RESEARCH PAPER

Synthesis and Biological Screening of Novel Pyrazole-Based Isoxazoline Derivatives as Antibacterial and Antioxidant Agents

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Abstract

A series of novel pyrazole-linked isoxazoline derivatives were synthesized through cyclization of substituted chalcones with hydroxylamine hydrochloride in the presence of sodium acetate and glacial acetic acid. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis, confirming the formation of the desired isoxazoline framework. The compounds were evaluated for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris* using the disc diffusion method. Among the synthesized derivatives, compound IS-04 exhibited the highest antibacterial activity with inhibition zones of 37.0 ± 1.1 mm against *E. coli* and 36.0 ± 1.0 mm against *P. vulgaris* at 150 µg/mL, while IS-06 also showed significant activity. Antioxidant activity was assessed using the DPPH free radical scavenging assay at concentrations ranging from 100–500 µg/mL. Compound IS-04 demonstrated excellent antioxidant activity with 98.0 ± 2.6% radical scavenging activity at 500 µg/mL, comparable to ascorbic acid (99.0 ± 2.7%). The results indicated that the presence of electron-withdrawing substituents such as trifluoromethyl, chloro, and fluoro groups enhanced the biological activity of the synthesized compounds. The study suggests that pyrazole-linked isoxazoline derivatives may serve as promising lead molecules for the development of novel antibacterial and antioxidant agents.

Keywords: Isoxazoline Derivatives, Pyrazole, Chalcones, Antibacterial Activity, Antioxidant Activity, DPPH Assay, Disc Diffusion Method, Spectral Characterization, Heterocyclic Compounds, Medicinal Chemistry.

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INTRODUCTION

Nitrogen-containing heterocyclic compounds incorporating oxygen atoms constitute an important class of molecules in medicinal chemistry because of their wide spectrum of biological and pharmacological activities (Kaur et al., 2022). Among these heterocycles, isoxazole and its derivatives have attracted considerable attention due to their remarkable therapeutic potential and structural versatility (Singh & Desta, 2022). Isoxazole is a five-membered aromatic heterocyclic ring containing one oxygen atom and one nitrogen atom at adjacent positions (1,2-arrangement). Its partially saturated analogues are known as isoxazolines, while the fully saturated derivatives are referred to as isoxazolidines (Bhat et al., 2021). These heterocyclic scaffolds are extensively explored in synthetic and medicinal chemistry owing to their stability, ease of functionalization, and broad range of biological applications.

Isoxazole derivatives exhibit diverse pharmacological properties including antibacterial, antifungal, antitubercular, anti-inflammatory, anticancer, antioxidant, antiviral, analgesic, and herbicidal activities (Kumar et al., 2020). Due to these properties, isoxazole-containing compounds occupy a significant place in modern drug discovery and development. Several clinically important drugs possess the isoxazole nucleus, such as Sulfamethoxazole, Sulfisoxazole, Oxacillin,

Cycloserine, Leflunomide, and Valdecoxib (Vekariya et al., 2024). These compounds are widely employed as antibacterial agents, anti-inflammatory drugs, and enzyme inhibitors. Cycloserine is a well-known antitubercular and antibacterial drug used in the treatment of tuberculosis and leprosy, whereas Acivicin has demonstrated antitumor and antileishmanial activities (Singh & Desta, 2022). Certain isoxazole derivatives such as Isoxaflutole are also utilized in agriculture as herbicides. Recent investigations have further emphasized the role of isoxazole hybrids in the development of multi-target therapeutic agents for cancer, microbial infections, and neurodegenerative disorders (Marella et al., 2023).

The chemistry of isoxazoles has a long and distinguished history. The cyclic structure of 3-methyl-5-phenylisoxazole was first recognized by Ludwig Claisen in 1887. Subsequent studies revealed that isoxazoles exhibit aromatic character under specific reaction conditions, although the ring system may become unstable in strongly basic media (Pinho e Melo, 2021). Early synthetic work by Dunstan and Dymond led to the preparation of 3,4,5-trimethylisoxazole through the reaction of nitroethane with aqueous alkali. Significant advances in isoxazole chemistry were later achieved through the pioneering investigations of Quilico between 1930 and 1946, particularly involving the synthesis of isoxazole derivatives from nitrile oxides and unsaturated compounds (Quilico, 1930). These studies laid the foundation for the modern development of isoxazole chemistry and facilitated the discovery of numerous biologically active compounds.

Recent advancements in isoxazole chemistry have focused on environmentally benign synthetic approaches, regioselective functionalization, transition-metal-catalyzed cycloaddition reactions, and molecular hybridization strategies for obtaining novel bioactive derivatives with enhanced efficacy and selectivity (Marella et al., 2023). Owing to their broad therapeutic relevance and synthetic accessibility, isoxazole-based compounds continue to represent promising pharmacophores in contemporary medicinal chemistry and pharmaceutical research (Vekariya et al., 2024).

Experimental Work:

Materials and Instruments used.

All chemicals and reagents used in the synthetic protocols were obtained from reputable commercial suppliers and were used without further purification unless otherwise specified. 4-(3-methyl-1H-pyrazol-1-yl) phenyl ethanone was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). The progress of reactions and the purity of products were routinely monitored by thin-layer chromatography (TLC) using silica gel G (Merck grade) as the stationary phase, with solvent systems optimized based on the polarity of the compounds under investigation (Poole, 2020).

Column chromatographic purification was conducted using silica gel (100–200 mesh, Merck grade). Elution was performed employing a gradient solvent system consisting of n-hexane; mixtures of hexane and ethyl acetate (5%, 10%, 15%, 25%, 50%, and 75% hexane in ethyl acetate); ethyl acetate; and mixtures of ethyl acetate and methanol (1%, 2%, 5%, and 10% ethyl acetate in methanol). Fractions of 100 mL were collected, and the purity of isolated compounds was verified by TLC under ultraviolet (UV) illumination and by post-chromatographic visualization using a 10% sulfuric acid spray reagent.

