Synthesis, Characterization and Cytotoxicity Activity Study of Some pyrazoline Derivatives from chalcone Derived from 2- (1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde

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Abstract
This research involved the synthesis of a number of novel pyrzolines derived from indol compounds. The condensation reaction of the chalcon derivatives (D1-D4) with hydrazine hydrateproduce pyrzoline derivatives (D5-D8). Spectroscopic methods were used to confirm and describe the structure of compounds (FT-IR, 1H-NMR). The cytotoxic activity of target substances at various doses against the human breast cancer cell line MCF7 was examined. The findings indicated that the substances had potential cytotoxic activity against the MCF7 cell line, particularly substance (D7), which had the strongest inhibition at a rate of 100 µg/ml among the substances tested at various concentrations.

Keywords: indol derivatives, chalcones, pyrzolines, cytotoxicity activity

1. Introduction

Due to its toxicity and potential for mutagenicity, indole has long been regarded as a typical N-heterocyclic aromatic pollutant (1). The biological significance of indole and its derivatives have found use in the pharmaceutical and other industries due to their wide range of naturally occurring biological activities (2). A significant class of chemicals called chalcones is present in a number of natural goods (3). The chalcone-containing chemical synthesis processes are straightforward, effective, practical, and high yielding (4). Chalcones, both synthetic and natural, have a range of intriguing medical functions (5, 6). Chemically, K they consist of an open chain in which the two aromatic rings are connected by a three-carbon α,β-unsaturated carbonyl system. In recent years, chemistry of chalcones has fascinated interest as these compounds have been found to exhibit several biological activities, such as cytotoxic (7), antimalarial (8), anti-inflammatory (9), anti-HIV (10), and as tyrosinekinase inhibitors (11). Pyrazoline is a five-membered heterocyclic compound having two adjacent nitrogen atoms within the ring. It has only one endocyclic double bond and is basic in nature. Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals (12). In the field of heterocyclic chemistry, the synthesis and characterization of pyrazoline derivatives were developing fields Due to the pyrazoline and ring's diverse properties, fairly assessable synthesis route, wide range of therapeutic activities, and variety of industrial applications, they became a center of interest for organic chemists (13,14). Because the pyrazoline ring is so stable, chemists have altered its structurally in numerous ways. This has sparked the creation of unique pyrazolines with a variety of pharmacological effects, including analgesic, anti-inflammatory, antibacterial, anticancer, and depressive properties (15). These nitrogen heterocycles are frequently found in nature as alkaloids, vitamins, pigments, and parts of the cells of both plants and animals (16).

2. CHEMISTRY PART

Materials and Methods

All of the chemicals and solvents needed to create the compounds came from several companies, including Merck, BDH, Fulka, and Sigma Aldrich. The chalcone compounds **[D_1-D_4]** were synthesized by the method previously described by reference [18], (Table 1). Using the tool Melting point SMP10, melting points were identified. College of Science, Diyala University. FT-IR spectrum were captured using a PERKIN ELMER SPEACTUM-65, JASCO infrared spectrophotometer, and KBr Disc at Diyala University's College of Science in the range [4000-400]. Deuterated DMSO was employed as the solvent and a BRUKUR 400 MHz spectrophotometer was utilized to record the ^{1}H-NMR spectra. The experiments were performed in the Central Lab. of the college of science at the University of Basra.

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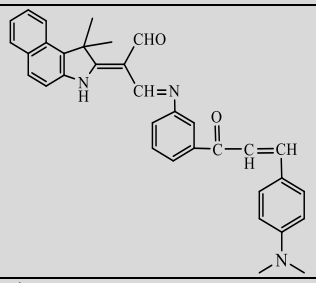
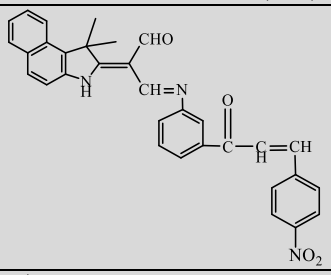
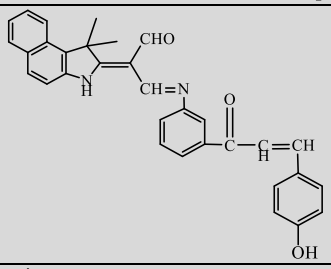
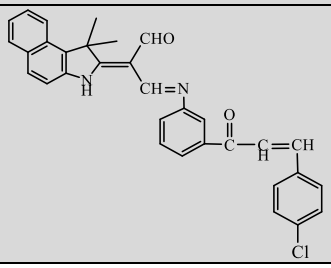
Table (1): Physical properties of the compounds (D_1-D_4)				
Comp. No.	Molecular formula	Yield%	Melting Point °C	Comp. name
D_1		52	154-156	2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-(dimethylamino)phenyl) acryloyl) phenyl)imino)propanal
D_2		62	180-182	2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-nitrophenyl) acryloyl) phenyl) imino)propanal
D_3		59	203-205	2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-hydroxyphenyl) acryloyl) phenyl) imino) propanal
D_4		68	140-142	3-((3-(3-(4-chlorophenyl) acryloyl) phenyl) imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal

3. Synthesis Methods

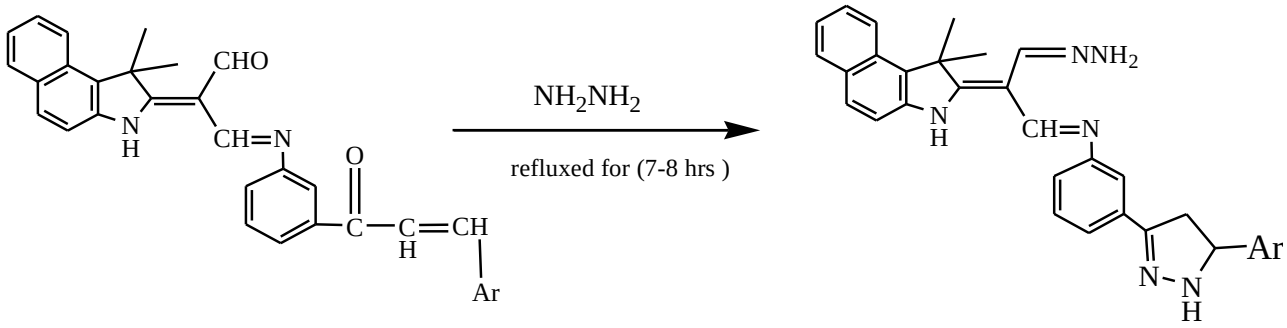
Synthesis of pyrazoline derivatives (D_5–D_8)

A mixture of chalcone **[D_1-D_4]** (0.0013mole) and hydrazine hydrate (0.0026 mole) in ethanol (20 mL) was heated under reflux for 7-8 hrs. Completion of the reaction was identified by TLC using Silica gel-G. (3:2) hexane: ethyl acetate, which gave one spot. After cooling to room temperature, the solution was filtered and then the precipitate appeared washed with water, and dried. FT-IR data in (cm^{-1}) of compound **[D_5]**: 3366 υ (NH_2), 3157 υ (NH),3094 υ (CH aromatic),2928-2865 υ (CH aliphatic),1621 υ (C=N),),1602 υ (C=N-Pyrazoline),1546-1519 υ (C=C) ,1204υ (CN),and 743 υ (CH bending).^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **[D_5]**: δ =13.43(s,1H,NH Indole),10.60(s,1H,NH Pyrazoline),8.60(s,2H,CH=N), 8.22-7.55 (m,14H,Ar-H)6.70(s,2H,NH_2),3.90(1H-Pyrazoline),3.04(s,6H,N(CH_3)$_2$),2.80-2.70(d,2H,CH_2 Pyrazoline),1.60 (s, 6H, 2xCH_3).

FT-IR data in (cm^{-1}) of compound **[D_6]**: 3358 υ(NH_2), 3192 υ(NH), 2930-2859 υ(CH aliphatic),1621 υ(C=N),1594 υ (C=N-Pyrazoline),1566-1459 υ(C=C), 1518-1388 υ(NO_2),1210 υ(C-N),and750 υ (CH bending).^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **[D_6]**: δ =13.43(s,1H,NH Indole),10.62(s,1H,NH-Pyrazoline),8.64(s,2CH,CH=N),7.58-8.22(m,14H,Ar-H),7.45(s,2H,NH2),3.92(1H-Pyrazoline),2.89-2.73(d,2H,CH2-Pyrazoline),1.67(s, 6H,2xCH_3).

