

Original Research

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Oxazole; Schiff bases; Thiazole; Antioxidant activity; DPPH; ABTS; Citrus limon.

Design, Synthesis, and In Vitro Antioxidant Evaluation of Novel Oxazole Derivatives

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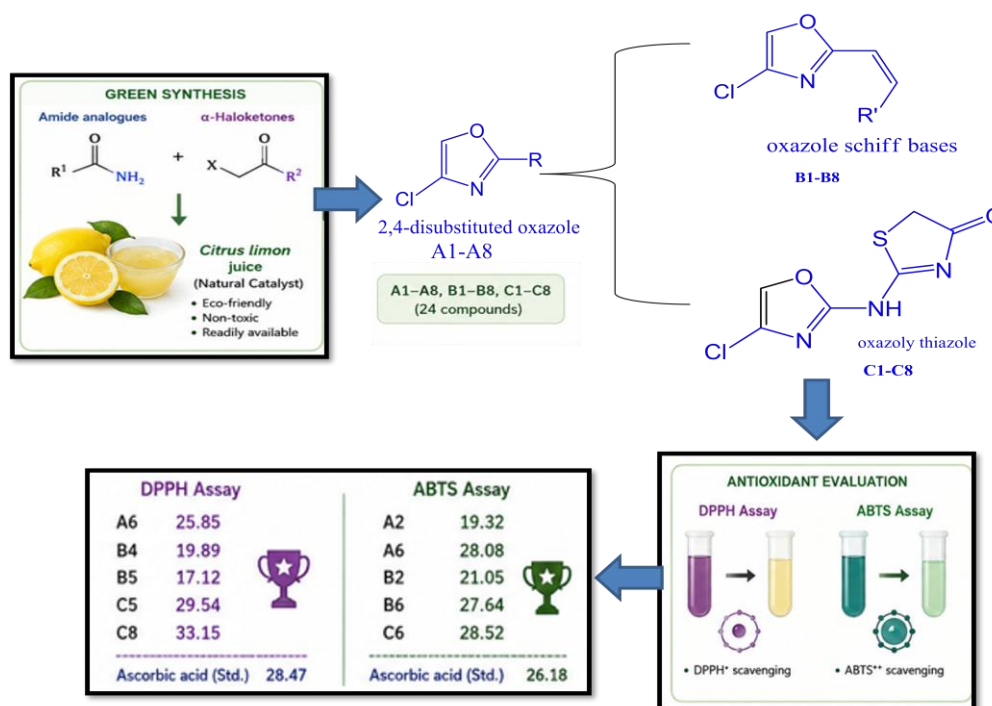
ABSTRACT: Background: Oxazole derivatives represent an important class of heterocyclic compounds with diverse biological activities, including antioxidant potential. The present study aimed to synthesize and characterize a series of 2,4-disubstituted oxazole derivatives and evaluate their in vitro radical-scavenging activity.

Methods: A series of 24 oxazole derivatives comprising A1–A8, B1–B8, and C1–C8 was synthesized using Citrus limon juice as a natural catalyst. The synthesized compounds were further developed into Schiff-base and thiazole analogues and characterized using Fourier-transform infrared spectroscopy (FT-IR), ¹H-nuclear magnetic resonance (¹H-NMR), ¹³C-nuclear magnetic resonance (¹³C-NMR), and mass spectrometry. Antioxidant activity was evaluated using DPPH and ABTS radical-scavenging assays, with ascorbic acid used as the reference standard.

Results: Several derivatives demonstrated appreciable radical-scavenging activity in both assays. In the DPPH assay, compounds B5, B4, A6, C5, and C8 exhibited IC₅₀ values of 17.12, 19.89, 25.85, 29.54, and 33.61 µg/mL, respectively, compared with 28.47 µg/mL for ascorbic acid. In the ABTS assay, A2, B2, B6, A6, and C6 showed IC₅₀ values of 19.32, 21.05, 27.64, 2, and 28.52 µg/mL, respectively, compared with 26.18 µg/mL for ascorbic acid. The differing activity profiles across the two assays indicate that antioxidant activity was influenced by the structural characteristics of the individual derivatives.

Conclusion: The study demonstrates that the synthesized oxazole derivatives possess promising in vitro radical-scavenging activity, with B5 and B4 showing the strongest DPPH activity and A2 and B2 showing the strongest ABTS activity. The findings support further investigation of selected oxazole derivatives to elucidate their structure–activity relationships and biological potential.

GRAPHICAL ABSTRACT



INTRODUCTION

Nucleic acids and cell membranes are harmed by free radical-induced oxidative stress, which causes pathological illnesses such as atherosclerosis [1], diabetes [2], Alzheimer's disease [3], and aging [4]. The discovery of compounds with antioxidant activity to stop oxidative damage has been the subject of extensive investigation. Therapeutic molecules having an oxazole core are still being investigated in medicinal chemistry as possible antioxidant agents [5]. Using trimethylamine, water as the solvent, and cyclodextrin (-CD) as the catalyst, Mahdavi et al. recently reported the synthesis of 1,3-oxazole in 2017 [6]. Yasaei et al. synthesized 5-(2-chloroquinolin-3-yl) oxazole in 2019. The synthesis reaction involving 2-chloroquinoline-3-carbaldehyde and TosMIC follows a Van Leusen pathway in the presence of Pd-catalyzed amidation [7]. Many oxazole compounds, both natural and synthetic, offer a wide range of druggable properties and pharmacological potentials [8]. Oxazole has a wide range of biological actions, such as antibacterial [9], antifungal [10], anticancer [11], vegetative growth factor [12], anti-tubercular, anti-leishmanial [13], antiprotozoal [14], anti-inflammatory [15], and more. Oxazoles, being weak bases, are employed in numerous chemical reactions to produce a diverse array of physiologically and medicinally active organic entities [16]. Among other things, several synthesis methods produce more dangerous byproducts, have low yields, and call for harsh reaction conditions. Furthermore, most oxazole compounds exhibited reduced antioxidant properties comparable to those of the employed standards [17, 18]. In this sense, the current research has been carried out in an attempt to get beyond the drawbacks of synthetic processes and create novel treatments with greater antioxidant capacity and reduced toxicity [19]. Because of the importance of oxazole and its derivatives, we have concentrated our research on the safe, affordable, and environmentally benign synthesis of oxazole using fruit juices as a natural catalyst [20]. The structures of the generated molecules were described using spectral analysis. The ability of the new compounds to scavenge free radicals was assessed after structural characterization. We are now concentrating on the synthesis of oxazole derivatives A1–A8, B1–B8 (oxazole substituted with thiazole), and C1–C8 (oxazole containing schiff bases). The antioxidant properties of each synthesized molecule were assessed. However, oxazoles and their biological effects has been the subject of many investigations [21]. Many of the synthesis methods require intense reaction conditions, which results in lower yields and more dangerous byproducts. Furthermore, most oxazole compounds displayed reduced antioxidant properties comparable to the employed standards [22]. In this sense, the current research has been carried out in an attempt to overcome the drawbacks of synthetic processes and create novel treatments with more antioxidant potentials and reduced toxicity. The Bredereck oxazole synthesis, which involves cyclizing α -haloketones with substituted amides in ethanol using citric acid as a catalyst, is used in this study to

demonstrate the synthesis of substituted oxazole derivatives. This subsequently interacted with aldehyde derivatives to generate Schiff's base and with α -haloketones to form thiazole substituted oxazoles. The structures of the generated molecules were described using spectral analysis. The novel compound's capacity to scavenge free radicals was assessed after structural characterization.

MATERIAL AND METHODS

General methods

All of the analytical-grade chemicals and reagents used in this investigation were bought from commercial vendors. The uncorrected melting points were determined using electrical melting point equipment. Silica-gel precoated plates (Merck, Germany) were used for TLC analysis; to see the spots, the dry plates were subjected to UV light and iodine fumes. An FT-IR spectrometer (SHIMADZU, Japan) was used to record IR (in KBr, $\bar{\nu}$ cm⁻¹). Bruker Advance Neo 500 MHz spectrometers (Bruker, Germany) were used to record ¹H and ¹³C NMR spectra. The chemical shifts (δ) were measured in dimethylsulfoxide (DMSO-d₆) in relation to tetramethylsilane (TMS, δ =0.0 ppm), which served as the internal standard. The results are expressed in parts per million. Agilent LC-MSD was used to record the compounds' mass spectra.

Chemical synthesis

Preparation of Citrus limon juice

Citrus limon is a species of citrus. Because of its high ascorbic acid content, it serves as an acid catalyst in organic synthesis. First, clean and pat dry the limes. The juice was filtered through cotton and then Whatman filter paper no. 1 to produce transparent juice that could be used as a catalyst [23].

General procedure for synthesis of 2, 4-disubstituted oxazole derivatives (A1-A8)

Amides (1.0 mmol) and α -haloketone (1.1 mmol) were added in the proper amounts to a dry beaker equipped with a mechanical stirrer. Ethanol (5 ml) was then gradually added to the mixture, drop by drop. This mixture was then supplemented with potassium carbonate. The resulting mixture is swirled for about twenty minutes at room temperature. Next, a lemon-based catalyst solution (1–3 ml) was gradually added to the reaction vessel. For three to four hours, the mixture was continuously churned at 60 to 80 degrees Celsius. Following this time, the reaction was examined by TLC, and the reaction mixture was then allowed to cool to room temperature. After adding a saturated aqueous solution of NaHCO₃ (5 ml), the product was extracted using 5 ml of ethyl acetate. The organic layers are mixed together, dried over anhydrous Na₂SO₄, filtered, and vacuum-concentrated. TLC (ethyl acetate: hexane (3:7)) was used to track the completion of the reaction, and the % yield was computed. FT-IR, NMR, and mass spectrometry were used to describe the product and verify its structure (Scheme 1).

General procedure for synthesis of oxazole based Schiff bases (B1-B8)

Aldehyde derivatives (0.1 mmol) and oxazole amine (0.1 mmol) were combined in an equal volume in a beaker. Lemon juice (2 ml) was added to this reaction mixture as a catalyst and left for a short while. For three hours, the reaction mixture was agitated in a water bath using a magnetic stirrer. The solid crude product appeared once the reaction was complete. It was refined by recrystallization with the least amount of ethanol after being washed with distilled water. The product was filtered and dried following recrystallization. Physical parameters were then determined and yield was computed (Scheme 2).

General procedure for synthesis of oxazole based thiazole derivatives (C1-C8)

Equimolar quantities of substituted amides (0.1 mmol) were reacted with α haloketones (0.1 mmol) further aqueous solution of potassium carbonate (0.01 mmol) and ethanol as a solvent was added. For three hours, the reaction mixture was agitated at 60 to 80 degrees Celsius using a magnetic stirrer. After cooling to room temperature, the reaction mixture was filtered and recrystallized from 95% ethanol. The product was filtered and dried following recrystallization. After reacting with an excess of 0.01 mmol of chloroacetyl chloride, these oxazole amines were heated to a high temperature in 10 milliliters of pyridine for two hours. Ethanol recrystallized the reaction mixture, which was then poured onto ice-cold water. Five milliliters of lemon juice were mixed with ethanol and heated for one to two hours while stirring. then crystallized from n-hexane after being filtered while heated. The product was filtered and dried following recrystallization. Physical parameters were then determined and yield was computed (Scheme 3). **Biological activity**

Antioxidant activity screening

The main method for evaluating a compound's antioxidant capacity is scavenging radicals. Numerous methods have been used to quantify the amount of synthetic radicals scavenged in different polar organic solvent solutions, such as methanol at room temperature. In this study, we assessed the antioxidant potential of compounds A1-A8, B1-B8, and C1-C8 using the DPPH and ABTS methods. These methods were selected because ascorbic acid is employed as a standard agent and they are good at evaluating compounds. The ability to scavenge radicals was computed using the following formula:

$$\% \text{ Scavenging activity} = \frac{A_{(\text{control})} - A_{(\text{sample})}}{A_{(\text{control})}}$$

The absorbance of the free radical in the presence of antioxidants is shown by $A_{(\text{control})}$ and $A_{(\text{sample})}$, respectively. The IC₅₀ values for each sample were obtained by plotting radical scavenging activity (% inhibition) against concentration. The IC₅₀ value indicates the concentration of an antioxidant that can scavenge 50% of the free radicals in the mixture under assay conditions. A low IC₅₀ value indicates higher antioxidant activity in the chemical sample.

DPPH radical scavenging activity

The capacity of 24 synthetic compounds to scavenge free radicals was evaluated in vitro using the DPPH test. A spectrophotometer was used to create an absorbance at 517 nm in a solution of DPPH (60 μM) and methanol. After that, 2 ml of this solution was combined with 1 ml of the sample at various concentrations (10–120 $\mu\text{g}/\text{ml}$), and the mixture was kept at room temperature in the dark. To prevent photodegradation, the prepared solution was always freshly mixed and kept in an amber-colored bottle. The test compounds' capacity to scavenge was evaluated spectrophotometrically by measuring the absorbance at 517 nm after a half-hour incubation period. Ascorbic acid (Vitamin C), a common antioxidant, was used as a positive control under identical conditions. Methanol free of test compounds served as the negative control, or blank. Each computation was done three times. Plotting the percentage of inhibition against concentration allowed for the computation of IC₅₀ values [24, 25, 26].

ABTS cation radical scavenging assay

The spectrophotometric investigation of ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] free radical scavenging activity was conducted in accordance with the method described in earlier studies, with a few minor adjustments. In this experiment, the ABTS^{•+} solution was made by mixing about equal amounts of 2.4 mM potassium persulfate (K₂S₂O₈) and 7 mM ABTS in distilled water. The mixture was then left to react for 12 hours at room temperature in the dark. Methanol was then added to this mixture in order to get an absorbance at 734 nm. The test chemical sample solutions (10–120 $\mu\text{g}/\text{ml}$) were made using methanol. The sample was incubated for seven minutes after various volumes of the ABTS solution were added. Ascorbic acid, also known as vitamin C, served as the reference standard antioxidant. Methanol without any test samples served as the control (blank). Spectrophotometry uses the absorbance of the ABTS-reduced solution at 734 nm to determine the antioxidant capacity of each molecule. Each computation was performed three times. Plotting the percentage of inhibition against concentration yielded IC₅₀ values [27, 28, 29].

Statistical Analysis

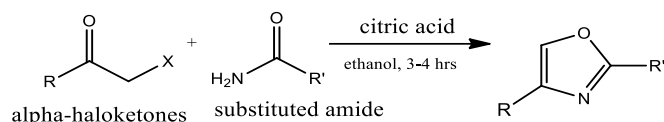
The mean $\pm$ S.D. (standard error mean) was used to display the results. The GraphPad prism was used for graphical interpretation. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test, with ascorbic acid as the reference control. The results were indicated as highly significant (**P < 0.001) and moderately significant (*P < 0.05 and **P < 0.01). Logarithmic regression analysis was used to determine the IC₅₀ value of synthetic compounds and conventional drugs based on the mean inhibitory value [30, 31].

RESULTS AND DISCUSSION

Synthesis and characterization of compounds

These compounds are characterized by FTIR, ¹³C-NMR, ¹H-NMR and mass spectrum attached in supplementary files. The synthesized derivative that was equipped is outlined in **Figure 2, 3 and 4**. A rational, modular design centred on an oxazole core synthesized under green, lemon-juice catalyzed conditions was used to choose the derivatives (A1–A8, B1–B8, and C1–C8). To isolate certain structural effects, a single oxazole intermediate was divided into three complimentary series:

heteroatom/thiazole insertion (C-series), extended π -conjugation via Schiff base creation (B-series), and direct 2, 4-substitution (A-series). Thin layer chromatography (TLC) was used to characterize the re-crystallized products. Table 1 provided the physical characterisation data in relation to their yield %. The biological activity of each heterocyclic derivative was then examined.



Scheme 1: Synthesis of substituted oxazole derivatives

Oxazole Formation Process (Figure 1)

Mechanistic procedures:

1. Nucleophilic attack: α -haloketone carbonyl carbon is attacked by amide nitrogen.
2. Cyclization: Five-membered oxazole rings are formed as a result of intramolecular nucleophilic assault.
3. Elimination: The aromatic oxazole ring is created when HX is lost.
4. Lemon juice's function: Its citric acid functions as a catalyst for Bronsted acid and aids in the activation of carbonyl groups. Because lemon juice contains organic acids like citric acid and ascorbic acid, it creates an acidic environment that enables the protonation of the carbonyl group and encourages nucleophilic cyclization that results in the creation of oxazoles.

