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Research Article

BIOCHEMICAL STUDY OF NEW CHALCONE BASED ON CHROMONYL THIADIAZOLINE DERIVATIVE

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ABSTRACT

Objectives: This study aimed to synthesize and characterize a novel chalcone compound (ZH) derived from chromonyl thiadiazoline and to assess its biological activities, including antibacterial, antioxidant, and anticancer properties, as well as its fluorescence characteristics and DNA cleavage capability.

Methods: The ZH molecule was synthesized and characterized structurally by elemental analysis, Fourier transform infrared (FT-IR) spectroscopy, multinuclear nuclear magnetic resonance (^{1}H, ^{13}C), and mass spectrometry. The acute toxicity was evaluated using Dixon's up-and-down approach to ascertain the lethal dose 50% (LD_{50}) value. The antibacterial efficacy was assessed using the minimum inhibitory concentration (MIC) method against Gram-positive bacteria (*Streptococcus aureus* and *Bacillus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The antioxidant activity was evaluated relative to the standard antioxidant butylated hydroxytoluene (BHT). The cytotoxic effect on MCF-7 breast cancer cells was assessed using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) test after 72 h of treatment. The compound's fluorescence characteristics and DNA cleavage activities were examined.

Results: The ZH compound demonstrated greater toxicity than its counterpart (2), as evidenced by a reduced LD_{50} value. It exhibited significant antibacterial efficacy, particularly against Gram-positive bacteria, with a MIC of 5 mg/mL. While ZH had notable antioxidant action, it was inferior to BHT (82%). The MTT assay demonstrated substantial cytotoxicity against MCF-7 cells, yielding an IC_{50} value of 45.54 µg/mL, which is markedly lower than that of the reference medication 5-Fluorouracil 5-FU (IC_{50} = 98.17 µg/mL). The molecule demonstrated significant fluorescence and efficient DNA cleavage activity.

Conclusion: The synthesized ZH molecule has considerable bioactive properties, notably its antibacterial and anticancer efficacy, indicating substantial pharmacological potential. These findings underscore its significance for forthcoming advancements in medicinal chemistry and pharmaceutical design.

Keywords: Acute toxicity, Antioxidant action, Chalcone, DNA cleavage, MTT viability assay, Thiadiazoline.

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INTRODUCTION

1,3,4-Thiadiazole is a significant five-membered heterocyclic compound, including two nitrogen atoms and one sulfur atom. Initially delineated in 1882 by Fischer, it was further advanced by Busch *et al*. The introduction of sulfur medicines and the subsequent discovery of mesoionic chemicals significantly expedited advancements in this domain [1]. 1,3,4-Thiadiazoles were systematically categorized into three subclasses: aromatic systems, which encompass the neutral thiadiazole; mesoionic systems, characterized as five-membered heterocycles that are neither covalent nor polar and contain a sextet of electrons associated with the five atoms forming the ring; and non-aromatic systems, including 1,3,4-thiadiazolines and tetrahydro-1,3,4-thiadiazolidines [2]. Thiadiazoline was synthesized through the reaction of aldehydes or ketones with semicarbazones or hydrazones [3]. Moreover, the thiadiazoline nucleus, characterized by a toxophoric (-N=C-S-) group, serves as a fundamental structural element in various categories of pharmaceuticals [4]. Thiadiazoline moieties are recognized for their role as hydrogen bonding domains, two-electron donor systems, and restricted pharmacophores [5]. The interest in 1,3,4-thiadiazole derivative preparation stems from the fact that they are used in the synthesis of heterocycles because of their broad variety of potential applications in materials science, pharmaceuticals, and agriculture [4,6]. The disubstituted 1,3,4-thiadiazoline exhibits pharmacological and multifunctional properties; for example, ligands containing electron-donating atoms such as nitrogen and sulfur can form complexes with a diverse array of transition metals [7]. 1,3,4-thiadiazoline derivatives participate in several reactions. They function as 1,3-dipoles or as a sulfur source, hence garnering significant interest in the synthesis of various organosulfur compounds [5,8].

Chalcones are chemicals characterized by the generic formula 1,3-diaryl-2-propen-1-one, synthesized through the condensation of aldehydes or ketones with a moiety possessing a terminal α-hydrogen. This reaction transpires through aldol condensation [9-11] in either a basic or acidic environment, yielding β-hydroxy ketones or β-hydroxy aldehydes. The intermediates rapidly eliminate a water molecule, resulting in the synthesis of α,β-unsaturated ketones or α,β-unsaturated aldehydes, respectively. A prevalent approach for synthesizing chalcones involves aldol condensation between benzaldehyde and acetophenone derivatives, utilizing a base as a catalyst. Egger and Schlogl; the researchers who synthesized the chalcones [12]. These chemicals are also known as benzyl acetophenone or benzylidene acetophenone. The Claisen–Schmidt condensation by enolate production is a pivotal technique for the synthesis of chalcones. This entails the reaction of equimolar amounts of ketones with a terminal α-hydrogen (*e.g.*, acetophenone or its derivatives) with aromatic aldehydes in the presence of an alcoholic basic solution, such as NaOH/EtOH [13]. The carbonyl carbon of the aldehyde attacks the ketone,

resulting in the formation of the intermediate enolate. Thereafter, the enolate eliminates a water molecule, producing the equivalent chalcone chemical.

Chalcones exhibit extensive medicinal and pharmacological applications owing to their significant biological activity. They serve as anti-inflammatory agents, antibacterials, antivirals, antifungals, antioxidants, anticancer agents, and therapies for malaria. Moreover, chalcones contribute to the regulation of blood glucose levels and cholesterol levels and function as antihypertensive agents [14-17].

In light of the potent pharmacological effects of chalcone derivatives, this study aims to explore their therapeutic potential. We have synthesized a novel chromonyl chalcone derivative using Claisen–Schmidt condensation of a Δ^2-1,3,4-thiadiazoline derivative with benzaldehyde. The compound's *in vivo* acute toxicity, antioxidant, antibacterial, DNA cleavage, anticancer, and fluorescence properties were also investigated.

EXPERIMENTAL SECTION

Materials

All the chemicals and solvents used were of analytical grade and supplied by BDH, Fluka, USP, Merck, and Aldrich. Thiosemicarbazide, 6-chloro-3-formylchromone, benzaldehyde, and glacial acetic acid, as well as butylated hydroxytoluene (BHT), were obtained from Sigma-Aldrich. Acetic anhydride, sodium hydroxide, ethanol, methanol, ethyl acetate, hydrochloric acid, n-hexane, benzene, and *β*-carotene were supplied from BDH and USP, respectively. Tween-20 (polyoxyethylene [20] sorbitan monolaurate) and linoleic acid were obtained from Fluka. Thin-layer chromatography (TLC) was carried out using an aluminum sheet coated with silica gel $60F_{254}$ (Merck), and iodine and ultraviolet (UV) light were used for visualizing TLC plates.

Instrumentation

The Fourier transform infrared (FT-IR) spectra of KBr discs were recorded in the range 4000–400 cm^{-1} using the Shimadzu FT-IR model 8400s instrument. The experimental values of ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra for the studied compounds were done in a Bruker spectrophotometer (500 and 75 MHZ, respectively) and using DMSO-d_6 as a solvent and TMS as an internal standard (Central Laboratory, University of Tehran, Iran). The mass spectra were measured by the EI technique at 70 eV using an Agilent Technologies 5975C spectrometer. Elemental analysis (C, H, N, S) was measured using CHNS-932 LECO Apparatus. Melting points were measured with a Bauchi 510 melting point apparatus and were uncorrected.

Synthesis

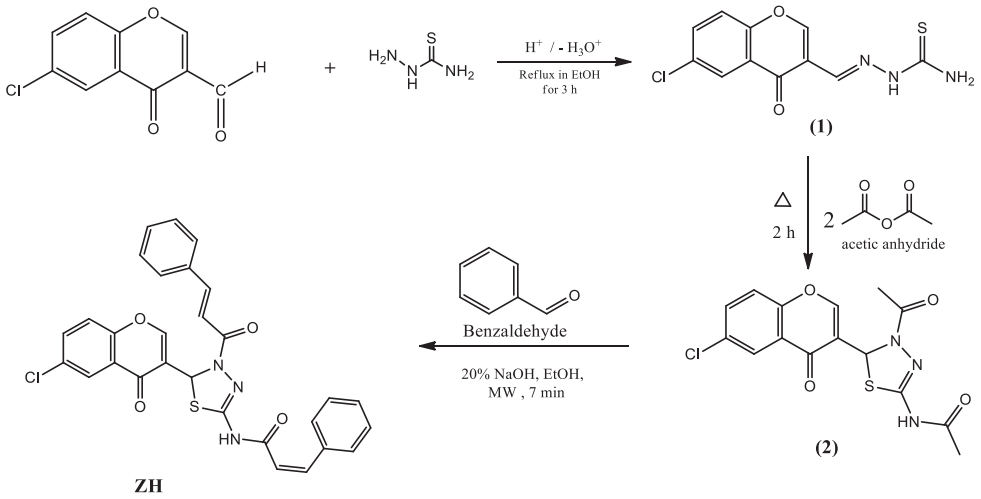
The compounds 2-((6-chloro-4-oxo-4H-chromen-3-yl)methylene) hydrazinecarbothioamide (**1**) and N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (**2**) were prepared and characterized as previously described in the literature [18]. This compound gave satisfactory elemental analysis and spectroscopic data and they are not reported. The synthetic procedure for the preparation of compounds **(1** and**2)** is presented in Scheme 1.

Synthesis of N-(5-(6-chloro-4-oxo-4H-chromen-3-yl)-4-cinnamoyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)-3-phenylacrylamide (ZH)

A mixture of compound **(2)** (0.5 mmol) and (1 mmol) of benzaldehyde with 20% NaOH (40 mg, 1.00 mmol) in ETOH (10 mL) was taken, and the mixture was stirred under microwave irradiation for 6–8 min. The reaction progress was monitored by TLC using (n-hexane: ethylacetate) (2:8). After completion of the reaction by stating materials spots disappeared, the mixture was poured onto ice–cold water and neutralized with diluted HCl. The solid compound obtained was filtered, washed several times with water, dried, and recrystallized from ethanol [19]. The synthetic procedures for the preparation of compound (ZH) are presented in Scheme 1.