FT-IR data in (cm^{-1}) of compound **[D_7]**: 3362 υ (OH), 3157 υ (NH), 3086 υ (CH aromatic), 2960 – 2928 υ (CH aliphatic),1621 υ(C=N),1601 υ(C=N-Pyrazoline),1546-1519 υ(C=C) ,and743 υ(CH bending).^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **[D_7]**: δ =13.43(s,1H,NH Indole),10.61(s,1H,NH-Pyrazoline),8.65(s,2H,CH=N),7.58-8.19(m,14H,Ar-H),6.74(s,2H,NH_2),3.91(1H-Pyrazoline),2.85-2.72(d,2H,CH_2-Pyrazoline),1.67(s, 6H,2xCH_3).

FT-IR data in (cm^{-1}) of compound **[D_8]**: 3430-3354 υ(NH_2), 3157 υ(NH),3094 υ(CH aromatic), 2968-2928 υ(CH aliphatic),1665 υ(C=N),1625 υ(C=N-Pyrazoline),1546-1519 υ(C=C), 1204 υ(CN),and743 υ(CH bending).^{1}H NMR (400 MHz, DMSO, δ in ppm) of compound **[D_8]**: δ =13.43(s,1H,NH Indole),10.60(s,1H,NH-Pyrazoline),8.67(s,2H,CH=N),7.22-8.19(m,14H,Ar-H),6.55(s,2H,NH_2),4.01(1H-Pyrazoline),2.89-2.76(d,2H,CH_2-Pyrazoline),1.67(s, 6H,2xCH_3).



Ar = 4-(dimethylamino)benzene, 4-nitrobenzene, 4-hydroxybenzene ,4-chlorobenzene

Scheme 1: Synthesis pyrazolines derivatives

2-BIOLOGICAL PART

Determination of the solubility of substances whose in vitro cytotoxicity was assessed. The cytotoxicity assay was conducted using the Freshney (2012) [20] technique using the crystal violate stain. Briefly stated, serum free media (SFM) was used to dilute the organic compounds after they had been dissolved in DMSO to create a range of concentrations between (50,100) g/ml. Human breast cancer cell line MCF7 and regular human (MEF) cell lines were the two types of cell lines employed. Tumor cells (1 x 105 cells/ml) were sown in 96-well microplates and cultured there for 24 hours at 37°C before the old media was replaced with fresh serum-free medium (SFM) that contained quantities of each substance. A humidified incubator with a temperature of 37°C and 5% CO2 was used to incubate the plate for 24 hours. the culture medium after incubation was discarded and 100 ìl of crystal violate was into each well and re-incubated 20 min at 37oC. The inhibition percentage was calculated by the following formula:

Inhibition (%) = (A-B/A) × 100

Where,

A = Absorbance of the control

B = Absorbance of the sample

4. Results and Discussion

New indole-containing pyrazolines were created and described using spectral analyses (^{1}H-NMR and FT-IR). Table(2) lists the physical characteristics of the new compounds, including melting point and yields

Table (2): Physical properties of the synthesized compounds

Comp. No.	Molecular formula	%Yield	Melting Point °C
D_5	$C_{34}H_{35}N_7$	76%	250-252
D_6	$C_{32}H_{29}N_7O_2$	61%	225-227
D_7	$C_{32}H_{30}N_6O$	71%	210-212
D_8	$C_{32}H_{29}ClN_6$	66%	216-218

FT-IR study

FT-IR spectra of the four new compounds(D_5-D_8) showed the appearance absorption bands of the new groups (NH) at 3192-3157 cm $^{-1}$and (C=N) of pyrazoline at 1625-1594, that approved the chemical composition of the manufactured compounds. The FT-IR spectrum of compound (D_7) shows absorption band of the group (-C=O and CH = CH-), which belongs to chalcones (D_7) have disappeared and appearance of the new absorption bands for group (NH) at 3157 cm $^{-1}$and (C=N) of pyrazoline at 1602, indicating that the production of this new compound are formed. In addition to appearing (NH_2) at 3366, (CH aromatic) at 3094 and (CH aliphatic) at 2865, also an absorption band at 1621 to the group (C=N) and in the last the band at 1546-1519 cm^{-1} which belonged to C=C group. The bands of other compounds are listed in **table 3**

Table (3): FT-IR spectral data for compounds (D_5-D_8).

