CHAPTER 5

Synthesis, characterization and lipoxygenase (LOX) inhibition assay of isoxazolines and isoxazoles

5.1 Introduction

Isoxazoles and isoxazolines are heterocyclic compounds bearing nitrogen and oxygen in adjacent position of five membered rings. The various isoxazole derivatives exist as lead component in bioactive natural products (e.g. muscimol and Ibotenic acid) and in many drugs available in market such as valdecoxib, cloxacillin and flucloxacillin (Fig. 1). Also the diversified substituent isoxazoles are demonstrated as analgesic, anti-inflammatory, antifungal, anti-bacterial and anti-cancer agents. Besides the therapeutic applications, the substituted isoxazoles are also served as an efficient substrates in synthesis useful building blocks such as γ-amino alcohols, (Müller and Jäger, 1982) β-hydroxy ketones, (Kozikowski and Stein, 1982) β-hydroxy nitriles. (Moersch et al., 1967) The isoxazolines produced from cycloaddition reaction of nitrile oxide and alkenes are also used as masked β-amino alcohol (Curran, 1983) and β-hydroxy carbonyl aldolate. (Anunziata et al., 1985)

Fig. 1 Structure of drugs bearing isoxazole and isoxazoline
**5.2 Synthesis of isoxazoles and isoxazolines: A literature survey**

The importance of isoxazoles and isoxazolines in both medicinal as well as organic chemistry promote many research groups to develop a new method for their synthesis. The [3+2] dipolar cycloaddition and cyclocondensation reaction are the most commonly utilized protocol for their preparation. The large numbers of simple and robust methods are available in literature for dipolar cycloaddition reaction between nitrile oxide (dipole) and alkynes (dipolarophile). Recently, multicomponent transition metal (Ti, Fe) catalyzed one pot methods have been employed for the synthesis of diversified isoxazoles (Dissanayake and Odom, 2012) and isoxazolines (Debleds et al., 2010) with the advantages of regioselectivity, short reaction time and high yield of product.

The 3,5-aryl/alky disubstituted isoxazoles are prepared from reaction of aldehydes and terminal alkynes using Cu(I) catalyzed highly regioselective one pot protocol. (Hansen et al., 2005) Initially, the aliphatic or aromatic aldehydes (1) are converted to oxime using hydroxylamine hydrochloride, followed by treatment of oximes with chloramines-T, which act as halogenating agent as well as base and afford nitrile oxides. Further, in the presence of copper catalyst, the generated nitrile oxides undergo cycloaddition with acetylene and afford alkyl or aryl substituted isoxazole (2) in excellent yield (Scheme 1).

\[
\begin{align*}
1. \text{NH}_2\text{OH.HCl, NaOH} \\
2. \text{TsN(Cl)Na.3H}_2\text{O} \\
3. \equiv-\text{R}^2, \text{Cu(I)} \\
\text{tert-BuOH.H}_2\text{O (1:1)}
\end{align*}
\]

\[R^1=R^2=\text{Et, n-Pr, t-Bu, C}_6\text{H}_5, p-\text{Me-C}_6\text{H}_4, p-\text{OMe-C}_6\text{H}_4, p-\text{NO}_2-\text{C}_6\text{H}_4, o-\text{Br-C}_6\text{H}_4.\]

**Scheme 1**
Recently, a ruthenium complex has proved as an efficient catalyst for dipolar cycloaddition reaction of internal or terminal alkynes (4) with hydroximoyl chloride (3) at room temperature. (Grecian and Fokin, 2008) The reaction gives 3,5-di and 3,4,5-trisubstituted isoxazoles (5) with excellent regioselectivity (Scheme 2).

\[
\begin{align*}
\text{3} & \quad \text{4} & \quad \text{5} \\
\text{R}= \text{H, Cl, OMe; } R^1= \text{C}_6\text{H}_5, \text{p-Me-C}_6\text{H}_4, \text{p-Cl-C}_6\text{H}_4, \text{-CH}_2\text{OH; } \\
R^2= \text{H, -CO}_2\text{Me, C}_6\text{H}_5, \text{CH}_2\text{OH; } \text{Cp}^*= \text{C}_5\text{Me}_5, \text{cod= cycloocta-1,5-diene}
\end{align*}
\]

