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Synthesis, Characterization, Cytotoxic Evaluation on MCF-7 Breast Cancer Cells, and Theoretical Studies of Novel 1,2,3-Triazoles

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ABSTRACT

The present study aims to synthesize, characterize, and evaluate the cytotoxic effects of novel 1,2,3-triazole derivatives (1-5) on MCF-7 breast cancer cells. Their theoretical studies were performed using DFT and docking computations. New five 1,2,3-triazole derivatives (**1-5**) were synthesized using different azide and maleimide moieties. The structural authenticity of the target compounds was elucidated using various analytical techniques, including ^{1}H and ^{13}C-NMR, FTIR, and mass spectra. By applying an MTT method, the synthesized triazoles was assessed to determine their cytotoxicity toward MCF-7 breast cancer cell line. DFT and molecular docking computations were applied to evaluate physicochemical properties and predict the potential interactions of the active compounds with relevant biological targets, respectively. Among the synthesized triazoles, compound **5** exhibited high activity towards the MCF-7 cell line with IC_{50} value of 5.03 µM compared to doxorubicin (a standard drug), whose IC_{50} value was 3.16 µM. Other derivatives **1-4** showed moderate activity with IC_{50} values of 82.45, 316.81, 328.48, and 348.34 µM, respectively. The molecular docking results showed that compound **5** has efficient interactions with breast cancer proteins (5KCV, 3ERT, and 4FX3), with low values of binding energy and RMSD. The experimental and theoretical findings suggest that the synthesized 1,2,3-triazole derivatives hold potential as effective agents in breast cancer therapy, and they are highly recommended to be a promising anti-breast cancer agent.

Keywords: 1,2,3-triazole, Breast cancer, Cytotoxicity, Cycloaddition, Molecular docking.

Introduction

Cancer refers to serious illnesses that typically result from normal cells turning into tumours.[1] When exposed to specific carcinogens (chemical, biological, or physical substances), the body's natural defence mechanisms are disrupted, leading to metamorphosis.[2] This occurrence is due to genetic factors.[3] Cancer is the second most common cause of mortality worldwide, behind only cardiovascular disease.[4] It is a multifactorial disease marked by unchecked cell development in the body.[5] Many anticancer medications are utilized in clinical settings, but cannot produce the desired therapeutic impact due to inadequate absorption, toxicity, or multidrug resistance.[6] Cancer is associated with several side effects, in addition to causing various difficulties.[7] Therefore, new anticancer medications that are highly effective against cancer cells and less harmful to healthy cells must be developed for the treatment of cancer problems.[8] Therefore, developing safer and more effective anticancer medications is clinically required.[9] In the drug discovery field, triazole derivatives are the most frequently suggested molecules.[10] Triazoles have been the subject of several investigations that have unraveled their unique anti-oxidant,[11] antifungal,[12] anti-inflammatory,[13] anti-tuberculosis,[14] anticancer,[15] and herbicidal activities.[16]

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Reports on the preclinical cancer tests of triazole derivatives suggest that these derivatives may impact the onset of breast cancer.[17]
The present study aimed to disclose new 1,2,3-triazole derivatives, their cytotoxic evaluation on the MCF-7 breast cancer cell line, and analyze their computational structures using density functional theory (DFT) and molecular docking.

Materials and Methods

Sources of materials
Sigma-Aldrich Company (USA) supplied the chemicals and solvents used in synthesizing compounds in this work. Trypsin and EDTA were purchased from Capricorn, USA. The culture media that included fetal bovine serum (FBS; 10%) complemented with RPMI-1640 (100 mg/mL) were obtained from Gibco (USA). In *an in vitro* cytotoxic test, an MTT stain was ordered from Sigma. The cell culture plates were received from Thermo Fisher Scientific (USA). Thin layer chromatography (TLC) plates with a silica gel 60 UV 254 (Merck) were utilized to monitor the reaction progress. UV light at 254 nm was utilized to detect and visualize the dots of all synthesized compounds. The purity of the target compounds was checked and acquired with a high percentage > 95%. In capillary tubes, melting points were measured using a melting point device (Gallenkamp). A Bruker inovo AV-400 spectrometer (Iraq) was employed to run ^{1}H and ^{13}C NMR spectra at room temperature using $CDCl_3$ and DMSO-d_6 as solvents. Chemical shifts (δ scale) were recorded in ppm, and the coupling constant (*J*) values were identified in Hz. The abbreviated terms (s for singlet), (d for doublet), (t for triplet), and (m for multiplet) are introduced to represent the splitting patterns. The IR device spectrophotometer (Shimadzu, Iraq) is utilized to acquire Fourier

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transform infrared (FTIR) spectra using KBr (1%) discs with a range of 4000-600 cm^{-1}. A micro-mass LCT-device-electrospray mode (ES-MS, Iran) is employed to gain the accurate mass of the synthesized derivatives.

