

Environmentally benign synthesis of isoxazolone derivatives using lemon juice under ultrasonic conditions

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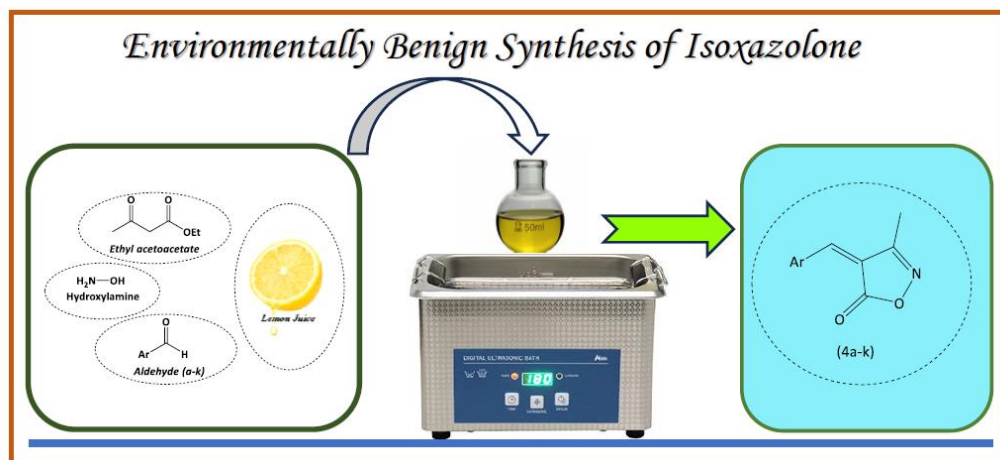
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Abstract

We report a novel, solvent-free synthetic methodology for the preparation of isoxazolone derivatives via the ultrasonic-assisted reaction of substituted aldehydes, ethyl acetoacetate (EAAC), and hydroxylamine in lemon juice. This approach is a natural acid-catalyzed multi-component reaction in an aqueous medium for the synthesis of isoxazolones in good to excellent yields. Furthermore, when ultrasonic radiation is also used, reaction rates are greatly accelerated and efficiency increases, allowing for quick synthesis with little energy input. This eco-friendly method reduces pollution in the environment by eliminating the need for dangerous chemical solvents. The resultant isoxazolone derivatives exhibit a broad spectrum of biological activities, including antimicrobial, anti-inflammatory, and anticancer properties, rendering them valuable scaffolds for pharmaceutical and medicinal development.



Keywords: Ultrasonic synthesis, Eco-friendly, Isoxazolone, Lemon Juice, Ethyl acetoacetate, Hydroxylamine

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Introduction

Researchers around the world have realized the fundamental importance of nitrogen-containing heterocycles in pharmaceutical and medicinal chemistry. These heterocycles are the core building blocks of many pharmaceutically active natural products. In fact, 59% of the small molecule drugs approved by the USFDA, contain nitrogen heterocycles, making them the most important heterocycles in medicinal chemistry.¹ Isoxazolone derivatives are a class of heterocyclic compounds that contain a five-membered ring structure consisting of carbon, nitrogen, and oxygen atoms. They are known for their diverse biological activities and have been studied for their potential applications in various fields like medicines, pesticides in crops, oils, dyes, high-temperature lubricants, semiconductors, liquid crystals etc.²⁻¹⁰ The chemical structures of bioactive compounds with the isoxazol-5(4*H*)-one nucleus, a pharmacological significant scaffold with a variety of biological activities, are depicted in Figure 1.

