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Synthesis, Characterization and Cytotoxicity Activity Study of Some Indole Derivatives

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Abstract

In this study, several new pyrazoline made from indole derivative were synthesized. pyrazoline derivatives are created via the condensation reaction of the chalcone derivatives (A1-A4) with 2,4-dinitrophenylhydrazine produce pyrazoline derivatives (A5-A8). The structure of substances was verified and described using the spectroscopic techniques (FT-IR and 1H-NMR), the reactions were followed by thin layer chromatography (TLC) to ensure that the preparation of these compounds is complete and their purity. Target compounds cytotoxic effects at different concentrations on the human breast cancer cell line MCF7 were investigated. The results showed that these chemicals had potential cytotoxic activity against the MCF7 cell line.

Keywords: Indole derivatives, chalcones, pyrazoline, cytotoxicity activity.

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دراسة التوليف والتوصيف والسمية الخلوية لبعض مشتقات الإندول

ضحى صالح محمد ووسن باقر علي

قسم الكيمياء – كلية العلوم – جامعة ديالى

الخلاصة

في هذه الدراسة تم تصنيع العديد من البايرزولين الجديد المصنوع من مشتق الإندول. تم إنشاء مشتقات البايزولين عن طريق تفاعل التكثيف لمشتقات الجالكون (A_1-A_4) مع مشتقات 2،4- ثنائي نيتروفينيل هيدرازين تنتج البيرزولين (A_5-A_8). تم التحقق من بنية المواد ووصفها باستخدام تقنيات التحليل الطيفي (FT-IR و^{1}H-NMR). أعقب التفاعلات كروماتوجرافيا الطبقة الرقيقة (**TLC**) للتأكد من اكتمال تحضير هذه المركبات ونقاوتها تم فحص التأثيرات السامة للخلايا للمركبات المستهدفة بتركيزات مختلفة على خط خلايا سرطان الثدي البشري MCF7 أظهرت النتائج أن هذه المواد الكيميائية لها نشاط سام للخلايا ضد خط خلية MCF7 .

الكلمات المفتاحية: مشتقات الإندول، الجالكون، البايرزولين، نشاط السمية الخلوية.

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]. The popularity of indole derivatives, a characteristic class of organic heterocyclic with a variety of biological and pharmacological effects, including anticancer, antihypertensive, antiviral and antitumor capabilities, has increased recently [3,4]. Pyrazolines are heterocyclic compounds with a five-membered unsaturated ring structure made up of two nitrogen atoms at positions 1 and 2 and three carbon atoms.A growing area in heterocyclic chemistry was the synthesis and Characteriztion of pyrazoline derivatives.This is because the pyrazoline ring attracted the attention of organic chemists due to their wide range of medicinal applications and very straightforward synthesis process[5,6].The derivatives of pyrozoline nucleus are known they possess antitumor, antibacterial, antifungal, antiviral, ant parasitic, antitubercular and insecticidal agents. Some of these compounds have also antiinflammatory, antidiabetic, anaesthetic and analgesic [7]. Pyrazolines remain privileged

heterocycles in drug discovery. 2-Pyrazoline scaffold has been proven as a ubiquitous motif which is present in a number of pharmacologically important drug molecules such as antipyrine, ramifenazone, ibipinabant, axitinib etc [8]. One group of electron-rich nitrogen carriers, pyrazolines are enjoying brisk growth, since they combine exciting electronic properties with the potential for dynamic applications. Concerning multi-functional applications, pyrazolines could provide a new range of applications that would revolutionize various fields. As reported in many studies, the potential applications of pyrazolines are vast and ever growing, but they have yet to make in-roads into real-life applications [9]. Pyrazolines have variety of methods for their synthesis but one of the popular methods is of Fischer and Knoevenagel i.e. the reaction of α, β-unsaturated ketones with phenyl hydrazine in acetic acid under refluxing condition [10].

Experimental part

Materials

All of the chemicals and solvents needed to create the compounds came from several companies, including Merck, BDH, Fulka and Sigma Aldrich.

Methods of Identification.

identification of the synthesized compounds was done by general methods, including:

- Thin Layer Chromatography (TLC).
- Melting Point

Devices used

- Fourier-Transform Infrared Spectroscopy (FT-IR): at Chemistry Department , College of Science, University of Diyala
- ^{1}H-NMR: ^{1}HNMR the spectra were recorded on a Bruke 400 MHz spectrometer at University of Science and Technology, College of Science, Tehran Iran and Central Lab. of the college of science at the University of Basra.

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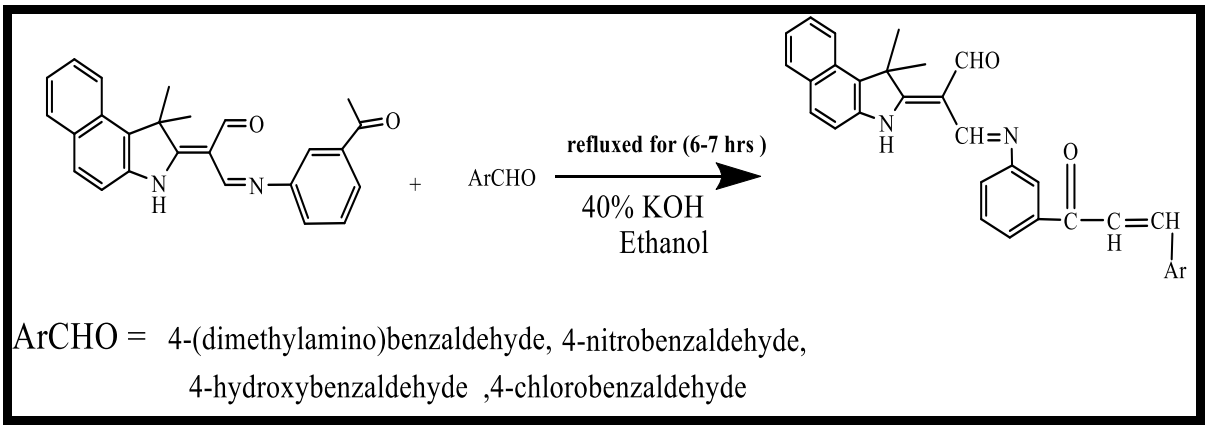
Synthesis methods

1. Synthesis of compounds (A_1-A_4)

The chalcone compounds (A_1-A_4). were synthesized by the method previously described by reference [11], (table 1). Equimolar quantity (0.5g,0.0013 mole) of (2E)-3-((3-acetylphenyl)imino)-2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal and appropriate aryl aldehyde (0.0013 mole) mixed and dissolved in 25 mL of ethanol absolute. To this, 40% potassium hydroxide solution (1 mL) added slowly and mixed occasionally for (3 hrs) at room temperature. the resulted mixture was refluxed for (6-8 hrs) at (78^{o}C) .Completion of the reaction was identified by TLC using Silica gel-G. (3:1) hexane: ethyl acetate, which gave one spot.



