Synthesis, Characterization and Study Biological Activity for New (Heterocyclic) Compounds from Chalcone Derivative

Tiba ibrahim[1], Shaimaa Adnan[2]
[1]Department of Chemistry, College of Science, University of Al-Qadisiyah, Iraq.
[2]Department of Chemistry, College of Education, University of Al-Qadisiyah Iraq.
Email: tiba.alsaman@qu.edu.iq

Abstract
This research involve synthesis heterocyclic compound by many steps. The first step react 2-amino-6-methoxy benzothiazole with 2,4-dimethoxy acetophenone to get azo compound (1) . The second step involve react (1) with 4-dimethyl amino benzaldehyde to get chalcone compound (2) . the last step is react (2) with hydrazine, phenyl hydrazine , to form pyrazol derivatives (3-4) and react compound (2) with malononitrile and ethylcyano acetate to form pyridine derivatives (5-6) and react compound (2) with thiourea to obtain the thiazine derivative (7). All prepared compounds have been diagnosed by (FT-IR and 1H-NMR) Spectroscopy and after diagnosis of spectra compound their biological effect was studied on two type anticancer and antioxidant

Add Keywords: Heterocyclic compounds, Azo compound, Chalcones , Pyrazol, Thiazine

1. Introduction

Heterocyclic compounds are considered one of the classes of organic compounds and are divided according to the type of atoms that make up that ring, either they are homocyclic, such as cyclobutane, cyclopropane and cyclopentane **[1]** Heterocyclic compounds have a wide range of applications. They are used as pharmaceuticals, veterinary products, and agricultural chemicals, as well as as corrosion inhibitors, antioxidants, and other functions **[2]** and their clinical applications are also used as antitumor, antifungal, antiviral, and anti-inflammatory drugs **[3]** Chalcones They are unsaturated ketone compounds containing the reactive ketoethylene group (CO-CH=CH-) which gives colored compounds due to the presence of the chromophore group(CO-CH=CH) **[4]** These compounds belong to the family of flavonoids known as 1,3-diarylprop-2-en-1-one, and exist in the form of α, β- unsaturated ketones with two aromatic rings (A and B) figure (1) **[5]** and are widely distributed in fruits, vegetables and tea spices, etc. They appear in multiple stereotypes and depend in their properties on the compensators of the aromatic rings, in addition to the carbonyl group, the alkene double bond appears The adjacent carbonyls are in two positions Trans (E) and Size (Z), where the trans position is considered to be the most thermodynamically stable **[6]** Shotter et al (1978) prepared chalcones derivatives using the Friedel-Kraft acyl reaction in Lewis acid as a catalyst **[7]** . Visakh Prabhakar and his group they used the Claisen-Schmidt reaction to prepare chalcones, and their effectiveness as antioxidants and anti-inflammatory was studied **[8]**.Chalcones can be used to prepare types of heterocyclic compounds through ring-closing reactions Given the critical importance of chalcones, attention has been paid to their preparation and study of their effectiveness, as well as the study of their applications by researchers, including **[9]** Xianwen Fang and his group who prepared a series of chalcones using the aldol reaction and study Its efficacy against microbes

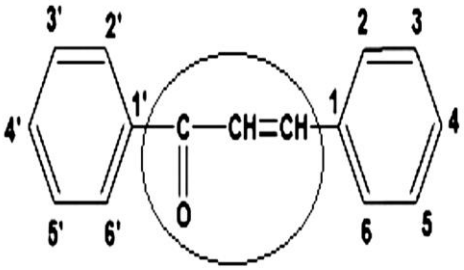

Figure (1) illustrate formula of chalcone

2. Material

All chemicals' compounds in this work were of a high purity, include: 2-amino-6-methoxy benzothiazole, 2,4-dimethoxy acetophenone ,4-dimethyl amino benzaldehyde, (Sigma Aldrich, Germany), hydrazine, phenyl hydrazine, thiourea (Riedel de Haen, Germany), malononitrile, ethyl cyanoacetate (Sigma Aldrich, Germany)

3. Instruments

A Shimadzu FT-IR 8400S KBr disk-shaped infrared was used to take FT-IR spectra (4000-400 cm^{-1}). ^{1}HNMR was measured on a Brucker Fourier transform spectrometer at (500 MHz) with DMSO measurements were measured at University of Tehran, Iran and melting points were measured using a digitizer (Stuart, UK).

