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Synthesis, Evaluation of anticancer and antimicrobial activities of some Schiff bases derivatives

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Keywords: Schiff base, tartaric acid, Thio carbohydrazide, and Anti-cancer effects.

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Abstract:

In this research, compound [1,2-Bis-(4-amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-ethane-1,2-diol] was used as the starting material for the synthesis of different Schiff's Bases compounds. The fusion of tartaric acid with Thio carbohydrazide produced the compound (C1), Schiff's bases (C2-C4) were created through the condensation of substances (C1) with different substituted benzaldehydes in the presence of glacial acetic acid as a catalyst. Thin layer chromatography (TLC) was used to confirm the compounds' purity, and spectroscopic methods were used to infer the compounds' structures (FTIR) and magnetic nuclear resonance spectroscopy (1H-NMR and 13C-NMR). The agar well diffusion method was used to test synthesized compounds for their antibacterial activity against K. pneumonia and S. aureus, and the findings were inconsistent. Target substances were tested for their ability to kill human breast cancer at concentrations of 50 and 100 g/mL. Human muscle tissue HC normal cell line, human cervical cancer Hela cell line, and HePG2 cell line. The results showed that the chemicals had potential cytotoxic activity against the Hela cell line, particularly compound (C4), which had the greatest inhibition at doses of 100 mg/mL among the examined substances.

Keywords: Schiff base, tartaric acid, Thio carbohydrazide, and Anti-cancer effects.

تحضير وتقييم الفعالية المضادة للسرطان والمضادة للميكروبات لبعض مشتقات قواعد شيف

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الخلاصة:

في هذا البحث استخدم المركب [١،٢--*BIS-(4-AMINO-5-MERCAPTO-4H-[1,2,4]TRIAZOL-3-YL)-ETHANE-1,2-DIOL*] كمادة يمكن من خلالها البدء بتحضير العديد من قواعد شيف. حضر المركب (*C1*) من خلال صهر حامض الترتريك *TARTARIC ACID* مع ثايوكربوهايدرازايد *THIOCARBOHYDRAZIDE* بينما المركبات (*C2 –C4*) حضرت من تفاعل المركب (*C1*) مع مركبات بنزالدهايد معوضة مختلفة بوجود حامض الخليك الثلجي كعامل مساعد. استخدمت كروموتوغرافيا الطبقة الرقيقة لتأكيد نقاوة المركبات، والطرائق الطيفية مثل مطيافية الأشعة تحت الحمراء (*FTIR*) والرنين النووي المغناطيسي *1H-MAGNETIC NUCLEAR RESONANCE SPECTROSCOPY)* (*NMR AND 13C-NMR*) لتأكيد تراكيب المركبات. استخدمت طريقة الانتشار في الوسط الزرعي لفحص الفعالية المضادة لبكتريا *K. PNEUMONIA AND S. AUREUS* اذ كانت النتائج غير متناسقة. كما استخدمت المركبات في هذا العمل لفحص قابلية قتل سرطان الثدي في خلايا النسيج العضلي المحدد بأنسجة العضلات البشرية ،وسرطان عنق الرحم البشري *HUMAN MUSCLE TISSUE HC NORMAL CELL LINE, HUMAN CERVICAL CANCER HELA CELL LINE, AND HEPG2 CELL LINE* عند تراكيز ٥٠ و١٠٠ ملي غرام / مليلتر. أثبتت هذه المركبات الفعالية الخلوية السمية ضد نوع *HELA CELL LINE* وبالأخص المركب (*C4*) والذي اعطى اعلى تثبيط عند التركيز ١٠٠ ملي غرام / ملليتر مقارنة بالمركبات الأخرى.

الكلمات المفتاحية: قواعد شف، حامض الترترك، ثايوكربوهايدرازايد، مضادات السرطان.

1. Introduction:

Kriging Synthesizing heterocyclic organic compounds is an important class that has been found to have many uses in medicinal chemistry. Most medicinally significant compounds with pharmacological actions ranging from antibacterial to anticancer contain nitrogen atoms in their heterocyclic structures **[1-3]**. An imine distinguishes Schiff bases. (C=N–) bond, commonly known as an azo methine compound, is typically made by easily condensing primary amines with carbonyl compounds, primarily aromatic aldehydes, and ketones. Schiff's bases are a great pharmacophore and are utilized as a starting material for the synthesis of several bioactive heterocyclic compounds. **[4-8]** for anti-inflammatory, anticancer, antibacterial, antifungal, antimalarial, antiviral, and antioxidant, Therapeutics that are cytotoxic, enzyme-inhibitory, and

anti-COVID-19 **[9, 10].** The azomethine group chemical is used as a synthetic intermediary in a variety of organic syntheses, including the preparation of 1,3-oxazepine by tricyclic addition with cyclic anhydride **[11].** The 1,2,4-triazoles and their derivatives are heterocyclic compounds having five members that include two carbon atoms and three nitrogen atoms. **[12].**

The 1,2,4-triazoles have a wide range of pharmacological possibilities, including antibacterial properties. Antifungal, anti-inflammatory, antioxidant, analgesic, and anticancer properties have garnered a great deal of interest during the past two decades, particularly. The biological significance of 1,2,4-triazole has led to the development of several techniques for the synthesis of this scaffold with biological activity **[13- 15]** and 1,2,4-triazoles have a unique position in the fields of medical and pharmaceutical chemistry as well as industrial **[16].**

