ZEAL SCIENTIFIC PUBLICATION

World Journal of
Chemical and Pharmaceutical Sciences

Journal homepage: https://zealjournals.com/wjcps/

ISSN: 2945-3186 (Online)

(RESEARCH ARTICLE)

Check for updates

Synthesis of five-membered heterocyclic rings from chalcone derivatives and evaluation of their biological activity

Bekhal Omer Mohammed *

Department of Chemistry, College of Science, University of Kirkuk, Kirkuk, Iraq

World Journal of Chemical and Pharmaceutical Sciences, 2026, 08(02), 004-015

Publication history: Received on 19 April 2026; revised on 29 May 2026; accepted on 01 June 2026

Article DOI: https://doi.org/10.53346/wjcps.2026.8.2.0017

Abstract

A series of aminochalcone and isoxazoline derivatives were synthesized and characterized using spectroscopic methods. The aminochalcones (C_1,C_2) were prepared through Claisen–Schmidt condensation of 4-aminoacetophenone with 4-nitrobenzaldehyde or 4-chlorobenzaldehyde in alkaline ethanolic medium. The obtained chalcones were then cyclized with hydroxylamine hydrochloride in the presence of sodium hydroxide to afford the corresponding 4,5-dihydroisoxazoline derivatives (I_1,I_2). The structures of the synthesized compounds were confirmed by melting points, FTIR, UV-Vis, and selected ^{1}H NMR spectral data. The FTIR spectra of chalcones showed characteristic absorption bands for NH_2, C=O, and C=C groups, while the isoxazoline derivatives showed disappearance of the chalcone carbonyl and olefinic bands with the appearance of new C=N and cyclic C–O absorption bands. These spectral changes confirmed successful cyclization of the chalcone system into the isoxazoline ring.

Keywords: Chalcone; Isoxazoline; Claisen–Schmidt Condensation; Hydroxylamine Hydrochloride

1. Introduction

Chalcones are an important class of naturally occurring and synthetic organic compounds belonging to the α,β-unsaturated ketone family. Their basic skeleton consists of two aromatic rings linked through a conjugated enone bridge, as shown in **Figure 1**. This structural arrangement gives chalcones distinctive chemical and biological properties and makes them attractive scaffolds in organic, medicinal, and heterocyclic chemistry. Many chalcone derivatives have been reported to exhibit a wide range of biological activities, including antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, antidiabetic, antiviral, antimalarial, and enzyme inhibitory effects [1–3]. The biological behavior of chalcones is strongly affected by the nature and position of substituents on the aromatic rings, which allows structural modification to improve their activity and selectivity [4].

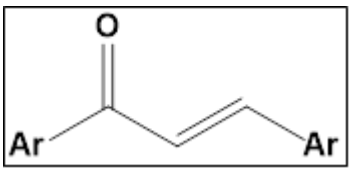

Figure 1 General structure of chalcone derivatives

* Corresponding author: Bekhal Omer Mohammed

The synthetic importance of chalcones is mainly related to the presence of the α,β-unsaturated carbonyl system. This conjugated system contains reactive centers that can interact with different nucleophilic reagents, making chalcones valuable intermediates for the synthesis of many heterocyclic compounds [5]. Chalcone-based heterocyclic derivatives have attracted increasing interest because the incorporation of nitrogen, oxygen, or sulfur atoms into the molecular framework may enhance chemical stability, polarity, binding ability, and biological performance [6]. Therefore, chalcones are widely regarded as useful synthons for constructing different five- and six-membered heterocyclic systems with potential pharmaceutical and industrial applications [7]. In addition to their medicinal relevance, chalcones have several important industrial and technological applications. Their extended π-conjugated system and easily tunable electronic properties make them useful in the design of fluorescent dyes, optical materials, nonlinear optical compounds, molecular probes, and chemical sensors [8]. Chalcone-based fluorescent molecules have been investigated for biological imaging, environmental monitoring, and detection of metal ions or small molecules [9]. Some chalcone derivatives have also been studied as corrosion inhibitors and functional organic materials, indicating that this class of compounds is not limited to drug-related research but also has value in materials science and applied chemistry [10].

Isoxazoline derivatives are another important class of organic compounds containing a five-membered heterocyclic ring with adjacent nitrogen and oxygen atoms, as illustrated in **Figure 2**. The isoxazoline nucleus is considered a valuable structural motif because it is present in many compounds with reported antibacterial, antifungal, anti-inflammatory, anticancer, antioxidant, insecticidal, acaricidal, and antiparasitic activities [11–13]. The presence of nitrogen and oxygen atoms in the ring contributes to the electronic character of these compounds and may improve their ability to interact with biological targets. For this reason, isoxazoline-containing molecules have become important in medicinal chemistry, agrochemical research, and veterinary medicine [14].

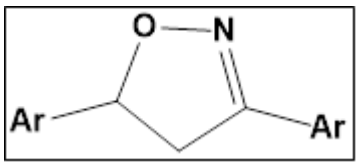

Figure 2 General structure of isoxazoline derivatives**.**

The practical importance of isoxazoline derivatives is clearly observed in the development of modern ectoparasiticidal agents. Several isoxazoline-based compounds have been used in veterinary medicine for the control of fleas and ticks, and this class has shown high relevance in parasite management and animal health [15]. In agrochemical chemistry, the isoxazoline scaffold has also been explored in the development of insecticidal and acaricidal agents, making it an important ring system for pesticide discovery [16]. These applications demonstrate the broad value of isoxazoline derivatives in both medical and non-medical fields.

The combination of chalcone and isoxazoline structural features is therefore of considerable interest. Chalcones provide a reactive and easily modified framework, while isoxazolines introduce a nitrogen–oxygen heterocyclic ring with recognized biological and applied significance. Chalcone-derived isoxazolines have been reported as promising molecules in antimicrobial, antioxidant, antiparasitic, and agrochemical studies [17,18]. Such hybrid structures may offer improved molecular diversity and provide useful platforms for further chemical and biological investigations.

The aim of this study was to synthesize selected aminochalcone derivatives and convert them into the corresponding isoxazoline derivatives using a simple and reliable synthetic route. The prepared compounds were characterized by available spectroscopic techniques, and the main structural features supporting the formation of chalcone and isoxazoline derivatives were discussed.

