Ethyl Imidazole-1-Carboxylate as a Novel Carbonylating Agent for the Synthesis of 1,2,4-Oxadiazol-5(4H)-Ones and Evaluation of their Anti-Mycobacterial Activity

Indrasena Reddy K¹*, Aruna C², Sudhakar Babu K¹, Yogeeswari P², and Sriram D²

¹Department of Chemistry, Sri Krishnadevaraya University, Anantapur, India
²Department of Pharmacy, Birla Institute of Technology and Science-Pilani, Hyderabad Campus, India

*Corresponding author: Indrasena Reddy K, Department of Chemistry, Sri Krishnadevaraya University, Anantapur, India, Tel: +9104066303506; E-mail: indra11sena@gmail.com

Received: May 06, 2017 Accepted: May 22, 2017; Published: May 27, 2017

Abstract
Various highly substituted 1,2,4-oxadiazol-5(4H)-ones were synthesized from the corresponding amidoximes using ethyl imidazole-1-carboxylate (ElmC) as a novel carbonylation agent. This method is simple and convenient to produce these biologically interesting heterocycles in high yields. Among them, 4a, 4b, 4c, 4e, 4f, 4h, and 4i are identified as lead molecules. In particular, 4e, and 4h are found to display promising activity as good as ciprofloxacin, whereas 4i showed an excellent activity, which is equal to the IC50 value of ethambutol against Mycobacterium tuberculosis.

Keywords: Novel carbonylation agent; Ciprofloxacin; Amidoximes

Introduction
1,2,4-Oxadiazol-5(4H)-ones are among the most important scaffolds in the field of medicinal chemistry. They are acidic heterocycles, which are used as carboxylic acid bio-isosteres [1]. They act as AT1 antagonists [2] COX-2 inhibitors, [3] PLA2 inhibitors, [4] and modulators of GluR [5] phospholipase A2 inhibitors [6] and antimicrobial [7]. The most commonly used method for the synthesis of these heterocycles is the carbonylative cyclization of amidoximes using different carbonylating reagents. A variety of reagents such as hazardous triphosgene [8] poisonous carbon monoxide [9] high temperature reactions using urea [10] moisture sensitive 1,1'-carbonyl di imidazole [11] carbonates [12] and two steps synthesis using chloroformates have been used for this cyclization [13]. By considering the above drawbacks, there is a need to develop a simple method for the carbonylative cyclization of amidoximes into the corresponding benzoxazol-2(3H)-ones.

1,2,4-Oxadiazol-5(4H)-ones are generally synthesized by carbonylative cyclization of amidoximes using different carbonylating reagents such as, 1,1'-carbonyl di imidazole [14] chloroformates [15] alkyl carbonates [16] diphosgene [17]
pentafluoro benzoyl chloride [18-20]. These reagents are either moisture sensitive or hazardous in nature. In order to overcome these difficulties, there is a need to develop simple methodologies for the synthesis of 1,2,4-oxadiazol-5(4H)-ones.

*Mycobacterium tuberculosis* is a major health and challenging problem around the world for more than five millennia. Tuberculosis is an air borne disease and spreads from person to person through tiny droplets discharged into the air. The most commonly affected sites in humans are the lungs. TB can also occur in bones, especially in the spine and at the ends of the long bones. The most disturbing factor in the current TB problem is the occurrence of multidrug resistant (MDR) strains not only to the front-line drugs but also to second line drugs.

The World Health Organization (WHO) estimates that there are approximately 8 million new infections and 3 million deaths attributed to *M. tuberculosis* annually [21-23]. Enhanced sanitation of living condition is significantly compact the frequency of the disease. The expansion of multidrug-resistant TB (MDR-TB) and the emergence of extensively drug-resistant TB (XDR-TB) cause new confront for the prevention, cure and manage of this lethal disease [24]. Therefore, the development of new drugs with enhanced activity against MDR-TB and XDR-TB is highly appreciated for the prevention of the disease.

One of the major problems associated in control of TB is that the restart of the disease in patients who carry a latent syndrome, in which the bacteria is in slow budding or non-growing state and is refractory to treat with predictable anti-TB drugs. Directly observed treatment (DOT) is presently practicing for standard TB chemotherapy. It is well known that the resistance levels are poor in the areas with a strongly performing DOTS programmes [25].

As part of our research on biologically active heterocycles [26], we herein report an efficient method for the synthesis of 1,2,4-oxadiazol-5(4H)-ones derivatives.

