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Original Research Article

Synthesis, Characterization, and Cytotoxic Evaluation of Novel 1,2,3-Triazole–Maleimide Hybrids against MCF-7 Breast Cancer Cells and their Theoretical Study

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ABSTRACT

Cancer remains a major global health challenge, causing over 9.6 million deaths annually. Although many anticancer drugs exist, their efficacy and safety are limited. Recently, 1,2,3-triazoles have emerged as versatile pharmacophores with diverse biological activities and promising therapeutic potential. The current research aimed to synthesize novel bis-1,2,3-triazole-maleimide hybrids, evaluate their cytotoxic activity against the MCF-7 (Michigan Cancer Foundation-7) breast cancer cell line, and elucidate their molecular interactions and electronic properties through theoretical investigations. The compounds were manufactured through a 1,3-dipolar cycloaddition reaction and characterized employing ^{1}H NMR (proton nuclear magnetic resonance), ^{13}C NMR (carbon-13 nuclear magnetic resonance) spectrometry, Fourier transform infrared (FTIR) spectroscopy and mass spectrometry. The anticancer efficacy of the compounds was assessed against the MCF-7 cancer cell line via the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The compounds were further analyzed through molecular docking simulations using the AutoDock Vina in PyRx, which provided insights into their binding energies and interactions with cancer-related receptors. The results revealed that all compounds exhibited cytotoxicity, with compound 3 showing the highest potency, with an IC_{50} value of 3.50 µM. Compound 3 showed superior binding affinity, corroborating its experimental cytotoxic activity. Density functional theory (DFT) calculations confirmed the stability and reactivity of the compounds, with compound 3 demonstrating the lowest energy gap and the highest electrophilicity, indicating its high chemical reactivity. The findings of the present study suggest that the bis-1,2,3-triazole-maleimide hybrids, particularly compound 3, hold promise as selective and potent anticancer agents for further development.

Keywords: Breast cancer, 1,3-dipolar cycloaddition, 1,2,3-triazole hybrids, Molecular docking, Cytotoxicity.

Introduction

Cancer remains the leading cause of death worldwide, accounting for more than 9.6 million deaths annually, according to a recent report from the World Health Organization (WHO).[1–4] It is a malignant disease characterized by uncontrolled and irregular cell proliferation.[5–7] Despite the development of numerous anticancer drugs in recent decades, many of these treatments exhibit limited efficacy and severe side effects, including drug-induced toxicity.[8-11] Moreover, the acquired resistance of cancer cells to chemotherapeutic agents poses a significant obstacle to effective cancer therapy.[12,13] Therefore, immediate attention is needed to develop novel, safe, and potent anticancer agents with improved selectivity and reduced adverse effects.[3,14]

Hypredic 1,2,3-triazoles, among the promising pharmacophores in medicinal chemistry, have attracted considerable attention due to their wide range of biological activities, including anticancer,[15,16] anti-inflammatory,[17,18] antioxidant,[19,20] antimicrobial,[21,22] and anti-infective drugs.[23,24]

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Conventionally, 1,2,3-triazole derivatives are synthesized through 1,3-dipolar cycloaddition between alkynes and organic azides, a reaction known for its efficiency and versatility. However, limited research has explored the integration of maleimide and organic azide moieties to synthesize novel 1,2,3-triazole hybrids, which may exhibit enhanced biological properties.[25,26]

In this context, the present study focuses on the synthesis of bis-1,2,3-triazole derivatives via a 1,3-dipolar cycloaddition reaction and the evaluation of their cytotoxic activity against the MCF-7 (Michigan Cancer Foundation-7) breast cancer cell line. Furthermore, theoretical investigations were conducted to elucidate the molecular interactions and electronic properties of the synthesized compounds. To the best of our knowledge, this investigation is the first report on the design and synthesis of hydrazin-maleimide-based bis-1,2,3-triazole derivatives for anticancer evaluation. This novel hybrid approach provides a new structural framework for the development of selective and potent anticancer agents. The combination of synthetic, biological, and theoretical methodologies ensures a comprehensive understanding of the structure–activity relationships of these compounds and highlights their potential for future drug development.