2. Experimental section

2.1. Reagents and Instruments

All chemicals and solvents were used as received or purified when necessary. The main starting materials included 4-aminoacetophenone, 4-nitrobenzaldehyde, 4-chlorobenzaldehyde, hydroxylamine hydrochloride, sodium hydroxide, ethanol, chloroform, and distilled water. FTIR spectra were recorded on Shimadzu FTIR spectrophotometers. ^{1}H NMR spectra were measured using a Bruker 300 MHz spectrometer in DMSO with TMS as internal standard. UV-Vis spectra were recorded using chloroform as solvent. Melting points were determined using a Gallen Kamp melting point apparatus.

2.2. General Synthetic Route

The synthetic pathway involved two main steps. First, aminochalcones(C_1,C_2) were synthesized by Claisen–Schmidt condensation of 4-aminoacetophenone with substituted benzaldehydes. Second, the prepared chalcones were treated with hydroxylamine hydrochloride in alkaline medium to give the corresponding isoxazoline derivatives (I_1,I_2).

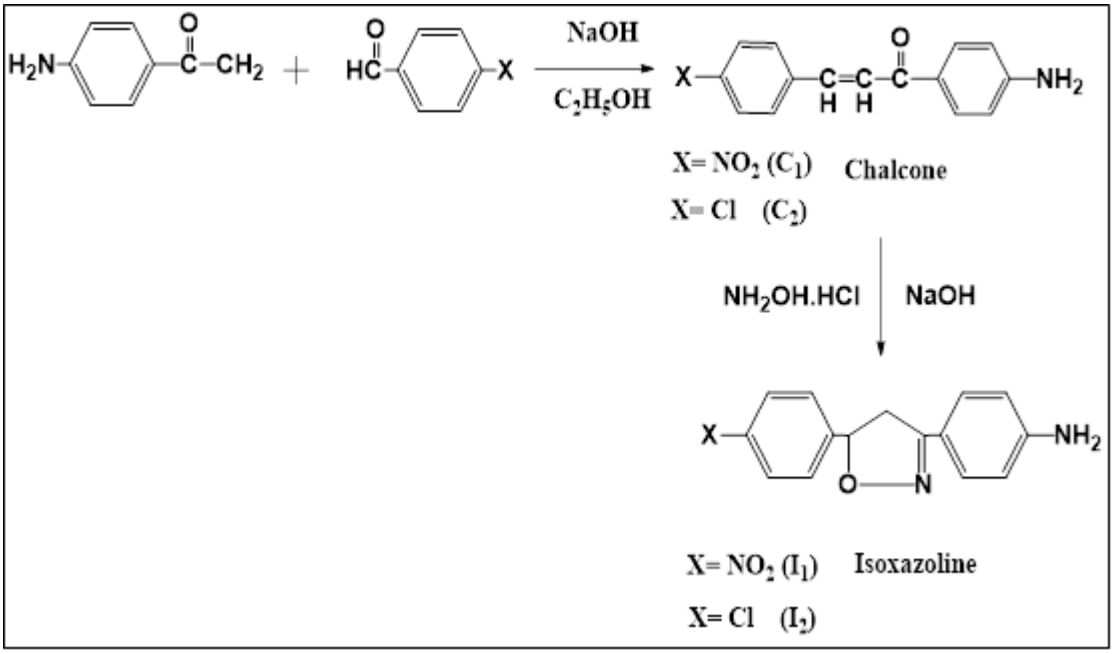

Figure 3 General synthesis of chalcones and isoxazoline derivatives

2.3. Synthesis of Chalcones

Equimolar amounts of 4-aminoacetophenone, 0.01 mol, 1.35 g, and either 4-nitrobenzaldehyde or 4-chlorobenzaldehyde, 0.01 mol, were dissolved in a minimum amount of ethanol. A sodium hydroxide solution, 0.02 mol, was added slowly with cooling. The reaction mixture was then poured slowly into 400 mL of ice water with continuous stirring and kept in a refrigerator for 22 h. The precipitated product was filtered, washed with water, and recrystallized from chloroform to give chalcones (C_1,C_2).

Compound (C_1): 4-[3-(4′-nitrophenyl)-2-propene-1-one]aniline. orange solid; yield: 72%; m.p.: 198 °C; molecular formula: $C_{15}H_{12}N_2O_3$. FT-IR (cm^{-1}): 3273–3483 (NH_2 asym. and sym.), 3105–3119 (=C–H), 1650 (C=O), 1635 (C=C, CH=CH). UV-Vis ($CHCl_3$): λmax = 306 nm. ^{1}H-NMR (DMSO): δ 7.6–8.2 (dd, 8H, aromatic protons), 8.3 (d, 1H, =CHAr), 6.6 (d, 1H, COCH=), 6.24 (s, 2H, NH_2).

Compound (C_2): 4-[3-(4′-chlorophenyl)-2-propene-1-one]aniline. Dark-Yellow solid; yield: 67%; m.p.: 172 °C; molecular formula: $C_{15}H_{12}NOCl$. FT-IR (cm^{-1}): 3273–3483 (NH_2 asym. and sym.), 3105–3119 (=C–H), 1650 (C=O), 1635 (C=C, CH=CH). UV-Vis ($CHCl_3$): λmax = 341.5 nm.

2.4. Synthesis of Isoxazoline Derivatives (I_1,I_2)

A mixture of chalcone C_1 or C_2, 0.02 mol, hydroxylamine hydrochloride, 0.02 mol, 1.39 g, and sodium hydroxide solution prepared from 0.5 g NaOH in 25 mL water was refluxed in 60 mL ethanol for 6 h. After completion of the reaction, the mixture was concentrated under vacuum and poured into ice water. The obtained precipitate was filtered, washed with water, and recrystallized from ethanol to give isoxazoline derivatives (I_1,I_2)

Compound (I_1): 4-[5-(4′-nitrophenyl)-4,5-dihydroisoxazol-3-yl]aniline. brown solid; yield: 43%; m.p.: 215 °C; molecular formula: $C_{15}H_{13}N_3O_3$. FT-IR (cm^{-1}): 3472, 3354 (NH_2 asym. and sym.), 3050 (C–H aromatic), 1628 (C=N endocyclic), 1597 (C=C aromatic), 1072 (C–O cyclic ether), 1504 (p-NO_2 stretching). UV-Vis ($CHCl_3$): λmax = 301.5 nm.

