EVALUATION STUDY OF THE EFFECTIVENESS FOR SOME ANTIBACTERIAL AGENT AGAINST DNA GYRASE ENZYME OF STAPHYLOCOCCUS AUREUS

Dr. Abdalkader Saeed Latif[a], Zena Abbas Fadel[b]

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Abstract: This study was dealt with evaluation of antibacterial effectiveness of each (Azithromycin, Chloramphenicol Gentamacin, Levofloxacin and Ciprofloxacin) computationally as antibacterial agents. This study was conducted by computational methodologies 'In silico'. The 3 dimensional (3D) structure model of DNA gyrase enzyme of Staphylococcus aureus was built by Homology Modeling method as target protein and the active site was visualized. The computational prediction showed molecule (Levofloxacin and Ciprofloxacin) has highest binding score (-45 Kcal/mol) (-40 Kcal/mol) respectively with active site of target protein and molecules (Azithromycin) binding score were (-12 Kcal/mol) meanwhile molecule (Gentamicin and Chloramphenicol) showed lowest binding score (-10 Kcal/mol) (-8 Kcal/mol) respectively with target DNA gyrase of Staphylococcus aureus bacteria .

Keywords: Staphylococcus aureus, DNA gyrase, Antibacterial agent, computational study

[a]. National University of Science and Technology / Dean of Health and Medical Technology College
[b]. National University of Science and Technology / Dean of Health and Medical Technology College

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INTRODUCTION

The most important bacteria to causes many diseases to human body is Staphylococcus aureus bacteria as its cause infection to skin and soft tissue such as furuncle and cellulites (1), The tendency of these bacteria to get severe infections is delicate to abolish, due to erratic antibiotic resistance in both community-acquired and hospitalized. Amidst methicillin-resistant Staphylococcus aureus (MRSA) indeed, customary antibiotics are no longer befit, Furthermore, the abundant and illogical use of antibiotics is an additional factor (2).

Pursuant World Health Organization the potential of Staphylococcus aureus to express a diversity of multidrug resistance is increasingly spread globally therefore it became a challenge to treat infection and mortality (3).

DNA gyrase enzyme of S. aureus bacteria is play a pivotal role in the multiplication of bacterial DNA, similarly, topoisomerase IV, both enzymes belong to type II topoisomerase that controls the topological state of DNA in cells (4,5). Structurally, DNA gyrase along with topoisomerase IV are Heterotetrameric enzymes are consist of two couples of subunits, where DNA gyrase is Consist of two A subunits and Two B subunits whilst Topoisomerase IV is consist of two ParC and two ParE. DNA gyrase subunits contain sequences of amino acids like the sequences that topoisomerase IV subunits have, hence they exhibit structural and functional similarities (6).

Functionally, the major responsible of GyrA/ParC is breakup and rejoining of the DNA due to the N-terminal domain of gyrA containing the active point tyrosine residues located in the catabolite-activator-protein like (CAP-suchlike) (tyr- 122) (7). Tyrosine is crucial for the breakage and 29elongation of the DNA due to forming an ester with 50 phosphate of the DNA, this Protein-DNA relation preserves the energy of DNA phosphodiester bond and allows revealing of the DNA by the attack of the OH-3 ends of the broken DNA. While in contrast, GyrB/ParE binds ATP, supplying through its hydrolysis, the power for the ligation procedure. Accordingly, DNA gyrase became a proper target for the development of antibacterial (8,9).

In last twenty years, the increasing of utilize the computational tool in drug discovery process to find and improve the ability of antibacterial agents against the resistance strain of bacteria (10), Wherein The molecular docking algorithm method can be used to visualize basic interactions between a small molecule (ligand) and a protein (target) at the molecular level, which aims to specify the conduct of small molecules in the active point of the target proteins besides illustrating the major biochemical processes (11). This process (docking) entails two steps, the prediction of the ligand verification along with its position and orientation inner these points (usually mention as pose) and estimation of the binding affinity energy (12). S. aureus's DNA gyrase enzyme is a target for numerous prime anticancer and antibacterial drugs. These drugs act in a treacherous way of action. Conversely to most other protein-targeted drugs, DNA gyrase targeted factors do not kill cells by abrasion them of captious enzymatic activities. Instead, they utilize the latent fatal identity of DNA gyrase and "toxin" these enzymes by augmentation of the steady-state proportion of DNA dissent compound (13). This activity prosody DNA gyrase to powerful physiological toxins that initiate DNA strands split in treated cells and prompt mortal events. Due to