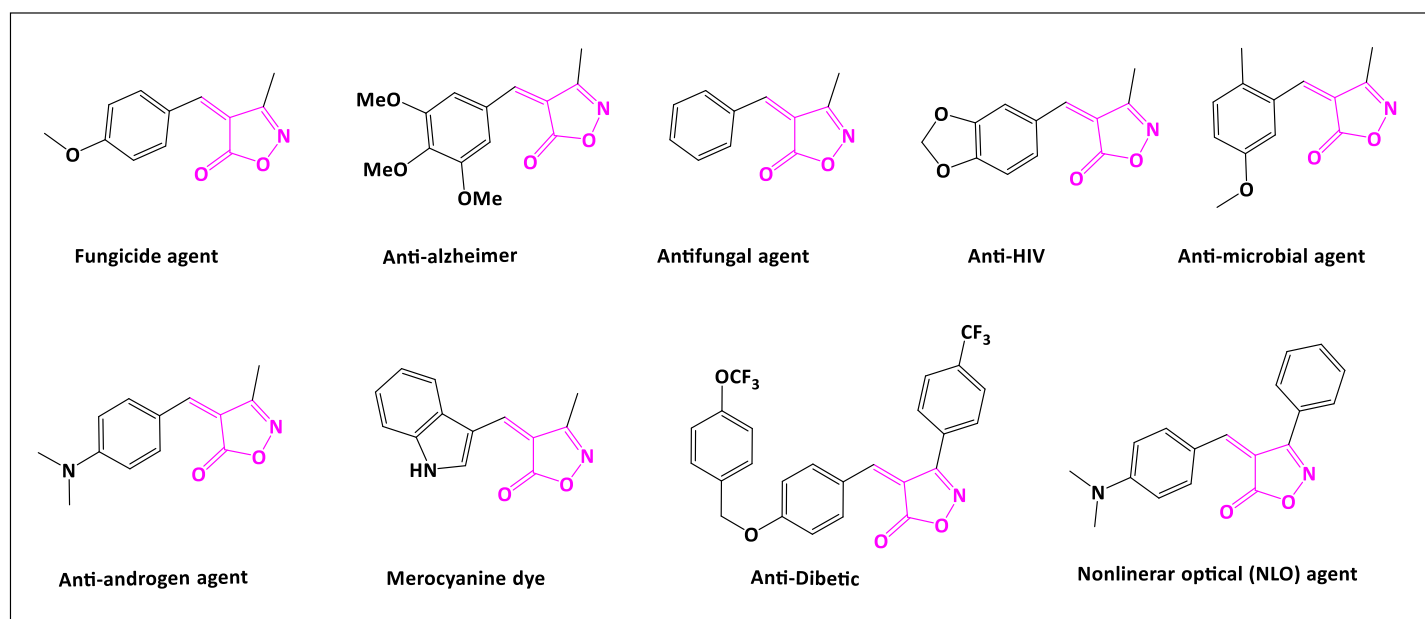


Figure 1. Isoxazolone derivatives are useful in various ways.

Several synthesis techniques have been described to create these heterocycles because of their significance. The synthesis of these heterocyclic compounds includes a multi-component condensation process including hydroxylamine, different aldehydes, and β -keto esters.

In the last few years, numerous researchers have been working on the synthesis of Isoxazolone derivatives. One of the most common strategies used involves a three-component (multi-component) reaction between various aldehydes, hydroxylamine, and dicarbonyl derivatives. This reaction is typically performed using different catalysts like sodium benzoate ($\text{C}_6\text{H}_5\text{CO}_2^- \text{Na}^+$),¹¹ 2-hydroxybenzoic acid (salicylic acid, 2-HO- $\text{C}_6\text{H}_4\text{CO}_2\text{H}$),¹² guanidine hydrochloride ($\text{H}_2\text{NC}=\text{NHNH}_2 \cdot \text{HCl}$),¹³ potassium phthalimide (PPI),¹⁴ nano- $\text{SiO}_2\text{-H}_2\text{SO}_4$,¹⁵ pyridine,¹⁶ boric acid (H_3BO_3),¹⁷ sodium silicate,¹⁸ pyridine/ultrasound,¹⁹ potassium carbonate,²⁰ sodium hypophosphite,²¹ sodium sulfide,²² ionic liquid [BMIM][PF₆],²³ Cu/TCH-pr@SBA-15 nano-composite,²⁴ citrazinic acid,²⁵ MnO_2 @Zeolite-Y nanoporous,²⁶ Montmorillonite nanoclay,²⁷ pyridinium *p*-toluenesulfonate (PPTS),²⁸ monosodium phosphate (NaH_2PO_4),²⁹ cobalt iron oxide (CoFeO),³⁰ 4-(dimethylamino)pyridine (DMAP),³¹ ZSM-

