

Original Research Article

Docking, dynamic simulation and ADME studies of new 1, 2, 3- triazole–based molecules for potential anticancer applications

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Sent for review: 3 January 2026

Revised accepted: 19 March 2026

Abstract

Purpose: To evaluate twelve 1,2,3-triazole derivatives as potential candidates in the management of cancer.

Methods: In silico analyses were conducted to predict the potential anticancer efficacy of twelve compounds (6a to 6l) via molecular docking technique. Methotrexate was used as a reference ligand as a suitable anticancer agent. The compounds were made to interact with the human estrogen receptor alpha (ER α , PDB ID: 3ERT), as a key therapeutic target. Molecular dynamics technique was also used to study the physical movements of atoms and molecules over time.

Results: Molecules 6b and 6c exhibited high affinity for the protein's active site (3ERT). Molecular dynamics showed that the 6b-3ERT complex remained well-structured during the 100-nanosecond simulation.

Conclusion: Molecule 6b has excellent in silico properties as an anticancer drug and should be studied further. The pharmacological action of the molecule is speculative and requires synthesis and in vitro or in vivo testing for further confirmation.

Keywords: Estrogen receptor α , Methotrexate, Molecular docking, Molecular dynamics simulation, 1,2,3-triazole

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Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The search for novel compounds that exhibit biological activity continues to play a vital role in drug discovery and development. The design of the corresponding chemical entities is informed by existing compounds that are known to exhibit related biological activities [1]. Biological activity is hypothesized to stem from the capacity to interact with critical amino-acid residues located within the target binding site. The stability of binding modes and structure–activity

relationships is examined using molecular docking in conjunction with molecular dynamics simulations [2]. Such studies have the potential to guide both the synthetic efforts and the optimization of biological activity of the proposed derivatives.

The choice of target compounds of interest is dictated by the presence of a 1,2,3-triazole. 1,2,3-Triazole derivatives have gained attention as substitutes for the widely used 1,2,4-triazoles due to their intriguing pharmaceutical properties

and the fact that they are part of various drugs registered by the Food and Drug Administration (FDA). A review of clinically used drugs reveals that 1,2,3-triazoles are the only type of triazole present in prescription medicines. Many synthesized 1,2,3-triazole derivatives exhibit significant anticancer, antimicrobial, antiviral, and anti-inflammatory activities. The 1,2,3-triazole-derived drug Aztreonam for treating gram-negative bacterial infections has been available for use for several years [3]. The derivatives were designed following click chemistry logic, which is an efficient method of molecular design and delivery that offers fast, modular, and selective covalent bond formation between diverse chemical entities; this concept has been employed to explore unexplored chemical fields [4]. In this study, twelve compounds of 1,2,3-triazole derivatives were designed as shown in Table 1. Pharmacokinetic properties of these compounds were studied and hypothetical activity was evaluated as anticancer drug candidate by molecular docking and molecular dynamics techniques. The suggested synthetic reaction scheme with the added catalysts and reaction medium is illustrated in Scheme 1. This study aimed to assess the potential inhibitory activity of twelve 1,2,3-triazole derivatives against a therapeutically relevant drug target, human estrogen receptor alpha (ER α). Figure 1 shows the chemical structure of 1,2,3-triazole.

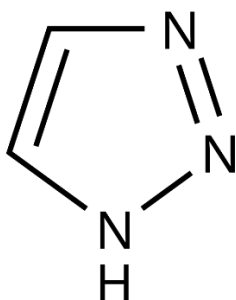


Figure 1: Chemical Structure of 1,2,3-triazole

METHODS

Synthesis

The synthesis followed the pathway illustrated, viz., starting with the compound (3; a benzimidazole derivative), which was first prepared by reacting γ -amino butyric acid with *o*-phenylenediamine. In Scheme 1, compound (3) was converted to compound (4) through reaction with chloroacetyl chloride, and then compound (4) was transformed into the azide derivative (5) by reaction with sodium azide. Subsequently,

compound (5) was utilized in a "Click Reaction" with various acetylene derivatives to yield a series of 1,2,3-triazole compounds (6a-l) [5].

Docking studies

The virtual compounds' docking results were calculated using the GOLD Suite (version 2025), a fully licensed genetic optimization program for ligand docking developed by the Cambridge Crystallographic Data Centre (CCDC). Using CCDC Hermes, protein-ligand hydrogen bonding interactions, short contacts and estimated bond lengths were assessed [6].