their mechanism of action, drugs that elevate levels of DNA gyrase-interfered DNA cleavage are cited as "Gyrase Poisons" to eminent them from drugs that stimulate inhibitors of these enzymes (14).

The quinolone family of drugs in particular (levofloxacin and ciprofloxacin) takes advantage of these properties, becoming mortal, distinguishable, and killing by raising the concentration of enzyme-DNA breakup complexes. Therefore, these drugs are called "topoisomerase toxins" because they transform gyrase and topoisomerase IV into cytotoxic (15). Where Quinolones compounds attach in a no covalent pattern at the enzyme-DNA cooperate in the breakup-ligation active point and these compounds connect with the protein and intercalate into the DNA at both split scissile bonds. As Quinolones improve the steady-state concentration of breakup complexes by conducting physical obstructions to ligation (16). Meanwhile, the macrolide family of drugs like (Azithromycin) is works one delay stop bacterial growth by hindering protein synthesis and translation for dealing with a wide range of bacterial infections, as its binds o the 23S rRNA of the bacterial 50S ribosomal subunit. It prevents bacterial protein synthesis by inhibiting the transpeptidation/translocation step of protein synthesis and by impeding the assembly of the 50S ribosomal subunit Label (17). But also there is another kind of drug (Chloramphenicol) that is used as a broad-spectrum antibacterial that is adequate against sort of sensitive and severe bacterial disorders but is not continually used because of its high hazard of bone marrow toxicity, Chloramphenicol is effective against an expansive range of microorganisms, but due to powerful side. Chloramphenicol prevents bacterial growth by attaching to the bacterial ribosome (shutting off peptidyl transferase) and deterring protein synthesis (18). Gentamicin is an aminoglycoside used to treat a wide variety of aerobic infections in the body. The mechanism of action that is hampering protein synthesis is a critical ingredient of aminoglycoside potency. Structural and cell biological studies propose that aminoglycosides shackle to the 16S rRNA in helix 44 (h44), near the A site of the 30S ribosomal subunit, changing the relation between h44 and h45 (19).

METHODS

Computational study

The essential step of a computational study is to sketch and visualize the five antibacterial compounds (Azithromycin, Chloramphenicol, Gentamicin, Levofloxacin, and Ciprofloxacin) which encode with (D1, D2, D3, D4, D5) respectively, those molecules were constructed by software Discovery.Studio.v2.5. Meanwhile, Protein Data Bank's DNA gyrase crystal form was uploaded. (https://www.rcsb.org/). The SWISS Dock Server (http://www.swissdock.ch/dock) was utilized for the Molecular Docking method. The software (Discovery.Studio.v2.5, Chimera 1.10.2, Python Molecule observer, Gaussian 03W) was utilized for the Molecular Docking algorithm results analysis (10).

In this study, the target protein structure preparation as a target, and this includes searching, downloading, optimization, and separation of nonstandard residues. The crystal structure of the DNA GYRASE protein of S. aureus was downloaded from the PDB server with code (O67108).

UCSF Chimera performed additional optimization, and Discovery found and displayed the active point. V2.5 of Format Studio (1). The next stage is to create the antibacterial agent as a ligand, which required fabricating 2D sketches, altering them into 3D structures (D1–D5), respectively, and underestimating the domain energy to satisfy the condition for the molecular docking algorithm. The third step is submitting to the molecular docking algorithm online by using the Swiss Dock Server. The terminal action is the probe of the computational modeling upshot docking by Chimera 1.10 software.

