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Novel Thiazolo-Triazole Derivative: Synthesis, Molecular Docking, and Biological Evaluation

Lina Saadi
Department of Pharmaceutical Chemistry, College of Pharmacy, University of Al-Qadisiyah
lena.saadi@qu.edu.iq

Abstract

Fused heterocyclic moieties have emerged as a focal point in recent years due to their unique structure. In this study, we synthesized a novel 5-Arylidene-thiazolididinone compound based on an imidazole scaffold. The structures of all synthesized derivatives were elucidated by spectral methods, FTIR, ^{1}H, and ^{13}C NMR. The target compound 6 was screened for its inhibitory activity as an antimicrobial agent and gained potent activity toward tested microorganisms (Staphylococcus aureus and Escherichia coli), which is more efficient than the standard drug, Amoxicillin. Additionally, the findings of the antioxidant study that was performed using DPPH for the new thiazolidinone derivative 6 revealed that it can trap the free radicals. Moreover, the molecular docking analysis showed that the target structure had an efficient binding interaction of 6 with the active site of the 6COX protein. Furthermore, the cytotoxicity assay results confirmed what the study found from the molecular docking study, which found that compound 6 has a significant activity against the MCF-7 cell line.

Keywords: *Anticancer; Antioxidant assay; Molecular Docking; Synthesis; Triazole*

Introduction

One of the essential objectives of medicinal and organic chemistry is the synthesis and design of molecules useful as human therapeutic agents, and because of a variety of circumstances, such as the rise in multidrug-resistant microbial infections, treating diseases still poses a significant and tough challenge(Helmy et al., 2023). The World Health Organization announced that the second most frequent cause of mortality in people is cancer, and breast cancer is the most dangerous type of cancer for women, in

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addition to oxidative stress diseases(Méndez-Valdés et al., 2023) . Numerous studies on the treatment of various diseases have shown that no drugs are now available that are 100% effective and have no side effects, especially for cancer (Anand et al., 2023). Therefore, researchers increasingly tend to design and discover new therapeutic agents to overcome the difficulties related to the effectiveness of the currently used drugs.

There are various biologically active compounds, such as five-membered rings that have two hetero atoms, among which is the 4-thiazolidinone and their arylidenes derivatives(Petrou, Fesatidou and Geronikaki, 2021). Heterocyclic compounds included N and S atoms have garnered a significant role in pharmaceutical chemistry studies, which is an important framework in many synthetic molecules with a wide range of pharmacological profiles, such as antibacterial (Saadi and Adnan, 2018 a; Tariq, Saadi and Adnan, 2020), anticancer(Roszczenko et al., 2022a; Tilekar et al., 2022), anti-inflammatory (Kaminskyy, Kryshchyshyn and Lesyk, 2017), antioxidant(Hosseini Nasab et al., 2022), antitubercular (Bhat et al., 2020), etc.(Kabir and Uzzaman, 2022).

Consequently, the combination of the scaffold, 5-Ene-4-thiazolidinone, with imidazole moiety which is also famous for having diverse pharmacologic activities (Sadeghian et al., 2022; Roy et al., 2022; Singha, Habib and Hossain, 2022; Li et al., 2023; Tolomeu and Fraga, 2023) in the same molecule is the goal of this study to discover the biological activity resulting from the incorporation of these moieties together and assessment of their biological activities in vitro, including antimicrobial and anticancer, and antioxidant activities.

Instrumentation and Materials

Melting points were registered on a digital (Stuart, UK) apparatus. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 400 MHz at 25°C in TMS (internal standard) and DMSO-d_6 (as solvent), parts per million units were used to report chemical shifts. FT-IR spectra were recorded on a Shimadzu 8400 (KBr disk) spectrophotometer. Thin layer chromatography (Merck, Germany), silica gel plates TLC 60 F254) to monitor all reactions. The spots were located using iodine vapour. All the solvents and reagents were provided in high purity. Ethanol 99% (Merck), Thionyl chloride 97%, Thiosemicarbazide, Sodium Methoxide, Pyridine (C.D.H), 4-Hydroxybenzaldehyde (TCL), Sodium acetate anhydrous, 3-Amino-4-methoxy benzoic acid, 4,5-Dichloroimidazole (Fluorochem), Hydrochloric acid (Himedia).

