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Synthesis, Characterization of Some New 1,2,4-Triazole derivatives as Antimicrobial and Study of their Molecular docking

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Abstract

This study outlines the synthesis of substituted 1,2,4-triazole derivatives through the cyclization reaction of thiourea derivatives. The process begins with the reaction of different halides with KSCN to produce isothiocyanate derivatives. then followed by a reaction with isonicotinic acid hydrazide to yield thioureas **(1-6)**, with a yield rate of (72-88%). Then, compounds **(1-6)** were treated with alkaline medium 4 N (NaOH) to produced 1,2,4-triazole derivatives **(7-12)** with a yield (51-69%).The structure of the prepared compounds was characterized using FTIR,^{1}HNMR and ^{13}CNMR spectroscopy. Some of the synthesized compounds were tested for antimicrobial activity when, compound 9 showed strong activity against gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*) and moderate activity against gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae*) compared with drug ciprofloxacin. Also, examined compounds showed strong inhibition against (*candida albicans)* except compound 10 showed weak activity. Furthermore, the molecular docking was studied by Gold suite to examining the reaction between target compounds and active binding section of GABA-AT protein was the PLP fitness values of the docking compounds on GABA-AT ranged (57.71-67.55).

Keywords: 1,2,4-Triazole , Thioureas , Isoniazid ,Docking the GABA-AT, Antimicrobial .

تحضير وتشخيص بعض مشتقات 4،2،1- ترايازول الجديدة كمضادات للميكروبات ودراسة التحامها الجزيئي

وسن كريم دمدوم [*,2,1], عدي هادي رؤوف الجيلاوي [1]
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الخلاصة :

في هذه الدراسة تم تحضير مشتقات 4,2,1- ترايازول المعوضة من خلال التفاعل الحلقي لمشتقات الثيوريا. تبدأ العملية بتفاعل الهاليدات المختلفة مع ثايوسيانات البوتاسيوم لينتج مشتقات الايزوثيوسيانات. ثم

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يتبعه التفاعل مع هيدرازيد حامض الايزونيكوتينيك للحصول على الثيوريا (**1–6**)،بنسبة منتوج (72–88%). ثم معاملة المركبات (**1–6**) بوسط قاعدي 4N (هيدروكسيد الصوديوم) لتنتج مشتقات 4,2,1–ترايزول (**7–12**) مع منتوج (51–69%). تم تشخيص تركيب المركبات المحضرة باستخدام التحليل الطيفي الاشعة تحت الحمراء, الرنين النووي المغناطيسي للبروتون والكاربون13 تم اختبار بعض المركبات المحضرة للنشاط المضاد للميكروبات حيث أظهر المركب 9 نشاطا قويا ضد بكتيريا جرام الموجبة (*Staphylococcus aureus, Staphylococcus epidermidis)* ونشاط معتدل ضد بكتريا جرام السالبة (*Escherichia coli, Klebsiella pneumoniae*) مقارنة مع عقار السيبروفلوكساسين. كما أظهرت المركبات المختبرة تثبيطاً قوياً ضد *(candida albicans)* باستثناء المركب 10 الذي أظهر نشاطاً ضعيفاً.علاوة على ذلك, تمت دراسة الالتحام الجزيئي بواسطة Gold suite لفحص التفاعل بين المركبات المستهدفة وقسم الارتباط النشط لبروتين GABA–AT حيث تراوحت قيم اللياقة PLP للمركبات الراسية على GABA–AT بمدى (67.55 – 57.71) .

1. Introduction

Isoniazid (isonicotinic acid hydrazide, INH) belongs to a category of natural compounds called hydrazides, characterized by using the presence of a hydrazide functional institution. This unique practical function finds applications throughout numerous industries, which include materials technological know-how, agrochemicals, and prescription drugs. Within the pharmaceutical area, hydrazides frequently function intermediates within the synthesis of various drug molecules, making them precious compounds in drug production approaches [1]. The INH molecule is utilized for the production of a diverse array of heterocyclic compounds. These compounds hold significant significance in several aspects of our everyday lives, as they provide notable contributions to both medicinal and industrial domains [2]. The compounds serve as fundamental components in both organic manufactured materials and natural products [3]. Five-membered nitrogen heterocyclic compounds are of significant importance as structural sources and are widely recognized as biologically active chemicals [4-8]. In recent years, there has been significant interest in the utilization of nitrogen-rich heterocyclic compounds derived from Imidazoles [9], Pyrazoles [10], Triazoles[11], tetrazoles [12] ,oxadiazoles [13] and oxazolone [14] for the development of novel energetic molecules. In the 1960s, made the first discovery of the newly compound belonging to class Triazole, also known as pyrrodiazole, is a nitrogenous heterocyclic moiety with the chemical formula $C_2H_3N_3$ [15]. Among important heterocyclic compounds triazoles are compounds that contain heteroatoms of nitrogen with carbon. Based on the placement of heteroatoms in five membered rings triazoles can be divided into two categories: 1,2,3-triazole and 1,2,4-triazole [16, 17]. The stability of the triazole ring is attributed to its aromaticity. Moreover, the triazole nucleus experiences resonance stabilization, allowing it to be shown through tautomeric forms [18]. There are multiple synthetic routes available for the preparation of triazole derivatives. One strategy involves the synthesis of thiourea intermediates, which play a pivotal role in organic synthesis reactions aimed at producing triazole derivatives. These compounds play vital roles, exhibiting significant applications in various biological systems as well as diverse industrial processes [19]. The incorporation of 1,2,4-triazoles has been documented to improve pharmacokinetic properties, including the absorption, distribution, metabolism, and excretion of drugs [20]. In the past few years, notable advancements have been made in the development of novel energy molecules for the treatment of cancer and various other diseases. One such promising chemical is carrier 1,2,4-triazole, which has shown potential as a therapeutic candidate [21]. Furthermore, 1,2,4-triazole derivatives have pharmacological properties such as antimicrobial [22-24], analgesic [25], antioxidant [26], anti-inflammatory [27, 28], anticancer [29, 30] and anticonvulsant [31, 32] different derivatives of 1,2,4-triazole can be used in the industry application such as corrosion inhibitors [33] and Mesomorphic properties [34]. The aim of this study to synthesis new 1,2,4-triazole derivatives from isoniazid as starting compound. The final derivatives

synthesized from two steps firstly, reaction of different halides with KSCN to obtain isothiocyanate derivatives then followed by without separation reaction with isonicotinic acid hydrazide to yield thioureas. Subsequently , cyclization reaction of thiourea compounds by 4N NaOH to give the desired 1,2,4-triazole derivatives.

