Synthesis, Characterization, Molecular docking, Antibacterial Activity, Antioxidant and Anticancer of New 1,2,4-Triazole Derivative

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Abstract

A novel 1,2,4-triazole-5-thiol derivative was designed through a series of reactions. Firstly, Azo derivative **1** was synthesized by a general method with two steps, a diazotization reaction in an acidic medium, and a coupling reaction in basic conditions. Secondly, Azo-carboxylic acid **1** reacted with $SOCl_2$ to produce acid chloride derivative **2**. Then carbothiamide derivative **3** was synthesized through substitution reaction by reacting **2** with 4-phenyl thiosemicarbazide in pyridine. In the next step, carbothiamide **3** was reacted with sodium hydroxide solution to afford target triazole derivative **4**. The structures of all synthesized derivatives were confirmed by techniques (^{1}H, ^{13}C NMR, IR). Moreover, the triazole derivative **4** was evaluated for its biological activity as an anticancer, antioxidant, and antibacterial. The results presented that compound **4** has moderate antibacterial activity and a high free radical scavenging effect (IC_{50} 30.2 µg/mL) compared to vitamin C (IC_{50} 21 µg/mL). The anticancer activity results presented that compound **4** has a toxic effect on a colon cancer cell line CaCo-2. The potential effect of compound **4** on the 5lqf protein was examined in silico studies.

Keyword: Azo, Acid chloride, Anticancer Carbothioamide, Triazole

Introduction

Many of 1,2,4-triazole derivatives exhibit significant pharmacological activities like antibacterial [1,2,3], anti-oxidant [4], anti-parasitic [5], antituberculosis [6], anticancer [7], etc.[8]. The 1,2,4-triazole ring has been included in a wide range of therapeutically pharmacological candidates, including rizatriptan, itraconazole, fluconazole, and ribavirin drugs [9].

There are many challenges facing individuals at the current time, including the resistance of microorganisms to most of the antibiotics currently available as the death rate increases because of this [10]. On the other hand, Cancer is one of the other challenges that cause an increase in the number of deaths because there is no effective drug [11], In addition to the many other diseases caused by oxidation stress [12]. Moreover, many of the currently existing medications have negative side effects [13], which amply supports the urgent necessity for the creation of novel antimicrobial, anticancer, and antioxidant agents.

According to these observations, triazole derivatives could be a suitable option as a secure antibacterial, anticancer, and antioxidant agent because of their widespread significance in the medical, pharmaceutical, and drug discovery fields [14].

Triazoles are desirable building blocks for the development of new antibacterial, antioxidant, and anticancer agents that target clinically significant infections due to their significant biological activity. Considering this, the purpose of this study is to synthesize a novel imidazole-based triazole derivative and assess its in vitro antibacterial effectiveness against antibacterial, antioxidant, and anticancer.

Instrumentation and Materials

All materials used in the research were supplied with a high degree of purity, 4,5-Dich1oroimidazole and *m*-Amino-*p*-methoxybenzoic acid (Fluorochem), 4-phenylthiosemicarbazide and ethanol 99% (Sigma), Sodium nitrite (Lobachemie), Hydrochloric acid 35% (Himedia), Sodium hydroxide (BDH), Thiony1 chloride

and pyridine (C.D.H). The instruments used in this research included silica gel TLC plates F 254 and iodine vapor for visualizing the products. Digital melting point apparatus was used to determine the melting points (Stuart, UK). ^{1}H and ^{1}3C NMR were performed on (Bruker 400 MHz) device. FT-IR spectrum were recorded on (Shimadzu FTIR-8400S).

Methods

Synthesis of 3-((4,5-dich1oro-l*H*-imidazol-2-y1) diazeny1)-4-methoxybenzoic acid (1)

The synthetic method included the steps of diazotization and coupling. In the first step, a cooled solution of sodium nitrite was prepared by dissolving (0.412 g, 5.98 mmol) of sodium nitrite in 20 m1 of distilled water, followed it was added dropwise to the amine solution prepared by dissolving (1 g, 5.98 mmol) of 3-amino-4-methoxybenzoic acid in 89 ml of distilled water and 22 ml of concentrated HCl and cooled to 0-5 ºC. After completing the addition of the nitrite solution, the solution continued to be stirred for half an hour at the same temperature to form a diazonium salt solution, which was added gradually directly in the coupling step to a cooled solution consisting of (0.819 g, 5.98 mmol) of 4,5-Dichlorimidazole dissolved at 30 mL ethanol and (74 mL, 10%) sodium hydroxide solution dissolved in distilled water. The mixture was stirred for two hours at a temperature of 0-5°C and a pH equal to 6. During this process, an orange precipitate was formed. The solution was left to settle until the next day [15]. The formed precipitate (orange) was filtered, washed with distilled water several times, dried, and recrystallized from ethanol. The yield 90%, m.p= 231-233°C.

