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Synthesis, Characterization For New (Heterocyclic) Compounds From 2-Amino-6-Methoxy Benzothiazole And Study Biological Activity

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ABSTRACT

The aim of the present study is 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 vaniline to get chalcone compound (2) . the last step is react (2) with hydrazine, phenyl hydrazine , 2,4-dinitro phenyl hydrazine to form pyrazol derivatives (3-5) and react compound (2) with malononitrile to form pyridine derivative (6) and react compound (2) with quandine hydrochloride to obtain the pyrimidine derivative (7) and react compound (2) with thiourea to obtain thiazine derivative(8) . All prepared compounds have been diagnosed by (FT-IR and ^{1}H-NMR) Spectroscopy and after diagnosis of spectra compound their biological effect was studied on two type anticancer and antioxidant .

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 [1]. Heterocyclic and heterogeneous, which is the type under study, which is one of the most important compounds that entered in many fields [2, 3]. Pyrazoline It is one of the heterogeneous organic compounds and is five-ring and consists of two nitrogen atoms and three adjacent carbon atoms and pyrazoline has only one double bond[4] . Pyrazolin and its derivatives are compounds that have a wide activity. It has been found to have pharmacological activities such as antimicrobials, anti-inflammatory, analgesics, antidepressants [5,6] antioxidants, antihy pertensives [7], anti-cancer, antispasmodic, in addition to its role as antipyretics.[8,9] pyridineIt is a heterocyclic organic compound with six atoms of one nitrogen atom and five carbon atoms, with the molecular formula C_5H_5N similar to benzene with the CH group replaced by a nitrogen atom Pyridine has a major role in stimulating chemical and biological reactions and in some enzymes such as NADP, which participates in some oxidation and reduction processes. Pyridine is present in the vitamins pyridoxine (vitamin B6) and niacin, as well as highly toxic alkaloids such as nicotine, and it is found in plants extensively as well [10,11].Pyrimidine It is a heterocyclic organic compound with a hexacyclic formula $C_4H_4N_2$ similar to pyridine and benzene, containing two nitrogen atoms at positions 1 and 3 of the hexagonal ring. Pyridazine has nitrogen atoms at positions 1 and 2 of the ring. [12]

MATERIAL

All chemicals compounds in this work were of a high purity , include : 2-amino-6-methoxy benzothiazole, 2,4-dimethoxy acetophenone ,vaniline,(Sigma Aldrich, Germany) , hydrazine, phenyl hydrazine , 2,4-dinitro phenyl hydrazine (Riedel de Haen, Germany) , malononitrile , quandine hydrochloride(Sigma Aldrich, Germany) ,thiourea ,(Sigma Aldrich, Germany)

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).

EXPERIMENTAL

1.Synthesis of Azo compound (1) [13]

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.

2.Synthesis of chalcone compound (2) [14]

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 0.152 g) of vanillin then added to the reaction mixture (10 ml) from NaOH 10% in the form of drops with continuous stirring for period 13 hours and at room temperature (25 c) 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 R_f = 0.34. The physical properties were shown in table 1. The general reaction for synthesis of chalcone compound (2) in Scheme 1.

3.Synthesis of pyrimidine compound (3)[15]

(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 of quandenehy drochloride and 0.002 mol of NaOH were added. The reaction mixture was escalated for 22 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 = 0.33. The physical properties were shown in table 1. The general reaction for synthesis of pyrimidine 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 pyridine compound (4)[16]

(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 0.002 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 (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 pyrazoline compounds (5-6)[17]

