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Article

Synthesis and Characterization of New Heterocyclic Compounds and Ring Fused and Evaluation of Activities as Anti-Bacterial and Anti-Cancer

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ABSTRACT

This research included the synthesis and characterization of some new heterocyclic compound derivatives of (triazole and thiazole) , were synthesized from the imidazole derivative 4,5-dichlorimidazole. The azo derivative (1) was prepared in the first step of this study by reacting the amino derivative (4-amino-3-nitro benzoic acid) in an acidic solution to produce diazonium salt; This molecule was then conjugated to the component (4,5-dichloroimidazole) in the basic intermediate of the derivative. Compound (1) was used to prepare the chemical acid chloride derivative (2) from the reaction. A mixture containing thionyl chloride $SOCl_2$. Next, the pyridine-containing thiosemicarbazide and the acid chloride derivative (2) are mixed to form derivative (3) . Reflexing this derivative in a basic media to get the triazole derivative (4) was the final step. The next step involved a ring-closure reaction between glacial acetic acid and acetic anhydride to produce the conjugated thiazole derivative (5). The (5) derivative was reacted with aromatic aldehydes(4-chlorobenzaldehyde,3,4-dimethoxybenzaldehyde,2-hydroxy-4-methoxy benzaldehyde, 2,4-dimethoxybenzaldehyde, 3-aminobenzaldehyde, 4-hydroxy-3-nitrobenzaldehyde, 1H-indole-3-carbaldehyde) in an acidic solution that contained acetic anhydride and glacial acetic acid to create thiazolidinone derivatives (6-12). The inquiry also included measurements of the melting points and analyses of all synthesized compounds using infrared spectroscopy ,^{13}CNMR and ^{1}HNMR. Additionally,

several biological studies were carried out, The prepared compounds showed high activity against *Staphylococcus aureus* bacteria, which are (1, 2, 3, 4, 9, 10, 12). As for *Escherichia coli* bacteria, compounds (2,3,4,7,10,11,12) showed high effectiveness. When measured as antioxidants, compound 10 showed the highest inhibitory activity at 75%, followed by compound 12 at 67%. It can be concluded from the values obtained that the prepared compounds have a good ability to inhibit free radicals, so the prepared compounds are considered to have antioxidant properties. The prepared compound 10 showed good toxic effect against liver cancer cell line. This effect is due to the fact that the prepared compound contains in its structure an imidazole ring which has high biological activity and resistance to cancer, and it also contains other heterogeneous rings such as triazole which may affect certain receptors present on the surfaces of cells.

Keywords: Azo compounds, Thiazol, Imidazole, Triazole.

Introduction

Heterocyclic compounds are cyclic compounds in which one or more carbon atoms are swapped out for non-carbon atoms in the ring structure, such as sulfur, nitrogen, or oxygen [1,2]. Because of their numerous pharmacological uses, these heterocycles are vital to drug development and materials science, among other domains Furthermore, heteromacrocycles—a class of heterocyclic compound with ten or more atoms in its cycle—contain elements such as nitrogen, sulfur, and oxygen. They are used to bind metallic cations and tiny molecules, acting as structural elements in complicated chemical structures and sensors [3]. Azo compounds, first discovered in 1858 by Griss, are characterized by the presence of an azo group (-N=N-) in their structure and are commonly used as dyes due to their vibrant colors ranging from yellow, red, blue, orange, to green, which are influenced by various functional groups like -N=N-, -N=O, (C=C in aromatic compounds), and (C=O and $-NO_2$). These compounds, which offer a broad color range and great colorfastness for textile applications, can be produced from precursors such as nitroanilines generated from 2,4,6-trinitrotoluene and 1,3,5-trinitrobenzene, according to research[4]. Moreover, azo compounds are used in the dyeing and printing of hydrophobic textiles, exhibiting superior color fastness, sweat resistance, and dye transfer capabilities[5]. Because of its many biological functions and availability in FDA-approved medications, thiazolidinone is a preferred pharmacophore in medicinal chemistry and a central topic for molecular hybridization research[6,7]. Following their synthesis and characterization, derivatives of thiazolidine-2,4-dione have demonstrated encouraging pharmacokinetic qualities, including strong gastrointestinal absorption and

limited blood-brain barrier permeability, which may result in better pharmacological profiles and decreased toxicity[8] . Analogs of thiazolidin-2,4-dione have hypoglycemic, antibacterial, and antioxidant properties via diverse molecular pathways, underscoring their importance in medication development for ailments such as diabetes, infections, and illnesses associated with oxidative stress [9] . Novel compounds with minimal cytotoxicity that are modeled after thiazolidin-4-one structures have shown strong antitubercular, antibacterial, and antifungal properties, suggesting that they may be useful antimicrobial agents [10]. There are two isomeric forms of triazole, a five-membered heterocyclic ring compound: 1,2,3-triazole and 1,2,4-triazole. Due to its numerous biological actions, such as its antibacterial, anti-inflammatory, analgesic, antitubercular, antiviral, antimalarial, and anticancer qualities, this scaffold is incredibly adaptable and has drawn a lot of interest [11]. Triazoles and their derivatives are essential components of many different types of drugs, including antioxidant, antihypertensive, antianxiety, antidepressant, and antitubercular medicines[12]. Triazoles' structural flexibility makes it possible to add various substituents, which opens up new avenues for the production of bioactive compounds with potential applications in medicine and other fields of study. Triazole compounds have been the subject of research on their synthesis techniques, pharmacological actions, and possible uses in medicinal chemistry, materials science, and epigenetics, among other areas, underscoring their promise significance in drug development and beyond[13].

Experimental Section

Materials

In this research, the chemicals utilized included 4-Amino-3-nitro benzoic acid (Fluorochem)and 4,5-Dichloroimidazole (Fluorochem). Thionyl dichloride,4-chlorobenzaldehyde , 3-aminobenzaldehyde , 4-hydroxy-3-, 1H-indole-3-carbaldehyde, Ascorbic acid, DMSO, Methanol and Ethanol 99% (Sigma Aldrich), 2,4-dimethoxybenzaldehyde, Acetic anhydride 99% (Scharlau), 3,4-dimethoxybenzaldehyde , hydrochloric acid and Sodium nitrite and Mueller–Hinton agar (Himedia), 2-hydroxy-4-methoxybenzaldehyde , Sodium hydroxide Anhydrous sodium acetate (Fluka) . 400–4000 cm–1 FT-IR spectra were captured on KBr disk using a Fourier transform SHIMADZU FTIR-8400S.. In the Department of Chemistry, University of Basra, ^{1}HNMR was recorded at a frequency of 500 MHz using DMSO-d6, and Varian Agilent USA was used.

Preparation

Preparation of azo derivative 1 [14]

The preparation method involved two steps. Ten milliliters of distilled water were used to dissolve 0.378 g (5.49 mmol) of $NaNO_2$. After dissolving 1 g (5.49 mmol) of 4-amino-3-nitrobenzoic acid in 40 milliliters of water and 10 mL of strong HCL, this was added dropwise to the amino solution, which was then cooled at 0 Celsius. The mixture was stirred at the same temperature for thirty minutes following the addition of the nitrite solution. In the coupling stage, a cooled solution containing 74 ml of 10% sodium hydroxide solution and 0.751 g, 5.49 mmol of 5,4-dichlorimidazole dissolved in 30 ml of ethanol was progressively mixed with diazonium salt solution. Solubled in distilled water . For two hours, the mixture was swirled at pH 6 and temperatures between 0 and 5)0C. During this period, a precipitate was observed to form with an orange tint. It was decided to postpone the decision until the following day. The precipitate is prepared by filtering, constantly washing with distilled water, drying, and recrystallization from ethanol. The yield was 93% and the melting point was 244–242°C.

Preparation of acid chloride derivative 2 [15]

After being weighed (0.3 g, 0.9 mmol), the azo derivative (1) was added to a two-hole flask with a rounded bottom. The beaker was then filled with 5 mL of thionyl chloride while being constantly stirred using a distillation funnel. In order to generate a vivid red precipitate, the mixture was heated to 70°C for eight hours, and any leftover SOCL2 was eliminated. There was a yield of 94% and a melting point of 188–191 0C.

Preparation of carbothioamide derivative 3 [16]

A solution of (0.1 g, 1.09 mmol thiosemicarbazide) in 4 ml pyridine was added after acid chloride derivative 2 (0.4 g, 1.09 mmol) was dissolved in 5 ml 1,4-dioxane in a round flask. TLC was used to monitor the reaction after the mixture was stirred for 27 hours straight at 25°C after 30 minutes of stirring at 0°C. The precipitate was filtered, cleaned with more distilled water, dried, and recrystallized with ethyl alcohol following the addition of 10 milliliters of distilled water to the mixture.

Preparation of triazole derivative 4 [17]

A round flask containing 0.3 g (0.85 mmol) of carbothioamide derivative was filled with a sodium hydroxide solution (15 ml, 2N). TLC technology was used to track the reaction as the mixture was heated to 90°C for 21 hours. After bringing the mixture to room temperature, acidify it to pH 4 using a diluted solution of (2N) HCl. Following precipitation, ethyl alcohol was used to collect, wash, dry, and recrystallize the product.

Preparation of Thiazolidinone derivative 5 [18]

In 25 milliliters of glacial acetic acid and acetic anhydride, 0.6 g of triazole derivative (4) and 0.14 g of anhydrous sodium acetate were combined with 0.16 g of chloroacetic acid (1.7 mmol).A quarter of a milliliter. TLC (gasoline:methanol, 1:4) was used to monitor the reaction after the mixture was heated for 25 hours to 95 degrees Celsius. The liquid was poured into ice grits once it had cooled to room temperature. After filtering, washing with distilled water, and recrystallizing from dioxane solvent, the precipitate (light brown precipitate) was obtained. The yield percentage was 82%, and Rf 0.72 was the melting point (292–294) ºC.