Compound (I_2): 4-[5-(4′-chlorophenyl)-4,5-dihydroisoxazol-3-yl]aniline. yellow solid; yield: 46%; m.p.: 161 °C; molecular formula: $C_{15}H_{13}N_2OCl$. FT-IR (cm^{-1}): 3410, 3325 (NH_2 asym. and sym.), 3050 (C–H aromatic), 1627 (C=N endocyclic), 1601 (C=C aromatic), 1080 (C–O cyclic ether), 1092 (p-Cl stretching). UV-Vis ($CHCl_3$): λmax = 293.5 nm. ^{1}H-

NMR (DMSO): δ 7.2–7.9 (m, 8H, aromatic protons), 6.0–6.3 (d, 1H, CH-isoxazoline), 5.6 (s, 2H, NH_2), 3.7–3.8 (dd, 1H, CH_2-isoxazoline), 3.4 (d, 1H, CH_2-isoxazoline).

2.5. Physical properties and elemental analysis of the synthesized compounds

The physical properties of the synthesized chalcone and isoxazoline derivatives are summarized in Table 1. The prepared compounds were obtained as colored solid products with melting points in the range of 152–230 °C. Chalcone derivatives (C_1,C_2) were obtained in good yields, while their cyclized isoxazoline derivatives (I_1,I_2) were obtained in moderate yields. The molecular formula, melting point, color, yield percentage, and elemental analysis values of carbon, hydrogen, and nitrogen are listed for each compound. The calculated elemental analysis values were consistent with the proposed molecular formulas of the synthesized compounds.

Table 1 Physical properties and elemental analysis of the synthesized chalcone and isoxazoline derivatives.

Comp.	X	Molecular formula	Melting point (°C)	Color	Yield %	C % Calculated (Found)	H % Calculated (Found)	N % Calculated (Found)
C_1	4-NO_2	$C_{15}H_{12}N_2O_3$	198	orange	72	67.16 (67.13)	4.51 (4.48)	10.44 (10.41)
C_2	4-Cl	$C_{15}H_{12}NOCl$	172	Dark Yellow	67	69.91 (69.88)	4.69 (4.66)	5.44 (5.41)
I_1	4-NO_2	$C_{15}H_{13}N_3O_3$	215	brown	43	63.60 (63.57)	4.63 (4.60)	14.83 (14.80)
I_2	4-Cl	$C_{15}H_{13}N_2OCl$	161	yellow	46	66.06 (66.03)	4.81 (4.78)	10.27 (10.24)

2.6. Biological activity study

The antibacterial activity of the synthesized chalcone derivatives [I]a,b and isoxazoline derivatives [II]a,b was evaluated against two pathogenic bacterial strains: Staphylococcus aureus as a Gram-positive bacterium and Escherichia coli as a Gram-negative bacterium. These bacterial strains are commonly used in antimicrobial screening studies because they represent two different bacterial cell wall structures and allow preliminary evaluation of the antibacterial potential of newly synthesized organic compounds [19,20]. The activity was determined using the agar well diffusion method, which is a simple and widely applied technique for estimating the inhibitory effect of compounds by measuring the diameter of the clear inhibition zone formed around each well after incubation [21,22]. Solutions of the synthesized compounds were prepared in dimethyl sulfoxide (DMSO) at concentrations of 25, 50, and 75 mg/mL. DMSO was used as a solvent because it is commonly employed for dissolving synthetic organic compounds in biological assays, while it should also be included as a negative control to ensure that the observed inhibition is related to the tested compounds rather than the solvent [23,24]. The prepared solutions were added into wells formed on agar plates previously inoculated with the tested bacteria. The plates were incubated at 37 °C for 24 h, and the antibacterial effect was evaluated by measuring the inhibition zone diameter in millimeters. Larger inhibition zones indicated stronger antibacterial activity. The obtained results were compared with a standard antibiotic used as a positive control, while DMSO was used as a negative control [25].

3. Results and discussion

3.1. Identification of chalcone compounds (C_1,C_2)

The synthesis of chalcone derivatives (C_1,C_2) involved the reaction between 4-aminoacetophenone and substituted aromatic aldehydes, namely 4-nitrobenzaldehyde or 4-chlorobenzaldehyde, in an alcoholic medium under basic conditions. The reaction was carried out through Claisen–Schmidt condensation using sodium hydroxide as a base, followed by dehydration to afford the corresponding α,β-unsaturated ketone derivatives. The original method reported that equimolar amounts of 4-aminoacetophenone and substituted benzaldehyde were dissolved in ethanol, followed by slow addition of sodium hydroxide solution, then precipitation in ice water and recrystallization from chloroform.

The synthesized chalcone compounds showed physical properties different from the starting materials, confirming the formation of new products. The compounds were obtained as colored solids with melting points of 198 °C for C_1 and 172 °C for C_2. The FTIR spectra of compound C_1, shown in **Figures 4**, exhibited characteristic absorption bands related

to NH_2, C=O, C=C, and aromatic/olefinic C–H groups. The appearance of the carbonyl band at 1650 cm^{-1} and the C=C band at 1635 cm^{-1} confirmed the formation of the chalcone system. The UV-Vis spectra of compounds C_1 and C_2, shown in **Figures 5 and 6**, displayed λmax values at 306 and 341.5 nm, respectively, indicating the presence of conjugated aromatic and α,β-unsaturated carbonyl systems.