N-(5-(6-chloro-4-oxo-4H-chromen-3-yl)-4-cinnamoyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)-3-phenylacrylamide (ZH)

Off-white powder; yield: 72%; Rf: 0.6; m.p: 177-178°C; Elemental Analysis for $C_{29}H_{20}ClN_3O_4S$ (542.00g/mol); Calcd: C, 64.26; H, 3.72; N, 7.75; S, 5.92. Found: C, 64.23; H, 3.77; N, 7.79; S, 5.94. IR (KBr) cm^{-1}: 3061ν(N-H),1622ν(C=O, Chromone ring),1594-1548 ν(C=O, chalcone groups), 1548 ν(C=C, chalcone groups), 1594ν(C=N, thiadiazoline ring), 1400–1394 ν(C=C),1138 ν(N-N, thiadiazoline ring), 1067 ν(C-O), 828 ν(C-Cl), 700$\nu_{str.}$(C-S-C, Asymmetrical),513$\nu_{str.}$(C-S-C, Symmetrical); ^{1}HNMR (500 MHz, DMSO-d_6)(δ/ppm): 11.78 (s, 1H, H-5), 8.025 (d, 1H, *J* = 5 Hz, H-33), 7.90 (d, 2H, *J* = 10 Hz, H-31), 7.77 (d, 1H, *J* = 10 Hz, H-34),7.70 (d, 4H, *J* = 10 Hz, H-7, H-11, H-22, H-26), 7.60 (d, 1H, *J* = 10 Hz, H-19), 7.51 (d, 1H, *J* = 10 Hz, H-3),7.39 (t, 4H, J_1= 5, J_2 = 10 Hz, H-23, H-25, H-8, H-10), 7.34 (t, 2H, J_1 = 5, J_2 = 10 Hz, H-9, H-24), 7.22 (d, 1H, *J* = 15 Hz, H-18), 6.81 (d, 1H, *J* = 10 Hz, H-2), 6.64 (s, 1H, H-16); ^{13}CNMR (500 MHz, DMSO-d_6) (δ/ppm): 175.00 (1C, C-29), 169.97 (1C, C-17), 168.49 (1C, C-1), 154.07 (2C, C-27, C-35), 151.97 (1C, C-13), 141.05 (2C, C-3, C-19), 135.09 (1C, C-21), 134.77 (1C, C-6), 133.91 (1C, C-33), 132.33 (3C, C-9, C-24, C-32), 130.81 (4C, C-23, C-25, C-8, C-10), 130.02 (2C, C-22, C-26), 128.63 (2C, C-7, C-11), 124.58 (1C, C-31), 123.94 (1C, C-28), 122.10 (1C, C-30), 120.73 (1C, C-2), 120.06 (1C, C-18), 117.74 (1C, C-34), 60.25 (1C, C-16); The EI-MS m/z (%): 542 $[M]^+$ (2), 412 $C_{20}H_{15}ClN_3O_3S^+$ (1), 279 $[C_{11}H_6ClN_3O_2S]^{\bullet+}$ (30), 213$[C_8H_{11}N_3O_2S]^{\bullet+}$ (3), 206$[C_{10}H_{10}N_2OS]^{\bullet+}$ (13), 180 $[C_9H_5ClO_2]^{\bullet+}$ (7), 154 $[C_7H_3ClO_2]^{\bullet+}$ (15), 126 $C_6H_4ClO^+$(12), 115 $C_5H_8NS^+$ (18),80 $[C_5H_4O]^{\bullet+}$ (100).



Scheme 1: Synthesis of chromonyl chalcone derivative using NaOH

Biological activity

Acute toxicity (lethal dose 50% [LD_{50}])

Healthy albino mice of either sex (male and female), aged from 7 to 9 weeks and whose body weight ranged between 23 and 33 g, were used to study the acute toxicity of chalcone derivative (ZH). The animals were injected intraperitoneally with the first dose of 500 mg/kg. The result was read as death X or life O after 24 h, and increases or decreases in the amount of dose were constant at 50 mg/kg and repeat dosing up or down for 4 mice after changing the resulting death to life and versa. LD_{50} was calculated based on the diagram and equation of Dixon (LD_{50} = Xf +Kd), where Xf is the last dose, K is the interval between dose levels, and d is the tabulated value (Table 1) [20].

Antibacterial activity

The compound's (ZH) antibacterial properties were examined *in vitro*. The panel of pathogens included *Staphylococcus aureus* and *Bacillus* as Gram-positive bacteria and *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria, utilizing the agar diffusion method [21]. The antibiotic tetracycline was utilized for calibration and comparison with the antibacterial agents. 0.2 mL of bacterial inoculum was evenly distributed using a sterile cotton swab over a sterile Petri dish containing Mueller–Hinton agar. The studied chemicals and tetracycline were dissolved in DMSO at doses of 1, 5, 25, 125, 250, and 500 mg/mL for compound ZH. Fifty microliters of compound ZH and tetracycline at dosages ranging from 1 to 500 mg/mL were introduced into each well (7 mm diameter holes punched in the agar gel, spaced 20 mm apart). The plates were incubated for 24 h at 36°C±1°C under erobic conditions. Following incubation, extensive bacterial proliferation was noted. The inhibition of bacterial growth was quantified in millimeters. Furthermore, the minimal inhibitory concentration (MIC) of the chemical was determined, which corresponds to the lowest concentration at which no bacterial growth was observed [22]. The MIC was documented due to the minimal concentration at which no discernible growth was evident.

Antioxidant activity (AA)

The *β*-carotene bleaching method was used to assess the chalcone derivative's (ZH) AA [23]. The *β*-carotene bleaching method relies on the disappearance of *β*-carotene's yellow hue due to its interaction with radicals generated from linoleic acid oxidation in an emulsion, consistent with prior methodologies. A *β*-carotene solution was formulated by dissolving 0.01 g of *β*-carotene in 50 mL of chloroform. Subsequently, 1 mL of this solution was transferred into a round-bottom rotating flask containing 0.02 mL of linoleic acid and 0.2 mL of Tween-20. Following the removal of chloroform using vacuum evaporation with a rotary evaporator at ambient temperature, 50 ml of distilled water was introduced into the flask with manual agitation as the initial stage. 3.8 mL of the emulsion was added to tubes containing 0.2 mL of the compound (ZH) and the reference compound (BHT), which was made by dissolving 0.01 g of BHT in 0.2 mL of DMSO. The absorbance was measured at 470 nm, and the samples were subsequently exposed to thermal autoxidation at 45°C in a water bath for 2 h. Absorbance was recorded at 15-min intervals. The AA was determined as a percentage of inhibition compared to the control, utilizing the equation [%AA = 1 - [(Ai – At)/(Ai* – At*)] × 100] where Ai represents the absorbance value of the sample measured at time zero. At denotes the absorbance value of the sample measured after incubation for 105 min at 45°C. Ai* represents the absorbance value of the control at time zero. At* denotes the absorbance value of the control determined after a 105-min incubation at 45°C.

Table 1: The tabulated Dixon values

Doses range	K represented serial tests started with				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
No. of dead mice*	X	XX	XXX	XXXX	

The symbols X and O represented the count of deceased and surviving mice, respectively, after each dosage, subsequently employing Dixon's values to compute the LD 50 .

Anti-breast cancer activity

3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) cellular viability test *in vitro*

The cytotoxicity of the sample on the MCF-7 cell line was assessed using the MTT cell viability assay [24]. Cells were seeded at a density of 1 × 10^4 cells/mL (100 µL/well) in 96-well plates and incubated overnight in a 5% CO_2 atmosphere at 37°C. Subsequently, they were exposed to varying concentrations (6.25, 12.5, 25, 50, 75, and 100 µg/mL) of the tested compound (ZH) alongside 5-Fluorouracil (5-FU) as a reference drug. A group consisting solely of culture medium was designated as the blank control. Each group consisted of three biological replicates. Following a 72-h dosing period, the cells were washed, and fresh medium (100 µL) containing 28 µL of a 2 mg/mL MTT solution was added to each well. Following a 2-h incubation in the dark at 37°C, the MTT solution was removed, and the remaining crystals in the wells were solubilized by adding 100 µL of DMSO, followed by a 15-min incubation at 37°C with shaking [25]. The optical density at 620 nm (OD620) for each well was quantified using a plate reader (Synergy H4: Bio-Tek, Winooski, VT, USA). The results are expressed as mean±standard deviation (SD). The survival rate of control cells treated with 0 M of the tested compound was 100%. Cell viability was determined using the equation: cell viability (%) = [(dosing cell OD – blank OD)/(control cell OD – blank OD)] × 100.

Acridine orange/ethidium bromide (AO/EB) staining

Morphological apoptosis of MCF-7 cells treated with different concentrations of the newly prepared compound (ZH) and standard (5-FU) was assessed using an AO/EB staining kit (Solarbio, Beijing, China, Cat No. CA1140). The density of 1 × 10^4 MCF-7 cells/mL was plated in 6-well plates (1 mL/well) and incubated overnight. The medium was replaced with the tested compound-containing (6.25, 12.5, 25, 50, 75, and 100 µg/mL) medium and incubated for 48 h under the same conditions mentioned before. Cells were washed with phosphate-buffered saline (PBS) and stained with AO/EB solution (20 µL AO/EB freshly mixed solution of equal volume in 1 mL PBS) for 2–3 min in the dark. After the successive washes, the fluorescent images were taken with an inverted fluorescence microscope (Olympus Corporation, Beijing, China) [26].