Preparation of compounds (6-12)(5-arylidene-4-thiazolidinone derivatives) [19]

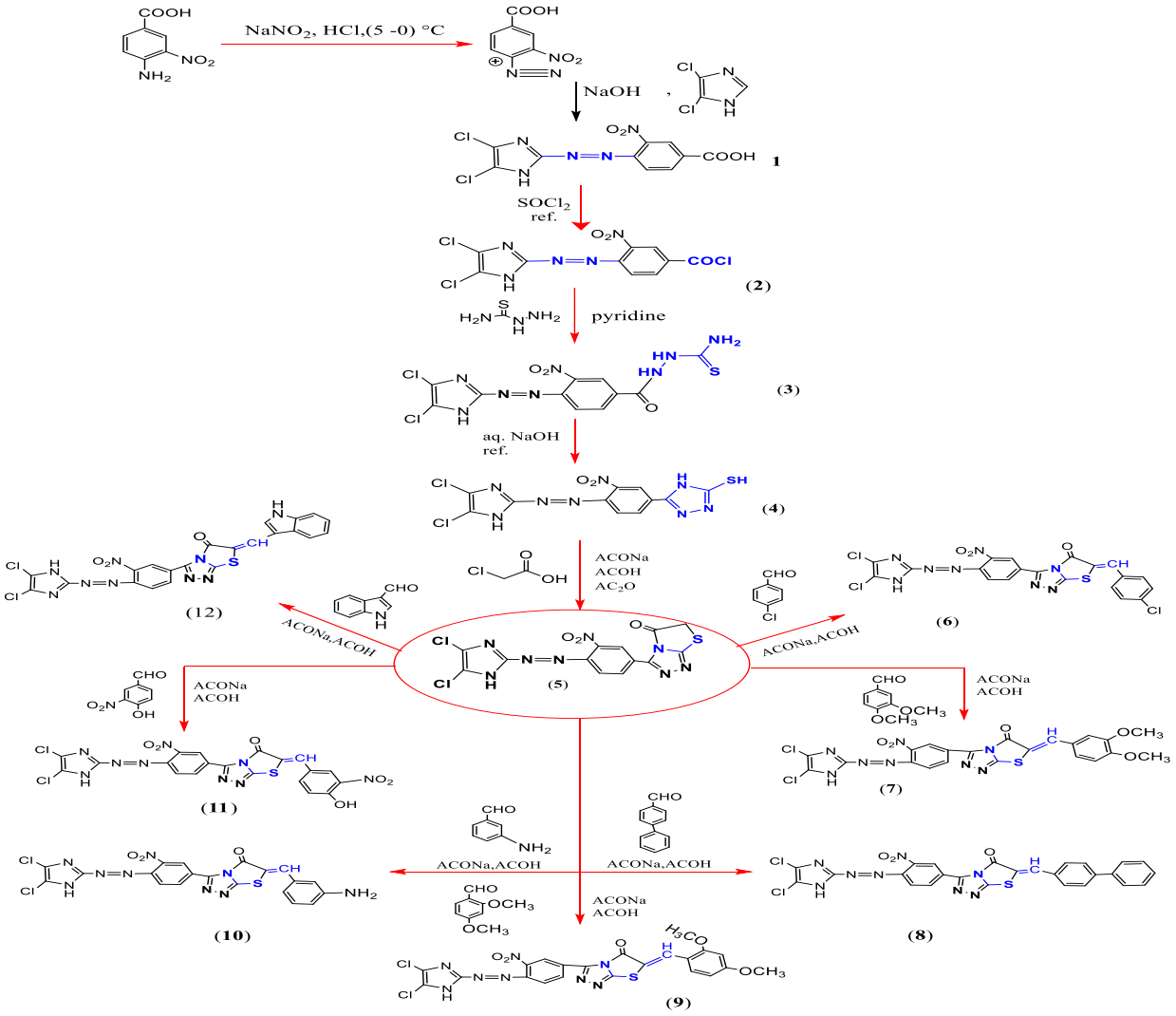
The thiazolidinone derivative (5) (0.1 g, 0.235 milli mol) was added to a mixture of sodium acetate (0.02 g, 0.235 mmol) and 4-chlorobenzaldehyde (0.032 g, 0.235 mmol), 3,4-dimethoxybenzaldehyde (0.039 g, 0.235 mmol), 2-hydroxy-4-methoxybenzaldehyde (0.035 g, 0.235 mmol), 2,4-dimethoxybenzaldehyde (0.039 g, 0.235 mmol), 3-aminobenzaldehyde (0.028 g, 0.235 mmol), 4-hydroxy-3-nitrobenzaldehyde (0.039 g, 0.235 mmol), 1H-indole-3-carbaldehyde (0.034 g, 0.235 mmol) respectively , in 15 ml of glacial acetic acid where the mixture was escalated at 95 degrees Celsius for a period ranging between (25-30 hours). After that, cooled water was added to the mixture. The sediment formed was washed with distilled water and filtered. The resulting compounds were recrystallized with ethanol .

Table (1) Shows Physical properties of the prepared compounds

Comp. No.	M.F.	M.Wt g/mole	M.P. °C	Color	Yield %	R_f
3	$C_{11}H_8Cl_2N_8O_3S$	403.20	132-134	Light orange	81 %	0. 56 (benzene: methanol,4:1)
4	$C_{11}H_6Cl_2N_8O_2S$	385.18	166-168	orange	78 %	0. 6 (benzene: methanol,4:1)
6	$C_{20}H_9Cl_3N_8O_3S$	547.75	188-190	Dark grey	49%	0.62 (chloroform:methanol,4:1)
7	$C_{23}H_{17}Cl_2N_7O_4S$	558.39	269-271	Light orange	81%	0.47 (benzene: methanol,4:1)
8	$C_{26}H_{14}Cl_2N_8O_3S$	589.41	238- 240	grey	57%	0.62 (chloroform:methanol,4:1)
9	$C_{23}H_{17}Cl_2N_7O_4S$	558.39	213- 215	brown	66%	0.48 (benzene: methanol,4:1)
10	$C_{20}H_{11}Cl_2N_9O_3S$	527.01	187-189	yellow	88%	0.58 (hexane:ethylacetate,6:1)
11	$C_{20}H_9Cl_2N_9O_6S$	574.31	219-221	brown	79%	0.52 (benzene: methanol,4:1
12	$C_{22}H_{11}Cl_2N_9O_3S$	552.35	187-189	Yellowish brown	67%	0.59 (benzene: methanol,4:1)

The method of action of the biological activity of an antibacterial

The prepared chemicals were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 200 µg/mL. Initially, the Mueller-Hinton agar medium was prepared using the procedure specified by the manufacturer. After dissolving 38 grams of the culture medium powder in 100 mL of distilled water and heating it in a water bath to dissolve, the powder was placed in an autoclave and left for a quarter of an hour. After sterilization at 121°C for one hour, the solution was cooled and poured into sterile Petri dishes. Bacteria were then spread using a sterile loop on the prepared medium. A well of 0.9 cm in diameter was made in the plate using a sterile punch. Approximately 0.5 mL of the prepared solutions were added, and the plates were incubated for 24 hours at 37°C. The inhibition zones for all the prepared compounds were measured



Scheme (1-1) Preparation of 5-arylidene-4-thiazolidinone derivatives

RESULTS AND MEASUREMENTS

Description of the compound 1

4-((4,5-.dichloro - 1H- imidazole -2-yl) diazinyl) -3- nitrobenzoic acid

The FT-IR spectra of the produced compound first reveal a large overlapping peak at 3440 cm^{-1} associated with the carboxylic acid (OH) and overlapping with the (-NH) peak. The peak values of Ar-H and CH in CH_3 were 3078 cm^{-1} and 2993 cm^{-1}, respectively. The values are as follows: 1697 cm^{-1} for (C=O), 1604 cm^{-1} for (C=N), 1543 cm^{-1} for (C=C), 1088 cm^{-1} for (C-Cl), and 1427 cm^{-1} for (N=N). ^{1}HMNR spectroscopic measurements for compound (1) show 13.6 ppm (S , 1 H,(OH)), 8.5 ppm (S , H, (NH)), and 8.1–8.2 ppm (M,3H, Ar-H).

Compound (1)'s ^{13} C-NMR spectra reveal the following: 175 ppm (C_{10}), 166 ppm (C_1), 150.8–150.4 ppm (C_2, C_3), 137 ppm (C_4), and 124–136 ppm (C_{Arom}).

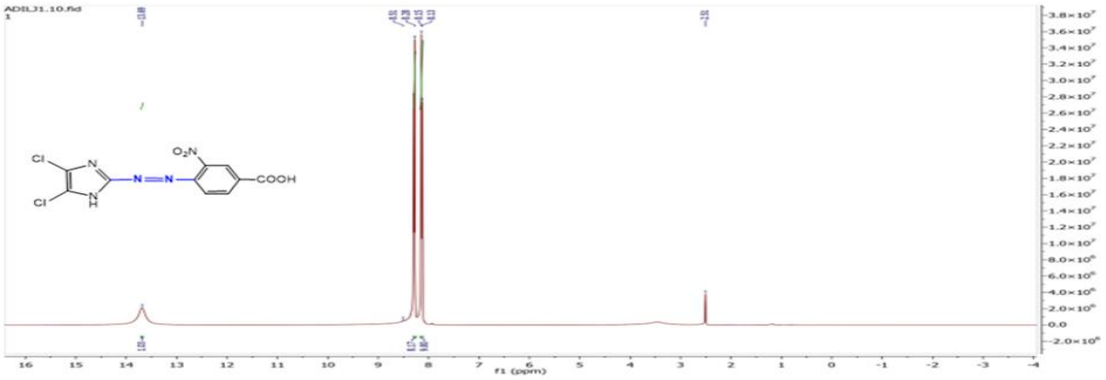


Fig (1) : ^{1}H-NMR spectrum of compound (1)

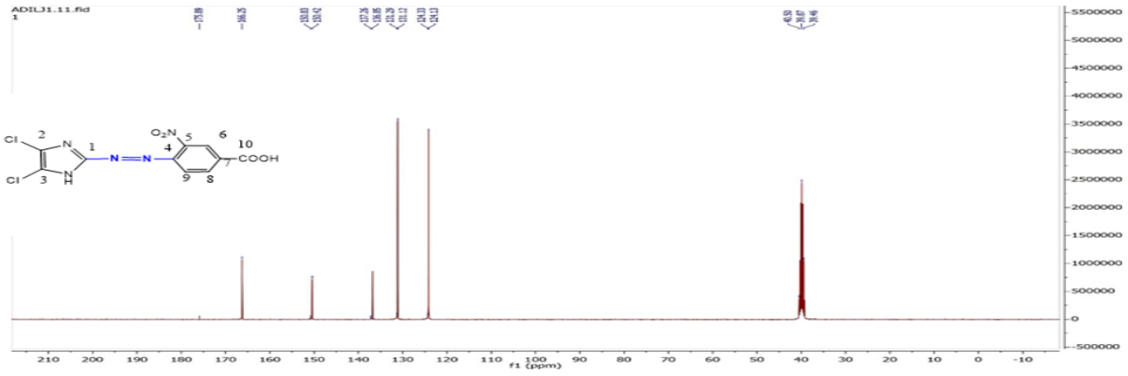


Fig (2) : ^{13}C-NMR spectrum of compound (1)