Cell lines and culture

The MDA-MB-231 as a human cell line (breast cancer type) was acquired from the National Cell Bank (Pasteur Institute, Iran). The cultured cells were maintained in a medium containing RPMI-1640 and 10% FBS and conducted in an antibiotic solution of 100 µg/mL for each penicillin and streptomycin. The cells were conserved and incubated under a humidified environment containing 5% CO_2 at 37 ℃. They were treated with a solution of trypsin/EDTA and phosphate-buffered saline (PBS) before further experiments.

Synthesis of 4-azidobenzoic acid

A solution of concentrated hydrochloric acid (2.25 mL) and water (4 mL) was added to 4-aminobenzoic acid (1.37 g, 0.01 mole). A dropwise addition of sodium nitrite solution (0.76 g, 0.011 mole) in water (2.5 mL) was made to the above mixture at 0 ºC. After the sodium nitrite solution was completed, it was followed by the dropwise addition of sodium azide (0.78 g, 0.012 mole) solution in water (2.5 mL) under continuous stirring for 20 min. The resulting solid was separated using absolute ethanol and re-crystallized.[18]

Synthesis of N-(3-nitorphenyl) maleimide

To a round bottom reaction flask containing DMF (30 mL), an equal mole (0.1 mol) of maleic anhydride (9.8 g) and nitroaniline (13.8 g) was added. After three hours of stirring at 25 ℃, the mixture was agitated. Crushed ice was used to cool the above mixture. After the yellow solid was precipitated, it was filtered and dried under a vacuum. The precipitated product was subjected to a purification step (recrystallization) from methanol to yield manamic acid (nitrilephryl; 70% at 145 ℃. The second step involved the production of *N*-(4-nitrophenyl) maleimide. In this case, 3-nitromaleimic acid was cyclodehydrated. This solution was treated with concentrated H_2SO_4, P_2O_5, and agitated for three hours at 65 ℃. After that, it was added to cold water or crushed ice until a yellow solid was precipitated. The final product was filtered, washed with water, and dried. By using ethyl alcohol, *N*-(4-nitrophenyl) maleimide was recrystallized successfully.[19]

Synthesis of N-(4-methylphenyl) maleimide

To synthesize *N*-(4-methylphenyl) maleimide, 1.96 g of maleic anhydride (20 mmol) or succinic anhydride in diethyl ether (25 mL) was added to a three-necked flask (100 mL) equipped with a dropping funnel and reflux condenser. A solution of aniline (20 mmol) in diethyl ether (5 mL) was added dropwise through a dropping funnel to the stirred solution of maleic anhydride. After stirring for one hour at 25 ºC, the thick suspension was formed, and it was chilled in an ice bath. The produced mixture was filtered and dried. The acidic product was then placed in a round flask containing acetic anhydride (6.7 mL) and anhydrous sodium acetate (0.65 g, 8 mmol), and the mixture was swirled over a steam bath for 30 minutes. The mixture was allowed to cool at room temperature and treated with 100 mL of an ice-water mixture. After the mixture was subjected to three rounds of ice-water washing, the precipitated product was collected, dried, and filtered. A mixture of ethanol and water was deemed a suitable eluent for purifying the crude N-substituted maleimide.[20]

General method for the synthesis of 1,2,3-triazole

To synthesize 1,2,3-triazole, 4-azidobenzene 3 (1.0 mmol) was gently added to an appropriate solution (1.0 mmol) of acetylacetone, acetylene dicarboxylic acid, dibenzoyl methane, *N*-(3-nitorphenyl) maleimide, and *N*-(4-methylphenyl) maleimide using a suitable solvent (10 mL). The above mixture was subjected to reflux for an appropriate time as indicated in Table 1. Thin layer chromatography layers were employed to monitor the resulting mixture till the initial substances were exhausted. The mixture was further cooled to induce the formation of crude products. The crude products were collected, washed with cool ethanol, and purified under a re-crystallization step from a mixture of ethyl acetate: hexane (1:1). After the drying process, the obtained uncontaminated hybrids **1-5** were collected.[21] The synthesized hybrids **1-5** were additionally purified to ensure the purity level was >95%. The structural analysis of the target products was verified by their ^{1}H-nuclear magnetic resonance (NMR), Fourier-transform infrared spectroscopy (FTIR), and mass spectrum data.

Table 1: The physical properties of synthesized compounds

Compound	Molecular formula	M.wt	Color	Reaction (h)	m.p (ºC)	Yield (%)
1	$C_{22}H_{17}N_3O_3$	369.11	light-yellow	2	175–176	53
2	$C_{12}H_{13}N_3O_3$	245.10	white	8	133–135	62
3	$C_{11}H_9N_3O_6$	277.19	white	2	187-190	59
4	$C_{17}H_{11}N_5O_6$	381.3	yellow	18	194-196	68
5	$C_{18}H_{14}N_4O_4$	350.33	light-yellow	15	180-183	77

4-(4-Benzoyl-5-phenyl-1H-1,2,3-triazole-1-yl) benzoic acid **(1)**

The compound was isolated as a light-yellow powder, yield: 53%; mp 175–176 ˚C; IR (KBr) cm^{-1}, ν: 3070 (CH $_{aromatic}$), 1681 (C=O), 1425 (N=N $_{triazole}$). ^{1}H NMR ($CDCl_3$) δ/ppm: 7.89-8.14 (m, 14H $_{aromatic}$), 11.74 (s, 1H, CO_2H). ^{13}C NMR ($CDCl_3$) δ/ppm: 76.7, 77.3, 118.9, 125.7, 128.5, 129.3. 129.5, 130.0, 130.2, 132.1, 133.8, 145.7, 161.6, 172.4. MS (ESI) m/z= 368.5$[M+H]^+$.