Flow cytometry

This method was conducted according to Zini and Agarwal [27] to estimate the effect of the selected compound (ZH) on breast cancer cell lines, as the AO detected % DNA fragmentation index (% DFI) using flow cytometry assay. MCF-7 breast cancer cell lines (2 × 10^5 cells/mL) were cultivated in Roswell Park Memorial Institute media containing 20% fetal bovine serum + insulin at 10 mL/petri dish. Upon the formation of a monolayer of cells, 100 µL of concentration (100 µg/mL) for each selected compound was added. After 24 h of incubation, cells were harvested by the addition of trypsin, centrifuged for 5 min at 1000 × g, and finally washed with PBS. Cells were stained according to the protocol and were analyzed. The sample was incubated and analyzed using a Calibur flow cytometer. The cell Quest software and MOdFit software were used to determine (% DFI). In this study, the negative control (DMSO) was also maintained against the positive control (5-FU). The determinations were performed in duplicates.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance to calculate the probability (p-value) with the Statistical Package for the Social Sciences (SPSS software version 21, IBM Corporation,

New York, USA). The half-maximum inhibitory concentration (IC_{50}) was determined by plotting cell viability (%) against drug concentration. The acquired values were expressed as mean±standard deviation. Values of $p<0.05$ and $p<0.01$ were deemed statistically significant.

RESULTS AND DISCUSSION

The chromonyl chalcone derivative (ZH) was synthesized using a Claisen–Schmidt reaction between a Δ^2-1,3,4-thiadiazoline derivative and benzaldehyde. The synthesized chalcone (ZH) is a solid chemical that often melts upon decomposition. Chalcone compounds exhibit stability in atmospheric conditions and are soluble in a majority of polar solvents. Scheme 2 illustrates the proposed mechanism for the base-catalyzed Claisen–Schmidt condensation reaction to synthesize chalcone chemicals [28]. The acetyl group of N-(4-acetyl-5-(6-chloro-4-oxo-4H-chromen-3-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide undergoes condensation with the carbonyl group of benzaldehyde in this reaction.

Spectroscopic analysis

Spectral studies, including the observed spectroscopic results for the title compounds, are discussed. The synthesized compound (ZH) gave a spectroscopic analysis consistent with the empirical structure.

Infrared spectra (FT-IR)

The infrared spectrum shows the position and the intensities of the peaks, which correspond to various groups present in the compound. The infrared of the prepared compound (**ZH**) shows a characteristic bond at 1622 cm^{-1} that can be attributed to the stretching vibration of the chromone ring carbonyl ν(C=O) [29]. In addition, the weak band at 3061 cm^{-1} can correspond to the ν(N-H) stretching vibration. The absorption bands around 1594–1548 cm^{-1} are related to the carbonyl group that conjugates with a double bond, which indicates the formation of chalcone [30,31]. The weak band at 1138 cm^{-1} can correspond to the ν(N-N) stretching vibration [18]. Furthermore, the spectrum was distinguished by the appearance of distinct absorption bands for ν(C-S-C) at the range 700 cm^{-1} and 513 cm^{-1}, which were assigned to asymmetrical and symmetrical stretching vibration, respectively, for the compound (**ZH**) [18]. The absorption band appears at 828 cm^{-1} and belongs to ν(C-Cl) stretching vibration [29] (Fig. 1).

^{1}HNMR and ^{13}CNMR spectra

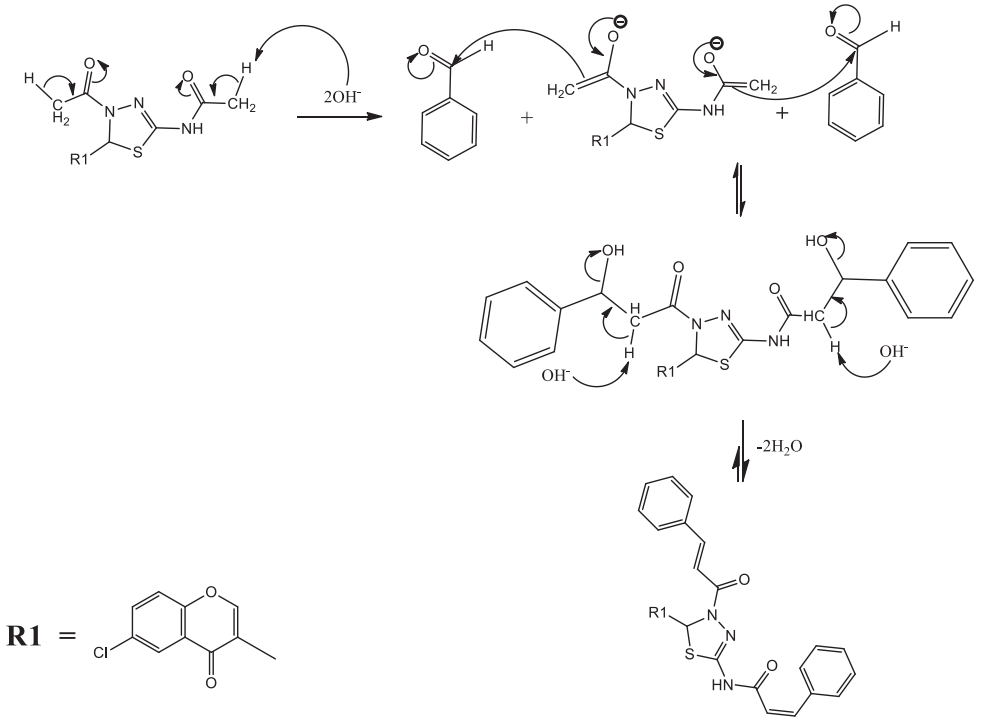
The ^{1}HNMR spectrum of compound (ZH) shows a singlet signal at δ (6.64) ppm, which is attributed to the H-16 proton of the thiadiazoline ring [32]. Furthermore, the compound (ZH) shows a singlet signal at δ (11.78) ppm, which is attributed to the NH proton [18]. The compound is characterized by showing four doublet signals at δ (6.81) ppm, δ (7.22) ppm, δ (7.51) ppm, and δ (7.60) ppm, which can be assigned to the double bond of the chalcone groups, respectively [31]. Furthermore, multiple signals that appear at δ (7.70–7.34) ppm can be attributed to the aromatic rings of the studied compound. Furthermore, the compound is characterized by showing doublet signals in the range δ (7.77–8.025) ppm, which can be assigned to double bonds of the chromone ring. Therefore, the ^{1}HNMR result supports the formation of chromonyl chalcone derivative (ZH) [29,30]. The spectrum of the compound (ZH) is shown in Fig. 2.

The ^{13}C-NMR spectrum of chromonyl chalcone derivative (ZH) shows signals at δ (168.49) ppm, δ (169.97) ppm, and signal at δ (175.00) ppm, which attribute to carbonyl carbon (C=O) of the chalcone groups and chromone ring, respectively [31,33]. Furthermore, the studied compound shows a signal at δ (151.97) ppm of the (C=N) carbon, and a signal appears at δ (60.25) ppm, which is attributed to the C-S carbon signal. Furthermore, the spectrum exhibited three signals at δ (120.06) ppm, δ (120.73) ppm, and δ (141.05) ppm, which can be assigned to double bonds (-CH=CH-CO), which indicates the formation of chalcone groups [31]. Furthermore, the signals of aromatic carbons of this synthesized compound are represented at δ (122.10–135.09) ppm. The spectrum of the compound (ZH) is shown in Fig. 3.

Electron ionization-mass

The mass spectrum of the studied compound detects the molecular ion peaks $[M]^+$, which are in excellent agreement with the suggested structure. Scheme 3 shows the potential suggested ion fragments with the appearance of the result of fragmentation of this synthesized compound ZH. Furthermore, the peak intensity gives an idea about the stability of fragments, primarily with the base peaks.

The mass spectrum of the compound (ZH) shows several fragment peaks at m/z 412, m/z 279, m/z 213, and m/z 206, and these peaks



Scheme 2: Mechanism of base-catalyzed Claisen–Schmidt condensation

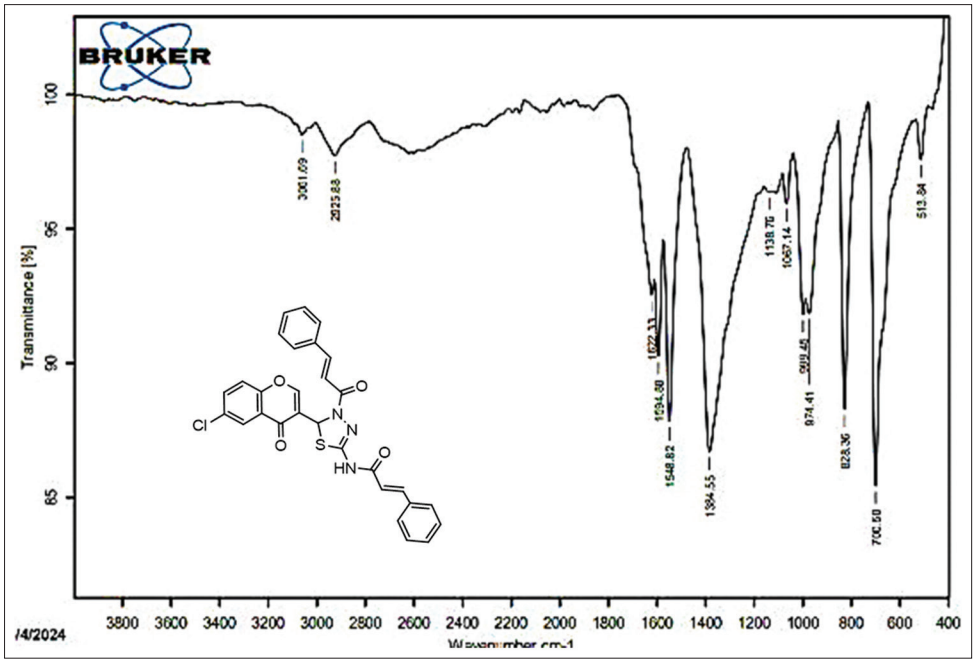


Fig. 1: Fourier transform infrared spectrum of compound (ZH)