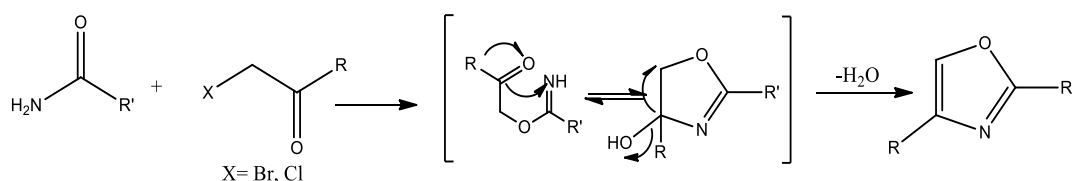


Figure 1: Mechanism for Oxazole Formation

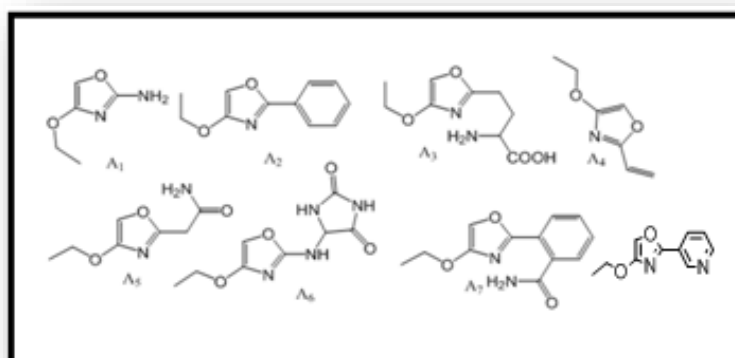
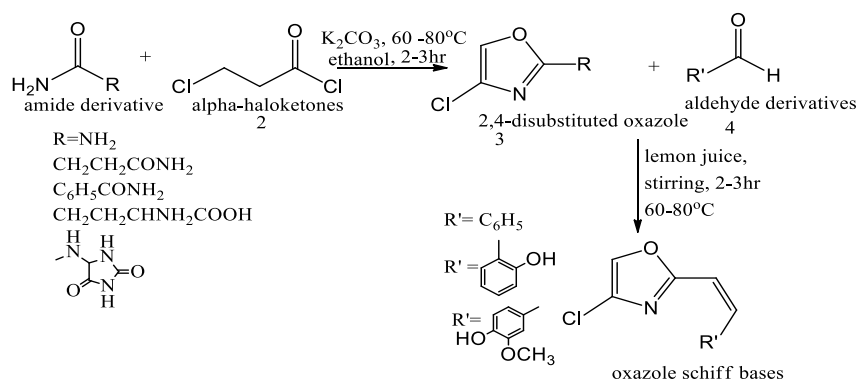


Figure 2: Structure of oxazole derivatives synthesized (A1-A8)



Scheme 2: Schematic diagram for the synthesis of oxazole based Schiff bases (B₁-B₈)

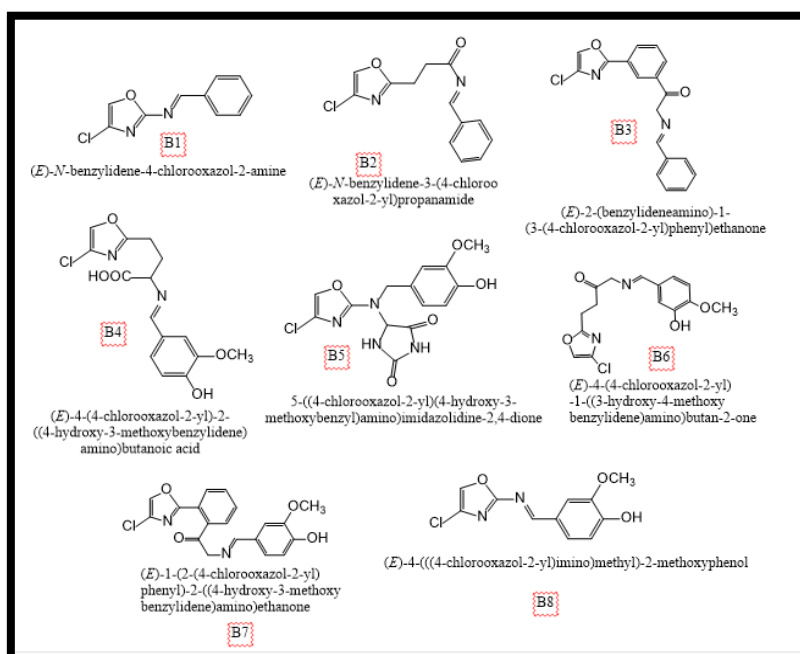
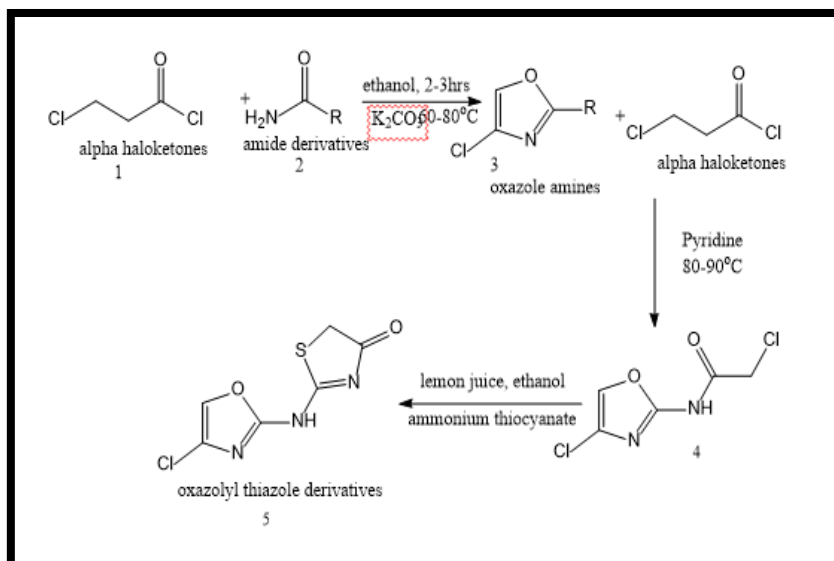


Figure 3: Structure of synthesized oxazole Schiff bases (B₁-B₈)



Scheme 3: Schematic diagram for the synthesis of oxazole based thiazole (C₁-C₈)

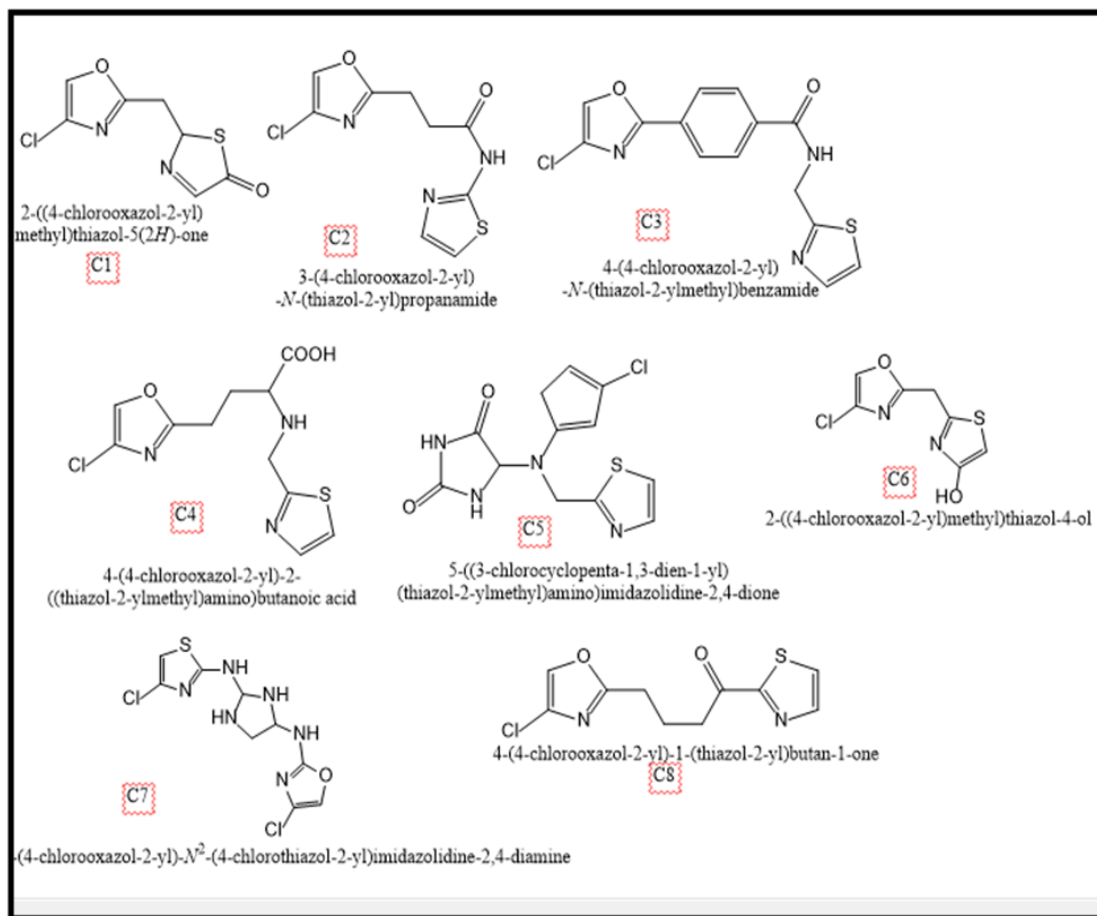


Figure 4: Structure of synthesized oxazole substituted thiazole (C₁-C₈)

Biological evaluation efficacy

In vitro antioxidant efficacy

The antioxidant activity of substances was evaluated in relation to ascorbic acid as a reference standard using the DPPH scavenging free radical activity test method and ABTS. Tables 2 and 3 present the data together with their IC₅₀ values and % inhibition. Radicals, the most unstable and extremely reactive species, are produced during a range of metabolic processes that damage living cells. For instance, they can cause strand breakage and damage DNA. In addition to its ability to scavenge radicals, antioxidants have lately been demonstrated to have anti-inflammatory, anti-cancer, anti-aging, and antidiabetic qualities. As a result, ascorbic acid (AA) was used as a control treatment in the DPPH and ABTS assay to evaluate the antioxidant capacity of the novel 24 compounds shown in Figure 5.

DPPH scavenging efficacy

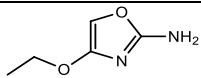
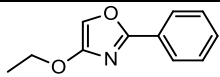
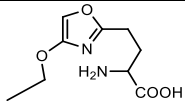
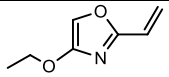
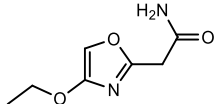
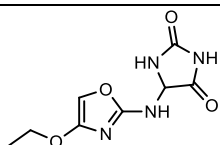
The DPPH assay revealed considerable variation in radical-scavenging activity among the synthesized derivatives. Several compounds showed substantially lower IC₅₀ values than the less-active members of the library, indicating a clear structure-dependent variation in activity. Among the 24 derivatives, B5 showed the strongest DPPH activity, with an IC₅₀ of 17.12 µg/mL, followed by B4 (19.89 µg/mL) and A6 (25.85 µg/mL). Compounds C5 (29.54 µg/mL) and C8 (33.15 µg/mL) also showed appreciable activity. Ascorbic acid exhibited an IC₅₀ of 28.47 µg/mL under the reported assay conditions. Thus, B5, B4, and A6 showed numerically lower IC₅₀ values than the reference standard, whereas C5 and C8 showed weaker activity than ascorbic acid. The relatively high activity of B5 may be associated with the combination of a phenolic hydroxyl group, methoxy substitution, and the conjugated Schiff-base framework present in this derivative. B4 possesses related structural features and also demonstrated strong DPPH activity. A6, which contains an imidazolidinedione-containing substituent and

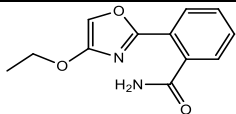
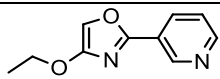
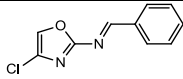
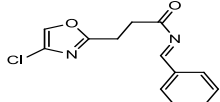
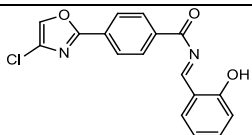
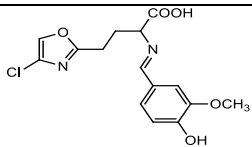
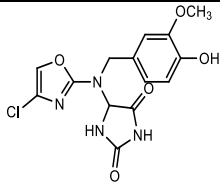
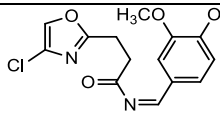
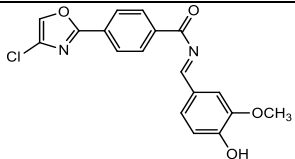
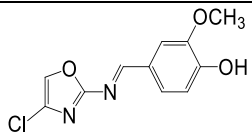
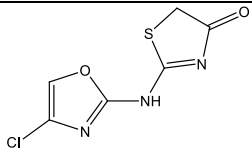
an amino linkage, also showed substantial activity. However, these structural associations should be considered qualitative SAR observations, because the present study does not independently establish the contribution of each functional group through controlled analogue comparisons. The DPPH results therefore indicate that antioxidant activity is influenced by the overall substitution pattern and molecular architecture of the derivatives rather than by the presence of a single functional group. Although electron-rich functionalities such as hydroxyl and methoxy groups may facilitate radical stabilization through hydrogen-atom or electron-transfer processes, the present dataset does not support a universal rule that electron-donating substituents always produce higher antioxidant activity.

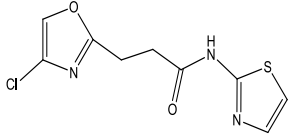
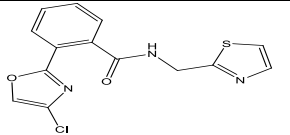
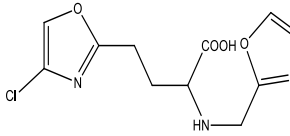
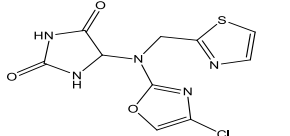
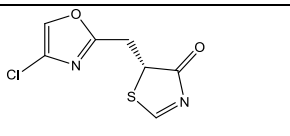
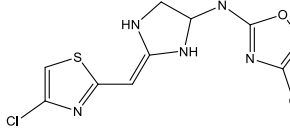
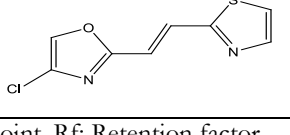
ABTS efficacy

With IC₅₀ values of 19.32, 28.08, 21.05, 27.64, and 28.52 µg/mL, respectively, the compounds A2, A6, B2, B6, and C6 had strong scavenging potential radical scavenging ability, according to ABTS assay results. These values were equivalent to ascorbic acid (IC₅₀ = 26.18 µg/mL). B2 exhibits the highest activity due to its extended conjugation and effective electron-donating ability, which facilitates stabilization of the radical intermediate; A2 increases the activity due to substitution of phenyl ring, B6 contains phenolic OH, conjugation through Schiff base linkage, and methoxy substituents in aromatic ring; C6 is substituted with thiazole ring substituted with OH group, which exhibits good activity compared to standard compounds; and A6 is substituted with electron-donating Imidazolidinedione and amino linker to oxazole. The ABTS results further demonstrated that structural modification of the oxazole scaffold produced assay-dependent changes in radical-scavenging activity. A2 and B2 were the most potent derivatives according to their IC₅₀ values, suggesting that their respective substitution patterns favor interaction with the ABTS radical under the experimental conditions. B6 and A6 also showed favorable activity, indicating that phenolic functionality, conjugation, and heterocyclic substitution may contribute to activity. However, the differences in compound ranking between the DPPH and ABTS assays indicate that antioxidant activity cannot be attributed to a single structural feature. The present findings therefore support a multifactorial SAR involving substituent electronics, hydrogen-atom/electron-donation capacity, conjugation, and molecular architecture.

Table 1. Physical parameters of synthesized compounds

Compounds	Structure	Yield (%)	M.P (°C)	Molecular formula	Molecular Weight (g/mol)	Rf value
A1		73%	135-136	C ₅ H ₈ N ₂ O ₂	128	0.35
A2		71%	126-128	C ₁₁ H ₁₁ NO ₂	189.21	0.50
A3		80%	104-106	C ₉ H ₁₄ N ₂ O ₄	214.22	0.41
A4		70%	130-131	C ₇ H ₉ NO ₂	139.15	0.45
A5		72%	158-160	C ₇ H ₁₀ N ₂ O ₃	170.12	0.48
A6		70%	180-182	C ₈ H ₁₀ N ₄ O ₄	226.19	0.51

A7		75%	126-127	C ₁₂ H ₁₂ N ₂ O ₃	232.24	0.55
A8		72%	132-133	C ₁₀ H ₁₀ N ₂ O ₂	190.20	0.40
B1		70%	222-224	C ₁₀ H ₇ ClN ₂ O	206.02	0.43
B2		68%	258-260	C ₁₅ H ₁₆ ClN ₂ O ₃	272.12	0.35
B3		65%	188-190	C ₁₈ H ₁₃ ClN ₂ O ₂	324.07	0.55
B4		67%	201-203	C ₁₅ H ₁₅ ClN ₂ O ₅	338.24	0.48
B5		78%	234-236	C ₁₄ H ₁₃ ClN ₄ O ₅	352.73	0.63
B6		72%	196-197	C ₁₄ H ₁₃ ClN ₂ O ₄	308.72	0.66
B7		60%	210-212	C ₁₈ H ₁₃ ClN ₂ O ₄	356.76	0.51
B8		68%	248-250	C ₁₁ H ₉ ClN ₂ O ₃	252.65	0.49
C1		72%	219-220	C ₆ H ₄ ClN ₃ O ₂ S	217.63	0.62

C2		75%	250-252	C ₉ H ₈ ClN ₃ O ₂ S	257.12	0.58
C3		70%	188-190	C ₁₄ H ₁₀ ClN ₃ O ₂ S	319.77	0.67
C4		70%	201-203	C ₁₁ H ₁₂ ClN ₃ O ₃ S	301.75	0.59
C5		62%	271-273	C ₁₀ H ₈ ClN ₅ O ₃ S	313.72	0.70
C6		70%	221-223	C ₇ H ₅ ClN ₂ O ₂ S	216.64	0.77
C7		72%	185-187	C ₁₀ H ₉ Cl ₂ N ₅ OS	318.18	0.71
C8		75%	269-271	C ₈ H ₅ ClN ₂ OS	212.66	0.68

M.P: Melting point, Rf: Retention factor.