Scheme 2

Since the use of toxic metal in drug synthesis will remain contact with the end products and cause severe health problems. (Rana, 2008) Therefore, the metal free methods involving cycloaddition reaction of nitrile oxide and olefins was utilized from past several years. The nitrile oxides are usually generated in-situ from hydroximinoyl chlorides, which are prepared from corresponding aldoximes using various electrophilic chlorine, bromine, iodine containing reagents such as NaOCl, (Kizer et al., 1999) Cl₂, (Kanemasa et al., 2000) \textit{N}-chlorosuccinimide (NCS), (Liu et al., 1980) \textit{N}-bromosuccinimide (NBS) (Baruah et al., 1988) and \textit{tert}-butyl hypoiodite (\textit{tert}-BuOI). (Minakata et al., 2011)

The sodium bromite in presence of catalytic amount of tributyl tin chloride used as efficient oxidizing agent for the preparation of isoxazolines (7) and isoxazoles (8) from aldoximes (6) through dipolar cycloaddition reaction (Scheme 3i). (Moriya et al., 1989) Later ceric ammonium nitrate (CAN) was demonstrated as efficient reagent for oxidative transformation of alkenes and alkynes (9) to 3-acetyl substituted isoxazolines (11) and
isoxazoles (10) respectively in good yield at reflux temperature (Scheme 3ii). (Itoh and Horiuchi, 2004)

\[ R^1 = \text{C}_6\text{H}_5, \text{CH}_3; R^2 = \text{C}_6\text{H}_5, \text{CO}_2\text{H}_3, \text{CO}_2\text{C}, \text{CN}; \\
R^3 = \text{H}, \text{Br}, \text{CO}_2\text{C}_2\text{H}_5; R^4 = \text{C}_6\text{H}_5, \text{OC}_6\text{H}_5 \\
(i) = \text{NaBrO}_2, \text{Bu}_3\text{SnCl}, \text{CH}_2\text{Cl}_2\cdot\text{H}_2\text{O} \]

\[ R^1 = \text{n-C}_4\text{H}_9, \text{n-C}_3\text{H}_{11}, \text{n-C}_6\text{H}_{13}, \text{n-C}_4\text{H}_9, \text{CH}_2\text{OCOMe}; R^2 = \text{Me}, \text{Ph} \\
(ii) = \text{CAN(IV)}, \text{acetone or acetophenone}, \Delta \]

**Scheme 3**

The environmentally benign solid phase method was also utilized for the synthesis of isoxazolines. (Shankar et al., 1998) The method involve the 1,3-dipolar cycloaddition reaction of resin bound \( p \)-hydroxy benzaldehyde. Initially, resin bound aldehyde (12) was converted to oxime (13) using hydroxylamine hydrochloride in pyridine at room temperature, followed by in-situ generation of nitrile oxide using \( N \)-chlorosuccinimide (NCS) and, to this substituted alkenes was added. Finally, the formed isoxazolines (14) are cleaved from resin using trifluoroacetic acid (Scheme 4).
The chloramine-T described as an effective agent for the preparation of isoxazoline (17) via 1,3-dipolar cycloaddition reaction of oxime (15) and safrole (16) (Scheme 5) (Hassner and Lokanatha Rai, 1989).

**Scheme 5**

The α,β-unsaturated oximes (19, prepared from corresponding chalcones 18) was also utilized as a useful substrate for the synthesis of isoxazoles (20) through the oxidative cyclization process, for instance, iodine/potassium iodide (Buechi and Vederas, 1972) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Desai and Tilve, 1999) have proved as best oxidizing agent for cyclization (Scheme 6).

**Scheme 6**

The convenient and step economic method was developed for the synthesis of 3,5-di and 3-substituted isoxazoles from easily accessible starting substrates. (Tang et al., 2009) The presented protocol involve the initial regioselective conjugate addition of N-hydroxy-4-
toluenesulfonamide (22) to various $\alpha, \beta$-unsaturated aldehydes and ketones (21 & 24), and final product isoxazole (23 & 25) was obtained after base mediated cyclization and dehydration of the intermediate obtained (Scheme 7).

