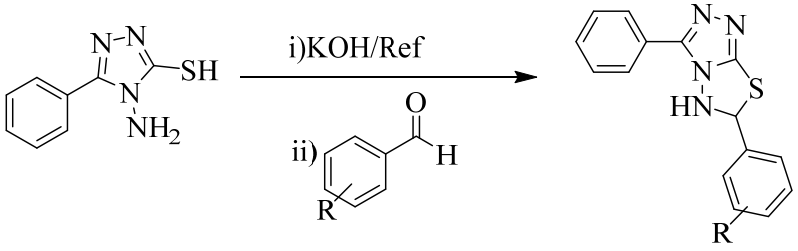
SYNTHESIS, CHARACTERIZATION, AND PHARMACEUTICAL ACTIVITY OF FUSED TRIAZOLOTHIADIAZOLE DERIVATIVES

Bassam A. Hassan[1], Fadil M. Hamed[2] and Athraa Hameed Mekky[3]

[1]Department of Pharmacetical Chemistry, College of Pharmacy, University of Thi-Qar, Thi-Qar, 64001, Iraq.
[2]Shatrah University, Thi-Qar, 64001, Iraq.
[3]Department of Chemistry, College of Science, University of Thi-Qar, Thi-Qar, 64001, Iraq.
e-mail: bassamalsafee@utq.edu.iq

Received 15.10.2024
Accepted 27.01.2025

***Abstract**: A series of fused heterocyclic compound Triazolothiadiazole Derivatives 4a-4f was produced by treating 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol (3) with aryl aldehydes in the presence of KOH. The new triazolothiadiazole derivatives demonstrated anticancer efficacy through docking with the EGFR tyrosine kinase receptor protein, evidenced by docking scores ranging from (-3.23) to (-3.99) kcal/mol in comparison to the control Xalkori's value of (-3.22) kcal/mol. The new compounds were assessed for their vitro cytotoxic activities and tested against the MCF-7 cell line. The synthesized compounds exhibited significant cytotoxic action against the MCF-7 cell line while demonstrating no cytotoxicity toward the normal HdFnd cell line. Compared to normal cells, the study revealed a considerable selectivity of the newly synthesized 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles for cancer cells. The study showed a good correlation between molecular docking and in vitro results for synthesized compounds towards the EGFR tyrosine kinase receptor protein.*



[3] **[4a-4f]**

R: **4a**: 3-OCH_3,4-OH ,**4b**: 2-NO_2 , **4c**: 2-OCH_3,3-OCH_3 ,
4d: 4-OCH_3, **4e**: 3-OCH_3, 4-OCH_3 , **4f**: 4-CH_3.

***Keywords:** heterocyclic compound, aryl aldehydes, triazolothiadiazole, anticancer, cytotoxic activity, molecular docking*
DOI: 10.32737/2221-8688-2025-4-465-475

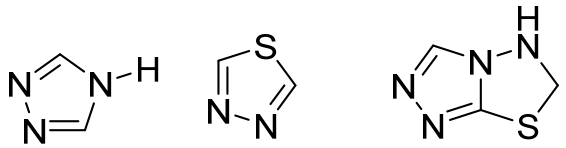
1. Introduction

In the past decades, traditional chemotherapy has used anticancer drugs to destroy cancer cells rapidly dividing throughout the body. Cytotoxic anticancer agents cannot distinguish between malignant cells and rapidly dividing normal cells, which may lead to one or more adverse consequences. Targeted anticancer agents preferentially attach to cancer cells [1], resulting in fewer side effects than cytotoxic medications that selectively target chemicals or proteins associated with the growth and spread of cancer cells. In recent decades, developing novel anticancer agents has become essential for cancer therapy [2]. The 1,2,4-triazole nucleus has been integrated into several therapeutically important molecules, mostly demonstrating anticancer activity [3].

In addition, the chemistry of fused heterocyclic 1,2,4-triazole derivatives has garnered substantial interest due to their pharmacological activity. A triazolothiadiazole system can be regarded as a cyclic analog of two

notable chemicals, thiosemicarbazide and biguanide, which typically exhibit antibacterial, anticancer, anti-tubercular, anti-inflammatory, analgesic, or anti-convulsant properties [4]. This work synthesizes a new class of heterocyclic compounds that include 1,3,4-thiadiazole and 1,2,4-triazole into a unified planar architecture and assesses their anticancer efficacy.

Triazolothiadiazole (3) is synthesized by the fused combination of two five-member heterocyclic systems of a 1,2,4-triazole (1) molecule with 1,3,4-thiadiazole (2) [5], as shown in Fig 1.



(1) (2) (3)

Fig. 1. Triazolothiadiazole heterocyclic compounds

Recent investigations into the synthesis of fused heterocyclic derivatives, including 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles, employing a range of methods characterized by high efficacy and selectivity against various cancer cell lines, have garnered significant attention owing to their remarkable pharmacological properties [6]. The properties encompass applications in cancer therapy, anti-HIV agents, antimicrobial compounds, β3-selective adrenergic receptor agonists, kinase inhibitors, various enzyme inhibitors, the β-lactam antibiotic tazobactam, and the cephalosporin cefatrizine. Derivatives of 1,2,3-triazole have garnered significant attention due to their wide-ranging biological functions, which include antifungal, antibacterial, antituberculosis, analgesic, antileishmanial, anti-inflammatory, CNS depressive medication, cancer prevention, antioxidant, anti-diabetic, anti-hepatitis B viral, molluscicidal, antihypertensive, diuretic, antimicrobial, anti-tubercular, and anti-convulsant properties [7].

Derivatives of triazolothiadiazoles find application in pharmacological advancements aimed at addressing a variety of conditions, including allergies, hypertension, inflammation, psychosis, bacterial and HIV infections, and hypnotics. More recently, they have been utilized in pain management, acting as fibrinogen receptor agonists with antithrombotic characteristics and as novel inhibitors of bacterial DNA gyrase B. We build upon our previous research by synthesizing innovative 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles [8].