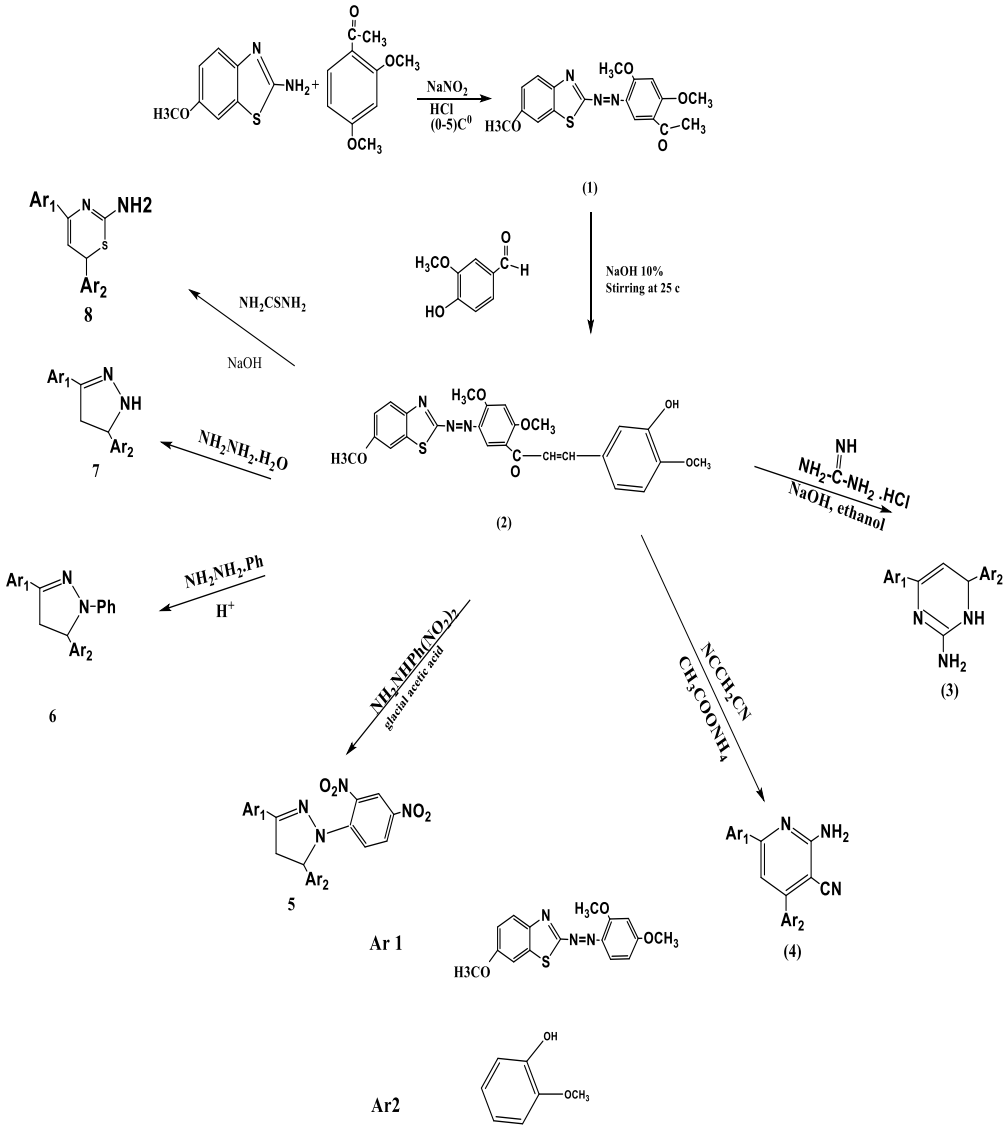
(0.001 mol 0.5 g) from compound (2) and dissolved in 35 mL absolute ethanol and the solution was stirred continuously until dissolved then added (0.1 ml)from phenyl hydrazine (0.2 g from 2,4-dinitro phenyl hydrazine and few drops from glacial acetic acid to reaction mixture. The reaction mixture was 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 R_f = 0.31The physical properties were shown in table 1. The general reaction for synthesis of pyrazoline 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.

6.Synthesis of pyrazoline compound (7)[18]

(0.001 mol 0.5 g) from compound (2) with (0.001 mol ,0.5 ml)from hydrazine and dissolved in 25 mL absolute ethanol and the solution was stirred continuously until dissolved. 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 = 0.32The physical properties were shown in table 1. The general reaction for synthesis of pyrazoline compound (7) in Scheme 1.then the precipitate was filtered off and recrystallized with absolute ethyl alcohol. The physical properties were shown in table 1" .

7.Synthesis of thiazine compound (8)[19]

(0.001 mol 0.5 g) from compound (2) dissolved in 10 mL alcoholic sodium hydroxide solution with (0.001 mol ,0.076 gm from thiourea. The reaction mixture was escalated for 23 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 thiazine compound (8) in Scheme 1.then the precipitate was filtered off and recrystallized with absolute ethyl alcohol. The physical properties were shown in table 1"