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Methods

Synthesis of Azo derivative (1)

Sodium nitrite (0.412 g, 5.98 mmol) was dissolved in 20 ml of distilled water. This solution was cooled and added drop by drop to 3-amino-4-methoxybenzoic acid (1 g, 5.98 mmol) in a mixture of (89 ml distilled water + 22 ml conc. HCl). Continue stirring the mixture in an ice bath at (0-5 °C) for 20 min to produce a diazonium salt solution. The formed salt was added gradually to a cooled solution composed of (0.819 g, 5.98 mmol) 4,5-dichloro imidazole in absolute ethyl alcohol, 30 mL and sodium hydroxide 10% solution. The mixture was continuously stirred for two hours at a low temperature of 0-5 °C. The product was an orange solid, followed by filtration, washing with distilled water many times, drying, and recrystallizing from absolute ethanol. Yield (90%), M.P (231-233°C). This derivative was synthesized and characterized previously in the literature (Saadi and Adnan, 2026).

Synthesis of acyl chloride derivative (2)

Thionyl chloride (6 mL) was added slowly by a dropping funnel with stirring to the synthesized carboxylic acid derivative 1 (0.3g, 1mmol) at 25°C, then refluxed at 70°C for seven hours (monitored by TLC). The excess $SOCl_2$ was removed by reduced pressure. The obtained red precipitate is used in the next step immediately. Yield 91 %, M.P (195-197 °C). This derivative was synthesized and characterized previously in the literature(Saadi and Adnan, 2023).

Synthesis of Carbothioamide derivative (3)

The synthesized acid chloride (0.3g, 0.89 mmol) in 5 ml of 1,4-dioxane was added to a solution containing (0.082g, 0.89 mmol) of thiosemicarbazide in 3 ml of pyridine. First, the mixture is stirred at 0 ºC for half an hour. Then, the reaction continues at room temperature for 26 hours. At the end, the mixture was quenched with 10ml of water (Saadi and Adnan, 2024). The obtained orange-yellowish solid was filtered, and recrystallization from ethanol. Yield 78 %, M.P (110-112 °C). FTIR, υ(cm^{-1}): 3425- 3186 (NH-NH_2), 3147 (NH), 3093 (C-H), 2947(C-H), 1688 (C=O), 1612(C=N), 1558-1519 (C=C), 1458(N_2), 1249(CS). ^{1}HNMR, δ (ppm): 4.01(s, 3H, OCH_3), 11.3 (brs. 1H, NH), 8.2-7.3 (m, 3H, =CH), 5.7 (s, 2H, NH_2), 8.6 (s, 1H, NH=CS), 9.9 (s, 1H, NH-CO). ^{13}CNMR, δ(ppm): 56, 113, 123, 126, 127, 129, 130, 135, 144, 153, 165, 181.

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Synthesis of 1,2,4-triazole-5-thiol (4)

Carbothioamide derivative 3 (0.3 g, 0.77 mmol) and (15ml, 2N) of NaOH were mixed in a round flask. The mixture was refluxed at 90 ℃ for 18 hours, then cooled to room temperature and acidified with diluted HCl (2N) to pH 4 (Saadi and Adnan, 2024). The resulting light brown solid was filtered and recrystallization from ethyl alcohol. Yield 70%, M.P (decompose: 285 °C). FTIR, υ(cm^{-1}): 3340 (NH), 3178 (NH), 2455(SH), 3055 (C-H), 2977 (C-H), 1668 (C=N), 1642 (C=N), 1604-1512 (C=C), 1427(N_2). ^{1}HNMR, δ (ppm): 4.01(s, 3H, OCH_3), 11.3 (brs. 1H, NH), 8.2-7.3 (m, 3H, =CH), 13 (s, 1H, NH), 12.9 (s, 1H, SH). ^{13}CNMR, δ(ppm): 56, 112, 121, 123, 124, 126, 129, 135, 142,144, 153, 167.

