

Original Research Article

***In silico* evaluation of binding interactions and ADME properties of new 1, 2, 3-triazole derivatives of benzimidazole as antibacterial agents**

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Abstract

Purpose: To predict the antibacterial effects of 1, 2, 3-triazole derivatives of benzimidazole (designated 5A, 5B, and 5C) against four different bacterial species, i.e., *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.

Methods: The ADME and pharmacokinetic properties of the designed compounds were predicted *in silico* on the SwissADME server, while Schrödinger suite was used for molecular docking and molecular dynamics simulations.

Results: The compounds obeyed Lipinski's rule of five and were orally absorbable, as predicted using SwissADME. Molecular docking studies predicted favorable binding for the compounds. Molecular dynamics simulations of the 3G7E–ligand complex revealed rapid equilibration, followed by stable structural behavior across the 100 ns trajectory.

Conclusion: These findings indicate that one of the compounds, i.e., 5C, is a promising candidate for further investigations as a potential antibacterial agent, and may serve as a starting scaffold for future pharmacological studies.

Keywords: 1,2,3-triazole benzimidazole, Antibacterial drugs, Design, Molecular docking, Molecular dynamics

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INTRODUCTION

Researchers can practically improve binding affinity and generate more effective pharmacological medicines using molecular modeling, a strategy which is one of the most appealing methodologies utilized in drug design [1]. Resistant microorganisms are a major issue in modern medicine. Consequently, it is crucial to screen and identify new antibacterial medications

that are chemically distinct from the known antibiotics [2]. Since benzimidazole is a significant pharmacophore in pharmacology and medicinal chemistry, poly-heterocycle synthesis based on benzimidazole and its derivatives has attracted the interest of pharmacists in the past few decades [3]. 1,2,3-Triazole is a five-membered, nitrogen-containing aromatic heterocycle composed of two carbon atoms and three nitrogen atoms, and its derivatives

represent important scaffolds for the development of therapeutic agents [3]. Indeed, derivatives of 1,2,3-triazole have attracted considerable interest in medicinal chemistry, particularly as potential antimicrobial agents. Both benzimidazole–triazole hybrids and the related triazole derivatives have shown promising activities in biological assays and molecular docking studies [3, 4]. For instance, Rostovtsev *et al.* reported that a copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) selectively produced 1,4-disubstituted 1,2,3-triazoles in high yields [5].

Computational methods are now routinely used at different stages of drug discovery, from identifying initial hits to optimizing lead compounds. Molecular docking is one of the most widely used approaches because it predicts how a small molecule may fit within the binding site of a target protein while providing an estimate of the favorability of the interaction. These predictions help researchers select the most promising compounds for further experimental testing [1]. Researchers can now view and study the complex atomic movements and interactions that govern the behavior of biological macromolecules using molecular dynamics (MD) simulation, a revolutionary step in computer science. The need to go beyond static

molecular representations and investigate the genuine dynamics in a simulated setting gave rise to MD in the late 20th century [6].