Description of the compound 2

4- ((4,5-.dichloro -1H- imidazole-2-yl)diazinyl)-3- nitrobenzol chloride Compound (2): 3109 cm^{-1} for (-NH), 3047 cm^{-1} for (Ar–H), 2862 cm^{-1} for (C-H)inCH_3, 1758 cm^{-1} for (C=O), 1604 cm^{-1} for (C=N), 1527 cm^{-1} for (C=C), 1064 cm^{-1} for (C-Cl), and 1404 cm^{-1} for (N=N) are the FT-IR spectrum data. ^{1}H-MNR spectrum measurements of compound (2) show 7.4 - 8.3 ppm (M, 3H, Ar-H) and 8.9 ppm (S, 1H, (NH)). . Compound (2) of the ^{13} C-NMR spectrum reveals: 171 ppm (C_{10}), 166 ppm (C_1), 150.9–150.4 ppm (C_2, C_3), and 124–136 ppm (C_{Arom})

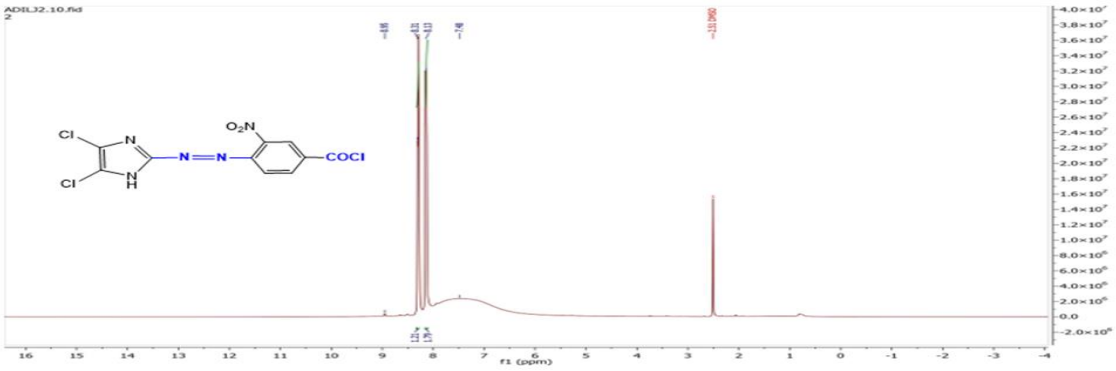


Fig (3) : ^{1}H-NMR spectrum of compound (2)

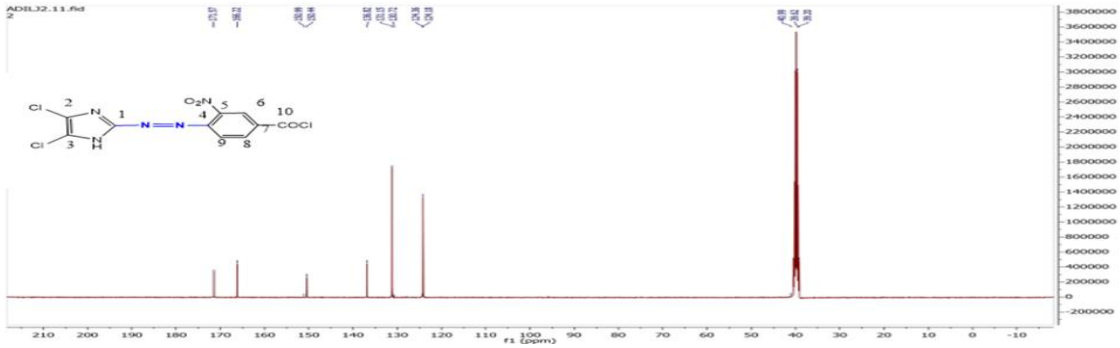


Fig (4) : ^{13}C-NMR spectrum of compound (2)

Description of the compound 3

2- (4-((4,5- dichloro -1H-imidazole -2-yl) diazenyl)-3-nitrobenzol) hydrazine-1-carbo thioamide

A peak at 3155 cm^{-1} for (Ar –H), a peak at (3402 and 3247)$cm^{-1}$for(-NH) , a peak at 2962 cm^{-1} for (C-H) in CH_3, 1674 cm^{-1} for (C=O), 1604 cm^{-1} for (C=N), 1519 cm^{-1} for (C=C), 1080 cm^{-1} for (C-Cl), and 1481 cm^{-1} for (N=N) are all visible in the compound (3)'s FT-IR spectrum data. ^{1}H-MNR spectrum measurements of compound (3) show 10.8 ppm imidazole (S, 1H, NH), 9.5–9.4 ppm (S,2H, NH), 6.1 ppm (S,2H, NH_2), and 7–8.8 ppm (M,3H, Ar-H).) . compound (3) of the ^{13}C-NMR spectrum reveal 182 ppm(C_{10}), 166ppm (C_{11}) ,164 ppm(C_1) , 150 ppm (C_2,C_3) , 123 -149 ppm (C_{Arom})

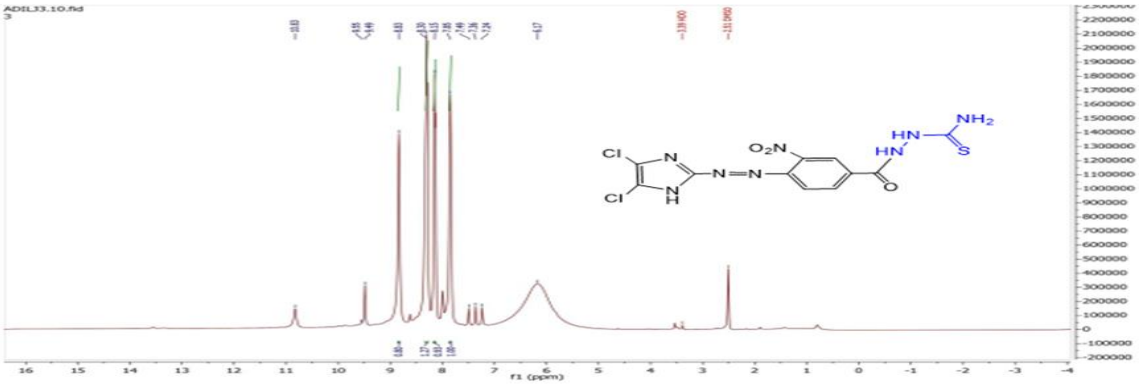


Fig (5) : ^{1}H-NMR spectrum of compound (3)

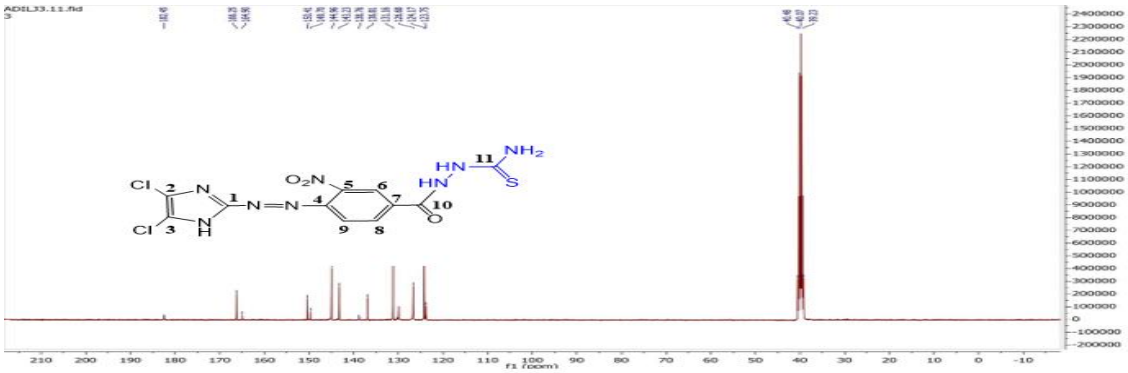


Fig (6) : ^{13}C-NMR spectrum of compound (3)

Description of the compound 4

5 - (4-((4,5-dichloro -1H-imidazole -2- yl) diazenyl)-3- nitrophenyl)-4H-1,2,4-triazole -3-thiol

Compound (4) exhibits a peak that can be seen at (3402) cm^{-1} for (NH), 3105 cm^{-1} for (Ar-H), 2952 cm^{-1} for (C-H) in CH_3, 1635 cm^{-1} for (C=N), 1527 cm^{-1} for (C=C), 1095 cm^{-1} for (C-Cl), and 1396 cm^{-1} for (N=N). These data are displayed in the FT-IR spectra. Measurements of compound (4)'s. The ^{1}H-NMR spectrum shows that it contains 8.7 ppm of imidazole (S,1H, NH), 8.3 ppm of tetrazole (S,1H, NH), 6.5 ppm of (S,1H, SH), and 7–8.1 ppm of (M,3H, Ar-H). .

compound (4) 's of The ^{13}C-NMR spectrum reveals : 166ppm (C_1), 150 ppm (C_2,C_3) ,136 ppm (C_{10}) ,132-131 ppm (C_{Arom})