![Scheme 7](image)

The rarely used oxidation reaction for the preparation of isoxazole (28), involve the simple two step reaction procedure such as amination of commercially available 3-Br-isoxazolines (26) followed by oxidation of 27 using iodine and imidazole (Scheme 8). (Girardin et al., 2009)

![Scheme 8](image)

The gem-dihalomethylarenes have shown the wide applications and efficiently replace the aromatic aldehydes in the synthesis of different kinds of biologically important heterocycles. The preparation of gem-dihalomethylarenes involves a cost effective method using easily available, low cost starting substrates and reagents such as methyl
arenes, N-bromosuccinimide and benzoyl peroxide as catalyst. As part of the synthetic applications, the gem-dibromomethylarenes are successfully used for the synthesis of aldehydes, (Wang et al., 2011) α, β-unsaturated carboxylic acids, (Augustine et al., 2007) oximes (Chandrappa et al., 2012) and, heterocycle such as benzimidazoles (Siddappa et al., 2010) and benzothiazoles. (Siddappa et al., 2011)

Lipoxygenases (LOX) belongs to the family of iron-containing enzymes. These enzymes effectively catalyze the addition reaction of oxygen to unsaturated fatty acids found in lipids. Lipoxygenases are found in plants, animals, fungi, bacteria and generates many signaling molecules and control the metabolic functions in cells. Further, Lipoxygenases are played an important role and responsible for the biosynthesis of leukotrienes. Leukotriene is the lipid mediators, the over production of these causes many diseases such as inflammation, allergy, cancer and cardiac arrest. Hence the development of potent therapeutics for the inhibition of LOX has wide scope in bioorganic and medicinal chemistry. This work aims for the synthesis of isoxazoline and isoxazole derivatives using 1,3-dipolar cycloaddition reaction and all the compounds evaluated for lipoxygenase inhibition.

5.3 Result and discussion

The isoxazolines (32, Table 1) were prepared using a convenient two step protocol such as (i) condensation reaction of aldehyde (29) with hydroxylamine hydrochloride in presence of triethylamine affords the oxime (30) and (ii) the 1,3-dipolar cycloaddition reaction of safrole (31) with nitrile oxide (generated in-situ from oximes using N-chlorosuccinimide and triethylamine). The product (32a-c) are obtained in good yield and evaluated for LOX inhibition assay.
The initial effort for the synthesis of isoxazole (35a) using one pot strategy was begin with the reaction of gem-dibromomethylarene (33a) and hydroxylamine hydrochloride (2.0 equiv.) in presence of triethylamine (2.0 equiv.) in anhydrous DMF (Scheme 10). The reaction affords the expected oxime after 4 h at 60 °C (monitored by TLC). The reaction mixture was cooled to room temperature, added N-chlorosuccinimide (1.2 equiv.) and stirred for 12 h. The solution of enamine of ethyl acetoacetate (34) and triethylamine in ethanol was added at 0 °C and stirred at room temperature. The reaction was monitored at regular interval and after 16 h the expected product (35a) was isolated in good yield (78%). The optimized reaction condition is utilized for the synthesis of 3-aryl-5-methyl isoxazoles (35, Table 2) through one pot strategy and isolated yield of all the compounds are in range of 71-88%. The plausible mechanism is discussed in scheme 11.
Scheme 10 The one pot synthesis of isoxazoles from *gem*-dibromomethylarene

Table 2 Synthesis of 3-ary-5-methyl Isoxazoles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
</tr>
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<tbody>
<tr>
<td>35a</td>
<td>78%</td>
</tr>
<tr>
<td>35b</td>
<td>83%</td>
</tr>
<tr>
<td>35c</td>
<td>71%</td>
</tr>
<tr>
<td>35d</td>
<td>84%</td>
</tr>
<tr>
<td>35e</td>
<td>86%</td>
</tr>
<tr>
<td>35f</td>
<td>88%</td>
</tr>
</tbody>
</table>

