Current Trends in Biotechnology and Pharmacy
Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

Molecular Dynamics Study of Quinazoline Compounds Complexed with Filamenting Temperature-Sensitive Z Protein and Gyrase Subunit B as Potential Antibacterials

Min Yue Yeong[1], Nik Danial Al-Fadli[1], Waheedah Abdul Hakeem[1], and Imtiaz Khalid Mohammed[1]*

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Cyberjaya, Selangor, Malaysia

*Corresponding author: imtiaz@cyberjaya.edu.my

Abstract

As the years go by, bacteria such as *Staphylococcus aureus* and *Mycobacterium tuberculosis* have developed resistance towards the current antibiotics which leads to ineffectiveness of antibacterial agents to kill or inhibit the bacteria. Thus, in order to overcome this issue, quinazoline derivatives have been proposed as the potential new antibacterial agents due to their antibacterial properties. Molecular dynamics, a computational technique, has been conducted in this study to determine the potential of quinazoline compounds as a novel antibacterial agent for *Staphylococcus aureus*'s DNA gyrase subunit B (GyrB) and *Mycobacterium tuberculosis*'s Filamenting temperature-sensitive Z (FtsZ) protein. Molecular dynamics simulation of the top 2 docked ligands of quinazoline for each FtsZ and GyrB were conducted by using Amber22 molecular dynamics simulation software. The analyses were conducted with *cpptraj*to evaluate the stability and binding interaction of the compounds with the target receptors.The dynamic studies of Q100 complexed with FtsZ show it is the most stable, with lower RMSD values (1.4Å for Q100 while FtzZ is 2.3Å) and lesser overall variation in RMSF. Although Q100 does not form a significant hydrogen bond with FtsZ, it has a higher negative free energy binding value (-25.48 kcal/mol) compared to Q56 with favourable hydrophobic and electrostatic interaction. While Q44 also shows the complex with GyrB is slightly more stable, with lower RMSF in residue 1 (3.0Å), stable RMSD (1.2Å for Q44 and 2.6Å for 3U2D), and a higher negative value of free binding energy (-23.21 kcal/mol) with favourable hydrophobic interaction. However, Q44 does not form a significant hydrogen bond as the occupancy is nearly zero. Q100 and Q44 have the most potential quinazoline derivative to act on the FtsZand GyrBrespectivelyto continue to the next step in drug design as a new antibacterial drug candidate.

Keywords: Molecular dynamics; Quinazoline derivatives; Filamenting Temperature-Sensitive Z Protein; DNA gyrase subunit B; AMBER

Introduction

As the years go by, bacteria have developed resistance towards the current antibiotics that were used in treating various types of bacterial infections through different mechanisms. The causes that enforce the bacteria to develop resistance against the current antibiotics has been reported is due to the excessive use and abuse of the antibiotics. In recent years, a large number of gram-negative and gram-positive bacteria have become unresponsive towards a wide range of antibiotics, and this leads to ineffectiveness of antibacterial agents to kill or inhibit the bacteria (1). Every year, more than 2.8 million antimicrobial-resistant infections occur and lead to more than 35,000 people die as a result in the United States based on the CDC's 2019 Antibiotic Resistance (AR) Threats Report. According

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

to the World Health Organization (WHO), in 2019, at least 1.27 million of the people worldwide were killed due to antimicrobial resistance. The WHO thus mentioned antimicrobial resistance as an urgent global public health threat (2).

Heterocyclic compounds represent a vital group of biologically active compounds that may be useful in drug discovery and development. Compounds that consist of quinazoline, a nitrogen-containing heterocyclic, as its moiety has drawn the scientist's attention in the last few years because of their significant biological activities(3). Quinazoline possesses a wide range of pharmacological activities including anticancer, antifungal, anticonvulsant, analgesic, antibacterial etc. (3). Quinazoline structures bind at multiple sites with a high affinity due to its structure which benefits in medically active compound discovery to produce a novel drug to treat a particular disease(4). Thus, more future research on quinazoline structure can be done toprovide further insight in the pharmaceutical field.