Synthesis of 3-((4,5-dichloro-l*H*-imidazol-2-yl) diazenyl)-4-methoxybenzoy1 chlori de (2)

Azo derivative (1) (0.3 g, 1 mmol) was weighed and placed in a round-bottomed flask with two necks, then thionyl chloride (6 ml) was added to it slowly through a dropping funnel with continuous stirring. The mixture was reflexed for 7 hours at 70°C then the excess of thionyl chloride was evaporated under reduced pressure to obtain a red precipitate [16]. The yield was 91% , m.p=195-197 °C.

Synthesis of 2-(3-((4,5-dich1oro-*lH*-imidazol-2-y1) diazenyl)-4-methoxybenzoyl)-N-phenylhydrazine-1-carbothioamide (3)

In a round flask, acid chloride derivative 2 (0.3 g, 0.89 mmol) was dissolved in 6 ml of 1,4-dioxane, and to a previous mixture was added a solution of 4-phenylthiosimarbazide (0.15 g, 0.89 mmol) in 4 mL of pyridine. The mixture was stirred at 0 °C for half an hour, then at 25 °C for 28 hours (monitored by TLC R_f 0.4 benzene: methanol, 5:1)). Distilled water (10 ml) was added to the mixture [17], and the precipitate formed was filtered, washed with distilled water, dried, and recrystallized from ethanol. Red precipitate, yield 75%, m.p= 128-130.

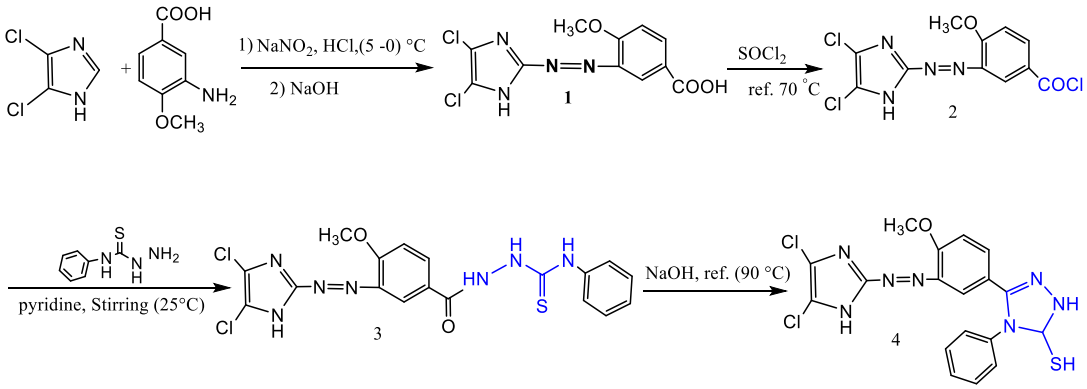
Synthesis of 3-(3-((4,5-dichloro-1*H*-imidazo1-2-y1) diazeny1)-4-methoxyphenyl)-4-phenyl-4,5-dihydro-1H-1,2,4-triazole-5-thiol (4)

A solution of sodium hydroxide (15 mL, 2 N) was added to (0.3 g, 0.65 mmol) of the carbothiamide derivative (3) in a round flask. The mixture was reflexed at 90 °C for 20 hours (monitored by TLC R_f 0.6 (hexane: ethylacetate,4:1)). The mixture was cooled to room temperature, then acidified with a dilute solution of (2N) HCl to pH 4 [18]. The formed precipitate was collected, washed with distilled water, dried, then recrystallized from absolute ethanol. Light orange precipitate, yield 69%, m. p= 342-344.

Results and Discussion

Triazole-5-thiol derivative **4** were synthesized by the reaction sequence as presented in the scheme 1. The starting compound 1 was synthesized according to a literature method with a high yield 90%. Azo-carboxylic acid **1** was treated with thionyl chloride to afford acyl chloride **2**. Acid chloride derivative **2** reacted with 4-phenylthiosemicarbazide in pyridine under cold conditions to afforded carbothiamide derivative **3** via nucleophilic substitution reaction [19]. The resulting 2-(3-((4,5-dichloro-l*H*-imidazo1-2-yl)diazenyl)-4-methoxybenzoy1)-N-phenylhydr azine-1-carbothioamide **3** was reacted with aqueous alkaline solution

of sodium hydroxide via cyclization reaction to afford 1,3,4-triazol-5-thiol derivative **4**.



scheme 1: Synthetic pathway of triazole derivative 4

The structures of (1–4) were assigned by FT-IR, ^{1}HNMR and ^{13}CNMR spectroscopy. The infrared spectra of the synthesized derivative **1** exhibited characteristic bands at 1450 cm^{-1} due to (N=N) and strong band at 1661 cm^{-1} belong to stretching vibration of the (C=O acid). In the ^{1}H-NMR spectrum pf compound 1 display a broad signal peak at 12.84 ppm assigned to (OH acid), while a single peak at 11.74 ppm belong to (NH ring), meanwhile,^{13}C-NMR spectra exhibited signal attributed to the carbon of COOH at 167.0 ppm.