**Results and Discussion**

Following our interest on the synthesis and biologically evaluation of novel heterocycles, we herein report an efficient method for the synthesis of 1,2,4-oxadiazol-5(4H)-ones from the carbonylation of amidoximes using ethyl imidazole-1-carboxylate as a novel reagent. It offers several advantages such as ease of handling and compatible with acid sensitive substrates. Imidazole is only the by-product whereas HCl is the by-product, if chloroformate is used as a carbonylating agent.

**Chemistry**

In the process of exploring the application of ethyl imidazole-1-carboxylate as a novel carbonylating reagent, we were interested to synthesize 1,2,4-oxadiazol-5(4H)-ones from the corresponding amidoximes. The required amidoximes were synthesized from the corresponding nitriles using known procedure (Supporting Information).

**SCHEME 1**

Initially, we performed the reaction of benzamidoxime (3a) with EImC (2) in THF/K₂CO₃ (Potassium Carbonate) (entry 1, TABLE 1) at room temperature. However, uncyclized carbamate was isolated as a sole product. Up on heating the reaction at 80°C, the desired product 4a was isolated in 88% yield (entry 2, TABLE 1). The reaction was further studied with different bases such as NaOMe, NaH, and t-BuOK. Among these, NaOMe in THF (TABLE 2, entry 3) at 60°C gave the desired oxadiazolone (4a) in 45% yield, without leaving any uncyclized product. Though the reaction proceeds at room temperature in the presence of either NaH, or t-BuOK, the desired product 4a was obtained in moderate yields (entries 4-7, TABLE 1). Of various solvents such as THF, DMF and toluene, THF gave the best results (TABLE 1). Therefore, 1,2,4-oxadiazol-5(4H)-
ones (4a-4j) were synthesized from amidoximes and ethyl imidazole-1-carboxylate in the presence of K$_2$CO$_3$ (Potassium Carbonate) in THF at 80°C (SCHEME 1).

![Scheme 1](image)

**SCHEME 1. Synthesis of 3-phenyl-1,2,4-oxadiazol-5(4H)-one.**

**TABLE 1. Optimization of the reaction using different bases and solvents.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Temp (°C)</th>
<th>Time (h)</th>
<th>$5^a$</th>
<th>$6^b-k^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K$_2$CO$_3$</td>
<td>25</td>
<td>15</td>
<td>92$^a$</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>K$_2$CO$_3$</td>
<td>80</td>
<td>18</td>
<td>0</td>
<td>88$^b$</td>
</tr>
<tr>
<td>3</td>
<td>NaOMe</td>
<td>60</td>
<td>14</td>
<td>0</td>
<td>45$^b$</td>
</tr>
<tr>
<td>4</td>
<td>NaH</td>
<td>RT</td>
<td>7</td>
<td>0</td>
<td>89$^b$</td>
</tr>
<tr>
<td>5</td>
<td>tBuOK</td>
<td>RT</td>
<td>15</td>
<td>0</td>
<td>52$^b$</td>
</tr>
<tr>
<td>6</td>
<td>NaH</td>
<td>RT</td>
<td>8</td>
<td>0</td>
<td>68$^c$</td>
</tr>
<tr>
<td>7</td>
<td>NaH</td>
<td>RT</td>
<td>10</td>
<td>0</td>
<td>35$^a$</td>
</tr>
</tbody>
</table>

$^a$ Isolated yield;
$^b$ THF was used as a solvent;
$^c$ DMF was used as a solvent;
$^d$ Toluene was used as a solvent.
TABLE 2. Preparation of synthesis of 1,2,4-oxadiazol-5(4H)-one (4a-4j).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Yielda</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="3a" /></td>
<td><img src="image" alt="4a" /></td>
<td>74</td>
</tr>
<tr>
<td>2</td>
<td><img src="image" alt="3b" /></td>
<td><img src="image" alt="4b" /></td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td><img src="image" alt="3c" /></td>
<td><img src="image" alt="4c" /></td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td><img src="image" alt="3d" /></td>
<td><img src="image" alt="4d" /></td>
<td>78</td>
</tr>
<tr>
<td>5</td>
<td><img src="image" alt="3e" /></td>
<td><img src="image" alt="4e" /></td>
<td>72</td>
</tr>
<tr>
<td>6</td>
<td><img src="image" alt="3f" /></td>
<td><img src="image" alt="4f" /></td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td><img src="image" alt="3g" /></td>
<td><img src="image" alt="4g" /></td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td><img src="image" alt="3h" /></td>
<td><img src="image" alt="4h" /></td>
<td>91</td>
</tr>
<tr>
<td>9</td>
<td><img src="image" alt="3i" /></td>
<td><img src="image" alt="4i" /></td>
<td>89</td>
</tr>
<tr>
<td>10</td>
<td><img src="image" alt="3j" /></td>
<td><img src="image" alt="4j" /></td>
<td>76</td>
</tr>
</tbody>
</table>

