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Preparation, Characterization, Anti-cancer and Antibacterial Evaluation of New Schiff base and Tetrazole Derivatives

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ABSTRACT

This work involved the synthesis of tetrazole derivatives 4,4'-(((sulfonylbis(4,1-phenylene))bis(diazene-2,1-dil))bis(2-(1-(4-R)phenyl]-4),5-Dihydro-1H-tetrazol-5-yl(phenol) (where R = OH or OCH_3) through the reaction of the substituted amine in THF as a solvent with sodium azide. FT-IR ^{1}H-NMR Spectrophotometric methods were used to characterize the prepared compounds. The antibacterial activity against four types of bacteria was studied. It was proven that tetrazole compounds were more effective than the compounds from which they were derived. The anticancer activity was measured against MCF-7 strains of human breast cells of the synthesized compounds.

1. Introduction

Schiff bases compounds containing the imine group were first described by Hugo Schiff as compounds resulting from the condensation reaction of aldehydes with ketones in the presence of primary amines in 1864. Usually, heat, acids, or bases will accelerate this reaction and consequently leads to an increase in the yield product of Schiff base [1]. Schiff bases are used as ligands to generate metal complexes with various structures or as intermediates in the production of amino acids [2,3]. Aliphatic aldehydes are unstable and undergo fast polymerization, while aromatic aldehydes, especially those with a conjugated system, form stable Schiff bases [4].

Recent years have witnessed an increase in interest in Schiff base compounds from which ligands can be prepared for metal complexes, allowing a wide range of compounds and their effectiveness, especially biological ones [5]. Hydrazone compounds (one type of Schiff bases) have shown a high selectivity for several anticancer drugs by acting as drug carrier systems [6].

Tetrazoles are heterocyclic organic compounds with high biological activity, as they contain four nitrogen atoms and one carbon atom [7]. These compounds possess biological activity and are also of great importance in the field of medicine [8], pharmacology, industry, agriculture and explosives [9]. This ring was first discovered in 1885 [10]. The reason for its high efficiency is due to the four nitrogen atoms, which have four free double electrons and are classified as an electronegative compound [11].

Recently, several methods have been found to synthesize tetrazole rings from amides. One of the most recent methods for this preparation is the reaction of sodium azide with Schiff bases [12].

There are also other methods, including preparing compounds using microwave [13] and ultrasound [14]. Imidoyl chloride is produced using chlorinating agents and is subsequently reacted with an azide to form unsubstituted tetrazole [15]. Tetrazole can also be synthesized from nitrile and an azide source using a type of reaction known as efflux [16]. Also, tetrazole derivatives are prepared by reacting hydrazones with sodium azide [17]. The tetrazole compounds possess three geometric isomers, giving them great importance as antibiotics and a great range of effectiveness [18,19].

2. Experiment

The chemicals used in this work were used without further purification. The end point of the reactions was determined for the prepared compounds. The purity of the prepared compounds was tested using thin layer chromatography (TLC).

A Shimadzu FT-IR 8400S spectrophotometer was used to record FT-IR spectra at the range (400–4000 cm-1) by KBr disk. The NMR spectra, a Varian device (400 MHz), was used by utilizing (DMSO-d6) as a solvent.

2.1 Synthesis of Schiff Bases [F1, F2]

The Schiff bases [F1,F2] compounds were prepared according to the literature [20,21]. The physical properties are shown in Table (1).