5 as a heterogeneous catalyst,³² sulfonated graphene oxide,³³ piperazine,³⁴ citric acid,³⁵ sodium chloride,³⁶ potassium bromide,³⁷ etc. as catalysss were used under various condition (room temperature to reflux).

Our current thinking is focused on how to use lemon juice for the construction of heterocyclic compounds. The citric acid derived from lemons has recently been utilized in various organic transformations or for the synthesis of organic derivatives fused Imidazoles,³⁸ substituted benzoxazoles,³⁹ bispyrazolyl methanes,⁴⁰ and bismuth nanoparticless.⁴¹ A few researchers have presented their investigation into the use of lemon juice for the synthesis of naturally or pharmaceutically active heterocyclic compounds.⁴²⁻⁴⁵ The text is about the importance of multicomponent reactions (MCRs) in modern synthetic chemistry. MCRs are important because they allow for the simultaneous formation of multiple chemical bonds, saving time, energy, and resources in the synthetic process of heterocycles.⁴⁶⁻⁴⁸ Based on the cases mentioned above, we are developing a more affordable, environmentally friendly, and efficient strategy for synthesizing isoxazolone derivatives using lemon juice. Lemon juice containing 5-6% of citric acid, facilitates an acid-catalyzed multi-component reaction in an aqueous medium.⁴⁹ This approach could prove to be a secure and cost-effective method, involving the use of lemon juice reaction under ultrasonic radiation.