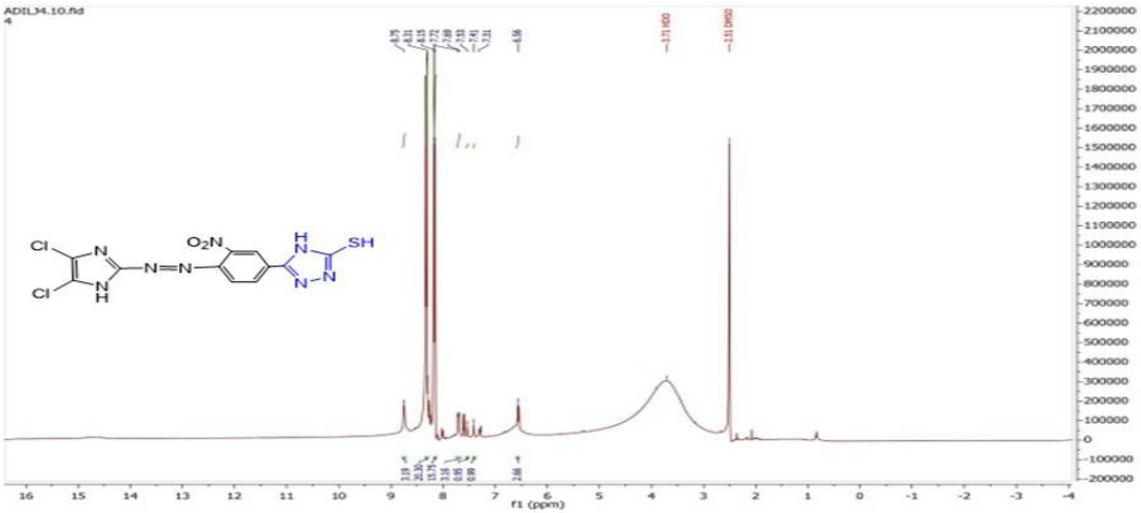


Fig (7) : ^{1}H-NMR spectrum of compound (4)

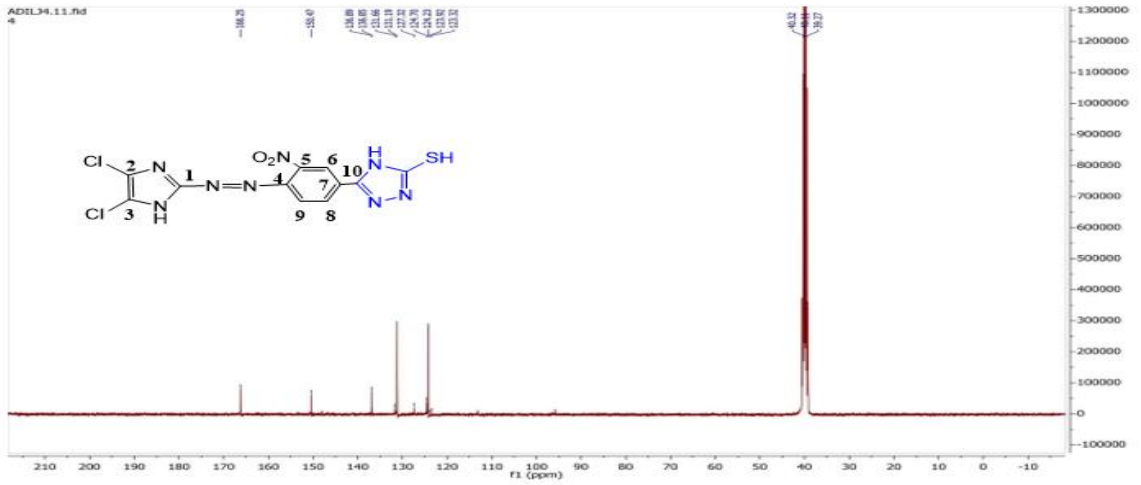


Fig (8) : ^{13}C-NMR spectrum of compound (4)

Description of the compound 5

3- (4-((4,5-dichloro -1H-imidazol-2-yl) diazinyl) -3- nitrophenyl) thiazolo [2,3-c] [1,2,4] triazol -5(6H) -one

Using the FT-IR spectra of compound (5), the following peak locations are identified: (-NH) at (3409) cm^{-1}, (Ar – H) at 3178 cm^{-1}, (C-H)inCH_3 at (2931 cm^{-1}), (C=O) at (1697 cm^{-1}), (C=N) at (1604 cm^{-1}), (C=C) at (1527 cm^{-1}), (C-Cl) at (1080 cm^{-1}), and (N=N) at (1427 cm^{-1}). The ^{1}H-NMR spectra of Compounds(5) show 8.9 ppm (S,1H, NH), 4.2 ppm (S, 2H, CH_2), and 7–8.1 ppm (M,3H, Ar-H).

Compound (5) of The ^{13}C-NMR spectrum reveals : 167 ppm (C_{11}) , 166ppm (C_1) , 61 ppm (C_{12}) ,150 ppm (C_2,C_3) ,164 ppm (C_{10}) ,142 ppm (C_{13}) ,118- 131 ppm (C_{Arom})

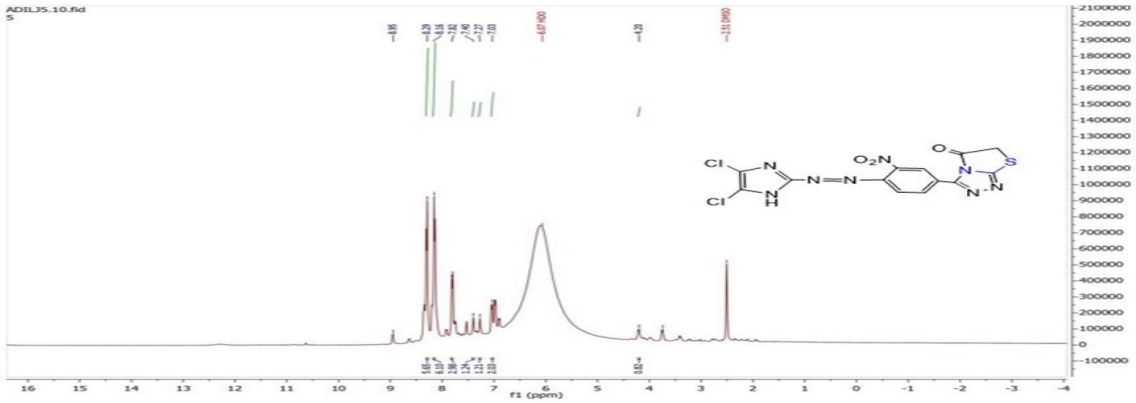


Fig (9) : ^{1}H-NMR spectrum of compound (5)

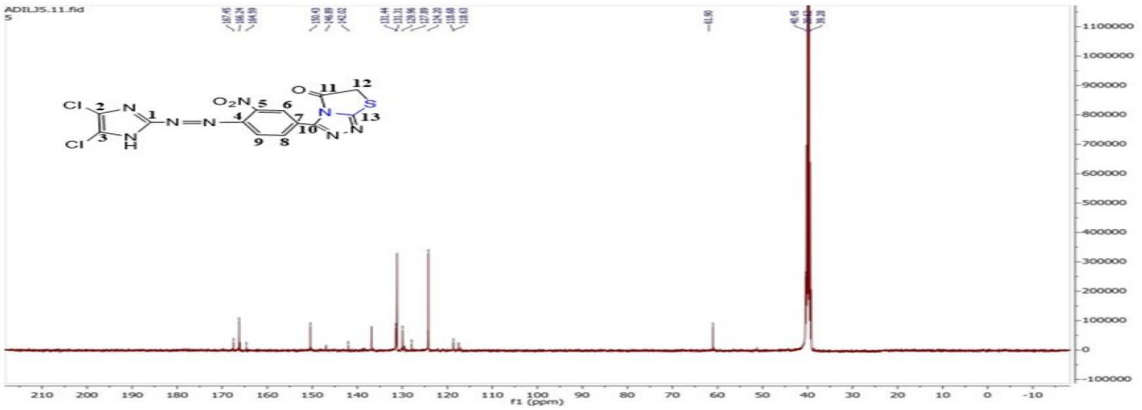


Fig (10) : ^{13}C-NMR spectrum of compound (5)

Description of the compound 6

6-((Z)-4-chlorobenzylidene)-3-(4-((4,5-dichloro-1H-imidazol-2-yl)diazenyl)-3-nitrophenyl)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one

Using the FT-IR spectra of compound (6), the following peak locations are identified: (-NH) at (3440) cm^{-1}, (=CH) at (3085) cm^{-1}, (Ar – H) at 3016 cm^{-1}, (C-H)inCH_3 at (2900 cm^{-1}), (C=O) at (1697 cm^{-1}), (C=N) at (1604 cm^{-1}), (C=C) at (1527 cm^{-1}), (C-Cl) at (1110 cm^{-1}), and (N=N) at (1437 cm^{-1}). The 1H-NMR spectra of Compounds(6) show 8.2 ppm (S,1H, NH), 3.7 ppm (S, H, CH), and 7.5–8.1 ppm (M,7H, Ar-H). Compound (6) of The ^{13}C-NMR spectrum reveals : 160 ppm (C_{11}) , 165ppm (C_{10}) , 170 ppm (C_{13}) , 168ppm (C_1), 107 ppm (C_{14}) ,156 ppm (C_4) ,101- 156 ppm (C_{Arom})

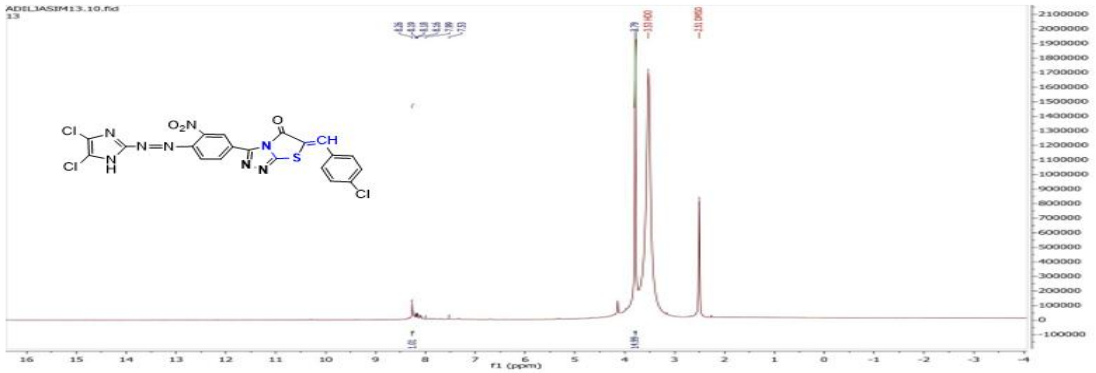


Fig (11) : ^{1}H-NMR spectrum of compound (6)