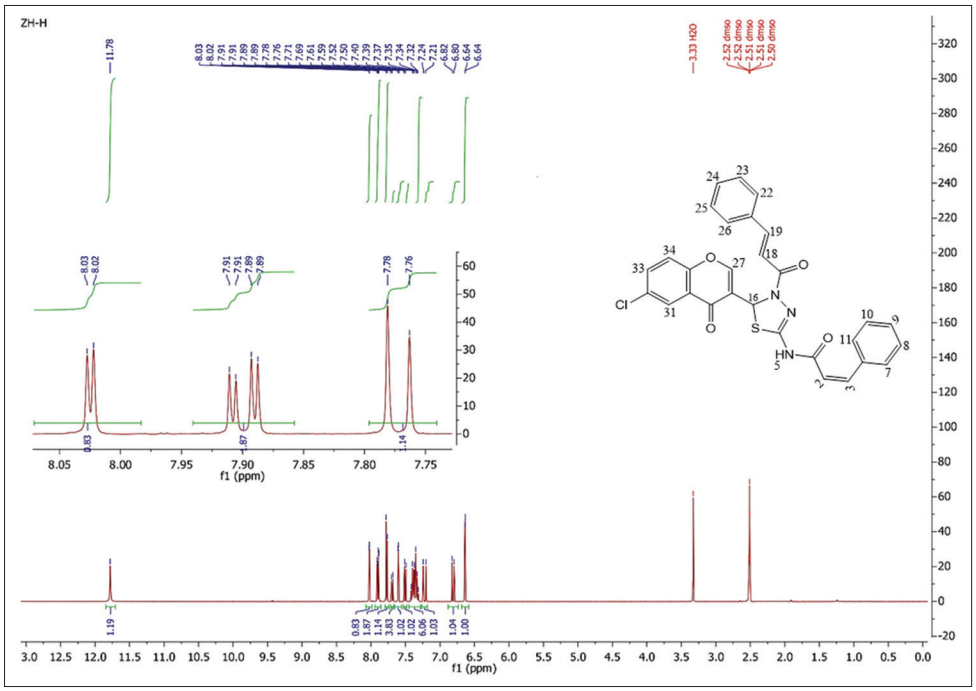


Fig. 2: ^{1}HNMR spectrum of compound (ZH)

can be assigned to $C_{20}H_{15}ClN_3O_3S^+$, $[C_{11}H_6ClN_3O_2S]^{\bullet+}$, $[C_8H_{11}N_3O_2S]^{\bullet+}$, and $[C_{10}H_{10}N_2OS]^{\bullet+}$ions, respectively. The base peaks at m/z 80 can be assigned to the $[C_5H_4O]^{\bullet+}$ ion for chalcone derivative (ZH). Successive degradation of the target compound and the appearance of different peaks due to various fragments are good evidence for the molecular structure of the investigated compound (Fig. 4).

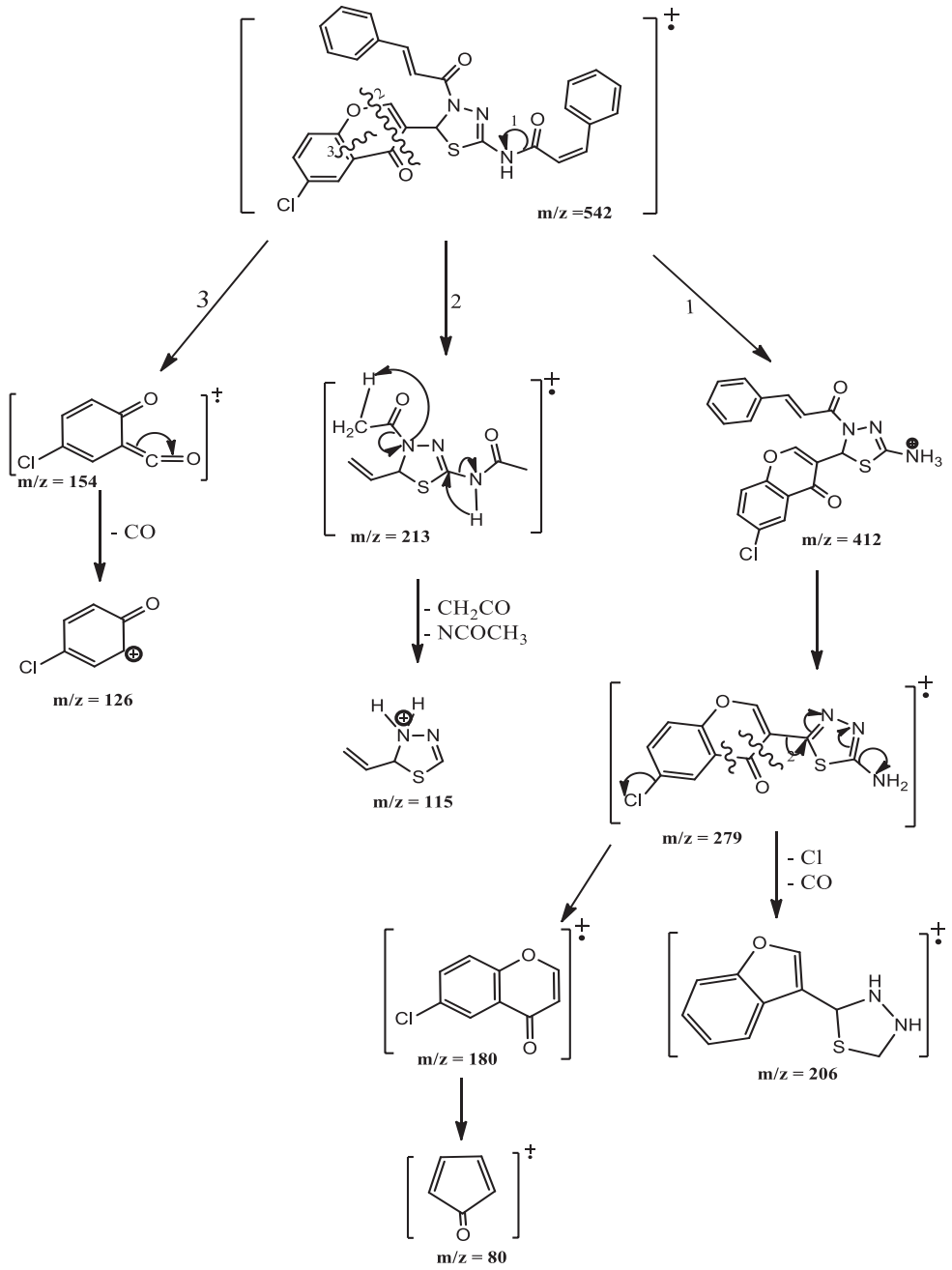
Biological activity

The median lethal dose (LD_{50})

The lethal dose (LD_{50}) of the examined chemical (ZH) *in vivo* was ascertained in mice using intraperitoneal injections of dosages between 500 and 750 mg/kg, with uniform intervals between concentrations. The LD_{50} value for the compound (ZH) was determined to be 758.45 mg/kg. The results may indicate the moderate toxic effects of the examined chemical and the clinical changes observed in the mice following the administration of varying doses. The deleterious symptoms exhibited in injected mice may present as behaviors including tremors, a rigid tail, salivation, urination, lacrimation, defecation, dyspnea, excitation, muscle fasciculations, capillary engorgement, convulsions, and the tortuous reflex in certain treatments, culminating in mortality at elevated toxic doses, as detailed in Table 2 [34,35].

Antibacterial activity

The sensitivity of four human pathogenic microorganisms (two Gram-positive bacteria, *S. aureus* and *Bacillus*, and two Gram-negative bacteria, *E. coli* and *P. aeruginosa*) to the novel synthetic heterocyclic



Scheme 3: The fragmentation pattern proposed for compound (ZH)

Table 2: Toxicity results (LD_{50}) of and toxic signs on mice

Test characterization	Results; ZH
Doses range	500–750=250 mg/kg
First dose	500 mg/kg
Last dose	750 mg/kg
Up and down dose	50 mg/kg
Median lethal dose (LD_{50}) mg/kg	758.45 mg/kg
Effective dose (LD_{50}/10) mg/kg	75.84 mg/kg
Number of mice	8 (XXOXOOXO)
Onset of toxic signs	5–24 min
Toxic signs	Salivation, dyspnea, convulsions, excitation, tremors, muscle fasciculation, death

compound (ZH) was evaluated and contrasted with that of the commercially available antibacterial antibiotic tetracycline. Our study validated that the chalcone compound exhibited antibacterial activity, which intensified with increasing compound concentration, against the examined bacteria. The MIC is defined as the lowest concentration of the compound in the medium that inhibits visible growth of the test organisms, with concentrations ranging from 1 to 500 mg/mL, as illustrated in Table 3 and Figs. 5-8.

All scientific research indicates that antibiotics serve as the primary foundation for the treatment of microbial infections. Conversely, the bacteria exhibited significant genetic variety, allowing them to swiftly circumvent the effects of antibiotics by gaining resistance. Moreover, the recent advancement in the capacity of harmful bacteria

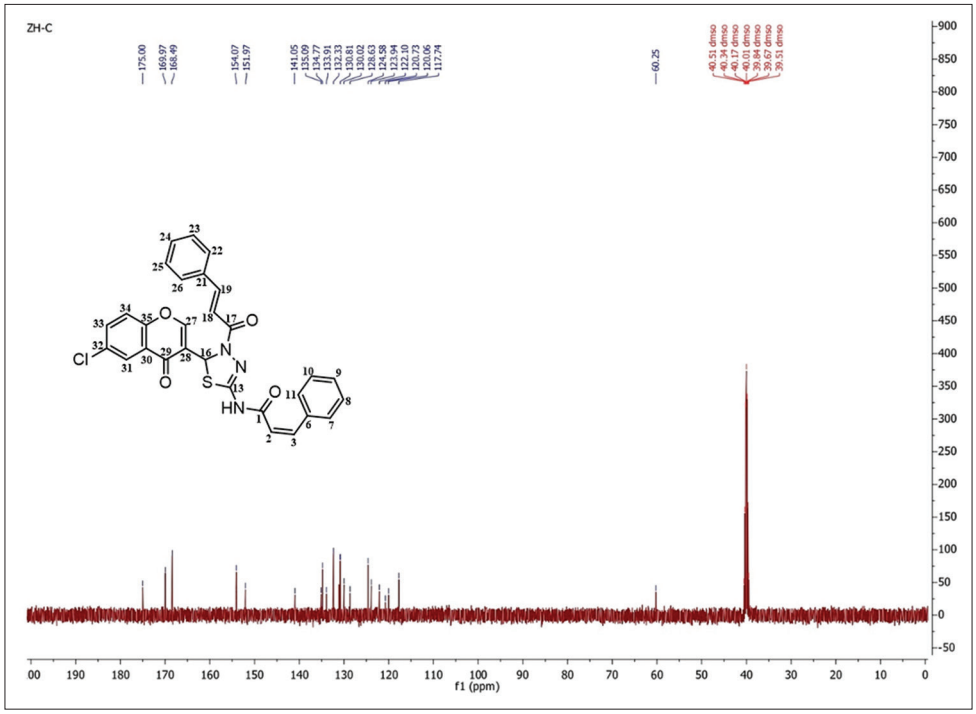


Fig. 3: ^{13}C NMR spectrum of compound (ZH)