Materials and Methods

Instrument and chemicals

Sigma-Aldrich and Alpha (USA) supplied the chemicals and solvents. The Fourier transform infrared (FTIR) spectra were acquired utilizing a Shimadzu FTIR Affinity-1 (Germany) at the College of Education for Pure Science, University of Basrah, Iraq. A Varian 500 spectrometer (Varian, Inc., USA) was utilized to ascertain the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra, with the chemical shifts (δ)

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represented in parts per million (ppm) employing tetramethylsilane (TMS) as a standard reference, and deuterated dimethyl sulfoxide (DMSO) serving as the solvent. The procedure was carried out at Tehran University. The mass spectra were analyzed utilizing a JEOL JMS-5X 10217 instrument (Japan) in the electron impact (EI) mode. The molecular and parent ions (m/z) were reported. The melting points were uncorrected and obtained using open capillary tubes with the Gallenkamp melting point instrument (ESL57725, UK). The synthesized compounds were evaluated against the breast cancer cell line at the Laboratories of Tehran University.

Synthesis of N-(phenylamino)maleimide

N-(phenylamino)maleimide was manufactured in two stages: maleic anhydride and phenylhydrazine, as delineated and illustrated in Figure 1.

N-(phenylamino)maleiamic acid (1)

Maleic anhydride (0.98 g, 0.01 mol) was added gradually to 10 mL of DMF (N,N-dimethylformamide) containing phenyl hydrazine (1.8 mL, 0.01 mol) in a flat-bottom flask, and the mixture was agitated for 3 hours at 25℃. This solution was immersed in ice-chilled water. The solid yellow colour was precipitated. The precipitate was subjected to filtration and subsequently dried at 70°C. The product was recrystallized from methanol, affording the desired compound in 75% yield.

N-(henylamino)maleimide (2)

A DMF solution of N-(phenylamino)maleamic acid (2.06 g, 0.01 mol) in 10 mL of DMF in a flat-bottom flask was stirred while P_2O_5 (0.74 g) and concentrated H_2SO_4 (0.5 mL) were added gradually. The solution was stirred for 2 hours at 70°C and then cooled. Subsequently, it was placed into crushed ice water, resulting in the formation of a yellow precipitate of N-(phenylamino)maleimide. The precipitate underwent filtration, thoroughly rinsed with water many times, and subsequently dried. The crude product was recrystallized utilizing methanol as the solvent. The product was 72%, and the melting point was 215ºC.[27,28]

Synthesis of organic azide derivatives

At 0°C, a solution of $NaNO_2$ (2.0 mmol dissolved in 5 mL of water) was added dropwise to a solution of aniline derivatives (1.0 mmol), concentrated hydrochloric acid (0.7 mL), and water (10 mL). After 15 minutes of stirring at 0°C, NaN_3 (2.0 mmol) was added to the liquid above, it was subsequently dissolved in 5 mL of water and agitated at 0 °C until solids began to form. Organic azide derivatives were obtained as solids by filtering, drying, and recrystallizing the collected precipitate in an appropriate solvent.[29]

Procedures for the synthesis of 1,2,3-triazole-hybrids (1-3)

A solution of suitable N-(phenylamino)maleimide (2.0 mmol) in chloroform was gradually supplemented with organic azide derivatives (1.0 mmol). The reaction mixture was subjected to reflux for the specified duration (Table 1). Thin-layer chromatography (TLC) was employed to analyze the resultant mixture until the initial reagents were depleted. To encourage the precipitation of crude products, the resulting reaction was cooled. The separated products were then recrystallized from a 1:1 volumetric ratio mixture of tetrahydrofuran and hexane after being cleaned with cooled ethanol, yielding unadulterated hybrids 1-3 at 55-75% efficiency. The purification of hybrid derivatives 1-3 achieved a purity of 95%.

1,1'-(oxybis(4,1-phenylene)) bis(5-(phenylamino)-3a,6a-dihydropyrrolo[3,4-d] [1, 2, 3] triazole-4,6(1H,5H)-dione) (1)

White solid. Yield 65%, m.p. 203-205ºC. IR spectrum (KBr, *v*, cm^{-1}): 3440 (N-H), 1725 (C=O), 1502 ($C=C_{aromatic}$), 1474 ($N=N_{triazole}$). ^{1}H NMR, *δ* (ppm): 5.27 (*J* = 10.1 Hz, d, 2H, 2CH), 5.97 (, *J* = 10.1 Hz, d, 2H, 2CH), 6.82 (s, 2H, 2NH), 7.13-7.65 (m, 18H, Ar-H). ^{13}C NMR, δ/ppm: 57.7, 83.3, 117.3, 119.50, 127.0, 129.0, 131.5, 135.5, 153.1, 169.7, 171.3. Mass (EI^+) (M+1) for $C_{34}H_{26}N_8O_5$: 628 (Figure 2).