Targeted anticancer agents preferentially attach to cancer cells, leading to reduced side effects compared to cytotoxic medications that seek to selectively target chemicals or proteins involved in the proliferation and spread of cancer cells. They have higher activity than traditional medicine and act as bactericidal anticancer agents, which have heterocyclic constituents like alkaloids and hesperidin but also cause a smooth decrease in blood pressure, like captopril medication tests. In the present study, we did a theoretical study by molecular docking to reduce side effects in the pharmaceutical step in drug development. All active compounds dissolve in ethanol like the maceration technique [9-14].

2. Experimental part

Materials. All the basic materials were purchased from Sigma-Aldrich with 97.5% -99 % purity.

Device. The following apparatus has identified and characterized the newly designed and synthesized compounds. Electric melting point equipment (31SMP) evaluated the compounds' melting points using open-glass capillary tubes. Infrared spectra Perkin Elmer toner 27 (Bruker) FT-IR spectrophotometer was utilized at Thiqar University/College of Science to examine the synthesized compounds' infrared spectra using a KBr disc in the range 4000-400 cm^{-1}. Proton Nuclear Magnetic Resonance Spectrum DMSO-d6 solvent was used to obtain ^{1}H NMR spectra at Albasrah University. ^{13}C NMR spectra were obtained using a Bruker BioSpin GmbH 100 MHz spectrometer at the

College of Education, Chemistry Department, Albasrah University, with DMSO-d6 as the solvent. The mass spectra of the prepared compounds were measured by a Network Mass Selective Detector (5973) using an energy of 70 eV at the University of Tehran/Iran.

Synthesis of benzo hydrazide. In a round-bottom flask, hydrazine hydrate (0.485 mL, 0.012 mole) was dissolved in absolute ethanol (50 mL), and methyl benzoate (1.36 g, 0.01 mole) was added dropwise. The mixture was heated under reflux for 5 hours. The solvent evaporated gently under a moderate temperature till the precipitate formed. The formed solid crystals were filtered, dried, and purified using the ethanol solvent, m.p 138-140 ºC, Yield 92%, Rf 0.84, Color white needle The FT-IR spectra of the benzo hydrazide showed an appeared absorption band at 3247, 3137, 3084, and 1648 cm^{-1} due to stretching of NH_2, NH, ArC-H, and carbonyl of benzohydrazide [15-22].

Synthesis of potassium 2-benzoyl hydrazine-1-carbodithioate. To synthesize the titled compound, a quantity of benzohydrazide (0.68 g, 0.005 moles) and potassium hydroxide (0.28 g, 0.005 mole) was dissolved in 50 ml of absolute ethanol in the round-bottom flask. CS_2 (0.3 ml, 0.005 mole) was added slowly at 0 °C to the mixture, and the yellow potassium 2-benzoyl hydrazine-1-carbodithioate was produced.

Synthesis of 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol. A mixture of compound 2 (1.07 g, 0.00565 mole) and hydrazine hydrate (0.24 mL, 0.005 mole) in a round-bottom flask in 50 mL of ethanol. The resulting mixture was refluxed for 5 hrs. The complete reaction was monitored by TLC using eluent hexane: ethyl acetate (6:4). After that, the solvent was concentrated and then acidified with 10% HCl. The formed precipitated materials were filtered, washed with water, and recrystallized (m.p. 189-191 ºC, yield 90%, R_f 0.66, color white and pinkish).

Synthesis of fused [1,2,4]triazolo[3,4-b]thiadiazole of derivatives [4a-4f]. An equimolar mixture consisting of 0.56 g (0.01 mole) of KOH and 2 g (0.01 mole) of compound **3** was heated under reflux, utilizing ethanol as the solvent (100 ml). Subsequently, a suitable aromatic aldehyde (4-hydroxy-3-methoxybenzaldehyde, 2-nitrobenzaldehyde, 2,3-dimethoxybenzaldehyde, 3,4-dimethoxybenzaldehyde, 4-methylbenzaldehyde) (0.01 mole), was introduced into the mixture. The solution was refluxed for 8 h. The reaction was monitored through thin-layer chromatography (TLC), employing a solvent mixture comprising 3 hexane and 7 parts ethyl acetate. The solvent was reduced to approximately 30 ml, and the mixture was subsequently cooled before being positioned atop crushed ice and subjected to agitation. The resultant solid was isolated through filtration and recrystallized using acetone to give the desired product.

4a: Yield 65%, color brown, m.p. 240–242°C,
4b: Yield 67%, color light brown, m.p. 248–251°C,
4c: Yield 68%, color light brown, m.p. 232–234°C,
4d: Yield 65%, color dark brown, m.p. 242–244°C
4e: Yield 67%, color dark brown, m.p. 234–236°C,
4f: Yield 66%, color light brown, m.p. 244–246°C