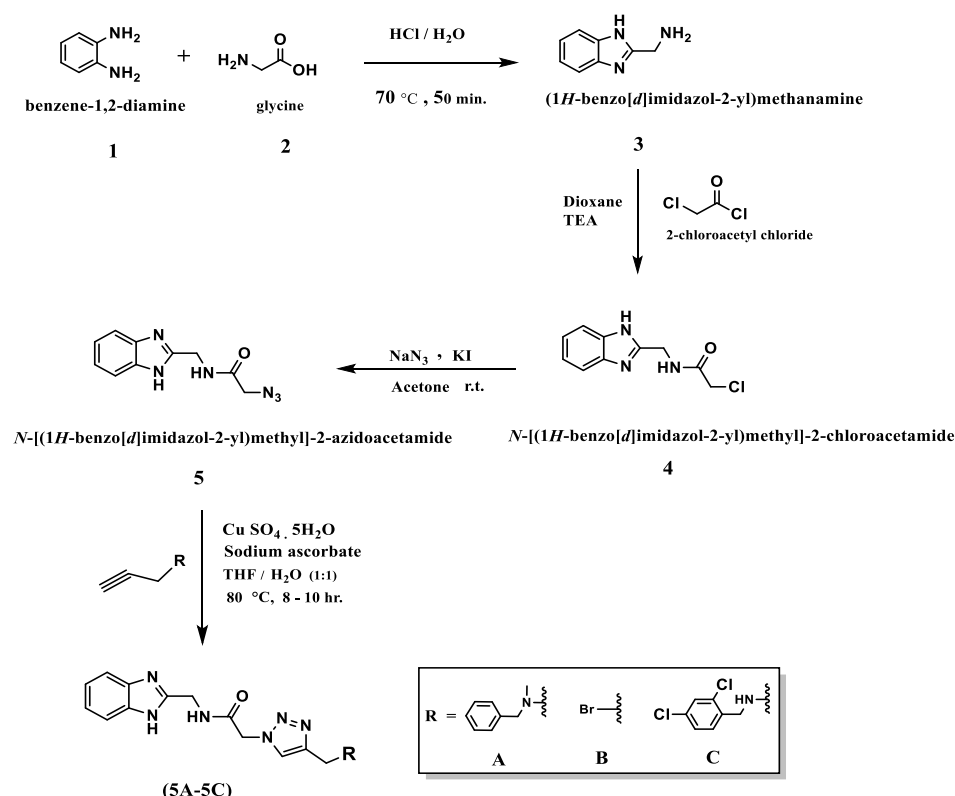
EXPERIMENTAL

Proposed synthetic route

The proposed synthetic route for compounds 5A–5C is shown in Scheme 1.

Computational methods

The prediction of the absorption, distribution, metabolism, and excretion (ADME) of the new compounds was conducted using SwissADME [7]. This docking method was used to predict the orientation and conformation of each ligand inside the binding site of the target [1]. The molecular blueprints of the novel compounds [5A, 5B and 5C] were created using ChemAxon's Marvin JS. These structures were given the SMILES moniker with the SwissADME algorithm in order to predict physicochemical and pharmacokinetic properties [7]. Finally, the BOILED EGG approach was used to investigate the polarity, lipophilicity, passive gastrointestinal absorption, and brain penetrability of the compounds [8].



Scheme 1: Proposed synthetic route for the designed benzimidazole–1,2,3-triazole derivatives

Molecular docking studies for the designed compounds

The 3D conformations of the three derivatives were obtained using the ChemDraw 16.0 application, which is part of the ChemOffice software suite. Thereafter, the SDF file was used to include the lowest-energy configurations of the molecules. In addition to the Monte Carlo approach, the optimization procedure made use of the Spartan 14.0 package, which comprised 200 optimizations involving 1500 interactions. The Glide program, which is part of Schrödinger software, was used for molecular drug design and docking assessment [9]. Virtual screening (VS) computer tools were used to choose compounds on the basis of target receptor binding capabilities. The designed compounds were docked onto the active sites of the receptors of Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria using crystal structures.

The orientation and binding affinity of each compound to amino acid residues were examined in virtual screening (VS). The five ligands evaluated in the docking study comprised the three designed derivatives (5A, 5B, and 5C) and two reference antibiotics (trimethoprim and cephalixin). The Protein Data Bank Identifiers (PDB IDs) of the four bacterial protein targets evaluated with these drugs were azobenzene reductase from *Bacillus subtilis* (1NNI), dihydrofolate reductase from *Staphylococcus aureus* (2W9S), DNA gyrase B from *Escherichia coli* (3G7E), and New Delhi metallo- β -lactamase-1 from *Klebsiella pneumoniae* (4HL2). The reduction and optimization tool, ProPrep, was used to carry out the processes necessary for the preparation of these proteins [10]. Using the LigPrep software, hydrogen atoms were added to the ligands, and the correct orientation and ionization state of each compound were determined. Moreover, the lowest energy conformation of each ligand was obtained using the OPLS 2005 force field. Then, the compounds were docked within the active site of the bacteria. The best-scoring pose for each ligand was selected based on the Glide docking score [9]. The three derivatives (5A, 5B, and 5C) were used in drug design evaluation.

