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Research Article

# Waste to Wealth Catalyzed Synthesis of 3-methyl-4-(hetero)arylmethyleneisoxazole-5(4*H*)-ones and *p*BR 322 DNA Cleavage Studies

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**Abstract:** Herein, we demonstrated the synthesis of pharmacologically significant 3-methyl-4-(hetero)arylmethylene isoxazole-5(4*H*)-one by employing the water extract of lemon fruit shell ash (WELFSA) and glycerol as a solvent medium. The present method explored WELFSA as an eco-friendly catalyst to obtain 3-methyl-4-(hetero)aryl methyleneisoxazole-5(4*H*)-one derivative by the reaction of substituted aryl/heterocyclic aldehyde, hydroxylamine hydrochloride and ethyl acetoacetate at 60 °C on oil bath stirring condition. The completion of the reaction was monitored by thin-layer chromatography, and then the reaction mixture was quenched with ice-cold water, the separated solid was filtered, recrystallized in ethanol, and confirmed by various spectroscopic techniques. Some of the selected derivatives are subjected to DNA cleavage studies against pBR322, and showed comparable activities.

***Keywords***: 3-methyl-4-(hetero)arylmethyleneisoxazole-5(4*H*)-one, agro-waste, faster reaction, glycerol, DNA cleavage

## 1. Introduction

Isoxazole is a five-membered heterocyclic moiety found in both natural and synthetic bioactive molecules [1,2]. These derivatives showed several biological activities such as antifungal, analgesic [3], anti-convulsant [4], anti-tumor [5], HDC-inhibitors [6], COX-2 inhibitors [7], anti-microbial [8], anti-inflammatory [9], anti-cancer [10], anti-viral and many more applications reported in the literature Figure 1 [11]. Further isoxazole skeletons extensively used for the construction of merocyanine dyes [12], optical recording and nonlinear optical research [13]. Furthermore, some of the ether moiety containing isoxazole skeleton found to be photochromic nature and liquid crystal properties [14,15] The major applications of this skeleton acting as an building blocks for numerous drug molecule constructions [16]. Some of the important marketing drug molecules are Valedecoxib is an anti-arthritic non-steroidal drug molecule selectively inhibits cyclooxygenase in the treatment of dysmenorrhea and acute pain [17]. Zonisamide is a sulfa drug used in the anti-convulsant treatment of partial seizures in adults and epilepsy patients [18]. Leflunomide available in the brand name 'Arava' is an important immunosuppressive inhibition pyrimidine synthesis [19]. Acetylsulfisoxazole showed a broad spectrum of antibacterial properties, it helps incorporation of PABA into dihydrofolic acid [20]. Isocarboxazid is an anti-depressant used to manage natural neurotransmitters in the brain, particularly person unable to respond to treatment with other drugs [21]. Drazoxol is often used to treat bacterial-infected disease [22]. Sulfamethoxazole in combination with two antibiotics used to treat pneumonia and bacterial disease [23]. Fungicide agent (Z)-4-(4-(dimethylamino)benzylidene)-3-methylisoxazol-5(4*H*)-one [7], nonlinear optical (NLO) agent


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(Z)-4-(4-(dimethylamino)benzylidene)-3-phenylisoxazol-5(4*H*)-one, and merocyanine benzodiazepine drug act on CNS helps to enrich effect of the GABA[24]. Compounds containing isoxazole ring in their structure shown leishmaniasis treatment[25], and also found in useful building blocks for various heterocyclic molecule construction such as imidazoles[16], pyridopyrimidines[26], quinolones and 1,3-oxazine-6-ones[27]. Isoxazole undergo kind of different chemical transformations like alkylation, *N*-methylation, reduction, epoxidation, reduction/bromination[17], reduction/hydroxylation, Reformatsky reaction[28] and addition of organ magnesium reagents[29]. Hence, 4-arylmethyleneisoxazole-5(4*H*)-one demonstrated wide range of applications[30], insights organic chemists to develop numerous synthetic routes *via* two step[31]. In the first step formation of the oxime intermediate take place by the reaction of ethyl acetoacetate and hydroxylamine hydrochloride followed by ring closure affords 3-methylene-isoxazole-5(4*H*)-one[32]. In the second step Knoevenagel condensation reaction between the aromatic aldehyde and 3-methyl-isoxazole-5(4*H*)-one gave the final product 3-methyl-4-arylmethyl-isoxazole-5(4*H*)-one[33]. Recently various catalysts reported for the synthesis such as sodium silicate pentahydrate[34], sodium sulfide[35], saccharin, tetra borate[36], *DL*-tartaric acid[37], DOWEX(R)50$W_4$/$H_2O$,[38], imidazole[39], citric acid[40], $Ni(OAc)_2$[41], Choline: urea (1:2) as eutectic mixture[42], Sn(II)Mont-$K_{10}$[43], sodium hypophosphite[44], and some of the novel methods such as solid-state grindstone, heating, water, and visible light assisted[45]. In general, these reported methods have their own disadvantages such as expensive catalyst[46], solvent use, elevated temperature[47], longer reaction time, poor yield, and tedious work-up[48]. As a result, there is still a need to develop a simple and efficient, eco-friendly methodology for the synthesis of isoxazoles. The development of agro-waste derived catalytic solvent medium gave added advantages such as inexpensive[49], solvent-free, environment benign, and faster reaction rate with easily isolation[50]. In this connection, the present work demonstrated application of the agro-waste-derived solvent medium for the synthesis of isoxazoles. The agricultural process generates high stock of waste, and improper disposal of this agro-waste leads to environmental problems[51]. So that, the proper management is the task in the present days[52]. Nowadays, there is a tremendous application of agro-waste demonstrated in the production of energy and biofuel[53]. Recently, agro-waste-derived solid materials and extract medium employed in various chemical transformations such as transesterification/bio-diesel production using banana plant waste[54], biomass-derived waste date pits, eggshell waste, rice husk ash, construction of the flow fuel cell by the agro-waste[55]. Also agro-waste-derived solvent catalytic medium were employed in various organic syntheses of coumarin-3-carboxylic acid[56], 2-amino-4*H*-chromene[57], amide and peptide bond formation, rice-husk ash and RHA-$SiO_2$(NPs)-$BO_3H_3$ derived catalysts employed for dihydropyrimidinone and thione synthesis, and many more applications are reported in the literature.

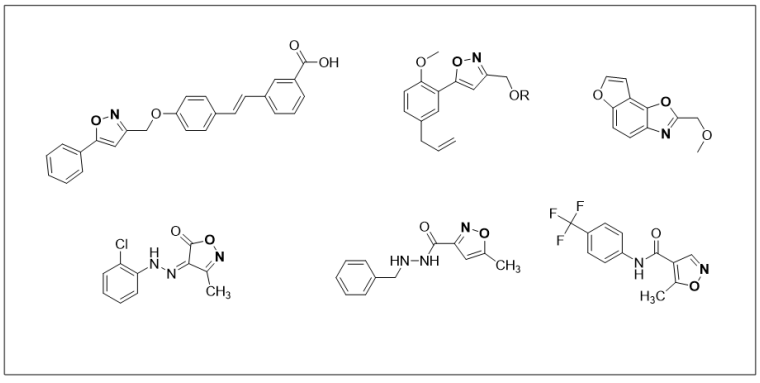



**Figure 1.** Important drug molecule containing isoxazole skeleton

DNA is involved in various crucial protein syntheses, and other genetic material production. Hence, the irregular DNA replication resulting in to growth of the tumors[58]. So, it is a challenge to prevent DNA replication, and it is necessary to employ a perfect DNA cleavage agent[59]. This is the base grasped by the researchers mind to develop efficient DNA cleavage molecules and their activity is dependent on the mode of affinity binding with DNA[60]. In this sense various experiments demonstrated that, DNA is the basic molecule for the intracellular target of drug molecules[61], interaction of drug molecules

leads to stop replication of the cell division ultimately contributing to cell death[62]. In this paper reported greener eutectic catalytic media (WELFSA/Glycerol) for the synthesis of 3-methyl-4-(hetero)arylmethyleneisoxazole-5(4*H*)-ones. Further selected isoxazole derivatives tested for their DNA cleavage studies against *p*BR322.

## 2. Materials and methods

### 2.1 *General*

All chemicals required for this work purchased from Sd-fine and AVRA chemicals, and used directly without further purification. Melting points determined by the open capillary method and uncorrected. Elemental analyses performed on an ESICO Microprocessor Flame Photometry model 1382. TLC performed using ethyl acetate hexane 50% mixture, the visualization observed in the UV chamber. FT-IR spectra recorded on a Thermo Fischer scientific using KBr pellet. The NMR spectra recorded in DMSO-$d_6$ on Agilent 400 MHz spectrometer operating at 400 and 100 MHz for $^{1}$H- and $^{13}$C-nuclei, respectively. All chemical shifts (δ values) given in parts per million (ppm), and homo coupling patterns presented in Hertz (Hz). HRMS (ESI) collected on a SYNAPT G2-Si spectrometer. The CRISPR-Cas12a software used to study the DNA cleavage.