Table 2 FTIR and UV-Vis spectral data of synthesized chalcone derivatives

Compound	**X**	**FTIR (KBr), cm^{-1}**	**λmax in $CHCl_3$ (nm)**
C_1	4-NO_2	3273–3483 ν(NH_2 asym., sym.); 3105–3119 ν(=C–H); 1650 ν(C=O); 1635 ν(C=C, CH=CH)	306
C_2	4-Cl	3273–3483 ν(NH_2 asym., sym.); 3105–3119 ν(=C–H); 1650 ν(C=O); 1635 ν(C=C, CH=CH)	341.5

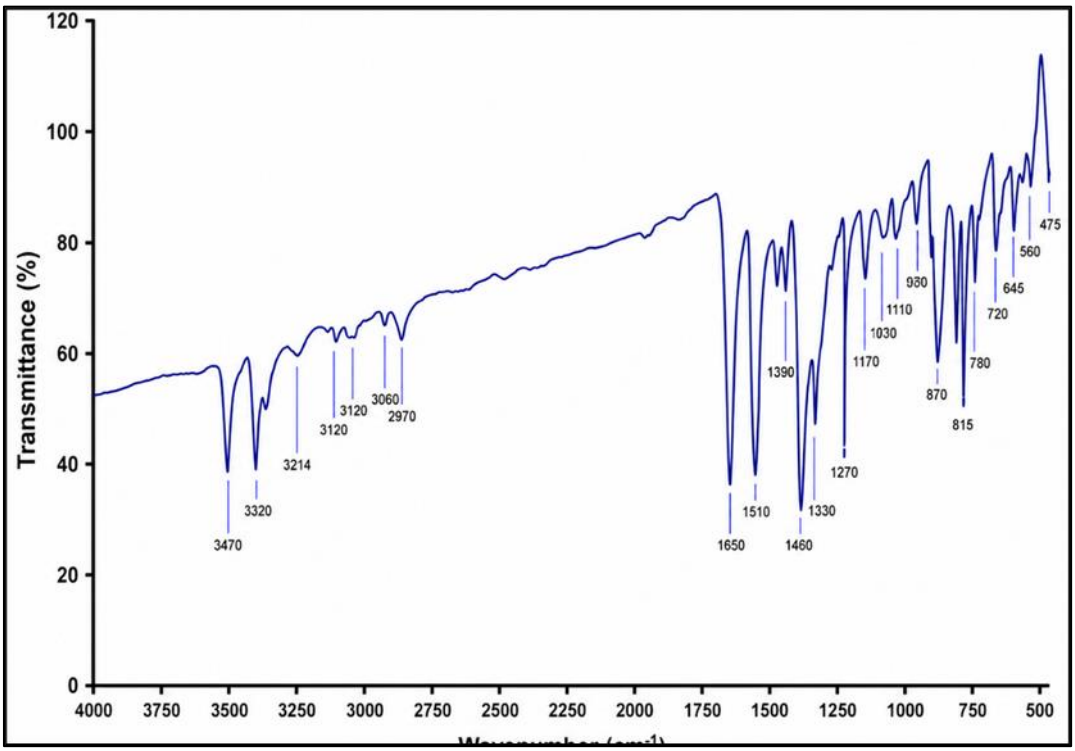

Figure 4 The FTIR spectrum of compound C_1.

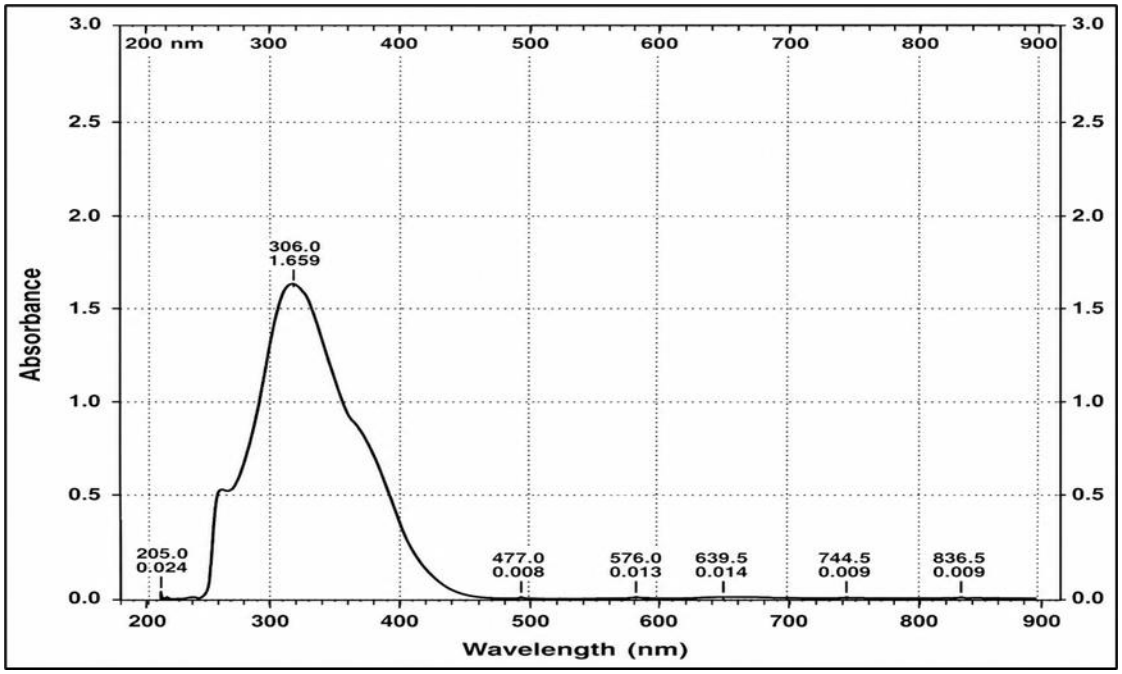

Figure 5 UV-Vis spectrum of chalcone derivative [I]a.