All the synthesized compounds were screened for lipoxygenase inhibition assay using linoleic acid as substrate and indomethacin as standard or control. The inhibition of enzyme activity at different concentration of synthesized compounds was determined using colorimetric method (Table 3). Among the tested compound, the isoxazoline (32c) bearing with 4-(2-pyridyl)-phenyl substituent showed good activity (IC₅₀ = 1.75 μM) while the other compounds such as 32b and 35b bearing P-CF₃-phenyl substituent at 3rd position of isoxazoline and isoxazole showed moderate activity. The percentage of lipoxygenase inhibition of all the synthesized compounds at different concentration is represented in Fig. 2.
Table 3 IC<sub>50</sub> values of the synthesized compounds

<table>
<thead>
<tr>
<th>Entry</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32a</td>
<td>2.98</td>
</tr>
<tr>
<td>32b</td>
<td>3.41</td>
</tr>
<tr>
<td>32c</td>
<td>1.75</td>
</tr>
<tr>
<td>35a</td>
<td>6.42</td>
</tr>
<tr>
<td>35b</td>
<td>4.15</td>
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<tr>
<td>35c</td>
<td>3.84</td>
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<td>35d</td>
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<tr>
<td>35e</td>
<td>8.91</td>
</tr>
<tr>
<td>35f</td>
<td>15.51</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Fig. 2 The lipoxygenase inhibition assay
5.4 Conclusion

The convenient one pot protocol was developed for the synthesis of 3-aryl-5-methyl-isoxazoles from gem-dibromomethylarenes. This method has several advantages compared to previous methods and here we have simplified the preparation of isoxazoles from low cost starting substrates by combining three reaction sequences (such as formation of oxime, chlorination of oxime and 1,3-dipolar cycloaddition reaction) in one pot method. Further, isoxazolines also were prepared from 1,3-dipolar cycloaddition reaction of safrole and nitrile oxides (which are generated *in situ* from oximes) using stepwise protocol. All the products obtained either in one pot or stepwise methods were isolated in good to excellent yield. The synthesized compounds were evaluated for lipoxygenase inhibition assay. Among the different tested compounds, the isoxazoline (32c) bearing with 4-(2-pyridyl)-phenyl substituent showed a potent activity and while remaining compounds are moderately inhibits the activity of lipoxygenase.

**Scheme 11** Proposed mechanism for one pot synthesis of isoxazoles
5.5 Experimental section

5.5.1 General procedure for the preparation of isoxazolines (32)
In a typical experiment the solution of aldehyde (29, 0.002 mol), hydroxylamine hydrochloride (0.002 mol) and triethylamine (0.0025 mol) in DMF was stirred at room temperature for 6 h. After completion of reaction (monitored by TLC), crushed ice was added to the reaction mixture and precipitated solid product (30) was filtered, washed with hexane and dried. N-chlorosuccinimide (0.0015 mol) and triethylamine (0.0015 mol) was added to the solution of oxime (30, 0.001 mol) and safrole (31, 0.0012 mol) in DMF at 0 °C. The reaction mixture was stirred at room temperature for 12 h. After completion of reaction water was added and extracted with ethyl acetate. The combined organic layer was dried and concentrated under reduced pressure. The obtained crude product (32) was purified using silica gel column chromatography.

5.5.2 General procedure for the preparation of 3-aryl-5-methyl isoxazoles (35)
In a typical experiment, the solution of gem-dibromomethylarenes (33, 0.002 mol), hydroxylamine hydrochloride (0.002 mol) and triethylamine (0.004 mol) in anhydrous DMF was refluxed at 90 °C for 4 h. The reaction mixture was cooled to 0 °C and added N-chlorosuccinimide (0.0025 mol). After overnight stirring at room temperature the solution of (E)-ethyl-3-(pyrrolidin-1-yl)-but-2-enoate (34, 0.0025 mol) and triethylamine (0.001 mol) in ethanol was added drop-wisely. The reaction mixture was allowed to stir at room temperature for 16 h. After completion of reaction (monitored by TLC), water was added and extracted with ethyl acetate. The combined organic layer was washed with brine solution, water and dried over anhydrous sodium sulfate. The solvent was
evaporated and obtained crude product was purified using silica gel column chromatography.