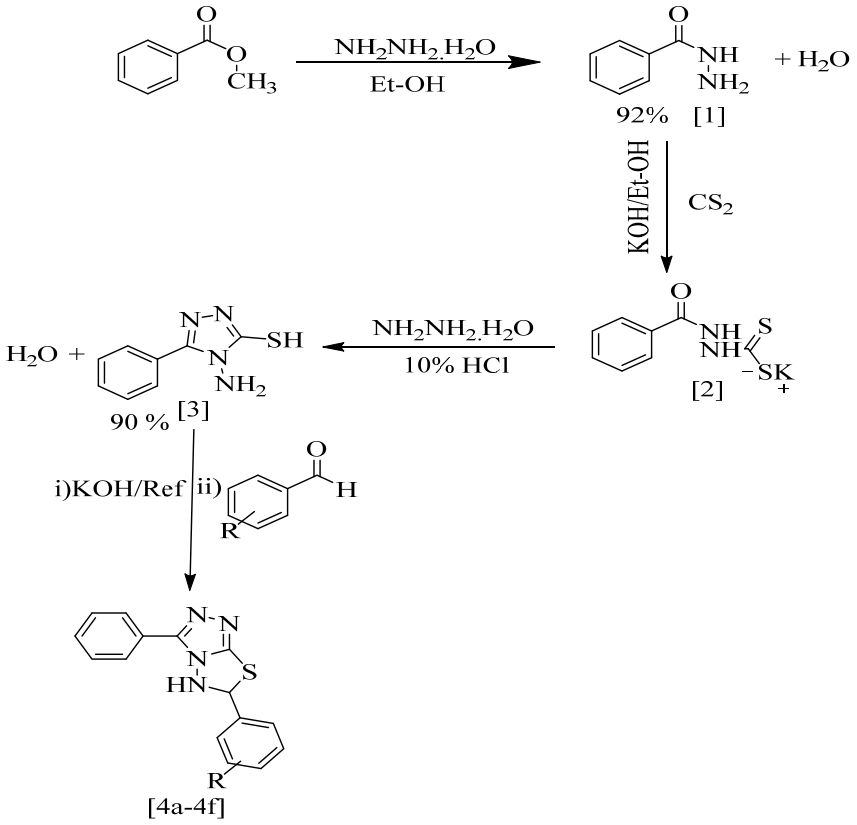
3. Results and discussion

The targeted [1,2,4]-triazolo[3,4-b]thiadiazole derivatives **4a-4f** were synthesized in four steps starting from methyl benzoate, as outlined in Scheme 1. Methyl benzoate was converted into (**3**) via benzohydrazide (**1**) and potassium 2-benzoylhydrazine-1-carbodithioate (**2**) according to the literature procedure [9]. Reaction **3** with aryl aldehydes in the presence of KOH afforded the target new fused triazolothiadazoles **4a-4f**. Elemental analyses for 4a-4f are represented in Table 1.

The structures **4a-4f** were obtained by spectral (FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analytical data [23-32].

2-methoxy-4-(3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)phenol (4a): Brown solid. FT-IR (KBr): 3417, 3123, 3036, 2992, 1614,1449, 1377 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6) δ 14.0965 (s, ^{1}H, NH), 10.1344 (s, 1H, OH), 7.8452 – 7.6344 (m, ArH,v2H), 7.4332 (s, ArH,v3H), 7.3541 (s, ArH, 2H), 7.2365 (d, J = 6.8 Hz, 1H), 6.8389 (s,

CH, 1H), 3.8244 (s, CH_3, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 164.4955, 162.4033, 151.6656, 148.3311, 148.1855, 130.6412, 128.7345, 128.1670, 125.5901, 123.1305, 115.7277, 55.541, 55.6004. A molecular ion peak at 326 of $[C_{16}H_{14}N_4O_2S]^+$ and 177.2 corresponded to the basic peak $[C_8H_7N_3S]^+$.



R: **4a**: 3-OCH_3,4-OH ,**4b**: 2-NO_2 , **4c**: 2-OCH_3,3-OCH_3 , **4d**: 4-OCH_3, **4e**: 3-OCH_3, 4-OCH_3 , **4f**: 4-CH_3.

Scheme 1. Synthesis of fused Triazolothiadiazoles [4a-4f]

Table 1. Elemental analyses for the 4a-4f compounds

Title	Found			Calculated		
	C%	H%	N%	C%	H%	N%
4a	59.02	4.47	17.19	58.88	4.32	17.17
4b	55.43	3.53	21.62	55.38	3.41	21.53
4c	60.04	4.78	16.55	59.98	4.74	16.46
4d	61.87	4.54	18.18	61.92	4.55	18.05
4e	59.88	4.67	16.32	59.98	4.74	16.46
4f	65.41	4.71	19.11	65.28	4.79	19.03

6-(2-nitrophenyl)-3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole(4b): Brown solid FT-IR (KBr): 3110, 2989, 2939, 1634,1479, 1356 cm^{-1}; ^1H NMR (400 MHz, DMSO-d6) δ 14.6622 (s, 1H, NH), 10.1342 (s, 1H, OH), 7.9456 – 7.8389 (m, ArH,2H), 7.5300 (s, ArH,3H), 7.4575 (s, ArH, 1H), 7.3368 (d, J = 7.8 Hz, 2H), 6.8358 (s, CH,1H). ^{13}C NMR (101 MHz, DMSO-d6) δ 164.4911, 162.4043, 151.6623, 148.33, 148.1898, 130.6466, 128.7340, 128.1613, 125.5960, 123.1363, 115.7255, 55.651, 55.6412

6-(2,3-methoxyphenyl)-3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4c): Light brown solid; FT-IR (KBr): 3112, 2936, 2938, 1662,1447, 1345 cm^{-1}; ^1H NMR (400 MHz, DMSO-d6) δ 14.2324 (s, NH,1H), 8.6510 (s, ArH, 1H), 7.8977

(dd, J = 6.7, 3.0 Hz, ArH, 2H), 7.5465 (d, J = 2.3 Hz, ArH, 1H), 7.4956 (s, ArH, 2H), 7.4654 (d, J = 9.6 Hz, ArH, 2H), 5.3234 (s, CH,1H), 3.8656 (s, CH_3, 3H), 3.756 (s, CH_3, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 167.0254, 162.3608, 152.9757, 150.7365, 146.1390, 128.9195, 127.9094, 126.1688, 110.2740, 107.7053,56.1241 55.9890, 54.5420, a molecular ion peak at 340.1 of $[C_{17}H_{16}N_4O_2S]^+$, and 177.3 corresponded to the basic peak $[C_8H_7N_3S]^+$.

6-(4-methoxyphenyl)-3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4d): Dark brown; FT-IR (KBr): 3101, 3066,2995,1607,1487, 1357 cm^{-1}, ^1H NMR (400 MHz, DMSO-d6) δ 14.4567 (s, NH, 1H), 8.0221 – 7.7932 (m, ArH, 2H), 7.7624 (d, J = 8.6 Hz, ArH, 2H), 7.7009 (d, J = 8.5 Hz, ArH, 2H), 7.5205 (d, J = 8.4 Hz, ArH, 2H), 6.8633 (s, CH, 1H), 3.5421 (d, J = 70.6 Hz, CH_3,3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 164.5811, 162.3131, 148.6246, 137.5777, 130.8409, 130.7634, 130.4255, 128.7650, 128.2932, 55.2431.