2.2 Synthesis of Tetrazole [F3, F4] [22, 23]

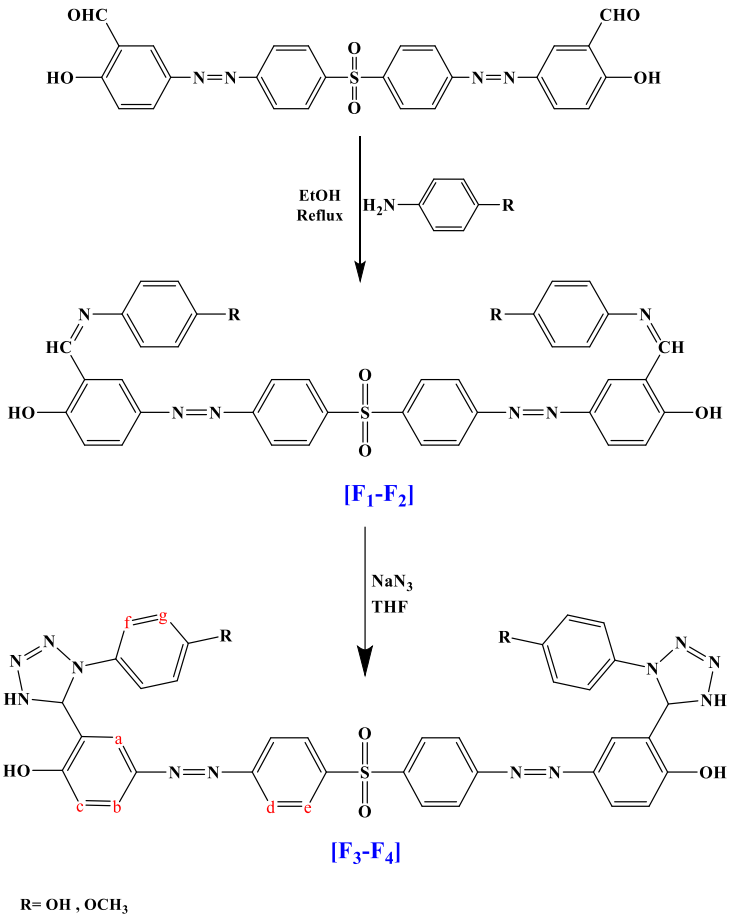
Sodium azide (0.004 mol, 0.26 g) was added to 0.002 mole of one of the prepared Schiff bases [F1, F2] in THF 30ml. The solution was refluxed on water-bath for (6-8) hrs. at 80 °C, and the precipitate was filtered. It was recrystallized with ethanol. The physical properties are shown in Table (1).

Table 1. Physical properties of prepared compounds [F_1-F_4]

Comp. No.	R	Molecular Formula/ M.Wt (g/mol)	Color	M.P (^{0}C)	T. Ref. (hours)	Yield (%)	R_f MeOH
F_1	OH	$C_{38}H_{28}N_6O_6S$ / 696.74	Orange	128-130	7	85	0.89
F_2	OCH_3	$C_{40}H_{32}N_6O_6S$ / 724.79	Light yellow	235-237	8	73	0.61
F_3	OH	$C_{38}H_{30}N_{12}O_6S$ / 782.80	Yellow	Gummy	8	70	0.73
F_4	OCH_3	$C_{40}H_{34}N_{12}O_6S$ / 810.85	White	240-242	6	80	0.65

3. Results and Discussion

The research included the synthesis of Schiff bases [F1, F2] and tetrazole [F3, F4] as in (**Scheme 1**). Spectroscopy methods, such as FT-IR and ^{1}H-NMR, were used to validate the structure of synthesized compounds.



Scheme 1. Prepared compounds [F_1-F_4]

3.1 Characterization of Schiff Bases Derivatives [F_1,F_2]

IR spectra of compounds [F_1,F_2] showed that the bands at (3336,3442) cm^{-1} were attributed to the OH groups. The disappearance of the band at (1730) cm^{-1} was attributed to the ν(C=O) aldehyde group [24], while the emergence of a new frequency at (1650,1654) cm^{-1} was attributed to the azomethine group ν(C=N) [25], as an evidence on the preparation of the Schiff base.

The stretching band at (3035,3091) cm^{-1} belonged to the aromatic (CH) group. Also, two bands at (1585,1580) cm^{-1}, (1488,1483) cm^{-1} referred to ν(C=C) aromatic. Furthermore, the azo (N=N) group displayed at (1442,1431) cm^{-1}. The ν(S=O) and ν(N–N) stretching bands displayed at (1392,1374) cm^{-1} and (1024,1064) cm^{-1} [26], respectively. Other bands are listed in Table 2.