Equation 1: Synthetic pathway of chalcones (D3-D6)

2. Synthesis of pyrazoline derivatives (A5-A8)

A mixture of chalcone [A_1-A_4] (0.0013mole) and 2,4-dinitrophenylhydrazine (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 as shown in equation 2.

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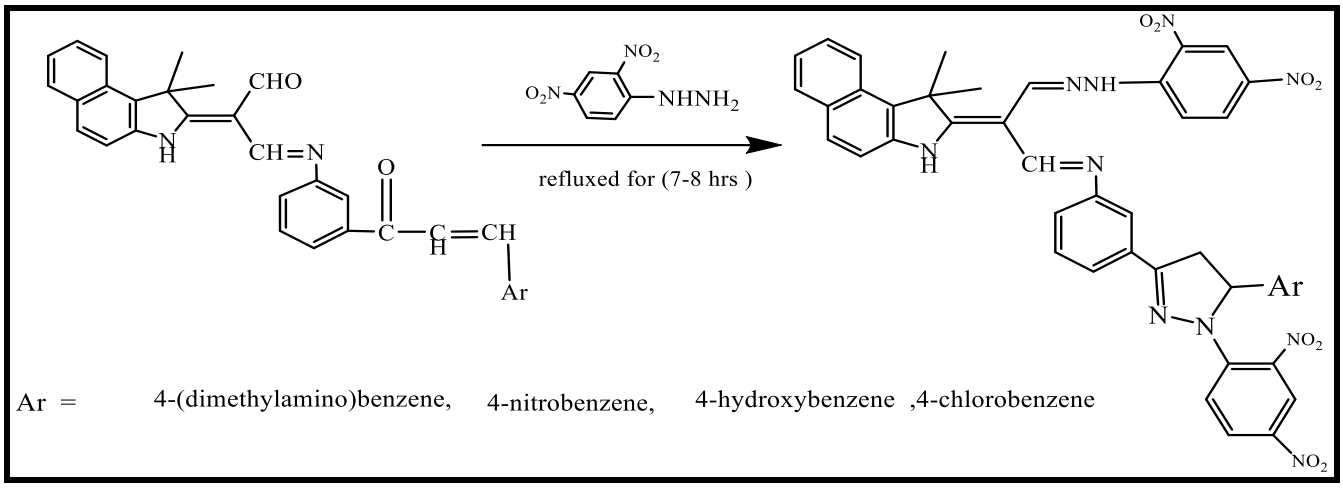

Equation 2: Synthesis pyrazolines derivatives

3. Determination of solubility of compounds tested for in vitro cytotoxicity

The cytotoxicity assay was carried out using the crystal violet stain according to the method of Freshney (2012) [12]. In brief, the organic compounds were dissolved in DMSO and diluted by serum free media (SFM) to prepare different concentrations range of (50,100) µg/ml. Two types of cell lines were used in human breast cancer cell line MCF7, and normal human (MEF) cell lines. The tumor cells (1 x 10^5 cell/ml) were cultured in 96-well microplate and incubated for 24 hrs at 37ºC, then previous media was changed with a new serum-free medium (SFM) containing concentrations of each compound. Plate was incubated for 24 hrs in humidified incubator at 37ºC containing 5% CO_2. After incubation, the culture medium was discarded and 100 µl of crystal violet was in to each well and re-incubated for 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

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Results and Discussion

1. Discussion results

The new synthesized compounds were subjected to spectral studies like ^{1}HNMR and FTIR, their results are discussed below. The Physical properties of the compounds (A1-A4), (A5- A8) are represented in table (1) and table (2) respectively

Table (1): Physical properties of the compounds (A_1-A_4)

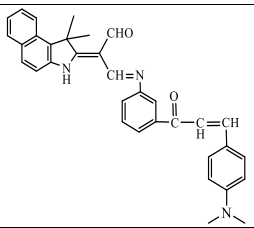
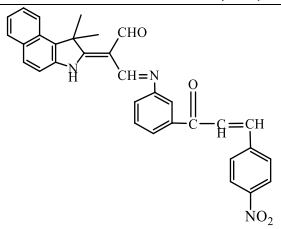
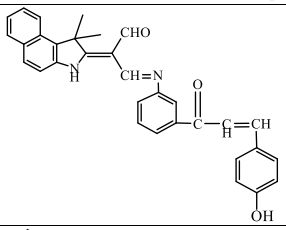
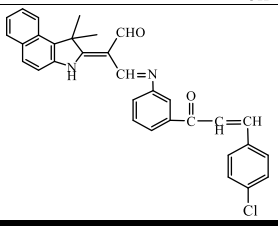
Comp. No.	Molecular formula	Yield %	Melting Point ºC	Comp. name
A_1		52	154-156	2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-(dimethylamino)phenyl)acryloyl)phenyl)imino)propanal
A_2		62	180-182	2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((3-(3-(4-nitrophenyl)acryloyl)phenyl)imino)propanal
A_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
A_4		68	140-142	3-((3-(3-(4-chlorophenyl)acryloyl)phenyl)imino)-2-(1,1-dime1thyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)propanal