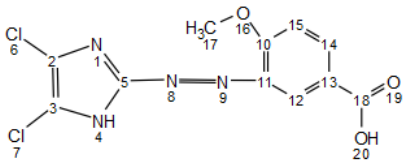
FT-IR spectrum of compound **2** showed disappearance of a band of hydroxyl of an acid in compound **1**. Further, the ^{1}H-NMR spectra of compound **2** show disappearance of OH of an acid. Whereas, ^{13}C-NMR showed significant signal attributed to the C=O group at 171.1 ppm.

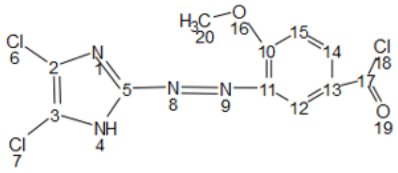
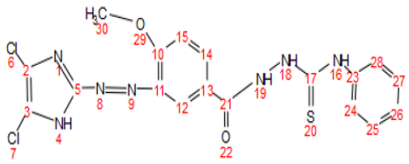
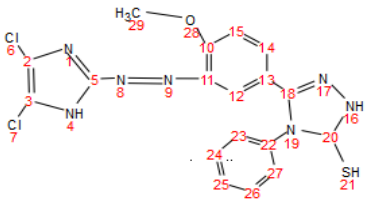
FT-IR spectrum of compound **3** exhibited new and strong band due to (C=O amide) at 1681 cm^{-1} with disappearance of carbonyl band of acid chloride **2**, also the spectrum shows many new bands at (3224-3394) cm^{-1} belong to stretching vibration of NH and NH_2 bonds characteristic to hydrazine thiosemicarbazide, in addition to other new band at 1257 cm^{-1} indicated to stretch (C=S) bond. Whereas ^{1}H-NMR spectra of compound 3 shows new peak at 8.8 ppm belong to NH-Ph proton, new peak at 9.6 ppm attributed to (-NH-C=S) and new peak at 10.3 ppm

due to (NH amide) proton. In the ^{13}C-NMR spectra of compound 3 shows new peaks at 173 ppm belong to C=O and new peak at 166 ppm attributed to C=S.

FT-IR spectrum of target compound **4** shows new characteristic bands, at 3342 cm^{-1} belong to NH triazole, new band at 2468 cm^{-1} belong to stretching vibration of SH band, and new band at 1663 cm^{-1} belong to (C=N triazole) as shown in Fig. 1. While ^{1}H-NMR spectra of compound 4 shows characteristic peaks at 10.6 ppm belong to NH proton of triazole ring, the resonances at 13.7 ppm attributed to SH proton, and peak at 5.8 belong to CH proton of triazole ring Fig. 2. Further, ^{13}C-NMR spectra of compound 4 showed disappearance of a peak of C=O at 173 ppm and appearance of new peak at 95 ppm belong to C_{20} (C-SH) as shown in Fig. 3. Table 1 illustrates the characterization of synthesized compounds 1-4. Target compound **4** were screened for their antimicrobial toward *Staphylococc us aureus* and *Escherichia coli*, antioxidant and anticancer activities. Table 1 shows the total spectrum of the synthesized compounds.

Table 1: Characterization of synthesized compounds

Compound	Characterization
Compound 1 	**FTIR** (KBr disk, ν cm^{-1}): 3173 (NH imidazole) 1450 (azo group), 3400-2500 (OH carboxylic acid), 1681 (C=O), 3070 (CH aromatic), 1575-1504 (C=C aromatic), 2993 (CH a1iphatic), 1604 (C=N imidazo 1e), 864 (C-Cl). **^{1}H-NMR** (400 MHz, DMSO-d_6, ppm): 12.84 (br, 1H, OH), 11.74 (s, l H, NH), 7.40-8.14 (m, 3H, aromatic), 4.06 s (s,3H, OCH_3). **^{13}CNMR** spectrum (400 MHz, DMSO-d_6, ppm): 56.8 (C17), 114.0 (C15), 117.9 (C13), 123.5 (C12), 128.5 (C2), 131.0 (C131), 134.8 (C14), 141.0 (C11), 150.9 (C5), 160.3 (C10), 167.0 (C18).