The ^{1}HNMR spectrum of compound [F_1] in Figure (1) showed two singlet signals at δ(10.38ppm, 1H, 10.21ppm, 1H) due to the O**H** group, and at δ(9.26ppm, 1H) due to (C**H**=N) group. The singlet at δ(8.30ppm, 1H) was attributed to (H_a). The appearance of two doublets at δ(8.14ppm, 2H), (7.58ppm, 2H) with coupling constant (^{3}J=7.21Hz) was attributed to (H_b, H_c), respectively. The two doublets at δ(8.21ppm, 2H, 8.03ppm, 2H) with coupling constant (^{3}J=5.27Hz) were attributed to (H_d, H_e), respectively. The spectrum showed two doublets at δ(7.23ppm, 2H, 7.01ppm, 2H) with coupling constant (^{3}J=8.97Hz), as attributed to (H_g, H_f), respectively.

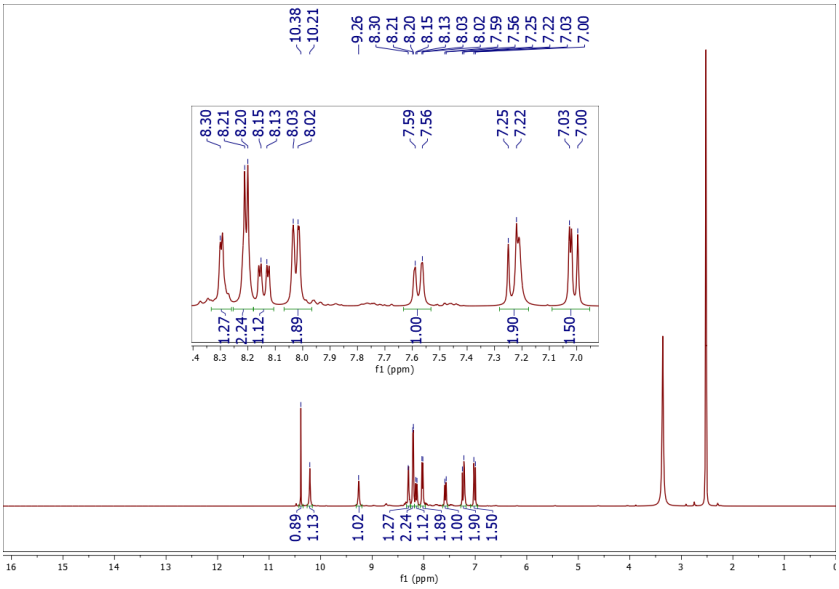


Fig. 1: H-NMR Spectrum of [F_1] compound

Table 2. FT-IR data of prepared compounds [F_1-F_4] (cm^{-1})

Comp. No.	R	νO-H νN-H	νC-H Ar.	ν S=O	νC=O C=N	ν C=C	ν N=N	νC-N νN- N	Others
F_1	OH	3336	3035	1392	---- 1650	1585 1488	1442	1168 1024	-----
F_2	OCH_3	3442	3091	1374	---- 1654	1580 1483	1431	1140 1064	ν(OCH_3) 2979, 2832 & ν(O-C) 1284
F_3	OH	3406 3213	3016	1385	----	1582 1491	1420	1145 1036	-----
F_4	OCH_3	3446 3222	3074	1375	----	1585 1483	1436	1108 1068	ν(OCH_3) 2929, 2850 & ν(O-C) 1282

The spectrum of compound [F_2] in Figure (2) displayed two peaks at δ(10.39ppm, 1H) and δ(9.17ppm, 1H), referring to protons of O**H** and (C**H**=N) groups, respectively. The appearance of a peak at δ(8.39ppm, 1H) was attributed to (H_a). The appearance of two doublets at δ(8.15ppm, 2H, 7.70ppm, 2H) with coupling constant (^{3}J=8.67Hz) was attributed to (H_b, H_c), respectively. The appearance of two doublets at δ(8.23ppm, 2H, 8.06ppm, 2H) with coupling constant (^{3}J=8.53Hz) was attributed to (H_d, H_e), respectively. The appearance of two doublets at δ(7.46ppm, 2H, 7.24ppm, 2H) with coupling constant (^{3}J=8.68Hz) was attributed to (H_g, H_f), respectively. The appearance of a singlet at δ(3.98ppm, 3H) was attributed to the proton of (OCH_3) group.