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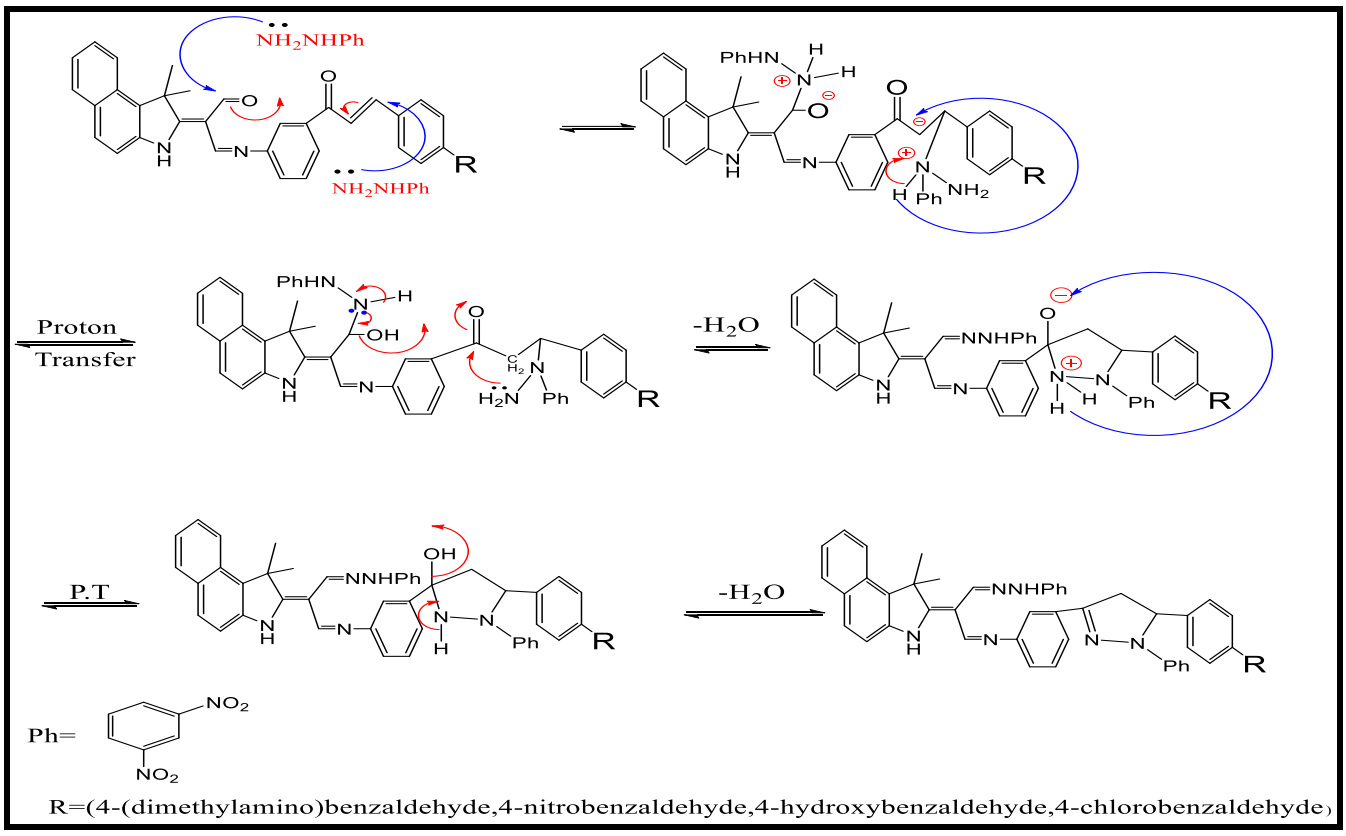
Table 2: Physical properties of the synthesized compounds (A_5-A_8)

Comp. No.	Yield %	Appearance	M. P. °C	Rf
A_5	61	Brown	108-110	0.45
A_6	67	Brown	158-160	0.41
A_7	73	Brown	160-162	0.32
A_8	64	Brown	121-123	0.39

The mechanism suggested by Chinnaraja, D., *et al.* [13] for the synthesis of compound (A_5-A_8) is given in scheme 1.



Scheme 1: Mechanism of formation of compound (A_5-A_8)

FT-IR and ^{1}H-NMR Study

in this part involves the synthesis of compounds **[A_5-A_8]** by reaction of chalcone derivatives **(A_1-A_4)** with 2, 4 - dinitrophenylhydrazine and this compound was identified by FT-IR which exhibits characteristic bands in the following range. The FT-IR of compound (A_5) demonstrate

the following: The bands at 3342 cm^{-1} assigned for the (N-H Indole) group, 3279 for the (N-H) group, 3057 cm^{-1} to the (C-H aromatic), 2914-2851 cm^{-1} to (C-H aliphatic), 1649 cm^{-1} to (C= N, pyrazoline) ,1594 cm^{-1} to (C= N), 1522-1435 cm^{-1} to (C=C). The spectrum of ^{1}H-NMR to compound (A_5) appears thefollowing data: 13.43 (S, 1H, NH Indole), 10.58 (S, 1H, N- NH), 8.92 (S, 2H, 2CH = N), 7.62-8.66 (m, 20H, Ar-H), 4.14 (t, 1H, Pyrazoline), 3.00 (S, 6H, N $(CH_3)_2$), 2.68 (d, 2H, CH_2 Pyrazoline), 1.94 (S, 6H, 2xCH_3). The FT-IR of compound **(A_6)** demonstrate the following stretching bands: at 3429 to the (NH Indole), 3110 to the (NH),3065 to the (CH aromatic), 2976-2922 to (CH aliphatic),1613 to (C=N-Pyrazoline), 1582 to (C=N), 1514-1455 to (C=C).