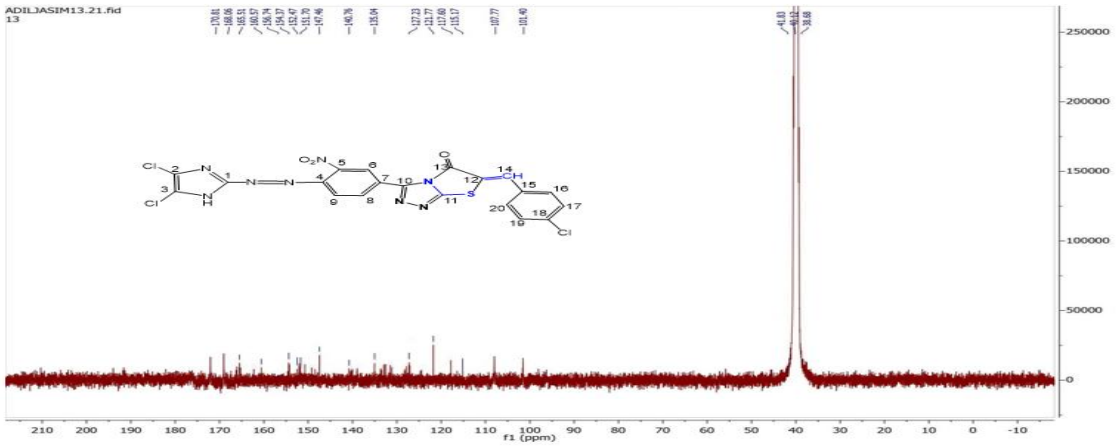


Fig (12) : ^{13}C-NMR spectrum of compound (6)

Description of the compound 7

3-(4-((4,5-dichloro-1H-imidazol-2-yl)diazenyl)-3-nitrophenyl)-6-((Z)-3,4-dimethoxy benzylidene)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one

Using the FT-IR spectra of compound (7), the following peak locations are identified: (-NH) at (3386) cm^{-1}, (=CH) at (3116) cm^{-1}, (Ar – H) at 3055 cm^{-1}, (C-H)inCH_3 at (2862 cm^{-1}), (C=O) at (1720 cm^{-1}), (C=N) at (1643 cm^{-1}), (C=C) at (1527 cm^{-1}), (C-Cl) at (1103 cm^{-1}), and (N=N) at (1473 cm^{-1}). The ^{1}H-NMR spectra of Compounds(7) show 9.8 ppm (S,1H, NH), 7.5 ppm (S, H, CH), 3.86,3.82 ppm (S, 6H, OCH_3), and 7.3–8.1 ppm (M,5H, Ar-H). Compound (7) of The ^{13}C-NMR spectrum reveals : 161 ppm (C_{11}) , 111ppm (C_{12}) , 192 ppm (C_{13}) , 56,55 ppm (C_{22},C_{21}) , 167ppm (C_1), 109 ppm (C_{14}) , 129- 156 ppm (C_{Arom})

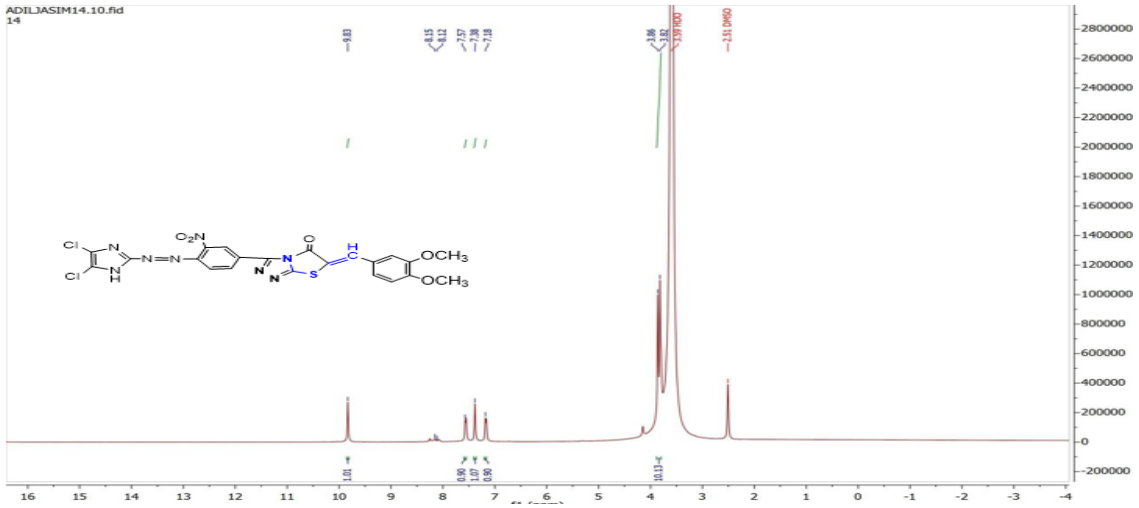


Fig (13) : ^{1}H-NMR spectrum of compound (7)

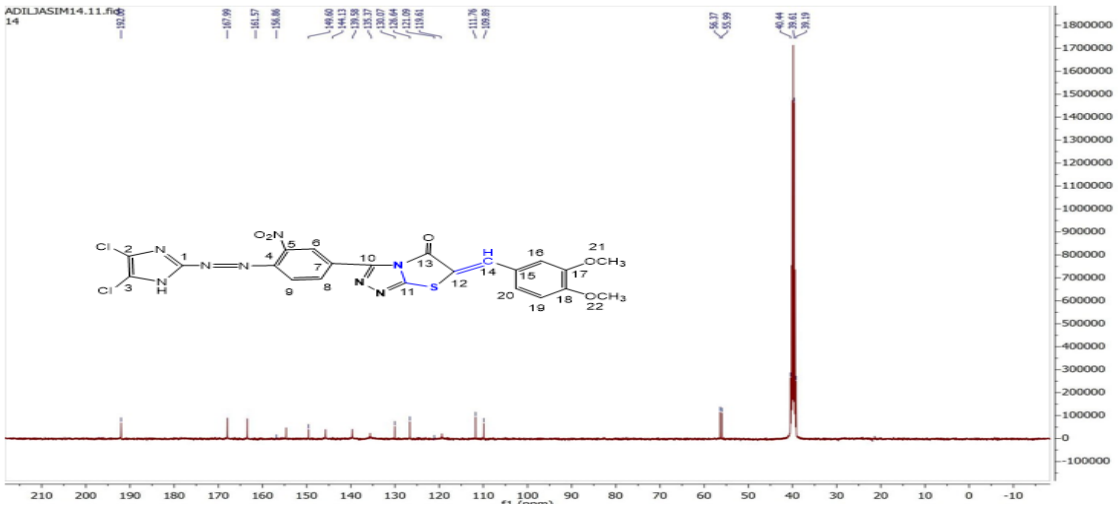


Fig (14) : ^{13}C-NMR spectrum of compound (7)

Description of the compound 8

(6Z)-6-([1,1'-biphenyl]-4-ylmethylene)-3-(4-((4,5-dichloro-1H-imidazol-2-yl) diazenyl)-3-nitrophenyl)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one

Using the FT-IR spectra of compound (8), the following peak locations are identified: (-NH) at (3425) cm^{-1}, (=CH) at (3150) cm^{-1}, (Ar – H) at 3020 cm^{-1}, (C

-H)inCH_3 at (2909 cm^{-1}), (C=O) at (1681 cm^{-1}), (C=N) at (1604 cm^{-1}), (C=C) at (1558 cm^{-1}), (C-Cl) at (1008 cm^{-1}), and (N=N) at (1411 cm^{-1}). The ^{1}H-NMR spectra of Compounds(8) show 10 ppm (S,1H, NH), 7.4 ppm (S, H,=CH), and 7.5–8.2 ppm (M,12H, Ar-H). Compound (8) of The ^{13}C-NMR spectrum reveals : 169 ppm (C_1) , 193ppm (C_{13}) , 144 ppm (C_{14}) , 155ppm (C_{10}), 127- 139 ppm (C_{Arom})

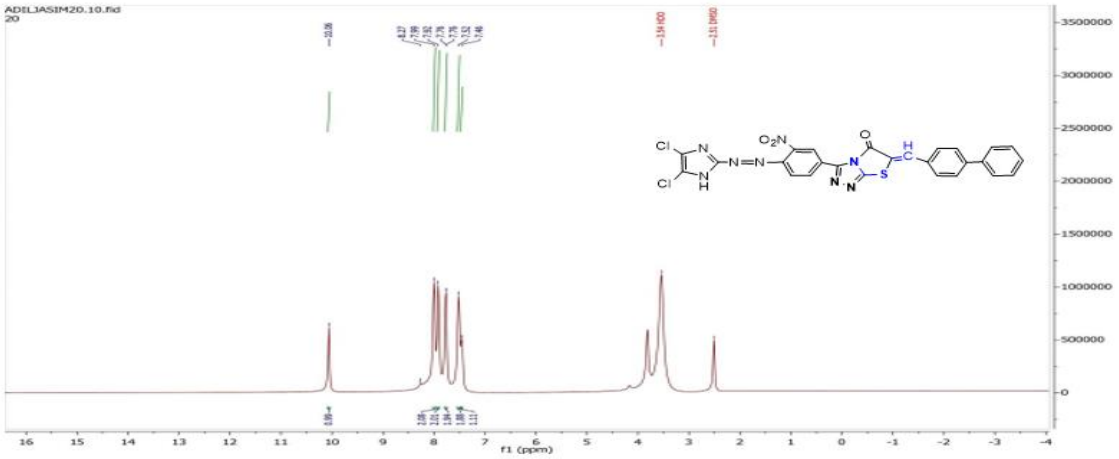


Fig (15) : ^{1}H-NMR spectrum of compound (8)