### 2.2 *Preparation of water extract*

Water extract catalyst prepared from lemon fruit peels (Citrus limon) collected in the local area of Karnataka, India. The preparation and characterization of the agro-waste extract catalytic medium described in the previously reported work by our group[55]. Briefly, the fruit shell cleaned in water to remove dirt and citric acid content, then chopped into small pieces, dried under sunlight, followed by burning on a Bunsen flame gave ash powder. Resulted ash powder (10 g) soaked in double distilled water (100 mL) with magnetically stirred for effective extraction about 1 h, then filtered and filtrate was named WELFSA. The solution was found basic in nature, due to the presence of high concentration of potassium and calcium oxide/carbonates. Usually, the carbonates are stable at high temperatures (1200 °C) and do not undergo decomposition on Bunsen flame (1000 °C), when the ash was soaked in water gave mixture of hydroxide and carbonates led to the basic nature of the solution. The complete elemental analysis and composition of the WELFSA prepared was established and reported.

The ash powdered EDX and SEM images were obtained on a CARL ZEISS OXFORD instrument, Japan.

### 2.3 *Typical procedure for the syntheses of 3-methyl-4-(het)arylmethyleneisoxazole-5(4H)-ones (4a-q)*

In a round-bottomed flask taken WELFSA (4 mL), and glycerol (0.2 mL) and heated with constant stirring in an oil bath for about 5 min to get eutectic mixture. Then substituted aromatic aldehyde (1) (2 mmol), ethyl acetoacetate (2) (2 mmol), and hydroxylamine hydrochloride (3) (2.2 mmol) added and continued stirring at 60 °C till completion of the reaction. The progress of the reaction was monitored by the separation of reactant and product spots separately in the TLC (ethyl acetate; hexane, 0.3:0.7, visualization of the spots in UV chamber). After the reaction, ice cold $H_2O$ poured in to reaction mixture, solid separated filtered, washed with cold water, and recrystallized by ethanol. The final product characterized for its homogeneity by FT-IR, $^{1}$H-, $^{13}$C-NMR, and HR-MS (ESI).

### 2.4 *DNA cleavage study*

DNA cleavage activity of the selected 3-methyl-4-(hetero)arylmethyleneisoxazole-5(4*H*)-one evaluated by agarose gel electrophoresis using isolated *p*BR322 DNA in a different concentration of the test compounds (30 µg/mL, 50 µg/mL, and 100 µg/mL) treated on isolated plasmid DNA, and allowed to incubate for 2 h at 37 °C. After incubation, treated plasmid DNA with control loaded on agarose gel electrophoresis monitored at 50 V constant for 30 min. After electrophoresis, the gel was stained with ethidium bromide (EB) for 30 min before being photographed under UV light[63].

## 2.5 *Structural characterization*

### 2.5.1 *(Z)-4-Benzylidene-3-methylisoxazol-5(4H)-one (4a)*

White crystalline; $R_f$ = 0.43 (Ethyacetate/Hexane, 3:7); Anal. calcd. for $C_{11}H_8ClNO_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.41; H, 4.66; N, 7.34; FT-IR: $\nu_{max}$ 3052 (CH stretching), 1748 (C=O stretching), 1868 (C=N stretching), 1553 (N-O stretching), 1420, 1420, 1258, 1141 $cm^{-1}$; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.29 (s, 3H), 7.24 (s, 1H), 7.41–7.57 (m, 3H, ArH), 7.68 (d, 1H), 8.64 (d, 1H), 8.73 (s, 1H); (100 MHz, DMSO-$d_6$) $\delta_C$ 11.5, 119.6, 128.8, 128.9, 130.3, 131.9, 132.2, 133.6, 133.8, 149.7, 160.9 and 167.7 ppm; HR-MS (ESI, methanol): m/z: found [M+1] 187.0600; requires 187.0600. The spectral data available in supporting information (Figures S1–S4).

### 2.5.2 *(E)-4-(4-Chlorobenzylidene)-3-methylisoxazol-5(4H)-one (4c)*

Yellow crystalline; $R_f$ = 0.37 (Ethyacetate/Hexane, 3:7); Anal. calcd. for $C_{11}H_8ClNO_2$: C, 59.61; H, 3.64; Cl,16.00; N, 6.32. Found: C, 59.71; H, 3.91; Cl,16.02; N, 6.22; FT-IR: $\nu_{max}$ 3047 (CH stretching), 2951, 1763, 1741 (C=O stretching), 1668 (C=N stretching), 1571, 1553 (N-O stretching), 1420, 1420, 1258, 1141 $cm^{-1}$; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.68 (s, 3H), 7.45 (s, 1H), 7.67 (t, 1H), 7.68 (d, 1H), 8.64 (d, 1H), 8.73 (s, 1H); (100 MHz, DMSO-$d_6$) $\delta_C$ 11.9, 121.7, 129.6, 131.5, 133.7, 136.8, 136.4, 146.5, 159.4 and 168.7 ppm; HR-MS (ESI, methanol): m/z: found [M+1] 222.2791; requires 221.6402.

### 2.5.3 *(E)-4-(4-Bromobenzylidene)-3-methylisoxazol-5(4H)-one (4d)*

White crystalline; $R_f$ = 0.37 (Ethyacetate/Hexane, 3:7) Anal. calcd. for $C_{11}H_8BrNO_2$: C, 49.65; H, 3.03; Br, 30.03; N, 5.26. Found: C, 49.41; H, 3.23; Br, 30.12; N, 5.41; FT-IR (KBr): $\nu_{max}$ 3056 (CH stretching), 2942, 1750 (C=O), 1777 (C=N), 1585, 1542 (N-O stretching), 1418, 1416, 1258, 1139, 755, 783, 697, 528, 512, 484 $cm^{-1}$; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.67 (s, 3H, $CH_3$), 7.43 (s, 1H, CH), 7.62 (t, $J$ = 8.3 Hz, 1H), 7.66 (d, $J$ = 7.79 Hz, 1H), 8.65 (d, $J$ = 7.79 Hz, 1H), 8.75 (s, 1H); (100 MHz, DMSO-$d_6$) $\delta_C$ 11.8, 121.4, 129.5, 131.7, 133.4, 136.5, 136.0, 146.0, 159.7 and 168.6 ppm; HR-MS (ESI, methanol): m/z: found [M+1] 265.2033; requires 264.9728. The spectral data available in supporting information (Figures S5–S8).

### 2.5.4 *(E)-4-(3,4-Dimethoxybenzylidene)-3-methylisoxazol-5(4H)-one (4j)*

White crystalline; $R_f$ = 0.45 (Ethyacetate/Hexane, 3:7) Anal. calcd. for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.21; H, 5.46; N, 5.88; FT-IR (KBr): $\nu_{max}$ 2939 (CH stretching), 1737 (C=O stretching), 1657 (C=N stretching), 1587, 1547 (N-O stretching), 1517, 1245, 1020, 992, 953, 740, 769, 602, 571 $cm^{-1}$; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.29 (s, 3H, $CH_3$), 3.98–4.02 (t, 6H, $OCH_3$), 6.96 (d, $J$ = 8.4 Hz, 1H, Ar), 7.09 (t, $J$ = 7.8 Hz, 1H, Ar), 7.56 (t, $J$ = 7.08 Hz, 1H, Ar), 6.95–6.47 (m, 1H), 7.59–7.61 (m, $J$ = 8.2 Hz, 1H, Ar), 8.76–8.77 (s, 1H); (100 MHz, DMSO-$d_6$) $\delta_C$ 16.2, 60.7, 115.3, 119.7, 121.0, 130.9, 135.6, 153.7, 154.2, 159.2 and 165.7 ppm; HR-MS (ESI, methanol): m/z: found [M+1] 248.0639; requires 247.0801. The spectral data available in supporting information (Figures S9–S12).

### 2.5.5 *(E)-4-(4-(Dimethylamino)benzylidene)-3-methylisoxazol-5(4H)-one (4o)*

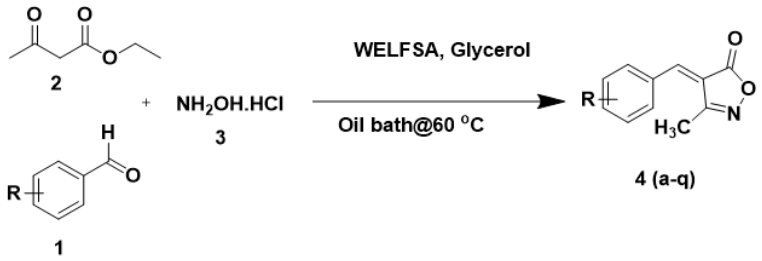
White crystalline; $R_f$ = 0.32 (Ethyacetate/Hexane, 3:7) Anal. calcd. for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 62.12; H, 4.69; N, 6.31; FT-IR (KBr): $\nu_{max}$ 2957 (CH stretching), 1736 (C=O stretching), 1652 (C=N stretching), 1556 (N-O stretching), 1498, 1385, 1344, 1104, 998, 775, 562 $cm^{-1}$; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.29 (s, 3H, $CH_3$), 4.05 (s, 3H), 6.39 (s, 1H), 7.00–7.02 (d, $J$ = 8.06 Hz, 1H, CH), 7.26–7.45 (d, $J$ = 8.8 Hz, 2H, Ar), 8.94–8.95 (s, 1H, OH); (100 MHz, DMSO-$d_6$) $\delta_C$ 16.1, 34.2, 60.9, 119.2, 119.3, 136.5 and 154.4 ppm; HR-MS (ESI, methanol): m/z: found [M+1] 234.0460; requires 233.2201. The spectral data available in supporting information (Figures S13–S16).