Molecular dynamics simulation

In this investigation, the Desmond software package was used to conduct all molecular dynamics simulations [11]. The default settings and standard procedures were used in order to ensure reliability and repeatability. In Maestro,

the structures were produced by adding missing hydrogen atoms, correcting protonation states, and removing unnecessary water molecules. Energy minimization was performed using Desmond's built-in technique to eliminate steric conflicts [11]. A TIP3P water model with a 10 Å buffer was used for solvation of the complexes, while neutralization was done with Na⁺ and Cl⁻ ions [12].

The interactions between molecules were accurately described using OPLS4 force field [13]. The NVT and NPT ensembles were used for equilibration to stabilize the temperature and pressure at 300 K and 1 atm, respectively [14]. System stability was assessed using RMSD, Rg, and hydrogen-bond analysis, and production MD simulations were run for 100 ns with a 2 fs time step [11, 14].

RESULTS

ADME data

The topological polar surface area (TPSA) values of the three compounds (5A, 5B and 5C) were below 140Å², signifying high degrees of permeability and bioavailability. It seems that the compounds are likely to be well absorbed, as the majority of ligands were absorbed well in the gastrointestinal system. In addition, the bioavailability score of the compounds was 0.55. Lipinski's "rule of five" states that compounds meant to be taken orally should possess certain properties, for example, molecular weight (MW) less than 500 Dalton, number of hydrogen bond donors below 5, hydrogen bond acceptors less than 10, and octanol-water partition coefficient (log P) less than 5. The results presented in Table 1 show that all the designed compounds met the requirements of the rule of five in terms of MW, H-bond acceptors, and TPSA.

Docking results interpretation

The binding affinity scores for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* were -3.443 to -2.385 kcal/mol, -5.91 to -5.224 kcal/mol, -6.487 to -6.089 kcal/mol, and -6.512 to -5.818 kcal/mol, respectively. Compound 5C showed the most favorable docking scores against *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, whereas compound 5A exhibited the most favorable docking score against *Staphylococcus aureus*. For 5C, the docking scores against *E. coli* and *K. pneumoniae* were -6.487 and -6.512 kcal/mol, respectively.

Table 1: Predicted ADME properties of the designed compounds (5A-5C)

Compound	5A	5B	5C
Formula	C ₂₁ H ₂₃ N ₇ O	C ₁₃ H ₁₃ BrN ₆ O	C ₂₀ H ₁₉ Cl ₂ N ₇ O
MW (g/mol)	389.46	349.19	444.32
H-bond acceptor	5	4	5
H-bond donor	2	2	3
Molar refractivity (m ³ /mol)	110	80.87	115.12
TPSA (Å ²)	91.73	88.49	100.52
GIT absorption	High	High	High
BBB permeability	No	No	No
Lipinski violation	0 violation	0 violation	0 violation
Bioavailability	0.55	0.55	0.55

The docking scores, binding orientations, and interactions with the selected bacterial protein targets are presented in Table 2 and Figures 1 and 2. The main interactions observed were hydrophobic, π -cation and π -bonding associations, which may contribute to the binding of the compounds to the selected protein targets.