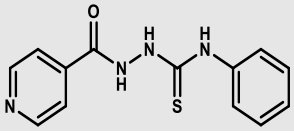
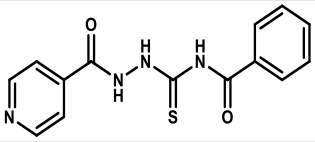
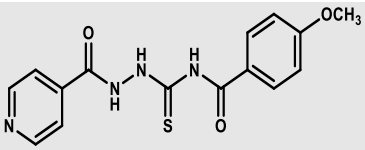
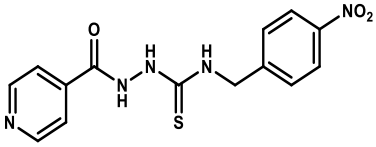
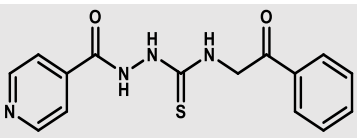
2.Materials and Methods

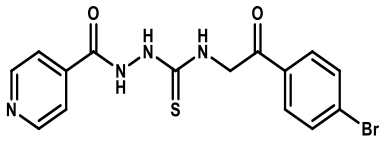
The melting points of the open glass capillaries were measured using a Gallenkamp capillary melting point instrument, without any adjustments made. A Bruker Vance 400 MHz spectrometer in (Department of Chemistry, college of pharmacy al Mansoura university, Egypt) was used to record the ^{1}HNMR and ^{13}CNMR spectral data. Using DMSO-d_6 as a reference and tetramethyl silane (TMS) as the internal standard, chemical shifts are reported in ppm downfield. Infrared spectral analysis was conducted using a Shimadzu 8400 FT-IR spectrometer to collect the vibrational spectral data. T.L.C (Silica gel -covered on the aluminum sheets) were utilized to monitor the reactions, and the eluent was utilized as a combination of hexane and ethanol and displayed with the use of iodine.

2.1 Synthesis of Thiourea Compounds (1-6) [35]

A solution containing 0.004 moles each of various halides and 0.35g (0.004 moles) of potassium thiocyanate in 15mL of acetone was stirred at room temperature for half-hour. This resulted inside the formation of isothiocyanate derivatives. Without separation 0.5g (0.004 moles) of isonicotinic acid hydrazide was added to the mixture, which was then refluxed between 3-5 hours to drive the reaction to completion. The reaction was monitored by TLC (hexane: ethanol, 3:2). The mixture was poured on crushed ice when the reaction completed, the solid product filtered, washed in distilled water. The Table (1) presents the physical characteristics and FTIR spectrum data.

Table1: The physical characteristics and FT-IR spectral data of thioureas (**1-6**)

Comp. code	Structure	Melting point °C	Yield %	color	Major FTIR Absorptions cm^{-1}
1		151-153	88	White	3236 ,3157 (NH), 3041(CH aromatic), 1681 (C=O), 1527,1485 (C=C aromatic),1180 (C=S)
2		180-183	72	White	3263,3120 (NH), 3049 (CH aromatic),1681,1668(C=O),1598,1550 (C=C aromatic),1255(C=S)
3		191-193	76	White	3269,3114 (NH), 3024 (CH aromatic), 2943, 2894 (CH aliphatic), 1672 (C=O),1595 1552(C=Caromatic),1253(C=S), 1217 asym, 1062 sym. (C-O)
4		88-90	81	Yellow	3261 (NH), 3002 (CH aromatic),2844,2941 (CH aliphatic),1677(C=O),1602,1573 (C=C aromatic), 1504 asym. 1313 sym. (NO_2),1251(C=S).
5		110-112	77	Off white	3334 (NH), 3056 (CH aromatic),2985,2937 (CH aliphatic), 1676 (C=O), 1587 ,1446 (C=C) ,1201 (C=S).

6		152-155	81	Yellow	3323 (NH), 3093 (CH aromatic), 2977, 2929 (CH aliphatic), 1722,1670 (C=O), 1581 (C=C) ,1203 (C=S).

2.2 Synthesis of 1,2,4-Triazole-3-thiol derivatives (7-12) [36]

Thiourea derivatives 1-6 (0.001 mol each) were separately refluxed in 30 mL of 4N sodium hydroxide solution for 7-11 h, until the end of the reaction as monitored by TLC using a hexane: ethanol solvent system ratio of 3: 2. After confirming completion of the reaction by TLC. Then, the solid compounds were filtered, washed with distilled water, and recrystallized with a suitable solvent. The physical properties , FTIR spectral data in Table (2) and ^{1}HNMR , ^{13}CNMR in Table (3) and Table (4).

Table 2: The physical characteristics and FTIR spectral data of 1,2,4-Triazole derivatives (**7-12**)

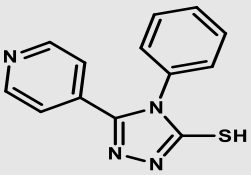
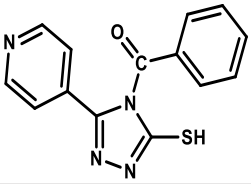
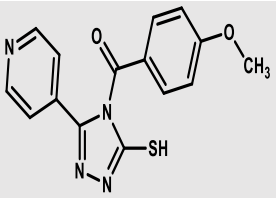
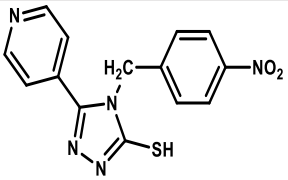
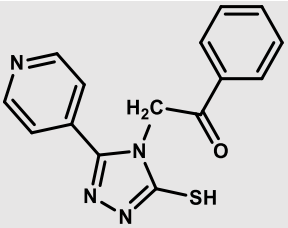
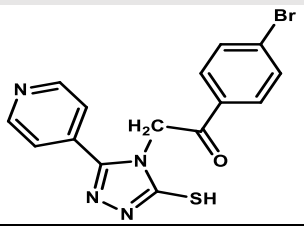
Comp. code	Structure	Melting point °C	Yield %	color	Major FTIR Absorptions cm^{-1}
7		196-198	69	White	3064, 3026 (CH aromatic), 2661 (SH), 1608 (C=N),1552 ,1496 (C=C).
8		265-268	52	Off white	3058,3020 (CH aromatic), 2657 (SH),1677 (C=O), 1652 (C=N), 1589,1554 (C=C).
9		230-232	60	White	3056 (C-H aromatic),2981 ,2839 (C-H aliphatic),2675 (SH), 1683 (C=O),1602 (C=N), 1575,1521 (C=C aromatic),1261,1024(C-O)
10		240-243	51	Red	3095,3002 (CH aromatic), 2975 ,2937 (CH aliphatic), 2671 (SH), 1641 (C=N),1585,1485 (C=C) , 1558 asym.1321sym. (NO_2)
11		130-132	66	Red	3080 ,3056 (C-H aromatic), 2981,2943 (C-H aliphatic), 2678 (SH),1685(C=O), 1604(C=N),1577 ,1521 (C=C).
12		233-235	68	Light yellow	3076,3026 (C-H aromatic), 2960,2929(C-H aliphatic), 2686 (SH),1722(C=O), 1598 (C=N),1546,1510 (C=C)