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4. Experimental

1.Synthesis of Azo compound (1) [10]

Compound (1) is prepared in two steps:
A- Preparation of diazonium salt : (0.01mol,1.8g) of 2-amino-6-methoxy benzothiazole was taken and dissolved in (30ml) of distilled water and (4ml) of concentrated HCl . (10ml) of a solution (0.01mol,0.68g) of sodium nitrite and then was added drop by drop with stirring for (20 min) and at a temperature ranging from (0-5°C) .
B - Preparation of Azo dye :
(0.01mol 1.8g) of 2,4-dimethoxy acetophenone was dissolved in (30ml) of absolute ethanol and (20 ml) of (5% NaOH) , then the diazonium salt formed in the first step was slowly added to the mixture with continuous stirring and at a temperature ranging from (0-5°C) , pH = 6 was made for the mixture and stirring for one hour , leaving the solution to the next day, filtering the precipitate and washing with distilled water several times and recrystallizing with absolute ethanol . The physical properties were shown in table 1. The general reaction for synthesis of Azo compounds (1) in Scheme 1 [1].

2.Synthesis of chalcone compound (2) [11]

In the second line: (0.001 mol 0.371 g) from compound (1) dissolved in (25) ml of absolute ethanol with stirring then add (0.001mol 0.149 g) of 4-dimethyl amino benzaldehyde then added to the reaction mixture (10 ml) from NaOH 10% in the form of drops with continuous stirring for period 14 hours and at room temperature (25 0C) after that the mixture was neutralized using hydrochloric acid , then the precipitate was collected after filtering , Wash with distilled water and dried and re-crystallized ethanol absolute . The reaction was followed up by TLC using the mobile phase (ethanol: benzene) at a ratio of (1:4). On , knowing that Rf = 0.31. The physical properties were shown in table 1. The general reaction for synthesis of chalcone compound (2) in Scheme 1.

3.Synthesis of pyrazoline compound (3)[12]

(0.001 mol 0.5 g) from compound (2) with (0.001 mol 0.05 ml) from hydrazine and dissolved in 25 mL absolute ethanol and the solution was stirred . The reaction mixture was escalated for 16 hour. The reaction was followed up by TLC using the mobile phase (ethanol: benzene) at a ratio of (1:4). On , knowing that R_f = 0.31The physical properties were shown in table 1. The general reaction for synthesis of pyrazoline compound (3) in Scheme 1.then the precipitate was filtered off and recrystallized with absolute ethyl alcohol. The physical properties were shown in table 1"

4-Synthesis of pyrazoline compounds (4)[13]

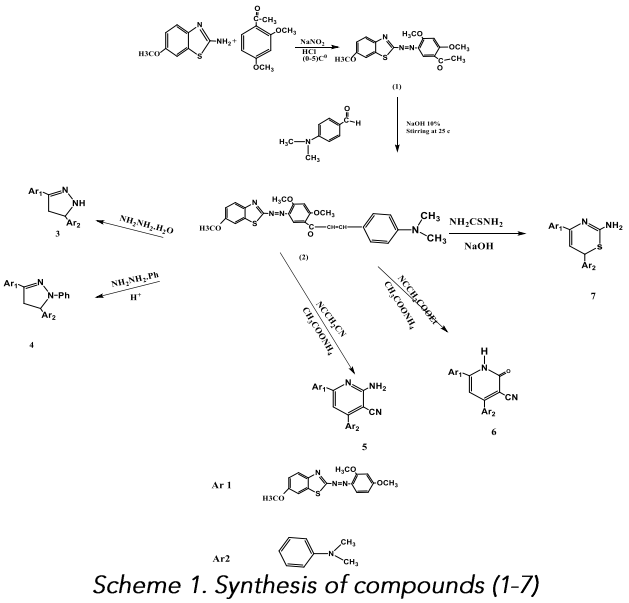
(0.001 mol 0.5 g) from compound (2) and dissolved in 35 mL absolute ethanol and the solution was stirred continuously until dissolved then added (0.1 ml)from phenyl hydrazine and few drops from glacial acetic acid to reaction mixture. The reaction mixture was escalated for 21 hour. The reaction was followed up by TLC using the mobile phase (ethanol: benzene) at a ratio of (1:4). On , knowing that Rf = 0.31The physical properties were shown in table 1. The general reaction for synthesis of pyrazoline compound (4) in Scheme 1.then the precipitate was filtered off and recrystallized with absolute ethyl alcohol. The physical properties were shown in table 1" .