Preparation of ligands and target

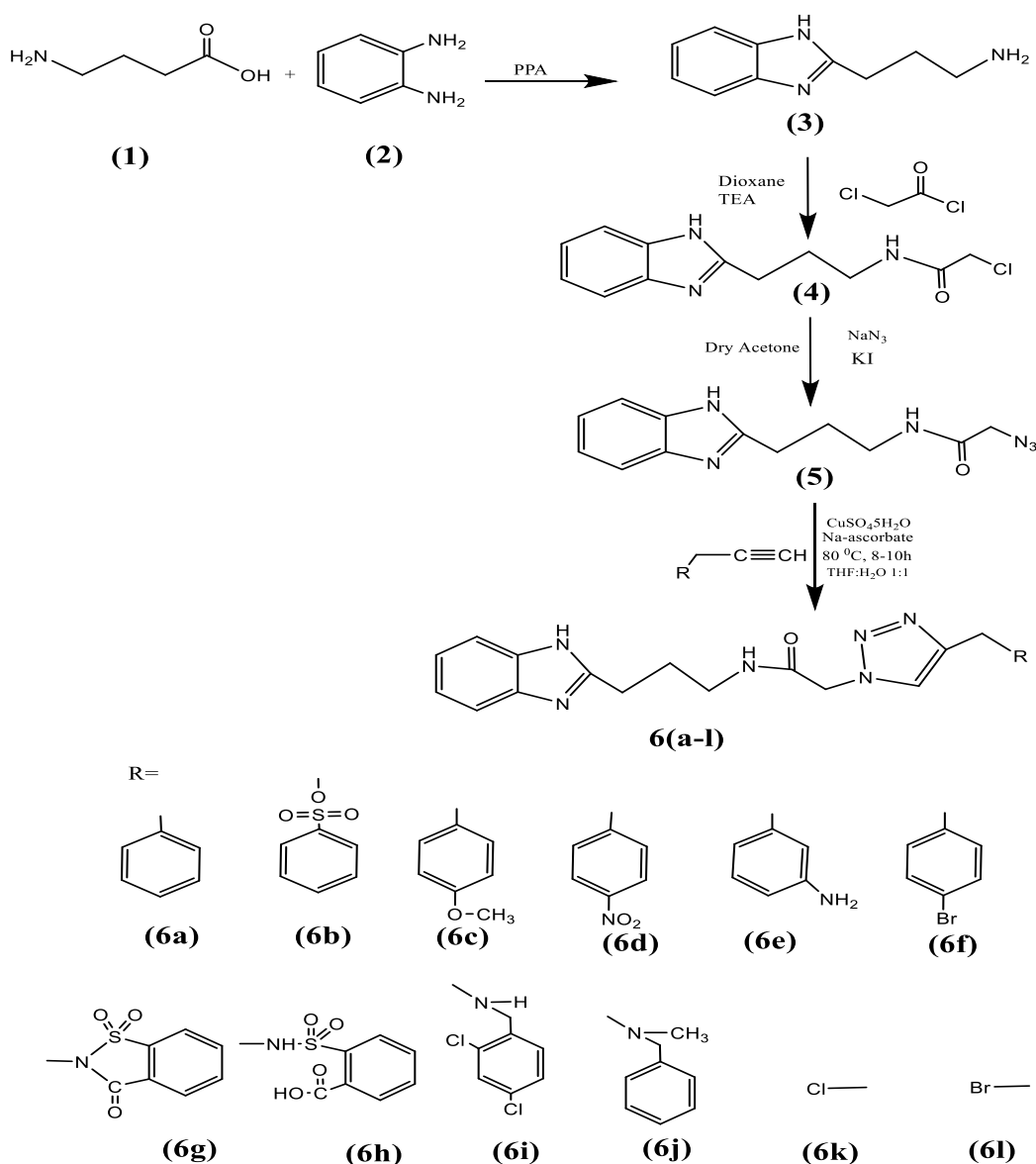
The human estrogen receptor alpha (ER α , PDB ID: 3ERT) was downloaded from the Protein Data Bank (PDB). The chemical structures of the designed compounds were generated using ChemOffice software (version 20; PerkinElmer) and converted into 3D structures using BIOVIA Discovery Studio 2021 visualizer software, which is useful for eliciting the active water molecules required for hydrogen bonding interactions with the 3ERT receptor binding site [7].

Molecular docking protocol

The protein identified as human estrogen receptor alpha (PDB code: 3ERT) was downloaded from the Protein Data Bank (PDB) website and prepared using the Hermes program inside CCDC GOLD suite 2025. Preparation of the protein was a multi-step process [8]. The first step was to include all the hydrogen atoms, which was crucial in correcting the ionization states of the amino acids (tautomerization). Then, water molecules, responsible for water-hydrogen bonding between ligands and the protein active site, were extracted. The third step in protein preparation was to remove any ligand or cofactor co-crystallized with the active core of proteins [9]. The active site prediction depends on the pocket remaining after the extraction of the co-crystal ligand. The protein binding site residues were characterized within a distance of 10 Å between the designed ligands and the 3ERT active site. Furthermore, BIOVIA Discovery Studio was used to elicit 2D interactions between the co-crystal ligand and 3ERT target protein, which helped to identify the amino acid residues in the target's active site, such as MET421 (2), MET388, ILE424, LEU391, LEU428, ALA350 (2), LEU387, MET343, GLU353, ARG394, TRP383, and LEU346.

Table 1: The chemical structures of the designed compounds with an amide origin

Compound no.	Chemical formula	Structure	Name of the compounds
6 a	C ₂₁ H ₂₂ N ₆ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-benzyl-1H-1,2,3-triazol-1-yl)acetamide
6 b	C ₂₁ H ₂₂ N ₆ O ₄ S		(1-(2-((3-(1H-benzo(d)imidazol-2-yl)propyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl benzenesulfonate
6 c	C ₂₂ H ₂₄ N ₆ O ₂		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(4-methoxybenzyl)-1H-1,2,3-triazol-1-yl) acetamide
6 d	C ₂₁ H ₂₁ N ₇ O ₃		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(4-nitrobenzyl)-1H-1,2,3-triazol-1-yl) acetamide
6 e	C ₂₁ H ₂₃ N ₇ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(3-aminobenzyl)-1H-1,2,3-triazol-1-yl)acetamide
6 f	C ₂₁ H ₂₁ BrN ₆ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(4-bromobenzyl)-1H-1,2,3-triazol-1-yl)acetamide
6 g	C ₂₂ H ₂₁ N ₇ O ₄ S		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-((1,1-dioxido-3-oxobenzo(d)isothiazol-2(3H-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide
6 h	C ₂₂ H ₂₃ N ₇ O ₅ S		2-(N-((1-(2-((3-(1H-benzo(d)imidazol-2-yl)propyl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)sulfamoyl)benzoic acid
6 i	C ₂₂ H ₂₃ Cl ₂ N ₇ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(((2,4-dichlorobenzyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetamide
6 j	C ₂₃ H ₂₇ N ₇ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-((benzyl(methyl)amino)methyl)-1H-1,2,3-triazol-1-yl)acetamide
6 k	C ₁₅ H ₁₇ ClN ₆ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(chloromethyl)-1H-1,2,3-triazol-1-yl) acetamide
6 l	C ₁₅ H ₁₇ BrN ₆ O		N-(3-(1H-benzo(d)imidazol-2-yl)propyl)-2-(4-(bromomethyl)-1H-1,2,3-triazol-1-yl) acetamide