Results and Discussion

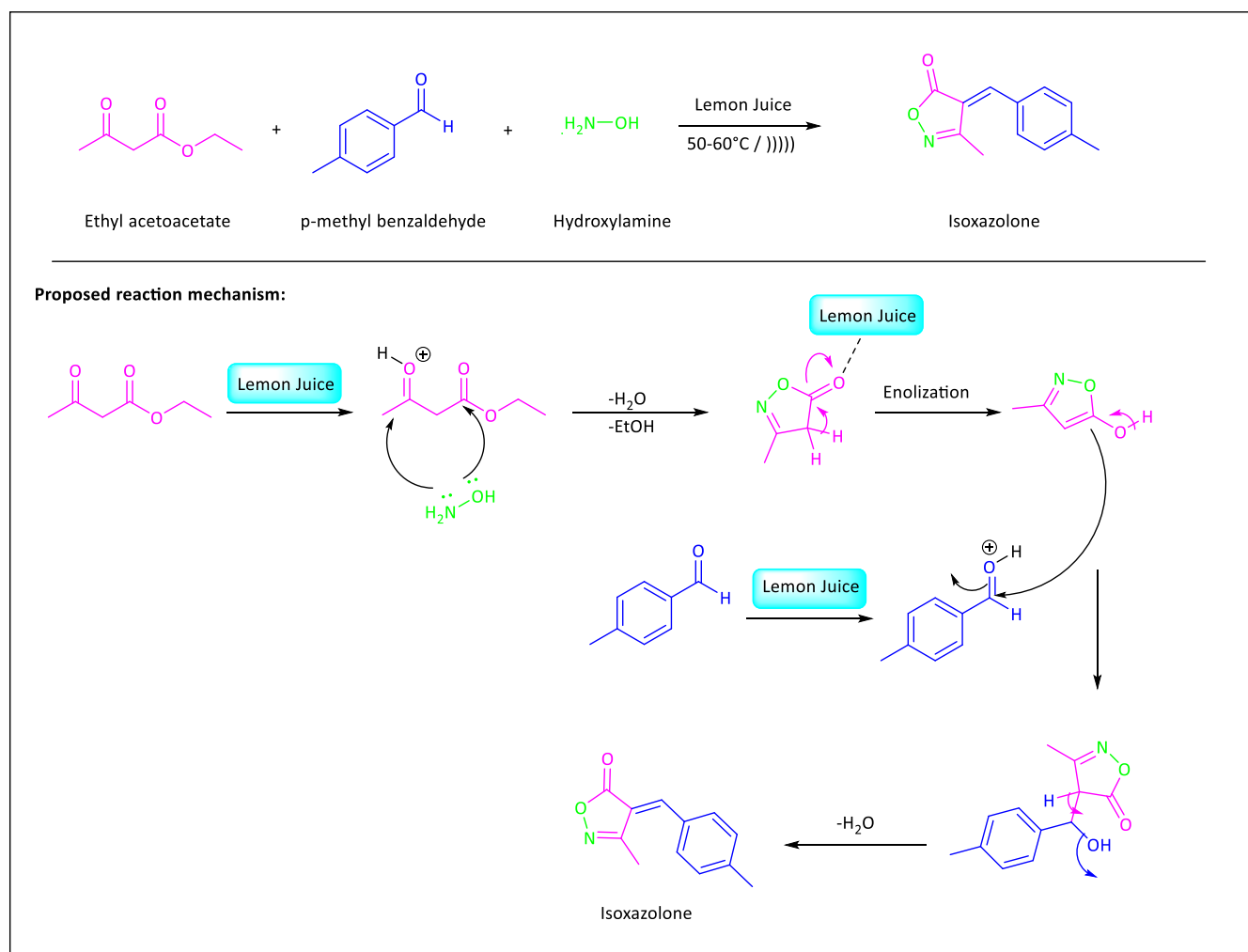


Figure 2. General reaction scheme and proposed reaction mechanism.

Based on a literature survey, we synthesized derivatives of (Z)-3-methyl-4-(4-methylbenzylidene)isoxazol-5(4H)-one employing a particular chemical reaction involving *p*-methyl benzaldehyde, ethyl acetoacetate, and hydroxylamine using lemon juice in an acid-catalyzed multi-component reaction in an aqueous medium. TLC we used to follow the progress of the reaction, and the proposed mechanism shown in Figure 2.

We have explored the reaction in citric acid and lemon juice, in the presence of water, alcoholic solvent and without solvent, for the synthesis of isoxazolone derivatives, and found feasible condition for synthesis (Z)-3-methyl-4-(4-methylbenzylidene)isoxazol-5(4H)-one from benzaldehyde, EAAC and hydroxylamine using lemon juice at 50-60 °C under ultrasonication and the result are summarized in Table 1.

Table 1. Optimization of condition for reaction

S No	Particulars	Temp.	Time	Yield
1	Citric acid /H ₂ O	25-35 °C	9-10 h	82%
2	Citric acid /H ₂ O/ Ultrasonic (US)	25-35 °C	4.0 h	83%
3	Citric acid /H ₂ O/ Ultrasonic (US)	50-60 °C	40 min	85%
4	Lemon Juice / H ₂ O / Ultrasonic (US)	25-35 °C	40 min	37%
5	Lemon Juice / H ₂ O / Ultrasonic (US)	50-60 °C	40 min	76%
6	Lemon Juice / Ethanol / Ultrasonic (US)	25-35 °C	35 min	38%
7	Lemon Juice / Ethanol / Ultrasonic (US)	50-60 °C	30 min	74%
8	Lemon Juice / Solvent free / Ultrasonic (US)	25-35 °C	30 min	56%
9	Lemon Juice / Solvent free / Ultrasonic (US)	50-60 °C	30 min	86%

Based on the above-mentioned information, we can infer that the maximal conversion of (Z)-3-methyl-4-(4-methylbenzylidene)isoxazol-5(4H)-one occurs at 50–60 °C in the absence of a solvent. Next, in order to get maximal conversion and yield, we optimize the quantity of lemon juice. The experimental results are listed in Table 2 below.