Compound 2 	**FTIR** (KBr disk, ν cm^{-1}): 3172 (NH),3085 (CH aromatic), 2846 (CH a1iphatic), 1751(C=O) ,1604 (C=N imidazo1e), 1535-1512 (C=C aromatic), 1442 (N=N), 825 (C-C1). **^{1}H-NMR** (400 MHz, DMSO-d_6), δ (ppm): 11.83 (s, 1H, NH), 7.25-8.09 (m,3H, aromatic), 3.91 (s, 3H, OCH_3). **^{13}C-NMR** (400 MHz, DMSO-d_6, ppm): 171.1 (C17), 155.8 (C10), 148.8 (C5), 142.2 (C11), 131.8 (C14), 131.5 (C3),129.8 (C12),128.7 (C-13), 122.7 (C2), 114.2 (C15) and 57.01 (C20).
Compound 3 	**FTIR** (KBr, ν (cm^{-1}): 3394,3288, 3224 (3N-H),3178 (N-H imidazo1e), 3062 (C-H aromatic) , 2962 (C-H aliphatic), 1681 (C=O amide),1604 (C=N imidazole), 1557-1488 (C=C aromatic), 1442 (N=N aromatic), 1257 (C=S), and 871 (C-C1). **^{1}H-NMR** (400 MHz, DMSO-d6), δ (ppm): 11.83 (s, 1H, NH), 7.19-8.33 (m,8H, aromatic), 3.8 (s, 3H, methoxy), 8.8 (NH-Ph), 9.6 (-NH-C=S),10.3(NH-C=O). **^{13}C-NMR** (400 MHz, DMSO-d_6, ppm): 173 (C-17), 113-134 (C2,3, 11-15,23-28),156(C10),145(C5),56 (C30),166 (C21).
Compound 4 	**FTIR (**KBr, ν cm^{-1}): 3342 (N-H triazole), 2468 (SH), 1663(C=N triazole), 1612 (C=N imidazole) 3193 (N-H imidazole), 2947 (C-H aliphatic),3093 (C-H aromatic), 1558-1519 (C=C aromatic), 1450 (N=N), and 848 (C-C1). **^{1}H-NMR** (400 MHz, DMSO-d_6), δ (ppm): 11 (s, 1H, NH), 6.8-8.2 (m,8H, aromatic), 3.8 (s, 3H, methoxy), 10.6 (NH triazole), 5.7 (CH triazole). **^{13}C-NMR** (400 MHz, DMSO-d_6, ppm):95 (C20),115-139 (C2,3,11-15,23-27),155 (C10), C5(145),C29(56),C20(95),C15(113),C18 (144)