Synthesis of Thiazolo [3,2-b] [1,2,4] triazol-6(5H)-one derivative (5)

Equimolar amounts of chloroacetic chloride, synthesized triazole 4, and anhydrous sodium acetate were dissolved and mixed in 3 ml of ethanoic anhydride and 25ml of anhydrous acetic acid. Refluxing the solution for 26 hours, then cooling and quenching with crushed ice. Light brown precipitate was collected, washed and purified with dioxane(Behalo, 2018). Yield 60%, M.P (312-310 °C). FTIR, υ(cm^{-1}): 3340 (NH), 3186 (NH), 2455(SH), 3093 (C-H), 2970, 2846 (C-H), 1612 (C=N), 1650 (C=N), 1558-1519 (C=C), 1458 (N_2). ^{1}HNMR, δ (ppm): 3.9 (s, 3H, OCH_3), 11.4 (brs. 1H, NH), 8.2-7.6 (m, 3H, =CH), 4.7 (s, 2H, CH_2).

Synthesis of 5-Arylidine-4-thiazolidinone derivative (6)

In a refluxing flask, equimolar amounts of the synthesized derivative 6, p-hydroxybenzaldehyde and anhydrous sodium acetate in 15 ml of glacial acetic acid were mixed and refluxed at 95 °C for 24 hours. After the reaction completed, the mixture was poured on cooled water to afford a brown solid, which was filtered and washed with D.W. then recrystallized from ethanol(Behalo, 2018). Yield 61%, M.P (Decompose: 202 °C). FTIR, υ(cm^{-1}): 3440 (OH), 3102 (=CH), 3170 (NH), 3016 (C-H), 2923 (C-H), 1683 (C=O), 1604 (C=C), 1666 (C=N), 1650 (C=N), 1589-1512 (C=C), 1450 (N_2). ^{1}HNMR, δ (ppm): 3.8 (s, 3H, OCH_3), 11.2 (brs. 1H, NH), 8.2-6.9 (m, 7H, =CH), 7.9 (s, 1H, =CH), 9.6 (br s, 1H, OH). ^{13}CNMR, δ(ppm): 56, 111, 116, 117, 119, 123, 126, 127, 128, 129, 135, 136, 141, 146, 155, 159, 160, 162, 164.

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Results and Discussion

Chemistry

The synthetic route of the target compound was achieved as outlined in Scheme 1. The synthetic pathway was carried out in six steps. The synthetic approach starts with synthesizing the Azo derivative 1 obtained in high yield 90% by two steps: Diazotisation of primary amine and coupling of the resulting diazonium salt with the imidazole derivative. The formation of 1 was confirmed by various spectral techniques by the presence of characteristic bands at 3400-2500 due to OH of the carboxylic group as a broad band, also a strong band at 1681cm^{-1} attributed to C=O, and 1450 cm^{-1} to N=N. while the ^{1}H NMR spectrum showed a characteristic broad peak at 12.84 due to the proton of OH. ^{13}C showed a signal at 167 ppm due to C=O.

The chlorination reaction of 1 with thionyl chloride gave the corresponding acid chloride 2 in 91 % yield. The formation of the -COCl fragment was approved by shifting the band position of C=O of the carboxylic group to a higher frequency at 1751 cm^{-l}. ^{1}H NMR showed the disappearance of the hydroxyl signal, whereas ^{13}C showed a characteristic signal at 171 ppm, an indication of the formation of acid chloride.

Hydrazine-1-carbothioamide derivative 3 was synthesized by reacting 2 with thiosemicarbazide in dry pyridine under cold conditions. Its structure was approved by the appearance of bands at the interval (3186-3425), 1688, 1249 cm^{-l} due to (NH, NH2), C=O amide, and C=S, respectively. In addition to the resonances at 5.7 (2H, NH2), 8.6(1H, NH-C=S), and 9.9 (1H, NH-C=O) ppm in the ^{1}HNMR spectrum, as well as resonances at 181 (C=S), 165 (C=O) ppm in ^{13}C NMR, there is clear proof for a successful synthesis process.