4-(4-Acetyl-5-methyl-1H-1,2,3-triazole-1-yl) benzoic acid **(2)**

The compound was isolated as a white powder, yield: 62%; mp 133–135 ˚C; IR (KBr) cm^{-1}, ν: 3066 (CH $_{aromatic}$), 2819, 2976 (CH_3), 1681 (C=O), 1506 (C=C $_{aromatic}$), 1427 (N=N $_{triazole}$). ^{1}H NMR (DMSO-d_6) δ/ppm: 2.50 (s, 3H, H1), 2.64 (s, 3H, H2), 7.55 (d, 2H, *J* = 8.1), 8.11 (d, 2H, *J* = 8.2), ^{13}C NMR (DMSO-d_6) δ/ppm: 10.24 (C-1), 28.12 (C-2), 124.85 (C-3, 3', 4,4'),130.69, 138.13, 143.34, 165.14 (C=O), 193.48 (CO_2H). MS (ESI) m/z= 245.2 $[M]^+$.

1-(4-Carboxyphenyl)-1H-1,2,3-triazole-4,5-dicarboxylic acid **(3)**

The compound was isolated as a white powder, yield: 59%; mp 187-190 ˚C; IR (KBr) cm^{-1}, ν: 3068 (CH $_{aromatic}$), 1687 (C=O), 1506 (C=C $_{aromatic}$), 1425 (N=N $_{triazole}$). ^{1}H NMR (DMSO-d6) δ/ppm: 7.225 (d, 2H, *J* = 4.2 Hz, H-1, 1'), 7.955 (d, 2H, *J* = 9.8 Hz, H-2,2'), 9.5 (s, 2H, H CO_2H); ^{13}C NMR (DMSO-d_6) δ/ppm: 120.7 (C-1), 123.8 (C-2), 127.5 (C-3), 131.1 (C-4), 135.2 (C-5), 139.5 (C-6), 144.4 (C-7), 162.0, 166.8, 166.9. MS (ESI) m/z= 377.2 $[M]^+$.

4-(5-(3-Nitrophenyl)-4,6-dioxo-4,5,6,6a-tetrahydropyrrolo[3,4-d][1,2,3]triazole-1(3aH)-yl)benzoic acid **(4)**

The compound was isolated as a yellow powder, yield: 68%; mp 194-196◦C; IR (KBr) cm^{-1}, ν: 3068 (CH $_{aromatic}$), 1724 (C=O), 1535 (C=C $_{aromatic}$), 1427 (N=N $_{triazole}$). 1H NMR (DMSO-d6) δ/ppm: 5.405 (d, 1H, J = 12 Hz, H-1), 6.065 (d, 1H, J = 12 Hz, H-2), 7.83–8.34 (d, and m, 8H, $H_{Aromatic}$),12.94 (CO_2H); 13C NMR (DMSO-d6) δ/ppm: 60.9 (C-6′), 57.8 (C-1), 84.29 (C-2), 115.29, 119.61, 125.38, 127.74, 131.70. 133.07, 134.31, 135.40, 143.34, 144.43, 148.28, 167.29, 169.51, 171.03; MS (ESI) m/z= 380.4 = $[M - 1]^+$.

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4-(4,6-Dioxo-5-(p-tolyl)-4,5,6,6a-tetrahydropyrrolo[3,4-d][1,2,3]triazole-1(3aH)-yl)benzoic acid **(5)**
The compound is a light-yellow powder, Yield: 77%; mp 180-183°C; IR (KBr) cm−1, ν: 3101.54 ($CH_{aromatic}$), 1724.36 (C=O), 1535 ($C=C_{aromatic}$), 1483.26 ($N=N_{triazole}$); ^{1}H NMR (DMSO-d6) δ/ppm: 2.34 (s, 3H, 1H), 5.345 (d, 1H, H2), 6.005 (d, 2H, *J* = 8.2 Hz, H6), 12.92 (s, 1H, CO_2H). ^{13}C NMR (DMSO-d_6) δ/ppm: 21.1 (C-1), 57.1 (C-2), 84.3 (C-3), 115.3, 125.9, 127.2, 127.7, 129.3, 129.9, 139.0, 143.3, 167.0, 167.7, 169.8, 171.5. MS (ESI) m/z= 350.3 $[M]^+$.