1,1'-(sulfonylbis(4,1-phenylene)) bis(5-(phenylamino)-3a,6a-dihydropyrrolo[3,4-d] [1,2,3]triazole-4,6(1H,5H)-dione) (2)

A cream solid. Yield 75%, m.p. 248-250ºC. FTIR spectrum (KBr, *v*, cm^{-1}): 3423 (N-H), 3061 ($C-H_{aromatic}$), 2976 ($C-H_{aliphatic}$), 1724 (C=O), 1502 ($C=C_{aromatic}$), 1377 ($N=N_{triazole}$). ^{1}H-NMR, *δ* (ppm): 5.27 (*J* = 10.1 Hz, d, 2H, 2CH), 5.97 (*J* = 10.1 Hz, d, 2H, 2CH), 6.82 (s, 2H, 2NH), 7.13-7.64 (18H, of Ar-H, m). ^{13}C-NMR, δ/ppm: 58.0, 83.6, 117.9, 117.5, 119.4, 126.9, 127.9, 129.5, 130.7, 134.9, 135.4, 153.2, 169.6, 171.2. Mass (EI^+) (M+1) $C_{32}H_{24}N_{10}O_6S$: 675 (Figure 3).

1,1'-(5-nitro-1,3-phenylene) bis(5-(phenylamino)-3a,6a-dihydropyrrolo[3,4-d] [1, 2, 3] triazole-4,6(1H,5H)-dione) (3)

A white solid. Yield 54%, m.p. 189-192ºC. FTIR spectrum (KBr, *v*, cm^{-1}): 3451.81 (N-H), 3053 ($C-H_{aromatic}$), 1726 (C=O), 1500 ($C=C_{aromatic}$), 1377 ($N=N_{triazole}$). ^{1}H NMR, *δ* (ppm): 5.25 (*J* = 10.1 Hz, d, 2H, 2CH), 5.94 (*J* = 10.1 Hz, d, 2H, 2CH), 7.11-7.64 (15H, m, $H_{aromatic}$ with 2H of NH). ^{13}C NMR, δ/ppm: 58.08, 83.63, 117.29, 117.53, 119.38, 119.53, 126.90, 127.99, 129.52, 130.70, 134.99, 135.41, 153.25, 169.57, 171.20. Mass (EI^+) (M+1) $C_{26}H_{19}N_{11}O_6$: 580 (Figure 4).

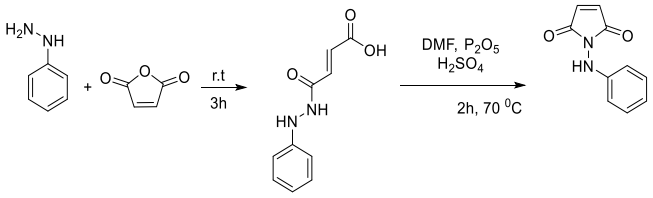

Figure 1: Preparation route of *N-(phenylamino)maleimide*.

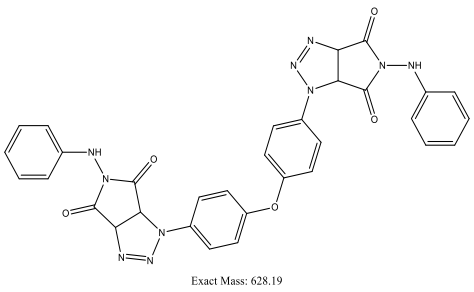

Figure 2: Structure of compound 1.

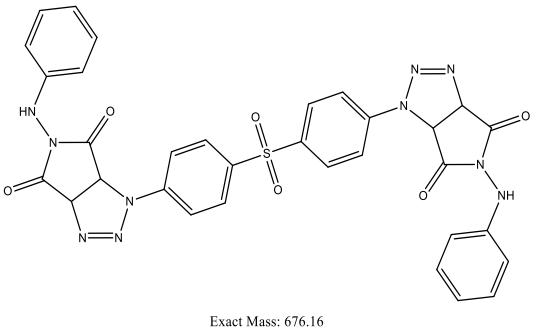

Figure 3: Structure of compound **2.**

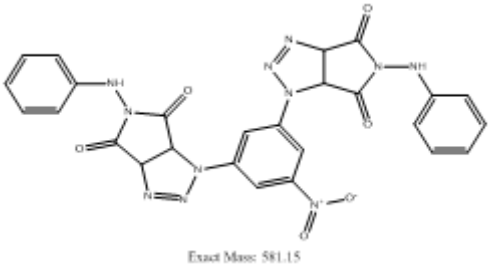

Figure 4: Structure of compound **3.**

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Source of cell line and cultivation

Human breast cancer cells, MCF-7 (Michigan Cancer Foundation-7) were obtained from the National Bank of Iran (Pasteur Institute, Iran). Using RPMI-1640 media (Gibco, USA) with 10% fetal bovine serum (FBS; Gibco, USA) culture medium, the cells were cultured and treated with antibiotics (100 µg/mL streptomycin and 100 U/ml penicillin). Trypsin/ethylenediaminetetraacetic acid (Gibco, USA) and phosphate-buffered saline (PBS) solution were used to culture cells at 37 °C in a humidified atmosphere with 5% CO_2.