Table 2. Optimization of lemon juice for reaction

S No	Conditions	Temp.	Time	Conversion
1	Lemon juice 0.5 mL/Ultrasonic (US)	50 °C	30 min	30%
2	Lemon juice 1.0 mL/Ultrasonic (US)	50 °C	30 min	64%
3	Lemon juice 1.5 mL/Ultrasonic (US)	50 °C	30 min	85%
4	Lemon juice 2.0 mL/Ultrasonic (US)	50 °C	30 min	91%

According to the information above, about 2.0 mL of lemon juice is needed for the synthesis of isoxazolone at 50 °C under ultrasonic circumstances in order to obtain optimal product transformation.

We explored the reaction without solvent using aryl aldehyde, hydroxylamine and varying EAA the results are tabulated below in Table 3.

Table 3. Optimization of equivalence study

S No	Eq. of EAAC	Eq. of aldehyde	Temp.	Time	Conversion
1	1.00	0.90	50 °C	30 min	74 %
2	1.00	1.00	50 °C	30 min	89 %
3	1.00	1.15	50 °C	30 min	89 %

Based on the above experiment data a low yield is produced using lower equivalents of aldehyde, while higher equivalents of aldehyde does not influence reaction yield.

Once the ideal conditions are determined, we synthesize the various isoxazolone derivative **4a-k**, which are depicted in Figure 3 and Table 4.

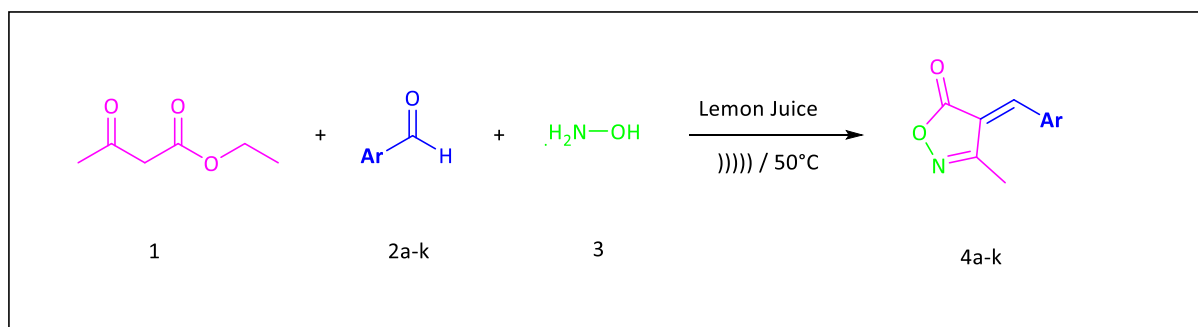
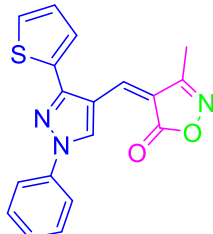
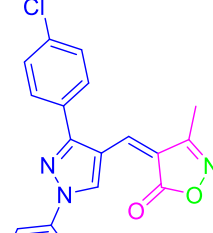
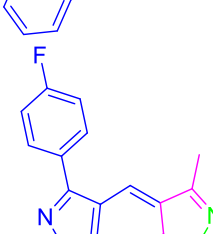
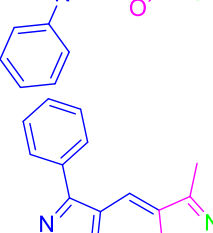


Figure 3. Synthesis of Isoxazolone derivative **4a-k**.