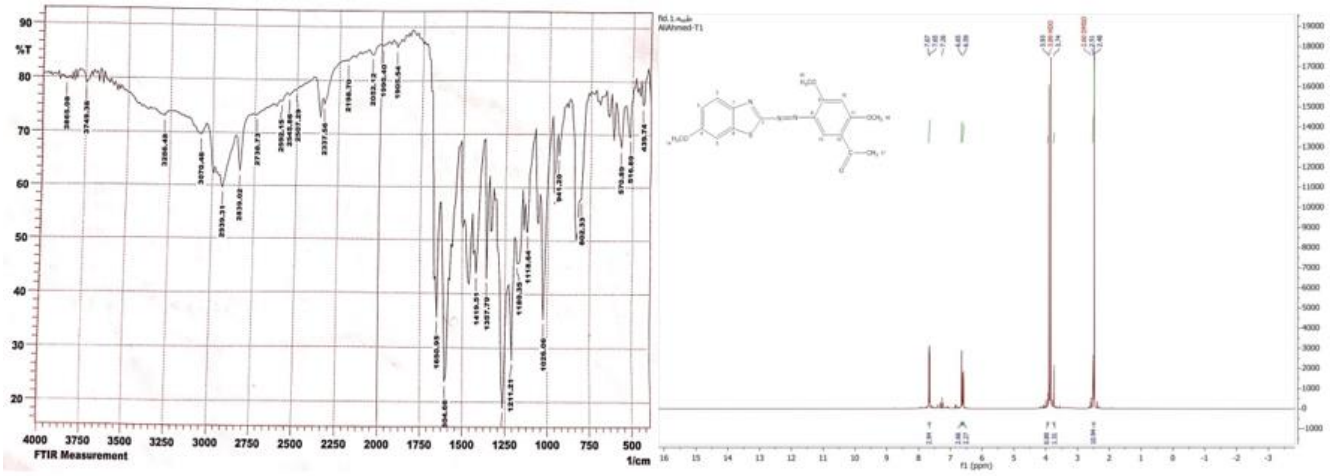


Scheme 1. Synthesis of compounds (1-8)

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 . ^{1}H-NMR of derivative (1) showed δ : (3.9 (S,3H,$(CH_3)_{17}$) , 3.7 (S,3H,$(OCH_3)_{16}$) , 2.5 (S,3H,$(OCH_3)_{14}$) , 2.4 (S,3H,$(OCH_3)_{15}$) and 6.5-7.6 (M, 5H,Ar-H) .

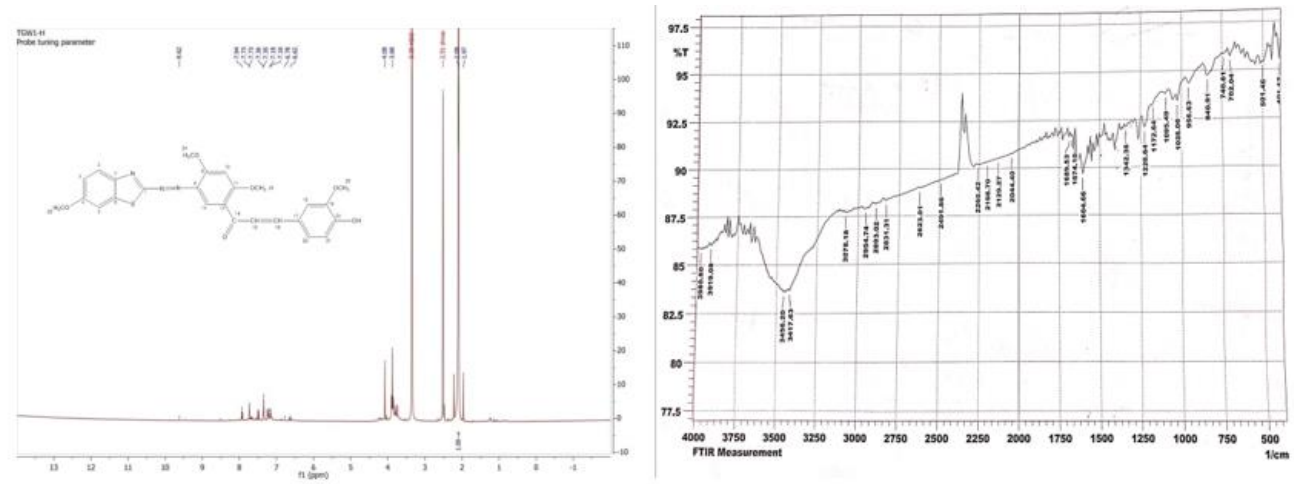


(a) (b)

Figure 1 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-hydroxy-3-methoxyphenyl) prop-2-en-1-one**

FT-IR showed band at 2954 cm^{-1} for C-H aliphatic, 3078 cm^{-1} for C-H aromatic, 1419 cm^{-}1 for N=N group, 1674 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 : (9.62 (S,1H,OH) , 1.9 (S,3H,$(OCH_3)_{23}$) , 2.01 (S,3H,$(OCH_3)_{24}$) , 3.8 (S,3H,$(OCH_3)_{26}$), 4.08 (S,3H,$(OCH_3)_{25}$, 6.5-7.9 (M,8H), 7.18,7.16 (d,2H,$(CH)_{15,16}$).

