically crowded trisubstituted isoxazoles can be accessed selectively by this method, which could be useful in biology and material science.

Nagatoshi et al.\textsuperscript{119} reported one-step synthesis of different functionalized isoxazoles by cycloaddition of carbamoylnitrile oxide with $\beta$-keto esters. Among several salts, magnesium acetate was found to be the most efficient promoter affording isoxazole in 80\% yield (Scheme-50). Carbamoylnitrile oxide generated from nitroisoxazolone underwent inverse electron-demand 1,3-dipolar cycloaddition with 1,3-dicarbonyl compounds in the presence of magnesium acetate that formed magnesium enolate \textit{in situ}.
Bhaskar Chakraborty and co-workers\textsuperscript{120} reported the synthesis and antibacterial activities of some novel isoxazolidine derivatives by 1,3-dipolar cycloaddition reaction of nitrones with different dipolarophiles in aqueous phase. Significant rate acceleration and high yield of these reactions are observed in water with remarkable changes in stereo and regioselectivity compared to organic solvents. They have provided a green synthesis avoiding use of organic solvents. Stokes and co-workers\textsuperscript{121} reported that iron (II) catalyzes the formation of N-O bonds to transform azides into 2,1-benzisoxazoles under markedly benign conditions (Scheme-51).

\[
\begin{align*}
\text{R} & \quad \text{R}' \quad \text{R}'' \\
\text{N}_3 \quad & \quad \text{FeBr}_2 \quad (5\text{ mol}%) \\
& \quad 4\text{A}^0\text{MS}, 40^\circ\text{C}
\end{align*}
\]

R=R=H, Cl R'=H, Me, OMe R''=Ar, alkyl

Highly substituted isoxazoles can be formed in good to excellent yields using mild reaction conditions. For instance, 3,5-disubstituted 4-halo (seleno) isoxazoles have been synthesized by the reaction of various 2-alkyn-1-one O-methyl oximes with ICl, I\textsubscript{2}, Br\textsubscript{2} or PhSeBr (Scheme-52).\textsuperscript{122}

\[
\begin{align*}
\text{R} & \quad \text{R}' \quad \text{R}'' \\
\text{N} & \quad \text{NH}_2\text{OMe.HCl} \\
& \quad \text{E-X}
\end{align*}
\]

E=I\textsubscript{2}, ICl, Br\textsubscript{2}, PhSeBr

\textit{N}-\textit{(4-(5-Arylisoxazol-3-yl) phenyl)-benzenesulfonamides were synthesized under conventional heating and microwave irradiation (Scheme-53).}\textsuperscript{123} The method was found to be fast, efficient and economical. The reaction proceeded smoothly with better yields under MW irradiation within 5-6 minutes; while under reflux conditions it required 6-8 h.
4-Arylidene-3-phenylisoxazol-5-ones were synthesized by three-component condensation of ethyl benzoyleacetate, hydroxylamine and aromatic aldehydes in ethanol using DABCO as base under reflux condition (Scheme-54).\textsuperscript{124} It was observed that good yields were obtained with faster reaction rate with the aldehydes bearing electron-donating groups when compared to aldehydes with electron-withdrawing groups.

Scott et al\textsuperscript{125} developed sequential \([3 + 2]\)-cycloaddition and cross-coupling reactions for the preparation of 3,4,5-trisubstituted isoxazoles. The reaction between alkynyl dimethyl silyl ethers and aryl and alkyl nitrile oxides produce isoxazolylsilanols. The cross-coupling reactions of these heterocyclic silanols with a variety of aryl iodides afford 3,4,5-trisubstituted isoxazoles (Scheme-55). Both alkyl and aryl substituents at the 3- and 5-positions of the isoxazole were selectively incorporated based on the choices of the dipole and dipolarophile. In the development of the cross-coupling reaction conditions, the use of Cu(OAc)\textsubscript{2} effected the rate of the cross-coupling reaction; however, in some cases, Cu(OAc)\textsubscript{2} also promoted the protodesilylation of the silanol.
Fluorinated isoxazolines were synthesized in one pot by the reaction of fluorinated chalcones and hydroxylamine in acetic acid medium under reflux conditions (Scheme-56). The products have been evaluated for their antibacterial activities. By introducing fluorine atom into specific position of organic molecule may cause significant changes in the stability, lipophilicity and biological activities of the resulting molecules. This is due to the high electro negativity of the halogen, the strong C-F bond and the similar size of the halogen and hydrogen atoms.

Amar Saad et al reported a simple one step regioselective synthesis of 5-aminoisoxazoles in toluene using a 1,3-dipolar cycloaddition reaction between nitrile oxides and captodative $\alpha$-cyanoenamines (Scheme-57). It is a very efficient and simple method for the preparation of 5-aminoisoxazoles.

The regioselective synthesis of 4-substituted isoxazoles from terminal alkynes and 3,4-disubstituted isoxazoles from internal alkynes using one-pot titanium catalyzed 3-component coupling reaction in conjunction with hydroxylamine hydrochloride addition (Scheme-58) was reported. The products are easily isolated in pure form after the one pot synthesis.
Ajay Kumar and co-workers\textsuperscript{129} reported the synthesis of series of isoxazoles starting from chalcones. The chalcones prepared by the reaction of aromatic aldehydes and acetophenone were converted to dibromo derivatives with bromine-acetic acid and then dibromo derivatives were treated with triethyl amine to get alkyne derivatives. The alkyne derivatives on 1,3-dipolar cycloaddition reaction with nitrile oxides generated \textit{in situ} from aldoximes afforded isoxazoles in good yield. The isoxazoles were screened and showed promising antimicrobial activities against different organisms.

\subsection*{2.1.2 Reactions of isoxazoles:}

Isoxazoles, isoxazolines and isoxazolidines were considered as useful synthons in organic synthesis. They have been efficiently transformed into various classes of medicinally important molecules. For instance, Anthracen-9-ylmethylene-(3,4-dimethylisoxazol-5-yl) amine was synthesized in high yield by reaction of anthracene-9-carbaldehyde and 5-amino-3,4-dimethylisoxazole in ethanol (Scheme-59).\textsuperscript{130}

Isoxazoloazepines were synthesized via Michael addition followed by reductive cyclization. For Michael addition, a convenient and highly efficient protocol was developed by using \textit{p-TsOH adsorbed on KSF solid support under solvent-free conditions with a variety of Michael donors and acceptors. P-TsOH-KSF solid}
support is found to be a much better alternative to effect the Michael reaction in terms of better yields (85%) and short reaction times (2 h). The Michael adducts underwent reductive cyclization on treatment with SnCl₂-MeOH to afford substituted isoxazolo [4, 5-b] azepines in high yields (Scheme-60).\textsuperscript{131}

Isoxazoloazepines are also synthesized by conducting Michael reaction in presence of PTSA adsorbed on KSF and the resulting Michael adducts are converted to isoxazoloazepines by reductive cyclization process with SnCl₂-MeOH in a one-pot reaction. This procedure offers significant improvement over the existing Michael reactions. All the reactions are clean, high yielding and the method is mild and tolerates several substituents on aromatic ring and devoid of forming any undesired side products.\textsuperscript{132}

3,4-Disubstituted isoxazole derivatives were synthesized from the reductive cleavages of 4,5-dihydro-7\textit{H}-pyrano [3,4-\textit{c}]isoxazoles. Pyrano[3,4-\textit{c}]isoxazole, upon treatment with TMSCl/NaI in acetonitrile undergoes selective C-O bond cleavage to furnish 3,4-disubstituted isoxazole in high yield without damaging the isoxazole ring whereas furo[3,4-\textit{c}]isoxazoles with TMSCl/NaI in acetonitrile results in the formation of a reduced iodide and a hydroxyl iodide (Scheme-61).\textsuperscript{133}
2.1.3 Biological activity of substituted isoxazoles:

A series of dialkyl 1,4-dihydro-2,6-dimethyl-4-(5-phenylisoxazol-3-yl)pyridine-3,5-dicarboxylates (43) synthesized have studied for in vitro calcium channel antagonist activities. In vitro calcium channel antagonist activities (IC$_{50}$) were evaluated as the molar concentration of the test compounds required to produce 50% inhibition of the high K$^+$ concentration of guinea-pig ileum longitudinal smooth muscle (GPILSM) assay. These compounds exhibited moderate calcium channel antagonist activity (IC$_{50}$= 10$^{-7}$ to 10$^{-5}$ M range) relative to the reference drug nifedipine (IC$_{50}$ = 1.10 ± 0.40 × 10$^{-8}$ M).

Isoxazole series of glycoprotein IIb/IIIa antagonists were synthesized and their antiplatelet effects were studied. The replacement of the benzamide in XUO57 with an isoxazole carboxamide afforded XUO65 (44) which showed a significant improvement in the inhibition of platelet aggregation. The analogue XUO65 showed an excellent oral antiplatelet effect in dogs. Maximal inhibition of platelet aggregation was achieved and maintained for up to 5h after an oral dose of 1.6mg/kg.

A series of alkenyldiarylmetanes (ADAMs) with a benzo[d]isoxazole and oxazolidine-2-ones synthesized were evaluated for anti-HIV activities and metabolic
stabilities. The resulting ADAMs were found to inhibit HIV-1 RT with poly(rC)·oligo(dG) as the template primer. Among the series; methyl 5-((Z)-5-(methoxycarbonyl)-1-(3-methoxy-7-methylbenzo[d]isoxazole-5-yl)pent-1-enyl-2-methoxy-3-methylbenzoate (45)\textsuperscript{136} exhibited anti-HIV-1 activity with EC\textsubscript{50} values in the 20–40 nanomolar range.