### 2.5.6 *(E)-4-(3-Hydroxy-4-methoxybenzylidene)-3-methylisoxazol-5(4H)-one (4p)*

Pale yellow crystalline; $R_f$ = 0.28 (Ethyacetate/Hexane, 3:7); Anal. calcd. for $C_9H_7NO_3$: C, 61.02; H, 3.98; N, 7.91. Found: C, 61.32; H, 4.12; N, 7.64; FT-IR (KBr): $\nu_{max}$ 3054 (CH stretching), 1765 (C=O stretching), 1647 (C=N), 1558 (N-O stretching), 1471, 1388, 1362, 1083, 785 $cm^{-1}$; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta_H$ 2.46 (s, 3H), 2.85 (s, 3H), 7.67 (d, 2H), 7.89 (s, 1H), 8.34 (d, 2H); (100 MHz, DMSO-$d_6$) $\delta_C$ 11.6, 24.0, 121.8, 131.0, 130.1, 146.2, 151.5, 162.5 and 169.6 ppm; HR-MS (ESI, methanol): m/z: found [M+1] 218.5637; requires 217.0768.

## 3. Results and discussions

The main objective of this work is to develop greener protocol for the 3-methyl-4-(hetero) arylmethyleneisoxazole-5(4*H*)-one synthesis *via* three component reaction of aromatic aldehyde, ethyl acetoacetate, and hydroxylamine hydrochloride in WELFSA and glycerol (Scheme 1). In a model reaction, benzaldehyde, ethyl acetoacetate, and hydroxylamine hydrochloride at 60 °C in an oil bath reaction progress monitored, and saw clear separation of the reactant and product spots in TLC. After the reaction, finely crushed ice cubes poured in to the reaction mixture gave separation of solid product, filtered and isolated good to excellent yield. To check the importance of the agro-waste catalytic medium, performed model reaction in the absence of WELFSA, surprisingly no product formation noticed even after 4 h. These trail reactions revealed that, the reaction requires catalyst for the forward reaction and added small amount of glycerol to facilitate tetracyclization. Before finalizing reaction optimization, further exercised different reaction conditions on a model reaction like on and off mechanochemical grinding for about 60 min, ultrasonication, and microwave irradiation at 180 W for about 5 min gave 29%, 68%, and no product formation, respectively. But the same reaction performed at 60 °C on an oil bath with stirring condition gave product isolation with excellent yield in 60 min and complete optimization studies tabulated in Table 1.




**Scheme 1.** Synthesis of (E)-4-benzylidene-3-methylisoxazol-5(4*H*)-one

**Table 1.** Optimization of the reaction

| S. No | Method | WELFSA + Glycerol (mL) | Time (min) | Yield (%) |
|---|---|---|---|---|
| 1 | Stirring at rt | 4.0 + 0.2 | 60 min | 47 |
| 2 | Mechanochemical | 4.0 + 0.2 | 60 min | 29 |
| 3 | Ultra-sonication | 4.0 + 0.2 | 60 min | 68 |
| 4 | MW 300 W | 4.0 + 0.2 | 60 min | nd |
| 5 | Heating@ 60 °C on oil bath | 4.0 + 0.2 | 60 min | 98 |

Further optimized WELFSA volume and glycerol required for the 1 mmol scale in a model reaction examined by performing series of different volumes of WELFSA and glycerol combination starting with 0, 1.0, 2.0, 3.0, 3.5, 4.0 & 4.5 mL of WELFSA, and 0 and 0.1 mL of the glycerol, and in another set kept volume of the WELFSA same, but changed glycerol volume to 0.2 mL. These reaction conditions revealed that, the reaction without WELFSA not showed product spot in TLC, and a gradual increase of the volume of WELFSA from 1 mL to 4.5 mL with 0.1 mL of glycerol noticed gradual increase of the product isolation at 60 °C in an oil bath stirring (S. No, 2–7, Table 2). Further, the ratio of the

glycerol increased to 0.2 mL with serial increase of the WELFSA in a model reaction gave gradual increase of the product isolation in excellent yield (S. No. 8–13, Table 2). This optimization reaction confirmed that 4 mL of WELFSA and 0.2 mL of glycerol eutectic mixture combination gave the best catalytic performance for the excellent product isolation. To check developed protocol tolerance to other substituted aryl and heterocyclic aldehydes performed reaction with 17 different substituted aromatic or heterocyclic aldehyde gave good to excellent product isolation, and their physical constant was tabulated in Table 3.

**Table 2.** Optimization of WELFSA and glycerol volume

| S. No | WELFSA (mL) | Glycerol (mL) | Time (min) | Yield (%) |
|---|---|---|---|---|
| 1 | 0.0 | 0.0 | 60 | ND |
| 2 | 1.0 | 0.1 | 60 | ND |
| 3 | 2.0 | 0.1 | 60 | ND |
| 4 | 3.0 | 0.1 | 60 | 20 |
| 5 | 3.5 | 0.1 | 60 | 29 |
| 6 | 4.0 | 0.1 | 60 | 40 |
| 7 | 4.5 | 0.1 | 60 | 61 |
| 8 | 1.0 | 0.2 | 60 | 34 |
| 9 | 2.0 | 0.2 | 60 | 41 |
| 10 | 3.0 | 0.2 | 60 | 67 |
| 11 | 3.5 | 0.2 | 60 | 85 |
| 12 | 4.0 | 0.2 | 60 | 94 |
| 13 | 4.5 | 0.2 | 60 | 94 |

**Table 3.** Synthesis and physical constant

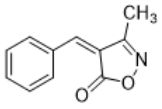
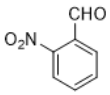
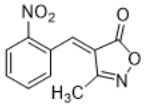
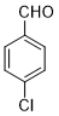
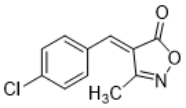
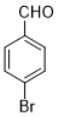
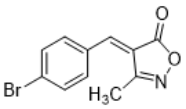
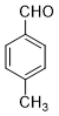
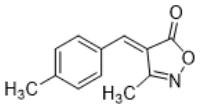
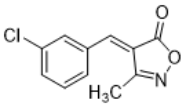
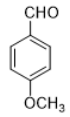
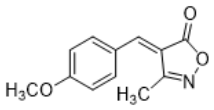
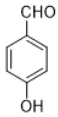
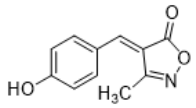
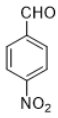
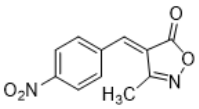
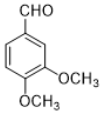
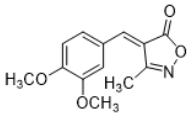
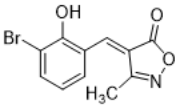
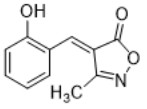
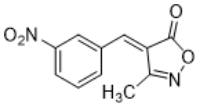
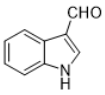
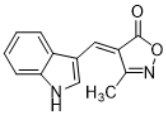
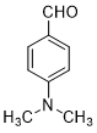
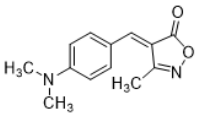
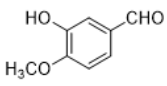
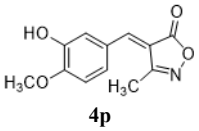
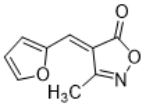
| No. | Aldehyde | Product | Yield (%) | m.p (°C) Obs./(Lit.) |
|---|---|---|---|---|
| 1 |  |  **4a** | 87 | 140–142 (141–143)[18] |
| 2 |  |  **4b** | 91 | 148–150 |
| 3 |  |  **4c** | 89 | 160–162 |
| 4 |  |  **4d** | 92 | 141–143 |
| 5 |  |  **4e** | 88 | 136–138 |
| 6 |  |  **4f** | 90 | 143–145 |

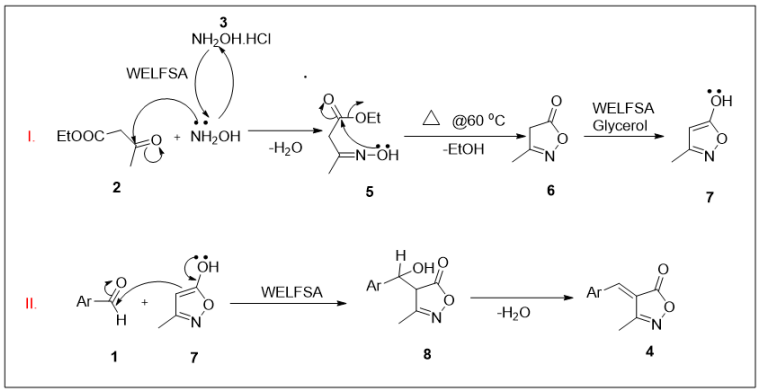
**Table 3.** *Cont.*

| No. | Aldehyde | Product | Yield (%) | m.p (°C) Obs./(Lit.) |
|---|---|---|---|---|
| 7 |  |  **4g** | 87 | 173–175 |
| 8 |  |  **4h** | 94 | 214–215 |
| 9 |  |  **4i** | 80 | 165–167 |
| 10 |  |  **4j** | 89 | 135–136 |
| 11 |  |  **4k** | 78 | 188–190 |
| 12 |  |  **4l** | 91 | 198–199 |
| 13 |  |  **4m** | 88 | 134–136 |
| 14 |  |  **4n** | 79 | 244–246 |
| 15 |  |  **4o** | 84 | 224–226 |
| 16 |  |  **4p** | 89 | 266–268 |
| 17 |  |  **4q** | 92 | 239–241 |

The excess of WELFSA and glycerol present in the reaction mixture removed by adding excess amount of dilution. We also recovered catalyst solvent medium after the reaction by one time wash with ethylacetate, and performed its catalytic activity in a model reaction revealed no product formation. So, it is confirmed that, the catalytic solvent medium has no reusability property after one-time use.