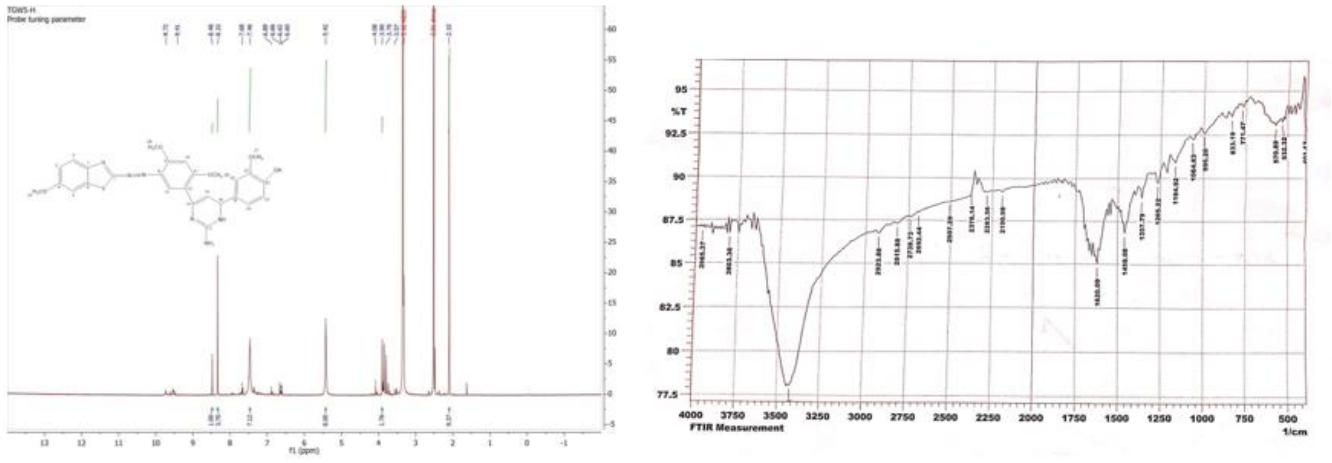


(a) (b)

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

3. Derivative (3) 4-(2-amino-6-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-3,4-dihydropyrimidin-4-yl)-2-methoxyphenol

FT-IR showed band at 3425 cm^{-1} for NH_2 , 2923 cm^{-1} for C-H aliphatic, 1458 cm^{-1} for N=N group ,1550 cm^{-1} for C=C aromatic , 1620 cm^{-1} for C=N pyrimidine ,1570 cm^{-1} for C=N thiazole . ^{1}H-NMR of derivative (3) showed δ : (9.7 (S,1H,OH) , 5.4(S,2H,NH_2) , 4.08 (S,3H,$(OCH_3)_{24}$) , 3.9 (S,3H,$(OCH_3)_{25}$) , 3.7 (S,3H,$(OCH_3)_{26}$), 3.5 (S,3H,$(OCH_3)_{27}$, 2.1 (d,1H,$(CH)_{16}$), 6.6 (d,1H,$(CH)_{15}$),6.8-8.4 (M,8H,Ar-H).