5-Synthesis of pyridine compound (5-6)[14]

(0.001 mol 0.5 g) from compound (2) dissolved in 30 mL absolute ethanol and the solution was stirred continuously until dissolved, then 0.001 mol 0.066 g from malononitrile and 0.1 ml from ethyl cyano acetate in addition to 0.002 mol of 0.15 g from Ammonium acetate as a catalyst were added. The reaction mixture was escalated for 20 hour. The reaction was followed up by TLC using the mobile phase (ethanol: benzene) at a ratio of (1:4). On , knowing that R_f = 0.3. The physical properties were shown in table 1. The general reaction for synthesis of pyridine compound (5-6) in Scheme 1.then the precipitate was filtered off and recrystallized with absolute ethyl alcohol. The physical properties were shown in table 1"[2]

6- Synthesis of thiazine compound (7)[15]

(0.01 mol 0.5 g) from compound (2) dissolved in 10 ml of alcoholic sodium hydroxyl solution with 0.001 mol 0.076 g from thiourea and the solution was stirred continuously until dissolved. The reaction mixture was escalated for 20 hour. The reaction was followed up by TLC using the mobile phase (ethanol: benzene) at a ratio of (1:4). On , knowing that Rf = 0.33. The physical properties were shown in table 1. The general reaction for synthesis of thiazine compound (7)[3] in Scheme 1.then the precipitate was filtered off and recrystallized with absolute ethyl alcohol. The physical properties were shown in table 1" .[3]



Scheme 1. Synthesis of compounds (1-7)

5. Results and Discussion

1. Derivative (1) 1-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)ethan-1-one

FT-IR showed band at 2939 cm-1 for C-H aliphatic, 3070 cm-1 for C-H aromatic, 1419 cm-1 for N=N group, 1650 cm-1 for C=O group, 1558 cm-1 for C=C aromatic and 1593 cm-1 for C=N . 1H-NMR of derivative (1) showed δ : (3.9 (S,3H,(CH3)17), 3.7 (S,3H,(OCH3)16) , 2.5 (S,3H,(OCH3)14) , 2.4 (S,3H,(OCH3)15) and 6.5-7.6 (M, 5H, Ar-H)

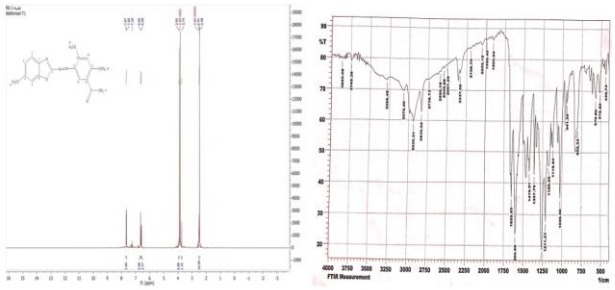


Figure 2 . a-^{1}H-NMR Spectrum of the Compound (1), b-FT-IR Spectrum of the Compound (1)

2. Derivative(2) 1-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-3-(4-(dimethylamino)phenyl)prop-2-en-1-one

FT-IR showed band at 2939 cm^{-1} for C-H aliphatic, 3170 cm^{-1} for C-H aromatic, 1434 cm^{-1} for N=N group, 1658 cm^{-1} for C=O group, 1512 cm^{-1} for C=C aromatic , 1604 cm^{-1} for C=N ,1550 cm^{-1} for C=C aliphatic . ^{1}H-NMR of derivative (2) showed δ : 4.08 (S,3H,$(CH_3)_{22}$) , 3.82 (S,3H,$(CH_3)_{23}$) , 3.79 (S,3H,$(CH_3)_{24}$), 3.05 (S,6H,$(CH_3)_{25,26}$ 6.7-7.9 (M,9H,Ar-H), 6.8 (d,1H,$(CH)_{15}$) 6.7 (d,1H,$(CH)_{16}$).