Table 3: The ^{1}HNMR spectral data of 1,2,4-Triazole derivatives (**7-10**)

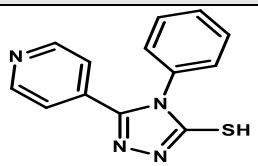
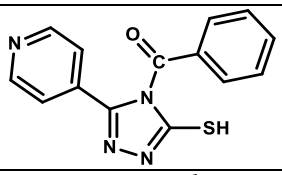
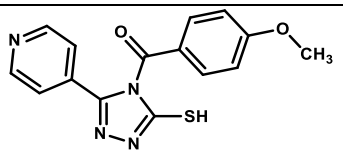
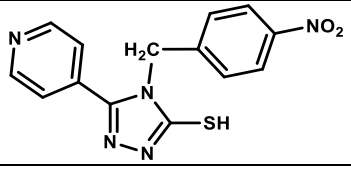
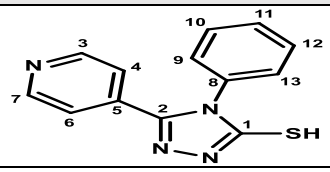
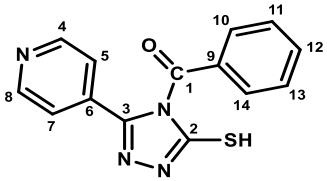
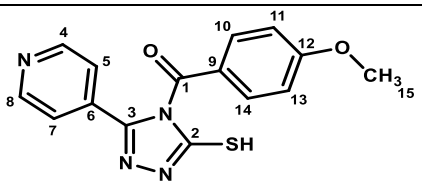
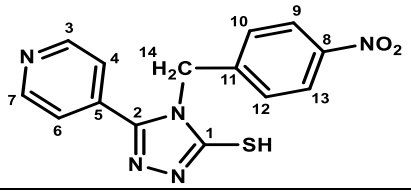
Comp. code	Structure	^{1}HNMR (400 MHz ,DMSO -d_6 ,δ , ppm)
7		13.83 (s,1H,S<u>H</u>),8.57-7.23(m, 9H,aromatic)
8		13.71(s,1H, S<u>H</u>), 8.77-7.45 (m, 9H,aromatic)
9		13.58 (s,1H,S<u>H</u>),8.76-7.01(d-d, 8H,aromatic)3.83 (s,3H,OC<u>H</u>$_3$).
10		14.14 (s,1H,S<u>H</u>),8.78-7.35(m, 8H,aromatic),5.58 (s, 2H, C<u>H</u>$_2$).

Table 4: The ^{13}CNMR spectral data of 1,2,4-Triazole derivatives (**7-10**)

Comp. code	Structure	^{13}CNMR (100 MHz, DMSO-d_6,δ,ppm)
7		169($C_{1\text{Triazole}}$),150 ($C_{2\ \text{Triazole}}$),148-122 (C_3-C_{13}).
8		167(C_1),156 ($C_{2\ \text{Triazole}}$), 154 ($C_{3\ \text{Triazole}}$),150-120 (C_4-C_{14}).
9		167(C_1),163 ($C_{2\ \text{Triazole}}$),161 ($C_{3\ \text{Triazole}}$) 151-114 (C_4-C_{14}),55(C_{15}).
10		159($C_{1\ \text{Triazole}}$),149 ($C_{2\ \text{Triazole}}$),147-114 (C_3-C_{13}),36 (C_{14}).

3. Molecular Docking study

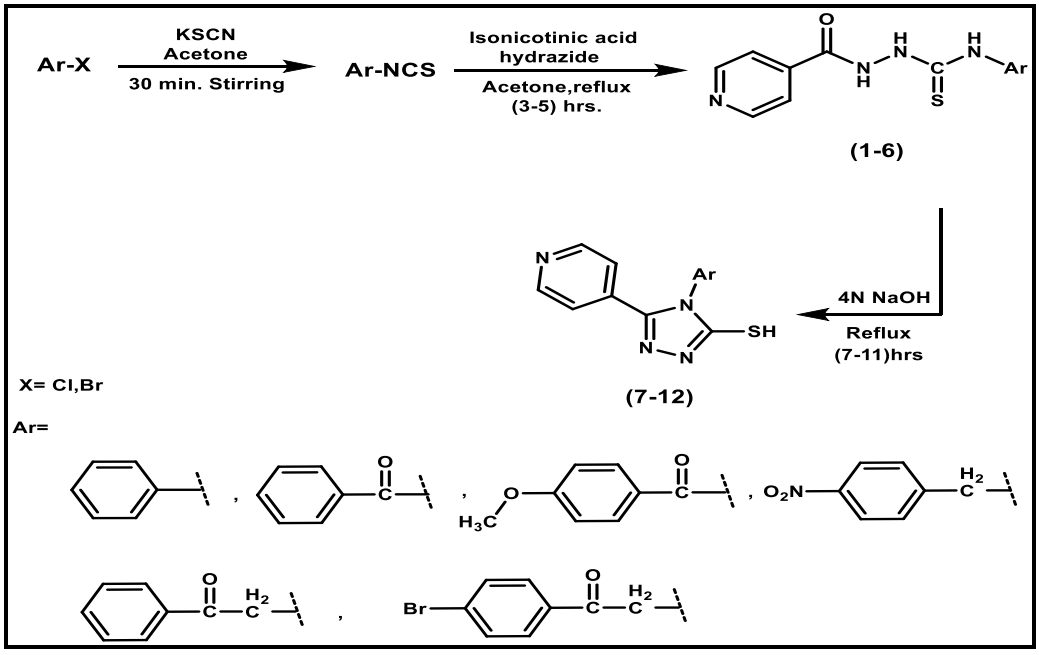
The GOLD genetic algorithm is employed for the purpose of docking flexible ligands into protein binding sites. Hermes provides the GOLD's graphical user interface, which is specifically developed to assist in input information preparation for GOLD docking, presentation of docking results, and computation of descriptors. The final synthesized compounds were successfully subjected to docking utilizing the GOLD Suite program.

4. Biological activity [37]

The antimicrobial activity test of some prepared compounds was performed according to the Agar wells diffusion method. The synthesized compounds were screened for their antimicrobial activity against four series of bacteria, including gram-positive, gram-negative and control strains, as well as a series of fungi. Plates were prepared using sterile nutrient agar media. A 0.1 ml aliquot from each culture was spread evenly onto the plates and allowed to dry at 37°C for 10 minutes to assist with microbial growth and replication. After drying, 5 mm wells are made with a cork borer. After that, the compounds to be tested is added to the well and incubated at 37 °C for 18 hours. The inhibition zones of evaluated compounds on microorganisms were measured.