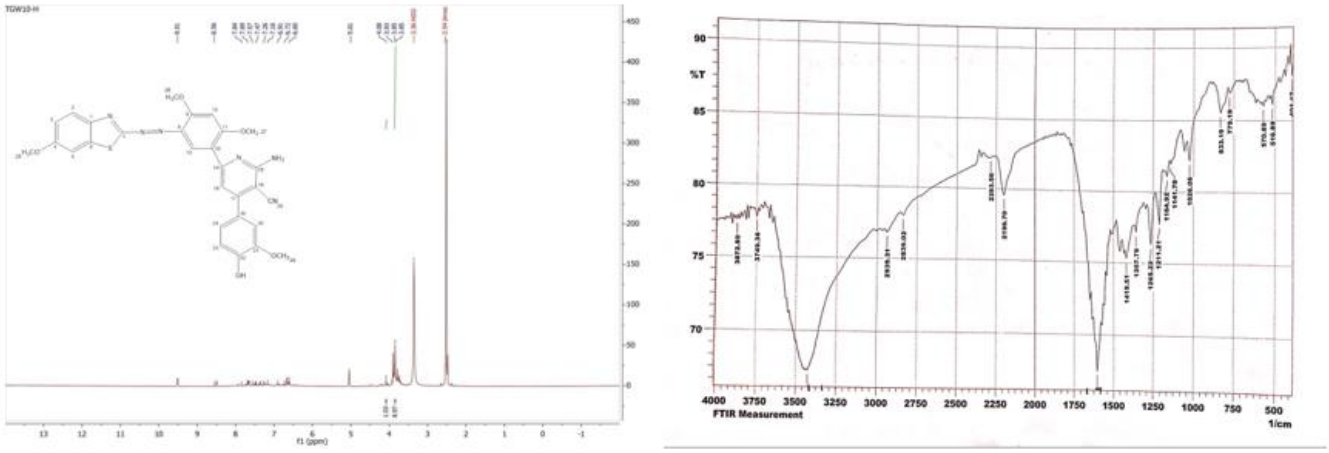


(a) (b)

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

4. Derivative (4) 2-amino-6-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-4-(4-hydroxy-3-methoxyphenyl)nicotinonitrile

FT-IR showed band at 3425 cm^{-1} for NH_2 , 2939 cm^{-1} for C-H aliphatic, 3008 cm^{-1} for C-H aromatic ,1419 cm^{-1} for N=N group, 1616 cm^{-1} for C=N pyrimidine , 1566 cm^{-1} for C=N thiazole ,1512 cm^{-1} for C=C aromatic ,2198 cm^{-1} for CN . ^{1}H-NMR of derivative (4) showed δ : 9.5 (S,1H,OH) , 5.01(S,2H,NH_2) , 4.08 (S,3H,$(OCH_3)_{25}$) , 3.93 (S,3H,$(OCH_3)_{26}$) , 3.85 (S,6H,$(OCH_3)_{27,28}$), 8.5 (S,1H,$(CH)_{18}$),6.6-7.8 (M,8H,Ar-H).

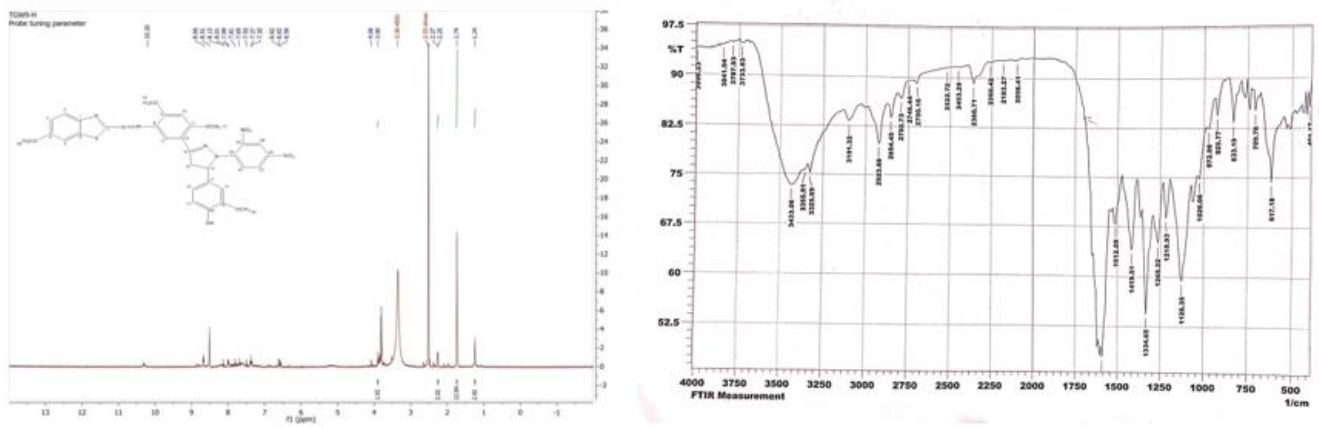


(a) (b)

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

5. Derivative (5) 4- (3- (2,4-dimethoxy-5-((6-methoxy benzo [d] thiazol-2-yl) diazenyl) phenyl) -1-(2,4-dinitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol

FT-IR showed band at 2923 cm^{-1} for C-H aliphatic, 3101 cm^{-1} for C-H aromatic ,1419 cm^{-1} for N=N group, 1620 cm^{-1} for C=N pyrazoline , 1545 cm^{-1} for C=N thiazole ,1512 cm^{-1} for C=C aromatic . ^{1}H-NMR of derivative (5) showed δ : 10.2 (S,1H,OH) , 4(S,3H,$(CH_3)_{29}$) , 3.9 (S,3H,$(CH_3)_{30}$) , 2.27 (S,3H,$(CH_3)_{31}$) , 2.27 (S,3H,$(CH_3)_{32}$) ,1.2 (d,2H,$(CH_2)_{16}$), 1.7 (t,1H,$(CH)_{15}$),6.5-8.6 (M,11H,Ar-H).

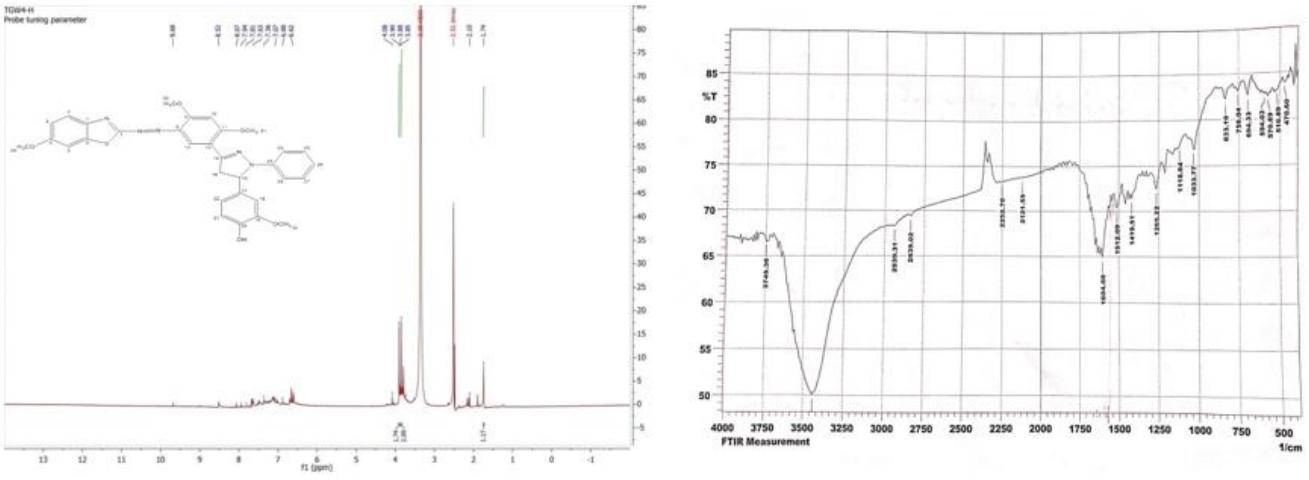


(a) (b)

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

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

FT-IR showed band at 2939 cm^{-1} for C-H aliphatic, 3001 cm^{-1} for C-H aromatic ,1419 cm^{-1} for N=N group, 1650 cm^{-1} for C=N pyrazoline , 1596 cm^{-1} for C=N thiazole ,1512 cm^{-1} for C=C aromatic . ^{1}H-NMR of derivative (6) showed δ : 4.08 (S,3H,$(OCH_3)_{32}$) , 3.9 (S,3H,$(OCH_3)_{31}$) , 3.88 (S,3H,$(OCH_3)_{30}$),3.85 (S,3H,$(OCH_3)_{29}$),2.1 (t,1H,$(CH)_{15}$), 1.7 (d,2H,$(CH_2)_{16}$, 6.6-8.5(M,13H,Ar-H)