Comp. No.	Characteristic bands of FT-IR spectra (ν in cm^{-1}, KBr disc)									
	NH_2	NH	C-H Ar.	C-H Alip.	CH=N	C=N Pyrazoline	C=C	CH_3	C-N	Others
7	3366	3157	3094	2928-2865	1621	1602	1546-1519	1334	1204	743 (C-H Bend)
8	3358	3192	2978	2930-2859	1621	1594	1566-1518	1340	1210	750 (C-H Bend)
9	over lapping	3157	3086	2928-2858	1621	1601	1546-1519	1330	-	3362 (C-OH) 743 (C-H Bend)
10	3354	3157	3094	2968-2928	1665	1625	1546-1519	1330	1204	C-Cl 743(C-H Bend)

NMR Study

^{1}H-NMR, spectrum were reported in DMSO (dimethyl sulfoxide) with chemical shifts in ppm and using TMS (tetramethylsilane) as standard. The ^{1}H-NMR results for compound **(D_5, D_6)** shown single signals at 13.43 ppm was belonged to proton of (NH) of indole ring. A singlet signal at 10.60 and 10.62 ppm was referred to proton atom of (NH)of pyrazoline. A singlet signal at 8.60 and 8.64 ppm was referred to proton of (CH=N) group. Signals were appeared in the region between (7.58-8.22) ppm were assigned to protons of aromatic ring for (D_5, D_6) compound. Signals was appeared in the 6.70and7.45 was attributed to (NH_2). singlet signal at 3.90 and 3.92 ppm was attributed to (1H) pyrazoline. singlet signal at 3.04 ppm was attributed to ($N(CH_3)_2$) group of compounds(D_5). Signals were appeared in the region between (2.89-2.70) ppm were assigned

to protons of (CH_2) of pyrazoline Finally peak at 1.60 and 1.67 ppm was belonged to six protons of two methyl groups.

Table (4): ^{1}H NMR spectral data for compounds (D_5-D_8).

No	NH Indole	NH Pyrazoline	CH=N	Ar-H	NH_2	H Pyrazoline	CH_2 Pyrazoline	2xCH_3	other
D_5	13.43	10.60	8.60	7.55- 8.22	6.70	3.90	2.80-2.70	1.60	3.04 N(CH_3)$_2$
D_6	13.43	10.62	8.64	7.58-8.22	7.45	3.92	2.89-2.73	1.67	-
D_7	13.43	10.61	8.65	7.58-8.19	6.74	3.91	2.85-2.72	1.67	9.52 OH
D_8	13.43	10.60	8.67	7.22- 8.19	6.55	4.01	2.89-2.76	1.67	-