The homogeneity of the isolated product was analyzed by several spectroscopic techniques. The FT-IR spectrum of compound **4a** showed prominent band at 3052 $cm^{-1}$ due to (CH) stretching, band at 1748 $cm^{-1}$ due to (C=O) stretching, band at 1868 $cm^{-1}$ due to (C=N stretching), band at 1553 $cm^{-1}$ due to (N-O stretching) Figure S1; The $^{1}$H-NMR spectrum of the product established δ at 2.29 singlet due to 3H of $CH_3$, 7.24 singlet 1H due to CH, 7.41–7.57 multiplet due to 3H of ArH, 8.32–8.35 triplet due to 2H of ArH Figure S2; $^{13}$C-NMR spectrum gave the peaks at 11.51, 119.64, 128.82, 128.94, 130.34, 131.96, 132.21, 133.69, 133.89, 149.76, 160.98 and 167.76 ppm Figure S3. HR-MS (ESI); m/z (Calcd.): 187.0600 Da; m/z (Obs.): 188.0471 $[M+H]^+$ Da Figure S4. Anal. Calcd for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.41; H, 4.66; N, 7.34 Figures (S1–S4).

The plausible mechanistic pathway Scheme 2 for the product formation will take place *via* hydroxylamine nucleophile attack on the carbonyl carbon of the β-ketoester lead to the formation of oxime intermediate under thermal condition, further cyclization and simultaneous elimination of ethanol take place. The Knoevengel condensation between intermediate and aryl aldehyde followed the loss of water molecule gave the expected product. The excess of WELFSA and glycerol present in the reaction mixture was removed by washing with excess amount of distilled water by dilution. We also concentrated the filtrate catalyst solvent and performed the catalytic activity in a model reaction found no formation of the expected product[56]. So it is confirmed that, the catalytic solvent has no reusability property after one-time use.




**Scheme 2.** Plausible mechanism for the synthesis of isoxazole

Also compared the present protocol demonstrated with various literature reported catalysts such as boric acid (10 mol%) for about 2.5 h afforded 93% yield (Table 4)[49], ion exchange resin DOWEX 1-X8 under rt about 6 h afford 95%, citric acid catalyzed for about 9 h gave 90% yield (1)[53]. The potassium phthalimide 10 mol% catalyzed reaction gave 96% (4) and sodium saccharin 10 mol% under rt afford 96% yield (5)[35]. nano-MMT functional catalyzed reaction under neat ultra-sonication required only 35 min and afford 96% yield (6). The modified $NH_2$-MMT under ultra-sonication afforded 97 % yield (7)[52]. The reaction was performed in sulfated polyborate and $ZnCl_2$ at autoclave toxic volatile organic solvent in a tedious reaction condition (8,9). The present developed method is eco-friendly and eliminates some of the critical reagent uses like heterogeneous catalysts, which have separation issues from the mother mixture. In such cases, separating the product from the mother reaction required volatile organic solvent a tedious process, and also contamination. The present agro-waste catalyzed has added advantages like facile, simple, and inexpensive compared to the literature reported (10).

**Table 4.** Comparative studies reported v/s present method

| S. No. | Catalyst | Time | Yield (%) |
|---|---|---|---|
| 1 | Boric acid 10 mol% | 2–3 h | 93 |
| 2 | DOWEX 1-x8OH, $H_2O$ r.t | 1–6 h | 95 |
| 3 | Citric acid (1 mmol) | 8 h | 90 |
| 4 | Potassium phthalimide 10 mol% | 2 h | 96 |
| 5 | Sodium saccharin (10 mol%) | 2 h | 96 |
| 6 | Nano-MMT-Sn 30 °C, US | 45 min | 96 |
| 7 | $NH_2$-MMT, 30 °C US | 1 h | 97 |
| 8 | Sulfated polyborate, 80 °C | 30 min | 85 |
| 9 | Autoclave/MW, $ZnCl_2$, 160–180 °C | 20 min | 81 |
| | **Present method** | | |
| 10 | WELFSA 4 mL, Glycerol 0.2 mL Heating@ 60 °C | 1 h | 94 |

In the present study performed interaction of the prepared derivatives with *p*BR322 DNA through agarose gel electrophoresis. In this study, untreated DNA taken as a control, and the sample treated with DNA as an experimental section. Different concentration of the test samples were treated on *p*BR322 DNA. In all sample treated DNA showed change in the band pattern of DNA by changing closed DNA (Form-I) to nicked circular DNA (Form-II), and linear form (Form-III). Generally fast movement of DNA occurs in control DNA, and in case of treated DNA cleavage slow migration occurs, due to the action of test compounds where supercoiled DNA changes with relaxation to produce nicked and linear form of DNA with breaks in DNA with smear appearance. Overall findings showed that, in all tested samples noticeable results were observed, and cleavage images are reproduced in Figure 2.

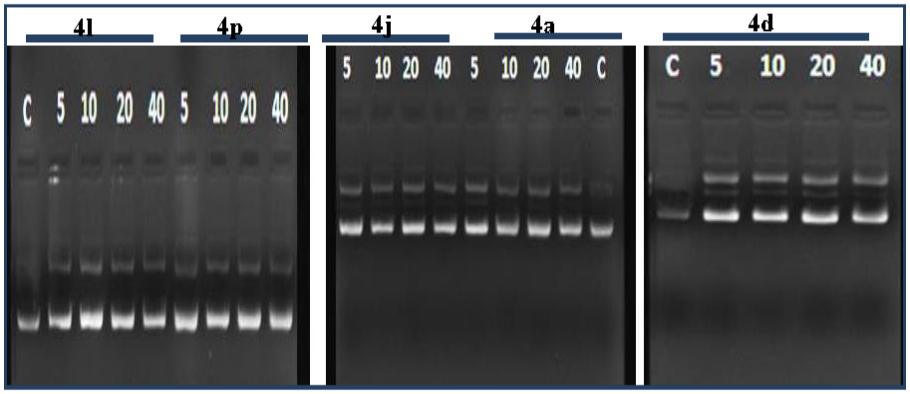



**Figure 2.** DNA Cleavage study of selected compounds with *p*BR322

## 4. Conclusions

Present work demonstrated agro-waste extracted WELFSA is an alternative greener catalytic medium towards the synthesis of 3-methyl-4-(hetero)arylmethyleneisoxazole-5(4*H*)-one derivatives synthesis using substituted aryl or heterocyclic aldehyde, ethyl acetoacetate and hydroxylamine hydrochloride. The developed method found added advantages like non-polluting, non-toxic, cheaper, simple reaction condition, and faster. The final product isolated chromatographically pure, and not required further purification. Further selected compounds were tested for DNA cleavage and showed comparable cleavage properties compared to the reported.

## Author contributions

Santosh Khatavi designed and conducted experiments, and analyzed and interpreted the data. Kantharaju Kamanna involved supervision, writing manuscript and processing publication.

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## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Supplementary Materials