Synthetic route of 1,2,3-triazole
4-(5-(3-Nitrophenyl)-4,6-dioxo-4,5,6,6a-tetrahydropyrrolo [3,4-d][1,2,3] triazol-1(3aH)-yl) benzoic acid (**4**), and 4-(4,6-dioxo-5-(p-tolyl)-4,5,6,6a-tetrahydropyrrolo[3,4-d][1,2,3] triazol-1(3aH)-yl) benzoic acid (**5**) were synthesized based on three steps. In the first step, 4-azido benzoic acid was prepared by treating 4-amino benzoic acid with concentrated HCl and $NaNO_2$ at 0–5 ° C. The resulting diazonium salt solution was utilized immediately for the following step without any purification. 4-Azido benzoic acid was produced when an aqueous NaN_3 solution was added to a diazonium salt solution. The second step involved the synthesis of maleimide derivatives from the reaction of maleic anhydride with 4-amino toluidine and 3-nitroaniline to produce maleimic acid derivatives. Then, 3-nitromaleamic acid was cyclized using P_2O_5 and H_2SO_4 to form *N*-(3-nitorphenyl) maleimide, while 4-methyl maleamic acid was cyclized by adding sodium acetate and acetic anhydride to form *N*-(4-methylphenyl) maleimide. In the final step, 4-azido benzoic acid **3** was reacted with *N*-(3-nitrophenyl) maleimide and *N*-(4-methyl phenyl) maleimide by stirring the mixture in chloroform to produce compounds **4** and **5**, respectively. Meanwhile, compounds **1** and **2** were synthesized by reacting 4-azidobenzoic acid with di-benzoyl methan and acetylacetone in ethanol with potassium carbonate at 70 °C for some time. The resulting mixtures were then treated with hydrochloric acid. Subsequently, the mixture was separated using chloroform and analyzed by TLC, employing a hexane: ethyl acetate (2:3) solvent system as the eluent for the crude reaction product. Product **3** was prepared by reacting 4-azidobenzoic acid with acetylene dicarboxylic acid in acetone. The synthetic pathway is depicted in Figure 1.