Compound 3 undergoes intramolecular cyclization in aqueous KOH to afford 1H-1,2,4-triazole-5-thiol derivative (4). The FTIR spectrum of 4 showed new bands at 3340 (NH triazole), 2545 (SH), 1642(C=N triazole) cm^{-1}. On the other hand, the ^{1}H NMR spectrum displayed signals at 13.1 (1H, NH triazole) and 12.9 (1H, SH) ppm, as well as the resonances at 167 ppm (C-SH) in the ^{13}CNMR, confirming the formed triazole ring.

The ring-closing reaction of 1,2,4-triazole thiol (4) with $ClCH_2COOH$ under reflux was achieved in one step in glacial acetic acid and acetic anhydride to afford thiazolotriazolone derivative (5). Spectral study presented a band at 1712 cm^{-1} for the

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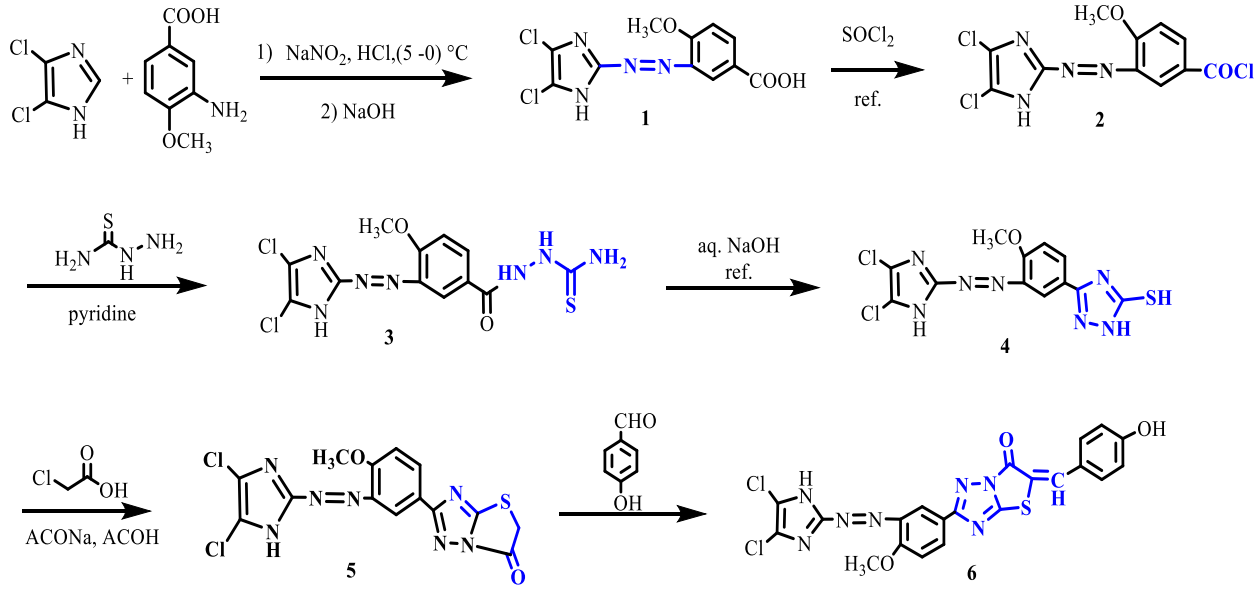
C=O of the thiazole ring in the FTIR spectrum, and a signal at 4.7 ppm in the ^{1}H NMR spectrum, confirming the presence of the thiazole ring.

Finally, the Knoevenagel condensation reaction of compound 5 on treatment with a selected benzaldehyde was carried out in the presence of ACONa-ACOH to afford the target compound thiazolo[3,2-b][1,2,4]triazol-6(5H)-one (6). The formation of target compound 6 is confirmed by the presence of bands at 1683 assigned to C=O of thiazole, 3402 cm^{-1}(OH phenolic), and 3102(=CH) cm^{-1}. Moreover, the resonances at 7.9 (=CH alkene), and a broad peak at 9.6 (OH) ppm in the ^{1}HNMR, and sharp signals at 136, and 117 ppm attributed to -CO=C- and =CH- of thiazole ring, respectively, in the ^{13}CNMR spectrum.