5. Result and Discussion

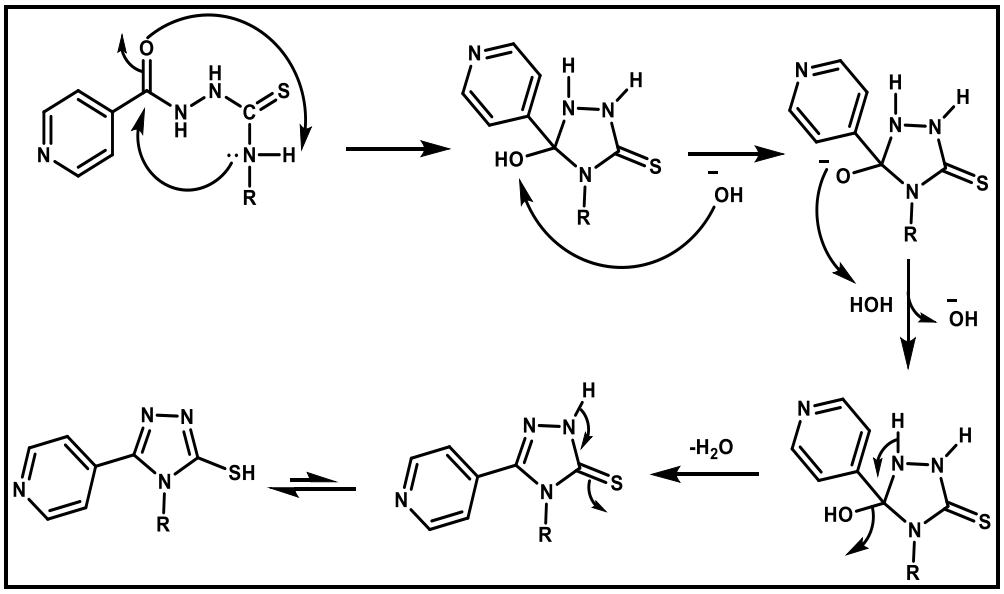
The study includes synthesis 1,2,4-triazol derivatives in two steps. The synthesis of different thiourea derivatives (1-6) and target compounds (7-12) are outlined in Scheme 1.



Scheme 1: Synthesis of 1,2,4-Triazol derivatives

The first step include synthesis of different thiourea derivatives (1-6) by reaction between different halide and potassium thiocyanate to obtain intermated compounds (potassium thiocyanate derivatives) then react without separating with isonicotinic acid hydrazide to obtain desired thiourea derivatives with yield (72-88%) via nucleophilic substituted reaction (addition-elimination). The FTIR spectra of the synthesized isothiocyanate derivatives showed disappearance of the υ(NH2) absorption band of isonicotinic acid hydrazide. A new peak appeared in the range of 3334-3114 cm^{-1}, indicating the formation of the υ(NH) group. Also, peaks were observed between 1180-1201 cm^{-1} due to the stretching and bending vibrations of the (C=S) group interacting with the (C-N) group, where the (C=S) is attached to a nitrogen atom [38-40]. In the second step, the thiourea derivatives were converted to the target 1,2,4-triazole derivatives (7-12) via a cyclization reaction upon refluxing with 4N NaOH solution. The FTIR spectra of the resulting 1,2,4-triazole compounds showed disappearance of the υ(NH) absorption band. A new, weak band appeared between 2686-2657 cm^{-1}, attributed to the (SH) group, in accordance with previous literature [41, 42]. Additionally, peaks in the range of 1652-1598 cm^{-1} corresponded to the (C=N) stretch, confirming formation of the 1,2,4-triazole ring [43]. The ^{1}HNMR spectral data of some

triazole derivatives show a disappearance absorption indicated to (NH) group and appearance singlet signal at δ (14.14-13.58) ppm belongs to (1H,-SH) [42, 44], a singlet signal at δ 5.58 ppm which belongs to 2H for (NCH_2) and singlet signal at 3.83 ppm belongs to 3H for (OCH_3) the presence of the chemical shifts proved the synthesis 1,2,4-triazol compounds. The ^{13}CNMR spectrum of some prepared compounds show signals belongs to (C=N) at rang (169-149)ppm which proved to synthesis triazole ring seen in mechanism Scheme (2)[45]. All data of prepared compounds of triazole listed in Tables (3,4) and Figures (1-8).



Scheme (2): Mechanism of synthesized compounds 1,2,4-Triazole

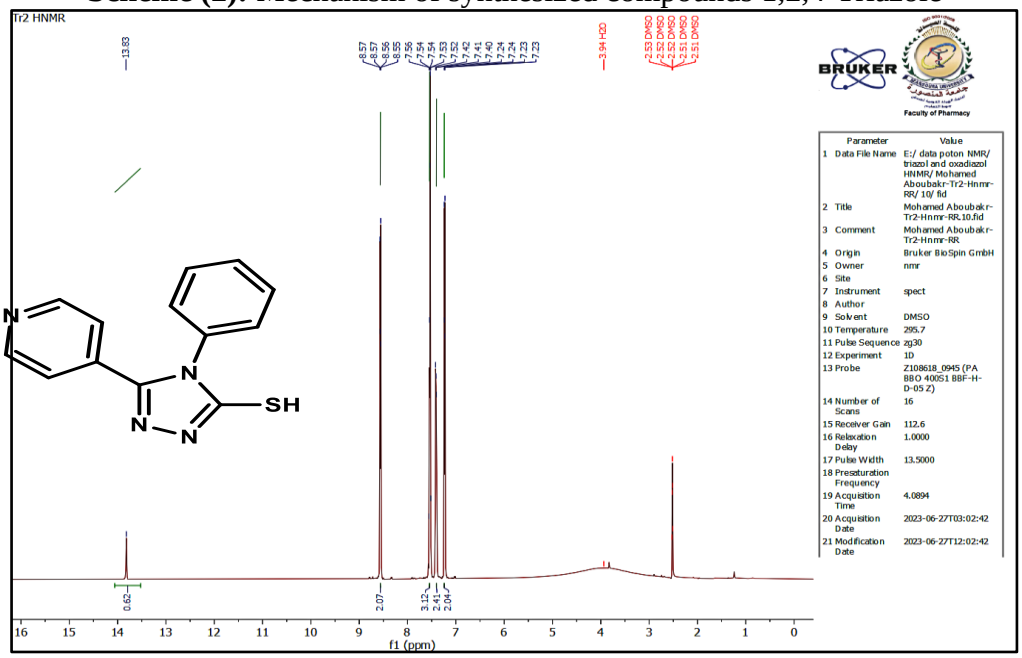


Figure 1 : ^{1}HNMR of derivative (7)