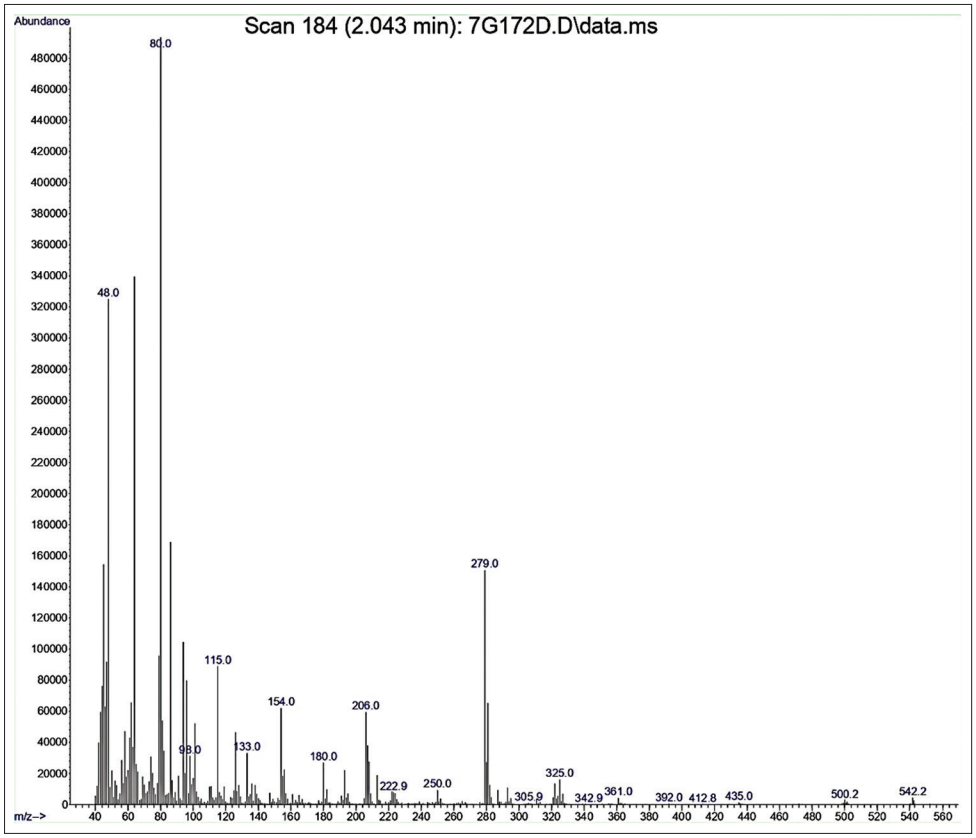


Fig. 4: Mass spectrum of the compound (ZH)

and parasites to withstand many medications has led to significant clinical challenges in the management of infectious disorders [36]. The toxicity of certain antimicrobial agents on host tissues and other difficulties has necessitated a focused search for novel antimicrobial compounds. *E. coli* is a highly pathogenic microbe responsible for numerous prevalent diseases in humans, commonly linked to urinary tract infections, particularly among stressed individuals and office workers utilizing shared restrooms, subsequently increasing the risk of *P. aeruginosa* infections, which are frequently associated with pediatric illnesses. The primary human bacterial agent responsible

Table 3: Sensitivity of human pathogenic selected microbes to the new synthetic heterocyclic compound

Compounds	Diameter of inhibition zone (mm) *Bacillus*; Concentration (mg/mL)							Compounds	Diameter of inhibition zone (mm) *Staphylococcus aureus*; Concentration (mg/mL)						
	1	5	25	125	250	500	MIC		1	5	25	125	250	500	MIC
ZH	NI	10	28	30	32	33	5	ZH	NI	30	36	26	29	29	5
Amoxicillin*	5	8	33	38	44	52	1	Amoxicillin*	10	23	40	49	51	58	1
Tetracycline*	5	11	14	22	30	50	1	Tetracycline*	NI	4	10	14	25	48	5
Compounds	**Diameter of inhibition zone (mm) *Escherichia coli*; Concentration (mg/mL)**							**Compounds**	**Diameter of inhibition zone (mm) *Pseudomonas aeruginosa*; Concentration (mg/mL)**						
	1	**5**	**25**	**125**	**250**	**500**	**MIC**		**1**	**5**	**25**	**125**	**250**	**500**	**MIC**
ZH	NI	27	30	25	28	29	5	ZH	NI	26	32	20	20	22	5
Amoxicillin*	NI	23	39	46	51	57	5	Amoxicillin*	NI	NI	NI	NI	NI	17	500
Tetracycline*	NI	8	11	15	21	44	5	Tetracycline*	NI	6	8	17	30	52	5

*Standard. NI: No inhibition, MIC: Minimum inhibitory concentration

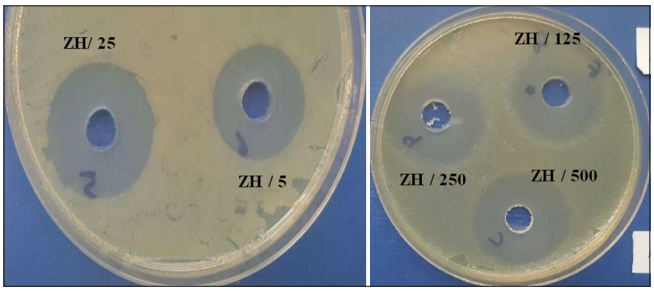


Fig. 5: Inhibition zones of the synthesis compound against *Escherichia coli* at concentrations (5–500 mg/mL)

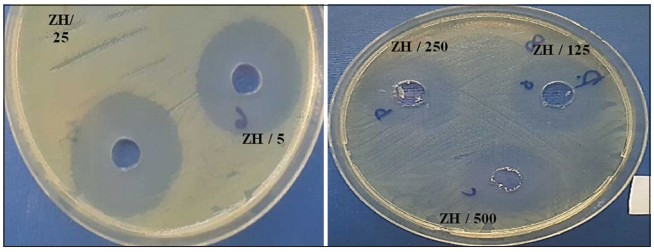


Fig. 6: Inhibition zones of the synthesis compound against *Pseudomonas aeruginosa* at a concentration (5–500 mg/mL)

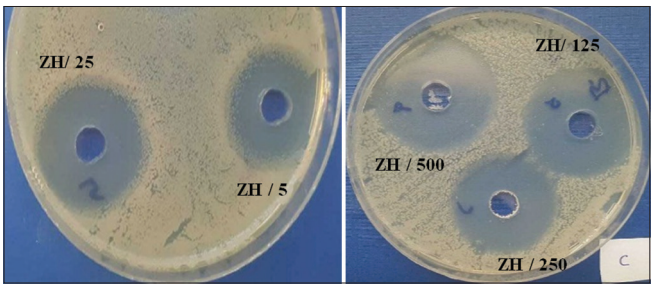


Fig. 7: Inhibition zones of the synthesis compound against *Bacillus* at a concentration (5–500 mg/mL)

for a range of potentially severe infections and clinical symptoms is *S. aureus*, particularly when it infiltrates the circulation or internal tissues [37].

The antibacterial activity of the novel synthetic chemical may be ascribed to the differences in cell wall composition and thickness between the two groups of bacteria. The efficacy of these novel chemicals in causing bacterial colony disintegration likely stems from their disruption of the bacterial cell wall, thereby impeding microbial development [37].

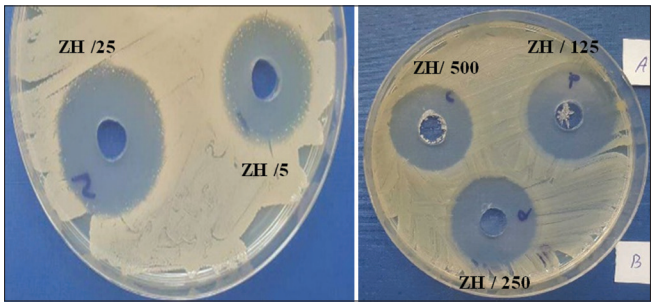


Fig. 8: Inhibition zones of the synthesis compound against *Staphylococcus aureus* at concentrations (5–500 mg/mL)

The novel synthetic heterocyclic drug, ZH, had superior efficacy compared to the positive control (tetracycline) against Gram-negative bacteria (*E. coli*), exhibiting inhibitory zones (IZ) of 27, 30, 25, and 28 mm at concentrations of 5, 22, 125, and 250 mg/mL, respectively. This outcome may stem from the presence of an outer membrane in Gram-negative bacteria, which contains lipopolysaccharides, enabling the substance to interact with the lipophilic layer and so augment the membrane's permeability to Gram-negative bacteria. In conclusion, the antibacterial efficacy of any drug may be correlated with the structural composition of bacterial cell walls, given the critical role of this wall in bacterial viability. Consequently, the efficacy of antibiotics in eradicating or suppressing bacterial proliferation may stem from the inhibition of a specific stage in the peptidoglycan production of gram-positive bacteria [38,39].