MTT assay for assessing cell viability in MCF7 cell lines

The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay provided by Sigma-Aldrich (USA) was employed to evaluate cellular proliferation and viability.

For monolayer culture, cancer cells were treated, collected, and controlled with trypsin at a density of 1.4×10^{-4} cells per well and cultured for 24 hours on a 96-well plate containing 200 µL of fresh media. After obtaining the monolayer, the cells were treated with 6-100 µg/mL of the synthesized compounds in a medium containing 5% CO_2 at 37°C. After 24 hours of treatment, the cultured monolayer was left immobile. Then, the added solution of 200 µL/well of MTT solution (0.5 mg/ml in PBS) was maintained at 37°C for an additional 4 hours. The MTT solution was prepared by adding DMSO (100 µL) to each well after removing the upper liquid from the cells. The viability of the cells was determined by quantifying the absorbance at 570 nm and using an enzyme-linked immunosorbent assay (ELISA) reader (Model Wave xs2, BioTek, USA). IC_{50} values, which represent the concentration of the chemical that kills cells by 50%, were determined from dose-response curves.

Statistical analysis

The GraphPad Prism 8.1 was utilized to determine the IC50 value. A P value of less than 0.0001 was considered statistically significant.

Density functional theory analysis

The density functional theory (DFT) calculations of the prepared compounds were conducted using the approximation of GGA and the Perdew–Burke–Ernzerhof (PBE)-level with a basis set of DNP using BIOVIA Materials Studio 2017 (17.1.0.48)/DMol-3. The global chemical reactivity descriptors (GCRD), including the highest occupied molecular orbital energy level (EHOMO), the lowest unoccupied molecular orbital energy level (ELUMO), and the concept of electron affinity, energy band gap ΔE, ionization potential, chemical hardness, electron negativity, global softness, and absolute softness, were estimated by DFT calculations.

Results and Discussion

Characteristics of the prepared compounds

All synthesized compounds 1, 2, and 3 were prepared in three steps. The first step involved the preparation of *N-(phenylamino)maleimide* by reacting phenyl hydrazine with maleic anhydride in chloroform to form maleamic acid. Then, the cyclization reaction of maleamic acid was achieved by reacting it with P_2O_5 and sulfuric acid in DMF to produce the maleimide,[27] as shown in Figure 1. In the second step, organic azides were prepared from the diazotization reaction of 4,4'-oxydianiline, 4,4'-diaminodiphenylsulfone, and 1-nitro-3,5-diaminobenzene, respectively. This step involves reacting primary amine derivatives with $NaNO_2$ and HCl at 0-5°C to form a diazonium salt, followed by the addition of NaN_3 to produce organic azides through a substitution reaction (Figure 5). The next step involved the manufacturing of new hybrid 1,2,3-triazole compounds 1–3 through a 1,3–dipolar cycloaddition reaction. New 1,2,3-triazole compounds 1–3 were prepared using a free catalytic process, and the reaction was monitored using TLC and the eluent chloroform: hexane (7:3). The R_f values are presented in Table 1. The model reaction was first conducted by refluxing N-(phenylamino)maleimide and organic azide derivatives for the necessary amount of time. Chloroform was selected as the most effective solvent for the manufacturing of novel bis-1,2,3-triazole derivatives 1-3, as depicted in Figure 6.

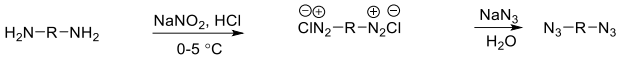

Figure 5: Preparation route of organic azide derivatives.