2. EXPERIMENTAL SECTION:

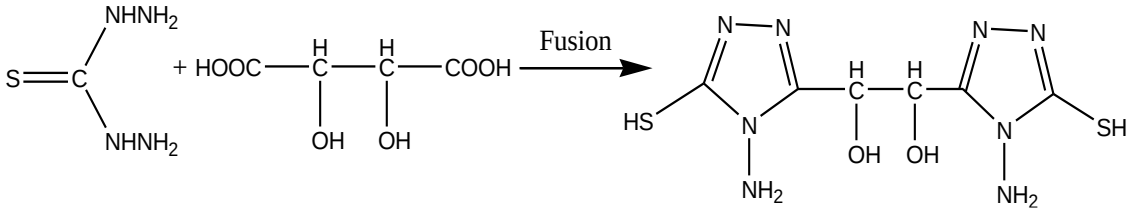
2.1 Materials

All the chemicals and solvents utilized during the synthesis of the compounds were bought from a variety of different suppliers, including Merck, BDH, Sigma Aldrich, and Fulka They weren't further purified; they were used as obtained. TLC sheets were used to verify the purity of the produced compounds, and FTIR and ^{1}H NMR instruments were used to analyze their chemical structures. The glassware laboratory was used in this investigation as well as the uncorrected Gallen Kamp (MFB-600) melting point apparatus, which was used to determine the melting points of compounds. Compounds' FT-IR spectra were obtained using a KBr Disc and a Perkin Elmer Speactum-65 in the Chemistry Department of Diyala University. TMS was applied as the internal standard for the ^{1}HNMR spectra, and deuterated DMSO was used as a solvent on a Bruker 400 MHz spectrophotometer.

2.2 Procedure:

2.2.1 Synthesis of 1,2-Bis-(4-amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-ethane-1,2-diol (C1)

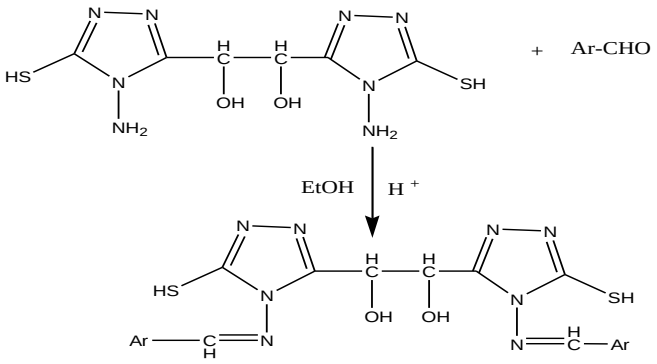
By mixing tartaric acid (0.003 mol) and Thio carbohydrazide (0.006 mol), which were placed in a round-bottomed flask and heated in an oil bath until the contents of the flask melted, compound (C1) was created **Scheme 1.** When the product was finished cooling, sodium bicarbonate solution was applied to neutralize any carboxylic acid that may have remained unreacted. The product was then recrystallized from (Ethanol/water) to afford the title compounds after being rinsed with water and collected by filtration **[17, 18]. Table 1**. Lists the physical characteristics of the created chemical (C1).



Scheme 1. Synthesis of compound C1

2.2.2 Synthesis of Schiff's bases (C2-C4)

The reaction of compound (C1) (0.01 mol) with several substituted benzaldehydes (0.02 mol) in absolute ethanol and (3–4 drops) of glacial acetic acid was refluxed for 6–8 hours to produce the Schiff bases **Scheme 2.** The precipitate was then cooled, filtered, and cleaned with diethyl ether before being recrystallized with the proper solvent **[19, 20]. Table 1**. Lists the synthetic compound's physicochemical characteristics (C2–C4).



Scheme 2. Synthesis of compounds C2-C4

Ar = 4-dimethyl amino benzene,4-nitrobenzene, 2,4-dichlorobenzene

Table 1: Physical properties of the prepared derivatives (C1-C4)

Compound. No	M.P°C	Yield	Recrystal. solvent
C1	231-233	75%	Ethanol + water
C2	160-162	93%	Ethanol
C3	150-152	91%	Ethanol
C4	189-192	92%	Ethanol

2.3 Biological evaluation

2.3.1 Antimicrobial assay

On blood agar and Mannitol salt agar, Staphylococcus aureus was cultivated and identified. On MacConkey agar and Rosin Methylene blue, an isolated K. pneumonia was grown and identified. Because the Macfarlane turbidity standard provided by the manufacturer (Biomatrix) yields an approximation of 1.5 x 108 cells/ml, it was utilized to calibrate the number of bacterial

cells. The Muller Hinton agar medium was made by dissolving 38 grams in 1L of distilled water, sterilizing it in an autoclave at 121 °C under 15 pounds of pressure for 15 minutes, letting it cool, and then pouring it onto sterile dishes. These dishes were then maintained in the refrigerator until they were needed.

2.3.2 Analysing synthetic chemicals' antibacterial properties using the agar diffusion method.

To prepare the suspended bacteria and place it in tubes with brain heart infusion broth to activate the bacteria, a few bacteria colonies were conveyed via a loop. The tubes were incubated at 37 °C for (18–24) hours. The conventional McFarland solution (1.5 x 108) cells/ml and the bacteria in suspension were compared. After that, the miller hinted that agar-containing plates on which the bacteria suspension had been previously dispersed were left to dry for a time. Using a cork borer that had been sanitized, holes with a diameter of 5 mm were created in the culture medium. By using a micropipette, 100 µl of the substance was added to each hole separately. After that, incubate the dishes for 24 hours at 37 °C. The diameter of the inhibition zone surrounding each hole was measured to gauge the potency of each concentration **[21]**

