RESEARCH ARTICLE

In silico and *In vitro* Evaluation of Some Synthesized Quinoline Derivatives into MexB Protein of *Pseudomonas aeruginosa*

Abdalkader Saeed Latif[1*], Majida G. Magtooph[2], Alia Essam Mahmood Alubadi[3]

[1]*Medical Laboratory Technology, Al-Farabi University, Baghdad, Iraq*
[2]*Biology, College of Science, University of Thi-Qar, Thi-Qar, Iraq*
[3]*Biology, College of Science, Mustansiriyah University, Baghdad, Iraq*

Received: 19th March, 2020; Revised: 20th April, 2020; Accepted: 28th May, 2020; Available Online: 25th June, 2020

ABSTRACT

Molecular docking performed to evaluate the effect of five quinoline derivatives on the MexB protein of *Pseudomonas aeruginosa* as a potential inhibitor by utilizing the 3D structure of each quinoline compounds (C1, C2, C3, C4, and C5), and the crystal structure of the protein, C4 showed the greatest potential with -31.4 kcal/mol binding energy, and the lowest potential was for C1 with (-18.5 kcal/mol) compared with ciprofloxacin. Fifty samples were collected from different sites from patients who are attending to the medical city of Baghdad and private Dhelal Beirut Center, Baghdad, 36 of the samples were diagnosed as *P. aeruginosa* by routine culture test and confirmed by VITEK2, and those isolates were subjected to the susceptibility test against carbapenems, carbenicillin, levofloxacin, and erythromycin by disc diffusion method. The isolates that showed resistance to all of four antibiotics were based to evaluate the activity of quinoline derivatives by using the agar well diffusion method, where compounds C4 and C5 showed the highest line of activity as the minimum inhibitory concentration (MIC) was 256 μg/mL, meanwhile, C1 showed the lowest activity with MIC of 1,024 μg/mL.

Keywords: MexB protein, Molecular docking, *Pseudomonas aeruginosa*, Quinoline derivatives.

International Journal of Drug Delivery Technology (2020); DOI: 10.25258/ijddt.10.2.2

How to cite this article: Latif AS, Magtooph MG, Alubadi AEM. *In silico* and *in vitro* evaluation of some synthesized quinoline derivatives into mexb protein of *Pseudomonas aeruginosa*. International Journal of Drug Delivery Technology. 2020;10(2):195-199.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Recently the big concerns of human health are the multidrug-resistant (MDR) bacteria, as contaminated with MDR bacteria could lead to life-threaten to elderly, children, and immunocompromised people. The cost of the treatment and the time consuming to recover from complications created a need to intensify the efforts to the development of novel antibacterial agents with different properties for recent therapies.[1] Lately, the molecular docking study (*in silico*) has become an important tool for drug discovery pipeline, where the molecular docking study can be used visualizing the main interaction between a small molecule (ligand) and a protein (target) at the molecular level, the study at this level allowed to characterize the behavior of ligands in the binding site of target proteins, as well as, to elucidate fundamental biochemical processes. The molecular docking process comprises two main steps: prediction of the ligand conformation, as well as, its position and orientation of functional groups within these sites (usually referred to as pose) and assessment of the binding affinity.[2]

P. aeruginosa is a human pathogen that causes extreme infections. Also, its internal resistance to different types of antibiotics and its ability to acquire high-level resistance that made these bacteria hard to kill.[3] The resistance mechanism mainly results from the cooperative of the expression of drug efflux pump systems and low-permeability outer membrane. Normally, the efflux pump systems comprise an inner-membrane component belonging to the resistance-nodulation-cell division (RND) superfamily of secondary transporters.[4] Recently, several systems participating in drug efflux have been identified in *P. aeruginosa*. The inner-membrane component of MexB is one of them, and it is responsible for substrate specificity,[5,6] and energy of the transport process.[7] Residues that involved in substrate binding are Gln125, Val47, and Ser48of A subunit, Ser180, Gly179, and Val177of B subunit, and Arg620 and Gln273 of C subunit. These residues are able to interact with the substrate via van der Waals interactions and hydrogen-bonding.[8]

Quinoline derivatives are heterocyclic compounds that have importance to humans as utilization of quinolones,

**Author for Correspondence: abdalkaderlatif@yahoo.com*

and its derivatives compound in medicinal chemistry area because of chemical and pharmacological properties.[9] Quinoline derivatives are interesting series of heterocyclic compounds, which have been shown to possess a variety of biological activities, such as, antibacterial and fungal agent and anticonvulsant, antitumor, anti-malarial, anti-platelet, antidepressant, antiulcer, and cardiac stimulant.[10]