Infrared spectroscopy was used to confirm the correctness of the compounds' preparation. The most important feature of the synthesized compounds' spectra was the disappearance of the azide group band at 2100-2180 cm^{-1} and the presence of bands in the unsaturated bond region at 1724-1726, 1500, and 1475 cm^{-1}, which are attributed to C=O, C=$C_{aromtic}$, and N=N, respectively. In addition, other bands could strengthen the correctness of the preparation. The effective manufacturing of novel bis 1,2,3-triazole hybrids 1-3 was definitively validated by their ^{1}H NMR analysis, which displayed two distinct doublet signals at δ 5.24–6.01 ppm corresponding to the fused 1,2,3-triazole hybrid protons (CH groups) in the expected region. The integration aligns with the number of protons in the synthesized molecules, alongside various signals in the aromatic region. The ^{13}C NMR exhibited signals at 53-84 ppm corresponding to the two carbon atoms linked to the triazole ring and signals at 169-171 ppm associated with the two carbon atoms of the carbonyl groups, along with various signals representing the carbon atoms in the aromatic region. The mass spectra were characterized by fragments at the molecular ion of the synthesized compounds, which enhances the validity of the prepared compounds.

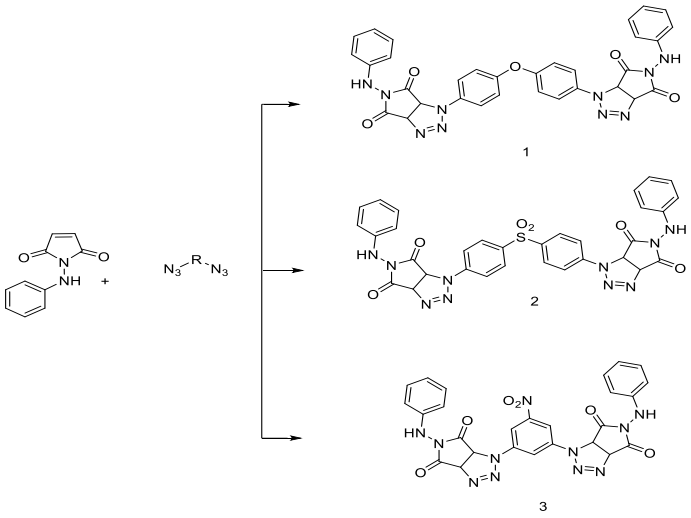

Figure 6: The synthesis route of new bis-1,2,3-triazole hybrids 1-3.

Cytotoxic effects of the synthesized compounds

The synthesized 1,2,3-triazole compounds 1-3 were evaluated for inhibition of breast cancer cell MCF-7 growth by cell viability assay. Following 24 hours of exposure to 6-100 µM of each molecule, Compounds 1 and 2 had moderate efficacy against the cancer cells. The efficacy of the screened compounds was compared to that of the drug doxorubicin (positive control), with DMSO acting as the negative control. The IC_{50} value represents the concentration required to inhibit 50% of cell viability. Table 2 and Figures 4-6 present the results of the cytotoxicity of the compounds.[30] Examination of the obtained data showed that all targets of synthesized compounds (1–3) had a significant effect on the MCF-7 cell line (IC_{50} = 8.72, 10.12, and 3.50 µM, respectively). Compound 3 was the most potent candidate for MCF-7, with an IC_{50} value of 3.50 µM. The triazole derivatives 2 (IC_{50} = 10.13) and 1 (IC_{50} = 8.72) exhibited the least potency compared to compound 3 against the MCF-7 cell line. The results demonstrated the association of structure and the effectiveness of synthesized compounds within substituted-[1,2,3]-triazole derivatives (1–3). For the cell line (MCF-7), the 3-nitrobenzene derivative 3 showed a higher potency (IC_{50} = 3.50) than the 4,4'-oxydibenzene derivative 1 (IC_{50} = 8.72) and the sulfonyldibenzene derivative 2 (IC_{50} = 10.12).

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Cytotoxicity and dose–response evaluation of hybrid 1,2,3-triazoles

The analysis of dose-response and derivation of IC_{50} values was conducted using GraphPad Prism 8.1 software. The estimated IC_{50} values for the hybrid 1,2,3-triazoles 1, 2, and 3 were 8.72, 10.12, and 3.50 µM, respectively, as illustrated in Figure 7. The variations in IC_{50} values may be ascribed to the orientation of the substituents and their respective positions. Figure 7 shows that different concentrations (6.26-100) of the synthetic compounds 1-3 were used. The viability of the cells at a concentration of 6.25 µg/mL was 65.88%. At a concentration of 12.5 µg/mL, the viability was 38.01%, and at 25 µg/mL, the viability was 17.93%. At concentrations of 50 and 100 µg/mL, the cell viabilities were 10.55% and 6.35%, respectively.