2.3.3 Cytotoxicity Assay

The following three cell lines were used in this study: cancer of the human breast Human cervical carcinoma and the HePG2 cell line Hela. While the human muscle tissue HC is a normal cell line. These cell lines were acquired from the tissue culture unit at the Al-Mustansirah University in Baghdad, Iraq's Iraqi Centre for Cancer, and Medical Genetics Research (ICC MGR). The evaluation of a compound's solubility before an in vitro cytotoxicity test. The cytotoxicity experiment was performed using the Freshly (2012) technique and the crystal violate stain. To prepare varied concentrations ranging from (50,100) g/mL, the organic compounds were first dissolved in DMSO and then diluted with serum-free media (SFM). The tumour cells were plated in 96-well microplates (1 x 105 cells/mL) and incubated for 24 hours at 37 °C., A fresh serum-free medium (SFM) containing concentrations of each drug was then used to replace the old media. A humidified incubator at 37 °C with (5% CO_2) was used to incubate the plate for 24 hours. Following incubation, 100 mL of crystal violate was added to each well, and they were again incubated for 20 min at 37 °C with the culture medium removed. The following equation was used to compute the inhibition percentage: The following three cell lines were used in this study: cancer of the human breast Human cervical carcinoma and the HePG2 cell line Hela. While the human muscle tissue HC is a normal cell line. These cell lines were acquired from the tissue culture unit at the Al-Mustansirah University in Baghdad, Iraq's Iraqi Centre for Cancer, and Medical Genetics Research (ICC MGR). The evaluation of a

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Percentage of cell inhibition = (absorbance reading of control cells - absorbance reading of treated cells for each concentration/absorbance reading of control cells) x 100

3. RESULTS AND DISCUSSION:

3.1 Chemistry results

Thio carbohydrazide was converted into 1,2-Bis-(4-amino-5-mercapto-4H- [1,2,4] triazol-3-yl)-ethane-1,2-diol (C1) through the cyclization of tartaric acid. **Scheme 1**. Schiff bases (C2-C4) were prepared by the condensation of compound (C1) with the appropriate aromatic aldehydes as shown in **Scheme 2.** The synthesized compounds' structures were established using ^{1}HNMR and FTIR spectroscopy. The product's TLC in each case revealed the existence of a single spot that was specific to that product.

3.2 FTIR spectroscopy for synthesis compounds [23, 24]

The compound (C1) was identified using FT IR, which displays distinctive bands in the following range. The bands at 3291-3190 cm^{-1}, which might be attributed to asymmetric and symmetric stretching vibration of (NH_2), absorption band at 3300 cm^{-1}, which was attributed to O-H group, 2936 cm^{-1} (C-H aliphatic), and 1617 cm-1 owing to (C= N) of triazole ring. The OH group was responsible for assigning the bands: (3159-33304 cm^{-1}) as the significant diagnostic bands of chemicals (C2-C4) in the FTIR spectra. About (3118-3136 cm^{-1}) is where the C-H aromatic stretching vibration bands of compounds (C2-C4) are found, The C-H aliphatic stretching vibration bands of these compounds at (2947-2955), whereas stretching vibration at 1633-1684 cm^{-1} was due to CH=N (imine), (1613-1628 cm^{-1}) for stretching vibration of C=N (triazole ring) and the disappearance stretching frequency of NH2 at 3291,3190 cm^{-1} is a good indicated to form Schiff bases compounds. Other bands of compound (C2-C4) are shown in **Table 2**.

Table 2: Compounds' FTIR measurements (C1-C4) FTIR spectrum characteristic bands (cm-1)

No	OH	SH	C-H Aromatic	C- H Aliphatic.	C=N	C=C Aromatic	Other
C1	3300	2660	-	2936	1617	-	NH2(3291,3190)
C2	3159	2688	3118	2947	1613	1591-1529	CH=N (1657)
C3	3271	2654	3136	2953	1618	1573-1485	CH=N (1633)
C4	3304	2680	3136	2955	1628	1584-1501	CH=N (1684)

3.3 NMR spectroscopy for the prepared compounds[25, 26]

The ^{1}H-NMR spectra were recorded in DMSO (dimethyl sulfoxide), with chemical shifts given in ppm and TMS (tetramethyl silane) used as the reference. The ^{1}H-NMR data for chemical (C1) revealed two protons of (SH) at 10.25 ppm in a single signal. Four (NH_2) group protons were identified by a singlet signal at 5.52 ppm. Signals that were detected at 5.22 ppm were attributed to protons of (2CH), while those at 4.51 ppm were related to two protons of (2OH), as shown in **Fig. 1.** The ^{1}H NMR data for compound (C2) in **Fig. 2**. Showed that two protons of (SH) were responsible for a single signal at 10.25 ppm. Two protons of the Schiff base group (CH=N) were identified by a singlet signal at 8.67 ppm. A signal that was detected in the range of 7.77 to 6.67 ppm was attributed to the aromatic ring proton. Signals at 5.31 ppm were attributed to protons of (2CH), those at 4.95 ppm to two protons of (2OH), and those at 3.10 ppm to a singlet signal of ($N(CH_3)_2$). While the single signals at 10.22 ppm in the ^{1}H NMR of compound (C3) **Fig. 3.** Were identified as the protons of (2SH). Two protons of the Schiff base group (CH=N) were identified by a singlet signal at 8.60 ppm. A signal that was visible in the range of (8.35-7.71) ppm was attributed to protons from aromatic rings. Protons of (2CH) were attributed to the signals that showed at 5.34 ppm, while two protons (2OH) were allocated to the signals at 4.32 ppm. The ^{1}HNMR results for compound (C4). **Fig. 4.** displays the chemical shifts in ppm: 10.25's, 2H, 2SH), 8.35's,2H,2 N=CH),8.01-7.40'm 6H,Ar),5.20 (d, 2H, 2CH), and 4.00 (d, 2H, 2OH). The ^{13}C NMR spectrum of compound (C1). Shows the following signals of carbon: 165.3, 150.4, 64.3. Whereas the ^{13}C NMR spectrum of compound (C2). Shows the following signals of carbon:166.8, 160.8, 152.3, 110.4 – 149.2, 64.9, and 60.6. The ^{13}C NMR spectrum of compound (C3). Shows the following signals of carbon: 169.2, 160.5, 159.6, 121.9 – 149.7, 64.8. Finally, the ^{13}C NMR spectrum of compound (C4). Shows the following signals of carbon:168.1, 160.7, 155.0, 127.3-149.8, 65.0.