Table 2. Antioxidant activity by the DPPH method for synthesized organic compounds.

Compound	% Radical scavenging activity (mean $\pm$ S.D)					IC ₅₀ μ g/ml
	10(μ g/ml)	20 (μ g/ml)	40 (μ g/ml)	80(μ g/ml)	100 (μ g/ml)	
A1	12.94 $\pm$ 1.41	18.28 $\pm$ 0.88	30.68 $\pm$ 0.40	42.35 $\pm$ 1.12	51.83 $\pm$ 0.81	112.99
A2	21.79 $\pm$ 1.33	41.75 $\pm$ 1.09**	56.63 $\pm$ 0.98	70.86 $\pm$ 0.73**	81.99 $\pm$ 0.82**	30.96
A3	16.41 $\pm$ 1.20*	28.72 $\pm$ 0.34	45.86 $\pm$ 0.22**	60.58 $\pm$ 1.15	71.12 $\pm$ 0.89*	48.57
A4	14.60 $\pm$ 0.06	26.05 $\pm$ 1.66	37.24 $\pm$ 0.55**	50.38 $\pm$ 0.52	59.20 $\pm$ 0.06	39.76
A5	16.92 $\pm$ 0.76	24.53 $\pm$ 0.84	31.51 $\pm$ 0.55*	42.92 $\pm$ 0.6	53.76 $\pm$ 0.78	102.06
A6	22.79 $\pm$ 0.41	44.84 $\pm$ 0.60*	58.89 $\pm$ 0.432	75.02 $\pm$ 0.71***	82.71 $\pm$ 0.601***	25.85
A7	18.09 $\pm$ 0.94	26.2 $\pm$ 0.84	35.235 $\pm$ 0.55*	47.3 $\pm$ 0.69	60.348 $\pm$ 0.78	68.92
A8	18.33 $\pm$ 0.57	26.57 $\pm$ 0.76	35.13 $\pm$ 0.50**	48.98 $\pm$ 0.05	55.62 $\pm$ 0.24	83.54
B1	20.15 $\pm$ 0.73	26.20 $\pm$ 0.75	36.21 $\pm$ 0.52**	42.68 $\pm$ 0.60	49.34 $\pm$ 0.70	115.87

B2	27.67±1.38**	35.44±0.61*	47.08±1.73**	58.52±1.29	72.45±1.48*	31.37
B3	18.25±1.01	27.12±1.88	38.38±1.14**	52.52±1.59	68.93±1.13	63.06
B4	31.04±0.89*	50.10±0.99***	58.11±1.02*	64.05±0.82*	77.39±0.60	19.89
B5	30.40±1.94***	52.87±0.67***	71.21±0.73	78.16±0.86***	86.41±1.12***	17.12
B6	22.59±1.34*	40.65±1.82	52.92±1.58	59.12±0.99	69.19±0.98	34.12
B7	21.69±1.31*	41.98±1.29*	52.70±1.48**	61.62±1.50*	75.16±1.80*	33.68
B8	30.03±1.49	39.67±1.34*	55.56±0.69*	60.35±2.60*	72.98±1.52*	36.91
C1	21.57±0.79	30.89±0.37	41.46±0.52*	48.74±2.17	55.50±0.99	86.72
C2	19.48±0.98	27.83±1.09	38.21±0.46	47.87±0.69	58.64±1.18	97.65
C3	21.37±0.75*	33.73±1.67	49.72±0.87	61.32±0.42*	74.32±1.17*	49.12
C4	16.34±0.67	28.76±1.19	39.75±1.12*	47.24±0.93	59.27±1.38	83.66
C5	23.36±1.77*	41.51±0.97	62.81±0.97	76.83±1.52***	82.40±0.92***	29.54
C6	18.64±1.41	29.13±1.72	45.82±0.89**	64.75±0.64***	73.69±1.41*	33.61
C7	13.71±0.83	24.35±0.83	47.12±1.45**	61.91±1.69*	72.44±1.12*	48.92
C8	28.35±1.99***	41.75±1.20**	57.81±1.11***	65.35±0.76***	74.13±1.71**	33.15
Ascorbic acid	28.09±0.78	46.33±0.99***	59.46±1.78***	71.27±0.79***	83.19±.65***	28.47

Values are expressed as mean $\pm$ SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. $P < 0.05$, $*P < 0.01$, and $P < 0.001$ were considered statistically significant.

Table 3. Antioxidant activity by the ABTS method for synthesized organic compounds.

Compound	% Radical scavenging activity (mean $\pm$ S.D)					IC50 μ g/ml
	10(μ g/ml)	20 (μ g/ml)	40 (μ g/ml)	80(μ g/ml)	100 (μ g/ml)	
A1	21.11±0.577	36.39±0.525	46.61±1.154	51.09±0.577	59.36±0.455	43.64
A2	31.40±0.266***	43.64±1.050***	57.92±1.57**	71.41±0.38***	78.93±0.82***	19.32
A3	16.41±1.20	28.38±0.69	39.86±0.225	51.24±0.61	58.11±0.622	38.96
A4	21.934±0.811	27.97±0.827	34.30±0.63	44.94±0.92	51.74±0.32	101.25
A5	16.92±0.764	25.86±0.35	30.81±0.60	39.92±0.69	52.75±0.78	94.83
A6	17.019±0.223	35.18±0.74*	51.05±0.73*	62.23±0.5**	81.03±0.5***	28.08
A7	12.709±0.41	25.84±0.60	40.89±0.432	52.02±0.71	68.71±0.601	41.23
A8	18.99±0.573	26.24±0.57	37.35±0.37	49.31±0.40	60.29±0.57	81.44
B1	11.40±0.34	19.62±0.57	32.92±0.848	47.46±0.57	55.88±0.61	90.14
B2	27.37±0.45**	49.49±2.07***	58.17±1.15***	62.05±1.63*	66.73±1.48	21.05
B3	18.12±0.83*	25.42±0.61	31.41±0.35	42.19±0.93	49.84±0.47	112.56
B4	18.251±1.011*	27.12±1.88	42.34±1.14	57.55±1.59	71.96±1.13	32.28
B5	19.58±0.80*	28.17±0.89	43.05±1.013	59.12±0.61	86.93±0.507***	28.91

B6	22.936±0.28***	41.35±0.32	54.92±0.73**	66.61±0.82***	81.57±0.671***	27.64
B7	13.72±0.55	27.19±0.52	35.28±0.50	40.67±0.506	47.99±0.89	110.55
B8	13.347±0.56	34.61±0.75*	47.901±0.65	55.23±0.54	69.32±0.34	34.49
C1	23.821±0.79***	35.31±0.37	45.18±0.529	54.88±2.17	57.47±0.99	36.18
C2	15.819±0.98	21.11±1.82	27.21±0.46	34.87±0.693	39.64±1.181	103.67
C3	28.123±0.756***	34.57±1.67	45.91±0.87	58.68±0.42	63.16±1.17	45.74
C4	16.34±0.67	26.09±0.71	41.75±0.81	52.58±0.36	60.54±0.041	39.27
C5	12.72±0.89	27.94±0.56	48.96±0.138	61.77±0.66	84.49±0.35**	41.32
C6	22.614±0.609***	31.89±0.77	55.95±0.45**	63.64±1.16**	69.43±0.72	28.52
C7	21.08±0.051*	33.97±0.618	47.13±0.941	65.21±0.24***	78.85±0.65	34.68
C8	24.131±0.511*	35.167±0.66	46.67±0.45	57.81±0.80	71.17±0.702	31.24
Ascorbic acid	24.78±1.30**	41.73±1.32***	57.32±2.12**	69.13±1.59***	84.39±1.05***	26.18

Values are expressed as mean $\pm$ SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. $P < 0.05$, $*P < 0.01$, and $P < 0.001$ were considered statistically significant.

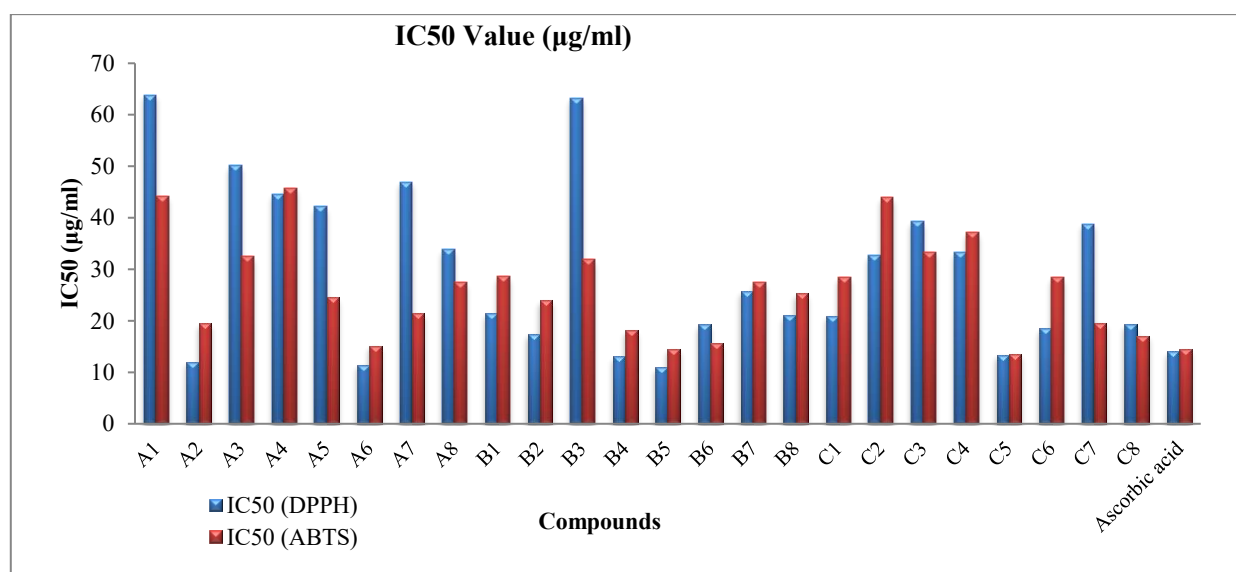


Figure 5: Graphical presentation of the IC₅₀ values in $\mu\text{g/mL}$ for the antioxidant activity of compounds A1-A8, B1-B8, and C1-C8 measured at various concentrations against DPPH and ABTS.

Comparison of DPPH and ABTS results revealed that compound potency was assay-dependent. B5 and B4 were among the most active derivatives in the DPPH assay, whereas A2 and B2 showed the lowest IC₅₀ values in the ABTS assay. A6 displayed favorable activity in both assays, providing comparatively consistent radical-scavenging behavior across the two screening systems. The differences in ranking may arise from differences in radical structure, reaction environment, and the physicochemical interactions governing radical quenching. Thus, evaluation using both assays provides a broader preliminary assessment than reliance on a single chemical antioxidant assay.

APPENDIX

4-ethoxyxazol-2-amine (A1)

White solid product was obtained with an isolated yield of 75%. The reaction was completed within 3 hours. The R_f value was recorded as 0.35 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. IR (KBr pellets, $\nu_{\text{max}}/\text{cm}^{-1}$): C=C (str) 1620.79, C=N (str) 1674, NH(str) 3346 primary amine NH₂, C-O-C (str) 1155, C-H (str) 2853, N-H bending (scissoring) 1650, C-N (str) 1150-1250, C-H bending (aromatic or heterocyclic) 700-900. ¹H NMR (500 MHz, DMSO) (ppm): 7.47-7.58 (1H, d, oxazole-H, 4 J = 0.8 Hz), 4.20-4.44 (Ar- NH₂), 4.10-4.20 (2H, quartet, J = 7.0 Hz -OCH₂- of ethoxy group), 1.11-1.13 (3H, triplet, J = 7.0 Hz, -CH₃ of ethoxy group). ¹³C NMR (500 MHz, DMSO) (ppm): 162.3 (C-2, C bonded to -NH₂ (electron-withdrawing), 128-135 (C-4, C bonded to ethoxy group (C-O) ³JHH (CH₂-CH₃, ethoxy), 116 (C-5, Carbon bearing the single proton), 64.3 (-OCH₂-, Ethoxy methylene carbon), 25 (-CH₃, ethoxy methyl carbon), while LCMS analysis of C₅H₈N₂O₂ is 128 [M + H] (m/z).

4-ethoxy-2-phenyloxazole (A2)

White solid product was obtained with an isolated yield of 71%. The reaction was completed within 3 hours. The R_f value was recorded as 0.50 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. IR (KBr pellets, $\nu_{\text{max}}/\text{cm}^{-1}$): Ar-C-H (str) 3175, C=C (str) 1624, C=N (str) 1660, C-O-C(str) 1142, 2980-2850 Aliphatic C-H stretch (ethyl group), 820-750 Aromatic C-H bending. ¹H NMR (500 MHz, DMSO) (ppm): 7.47-7.58 (m, 1H, d, oxazole-H, 4 J = 0.8 Hz), 7.11-7.16 (3H, m, (Aromatic protons (Ph, meta & para), 7.4-7.6, (2H, o, multiplet), 3.70-3.72 (2H, s, J = 7.0 Hz -OCH₂- of ethoxy group), 2.35 (3H, triplet, J = 7.0 Hz, -CH₃ of ethoxy group). ¹³C NMR (100 MHz, DMSO) (ppm): 164 (Oxazole C=N), 138.1 (quaternary aromatic carbon), 125 (C-5, Carbon bearing the single proton), 128-139 (C-benzene), 64.3 (C-4, C bonded to ethoxy group (C-O) ³JHH (CH₂-CH₃, ethoxy), 14.5 (-CH₃, ethoxy methyl carbon), while LCMS analysis of C₁₁H₁₁NO₂ is 190.12 [M + H] (m/z).

2-amino-4-(4-ethoxyoxazol-2-yl)butanoic acid (A3)

White solid product was obtained with an isolated yield of 82%. The reaction was completed within 3 hours. The R_f value was recorded as 0.41 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. IR (KBr pellets, $\nu_{\text{max}}/\text{cm}^{-1}$): C=C (str) 1634, C=N (str) 1651.74, C-O-C (str) 1130, Ar-C-O-C (str) 1003, C-H (str) 2931, NH (str) 3320.95, COOH (str) 1773.78. ¹H NMR (400 MHz, DMSO) (ppm): 1.30 (t, J ≈ 7 Hz, 3H, CH₃ of ethoxy group), 3.70-3.72 (q, J ≈ 7 Hz, 2H, CH₂ of ethoxy group), 0.9-1.11 (2H, m, β-CH₂), 1.9-2.4 (m, 2H, γ-CH₂), 3.6-4.0 (d, 1H, α-CH (next to NH₂ and COOH), 7.47-7.58 (d, 1H, Oxazole C5-H, J = 0.8 Hz), 8.5 (2H, NH₂), 11 (broad, 1H, COOH proton). ¹³C NMR (100 MHz, DMSO) (ppm): 174.7, (COOH), 155 (Oxazole C=N and C-O carbons), 125 (C-5, C-4), 138.1 (C-O), 55.5 (CH, α-carbon of amino acid) 63 (C-4, C bonded to ethoxy group (C-O) ³JHH (CH₂-CH₃, ethoxy), 27-34.4 (2-C side chain), 14.5 (ethoxy methyl), while LCMS analysis of C₉H₁₄N₂O₄ is 214.22 [M + H] (m/z).