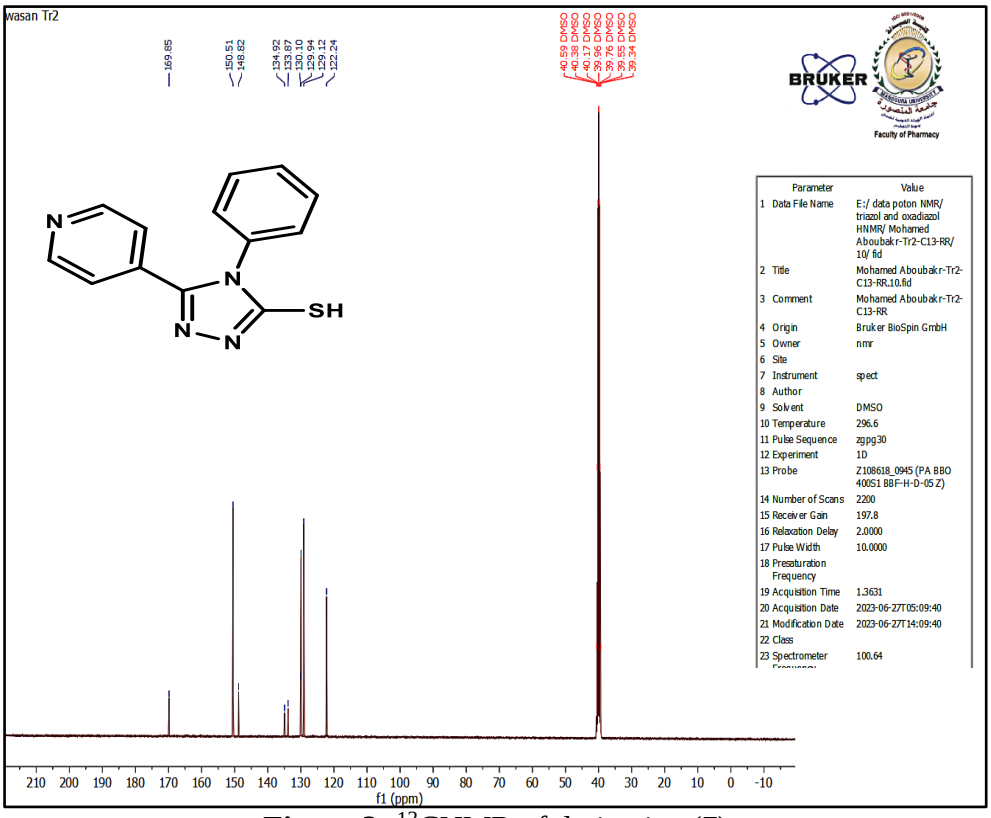


Figure 2: ^{13}CNMR of derivative (7)

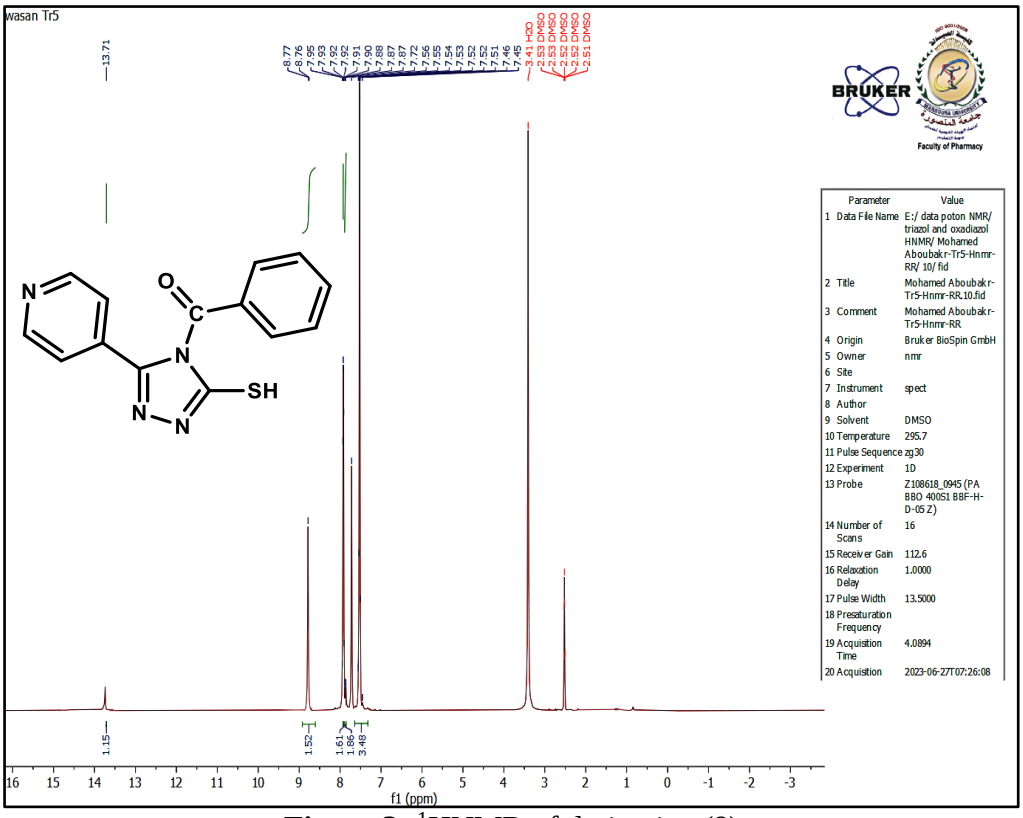


Figure 3: ^{1}HNMR of derivative (8)