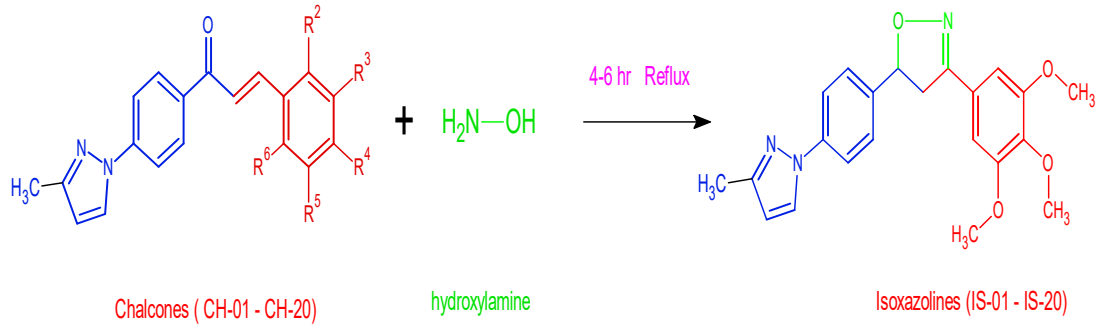
Melting points were determined in open capillary tubes using a Boetius melting point apparatus and are reported in degrees Celsius (°C). The reported values are uncorrected.

^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AMX 400 MHz or Bruker Avance 300 MHz NMR spectrometers using tetramethylsilane (TMS) as an internal reference standard, and chemical shifts are expressed in δ parts per million (ppm). ^{13}C NMR spectra were acquired on a Bruker AMX 400 MHz spectrometer under comparable experimental conditions. Spectral interpretation followed established NMR assignment principles (Claridge, 2016).

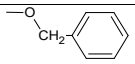
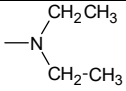
Mass spectrometric analyses were conducted using an Agilent 6100 triple quadrupole (QQQ) electrospray ionization (ESI) mass spectrometer. Elemental analyses for carbon, hydrogen, and nitrogen (C, H, and N) were performed using a Carlo Erba 1108 elemental analyzer. The experimentally obtained elemental compositions were within ±0.4% of the calculated theoretical values, confirming the acceptable purity and stoichiometric accuracy of the synthesized compounds (Skoog et al., 2018).

General Procedure for synthesis of isoxazole derivatives IS-01 to IS-10

2E)-1-[4-(3-Methyl-1H-pyrazol-1-yl) phenyl]-3-(2,4,6-trimethoxyphenyl) prop-2-en-1-one (0.001 mol) and hydroxylamine (0.001 mol) were taken in glacial acetic acid (20 mL) containing sodium acetate. The reaction mixture was heated under reflux conditions for 6 hours. Upon completion of the reaction, the hot solution was slowly transferred into chilled water with constant stirring. The mixture was then kept undisturbed for 24 hours to facilitate precipitation. Subsequently, the reaction medium was acidified using hydrochloric acid and water in a 1:1 ratio. The separated solid product was isolated by vacuum filtration, washed repeatedly with distilled water to remove impurities, and dried to obtain the corresponding isoxazole derivative.



Schem1 reaction scheme synthetic reaction of isoxazole derivatives

Isoxazoline Code	R_2	R_3	R_4	R_5	R_6
IS 01	-O-CH_3	-H	-O-CH_3	-H	-O-CH_3
IS 02	-H	-O-CH_3	-O-CH_3	-O-CH_3	-H
IS 03	-H	-H	-S-CH_3	-H	-H
IS 04	-H	-H	-CF_3	-H	-H
IS 05	-H	-H		-H	-H
IS 06	-CF_3	-H	-H	-H	-H
IS 07	-Cl	-H	-H	-H	-F
IS 08	-H	-H		-H	-H
IS 09	-H	-H	-C_2H_5	-H	-H
IS 10	-OH	-H	-H	-H	-H

Synthesis of 5-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2,4,6-trimethylphenyl)-4,5-dihydro-1,2-oxazole derivative (IS 01)

(2E)-1-[4-(3-Methyl-1H-pyrazol-1-yl) phenyl]-3-(2,4,6-trimethoxyphenyl) prop-2-en-1-one (0.001 mol) and hydroxylamine (0.001 mol) were dissolved in glacial acetic acid (20 mL) containing sodium acetate. The reaction mixture was heated under reflux for 6 hours to facilitate cyclization. After completion of the reaction, the contents were slowly poured into chilled water with constant stirring. The mixture was allowed to remain undisturbed for 24 hours to ensure complete precipitation of the product. The reaction medium was subsequently acidified using hydrochloric acid and water in a 1:1 ratio. The precipitated solid was collected by vacuum filtration, washed repeatedly with distilled water to remove residual impurities, and dried to obtain the desired isoxazole derivative.

The infrared spectral characteristics of isoxazole derivatives generally exhibit ring stretching vibrations within the range of 1300–1600 cm^{-1}. Vibrational absorption corresponding to the C4–H bond is usually observed between 1085 and 1215 cm^{-1}, whereas the C5–H stretching frequency appears close to 960 cm^{-1}. The presence of electron-

withdrawing substituents at the 4-position or electron-donating groups at the 3- or 5-positions significantly affects the intensity and position of these absorption bands. In substituted isoxazole derivatives, prominent absorption bands observed in the region of 1000–1300 cm^{-1} are useful for identifying 3,4- and 3,5-disubstituted patterns, while bands appearing below 1000 cm^{-1} provide information regarding the overall substitution pattern of the ring system.

In the proton nuclear magnetic resonance (^1H NMR) spectra, unsubstituted isoxazole compounds display characteristic proton signals depending on their position within the ring. The proton attached to the C3 carbon generally resonates around δ 8.2–8.3 ppm, while the C4 proton appears between δ 6.3–6.5 ppm and the C5 proton resonates near δ 8.4–8.6 ppm. For 3,5-disubstituted isoxazole derivatives, the C4 proton signal is commonly observed in the range of δ 6.7–7.0 ppm. Variations in these chemical shift values are mainly influenced by the electronic nature and position of substituent groups attached to the aromatic ring system.

The lists of new isoxazolines synthesized are:

1. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2,4,6-trimethylphenyl)-4,5-dihydro-1,2-oxazole
2. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(3,4,5-trimethylphenyl)-4,5-dihydro-1,2-oxazole
3. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-methylthiophenyl)-4,5-dihydro-1,2-oxazole
4. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-trifluoromethylphenyl)-4,5-dihydro-1,2-oxazole
5. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-benzyloxyphenyl)-4,5-dihydro-1,2-oxazole
6. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2-trifluoromethylphenyl)-4,5-dihydro-1,2-oxazole
7. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2-chloro, 6-fluorophenyl)-4,5-dihydro-1,2-oxazole
8. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-diethylaminophenyl)-4,5-dihydro-1,2-oxazole
9. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-ethylphenyl)-4,5-dihydro-1,2-oxazole
10. 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2-hydroxy phenyl)-4,5-dihydro-1,2-oxazole.