Table 4. Summary synthesis of Isoxazolone derivatives **4a-k**

S No	Compound	Time	Colour	Yield	M.P. °C		Ref
					Found	Reported	
4a		25 min	Brown solid	86%	148	159-161	51
4b		30 min	Yellow solid	90%	175	-	-
4c		30 min	Pale yellow solid	86%	162	166-168	52
4d		25 min	Light yellow solid	92%	133	132-133	51
4e		25 min	Yellow solid	89%	175	177-179	51
4f		20 min	Brown solid	89%	232	226-227	37
4g		30 min	Light yellow solid	86%	214	210	50

Table 4. Continued

S No	Compound	Time	Colour	Yield	M.P. °C		Ref
					Found	Reported	
4h		20 min	Reddish solid	85%	198	192	50
4i		30 min	Yellow solid	86%	226	228	50
4j		30 min	Yellow solid	82%	210	204	50
4k		20 min	Pale yellow solid	90%	205	208	50

We have reported an efficient strategy and effective conditions for the synthesis of isoxazole derivatives and we highlight the effectiveness of this method, compared with previous methods as summarized below in Table 4.

S. No	Condition	Temp.	Time	Yield	Ref
1	Citric acid / Water	r.t	8.0 h	90	35
2	Pyridine / Ethanol	Reflux	2.0 h	52.5	16
3	Pyridine / Water / Ultrasound	-	1.5 h	67	19
4	PTSA / Water	Reflux	2.0 h	62	28
5	Monosodium phosphate	80 °C	3.0 h	68	29
6	K ₂ CO ₃ / water	Reflux	3.0 h	72	20
7	Sodium hypophosphite / Ethanol	r.t.	10 min	80	21
8	2,2'-Bipyridine, perchlorate / Water	Reflux	65 min	84	23
9	Boric acid B(OH) ₃ / Water	80°C	30 min	86	17
11	Cu/TCH-pr@SBA-15 nanocatalyst	80°C	8.0 h	95	24
12	Potassium phthalimide / Water	r.t.	1.5 h	70	14
13	Sodium silicate / Water	r.t.	2.5 h	89	18
14	Sodium benzoate / Water	r.t.	1.5 h	87	11
15	Sodium sulfide / Ethanol	r.t.	2.5 h	80	22
16	[BMIM][PF ₆] / Ethanol	Reflux	1.0 h	85	23
17	Lemon Juice / Ultrasonic radiation	50 °C	30 min	91	Current work

Conclusions

We have developed a simple and effective method for synthesizing some isoxazol-5(4*H*)-one derivatives by combining an aryl aldehyde, ethyl acetoacetate (EAA), and hydroxylamine in lemon juice under ultrasonic affordable, readily available, biodegradable, safe, and simple irradiation at 50-60 °C. Lemon juice has the potential to be a reactant as well as a solvent for reaction. In another aspect, it aligns with the concept of green chemistry. This method offers several advantages, including avoiding the use of metal catalysts, easy work-up, clean reactions, shorter reaction times, and excellent yields, contributing to green chemistry. Performing the reaction at 50-60 °C under ultrasonic irradiation is significant as it reduces hazardous waste and energy consumption, eliminates the need for solvents, minimizes water pollution, and helps conserve biodiversity and the ecosystem.

Experimental Section

General. All the ingredients, reagents, and solvents used in this experiment were sourced from reputable commercial providers. We used borosilicate glassware and a 6.5-liter ultrasonic bath manufactured by Athena technology model 220/240 V, AC/50Hzs for sonication. Silica gel served as the stationary phase in TLC analysis, which allowed us to monitor the progress and conversion of the product. The characteristics of each spot were visible under ultraviolet light. Melting points were determined in open capillary tubes using a paraffin oil bath and were uncorrected. IR spectra were recorded using a Bruker FT-IR spectrometer, ^1H NMR spectra were acquired using a Bruker 400 and 500 MHz spectrometer, and ^{13}C NMR spectra were recorded using a Bruker 100 & 125 MHz spectrometer. Additionally, the Agilent 7800 system was used to analyses the mass of every compound.