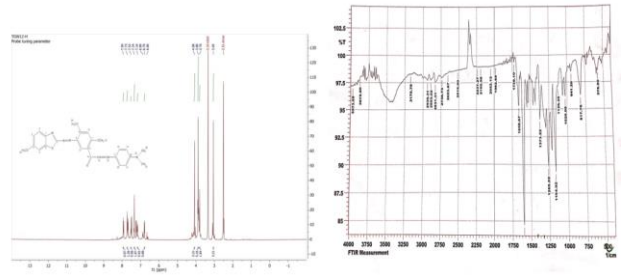


Figure 3a-1H-NMR Spectrum of the Compound (2), b-FT-IR Spectrum of the Compound (2)

3. Derivative (3) 4-(3-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl) diazenyl)phenyl)-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimethylaniline

FT-IR showed band at 3440 cm^{-1} for NH , 2916 cm^{-1} for C-H aliphatic, 3085 cm^{-1} for C-H aromatic ,1419 cm^{-1} for N=N group, 1550 cm^{-1} for C=N thiazole , 1604 cm^{-1} for C=N pyrazoline ,1519 cm^{-1} for C=C aromatic . ^{1}H-NMR of derivative (3) showed δ : 4.08 (S,3H,$(CH_3)_{23}$) , 3.8 (S,3H,$(CH_3)_{24}$), 3.7 (S,3H,$(CH_3)_{25}$), 3 (S,6H,$(CH_3)_{26,27}$) , 8.5 (S,1H,NH) , 2.3 (t,1H ,$(CH)_{14}$, 2.1 (d,2H,$(CH_2)_{15}$), 6.6-7.9 (M,9H,Ar-H).

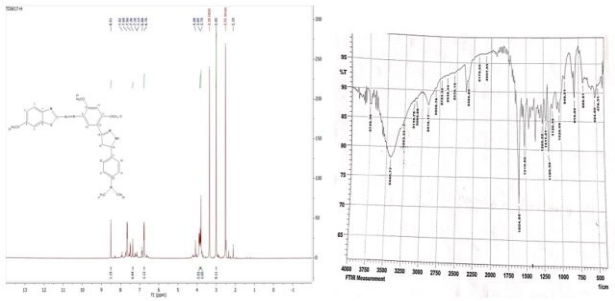


Figure 4. a-^{1}H-NMR Spectrum of the Compound (3), b-FT-IR Spectrum of the Compound (3)

4. Derivative (4) 4-(3-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimethylaniline

FT-IR showed band at 2931 cm^{-1} for C-H aliphatic, 3085 cm^{-1} for C-H aromatic ,1419 cm^{-1} for N=N group, 1650 cm^{-1} for C=N pyrazoline , 1558 cm^{-1} for C=N thiazole ,1520 cm^{-1} for C=C aromatic . ^{1}H-NMR of derivative (4) showed δ : 4.08 (S,3H,$(CH_3)_{29}$) , 3.9 (S,3H,$(CH_3)_{30}$) , 3.8 (S,3H,$(CH_3)_{31}$), 3.05 (S,6H,$(CH_3)_{32,33}$, 6.8-7.9(M,14H,Ar-H),1.2 (t,1H,$(CH)_{15}$,1.9 (d,2H,$(CH_2)_{16}$

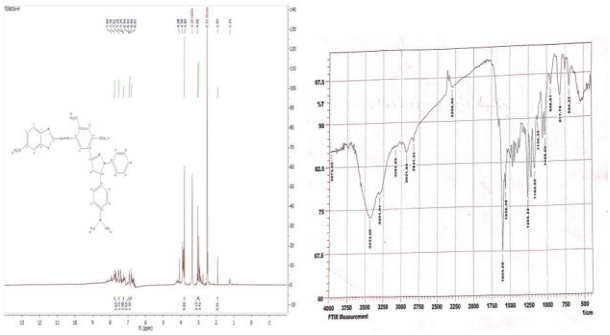


Figure 5 . a-1H-NMR Spectrum of the Compound (4), b- FT-IR Spectrum of the Compound (4)

5. Derivative (5) 2-amino-6-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-4-(4-(dimethylamino)phenyl)nicotinonitrile

FT-IR showed band at 3433 cm^{-1} for NH_2 , 2931 cm^{-1} for C-H aliphatic, 3078 cm^{-1} for C-H aromatic ,1414 cm^{-1} for N=N group, 1604 cm^{-1} for C=N pyridine , 1558 cm^{-1} for C=N thiazole ,1512 cm^{-1} for C=C aromatic ,2214 cm^{-1} for CN . ^{1}H-NMR of derivative (5) showed δ : 4 (S,3H,$(CH_3)_{25}$) , 3.9 (S,3H,$(CH_3)_{26}$) , 3.7 (S,3H,$(CH_3)_{27}$), 3.12 (S,6H,$(CH_3)_{29,30}$) , 5.02 (S,2H,,NH_2), 6.8-8.07 (M,10H,Ar-H).