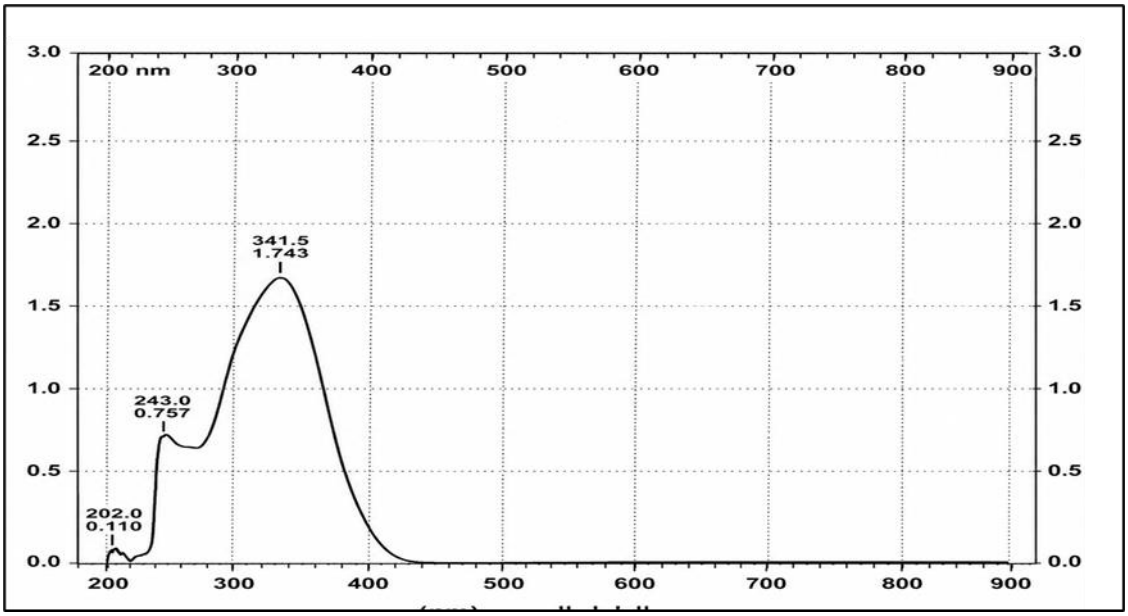

Figure 6 UV-Vis spectrum of chalcone derivative [I]b

The ^{1}H-NMR spectra of chalcone derivatives [I]a,b were recorded using DMSO as solvent, and the spectral data are summarized in Table 3. The spectrum of compound C_1 is shown in **Figure 7.** The spectra showed characteristic signals corresponding to aromatic, olefinic, and amino protons. For compound C_1, the aromatic protons appeared in the region δ 7.6–8.2 ppm, while the olefinic protons appeared at δ 6.6 and 8.3 ppm. The NH_2 protons appeared as a singlet at δ 6.24 ppm. Compound C_2 showed similar spectral behavior, with aromatic proton signals in the region δ 7.2–7.9 ppm, olefinic proton signals at δ 6.5 and 8.1 ppm, and NH_2 protons as a singlet at δ 6.18 ppm. These spectral data supported the formation of chalcone derivatives containing aromatic rings, amino groups, and α,β-unsaturated carbonyl systems.

Table 3 ^{1}H-NMR spectral data of chalcone derivatives

Compound	^{1}H-NMR spectral data, δ ppm
[I]a	6.24 (s, 2H, NH_2); 6.6 (d, 1H, COCH=); 7.6–8.2 (dd, 8H, aromatic protons); 8.3 (d, 1H, =CHAr)
[I]b	6.18 (s, 2H, NH_2); 6.5 (d, 1H, COCH=); 7.2–7.9 (dd, 8H, aromatic protons); 8.1 (d, 1H, =CHAr)

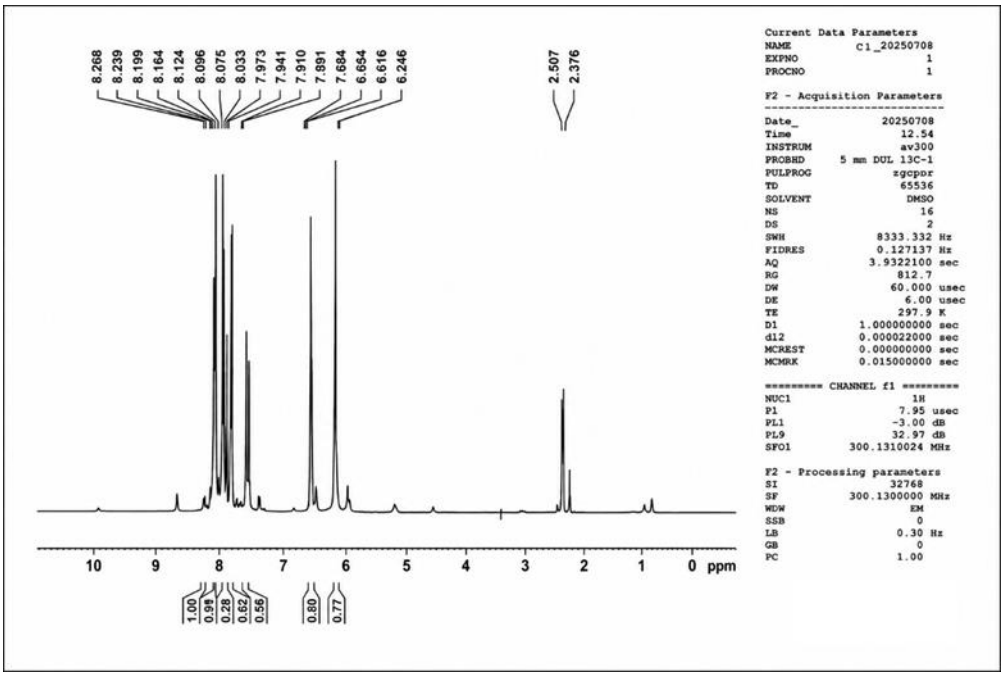

Figure 7 ^{1}H-NMR spectrum of chalcone derivative C_1.

3.2. Identification of isoxazoline compounds

Isoxazoline derivatives (I_1,I_2) were synthesized by cyclization of chalcone derivatives C_1,C_2 with hydroxylamine hydrochloride in alkaline ethanolic medium. The reaction was carried out under reflux for 5 h in the presence of sodium hydroxide. This reaction converts the open-chain α,β-unsaturated carbonyl system of chalcones into a five-membered isoxazoline ring containing nitrogen and oxygen atoms. The experimental method reported the use of chalcone C_1 or C_2, hydroxylamine hydrochloride, and sodium hydroxide solution in ethanol, followed by concentration, precipitation in ice water, filtration, washing, and recrystallization from ethanol.