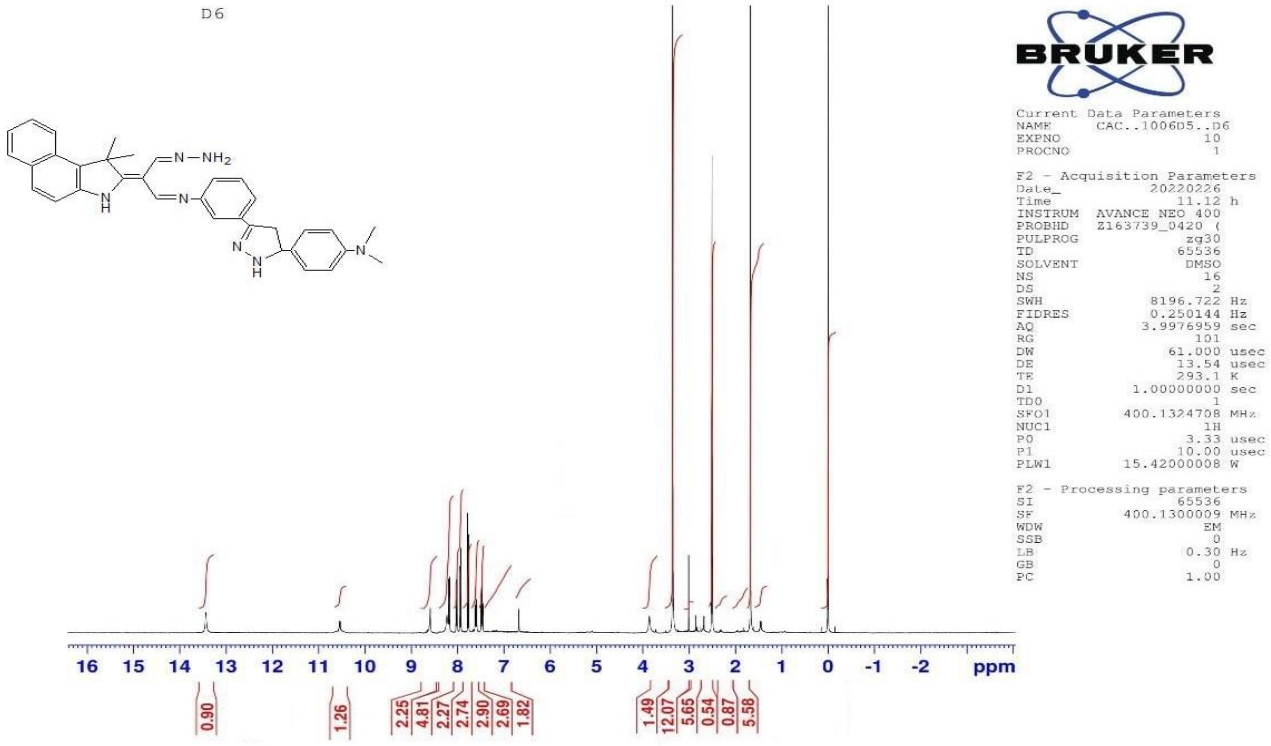


Fig (1): ^{1}H NMR spectrum of compound (D_5)