Computer-Aided Drug Design (CADD) is a widely used technology that utilises computational methods to find, produce, and evaluate pharmaceuticals and other biologically active chemicals as well as tools for the modelling of compounds. By using CADD, researchers are able to study interactions between drugs and receptors with a lesser cost and effort in the pharmaceutical field in the drug discovery and development stage (5) thus allowing the development of new antibacterial to overcome the current situation of antibacterial resistance. CADD can be applied in molecular modelling which further aids in molecular docking as well as molecular dynamics (6).

Methodologies

The overview of this study is shown in Figure 1. The target structures which are

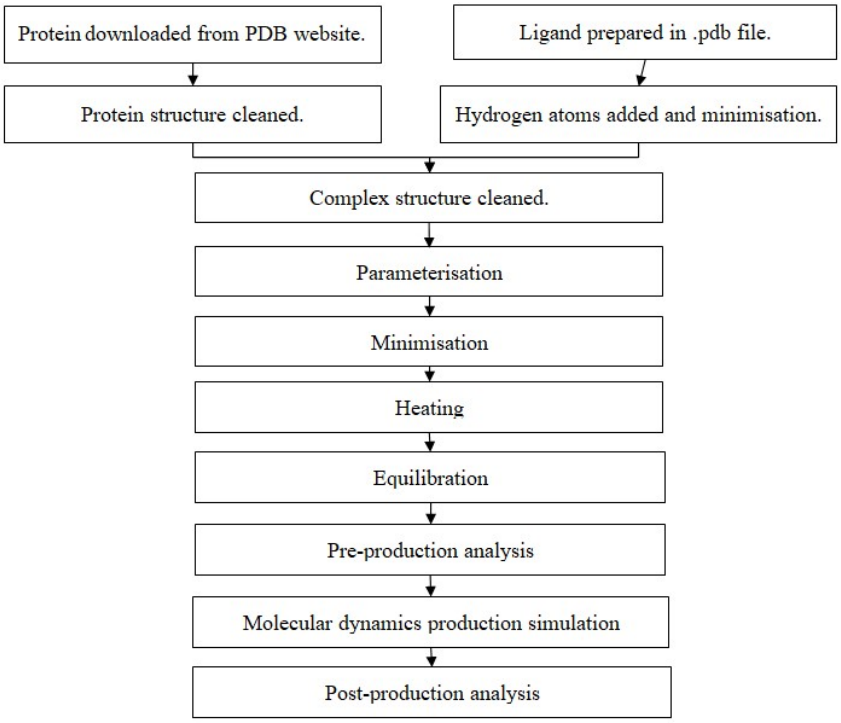

Figure 1: Overview of study

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

FtsZ (PDB ID: 6Y1U) and bacterial GyrB (PDB ID: 3U2D) were downloaded from Protein Bank Data (PDB) website developed by Research Collaboratory Structural Bioinformatics (RCSB) and the non-amino acid residues such as water molecules and ligands which are present were removed. The top 2 ranked ligands for each target protein were extracted from molecular docking results (7) and hydrogen atoms were added into the ligand molecules. The files of both target protein structure and docked ligand obtained were merged in order to form the complex by using text editor. The files were then saved as ".pdb" format and were visualised to ensure complex structures were merged correctly by using Discovery Studio Visualizer (DSV). Molecular dynamics simulation was performed using the Amber22 and after preparing the topology and coordinate files in the software which ff19SB and GAFF force field was applied. The complex structures were solvated in the OPC water model explicitly and neutralised by the counterionssubsequently. Minimization was conducted and the complexes was heated up to 310K for a total of 30 ps while SHAKE algorithm is applied to constraint the hydrogen atoms with the cut off distance of 9Å. The complexes underwent equilibration steps with constant pressure for a total of 300 ps. Perl script was used in order to conduct pre-production analysis including the properties such as density, total energy, potential energy, kinetic energy, temperature, volume, and pressure to assess the stability of the complexes before further continuation to the production run. 5 ns of molecular dynamics production run was performed for each complex respectively and the trajectories were recorded for the final post-production analysis which includes the parameters such as H-bonds analysis, root mean square deviation (RMSD), root mean square fluctuation (RMSF), and Molecular Mechanics with Generalised Born and Surface Area Solvation (MM/GBSA) by using cpptraj module in the software used.