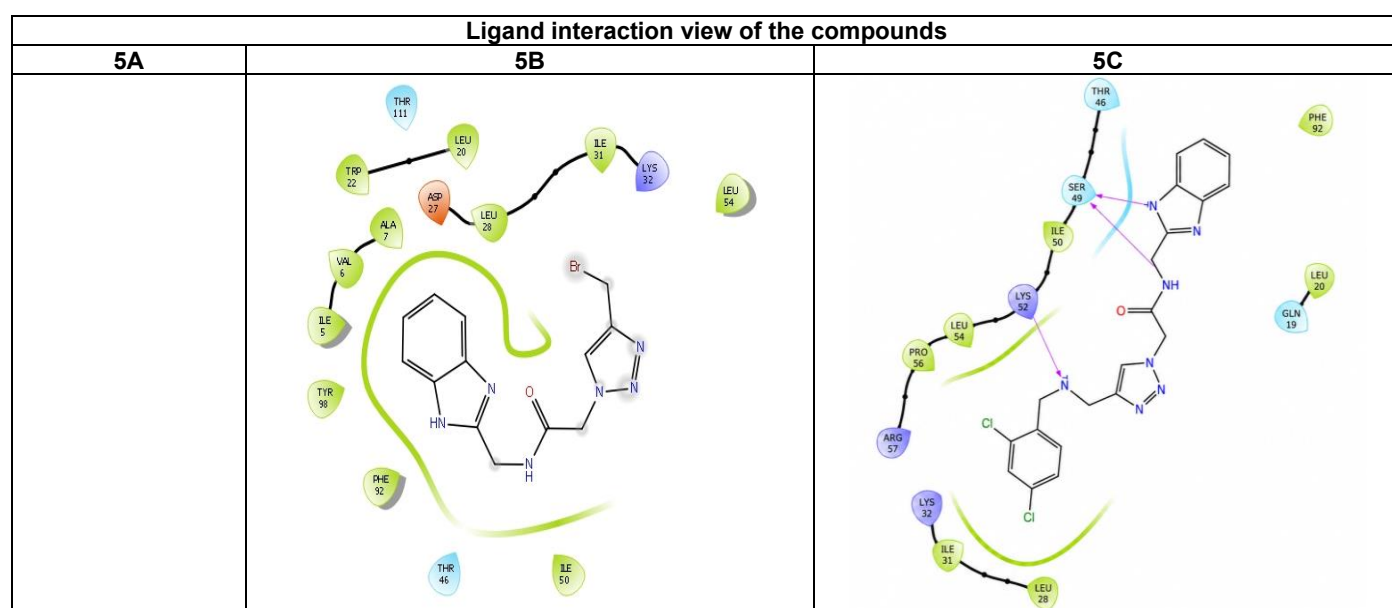
Molecular dynamics of the designed compounds

The results showed that the RMSD distance from the reference structure of 3G7E–ligand system rose initially from ≈ 0 at 0 ns to an early-window

mean of ~ 1.69 Å. This adjustment brought the RMSD to a plateau of ~ 2.43 Å, with a standard deviation (SD) of ~ 0.32 Å, where the fluctuations were bounded around an equilibrated state. The values varied in the range of ≈ 0 to -3.19 Å, with the last part of the simulation yielding ~ 2.79 Å, which was still within the established plateau range, and the last value was ~ 3.01 Å. In summary, the RMSD profile indicates that the RMSD of the 3G7E complex reached a fairly stable form in the initial adjustment stage and changed only slightly during the subsequent simulation period.

Table 2: *In silico* docking scores (kcal/mol) of the designed compounds and reference antibiotics

Compound	G-positive bacteria		G-negative bacteria	
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumonia</i>
5A	-2.385	-5.91	-6.283	-5.818
5B	-3.101	-5.224	-6.089	-6.025
5C	-3.443	-5.513	-6.487	-6.512
Trimethoprim	-3.045	-9.486	-5.971	-5.43
Cephalexin	-4.245	-5.361	-6.472	-6.864

**Figure 1:** The interaction diagrams of compounds 5A, 5B, and 5C within the active site of dihydrofolate reductase from *Staphylococcus aureus* (PDB ID: 2W9S)