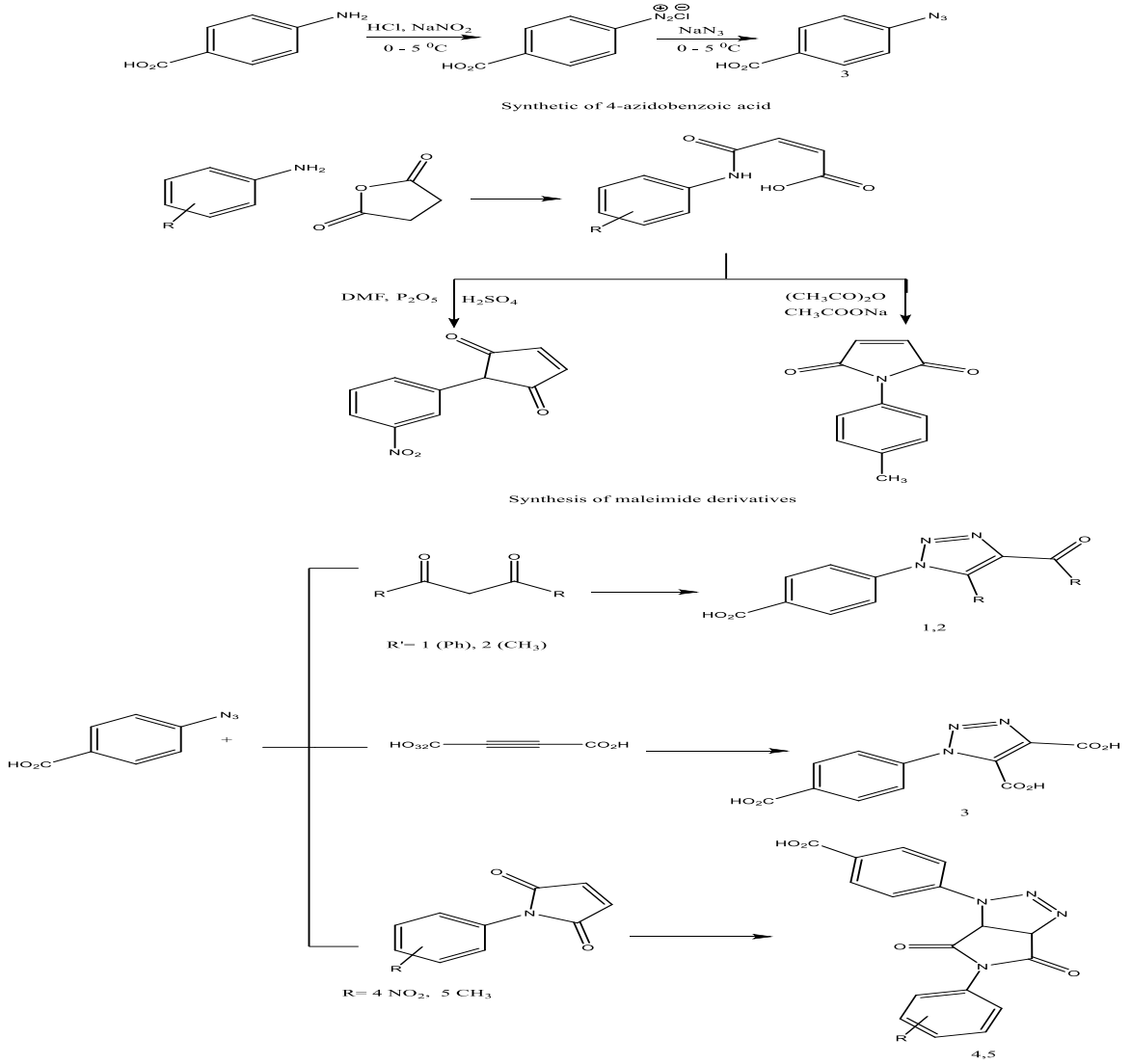

Figure 1: Synthetic pathway of 1,2,3-triazole compounds.

MTT assay of cell viability
The MTT test in *an in vitro* study was employed to verify cell proliferation and cell viability.[22] Concisely, the cultured cells were treated with trypsin and further adjusted to 1.4×10^4 cells per well. They were then placed in 96-well plates containing 200 µL of a culture medium in each well and incubated for 24 hours. After the cells were seeded as a monolayer in each well, they were treated with DMSO at concentrations ranging from 12.5 to 200 µg/. This exposure was carried out at 37 °C for 24 hours under a humid environment with 5% CO_2. After the treatment period (24 hours), the monolayer culture remained undisturbed in the original plate. The supernatant was removed and MTT solution (0.5 mg/ml in PBS) was added to each 200 µl/well. An

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additional incubation (4 hours) was conducted on the treated plates at 37 °C. The MTT solution was removed from the supernatant of the cells and they were treated with 100 µL of DMSO. The treated cells were placed in a shaker and incubated at 37°C until the formed crystals were fully dissolved. Cell viability was assessed by measuring absorbance at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Model Wave xs2, BioTek, USA). The IC_{50} values were determined based on the corresponding dose-response curves.

Computational experiments

DFT-assisted computations was employed for studying the geometries and energies of the synthesized compounds (**1-5**) at the BL3YP level of theory,[23] with a standard DND basis set via the DMol3-Material Studio (version 2017) program.[24] A molecular docking study of compound **5** was conducted using MOE 2019. A Protein Data Bank (PDB) was considered the main source for supplying the crystal structure of the selected proteins.

Results and Discussion

The successful synthesis of novel 1,2,3-triazole derivatives 1–5 was demonstrated by their analysis using ^{1}H NMR. Compound **1** exhibited a value of integration in the aromatic region between 7.89 and 8.141 ppm, indicating the presence of 14 protons. Compound **2** displayed two separate signals at 2.509 and 2.645 ppm, which are attributed to CH_3 groups. Compound **3** was characterized by the appearance of signals at 13 ppm, indicating the presence of two carboxylic groups fused with 1,2,3-triazole. Compounds **4** and **5** exhibited distinct doublet signals between δ 5.24 and 6.01 ppm, corresponding to the fused 1,2,3-triazole-maleimide protons (CH groups) in the suggested region.

In FTIR spectra, the appearance of absorption bands in the predicted region verified the production of compounds **1**–**5**. The FTIR spectra also indicated the absence of an azido group at 2121 cm^{-1} and the presence of intense absorption bands at 1377–1386 cm^{-1}, which are assigned to the azo group (N=N) of the 1,2,3-triazole moiety. Furthermore, a novel carbonyl group is present in the compounds, observed at 1700. Moreover, the mass spectra data of all 1,2,3-triazole derivatives **1**–**5** are in line with the anticipated structures.

Cytotoxicity of the 1,2,3-triazole derivatives

The efficacy of the target derivatives in inhibiting the proliferation of MDA-MB231 human breast cancer cells was confirmed through cell viability screening.[25] After 24 hours of incubation with 200 µM for each derivative, compounds **1-5** exhibited average performances against cancer cell lines (Table 2). Evaluation of the cytotoxicity of the target compounds was achieved using an MTT assay. For calculating the IC_{50} values and analyzing the dose response, GraphPad Prism 8.1 software was ultimately employed. The results are displayed in Figure 2. The noted discrepancies in the IC_{50} values are interpreted as the nature of the substitutions and their positions.[26] Compound **1** retained two phenyl groups in the triazole cycle, compound **2** retained two methyl groups, and compound **3** retained two carboxylic groups. Meanwhile, compounds **4** and **5** were produced by cyclized 4-azidobenzoic acid with *N*-(3-nitrophenyl) maleimide and *N*-(4-methyl phenyl) maleimide, respectively. The IC_{50} values of 1,2,3-triazole derivatives **1-5** were 328.48, 348.34, 316.81, 82.45, and 5.03 µM, respectively, as represented in the order 2<1< 3<4<5. Compound **5** displayed a higher IC_{50} value than compounds **1-4** (Figure 2). The structural groups were named as *N*-(4-methylphenyl) maleimide (compound **5**) and the *N*-(3-nitrophenyl) maleimide group (compound **4**). The increase in IC_{50} values for cytotoxicity was attributed to the presence of functional groups in the target compounds. The lower IC_{50} value of compound **5** is highly expected due to its tendency to trigger significant activities and its lipophilicity in membrane partitioning inclinations. These functional groups are highly expected to be a responsible factor for enhancing cytotoxicity.[27]