2-(4-ethoxyoxazol-2-yl)acetamide (A4)

White solid product was obtained with an isolated yield of 70%. The reaction was completed within 3 hours. The R_f value was recorded as 0.45 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. C=C (str) 1652, C=N (str) 1694.92, C-O-C (str) 1050.4, C-H (str) 2895, C=O (str) 1704.83, NH(str) 3381, C-H bending 780. ¹H NMR (400 MHz, CDCl₃) (ppm): 1.35-1.5 (t, J ≈ 7 Hz, 3H, CH₃ of ethoxy group), 2.15-2.4 (1H, CH), 3.70-3.72 (2H, d, -CH₂ protons), 4.5-5.12 (q, J ≈ 7 Hz, 2H, CH₂ of ethoxy group), 7.47-7.58 (s, 1H, d, oxazole-H, 4 J = 0.8 Hz), ¹³C NMR (500 MHz, DMSO) (ppm): 180, (CO), 150 (Oxazole C=N and C-O carbons), 138.1 (C-O), 125 (C-5, C-4), 40-45 (CH₂, Methylene between oxazole and amide), 68 (C-4, C bonded to ethoxy group (C-O) ³JHH (CH₂-CH₃, ethoxy), 14.5 (ethoxy methyl) while LCMS analysis of C₇H₁₀N₂O₃ is 139.4 [M + H] (m/z).

4-ethoxy-2-vinyloxazole (A5)

White solid product was obtained with an isolated yield of 78%. The reaction was completed within 3 hours. The R_f value was recorded as 0.48 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. C=C (str) 1621.53, C=N (str) 1673, C-O-C (str) 1136.21, C-H (str) 2853.64, 3100 =C-H stretch (vinyl group), 900-990 =C-H bending (vinyl out-of-plane), 750-850 (Ar./heteroaromatic C-H bending). ¹H NMR (500 MHz, DMSO) (ppm): 0.7-1.32 (t, J ≈ 7 Hz, 3H, CH₃ of ethoxy group), 3.90-4.80 (q, J ≈ 7 Hz, 2H, CH₂ of ethoxy group), 5.65 (dd, J ≈ 17, 1 Hz), 6.60(dd, J ≈ 17, 10 Hz, Vinyl CH (central), 7.0-7.6 (s, CH, C5-H of oxazole ring). ¹³C NMR (500 MHz, DMSO) (ppm): 165, (CO), 155-157 (Oxazole

C=N and C–O carbons), 125–130 (Vinyl CH and oxazole C5–H), 115 (CH₂), 78 (C-4, C bonded to ethoxy group (C–O) ³J_{HH} (CH₂–CH₃, ethoxy), 18 (ethoxy methyl) while LCMS analysis of C₇H₉NO₂ is 170 [M + H] (m/z).

5-(4-ethoxyoxazol-2-yl)aminoimidazolidine-2,4-dione (A6)

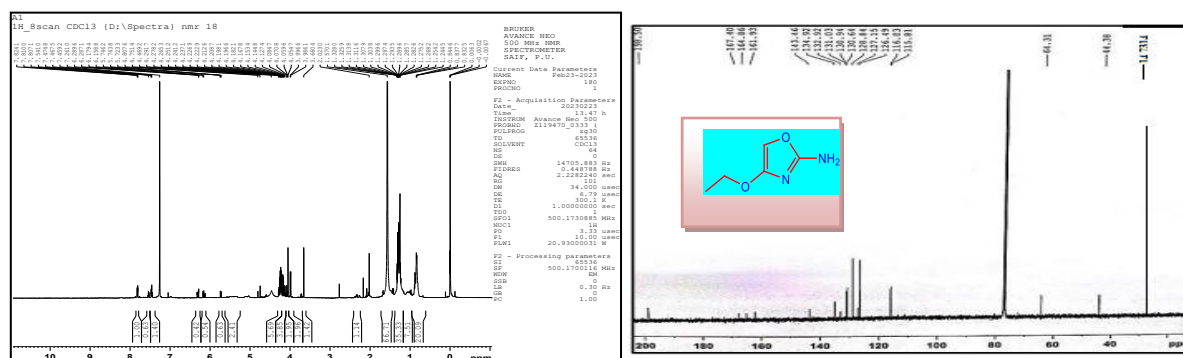
White solid product was obtained with an isolated yield of 72%. The reaction was completed within 3 hours. The R_f value was recorded as 0.51 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. C=C (str) 1603.13, C=N (str) 1654.13, C–O–C (str) 1060.32, C=O (str) 1716.74, NH(str) 3190, Ar–NH (str) 1532, C–H (str) 2850. ¹H NMR (500 MHz, DMSO) (ppm): 1.85 (s, J ≈ 7 Hz, 3H, CH₃ of ethoxy group), 3.95 (d, J ≈ 7 Hz, 2H, CH₂ of ethoxy group), 7.0–7.3 (t, CH, C5–H of oxazole ring), 7.20–7.60 (d, NH). ¹³C NMR (500 MHz, DMSO) (ppm): 162.3 (CO), 134.4 (Oxazole C=N and C–O carbons), 126.3 (C =C in oxazole), 21.56 (CH₃), 64.3 (CH₂), 21.56 (CH₃), while LCMS analysis of C₈H₁₀N₄O₄ is 226.0 [M + H] (m/z).

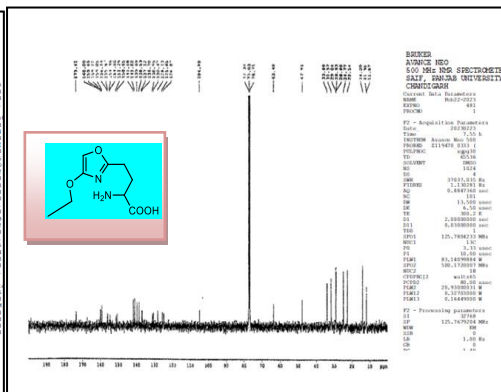
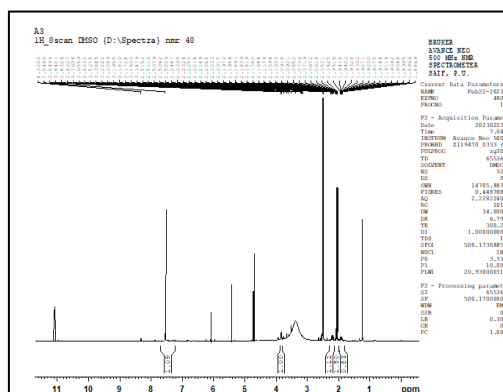
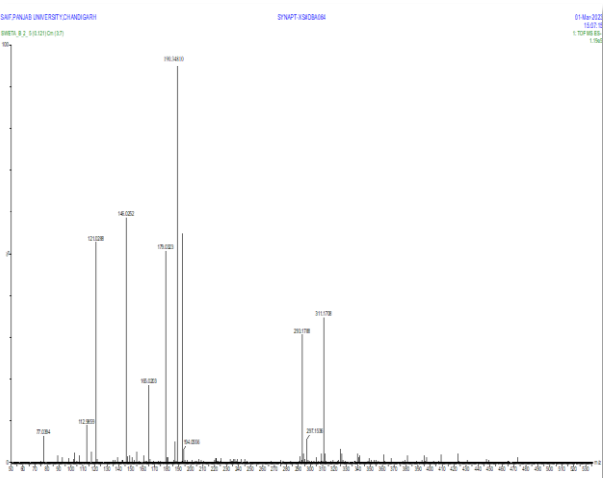
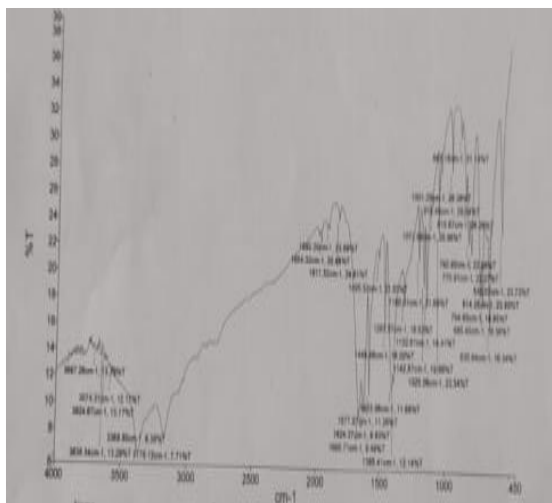
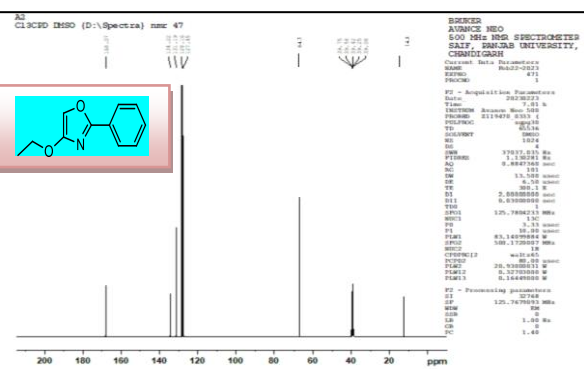
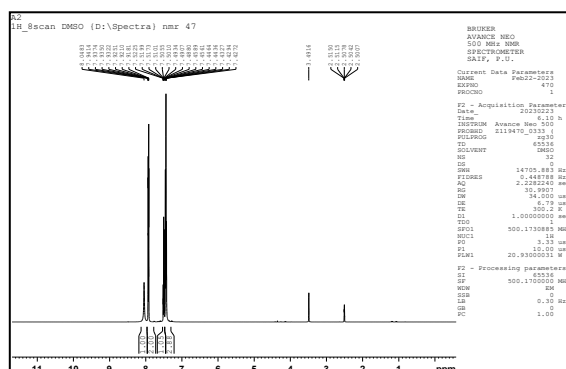
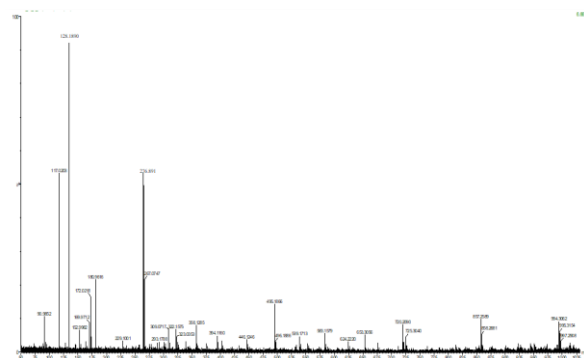
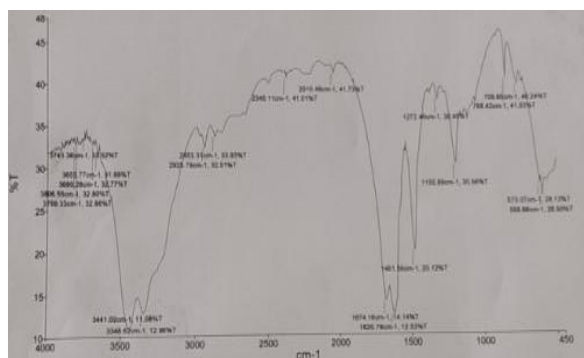
2-(4-ethoxyoxazol-2-yl)Benzamide (A7)

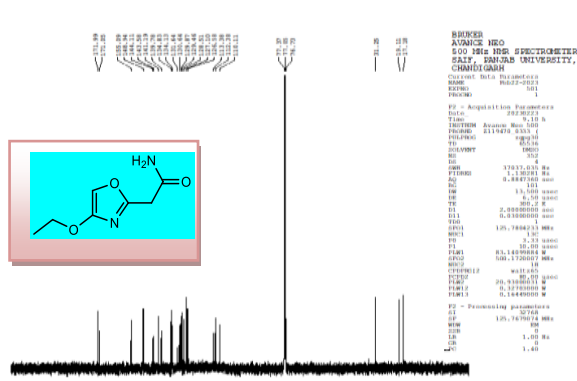
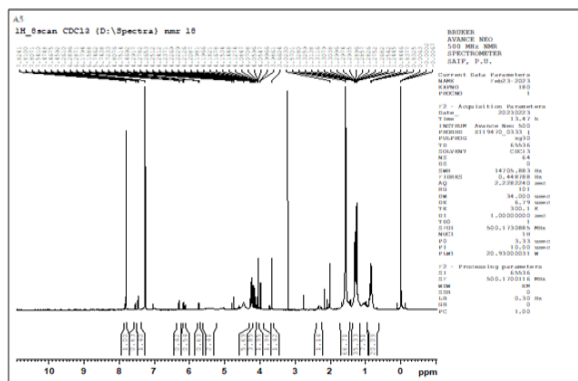
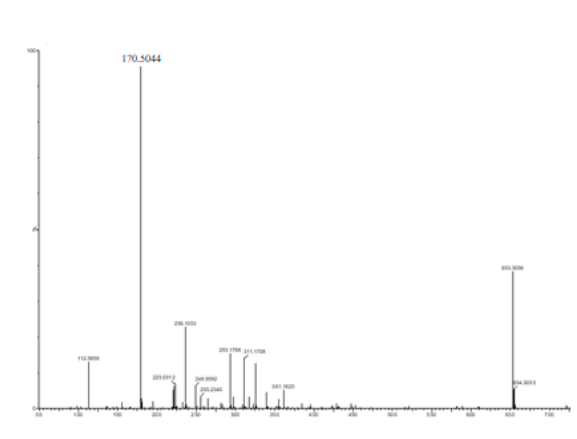
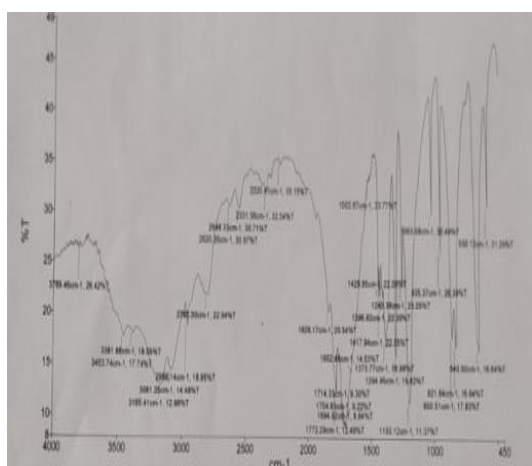
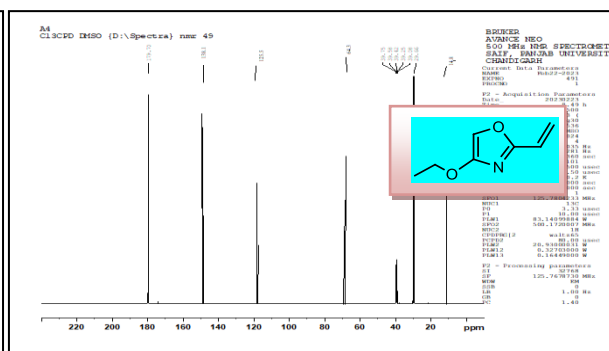
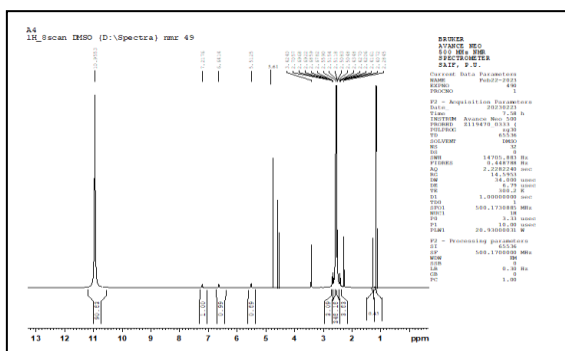
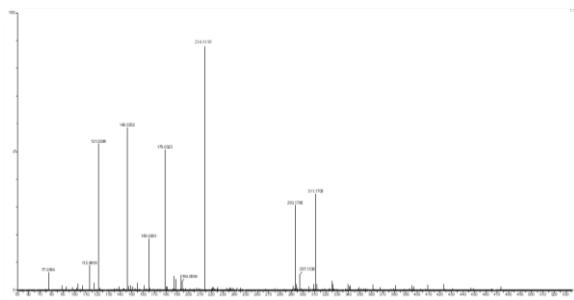
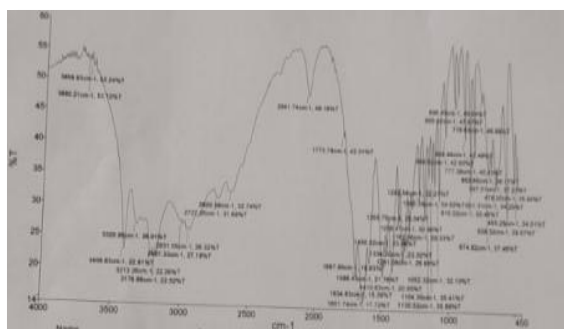
White solid product was obtained with an isolated yield of 80%. The reaction was completed within 3 hours. The R_f value was recorded as 0.55 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. C=C (str) 1605.31, C=N (str) 1732, C–O–C (str) 1250.41, Ar–C–H (str) 3206, C=O (str) 1732, NH(str) 3459.92, C–O (str) 1065. ¹H NMR (500 MHz, DMSO) (ppm): 1.65 (s, J ≈ 7 Hz, 3H, CH₃ of ethoxy group), 3.95 (d, J ≈ 7 Hz, 2H, CH₂ of ethoxy group), 7.22–7.38 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.20–8.0 (m, 4H, benzene), 7.5–8.1 (2H, CONH₂). ¹³C NMR (500 MHz, DMSO) (ppm): 165.3 (CO–NH₂), 161.3 (C-2 oxazole), 136.1 (C–O), 125.4 (CH oxazole ring), 76.7 (C-4, C bonded to ethoxy group (C–O)), 40.44 (CH₃, ethoxy methyl) while LCMS analysis of C₁₂H₁₂N₂O₃ is 232.08 [M + H] (m/z).