Scheme 1. Pathway of the synthesis of Thiazolo-Triazole derivative

Biological Tests

Antimicrobial assessment

The antibacterial activity of the target compound 6 was scanned against two types of bacteria, Gram-negative bacteria (Escherichia coli) and Gram-positive bacteria (Staphylococcus aureus). Compound 6 showed very strong antibacterial activity against

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both Gram-negative and Gram-positive microorganisms compared to the standard drug, Amoxicillin.

The structural activity relationship of the target synthesized thiazolotriazole 6 with derivative 5 and achieved antimicrobial results showed that compound 6, with a substitution on active carbon C5 in the thiazole ring, has higher activity toward tested bacteria than derivative 5, which has no substitution at the thiazole ring. The reason may be attributed to the presence of a hydroxyl group on the benzene ring of the Thiazolotriazole 6 derivative that promoted the antibacterial effectiveness against both bacterial strains that were examined. Table 1 demonstrates the values of inhibition zones.

Table 1. Antimicrobial activity inhibition zone of 5 and 6 derivatives

Comp.	***Inhibition zone diameter, mm***	
	E. coli (-)	***Staphylococcus (+)***
5	10	8
6	21	20
S1(Amoxicillin drug)	15	14
DMSO	-	-

Antioxidant activity

Removing harmful free radicals from the biological system is an essential strategy. Therefore, there are many methods for evaluating antioxidant activity that include the removal of these free radicals. In this study, the DPPH method was selected to evaluate the effectiveness of the synthesized compound. This method takes place through the mechanism of breaking the chain, so it is considered one of the most important methods for estimating the concentration of free radical scavengers. This method depends on the colour change of the DPPH radical from purple to yellow as a result of the reduction of free radicals by antioxidants (Baliyan et al., 2022).

In this research, the antioxidant activity of the newly synthesized derivative 6 and Ascorbic acid (standard antioxidant) was performed using the DPPH method protocol. The samples were tested for their radical scavenging ability at different concentrations ranging between 12.5 - 200 µg/ml. The reaction was achieved at room temperature. Absorbance was measured at 517 nm using a spectrophotometer. The results of the antioxidant showed that the tested compound 6 has moderate activity with an inhibition percentage 56.67% at the highest dose and the half inhibitory concentration (IC_{50}) equal

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to 190.4 µg/ml. This activity may be due to the structure of Thiazolo-Triazole derivative 6, which contains a hydroxyl group that can trap the free radical by donating a proton to the DPPH radical and converting it to a stable molecule. The significant variance in IC_{50} values between target compound 6 and vitamin C can be rationalized by steric and structural factors. Compared to the ascorbic acid which is flexible and small molecule, compound 6 characterized by rigid and bulky framework due to the presence of multiple conjugated heterocyclic rings which act as steric hindrance restricts its accessibility to capture free radicals. A representation diagram of antioxidant activity and radical scavenging activity (mean and SD) was illustrated in Table 2 and Figure 1.

Table 2. Antioxidant activity of novel Thiazolo-Triazole derivative

Concentration	*DPPH Radical Scavenging Activity %*			
	Compound 6		*Ascorbic acid*	
	Mean	***SD***	***Mean***	***SD***
200	56.67	3.834	80.36	2.869
100	44.98	2.275	69.48	3.708
50	29.55	0.942	54.48	2.412
25	31.10	5.840	40.43	7.081
12.5	23.42	2.257	17.63	7.196
IC_{50}	190.4		21.04	