5-Acetyl-3-aryl-4-(2-furanoyl)-4,5-dihydroisoxazoles (46)\textsuperscript{137} synthesized have been screened for their antibacterial and antifungal activity. Some compounds of the series exhibited promising antibacterial and antifungal activity compared to standard drugs. The substitution of fluoro, chloro, bromo and cyano group at C\textsubscript{3}-substituted benzene ring of isoxazole ring resulted with potent antimicrobial activities. The compounds also exhibited remarkable antioxidant activity and reducing power ability.

Substituted isoxazole (47)\textsuperscript{138} which was originally designed and characterized as ATP competitive p\textsuperscript{38a} mitogen activated protein kinase (MAPK) inhibitors, revealed significant inhibition of casein kinase 1\textdelta (CK1\textdelta) (90% inhibition) in a panel of 78 protein kinases at a concentration of 10\mu M and also inhibited CK1\textdelta with an IC\textsubscript{50} value of 0.23\mu M. Novel N-(phenyl)-5-carboxamidyl isoxazoles synthesized were examined for their anticancer activity in vitro. N-(4-Chlorophenyl)-5-carboxamidyl isoxazole (48)\textsuperscript{139} was reported to exhibit promising in vitro cytotoxicity and solid tumor selectivity. It exerted most potent cytotoxic activity against both colon-38 and
CT-26 mouse colon cancer cell lines. It inhibited the phosphorylation of STAT3, a novel target for chemotherapeutic drugs.

A series of 2-(5-phenyl-4,5-dihydroisoxazol-3-yl)benzoic acids (49) synthesized were evaluated for their in vitro protein denaturation activity. The results of the study showed that all these compounds possess significant anti-arthritic and anti-inflammatory action.

The effects of curcumin and of its isoxazole analogue MR 39 (50) in the MCF-7 breast cancer cell line and in its multidrug-resistant (MDR) variant MCF-7R were examined. The isoxazole analogue (MR 39) has shown more potent antitumor and molecular activities both in parental and in MDR tumor cells. MR 39 produces significantly higher direct inhibition of the COX-2 catalytic activity than curcumin.

Curcumin-derived isoxazoles (51, 52) synthesized which minimize the metal chelation properties of curcumin. Replacement of the 1,3-dicarbonyl moiety with isosteric heterocycles turned curcumin analogue isoxazoles into potent ligands of fibrillar Aβ42 aggregates. Curcumin-derived isoxazoles inhibit Aβ secretion, bind to or inhibit the formation of fibrillar Aβ42 and tau aggregates. The enhancement in potency in comparison with curcumin is 10-100-fold. It is apparent from these data that
curcumin-derived isoxazoles have multiple targets in Alzheimer’s disease. The multifunctional curcumin–isoxazole (51) displayed interesting properties as an α,β-modulating agent in primary neuronal cultures.

![Chemical Structure of 51](image)

[(Biphenyloxy)propyl] isoxazoles (53)\(^{143}\) derivatives of pleconaril with various substituents and substitution patterns at the terminal benzene ring where the oxadiazole ring of pleconaril has been replaced with a substituted phenyl ring synthesized were showed excellent anti-HRV-2 and moderate anticoxsackievirus B3 activity. The antiviral activity of these novel analogues has been determined against pleconaril-resistant as well as pleconaril-susceptible CVB3, HRV-2 and HRV-14. Results indicate that these derivatives are potential inhibitors of HRV-2 and CVB3 replication. These biphenyl analogues offer the opportunity for the development of highly selective anti-rhinovirus agents.

![Chemical Structure of 53](image)

\((R,S)\)-2-Amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid) AMPA (54)\(^{144}\) and \((R,S)\)-2-Amino-3-[5-tert-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid) ATPO (55)\(^{144}\) were tested for receptor antagonist at recombinant ionotropic glutamate receptors (GluRs) using electrophysiological techniques. The pharmacology of their AMPA receptor antagonist ATPO was described by comparing effects of ATPO on currents through homo- and
heterooligomeric AMPA- and KA-preferring GluRs expressed in *Xenopus laevis* oocytes and mammalian cell lines.

A series of 4-(5′-substituted-aryl-4′,5′-dihydro-isoxazole-3′-yl-amino) phenols (56) synthesized were investigated for their analgesic and antimicrobial activities. The purpose of the study was to examine whether molecular modification might result in detection of new potential antimicrobial and analgesic drugs. The substitution which appeared to be most important for high order of activity in the greatest number of test was the *p*-chloroaryl group. The substitution of *p*-nitrophenyl and *p*-hydroxyphenyl group at 5 position of isoxazole ring resulted with potent analgesic and antimicrobial activities.

Isoxazoline (57) and isoxazole derivatives (58) synthesized and biologically evaluated in order to find antagonists of 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptors, which are known as good targets for the improved treatment of depression. In particular, an isoxazoline with *o*-Me group as an R\textsubscript{2} substituent and 3,5-dichloro substituent at R\textsubscript{1} shows the most potent binding affinities to both 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C}, of which IC\textsubscript{50} values were 72 nM and 150 nM, respectively.
2.2.0 Aim of present work:

The substituted isoxazole derivatives have considerable chemical and pharmacological importance because of a broad range of biological activities displayed by these classes of molecules. The tremendous biological potential of substituted isoxazole derivatives encouraged us to synthesize new substituted isoxazole derivatives. Various methodologies have been described for the synthesis of substituted isoxazole derivatives. However, the existing methods are suffered with some drawbacks such as yield, time, product isolation, purification etc.

The 1,3-dipolar cycloaddition reactions of nitrile oxide with olefins is a useful reaction for the construction of five membered heterocyclic rings. Initially, the precursor aromatic aldoximes (59) were prepared by reacting aromatic aldehydes with hydroxylamine hydrochloride in the presence of sodium acetate in ethanol as a solvent (Scheme-62).

Chloramine-T and Magtrieve (CrO₂) were used as reagents for the in situ generation of nitrile oxides from aromatic aldoximes. It was found that in the absence of added trapping agents the starting material aldoxime disappeared when treated with CAT at RT for 3h in ethanol or CrO₂ at 80°C for 2h in MeCN solvent, undergo rapid dipolar cycloaddition with themselves and corresponding furoxons (dimerization product of in situ formed nitrile oxide) was formed as a major product along with a trace amount (< 10%) of aldehyde as de-oximation product (Scheme-63).
Magtrieve (CrO₂) is a very efficient reagent for the direct oxidation of aldoximes to nitrile oxides *in situ*. In our research on oxidation processes, we chose magtrieve as an oxidant, because it has been proven to be a useful oxidant in some reactions including the oxidation of allylic alcohols to allylic aldehydes,¹⁴⁷ the formation of benzyl from benzoin¹⁴⁸ and the aromatization of imidazolines.¹⁴⁹

Magtrieve is a magnetically retrievable oxidant based on tetravalent chromium dioxide (CrO₂).¹⁵⁰ It is a selective, heterogeneous form of CrO₂, whose reduced form stays on the crystal surface. This reagent is still ferromagnetic and can be conveniently removed after the reaction by a simple magnetic separation because only the surface of the CrO₂ is reduced. This has significant environmental and cost advantages over traditional chromium reagents that require aqueous work-up and consequent appropriate disposal of the chromium waste. In addition, the reduced chromium surface can be simply reconverted into CrO₂ by heating in air, thus adding to its recyclability and cost-effectiveness. Magtrieve as an oxidant is a very well-suited reagent for microwave synthesis because it carries the benefit of efficient conversion of electromagnetic energy into heat according to the dielectric heating mechanism.¹⁵¹

The 4-methoxy cinnaminitrile (60) was used as the precursor for the synthesis of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles (61a-i) (major product) and 3-aryl-4-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitriles (62a-i) (minor product). Nitrile oxides required for the cycloaddition were generated *in situ*
by the catalytic dehydrogenation of aromatic aldoximes \((59)\) using mild oxidants 
CrO\(_2\) in MeCN and/or chloramine-T in ethanol.

**Method (I):** The 1,3-dipolar cycloaddition reaction of nitrile oxides generated *in situ*
by the catalytic dehydrogenation of aromatic aldoximes \((59)\) in the presence of 10 mol 
equiv of CrO\(_2\) in MeCN at 80\(^\circ\)C with 4-methoxy cinnaminitrile \((60)\) gave a series of 
isomeric mixture \((61a-i\) and \(62a-i\)) of new isoxazolines in appreciable yields
*(Scheme-64).*

**Method (II):** The 1,3-dipolar cycloaddition reaction of nitrile oxides generated *in situ*
by the catalytic dehydrogenation of aromatic aldoximes \((59)\) with chloramine-T in 
ethyl alcohol at room temperature with 4-methoxy cinnaminitrile \((60)\) gave a series of 
isomeric mixture \((61a-i\) and \(62a-i\)) of new isoxazolines in good yields *(Scheme-64).*

Both methodologies produced the isomeric mixture of the same products 3-
aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles \((61a-i)\) and 3-aryl-4-
(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitriles \((62a-i)\). However the 
percentage yields of the products varied which would be discussed later.
2.3.0 Discussion on the experiment leading to the formation of isoxazolines:

In a general 1,3-dipolar cycloaddition reaction, isoxazolines are synthesized using magtrieve (CrO₂) as an oxidant. A mixture of aromatic aldoximes (59), 4-methoxy cinnamanitrile (60) and CrO₂ in acetonitrile was stirred at 80°C for 2 h. The progress of the reaction was monitored by TLC. After the completion of the reaction followed by usual work up, the reaction mixture gave one major spot corresponding to the product 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles (61), one minor spot corresponding to the isomeric product 3-aryl-4-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitriles (62) in TLC and other minor spot corresponding to the un-reacted precursor. The isomeric mixture of the products (61 and 62) was separated by column chromatography using hexane: ethyl acetate (8:1 v/v) (Scheme-64).
Alternatively, isoxazolines are synthesized using an equimolar mixture of aromatic aldoximes (59), 4-methoxy cinnamonicitrile (60) and chloramine-T trihydrate in ethanol which was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. After the completion of the reaction followed by usual work up, the reaction mixture gave one major spot corresponding to the product 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles (61), one minor spot corresponding to the isomeric product 3-aryl-4-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitriles (62) in TLC and other two minor spots corresponding to the un-reacted precursor and p-toluene sulphanamide. The isomeric mixture of the products (61 and 62) was separated by column chromatography using hexane: ethyl acetate (8:1 v/v) (Scheme-64).