The FTIR and UV-Vis spectral data of the synthesized isoxazoline derivatives I_1,I_2 are summarized in Table 3. The FTIR spectra of compounds I_1 are shown in **Figures 8**. The most important evidence for cyclization was the disappearance of the C=O and CH=CH bands of the starting chalcones, together with the appearance of new absorption bands for endocyclic C=N and cyclic ether C–O groups. Compound I_1 showed bands at 1628 cm^{-1} for C=N and 1072 cm^{-1} for cyclic C–O, while compound I_2 showed bands at 1627 cm^{-1} and 1080 cm^{-1} for the same groups. The UV-Vis spectra of compounds I_1 and I_2 are shown in Figures 9 and 10, respectively, with λmax values at 301.5 and 293.5 nm, indicating electronic transitions within the aromatic and heterocyclic systems. These spectral changes confirmed the formation of the isoxazoline ring.

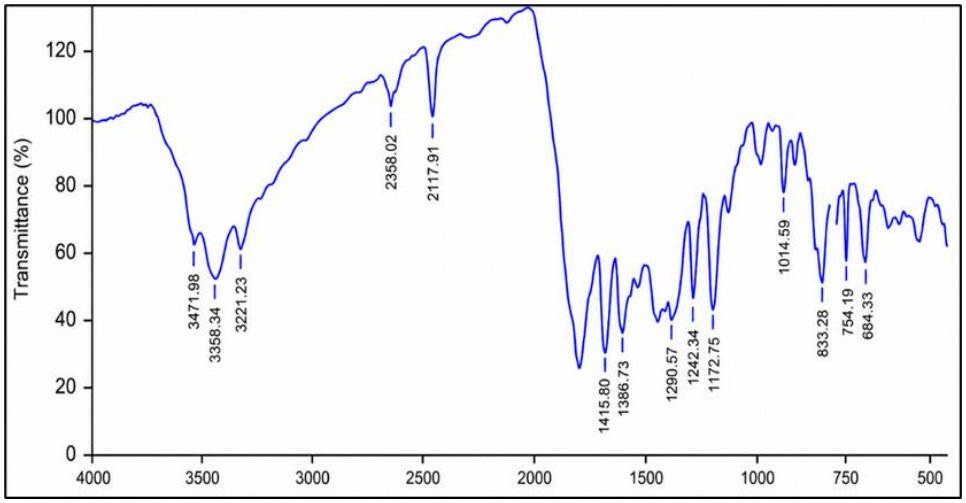

Figure 8 FT-IR spectrum of isoxazoline derivative **I_1**

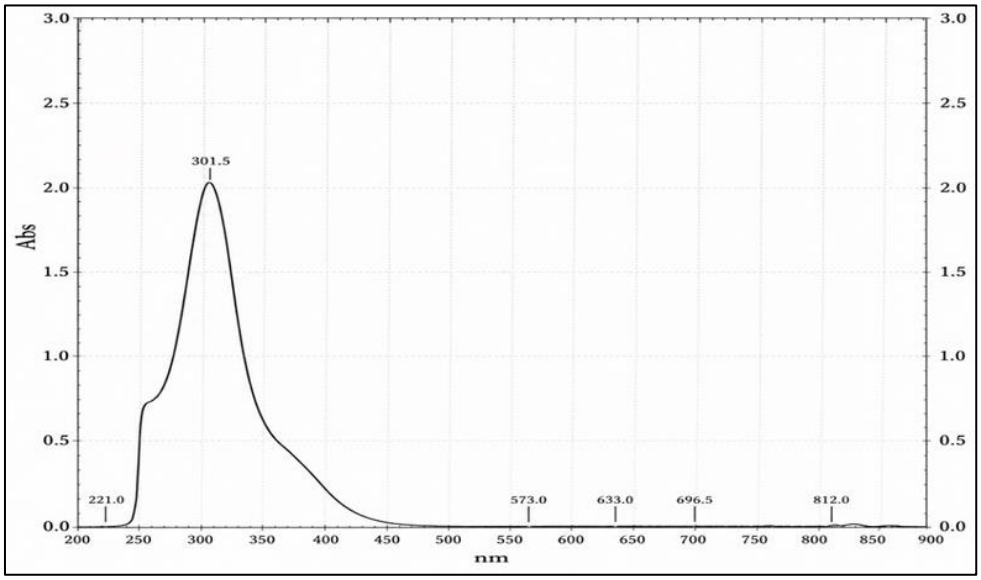

Figure 9 UV-Vis spectrum of isoxazoline derivative I_1

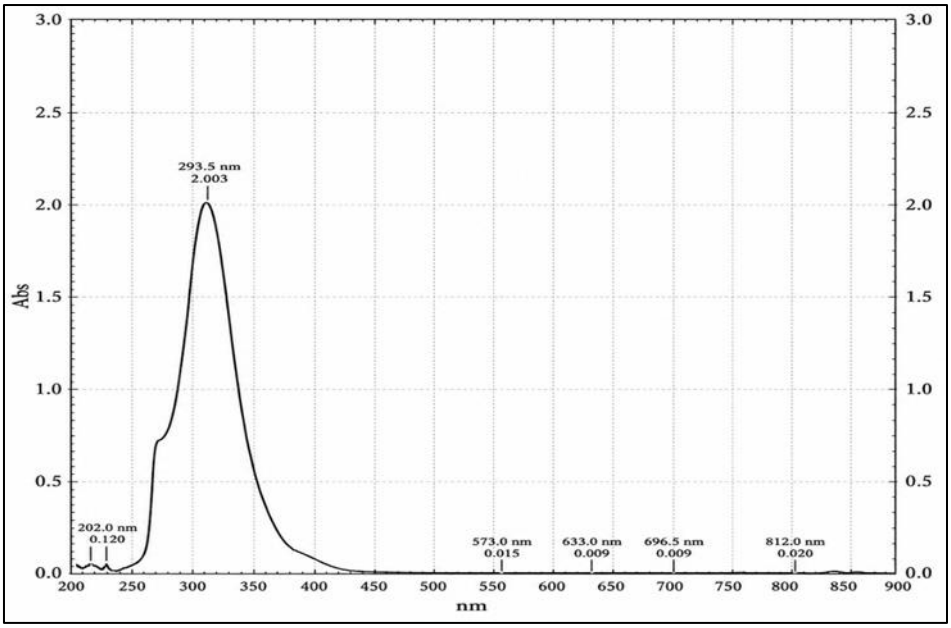

Figure 10 UV-Vis spectrum of isoxazoline derivative I_2

The ^{1}H-NMR spectral data of compounds I_1 and I_2 are summarized in Table 4, while their corresponding spectra are shown in **Figures11**. The spectra showed characteristic signals confirming the formation of the isoxazoline derivatives. The aromatic protons appeared as multiplet signals in the region δ 6.80–8.05 ppm, as listed in Table 4. The disappearance of the olefinic proton signals of the chalcone precursors, together with the appearance of new isoxazoline ring proton signals, confirmed the cyclization reaction. The ^{1}H-NMR spectrum of compound I_2 is presented in Figure 11.