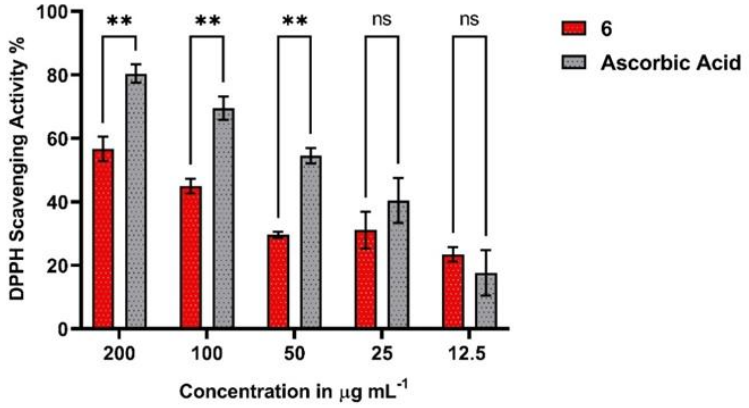

Figure 1. DPPH radical scavenging activity of thiazolo-triazole derivative 6

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Molecular docking study

Many heterocycle molecules have been extensively studied; among them, 4-Thiazolidinone derivatives were reported to possess anticancer efficacy (Roszczenko et al., 2022b). Thus, in the current study, docking studies were performed to estimate the binding affinity of Thiazolo-Triazole compound (6) to the selected receptor (PDB code:6COX), which is well known for playing a role in the spread of cancer. Regarding the choice of 6COX as a target from its established over-expression in the MCF-7 breast cancer cell line and its pivotal role in tumor angiogenesis and survival (Maanasi Vinay Nair *et al.*, 2021). *Moreover compound 6 incorporates specific heterocyclic moieties that are structurally a well-known anti-inflammatory and antiprolerative agents targeting the COX 2 enzyme cavity.*

Using the Chem-Draw Professional 16 program, the ligand structure (5-Arylidine-thiazolidinone) was created in mol format. Discovery Studio (DS) Visualizer was then used to convert the mol format to a 2D structure. To detect torsions during docking, the ligand was saved in PDBQ format.

The COX-2 crystal structure (PDB ID: 6COX) was acquired from the Protein Data Bank. The binding sites were cleared of all residues and non-standard ligands. All water molecules were removed from the protein structures, and the protein structure was enhanced with partial atomic charges. Protein data are stored in (Pdb) format; The atomic solubility coefficients were calculated and then transformed into (Pdbqs) format. Docking interaction of 4-Arylidine-thiazolotriazolone with human 6COX protein produced the lowest binding energy, which is equal to 10.94 kcal/mol. Molecular docking results of the ligand have presented a preferred interaction by the formation of hydrogen bonds with (ASN43A, ARG44A, GLU46A, CYS47A, TYR130A, GLY135A, LYS137A, LYS137A, LYS468A) and hydrophobic interactions with (ARG44A(3.50), GLU46A(3.31), LEU152A(3.41)). For comparison purposes, a literature-based comparative molecular docking study was instituted against Cyclooxygenase-2 (PDB ID: 6COX). Compared to the standard selective COX-2 inhibitor, Celecoxib, which displays a binding energy of -10.49 kcal/mol under similar grid parameters as reported recently (Fayez et al., 2026), compound 6, exhibited a highly competitive and promising binding affinity of -10.9. This striking structural and energetic alignment strongly underscores the potential of compound 6 to efficiently accommodate and inhibit the COX-2 enzymatic cavity, aligning with its promising in vitro anti-cancer profiling.

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Although the static molecular docking profiles provided competitive binding energies and favourable orientations for the synthesized derivatives, further molecular dynamics (MD) simulations are needed in future studies to rigorously validate the thermodynamic stability and structural fluctuations of the compound 6 / 6COX complex within a simulated biological environment. These interactions may explain the high binding energy of inhibitor 6 and its anticancer activity. More details are illustrated in Table 3, 4 and Figure 2.