6-(3,4-methoxyphenyl)-3-phenyl-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4e): Brown solid; FT-IR (KBr): 3160, 2906, 1626, 1403 cm^{-1}, ^1H NMR (400 MHz, DMSO-d6) δ 14.4431 (s, NH, 1H), 7.8785(s, ArH, 2H), 7.4744 (d, J = 6.920, 3.1 Hz, ArH, 3H), 6.9995 (d, J = 6.0, 3.1 Hz, ArH, 2H), 6.9480 (d, *J* = 6.4 Hz, 1H) 5.3321 (s, CH,1H), 3.8690 (s, CH_3, 3H), 3.7993 (s, CH_3, 3H). ^{13}C NMR (101 MHz, DMSO-d6) δ 164.9421, 162.3670, 149.1713, 148.3940, 130.6544, 128.7181, 128.2050, 125.5545, 124.4548, 124.3805, 111.6360, 109. 4679, 55.7933, 55.5341

3-phenyl-6-(p-tolyl)-5,6-dihydro-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (4f): Brown solid; FT-IR (KBr): 3130, 2833, 1600,1510 cm^{-1}; ^1H NMR (400 MHz, DMSO-d6) δ 14.4062 (s, NH, 1H), 8.0233 (s, ArH, 1H), 7.8618-7.6560 (d, J = 7.0, 3.4 Hz, ArH, 5 H), 7.3232-6.9390 (d, J = 6.6 Hz, ArH, 4H), 5.3154 (s, CH, 1H), 2.0889 (s, CH_3, 3H).^{13}C NMR (101 MHz, DMSO-d6) δ 167.8009, 162. 3730, 148.8316, 130.0867, 130.2643, 128.7324, 128.1657, 125.5560, 124.3312, 113.6023, 55.5840. 38.7522

The newly synthesized compounds were assessed for their in vitro cytotoxic activities against the MCF-7 cell line. The synthesized compounds exhibited significant cytotoxic action inhibitory effects on the MCF-7 cell line, exhibiting Low cytotoxicity towards the normal HdFnd cell line. The study showed a considerable selectivity of newly synthesized 1,2,4-triazolo[3,4-b][1,3,4]thiadiazoles for cancer cells compared to normal cells. The study revealed a strong association between molecular docking and in vitro outcomes for manufactured drugs targeting the EGFR tyrosine kinase receptor protein, elucidating the specific [1,2,4]-triazolo[3,4-b]thiadiazole derivatives. The potential of 4a-4f as novel cancer agents is feasible.

***Molecular docking*.** Targeted therapy is a pivotal modality in cancer treatment and is regarded as a crucial answer in anticancer therapy. Targeted anticancer medications specifically bind to cancer cells, leading to fewer unwanted effects than cytotoxic drugs, which try to selectively target chemicals or proteins engaged in the dissemination and expansion of cancer cells. Conventional chemotherapy employs antineoplastic agents to eradicate rapidly proliferating neoplastic cells across the organism and molecular help to discover new Drugs like Captopril [33-34]. Cytotoxic anticancer medications cannot differentiate between cancerous cells and naturally quickly dividing normal cells, potentially resulting in one or more side effects. Targeted anticancer treatments specifically bind to cancer cells, resulting in diminished side effects relative to cytotoxic drugs that selectively inhibit the chemicals or proteins associated with the proliferation and dissemination of cancer cells. Molecular docking plays an increasingly important part in the targeted drug discovery and development process since it saves time, cost, and research effort and reduces side effects of anticancer drugs. Molecular docking is becoming a more essential method for drug development. The key objectives are to assess ligand-protein affinity and to attain a ligand-receptor complex with optimum shape and reduced binding free energy. As the molecular docking study shows, the newly synthesized compounds exhibited an anti-cancer effect. The anticancer effects of these compounds are on the EGFR tyrosine kinase receptor, with varied scores. Their docking scores range from (-3.23) to (-3.99) kcal/mol,

whereas Xalkori binding affinity is (-3.22) kcal/mol.

Furthermore, Compound 4e exhibited the most significant binding affinity with a -3.99 kcal/mol value. When these chemicals are placed into the EGFR tyrosine kinase receptor, they exhibit anticancer action with varying binding affinity, as illustrated in Table 2 and Fig. 2 [33].

Table 2. Results of molecular interaction between EGFR tyrosine inhibitor compounds (4a-4f).

Title	Docking score on ER – (Kcal/mol)	H-bond	Others bonds
4a	- 3.699	LYS 34, GLN 69, ARG 73	ARG 76
4b	- 3.835	--	ARG 73, LYS 60
4c	- 3.235	GLY 79	--
4d	- 3.669	ARG 73, LYS 34	--
4e	- 3.999	LYS 34, ARG 73	ARG 76
4f	- 3.413	LYS 60	ARG 73, LYS 60