Scheme 1: The suggested synthetic reactions with the added catalysts and reaction medium

Two-dimensional interactions between the co-crystal ligand and residues in 3ERT binding pocket are reported in Figure 2. For other parameters of docking process, the default values were used. The default setting for the number of produced postures was 10 and the top-ranked solution was left unchanged. The twelve ligands were added in mol2 file format. Chemscore kinase served as the basis for the setup. Meanwhile, the extent of fitness is equivalent to binding intensity: GOLD's fitness functions (PLP, ChemPLP, GoldScore, and ChemScore) were amplified such that a higher numerical score indicated a more energetically favorable and consequently stronger predicted binding interaction (higher affinity) between the ligand and the protein [10]. In this study, methotrexate (a suitable anticancer agent) was

used as a reference ligand to compare with the twelve designed compounds.

Pharmacokinetic study

Pharmacokinetic properties: Absorption, Distribution, Metabolism, and Elimination (ADME) were studied along with other physicochemical properties of the newly designed compounds using the SwissADME server. This server is an accessible website used to study physicochemical parameters, medicinal chemistry and bioavailability, which are important parameters for the design and synthesis of new compounds. Chemical structures of the newly generated compounds were transformed into SMILES format using ChemAxon's Marvin JS, while pharmacokinetic properties were evaluated using the SwissADME web tool [11].

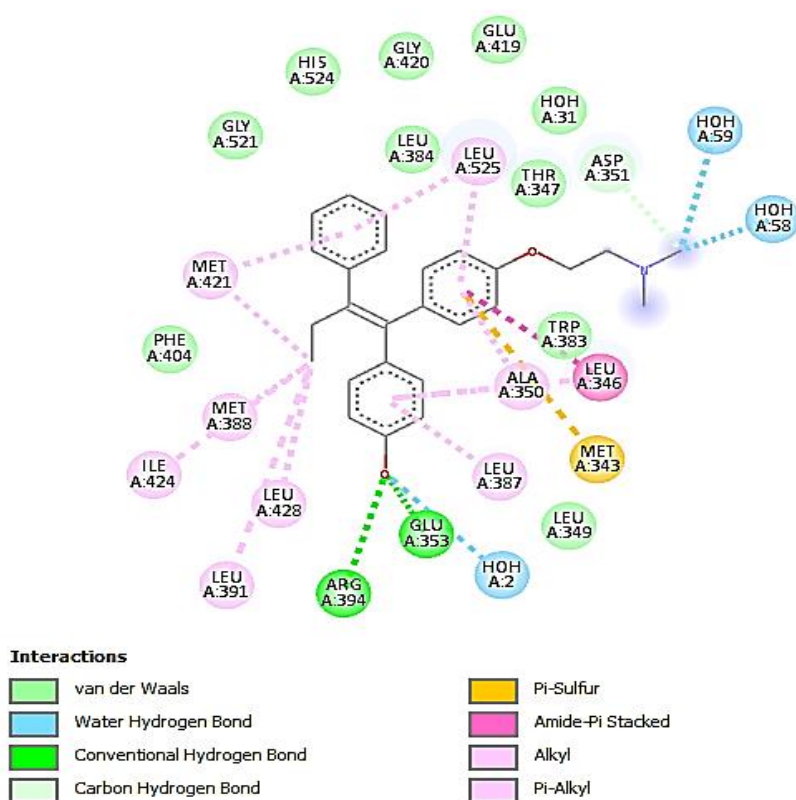


Figure 2: Interactions of the co-crystal ligand with the predicted binding pocket residues of the human estrogen receptor alpha (PDB ID: 3ERT)

Molecular dynamics simulation setup

All molecular dynamics simulations in this investigation were run using Desmond software using the normal methods and default parameters to ensure reliability and reproducibility. Protein 3ERT and the custom-designed ligand complex were the beginning structures. After selecting the best binding posture of ligand-6b pose 1 from molecular docking studies, the protein–ligand pairings were imported into Desmond's Maestro interface for system setup and simulation to create this complex. The complex was rigorously prepared in Maestro after import. Hydrogen atoms were added to correct protonation states and appropriately adjusted to achieve physiological pH. The system was inspected for correctness and removal of incomplete residues or atoms. All non-essential crystallographic water molecules and heteroatoms were removed to enable focus on primary protein–ligand interactions, although structurally relevant water molecules were allowed where needed. Energy minimization employing Desmond's internal algorithms relaxed local strains and resolved undesirable system connections. To imitate the aqueous biological milieu, the protein–ligand interaction was placed in an orthorhombic simulation box solvated