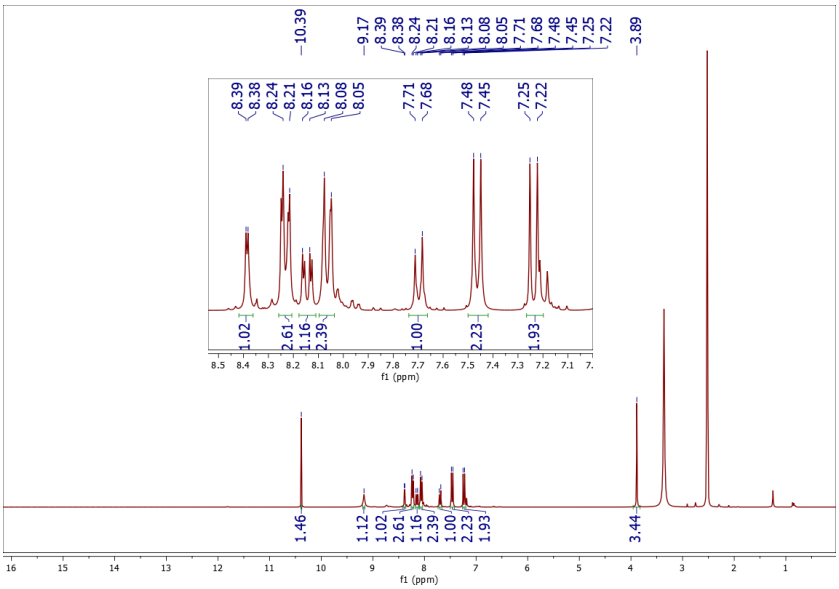


Fig. 2: H-NMR Spectrum of [F_2] compound

3.2 Characterization of Tetrazole Derivatives [F_3,F_4]

The IR spectra of the compounds [F_3,F_4] showed the disappearance of the (C=N) band and the presence of a band at (3406,3446) cm^{-1} and (3213, 3222) cm^{-1} due to the (OH) and (NH) groups, respectively [26]. The spectra displayed at (3075,3016) cm^{-1} referred to the ν(CH-Aromatic) group. The band shown at (1582,1585) cm^{-1} and (1491,1483) cm^{-1} was attributed to ν(C=C) groups. Also, a band at (1420,1436) cm^{-1} belonged to the (N=N) group [24]. The ν(S=O) and ν(N–N) stretching bands were displayed at (1385,1375) cm^{-1} and (1036-1068) cm^{-1}, respectively [26]. Other bands are listed in Table 2.

^{1}HNMR spectrum of compound [F_3] in figure (3) showed two singlet signals at δ(10.31ppm, 1H) and (10..15ppm, 1H) due to the OH group. The peak at δ(9.22ppm, 1H) was attributed to (C**H**=N) group, while the peak at δ(8.74ppm, 1H) was attributed to the proton of (NH) group. The appearance of a singlet at δ(8.34ppm, 1H) was attributed to (H_a), while the appearance of two doublets at δ(8.14ppm, 2H) and (7.58ppm, 2H) with coupling constant (^{3}J=7.83Hz) was attributed to (H_b, H_c), respectively. The appearance of two doublets at δ(8.22ppm, 2H) and (8.08ppm, 2H) with coupling constant (^{3}J=6.34Hz) was attributed to (H_d, H_e), respectively. As for the two doublets at δ(7.28ppm, 2H) and (7.09ppm, 2H) with coupling constant (^{3}J=6.06Hz), they were attributed to (H_g, H_f), respectively [25].