5.5.3 General procedure for the preparation of (E)-ethyl-3-(pyrrolidin-1-yl)-but-2-enoate (34)

In a typical experiment, the solution of ethyl-2-butynoate (0.01 mol) and pyrrolidine (0.01 mol) in tert-butanol was refluxed at 70 °C for 15 h. After completion of reaction solvent was removed under reduced pressure and obtained product (34, brown liquid) was utilized directly for preparation of 35.

5.5.4 X-ray crystal structure determination of compound 32c

A single crystal of the compound (32c) with dimensions of 0.30 × 0.25 × 0.20 mm was chosen for X-ray diffraction studies. The data were collected on a Bruker SMART APEX II X-ray diffractometer with graphite monochromated MoKα radiation, operating at 50 kV and 30 mA. Raw data was processed and reduced by using APEX2 and SAINT. The crystal structure was solved by direct methods using SHELXS-97. All non-hydrogen atoms were revealed in the first Fourier map itself. Anisotropic refinement of non-hydrogen atoms was started at this stage. Subsequent refinements were carried out with anisotropic thermal parameters for non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms which were placed at chemically acceptable positions. Full-matrix least squares refinement was carried out using SHELXL-97 with a final residual values of R1 = 0.0523. The ortep diagram of the molecule (32c) is represented in Fig. 3. The crystal structure analysis showed that the compound (32c) crystallizes in monoclinic system under the space group P2₁/c, with cell parameters a = 17.548(5) Å, b = 8.719(3) Å, c = 12.237(4) Å, β = 108.380(9)° and Z=4. The bond lengths and bond angles agree
with the standard values and are comparable to a related structure. The benzodioxole and isoxazoline is bridged by the carbon atom; makes a dihedral angle of 38.03 (9)º. The isoxazoline is in same plane with the benzene ring as indicated by the dihedral angle angle of 17.32 (10)º.

**Fig. 3** ORTEP diagram of the molecule 32c at 50 % probability

### 5.5.5 Lipoxygenase inhibition assay of the synthesized compounds

The assay mixture contained 80 mM linoleic acid as a substrate and sufficient amount of soya bean lipooxygenase enzyme in 50 mM sodium phosphate buffer (pH-6.3). The reaction was initiated by the addition of enzyme buffer mixture to substrate (linoleic acid) and the enzyme activity was monitored using UV-visible spectrophotometer at 234 nm. In the inhibition studies, the activities were measured by incubating various concentrations of test compounds with enzyme buffer mixture for 2 minutes before addition of the substrate. The assay was performed in triplicate and mean values were used for the calculation. The IC$_{50}$ values (Table 3) were calculated using one way Annova method. Percentage inhibition was calculated by comparing decrease in absorbance of the test compounds with that of control enzyme activity (Fig. 1). The activity of LOX was compared with the standard positive control, Indomethacin. (Shinde et al., 1999)
5.6 Characterization data

5-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (32a). (Rai et al., 1992)

Obtained as white solid from 29a; $^1$H NMR (400 MHz, CDCl$_3$) $\delta =$ 6.87 (s, 2H, Ar-H), 6.76 (d, $J = 5.6$ Hz, 2H, Ar-H), 6.70 (d, $J = 8$ Hz, 1H, Ar-H), 5.94 (s, 2H, -CH$_2$-), 4.94 (t, $J = 8.4$ Hz, 1H, -CH), 3.87 (s, 9H, -(OCH$_3$)$_3$), 3.30 (dd, $J = 10$ and 16.4 Hz, 1H, -CH), 2.98-3.10 (m, 2H, -CH$_2$), 2.81 (dd, $J = 7.2$ and 14 Hz, 1H, -CH) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta =$ 149.90, 149.61, 154.15, 147.43, 134.18, 129.35, 129.21, 129.16, 124.11, 122.25, 121.07, 120.15, 107.83, 78.26, 55.62, 50.11, 40.12 ppm.

5-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-(trifluoromethyl)phenyl)-4,5-dihydroisoxazole (32b).