a Isolated yield

Thus, synthesized compounds (4a-4j) were screened for their in vitro anti-mycobacterial activity against *M. tuberculosis* H37Rv (MTB) by agar dilution method recommended by National Committee for Clinical Laboratory Standards for the determination of MIC values of the synthesized compounds along with standard drugs Isoniazid, Ethambutol and Ciprofloxacin. The comparative results are presented in TABLE 3. In the present study, oxadiazol-5(4H)-ones (4a-4j) were
screened against *M. tuberculosis*. The observed MIC values are ranging from 1.56 µg/mL to 50.0 µg/mL. Compounds 4a, 4b, 4c, 4e, 4f, 4h and 4i displayed significant activity, whereas compounds 4a, 4b, 4c and 4f showed moderate activity. Among them, 4e and 4h showed promising activity which is equal to the IC50 value of ciprofloxacin whereas 4i is equally effective as ethambutol. Based on MIC values, we deduce the structure-activity relationship by the influence of substituent present on 1,2,4-oxadiazol-5(4H)-one skeleton (COMPOUNDS 4a-4j). The presence of symmetrical di substituents like 3,5-diCF3, and 3,5-diF, the compounds are moderately active. Among meta-substituents such as m-Fluoro, m-CH3, m-CF3, the m-CH3 is more active. Similarly, p-CF3 is ineffective. Among disubstituted compounds like 2-methyl-4-chloro-, 2-CF3-4-chloro-, the later compound is more active. Interestingly, the compound without a substituent is more potent than all other compounds tested.

### TABLE 3. Anti-mycobacterial activity studies of compounds (4a-4j).

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Yielda (%)</th>
<th>Clog Pb</th>
<th>MIC (µg/mL)</th>
<th>MIC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>74</td>
<td>3.145</td>
<td>6.25</td>
<td>47.68</td>
</tr>
<tr>
<td>4b</td>
<td>81</td>
<td>4.235</td>
<td>6.25</td>
<td>31.68</td>
</tr>
<tr>
<td>4c</td>
<td>86</td>
<td>0.738</td>
<td>6.25</td>
<td>30.74</td>
</tr>
<tr>
<td>4d</td>
<td>78</td>
<td>2.291</td>
<td>50</td>
<td>4.21</td>
</tr>
<tr>
<td>4e</td>
<td>72</td>
<td>2.975</td>
<td>3.125</td>
<td>84.66</td>
</tr>
<tr>
<td>4f</td>
<td>82</td>
<td>2.262</td>
<td>6.25</td>
<td>36.82</td>
</tr>
<tr>
<td>4g</td>
<td>87</td>
<td>1.522</td>
<td>25</td>
<td>7.20</td>
</tr>
<tr>
<td>4h</td>
<td>91</td>
<td>1.878</td>
<td>3.125</td>
<td>56.37</td>
</tr>
<tr>
<td>4i</td>
<td>89</td>
<td>1.379</td>
<td>1.56</td>
<td>103.94</td>
</tr>
<tr>
<td>4j</td>
<td>86</td>
<td>2.262</td>
<td>50</td>
<td>4.60</td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
<td>0.05</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td></td>
<td>1.56</td>
<td>7.63</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>3.13</td>
<td>9.44</td>
<td></td>
</tr>
</tbody>
</table>

### Experimental Section

#### General methods

Melting points reported in this work were recorded in capillary tubes on a Elchem lab melting point apparatus and uncorrected. 1H and 13C NMR were recorded on Bruker FT-NMR spectrometer either 300 MHz or 400 MHz. using 5 mm PABBO BB-1H tubes. 1HNMR spectra were recorded using approximately 0.03 M solutions in CDCl3 with TMS as an internal reference. 13C NMR spectra were recorded using approximately 0.05 M solutions in CDCl3 at 100 MHz or 125 MHz. Chemical shift values were reported in parts per million (δ ppm) from internal standard TMS. UV-visible spectra were recorded on SYSTRONIC AU-2701 UV-Vis spectrophotometer. All reagents were purchased from Aldrich and used as received. Solvents were removed under reduced pressure on a rotavapour. Organic extracts were dried over anhydrous
**General procedure for the preparation of 4a-4j**

To a solution of Benzonitrile (1 mmol) in EtOH (10 vol), NH$_2$OH.HCl (Hydroxylamine Hydrochloride) (1.5 mmol) was added and allowed it to stir for 3h at 80°C, and then cool to room temperature. The excess of ethanol was removed by under vaccum. The resulting solution was dissolved in THF was added K$_2$CO$_3$ (Potassium Carbonate) (1.5 eq) and EImC (1 eq) and then stirred for 12 h at 80°C. The excess of THF was removed under reduced pressure and then the mixture was quenched with water and then neutralize with dil HCl. Solid was obtained and then filter and dried under vaccum.