Results and Discussion

Pre-production Analysis

Results of the pre-production analysis which emphasis density, total energy, potential energy, kinetic energy, temperature, volume, and pressure for the top two quinazoline derivatives complexed with the respective target protein, DNA gyrase subunit B (3U2D) and FtsZ (6Y1U) are shown in Figure 2. The findings indicate that the quality of simulation isassured, and the complexes are stable throughout the simulation run evidenced by the equilibrated graphs presented in the figure (8).

Post-production Analysis

Root Mean Square Deviation (RMSD)

RMSD of the quinazoline derivatives complexed with the target proteins throughout 5 ns of production run are shown in Figure 3. The fluctuation of RMSD ranging from 0.5Å to 2.6Å for 3U2D receptor and 0.2Å to 1.2Å for Q44 compound indicates that the 3U2D-Q44 complex is stable as the values are within the range of 3.4Å (9). Meanwhile, the RMSD profile of another ligand, Q48, complexed with 3U2D shows that the fluctuation for the receptor is ranging from approximately 0.5Å to 2.5Å while the deviation of the Q48 compound is ranging from 0.1Å to 1.2Å. The deviations in the latter complex are also shown to be within the range (9), indicating that the 3U2D-Q48 are also stable throughout the MD simulation. In comparison, 3U2D-Q44 and 3U2D-Q48 complexes are stable and do not differ much in deviation of both ligand and receptor.

The RMSD of 6Y1U is within the range of 0.5Å to 2.6Å while Q100 compounds exhibit RMSD ranging from 0.2Å to 1.5Å. Since the results fall within the range of less than 3.4Å, the variation suggests that the 6Y1U-Q100 complex is stable (9). The findings for 6Y1U-Q56, indicate that the RMSD of the receptor is roughly between 0.5Å to 2.6Å, while ligand is ranging from 0.2Å to 2.3Å. Hence, 6Y1U-Q56 complex is considered stable throughout the

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

5ns of MD simulations as the values show is within the acceptable range of less than 3.4Å (9) although significant deviation is observed in the RMSD of Q56. In contrast to the 6Y1U-Q56 complex, the 6Y1U-Q100 complex exhibits a slightly smaller variation of RMSD. Therefore, 6Y1U-Q100 is somewhat more stable than the other complex.

Root Mean Square Fluctuation (RMSF)

Based on the graph in Figure 4, the RMSF value at residue 1 in 3U2D-Q48 complex (4.1Å) is significantly higher than 3U2D-Q44 complex (3.0Å). Residue 184 in 3U2D-Q48 complex is also slightly higher with the value of 1.8Å while in 3U2D-Q44 is 1.2Å. Thus, residue 1 and residue 184 have a higher flexibility in 3U2D-Q48 complex during the simulation. Although residue 1 in both complexes has significantly higher peaks which suggest more flexibility than other residues in the complexes, only the RMSF values for 3U2D-Q44 are still within the permitted range of 3.4Å (9). Hence, Q44 is slightly more stable throughout the MD simulation than Q48 when binding to DNA gyrase subunit B.