The spectrum of ^{1}H-NMR to compound **(A_6)** appears the following data: 13.44 (S, 1H, NH Indole), 10.52(S, 1H, N-NH), 8.93 (S, 2H, 2CH=N), 7.23 - 8.59 (m, 20H, Ar-H), 4.21 (t, 1H, Pyrazoline), 3.01 (d, 2H, CH_2 Pyrazoline),1.99(S, 6H, 2xCH_3). The FT-IR spectrum of compound **(A_7)** appears to show the following data: The bands at 3433 assigned for the (OH),3362 to the (NH Indole), the stretching vibration of (NH) appear at 3102 ,2928-2856 to the (CH aliphatic),1617 to (C=N-Pyrazoline), 1590 to (C=N), 1519-1424 to (C=C) stretching vibration of double bonds. the ^{1}H- NMR spectrum of compound (A_7) was extracted the following data: 13.30 (S, 1H, NH Indole), 10.25 (S, 1H, N-NH), 9.50 (S,1H, OH), 8.90 (S, 2H, 2CH = N), 7.30 - 8.60 (m, 20H, Ar-H), 4.00 (t, 1H, Pyrazoline), 2.80 (d, 2H, CH_2 Pyrazoline), 1.80 (S, 6H, 2xCH_3). The FT-IR spectrum of compound (A_8) demonstrate the following: 3362 the stretching vibration of (NH), 3054 to (CH aromatic), 2968 -2928 to (CH aliphatic), 1661 to (C=N-Pyrazoline), 1617 to (C=N), 1515-1487 to (C=C). The spectrum of ^{1}H-NMR of compound (A_8) appears to show the following data: 13.43(S, 1H, NH Indole), 11.49 (S, 1H, N-NH), 8.84 (S, 2H, 2CH=N), 7.50-8.59 (m, 20H, Ar-H), 4.02 (t, 1H, Pyrazoline), 2.89 (d, 2H, CH_2 Pyrazoline), 1.94 (S, 6H, 2xCH_3).

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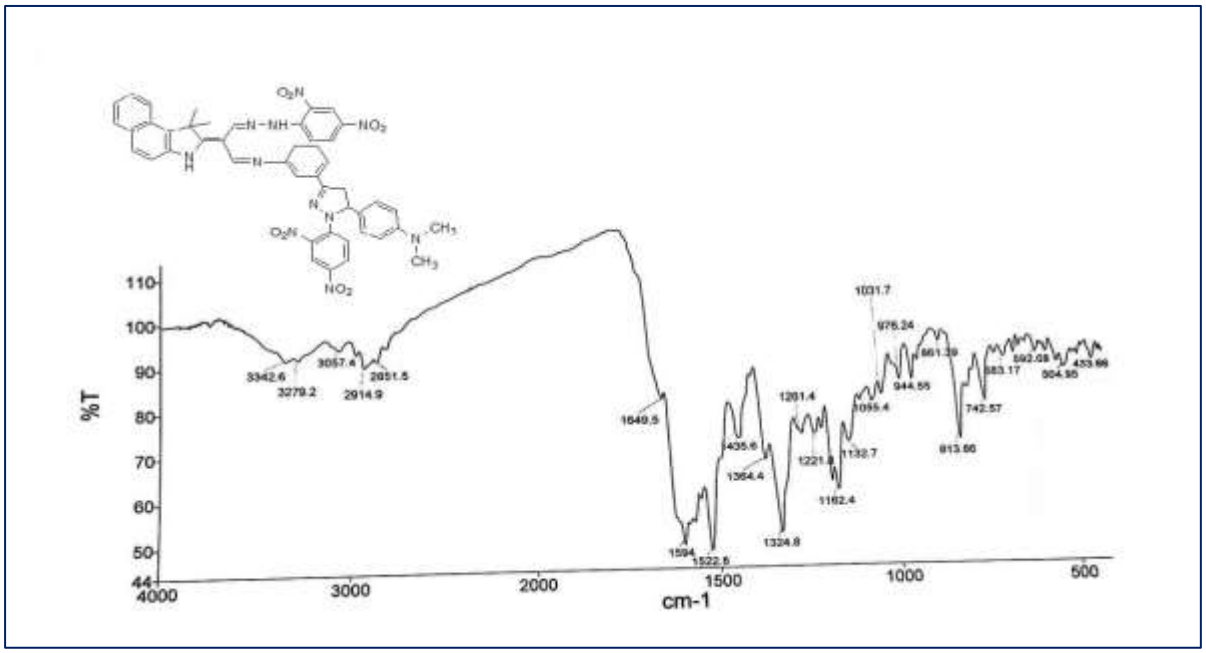

Figure 1: The FT-IR spectra of the compound (A_5)

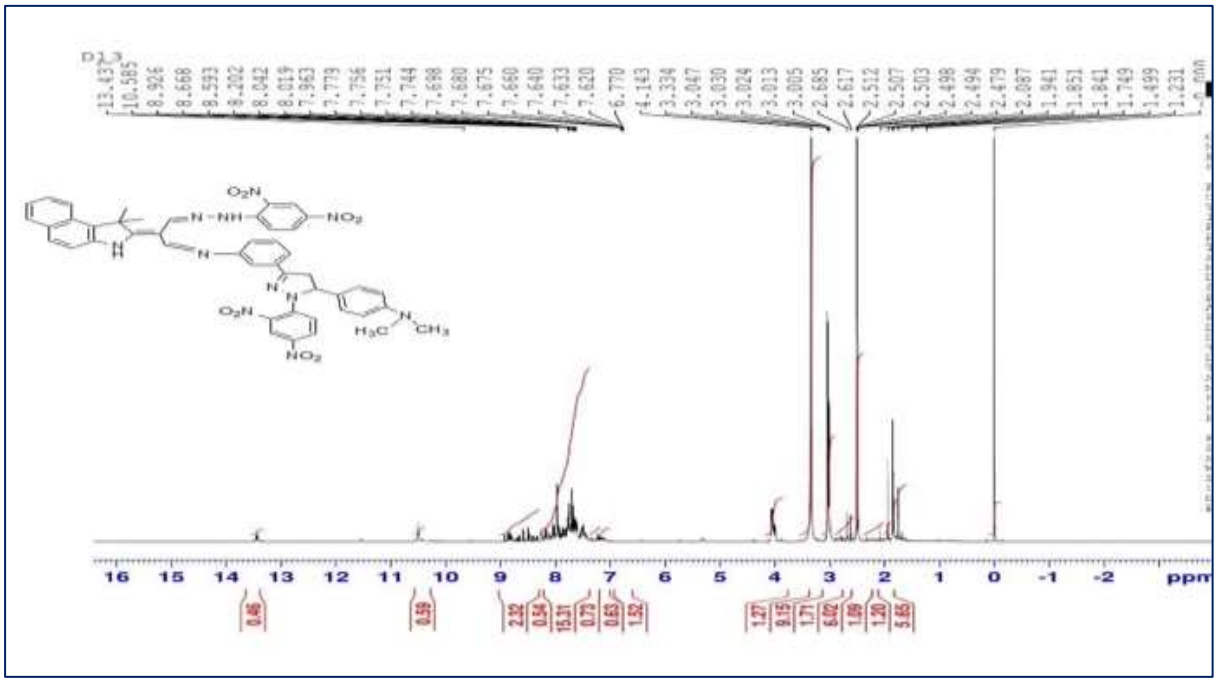

Figure 2: The ^{1}H-NMR spectra of the compound (A_5)