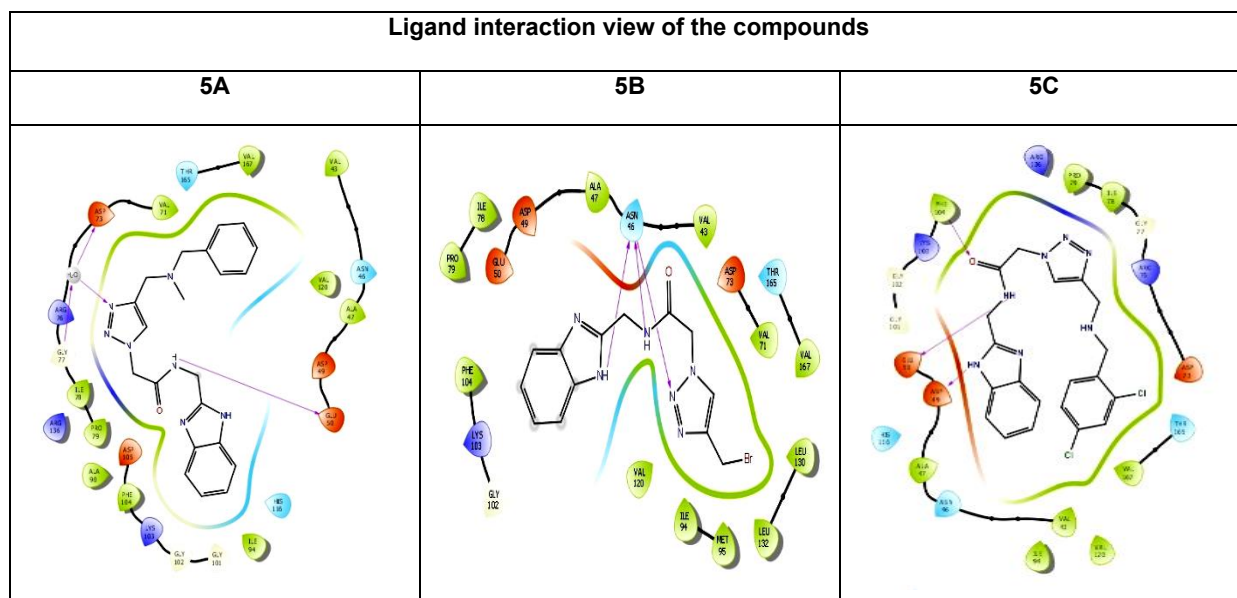


Figure 2: The interaction diagrams of compounds 5A, 5B, and 5C within the active site of DNA gyrase B from *Escherichia coli* (PDB ID: 3G7E)

The interaction profile of 3G7E–ligand system revealed that non-covalent contacts were maintained during all the simulations. The π -cation interactions were less common, with an average of ~ 0.18 occurring per frame, and a maximum of two occurring simultaneously. In contrast, π - π stacking and halogen bonding were either absent or very weak. Overall, hydrogen bonding was the most important interaction during the simulation, while π -cation contacts were found only occasionally. The per-residue RMSF profile indicated low RMSF values for most of the residues, although high RMSF values were observed in few regions with high flexibility. The average fluctuation was ~ 1.01 Å (median = ~ 0.78 Å), with most residues remaining within the 0.5–1.0 Å range, suggesting limited residue-level flexibility during the simulation. A limited set of amino acid residues showed clear peaks, particularly residues 22

(~ 2.41 Å), 23 (~ 2.38 Å), and 16 (~ 1.76 Å). The RMSF peaks were localized rather than widespread, while most of the remaining residues maintained relatively low RMSF values, in line with the overall stability observed in the RMSD profile. The RDF describes the spatial distribution of atoms around the reference group. For the 3G7E–5C protein–ligand complex, a sharp first peak was observed at ~ 1.1 Å, with $g(r) \approx 117$, indicating a preferred short-range separation. After this peak, $g(r)$ decreased to its first minimum at ~ 1.9 Å, which marked the end of the first coordination shell. Beyond ~ 2 Å, the RDF function for the 3G7E–5C protein–ligand complex exhibited smaller oscillations and gradually approached a baseline. Overall, the RDF profile revealed strong and specific local contacts anchoring the ligand. The RMSD plot, the interaction plot, the RMSF plot, and the RDF plot are shown in Figures 3–6 below.