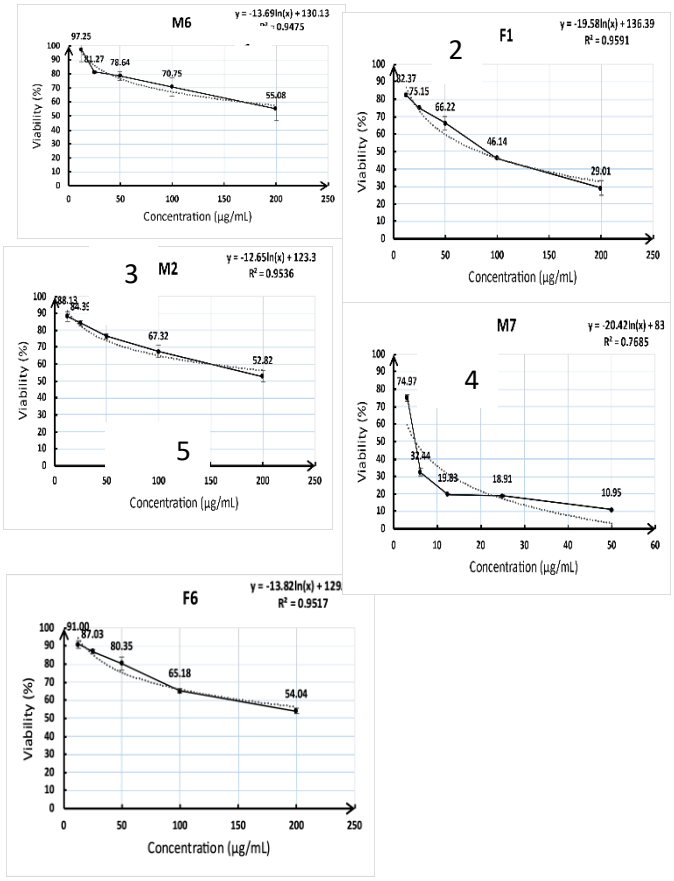

Figure 2: IC_{50} values of 1, 2, 3, 4, and 5 compounds against MDA-MB231 breast cancer cells after 24

Computational analysis of the 1,2,3-triazole derivatives

The geometry optimization of compounds **1-5** was carried out using the density function theory.[28] Figure 3 shows the optimized structure of the studied molecules, and Table 3 presents the binding energy, HOMO-LUMO energy gap, heat of formation, and computed dipole moment. The chemical activity of the molecule is shown by the value of dipole moment and energy gap ($\Delta E_{LUMO-HOMO}$).[29] Based on the values listed in Table 3, higher reactivity was associated with molecule **5** ($\Delta E_{LUMO-HOMO}$ = 2.201 eV, and µ = 4.682 Debye). This observation is in line with the results of the biological study. As for the values of the heat of the formation and the binding energy, it was found that compound **1** is the most stable, and the stability is because it contains three phenyl rings.

Molecular docking analysis

Molecular docking studies are one of the most important methods used to determine the susceptibility of compounds to interact with different receptors, such as the protein that causes a particular disease theoretically using molecular docking programs.[30] The biological activities of the compounds are indicated by their binding to the disease protein, indicating their potential as an inhibitor or antidote to the disease. Molecular docking studies allow researchers to visualize compound interactions, predict the binding between active groups of the ligand and protein, and calculate binding energies and the number of hydrogen bonds formed. These factors provide strong indications of the potential use of the ligand as a treatment for diseases associated with the target protein.

The study of the molecular docking of compound **5** was tested against breast cancer proteins, which included ERα (PDP: 3ERT), EGFR (PDP: 2J6M), PR (PDP: 4OAR), CDK2 (PDP: 4FX3), mTOR (PDP: 4DRH), CDK6 (PDP: 3NUP), and Akt (PDP: 5KCV).[31] Strongest interactions were found between 5KCV, 3ERT, and 4FX3 proteins, where compound **5** exhibited the highest affinity energy (S) and the lowest values of RMSD.[32] Table 4 shows the data of molecular docking

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analysis of compound **5** with the target proteins and Figure 4 highlights the 2D and 3D representations of the noted interactions. From Figure 4, two binding interactions were elucidated between compound **5** and 5KCV protein. The first was donor hydrogen bonding between the oxygen atom and Val-271, and the second was π-π interaction with Trp-80. Meanwhile, compound **5** interacted with protein 3ERT through a donor H-bonding with amino acid Met-343. In addition, two π-π interactions are observed between a hydrophobic phenyl moiety and the amino acids Thr-347 and Leu-39, respectively. On the other hand, it was noted that compound **5** docked with the protein 4FX3 by forming acceptor H-bonds with the amino acid Lys-33 through an oxygen atom and π-interaction with the amino acid Phe-80.[33]