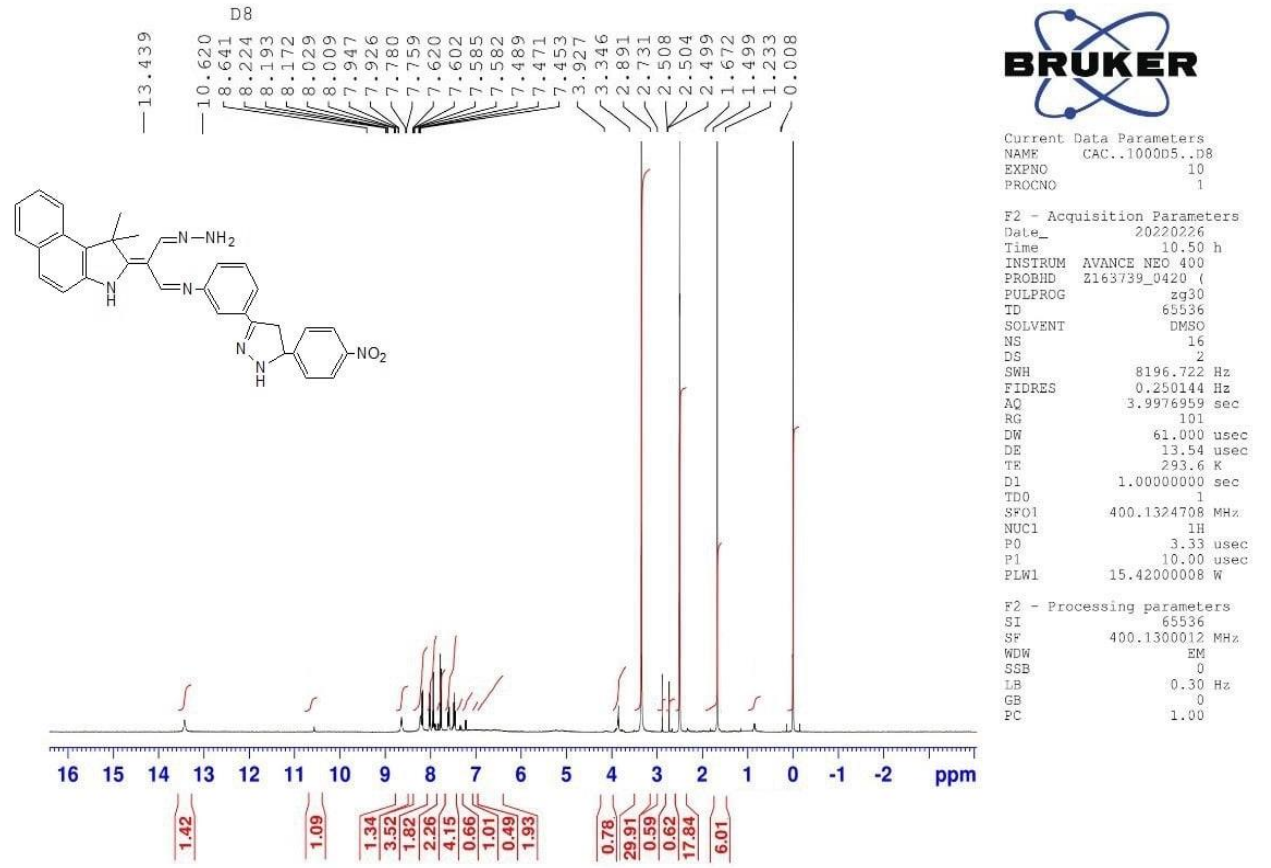


Fig (2): ^{1}H NMR spectrum of compound (D_6)

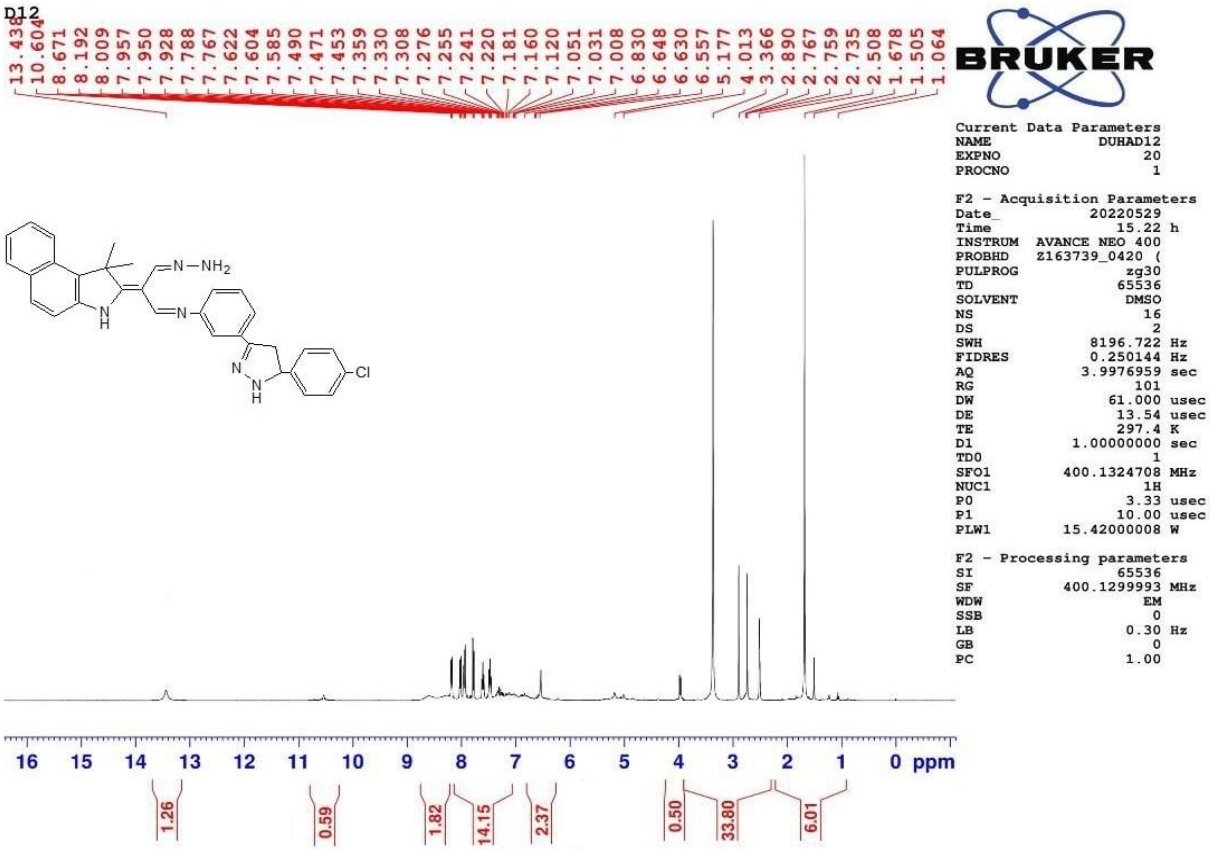


Fig (3): ¹H NMR spectrum of compound (D_8)