3.4 Antimicrobial activity evaluation

The agar well diffusion method was used on Muller Hinton agar medium to test the antibacterial activity of novel compounds using 10 mg/ml of dimethyl sulfoxide (DMSO) as a

solvent, with McFarland turbidity as a reference solution. The studied drugs' zones of inhibition were measured in millimeters (mm). The results are reported in **Table 3**. According to the screening results, the compounds (C3- C4) have high activity against S. aureus bacteria at a concentration of 300 ppm, whereas compound C1 has no activity at a concentration (100, 200) ppm against both S. aureus and K. pneumonia. In vitro anticancer activity.

Three human cell lines—the human cervical cancer Hela cell line, the human breast cancer HePG2 cell line, and the human—were tested to determine whether each substance had any harmful effects in vitro. The human muscle tissue HC is a normal cell line that was exposed to two different concentrations—50 and 100 g/mL—for 24 hours at 37 degrees Celsius. Results showed that compound (C2) had the highest cytotoxic activity with the lowest inhibition rate (88.70%) at a concentration of 100 g/mL on the HePG2 cell line, compound (C4) had the lowest inhibition rate (76.16%) at the same concentration on the HePG2 cell line, and compound (C3) had the highest inhibition rate (79.67%) at the same concentration on the Hela cell line. While compound (C4) had the highest level of cytotoxicity on the Hela cell line, with an inhibition rate of (89.50%) at a dose of 100 g/mL. The other results are summarized in **Table 4** and **Fig. (9-15)**.

Table 3: The Biological activity compounds (C1-C4)

	S. aureus			*K. pneumonia*		
No	100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm
C1	-	-	12 mm	-	-	-
C2	-	11 mm	-	-	-	-
C3	11mm	14 mm	22mm	-	-	12 mm
C4	14 mm	13 mm	17 mm	-	-	12 mm

Table 4: The in vitro cytotoxicity of produced compounds on several cell lines at 50 and 100 g/mL following a 24-hour incubation at 37 °C.

No.	Inhibition ratio100% Normal Cell Line HC Con. µg/mL		Inhibition ratio100% Cell Line cancer HepG2 Con. µg/mL		Inhibition ratio100% Hela cell line Con. µg/mL	
	50	100	50	100	50	100
C1	18.40	19.70	69.47	78.43	78.65	89.48
C2	9.12	11.78	71.13	88.7	81.10	87.34
C3	8.11	13.57	57.45	78.53	67.55	79.67
C4	10.12	16.22	63.67	76.16	78.69	89.50

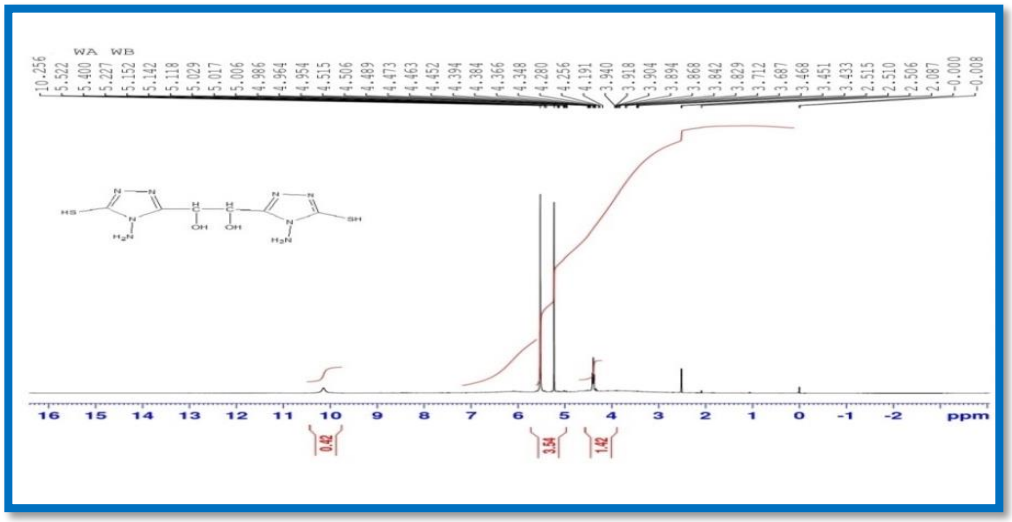

Fig. 1: The ^{1}H-NMR spectrum of the compound (C1).

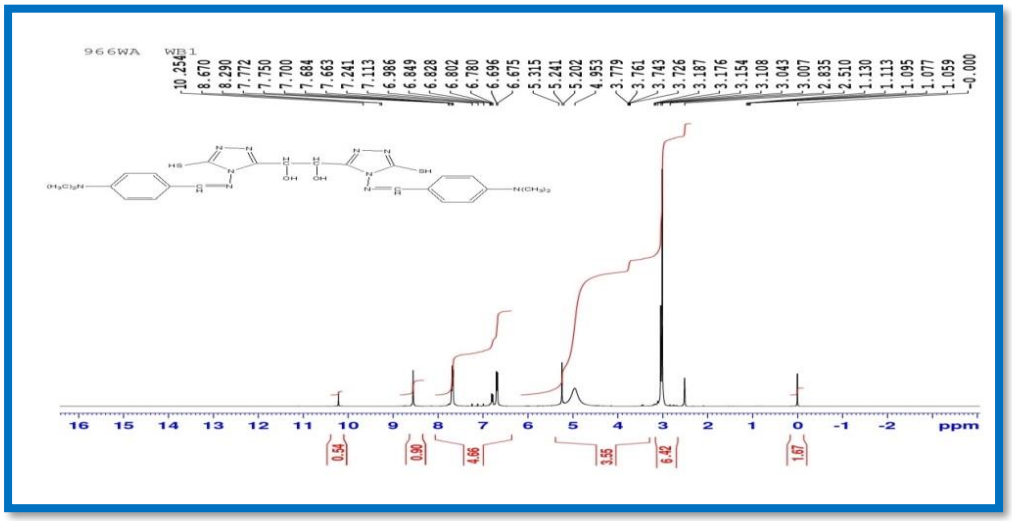

Fig. 2: The ^{1}H-NMR spectrum of the compound (C2).