Table 1: Physical properties of synthesized compounds 1-3.

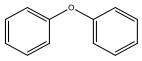
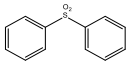
Compound	R	Reaction time (hour)	M.p. (°C)	Yield (%)	R_f
1		17	203-205	65	0.75
2		19	248-250	75	0.9
3		23	189-192	54	0.7

Table 2: IC_{50} values of the target derivatives 1–3.

Compound	IC_{50} (µM) MCF-7
1	**8.72**
2	**10.12**
3	**3.50**

Based on the IC_{50} concept, which represents the level that prevents 50% of cell viability, the IC_{50} value of compound 1 was determined to be 8.72 µg/mL.

Compound 2, as shown in Figure 7, indicated that the cell viability at a concentration of 6.25 µg/mL was 69.57%. At a concentration of 12.5 µg/mL, the viability was 41.80%, and at 25 µg/mL, the viability was 19.25%. For concentrations of 50 µg/mL and 100 µg/mL, the viabilities were 12.09% and 9.53%, respectively. The IC_{50} value of compound 2 was 10.12%. Compound 3, as shown in Figure 7, indicated that the cell viability at a concentration of 6.25 µg/mL was 52.97%. At a concentration of 12.5 µg/mL, the viability was 22.85%, and at 25 µg/mL, the viability was 20.208%. For concentrations of 50 µg/mL and 100 µg/mL, the viabilities were 18.34% and 11.73%, respectively. The IC_{50} value of compound 3 was 3.50%.

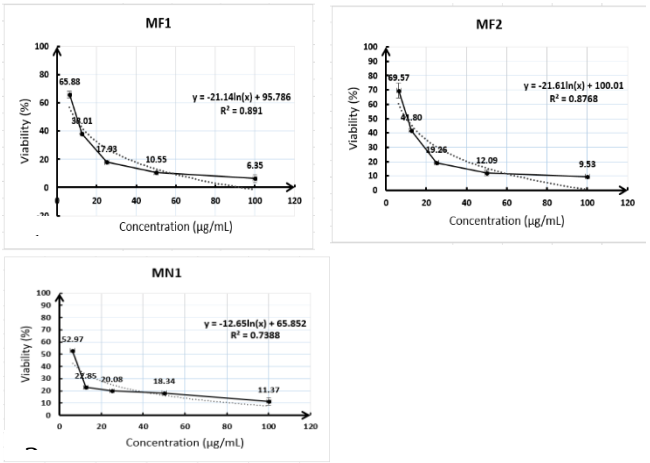


Figure 7: Assessment of cell viability in the MCF-7 cell line for the synthesized compounds 1-3.

Global reactivity descriptors

A compound is stable if the value of the molecular orbital energy is negative.[31] Higher energy gaps make molecules less reactive and more stable. A lower energy gap is easily polarized, often associated with reduced kinetic stability, and exhibits high chemical reactivity. Soft molecules have a low orbital energy gap.[32] The chemical descriptors of the prepared compounds, calculated by DFT at the DMol-3/DNP level of theory, are listed in Table 3. This indicates that the compounds are stable, as evidenced by the negative values of E_{LUMO} and E_{HOMO} energies. The chemical characteristics of the molecules are related to the energy gap ΔE, chemical hardness (η), absolute softness (σ), and global softness (S). Compound 2 has the highest value of ΔE at 2.598 eV. It also showed high hardness η. Moreover, their absolute softness and softness values are lower. These two compounds have comparatively stable and hard molecules compared to other compounds. Conversely, compounds 1 and 3 exhibit higher reactivity than the others, as indicated by their low chemical hardness and ΔE values, along with high absolute softness and softness parameters. As a result, their application in biological contexts can be flexible.[33]

Based on the chemical potential (µ), which reflects the tendency of electrons to leave a stable system and is expressed as a negative value, the compound can be considered stable. It does not decompose spontaneously into its elements. Furthermore, the chemical potential is the HOMO and LUMO orbitals' combined average energy value. The electronegativity (χ) is an indicator of an atom's attraction to electrons in a covalent bond; it is a negative value of the chemical potential and is a crucial factor that aids in determining the performance of inhibition at a molecular level. The dipolar degree of the molecular level is evaluated using these two factors of µ and χ.[34] The findings confirm agreement with those of the anti-cancer study. The dipole moment of compound 3 is higher than that of the other compounds. The global electrophilicity (ω) quantifies the energy stabilization that occurs when a system accepts an additional electronic charge. Electrophilicity has two aspects: the ability of a species to accept additional electronic charge and the system's tendency to resist the transfer of charge to its surroundings, making it a more reliable descriptor of overall chemical reactivity.[35] A molecule is an electrophile if its global electrophilicity is high. Compound 3 was more electrophilic than the other compounds because of the presence of nitro groups in these compounds.