Table 4 ^{1}H-NMR spectral data of compounds.

Compound	**^{1}H-NMR spectral data, δ ppm**
I_1	3.45–3.80 (dd, 2H, CH_2 of isoxazoline ring); 5.10 (d, 1H, CH of isoxazoline ring); 5.40 (br s, 2H, NH_2); 6.85–7.95 (m, 8H, aromatic protons)
I_2	3.50–3.85 (dd, 2H, CH_2 of isoxazoline ring); 3.78 (s, 3H, OCH_3); 5.18 (d, 1H, CH of isoxazoline ring); 5.45 (br s, 2H, NH_2); 6.80–8.05 (m, 8H, aromatic protons)

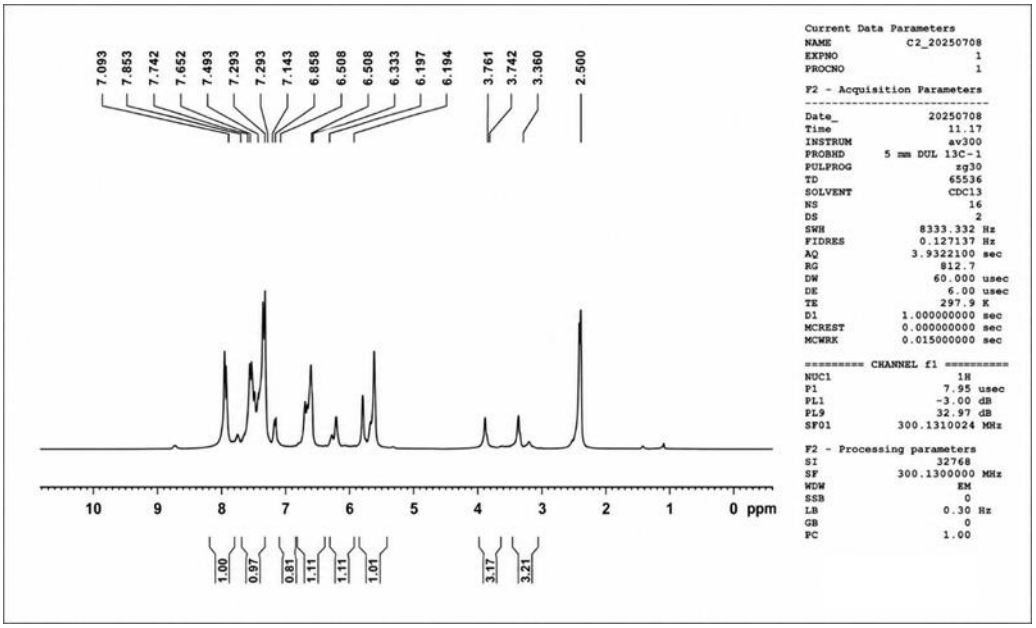

Figure 11 ^{1}H-NMR spectrum of chalcone derivative I_2

3.3. Biological Activity of the Synthesized Compounds

The antibacterial activity of the synthesized compounds I1, C1, I2, and C2 was evaluated against Escherichia coli and Staphylococcus aureus at concentrations of 25, 50, and 75 mg/mL. As shown in Table 5 and Figure 12, the inhibition zones increased with increasing concentration. Compound I1 showed the highest activity among the prepared compounds, especially at 75 mg/mL, while the blank disk showed no inhibition.

Table 5 Biological effectiveness of prepared compounds and control treatments

Comp. No.	***Escherichia coli***			***Staphylococcus aureus***		
Conc. mg/mL	25	50	75	25	50	75
I_1	1	3	6	2	4	7
C_1	0	2	5	1	3	5
I_2	0	1	3	1	2	4
C_2	0	1	2	0	1	3
Amoxicillin	2	4	5	2	5	7
Ampicillin	2	3	5	2	4	6
Ciprofloxacin	1	2	5	2	3	5
Blank disk	0	0	0	0	0	0

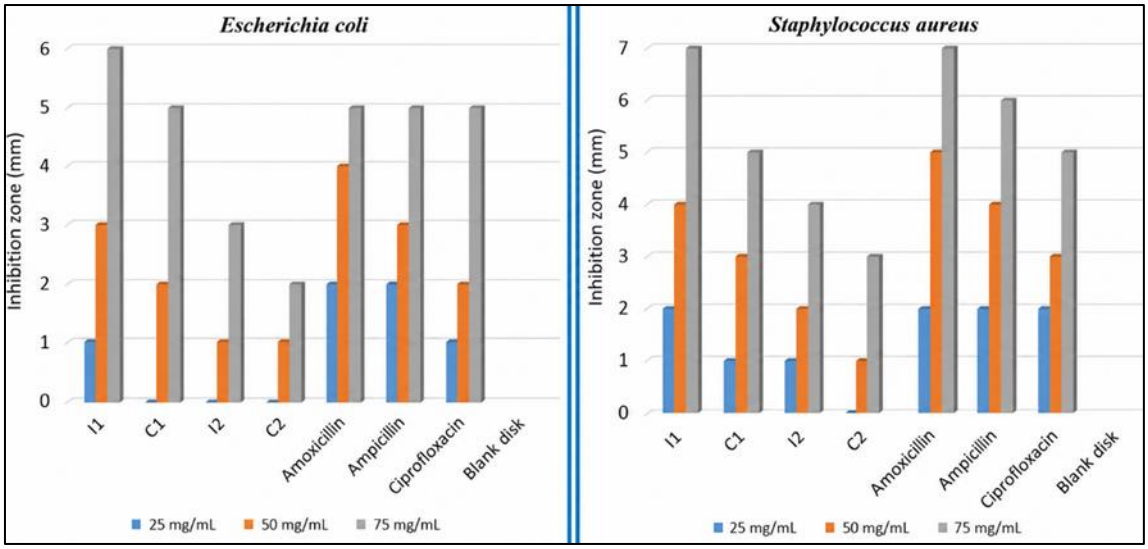

Figure 12 Inhibitory activity values of the prepared compounds against *E. coil* and *S. aureus*