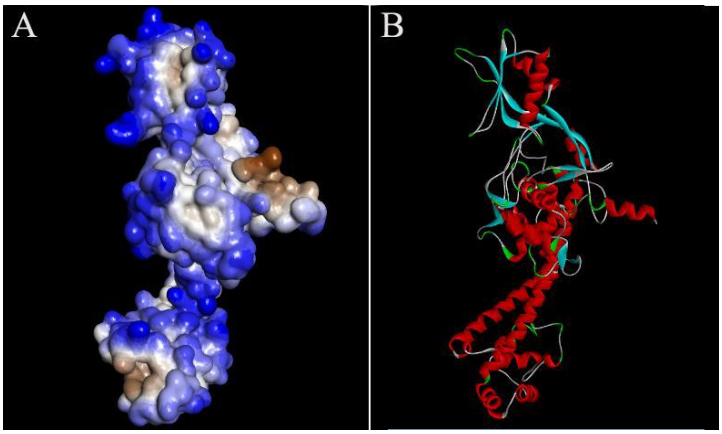

Figure 1. DNA gyrase subunit A protein (A: protein model with Hydrophobicity surface. B: protein model with secondary structure)

RESULTS

A computational study (Molecular Docking) was operated to estimate the efficacy of five molecules (D1-D5) against DNA gyrase of S. aureus. The Gibbs energy (G), binding free energy, and complete fitness is the main proper score that recollects the affinity power of the ligand-binding protein complex, Thus the lower the energy required for the bond, the higher the binding affinity gets stable. Automatic calculation of molecular docking process was done and the final results of molecular docking for all five molecules were showed in Table (1) and figure (2).

Table 1. Molecular Docking Results of Five Molecules

Molecule Symbol	Total binding Energy (Kcal/mol)	Full Fitness (kcal/mol)	Estimated ΔG (kcal/mol)
Azithromycin	-12.81	-2936.31	-7.54
Chloramphenicol	-8.02	-2940.68	-7.62
Gentamicin	-10.06	-2924.21	-6.36
Levofloxacin	-45.26	-3006.16	-8.05
Ciprofloxacin	-40.43	-2946.51	-6.56

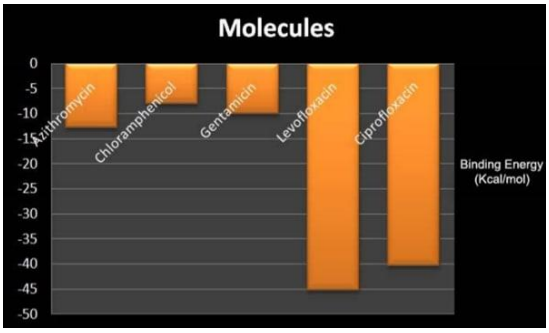

Figure 2. The values of the binding Energy (kcal/mol) of the molecules

The results of the molecular docking algorithm showed that five molecules (D1-D5) have a quite useful possible affinity energy to bind to the select active point in GyrA of the DNA gyrase enzyme, and this binding occupies the allosteric form of the active site and blocks it and averts the active point from binding to another substrate, all These occurrences preside to loss of enzyme role and disturbances of the main process of the protein. Where the molecule (D4) showed the highest binding energy (-45.26 kcal/mol), the lowest (D1) with (-8.02kcal/mol). Molecular docking simulations pictured that a beneficial indicator could be seen by corresponding the valuations of binding free energy, full fitness, and Gibbs energy (G), depending on the Lipinski rule, as shown in (Table 1). The bond formation can output a strong compound illustrated by insufficient binding energy, ΔG value, complete fitness, and numerals, of hydrogen interactions with the amino acid residue edge chain in the active site of GyrA at 122 amino acid positions of the DNA gyrase as shown in Figure (3).

Established on the molecular docking simulation results, the five molecules (D1-D5) have very sufficient index parameters based on (Table 1) and can be used as nominee enzyme impeding to block the main function of DNA gyrase enzyme and inhibit the process in S .aureus bacteria.