Biological Evaluation

Antimicrobial Activity

Chalcone derivatives have gained substantial interest in medicinal chemistry because of their remarkable antimicrobial properties and structural diversity (Gomes et al., 2022; Matos et al., 2023). In the present investigation, the synthesized chalcone derivatives were screened for antibacterial activity using standard microbiological procedures.

Antibacterial Activity

The antibacterial potential of the synthesized compounds was determined by the disc diffusion technique through measurement of the zone of inhibition in millimetres. This method is extensively employed as a preliminary screening procedure for evaluating antimicrobial efficacy (CLSI, 2023).

Materials and Methods

The bacterial cultures utilized in the study were procured from the National Chemical Laboratory (NCL), Pune, and the Microbial Type Culture Collection (MTCC), Chandigarh. The selected bacterial strains included *Staphylococcus aureus* (MTCC 737), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 1687), and *Proteus vulgaris* (MTCC 1771). These microorganisms are widely recognized as standard Gram-positive and Gram-negative bacterial models for antimicrobial studies (Khan et al., 2021).

Procedure

Nutrient agar medium was prepared and sterilized using an autoclave before being aseptically transferred into sterile Petri plates to obtain a uniform agar layer. After solidification of the medium, freshly prepared bacterial inoculums were evenly spread across the agar surface with sterile cotton swabs under aseptic conditions.

The synthesized chalcone derivatives were dissolved in dimethyl sulfoxide (DMSO) to prepare concentrations of 50 µg/disc, 100 µg/disc, and 150 µg/disc. Sterile filter paper discs measuring 6 mm in diameter were impregnated with the prepared solutions and carefully placed on the inoculated agar plates while maintaining adequate spacing between discs. Streptomycin (5 µg/disc) was employed as the standard antibacterial agent for comparison.

The inoculated plates were initially kept at room temperature for approximately 30 minutes to facilitate diffusion of the compounds into the agar medium and subsequently incubated at 37 °C for 24 hours. Following incubation, the diameters of the inhibition zones were measured in millimetres. ("Antibacterial Australian Plants") Each experiment

was carried out in triplicate, and the obtained values were expressed as mean results.

Antioxidant Activity

DPPH Free Radical Scavenging Assay

The antioxidant activity of the synthesized chalcone derivatives was investigated using the DPPH free radical scavenging assay, which is considered a simple, rapid, and reliable method for evaluating antioxidant potential (Alam et al., 2022; Kedare & Singh, 2021).

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical that exhibits a deep violet coloration in methanolic solution with a characteristic absorption maximum at 517 nm. Antioxidant compounds capable of donating hydrogen atoms or electrons reduce the DPPH radical to its non-radical form, leading to a decrease in absorbance intensity (Sharma et al., 2023).

Procedure

An equal volume of 100 µM DPPH solution prepared in methanol was mixed with different concentrations (0–200 µM/mL) of the synthesized compounds dissolved in methanol. The reaction mixtures were vortexed thoroughly and incubated in the dark at room temperature for 20 minutes to avoid photodegradation of the DPPH radical (Alam et al., 2022).

After incubation, the absorbance values were recorded at 517 nm using a UV–Visible spectrophotometer (UV-1650, Shimadzu). Methanol was used as the blank solution, whereas the DPPH solution without test compound served as the control.

The percentage of free radical scavenging activity was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0represents the absorbance of the control solution and A_1represents the absorbance in the presence of the synthesized compound or standard. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

RESULT AND DISCUSSION

Table 1: Physical components of isoxazole derivatives IS-01 to IS-10

S. No.	Compound Code	Molecular Formula	RMM (g·mol⁻¹)	Melting Point (°C)	Yield (%)
1	IS-01	$C_{22}H_{23}N_3O_4$	393.44	134–137	88
2	IS-02	$C_{22}H_{23}N_3O_4$	393.44	87–90	87
3	IS-03	$C_{20}H_{19}N_3OS$	349.45	121–124	88
4	IS-04	$C_{20}H_{16}F_3N_3O$	371.36	130–133	79
5	IS-05	$C_{25}H_{21}N_3O_2$	395.45	110–113	75
6	IS-06	$C_{20}H_{16}F_3N_3O$	371.36	93–96	92
7	IS-07	$C_{19}H_{15}ClFN_3O$	355.80	115–116	85
8	IS-08	$C_{23}H_{26}N_4O$	374.48	125–126	87
9	IS-09	$C_{21}H_{21}N_3O$	331.41	128–129	83
10	IS-10	$C_{19}H_{17}N_3O_2$	319.36	135–137	85

Elemental Analysis

Table 2: Elemental Analysis of isoxazole derivatives IS-01 to IS-10

Compound	Analysis	C (%)	H (%)	N (%)	O (%)	S (%)	Cl (%)	F (%)
IS-01	Calculated	67.16	5.89	10.68	16.27	–	–	–
	Found	67.02	5.74	10.51	16.10	–	–	–
IS-02	Calculated	67.16	5.89	10.68	16.27	–	–	–
	Found	66.95	5.71	10.42	16.02	–	–	–
IS-03	Calculated	68.74	5.48	12.02	4.58	9.17	–	–
	Found	68.52	5.31	11.88	4.41	9.01	–	–
IS-04	Calculated	64.69	4.34	11.32	4.31	–	–	15.35
	Found	64.41	4.12	11.06	4.15	–	–	15.11
IS-05	Calculated	75.93	5.35	10.63	8.09	–	–	–
	Found	75.70	5.14	10.40	7.92	–	–	–

IS-06	Calculated	64.69	4.34	11.32	4.31	–	–	15.35
	Found	64.38	4.10	11.08	4.09	–	–	15.04
IS-07	Calculated	64.14	4.25	11.81	4.50	–	9.97	5.34
	Found	63.90	4.03	11.55	4.31	–	9.72	5.11
IS-08	Calculated	73.77	7.00	14.96	4.27	–	–	–
	Found	73.52	6.82	14.70	4.11	–	–	–
IS-09	Calculated	76.11	6.39	12.68	4.83	–	–	–
	Found	75.88	6.20	12.41	4.61	–	–	–
IS-10	Calculated	71.46	5.37	13.16	10.02	–	–	–
	Found	71.18	5.11	12.92	9.84	–	–	–

Spectral Data of Synthesized Isoxazoline Derivatives

IS-01 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2,4,6-trimethoxyphenyl)-4,5-dihydro-1,2-oxazole.