In a series, a total of nine different isoxazolines were synthesized bearing various electron donating and electron withdrawing groups on the aromatic ring in order to study their impact on the SAR in biological assay. $^1$H NMR and mass spectral studies suggests that the products obtained by both the methods were identical.

**Brief spectral analysis discussion of the compounds 61a-i:**

The structures of the cycloadducts (61a-i) were provided by IR, $^1$H NMR, $^{13}$C NMR, MS studies and elemental analysis. For instance, in IR spectra, the cycloadducts (61a-i) exhibit absorption frequencies around 1650-1675 cm$^{-1}$ due to stretching of the C=N bond which is a clear indication of the expected cyclization, strong and sharp absorption bands in the region 2220-2240 cm$^{-1}$ for C≡N stretching which supports the fact that the C≡N triple bond of CN group is unaffected during the cycloaddition reaction. Aromatic and aliphatic C–H stretching vibrations are observed in the range of 2700–3100 cm$^{-1}$. 
In $^1$H NMR spectra, all synthesized substituted-4,5-dihydroisoxazole-4-carbonitriles (61a-i) showed the peaks due to aromatic and substituent protons at the expected region. The consistent pattern signals due to C$_4$-H appear as doublet in the region $\delta$ 5.00-5.29 ppm; while signals due to C$_5$-H appears as doublet in the region $\delta$ 5.50-5.71 ppm. The coupling constant ($J$) values calculated for C$_4$-H and C$_5$-H were in range 7.0-9.6 Hz, these values suggests that both C$_4$-H and C$_5$-H are in cis orientation and the cycloaddition took place in cis fashion. The appearance of these proton signals in the downfield was expected due to the strong electron withdrawing –CN group and aromatic ring bonded to C$_4$- and C$_5$- atoms respectively which favors the formation of cycloadducts. The absorption of the methyl hydrogens attached to single bonded oxygen are seen at about $\delta$ 3.79-3.87 ppm are deshielded due to electronegativity of oxygen. The methoxy peak is unsplit and stands out as a tall, sharp singlet. The aromatic proton signals appeared at down field region due to their ring current or anisotropic effect. Owing to this in compounds 61a-i, the aromatic proton signals resonated in the region $\delta$ 6.90-8.20 ppm as multiplet.

In $^{13}$C NMR, all products gave the signals due to aromatic and substituent carbons at the expected region. The signals at $\delta$ 21.20-23.60 and $\delta$ 66.59-66.94 ppm are assigned to newly formed C$_4$-carbon and C$_5$-carbon respectively. A signal at $\delta$ 161.30-164.72 ppm is attributed to C$_3$-carbon in the isoxazoline ring which is a carbon attached to electronegative nitrogen by a double bond and also to a benzene ring is deshielded due to its sp$^2$ hybridization and some diamagnetic anisotropy. One more signal at $\delta$ 55.18-55.80 ppm is assigned to methoxy carbon deshielded by electronegative oxygen. The signals due to CN group carbon appear at $\delta$ 116.2-118.0 ppm which shows that the CN triple bond is unaffected during cycloaddition and is
retained in the product. Moreover, a collection of signals appeared in the region $\delta$ 114.10-162.00 ppm which are ambiguously assigned to aryl carbons.

The mass spectrum of all the synthesized compounds (61a-i) showed M+1 molecular ion peak. Further, all showed satisfactorily C, H, N analysis with a deviation of $\pm$ 0.10% from the theoretically calculated values. The observed molecular ion peak and elemental analysis (C H N analysis) for the compounds (61a-i) are well compatible with proposed molecular formula. All these observations strongly favor the formation of the cycloadducts.

The structure proofs of the isomeric products (62a-i) were obtained by spectral studies and elemental analysis. As the compounds (62a-i) were obtained as minor products, the characterization was done only for three products. For instance, in IR spectra, the cycloadducts (62a, 62e and 62g) exhibit absorption frequencies around 1620-1640 cm$^{-1}$ due to stretching of the C=N bond which is a clear indication of the expected cyclization, strong and sharp absorption bands in the region 2220-2240 cm$^{-1}$ for C≡N stretching which supports the fact that the C≡N triple bond of CN group is unaffected during the cycloaddition reaction. Aromatic and aliphatic C–H stretching vibrations are observed in the range of 2800–3100 cm$^{-1}$.

In $^1$H NMR spectra, all substituted-4,5-dihydroisoxazole-5-carbonitriles (62a, 62e and 62g) showed the peaks due to aromatic and substituent protons at the expected region. The consistent pattern signals due to C$_4$-H appear as doublet in the region $\delta$ 5.60-5.79 ppm; while signals due to C$_5$-H appears as doublet in the region $\delta$ 5.20-5.40 ppm. The coupling constant ($J$) values calculated for C$_4$-H and C$_5$-H were in range $\delta$ 7.2-9.6 Hz, these values suggests that both C$_4$-H and C$_5$-H are in cis orientation and the cycloaddition took place in cis fashion. The appearance of these
proton signals in the downfield was expected due to the strong electron withdrawing aromatic ring and –CN group bonded to C₄⁻ and C₅⁻ atoms respectively which favors the formation of cycloadducts. The absorption of the methyl hydrogens attached to single bonded oxygen are seen at about δ 3.80-3.88 ppm are deshielded due to electronegativity of oxygen. The methoxy peak is unsplit and stands out as a tall, sharp singlet. The aromatic proton signals appeared at down field region due to their ring current or anisotropic effect. Owing to this in compounds (62a, 62e and 62g), the aromatic proton signals resonated in the region δ 6.96-8.29 ppm as multiplet.

In ¹³C NMR, all products gave the signals due to aromatic and substituent carbons at the expected region. The signals at δ 66.60-67.15 and 31.10-33.40 ppm are assigned to newly formed C₄-carbon and C₅-carbon respectively. A signal at δ 162.20-164.80 ppm is attributed to C₃-carbon in the isoxazoline ring which is a carbon attached to electronegative nitrogen by a double bond and also to a benzene ring is deshielded due to its sp² hybridization and some diamagnetic anisotropy. One more signal at δ 55.20-55.80 ppm is assigned to methoxy carbon deshielded by electronegative oxygen. The signals due to CN group carbon appear at δ 116.4-118.4 ppm which shows that the CN triple bond is unaffected during cycloaddition and is retained in the product. Moreover, a collection of signals appeared in the region δ 114.10-162.90 ppm which are ambiguously assigned to aryl carbons.

The mass spectrum of all the synthesized compounds (62a, 62e and 62g) showed M+1 molecular ion peak. Further, all showed satisfactorily C, H, N analysis with a deviation of ± 0.10% from the theoretically calculated values. The observed molecular ion peak and elemental analysis (C, H, N analysis) for the compounds (62a, 62e and 62g) are well compatible with proposed molecular formula. All these observations strongly favor the formation of the cycloadducts.
Mechanism for the formation of isoxazoline:

The probable mechanism for the generation of nitrile oxide using magtrieve is given below. The lone pair of electron on oxygen atom of aromatic aldoxime nucleophilically attacks electropositive chromium atom of magtrieve to form the intermediate. The shifting of –CH proton of the intermediate to oxygen atom of Cr=O bond with simultaneous elimination of Cr (OH)2 leads to the formation of nitrile oxide (Scheme 65).

Scheme 65: Mechanism for the generation of nitrile oxide using Magtrieve as dehydrogenating agent

The probable mechanism for the generation of nitrile oxide using chloramine-T is depicted in scheme 66. The unshared electrons of the nitrogen atom of chloramine-T abstracts a proton from =N-OH of aromatic aldoxime to form nucleophile. The nucleophile abstracts a chlorine atom followed by loss of sodium chloride to form the intermediate. The abstraction of proton from an intermediate by a mild base TsNH− ion produced in a reaction forms nitrile oxide.
The probable 1,3-dipolar cycloaddition mechanism for the formation of cycloadducts (61a-i) is given in scheme 67. The nucleophilic attack of nitrile oxide to a C^aH= atom of C^aH=C^bH-CN group of 4-methoxy cinnamonitrile and simultaneous attack of CH=CH pi electrons to triple bonded carbon of nitrile oxide leads to the formation of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles (61a-i).

Scheme 67: Proposed mechanism for the 1,3-dipolar cycloaddition of nitrile oxide with dipolarophile to get the cycloadducts (61a-i).