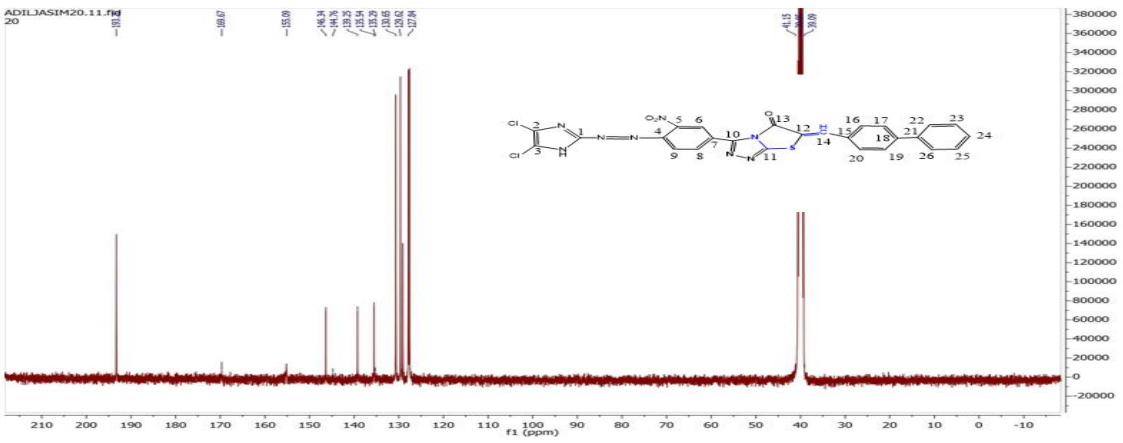


Fig (16) : ^{13}C-NMR spectrum of compound (8)

Description of the compound 9

3-(4-((4,5-dichloro-1H-imidazol-2-yl)diazenyl)-3-nitrophenyl)-6-((Z)-2,4-dimethoxy benzylidene)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one

Using the FT-IR spectra of compound (9), the following peak locations are identified: (-NH) at (3249) cm^{-1}, (=CH) at (3116) cm^{-1}, (C-H)inCH_3 at (2970 cm^{-1}), (C=O) at (1681 cm^{-1}), (C=N) at (1612 cm^{-1}), (C=C) at (1573 cm^{-1}), (C-Cl) at (1141 cm^{-1}), and (N=N) at (1427 cm^{-1}). The ^{1}H-NMR spectra of Compounds(9) show 9.9 ppm (S,1H, NH), 7.1 ppm (S, H, CH), 1.9 , 2. 2 ppm (S, 6H, OCH_3), and 7.3–7.6 ppm (M,6H, Ar-H). Compound (9) of The ^{13}C-NMR spectrum reveals : 144 ppm (C_{10}) , 193 ppm (C_{13}) , 20,19 ppm (C_{22},C_{21}) , 166ppm (C_1), 141 ppm (C_{14}) , 127- 141 ppm (C_{Arom})

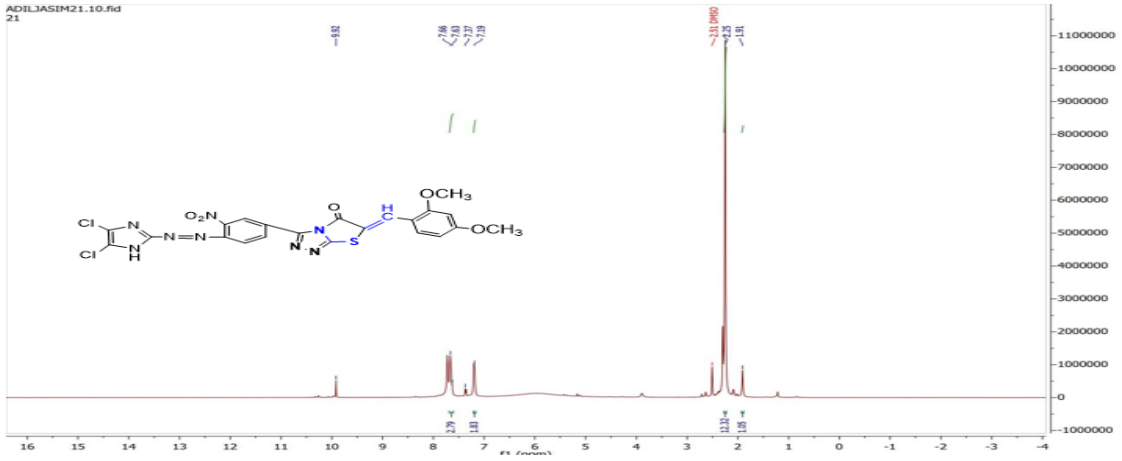


Fig (17) : ^{1}H-NMR spectrum of compound (9)

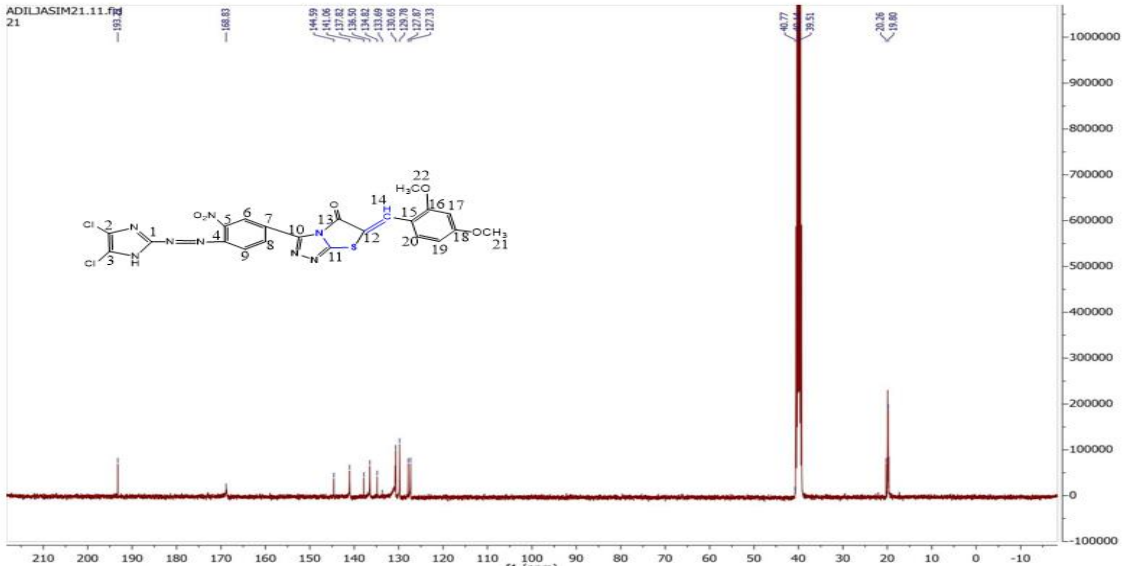


Fig (18) : ^{13}C-NMR spectrum of compound (9)

Description of the compound 10

6-((Z)-3-aminobenzylidene)-3-(4-((4,5-dichloro-1H-imidazol-2-yl)di azenyl)-3-nitrophenyl)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one Using the FT-IR spectra of compound (10), the following peak locations are identified: (-NH, NH_2) at (3425,3287) cm^{-1}, (=CH) at (3186) cm^{-1}, (Ar – H) at 3016 cm^{-1}, (C-H)inCH_3 at (2839 cm^{-1}), (C=O) at (1697 cm^{-1}), (C=N) at (1640 cm^{-1}), (C=C) at (1558 cm^{-1}), (C-Cl) at (1018 cm^{-1}), and (N=N) at (1419 cm^{-1}). The ^{1}H-NMR spectra of Compounds(10) show 10.4 ppm (S,1H, NH)at imidazole. 4.1 ppm (S, 2H, NH_2). 3.7 ppm (S, 1H, CH=), and 7.8–8.1 ppm (M,6H,Ar-H). Compound (10) of The ^{13}C-NMR spectrum reveals : 149 ppm (C_{11}) , 193 ppm (C_{13}), 137 ppm (C_{14}) , 169ppm (C_1), 158 ppm (C_{10}) , 119- 132 ppm (C_{Arom}) , 140 ppm (C_{12})

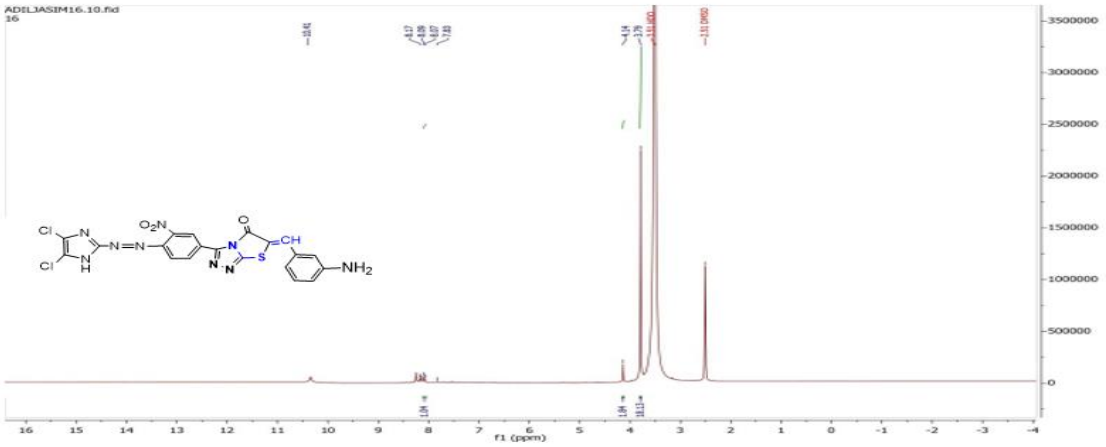


Fig (19) : ^{1}H-NMR spectrum of compound (10)

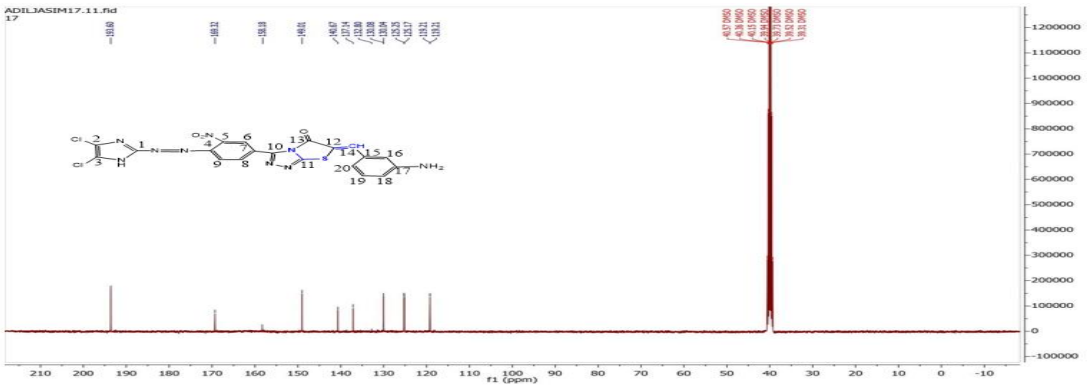


Fig (20) : ^{13}C-NMR spectrum of compound (10)