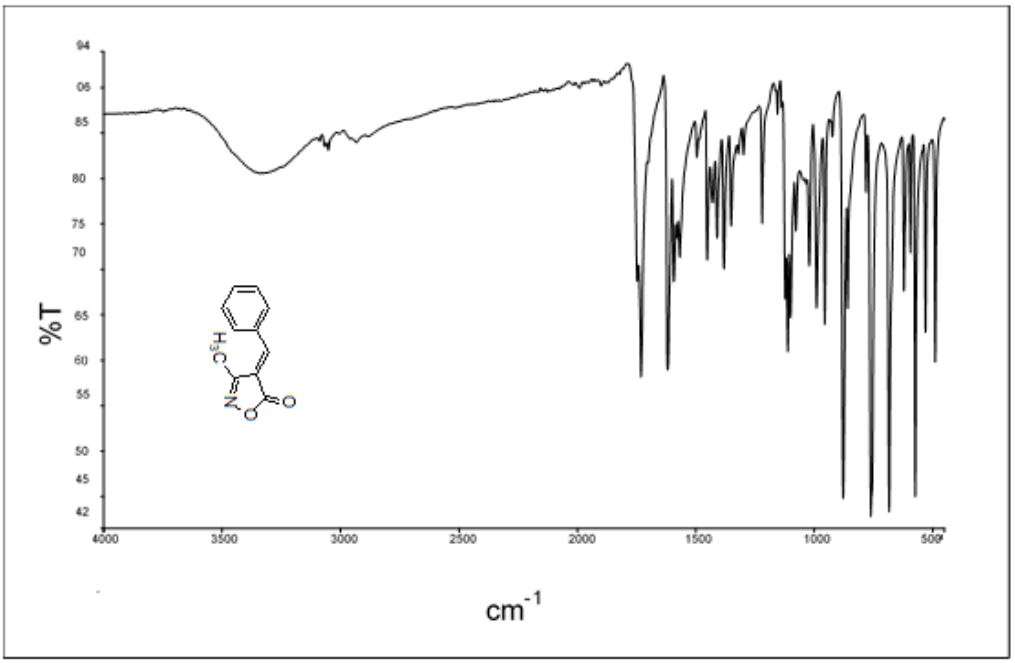


**Figure S1.** FT-IR Spectrum of benzylidene-3-methylisoxazol-5(4*H*)-one (**4a**)

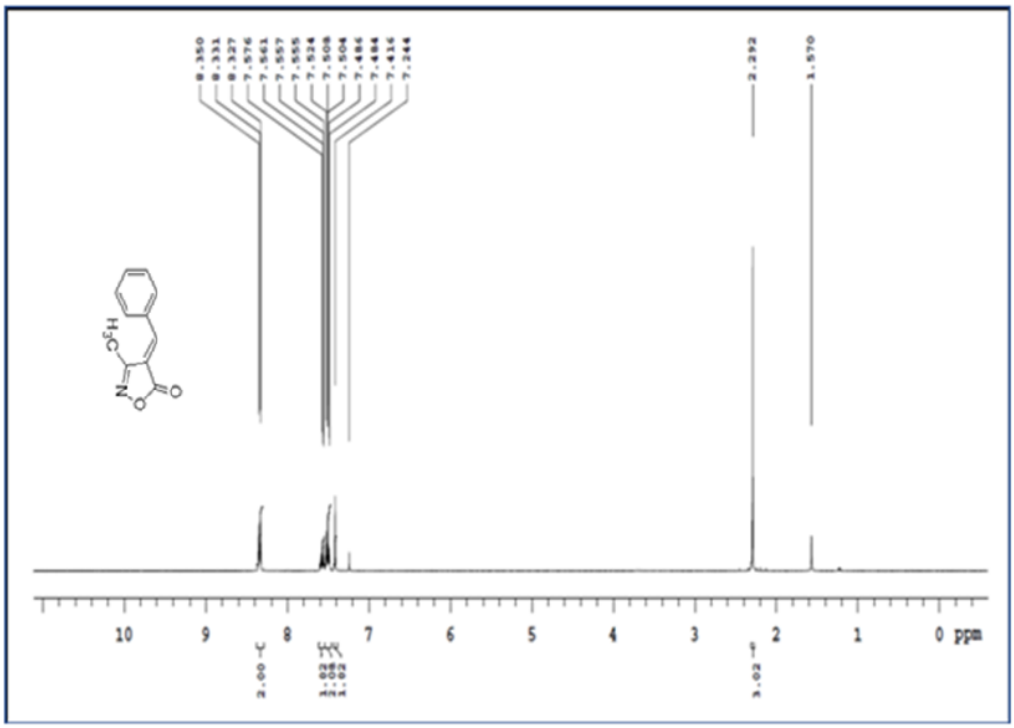


**Figure S2.** [1]H-NMR Spectrum of benzylidene-3-methylisoxazol-5(4*H*)-one (**4a**)

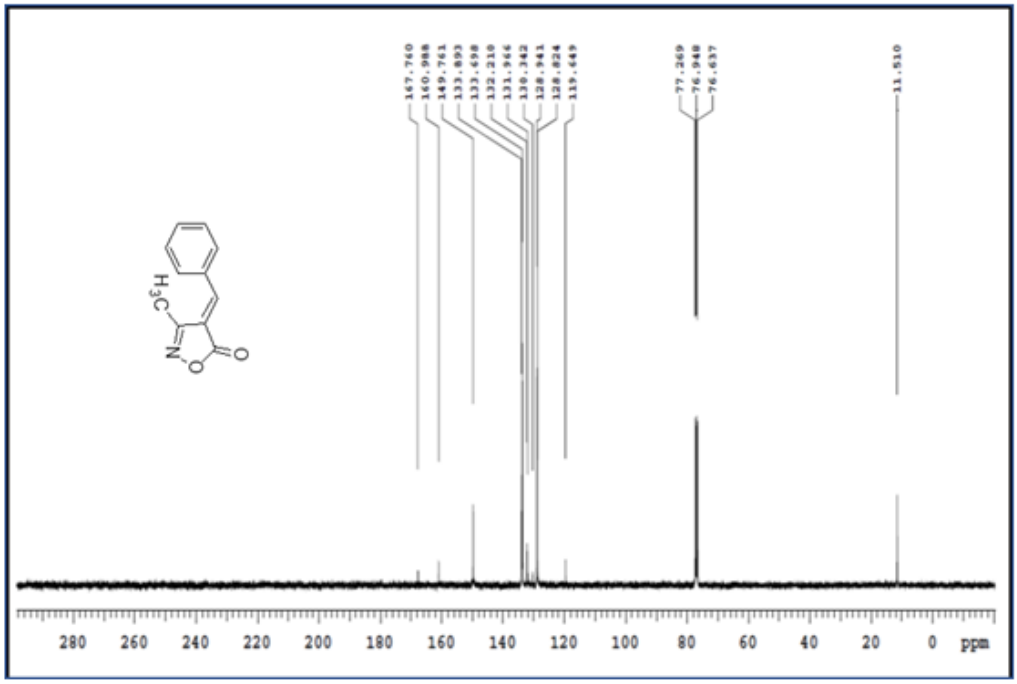


**Figure S3.** $^{13}$C-NMR Spectrum of benzylidene-3-methylisoxazol-5(4*H*)-one (**4a**)

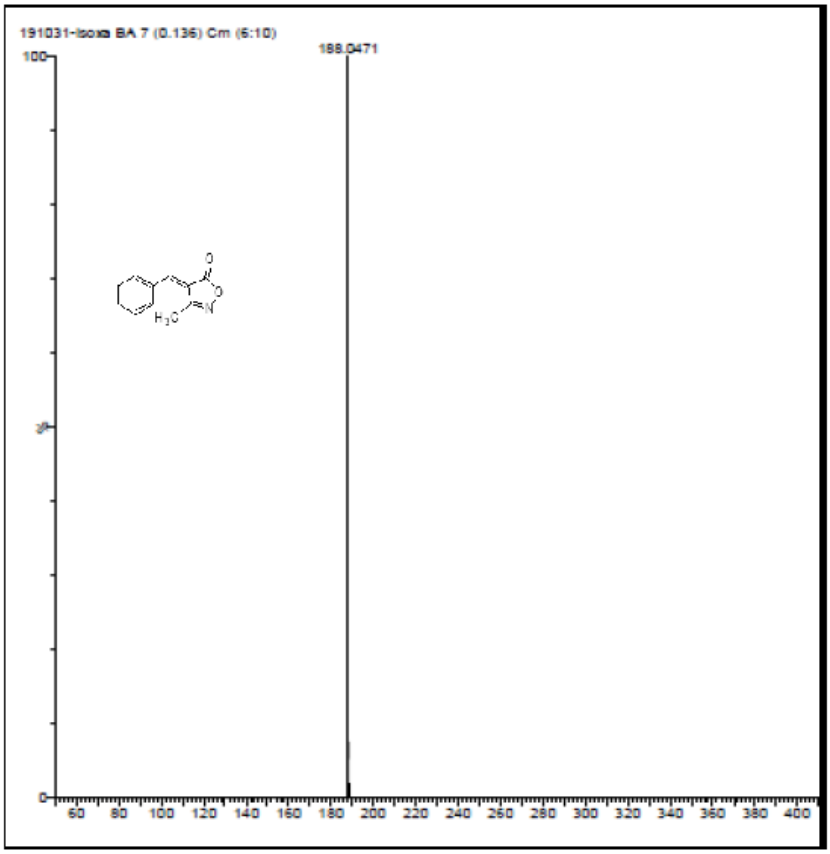


**Figure S4.** HR-MS Spectrum of benzylidene-3-methylisoxazol-5(4*H*)-one (**4a**)

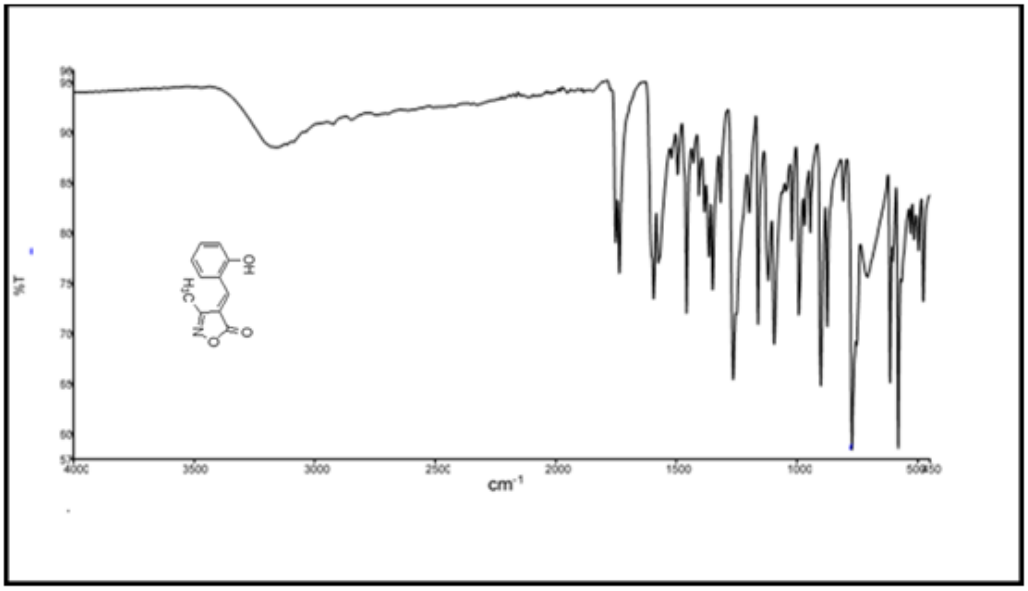