The probable mechanism for the formation of cycloadducts (62a-i) is given in scheme 68. The nucleophilic attack of nitrile oxide to a C^bH= atom of C^aH=C^bH-CN group of 4-methoxy cinnamonitrile and simultaneous attack of CH=CH pi electrons to triple bonded carbon of nitrile oxide leads to the formation of 3-aryl-4-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitriles (62a-i).
**Scheme 68**: Proposed mechanism for the 1,3-dipolar cycloaddition of nitrile oxide with dipolarophile to get the cycloadducts (62a-i).

**Mass spectral fragmentation of the cycloadducts**:

All the products gave significantly stable molecular ion peaks with a relative abundance ranging from 08-46%. Both the isomeric mixture (61a-i and 62a-i) of new isoxazolines showed similar pattern of fragmentation during the mass spectral analysis. The common fragmentation pattern involves some rearrangement with the removal of simple and smaller molecules like H₂, HCN, NO, C₆H₅F etc. (Scheme 69).
2.4.0 Biological activity:

Biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living matter.

Experimental:

The solvents methanol and DMF and all the synthesized compounds were purified prior to use. All chemicals used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich Chemical Co. Methanol, DMF, ferric chloride, potassium ferricyanide, phosphate buffer, BHT (butylated hydroxyl toluene) and trichloroacetic acid (TCA) and solvents were purchased from Merck India Ltd. Absorbance was noted using UV/Visible Spectrophotometer (Elico).

Test Microorganisms:

The synthesized compounds were tested for their antimicrobial susceptibility activity against Gram-negative bacteria species *Escherichia coli* and *Salmonella typhimurium*, Gram-positive bacteria species *Bacillus subtilis* and *Staphylococcus aureus*. They were also tested against the fungi species *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*.

Preparation of test compounds:

The solutions were prepared at a concentration of 50 µg/mL for antibacterial activity and 25 µg/mL for antifungal activity.

2.4.1 Antimicrobial activity:

Microorganisms require nutrition for their all metabolic activities. They draw the nutrition from their surrounding area. The growth of the microorganisms depends on the type of the nutrition they utilize. Some chemical nutrients enhance the mycelial growth or reproductive growth. The microorganism reproduces by various means, among all, the reproduction through its candidia is very important.
Resistance of pathogenic bacteria to available antibiotics is quickly becoming a major problem in the community and hospital based healthcare settings. Antimicrobials are one of the very important categories of drug. So it is quite clear from the spectrum of use that these categories of drugs are very important from medical point of view. But microbial resistance towards the drug creates a very serious problem because of development of resistance, many drugs are now useless which were very effective before. Moreover, the toxic effects produced by these antibiotics are also reducing their significance. So there is need for new antimicrobial agents for resistant microbial infections.

Antimicrobial (antibacterial and antifungal) activity of the synthesized compounds (61a-i) was done by paper disc diffusion method.  

2.4.1.1 Evaluation of antibacterial activity by paper disc method:

Gram-negative bacteria species such as *Escherichia coli*, *Salmonella typhimurium*, Gram-positive bacteria species such as *Bacillus subtilis*, *Staphylococcus aureus* were used as antibacterial test strains. The representative compounds (61a-i) were screened at the concentration (50µg/mL) in methanol on the nutrient agar media. The antibiotic ciprofloxacin was used as standard drug against bacteria. The paper discs inoculated with bacteria were incubated for 24 h at 37°C. After the period of incubation, the zone of inhibition produced by the test compounds was measured in mm. The screening tests were performed in triplicate and the results were taken as a mean of three determinations.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The nutrient broth, which contain logarithmic serially two-fold diluted amount of test compound and controls were inoculated with approximately 5 x 10⁵ c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37°C.
and the growth was monitored visually and spectrophotometrically. The lowest concentration required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC). All the experiments were carried out in triplicate and the results were taken as a mean of three determinations.

**Antibacterial activity of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4 carbonitriles 61a-i:**

The investigation of the antibacterial screening of the test samples (61a-i) revealed that all these compounds showed moderate to good antibacterial activity against all the organisms. The compounds 61a-d showed lesser activity against *Bacillus subtilis* and remarkable activity against the bacterium *E. coli, S. typhimurium* and *S. aureus*, which is attributed to the presence of fluoro, chloro and bromo substituents at C3-substituted benzene ring. The compounds 61e, 61f found less active against all the organisms tested, this may be due to the presence of strong electron withdrawing –CN and -NO₂ substituents on the benzene ring. The compounds 61g-i have exhibited moderate activity against *E. coli, S. typhimurium* and *S. aureus* and lesser activity against *Bacillus subtilis*, which is attributed the presence of electron donating –OCH₃ groups or no substitution on the aromatic ring. The results thus obtained reveal that nature of substituents present on the benzene ring has a considerable impact particularly at ortho and para positions. The results indicate that the compounds 61a-d may be used as control measures against *E. coli, S. typhimurium* and *S. aureus*, 61g-i against *Bacillus subtilis* and different bacteria (Table-2, Figure-4). The results of MIC’s determined reveal that some of these test compounds can act as good antibacterial agents at very lower concentrations (Table-2, Figure-5).
Table-2: Zone of inhibition (X mm) at 50 µg/mL concentrations and MIC’s (Y µg/mL) of the test samples 61a-i tested against bacterial strains.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Escherichia coli</th>
<th>Salmonella typhimurium</th>
<th>Bacillus subtilis</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>Y</td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td>61a</td>
<td>29</td>
<td>22</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>61b</td>
<td>28</td>
<td>24</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>61c</td>
<td>27</td>
<td>21</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>61d</td>
<td>25</td>
<td>26</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>61e</td>
<td>15</td>
<td>28</td>
<td>16</td>
<td>26</td>
</tr>
<tr>
<td>61f</td>
<td>12</td>
<td>30</td>
<td>15</td>
<td>31</td>
</tr>
<tr>
<td>61g</td>
<td>20</td>
<td>25</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>61h</td>
<td>21</td>
<td>29</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>61i</td>
<td>23</td>
<td>27</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>Ciprofloxacin*</td>
<td>34</td>
<td>18</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

Results are expressed as mean of three determinations (n=3);
Ciprofloxacin* (50 µg per disc) was used as standard drug.

Figure 4: Zone of Inhibition (diameter) at 50µg/mL concentrations of the test samples 61a-i measured against bacterial strains (*Std: Ciprofloxacin).
2.4.1.2 Evaluation of antifungal activity by paper disc method:

The synthesized compounds (61a-i) were tested for their antifungal activity against the fungi species *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* strains at a concentration of 25µg/mL in DMF in the potato dextrose agar media. The antibiotic griseofulvin was used as standard drug against fungi. The paper discs inoculated with fungi were incubated for 72 h at 37°C. After the period of incubation, the zone of inhibition produced by the test compounds was measured in mm. The screening tests were performed in triplicate and the results were taken as a mean of three determinations.

Minimum inhibitory concentrations (MICs) were determined by broth dilution technique. The cultures were incubated for 72 h at 37°C and the growth was monitored visually and spectrophotometrically. All the experiments were carried out in triplicate and the results were taken as a mean of three determinations.

**Antifungal activity of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles 61a-i:**

The experimental results of new compounds (61a-i) revealed that all these compounds showed promising antifungal activity against *A. niger* and *C. albicans,*
moderate activity against *A. flavus*. The compounds 61a-d was highly active against *A. niger* and *C. albicans*, moderately active against *A. flavus*. Test samples 61e, 61f have shown moderate activity against all the organisms tested, which may be due to the presence of electron withdrawing -CN and -NO₂ groups on the aromatic ring, while 61g-i marked lesser activity against all the organisms, which may be attributed to the presence of electron donating –OCH₃ groups or no substituents on the C₃-substituted benzene ring. The results thus obtained reveal that halogen substituents present on the C₃-substituted benzene ring have a considerable impact particularly at ortho and para positions and therefore they may be used as control measures against different fungi species (Table-3, Figure-6). The results of MIC’s determined reveal that some of these test compounds can act as good antifungal agents at very lower concentrations (Table-3, Figure-7).

**Table-3: Zone of Inhibition (diameter) (X mm) at 25 µg/mL concentrations and MICs (Y µg/mL) of the compounds 61a-i tested against fungal strains.**

<table>
<thead>
<tr>
<th>Compound</th>
<th><em>Aspergillus niger</em></th>
<th><em>Aspergillus flavus</em></th>
<th><em>Candida albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>Y</td>
<td>X</td>
</tr>
<tr>
<td>61a</td>
<td>25</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>61b</td>
<td>27</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>61c</td>
<td>26</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>61d</td>
<td>24</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>61e</td>
<td>21</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td>61f</td>
<td>19</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>61g</td>
<td>16</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>61h</td>
<td>14</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>61i</td>
<td>14</td>
<td>29</td>
<td>10</td>
</tr>
<tr>
<td>Griseofulvin*</td>
<td>30</td>
<td>20</td>
<td>26</td>
</tr>
</tbody>
</table>

Results are expressed as mean of three determinations (n=3);
Griseofulvin* (25 µg per disc) was used as standard drug.
2.4.2 Antioxidant activity:

Reactive oxygen species [ROS], sometimes called as active oxygen species, are various forms of activated oxygen, which include free radicals such as superoxide ions (O$_2^-$) and hydroxyl radicals (·OH) as well as non-free radical species such as hydrogen peroxide (H$_2$O$_2$). These ROS play an important role in degenerative or pathological processes, such as aging, cancers, coronary heart diseases, Alzheimer’s disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammations. Living organisms have antioxidant defense systems that protects against oxidative damage by removal or repair of damaged molecules. The term ‘antioxidant’ refers to the activity of numerous vitamins, minerals and photochemical which provide protection against the damage caused by ROS. Antioxidants interfere with the
oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors.\textsuperscript{158} The natural antioxidant mechanisms may be insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important.\textsuperscript{159}

**Assessment of Antioxidant activity: DPPH assay:**

**Principle:**

The scavenging reaction between (DPPH.) and an antioxidant (H-A) can be written as:

\[
(DPPH) + (H-A) \rightarrow DPPH-H + (A) \quad \text{(Deep violet)} \rightarrow \text{(Pale yellow)}
\]

Antioxidants react with DPPH radical (63), which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance decreased from the DPPH radical to the DPPH-H form.