Description of the compound 11

3-(4-((4,5-dichloro-1H-imidazol-2-yl)diazenyl)-3-nitrophenyl)-6-((Z)-4-hydroxy-3-nitrobenzylidene)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one

Using the FT-IR spectra of compound (11), the following peak locations are identified: (OH) at (3425) cm^{-1}, (-NH) at (3180) cm^{-1}, (=CH) at (3012) cm^{-1}, (Ar – H) at 3001 cm^{-1}, (C-H)inCH_3 at (2947 cm^{-1}), (C=O) at (1681 cm^{-1}), (C=N) at (1604 cm^{-1}), (C=C) at (1558 cm^{-1}), (C-Cl) at (1072 cm^{-1}),(NO_2)at (1512,1350 cm^{-1}), and (N=N) at (1419 cm^{-1}). The ^{1}H-NMR spectra of Compounds(11) show 9.4 ppm (S,1H, OH). 8.6 ppm (S, 1H, NH). 6.4 ppm (S, 1H, =CH), and 7.4–8.2 ppm (M,6H,Ar-H). Compound (11) of The ^{13}C-NMR spectrum reveals : 151 ppm (C_{11}) , 188 ppm (C_{13}) , 171ppm (C_1), 169 ppm (C_{10}) , 114- 135 ppm (C_{Arom}) , 137 ppm (C_{12})

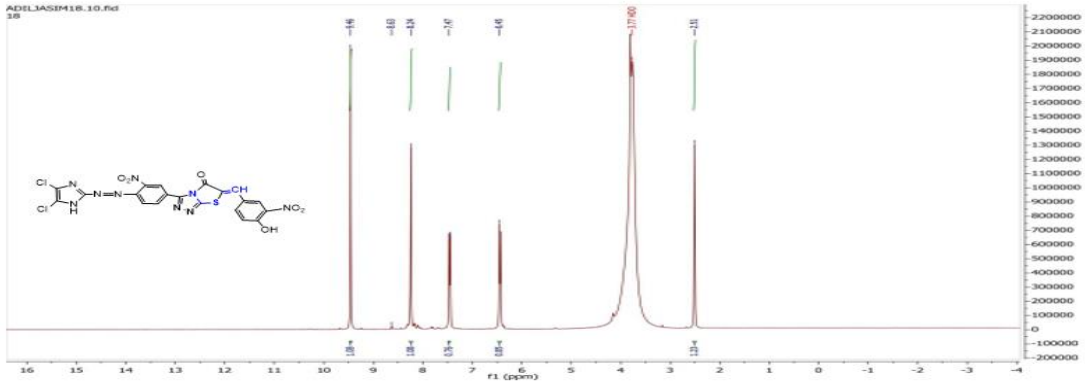


Fig (21) : ^{1}H-NMR spectrum of compound (11)

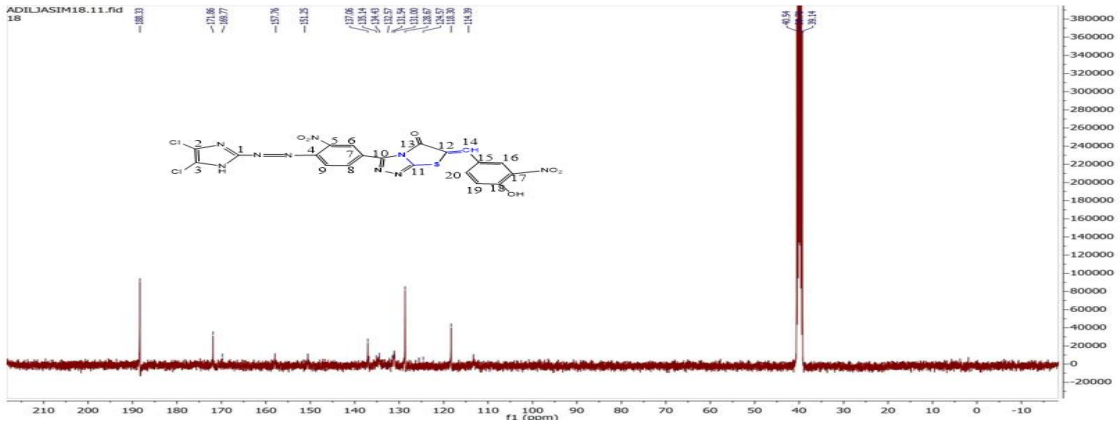


Fig (22) : ^{13}C-NMR spectrum of compound (11)

Description of the compound 12

(6E)-6-((1H-indol-3-yl)methylene)-3-(4-((4,5-dichloro-1H-imidazol-2-yl)diazenyl)-3-nitrophenyl)thiazolo[2,3-c][1,2,4]triazol-5(6H)-one

Using the FT-IR spectra of compound (12), the following peak locations are identified: (-NH) at (3425) cm^{-1}, (=CH) at (3286) cm^{-1}, (Ar – H) at 3193 cm^{-1}, (C-H)inCH_3 at (2977 cm^{-1}), (C=O) at (1651 cm^{-1}), (C=N) at (1558 cm^{-1}), (C=C) at (1527 cm^{-1}), (C-Cl) at (1112 cm^{-1}), and (N=N) at (1411 cm^{-1}). The ^{1}H-NMR spectra of Compounds(12) show 9.9 ppm (S,1H, NH)at imidazole. 9.6 ppm (S,1H, NH)at pyrrole. 7.1 ppm (S, 1H, CH). , and 7.2–8.2 ppm (M,8H, Ar-H). Compound (12) of The ^{13}C-NMR spectrum reveals : 137 ppm (C_{11}) , 185 ppm (C_{13}) , 139ppm (C_1), 138 ppm (C_2,C_3) , 113- 124 ppm (C_{Arom}) , 45 ppm (C_{16}) .

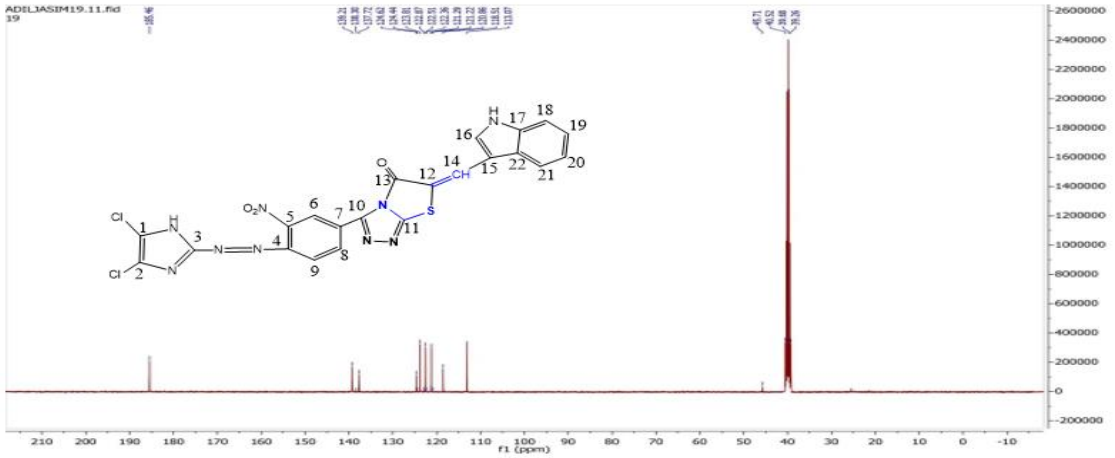


Fig (23) : ^{1}H-NMR spectrum of compound (12)

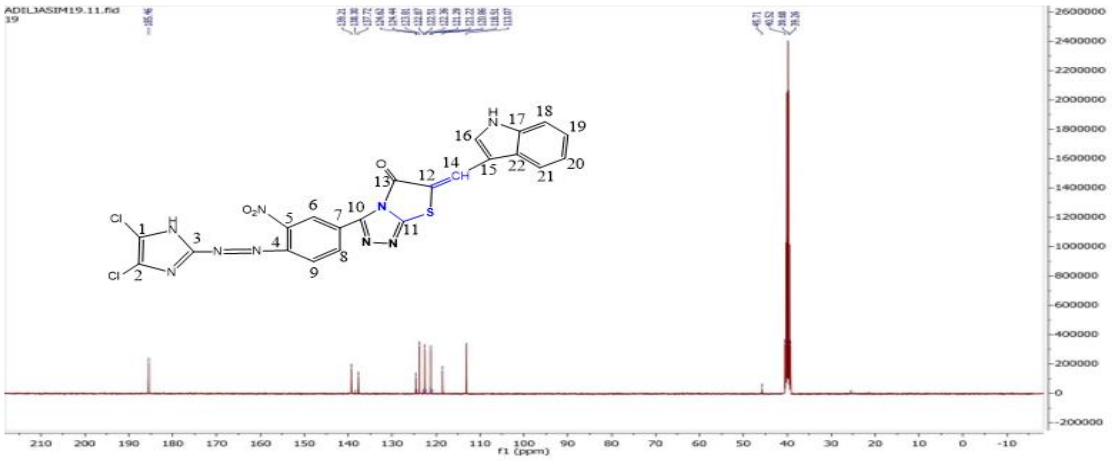


Fig (24) : ^{13}C-NMR spectrum of compound (12)

The Bioactivity

Antibacterial

Two types of pathogenic bacteria isolated from clinical patients after laboratory diagnosis were used to test the biological efficacy of the prepared chemicals. *Escherichia coli* is a type of gram-negative bacteria, and the prepared compounds under study showed strong inhibitory results against gram-negative bacteria, where compounds (2,3,4,7,10,11,12) showed excellent inhibitory activity. As for the other class of bacteria, which is gram-positive *Streptococcus*, the results also indicated that the prepared compounds show good efficacy, especially compounds (1,2,3,4,9,10,12) are more effective than other compounds. This is because the prepared compounds under study are biologically active due to the active groups that the prepared compounds possess.

Table (2) Shows the antibacterial activity values of the prepared compounds from1 to 12.