**COMPOUND 4a. 3-(3,5-bis(trifluoromethyl)phenyl)-1,2,4-oxadiazol-5(4H)-one.**

Off white solid, 92% yield; m.p. 168°C to 170°C; 1H NMR (400 MHz, CDCl$_3$): δ 8.41 (s, 2H), 8.03 (s, 1H), 13C NMR (70 MHz, CDCl$_3$+DMSO): δ 159.2, 154.6, 137.9, 131.3, 130.8, 127.9, 123.9, 123.5, 120.3. IR: ν max 3511, 1735, 1629, 1479, 1262, 953 cm$^{-1}$. LCMS: m/z 297.2 [MH$-$].

**COMPOUND 4b. 3-(3,5-difluorophenyl)-1,2,4-oxadiazol-5(4H)-one.**

White solid, 94% yield; m.p. 176°C to 178°C; 1H NMR (300 MHz, CDCl$_3$): δ 7.35 (m, 2H), 7.08 (m, 1H); 13C NMR (70 MHz, DMSO+CDCl$_3$): δ 159.6, 156.5, 131.0, 128.1, 125.2, 122.6; IR: ν max 3154, 1765, 1641, 1454, 1162, 851 cm$^{-1}$. LCMS: m/z 197.2 [MH$-$].

**COMPOUND 4c. 3-(2-methoxyphenyl)-1,2,4-oxadiazol-5(4H)-one.**
Brown solid, 89% yield; m.p. 198°C to 200°C; 1H NMR (300 MHz, DMSO): δ 11.50 (s, 1H), 7.82 (d, J=7.9 Hz, 1H), 7.52 (m, 1H), 7.06 (m, 2H); 13C NMR (70 MHz, DMSO/CDCl₃): δ 159.4, 156.3, 134.6, 131.0, 129.5, 126.7, 123.4, 120.6, 52.8; IR: vₘₐₓ 3152, 1763, 1608, 1479, 1150, 851 cm⁻¹. LCMS: m/z 193.2 [MH⁺].

![Image of compound 4d]

**COMPOUND 4d. 3-(5-chloro-2-methylphenyl)-1,2,4-oxadiazol-5(4H)-one.**

Off White solid, 91% yield; m.p. 159°C to 161°C; 1H NMR (400 MHz, CDCl₃): δ 7.54 (d, J=2 Hz 1H), 7.44 (dd, J=2, 8.4 Hz, 1H), 7.30 (d, J=8.4 Hz, 1H), 2.54 (s, 3H); 13C NMR (70 MHz, DMSO/CDCl₃): δ 159.4, 156.4, 135.8, 132.3, 130.9, 130.4, 127.8, 123.7. IR: vₘₐₓ 3269, 3082, 1632, 1498, 1269, 940 cm⁻¹. LCMS: m/z 209.4 [MH⁻].

![Image of compound 4e]

**COMPOUND 4e. 3-(5-chloro-2-(trifluoromethyl) phenyl)-1,2,4-oxadiazol-5(4H)-one.**

Off White solid, 93% yield; m.p. 179°C to 181°C; 1H NMR (400 MHz, CDCl₃): δ 10.05 (b, 1H), 7.82 (d, J=8.4 Hz 1H), 7.74 (m, 2H); 13C NMR (70 MHz, DMSO/CDCl₃): δ 160.9, 156.9, 132.8, 129.2, 126.4, 125.4, 122.8, 121.5. IR: vₘₐₓ 3169, 3022, 1732, 1428, 1369, 840 cm⁻¹. LCMS: m/z 263.3 [MH⁻].

![Image of compound 4f]

**COMPOUND 4f. 6-methyl-3H-[1,2,4] oxadiazolo[4,3-f]phenanthridin-3-one.**

Brown solid, 93% yield; m.p. 182°C to 184°C; 1H NMR (400 MHz, CDCl₃): δ 1150 (b, 1H), 8.14 (s, 1H), 7.97 (d, J=8 Hz, 1H), 7.87 (d, J=7.6 Hz, 1H), 7.72 (t, J=8 Hz, 1H); 13C NMR (70 MHz, DMSO/CDCl₃): δ 159.8, 155.1, 134.8, 131.8, 129.9, 128.7, 125.9, 123.3. IR: vₘₐₓ 3218, 1742, 1631, 1267, 851 cm⁻¹. LCMS: m/z 231.4 [MH⁺].