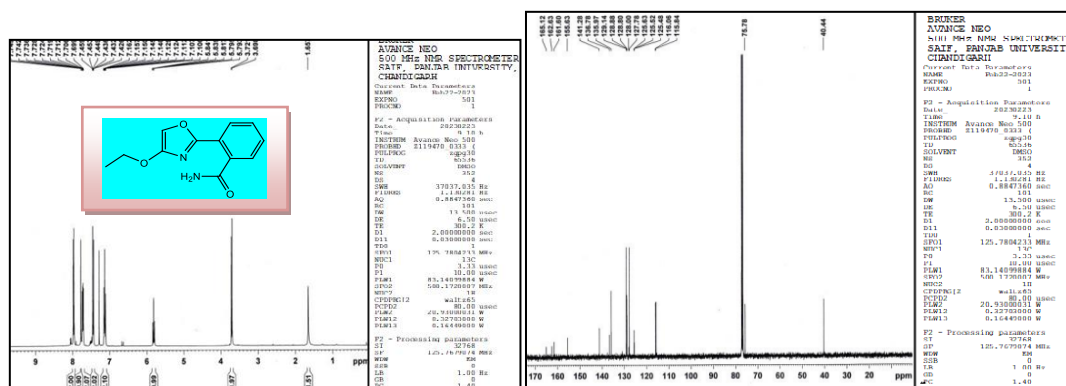
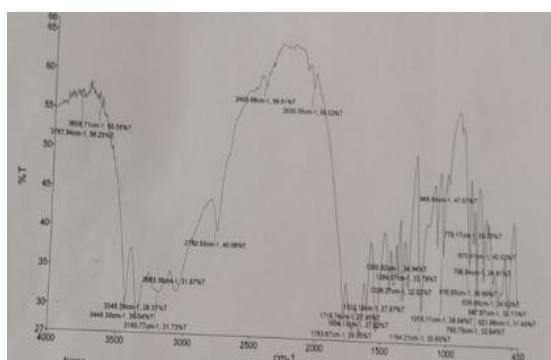
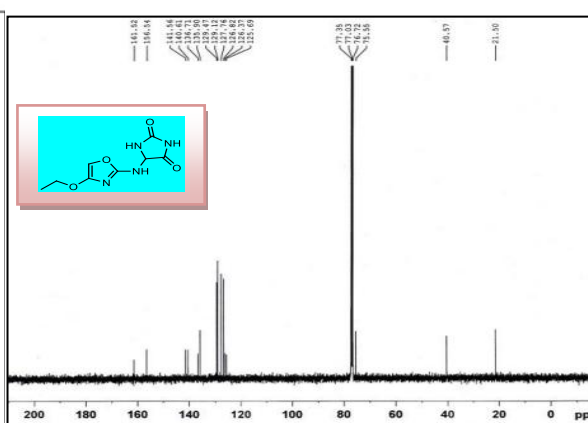
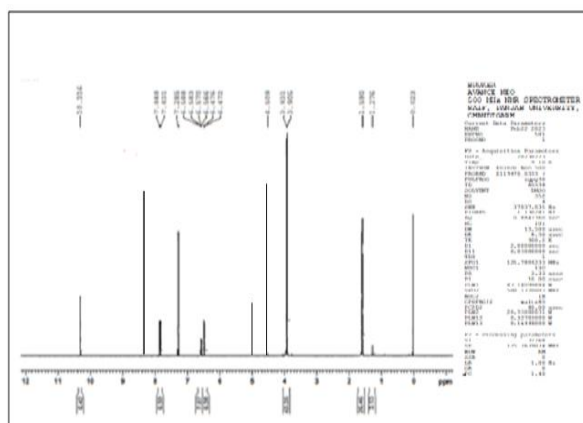
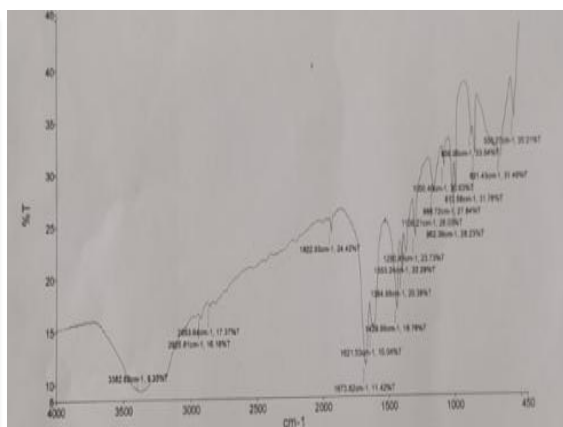
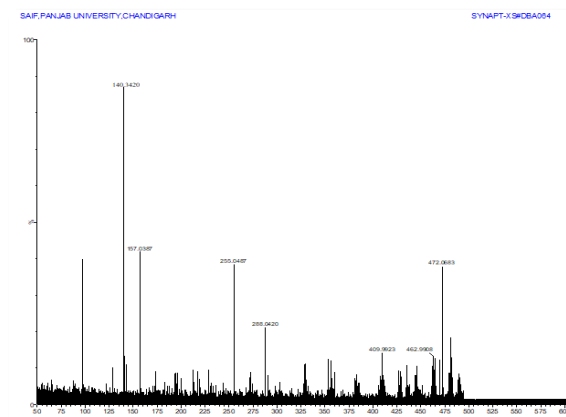
4-ethoxy-2-(pyridin-3-yl)oxazole (A8)

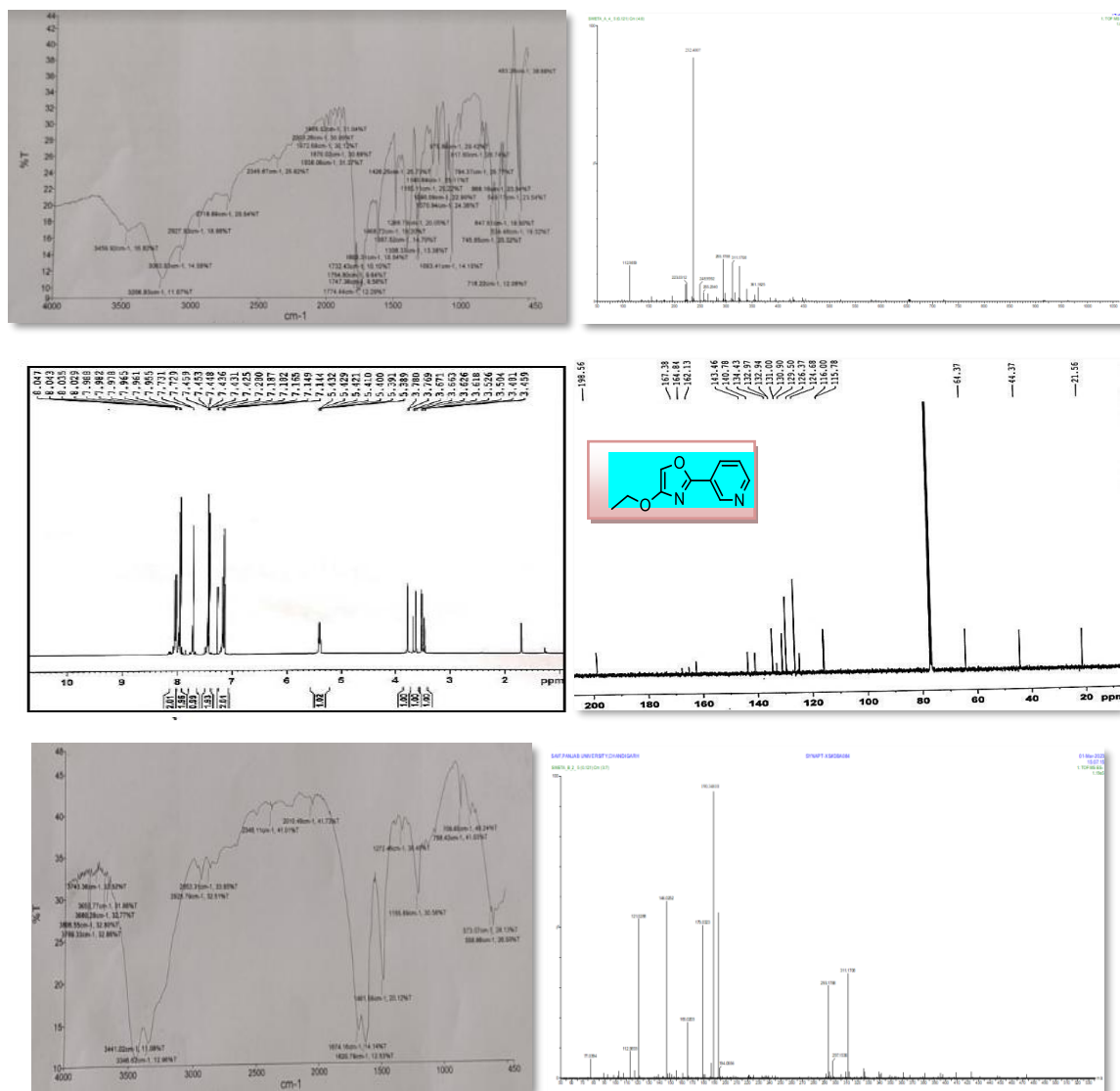
White solid product was obtained with an isolated yield of 72%. The reaction was completed within 3 hours. The R_f value was recorded as 0.40 using a solvent system of n-hexane and ethyl acetate in a 7:3 ratio, respectively. Ar–C–H (str) 3050, C–H (str) aliphatic 2950, 2870, C=N (str) 1650, C–O (str) 1260.41, C–O–C (str) 1040.32, 750–850 Aromatic/heteroaromatic C–H bending. ¹H NMR (400 MHz, CDCl₃) (ppm): 1.35 (t, J ≈ 7 Hz, 3H, CH₃ of ethoxy group), 4.10 (q, J ≈ 7 Hz, 2H, CH₂ of ethoxy group), 6.8–7.20 (s, CH, C5–H of oxazole ring), 7.20–8.0 (m, 4H, benzene) 10 (s, 1H, NH amide). ¹³C NMR (100 MHz, CDCl₃) (ppm): 165, (CO amide), ~155 (Oxazole C=N and C–O carbons), 135 (aromatic carbon), 110–120 (CH oxazole ring), 63 (C-4, C bonded to ethoxy group (C–O)), 14 (CH₃, ethoxy methyl) while LCMS analysis of C₁₂H₁₂N₂O₃ is 190.20 [M + H] (m/z).











H), 115 (C4 of oxazole (bearing Cl), 30 (CH₂ next to amide carbonyl), 40 (CH₂) while LCMS analysis of C₁₂H₁₂N₂O₃ M⁺ = 270 [M + H] (m/z).

(E)-2-(benzylideneamino)-1-(3-(4-chlorooxazol-2-yl)phenyl)ethanone (B3)

Light yellow product was obtained with an isolated yield of 65%, and the reaction was completed in 3 hours. A value of 0.55 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. Ar C-H (str) 3010, 2980–2850 Aliphatic C–H stretch, 1675–1650 C=O (str), C=C (str) 1634, C=N (str) 1651.74, 1250–1050 (C–N and C–O str). ¹H NMR (500 MHz, DMSO) (ppm): 8.79 (s, 1H, (–CH=N–), 7.82 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.55–7.58 (4H, aromatic ring), 3.40–3.55 (2H, d, –CH₂=O, protons), 1.77 (2H, d, –CH₂, protons). ¹³C NMR (500 MHz, DMSO) (ppm): 180 Carbonyl carbon (C=O of ethanone), 165, Imine carbon (–CH=N–) 145 (C2 Oxazole), 122 (C5 of oxazole (bearing the H), 125 (C4 of oxazole (bearing Cl) 36.1 (–CH₂– of ethoxy group) 25.2(CH₂ side chain), while LCMS analysis of C₁₂H₁₂N₂O₃ M⁺ = 327.0 [M + H] (m/z).

(E)-4-(4-chlorooxazol-2-yl)-2-((4-hydroxy-3-methoxybenzylidene)amino)butanoic acid (B4)

Light yellow product was obtained with an isolated yield of 65% and the reaction was completed in 3 hours. A value of 0.55 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3400–3300 Broad O–H (phenolic and carboxylic acid), Ar-C-H (str) 3075, 2950 aliphatic C-H, 1700 C=O (str), C=C (str) 1624, C=N (str) 1620, 1510–1450 Aromatic C=C and C–O stretches, 1250–1020 C–O (methoxy), C–N (imine, oxazole). ¹H NMR (500 MHz, DMSO) (ppm): 8.79 (s, 1H, (–CH=N–), 7.82 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.55–7.58 (4H, aromatic ring), 3.40–3.55 (2H, d, –CH₂=O, protons), 1.77 (2H, d, –CH₂, protons). ¹³C NMR (500 MHz, DMSO) (ppm): 180 Carbonyl carbon (C=O of ethanone), 165, Imine carbon (–CH=N–) 145 (C2 Oxazole), 122 (C5 of oxazole (bearing the H), 125 (C4 of oxazole (bearing Cl) 36.1 (–CH₂– of ethoxy group) 25.2(CH₂ side chain), while LCMS analysis of C₁₂H₁₂N₂O₃ M⁺ = 338.0 [M + H] (m/z).

5-(4-chlorooxazol-2-yl)(4-hydroxy-3-methoxybenzyl)amino)imidazolidine-2,4-dione (B5)

Yellow product was obtained with an isolated yield of 78% and the reaction was completed in 3 hours. A value of 0.63 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3400–3300 N–H stretch (secondary amine), 3200–3600 (broad) O–H stretch (phenol), ~1720 and ~1760 (strong) C=O, ~1600 C=N or C–O (oxazole ring) ~1000–1300 C–O (methoxy), 1510–1450 Aromatic C=C and C–O stretches, 1250–1020 C–O (methoxy). ¹H NMR (500 MHz, DMSO) (ppm): 10.0 (s, OH (phenolic) 5.8 (br, m, NH (hydantoin and amine), 7.82 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.84–7.94 (3H, aromatic ring), 1.76 (2H, CH₂), 3.82 (OCH₃), 2.63 (Cl). ¹³C NMR (500 MHz, DMSO) (ppm): 156.9 C=O (carbonyl, hydantoin ring), 147.5 (Aromatic C–OH, C–OMe), 150 Oxazole ring (C=N, C–Cl), 138.1 Quaternary aromatic (linked to CH₂), 125.4 (Aromatic CH carbons), 120 (C5 of oxazole (bearing the H), 78 (Methoxy carbon (–OCH₃), while LCMS analysis of C₁₂H₁₂N₂O₃ M⁺ = 353.02 g/mol [M + H] (m/z).

(E)-4-(4-chlorooxazol-2-yl)-1-((3-hydroxy-4-methoxybenzylidene)amino)butan-2-one (B6)

Yellow product was obtained with an isolated yield of 72% and the reaction was completed in 3 hours. A value of 0.66 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 1680–1720 C=O (ketone), ~1610–1660 C=N (imine), 3200–3600 O–H (phenolic), 3000–3100 C–H (aromatic), 1000–1300 C–O (methoxy), 600–800 C–Cl (aryl halide), 1500–1600 C–N, C=N (oxazole ring). ¹H NMR (500 MHz, DMSO) (ppm): 9.0 (s, Phenolic OH) 8.0 (CH=N (imine), 7.3–7.6 (d, dd, Aromatic protons (benzene ring), 3.7 (s, OCH₃ (methoxy group), 2.4–2.7 (CH₂ next to C=O), 2.2 (CH₂). ¹³C NMR (500 MHz, DMSO) (ppm): 179.8 (C=O (ketone)), 163.7 (C=N (imine), C–OH, C–OMe), 157.6 Oxazole ring (C=N, C–Cl), 125.4 (Aromatic C=C), 138 (Aromatic C= carbons), 127.3–130 (C5 of oxazole (bearing the H), 56 (Methoxy carbon (–OCH₃), 25.2–36.1 (CH₂). while LCMS analysis of C₁₂H₁₂N₂O₃ M⁺ = 307.12 g/mol [M + H] (m/z).