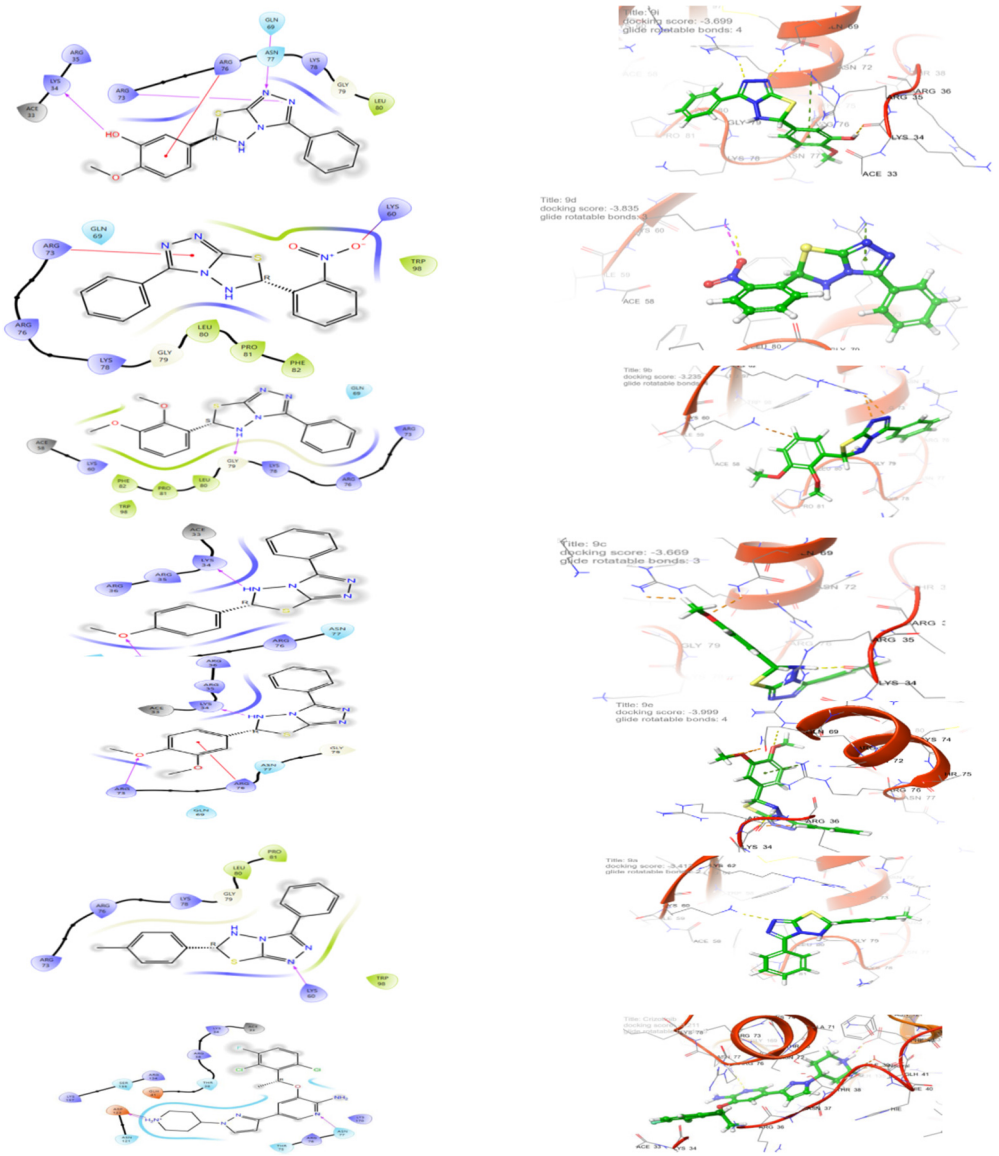


Fig. 2. 2D and 3D dimensional représentations of molecular interactions between 4a, 4b, 4d, 4d, 4e, 4f, and Xa compound and EGFR tyrosine inhibitor

Cytotoxicity screening. The synthesized compounds (4a and 4b) were assessed for cytotoxicity against MCF-7 and HdFn cell lines using the MTT test. This assay relies on the color transition of 3-(4,5-dimethyl-2-thiazolyl)bromide-2,5-diphenyl-2H-tetrazolium from yellow to purple after the apoptosis of live cells. Following a 24-hour incubation of the plates at physiological temperature and in a CO_2 environment, different concentrations (25, 50, 100, 200, and 400 $\mu g \cdot mL^{-1}$) of the produced compounds (4a, 4b) were introduced.

Table 3. Cytotoxic effects of 4a at different concentrations on MCF-7 and HdFn.

4a	**HdFn**		**MCF-7**	
Concentration, µg/mL	mean	Stander D.	mean	Stander D.
400	80.267	1.201014	51.231	4.524195
200	87.133	1.415646	52.453	4.979715
100	93. 574	0.405963	56.549	2.655238
50	95.658	0.910287	61.692	1.758341
25	96.827	0.501827	74.451	4.27583

Table 4. Cytotoxic effects of 4b at different concentrations on MCF-7 and HdFn

4b	**HdFn**		**MCF-7**	
Concentration, µg/ml	mean	Stander D	mean	Stander D.
400	83.726	0.030019	48.590	3.22407
200	89.313	2.264514	58.66749	4.105708
100	95.547	1.409625	70.38506	2.760063
50	96.683	1.902860	85.13460	1.934432
25	98.270	0. 582961	92.30377	2.58431

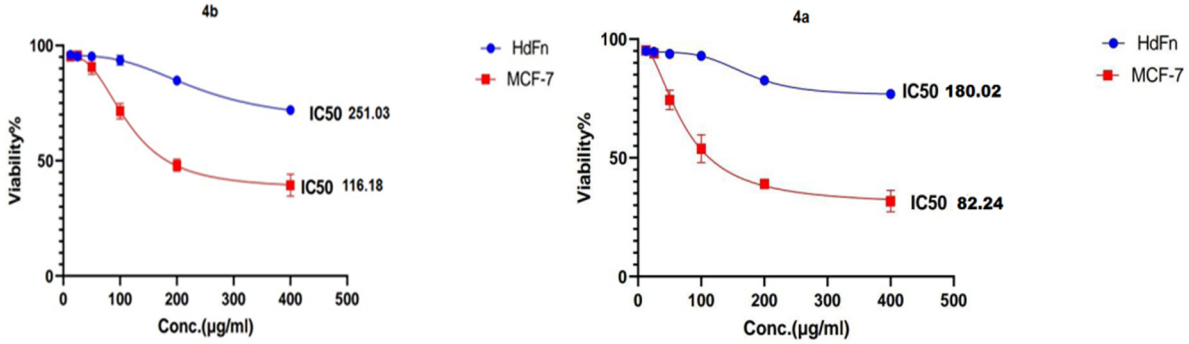


Fig 3. Dose-dependent cytotoxic effect of *4a* and *4b* on MCF-7 and HdFn cell lines

The results of IC_{50}, which represents the half maximal inhibitory concentration, indicated that the synthesized compounds (4a, 4b) exhibit anticancer activity. However, the activity level varies depending on the functional substitution group of the compounds. The efficacy of anticancer activity is directly related to the concentration of the administered medications,

with low concentrations showing minimal effectiveness. Additionally, the lipophilicity and van der Waals volume are crucial considerations. Display the findings of the anticancer activity of (4a and 4b) [35-44]. The synthesized compounds (4a, 4b) showed considerable cytotoxicity towards the MCF-7 cell line at 82.24 µg/mL and 116.18 µg/mL doses.