explicitly with transferable intermolecular potential 3 points (TIP3P) water molecules after energy minimization. To avoid artificial interactions from periodic boundary conditions, the box enclosed the solute with at least 10 Å of padding. To simulate physiological circumstances, sodium and chloride ions were increased to 0.15 M and counter-ions were supplied as needed to ensure charge neutrality. For all molecular representations, the OPLS4 force field provided accurate and current parameterization for proteins and ligands. The system was then equilibrated and simulated using the default Desmond workflow, which starts with a constant number of particles, volume, and temperature (NVT; a specific thermodynamic ensemble where the physical conditions of the system are kept constant throughout the simulation). The three variables that are fixed: Constant Number of atoms/particles (N), Constant Volume (V; the simulation box size) and Constant Temperature (T; controlled via a thermostat) ensemble equilibration at 300 K were then switched to number of particles, constant pressure and constant temperature (NPT) ensemble equilibration at 1 atmosphere and 300 K. This two-step equilibration process achieved heat and pressure equilibration, to ensuring that a realistic

system equilibrium before production run. The production molecular dynamics simulations were run exclusively under the NPT ensemble, the standard for simulating biological systems due to its constant pressure and temperature, which mimics living cells and tissues. Trajectory data were captured for comprehensive examination after each 100-nanosecond production run with a 2-femtosecond time step. The protein backbone and ligand root mean square deviation (RMSD) were calculated to track conformational changes

and stability. Protein compactness was assessed using the radius of gyration (Rg), while binding strength and specificity were measured using the quantity and kind of hydrogen bonds between ligand 6b-pose 1 and protein binding sites. Additional interaction investigations were determined and quantified via crucial molecular contact persistence. All analyses were carried out using built-in Desmond and Maestro visualization modules.

Table 2: Binding energies ranking of methotrexate and the twelve derivatives docked with 3ERT, along with the type of interactions and interacting amino acid residues of 3ERT

Compound	Piecewise Linear Potential (PLP) fitness	H-bond acceptor amino acid residues	Short contact amino acid residues
6 b	94.714	THR 347	TYR 526 (2), LYS 529, MET 528 (2), LEU 525 (3), THR 347 (6), TRP 383 (3).
6 c	91.633	THR 347(2) through water molecule (2)	CYS 530, THR 347, TRP 383 (5), VAL 534, LEU 391, MET 343, LYS 529 (3), LYS 530.
6 j	85.876	CYS 530, LEU 536	ASP 351 (2), THR 347, CYS 530, PRO 535 (3), LEU 356 (2).
6 d	84.547	CYS 530, THR 347, GLU 353, ARG 394, VAL 534.	CYS 530 (2), LYS 529 (2), LYS 530 (2), LEU 525 (2), MET 343, LEU 346, TYR 526, GLU 535 (2), MET 522, LEU 387, LEU 384.
6 g	82.104	TYR 526 through water molecule 2, LEU 525, MET 522, LEU 536.	MET 522, LEU 536, GLU 380, LYS 529, MET 528, ALA 350, LEU 346, GLU 353, LEU 349, PHE 404, ARG 394, LEU 391, LEU 387, LEU 384.
6 i	81.564	TYR 526 (2) through water molecule (58), MET 522.	PRO 535, LYS 529, VAL 534, VAL 533, CYS 530, LEU 536, LEU 384, LEU 346, LEU 387, LEU 525, THR 347, MET 343, ASP 351, MET 528.
6 a	81.538	THR 347, CYS 530 through water molecule (58).	CYS 530 (3), PRO 535, VAL 534, VAL 533 (2), LEU 536, LYS 529 (2), LEU 525, LEU 349, LEU 387, LEU 348, LEU 346 (2), ALA 350, THR 347, MET 343, ASP 351, MET 528.
6 e	79.380	THR 347	LEU 536, PRO 535, VAL 533 (2), VAL 534, CYS 530 (4), LYS 529 (2), TRP 383, MET 528 (2), LEU 384, LEU 387, LEU 391, PHE 404, ARG 394, LEU 349, GLU 353, LEU 346, ALA 350.
6 h	78.629	THR 347 (2).	VAL 533 (2), VAL 534, PRO 535 (3), LEU 536 (2), MET 343, MET 528, LEU 525, ASP 351, THR 347, TRP 383, ALA 350, LEU 346, LEU 387, LEU 384, LEU 349, LEU 391, PHE 404, MET 421.
6 f	77.274	THR 343	MET 343, TRP 383, THR 347, ALA 350, ASP 351, TRP 526, MET 522, MET 528, CYS 530, LYS 529, LEU 536, PRO 535, VAL 534, VAL 533.
Methotrexate	70.544	THR 347 through water molecule (59), ARG 394, GLU 353.	ASP 351, THR 347, LEU 525, LEU 384, MET 421 (2), PHE 404, MET 388, ILE 424, LEU 428, LEU 391, LEU 387, LEU 349, ALA 350 (2), MET 343, TRP 383, LEU 346.
6 k	69.411	THR 347 through water molecule (58), ARG 394	LEU 387, LEU 346, LEU 384, MET 343 (2), ALA 350, THR 347, ASP 351, LEU 525 (2), MET 528, TYR 526, MET 522 (2), GLU 380, LEU 536, LYS 536.
6 l	69.262	THR 347 through water molecule (58), ARG 394	LEU 387, LEU 346, LEU 384, THR 347, MET 343 (2), MET 528, ASP 351, TRP 383, MET 522, TYR 526, LEU 536, LEU 525 (3).