Molecular docking analysis

The molecular docking (MD) analysis was performed for the compounds 1, 2, and 3, against the proteins of the MCF-7 cell line: (PDB:1SA0) and (PDB: 3ERT), which were downloaded from the PDB bank, by using Molecular Operating Environment (MOE) software (2019.0102).[36] The affinity energy, root mean square deviation

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(RMSD), and interaction information for the compounds 1, 2, and 3 are presented in Table 4. The RMSD was employed to compare the conformation of docked structures to that of the reference-docked conformation. The S value is a scoring number that assesses the affinity of the ligand with the receptor. The compound can be considered a potential inhibitor if it has low RMSD and high S values.[37] The values of RMSD with more than two are not considered. Re-docking ligands confirmed the docking process, and RMSD values were less than two.

The compound 1 exhibits a π-interaction between the hydrophobic phenyl moiety and the amino acid Thr340 of the 1SA0 receptor, as illustrated in Figure 8. Compound 2 exhibited three interactions with the 3ERT receptor; the first interaction is a π-interaction of the hydrophobic phenyl moiety with the Phe404 amino acid, and the second and third interactions are hydrogen bonds with the Met343 (2.93 Å) and Met522 (2.61 Å) amino acids.[38] The compound 3 binds to receptors 1SA0 and 3ERT and has a good conformation.