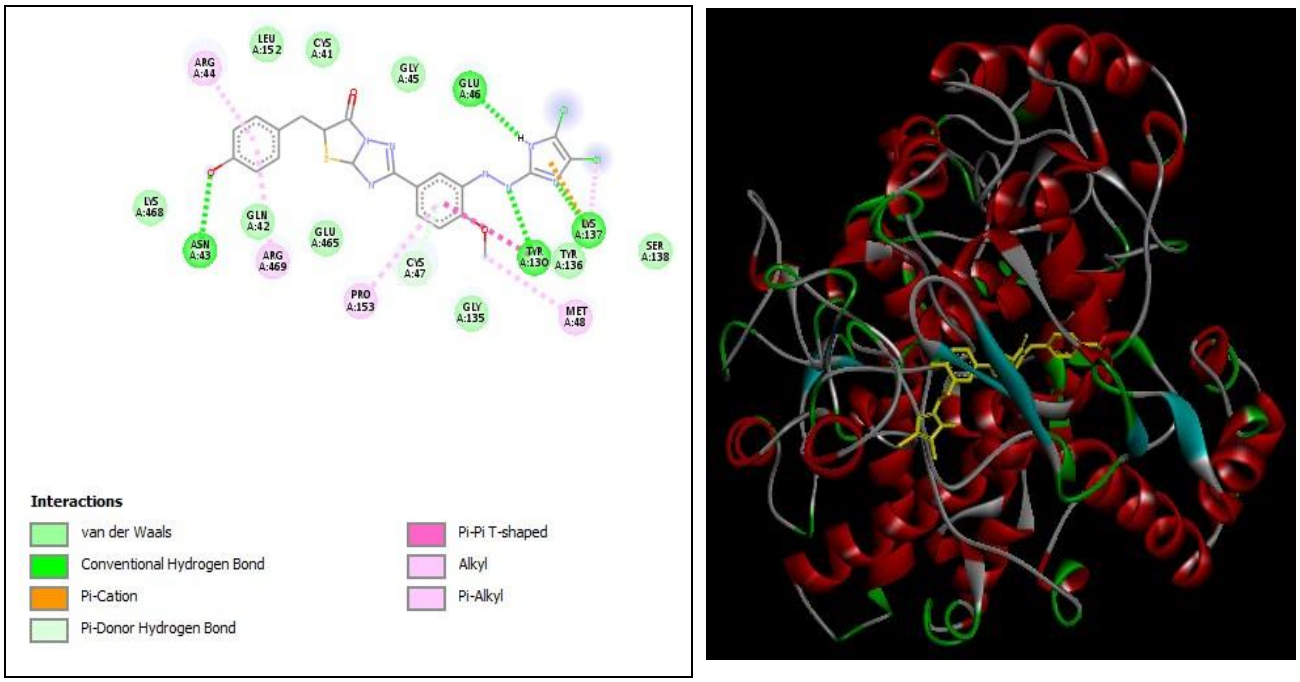

Figure 2. 2D and 3D for interaction of Thiazolo-Triazole (6) inside the active site (PDB ID: 6COX)

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Table 3. shows the H-bonds of Heterocycles compound 6 with the protein responsible for breast cancer, 6COX

Compound	Lowest bending energy	Run	Distance H-A	H-Bonds NO.	Hydrogen- bending Interaction
6	-10.94	37	(2.01-3.34)	٩	O: ASN43A , O: ARG44A , O: GLU46A , N: CYS47A , N: TYR130A , O: GLY135A, N: LYS137A, N: LYS137A, O: LYS468A

Table 4. Displays the hydrophobic interactions, halogen bonds, π bond grouping, and π-cation interactions of compound 6 with protein 6COX

Compound No.	Lowest bending energy	π-Cation Interactions	π-Stacking	Halogen Bonds	Hydrophobic Interactions
6	-10.94	LYS137A	TYR130A	-	ARG44A(3.50), GLU46A(3.31), LEU152A(3.41)

Anticancer Activity

MTT assay in vitro was used to examine the cytotoxic activity of the target compound 6 against MCF-7 (breast cell line) and normal cells, Human Dermal Fibroblasts-Neonatal (HdFn). This method includes incubating at 37 ◦C for one day. The sample was prepared in five dose concentrations (12.5, 25, 50, 100, 200 µg/ml). The synthesized compound under investigation prevented the proliferation of the MCF-7 and HdFn cells at the higher dose (200 µg/ml), with a cytotoxic potential of 63 per cent of cancer cells. While the normal cell line (HdFn), at the same concentration, the cytotoxic effect was 37.96 per cent. With respect to human cells, the sample showed selective cytotoxicity against cancer cell lines with an IC_{50} value of 35.2 g/ml and was less affected against normal cell lines with an IC_{50} value of 49.67 g/ml. This pronounced biological potency strongly corroborates our molecular docking insights, where compound 6 achieved a high binding affinity of -10.94 kcal/mol within the 6COX pocket.