RESULTS

Molecular docking

Docking results proved that ten of the twelve compounds possessed lead candidates. These were compounds (6a-6j) that showed high piecewise linear potential (PLP) fitness comparable to the reference compound (methotrexate), which had PLP fitness of 70.544 (Table 2). A higher score signified a more favorable interaction between the ligand and the protein, suggesting a more stable and energetically preferred binding pose. The PLP fitness scores for the designed compounds and methotrexate, with hydrogen bond interactions and short contact at the 3ERT active site, were shown, followed by 3D pictures of the designed compounds interacting with the target receptor (3ERT) using GOLD suite software as a molecular docking tool, along with methotrexate as a reference ligand (Figure 3).

Pharmacokinetic results interpretation

The Swiss ADME server was used to evaluate the physicochemical parameters of the synthesized compounds. The ADME features may be detected before synthesis and biological testing using this affordable and helpful technology. This helps to exclude ligands that are insufficient or have an unsatisfactory pharmacokinetic profile [11]. Among these factors is the topological polar surface area (TPSA), which is used to describe the capacity of

drugs to permeate cells. Compounds with $TPSA < 140 \text{ \AA}^2$ have high cellular permeability and bioavailability; a low value of $TPSA < 140 \text{ \AA}^2$ is a good indicator of good human oral bioavailability [11,12]. From the SwissADME results, it was found that all the synthesized compounds have $TPSA < 140 \text{ \AA}^2$, which means the twelve compounds are expected to be absorbed passively and thoroughly from the gastrointestinal tract (GIT) but will not pass through the blood-brain barrier. The Lipinski's "rule of five" (RO5) is a common guideline for predicting if a compound will be orally absorbed [13]. The compound will require a molecular mass less than 500 Daltons, no more than 5 hydrogen bond donors, and no more than 10 hydrogen bond acceptors, along with a log P value below 5. In this study, ten compounds satisfied the RO5 criteria [13]. The BOILED-Egg model (Figure 4) is a graphical representation used to assess pharmacokinetic properties such as gastrointestinal absorption and blood-brain barrier permeability [14]. The white area represents molecules that are passively absorbed through the GIT, while the yolk area of the egg represents molecules that pass the blood-brain barrier (BBB). The red dots represent molecules that were not effluxed from the BBB by p-glycoprotein, while the blue dots represent molecules expected to be effluxed by p-glycoprotein. All of the compounds appeared as blue dots and were located within the egg yolk, as shown in Table 3. Note that methotrexate appeared outside the range, indicating low GI passive absorption and low BBB penetration.

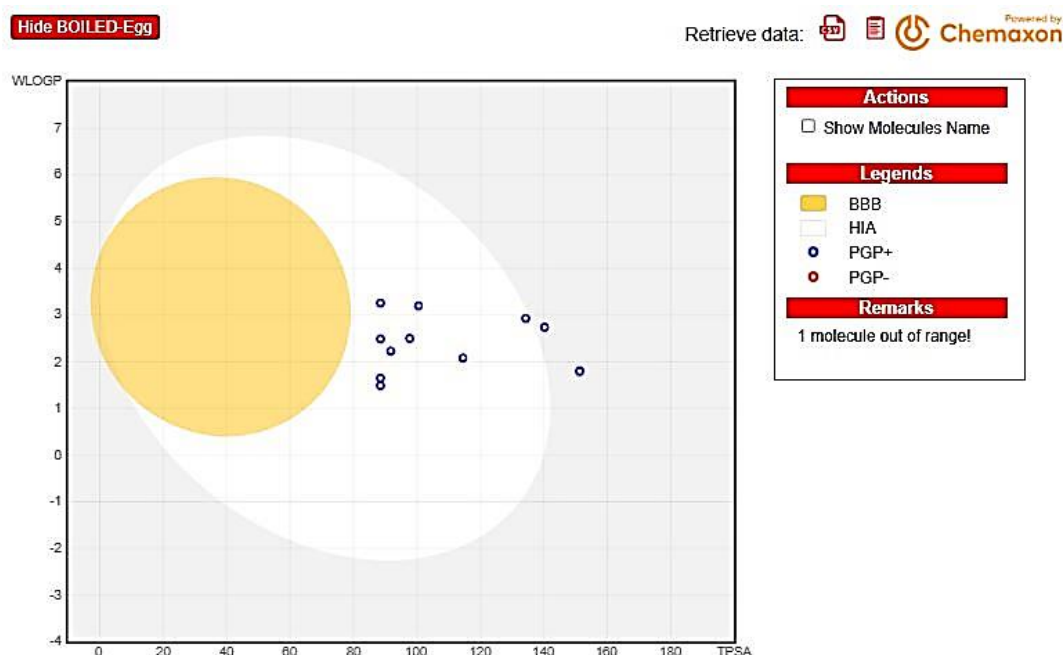


Figure 4: The Boiled-Egg analysis for compounds (6a-l) along with methotrexate. *BBB = blood brain barrier permeability, HIA = Human Intestinal Absorption, PGP = P-glycoprotein efflux pump