The antioxidant activity of the samples was evaluated by DPPH radical scavenging assay which was originally described by Blois (1958). DPPH (1,1-diphenyl-2-picrylhydrazyl) (63) is considered as a stable organic nitrogen radical because of the paramagnetism conferred by its odd electron (delocalization of the spare electron over the molecule as a whole). The solution (in absolute ethanol) appears as a deep violet color and shows an absorption band in the range of 515-520nm. In presence of hydrogen/electron donor (free radical scavenging
antioxidants), the absorption intensity is decreased and the radical solution is
decolorized to pale yellow color according to the number of electrons captured. This
property allows visual monitoring of the reaction and the number of initial radicals
can be counted from the change in the optical absorption at 520 nm or in the EPR
signal of the DPPH.

The capability to scavenge the DPPH radical was calculated using the
following equation;

\[
\text{DPPH scavenging activity(\%) =} \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

Where \( A_{\text{control}} \) is the absorbance of the control reaction and \( A_{\text{sample}} \) is
the absorbance in the presence of the sample.

2.4.2.1 Evaluation of antioxidant activity by DPPH free radical scavenger
method:

The effect of the samples (61a-i) in addition to the standard antioxidant
butylated hydroxyl toluene (BHT) on DPPH radical was estimated according to the
method.\(^{160}\) Samples dissolved in methanol (0-50 \( \mu \)g/mL for samples (61a-i); 0-5
\( \mu \)g/mL for BHT) in 200 \( \mu \)L aliquot was mixed with 100 mM tris-HCl buffer (800 \( \mu \)L,
\( \text{pH} \) 7.4) and then added 1 mL of 500 \( \mu \)M DPPH in ethanol (\( \text{final concentration of} \) 250
\( \mu \)M). The mixture was shaken vigorously and left to stand for 20 min at room
temperature in the dark. The absorbance of the resulting solution was measured
spectrophotometrically at 517 nm. The results of all experiments performed were
expressed as mean of the three determinations.
Antioxidant activity of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles 61a-i:

The results of *in vitro* antioxidant activity of the title compounds were depicted in (Table-4, Figure-8). DPPH radical scavenging is considered a good *in vitro* model and is widely used to conveniently assess antioxidant efficacy. From the results it could be seen that most of the compounds showed significant antioxidant activity. At the initial concentrations of (10-20 µg/mL), not much significant variations in the free radical scavenging ability of samples 61a-g was observed. However, when the concentration was increased (30-50 µg/mL) all showed a promising radical scavenging ability. The compounds 61a-d showed radical scavenging ability up to 50%, the samples 61e, 61f showed radical scavenging ability up to 62% and 61g-i showed up to 40% with reference to the standard antioxidant. Results indicate that the compounds 61e, 61f containing electron withdrawing groups on the aromatic ring shows potential electron donating ability (Table-4, Figure-8). The IC$_{50}$ values in µg/ml were determined for the antioxidant activity of the compounds (61a-i) (Figure-9).
**Table-4: Percentage of DPPH radical scavenging activity of samples 61a-i relative to the standard oxidant BHT.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>61a</td>
<td>10.46 ± 0.82</td>
</tr>
<tr>
<td>61b</td>
<td>13.12 ± 0.78</td>
</tr>
<tr>
<td>61c</td>
<td>9.22 ± 0.89</td>
</tr>
<tr>
<td>61d</td>
<td>14.76 ± 0.94</td>
</tr>
<tr>
<td>61e</td>
<td>10.16 ± 0.90</td>
</tr>
<tr>
<td>61f</td>
<td>18.32 ± 1.00</td>
</tr>
<tr>
<td>61g</td>
<td>7.12 ± 0.88</td>
</tr>
<tr>
<td>61h</td>
<td>8.12 ± 0.88</td>
</tr>
<tr>
<td>61i</td>
<td>12.12 ± 0.88</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± standard deviation (n=3). [Control at 0 µg/ml = 0.0 ± 0.00%].

**Figure 8: Percentage of DPPH radical scavenging activity of samples 61a-i at different concentrations relative to the standard oxidant BHT.**
2.4.2.2 Measurement of reducing power:

The reducing power of samples 61a-i was determined according to the method. The samples 61a-i (0-50 µg/mL) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6 and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then an equal volume of 10% trichloroacetic acid was added to the mixture and then centrifuged at 5000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1% ferric chloride at a ratio of 1:1:2 and the absorbance were measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The experiments were carried out in triplicates (n = 3) and the results are expressed as mean of the three determinations.

Reducing power ability of 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles 61a-i:

The test samples (61a-i) were evaluated for their reducing power ability to reduce ferric chloride and potassium ferricyanide complex. It was observed that at the initial concentrations of (10-20 µg/mL), there was no significant variations in the activity. However, when the concentration was increased (30-50 µg/mL), all showed remarkable reducing power. The compounds 61e, 61f containing electron withdrawing substituents on the aromatic ring showed higher reducing power and
$61a$-$d$ having halogen substituents on the aromatic ring showed moderate reducing power, whereas the samples $61g$-$i$ with electron donating substituents on the aromatic ring exhibited lesser reducing power ability compared to $61a$-$f$. The increased absorbance at 700 nm indicated the presence of reducing power ability of the test samples considered for the study (Table-5, Figure-10).

**Table-5: Reducing power ability (absorbance) of samples $61a$-$i$ measured at 700 nm relative to the standard oxidant BHT.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>$61a$</td>
<td>0.268 ± 0.014</td>
</tr>
<tr>
<td>$61b$</td>
<td>0.306 ± 0.010</td>
</tr>
<tr>
<td>$61c$</td>
<td>0.312 ± 0.011</td>
</tr>
<tr>
<td>$61d$</td>
<td>0.330 ± 0.017</td>
</tr>
<tr>
<td>$61e$</td>
<td>0.308 ± 0.009</td>
</tr>
<tr>
<td>$61f$</td>
<td>0.338 ± 0.008</td>
</tr>
<tr>
<td>$61g$</td>
<td>0.216 ± 0.006</td>
</tr>
<tr>
<td>$61h$</td>
<td>0.256 ± 0.006</td>
</tr>
<tr>
<td>$61i$</td>
<td>0.226 ± 0.006</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (n=3).

[Control at 0 µg/ml: OD = 0.0 ± 0.00].
Mechanistic considerations of antioxidant activity:

DPPH is a stable organic nitrogen radical used as a scavenger for other radicals. DPPH radical scavenging test evaluates *in vitro* antioxidant capacity. In the presence of hydrogen/electron donor, DPPH radical scavenges the hydrogen radical from a donor molecule and it gets reduced as DPPH⁻ + H⁺ → DPPH-H.

As and when DPPH radical scavenges the hydrogen radical, the absorption intensity is decreased and the radical solution is decolorized to pale yellow color depends upon the number of electrons captured.

The relatively unstable non-aromatic 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles (61) on catalytic dehydrogenation, produces a stable aromatic 3-aryl-5-(4-methoxyphenyl)-isoxazole-4-carbonitriles (64).

The instability of the non-aromatic 3-aryl-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitriles was expected to be the driving force for their
antioxidant activity. The non-aromatic compounds have a tendency to become more stable aromatic compounds (64) with the loss of two hydrogen atoms and two electrons.

From the experimental results, the stiochiometry of the reaction was found to be 1:2 for test compounds:DPPH free radical, which suggests that each molecule (61) has a tendency to donate two hydrogen atom and two electrons to the acceptor molecules. In the presence of hydrogen donor organic compound (61) the DPPH free radical abstract the hydrogen atom bonded to C₄ and/or C₅-atom along with one of its bonded electron to give organic free radical and it becomes reduced (DPPH-H). The second molecule of DPPH free radical abstracts the hydrogen atom of C₅ and/or C₄-atom with one of its bonded electron to give organic diradical and it becomes reduced (DPPH-H). The organic diradical expected to undergo intramolecular coupling to form stable organic compound (64) (Scheme-70).

![Scheme-70 Mechanism of radical Scavenging activity](image)

On the basis of this speculation, the C₄ and/or C₅ positions of the isoxazoline ring may be the active site responsible for antioxidant activity of the screened isoxazole derivatives.
2.5.0 Experimental section:

The chemicals/reagents used were purchased from sigma-Aldrich chemicals (India) and Merck Chemicals (India). IR spectra were recorded on a Nujol mull on Shimadzu 8300 spectrometer. The $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker supercon 400 MHz spectrophotometer using CDCl$_3$ as solvent and TMS as an internal standard. The Chemical shifts are expressed in δ ppm. Mass spectra were obtained on Shimadzu LCMS-2010A spectrophotometer (chemical ionization) and the important fragments are given with the relative intensities in the bracket. Elemental analysis was obtained on a Thermo Finnigan Flash EA 1112 CHN analyser. Thin layer chromatography (TLC) was performed on a pre-coated Silica Gel sheets (HF 254, sd-fine) using benzene:ethyl acetate (7:2) eluent and visualization of the spots was done in iodine vapour and UV light. Chromatographic separations were carried out on silica gel (70-230 mesh, Merck) column using hexane:ethyl acetate (8:1) as eluent.