Obtained as white solid from 29b; IR (KBr) 2926, 2858, 1588, 1569, 1254 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta =$ 7.75 (d, $J = 8$ Hz, 2H, Ar-H), 7.64 (d, $J = 8$ Hz, 2H, Ar-H), 6.76 (d, $J = 5.2$ Hz, 2H, Ar-H), 6.70 (d, $J = 8$ Hz, 1H, Ar-H), 5.94 (s, 2H, -CH$_2$-), 4.99 (t, $J = 8.4$ Hz, 1H, -CH), 3.32 (dd, $J = 10.4$ and 16.8 Hz, 1H, -CH), 3.01-3.10 (m, 2H, -CH$_2$), 2.84 (dd, $J = 6.8$ and 14 Hz, 1H, -CH) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta =$ 149.89, 149.58, 147.38, 133.47, 132.10, 129.24, 129.18, 129.08, 127.48, 124.14, 123.78, 120.11, 107.71, 78.28, 50.07, 40.15 ppm; HRMS (ESI) m/z calcd for C$_{18}$H$_{14}$F$_3$NO$_3$ [M]$^+$ 349.0926; Found 372.0854.
5-(benzo[d][1,3]dioxol-5-ylmethyl)-3-(4-(pyridin-2-yl)phenyl)-4,5-dihydroisoxazole (32c).

Obtained as white solid from 29c; IR (KBr) 2922, 2853, 1583, 1571, 1240 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 8.71\) (d, \(J = 4.4\) Hz, 1H, Ar-H), 8.04 (d, \(J = 8.6\) Hz, 2H, Ar-H), 7.75 (t, \(J = 7\) Hz, 4H, Ar-H), 6.71-6.78 (m, 4H, Ar-H), 5.94 (s, 2H, \(-\text{CH}_2\)-), 4.96 (t, \(J = 10.2\) and 16.6 Hz, 1H, -CH), 3.05-3.12 (m, 2H, \(-\text{CH}_2\)-), 2.73 (s, 3H, \(-\text{CH}_3\)) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta = 149.91, 149.67, 147.45, 141.41, 133.62, 133.56, 132.12, 129.20, 129.13, 127.89, 124.07, 123.89, 120.41, 107.68, 78.25, 50.01, 40.12 ppm; HRMS (ESI) \(m/z\) calcd for C\(_{22}\)H\(_{18}\)N\(_2\)O\(_3\) [M]+ 358.1317; Found 358.1317 (M+Na).

Ethyl 5-methyl-3-phenylisoxazole-4-carboxylate (35a). (Zhu et al., 2011)

Obtained as yellow oil from 33a; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.61\) (d, \(J = 7.6\) Hz, 2H, Ar-H), 7.45 (t, \(J = 6.8\) Hz, 3H, Ar-H), 7.77-7.72 (m, 4H, Ar-H), 7.24 (t, \(J = 2.6\) Hz, 2H, Ar-H), 4.26-4.21 (q, \(J = 7.2\) and 14.4 Hz, 2H, O-\(\text{CH}_2\)), 2.73 (s, 3H, -CH\(_3\)), 1.22 (t, \(J = 7.2\) Hz, 3H, -CH\(_3\)) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta = 175.75, 162.58, 161.98, 129.69, 129.37, 128.52, 127.94, 108.49, 60.68, 13.95, 13.54 ppm;

Ethyl 5-methyl-3-(4-(trifluoromethyl)phenyl)isoxazole-4-carboxylate (35b).

Obtained as white solid from 33b; IR (KBr) 2954, 2861, 1958, 1732, 1314, 1242 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.76\) (d, \(J = 8\) Hz, 2H, Ar-H), 7.70 (d, \(J = 8\) Hz, 2H, Ar-H), 4.25 (q, \(J = 7.2\) and 14 Hz, 2H, O-\(\text{CH}_2\)), 2.75 (s, 3H, -CH\(_3\)), 1.24 (t, \(J = 7.2\)
Hz, 3H, -CH₃) ppm; ¹³C NMR (100 MHz, CDCl₃) δ = 176.17, 161.64, 132.15, 131.86, 131.53, 129.87, 125.26, 124.92, 108.44, 60.91, 13.95, 13.61 ppm; HRMS (ESI) m/z calcd for C₁₄H₁₂F₃NO₃ [M+H]⁺ 300.04; Found 300.54.