Compound ZH exhibited antibacterial activity against Gram-positive bacteria (*S. aureus* and *Bacillus*) that ranged from high to moderate. Our findings demonstrated that compound ZH exhibited the most significant antibacterial efficacy against Gm+Ve (*S. aureus*), with inhibition zones measuring (30, 36, 26, 29 mm) at concentrations of (5, 25, 125, and 250 mg/mL). The **ZH** compound exhibited more potency than the positive control (IZ= 4–25 mm) at the same concentration. Conversely, our data indicated that compound **ZH** exhibited significant antibacterial activity against Gram-positive (*Bacillus*), with an inhibition zone (IZ) extending from 28 to 32 mm, in comparison to tetracycline, which had an IZ of 14–30 mm at concentrations of 25–250 mg/mL.

The antibacterial action of this novel synthetic heterocyclic molecule may be related to its structural characteristics, primarily the presence of electron-withdrawing groups such as chlorine. The presence of a halogen atom in the molecule enhances its lipophilicity and promotes hydrophobic interactions with specific binding sites on receptors or enzymes. Moreover, the Cl ion in the molecule can augment antibacterial activity by either exterminating germs or suppressing their proliferation through the obstruction of their active site. Furthermore, the inclusion of heteroatoms led to an enhancement in antibacterial activity [40,41].

The MIC of the tested compound ZH in this study against the test organisms ranged between 5 and 500 mg/mL (Table 3). Antimicrobial agents with low activity against an organism had a high MIC, while a highly active antimicrobial agent gave a low MIC. The most resistant microorganisms were *E. coli* and *P. aeruginosa*, whereas the most sensitive microorganisms were *S. aureus* and *Bacillus*. The lowest MIC value of (5) mg/mL was recorded on *S. aureus* with compound ZH, whereas the lowest MIC value of (5) mg/mL was obtained on *Bacillus*. The results of the present study suggest that the ZH possesses remarkable toxic activity against bacteria and may assume pharmacological importance [42].

AA

Reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, hydroxyl radicals, and nitric oxide radicals, are produced during bioorganic redox processes and normal cellular metabolism. They significantly contribute to oxidative stress associated with the development and pathogenesis of various life-limiting diseases, including cancer, diabetes mellitus, arteriosclerosis, and rheumatoid arthritis [18]. Scientific evidence indicates that exposure of normal cells to free radicals results in structural damage by disrupting the functioning of enzymes and essential macromolecules, including lipids, proteins, and nucleic acids. Antioxidants are either synthetic or natural compounds that can prevent or postpone certain forms of cellular damage resulting from oxidative stress created by free radicals. Over the past decade, medical chemists, food chemists, and biologists have predominantly concentrated on researching and evaluating various novel and efficacious natural or synthetic antioxidants as a preventive measure against human diseases to mitigate and/or inhibit oxidative damage associated with free radical reactions [43].

The current work assessed the AA of the novel synthesized compounds using the *β*-carotene bleaching method. This process involves the oxidation of linoleic acid, resulting in the formation of unstable hydroperoxides that readily oxidize *β*-carotene molecules, rich in double bonds, leading to a rapid loss of color and double bonds in the β-carotene molecule. This approach involves the oxidation of linoleic acid to form unstable hydroperoxides, which readily react with and oxidize the double-bond-rich *β*-carotene molecules, resulting in fast decolorization and the loss of their double bonds. Consequently, the presence of antioxidant compounds can impede the degree of *β*-carotene degradation by neutralizing linoleate free radicals and other free radicals generated within the system [44]. The absorbance values diminished swiftly in samples lacking antioxidants, whereas the inclusion of an antioxidant allowed them to maintain their color, resulting in sustained high absorbance over an extended duration [45]. The results in Table 4 and Fig. 9 indicated an increase in the AA of the synthetic compound and standards in the order of **ZH**<BHT with corresponding percentage values of 18.1% and 80.6%. A possible explanation for the higher AA of this compound ZH might be that the compound ZH has a thiadiazole ring, which can act as a scavenger for radicals to prevent oxidative cellular damage and thus enhance antioxidant properties [46].

The finding that compound ZH possessed a strong protective effect is interesting and points to the potential use of this new compound as an agent to overcome oxidative stress that is associated with cellular metabolism and disease conditions [46,47]. The mechanism by which ZH protects the body's cells from oxidative damage may require further study and investigation.

Interestingly, previous studies showed that some thiazole antibiotics, such as febuxostat and ebselen, possess a relative AA against ROS [46,48]. Furthermore, the ring of thiadiazole that can initiate the free radical scavenging activity may be due to its C-S-C moieties [4,18].

Notably, scientific studies have confirmed that compounds in general, including those that have antioxidant properties, may be subjected to metabolism *in vivo* through specialized enzymatic systems in the body, which often convert lipophilic chemical compounds into polar products that are easily secreted. Moreover, the metabolism of any compound can result in an increase or a decrease in its toxicity [43]. Therefore, we expect ZH to enter different metabolic pathways in the body that may differently modify its structure and/or toxicity, and this requires further research. Again, the possible exact mechanism through which compound ZH and the new other synthetic compounds protect against oxidative damage will be a matter of future studies and must be confirmed in a more controlled experimental design [44].

Cell cytotoxicity (anticancer) study

The primary objective of researchers and scientists is to identify and formulate a novel anticancer drug that demonstrates high efficacy while minimizing the side effects associated with existing chemotherapy agents. Consequently, the demand for a time-efficient, cost-effective, high-throughput system for drug efficacy testing has resulted in the development of an *in vitro*. Cytotoxicity testing using human cancer cell lines [49,50] demonstrated results comparable to those of 5-FU (positive control).

This study evaluated the cytotoxic effects of the synthesized compound on the breast cancer cell line (MCF-7), using 5-FU as a reference cytotoxic agent. The IC_{50} and cell viability percentages of MCF-7 cancer cells at various concentrations from 6.25 to 100 µg/mL are presented in Table 5 and Figs. 10-13. The findings indicate that compound ZH (IC_{50} = 45.54 µg/mL) exhibits greater cytotoxicity compared to 5-FU (IC_{50} = 98.17 µg/mL), as presented in Table 5. The tested compound demonstrated anticancer activity across all concentrations, with dose-dependent effects. Specifically, as the concentration in the culture media increased, the percentage of cell viability decreased, indicating

Table 4: Antioxidant activity of prepared compound, the values are the mean±standard deviation

Compound symbol	Aj	At	Aj*	At*	AA%
BHT	0.582±0.01	0.544±0.011	0.456±0.031	0.241±0.016	82.3
ZH	0.535±0.027	0.359±0.008	0.456±0.031	0.241±0.016	18.1

Data are presented as mean±SD. BHT: Butylated hydroxyl toluene, SD: Standard deviation

Table 5: The IC_{50} values and the percent of cell viability of the tested compound in breast cancer cell line MCF-7, the values are the mean±standard deviation

Compound	Cell viability%						IC_{50} (µg/mL)
	Concentration (µg/mL)						
	6.25	12.5	25	50	75	100	
ZH	83.66±1.16	56.63±1.05	52.63±1.25	48.55±0.93	47.87±1.53	41.58±0.07	45.54
5FU	83.13±0.86	80.69±1.07	72.76±0.86	66.57±1.06	58.93±0.61	49.29±0.06	98.17

Data are presented as mean±SD. SD: Standard deviation, 5FU: 5-Fluorouracil

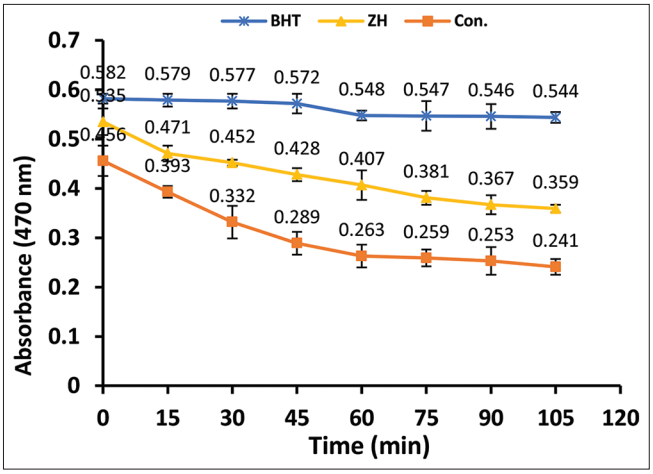


Fig. 9: Antioxidant activity of compound ZH

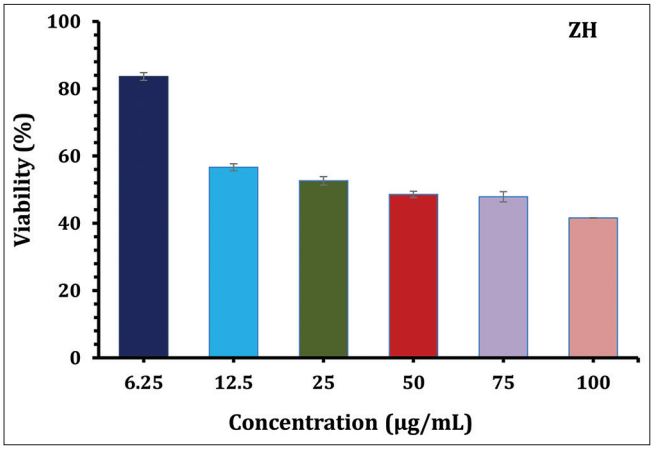


Fig. 10: Anticancer activity of compound ZH at (6.25–100) µg/mL

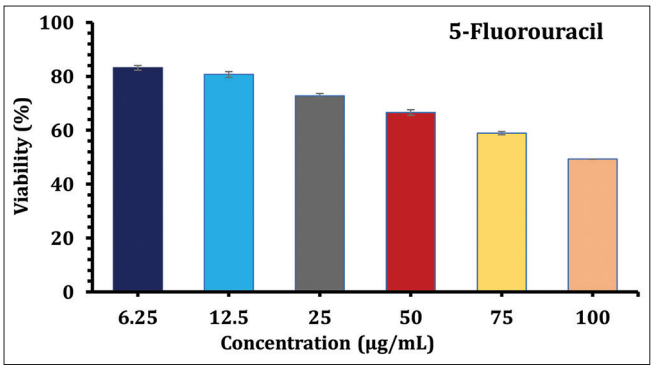


Fig. 11: Anticancer activity of drug 5-fluorouracil at (6.25–100) µg/mL

an increase in the percentage of dead cells. IC_{50} values varied between 45.5 and 98.17 µg/mL. The cytotoxic activity of compound ZH was observed to be greater in cancerous cells compared to 5-FU, particularly at a concentration of 12.5 µg/mL.