The results of RMSF of the residues of the FtsZ is also demonstrated in Figure 4 which significant peaks are clearly shown in residue 56 in 6Y1U-Q56 and residue 161 in both 6Y1U-Q100 and 6Y1U-Q56 complex which suggests higher flexibility in these residues compared to the others. While the RMSF of most of the residues does not differ much in both complexes, 6Y1U-Q100 complex has shown more balanced peaks while 6Y1U-Q56 exhibits more significant fluctuations especially in residue 161 that reaches 2.5Å. However, the fluctuations in both complexes are still within the permitted range of 3.4Å (9). Hence, reveal that both complexes are stable throughout the MD simulations.

Binding Free Energy

Table 1 shows all quinazoline derivatives have negative binding free energy with the respective target proteins indicating

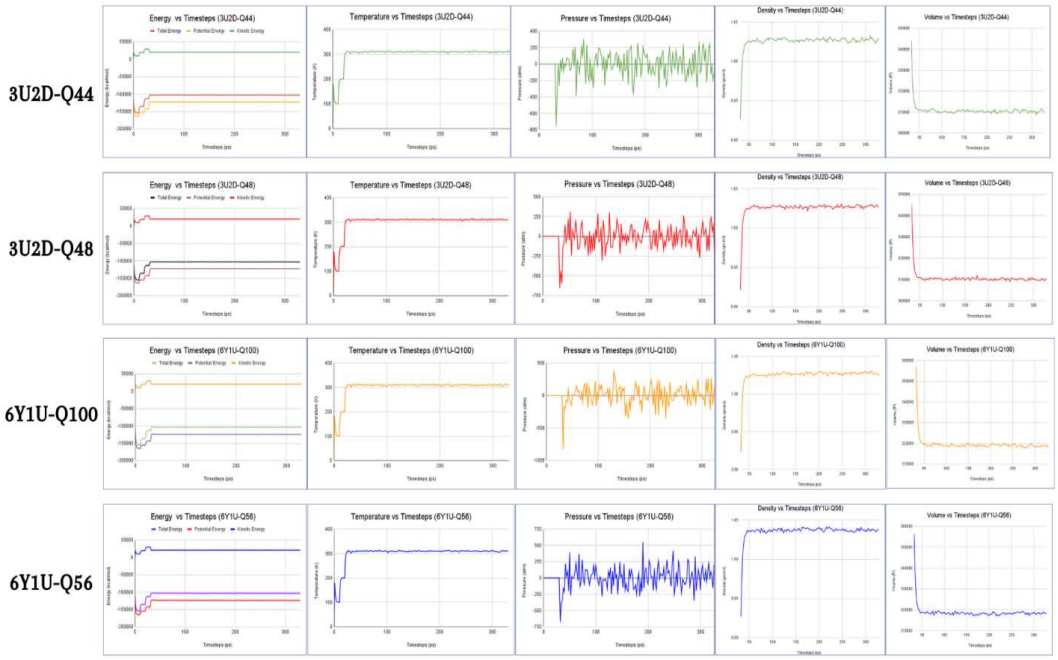

Figure 2: Pre-production analysis of quinazoline derivatives with the respective target proteins