The quinoline compounds occur in various natural products, especially in alkaloids, as they are used for design of many synthetic compounds with diverse pharmacological properties.[11] There are number of natural products of quinoline skeleton utilize as a medicine or development novel potential molecules.[12]

METHODS

In silico Study

The 3D structure of quinoline derivatives (C1–C5) are shown in Figures 1a to e, respectively, comparative ligands were drawn by the software Discovery Studio v2.5. While the crystal structure MexB protein with the code (3W9I) was downloaded from the protein data bank (https://www.rcsb.org/pdb/home) (Figure 2). The tools used are server and software. Servers are Swiss Dock (http://www.swissdock.ch/dock) for molecular docking process, and software consisting of Discovery Studio v2.5, Chimera 1.10.2, Python Molecule Viewer, and Gaussian 03W.

The first stage is the preparation of macromolecular structures targets, includes search, download, optimization, and separation of residues nonstandard. Macromolecular structure downloaded from the web Protein Data Bank (PDB) server with code (3W9I) for MexB protein of *P. aeruginosa*. Further optimization is done by using UCSF Chimera, also the binding site determined and visualized by Discovery Studio software (Figure 2). The second stage is the preparation of the ligand, which includes the stretch 2D structures, changes the structure into 3D, as well as, minimizes the force field energy. The third stage is done by submitting the molecules into molecular docking online by using the Swiss Dock server. The last stage is the analysis of docking simulation results by Chimera 1.10.

Bacterial Isolation and Identification

All of the isolates involved in this study were gained from sputum, urine burns, wounds, and blood samples from Iraqi patients attending to Medical City of Baghdad and private Dhelal Beirut Center (DBC), Baghdad during June to October 2019. The thirty-six isolates of *P. aeruginosa* were identified depending on the morphology, cultural characteristics, and VITEK2 (bioMérieux) apparatus was utilized to confirm the identification of bacteria.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the *P. aeruginosa* isolates was performed by Kirby-Bauer (disc diffusion method) against carbapenems, carbenicillin, levofloxacin, and erythromycin, based on Clinical Laboratory Standard Institution (CLSI) guide book.

Antimicrobial activity of synthesized quinoline derivatives was evaluated by agar well diffusion method. In this method, bacterial suspension [(1.5×10^8 CFU/μL) gained 0.5 McFarland standard solution for turbidity] was inoculated sterilely into Muller Hinton agar plates by spreading method. After drying to remove excess moisture by sterilized hood, wells have been made with suitable depth to involve 50 μL of dissolved compounds.