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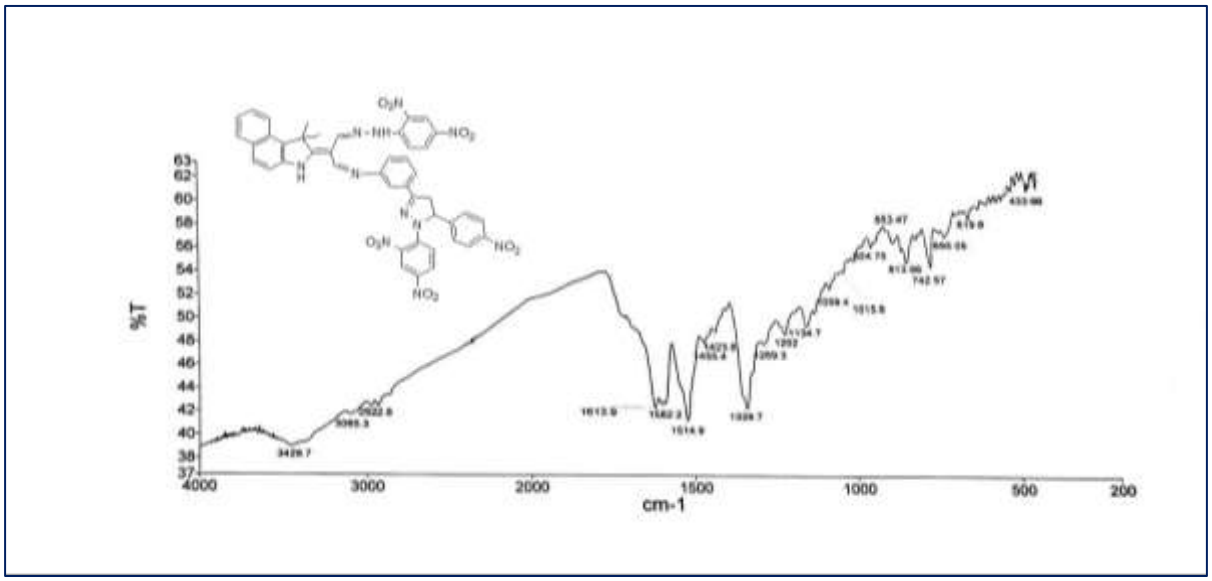

Figure 3: The FT-IR spectra of the compound (A_6)

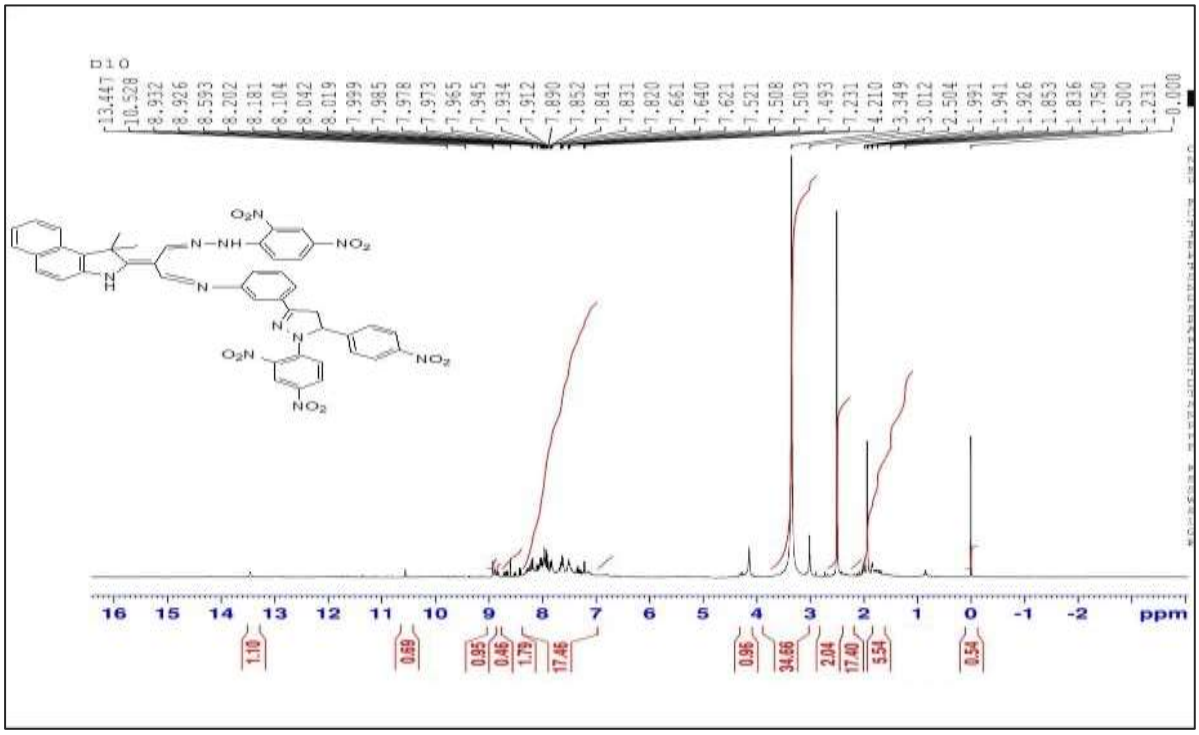

Figure 4: The ^{1}H-NMR spectra of the compound (A_6).