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The powerful anticancer efficacy of compound 6 against the human breast cancer cell line MCF-7 with IC_{50}=35.2 µg/ml can be structurally and biochemically rationalized through its targeted interactions within the active site of the COX-2 enzyme (PDB ID: 6COX). Overexpression of COX-2 is clinically established to drive breast cancer progression, angiogenesis, and apoptosis evasion in MCF-7 cells. Mechanistically, the molecular docking data clearly explain this biological potency. Compound 6 possesses a highly optimized architectural framework consisting of a core thiazole ring and adjacent heteroatoms, which distribute electron density favourably to engage the enzyme's binding pocket. The exceptional binding energy of -10.94 kcal/mol is driven by a network of non-covalent forces. Specifically, the formation of nine hydrogen bonds (stabilized by a close-range distance profile of 2.01-3.34 Å) with essential amino acids such as ARG44A, GLU46A, and TYR130A severely anchors the ligand. Furthermore, the stabilization is supplemented by robust pi-cation interactions with LYS137A and pi-stacking with TYR130A, alongside distinct hydrophobic interactions.

Consequently, this comprehensive electronic and geometric fit locks the COX-2 catalytic channel, effectively shutting down downstream oncogenic signalling cascades. This enzymatic blockade triggers cellular stress and selective cytotoxicity in the malignant MCF-7 phenotype while preserving a safer threshold for normal HdFn fibroblasts (IC_{50} = 49.67, µg. mL^{-1}).

This promising inhibition activity encourages further efforts for deep investigations into their inhibition mechanism. Table 5 shows the cytotoxic effect of 6 on HdFn and MCF-7 cell lines, as well as the values of IC_{50}. The figure 3 shows the viability rates of MCF-7 cells and HdFn cells with compound 6.

Table 5. The cytotoxic effect of 6 on HdFn and MCF-7 cell lines.

Concentration µg mL^{-1}	***Cell Viability***	
	Normal Cell line	***Cancer cell line***
	HdFn	***MCF-7***
	Mean(%) ± SD	***Mean(%) ± SD***
200	53.8±4.76	37.963±3.9
100	61.3±2.6	51.65±2.98
50	70.98±3.3	61.612±4.87
25	86.07±2.7	74.344±4.1
12.5	95.79±2.68	85.571±1.9
IC_{50}	49.67	35.2

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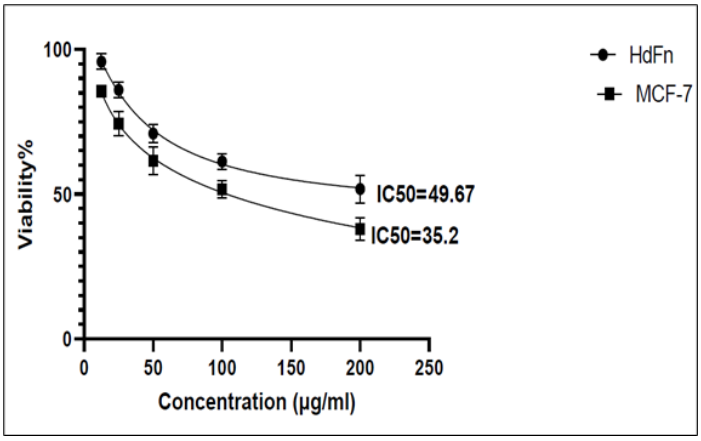

Figure 3. IC_{50} of compound 6 in the breast cancer cell line (MCF-7) and the normal cell line (HdFn)

Conclusions

A novel 5-Arylidine-thiazolidinone derivative was designed using a general synthesis protocol, bearing an imidazole moiety. The structure and properties of thiazolidinone 6 were confirmed by various spectral techniques such as ^{1}HNMR, ^{13}CNMR, and FT-IR. Evaluation assays were performed for the target compound 6 as inhibitory activity against Staphylococcus aureus and Escherichia coli, free radical scavenging ability, and anticancer activity. The antibacterial activity showed that thiazolidinone derivative 6 exhibited excellent activity compared to Amoxicillin as a standard drug, while the ability to scavenge free radicals was moderate relative to Ascorbic acid. Results of cytotoxicity using MTT assay toward breast cancer cell line MCF-7 and normal human cells (HdFn) showed that derivative 6 can inhibit the growth of cancer cells. The cytotoxicity results are compatible with the molecular docking results of the tested compound, which presented a strong interaction with the 6COX protein

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