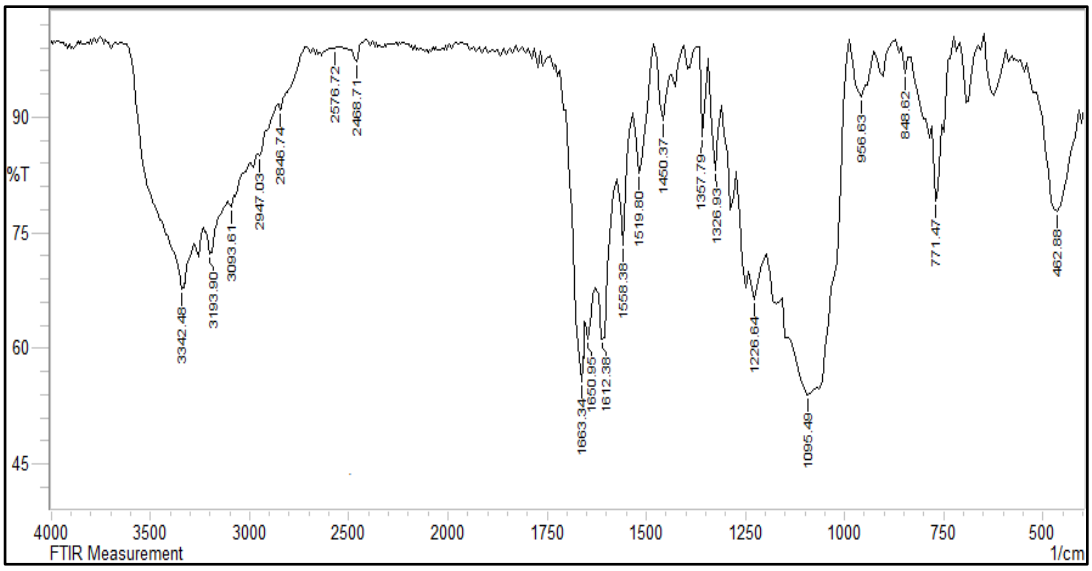


Fig. 1: FT-IR spectrum of compound 4

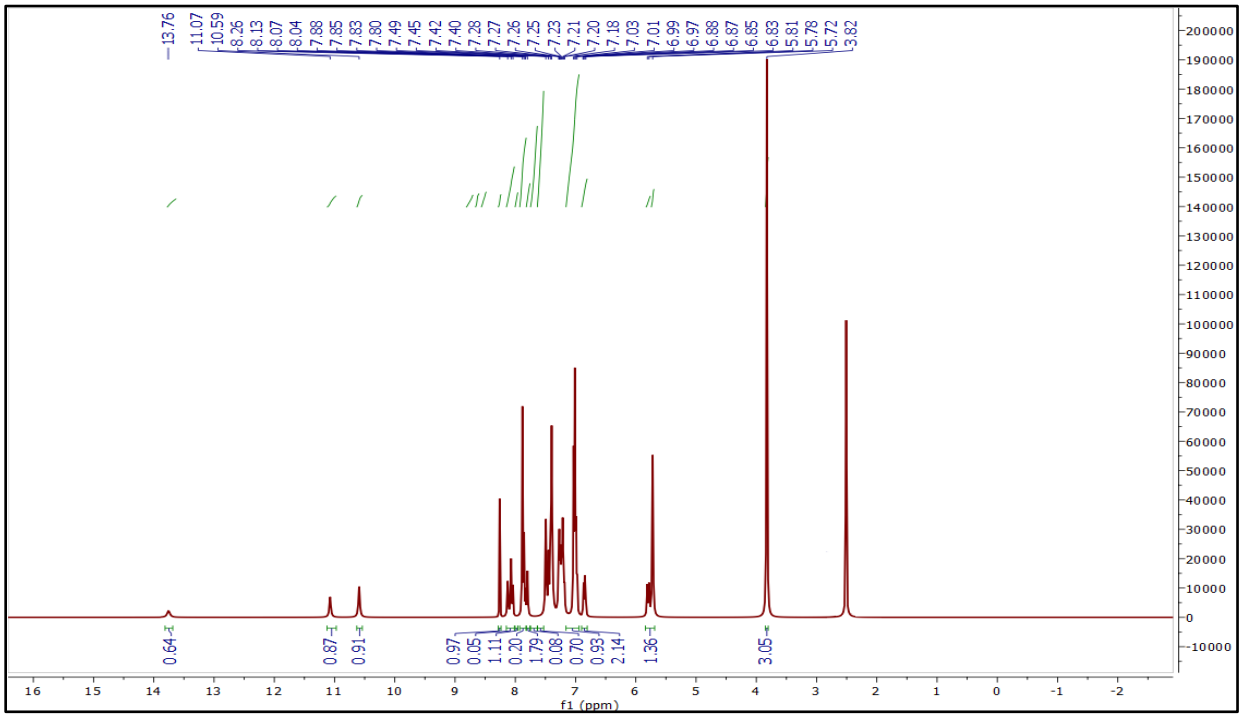


Figure 2: ^{1}H-NMR spectrum of compound 4

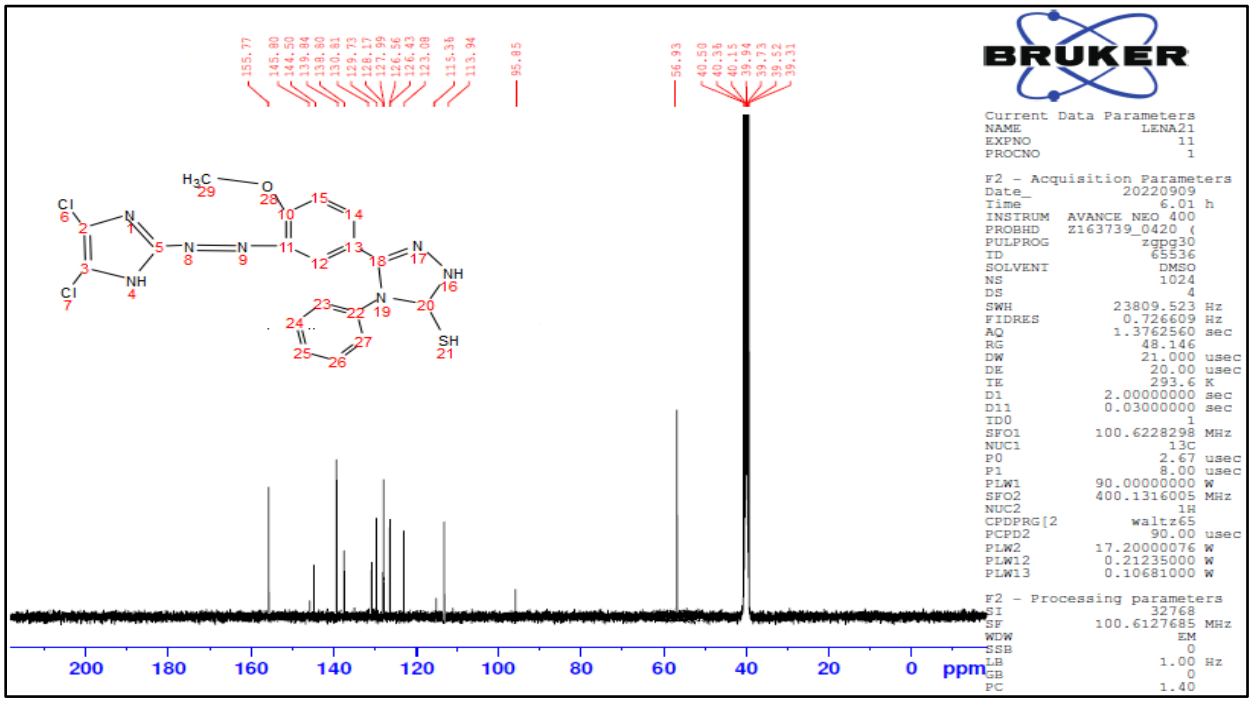


Figure 3: ^{13}C-NMR spectrum of compound 4

Antimicrobial activity

The result of antibacterial action of triazole derivative **4** was investigated utilizing disc diffusion method. Two types of bacteria were used to determine the activity of target compound **4**, *Staphylococcus aureus* and *Escherichia coli*). solution of the triazole derivative prepared with concentration 0.2mg/mL in DMSO as solvent. Muller Hinton agar was used as medium for growing the bacterial strains that are incubated in 37 °C for 24 h. the diameter of inhibition was calculated in comparison to standard drug (Amoxycillin). The triazole derivative **4** showed moderate activity against both types of bacteria *Staphylococcus aureus* and *Staphylococcus aureus* and *Escherichia coli* with inhibition zone 17 mm and 16 mm, respectively as illustrates in Table 2. In addition, all strains were unaffected to DMSO (negative contro1). The antibacterial activity was classified into: <5mm (No active), 5-10 mm (low), 10-20 mm (moderate), >20 mm (strong) [20].

Table (2): Antibacterial activity of synthesized compound 4a

Compound	Inhibition zone diameter, mm	