Chalcone (ZH) demonstrated its medicinal importance as an anticancer agent [51]. A variety of anticancer chalcones are presently employed in cancer treatment, including anthracyclines, bleomycin, mitomycin C, dactinomycin, and mithramycin. The primary mechanism of action for these anticancer thiadiazolines is the suppression of cell wall synthesis, DNA intercalation, or the inhibition of DNA synthesis [50,52]. The thiadiazoline ring in the chemical structure of compound ZH is associated with anticancer activity through the inhibition of the transpeptidase enzyme, which catalyzes the cross-linking of peptidoglycan strands during the production of the cancer cell wall. The thiazolidinone ring can interact with the active site of the transpeptidase enzyme due to its structural similarity to the substrate, namely the terminal D-ala-D-ala dipeptide of each monomer unit's pentapeptide [53]. The D-ala-D-ala dipeptide of the substrate can adopt several conformations due to rotation around the C–C single bonds. Still, the thiadiazoline molecule exhibits a restricted range of conformations owing to the stiffness of its five-membered ring [54]. Among the several conformations of the terminal dipeptide, the one that interacts with the enzyme mimics the thiadiazoline ring structure, allowing for competitive binding to the enzyme's active site. Banik *et al*. demonstrate that thiadiazoline with polyaromatic substituents induces tumor cell death in various breast cancer cell lines [49,52]. The presence of (-S-C-N-) moieties in the examined drug correlates with anticancer activity through contact with the protein's active site through hydrogen bonding, hence impeding cell proliferation [49]. Nonetheless, other novel families of thiadiazolines have demonstrated anticancer effects as well [55].

On the other hand, the present results clearly indicated that the compound **ZH** could induce apoptosis of MCF-7 Cells, as illustrated in Fig. 13. AO is a vital dye that will stain the nuclei of both live and dead cells green, while EB will stain only cells that have lost membrane integrity to red. Thus, live cells will appear uniformly green, while early apoptotic cells will have condensed or fragmented nuclei with a bright green color. Late apoptotic cells will show condensed and fragmented orange chromatin. The results showed that increased compound **ZH** concentration resulted in gradual increases in orange and red staining accompanied by reductions in green staining of nuclei, indicating cell damage and apoptosis (Fig. 13). Therefore, a high concentration (100 µg/mL) of **ZH** could cause serious membrane damage in around 59% of cells. Moreover, these results indicate that the apoptotic rate gradually increases with the ZH concentrations and treatment time. It is verified that at around 25 µg/mL, ZH can induce half of the cells to undergo apoptosis at 48 h, consistent with the IC_{50} results.

DNA cleavage study (Genotoxicity assay)

This study employed flow cytometry and AO labelling to assess the genotoxicity of the selected compound ZH on the MCF-7 breast cancer cell line. AO interacts with DNA by intercalation and with RNA through electrostatic attraction. This dye is capable of permeating cells and interacting with double-stranded DNA through intercalation, emitting green fluorescence, whereas electrostatically charged single-stranded RNA and DNA exhibit red fluorescence. AO staining is effective for quantifying apoptosis, the programmed cell death process in multicellular organisms [56]. We evaluated the capacity of the examined chemical **ZH** to induce DNA fragmentation. The MCF-7-treated cells exhibited DNA fragmentation, a hallmark of apoptosis documented in other research [57]. The outcomes following 24 h of incubation at a concentration of 100 µg/mL are presented in Table 6 and Figs. 14-16. The examined compound exhibited a significant DNA fragmentation index (DFI %), as detailed in Table 6, which compares the DFI % of the selected compound with that of the anti-tumor agent 5-FU (positive control). The results indicated that the DFI % is dose-dependent, and the viable cells (bottom left, Figs. 14-16) significantly decreased with increasing overlap of this chemical on MCF-7 compared to the negative control at a concentration of 100 µg/mL.

The results indicated that compound ZH exhibited a greater percentage, 56.1%, in comparison to 5-FU, which was 46.95%.

Previous investigations reveal the biological action of chalcone chemicals, including the inhibition of DNA and RNA [58]. Thiadiazolines, capable of binding or cleaving DNA, are currently under significant scrutiny due to their relevance in the formulation of anticancer agents. Furthermore, the data elucidating the *in vitro* percentage of DFI indicates that a DFI below 15% signifies an exemplary standard