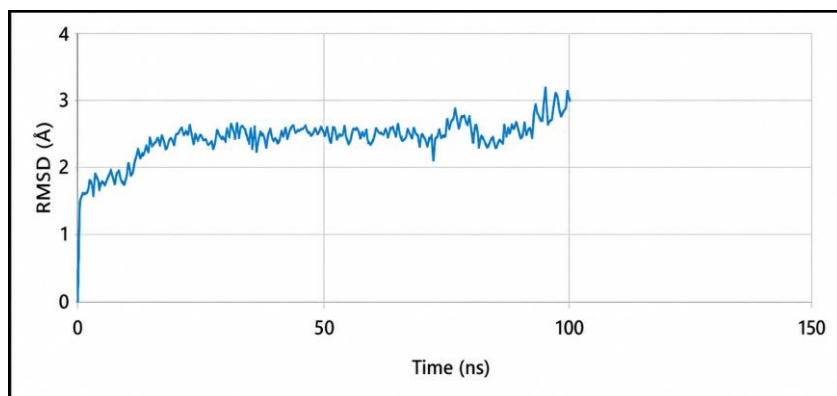


Figure 3: The RMSD plot of the 3G7E- ligand complex

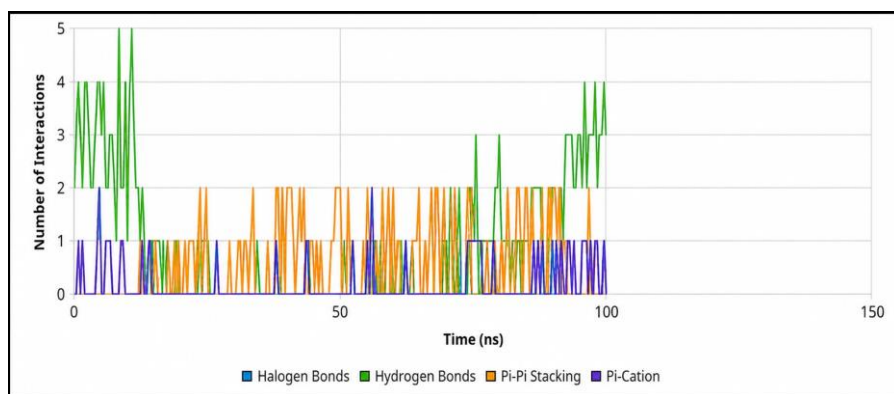


Figure 4: The interaction plot of the 3G7E- ligand complex

The RMSD plot of 3G7E-ligand complex indicates early equilibration with stable fluctuations within $\sim 2.4 - 2.9 \text{ \AA}$, but without any signs of unfolding or drift in plot. This means that the 3G7E-ligand complex had a stable conformation during the 100 ns simulation.

The 3G7E-ligand complex interaction plot indicates that hydrogen bond interactions were the most common for the entire simulation, with some π -cation contacts present. In contrast, π - π stacking and the halogen bonding interactions were either negligible or absent. In general,

these contacts did not produce any large changes in binding during the 100 ns of simulation.

The RMSF plot revealed minimal fluctuations in most residues (approximately $0.5 - 1.0 \text{ \AA}$), suggesting a generally rigid structure of the protein. The B-factors agreed with the appearance of higher flexibility ($\sim 2.5 \text{ \AA}$) only in loop and terminal regions. These findings validate a stable structural scaffold and indicate some dynamic flexibility in the 3G7E-ligand complex at a local level.