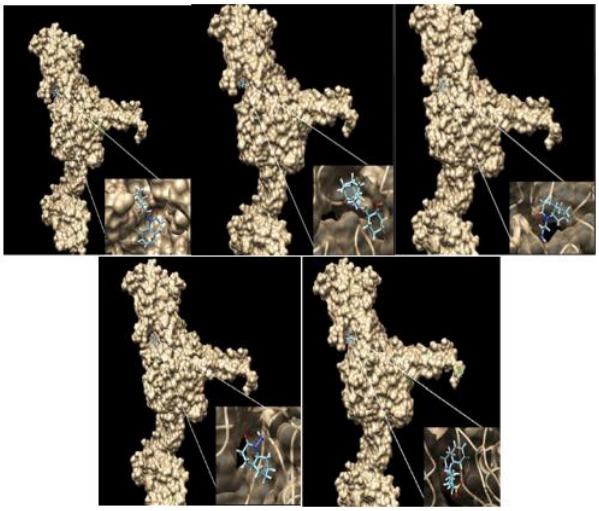

Figure 3. Molecular docking of (D1-D5) respectively

CONCLUSION

Structure based drug design (SBDD) strategy that was used to predict and design molecules had a success score. The DNA gyrase protein was a good target for bacterial growth suppression. Compounds (Levofloxacin and Ciprofloxacin) showed highest antibacterial activity, meanwhile compounds (Azithromycin and Gentamicin) showed moderate level of activity at (512 µg/ml) and compound (Chloramphenicol) showed no activity against DNA gyrase anzyme.

REFERENCES

i. Infection disease epidemiology, prevention and contro, 651.201,5414-TDD/TTY. 651-201-5797. www.health.state.mn.us.

ii. Hussain MS, Naqvi A, Sharaz M. Methicillin resistant staphylococcus aureus (MRSA); prevalence and susceptibility pattern of (MRSA) isolated from pus in tertiary care of district hospital of Rahim Yar Khan. Professional Med J 2019; 26(1):122-127. DOI: 10.29309/TPMJ/2019.26.01.2510.

iii. Dell'Annunziata, F.; Folliero, V.; Palma, F.; Crudele, V.; Finamore, E.; Sanna, G.; Manzin, A.; De Filippis, A.; Galdiero, M.; Franci, G. Anthraquinone Rhein Exhibits Antibacterial Activity against Staphylococcus aureus. Appl. Sci. 2022, 12, 8691. https://doi.org/10.3390/app12178691.

iv. Ann E. Eakin, Oluyinka Green, Neil Hales, Grant K. Walkup, Shanta Bist, Alok Singh, George Mullen, Joanna Bryant, Kevin Embrey, Ning Gao, Alex Breeze, Dave Timms, Beth Andrews, Maria Uria-Nickelsen, Julie Demeritt, James T. Loch III, Ken Hull, April Blodgett, Ruth N. Illingworth, Bryan Prince, P. Ann Boriack-Sjodin, Sheila Hauck, Lawrence J. MacPherson, Haihong Ni, and Brian Sherer. Pyrrolamide DNA Gyrase Inhibitors: Fragment-Based Nuclear Magnetic Resonance Screening To Identify Antibacterial Agents. American Society for Microbiology Antimicrobial Agents and Chemotherapy Volume 56, Issue 3, March 2012, Pages 1240-1246. https://doi.org/10.1128/AAC.05485-1.

v. Tabassum Khan, Kaksha Sankhe, Vasanti Suvarna, Atul Sherje, Kavitkumar Patel, Bhushan Dravyakar, DNA gyrase inhibitors: Progress and synthesis of potent compounds as antibacterial agents, Biomedicine & Pharmacotherapy, Volume 103, 2018, Pages 923-938, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2018.04.021.

vi. Davide Benedetto Tiz, Žiga Skok, Martina Durcik, Tihomir Tomašič, Lucia Peterlin Mašič, Janez Ilaš, Anamarija Zega, Gábor Draskovits, Tamás Révész, Ákos Nyerges, Csaba Pál, Cristina D. Cruz, Päivi Tammela, Dušan Žigon, Danijel Kikelj, Nace Zidar. An optimised series of substituted N-phenylpyrrolamides as DNA gyrase B inhibitors, European Journal of Medicinal Chemistry, Volume 167, 2019, Pages 269-290, ISSN 0223-5234, https://doi.org/10.1016/j.ejmech.2019.02.004.