Nonetheless, it had minimal cytotoxic effects on the human normal cell line (HdFn Cell line) at 180.02 µg/mL and 251.03 µg/mL doses. However, the study proved a good correlation between molecular docking and *in vitro* results for synthesized compounds towards the EGFR tyrosine kinase receptor, as seen in Fig. 3 and Tables 3 and 4.

Conclusions

This study involved the synthesis of six new fused 1,2,4-triazolo[4,3-b][1,2,4,5]thiadiazole derivatives. The synthesized compounds exhibited significant cytotoxic action against the MCF-7 cell line while demonstrating low cytotoxic effects on the normal HdFnd cell line. The study showed a considerable selectivity of newly synthesized fused 1,2,4-triazolo[3,4-b] [1,3,4]thiadiazoles for cancer cells compared to normal cells. The study exhibited a strong association between molecular docking and in vitro outcomes for manufactured drugs targeting the EGFR tyrosine kinase receptor protein. The molecular docking findings aligned with in vitro laboratory data, indicating the potential of these new compounds as novel cancer therapies.

Acknowledgments

This study was supported by the College of Pharmacy, Thiqar University, Ministry of Higher Education and Scientific Research, Iraq.

References

1. Mekky A.H., Hamed F.M., Hassan B.A. Synthesis, Characterization, Molecular Docking Studies and Pharmaceutical Evaluation of some Novel [1,2,4]Triazolo[3,4-B][1,3,4]Thiadiazole. *Jurnal Kimia Valensi*., 2024, **Vol. 10(2)**, p. 304-314. DOI: 10.15408/jkv.v10i2.40043
2. Hassan B.A., Fadil M.H. Synthesis And Pharmaceutical Activity Of Triazole Schiff Bases With Theoretical Characterization. *Chemical Problems*. 2024, **Vol. 22(3)**, p. 332-341. DOI: 10.32737/2221-8688-2024-3-332-341.
3. Farzaliyev V.M., Aliyev Sh.R., Babai R.M., Mammadova R.F., Guliyeva G.M., Mammadov A.M., Eivazova G.Sh. Synthesis of aminomethyl derivatives of 3- mercapto-2-hydroxypropyl-1-isobutyl ether and their study as protective additives to lubricant oils. *Chemical Problems*, 2023, **no. 1**, p. 48-56.
4. Kamel M.M., Abdo N.Y.M. Synthesis of Novel 1, 2, 4-Triazoles, Triazolothiadiazines And Triazolothiadiazoles As Potential Anticancer Agents. *European Journal Of Medicinal Chemistry*, 2014, **Vol. 86**, p. 75-80. DOI: 10.1016/j.ejmech.2014.08.047
5. Daoud Kh., Mohamed Sh.R., Al-Niami N.M.Z. Synthesis Of Some Substituted 1, 3, 4-Oxadiazoles, 1, 3, 4-Thiadiazoles And 1, 2, 4-Triazoles From 2-(2, 3-Dimethylphenyl Amino) Benzoic Acid. *Journal of Education and Science*, 2009, **Vol. 22(3)**, p. 1-10. DOI:10.33899/edusj.2009.57764
6. Aday H.A. Synthesis And Characterization of The Triazole Derived From Thiosemicarbazide, 4-Amino-5-Phenyl-4h-1, 2, 4-Triazole-3-Thiol And Their Copper (II) And Nickel (Ii) Complexes. *Engineering And Technology Journal*, 2013, **Vol. 31(2)**, p. 216-221.
7. Dalal M.J., Athraa H.M. Synthesis, Characterization And Antioxidant Evaluation Of Some Tetrazole Derivatives. *Indonesian Journal of Chemistry*, 2022, **Vol. 22(6),** p. 1596-1604. DOI: 10.22146/ijc.74912
8. Flifel I.A., Ajeel K.A., Al-Jabery A.N. Synthesis, Characterization And Antibacterial Study 0f New 2-Ethyl-5-{[(3-Phenyl-5-Sulfanyl-4h-1, 2, 4-Triazol-4-Yl) Imino] Methyl} Benzene-1, 4-Diol, And Their