**Ethyl 5-methyl-3-(4-(pyridin-2-yl)phenyl)isoxazole-4-carboxylate (35c).**

Obtained as white solid from 33c; IR (KBr) 2950, 2855, 1961, 1728, 1324, 1238 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ = 8.70 (t, J = 2.2 Hz, 1H, Ar-H), 8.06 (t, J = 5.2 Hz, 2H, Ar-H), 7.77-7.72 (m, 4H, Ar-H), 7.24 (t, J = 2.6 Hz, 2H, Ar-H), 4.26-4.21 (m, 2H, O-CH₂), 2.72 (s, 3H, -CH₃), 1.24-1.20 (m, 3H, -CH₃) ppm; ¹³C NMR (100 MHz, CDCl₃) δ = 175.90, 162.14, 161.95, 156.57, 149.62, 140.38, 136.94, 129.82, 129.10, 126.44, 122.46, 120.71, 108.53, 60.74, 14.03, 13.54 ppm; HRMS (ESI) m/z calcd for C₁₈H₁₆N₂O₃ [M+H]⁺ 309.10; Found 309.48.

**Ethyl 5-methyl-3-(naphthalen-1-yl)isoxazole-4-carboxylate (35d).**

Obtained as white solid from 33d; IR (KBr) 2943, 2861, 1757, 1440, 1217 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ = 7.94 (t, J = 4.8 Hz, 1H, Ar-H), 7.88 (d, J = 7.6 Hz, 1H, Ar-H), 7.63 (d, J = 8 Hz, 1H, Ar-H), 7.52-7.42 (m, 4H, Ar-H), 3.87 (q, J = 8 and 14 Hz, 2H, -OCH₂), 2.81 (s, 3H, -CH₃), 0.61 (t, J = 7 Hz, 3H, -CH₃) ppm; ¹³C NMR (100 MHz, CDCl₃) δ = 175.60, 161.72, 161.65, 133.19, 132.09, 129.79, 128.22, 127.64, 126.69, 126.39, 125.95, 125.21, 124.82, 110.29, 60.25, 13.27, 13.10 ppm; HRMS (ESI) m/z calcd for C₁₇H₁₅NO₃ [M+H]⁺ 282.11; Found 282.57.
Ethyl 3-(4-chlorophenyl)-5-methylisoxazole-4-carboxylate (35e). (Zhu et al., 2011)

Obtained as white solid from 33e; \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.62\) (s, 1H, Ar-H), 7.52-7.50 (m, 1H, Ar-H), 7.44-7.41 (m, 1H, Ar-H), 7.37-7.34 (m, 1H, Ar-H), 4.23 (q, \(J = 7.4\) and 14.2 Hz, 2H, -OCH\(_2\)), 2.72 (s, 3H, -CH\(_3\)), 1.23 (t, \(J = 7\) Hz, 3H, -CH\(_3\)) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta = 176.18, 161.69, 161.33, 133.85, 129.78, 129.60, 129.23, 108.40, 60.84, 13.92, 13.52\) ppm;

Ethyl 3-(4-bromophenyl)-5-methylisoxazole-4-carboxylate (35f).

Obtained as white solid from 33f; IR (KBr) 2957, 2849, 1738, 1434, 1214 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta = 7.56\) (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.50 (d, \(J = 8.4\) Hz, 2H, Ar-H), 4.23 (q, \(J = 7.2\) and 14Hz, 2H, -OCH\(_2\)), 2.72 (s, 3H, -CH\(_3\)), 1.24 (t, \(J = 7.2\) Hz, 3H, -CH\(_3\)) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta = 176.0, 161.75, 161.67, 131.21, 131.0, 127.45, 124.28, 108.34, 60.81, 14.01, 13.58\) ppm; HRMS (ESI) m/z calcd for C\(_{13}\)H\(_{12}\)BrNO\(_3\) [M+H]\(^+\) 310.0; Found 310.06.
5.7 References


APPENDICES
$^{1}$H and $^{13}$C spectra of compound 32c
Mass spectrum of compound 32c

PerkinElmer Spectrum Version 10.03.09
Saturday, February 14, 2015 3:40 PM

IR spectrum of compound 32c
$^1$H and $^{13}$C spectra of compound 35a
$^1$H and $^{13}$C spectra of compound 35f
Mass spectrum of compound 35f