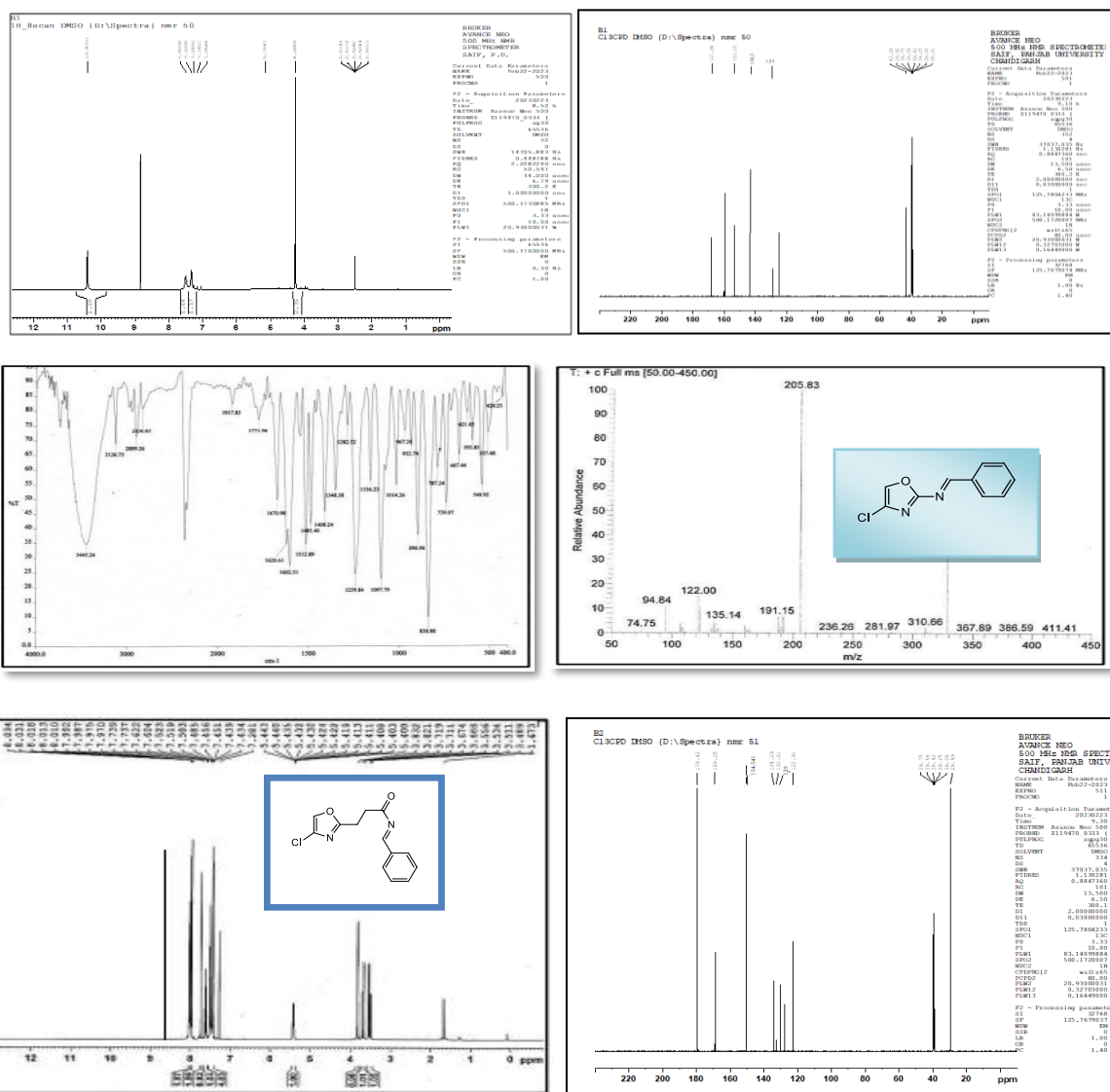
(E)-1-(2-(4-chlorooxazol-2-yl)phenyl)-2-((4-hydroxy-3-methoxybenzylidene)amino)ethanone (B7)

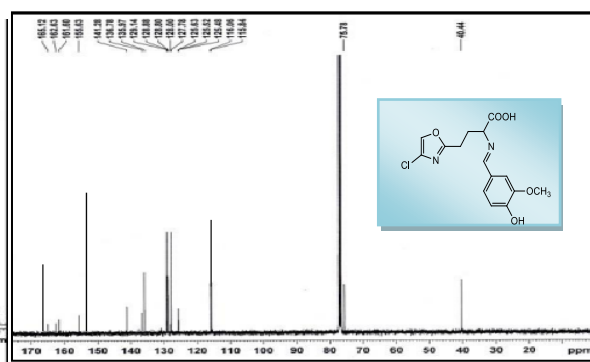
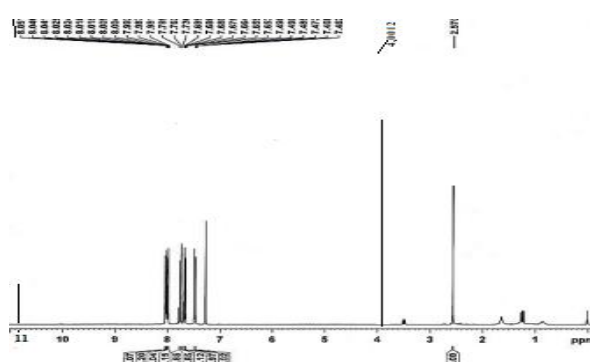
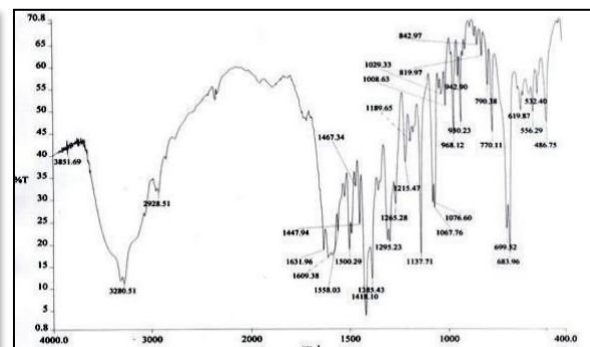
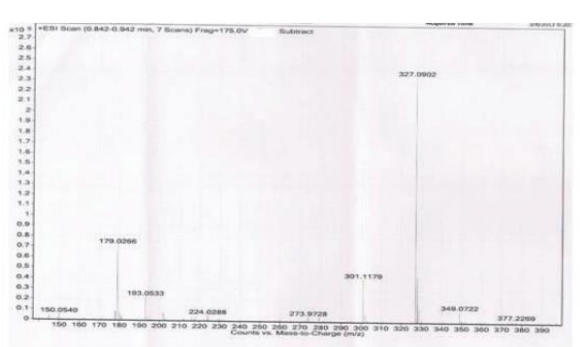
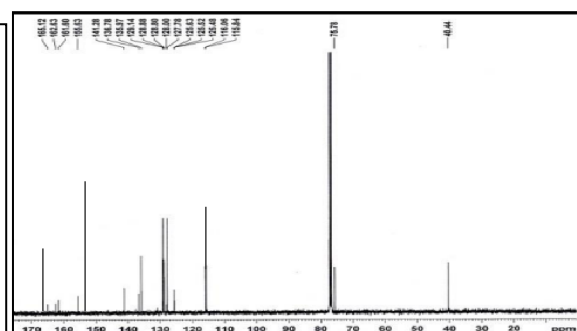
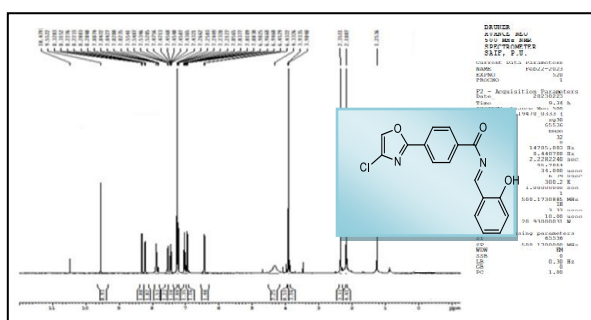
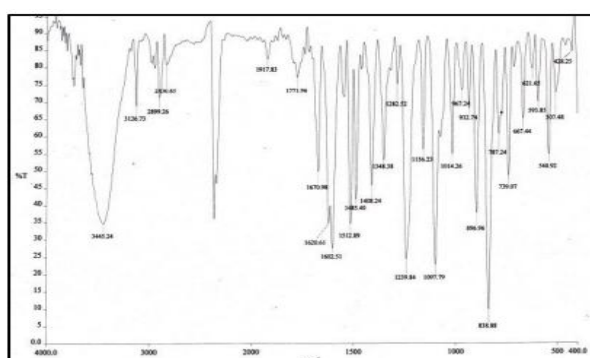
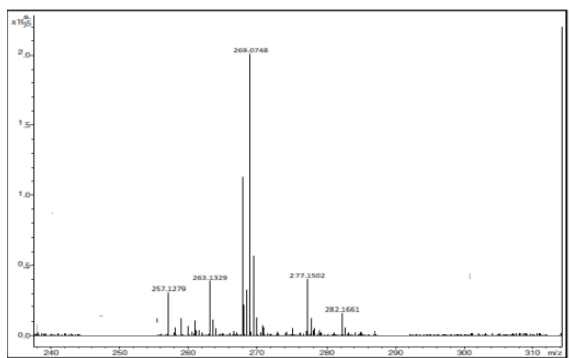
Yellow product was obtained with an isolated yield of 80% and the reaction was completed in 3 hours. A value of 0.51 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3365 O–H stretch (phenol), 3052 Ar C-H (str), 1668 C=O (str), 1615 C=N (str, imine), 1512 Aromatic C=C (str), 1252 C–O (str), 1135 C–Cl stretch. ¹H NMR (500 MHz, DMSO) (ppm): 3.67 (s, 3H, OCH₃), 5.32 (d, 1H, Ar–H (methoxybenzene ring), 7.44–7.54 (d, 1H, Ar–H), 7.12 (t, 1H, Ar–H), 7.82 (t, 1H, Ar–H), 7.65 (s, 1H, –CH=N–) (imine proton), 8.2 (s, 1H, –OH (phenolic, D₂O exchangeable). ¹³C NMR

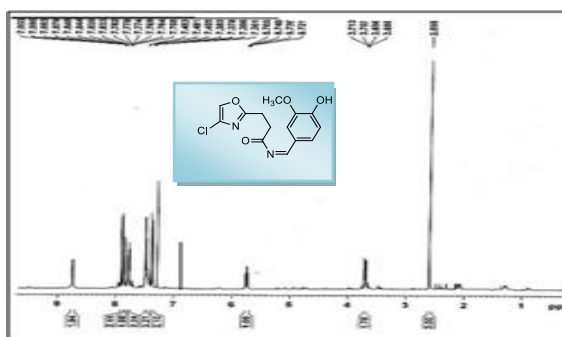
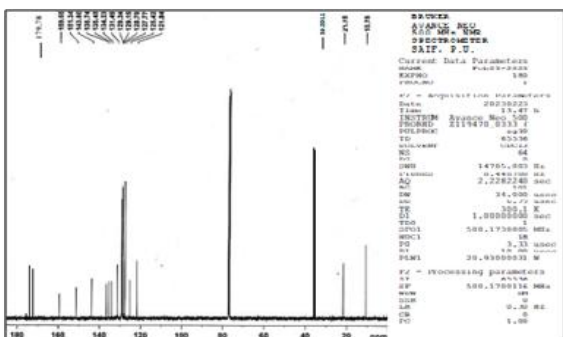
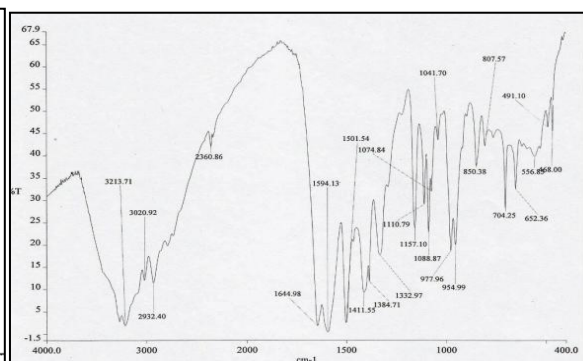
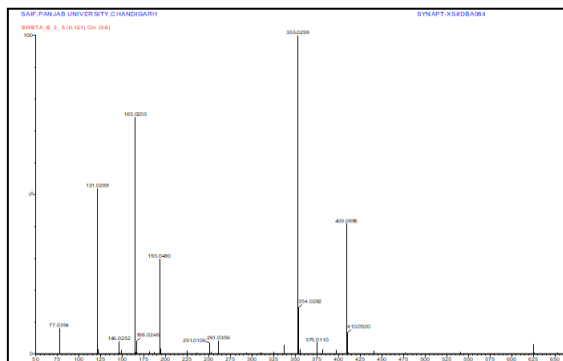
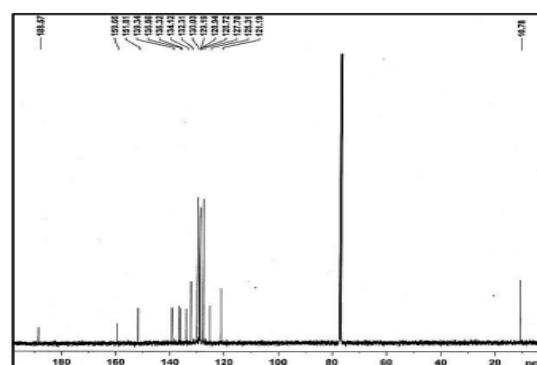
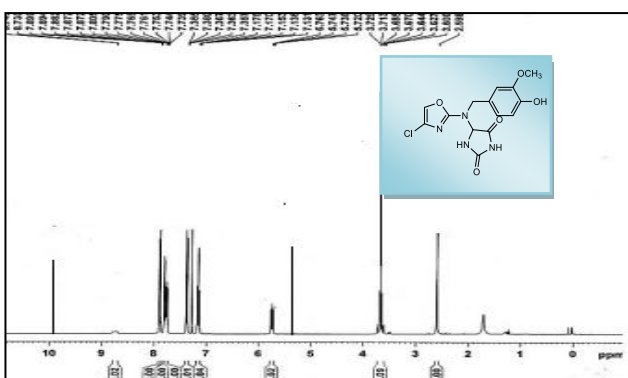
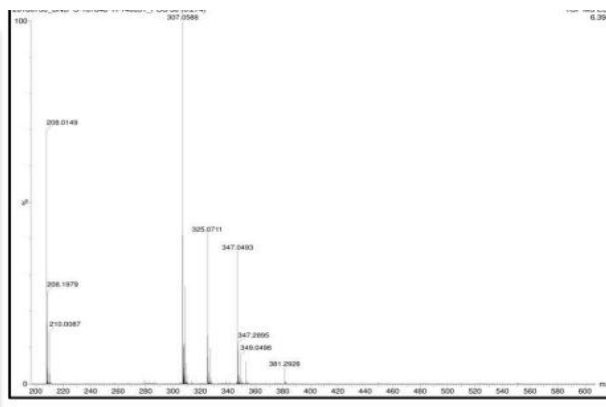
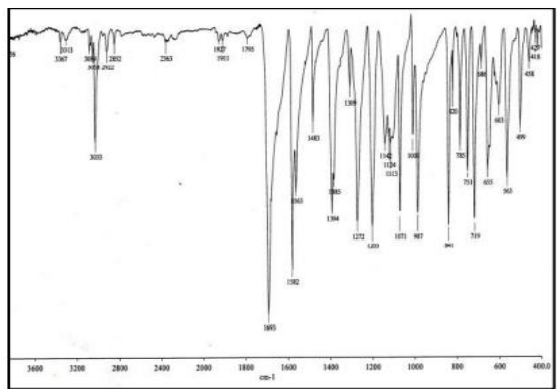
(500 MHz, DMSO) (ppm): 117.9-127.3 (Ar. C), 127–133 (Substituted Ar. oxazole ring C), 149.8 (C=N (imine), 159.6 (C–OH (phenolic), 163.7 (Oxazole C–O), 174.4 C=O (carbonyl of ketone) $M^+ = 356.42$ m/z [M + H] (m/z).