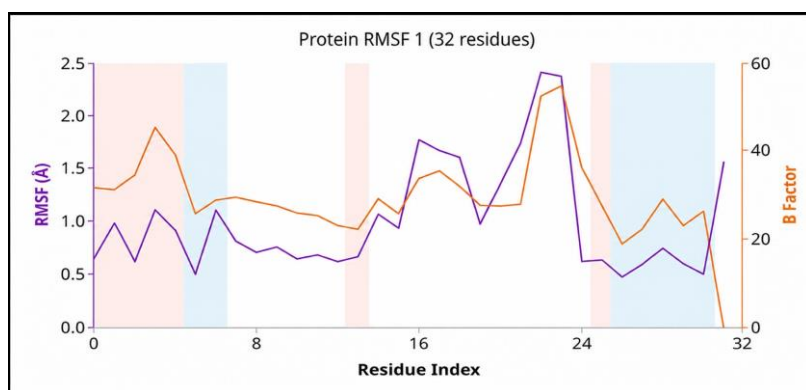


Figure 5: The RMSF plot of the 3G7E-ligand complex

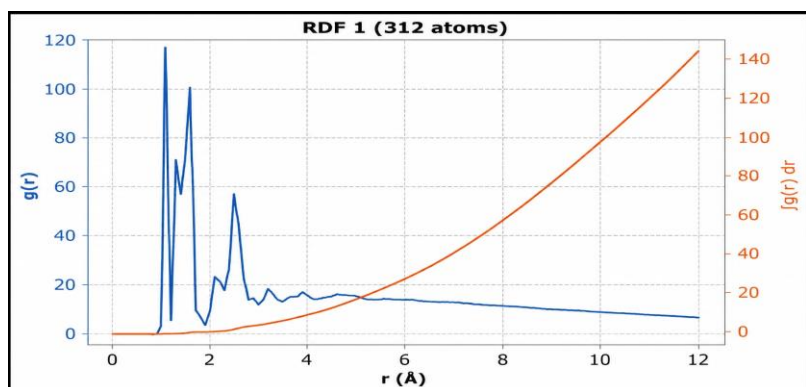


Figure 6: The RDF plot of the 3G7E- ligand complex

The RDF plot displayed a strong peak at ~ 1.1 Å ($g(r) > 110$) in the short-range region, which signifies the presence of strong short-range interactions within the first coordination shell. Secondary peaks were smaller than the main peak at 1.5–3.0 Å, indicating weaker additional shells, whereas at > 3.5 –4.0 Å, the curve tended towards a flat baseline, which is characteristic of disordered solvent. The cumulative integral confirms that most neighboring density lay within the first solvation shell.

DISCUSSION

The results from the present study indicate that the three designed benzimidazole–1,2,3-triazole derivatives had favorable predicted drug-likeness and target-dependent binding profiles against four bacterial proteins. This finding is in agreement with the report of broad anti-infective potential of benzimidazole–triazole hybrids by Marinescu [3]. In addition, Mendapara *et al.* reported that the related benzimidazole–1,2,3-triazole hybrids displayed *in vitro* antimicrobial activity together with favorable molecular docking interactions [15]. Similarly, Alkhaldi *et al.* have described antimicrobial effects of 1,2,3-triazole derivatives in a study supported by molecular docking against glyceraldehyde-3-phosphate dehydrogenase [4]. These previous findings support the selection of the benzimidazole–triazole scaffold in the present study. However, unlike in the previous investigations cited, the antibacterial potential of the present derivatives was evaluated computationally. Therefore, the current results support the prioritization of these compounds for further investigations, but do not constitute experimental confirmation of antibacterial properties.

The docking findings also suggest that the binding behavior of the designed derivatives depends on both the substituent and the bacterial target. The predicted interactions with *Staphylococcus aureus* dihydrofolate reductase (PDB ID: 2W9S) are consistent with the findings of Haque *et al.*, who investigated benzimidazole derivatives as potential bacterial dihydrofolate reductase inhibitors [16]. Compound 5C was further evaluated in complex with *Escherichia coli* DNA gyrase B (PDB ID: 3G7E) using molecular dynamics simulation. This bacterial target was also investigated by Maurya *et al.*, who reported *in vitro* antibacterial activity of benzimidazole hybrids and predicted DNA gyrase inhibition [17]. Together, these data indicate that benzimidazole-based hybrids may accommodate different bacterial binding pockets. However, molecular docking does not suffice to confirm multi-target inhibition: direct enzyme-inhibition

assays are needed to validate the proposed mechanisms. Other computational studies have been conducted on azobenzene reductase from *B. subtilis* [18] and NDM-1 from *K. pneumoniae* [19]. Moreover, Shah *et al.* used computational design to study the antimicrobial activities of triazolo-benzimidazole derivatives [20].