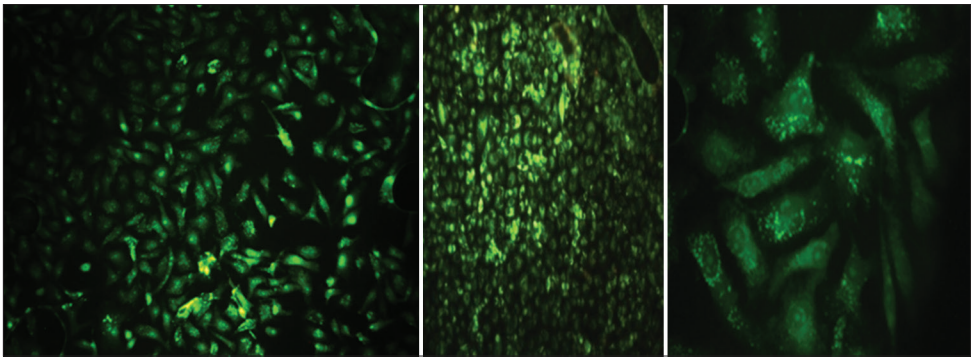


Fig. 12: Anticancer activity of control

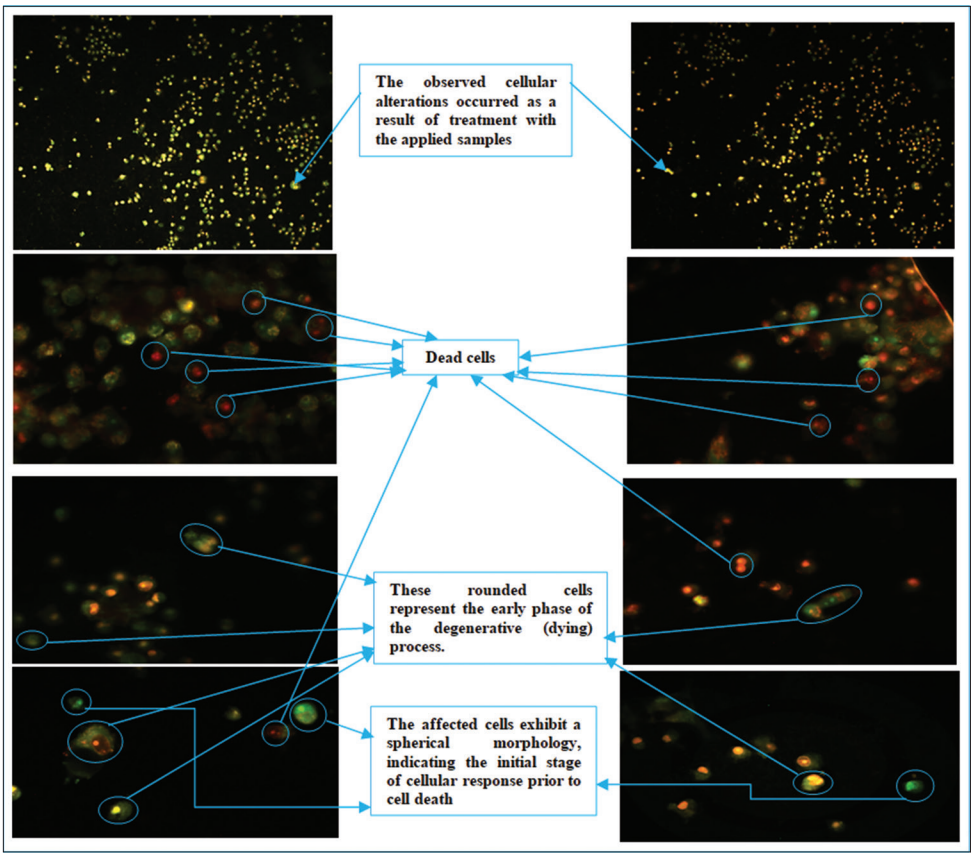


Fig. 13: Anticancer activity of compound ZH at 40 µg/mL

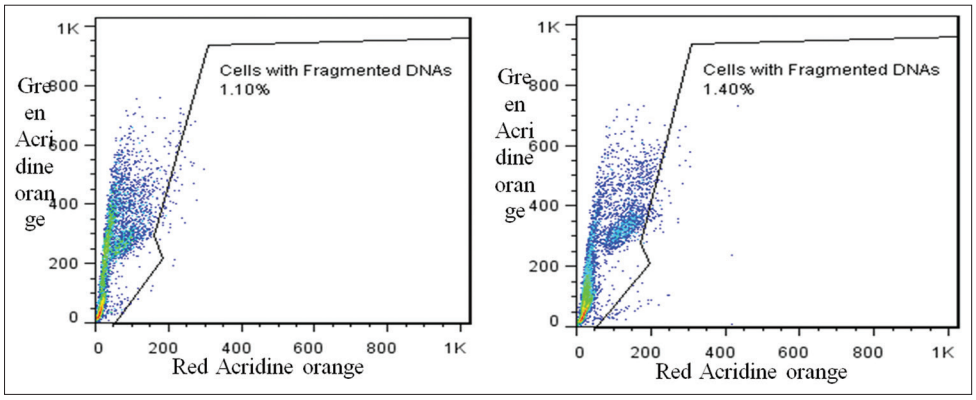


Fig. 14: DNA fragmentation of negative control

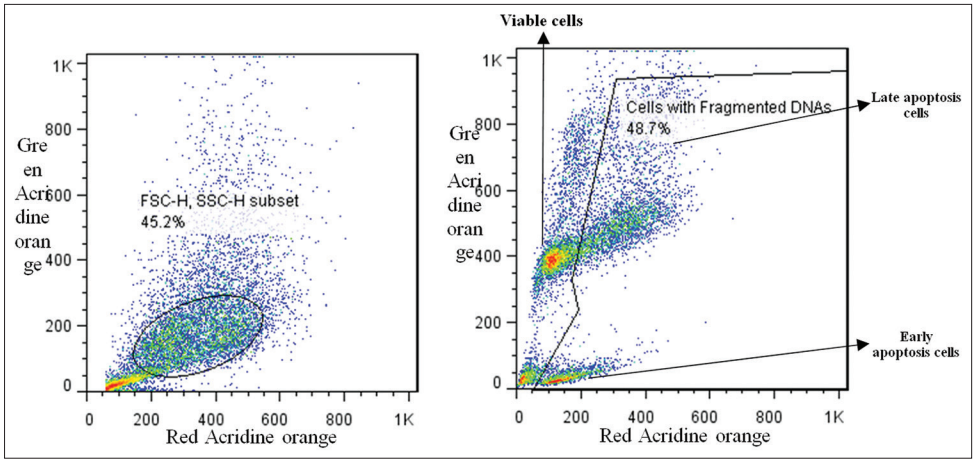


Fig. 15: DNA fragmentation of positive control

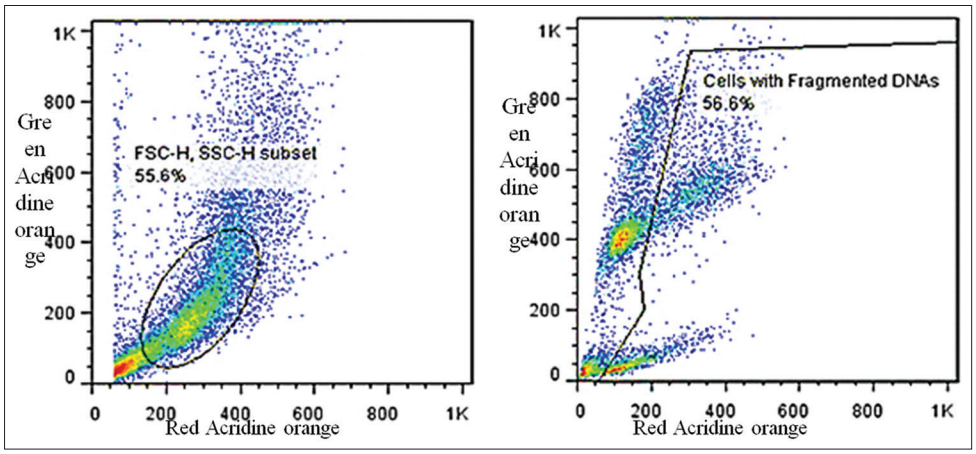


Fig. 16: DNA fragmentation of the compound ZH at (100) µg/mL

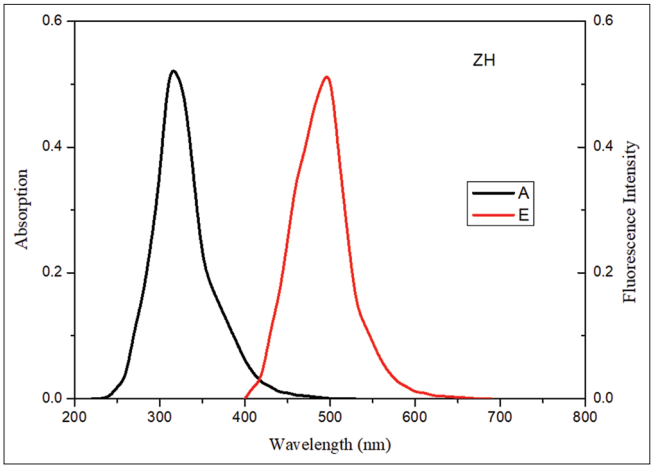


Fig. 17: The absorption and emission spectra of ZH

Table 6: DNA Fragmentation percentage (% DFI) of compound ZH using MCF-7breast cancer cell line

Sample	Concentration (100 µg/mL)		
	DFI (%) (1)	DFI (%) (2)	DFI (%) average
ZH	56.6	55.6	56.1
Positive control (5FU)	45.2	48.7	46.95
Negative control	1.10	1.40	1.25

5FU: 5-Fluorouracil, %DFI: DNA fragmentation index

Table 7: Ultraviolet and fluorescence data of synthesized compound ZH

Compound symbol	λ_{ex} (nm)	λ_{em} (nm)	Stokes shift nm (cm^{-1})
ZH	310	500	190 (12258)

for high DNA integrity [59]. These findings endorse the utilization of this compound in biomedical applications, nanomedicine, and gene delivery systems. In conclusion, the results indicate that the evaluated compounds demonstrate significant promise as anticancer agents based on the anticancer and DNA fragmentation studies.

Electronic spectra and fluorescence study

As the demand for biological imaging and fluorescence microscopy continues to rise due to their wide range of applications in biochemistry and medicine, the development of fluorescent organic dyes with large Stokes shifts and high photostability becomes crucial. A small Stokes shift indicates minimal changes in the dipole moment between the ground and excited states of the compound. When a derivative exhibits a smaller Stokes shift than the original molecule, this can be described as a blue shift. For effective biological and medical applications, probes must have a larger Stokes shift to reduce spectral overlap between absorption and emission, minimizing fluorescence interference and quenching. This ensures a stronger and clearer signal for biological imaging [60].

Consequently, advancements in fluorescent organic dyes exhibiting a substantial Stokes shift (generally over 80 nm) might reduce cross-talk between the excitation source and the fluorescent emission, hence enhancing the signal-to-noise ratio in cellular imaging. Conversely, fluorescent dyes exhibiting high photostability are advantageous for non-invasive long-term cellular imaging, which is crucial for examining biological processes, disease pathways, and therapeutic effects over extended durations [61].

Furthermore, numerous conventional fluorophore dyes, including fluorescein, rhodamine, cyanine, and Nile red, demonstrate minimal Stokes shifts ($\Delta\lambda$ <70 nm), resulting in the reabsorption of emitted photons and causing unwanted background interference. Significant efforts have been devoted to this topic, resulting in the development of several prominent fluorophore dyes with a substantial Stokes shift ($\Delta\lambda$ >80 nm). However, challenges arise due to their intricate architectures, multi-step reactions, and limited yields. Conversely, photostability is a critical factor in assessing the practical utility of fluorescent dyes for bio-imaging. The exceptional photostability is advantageous when employing a fluorescent dye for bio-imaging, particularly for prolonged illumination in the study of biological processes [62].

Herein, the UV-visible and photoluminescence properties of the synthesized compound **ZH** were studied at room temperature at a concentration of 10^{-4} M in DMSO solvent, with an excitation at 390 nm.

Table 7 and Fig. 17 illustrate the absorption (λ_{ex}), emission (λ_{em}), and stock shift of the prepared compound. In general, aromatic systems exhibit fluorescence due to $\pi \rightarrow \pi^*$ transitions [63]. Furthermore, the results presented in (Table 7) indicated that the ZH compound exhibits a very small overlap between the absorption and fluorescence spectrum and a very large Stokes shift ($\Delta\lambda$=190 nm, or $\Delta\bar{\upsilon}$=12258 cm^{-1}). This is a very good property, as indicated by scientific studies, because the large Stokes shift is beneficial to the practical application since it can reduce self-quenching that results from molecular self-absorption. The creation of fluorescent organic dyes exhibiting a significant Stokes shift is crucial for use in biological applications. Recent developments in fluorescent organic dyes featuring a significant Stokes shift (usually exceeding 80 nm) can effectively reduce cross-talk between the excitation source and the fluorescent emission, enhancing cellular imaging with a high signal-to-noise ratio [64]. The compound ZH can serve as a probe for bioimaging applications.

Finally, our data (Table 7) demonstrate that compound ZH exhibited good anti-DNA cleavage and anticancer efficacy. Further investigation and research are required to validate the efficacy of this chemical against various cancer cell types.

CONCLUSION

The present study concluded that the chalcone compound **ZH,** derived from Δ^2-1,3,4-thiadiazoline derivative, was prepared, characterized, and biologically evaluated as antibacterial; the chalcone group in the studied compound likewise assumed a significant role in the restraint of receptor enzyme. The presence of the -N=C-S- in the biologically active molecules has appeared to play a vital job in their antioxidant and anticancer agents. The compound shows moderate antibacterial activities against *S. aureus*, *Bacillus*, *E. coli,* and *P. aeruginosa*. The most elegant result, as antibacterial activity, was obtained for compound ZH, while the synthesized compound ZH showed low activity as an antioxidant agent. Compound ZH has greater anticancer activity, and the percentage inhibition of cell viability by the compound was 48.55% at a concentration of 50 µg/mL. The results showed that Compound ZH has a higher percentage with a % DFI (56.1%) compared with 5-FU (46.95 %). The present study reported moderate *in vivo* toxic effects by LD_{50} measurement of a new compound (ZH). On the other hand, our results confirm that the tested compound ZH has fluorescence properties. Meanwhile, the compound ZH produced a good stock shift at (190 nm), which further shows promise in biological and medicinal applications fields.

ETHICAL APPROVAL

There is no ethical issue.

ACKNOWLEDGMENTS

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AUTHOR'S CONTRIBUTION

Zainab Kadhim Al-Khazragie contributed to the conception and design of the study, performed the synthesis of the chalcone derivative, coordinated the biochemical assays, and prepared the initial manuscript draft. Hanan A. Al-Hazam was responsible for the antimicrobial and antioxidant evaluations, data collection, and analysis of experimental outcomes. Sabah Abbas Malik contributed to the statistical analysis, data interpretation, and critical revision of the manuscript for intellectual content. All authors reviewed and approved the final version of the manuscript for publication.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

SOURCE OF FUNDING

This research was conducted as a personal endeavor. The synthesis of the compound was conducted at the Department of Chemistry, College of Science, University of Basrah. All subsequent analyses were performed at personal expense, including those conducted in Iran and at the College of Education.

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