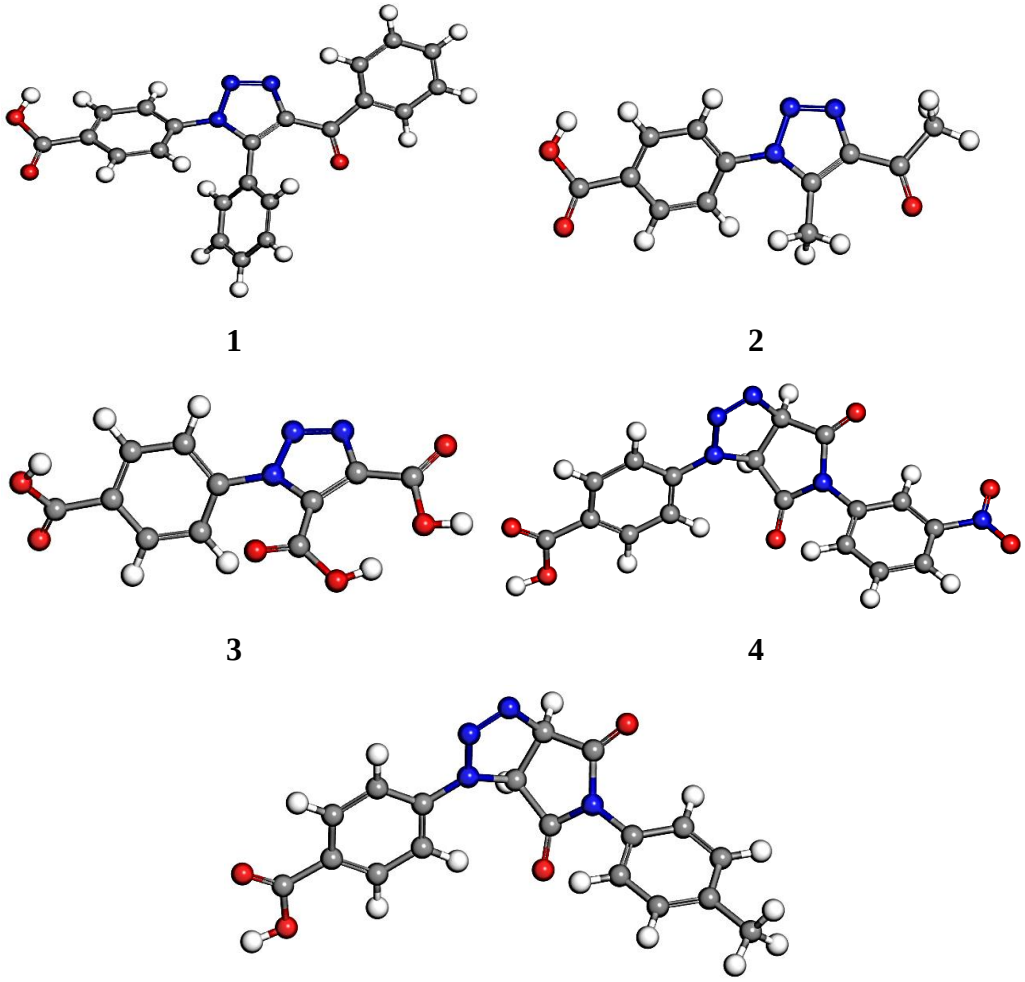

Figure 3: Optimization of geometrical structures of 1,2,3-triazole compounds (**1-5**).

Table 2: Cell viability after 24 hours of treatment with 200 µM for each 1,2,3-triazole derivatives

Sample	Viability% (1)	Viability % (2)	Viability % (3)	Viability % (4)	Viability % (5)	Average %
Control	570	570	570	570	570	570
1	88.13	84.39	76.38	67.32	52.82	73.808
2	97.25	81.27	78.64	70.75	55.08	76.598
3	91.00	87.03	80.35	65.18	54.04	75.52
4	82.37	75.15	66.22	46.14	29.01	59.778
5	74.97	32.44	19.83	18.91	10.95	31.42

Table 3: Calculated energies of molecules.

Molecules	HOMO energy (eV)	HUMO energy (eV)	$\Delta E_{LUMO-HOMO}$ (eV)	µ (Debye)	ΔH_f (Kcal/mol)	Binding energy (eV)
1	-5.459	-2.784	2.675	1.883	-214.74	-226.836
2	-5.620	-2.820	2.800	1.683	-74.90	-144.084
3	-6.446	-3.487	2.959	4.139	-93.77	-140.753
4	-5.484	-2.583	2.901	3.638	-144.34	-201.636
5	-5.754	-3.553	2.201	4.682	-187.59	-205.051

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Table 4: Molecular docking data of the compound **5** with target 5KCV, 3ERT, and 4FX3 proteins