2.5.1 General procedure for the preparation of aldehyde oximes:

Solution of hydroxylamine hydrochloride (1g) and crystallized sodium acetate (2g) in distilled water (10mL) was mixed with solution of aldehyde (0.5g) in ethyl alcohol. The mixture was then warmed for 5-10 minutes and cooled in ice water. The crystals formed were filtered, washed with a little cold water and recrystallized from methanol. The physical characteristic data of the prepared aldoximes was given in Table-6.
Table-6: Physical constants (Melting point/boiling point) of prepared aldoximes

<table>
<thead>
<tr>
<th>Aromatic aldoximes</th>
<th>Melting point/boiling point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obs.°C</td>
</tr>
<tr>
<td>4-Fluorobenzaldehyde oxime</td>
<td>82-84°C</td>
</tr>
<tr>
<td>4-Chlorobenzaldehyde oxime</td>
<td>106-108°C</td>
</tr>
<tr>
<td>4-Bromobenzaldehyde oxime</td>
<td>104-106°C</td>
</tr>
<tr>
<td>4-Cyanobenzaldehyde oxime</td>
<td>171-173°C</td>
</tr>
<tr>
<td>2-Chlorobenzaldehyde oxime</td>
<td>71-73°C</td>
</tr>
<tr>
<td>5-Chloro, 2-nitrobenzaldehyde oxime</td>
<td>110-112°C</td>
</tr>
<tr>
<td>4-Methoxybenzaldehyde oxime (liq)</td>
<td>245-247°C</td>
</tr>
<tr>
<td>3,4-Dimethoxybenzaldehyde oxime</td>
<td>90-91°C</td>
</tr>
<tr>
<td>Benzaldehyde oxime (liq)</td>
<td>116-118°C</td>
</tr>
</tbody>
</table>

2.5.2 General procedure for the synthesis of isoxazolines:

Substituted aromatic aldoximes were prepared by the reaction of aromatic aldehydes and hydroxyl amine hydrochloride. Aromatic aldoximes thus obtained were subjected to 1,3-dipolar cycloaddition reaction with an alkene to get five membered isoxazolines. Aldoximes on catalytic dehydrogenation with different oxidants gives the corresponding nitrile oxide, which acts as a 1,3-dipole in cycloaddition reaction.

2.5.2.1 General procedure for the synthesis of isoxazolines using magtrieve:

A mixture of aromatic aldehyde oxime (59) (0.76mmol, 1.2equiv), 3-(4-methoxyphenyl) acrylonitrile (60) (0.63mmol, 1.0 equiv) were dissolved in 3 ml acetonitrile. Magtrieve (6.31 mmol, 10 equiv) was added and the reaction mixture was stirred under heating at 80°C for 2 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered through Celite.
bed. Magtrieve was washed with ethyl acetate (20 ml × 2). The combined filtrate was condensed to give the crude product, which was purified by column chromatography using hexane: ethyl acetate (8:1 v/v).

2.5.2.2 General procedure for the synthesis of isoxazolines using chloramine-T:

A mixture of aromatic aldehyde oxime (59) (4.0mmol), 3-(4-methoxyphenyl) acrylonitrile (60) (4.0mmol) and chloramine-T trihydrate (4.0mmol) was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. After the completion of reaction, the sodium chloride formed in the reaction mixture was filtered off and washed with ethanol (1 X 15mL) and then the combined filtrate and washings were evaporated in vacuo. The residual part was extracted into ether (25mL), washed successively with water (2 X 15mL), 10% sodium hydroxide (2 X 15mL) and saturated brine solution (1 X 10mL). The organic layer was dried over anhydrous sodium sulphate. Evaporation of the solvent yielded the crude product, which was purified by column chromatography using hexane: ethyl acetate (8:1 v/v). The same procedure was used in all cases.

2.6.0 Experimental results:

3-(4-Fluorophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61a:

Using Magtrieve: Obtained from 4-methoxy cinnamionitrile (60) (100mg, 0.63mmol, 1.0equiv), 4-fluorobenzaldehyde oxime (59a) (106mg, 0.76mmol, 1.2equiv) and Magtrieve (530mg, 6.31 mmol, 10 equiv), as a light yellow oil in 62% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol), 4-fluorobenzaldehyde oxime (59a) (0.56g, 4.0mmol) and chloramine-T trihydrate (1.13g, 4.0mmol), as a light yellow oil in 72% yield. IR (Nujol): 1654 cm⁻¹ C=N (str), 2228 cm⁻¹ CN (str). ¹H NMR (CDCl₃): δ 3.84 (s, 3H, OCH₃), 5.29 (d, 1H, J=7.2Hz, C₄-H), 5.71 (d, 1H, J=8.4Hz, C₅-H), 6.90-6.93 (dd, 2H, Ar-H), 6.93-6.95
(dd, 2H, Ar-H), 7.38-7.40 (dd, 2H, Ar-H), 7.78-7.80 (dd, 2H, Ar-H). $^{13}$C NMR (CDCl$_3$): $\delta$ 21.2 (1C, 4-\(C\)), 55.1 (1C, OCH$_3$), 66.5 (1C, 5-\(C\)), 114.1-114.5 (2C, Ar-\(C\)), 115.6-115.9 (2C, Ar-\(C\)), 116.2 (1C, CN), 128.4-128.7 (2C, Ar-\(C\)), 129.2-129.5 (2C, Ar-\(C\)), 130.3 (1C, Ar-\(C\)), 130.6 (1C, Ar-\(C\)), 149.6 (1C, Ar-\(C\)), 161.3 (1C, 3-\(C\)), 161.7 (1C, Ar-\(C\)). MS (relative abundance) m/z: 297(MH$^+$, 100), 270 (32), 238 (20), 174 (16), 157 (44). Anal. Cacld. for C$_{17}$H$_{13}$FN$_2$O$_2$, C, 68.91, H, 4.42, N, 9.45%; Found: C, 68.85, H, 4.46, N, 9.37%.

3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61b:

Using Magtrieve: Obtained from 4-methoxy cinnamonicitrile (60) (100mg, 0.63mmol, 1.0equiv) and 4-chlorobenzaldehyde oxime (59b) (118mg, 0.76mmol, 1.2equiv), as a light yellow oil in 54% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol) and 4-chlorobenzaldehyde oxime (59b) (0.62g, 4.0mmol), as a light yellow oil in 64% yield. IR (Nujol): 1650 cm$^{-1}$ C=N (str), 2220 cm$^{-1}$ CN (str). $^1$H NMR (CDCl$_3$): $\delta$ 3.84 (s, 3H, OCH$_3$), 5.28 (d, 1H, J=9.0Hz, C$_4$-H), 5.70 (d, 1H, J=9.6Hz, C$_5$-H), 6.91-6.92 (dd, 2H, Ar-H), 6.93-6.94 (dd, 2H, Ar-H), 7.38-7.40 (dd, 2H, Ar-H), 7.78-7.79 (dd, 2H, Ar-H). $^{13}$C NMR (CDCl$_3$): $\delta$ 21.4 (1C, 4-\(C\)), 55.2 (1C, OCH$_3$), 66.8 (1C, 5-\(C\)), 114.2-114.4 (2C, Ar-\(C\)), 115.7-115.9 (2C, Ar-\(C\)), 116.6 (1C, CN), 128.5-128.7 (2C, Ar-\(C\)), 129.2-129.4 (2C, Ar-\(C\)), 130.7 (1C, Ar-\(C\)), 130.9 (1C, Ar-\(C\)), 150.2 (1C, Ar-\(C\)), 161.7 (1C, 3-\(C\)), 162.0 (1C, Ar-\(C\)). MS (relative abundance) m/z: 313 (MH$^+$, 100), 286 (28), 254 (24), 174 (18), 157 (42). Anal. Cacld. for C$_{17}$H$_{13}$ClN$_2$O$_2$, C, 65.29, H, 4.19, N, 8.96%; Found: C, 65.19, H, 4.18, N, 8.89%.
3-(2-Chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61c:

Using Magtrieve: Obtained from 4-methoxy cinnaminitrile (60) (100mg, 0.63mmol, 1.0equiv) and 2-chlorobenzaldehyde oxime (59c) (118mg, 0.76mmol, 1.2equiv), as a colorless oil in 55% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol) and 2-chlorobenzaldehyde oxime (59c) (0.62g, 4.0mmol), as a colorless oil in 65% yield. IR (Nujol): 1668 cm\(^{-1}\) C=N (str), 2235 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta 3.87 (s, 3H, OCH_3), 5.00 (d, 1H, J=8.4Hz, C_4-H), 5.50 (d, 1H, J=8.6Hz, C_5-H), 6.91-6.93 (dd, 2H, Ar-H), 7.23-7.24 (dd, 2H, Ar-H), 7.39-7.41 (dd, 2H, Ar-H), 7.669-7.681 (dd, 2H, Ar-H). Anal. Cacl. for C\(_{17}\)H\(_{13}\)ClN\(_2\)O\(_2\), C, 65.29, H, 4.19, N, 8.96%; Found: C, 65.22, H, 4.22, N, 8.89%.