	E. coli (-)	*Staphylococcus* (+)
4	17	16
(Amoxicillin drug)	15	14
DMSO	-	-

Antioxidant Activity

Antioxidant activity of synthesized triazole derivative 4 was investigated utilizing the DPPH• free radical scavenging. DPPH• scavenging effect of compounds was performed according previous procedure [21]. From the DPPH solution (0.01g dissolve in mixture of Methanol-DMSO (9:1) (v: v)) was taken 3 ml and added to one milliliter of each prepared concentration of synthesized triazole **4** and vitamin C solution (200, 100, 50, 25,12.5 μg mL^{-1}) in mixture of solvents (Methanol-DMSO (9:1). Incubated the sample for one hour at 37°C. The basic of this assay is that the DPPH radical can be reduced to form stable molecular in presence of a substance that can donate an electron or hydrogen atom to it, and as a result the purple color of free radical converted to yellow, which can be measured in the visible spectrum to give a strong absorption at 517 nm [22].

The study that performed on compound 4 is compared to standard antioxidant (Ascorbic acid). The results exhibited that triazole derivative 4 high antioxidant activity about 78.09 % as compared to ascorbic acid 80% at the highest dose. Also, the results showed that triazole derivative 4 has IC_{50} equal to 30.2 μg/mL and is close to Ascorbic acid IC_{50} 21.04 μg/mL. The data are illustrated id Fig.4 and table 3.