Study of Quinazoline Compounds Complexed

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

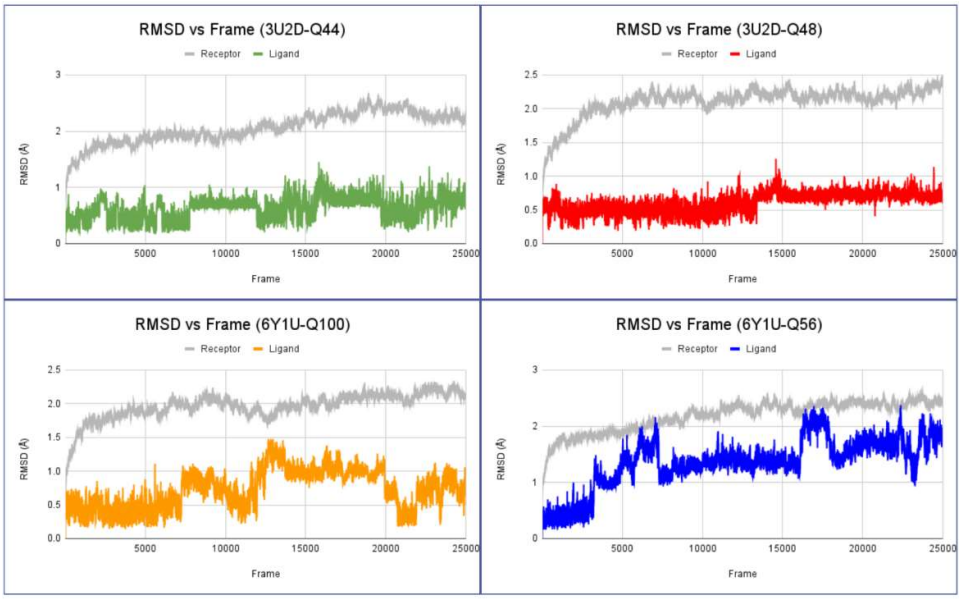

Figure 3: RMSD of the quinazoline derivatives complexed with the target proteins

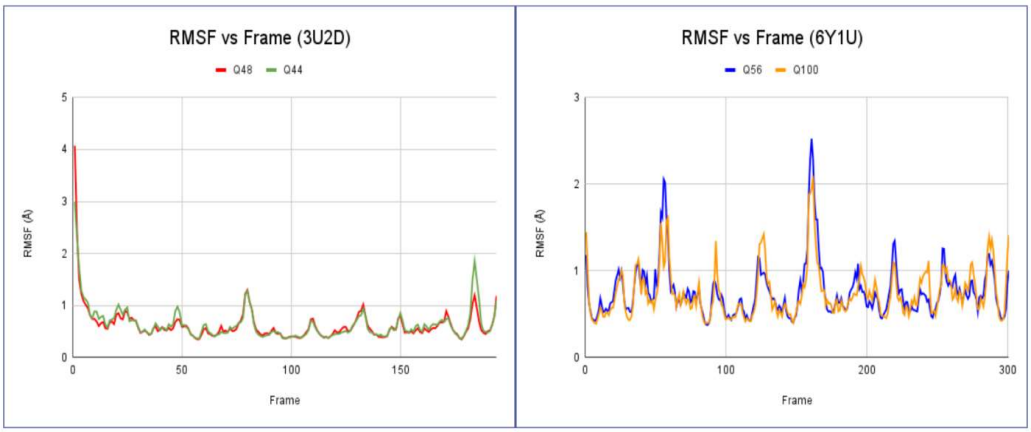

Figure 4: RMSF of the quinazoline derivatives complexed with the target proteins

that the binding of the compounds studied to 3U2D and 6Y1U respectively are favourable. Q44 with a slightly higher negative binding free energy of -23.21kcal/mol has a stronger binding to 3U2D receptor compared than Q48 with the binding free energy of -20.50 kcal/mol (10) which is mainly contributed by favourable Van der Waals energy in the system which is associated with hydrophobic interactions while the negative binding free energy for Q48 is contributed by favourable Van der Waals and electrostatic energy. The binding free energy findings for both compounds with 3U2D exhibit a good agreement with the docking findings (7) although the values are more negative than

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

Table 1: MM/GBSA analysis of quinazoline derivatives complexed with target proteins

Quinazoline Derivatives Complexed with 3U2D						
Compound	Ebonded	EvdW	EEL	EGB	ESURF	ΔGbind
Q44	0.0027	-44.7601	1.5781	25.6118	-5.6407	-23.2082
Q48	0.0027	-46.3257	-3.2930	34.7930	-5.6800	-20.5031
Quinazoline Derivatives Complexed with 6Y1U						
Compound	Ebonded	EvdW	EEL	EGB	ESURF	ΔGbind
Q100	0.0008	-43.6219	-12.5674	36.0579	-5.3458	-25.4763
Q56	-0.0015	-34.1470	-18.5728	32.3359	-4.6551	-25.0406

Note: All values are given in kcal/mol. Ebonded: bonded energy; EvdW: Van der Waals Energy; EEL: Electrostatic energy; EGB: Polar solvation energy; ESURF: Non-polar solvation energy; ΔGbind: Binding free energy

molecular docking due to conformational changes in dynamics simulation.