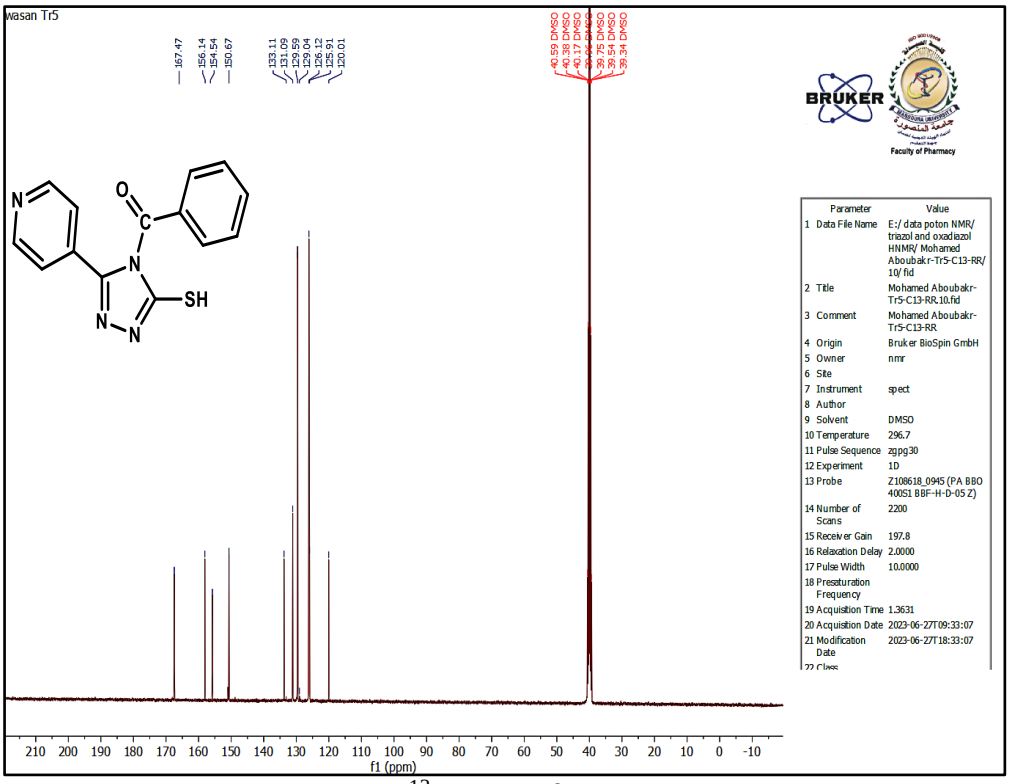


Figure 4 : ^{13}CNMR of derivative (8)

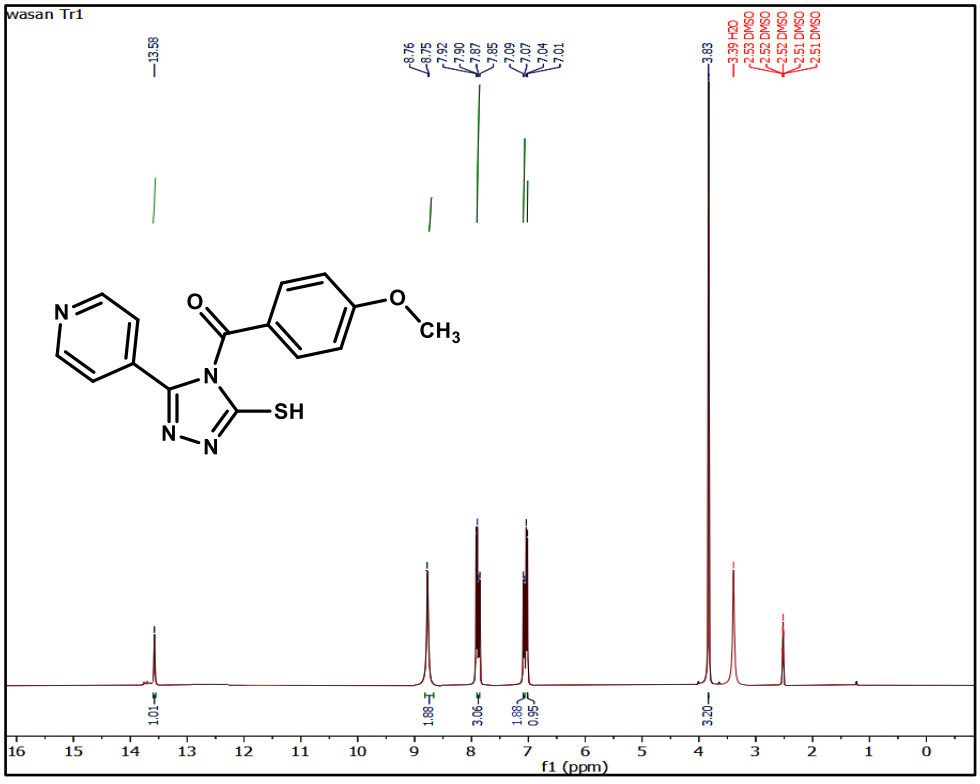


Figure 5 : ^{1}HNMR of derivative (9*)

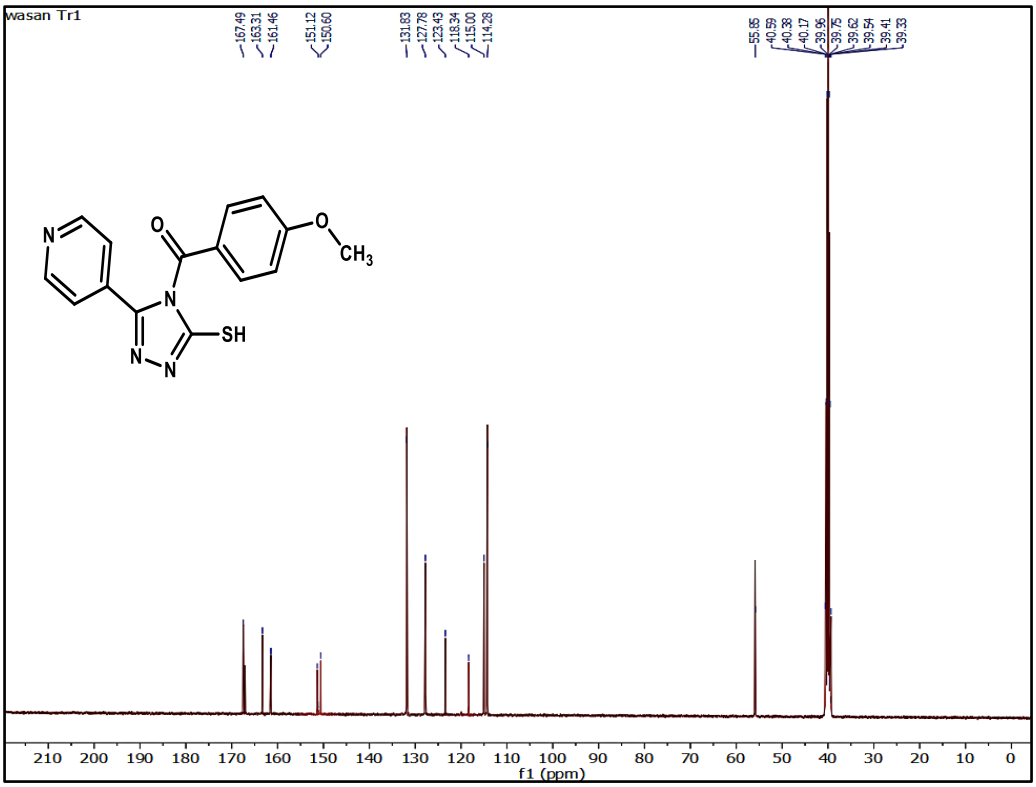


Figure 6 : ^{13}CNMR of derivative (9)