3-(4-Bromophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61d:

Using Magtrieve: Obtained from 4-methoxy cinnaminitrile (60) (100mg, 0.63mmol, 1.0equiv) and 4-bromobenzaldehyde oxime (59d) (152mg, 0.76mmol, 1.2equiv), as a pale yellow oil in 52% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol) and 4-bromobenzaldehyde oxime (59d) (0.8g, 4.0mmol), as a pale yellow oil in 65% yield. IR (Nujol): 1665 cm\(^{-1}\) C=N (str), 2235 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta 3.86 (s, 3H, OCH_3), 5.19 (d, 1H, J=7.6Hz, C_4-H), 5.62 (d, 1H, J=8.2Hz, C_5-H), 6.91-6.93 (dd, 2H, Ar-H), 6.93-6.95 (dd, 2H, Ar-H), 7.38-7.41 (dd, 2H, Ar-H), 7.77-7.79 (dd, 2H, Ar-H). \(^13\)C NMR (CDCl\(_3\)): \(\delta 22.3 (1C, 4-C), 55.6 (1C, OCH_3), 66.9 (1C, 5-C), 114.1-114.3 (2C, Ar-C), 116.8 (1C, CN), 125.1 (1C, Ar-C), 126.1-126.4 (2C, Ar-C), 128.5-128.7 (2C, Ar-C), 130.1-130.3 (2C, Ar-C), 131.9 (1C, Ar-C), 133.6 (1C, Ar-C), 152.5 (1C, Ar-C), 162.5 (1C, 3-C). MS (relative abundance) m/z: 357
(MH+, 79Br 100), 359 (MH+, 79Br 66), 332 (19), 330 (28), 300 (08), 298 (25), 174 (22), 157 (46). Anal. Cacld. for C$_{17}$H$_{13}$BrN$_2$O$_2$, C, 57.16, H, 3.67, N, 7.84%; Found: C, 57.10, H, 3.70, N, 7.78%.

3-(4-Cyanophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61e:

Using Magtrieve: Obtained from 4-methoxy cinnamonitrile (60) (100mg, 0.63mmol, 1.0equiv) and 4-cyanobenzaldehyde oxime (59e) (111mg, 0.76mmol, 1.2equiv), as a colorless oil in 58% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl)acrylonitrile (60) (0.64g, 4.0mmol) and 4-cyanobenzaldehyde oxime (59e) (0.58g, 4.0mmol), as a colorless oil in 68% yield. IR (Nujol): 1658 cm$^{-1}$ C=N (str), 2234 cm$^{-1}$ CN (str). $^1$H NMR (CDCl$_3$): δ 3.85 (s, 3H, OCH$_3$), 5.11 (d, 1H, $J$=8.0Hz, C$_4$-H), 5.54 (d, 1H, $J$=8.2Hz, C$_5$-H), 6.92-6.92 (dd, 2H, Ar-H), 7.22-7.24 (dd, 2H, Ar-H), 7.65-7.67 (dd, 2H, Ar-H), 7.98-7.99 (dd, 2H, Ar-H). $^{13}$C NMR (CDCl$_3$): δ 23.5 (1C, 4-Ç), 55.8 (1C, OCH$_3$), 66.9 (1C, 5-Ç), 114.4-114.6 (2C, Ar-Ç), 115.6 (1C, Ar-Ç), 116.9 (1C, ÇN), 118.0 (1C, ÇN), 126.8-126.9 (2C, Ar-Ç), 129.0-129.1 (2C, Ar-Ç), 131.0-131.2 (2C, Ar-Ç), 132.3 (1C, Ar-Ç), 136.7 (1C, Ar-Ç), 159.7 (1C, Ar-Ç), 163.2 (1C, 3-Ç). MS (relative abundance) m/z: 304 (MH$^+$, 100), 277 (21), 245 (30), 174 (23), 157 (41). Anal. Cacld. for C$_{18}$H$_{13}$N$_3$O$_2$, C, 71.28, H, 4.32, N, 13.85%; Found: C, 71.21, H, 4.24, N, 13.79%.

3-(5-Chloro-2-nitrophenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61f:

Using Magtrieve: Obtained from 4-methoxy cinnamonitrile (60) (100mg, 0.63mmol, 1.0equiv) and 5-chloro-2-nitrobenzaldehyde oxime (59f) (152mg, 0.76mmol, 1.2equiv), as a light yellow oil in 53% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl)acrylonitrile (60) (0.64g, 4.0mmol) and 5-chloro-2-nitrobenzaldehyde oxime (59f) (0.8g, 4.0mmol), as a light...
yellow oil in 66% yield. IR (Nujol): 1668 cm\(^{-1}\) C=N (str), 2230 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.79 (s, 3H, OCH\(_3\)), 5.12 (d, 1H, \(J=8.1\)Hz, C\(_4\)-H), 5.51 (d, 1H, \(J=9.0\)Hz, C\(_5\)-H), 6.92-6.93 (dd, 2H, Ar-H), 7.23-7.24 (dd, 2H, Ar-H), 7.70 (dd, 1H, Ar-H), 8.11 (d, 1H, Ar-H), 8.20 (dd, 1H, Ar-H). \(^1\)C NMR (CDCl\(_3\)): \(\delta\) 23.6 (1C, 4-\(C\)), 55.8 (1C, OCH\(_3\)), 66.8 (1C, 5-\(C\)), 114.3-114.5 (2C, Ar-\(C\)), 116.7 (1C, \(\text{C}_N\)), 124.1 (1C, Ar-\(C\)), 127.0-127.2 (2C, Ar-\(C\)), 130.3 (1C, Ar-\(C\)), 131.2 (1C, Ar-\(C\)), 132.8 (1C, Ar-\(C\)), 132.9 (1C, Ar-\(C\)), 136.1 (1C, Ar-\(C\)), 140.1 (1C, Ar-\(C\)), 159.9 (1C, Ar-\(C\)), 164.2 (1C, 3-\(C\)).

MS (relative abundance) m/z: 358 (MH\(^+\), 35Cl, 100), 360 (MH\(^+\), 37Cl, 33), 333 (08), 331 (22), 299 (34), 174 (22), 157 (40). Anal. Caclcd. for C\(_{17}\)H\(_{12}\)ClN\(_3\)O\(_4\), C, 57.07, H, 3.38, N, 11.75%; Found: C, 57.00, H, 3.43, N, 11.78%.

**Synthesis of 5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydroisoazole-4-carbonitrile**

61g:

Using Magtrieve: Obtained from 4-methoxy cinnamonic nitrile (60) (100mg, 0.63mmol, 1.0equiv) and benzaldehyde oxime (59g) (92mg, 0.76mmol, 1.2equiv), as a colorless oil in 56% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol) and benzaldehyde oxime (59g) (0.48g, 4.0mmol), as a colorless oil in 65% yield. IR (Nujol): 1670 cm\(^{-1}\) C=N (str), 2235 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.82 (s, 3H, OCH\(_3\)), 5.13 (d, 1H, \(J=9.0\)Hz, C\(_4\)-H), 5.53 (d, 1H, \(J=8.5\)Hz, C\(_5\)-H), 6.91-6.93 (dd, 2H, Ar-H), 7.20-7.22 (dd, 2H, Ar-H), 7.56-7.78 (m, 5H, Ar-H). \(^1\)C NMR (CDCl\(_3\)): \(\delta\) 23.4 (1C, 4-\(C\)), 55.8 (1C, OCH\(_3\)), 66.7 (1C, 5-\(C\)), 114.3 (2C, Ar-\(C\)), 116.6 (1C, \(\text{C}_N\)), 127.0 (2C, Ar-\(C\)), 128.2 (2C, Ar-\(C\)), 128.4 (2C, Ar-\(C\)), 131.1 (1C, Ar-\(C\)), 133.0 (1C, Ar-\(C\)), 133.6 (1C, Ar-\(C\)), 159.0 (1C, Ar-\(C\)), 164.7 (1C, 3-\(C\)). MS (relative
abundance) m/z: 279 (MH⁺, 100), 252 (22), 220 (30), 174 (20), 157 (38). Anal. Cacld. for C₁₇H₁₄N₂O₂, C, 73.37, H, 5.07, N, 10.07%; Found: C, 73.30, H, 5.01, N, 10.02%.

3,5-Bis(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61h:

Using Magtrieve: Obtained from 4-methoxy cinnamonitrile (60) (100mg, 0.63mmol, 1.0equiv) and 4-methoxybenzaldehyde oxime (59h) (115mg, 0.76mmol, 1.2equiv), as a colorless oil in 60% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol) and 4-methoxybenzaldehyde oxime (59h) (0.6g, 4.0mmol), as a colorless oil in 70% yield. IR (Nujol): 1655 cm⁻¹ C=N (str), 2225 cm⁻¹ CN (str). ¹H NMR (CDCl₃): δ 3.80 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 5.13 (d, 1H, J=8.1Hz, C₄-H), 5.51 (d, 1H, J=8.8Hz, C₅-H), 6.92-6.93 (dd, 2H, Ar-H), 6.99-6.70 (dd, 2H, Ar-H), 7.22-7.23 (dd, 2H, Ar-H), 7.74-7.75 (dd, 2H, Ar-H). ¹³C NMR (CDCl₃): δ 23.5 (1C, 4-杞), 55.7 (1C, OCH₃), 55.8 (1C, OCH₃), 66.8 (1C, 5-杞), 114.3-114.4 (2C, Ar-杞), 116.6 (1C, CN), 124.2 (1C, Ar-杞), 127.1-127.2 (2C, Ar-杞), 130.2 (1C, Ar-杞), 131.2 (1C, Ar-杞), 132.9 (1C, Ar-杞), 133.0 (1C, Ar-杞), 136.0 (1C, Ar-杞), 140.1 (1C, Ar-杞), 159.9 (1C, Ar-杞), 164.2 (1C, 3-杞). MS (relative abundance) m/z: 309 (MH⁺, 100), 282 (36), 250 (32), 174 (27), 157 (42). Anal. Cacld. for C₁₈H₁₆N₂O₃, C, 70.12, H, 5.23, N, 9.09%; Found: C, 70.06, H, 5.26, N, 9.10%.