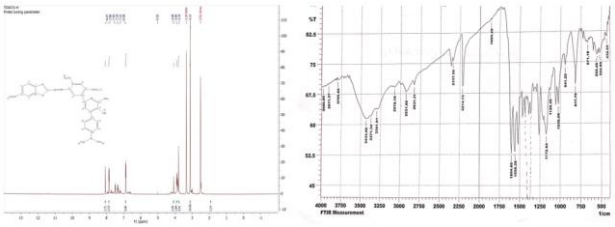


Figure 6 . a-^{1}H-NMR Spectrum of the Compound (5), b-FT-IR Spectrum of the Compound (5)

6. Derivative (6) 6-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl) diazenyl)phenyl)-4-(4-(dimethylamino)phenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile

FT-IR showed band at 3425 cm^{-1} for NH , 2908 cm^{-1} for C-H aliphatic, 3078 cm^{-1} for C-H aromatic ,1434 cm^{-1} for N=N group, 1710 cm^{-1} for C=O , 1658 cm^{-1} for C=N thiazole ,1596 cm^{-1} for C=C aromatic ,2214 cm^{-1} for CN . ^{1}H-NMR of derivative (6) showed δ : 4 (S,3H,$(CH_3)_{25}$) , 3.9 (S,3H,$(CH_3)_{26}$) , 3.8 (S,3H,$(CH_3)_{27}$), 2.99 (S,6H,$(CH_3)_{28,29}$) , 9.6 (S,1H,NH), 8.11 (S,1H,$(CH)_{18}$), 6.8-7.9 (M,9H,Ar-H).

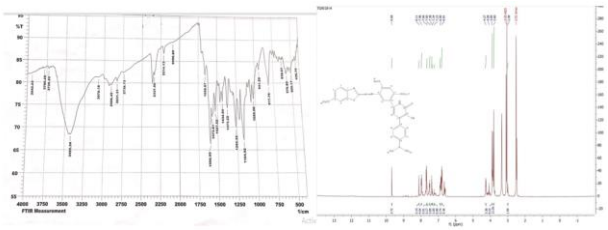


Figure 7 . a-^{1}H-NMR Spectrum of the Compound (6), b-FT-IR Spectrum of the Compound (6)

7. Derivative (7) 4-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-6-(4-(dimethylamino)phenyl)-6H-1,3-thiazin-2-amine

FT-IR showed band at 3394 cm^{-1} for NH_2 , 3178 cm^{-1} for C-H aromatic ,2977 cm^{-1} for C-H aliphatic, 1458 cm^{-1} for N=N group, 1650 cm^{-1} for C=N thiazine , 1589 cm^{-1} for C=N thiazole,1550 cm^{-1} for C=C aromatic, 1026 cm^{-1} for C-S . ^{1}H-NMR of derivative (7) showed δ : 4.08 (S,3H,$(CH_3)_{24}$) , 3.97 (S,3H,$(CH_3)_{25}$) , 3.79(S,3H,$(CH_3)_{26}$), 3 (S,6H,$(CH_3)_{27,28}$),1.2 (d,1H,$(CH)_{15}$) , 2.1(d,1H,$(CH_3)_{16}$), 6.8-7.9(M(9H),Ar-H , 5 (S,2H,NH_2).

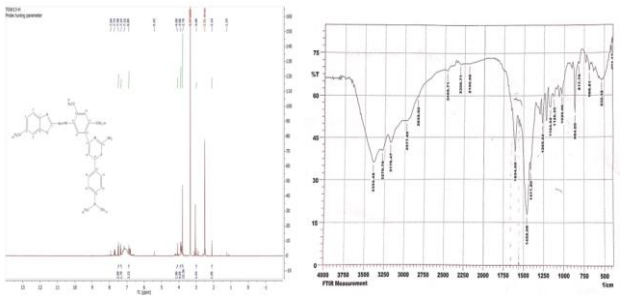


Figure8. a-1H-NMR Spectrum of the Compound (7), b-FT-IR Spectrum of the Compound (7)