In the present study, molecular dynamics simulation was performed for 100 ns in order to gain more insight into the stability of the 3G7E–5C protein–ligand complex. After one initial adjustment in the structure, the RMSD was found to lie within a bounded plateau with a global mean of about 2.43 Å and a standard deviation of about 0.32 Å. Majority of the residues had low RMSF (around 1.01 Å), with only a small number of residues having higher RMSF values. The most stable interactions were hydrogen bonds, with an average of almost one bond per frame and a maximum of five simultaneously formed bonds. Halogen bonding and π – π stacking were negligible, while π -cation interactions were not frequent. In addition, a strong first RDF peak appeared near 1.1 Å, while a first RDF minimum occurred near 1.9 Å, suggesting localized contacts around compound 5C. Altogether, these observations are compatible with a predicted binding mode which were maintained during the simulated time. However, stability on one molecular dynamics trajectory alone is not sufficient for establishing antibacterial efficacy and sampling convergence. This interpretation would be strengthened through replication of simulations and appropriate uncertainty analyses [6,14]. All three designed derivatives (5A–5C) showed good gastrointestinal absorption and fulfilled Lipinski's rule of five, as was predicted using SwissADME [7]. These properties indicate the suitability of these ligands for use in further screening, but are not meant to be taken as an indication of experimental confirmation of oral bioavailability, metabolism and safety.

Study limitations

The principal limitation in the present study is the absence of biological validation. Thus, there is need for minimum inhibitory concentration and minimum bactericidal concentration assays, in addition to direct inhibition studies against the selected bacterial enzymes, mammalian-cell cytotoxicity testing, and experimental pharmacokinetic evaluation. Notwithstanding these limitations, compound 5C represents a rational candidate for further investigations because the predicted binding mode within DNA gyrase B was maintained throughout the 100-ns simulation. Future studies using appropriate *in vitro* and *in vivo* assays will be focused on

evaluation of the pharmacological and biological activities of compounds 5A–5C, in addition to potential adverse effects and toxicity.

CONCLUSION

Among the designed derivatives, compound 5C showed the most favorable overall binding profile against the investigated bacterial targets. The predicted binding for 5C was comparable to that of cephalexin for some of the investigated targets, particularly *E. coli*. Overall, compound 5C showed promising predicted binding across the investigated bacterial targets and may therefore be considered a suitable candidate for further investigations. *In silico* ADME analysis using SwissADME indicated that compounds 5A, 5B, and 5C complied with Lipinski's rule of five, although gastrointestinal absorption was computationally predicted rather than experimentally measured.

DECLARATIONS

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Ethical approval

Not applicable.

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.

Asmaa Adnan Abdulnabi conceived and designed the study, performed the molecular docking and ADME analyses, collected and analyzed the data, and contributed to manuscript writing.

Kanar M. Alawad (Corresponding Author) supervised the work, validated the methodology, revised the manuscript, and handled communication and submission to the journal.

Jessica Shlmoon Hanna performed the molecular dynamics simulations and contributed to the interpretation of the simulation results.

Kany A. Abdulqader assisted in docking simulations, data organization, and visualization. Tiba M. Hameed contributed to literature review, manuscript formatting, and technical editing.

Zainab M. Alawad assisted in ADME profiling, proofreading, and minor revisions.

Humam L. Qusay provided scientific consultation, contributed to interpretation of the results, and participated in reviewing the final version of the manuscript. All authors read and approved the manuscript for publication.

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