Transition Metal Complexes. *University of Thi-Qar Journal*, 2017, **Vol. 12(4)**, p. 1-20.
9. Soleiman-Beigi M., Fariba M. A Novel Copper-Catalyzed, One-Pot Synthesis Of Symmetric Organic Disulfides From Alkyl And Aryl Halides: Potassium 5-Methyl-1, 3, 4-Oxadiazole-2-Thiolate As A Novel Sulfur Transfer Reagent. *Tetrahedron Letters*, 2012, **Vol. 53(52)**, p. 7028-7030/ DOI: 10.1016/j.tetlet.2012.10.016
10. Shah A.P., Kher M.N., Beladiya J.V., Khedkar V.M., Kapadiya K.M. Dibenzyl amine as an ammonia surrogate in the Ugi tetrazoles: Design, synthesis and impactful antioxidant activity. *Results in Chemistry*, 2024, **Vol. 7**, 101272. DOI: 10.1016/j.rechem.2023.101272
11. Shalaal S.H., Halail A.T., Hamed F.M., Hasan B.A. Maceration Techniques Extraction Of Thymus Vulgaris And Laurel (Laurus Nobilis) Leaves With Antibacterial Study. *Plant Archives*, 2019, **Vol. 19(2)**, p. 4041-4044.
12. Mohamed S.A., Hussein M.S., Al-badrany K.A. Synthesis and characterization of pyrazolines and oxazapine derivatives using chalcones as precursor and evaluating their biological activity. *Samarra Journal of Pure and Applied Science*, 2022, **Vol. 4(4)**, p. 17-31
13. Faraj E.M., Jumaa F.H. Preparation, diagnostics and biological evaluation of new Schiff base and tetrazole derivatives. *Materials Today: Proceedings*, 2022, **Vol. 49**, p. 3549-3557. DOI: 10.1016/j.matpr.2021.08.061
14. Dhasarathan S., Shunmugaperumal S., Selvaraj P.K. Synthesis of polydentate, multi-metal ion sensing, unsymmetrical Schiff bases with complimented antifungal activity. *Turkish Journal of Chemistry*, 2022, **Vol. 46(4)**, p. 1024-1041. DOI: 10.55730/1300-0527.3412
15. Abdulridha M.M., Hassan B.A., Hamed F.M. Synthesis And Antibacterial Evaluation of 1,3, 4-Thiadiazole Containing 1, 3, 4-Oxadiazole Bearing Schiff Bases. *International Journal of Pharmaceutical Research*, 2018, **Vol. 10(4)**, p. 12
16. Ahmed A.S., Ahmed A.J.M. New pAminodiphenylamine Amide Compounds: Design, Synthesis and Anti β-lactamases Activity Evaluation. *Chemical Problems*, 2024, **Vol. 22(1)**, p. 20-32. DOI:10.32737/2221-8688-2024-1-20-32.
11. Hassan B.A., Abdulridha M.M., Hamed F.M. Design And Antibacterial Activity Of 3, 6-Diphenyl-1, 5, 6, 7, 8, 8 Ahexahydro [1, 2, 4]Triazolo [4, 3-B][1, 2, 4, 5] Tetrazine As Fused Heterocyclic Compounds. *Biochemical & Amp; Cellular Archives*, 2020, **Vol. 20(1)**, p. 1499-1502.
17. Banerjee A., Kundu S., Bhattacharyya A., Sahu S., Maji M.S. Benzannulation Strategies For The Synthesis Of Carbazoles, Indolocarbazoles, Benzocarbazoles, And Carbolines. *Organic Chemistry Frontiers*, 2021, **Vol. 8(11)**, p. 2710-2771. DOI: 10.1039/D1QO00092F
18. Hassan B.A., Nasera H.N., Abdulridha M.M. Synthesis And Antimicrobial Evaluation Of Fused Heterocyclic Compound [1, 2, 4] Triazolo [4, 3-B][1, 2, 4, 5] Tetrazine. *International Journal of Research In Pharmaceutical Sciences*, 2019, **Vol. 10(2)**, p. 1254-1258.
19. Hassan B.A., Baqer F.M., Abdulridha M.M. Design, Synthesis And Characterization Of Benzoxazepine Thiourea New Derivatives. *International Journal of Drug Delivery Technology*, 2021, **Vol. 11(3)**, p. 874-876.
20. Baqer F.M., Hassan, B.A., Hamed F.M M. Quercetin Isolated from Dried Ginkgo Biloba leaves with the Phytochemical Screening. *International Journal of Pharmaceutical Research* (09752366), 2021, **Vol. 13(1),** p. 22-32. DOI: 10.31838/ijpr/2021.13.01.352
21. Bassam A.H.A., Abdulridha M.M. Preparation And Characterisation Of Some Transition Metal Complexes Of New 4-[(5-Ethyl-1, 3, 4-Oxadiazol-2-Yl) Sulfanyl] Aniline. *The Swedish Journal Of Scientific Research*, 2014, **Vol. 1(6)**, p. 11-23.
22. Hasan B.A., Abdulridha M.M. Preparation And Characterisation Of Sometransation Metal Complexes Of New [Butanal (5-Ethyl-1, 3, 4-Oxadia-Zol-2-Yl) Hydrazone]. *The Swedish Journal Of Scientific Research*, 2014, Vol. **1(5)**, p. 23-36.
23. Hassan B.A. Mekky A.H. Synthesis, molecular docking, and anticancer study of some new [1,2,4]triazolo[4,3-b][1,2,4,5]tetrazines. *Indian J. Heterocycl.*