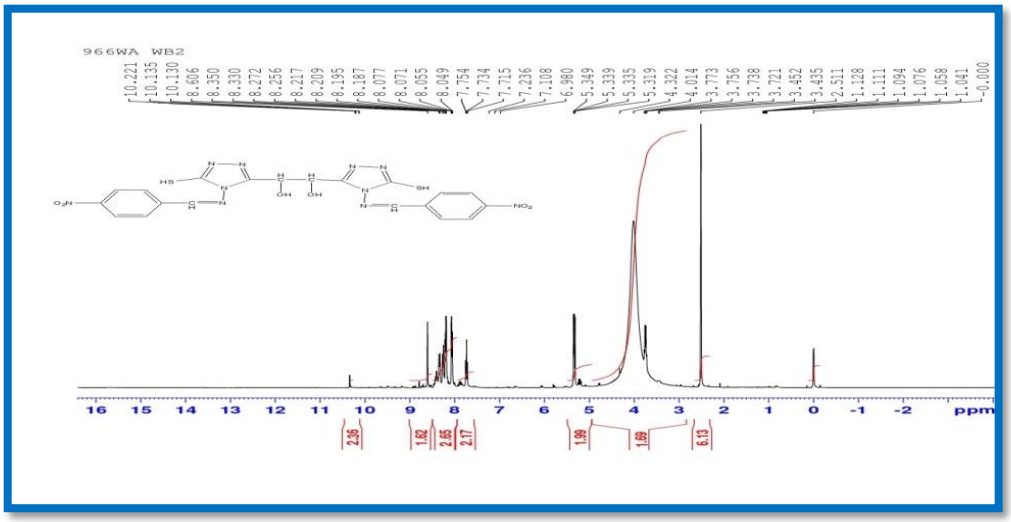

Fig. 3: The ^{1}H-NMR spectrum of the compound (C3).

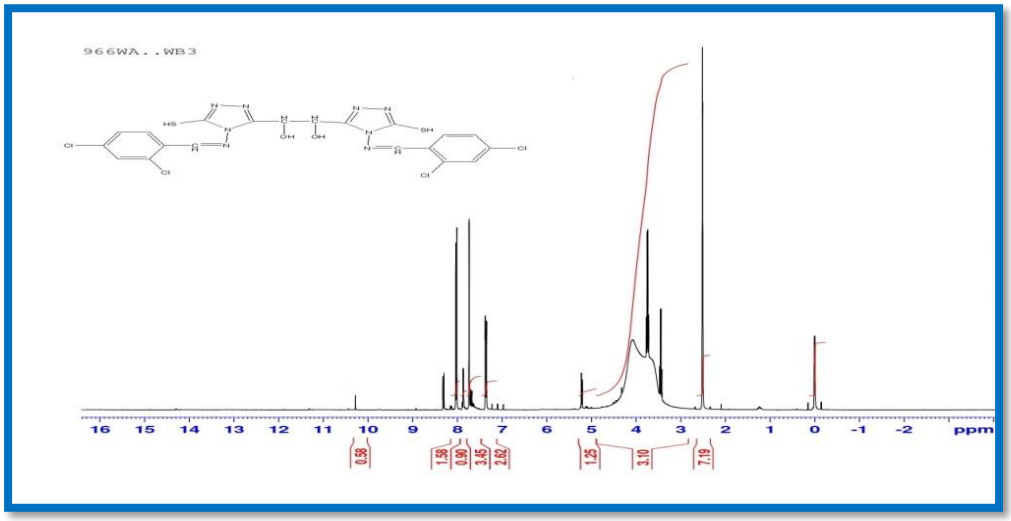

Fig. 4: The ^{1}H-NMR spectrum of the compound (C4).

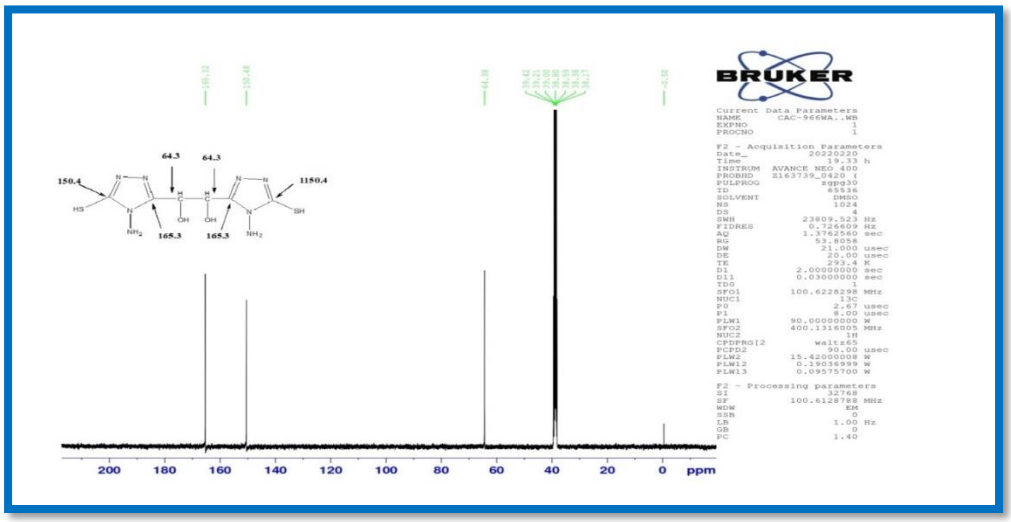

Fig. 5: The ^{13}C-NMR spectrum of the compound (C1).