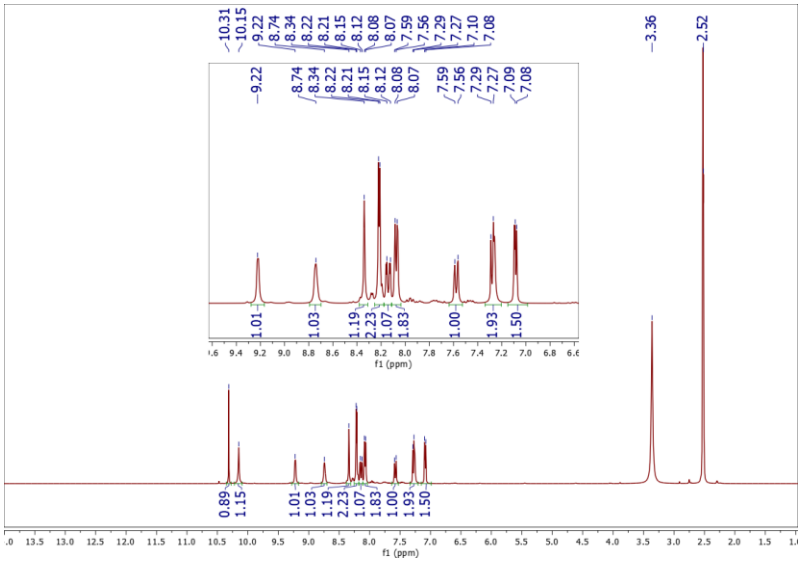


Fig. 3: H-NMR Spectrum of [F_3] compound

^{1}HNMR spectrum of the compound [F_4] in figure (4) showed a singlet signal at δ(10.43ppm, 1H), which was attributed to the OH group. The peak at δ(9.28ppm, 1H) was attributed to (C**H**=N) group, while the singlet at δ(8.73ppm, 1H) was attributed to the (NH) proton. The appearance of a singlet at δ(8.40ppm, 1H) was attributed to (H_a). Concerning the two doublets at δ(8.14ppm, 2H) and (7.75ppm,

2H) with coupling constant (^{3}J=8.69Hz), they were attributed to (H_b, H_c), respectively. The two doublets at δ(8.21ppm, 2H) and (8.06ppm, 2H) with coupling constant (^{3}J=7.90Hz) were attributed to (H_d, H_e), respectively. While the two doublets at δ(7.48ppm, 2H) and (7.27ppm, 2H) with coupling constant (^{3}J=8.68Hz) were attributed to (H_g, H_f), respectively. The singlet at δ(3.98ppm, 3H) was attributed to the proton of (OCH_3) group [25,26].

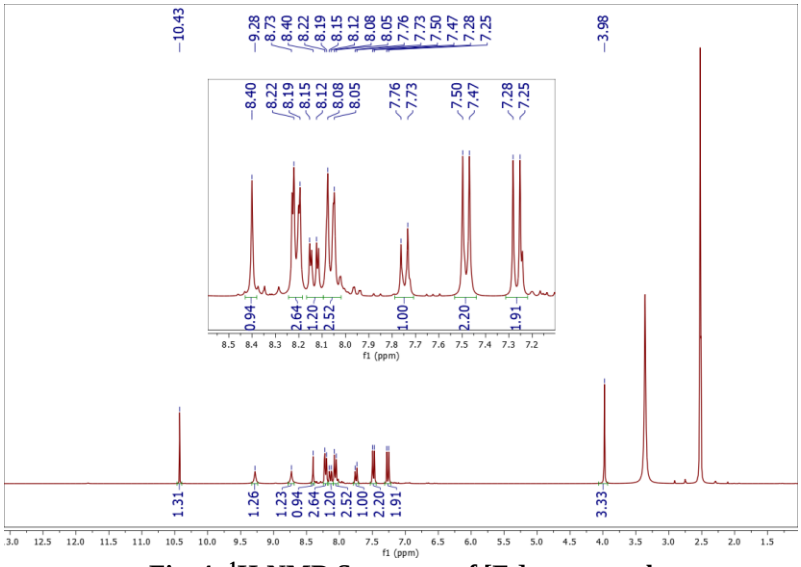


Fig. 4: ^{1}H-NMR Spectrum of [F_4] compound

3.3 Anticancer for [F_3] and [F_4]

The activity of the prepared compounds (F_4, F_3) was measured as inhibiting cell growth against the breast-cell line (MCF-7). The results were compared with cisplatin. The effect of the two compounds on the development of MCF-7 after 24 hours was studied and recorded. The calculated IC values (refer to Tables 3 and 4) shown in (Fig. 5) were recorded. Compounds [F_3, F_4] had IC_{50} values (52.14 ± 1.72 mM) and (12.18 ±1.05µM), respectively, with the activity of the two compounds measured less than cis-platin (4.46 ±0.04µM). The result showed the reticence of the growth of tumour cells according to the concentration of the two compounds. Hence, the compound [F_3] showed the least inhibition result on the proliferation of cancer cells, while the compound [F_4] showed the highest inhibition effect due to the presence of methoxide groups in the structure, which increased the cytotoxic activity [27-29].

Table 3: viability (%) of [F_3, F_4] comp.