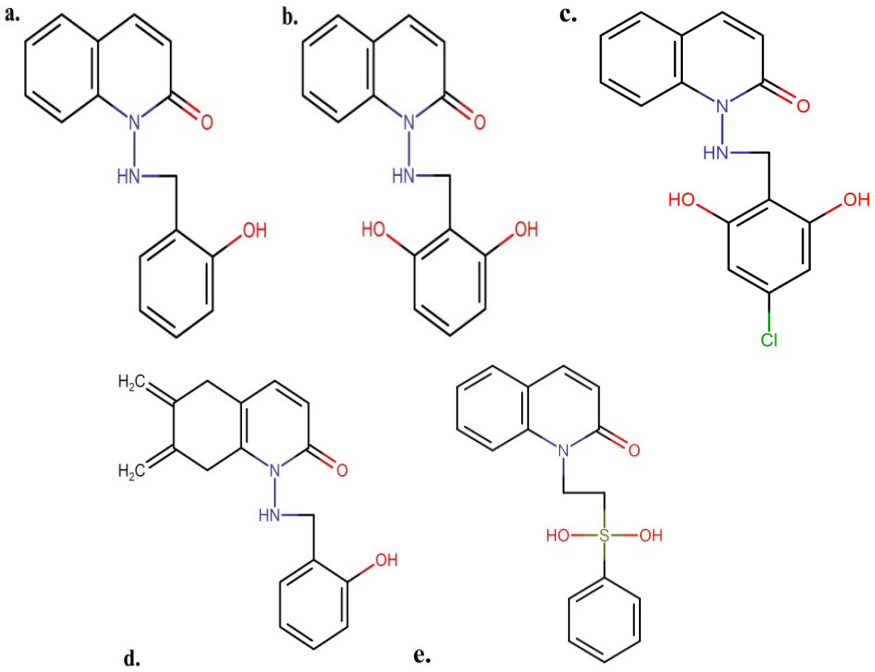

Figure 1: Molecule structure of quinoline derivatives

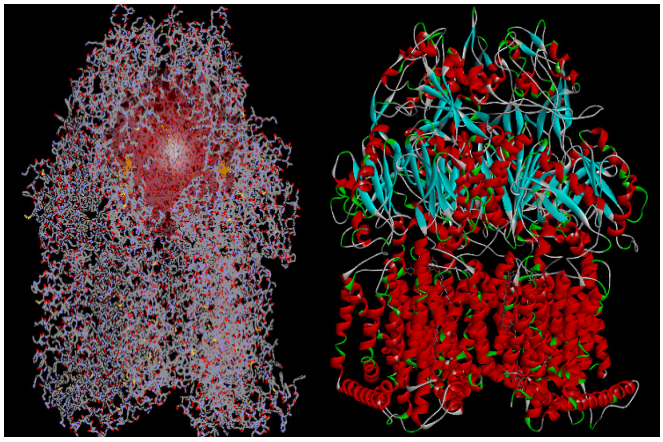

Figure 2: Crystal structure of MexB protein and binding site visualization

Table 1: Molecular docking results of quinoline derivative molecules

Molecule symbol	*Estimated ΔG (kcal/mol)*	*Full fitness (kcal/mol)*	*Amount of hydrogen bond*
C1	-8.67	-3,375	2
C2	-9.76	-3,885	3
C3	-11.44	-3,969	3
C4	-12.46	-4,165	4
C5	-11.91	-4,056	3
Levofloxacin	-14.55	-4,671	4

RESULTS

In silico Study

The docking studies were achieved to evaluate the effect of five quinoline derivatives molecules (C1–C5) against the macromolecules MexB protein of *P. aeruginosa*. The binding free energy, full fitness, and the Gibbs energy (ΔG) are mainly the functional score that reflects the interaction energy between the ligand-protein complex in which the molecule, which has the lowest energy, showed more stable interactions. Docking simulation results can be seen in Figure 3.

Docking algorithm simulation data above indicate that five of 1uinoline derivatives molecules (C1, C2, C3, C4, and C5) showed a good potential to bind with the binding site of MexB protein and block it, and that led to inhibition the main function of MexB protein (transport). Derivative compounds which have the greatest potential as a MexB protein inhibitor is C4 with -31.4 kcal/mol binding energy, and lowest is C1 with -18.5 kcal/mol binding energy in addition to the other indicators compared with levofloxacin (-49.5 kcal/mol).

Docking simulations showed good indication could be seen by comparing the values of the binding free energy, full fitness, the Gibbs energy (ΔG), and the amount of hydrogen interaction as standard inhibitor can be seen in Table 1.

A bond-forming created a strong complex that is characterized by low binding energy, ΔG value, and the number hydrogen interactions with the side chain of amino acid residues of each Gln125, Ser48, Val47 of A subunit, Ser180 Gly179, Val177 of B subunit, Arg620, and Gln273 of C

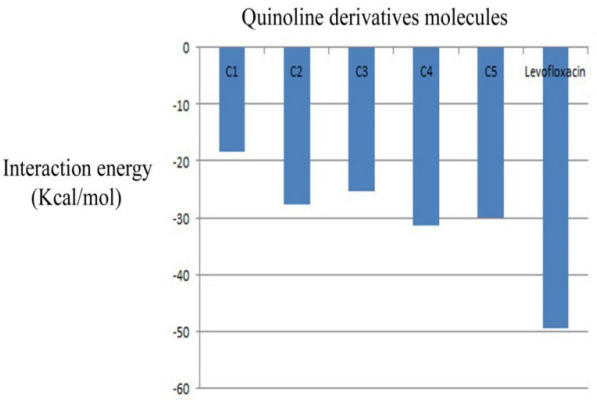

Figure 3: Value of the binding interaction energy (kcal/mol) of quinoline derivatives molecules, and levofloxacin

Table 2: Distribution of bacterial isolates according to the source of samples

Source	*No. of isolates*	*Percentage (%)*
Burns	14	38%
Wounds	9	26%
Blood	2	5%
Sputum	8	24%
Urine	3	7%
Total	36	100%

subunit, as shown in Figure 4. Based on the simulation results of docking, five quinoline derivatives molecules (C1–C5) have pretty good indicator criteria based on Figure 3 and Table 1, and they can potentially be used as MexB protein inhibitors candidates to block the main function of protein in *P. aeruginosa* bacteria.