In vitro cytotoxic activity

The human breast cancer cell line MCF7 was tested in vitro using the three new composite vehicles (1, 2, 6, and 7) at two different concentrations—50 and 100 µg/mL—over the course of 24 hours at a temperature of 37 degrees. Our findings demonstrated that compound [D_7] had the highest level of cytotoxicity, with an inhibition rate of 77.45 percent at a concentration of 100 µg/ml. among the other vehicles that have various concentrations installed. The outcomes for compound [D_1] were made public. Reliance on them to focus consistently resulted in inhibition rates of 20.07 and 56.45 percent for concentrations of 50 and 100 µg/ml, respectively. The inhibition rates for Compound [D_2] were 30.13 and 48.46 percent for 50 concentrations and 100 µg/ml, respectively. The compound [D_6] gave inhibition rates of 22.6 and 33.41% for concentrations 50 and 100 µg/ml, respectively.

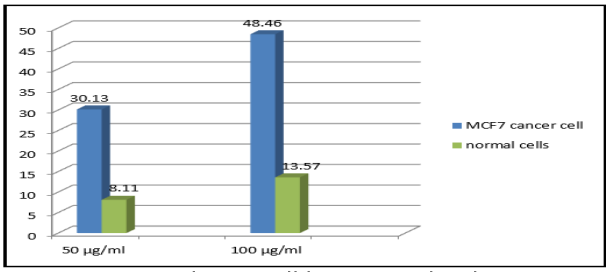


Fig (5): MCF7 and MEF cell line treated with compound (D_2).

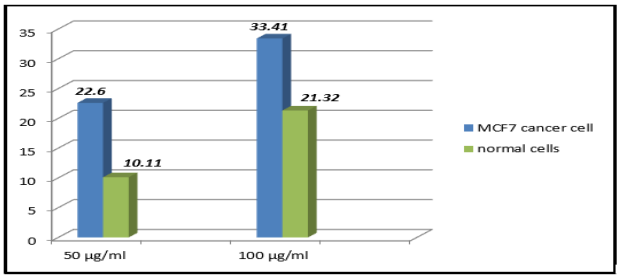


Fig (6): MCF7 and MEF cell line treated with compound (D_6).

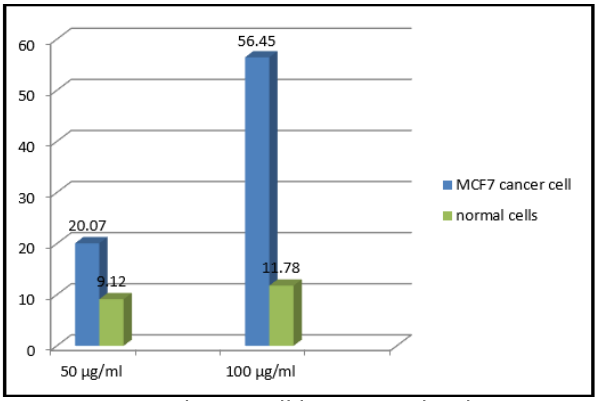


Fig (4): MCF7 and MEF cell line treated with compound (D_1).

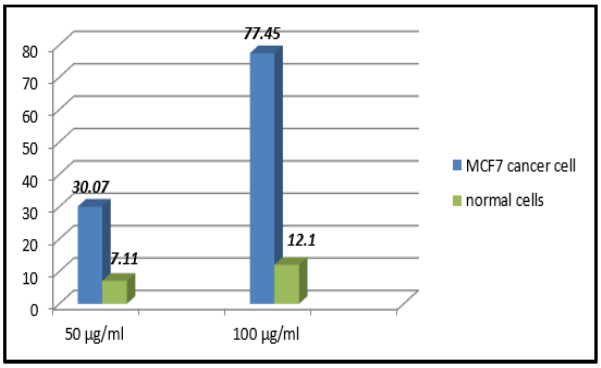


Fig (7): MCF7 and MEF cell line treated with compound (D_7)

5. Conclusion

In the current effort, novel derivatives of chalcones and pyrazoline derivatives were produced. A variety of spectroscopic techniques, including FT-IR and ^{1}H-NMR, as well as measurements of some of these compounds' physical properties, were used to characterize them. The cytotoxic efficacy of target substances against the human breast cancer cell line MCF7 was examined. The findings indicated that the substances had potential cytotoxic effects on the MCF7 cell line, particularly compound D_7, which had the greatest inhibition at a rate of 100 g/ml among the substances examined at various doses.

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