Comp.NO	*Streptococcus*	Mm	*Escherichia Coli*	Mm
1	++	12	+	10
2	++	13	++	12
3	++	14	++	11
4	++	11	++	19
5	+	9	+	9
6	+	7	+	10
7	+	6	++	14
8	+	10	+	10
9	++	13	+	9
10	++	16	++	13
11	+	10	++	15
12	++	17	++	12

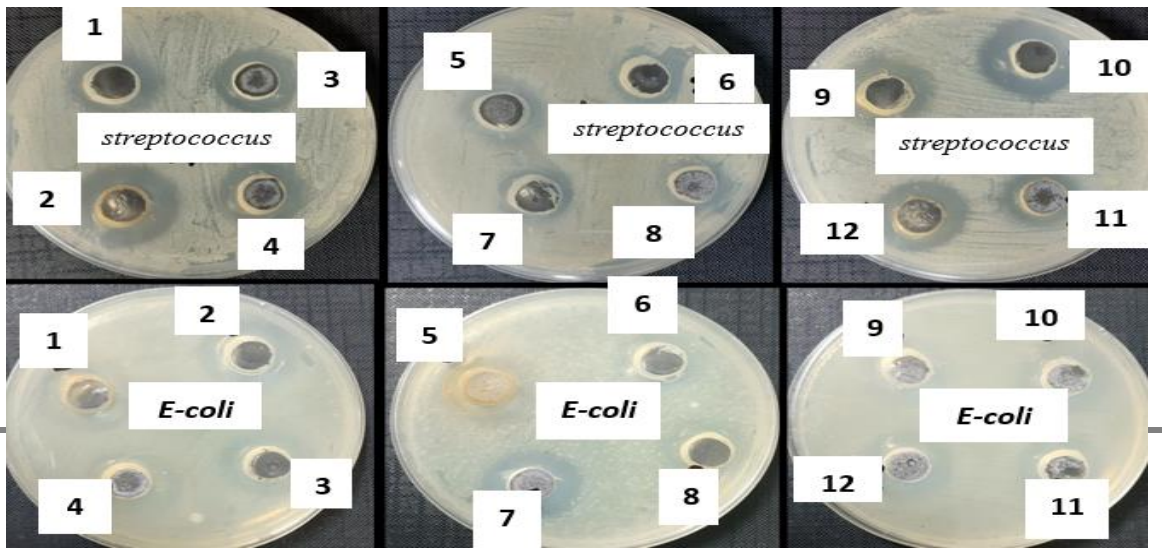


Fig (25) : shows the effect of the prepared compounds on (*Streptococcus*) against (*E.Coli*)

Antioxidant activity

The antioxidant activity of the prepared Thiazolidinone derivative containing the amin group was evaluated using the rapid method (DPPH radical scavenging) in which the 2,2-diphenyl-1-picrylhydrazyl radical is characterized by its strong absorption and at a wavelength of 517 nm, which changes colour in the presence of an oxidizing substance from violet to yellow. Methanol-DMSO mixture was used as the sample solvent. The DPPH test for free radicals was used to determine the biological activity of the prepared compounds, as this test is characterized by simplicity and ease. The DPPH test depends on the rate of return of radicals in the presence of antioxidants that have the ability to give up hydrogen or electrons. The change is observed through the change in color from violet of the free radical to yellow, and it turns into a stable molecule.

Table (3) Shows the free radical inhibition rate of compound 10.

DPPH Radical Scavenging Activity %				
Concentration µg/ml	Compound 10		Ascorbic acid	
	Mean	SD	Mean	SD
200	75.733	2.100394	77.23767	2.400792
100	69.56	4.235175	67.97833	3.018565
50	58.796	6.127538	54.47533	2.411951
25	43.943	3.354899	40.43233	7.08072
12.5	38.503	1.860291	17.63133	7.196421

The results became clear after conducting a DPPH test for the compounds under study (10,12). It was clear that the prepared compounds under study possess biological activity as antioxidants, as they were compared with ascorbic acid, which is considered a standard antioxidant. Different concentrations of the prepared compounds under study were used to measure their biological activity (12.5, 25, 50, 100, 200) g/mlµ, where the prepared compound (10) showed high inhibitory activity reaching (75.73%) at the highest concentration (200 g/mlµ). Compound (12) also gave a high inhibitory activity, reaching (67.63%). The results show that the ability of these compounds to prevent the formation of free radicals results from the presence of electronic doubles, as well as their possession of groups that give free radicals to hydrogen atoms and transform

them into stable molecules. Examples of these groups are NH group) as shown in the following Figs

Table (4) Shows the free radical inhibition rate of compound 12.

DPPH Radical Scavenging Activity %				
Concentration µg/ml	Compound 12		Ascorbic acid	
	Mean	SD	Mean	SD
200	67.014	1.304636	77.23767	2.400792
100	67.631	0.240913	67.97833	3.018565
50	50.656	4.422794	54.47533	2.411951
25	42.70833	1.632872	40.43233	7.08072
12.5	31.01867	1.104263	17.63133	7.196421

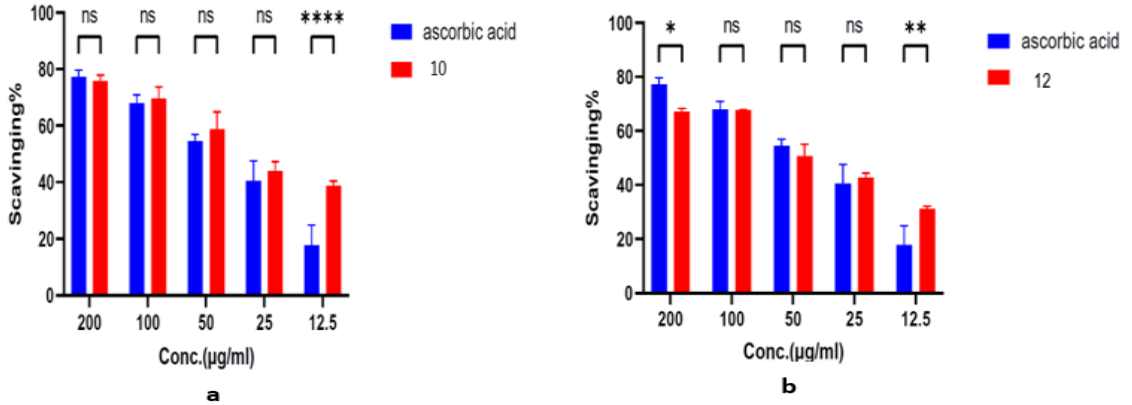


Fig (26)(a) : shows the antioxidant activity of compound 10 and ascorbic acid.

Fig (26)(b) : shows the antioxidant activity of compound 12 and ascorbic acid.

Toxic efficacy

The cytotoxicity test of the prepared compounds under study was used using the MTT test. The liver cancer cell line (HEPG-2) was used to identify the biological activity of the compounds under study. Compound (10) was chosen to conduct the test. The results showed that compound (10) has an effective toxic effect on the growth of cancer cells. The reason for this effect is attributed to the prepared compound containing the imidazole ring in its structure, which has high

biological activity and resistance to cancer, as well as the compounds (10) containing other heterogeneous rings such as triazole, thiazole, which may affect certain receptors present on the surfaces of cells. It was noted that the inhibition rates of the compound prepared under study increased with increasing concentration. It was noted from the results obtained that compound 10 has the highest inhibition rate at a concentration of 500 µg/ml, where the inhibition rate reached 82% for the infected line and the percentage of remaining cancer cells at this concentration was 18%. As for the healthy HdFn cells, the inhibition rate when using the same concentration of compound 6 was 21% and the percentage of remaining healthy cells for compound 10 at the same concentration was 79%. and the table below shows the results obtained. On the other hand, it was found that the half inhibitory concentration (IC50) (the concentration that reduces cell survival to 50%) reached 116 µg/ml at a concentration of µg/ml. The results are shown in Fig (27)

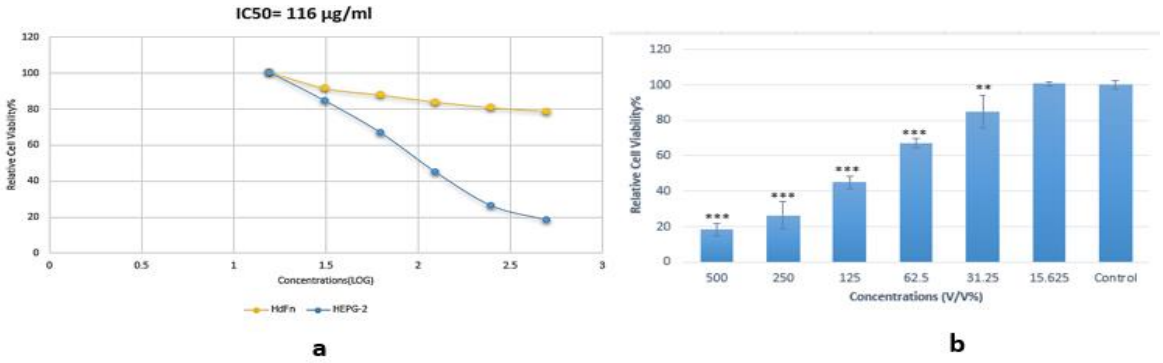


Fig (27)(a,b): It shows the toxic effect of the compound (10) on the cancerous line of the type(HEPG-2)

Conclusions

1- The structural formula of the prepared compounds was determined after conducting infrared spectroscopy, proton ^{1}HNMR spectroscopy and ^{13}C-NMR spectroscopy.
2- Some of the prepared compounds have high activity against the bacteria (*Staphylococcus aureus*), which are compounds (1,2,3,4,9,10,12). As for the bacteria (*Escherichia coli*), the compounds (2,3,4,7,10,11,12) showed High effectiveness.
3- Compound 10 showed the highest inhibitory activity of 75%, followed by compound 12 with 67%. It can be concluded from the obtained values that the prepared compounds have good ability to inhibit free radicals, so the prepared compounds are considered to have antioxidant properties.

4- When conducting the reactions, it was observed that there was a difference in the time period to complete the reaction, and the reason was the difference in the substituting totals of the reactants
5- The prepared compound 10 showed good toxic effect against liver cancer cell line. This effect is due to the fact that the prepared compound contains in its structure an imidazole ring which has high biological activity and resistance to cancer, and it also contains other heterogeneous rings such as triazole which may affect certain receptors present on the surfaces of cells.
6- The possibility of studying these compounds on other types of bacteria and parasites and comparing them with some other antibiotics

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