Concentration (µM)	Cell viability (%)	
	F_3	F_4
0.000	100	100
0.100	91.77	93.32
1.000	86.31	80.1
10.00	72.36	53.13
100.0	52.26	25.65
200.0	25.83	11.71
400.0	8.67	5.38

Table 4: IC50 value of [F_3, F_4] against (MCF-7) cell line related to cisplatin

Compound	IC_{50} value (µM)
F_3	52.14± 1.72
F_4	12.18 ± 1.05
Cis-platin	4.46 ± 0.04

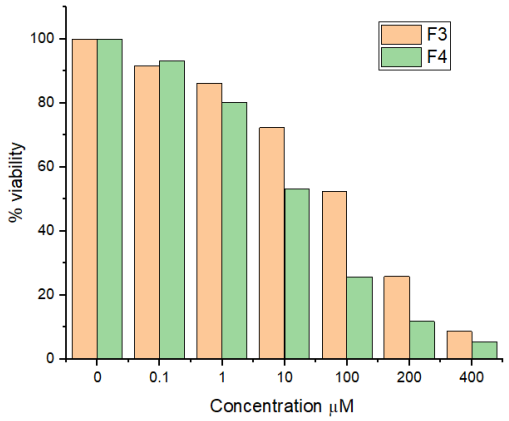


Fig. 5: %viability of (MCF-7) cell after treated with [F_3, F_4] (P value = 0.005 in each case)

3.4 Antibacterial Activity

The tetrazoles showed antibacterial activity more than the Schiff bases derived from them. The compounds [F3, F4] showed higher activity against *Pseudomonas aeruginosa* at the lowest inhibitory concentration. Compound [F4] gave higher activity against *E. coli* and *Staphylococcus aureus*, while compound [F3] gave the highest inhibition against *Streptococcus faecalis* at the lowest inhibitory concentration.

Table 5: Inhibitory activity of some complexes prepared on four types of positive and negative bacteria (the diameter of inhibition is measured in mm)

Comp.	Concentration mg/ml	*Pseudomonas aeruginosa* -	*Escherichia coli* -	*Staphylococcus aureus* +	*Streptococcus faecalis* +
F1	0.01	8	10	20	16
	0.001	6	7	16	13
	0.0001	3	5	11	10
F2	0.01	5	15	17	13
	0.001	2	11	14	12
	0.0001	1	8	12	10
F3	0.01	10	14	23	17
	0.001	9	11	18	15
	0.0001	7	8	15	12
F4	0.01	13	16	22	13
	0.001	11	14	19	12
	0.0001	7	11	17	11

Conclusions

The correctness and validity structures of the produced compounds were determined using spectroscopic methods and physical measures. At laboratory temperatures, these chemicals did not decompose or alter the color. In comparison to cis-platin, the compounds [F_3] and [F_4] had limited activity against human breast cell lines (MCF-7). The antibacterial study also showed a clear inhibitory activity of tetrazole derivatives [F_3] and [F_4] higher than compounds [F_1] and [F_2] on the bacterial species studied.

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تحضير وتشخيص وتقييم الفعالية المضادة للسرطان والمضادة للبكتيريا لقواعد شيف جديدة ومشتقات التترازول

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[3] المديرية العامة للتربية في الانبار ، وزارة التربية والتعليم ، العراق

الملخص

تضمن هذا البحث تحضير مشتقات تترازول 4,4'-(((سلفونايل بس(4،1-فينايلين))بس(دايازين-2،1-دايل))بس(2-(1-(4-معوضR)فنيل]-4)،5-ثنائي هيدرو-H1-تترازول-5-ايل(فينول) (حيث R = OH أو OCH_3) من خلال تفاعل معوضات الأمين في THF كمذيب مع أزيد الصوديوم. تم استخدام الطرق الطيفية FT-IR و ^{1}H-NMR لتشخيص المركبات المحضرة ، وتمت دراسة الفعالية المضادة للبكتيريا ضد أربعة أنواع من البكتيريا حيث تم إثبات أن مركبات التترازول أكثر فعالية من المركبات التي تم اشتقاقها منها ، كما تم قياس النشاط المضاد للسرطان ضد MCF-7 من خلايا الثدي البشرية للمركبات المحضرة.