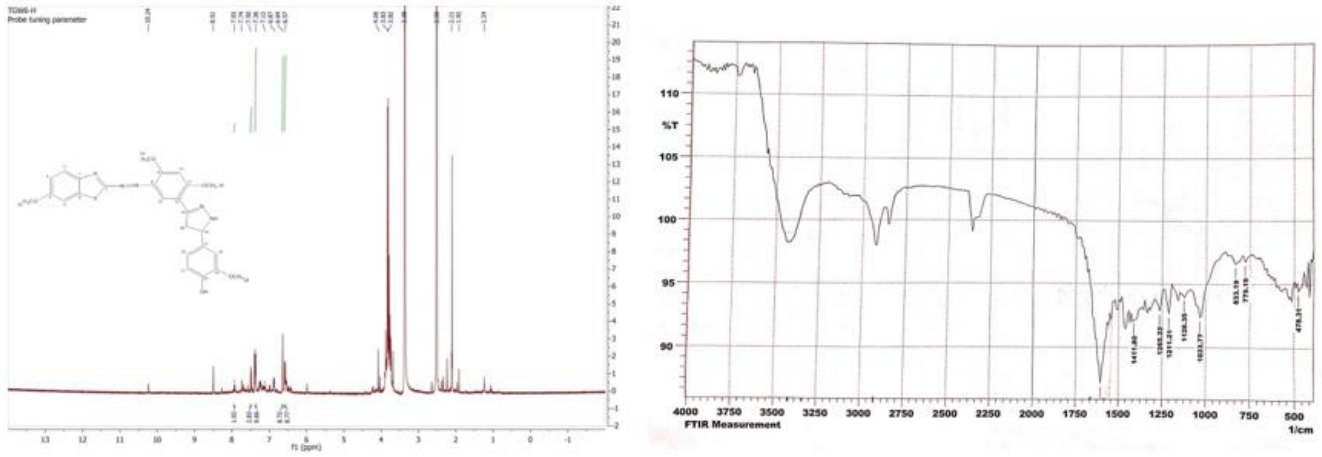


(a) (b)

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

7. Derivative (7) 4-(3-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol

FT-IR showed band at 3425 cm^{-1} for NH , 2916 cm^{-1} for C-H aliphatic, 3001 cm^{-1} for C-H aromatic ,1411 cm^{-1} for N=N group, 1550 cm^{-1} for C=N thiazole , 1650 cm^{-1} for C=N pyrazoline ,1512 cm^{-1} for C=C aromatic . ^{1}H-NMR of derivative (7) showed δ : 10.2 (S,1H,OH) , 8.5 (S,1H,NH) , 4.06 (S,3H,$(OCH_3)_{23}$) , 3.83 (S,3H,$(OCH_3)_{24}$) , 3.82(S,3H,$(OCH_3)_{25}$),2.11(S,3H,$(OCH_3)_{26}$,1.2 (d,2H,$(CH_2)_{16}$), 1.9 (t,1H ,$(CH)_{15}$,6.5-7.9 (M,9H,Ar-H).

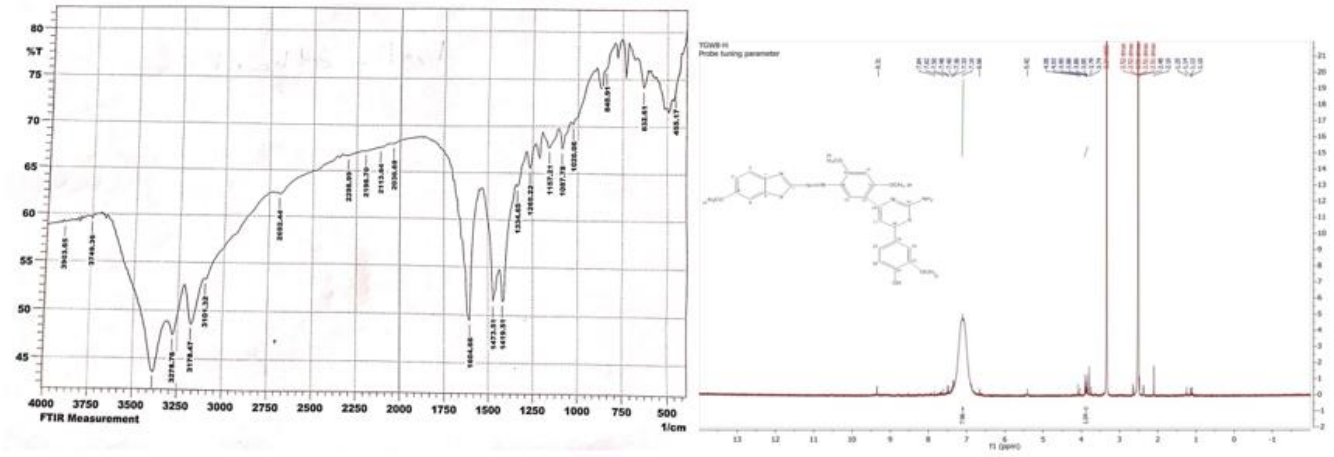


(a) (b)