Synthesis of Isoxazolone derivatives 4a-k. A mixture of EAAC (2.014 mmol), aryl aldehyde (or its derivative) (2.014 mmol), and hydroxylamine (2.014 mmol) was added within a sealed tube. To this mixture lemon juice (2.0 ml) was added and the resultant mixture was kept at 50 °C, under ultrasonic radiation for an appropriate time. Completion of the reaction was determined by TLC (Ethyl acetate: Hexane, 95:5), and the resulting reaction mixture was filtered and washed with water times. Finally, this crude product was recrystallized from EtOH to afford the pure product.

4-(4-Fluorobenzylidene)-3-methylisoxazol-5(4H)-one (4a). Brown Solid (Yield-86%); M.P. 158 °C; ^1H NMR (d_6 -DMSO, 400 MHz) δ : 8.54 (m, 2H, Ar-H), 7.98 (s, 1H, CH), 7.48-7.44 (m, 2H, Ar-H), 2.30 (s, 3H, CH₃); ^{13}C NMR (CDCl₃, 100 MHz) δ : 168.0, 167.2, 164.7, 161.0, 148.2, 136.8, 136.7, 128.9, 116.6, 116.4, 11.6; FT-IR cm⁻¹ (KBr):3047.55, 1749.04, 1621.19, 1507.35, 1423.31, 1297.26, 1169.36, 960.34, 830.69; MS: m/z = 206.23 [M+1].

4-(2,4-difluorobenzylidene)-3-methylisoxazol-5(4H)-one (4b). Yellow Solid (Yield-90%); M.P. 175 °C; ^1H NMR (d_6 -DMSO, 400 MHz) δ : 7.96-7.94 (m, 1H, Ar-H), 7.50-7.48, (m, 1H, Ar-H), 7.31-7.21 (m, 1H, Ar-H), 7.06 (s, 1H, CH) 1.90 (s, 3H, -CH₃); ^{13}C NMR (CDCl₃, 100 MHz) δ : 167.8, 166.9, 165.4, 161.1, 148.2, 138.7, 138.6, 135.4, 116.6, 116.4, 11.6; FT-IR cm⁻¹ (KBr):3433.36, 3072.47, 1672.25, 1496.98, 1367.09, 990.39, 874.42; MS: m/z = 224.03 [M+1].

4-(4-chlorobenzylidene)-3-methylisoxazol-5(4H)-one (4c). Pale yellow solid (m, 1H, Ar-H), 7.56-7.37 (m, 1H, Ar-H), 7.26 (s, 1H, CH), 2.33 (s, 3H, -CH₃); ^{13}C NMR (CDCl₃, 100 MHz) δ : 167.5, 160.9, 148.0, 147.8, 135.0, 133.6, 131.8, 130.3, 129.8, 121.2, 11.6; FT-IR cm⁻¹ (KBr):3148.00, 3056.70, 1734.91, 1595.56, 1517.52, 1335.33, 1123.5, 872.5; MS: m/z = 222.05 [M+1].

4-(4-methylbenzylidene)-3-methylisoxazol-5(4H)-one (4d). Light yellow Solid (Yield-92%); ; M.P. 133 °C; ^1H NMR (CDCl₃, 500 MHz) δ : 8.28-8.27 (d, 2H, Ar-H), 7.39 (s, 1H, CH), 7.32-7.26 (d, 2H, Ar-H), 2.44 (s, 3H, Ar-CH₃), 2.28 (s, 3H, -CH₃); ^{13}C NMR (CDCl₃, 125 MHz) δ : 168.2, 161.2, 150.0, 145.7, 134.1, 131.0, 130.0, 129.9, 129.7, 118.4, 22.1, 11.6; FT-IR cm⁻¹ (KBr):3010.19, 2935.37, 1968.19, 1612.12, 1423.54, 1264.30, 987.14, 872.95; MS: m/z = 202.10 [M+1].