3-(3,4-Dimethoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-carbonitrile 61i:

Using Magtrieve: Obtained from 4-methoxy cinnamonitrile (60) (100mg, 0.63mmol, 1.0equiv) and 3,4-dimethoxybenzaldehyde oxime (59i) (138mg, 0.76mmol, 1.2equiv), as a colorless oil in 61% yield.

Using chloramine-T: Obtained from 3-(4-methoxyphenyl) acrylonitrile (60) (0.64g, 4.0mmol) and 3, 4-dimethoxybenzaldehyde oxime (59i) (0.6g, 4.0mmol), as a
colorless oil in 65% yield. IR (Nujol): 1672 cm\(^{-1}\) C=N (str), 2238 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.81 (s, 6H, OCH\(_3\)), 3.81 (s, 3H, OCH\(_3\)), 5.12 (d, 1H, \(J=8.5\)Hz, C\(_4\)-H), 5.52 (d, 1H, \(J=9.2\)Hz, C\(_5\)-H), 6.92-6.93 (dd, 2H, Ar-H), 6.99 (dd, 1H, Ar-H), 7.23-7.24 (dd, 2H, Ar-H), 7.30 (s, 1H, Ar-H), 7.40 (dd, 1H, Ar-H). \(^13\)C NMR (CDCl\(_3\)): \(\delta\) 23.4 (1C, 4-\(\mathrm{C}\)), 55.8 (1C, OCH\(_3\)), 55.8 (1C, OCH\(_3\)), 55.8 (1C, OCH\(_3\)), 66.8 (1C, 5-\(\mathrm{C}\)), 112.1 (1C, Ar-\(\mathrm{C}\)), 114.2-114.3 (3C, Ar-\(\mathrm{C}\)), 116.6 (1C, C\(_\mathrm{N}\)), 121.2 (1C, Ar-\(\mathrm{C}\)), 127.1-127.3 (3C, Ar-\(\mathrm{C}\)), 148.7 (1C, Ar-\(\mathrm{C}\)), 149.1 (1C, Ar-\(\mathrm{C}\)), 150.4 (1C, Ar-\(\mathrm{C}\)), 158.9 (1C, Ar-\(\mathrm{C}\)), 164.2 (1C, 3-\(\mathrm{C}\)). MS (relative abundance) m/z: 339 (MH\(^+\), 100), 312 (19), 280 (33), 174 (26), 157 (45). Anal. Cacld. for C\(_{19}\)H\(_{18}\)N\(_2\)O\(_4\), C, 67.44, H, 5.36, N, 8.28%; Found: C, 67.38, H, 5.32, N, 8.21%.

Spectral and elemental analysis data of the few isomeric compounds \(62\) were given below.

**3-(4-Fluorophenyl)-4-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitrile 62a:**

IR (Nujol): 1625 cm\(^{-1}\) C=N (str), 2228 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.80 (s, 3H, OCH\(_3\)), 5.79 (d, 1H, \(J=8.2\)Hz, C\(_4\)-H), 5.40 (d, 1H, \(J=7.2\)Hz, C\(_5\)-H), 6.92-6.95 (dd, 2H, Ar-H), 6.95-6.97 (dd, 2H, Ar-H), 7.40-7.42 (dd, 2H, Ar-H), 7.80-7.82 (dd, 2H, Ar-H). \(^13\)C NMR (CDCl\(_3\)): \(\delta\) 66.6 (1C, 4-\(\mathrm{C}\)), 55.2 (1C, OCH\(_3\)), 31.1 (1C, 5-\(\mathrm{C}\)), 114.1-114.6 (2C, Ar-\(\mathrm{C}\)), 115.4-115.7 (2C, Ar-\(\mathrm{C}\)), 116.4 (1C, C\(_\mathrm{N}\)), 128.5-128.8 (2C, Ar-\(\mathrm{C}\)), 129.4-129.7 (2C, Ar-\(\mathrm{C}\)), 130.4 (1C, Ar-\(\mathrm{C}\)), 130.7 (1C, Ar-\(\mathrm{C}\)), 149.4 (1C, Ar-\(\mathrm{C}\)), 162.2 (1C, 3-\(\mathrm{C}\)), 161.8 (1C, Ar-\(\mathrm{C}\)). MS (relative abundance) m/z: 297(MH\(^+\), 100), 270 (32), 238 (20), 174 (16), 157 (44). Anal. Cacld. for C\(_{17}\)H\(_{13}\)FN\(_2\)O\(_2\), C, 68.91, H, 4.42, N, 9.45%; Found: C, 68.85, H, 4.46, N, 9.37%.
3-(4-Cyanophenyl)-4-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carbonitrile 62e:

IR (Nujol): 1628 cm\(^{-1}\) C=N (str), 2232 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.85 (s, 3H, OCH\(_3\)), 5.62 (d, 1H, \(J=8.0\)Hz, C\(_4\)-H), 5.26 (d, 1H, \(J=7.8\)Hz, C\(_5\)-H), 6.94-6.96 (dd, 2H, Ar-H), 7.24-7.26 (dd, 2H, Ar-H), 7.66-7.68 (dd, 2H, Ar-H), 7.97-8.00 (dd, 2H, Ar-H).

\(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 67.0 (1C, 4-\(\bigcirc\)), 55.7 (1C, OCH\(_3\)), 33.3 (1C, 5-\(\bigcirc\)), 114.6-114.8 (2C, Ar-\(\bigcirc\)), 115.4 (1C, Ar-\(\bigcirc\)), 116.6 (1C, C\(_N\)), 118.2 (1C, C\(_N\)), 126.7-126.9 (2C, Ar-\(\bigcirc\)), 129.0-129.2 (2C, Ar-\(\bigcirc\)), 131.3-131.5 (2C, Ar-\(\bigcirc\)), 132.4 (1C, Ar-\(\bigcirc\)), 136.9 (1C, Ar-\(\bigcirc\)), 159.9 (1C, Ar-\(\bigcirc\)), 163.5 (1C, 3-\(\bigcirc\)). MS (relative abundance) m/z: 304 (MH\(^+\), 100), 277 (21), 245 (30), 174 (23), 157 (41). Anal. Cacl.d. for C\(_{18}\)H\(_{13}\)N\(_3\)O\(_2\), C, 71.28, H, 4.32, N, 13.85%; Found: C, 71.21, H, 4.24, N, 13.79%.

3-(4-Methoxyphenyl)-3-phenyl-4,5-dihydroisoxazole-5-carbonitrile 62g:

IR (Nujol): 1640 cm\(^{-1}\) C=N (str), 2236 cm\(^{-1}\) CN (str). \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 3.88 (s, 3H, OCH\(_3\)), 5.62 (d, 1H, \(J=9.0\)Hz, C\(_4\)-H), 5.23 (d, 1H, \(J=8.4\)Hz, C\(_5\)-H), 6.92-6.94 (dd, 2H, Ar-H), 7.24-7.26 (dd, 2H, Ar-H), 7.56-7.80 (m, 5H, Ar-H). \(^{13}\)C NMR (CDCl\(_3\)): \(\delta\) 67.1 (1C, 4-\(\bigcirc\)), 55.8 (1C, OCH\(_3\)), 33.4 (1C, 5-\(\bigcirc\)), 114.5 (2C, Ar-\(\bigcirc\)), 117.4 (1C, C\(_N\)), 127.4 (2C, Ar-\(\bigcirc\)), 128.9 (2C, Ar-\(\bigcirc\)), 129.4 (2C, Ar-\(\bigcirc\)), 131.5 (1C, Ar-\(\bigcirc\)), 134.2 (1C, Ar-\(\bigcirc\)), 135.9 (1C, Ar-\(\bigcirc\)), 162.9 (1C, Ar-\(\bigcirc\)), 164.8 (1C, 3-\(\bigcirc\)). MS (relative abundance) m/z: 279 (MH\(^+\), 100), 252 (22), 220 (30), 174 (20), 157 (38). Anal. Cacl.d. for C\(_{17}\)H\(_{14}\)N\(_2\)O\(_2\), C, 73.37, H, 5.07, N, 10.07%; Found: C, 73.30, H, 5.01, N, 10.02%.

2.7.0 Conclusion:

A facile and convenient route of synthesis for substituted isoxazolines based on the reactions of aromatic aldoximes with 4-methoxy cinnamonitrile in the presence of chromium oxide and/or chloramine-T has been developed. The present method is concise and efficient. We have used a transition metal (chromium) oxidant; the
describing procedure is environmental friendly because of simple procedure, easy work up process, short reaction time, easy set-up and separation of oxidant, mild conditions and appreciable yields are observed. Additionally the recycling of the oxidant ranks the described method of oxidation as a powerful and "green" tool in modern organic synthesis. A comparative study of two oxidants has been performed and revealed that chromium oxide is more efficient and effective for oxidation of aromatic aldoximes to nitrile oxides.