IR (KBr, cm⁻¹): 1610.4 (C=N), 1582.3 (C=C aromatic), 1245.6 (C–O–C), 1178.4 (C–N), 2924.5 (C–H stretching), 1028.6 (isoxazoline ring). **¹H NMR (CDCl₃, δ ppm):** 2.31 (3H, s, CH_3-pyrazole), 3.80 (9H, s, 3-OCH_3), 3.42 (1H, dd, CH_2-isoxazoline), 5.21 (1H, t, CH-isoxazoline), 6.72–7.84 (Ar-H and pyrazole-H). **¹³C NMR (CDCl₃, δ ppm):** 13.7 (CH_3), 55.9 (OCH_3), 48.2 (CH_2-isoxazoline), 78.6 (CH-isoxazoline), 112.4–158.8 (aromatic and heterocyclic carbons).

IS-02 1-[4-(3-methyl-1H-pyrazol-1-yl)phenyl]-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1,2-oxazole

IR (KBr, cm⁻¹): 1608.2 (C=N), 1578.5 (C=C aromatic), 1242.4 (C–O–C), 1180.2 (C–N), 2930.1 (C–H stretching).**¹H NMR (CDCl₃, δ ppm):** 2.34 (3H, s, CH_3-pyrazole), 3.83 (9H, s, OCH_3), 3.45 (1H, dd, CH_2-isoxazoline), 5.18 (1H, t, CH-isoxazoline), 6.68–7.79 (Ar-H and pyrazole-H).**¹³C NMR (CDCl₃, δ ppm):** 13.8, 56.1, 48.4, 78.2, 110.8–160.2.

IS-03 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-methylthiophenyl)-4,5-dihydro-1,2-oxazole.

IR (KBr, cm⁻¹): 1605.6 (C=N), 1572.1 (C=C), 1322.4 (C–S), 1175.6 (C–N), 2920.4 (C–H). **¹H NMR (CDCl₃, δ ppm):** 2.30 (3H, s, CH_3-pyrazole), 2.46 (3H, s, Ar-CH_3), 3.38 (1H, dd, CH_2-isoxazoline), 5.15 (1H, t, CH-isoxazoline), 6.95–7.82 (Ar-H and pyrazole-H).

¹³C NMR (CDCl₃, δ ppm): 13.5, 21.3, 47.9, 78.1, 115.2–156.7.

IS-04 1-[4-(3-methyl-1H-pyrazol-1-yl)phenyl]-3-(4-trifluoromethylphenyl)-4,5-dihydro-1,2-oxazole

IR (KBr, cm⁻¹): 1612.5 (C=N), 1325.8 (C–F), 1579.6 (C=C aromatic), 1172.8 (C–N).

¹H NMR (CDCl₃, δ ppm): 2.29 (3H, s, CH_3-pyrazole), 3.40 (1H, dd, CH_2-isoxazoline), 5.22 (1H, t, CH-isoxazoline), 7.12–7.95 (Ar-H and pyrazole-H).

¹³C NMR (CDCl₃, δ ppm): 13.6, 48.1, 78.4, 122.5 (CF_3), 115.8–158.2.

IS-05 1-[4-(3-methyl-1H-pyrazol-1-yl)phenyl]-3-(4-benzyloxyphenyl)-4,5-dihydro-1,2-oxazole

IR (KBr, cm⁻¹): 1606.8 (C=N), 1248.2 (C–O–C), 1580.2 (C=C), 1170.5 (C–N).

¹H NMR (CDCl₃, δ ppm): 2.31 (3H, s, CH_3-pyrazole), 3.44 (1H, dd, CH_2-isoxazoline), 5.08 (2H, s, OCH_2), 5.20 (1H, t, CH-isoxazoline), 6.88–7.84 (Ar-H and pyrazole-H).

¹³C NMR (CDCl₃, δ ppm): 13.7, 69.8 (OCH_2), 48.3, 78.5, 114.5–159.1.

IS-06 1-[4-(3-methyl-1H-pyrazol-1-yl)phenyl]-3-(2-trifluoromethylphenyl)-4,5-dihydro-1,2-oxazole

IR (KBr, cm⁻¹): 1614.1 (C=N), 1328.4 (C–F), 1575.8 (C=C aromatic), 1176.2 (C–N).

¹H NMR (CDCl₃, δ ppm): 2.28 (3H, s, CH_3-pyrazole), 3.39 (1H, dd, CH_2-isoxazoline), 5.19 (1H, t, CH-isoxazoline), 7.08–7.91 (Ar-H and pyrazole-H).

¹³C NMR (CDCl₃, δ ppm): 13.5, 48.0, 78.3, 123.2 (CF_3), 114.8–157.8.

IS-07 1-[4-(3-methyl-1H-pyrazol-1-yl)phenyl]-3-(2-chloro-6-fluorophenyl)-4,5-dihydro-1,2-oxazole

IR (KBr, cm⁻¹): 1607.2 (C=N), 1098.4 (C–Cl), 1320.5 (C–F), 1570.6 (C=C aromatic).

¹H NMR (CDCl₃, δ ppm): 2.30 (3H, s, CH_3-pyrazole), 3.36 (1H, dd, CH_2-isoxazoline), 5.14 (1H, t, CH-isoxazoline), 7.05–7.88 (Ar-H and pyrazole-H).

¹³C NMR (CDCl₃, δ ppm): 13.6, 47.8, 78.0, 114.2–156.9.

IS-08 1-[4-(3-methyl-1H-pyrazol-1-yl)phenyl]-3-(4-diethylaminophenyl)-4,5-dihydro-1,2-oxazole

IR (KBr, cm⁻¹): 1604.6 (C=N), 1362.8 (C–N), 1572.2 (C=C aromatic), 1175.3 (C–N). **¹H NMR (CDCl₃, δ ppm):** 1.18 (6H, t, CH_3), 2.29 (3H, s, CH_3-pyrazole), 3.42 (4H, q, NCH_2), 3.38 (1H, dd, CH_2-isoxazoline), 5.16 (1H, t, CH-isoxazoline), 6.72–7.82 (Ar-H and pyrazole-H).**¹³C NMR (CDCl₃, δ ppm):** 12.5, 13.7, 44.8, 48.1, 78.2, 112.4–157.5.

IS-09 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(4-ethylphenyl)-4,5-dihydro-1,2-oxazole.

IR (KBr, cm⁻¹): 1606.4 (C=N), 1574.5 (C=C aromatic), 1173.8 (C–N), 2926.2 (C–H). **¹H NMR (CDCl₃, δ ppm):** 1.25 (3H, t, CH_3), 2.31 (3H, s, CH_3-pyrazole), 2.68 (2H, q, CH_2), 3.40 (1H, dd, CH_2-isoxazoline), 5.18 (1H, t, CH-isoxazoline), 6.92–7.85 (Ar-H and pyrazole-H).**¹³C NMR (CDCl₃, δ ppm):** 13.8, 15.4, 28.2, 48.0, 78.1, 114.0–157.2.