Table 3: ADME results of designed compounds along with methotrexate

Compound	Molecular weight (g/mol)	H-donor	H-acceptor	MR	TPSA (Å ²)	GI-absorb	BBB permeability	Bioavailability	Lipinski's violation
Methotrexate	454.44	5	9	118.40	210.54	low	No	0.11	Yes, N or O > 10
6 a	374.44	2	4	107.11	88.49	High	No	0.55	0
6 b	454.50	2	7	116.32	140.24	Low	No	0.55	0
6 c	404.46	2	5	113.60	97.72	High	No	0.55	0
6 d	419.44	2	6	115.93	134.31	High	No	0.55	0
6 e	389.45	3	4	111.51	114.51	High	No	0.55	0
6 f	453.34	2	4	114.81	88.49	High	No	0.55	0
6 g	479.51	2	7	125.32	151.32	Low	No	0.55	Yes, N or O > 10
6 h	479.51	2	7	125.32	151.32	Low	No	0.55	Yes, N or O > 10
6 i	472.37	3	5	124.73	100.52	High	No	0.55	0
6 j	417.51	2	5	119.62	91.73	High	No	0.55	0
6 k	332.79	2	4	87.41	88.49	High	No	0.55	0
6 l	377.24	2	4	90.49	88.49	High	No	0.55	0

Molecular dynamics results interpretation of molecule 6b

Protein backbone and ligand root mean square deviation (RMSD)

Table 4 displays fifteen 100-nanosecond (ns) simulation frames. Each frame has protein-ligand complex RMSD and simulation time. The RMSD measurements demonstrate how much the structure changes along the way from the initial place. Lower RMSD values indicate that the structure is comparable to the starting shape, whereas higher values indicate that it is altering or relaxing to become more stable later in the simulation [15]. The RMSD values rise from a low deviation area to a steady band along the 0 – 100 ns trajectory and stay within a restricted range. This trend indicates that the 6b–3ERT complex is balanced. Large spikes or an upward trend indicates unfolding or structural rearrangements. This suggests that 6b forms a persistent binding to the 3ERT active site during simulation. Protein-ligand conformational correction occurs in the early phase of equilibration due to the fast rise in RMSD. After 20 ns, RMSD values stabilized between 2.0 Å and 3.2 Å. This suggests that the structure was steady and rarely changed. The simulation indicates that the 6b ligand remained coupled to 3ERT's active site, indicating structural stability and equilibrium.

Table 4: The RMSD of compound 6b

Frame	Time (ns)	RMSD 1 (673 atoms)
14	3.25	2.049874
41	10.0001	1.827769
68	16.7501	2.142227
94	23.2502	2.344702
121	30.0002	2.446731
148	36.7503	2.212112
174	43.2503	3.024413
201	50.0004	2.857583
228	56.7505	3.084672
254	63.2505	2.728996
281	70.0006	2.674199
308	76.7506	2.867378
334	83.2507	2.734707
361	90.0007	2.696688
388	96.7508	3.301241

Interactions matter

Table 5 lists fifteen 0 – 100 ns ideal frames. The three columns "Hydrogen Bonds," "Pi-Pi Stacking," and "Pi-Cation" illustrate how many interactions 6b and 3ERT have at each frame. Most examined frames had hydrogen bonds, usually one or multiple connections. So 6b is still

polar fixed in the binding pocket. Regular Pi–Pi stacking interactions keep the ligand and protein aromatic rings face-to-face or edge-to-edge, thereby stabilizing the complex. Periodic pi-cation linkages improve electrostatic–aromatic stability. As hydrogen bonds persist and π -based interactions often occur in the 0 – 100 ns region, 6b stably binds to 3ERT, as indicated by RMSD analysis plateau behavior. Figure 5 illustrates compound 6b–3ERT interactions during 100 ns. The blue line indicates hydrogen bonding, the green line π - π stacking, and the orange line π -cation interactions. The simulation indicated that the ligand constantly interacted with several residues at the binding site, as hydrogen bonds and π - π stacking contacts vary. The stability of interactions, including π - π stacking and hydrogen bond formation, indicates that 6b is well-anchored in the protein pocket. The result showed that compound 6b maintained strong molecular contact with 3ERT throughout the interaction, which resulted in a stabilized complex.

Root Mean Square Fluctuation (RMSF)

Table 6 displays representative RMSF values for equally sampled residues along the simulation course. In the raw file, each column tells how much each residue or atom changes. Keeping all dataset details made it easy to compare patterns across datasets. The 6b–3ERT complex had a rigid core with few flexible components due to its moderate RMSF values. Low RMSF around many residue indices indicated a strong backbone and well-anchored binding pocket. This was supported by stable RMSD and high interaction counts. Mobile surface loops or terminal segments exposed to solvents exhibited slightly higher RMSF areas,

Table 5: Interaction count of compound 6b

Frame	Time (ns)	Hydrogen bonds	Pi-Pi stacking	Pi-cation
1	0	0	0	0
8	1.75	1	2	0
15	3.5	1	0	1
22	5.25	1	0	0
29	7.0001	3	1	0
36	8.7501	2	0	0
43	10.5001	2	0	0
50	12.2501	2	1	0
58	14.2501	1	1	0
65	16.0001	2	0	0
72	17.7501	2	1	0
79	19.5002	2	0	0
86	21.2502	2	0	0
93	23.0002	2	1	0
100	24.7502	2	1	0