In vitro Study

Fifty samples were collected from many sites ranged between wounds, burns, blood, sputum, and urine. Thirty-six isolates were identified as gram negative bacteria *P. aeruginosa* and distributed as follows: 14 from burns, 9 from wounds, 2 from blood, 8 from sputum, and 3 from urine, as seen in Table 2. All 36 isolates were identified by routine cultural tests and VITEK 2 protocol. The antibiotic susceptibility test of 36 isolates was done against carbapenems, carbenicillin, levofloxacin, and erythromycin by disc diffusion method; the results showed that 43% of 36 isolates were resistant to carbenicillin, 29% of isolates were resistant to erythromycin, 8% of isolates were resistant to levofloxacin, and 20% of 36 isolates were resistant to erythromycin. Most *P. aeruginosa* isolates showed resistance to the different types of antibiotics caused by decreased membrane permeability, antibiotic inactivation enzyme production, and expression of efflux pump system.[13,14]

Antibacterial activity evaluation of quinoline derivative compounds was conducted by dissolving all five compounds in solvent and prepared a serial dilution (0.5–2,048 µg/mL), ciprofloxacin used as control. The evaluation study showed

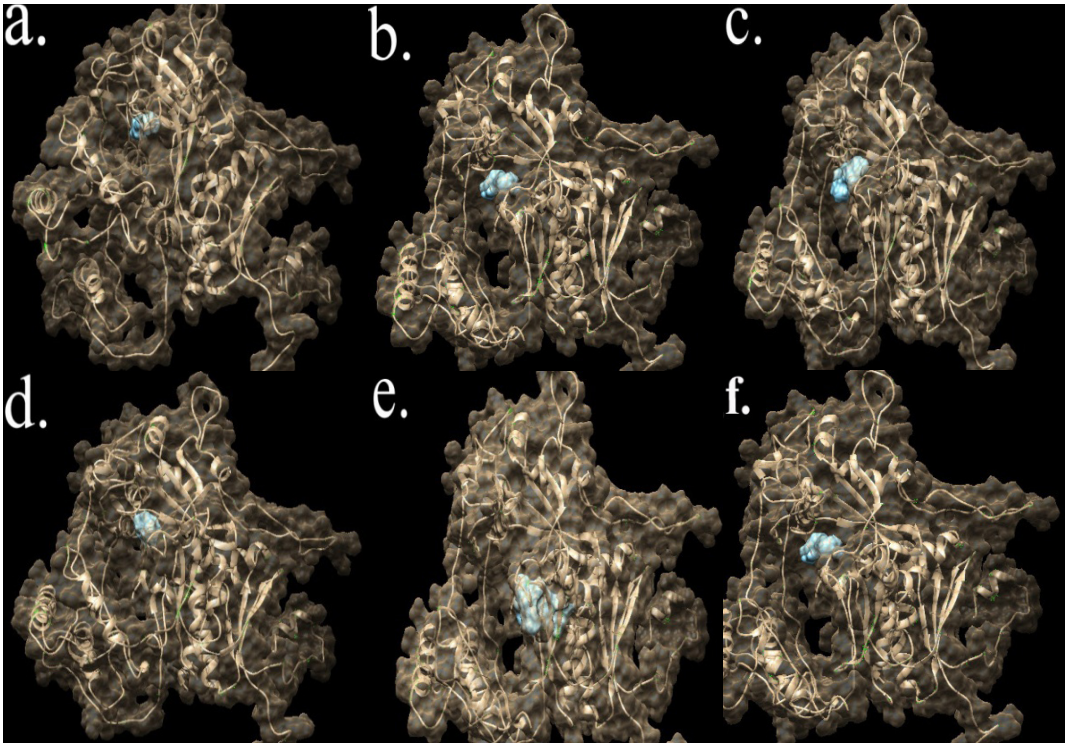

Figure 4: Interactions of MexB binding site residues with quinoline derivatives, as well as, comparative ligand; **(a)** C1; **(b)** C2; **(c)** C3; **(d)** C4; **(e)** C5; **(f)** Levofloxacin

that compounds C4 and C5 exhibit the highest line of antibacterial activity against *P. aeruginosa* isolates, where the MIC value was 256 µg/mL. Compounds C2 and C3 exhibit the medium line of antibacterial activity at concentration 512 µg/mL. Finally, the compound C1 exhibits the lowest line of antibacterial activity at concentration 1,024 µg/mL against clinical isolates compared with ciprofloxacin. The low antibacterial activity of compound may be explained that C1 has lower functional group like 2-hydrogen bonds, unlike other compounds, meanwhile, compounds C4 and C5 have 4-, 3-hydrogen bonds, respectively, and the low molecular weight with an appropriate orientation that enable these compounds to make covalent bonding to the main residues of the binding site in MexB protein and block it.[15,16] Although compounds C2 and C3 have a good number of hydrogen donor and acceptor with good molecular weight, but the orientation of functional group may be in a position not complementary with the residue of the binding site of MexB protein of bacteria, that required increasing the concentration of the compounds to be able binding with MexB protein. The *in vitro* studies results showed that harmonized with the *in silico* studies results as compounds C4 and C5 exhibit the highest functional score, respectively, and compound C1 exhibit the lower functional score.