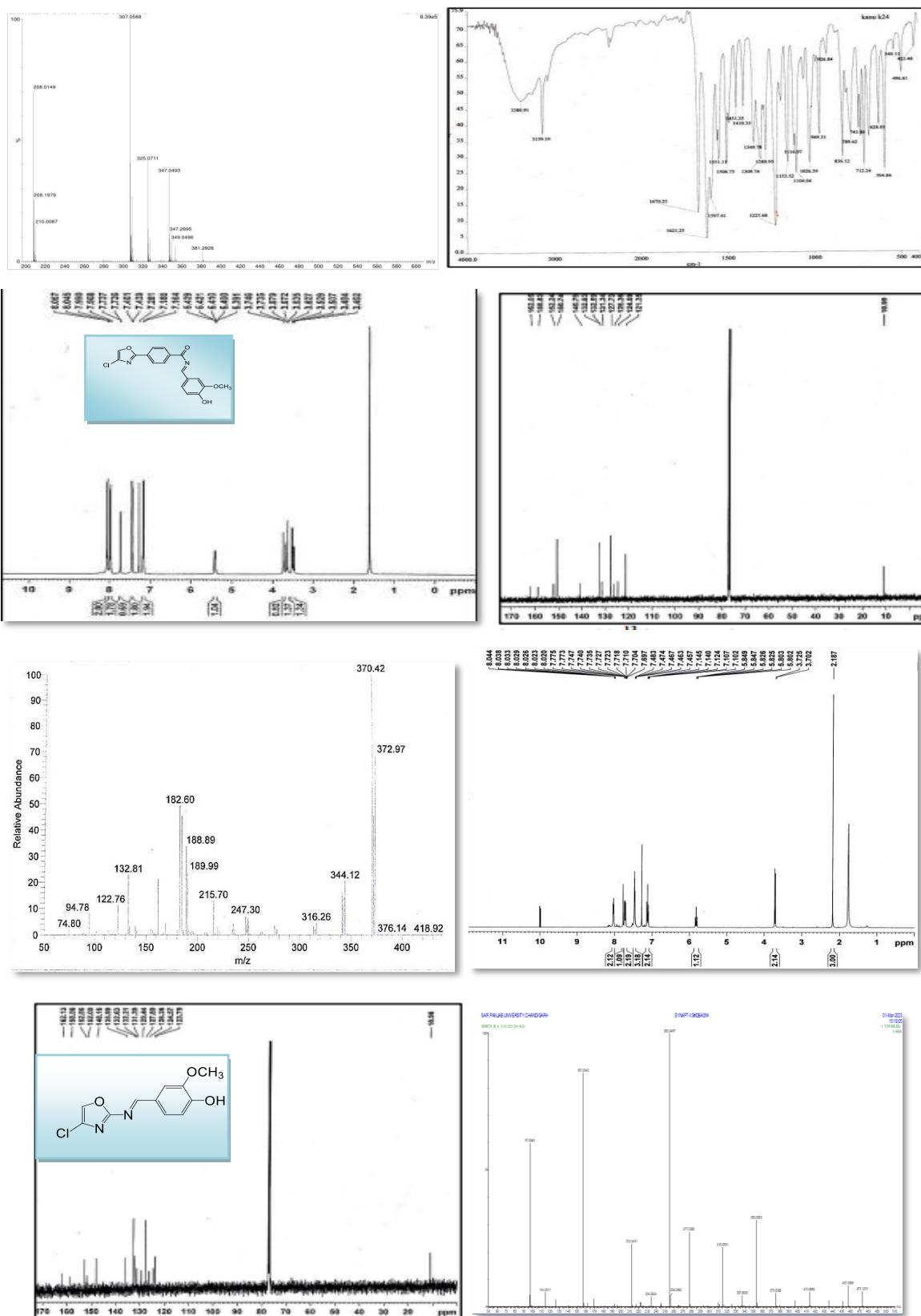
(E)-4-(((4-chlorooxazol-2-yl)imino)methyl)-2-methylphenol (B8)

Yellow product was obtained with an isolated yield of 68% and the reaction was completed in 3 hours. A value of 0.49 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3432 (O–H, str), 3015 Ar C–H (str), 1618 C=N (str, imine), 1572, 1510 Aromatic C=C (str), 1280 C–O (str), 1145 C–Cl stretch. ¹H NMR (500 MHz, DMSO) (ppm): 2.31 (s, 3H, CH₃), 5.96 (m, 3H, Ar–H), 8.01 (s, 1H, –CH=N–) (imine proton), 10.12 (s, 1H, phenolic OH). 7.20 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.44-7.54 (3H, aromatic ring). ¹³C NMR (500 MHz, DMSO) (ppm): 10.3 (–CH₃), 125-132 – (Ar. C), 138.1 (C=N (imine), 150.0 (C–OH (phenolic), 155 (C=N), 112.0-130.8 (C–Ar), 56.1 (CH₃). $M^+ = 252.85$ m/z [M + H] (m/z).









2-(4-chlorooxazol-2-yl)methylthiazol-5(4H)-one (C1)

White crystalline product was obtained with an isolated yield of 72% and the reaction was completed in 3 hours. A value of 0.62 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3115–3030 C–H stretch (aromatic/heterocyclic), 1725–1735 C=O (lactam/thiazolone), 1570–1590 C=N and C=C (oxazole/thiazole), 1245 (C–O oxazole), 1045–1120 C–Cl (aryl chloride). ^1H NMR (500 MHz, DMSO) (ppm): 3.90 (s, 2H, $-\text{CH}_2$ (methylene bridge between oxazole and thiazolone), 7.10 (s, 1H, oxazole proton), 7.45–7.65 (s, 1H, thiazole proton), 8.20 (NH, s), 2.73 (Cl). ^{13}C NMR (100 MHz, CDCl_3) (ppm): 38.7 ($-\text{CH}_2$), 126.4 (Ar. C), 135 (thiazole C–H), 150–160 (C=N, C–O), 160 (C=O). $M^+ = 216.07$ m/z $[\text{M} + \text{H}]$ (m/z).

4-(4-chlorooxazol-2-yl)-1-(thiazol-2-yl)butan-2-one (C2)

White crystalline product was obtained with an isolated yield of 75% and the reaction was completed in 3 hours. A value of 0.58 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3050–3100 C–H stretch (aromatic/heterocyclic), 1715–1725 C=O (lactam/thiazolone), 1575–1600 C=N and C=C (oxazole/thiazole), 1250–1300 (C–O oxazole), 1040–1120 C–Cl (aryl chloride), 800–850 Out-of-plane C–H bend. ^1H NMR (500 MHz, DMSO) (ppm): 2.45–2.55 (m, 3H, $-\text{COCH}_3$ (methyl of ketone), 2.80–3.10 (t 2H, $-\text{CH}_2-\text{CO}$), 3.45–3.60 (2H, t, $-\text{CH}_2-\text{Oxazole}$), 7.10–7.32 (s, 1H, Thiazole proton ($\text{C}_5\text{--H}$), 7.40–7.60 (s, 1H, $\text{C}_5\text{--H}$), 11.09 (CONH). ^{13}C NMR (500 MHz, DMSO) (ppm): 25.7 ($-\text{CH}_2$), 173.7 (C=O, Ketone), 125 Oxazole/Thiazole ring CH carbons, 162.7 (C=N, C–O, C–Cl ring carbons), 154.6 (C–NH). $M^+ = 255$ m/z $[\text{M} + \text{H}]$ (m/z).

4-(4-chlorooxazol-2-yl)-N-(thiazol-2-ylmethyl)Benzamide (C3)

White crystalline product was obtained with an isolated yield of 70% and the reaction was completed in 3 hours. A value of 0.67 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 1640–1680 cm^{-1} (C=O stretch), 3200–3400 cm^{-1} N–H stretch (amide), 1450–1600 cm^{-1} (Aromatic C=C), 1200–1350 cm^{-1} C–N (amide and heterocycles), 600–800 cm^{-1} C–Cl (aromatic), 1050–1250 cm^{-1} (C–O, C–N). ^1H NMR (500 MHz, DMSO) (ppm): 7.32 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.84–7.96 (2H, CH), 5.23 (s, 1H, Thiazole proton ($\text{C}_5\text{--H}$), 13.77 (CONH), 2.98 (s, d, CH_2). ^{13}C NMR (100 MHz, CDCl_3) (ppm): 44 ($-\text{CH}_2$), 127.6–135.3 (C–Ar), 167.8 (C=O, amide), 138.1 (C–O), 118.1 (C-5 thiazole). $M^+ = 320$ m/z $[\text{M} + \text{H}]$ (m/z).

4-(4-chlorooxazol-2-yl)-2-((thiazol-2-ylmethyl)amino)butanoic acid (C4)

White crystalline product was obtained with an isolated yield of 70% and the reaction was completed in 3 hours. A value of 0.59 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 2500–3300 (COOH), 3300–3400 (N–H stretch (2° amine), 1700–1725 (C=O (carboxylic acid), 1550–1600 cm^{-1} C–N (C=N (oxazole/thiazole), 600–800 cm^{-1} C–Cl (aromatic), 1050–1250 cm^{-1} (C–O, C–N). ^1H NMR (500 MHz, DMSO) (ppm): 7.32 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.0–8.0 (s, 1H, Thiazole proton ($\text{C}_5\text{--H}$), 4.0 (s, 2H, CH_2), 2.8 CH (α to NH, COOH), 1.5–1.8 (m, 2H, CH_2), 11 (s, COOH), 8.01 (b, NH). ^{13}C NMR (500 MHz, DMSO) (ppm): 48.5 (CH_2), 66.7 (CH) (α to amine/carboxylic), 140–145 (–Cl), 125 (Ar. C), 173.8 (Carboxylic acid (C=O), 168.7 (C1- thiazole), $M^+ = 303.0$ m/z $[\text{M} + \text{H}]$ (m/z).

5-(3-chlorocyclopenta-1,3-dien-1-yl)(thiazol-2-ylmethyl)aminoimidazolidine-2,4-dione (C5)

White crystalline product was obtained with an isolated yield of 80% and the reaction was completed in 3 hours. A value of 0.70 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3200–3400 (N–H (imidazolidine), 1700–1750 (C=O (ureide/2,4-dione), 1600–1650 (C=C (cyclopentadiene), 1200–1600 (C=N, C–N (thiazole, imidazolidine), 700–800 (C–Cl). ^1H NMR (500 MHz, DMSO) (ppm): 4.42 (s,d, N– CH_2 (thiazolylmethyl), 6.8 (m, 3H, Cyclopentadienyl protons (C=C–H), 6.82 (s, NH (ureide), 7.381 (1H, d, oxazole-H, 4 J = 0.8 Hz), 10.031 (2H, NH). ^{13}C NMR (500 MHz, DMSO) (ppm): 170.1 (C=O), 168.61 (Thiazole), 157.3 Carbonyls (C=O, imidazolidine), 141.9 (CH), 125.4 (C–Cl), 118.7 (Cyclopentadiene C=C), 89.2 (CH–N), 54.2 (N– CH_2 (thiazolylmethyl). $M^+ = 311$ m/z $[\text{M} + \text{H}]$ (m/z).

2-(5-chlorooxazol-2-yl)methylthiazol-4-ol (C6)

White crystalline product was obtained with an isolated yield of 70% and the reaction was completed in 3 hours. A value of 0.77 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3200–3400 (N–H (imidazolidine),

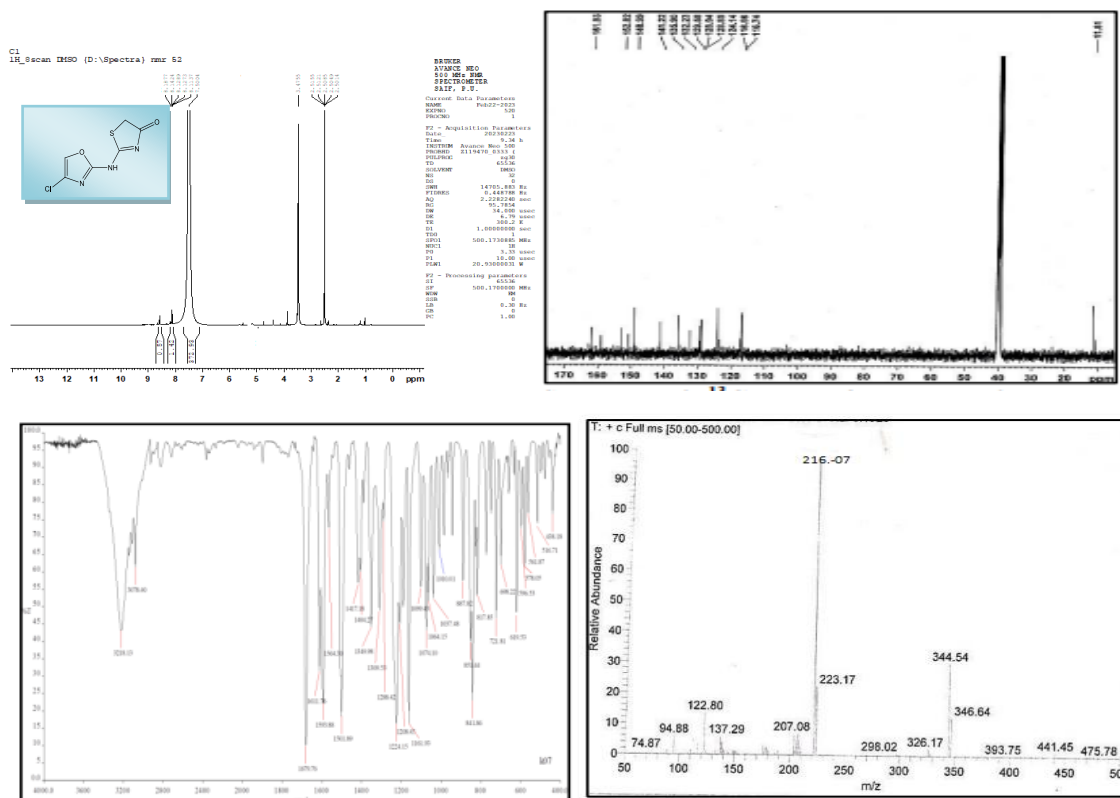
1700–1750 (C=O (ureide/2,4-dione), 1600–1650 (C=C (cyclopentadiene), 1200–1600(C=N, C–N (thiazole, imidazolidine), 700–800 (C–Cl). ¹H NMR (500 MHz, DMSO) (ppm): 7.69 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.50 (1H, CH), 3.63 (2H,CH₂ thiazole), 3.78 (1H, CH, thiazole). ¹³C NMR (500 MHz, DMSO) (ppm): 31.3 (CH₂), 57.6 (CH), 125.4 (C–Cl), 138.1 (C–O), 154.3 (C–NH), 162.8 (CH–N thiazole), 186.5 (C=O). m/z 215.12 (M+H).

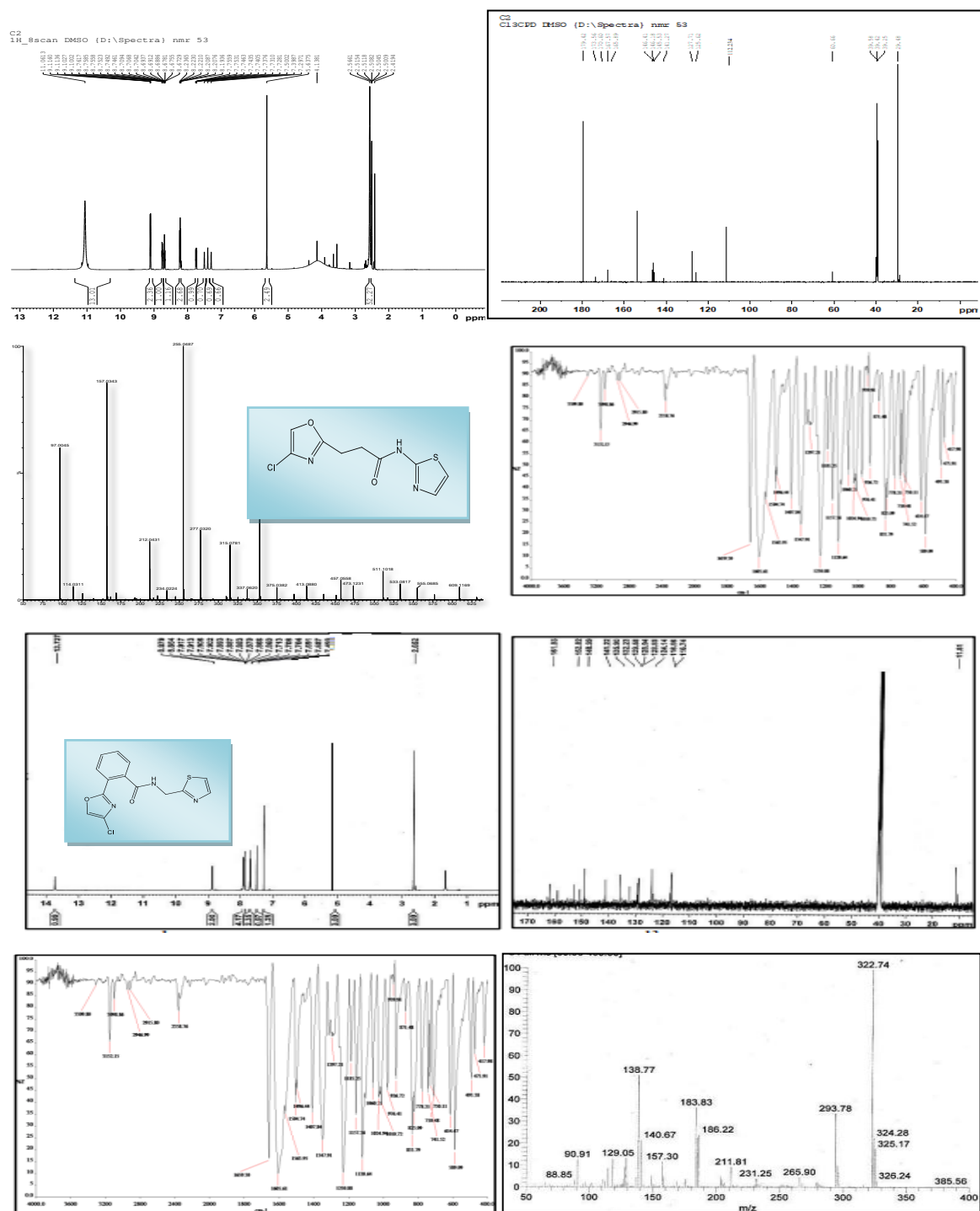
N4-(4-chlorooxazol-2-yl)-N2-(4-chlorothiazol-2-yl)imidazolidine-2,4-diamine (C7)