Protein (Receptor)	RMSD*	Affinity Energy (S) Kcal/mol	Interaction		
			Type	Amino acid	Distance (Å)
5KCV	1.0977	-7.4072	H-donor	Val271	3.15
			π-π	Trp80	3.83
3ERT	1.8307	-7.2627	H-donor	Met343	3.70
			π-H	Thr347	4.49
			π-H	Leu391	3.69
4FX3	1.4506	-7.0145	H-acceptor	Lys33	3.04
			H-π	Phe80	3.88

*: Root means square deviation

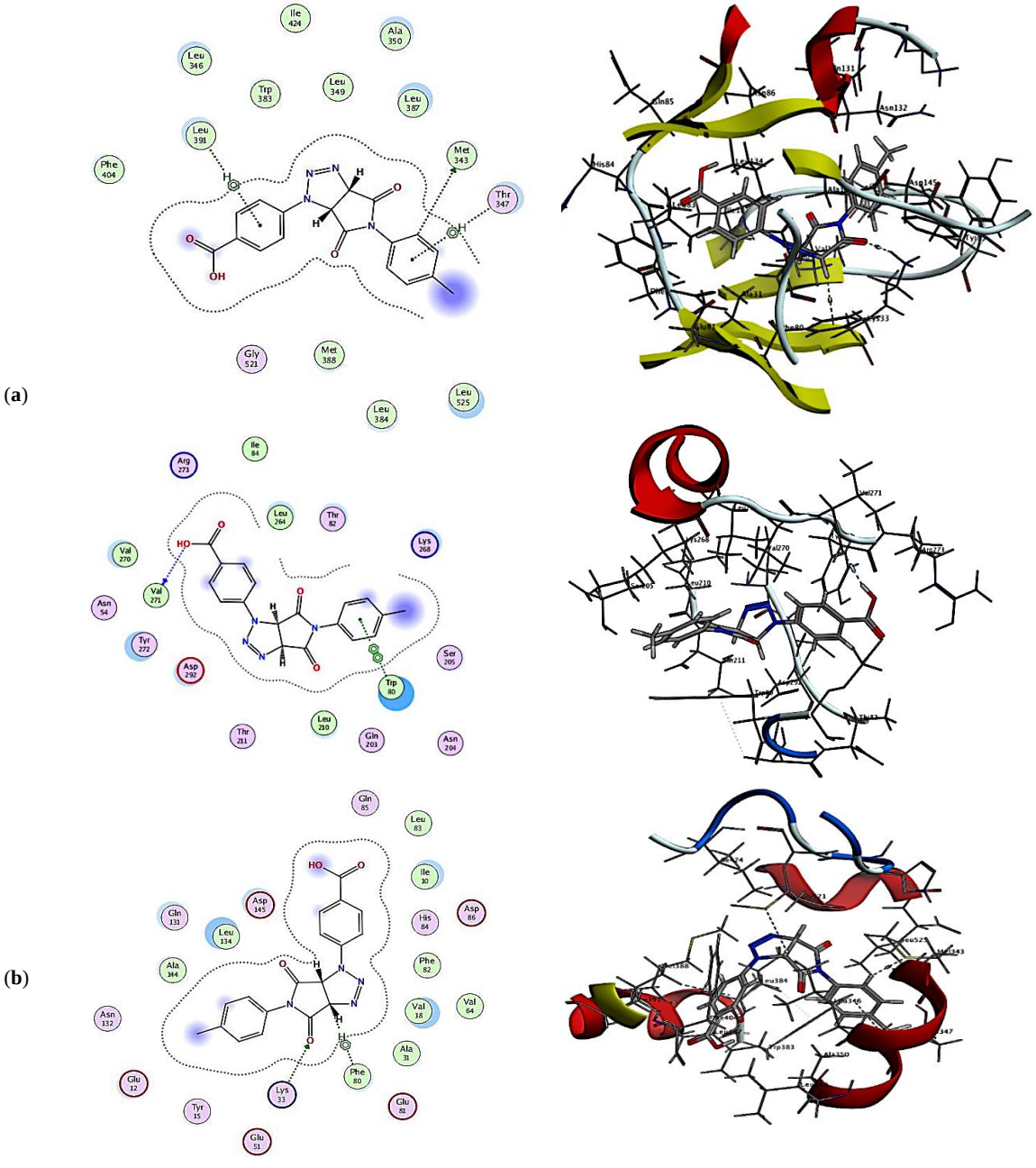

Figure 4: 2D and 3D representations for binding compound **5** with the target proteins.
a: 5KCV; **b**: 3ERT, and **c**: 4FX3.

Conclusion

Under a 1,3-dipolar cycloaddition reaction, new 1,2,3-triazole derivatives were successfully synthesized. Compound **5** was found to have higher cytotoxic effectiveness against the breast cancer MCF-7 line than the other compounds, while other synthesized compounds showed weak effectiveness. Additionally, the DFT study revealed that compound 5 exhibited the strongest interactions with the selected proteins, suggesting it effectively targets AKT, Erα, and CDK2 proteins in MCF-7 breast cancer cells. Therefore, the synthesized compounds are considered a promising agent against breast cancer.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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