Chem., 2024, **Vol. 34(3),** p. 391–398. DOI: 10.59467/IJHC.2024.34.391.
24. Pricopie A.I., Focșan M., Ionuț I., Marc G., Vlase L., Găină L.I., Vodnar D.C., Elemer S., Barta G., Pîrnău A., Oniga O. Novel 2, 4-Disubstituted-1, 3-Thiazole Derivatives: Synthesis, Anti-Candida Activity Evaluation And Interaction With Bovine Serum Albumin. *Molecules.* 2020, **Vol. 25(5)**, 1079. DOI: 10.3390/molecules25051079
25. Lelyukh M., Paliy A., Zhukrovska M., Kalytovska M., Chaban I., Shelepeten L., Chaban T. A Review On Synthetic Approaches For Obtaining And Chemical Modification Of 1, 2, 4-Triazolo [3, 4-B][1, 3, 4] Thiadiazole Based Heterocyclic Compounds. *Current Chemistry Letters*, 2024, **Vol. 13(4)**, p. 737-752. DOI: 10.5267/j.ccl.2024.3.007
26. Abdel-Aal M.T., El-Sayed W.A., El-Kosy S.M., El-Ashry el S.H. Synthesis and antiviral evaluation of novel 5-(N-Aryl-aminomethyl-1,3,4-oxadiazol-2-yl)hydrazines and their sugars, 1,2,4-triazoles, tetrazoles and pyrazolyl derivatives. *Arch Pharm (Weinheim)*, 2008, **Vol. 341(5)**, p. 307-13. DOI: 10.1002/ardp.200700154.
27. Abass A.A., Muhsin S.N., Hasan Sh.A., Hassan B.A. Efficacy Study Of Captopril On Some Liver Function Tests In Hypertensive Patients. *Revista Latinoamericana De Hipertension*, 2023, **Vol. 18(3)**, p. 142-146. DOI: 10.5281/zenodo.8052329
28. Hassan A.Y., Kadh M.S., Saleh N.M., Abou-Amra E.S. Synthesis Of Novel [1, 2, 4] Triazolo [3, 4-B][1, 3, 4] Thiadiazole-6 (5h)-Thione, 5, 8-Dihydro-[1, 2, 4] Triazolo [4, 3-B][1, 2, 4, 5] Tetrazine And 5, 10-Dihydro-[1, 2, 4] Triazolo [4, 3-B][1, 2, 4] Benzotriazine Derivatives And Study Their Biological Activity. *Int. J. Adv. Res*, 2016, **Vol. 4(8)**, p. 335-347. DOI**:** 10.21474/IJAR01/1222
29. Al alawy H., Flifel I.A. Synthesis, Characterization And Anticancer Study Of New3-[(2z)-2 (2-Hydroxybenzylidene) Hydrazinyl]-5-(2-Hydroxyphenyl)-1, 3, 4-Oxadiazol-3-Ium And Its Transition Metal Complexes. *University Of Thi-Qar Journal Of Science*, 2023, **Vol. 10**, p. 98-102. DOI: 10.32792/utq/utjsci/v10i2.1094
30. Hameed A.A., Hassan F. Synthesis, Characterization And Antioxidant Activity Of Some 4-Amino-5-Phenyl-4h-1, 2, 4-Triazole-3-Thiol Derivatives. *Int J Appl*, 2014, **Vol. 4**, p. 202-11. DOI: 10.13140/RG.2.2.18501.96484.
31. El-Reedy A.A., Soliman N.K. Synthesis, Biological Activity And Molecular Modeling Study Of Novel 1, 2, 4-Triazolo [4, 3-B][1, 2, 4, 5] Tetrazines And 1, 2, 4-Triazolo [4, 3-B][1, 2, 4] Triazines. *Scientific Reports*, 2020, **Vol. 10(1),** p. 6137. DOI: 10.1038/s41598-020-62977-x.
32. Pandey S.K., Singh A., Singh A., Nizamuddin. Antimicrobial Studies Of Some Novel Quinazolinones Fused With [1, 2, 4]-Triazole,[1, 2, 4]-Triazine And [1, 2, 4, 5]-Tetrazine Rings. *European Journal Of Medicinal Chemistry*, 2009, **Vol. 44(3)**, p. 1188-1197. DOI: 10.1016/j.ejmech.2008.05.033.
33. Mousa H., Abd Al-Amir T. The Infections In Urinary Tract Among Pregnant Women In Nasiriya City, Iraq: Bacterial Urinary Tract Infections Among Pregnant Women. *University Of Thi-Qar Journal Of Science,* 2023, **Vol. 10(1)**, p. 117-121. DOI: 10.32792/utq/utjsci/v10i1.1039
34. Al-Hilali R.M., Al-Mozan H.D.K. Isolation And Identification of Negative Bacteria That Cause Diarrhea. *University Of Thi-Qar Journal Of Science,* 2023, Vol. **10(1)**, p. 175-180. DOI: 10.32792/utq/utjsci/v10i1.1059
35. Musa M. The Prevalence And The Significance Of The Pulmonary Bacterial Super-Infections Among Hospitalized Covid-19 Patients: A Scoping Review. *University Of Thi-Qar Journal Of Science,* 2023, Vol. **10(1)**, p. 66-72. DOI: 10.32792/utq/utjsci/v10i1.930
36. Kleef D.G., Hussein K.R. Isolation Of Bacterial Causative Agents For Diabetic Foot Patients And Antibiotic Susceptibility Test Against Bacterial Isolates. *University Of Thi-Qar Journal Of Science,* 2023, Vol. **10(1(S.I.)**, p. 201-204. DOI: 10.32792/utq/utjsci/v10i1(SI).1032
37. Hanan Z.K., Saleh M.B., Mezal E.H. Antimicrobial Resistance Pattern And Plasmid Profile Of Salmonella Enterica Isolated From Diarrheal Children In Thi-Qar

Province/Iraq. *University Of Thi-Qar Journal Of Science,* 2020, **Vol. 7**, p. 49-53. DOI: 10.32792/utq/utjsci/v7i2.711

38. Stetsiuk O., Abhervé A., Avarvari N. 1, 2, 4, 5-Tetrazine Based Ligands And Complexes. *Dalton Transactions*, 2020, **Vol. 49(18)**, p. 5759-5777. DOI: 10.1039/D0DT00827C.
39. Abualnaja M.M., Alalawy A.I., Alatawi O.M., Alessa A.H., Qarah A.F., Alqahtani A.M., El-Metwaly N.M. Synthesis of tetrazole hybridized with thiazole, thiophene, or thiadiazole derivatives, molecular modeling, and antimicrobial activity. *Saudi Pharmaceutical Journal*, 2024, **Vol. 32(3),** 101962. DOI: 10.1016/j.jsps.2024.101962
40. Abdul-Rida N.A., Talib K.M. New Chalcone Derivatives As Anticancer And Antioxidant Agents: Synthesis, Molecular Docking Study And Biological Evaluation. *Chemical Problems*, 2024, **Vol. 22(2)**, p. 177-186. DOI: 10.32737/2221-8688-2024-2-177-186
41. Xu F., Yang Z.Z., Jiang J.R., Pan W.G., Wu J.Y., Zhu Y., Wang J., Shou Q.-Y., Wu H.G. Synthesis, Antitumor Evaluation And Molecular Docking Studies Of [1, 2, 4] Triazolo [4, 3-B][1, 2, 4, 5] Tetrazine Derivatives. *Bioorganic & Medicinal Chemistry Letters*, 2016, **Vol. 26(13)**, p. 3042-3047. DOI: 10.1016/j.bmcl.2016.05.007
42. Suleymanova A.B., Aliyeva K.T., Nasirova A.E. Chemical Composition Of Extracts And Essential Oils Obtained From Common Juniper (Juniperus Communis L.) In Azerbaijan. *Chemical Problems*, 2024, **Vol. 22(2)**, p. 211-220. DOI: 10.32737/2221-8688-2024-2-211-220
43. Mohammed H.M. Synthesis And Biological Evaluation of Newly Synthesized Triazolotriazines And Triazolotraizines Derivatives. *Journal Of University Of Anbar For Pure Science*, 2017, **Vol. 11(3)**, p. 25-33
44. Nabeel A.A., Islam H.T. Synthesis, Characterization In Silico And In Vitro Study Of New 1,2,3- Triazole Derivatives As Antioxidant Agents. *Chemical Problems*, 2023, **Vol. 21(4),** p. 343-352. DOI: 10.32737/2221-8688-2023-4-343-352.