TABLE 1. Physical properties of the derivatives (1-7)

Derivatives	Colour	M.P (°C)	M,Wt (g/mol)
1	Brown	53-55	372
2	Dark Brown	196-198	502.59
3	Brown	235-237	516.62
4	Red	184-186	592.72
5	Brown	175-177	565.65
6	black	Sticky	566.64
7	Brown	207-209	560.69

6. Biological Study

The purpose of this part of study, was to investigate cell proliferation under multiple conditions and to draw attention to the importance of cellular NAD+ in prostate cancer cells to find a link between the change in intracellular NAD+ levels and compound (7) . To do this, a PC3 as prostate cancer cell line and WRL68- as non-cancerous cells were used. Cells were treated with compound (7) for 24 hours (Figure 10) to find the effect of supplementation with compound (7) or decrease its degradation on cancer cell proliferation. The results showed anti-proliferative effect, it is clearly confirmed that cell proliferation (cell vitality) was decrease with increase of concentration of compound (7)

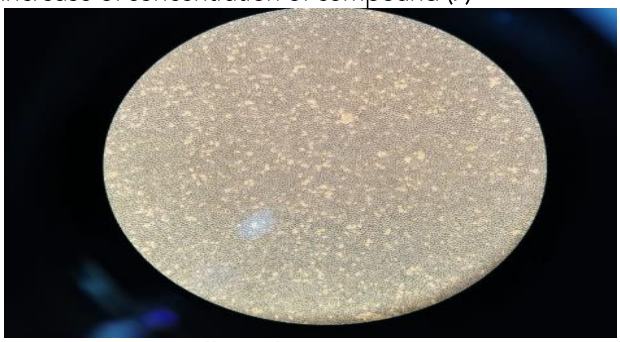


Figure (9) Effect of treatment on living cells

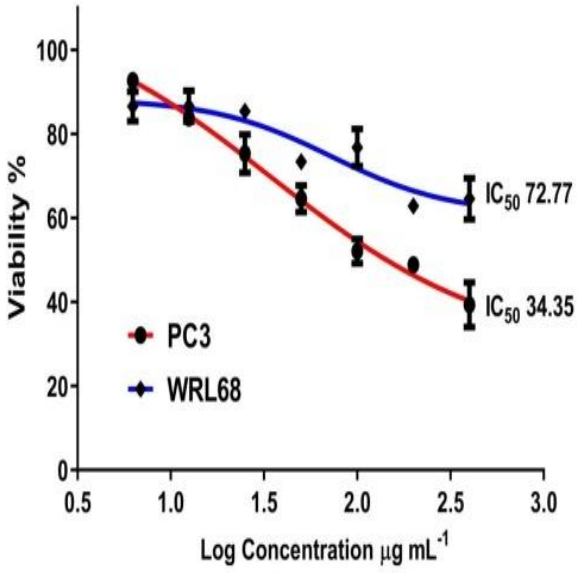


Figure (10) IC50 for comp.7

And study antioxidant The purpose of this part of study, was to investigate lipid peroxides levels under multiple conditions by use different concentrations of compound 7 with Ascorbic acid (Figure 11) Table (2) show that in high concentration Scavenging activity increase

TABLE 2. treatment different concentration of compound (7) with Ascorbic acid and comp. (2)					
Conc.	PC3			WRL68	
	Mean	SD		Mean	SD
400.00	39.31	5.33	3.00	64.54	4.88
200.00	48.84	0.12	3.00	62.85	1.95
100.00	52.08	2.92	3.00	76.78	4.45
50.00	64.55	3.14	3.00	73.34	1.29
25.00	75.31	4.56	3.00	85.38	1.14
12.50	83.80	1.86	3.00	86.57	3.71
6.25	92.67	1.05	3.00	86.54	3.54

TABLE 3. treatment different concentration of compound (7) with comp. (2)					
Number of values	200	100	50	25	12.5
	3	3	3	3	3
Mean	74.27	59.22	40.63	28.97	20.60
Std. Deviation	5.374	5.045	3.713	4.263	6.559
Std. Error of Mean	3.103	2.913	2.144	2.461	3.787

7. Conclusion

1- Stable heterocyclic derivatives can be prepared
2- Derivative (2) has good biological activity in anti-cancer and antioxidant.
3- The effect of the substituted group on the reaction time

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