Meanwhile, Q100 with a higher negative score, binds stronger to 6Y1U compared to Q56 (10). The highly negative binding free energy of Q100 and Q56 is mainly contributed by favourable electrostatic and Van der Waals energy in the system while the latter energy is associated with hydrophobic interactions. The binding free energy findings for both complexes exhibit a good agreement with the docking findings (7).

Binding Interaction

Q44 does not form significant hydrogen bonds as the occupancy of hydrogen bond form throughout the MD simulation, shown in Table 2, is less than 10% which should not be considered according to Nada et al. (2022). However, as shown in Table 1 previously, other binding interactions such as Van der Waals interaction (-44.76 kcal/mol) which is associated with hydrophobic interactions is present in the system but not electrostatic interaction due to positive value of the EEL energy (1.58 kcal/mol) which indicates the receptor and ligand slightly repels each other. Thus, in a nutshell, EEL is unfavourable while the hydrophobic interactions are strongly exhibited throughout the MD simulation of 3U2D-Q44 complex. These findings of the binding interactions specifically hydrogen bond interaction and electrostatic interaction do not demonstrate a good agreement with the docking findings (7) that mentioned hydrogen bonds should form in Ile51, Arg81, Arg84, Arg144 while Glu58 should form electrostatic interaction with Q44. This may be contributed by the ligand and protein dynamics and conformation changes over time of MD simulation (11). Meanwhile, significant hydrogen bonds are formed between O1 (11.2%) and O33 (31.6%) of Q48 with Arg144 as shown in Table 2. The presence of favourable electrostatic attraction and Van der Waals interaction are observed in previous Table 1 due to the negative value of EEL (-3.29 kcal/mol) and EvdW (-46.33 kcal/mol). In comparison with the results from docking study (7), partial agreement is

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

Table 2: Occupancy of hydrogen bonds between quinazoline derivatives and 3U2D and 6Y1U

Quinazoline Derivatives Complexed with 3U2D			
Compound	Acceptor	Donor	Occupancy (%)
Q44	No significant occupancy		
Q48	LIG@O33	ARG_144@NH1	31.6
	LIG@O1	ARG_144@NH1	11.3
Quinazoline Derivatives Complexed with 6Y1U			
Compound	Acceptor	Donor	Occupancy (%)
Q100	No significant occupancy		
Q56	ASP_178@OD1	LIG@O2	44.5
	ASP_178@OD1	LIG@O1	32.0

Note: Only hydrogen bonds with occupancy >10% is displayed in this table

achieved in which hydrogen bond is formed in Arg144 except Arg84 with the ligand. Furthermore, presence of favourable electrostatic interaction and hydrophobic interactions can be observed in MD simulation which corresponds in the docking results for Q48 complexed with 3U2D.

Absence of significant hydrogen bond between Q100 and 6Y1U as the occupancy of between Q100 and 6Y1U are less than 10% which should not be considered (Nada et al., 2022) based on Table 2. Although no significant hydrogen bond is formed throughout the MD simulation in 6Y1U-Q100 system, favourable hydrophobic interaction (-43.62 kcal/mol) and electrostatic interaction (-12.57 kcal/mol) shown in Table 1 previously give rises to its favourable binding. Based on the intermolecular interaction result in the previous docking study (7), the hydrogen bond analysis in MD simulation demonstrates good agreement with the docking study that no conventional or carbon hydrogen is formed. The presence of favourable hydrophobic interactions can also be observed in both docking and dynamics study except electrostatic interactions where it is absent in the docking study. However, for Q56, specifically the O2 and O1, forms significant hydrogen bonds Asp178 of 6Y1U with the occupancy approximately 44.5% and 32% respectively as shown in (Figure 5).