vii. Cristina Nastasă, Dan C. Vodnar, loana lonuț, Anca Stana, Daniela Benedec, Radu Tamaian, Ovidiu Oniga, Brînduşa Tiperciuc. Antibacterial Evaluation and Virtual Screening of New Thiazolyl-Triazole Schiff Bases as Potential DNA-Gyrase Inhibitors. Int. J. Mol. Sci. 2018, 19(1), 222; https://doi.org/10.3390/ijms19010222.

viii. Coba-Males, M.A.;Santamaría-Aguirre, J.; Alcívar-León,C.D. In Silico Evaluation of New Fluoroquinolones as Possible Inhibitors of Bacterial Gyrases in Resistant Gram-Negative Pathogens.Chem. Proc. 2022, 8, 43. https://doi.org/10.3390/ecsoc-25-11753.

ix. Elisabetta Leo, Katherine A. Gould, Xiao-Su Pan, Giovanni Capranico, Mark R. Sanderson, Manlio Palumbo, L. Mark Fisher. Novel Symmetric and Asymmetric DNA Scission Determinants for Streptococcus pneumoniae Topoisomerase IV and Gyrase Are Clustered at the DNA Breakage Site, Journal of Biological Chemistry, Volume 280, Issue 14, 2005, Pages 14252-14263, ISSN 0021-9258, https://doi.org/10.1074/jbc.M500156200.

x. Abdalkader Saeed Latif, Essam Fadel Al-Jumaily. Evaluation study of Coumarin molecules against Glycosyltransferase enzyme of Streptococcus pneumonia. International Journal of Pharmaceutical Research. (2020). Supplementary Issue 1, PP 808-813. ISSN: 0975-2366. http://www.ijpronline.com/ViewSpecialArticleDetail.aspx?ID=207 .

xi. Abdal- Kader S L, Essam F J, Redha B. (2016). Effect of a new Quinoline-2-one Derivatives (Compound 3) On Purified DNA gyrase from clinical isolate Pseudomonas aeruginosa PA31. IOSR Journal Of Pharmacy. 2250-3013, (p)-ISSN: 2319-4219.

xii. Matos M J, Vina D, Vazquez-Rodriguez S, Uriarte E, Santana L. (2012). Focusing on new monoamine oxidase inhibitors: differently substituted coumarins as an interesting scaffold. Current Topics in Medicinal Chemistry (Sharjah, United Arab Emirates). 12(20):2210-39.

xiii. Osheroff, N. and Bender, R. P. (2008). DNA topoisomerases as targets for the chemotherapeutic treatment of cancer. In: Dai W, editor. Checkpoint Responses in Cancer Therapy. New Jersey: Humana Press, Totowa; 57–91.

xiv. Osheroff, N. and Baldwin, E. L. (2005). Etoposide, topoisomerase II and cancer. Curr. Med. Chem. Anticancer Agents.5 (4) :363–372.

xv. Hiasa, H.; Drlica, K.; Kerns, R.; Malik, M.; Mustaev, A. and Zhao, X. (2009) Quinolones: Action and resistance updated. Curr. Top. Med. Chem. 9(11): 981−998.

xvi. Abdal- Kader Saeed Latif, Essam Fadel Al-Jumaily and Redha Al-Bayati. Effect of a new Quinoline-2-one Derivatives (Compound 3) on Purified DNA gyrase from clinical isolate Pseudomonas aeruginosa PA31. IOSR Journal Of Pharmacy. (2016). V 6, Issue 12 Versions. PP. 12-18.

xvii. Liou, G. F.; Pitts, S. L.; Mitchenall, L. A.; Burgin, A. B.; Maxwell, A.; Neuman, K. C. and Osheroff, N. (2011). Use of divalent metal ions in the DNA cleavage reaction of topoisomerase IV. Nucleic Acids Res. 39(11): 4808−4817.

xviii. Kroemer, R. T. (2007). Structure-based drug design: Docking and scoring. Curr. Protein Pept. Sc.8 (4), 312-328.