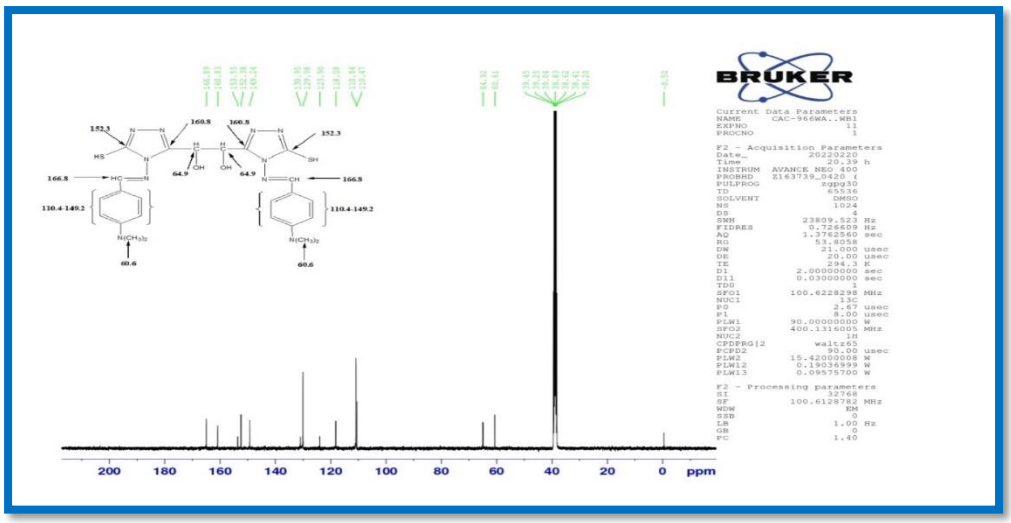

Fig. 6: The ^{13}C-NMR spectrum of the compound (C2).

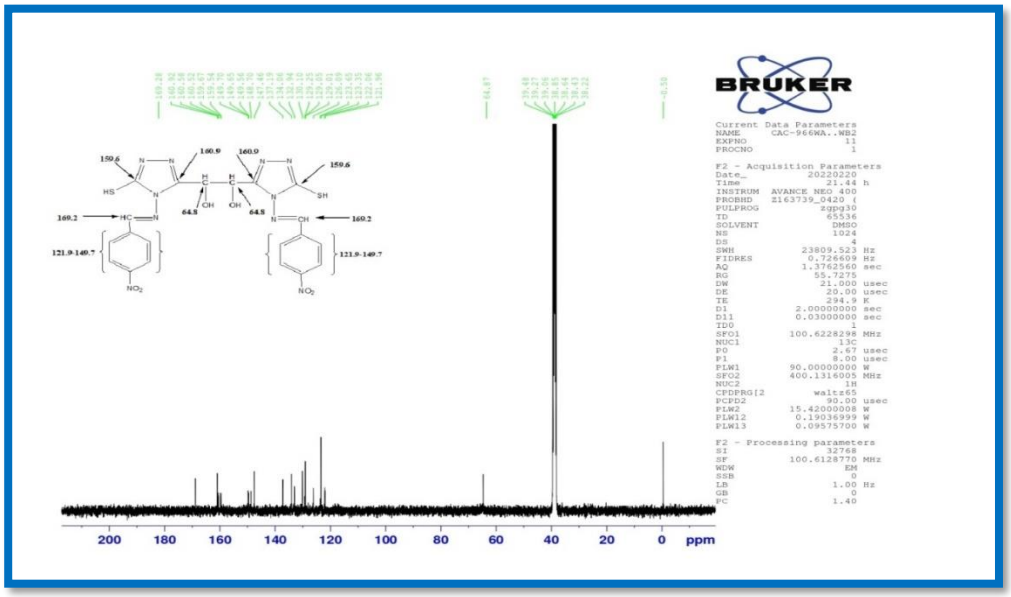

Fig. 7: The ^{13}C-NMR spectrum of the compound (C3).

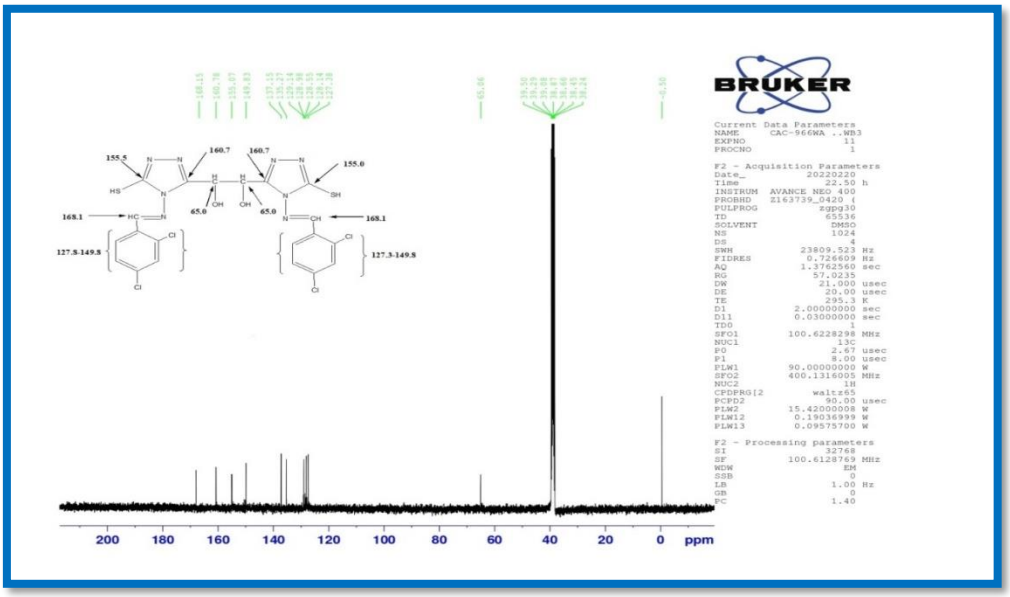

Fig. 8: The ^{13}C-NMR spectrum of the compound (C4).