Table 3. Antioxidant activity of synthesized compound 4a

Compound	Inhibition of DPPH, %					IC_{50}, µg/mL
	200µg/mL	100µg/mL	50µg/mL	25µg/mL	12.5µg/mL	
4	78.09	69.71	55.48	43.17	27.59	30.2
Ascorbic acid (standard)	80.36	69.48	54.48	40.43	17.63	21.04

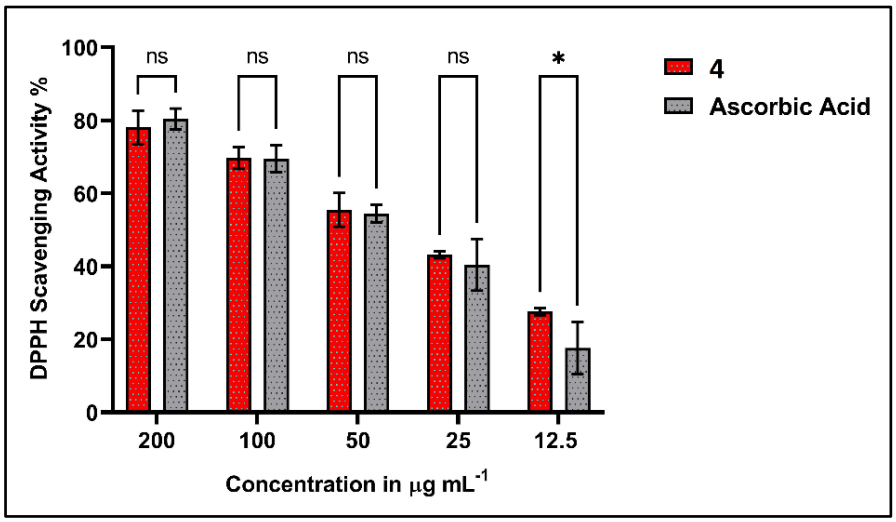


Fig. 4: Representation of DPPH Scavenging Activity of compound 4

Molecular docking studies

The Triazole structure was created using Chem Draw 2016 as mol format and converted to two dimensions. The structure of 5lqf is downloaded from Protein Data Bank, and the Protein is saved in Pdb format. The analysis of hydrogen bond and non-bonded formation between residues amino acid and ligand in the active site of 5lqf was performed using AutoDock 4.2.6 program.

The target 5lqf inhibitor 3-(3-((4,5-dichloro-1H-imidazo1-2-y1) diazeny1)-4-methoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiol with 5lqf generated a binding energy that is equal to 7.35 kcal/Mol. The interaction between ligand and 5lqf is preferred by the formation of Hydrogen bonding with the (VAL197B, GLY199B, TYR334B, ASP335B, ASP335B) and hydrophobic interactions with (ASN200B, LYS385B, MET389B, THR198B, GLY194B). These

interactions of triazole derivative 4 may clarify the higher binding free energy and anticancer activity (Table 4 ,Fig. 5 and 6).

Table 4: Values of interaction energy and H-bonding of triazole derivative with 5lqf protein

Compound No.	Lowest bending energy	Run	Distance H-A	H-Bonds NO.	Hydrogen- bending Interaction
21	-7.35	39	(1.91-2.76)	5	O: VAL197B, N: GLY199B, N:TYR334B,O: ASP335B, O: ASP335B

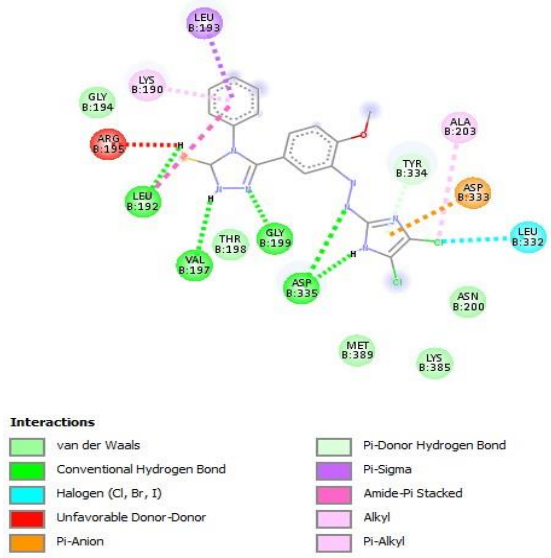


Figure (5): 2D representation of the intermolecular interactions with the 5lqf protein

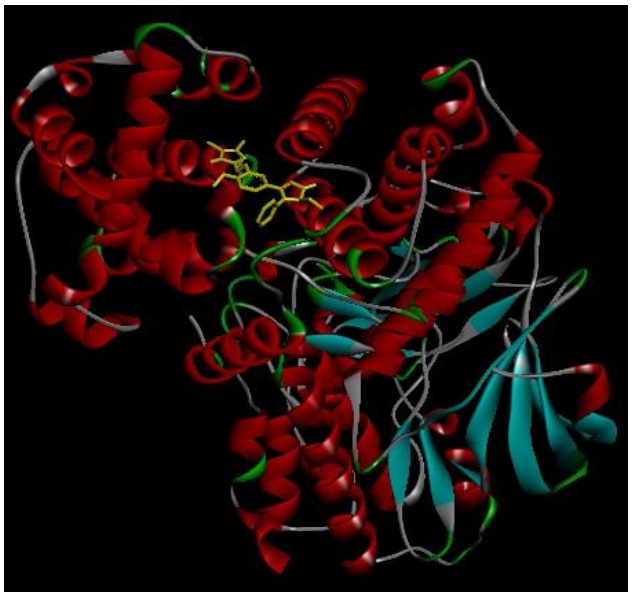


Figure (6): 3D representation of intermolecular interactions with the 5lqf protein