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

8. Derivative (8)4-(2-amino-4-(2,4-dimethoxy-5-((6-methoxybenzo[d]thiazol-2-yl)diazenyl)phenyl)-6H-1,3-thiazin-6-yl)-2-methoxyphenol

FT-IR showed band at 3278 cm^{-1} for NH , 3101 cm^{-1} for C-H aromatic ,1419 cm^{-1} for N=N group, 1604 cm^{-1} for C=N thiazine , 1087 cm^{-1} for C-S . ^{1}H-NMR of derivative (8) showed δ : 9.3 (S,1H,OH) , 5.4 (S,2H,NH_2) , 4 (S,3H,$(CH_3)_{24}$) , 3.8 (S,3H,$(CH_3)_{25}$) , 3.7(S,3H,$(CH_3)_{26}$),2.4 (S,3H,$(CH_3)_{27}$,1.1 (d,1H,$(CH)_{16}$), 2.1(d,1H,$(CH)_{17}$).



(a) (b)

Figure 8. a-^{1}H-NMR Spectrum of the Compound (8) **,** b-FT-IR Spectrum of the Compound (8)

TABLE 1. Physical properties of the derivatives (1-8)

Derivatives	Color	M.P (ºC)	M,Wt (g/mol)
1	Brown	53-55	372
2	Dark Brown	217-219	505
3	Desert	300-302	545
4	Dark	296-298	568
5	Light Brown	190-192	685
6	Brown	191-193	595
7	Brown	296-298	519
8	Light Brown	271-273	563

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 breast cancer cells to find a link between the change in intracellular NAD^+ levels and compound (8) . To do this, a MCF-7 as breast cancer cell line and WRL68- as non-cancerous cells were used. Cells were treated with compound (8) for 24 hours (Figure 10) (Table 2) to find the effect of supplementation with compound (8) 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 (8)

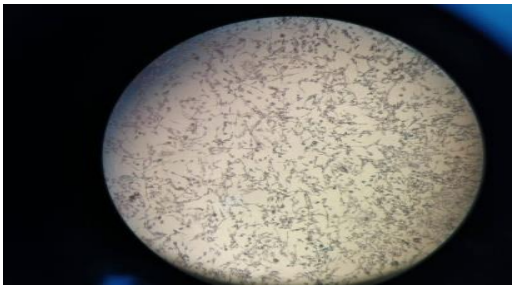


Figure (9) Effect of treatment on living cells

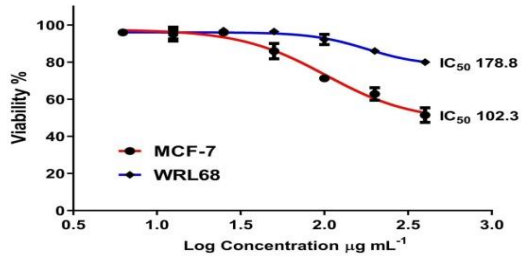


Figure (10)IC50 for comp. 8

Table (2) treatment different concentration of compound (8) with PC3 and WRL68

	MCF-7		WRL68	
Concen.	**Mean**	**SD**	**Mean**	**SD**
400.00	51.47	3.94	79.98	1.80
200.00	62.85	3.42	86.00	1.95
100.00	71.26	1.54	92.21	2.78
50.00	85.92	4.14	96.49	1.29
25.00	95.99	0.87	96.95	1.14
12.50	95.64	3.16	94.29	2.98
6.25	95.99	0.96	96.07	0.12

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 8 with Ascorbic acid (Figure 11) Table (3) show that in high concentration DPPH Scavenging activity increase

Table 3. treatment different concentration of compound (8) with Ascorbic acid

	200	**100**	**50**	**25**	**12.5**
Number of values	**3**	**3**	**3**	**3**	**3**
Mean	81.37	72.73	57.60	39.00	22.90
Std. Deviation	1.106	2.411	2.207	1.732	1.836
Std. Error of Mean	0.6386	1.392	1.274	1.000	1.060

Table 4. treatment different concentration of compound (8)

	200	**100**	**50**	**25**	**12.5**
Number of values	3	3	3	3	3
Mean	73.62	52.12	42.90	40.43	17.63
Std. Deviation	1.580	2.123	2.412	7.081	7.196
Std. Error of Mean	0.9122	1.226	1.393	4.088	4.155

CONCLUSION

1- The ability to prepare compounds in their stable form

2 The prepared compounds have a high biological activity.

3- The substituted groups significantly affect the reaction time

ACKNOWLEDGMENTS

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