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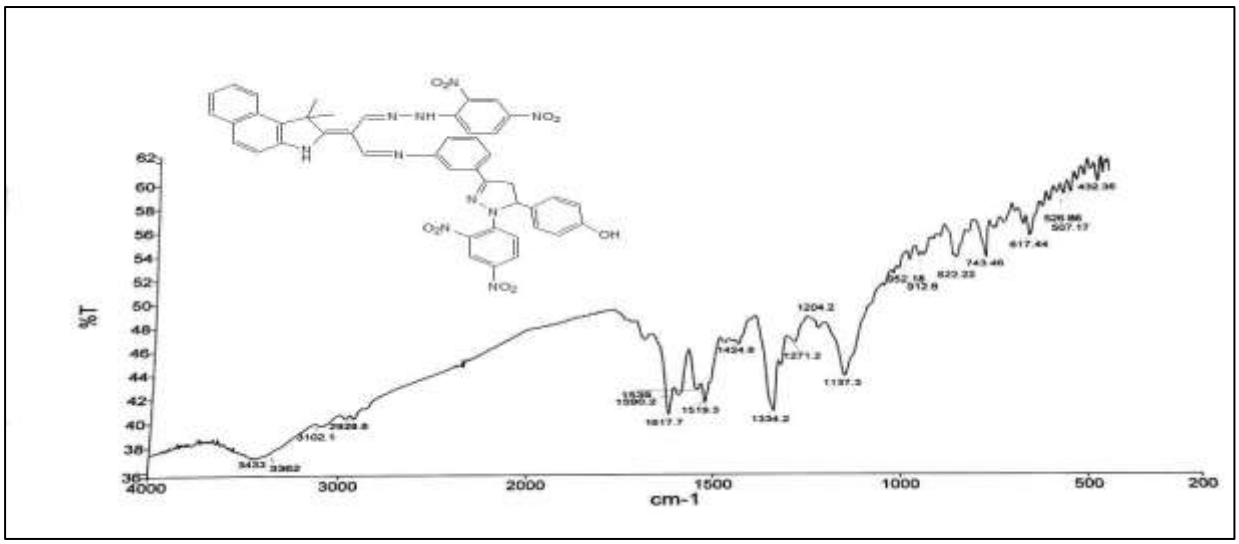

Figure 5: The FT-IR spectra of the compound (A_7)

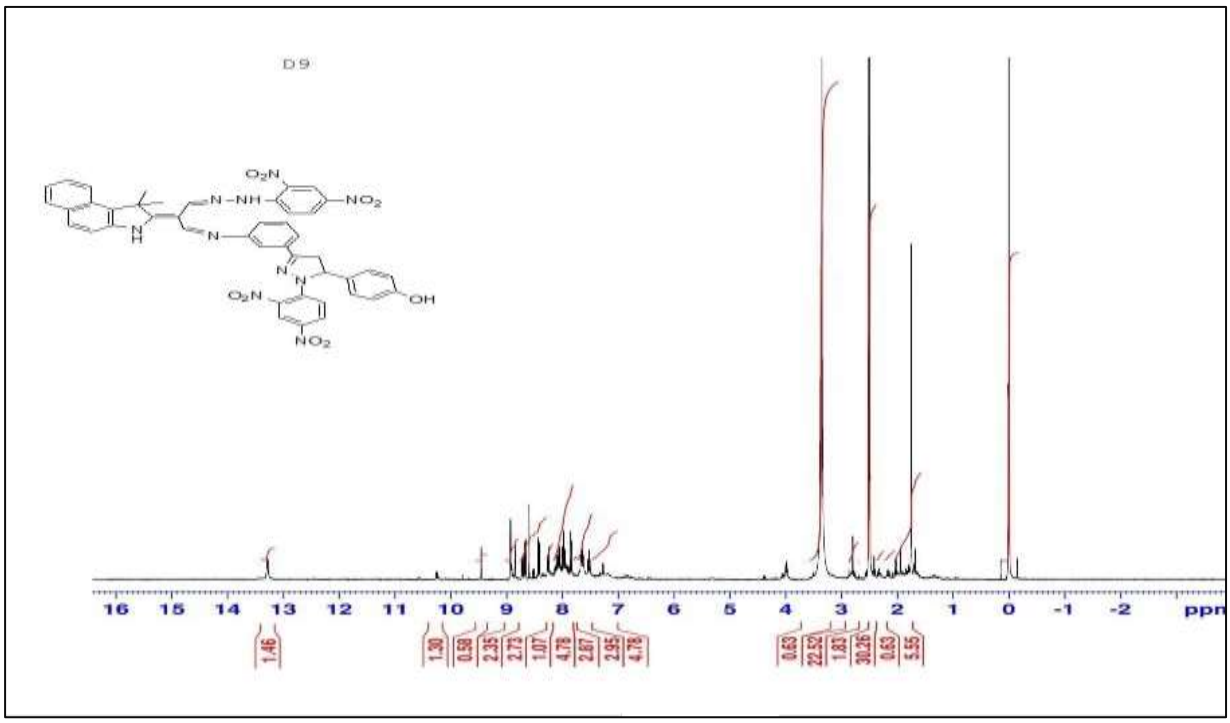

Figure 6: The ^{1}H-NMR spectra of the compound (A_7).