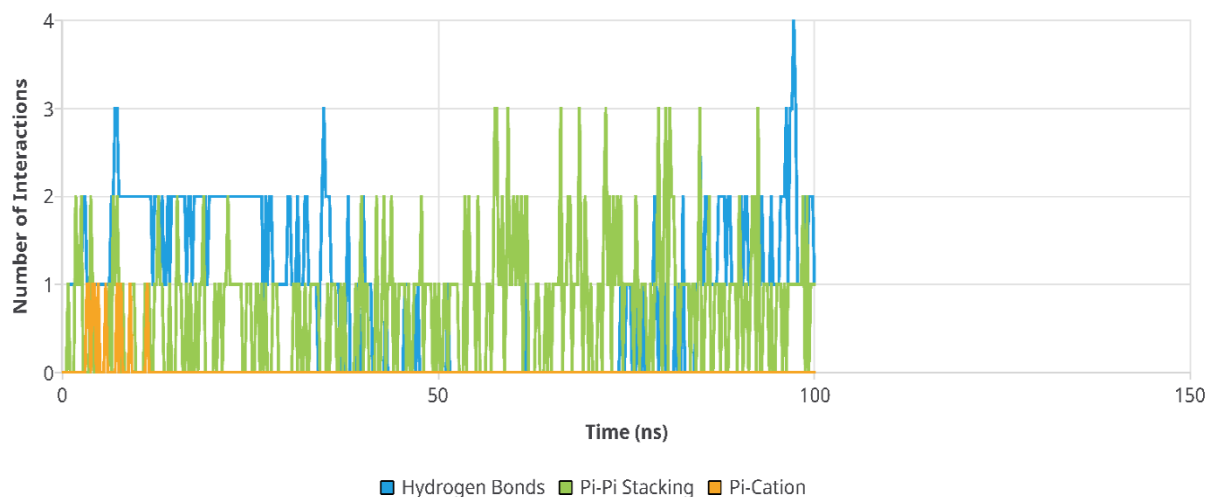


Figure 5: Number of interactions vs. time of compound 6b

although these differences were tiny and didn't impact the structure. This showed that 6b stabilized binding-site residues in a robust complex while keeping peripheral loops flexible. The RMSF map (Figure 6) illustrates how 6b–3ERT residues varied during simulation. The purple line displays RMSF values (in Å) for each residue, whereas the orange line indicates atom mobility (B-factor). The graph indicates that the protein backbone was stable, with residues exhibiting low to moderate fluctuations (less than 3 Å). Peaks around residue indices 45 and 90 indicated surface loops or terminal sections with little flexibility. The central remnants were stiff, but these portions were flexible. Throughout the simulation, ligand 6b maintained a stable and compact protein–ligand pair.

Gyration radius

Table 7 displays fifteen 6b–3ERT trajectory frames. Each frame stored the index, simulation time, and complex "Radius of Gyration" value. This showed how the protein-ligand combination grew or shrank during simulation. The radius of gyration values did not change significantly between measured frames. The size and compactness of 6b–3ERT complex remained basically the same throughout the procedure. There was no significant increase or decline, suggesting that the protein was unfolding or collapsing. Instead, the complex varied around a constant Rg. This indicated a well-folded structure and stable 6b in the 3ERT binding pocket. The radius of Gyration (Rg) measures protein packing during dynamics. In the simulation, Rg barely changed from 12.0 to 12.8 Å (Table 7). This stability meant that the complex was still tight and well-folded and the protein structure hadn't changed. Small oscillations

reveal proteins are breathing, and the curve's consistency shows that 6b binding does not structurally disrupt 3ERT throughout simulation. Table 8 displays 15 frames from 6b–3ERT complex's molecular dynamics trajectory with Molecular Surface Area (MSA) values for different time periods. This data showed how the protein–ligand complex's exposed surface area changed over time. The MSA values varied between sample frames, usually within a limited range. Solvent exposure remained constant throughout the simulation. This indicates that the compound remained compact and solvated without disintegrating. The 100 ns simulation shows the 6b–3ERT complex remained well-structured and stable in solvent. Minor conformation breathing motions or side-chain reorientations may boost MSA at particular frames.

Table 6: Root Mean Square Fluctuation (RMSF) of compound 6b

Frame	Residue Index	Residue	RMSF (B; Per Residue)
0	0	A: GLU_323	0.98228
6	6	A: SER_329	1.565756
13	13	A: ALA_350	0.74535
20	20	A: MET_357	0.903327
27	27	A: LEU_379	1.082907
34	34	A: ILE_386	0.657023
41	41	A: TRP_393	0.621654
48	48	A: PHE_404	0.963795
54	54	A: LEU_410	1.198378
61	61	A: MET_421	1.414174
68	68	A: LEU_428	0.556868
75	75	A: HIS_516	1.015565
82	82	A: GLU_523	1.724791
89	89	A: LYS_529	5.234677
96	96	0	2.570453