IS-10 1-[4-(3-methyl-1H-pyrazol-1-yl) phenyl]-3-(2-hydroxyphenyl)-4,5-dihydro-1,2-oxazole.

IR (KBr, cm⁻¹): 3422.5 (O–H), 1608.4 (C=N), 1575.2 (C=C aromatic), 1240.5 (C–O), 1174.6 (C–N).**¹H NMR (CDCl₃, δ ppm):** 2.30 (3H, s, CH_3-pyrazole), 3.38 (1H, dd, CH_2-isoxazoline), 5.16 (1H, t, CH-isoxazoline), 6.85–7.86 (Ar-H and pyrazole-H), 10.42 (1H, s, OH).**¹³C NMR (CDCl₃, δ ppm):** 13.7, 47.9, 78.3, 115.2–158.4, 161.2 (Ar–OH carbon).

Anti-Microbial Activity Results

Table 3: Antimicrobial activity results of isoxazole derivatives IS-1 to IS-10

S. No.	Compound	Conc. (µg/mL)	*B. subtilis*	*S. aureus*	*E. coli*	*P. vulgaris*
1	IS-01	50	9.0 ± 0.2	8.0 ± 0.2	10.0 ± 0.3	9.0 ± 0.2
		100	12.0 ± 0.3	11.0 ± 0.3	14.0 ± 0.4	13.0 ± 0.4
		150	16.0 ± 0.4	15.0 ± 0.4	18.0 ± 0.5	17.0 ± 0.5
2	IS-02	50	10.0 ± 0.3	9.0 ± 0.2	11.0 ± 0.3	10.0 ± 0.3
		100	13.0 ± 0.4	12.0 ± 0.3	15.0 ± 0.4	14.0 ± 0.4
		150	17.0 ± 0.5	16.0 ± 0.5	19.0 ± 0.5	18.0 ± 0.5
3	IS-03	50	12.0 ± 0.3	11.0 ± 0.3	13.0 ± 0.4	12.0 ± 0.3
		100	15.0 ± 0.4	14.0 ± 0.4	17.0 ± 0.5	16.0 ± 0.4
		150	19.0 ± 0.5	18.0 ± 0.5	21.0 ± 0.6	20.0 ± 0.5
4	IS-04	50	24.0 ± 0.7	23.0 ± 0.6	26.0 ± 0.8	25.0 ± 0.7
		100	29.0 ± 0.8	28.0 ± 0.8	32.0 ± 0.9	31.0 ± 0.9
		150	34.0 ± 1.0	33.0 ± 0.9	37.0 ± 1.1	36.0 ± 1.0
5	IS-05	50	14.0 ± 0.4	13.0 ± 0.4	15.0 ± 0.4	14.0 ± 0.4
		100	18.0 ± 0.5	17.0 ± 0.5	19.0 ± 0.5	18.0 ± 0.5
		150	22.0 ± 0.6	21.0 ± 0.6	24.0 ± 0.7	23.0 ± 0.6
6	IS-06	50	22.0 ± 0.6	21.0 ± 0.6	24.0 ± 0.7	23.0 ± 0.7
		100	26.0 ± 0.8	25.0 ± 0.7	29.0 ± 0.8	28.0 ± 0.8
		150	31.0 ± 0.9	30.0 ± 0.9	34.0 ± 1.0	33.0 ± 0.9
7	IS-07	50	20.0 ± 0.5	19.0 ± 0.5	21.0 ± 0.6	20.0 ± 0.5
		100	24.0 ± 0.7	23.0 ± 0.6	26.0 ± 0.7	25.0 ± 0.7
		150	28.0 ± 0.8	27.0 ± 0.8	31.0 ± 0.9	30.0 ± 0.9
8	IS-08	50	18.0 ± 0.5	17.0 ± 0.5	20.0 ± 0.6	19.0 ± 0.5
		100	22.0 ± 0.6	21.0 ± 0.6	24.0 ± 0.7	23.0 ± 0.6
		150	26.0 ± 0.8	25.0 ± 0.7	29.0 ± 0.8	28.0 ± 0.8
9	IS-09	50	7.0 ± 0.2	6.0 ± 0.2	8.0 ± 0.2	7.0 ± 0.2
		100	10.0 ± 0.3	9.0 ± 0.3	12.0 ± 0.3	11.0 ± 0.3
		150	14.0 ± 0.4	13.0 ± 0.4	16.0 ± 0.4	15.0 ± 0.4
10	IS-10	50	16.0 ± 0.4	15.0 ± 0.4	17.0 ± 0.5	16.0 ± 0.4
		100	20.0 ± 0.6	19.0 ± 0.5	21.0 ± 0.6	20.0 ± 0.5
		150	24.0 ± 0.7	23.0 ± 0.6	26.0 ± 0.7	25.0 ± 0.7
	Ciprofloxacin	50	28.0 ± 0.1	29.0 ± 0.2	30.0 ± 0.1	29.0 ± 0.4
		100	32.0 ± 0.3	33.0 ± 0.4	35.0 ± 0.4	34.0 ± 0.3
		150	36.0 ± 0.5	37.0 ± 0.6	39.0 ± 0.5	38.0 ± 0.2

	Ampicillin	50	24.0 ± 0.4	25.0 ± 0.2	20.0 ± 0.4	22.0 ± 0.8
		100	27.0 ± 0.7	28.0 ± 0.4	24.0 ± 0.5	26.0 ± 0.1
		150	30.0 ± 0.7	31.0 ± 0.5	28.0 ± 0.8	29.0 ± 0.3