In addition to hydrogen bond interaction, the findings in Table 1 show negative values of electrostatic energy (-18.57 kcal/mol) and VDW energy (-34.15 kcal/mol). Hence, indicates that favourable electrostatic and hydrophobic interactions are present within the 6Y1U-Q56 complex. This demonstrates a good correspondence with the docking study (7) specifically the presence of all hydrogen, electrostatic, and hydrophobic interactions although the number and type of hydrogen bonding residues are slightly different between docking study and dynamics. According to Zarezade V et al., 2021, the contribution of

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

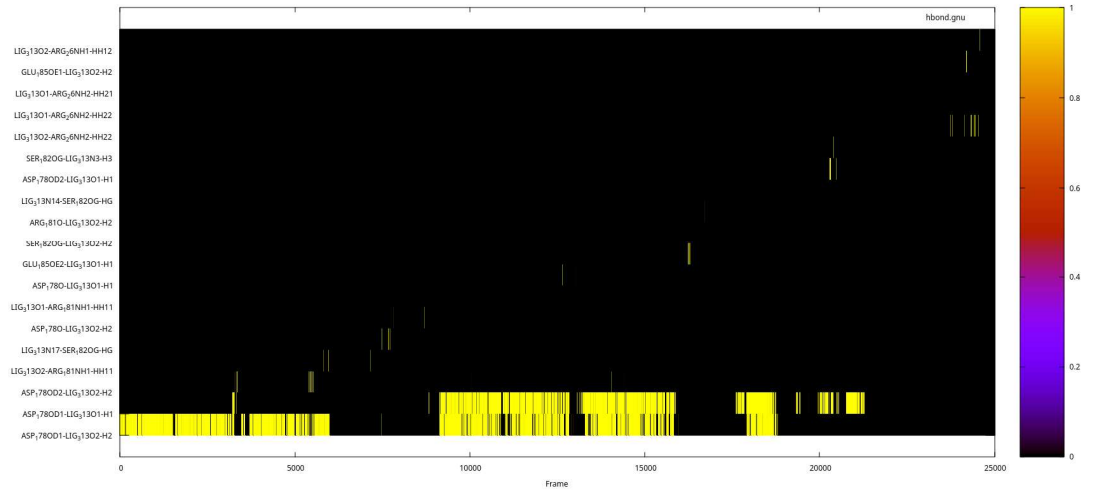

Figure 5: Hydrogen bond analysis of 6Y1U complexed with Q56

these different findings is due to conformational changes throughout the MD simulation.

Conclusion

Molecular dynamics simulation was conducted in this study to determine the potential of novel quinazoline derivatives as antibacterial agents by determining the stability, binding free energy, and binding interaction between the novel quinazoline derivatives and target proteins. All the compounds studied in this research have shown favourable binding free energy and inhibition constant in the docking study due to various intermolecular interactions between the quinazoline derivative compounds and target proteins. Compound Q44 demonstrates that the complex with DNA gyrase subunit B is slightly more stable than compound Q48, due to lesser fluctuation of tyrosine residue although both do not differ much in RMSD. Compound Q44 does not form significant hydrogen bonds or favourable electrostatics. However, its high negative value of vdW energy (-44.76kcal/mol) contributes to its favourable more negative binding free energy (-23.21kcal/mol). Meanwhile, Compound Q100 complexes with FtsZ show that it is more stable, with lower RMSD values in the ligand (1.5Å) while no significant difference in receptor compared to Compound Q56 (2.6Å). More balance peaks in RMSF are observed in Compound 100 and the higher negative free energy binding value (-25.48 kcal/mol) also contributes to its higher stability. However, no significant hydrogen bond is formed throughout the MD simulation. Thus, its low binding free energy is mostly contributed by hydrophobic interactions (-43.62 kcal/mol) and electrostatic interactions (-12.57 kcal/mol).