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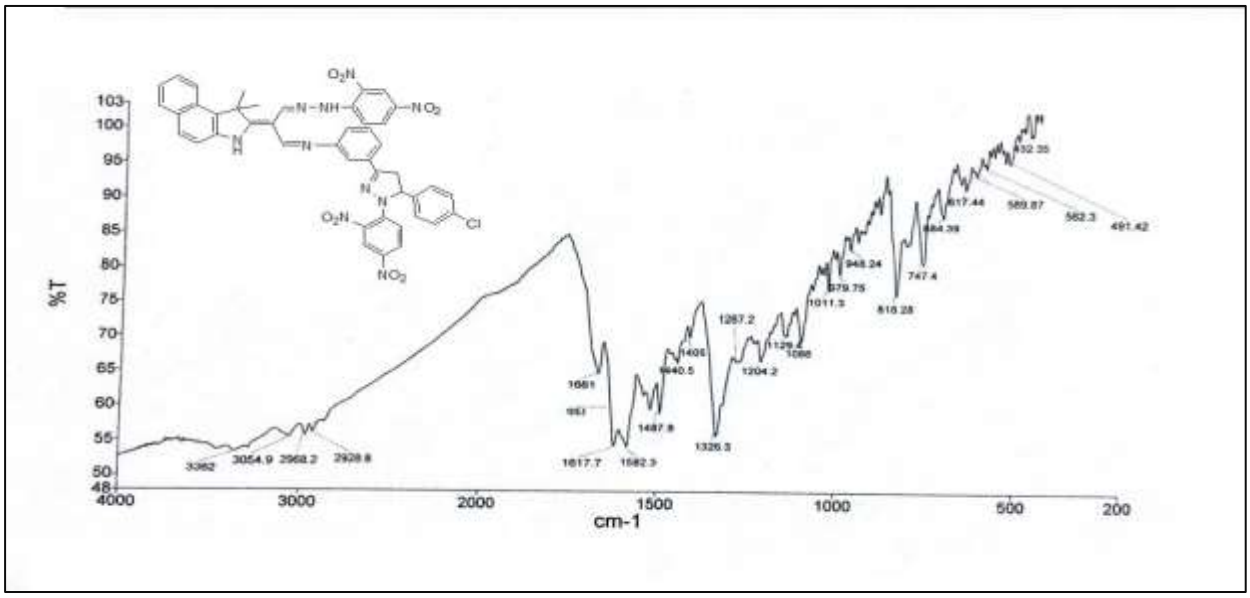

Figure 7: The FT-IR spectra of the compound (A_8)

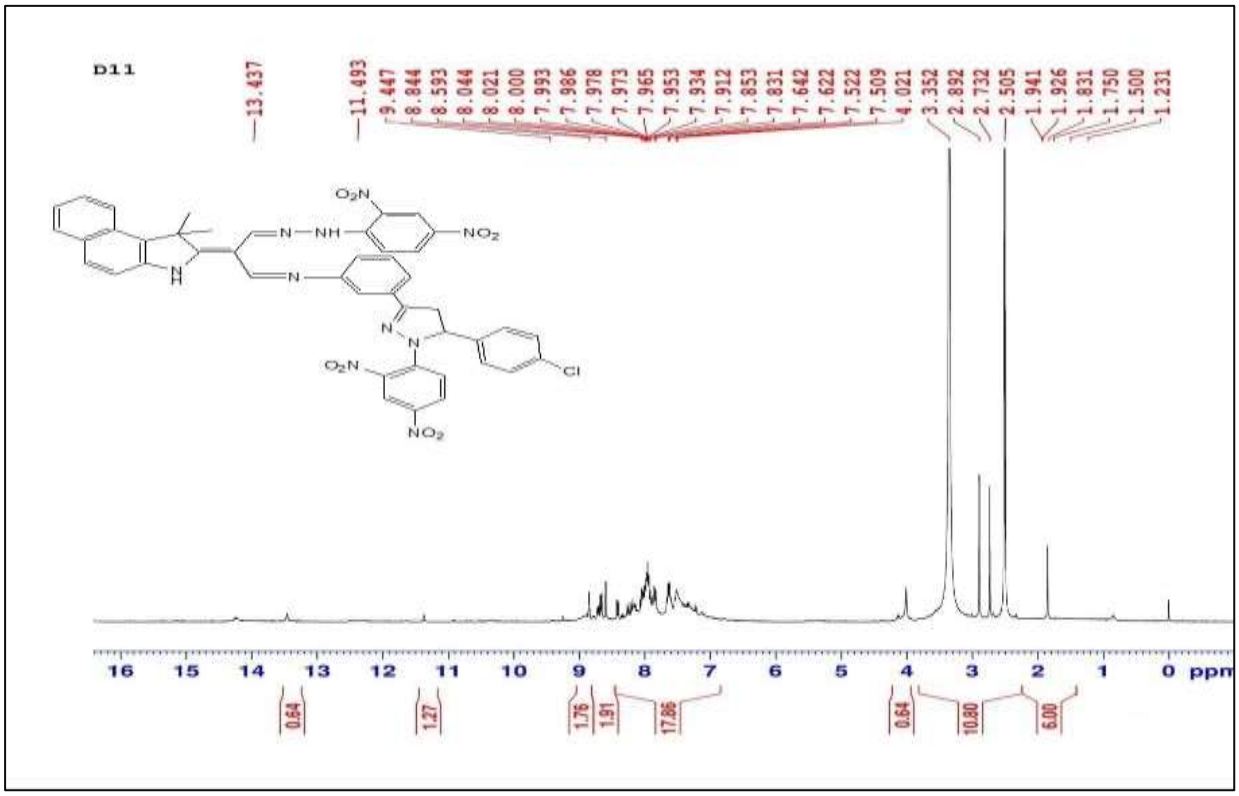

Figure 8: The ^{1}H-NMR spectra of the compound (A_8)

2. Biological results

The three new composite vehicles (A_3, A_6 and A_7) were tested in vitro on the human breast cancer cell line MCF7 at two different concentrations (50 and 100 µg/mL) over the course of 24 hours at 37 degrees. Our results showed that among the other vehicles with different concentrations installed, compound [A_3] had the highest level of cytotoxicity, with an inhibition rate of 46.33 percent at a concentration of 100 µg/ml. Results for compound [A_6] were released. For concentrations of 50 and 100 µg/ml respectively, reliance on them to focus produced inhibition rates of 20.21 and 31.67 percent. At 50 concentrations and 100 µg/ml, in the last the Compound [A_7] showed inhibition rates of 20.07 and 36.45 percent respectively.