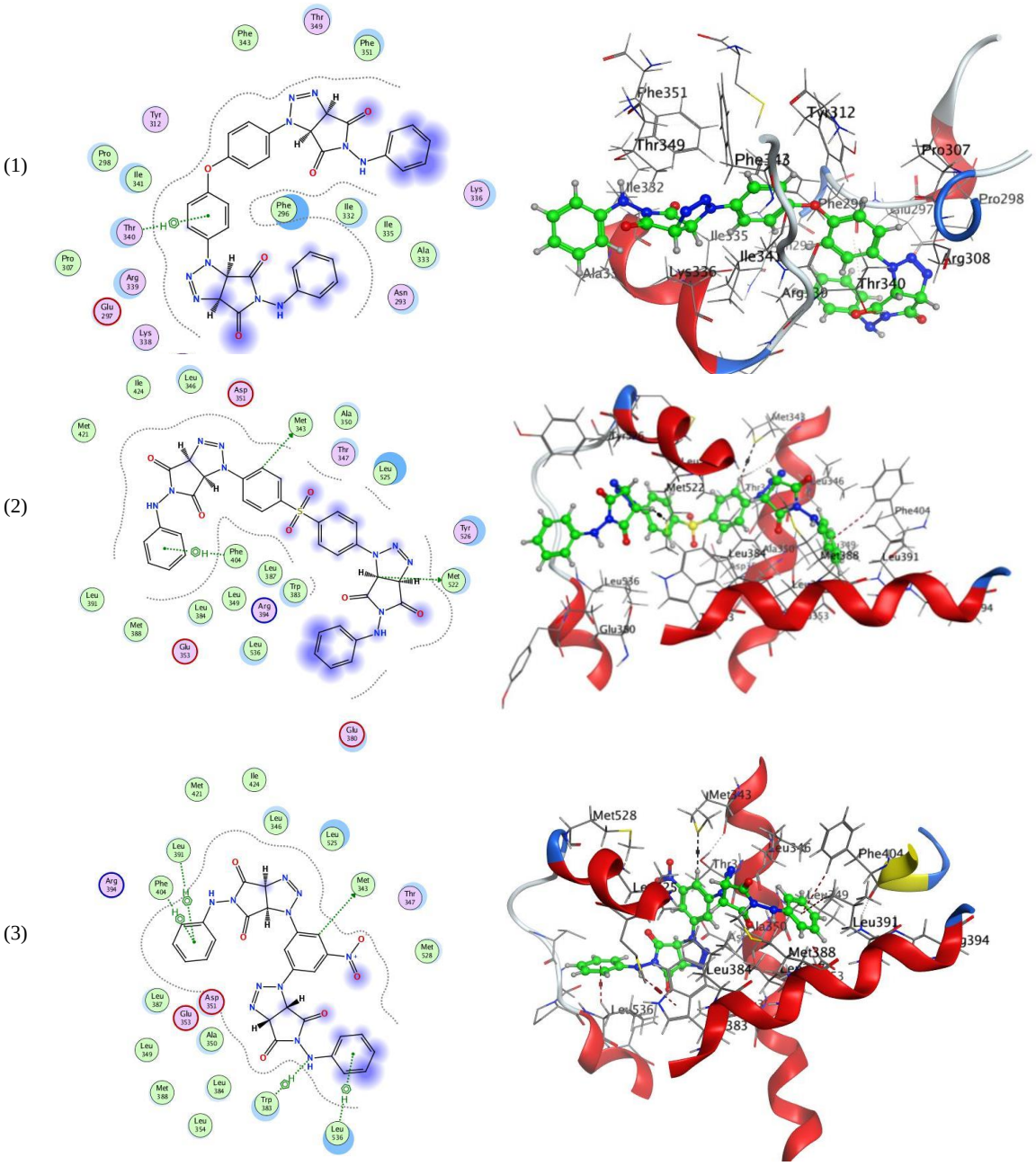

Figure 8: 2D and 3D forms of target protein 1SA0 with compounds 1 and 2 and target protein 3ERT with compound 3.

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Table 3: Chemical descriptors of the prepared compounds.

Compound	E_{LOMO} (eV)	E_{HOMO} (eV)	Energy gap (ΔE) (eV)	Electron affinity (EA) (eV)	Ionization potential (IE) (eV)	Chemical hardness (η) (eV)	Electronegativity (χ) (eV)	Chemical Potential (μ) (eV)	Global softness (S) (eV^{-1})	Global electrophilicity (ω) (eV^{-1})	Absolute softness (σ) (eV^{-1})	Dipole moment (dyb)
1	-2.393	-4.891	2.498	4.891	2.393	1.249	3.642	-3.642	-0.625	5.310	0.801	5.039
2	-2.848	-5.446	2,598	5.446	2.848	1.299	4.147	-4.147	-0.650	6.620	0.770	3.781
3	-3.441	-5.539	2.098	5.539	3.441	1.049	4.490	-4.490	-0.525	9.609	0.953	8.638

There is hydrogen bonding between the oxygen atom of the carbonyl group in compound 3 and amino acid Ile341 of the 1SA0 protein receptor, with a bonding distance of 2.33 Å. While it has five interactions with the 3ERT protein, four of them are pi-interactions of the hydrophobic phenyl moiety with Leu536, Trp383, Phe404, and Leu391 amino acids, and the fifth one is hydrogen bonding with the Met343 (2.85 Å) amino acid. Based on the docking results, compound 3 exhibits more interactions with the 3ERT and 1SA0 receptors, and it has a favourable conformation that enables the carbonyl group and nitrogen atom to form maximum intermolecular hydrogen bonding with the 3ERT receptor. The docking results agree with the DFT calculations of the chemical descriptors.[39]

Table 4: Docking results of the prepared compounds with protein receptors.

Compound	Receptor	RMSD	Affinity energy (S) Kcal/mol	Interaction		
				Type	Amino acid	Bonding distance Å
1	1SA0	1.859	-8.853	*pi*-interaction	Thr340	-
	3ERT	2.032	-10.046			
2	1SA0	2.631	-7.751			
	3ERT	1.875	-10.124	*pi-interaction*	Phe404	-
				H-Bonding	Met343	2.93
				H-Bonding	Met522	2.61
3	1SA0	1.926	-7.134	H-Bonding	lle341	2.33
	3ERT	1.776	-9.621	*pi*-interaction	Leu536	-
				pi-interaction	Trp383	-
				pi-interaction	Phe404	-
				pi-interaction	Leu391	-
				H-Bonding	Met343	2.85

Conclusion

Bis-1,2,3-triazole hybrids were synthesized via a 1,3-dipolar cycloaddition reaction and were assessed *in vitro* for their efficacy against MCF-7 breast cancer. The findings demonstrated that all hybrid compounds were effective as anti-breast cancer agents. Hybrid 3 demonstrated significant growth inhibition of breast cancer cells (MCF-7) with an IC_{50} value of 3.50 μM. It is expected to be a promising agent against breast cancer. The DFT calculations and MD studies demonstrated that compound 3 is more effective than the other compounds, aligning well with previous anticancer research. The findings of the current study suggest that these compounds hold promise as potential therapeutic agents for various diseases. The study holds promising future prospects in developing selective, potent anticancer agents, particularly compound 3, for further preclinical and clinical trials. Additionally, the novel hybrid approach could inspire new drug designs targeting various cancer types.

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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