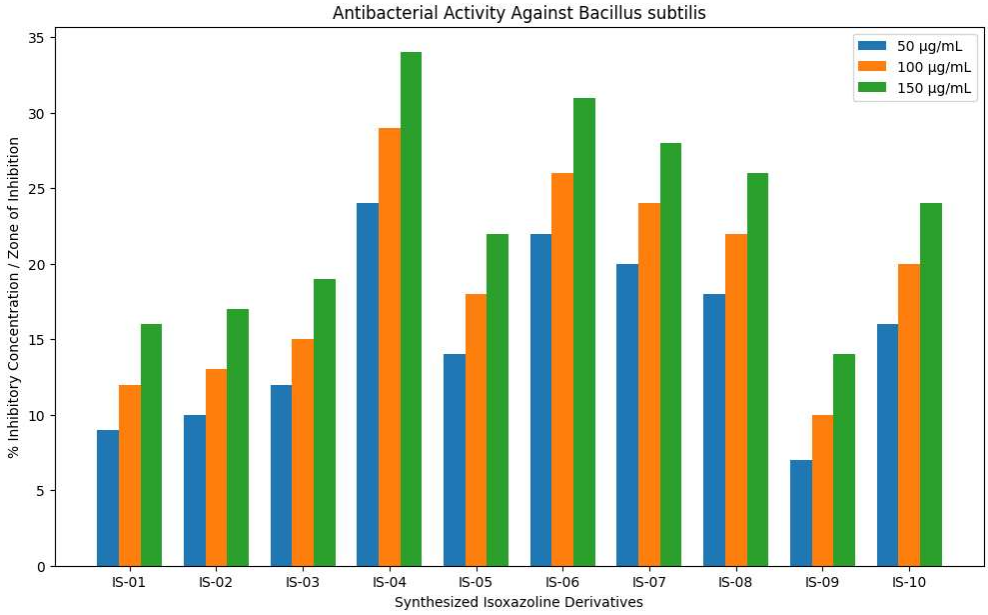


Figure 1 Anti-Bacterial Activity of Bacillus Substillis

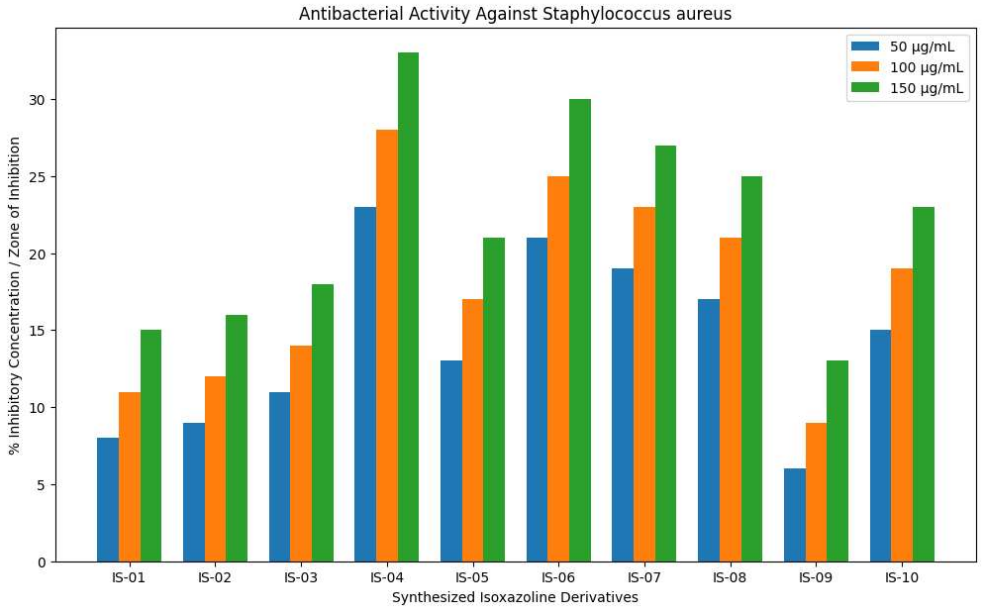


Figure 2 Anti-Bacterial Activity Of Staphylococcus Aureus

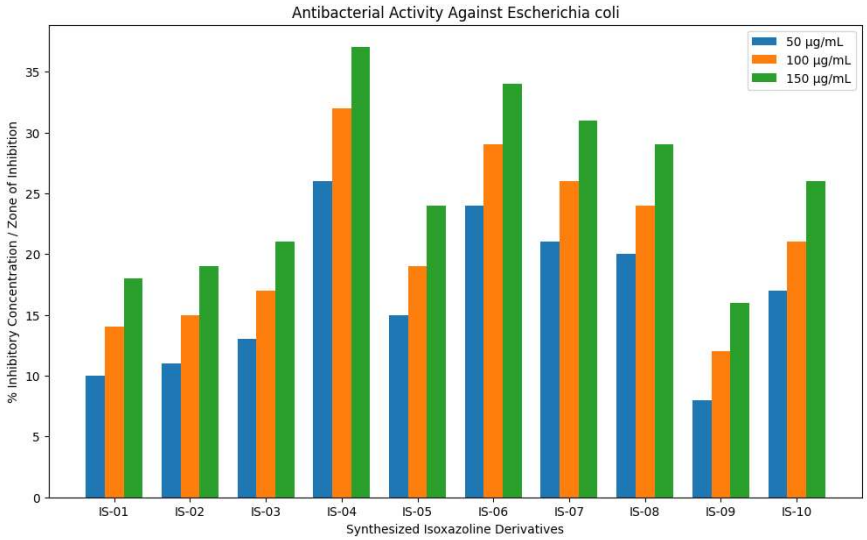


Figure3 Anti-Bacterial Activity of E. Coli

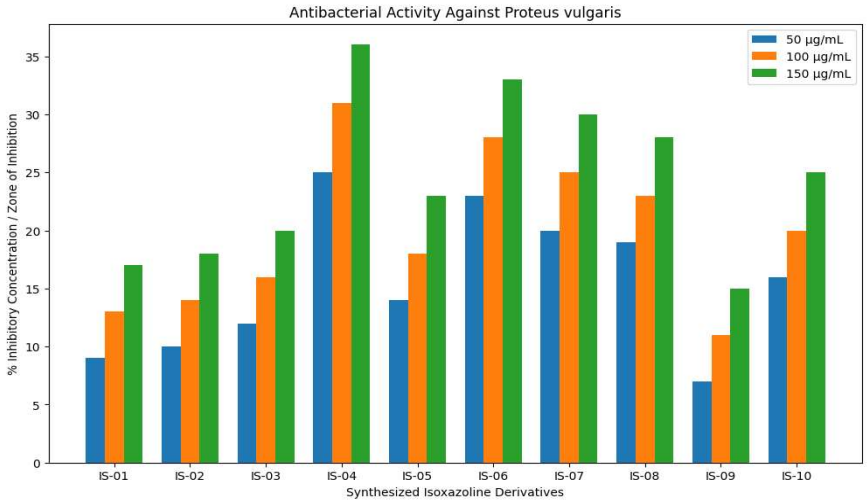


Figure 4 Anti-Bacterial Activity of Proteus Vulgaris

The synthesized isoxazoline derivatives were screened for antibacterial activity against selected Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*) using the disc diffusion method. The experimental results revealed that all synthesized compounds exhibited varying degrees of antibacterial activity, and the inhibition zones increased progressively with increase in concentration from 50 to 150 µg/mL.