CONCLUSION

The *in silico* and *in vitro* studies showed that quinoline derivatives (C1, C2, C3, C4, and C5) have potential MexB protein inhibitor candidates with different levels of antibacterial activity compared with ciprofloxacin. Molecular docking simulations (*in silico*) reflecting the initial step in the development of the discovery of new drug candidates. Furthermore, the research needs to be done by *in vivo* study to assess the potential quinoline derivatives as a drug candidate in the treatment of resistance *P. aeruginosa* strain.

ACKNOWLEDGMENT

Authors are extremely grateful to all the people for their cooperation and help to get data and also thank the Department of Medical Laboratory Technology, Al-Farabi University.

REFERENCES

1. Chow V, Sakharkar K and Sakharkar M. Novel genomics approach for the identification of drug targets in pathogens with special reference to Pseudomonas aeruginosa. In Silico Biol. 2004;4(3):355–360.
2. McConkey BJ, Sobolev V, Edelman M. The performance of current methods in ligand-protein docking. Current Science. 2002; 83:845–855.
3. Kapetanovic. Computer-aided dr ug discover y and development (caddd): *In silico* chemico-biological approach. Chemico-Biological Interactions. 2008;171(2):165–176.
4. C- Sandhu, N. S.; Shaikh, T.; Jain, G. and Jayaram, B. From Drug Target to Leads Sketching A Physicochemical Pathway for Lead Molecule Design *In silico*. Current Pharmaceutical Design. 2007;13:3454-3470.
5. Poole K. Srikumar R. Multi drug efflux in *Pseudomonas aeruginosa*: components, mechanisms and clinical significance. Curr. Top. Med. Chem. 2001;1:59–71.
6. Nikaido, H. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. Semin. Cell Dev. Biol. 2001;12:215–223.

7. Tikhonova, E. B., Wang, Q. and Zgurskaya, H. I. Chimeric analysis of the multicomponent multi- drug efflux transporters from Gram-negative bacteria. J. Bacteriol. 2002;184:6499–6507.
8. Elkins CA, Nikaido H. Substrate specifi- city of the RND-type multidrug efflux pumps AcrB and AcrD of Escherichia coli is determined predomi- nantly by two large periplasmic loops. J. Bacteriol. 2002;184:6490–6498.
9. Zgurskaya, H. I. and Nikaido, H. Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of Escherichia coli. Proc. Natl Acad. Sci. USA. 1999;96:7190–7195.
10. Sennhauser G, Bukowska MA, Briand C, Grütter MG. Crystal structure of the multidrug exporter MexB from Pseudomonas aeruginosa. Journal of molecular biology. 2009 May 29;389(1):134-145.
11. Sukhen S. Design and Synthesis of some and 2-Quinolones for Antibacterial activity. Journal of Pharma Research. 2015; 4(3):1110-1116.
12. Redha, I. Al-Bayati.; Shakeeb, M.; Hameed and Hiba, Ibrahim. Synthesis of new schiff 's bases 4-chloro coumarin derivatives'. World WJPR. 2015;4(10):2695-2701.
13. Anukumari G, Anand M, Dubey P. Synthesis and antibacterial activities of some substituted 2-styrylquinolines. Der Pharma Chemica. 2014;6(2):217-220.
14. Latif A. Design and Evaluation of Antibacterial Activity (*In silico*, *In vitro* a nd *In vivo*) of New Q uinoline-2-one Derivatives against clinical *Pseudomonas aeruginosa*. Ph.D. Thesis, Department of Biotechnology, University of Baghdad. (2016). Thesis.
15. Lambert P., Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J R Soc Med. 2002;41:22-26.
16. Abdalkader S L, Essam F A.. Evaluation study of Coumarin molecules against Glycosyltransferase enzyme of *Streptococcus pneumonia*. International Journal of Pharmaceutical Research | Jan - Jun 2020 | Supplementary Issue 1. Available from DOI: https://doi.org/10.31838/ijpr/2020.SP1.126