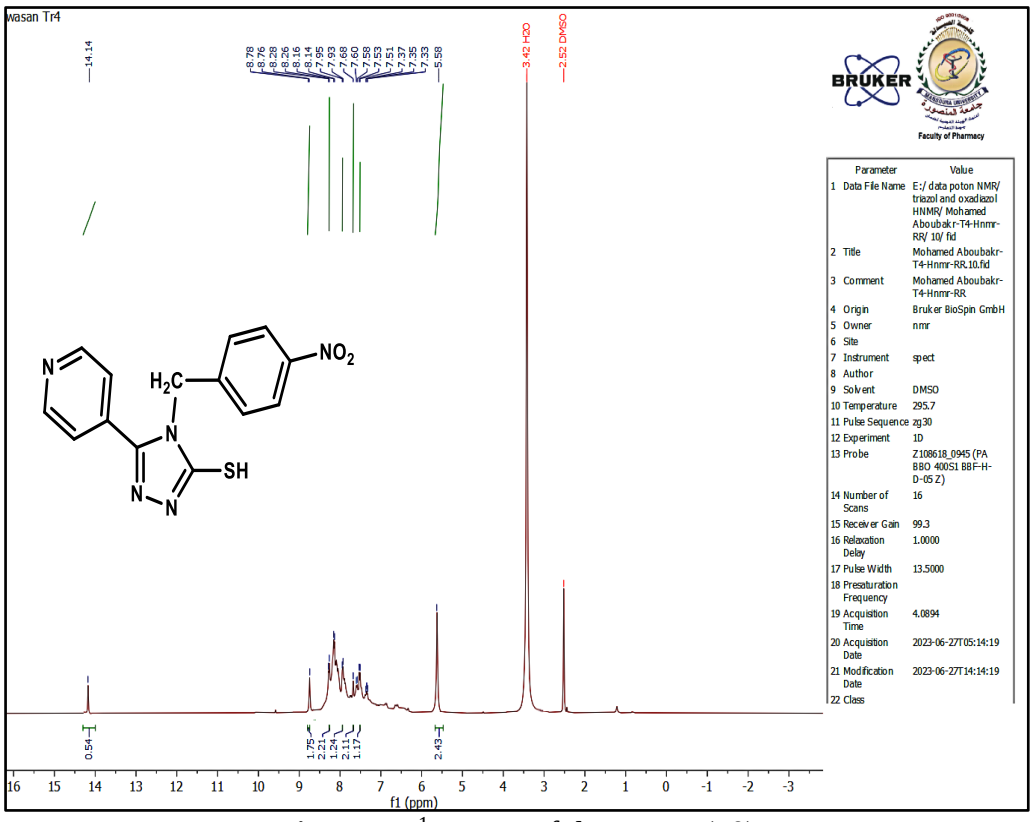


Figure 7 : ^{1}HNMR of derivative (10)

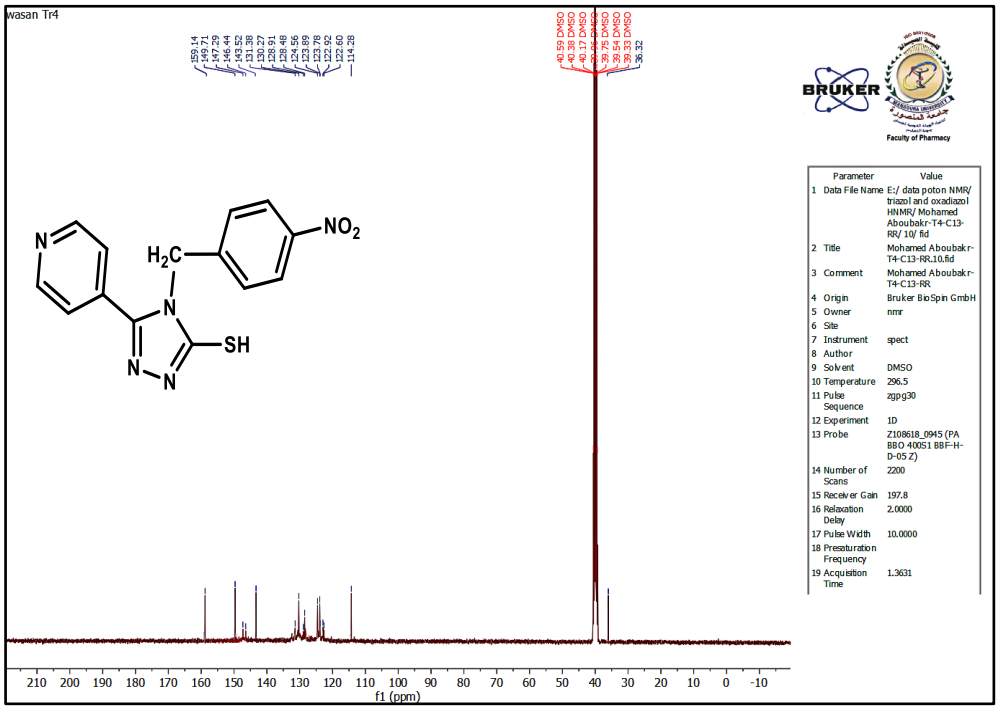


Figure 8 : ^{13}CNMR of derivative (10)

6. Molecular docking study

The GOLD Suite has demonstrated impeccable accuracy in posture prediction and exceptional results in virtual screening. GOLD is a constituent of CSD-Discovery, which includes supplementary software packages like Hermes, CSD python, Mercury, Isostar, ConQuest, Mogul, and others. Molecular docking simulations of all synthesized compounds were performed using GOLD Suite software. Energy minimization of the ligands was conducted to attain minimal energy conformations by optimizing molecular geometries and relieving internal strains. This process corrects any distorted structures. By analyzing ligand-protein interactions at the active binding site, docking data provides an estimation of ligand selectivity and binding affinity for GABA-aminotransferase (GABA-AT). The enzymatic structure used was the (E)-(1S,3S)-3-Amino-4-fluoromethylenyl-1-cyclopentanoic acid-inhibited GABA-AT with PDB code 4ZSW. The ligand-enzyme interaction energies and binding poses were evaluated to predict inhibitory potential against the GABA-AT target. The compounds and reference medicines were evaluated and rated according to their PLP fitness. The PLP fitness values of the docked compounds on GABA-AT ranged from 57.71 to 67.55, as indicated in Table 1 and Figure 1. The GOLD software provides the precise measurement of the hydrogen bonding distance between our ligands and a target protein. [46].