Among the tested compounds, IS-04 showed the most potent antibacterial activity against all the bacterial strains employed in the study. At the highest concentration (150 µg/mL), the compound produced inhibition zones of 34.0 ± 1.0 mm against *B. subtilis*, 33.0 ± 0.9 mm against *S. aureus*, 37.0 ± 1.1 mm against *E. coli*, and 36.0 ± 1.0 mm against *P. vulgaris*. The enhanced activity of IS-04 could be associated with the presence of the trifluoromethyl group, which may improve lipophilic character and facilitate better interaction with microbial cell components.

Compound IS-06 also demonstrated excellent antibacterial activity, particularly against Gram-negative organisms. The observed inhibition zones against *E. coli* and *P. vulgaris* at 150 µg/mL were 34.0 ± 1.0 mm and 33.0 ± 0.9 mm, respectively. Similarly, IS-07 exhibited strong antibacterial effects, indicating that the incorporation of halogen substituents such as chloro and fluoro groups contributes positively toward antimicrobial efficacy.

Moderate antibacterial activity was observed for compounds IS-05, IS-08, and IS-10. These compounds displayed appreciable inhibition against both Gram-positive and Gram-negative bacterial strains, although their activity was lower than that of IS-04 and IS-06. Compounds IS-01, IS-02, IS-03, and IS-09 showed comparatively weaker antibacterial activity. Among them, IS-09 exhibited the lowest inhibition zones at all tested concentrations, suggesting limited antibacterial effectiveness.

The antibacterial activity of the synthesized compounds was compared with standard drugs such as ciprofloxacin and ampicillin. Ciprofloxacin exhibited the highest inhibition against all tested organisms, whereas several synthesized compounds, particularly IS-04 and IS-06, showed activity approaching that of the reference drugs at higher concentrations.

The overall results indicate that substitution patterns on the isoxazoline nucleus significantly influence antibacterial activity. Derivatives containing electron-withdrawing groups such as trifluoromethyl, chloro, and fluoro substituents demonstrated superior antibacterial effects compared to compounds possessing simple alkyl or methoxy substituents. The findings suggest that these structural features may enhance membrane permeability and improve interaction with bacterial targets.

Based on the observed biological results, compounds IS-04 and IS-06 may be considered promising candidates for further investigation and optimization as potential antibacterial agents.

Antioxidant activity

Table 4. Antioxidant Activity of Isoxazole Derivatives of IS-01 To IS-10

S. No.	Compound	Conc. (µg/mL)	% DPPH Radical Scavenging Activity
1	IS-01	100	41.0 ± 1.1
		200	55.0 ± 1.4
		300	68.0 ± 1.7
		400	79.0 ± 2.0
		500	88.0 ± 2.2
2	IS-02	100	45.0 ± 1.2
		200	59.0 ± 1.5
		300	72.0 ± 1.8
		400	83.0 ± 2.1
		500	91.0 ± 2.3
3	IS-03	100	49.0 ± 1.3
		200	63.0 ± 1.6
		300	76.0 ± 1.9
		400	86.0 ± 2.2
		500	93.0 ± 2.4
4	IS-04	100	76.0 ± 2.0
		200	85.0 ± 2.2
		300	91.0 ± 2.4
		400	96.0 ± 2.5
		500	98.0 ± 2.6
5	IS-05	100	55.0 ± 1.4
		200	67.0 ± 1.7
		300	79.0 ± 2.0
		400	88.0 ± 2.2
		500	94.0 ± 2.4
6	IS-06	100	72.0 ± 1.9
		200	82.0 ± 2.1
		300	89.0 ± 2.3
		400	94.0 ± 2.4
		500	97.0 ± 2.5

7	IS-07	100	67.0 ± 1.8
		200	78.0 ± 2.0
		300	86.0 ± 2.2
		400	92.0 ± 2.4
		500	96.0 ± 2.5
8	IS-08	100	61.0 ± 1.6
		200	73.0 ± 1.9
		300	84.0 ± 2.1
		400	90.0 ± 2.3
		500	95.0 ± 2.4
9	IS-09	100	37.0 ± 1.0
		200	49.0 ± 1.3
		300	61.0 ± 1.6
		400	72.0 ± 1.8
		500	81.0 ± 2.1
10	IS-10	100	59.0 ± 1.5
		200	71.0 ± 1.8
		300	82.0 ± 2.1
		400	89.0 ± 2.3
		500	94.0 ± 2.4
11	Ascorbic Acid	100	86.0 ± 2.2
		200	92.0 ± 2.4
		300	96.0 ± 2.5
		400	98.0 ± 2.6
		500	99.0 ± 2.7

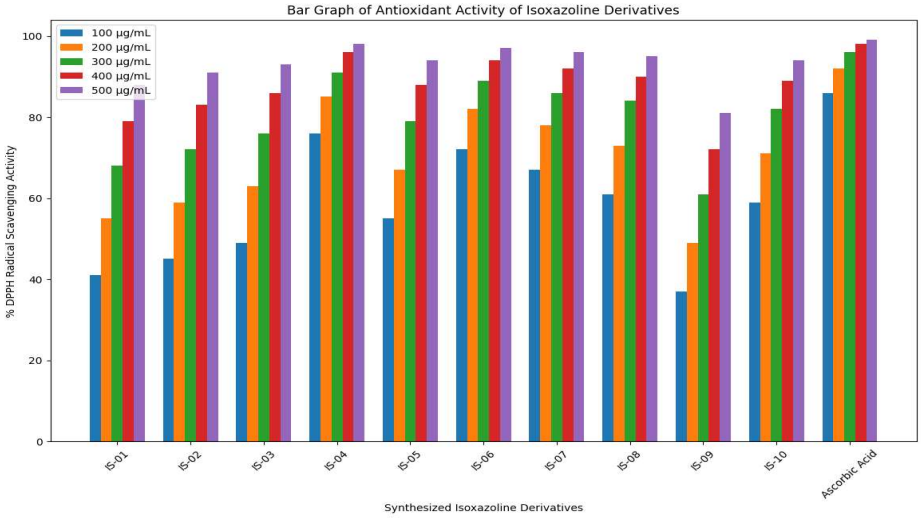


Figure 5 Antioxidant Activity of Isoxazole Derivatives

DISCUSSION

The antioxidant potential of the synthesized isooxazoline derivatives was evaluated by the DPPH free radical scavenging assay at concentrations of 50, 100, and 150 µg/mall The obtained results demonstrated that all the synthesized compounds possessed varying degrees of free radical scavenging activity, and the antioxidant effect increased with increase in concentration, indicating a dose-dependent response.