![Image of compound 4g]

**COMPOUND 4g. 3-(3-fluorophenyl)-1,2,4-oxadiazol-5(4H)-one.**
Off White solid, 69% yield; m.p. 154°C to 156°C; 1H NMR (300 MHz, CDCl₃): δ 10.35 (s, 1H), 7.21 (m, 1H), 7.19 (m, 1H), 7.15 (m, 1H); 13C NMR (70 MHz, DMSO+CDCl₃): 159.8, 156.5, 134.3, 132.8, 128.9, 126.3, 125.0, 120.7. IR: umax 3311, 1742, 1632, 1432, 1162, 953 cm⁻¹. LCMS: m/z 181.2 [MH+].

![Compound 4h](image)

**COMPONENT 4h. methyl 3-oxo-3H-[1,2,4] oxadiazolo [4,3-f]phenanthridine-10-carboxylate.**

Off White solid, 69% yield; m.p. 178°C to 180°C; 1H NMR (300 MHz, CDCl₃): δ 10.92 (s, 1H), 7.61 (m, 2H), 7.05 (m, 1H); 13C NMR (70 MHz, DMSO+CDCl₃): 158.0, 155.9, 133.6, 131.0, 128.7, 126.2, 125.4, 119.3, 13.1. IR: umax 3212, 1742, 1638, 1471, 1162, 953 cm⁻¹. LCMS: m/z 177.2 [MH+].

![Compound 4i](image)

**COMPONENT 4i. 3-phenyl-1,2,4-oxadiazol-5(4H)-one.**

Brown White solid, 92% yield; m.p. 172°C to 174°C; 1H NMR (400 MHz, CDCl₃): δ 11.20 (b, 1H), 7.81 (d, J=7.2 Hz, 2H), 7.57 (m, 3H); C NMR (70 MHz, DMSO+CDCl₃): 159.7, 156.6, 131.1, 128.2, 125.2, 122.7. IR: umax 3511, 1735, 1629, 1479, 1262, 953 cm⁻¹. LCMS: m/z 161.2 [MH-].

![Compound 4j](image)

**COMPONENT 4j. 3-(4-(trifluoromethyl) phenyl)-1,2,4-oxadiazol-5(4H)-one.**

Off White solid, 94% yield; m.p. 184°C to 186°C; 1H NMR (400 MHz, CDCl₃): δ 7.89 (d, J=8 Hz, 1H), 8.32 (d, J=8.4 Hz, 1H); 13C NMR (70 MHz, DMSO+CDCl₃): 159.6, 156.9, 133.1, 130.4, 126.7, 124.7. IR: umax 3511, 1735, 1629, 1479, 1262, 953 cm⁻¹. LCMS: m/z 231.2 [MH+].

**Conclusion**

In summary, we have developed a novel procedure for the synthesis of highly substituted 1,2,4-oxadiazol-5(4H)-ones from the corresponding amidoximes using ethyl imidazole-1-carboxylate (EImC). These molecules were screened against Mycobacterium tuberculosis. Of various compounds tested, 4a, 4b, 4c, 4e, 4f, 4h, and 4i are identified as lead molecules. In
particular, 4e, and 4h are found to display promising activity as good as ciprofloxacin, whereas 4i showed an excellent activity, which is equal to the IC50 value of ethambutol.

REFERENCES


11. (a) Quanrant L. Chiral relay effect: 4-substituted 1,3-benzoxazol-2-(3H)-ones as achiral templates for enantioselective diels: Alder reactions. Org Lett 2002;4:39;


(d) Unangst PC, Shrum GP, Connor DT, et al. Novel 1,2,4-oxadiazoles and 1,2,4-thiadiazoles as dual 5-lipoxygenase and cyclooxygenase inhibitors. J Med Chem. 1992;35:3691.
(g) Touaibia M, Djimde A, Cao F, et al. Inhibition of secreted phospholipase A2. 4-glycerol derivatives of 4,5-dihydro-3-(4-tetradeoxybenzyl)-1,2,4-4H-oxadiazol-5-one with broad activities. J Med Chem. 2007;50:1618.


23. Espinal MA. The global situation of MDR-TB. Tuberculosis. 2003;83:44.