Anticancer Activity

The cytotoxicity of the synthesized compound 4 was examined using the MTT assay and using two types of cell lines: human normal cell line (HdFn) and colon cancer cell line (CaCo-2). Where the cell line (HdFn) was used for comparison with cancer cell lines. The cytotoxicity results showed that compound 4 under

study has a toxic effect on the growth of cancer cells, and its effect on healthy cells is less. This effect may be attributed to the fact that the prepared compound 4 contains an imidazole ring in its structure which has anti-cancer efficacy [23]. In addition, another heterocyclic ring triazole may affect certain receptors on cell surfaces.

The results conducted for compound **4** on the growth of colon cancer cells (CaCo-2) showed that compound **4** has a toxic effect on the growth of cancer cells, as the number of living cells remaining after reacting is (48.39), and the inhibition rate is 51.61% at the highest dose (200 µg mL^{-1}) as shown in table 5 and fig. 7. On the other hand, it was observed that the half-inhibitory concentration (IC_{50}) is within the concentrations used and is equal to 65.1 µg/ml for the Colon cancer cell line, and it has a higher value to the healthy cell line (HdFn) and is equal to 76.85 µg/ml. This is a good result because the compound kills cancer cells and needs a higher concentration to inhibit healthy cells, thus its effect on them will be less of them.

Table (5): The cytotoxic effect of 4 on HdFn and CaCo-2 cell line

Concentration µg mL^{-1}	Cell Viability	
	Normal Cell line	Cancer cell line
	HdFn	CaCo-2
	Mean viability (%) ± SD	Mean viability (%) ± SD
200	72.64±1.95	48.395±3.3
100	80.20±3.11	68.94±8.7
50	85.64±3.32	82.83±1.54
25	94.17±0.77	91.44±0.87
12.5	96.18±0.23	93.145±1.3
IC50	76.85	65.1

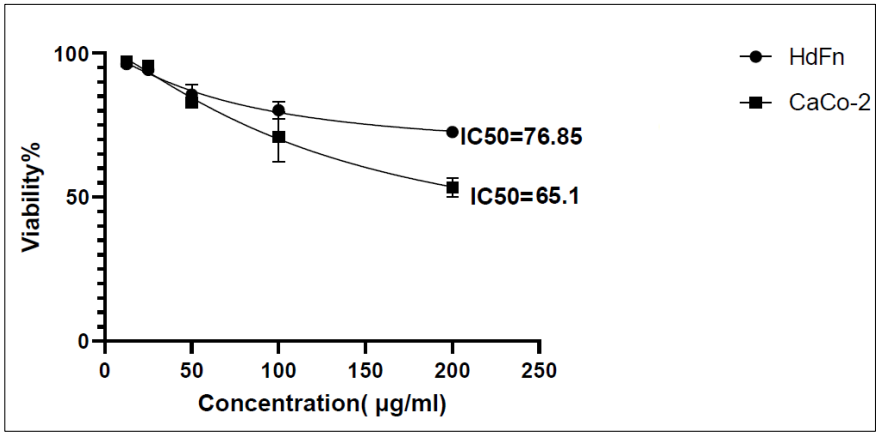


Fig. 7: Half inhibitory concentration of colon cancer cell line (CaCo-2) and normal cell line (HdFn) for compound 4

Conclusion

In this study, the new triazole derivative bearing imidazole core was successfully synthesized and characterized utilizing spectroscopic techniques (FT-IR, ^{1}H, and ^{13}C NMR). Also, in vitro biological activities of triazole derivative 4 were performed. In general, compound 4 showed moderate antibacterial activity. While the Free radical scavenging effect of 3-(3-((4,5-dichloro-1H-imidazol-2-yl)diazenyl)-4-methoxyphenyl)-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-5-thiol **(4)** showed good activity comparable to Vitamin C with IC_{50} value of 30.2 µg mL^{-1}. In silico study, the new triazole compound 4 showed a variety of powerful interactions with the effective binding sites of the receptor of colon cancer and may be considered a good inhibitor of 5lqf protein. Further, the results of triazole derivative 4 were observed to be toxic to colon cancer cells with IC_{50} equal to 65.1 µg mL^{-1}.

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