**Figure S5.** FT-IR Spectrum of (2-hydroxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4d**)

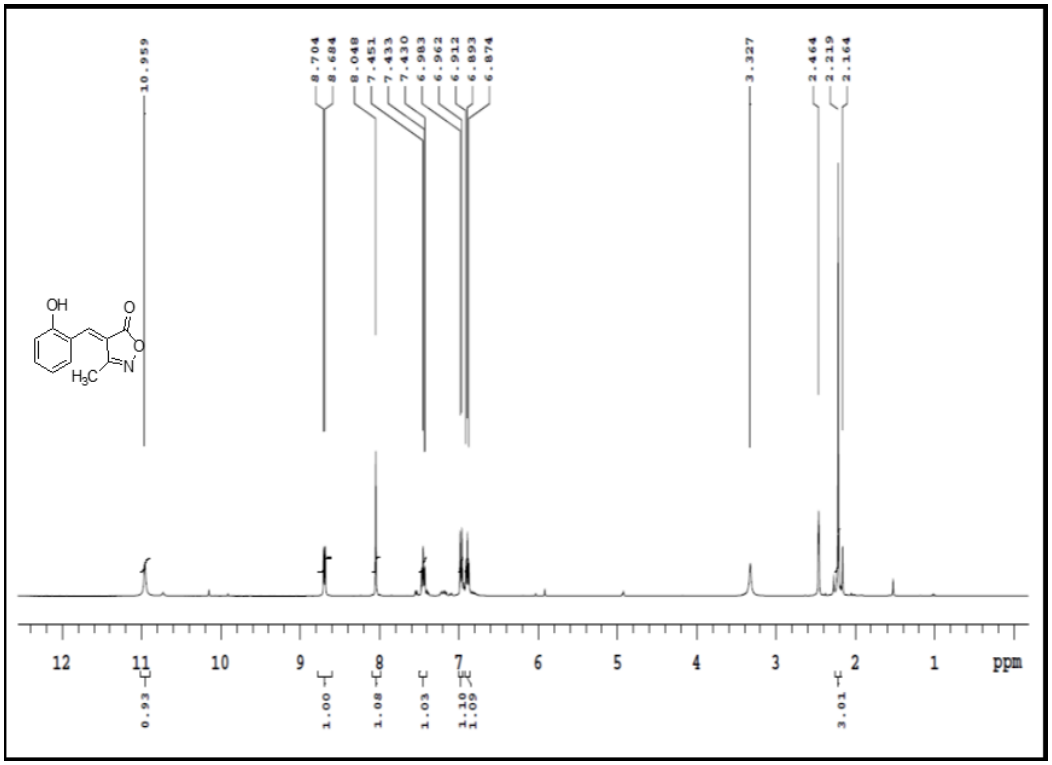


**Figure S6.** FT-IR Spectrum of (2-hydroxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4d**)

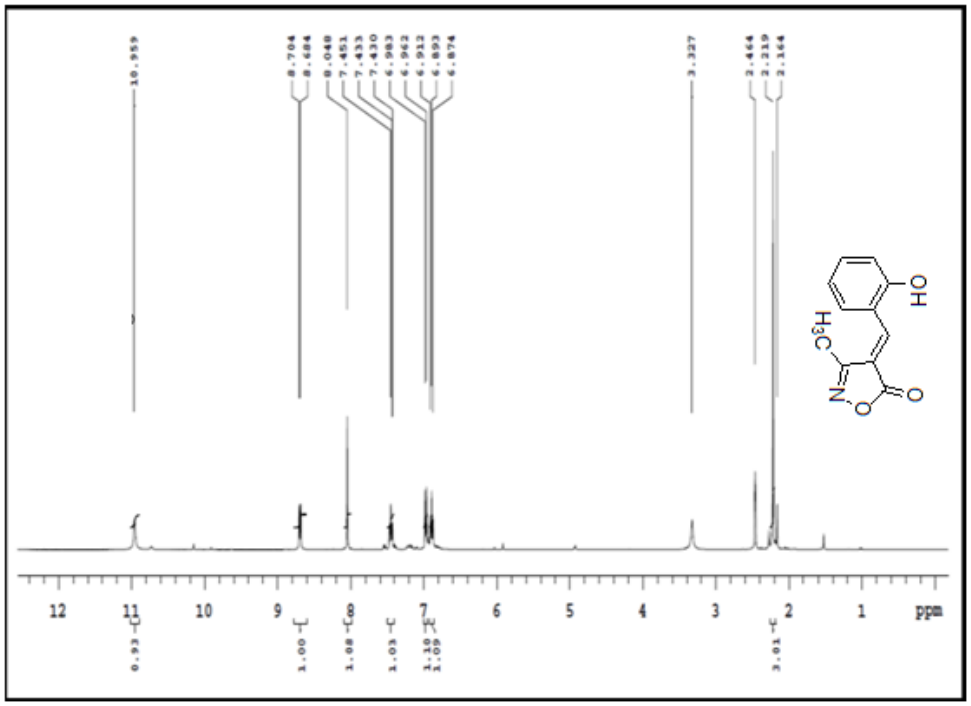


**Figure S7.** *13*C-NMR Spectrum of (2-hydroxybenzylidene)-3-methylisoxazol-5(4*H*)-one **4d**

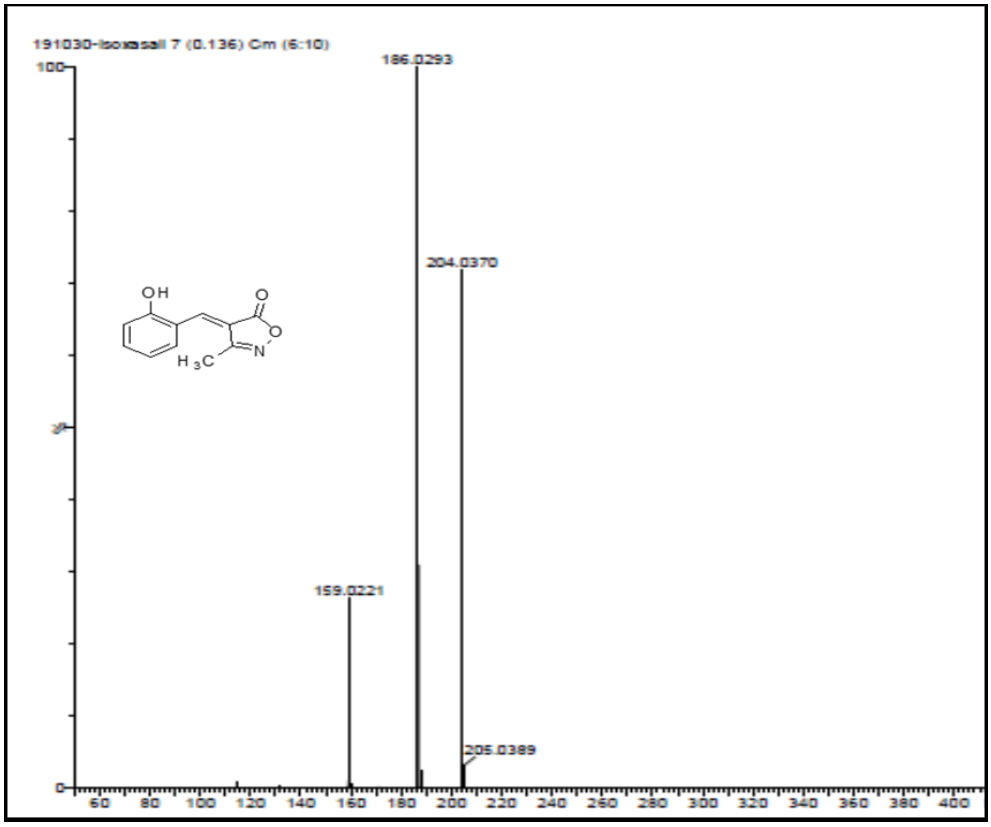


**Figure S8.** $^{13}$C-NMR Spectrum of (2-hydroxybenzylidene)-3-methylisoxazol-5(4*H*)-one **4d**

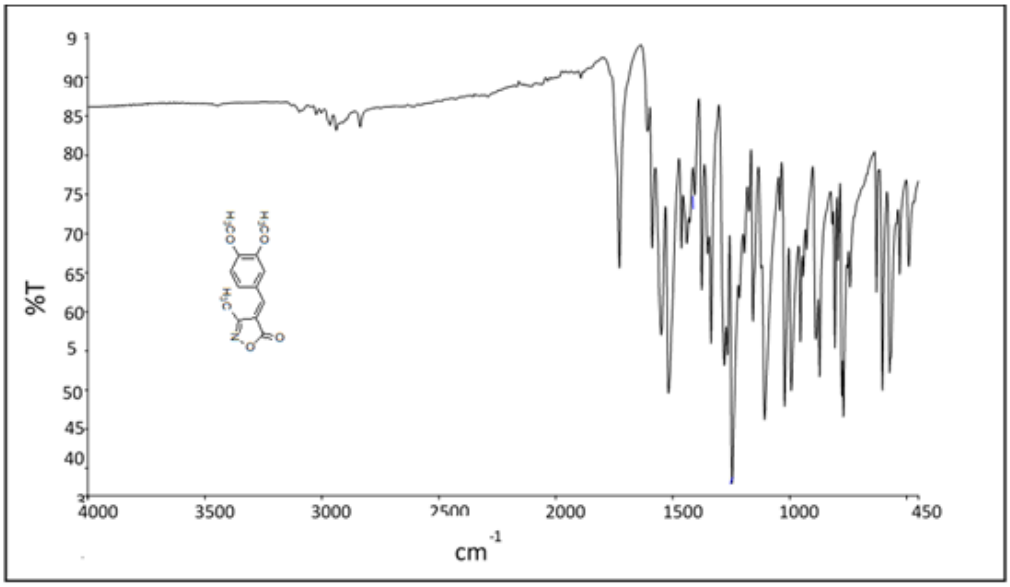