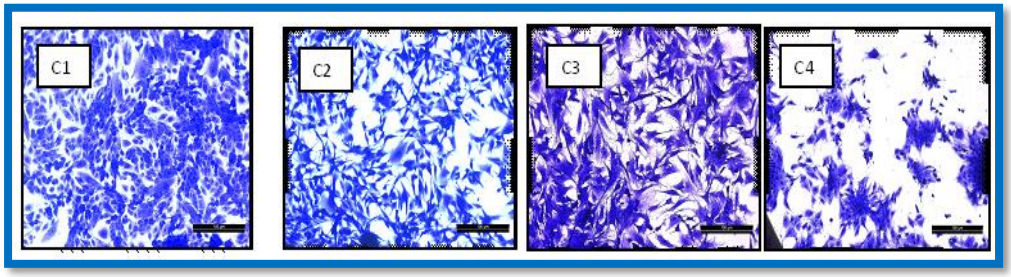

Fig. 9: The Inhibition of cell line (HepG2) by compounds C1-C4 in 50 µg/mL

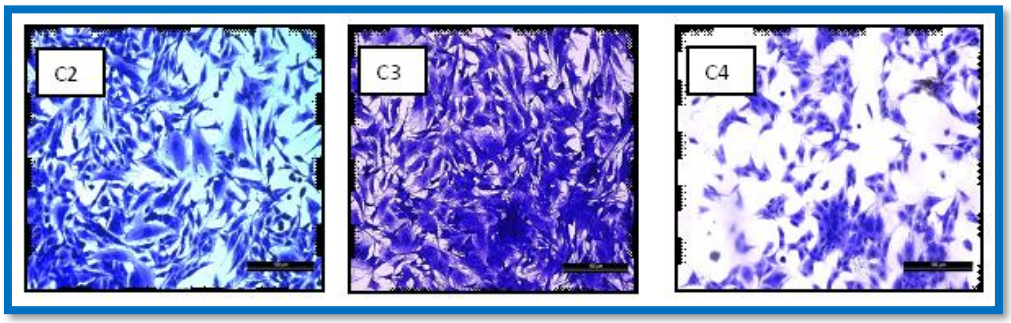

Fig. 10: The Inhibition of cell line (HepG2) by compounds C2-C4 in 100 µg/mL

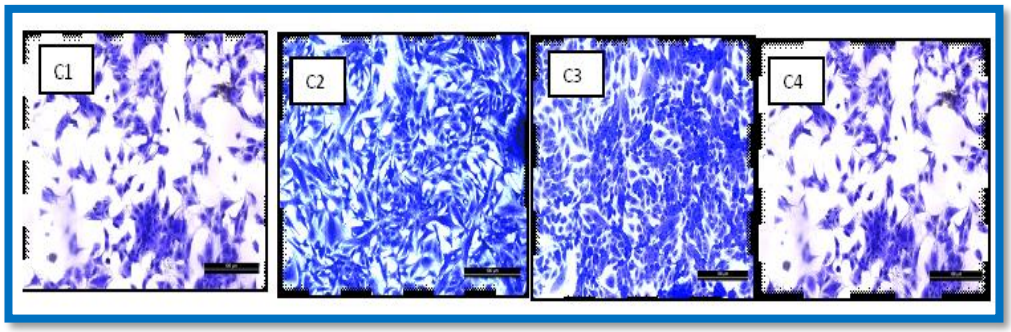

Fig. 11: The Inhibition of cell line (Hela) by compounds C1-C4 in 50 µg/mL

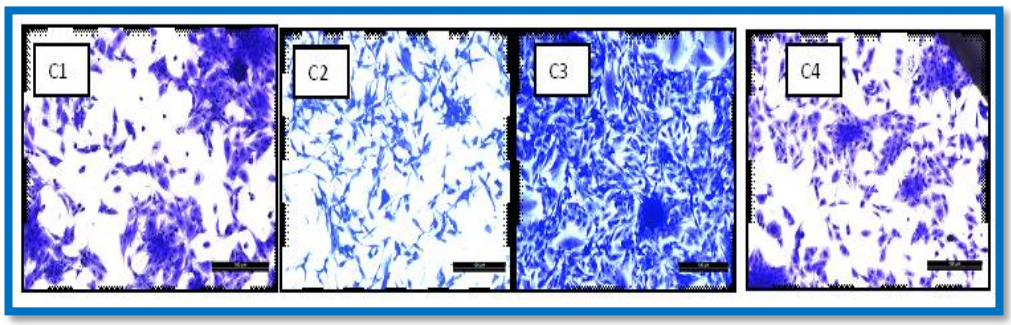

Fig. 12: The Inhibition of cell line (Hela) by compounds C1-C4 in 100 µg/mL

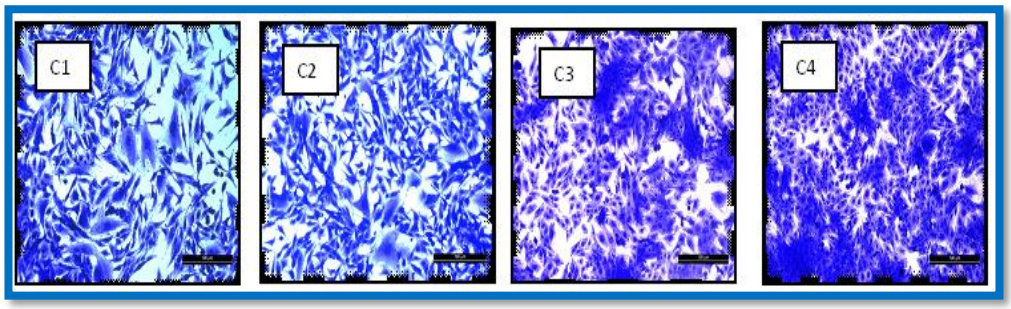

Fig. 13: The Inhibition of Normal cell line (HC) by compounds C1-C4 in 100 µg/mL

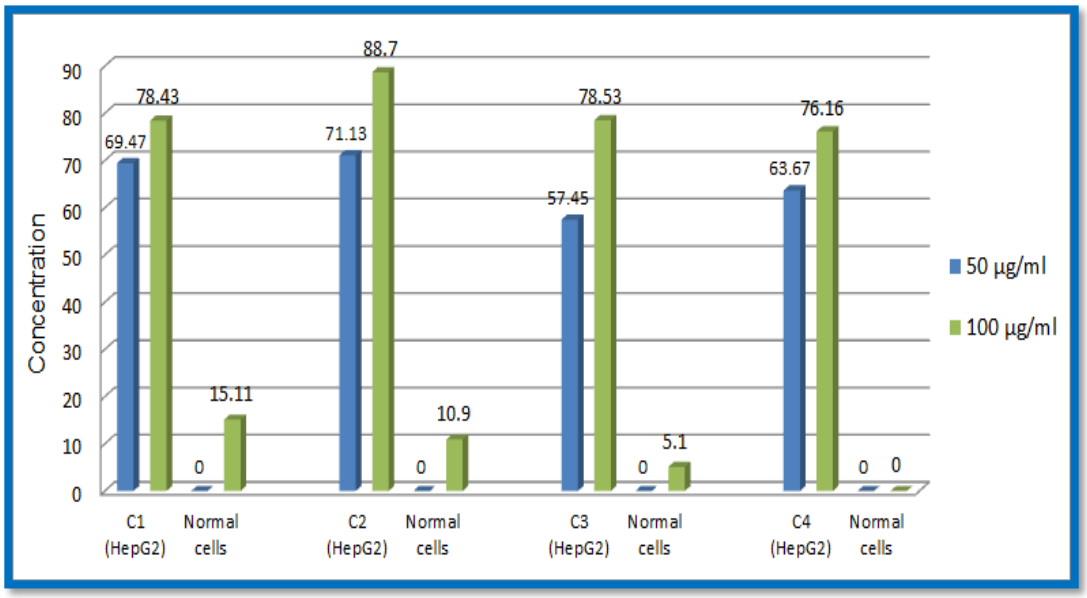

Fig. 14: The HepG2 and HC cell line treated with compounds C1-C4

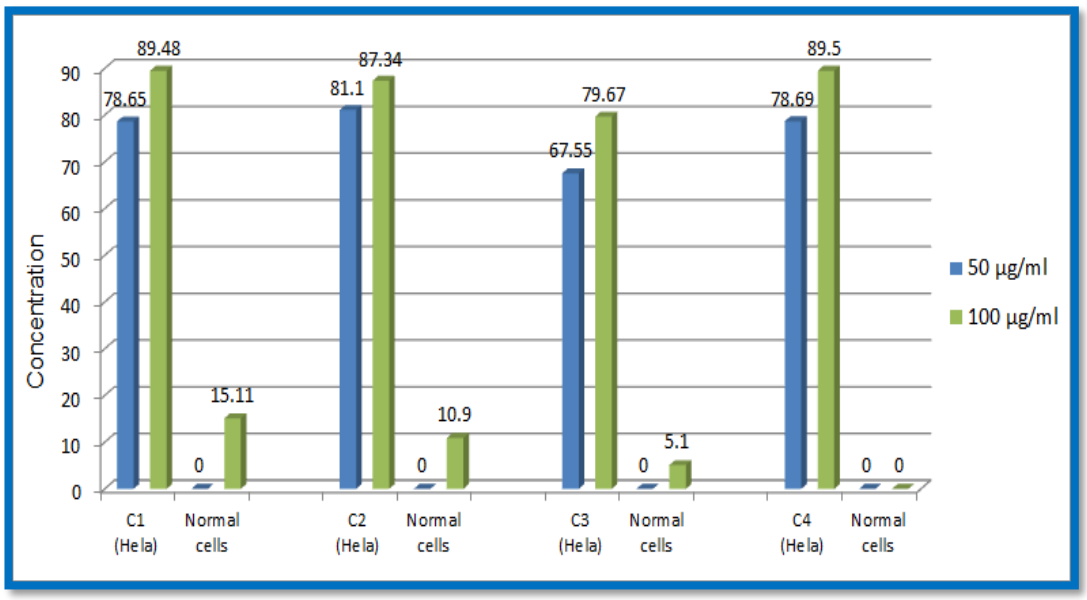

Fig. 15: The Heal and HC cell line treated with compounds C1-C4

4. CONCLUSION

Schiff bases compounds (C1 and C2) were prepared by earlier method describes by Sreenu,p.; et al [17]. while C3 and C4 Schiff bases were synthesized in the present work. Many techniques were used to characterize these new compounds such as FTIR, 1HNMR and 13CNMR. In addition to physical properties. Evaluated the produced compounds' antibacterial properties and found that they had good to acceptable antibacterial activity against the two species of bacteria used—K. Pneumonia, a gram-negative bacterium, and S. aureus, a gram-positive bug. The cytotoxic potential of a few title compounds was also tested against three

human cell lines: breast cancer in humans' cervical cancer in humans, HePG2 cell line Muscle from humans, and Hela cells In two distinct concentrations of 50 and 100 g/mL, the HC cell line is normal. The findings showed that chemical (C4) had the maximum level of cytotoxic action against the Hela cell line at a dose of 100 g/mL, with an inhibition rate of (89.50%).

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