Table 1: The contacts between target and the active binding section of the protein GABA-AT

Com.	(PLP fitness) bending energy	Number of H-bonds	Amino acids involved in H-bonds interaction	Length of bonding
8	57.71	2	GLU 265	1.783
			GLU 270	2.729
			GLN 301	3.002
		3	LYS 329	2.065
				3.378
				2.052
		2	HIS 190	1.723
				2.142
9	66.75	2	GLY191	2.275
		2	GLN301	3.034
		3	SER137	2.872
			LYS 329	2.153
			VAL 300	1.697
			SER328	3.023
10	65.34	4	GLN301	2.304
				2.391
				1.804
				1.240
		4	SER 137	2.915
				2.266
			LYS 329	2.379
				2.608
				1.670
		4	SER 328	2.596
				2.279
				2.198
				1.377
12	67.55	1	LYS 329	2.760
			SER 137	2.601
		1	VAL 300	2.023
			SER 328	1.855
			GLY 136	1.703

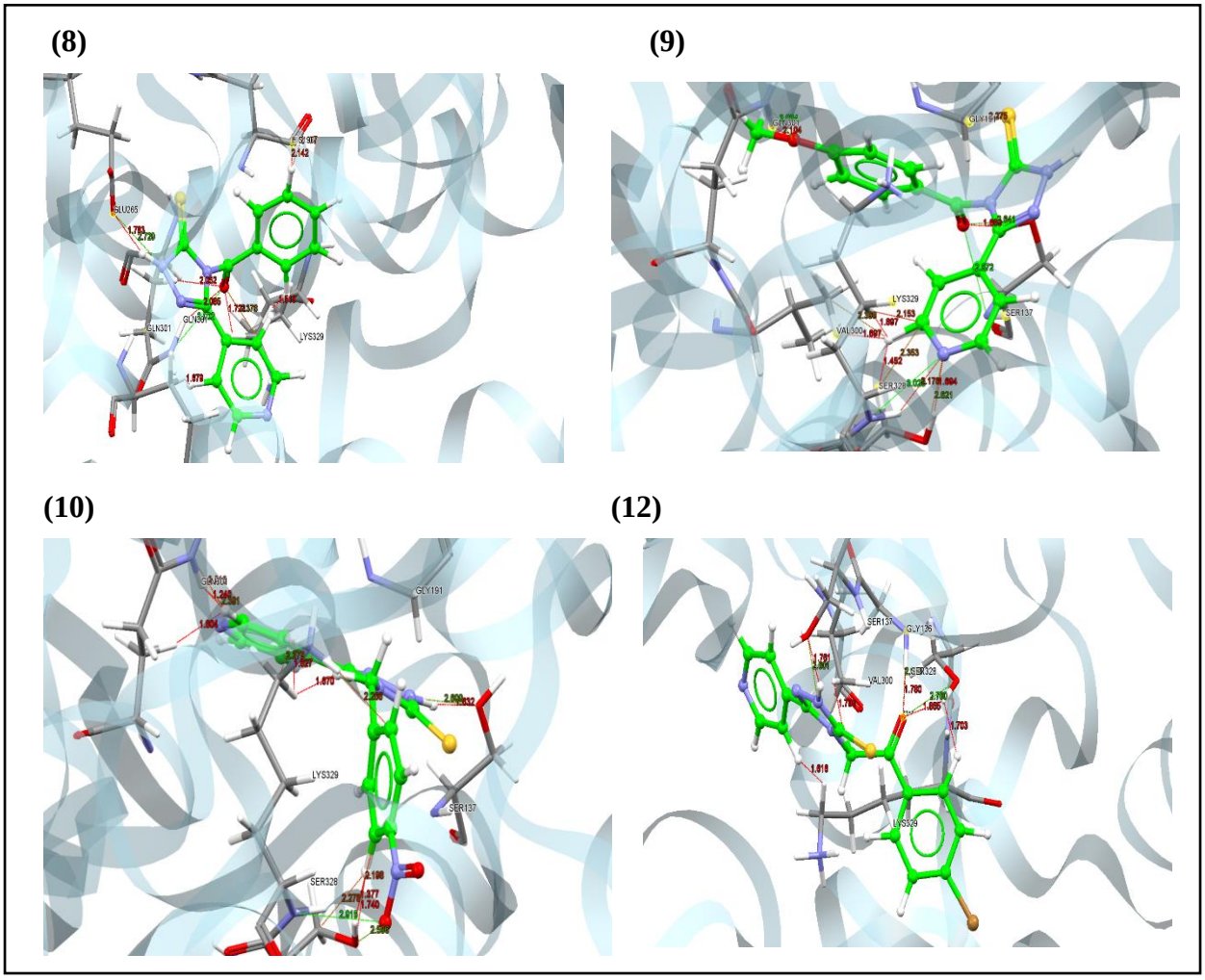


Figure 9: Interaction between ligands and target protein GABA-AT

7. Microbial activity

The antimicrobial activity of some 1,2,4-Triazole compounds was assessed on two Gram-Positive bacteria, namely ***Staphylococcus aureus*** and ***Staphylococcus epidermidis***, as well as two Gram-Negative bacteria, ***Escherichia coli*** and ***Klebsiella pneumoniae***. Table 5 displays the statistics pertaining to biological activity. All the tested compounds (7,9,10 and 12) showed inhibition against (***Staphylococcus aureus*** and ***Staphylococcus epidermidis***), compound 9,12 have highest inhibition against (***Staphylococcus aureus***) which contain methoxy group and bromo group in structure of the compounds and compound 9 highest inhibition against (***Staphylococcus epidermidis)*** compare with drug ciprofloxacin. On the other hand, compounds (7, 9, 10, and 12) were less effective compared to the drug against Gram-negative bacteria (***Escherichia coli, Klebsiella pneumoniae)***. While the compound 12 have no inhibition against (***Escherichia coli***). As for the activity of the prepared compounds against fungal, the tested compounds (7,9 and 12) showed strong activity against (***Candida albicans)*** compared with drug clotrimazole where compound 9 gave the highest activity.

Table 5: Results of Antimicrobial test of some prepared compounds.

Comp.100 µg/ml	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella pneumoniae***	***Candida albicans***
7	14	15	12	15	19
9	20	24	11	11	27
10	11	14	11	14	10
12	21	18	-	13	23
Ciprofloxacin	20	21	40	30	

Clotrimazole					12

Conclusion

The synthesis of the 1,2,4-triazole derivatives has been achieved successfully, with yields ranging from (51-69%). The chemical structures of synthesized compounds were confirmed by FTIR, ^{1}HNMR and ^{13}CNMR spectroscopy. Synthesized derivative compound (9) was subjected to testing for their antimicrobial activity which exhibited antibacterial activity for ***Staphylococcus aureus*** and ***Staphylococcus epidermidis***. All tested compounds show less activity against ***(Escherichia coli, Klebsiella pneumoniae***) compared with drug Ciprofloxacin. Also, compounds (7, 9 and 12) showed strong inhibition against ***Candida albicans***.as well as molecular docking study the GOLD Suite has demonstrated impeccable performance in pose prediction, yielding outstanding results.

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