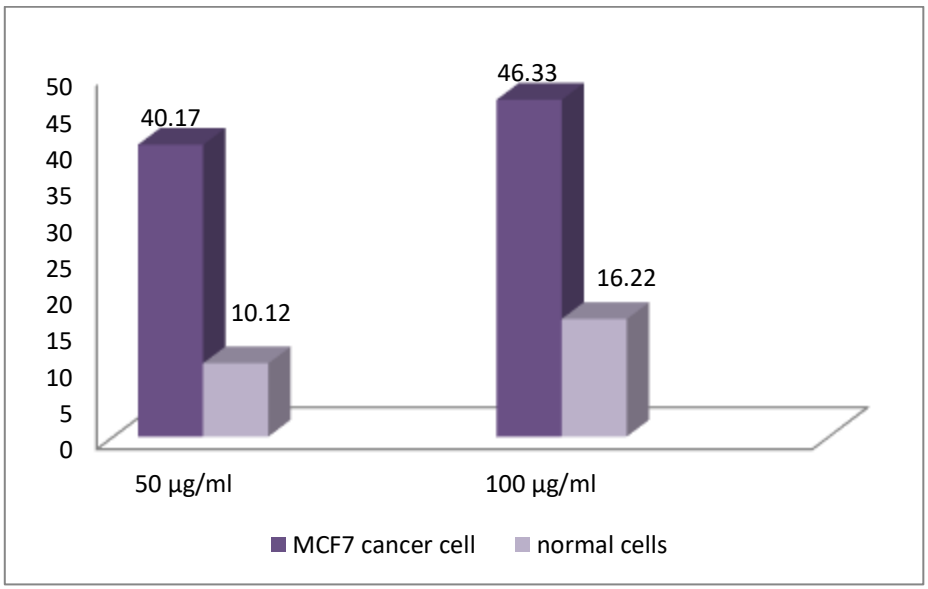

Figure 9: MCF7 and MEF cell line treated with compound (A_3) concentrations (50 and 100) µg/ml for 24 hours

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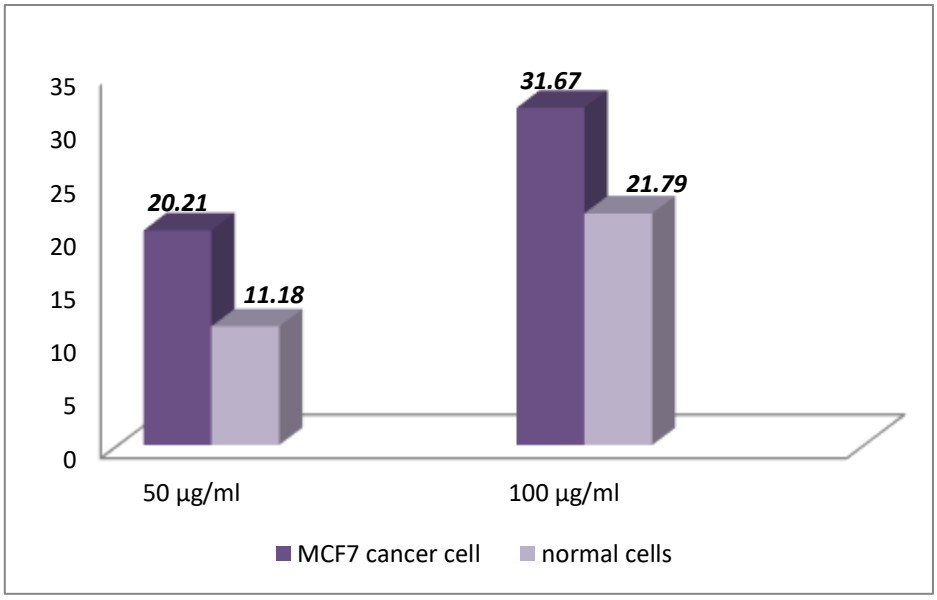

Figure 10: MCF7 and MEF cell line treated with compound (A_6) concentrations (50 and 100) µg/ml for 24 hours

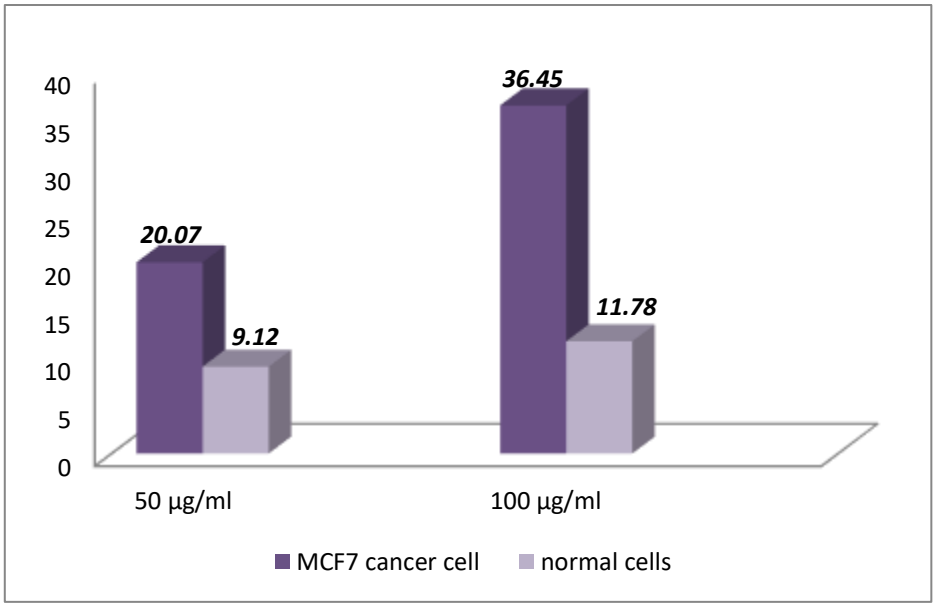

Figure 11: MCF7 and MEF cell line treated with compound (A_7) concentrations (50 and 100) µg/ml for 24 hours

Conclusions

The current endeavor resulted in the production of novel chalcone derivatives and pyrazoline derivatives. These compounds were characterized by a number of spectroscopic methods, including FT-IR and ^{1}H-NMR and assessments of some of their physical properties. The MCF7 human breast cancer cell line was used to test the effectiveness of target compounds as cytotoxic agents. The results showed that this compound may have had cytotoxic effects on the MCF7 cell line.

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