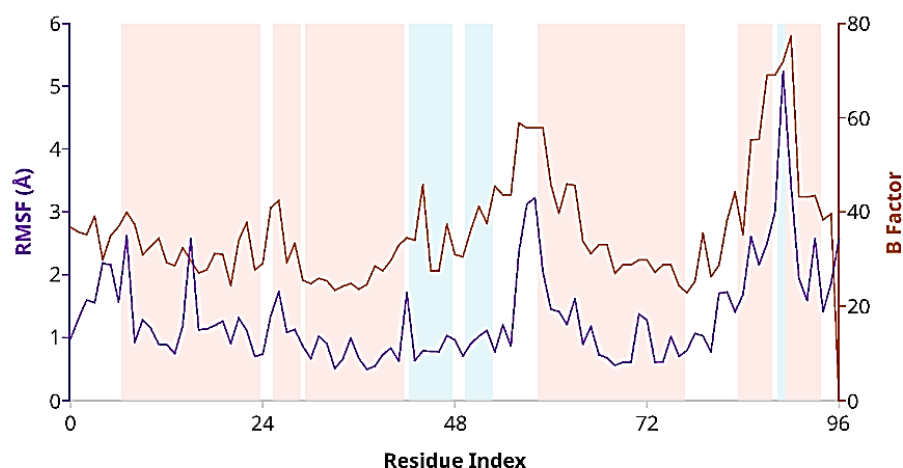


Figure 6: RMSF map of compound 6b

Table 7: Radius of gyration of compound 6b

Frame	Time (nanoseconds)	Radius of Gyration 1 (673 atoms)
1	0	12.11714
30	7.2501	12.05146
58	14.2501	12.35049
87	21.5002	12.31348
115	28.5002	12.51474
144	35.7503	12.4484
172	42.7503	12.38411
201	50.0004	12.36494
230	57.2505	12.35703
258	64.2505	11.9965
287	71.5006	12.3125
315	78.5006	12.76668
344	85.7507	12.72671
372	92.7507	12.67472
401	100	12.58187

Table 8: Molecular Surface Area (MSA) of compound 6b

Frame	Time (nanoseconds)	Molecular Surface Area 1 (673 atoms)
1	0	4198.302
29	7.0001	4517.377
58	14.2501	4565.545
86	21.2502	4649.206
115	28.5002	4595.42
143	35.5003	4491.191
172	42.7503	4418.214
201	50.0004	4589.777
229	57.0005	4526.518
258	64.2505	4085.808
286	71.2506	4300.547
315	78.5006	4734.898
343	85.5007	4777.426
372	92.7507	4817.206
401	100	4622.188

Polar surface area

Table 9 shows 15 molecular dynamics (MD) trajectory frames. All columns, including time,

frame, and polar surface area, remained. At several times in the 100 ns simulation, the solvent exposed 6b–3ERT complex's polar region reacts. The solvent remained because the polar surface area changed across several measurement frames. Protein surface side-chain and loop motions generated modest up-and-down alterations without structural rearrangements. The stable PSA profile, Rg and RMSD suggested that 6b–3ERT complex maintained a hydrated, structured binding environment throughout the simulation. The 6b–3ERT complex's Polar Surface Area (PSA) varied during the 100 ns molecular dynamics simulation (Table 9). The PSA levels fluctuated between 1500 - 1850 Å² over the course of the study. Despite some side chain or loop movements, the polar portions of the complex remained exposed to solvents. The 6b–3ERT system's stable and non-changing polar surface provides stable hydrogen bonding and good solvation throughout the simulation.

Table 9: Polar Surface Area of compound 6b

Frame	Time (ns)	Polar Surface Area 1 (673 atoms)
1	0	1512.278
29	7.0001	1624.787
58	14.2501	1616.985
86	21.2502	1720.055
115	28.5002	1720.945
143	35.5003	1665.094
172	42.7503	1728.507
201	50.0004	1740.059
229	57.0005	1705.504
258	64.2505	1588.87
286	71.2506	1698.026
315	78.5006	1741.805
343	85.5007	1720.7
372	92.7507	1703.803
401	100	1730.523

DISCUSSION

Molecular dynamics (MD) simulation revolutionized computational science by allowing researchers to see and understand biological macromolecules' complex atomic movements and interactions. Molecular dynamics was founded in the late 20th century to examine the actual dynamics of molecules in a simulated environment. Over the past decades, processing power, algorithms, and force fields have allowed simulations of more complicated systems, from tiny peptides to virus particles, in nanoseconds, microseconds, and milliseconds [16]. Molecular dynamics simulation offers an unmatched view of a biomolecule's "life" rather than just a computational curiosity. MD examines how each atom moves under the influence of other atoms and the environment (such as water and ions) by calculating Newton's equations of motion for thousands to millions of atoms [17]. This technology allows atomic-level observation of molecular flexibility, conformational changes, folding and unfolding, and interaction formation [18]. Experimental methods alone cannot do this. This ability is crucial since enzymes, receptors, and nucleic acids are dynamic entities whose activity often depends on their ability to sample different structural states [19]. MD is crucial to modern bioscience. It bridges the gap between static structures like X-ray crystallography and cryo-EM and the complex, dynamic surroundings of biomolecules [20].

In this study, a new set of twelve 1,2,3-triazole derivatives was developed using three *in silico* study techniques. The molecular docking results proved that 10 compounds (6a-6j) showed high PLP fitness comparable to the reference compound (methotrexate) [21], which had a PLP fitness of 70.544. A higher score signifies a more favorable interaction between the ligand and the protein, suggesting a more stable and energetically preferred binding pose. The pharmacokinetic results reveal that all of the designed compounds were well absorbed from the GI tract, did not penetrate the blood-brain barrier and could be administered orally [22]. The molecular dynamic simulation results confirmed the docking results for compound 6b and proved that 6 b ligand remains coupled to 3ERT's active site, indicating high structural stability and equilibrium.

CONCLUSION

In silico findings of twelve novel 1,2,3-triazole molecules for potential use as drug candidates have been reported. The 6b molecule was found to have excellent *in silico* results as anticancer

drug candidate. Further studies would be required to explore the synthetic and characterization profiles, as well as *in vitro* and *in vivo* studies.

DECLARATIONS

Acknowledgement/Funding

The authors are grateful to Al-Nahrain University, College of Pharmacy in Baghdad, Iraq, for their assistance in completing this study.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. TMH and KMA conceived and designed the study, KAA and AAH collected and analyzed the data, and AAA wrote the manuscript. All authors read and approved the manuscript for publication.

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