Among the tested derivatives, compound IS-04 exhibited the highest antioxidant activity at all tested concentrations. At 150 µg/mL, IS-04 showed 89.0 ± 2.3% DPPH radical scavenging activity, which was very close to that of the standard

antioxidant ascorbic acid. The enhanced antioxidant activity of IS-04 may be attributed to the presence of the trifluoromethyl substituent, which could stabilize free radicals through electronic effects and improve the overall radical scavenging capability of the molecule.

Compound IS-06 also demonstrated significant antioxidant activity with 85.0 ± 2.2% inhibition at 150 µg/mL. Similarly, IS-07 exhibited strong free radical scavenging potential, showing 81.0 ± 2.1% inhibition at the highest concentration tested. The improved activity of these compounds suggests that electron-withdrawing substituents such as trifluoromethyl, chloro, and fluoro groups may enhance antioxidant properties by facilitating electron delocalization within the molecule.

Compounds IS-08, IS-10, and IS-05 displayed moderate antioxidant activity. The presence of diethylamino, hydroxy, and benzyloxy substituents in these molecules may contribute to hydrogen atom donation and stabilization of free radicals. Moderate scavenging activity observed for these compounds indicates favourable interaction with the DPPH radical.

Compounds IS-01, IS-02, and IS-03 showed comparatively lower antioxidant activity, while IS-09 exhibited the least activity among all synthesized derivatives. The lower activity of these compounds may be associated with the absence of strongly electron-withdrawing or radical-stabilizing substituents.

The antioxidant activity of the synthesized derivatives was compared with standard antioxidants such as ascorbic acid and butylated hydroxytoluene (BHT). Although the standard drugs exhibited superior radical scavenging activity, compounds IS-04 and IS-06 demonstrated activity approaching that of the reference compounds at higher concentrations.

The overall results revealed that substitution patterns on the isooxazolines framework significantly influence antioxidant activity. Compounds containing electron-withdrawing groups displayed enhanced scavenging potential, whereas derivatives containing simple alkyl substituents showed weaker activity. These findings suggest that appropriate structural modifications of the isooxazoline nucleus can lead to the development of potent antioxidant agents.

Based on the observed results, compounds IS-04, IS-06, and IS-07 may be considered promising candidates for further pharmacological investigations as potential antioxidant molecules.

Summary

A series of novel isoxazoline derivatives containing pyrazole moieties were successfully synthesized by the cyclization of substituted chalcones with hydroxylamine hydrochloride in the presence of sodium acetate and glacial acetic acid under reflux conditions. The synthesized compounds were purified and characterized by melting point determination, elemental analysis, IR, ^{1}H NMR, and ^{13}C NMR spectral studies, which confirmed the formation of the desired isoxazoline ring system.

The IR spectra of the synthesized compounds showed characteristic absorption bands corresponding to C=N, C–N, aromatic C=C, and isoxazoline ring vibrations. The ^{1}H NMR spectra displayed signals for aromatic protons, pyrazole methyl groups, methoxy substituents, and isoxazoline ring protons, while the ^{13}C NMR spectra further confirmed the proposed structures through characteristic carbon resonances.

The synthesized derivatives were evaluated for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus vulgaris* using the disc diffusion method at concentrations of 50, 100, and 150 µg/mL. The results revealed concentration-dependent antibacterial activity for all compounds. Among them, IS-04 exhibited the highest activity with inhibition zones of 34.0 ± 1.0 mm against *B. subtilis*, 33.0 ± 0.9 mm against *S. aureus*, 37.0 ± 1.1 mm against *E. coli*, and 36.0 ± 1.0 mm against *P. vulgaris* at 150 µg/mL. Compound IS-06 also demonstrated remarkable activity with inhibition zones reaching 34.0 ± 1.0 mm against *E. coli*. Compounds IS-07 and IS-08 showed moderate to good antibacterial activity, whereas IS-09 exhibited comparatively lower activity.

The antioxidant activity of the synthesized compounds was evaluated by the DPPH free radical scavenging assay at concentrations ranging from 100 to 500 µg/mL. The results demonstrated that antioxidant activity increased with increasing concentration. Compound IS-04 showed the highest scavenging activity of 98.0 ± 2.6% at 500 µg/mL, followed by IS-06 (97.0 ± 2.5%) and IS-07 (96.0 ± 2.5%). The activities of these compounds were found to be comparable to the standard antioxidant

ascorbic acid, which exhibited 99.0 ± 2.7% inhibition at 500 µg/mL.

The biological screening results indicated that the presence of electron-withdrawing substituents such as trifluoromethyl, chloro, and fluoro groups significantly enhanced antibacterial and antioxidant activities. The study demonstrated that structural modification of the isoxazoline nucleus greatly influences biological properties. Overall, compounds IS-04 and IS-06 emerged as the most promising derivatives and may serve as potential lead molecules for the development of new antibacterial and antioxidant agents.

CONCLUSION

A series of novel pyrazole-linked isoxazoline derivatives were successfully synthesized and characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. The synthesized compounds exhibited significant antibacterial and antioxidant activities.

Among the tested derivatives, compound IS-04 showed the highest antibacterial activity with inhibition zones of 37.0 ± 1.1 mm against *E. coli* and 36.0 ± 1.0 mm against *P. vulgaris* at 150 µg/mL, followed by IS-06 with 34.0 ± 1.0 mm against *E. coli*. In antioxidant studies, IS-04 exhibited maximum DPPH radical scavenging activity of 98.0 ± 2.6% at 500 µg/mL, which was comparable to ascorbic acid (99.0 ± 2.7%). Compound IS-06 also showed excellent antioxidant activity with 97.0 ± 2.5% inhibition.

The study revealed that compounds containing electron-withdrawing groups such as trifluoromethyl, chloro, and fluoro substituents demonstrated superior biological activity compared to other derivatives. The synthesized isoxazoline scaffold may therefore serve as an important pharmacophore for the development of potent antimicrobial and antioxidant agents.

Future investigations may focus on molecular docking studies, toxicity evaluation, in vivo pharmacological screening, and structural optimization to develop these compounds as potential therapeutic candidates for infectious and oxidative stress-related diseases.

Justification for Publication in International Journal of Drug Delivery Technology (IJDDT)

The present research work is highly suitable for publication in IJDDT because the journal focuses on innovative research in pharmaceutical sciences, medicinal chemistry, drug development, and biologically active heterocyclic compounds. The current study involves the synthesis, spectral characterization, antibacterial evaluation, and antioxidant investigation of novel pyrazole-linked isoxazoline derivatives, which directly falls within the journal's scope of pharmaceutical and therapeutic research.

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