**Figure S9.** FT-IR Spectrum of (2,4-dimethoxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4j**)

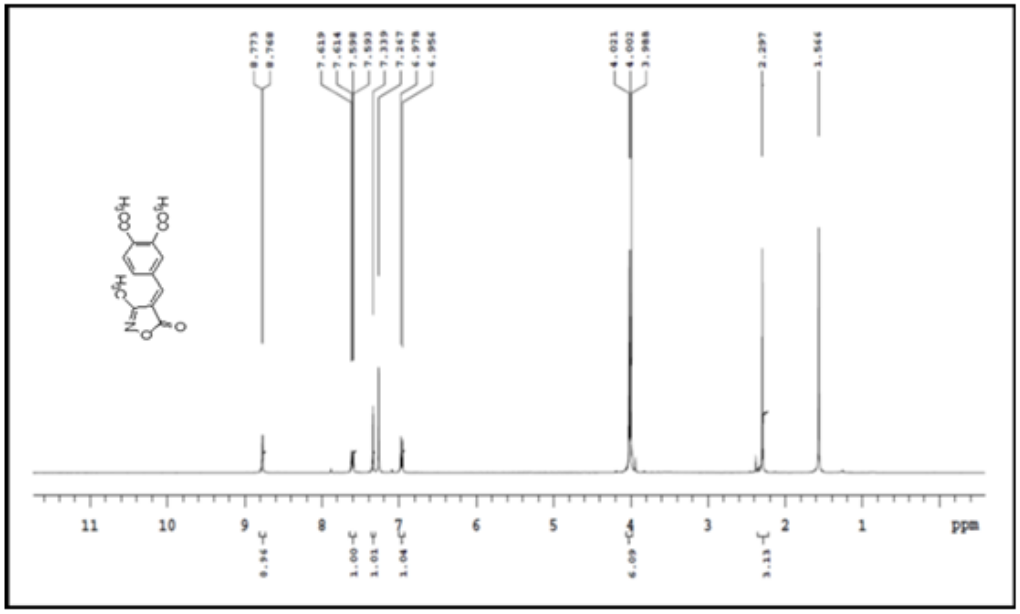


**Figure S10.** FT-IR Spectrum of (2,4-dimethoxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4j**)

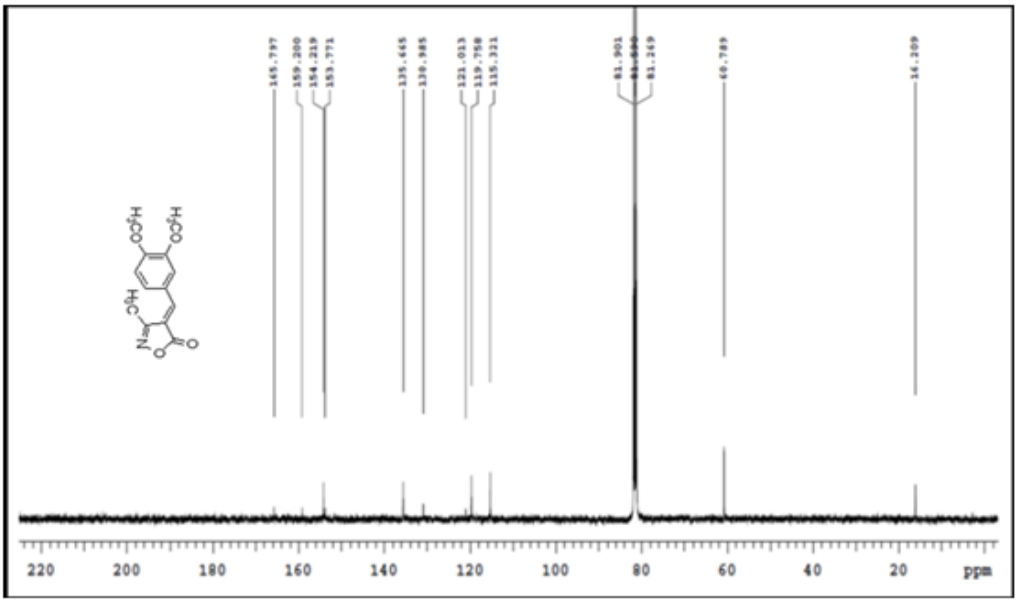


**Figure S11.** $^{13}$C-NMR Spectrum of (2,4-dimethoxybenzylidene)-3-methylisoxazol-5(4*H*)- one (**4j**)

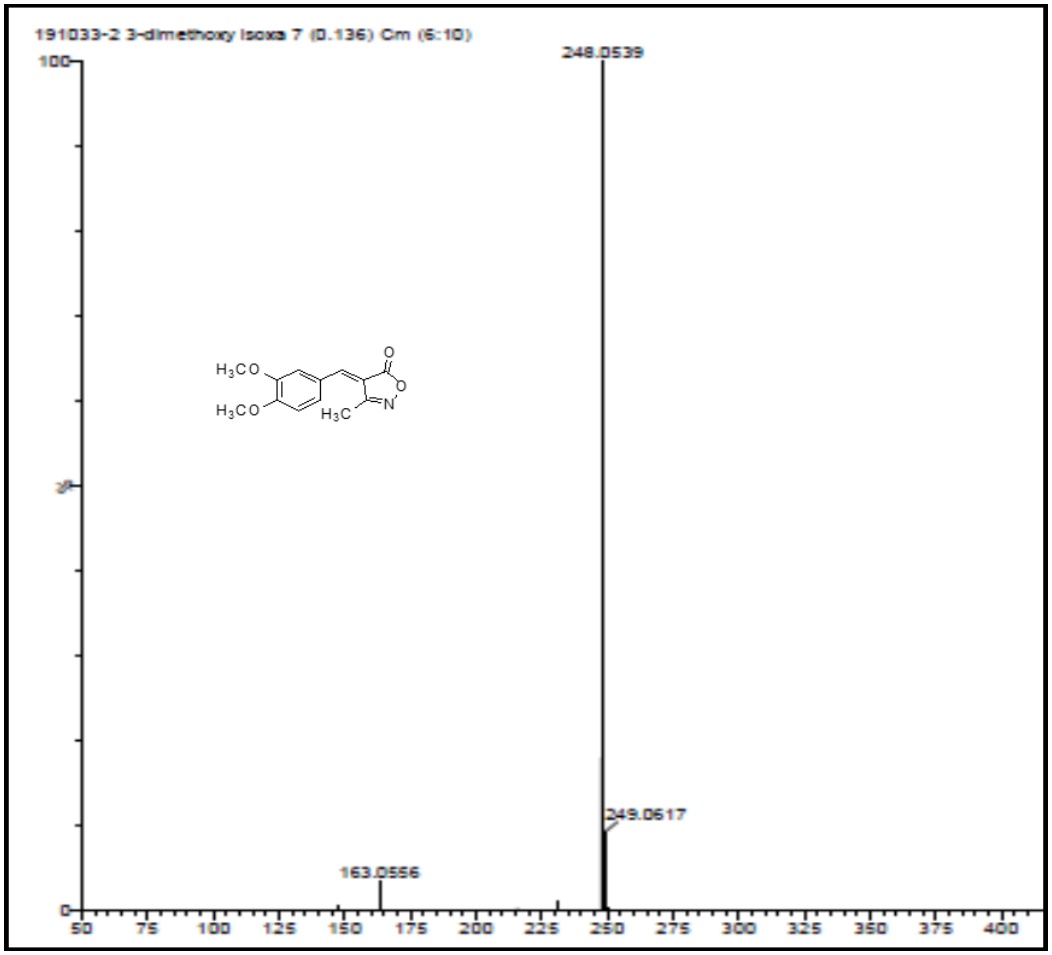


**Figure S12.** HR-MS (ESI) Spectrum of (2,4-Dimethoxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4j**)

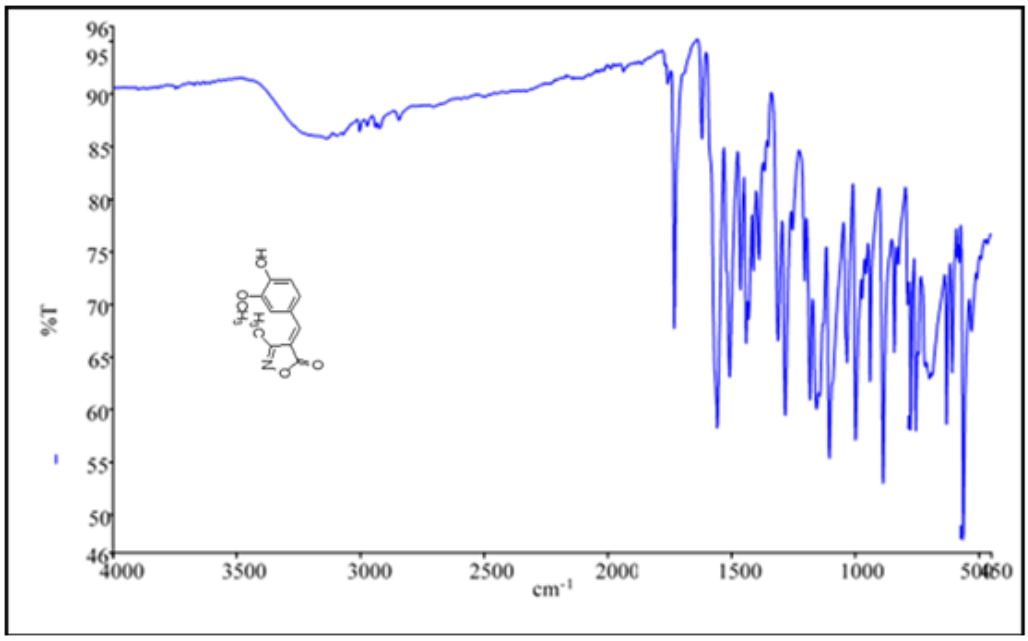