In summary, compound Q100 and Q44 have the most potential as the new drug candidates for its antibacterial properties and continue to the next step in drug design.

Contributions

MYY and IKM designed the experiments, MYY carried out the experiments, MYY has written the initial draft. MYY, and IKM, refined the manuscript. All authors have read and approved the manuscript.

References

1. Bandyopadhyay, M., &Muthuirulan, P. (2018). *Mini Review Promising Targets for Prospective Antibacterial Therapy*. EC Microbiology, Antimicrobial resistance.

Vol. 17 (Supplementry Issue 3s) 191 - 199, July-Sept 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2024.3s.14

2. Antimicrobial resistance. (2021). World Health Organization. Retrieved from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
3. Karan, R., Agarwal, P., Sinha, M., & Mahato, N. (2021). *Recent advances on quinazoline derivatives: A potential bioactive scaffold in medicinal chemistry.*Chem Engineering, 5(4), MDPI.
4. Jafari, E., Khajouei, M. R., Hassanzadeh, F., Hakimelahi, G. H., &Khodarahmi, G. A. (2016). Quinazolinone and quinazoline derivatives: recent structures with potent antimicrobial and cytotoxic activities. Research in Pharmaceutical Sciences, 11(1), 1-14.
5. Hoque, I., Chatterjee, A., Bhattacharya, S., & Biswas, R. (2017). *An* Approach of Computer-Aided Drug Design (CADD) Tools for In Silico Pharmaceutical Drug Design and Development. *International Journal of Advanced Research in Biological Sciences*, 4(2), 60–71.
6. Sari, S. (2020). Molecular Modelling and Computer-Aided Drug Design: The Skill Set Every Scientist in Drug Research Needs and Can Easily Get. *Hacettepe University Journal of the Faculty of Pharmacy*, 40.
7. Nik Danial. (2023). Molecular Docking Studies of Quinazoline Compounds Complexed With Filamenting Temperature-Sensitive Z Protein And Gyrase Subunit B as Potential Antibacterials.
8. Murdock, S. E., Tai, K., Ng, M. H., Johnston, S., Wu, B., Fangohr, H., Laughton, C. A., Essex, J. W., & Sansom, M. S. (2006). *Quality assurance for Biomolecular Simulations. Journal of Chemical Theory and Computation*, 2(6), 1477–1481.
9. Kumar, A., Prasun, C., Rathi, E., Nair, M. S., & Kini, S. G. (2023). *Identification of potential DNA gyrase inhibitors: virtual screening, extra-precision docking and molecular dynamics simulation study. Chemical Papers*, 77(11), 6717–6727.
10. Owoloye, A. J., Ligali, F. C., Enejoh, O. A., Musa, A. Z., Aina, O., Idowu, E. T., &Oyebola, K. M. (2022). *Molecular docking, simulation and binding free energy analysis of small molecules as Pf HT1 inhibitors*. PLoS ONE, 17(8 July).
11. Zarezade, V., Rezaei, H., Shakerinezhad, G., Safavi, A., Nazeri, Z., Veisi, A., Azadbakht, O., Hatami, M., Sabaghan, M., &Shajirat, Z. (2021). The identification of novel inhibitors of human angiotensin-converting enzyme 2 and main protease of SARS-COV-2: A combination of in silico methods for treatment of COVID-19. *Journal of Molecular Structure*, 1237, 130409.
12. Nada, H., Elkamhawy, A., & Lee, K. (2022). Identification of 1H-purine-2, 6-dione derivative as a potential SARS-CoV-2 main protease inhibitor: molecular docking, dynamic simulations, and energy calculations. *Peer J*, 10.