4. Conclusion

In this study, two aminochalcone derivatives and their corresponding isoxazoline derivatives were synthesized and characterized. Chalcones (C_1,C_2) were obtained through Claisen–Schmidt condensation of 4-aminoacetophenone with 4-nitrobenzaldehyde or 4-chlorobenzaldehyde. These chalcones were successfully cyclized with hydroxylamine hydrochloride in alkaline ethanolic medium to give isoxazolines (I_1,I_2). The structures of the synthesized compounds were supported by FTIR, UV-Vis, and ^{1}H NMR spectral data. The disappearance of C=O and CH=CH bands from the chalcone spectra and the appearance of C=N and cyclic C–O bands in the isoxazoline spectra confirmed the formation of the heterocyclic ring. The results demonstrate that aminochalcones are useful intermediates for the synthesis of nitrogen–oxygen-containing heterocyclic compounds.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of competing financial interests.

References

[1] Elkanzi NAA, Hrichi H, Alolayan RA, Derafa W, Zahou FM, Bakr RB. Synthesis of chalcones derivatives and their biological activities: a review. *ACS Omega.* 2022;7:27769–27786.

[2] Dhaliwal JS, Moshawih S, Goh KW, Loy MJ, Hossain MS, Hermansyah A, et al. Pharmacotherapeutics applications and chemistry of chalcone derivatives. *Molecules.* 2022;27:7062.

[3] Mezgebe K, Melaku Y, Mulugeta E. Synthesis and pharmacological activities of chalcone and its derivatives: a review. *ACS Omega.* 2023;8:19194–19211.

[4] Zhuang C, Zhang W, Sheng C, Zhang W, Xing C, Miao Z. Chalcone: a privileged structure in medicinal chemistry. *Chem Rev.* 2017;117:7762–7810.

[5] Albuquerque HMT, Santos CMM, Cavaleiro JAS, Silva AMS. Chalcones as versatile synthons for the synthesis of 5- and 6-membered nitrogen heterocycles. *Curr Org Chem.* 2014;18:2750–2775.

[6] Kotra V, Ganapaty S, Adapa SR. Chalcones: versatile intermediates in heterocyclic synthesis. *Synth Commun.* 2010;40:2479–2511.

[7] Eicher T, Hauptmann S, Speicher A. *The Chemistry of Heterocycles: Structures, Reactions, Synthesis, and Applications.* 3rd ed. Weinheim: Wiley-VCH; 2012.

[8] Mellado M, Madrid A, Peña-Cortés H, López R, Jara-Gutiérrez C. Synthesis of fluorescent chalcones, photophysical properties and applications. *Spectrochim Acta A Mol Biomol Spectrosc.* 2023;292:122427.

[9] Wangngae S, et al. Photophysical study and biological applications of synthetic chalcone-derived fluorescent dyes. *Molecules.* 2021;26:3646.

[10] Menezes RA, et al. Focused review on applications of chalcone-based materials. *Discover Applied Sciences.* 2025.

[11] Ibrahim NK, Ibrahim WA, Farhan MA. Synthesis and pharmacological significance of isoxazoline derivatives: a mini review. *Academic Science Journal.* 2024;2:1–18.

[12] Wang X, Hu Q, Tang H, Pan X. Isoxazole/isoxazoline skeleton in the structural modification of natural products: synthesis and biological activity. *Pharmaceuticals.* 2023;16:228.

[13] Alshamari A, Al-Qudah MA, Hamadeh F, Al-Momani LA, Abu-Orabi ST. Synthesis, antimicrobial and antioxidant activities of 2-isoxazoline derivatives. *Molecules.* 2020;25:4271.

[14] Chen MT, et al. The green and effective synthesis of isoxazole-based derivatives and their biological applications. *Pharmaceuticals.* 2025;18:1179.

[15] Zhou X, Hohman AE, Hsu WH. Current review of isoxazoline ectoparasiticides used in veterinary medicine. *J Vet Pharmacol Ther.* 2022;45:1–15.

[16] Kong L, et al. Isoxazoline: an emerging scaffold in pesticide discovery. *J Agric Food Chem.* 2025.

[17] Al-Rubaye HI, Abbas ZM, Jinzeel NA, Askar FW. Synthesis and preliminary biological study of new chalcone derivatives containing isoxazoline moiety. *J Kufa Chem Sci.* 2024;3:304–319.

[18] Salim I. New 2-isoxazoline derivatives containing furan moieties synthesized from chalcones and evaluation of antimicrobial activity. *J Turk Chem Soc Sect A Chem.* 2024;11:1–10.

[19] Nawaz T, et al. Synthesis, antibacterial, antibiofilm, and docking studies of chalcone derivatives. *BMC Chem.* 2024.

[20] Salim I. New 2-isoxazoline derivatives containing furan moieties synthesized from chalcones and evaluation of antimicrobial activity. *J Turk Chem Soc Sect A Chem.* 2024;11:1–10.

[21] Gajic I, Kabic J, Kekic D, Jovicevic M, Milenkovic M, Mitic-Culafic D, et al. Antimicrobial susceptibility testing: a comprehensive review of currently used methods. *Antibiotics.* 2022;11:427.

[22] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing.* 33rd ed. Wayne, PA: CLSI; 2023. CLSI supplement M100.

[23] Summer K, Browning DF, Busby SJW. Out of control: the need for standardised solvent approaches in antimicrobial assays. *J Antimicrob Chemother.* 2022;77:2461–2468.

[24] Da Silva GN, et al. Standardization of the safety level of the use of DMSO in biological assays. *Braz J Biol Sci.* 2016.

[25] Alshamari A, Al-Qudah MA, Hamadeh F, Al-Momani LA, Abu-Orabi ST. Synthesis, antimicrobial and antioxidant activities of 2-isoxazoline derivatives. *Molecules.* 2020;25:4271.