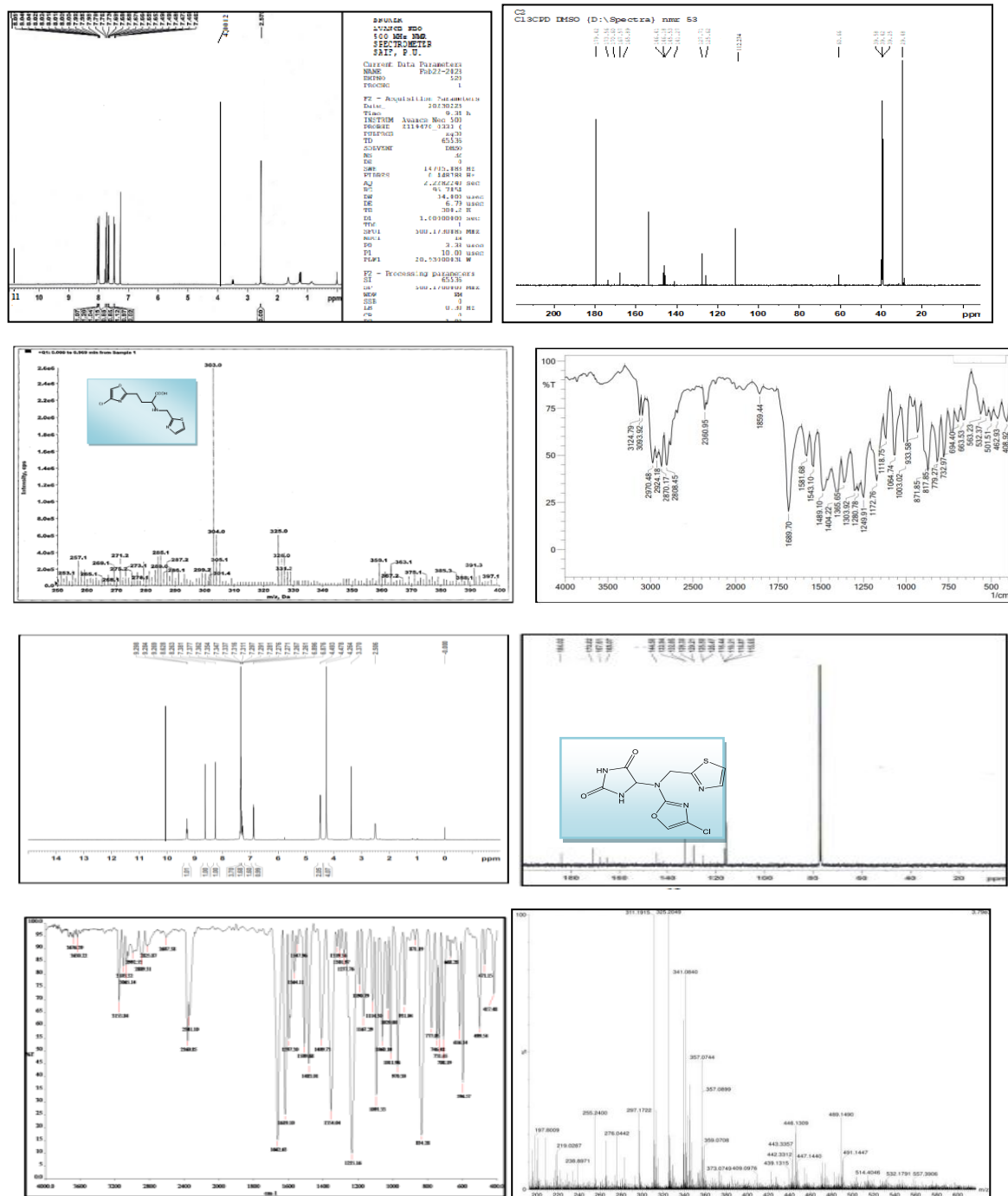
White crystalline product was obtained with an isolated yield of 72% and the reaction was completed in 3 hours. A value of 0.71 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. 3300–3400 (broad, N–H stretch), 1600–1650 (C=N stretch (azole rings), 1200–1350 (C–O (C–N stretch (amines), 1000–1200 (C–O–C stretch (oxazole), 750 (C–Cl). ¹H NMR (500 MHz, DMSO) (ppm): 7.69 (1H, d, oxazole-H, 4 J = 0.8 Hz), 3.23 (2H,CH₂ thiazole), 3.98 (1H, CH, thiazole), 4.12 (1H, NH). ¹³C NMR (500 MHz, DMSO) (ppm): 160–168.5 C=N, aromatic C (N/O/S bearing), 160.5 (CH), 137.5 (C–Cl), 125.4 (C–Cl), 86.1 (CH), 59.8 (CH₂), m/z 318.98 (M+H).

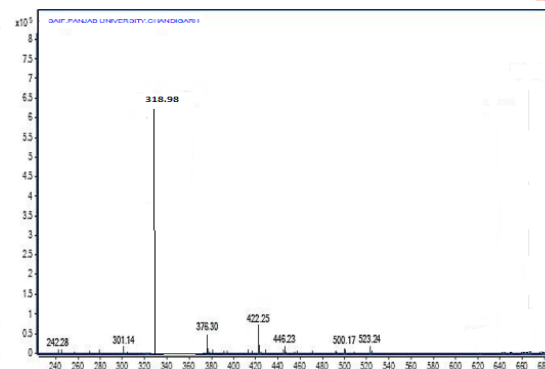
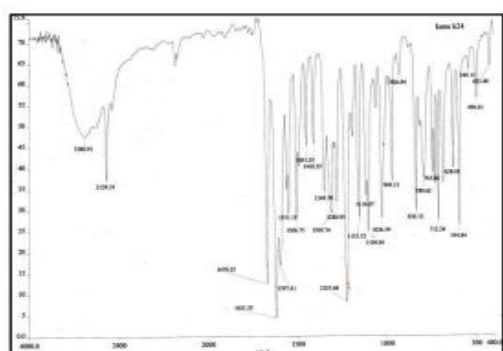
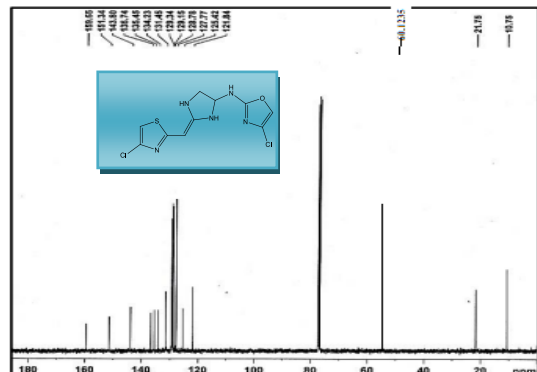
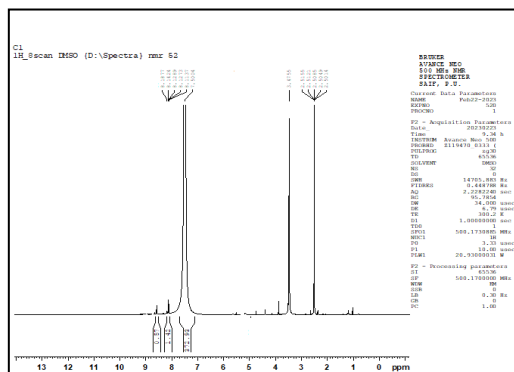
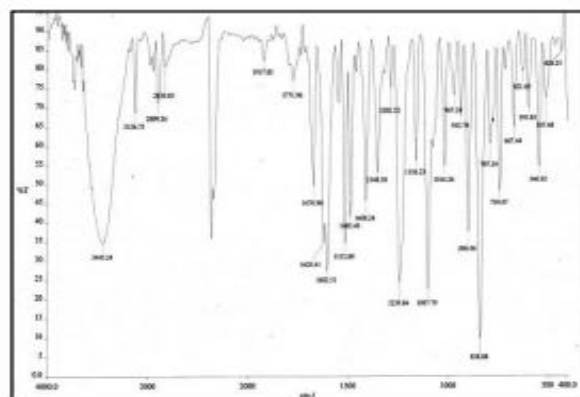
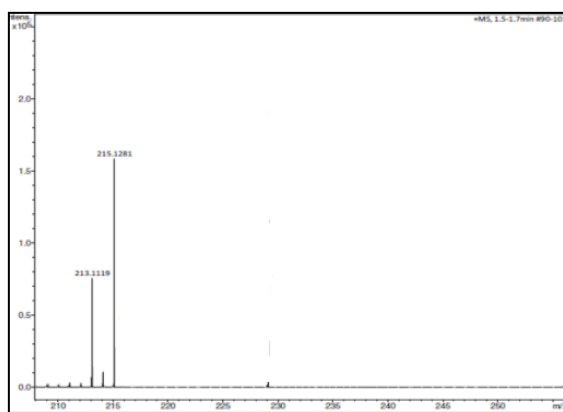
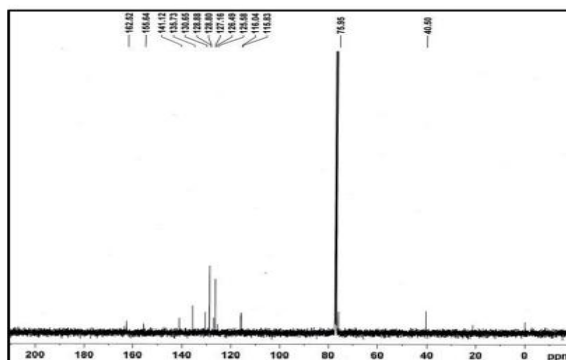
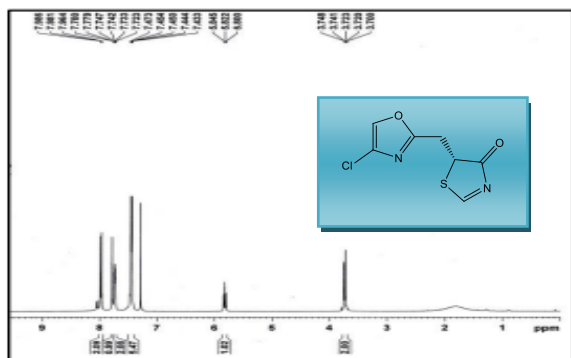
4-(4-chlorooxazol-2-yl)-1-(thiazol-2-yl)butan-1-one (C8)

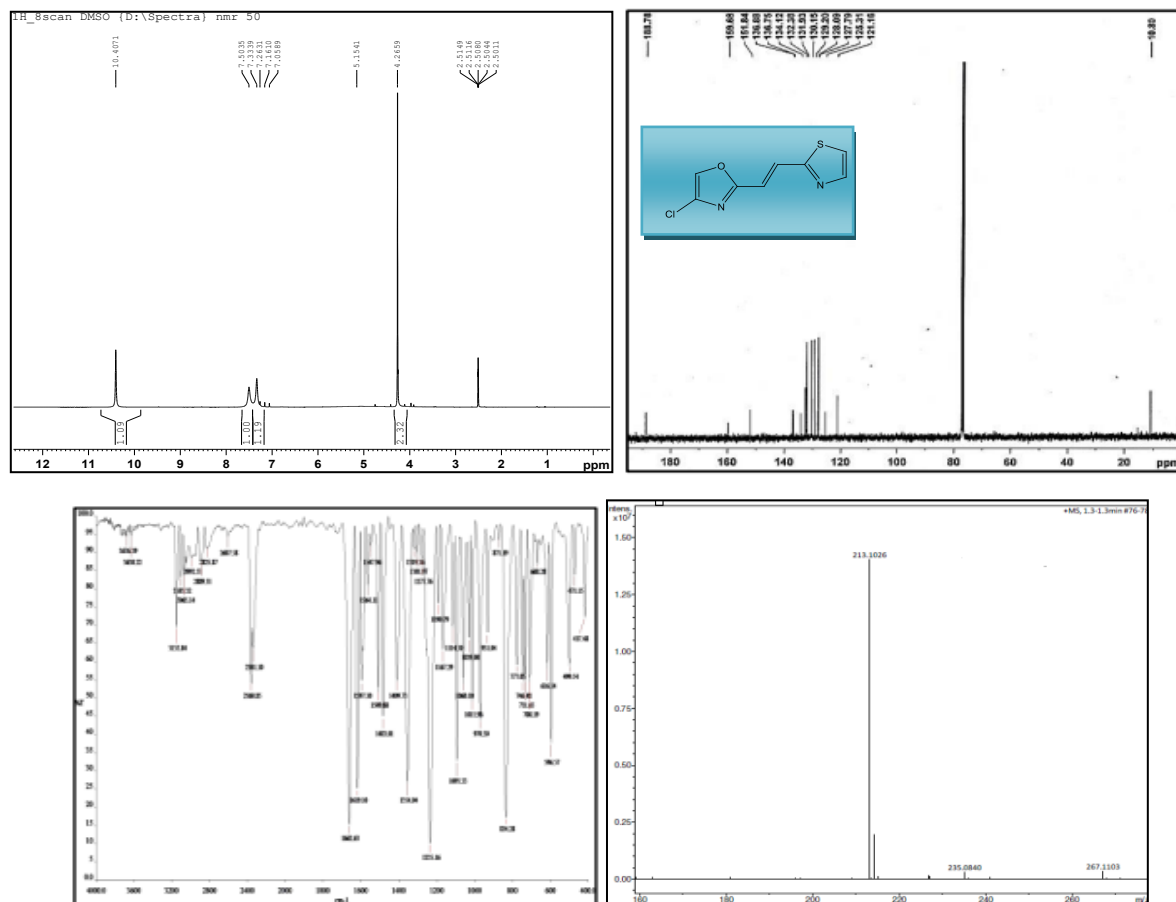
White crystalline product was obtained with an isolated yield of 75% and the reaction was completed in 3 hours. A value of 0.68 was recorded as R_f using n-hexane and ethyl acetate in the ratio of 7:3, respectively. IR (KBr pellets, ν_{max}/cm⁻¹): 1700–1725 (**C=O stretch** of ketone), 1600 (**C=N or aromatic C=C** in heterocycles), 3100–3000 (Aromatic C–H stretch), 2900–2850 Aliphatic C–H, 1050–1250C–O or C–N in oxazole/thiazole rings, 750 (C–Cl). ¹H NMR (500 MHz, DMSO) (ppm): 7.69 (1H, d, oxazole-H, 4 J = 0.8 Hz), 7.74–7.78 (2H,CH thiazole), 4.31–4.42 (2H, CH=CH), 2.3 (CH₂–CH₂). ¹³C NMR (500 MHz, DMSO) (ppm): 166.2 (CH thiazole), 164.3 (C–NH), 143.9 (2CH thiazole), 138.1 (C–O), 125.4 (C–Cl). m/z (M+H) 212.66.











CONCLUSION

A library of 24 oxazole derivatives was synthesized using Citrus limon juice as a reaction catalyst and evaluated using DPPH and ABTS radical-scavenging assays. Several derivatives displayed IC_{50} values within or below the range of the ascorbic-acid reference under the respective assay conditions. B5 and B4 were the strongest DPPH-active derivatives, whereas A2 and B2 were the strongest ABTS-active derivatives. A6 showed favorable activity in both assays and was therefore selected, together with B5 and C6, for subsequent biological investigation. FT-IR, ^1H -NMR, ^{13}C -NMR, and mass spectroscopy. Compounds A6, B4, B5, C5, and C8 showed significant antioxidant activity with IC_{50} values of 25.85, 19.89, 17.12, 29.54, and 33.15 $\mu g/mL$, respectively, when compared to the Ascorbic acid IC_{50} value of 28.47 $\mu g/mL$ for DPPH. Compounds A2, A6, B2, B6, and C6 were found to have considerable antioxidant activity with IC_{50} values of 19.32, 28.08, 21.05, 27.64, and 28.52 $\mu g/mL$, respectively, when compared to the conventional Ascorbic acid IC_{50} value of 26.18 $\mu g/mL$ for the ABTS assay. The current compounds show greater efficacy and wider SAR coverage when compared to oxazole antioxidant literature studies, setting a useful standard for future oxazole-based antioxidant research. These results strongly imply that the novel oxazole compounds are intriguing lead candidates for therapeutic applications against oxidative stress-related illnesses (such as cancer, diabetes, neurodegeneration, and inflammation). The SAR indicates that antioxidant activity is influenced by the combined effects of substituent electronics, hydrogen-atom/electron donation, conjugation, and scaffold architecture. These findings support further mechanistic and biological evaluation of the most active derivatives.

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Competing interest statement

The authors declare that they have no conflicts of interest.

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Author contribution

Sweta Joshi conceptualized and designed the study, conducted the experimental work, performed data acquisition and analysis, and drafted the manuscript. Jyoti M. Kalra^{1*} provided supervision throughout the research process, contributed to the study design and interpretation of data.

Abbreviations

ABTS- Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic acid)

DPPH- 2,2-Diphenyl-1-Picrylhydrazyl

IC₅₀-Inhibitory Concentration 50

TLC- Thin Layer Chromatography

NMR- Nuclear Magnetic Resonance

FT-IR- Fourier Transform Infrared Spectroscopy

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