4-(4-methoxy benzylidene)-3-methylisoxazol-5(4H)-one (4e). Yellow Solid (Yield-89%); M.P. 175 °C; ^1H NMR (CDCl₃, 500 MHz) δ : 8.45-8.42 (d, 2H, Ar-H), 7.34 (s, 1H, CH), 7.01-6.99 (d, 2H, Ar-H), 3.91 (s, 3H, OCH₃), 2.27 (s, 3H, -CH₃); ^{13}C NMR (CDCl₃, 125 MHz) δ : 168.8, 164.6, 161.3, 149.3, 137.0, 125.8, 116.4, 114.7, 55.7, 11.6; FT-IR cm⁻¹ (KBr):2929.47, 2838.08, 1751.41, 1591.24, 1484.96, 1324.64, 1250.74, 986.50, 815.00; MS: m/z = 218.07 [M+1].

4-(4-(dimethylamino)benzylidene)-3-methylisoxazol-5(4H)-one (4f). Brown Solid (Yield-89%); M.P. 232 °C; ^1H NMR (CDCl₃, 500 MHz) δ : 8.40-8.39 (d, 2H, Ar-H), 7.21 (s, 1H, CH), 6.74-6.72 (d, 2H, Ar-H), 3.15 (s, 6H, -

$N(CH_3)_2$, 2.23 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ :170.1, 161.5, 154.1, 149.2, 137.6, 121.8, 111.7, 111.4, 40.2, 11.7; FT-IR cm^{-1} (KBr):2927.23, 1662.28, 1594.03, 1556.25, 1412.26, 1298.31, 1149.85, 912.48, 864.75.

3-methyl-4-((1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)methylene)isoxazol-5(4H)-one (4g). Light yellow solid (Yield-86%); M.P. 214 °C; 1H NMR (d_6 -DMSO, 400 MHz) δ : 7.94-7.92 (d, 2H, Ar-H), 7.68-7.60 (m, 5H, Ar-H), 7.51 (s, 1H, CH), 7.49-7.40 (m, 2H, Ar-H), 2.49 (s, 3H, CH_3), 2.23 (s, 3H, Ar- CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ :168.9, 161.7, 140.0, 139.2, 138.4, 133.5, 130.0, 129.7, 129.1, 128.2, 127.6, 119.6, 115.1, 114.7, 20.9, 10.9; FT-IR cm^{-1} (KBr):3144.64, 2979.94, 1734.55, 1611.86, 1596.65, 1454.70, 1334.46, 1231.35, 996.54, 831.85; MS: m/z = 244.39 [M+1].

3-methyl-4-((1-phenyl-3-(thiophen-2-yl)-1H-pyrazol-4-yl)methylene)isoxazol-5(4H)-one (4h). Reddish solid (Yield-85%); M.P. 198 °C; 1H NMR (d_6 -DMSO, 400 MHz) δ : 7.89-7.87 (m, 2H, Ar-H), 7.88-7.75 (m, 1H, Ar-H), 7.68-7.66 (m, 1H, Ar-H), 7.62-7.58 (m, 2H, Ar-H), 7.48-7.45 (m, 1H, Ar-H), 7.29-7.26 (s, 1H, CH), 2.31 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ :168.8, 161.0, 150.3, 138.7, 138.1, 133.5, 131.8, 129.8, 128.7, 128.5, 128.4, 128.2, 119.4, 115.2, 114.9, 79.2, 78.8, 78.5, 10.9; FT-IR cm^{-1} (KBr):3145.20, 3109.20, 1744.93, 1613.44, 1498.64, 1460.42, 1297.09, 1128.987, 992.60, 871.71; MS: m/z = 236.35 [M+1].

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Supplementary Material

For spectral analysis of synthesised compounds **4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **4g** & **4h**. Please find the Spectral data in supplementary information (SI).

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