**Figure S13.** FT-IR Spectrum of (3-hydroxy-4-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4o**)

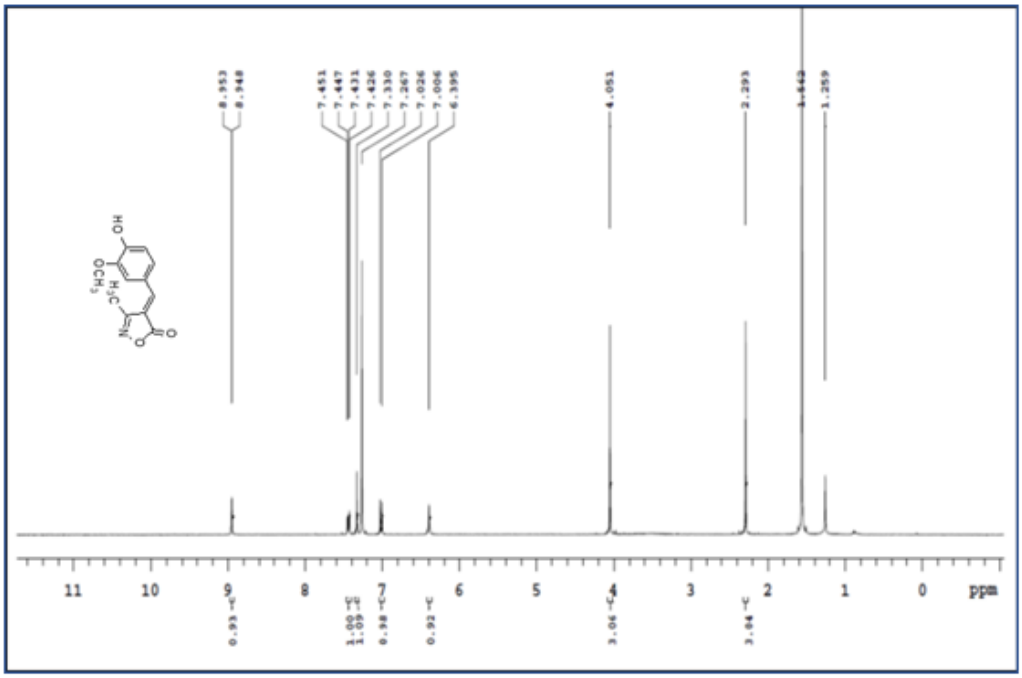


**Figure S14.** [1]H-NMR Spectrum of (3-hydroxy-4-methoxybenzylidene)-3- methylisoxazol-5(4*H*)-one (**4o**)

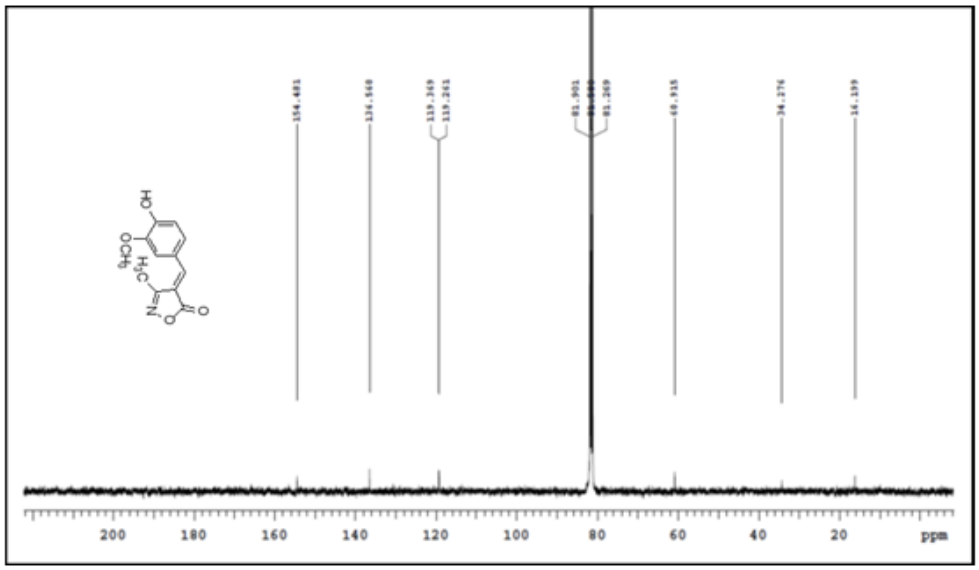


**Figure S15.** $^{1}$H-NMR Spectrum of (3-hydroxy-4-methoxybenzylidene)-3- methylisoxazol-5(4*H*)-one (**4o**)

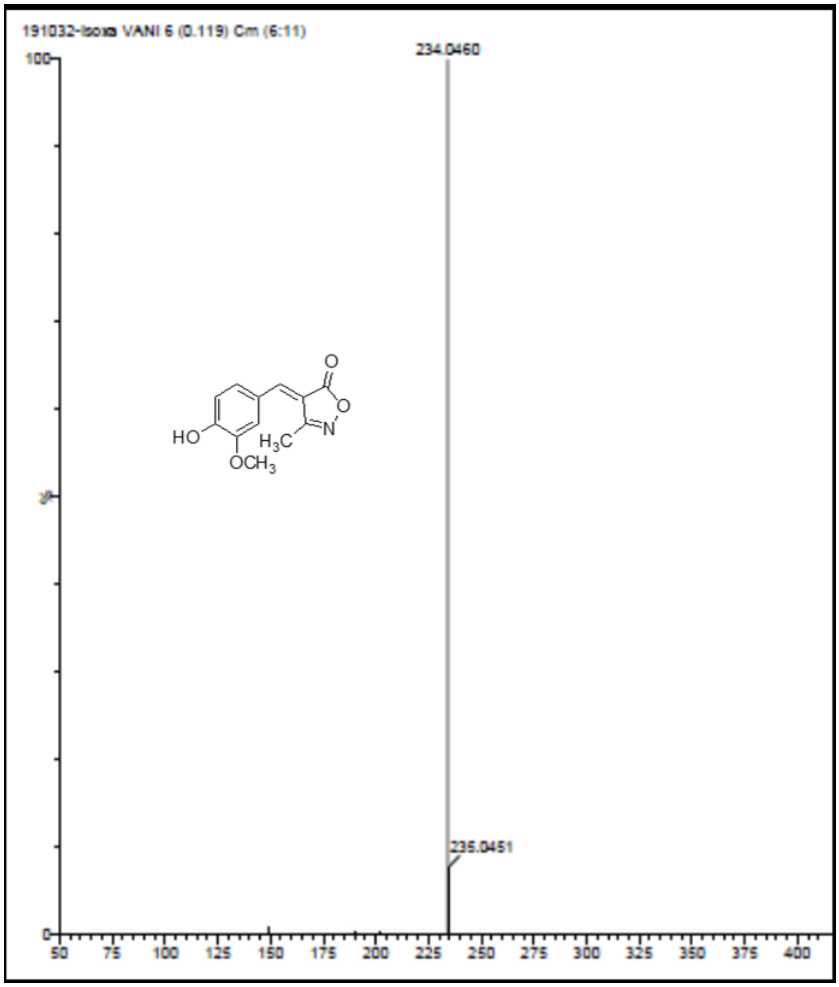


**Figure S16.** HR-MS(ESI) Spectrum of (3-hydroxy-4-methoxybenzylidene)-3-methylisoxazol-5(4*H*)-one (**4o**)