**Turkish Computational and Theoretical Chemistry**

*Turkish Comp Theo Chem (TC&TC)*

***Volume(Issue): 7(3) – Year: 2023 – Pages: 10-16***

***e-ISSN: 2602-3237***

***https://doi.org/10.33435/tcandtc.1196422***

***Received***: 29.10.2022 ***Accepted***: 31.01.2023 *Research Article*

# *In silico ADME, toxicological analysis, molecular docking studies and Molecular dynamics simulation of Afzelin with potential antibacterial effects against Staphylococcus aureus*

**Elhafnaoui Lanez, Nadjiba Zegheb, Touhami Lanez**[1]

University of El Oued, Faculty of Sciences, Chemistry Department, VTRS Laboratory, B.P.789, 39000, El Oued, Algeria

**Abstract:** Afzelin has been designed and tested for its in silico antibacterial activity against DNA gyrase complex of Staphylococcus aureus. The results of the toxicity study indicate that afzelin displayed moderate antibacterial potential against staphylococcus aureus with LD50 = 5000 mg/Kg, which is almost four times and a half weaker than that obtained for the commercial antibiotic chloramphenicol. The afzelin and the commercial antibiotic chloramphenicol were subjected to docking studies to understand their interaction with DNA gyrase complex of Staphylococcus aureus. Results indicated a good affinity of afzelin to the chosen target with the formation of four hydrogen bonds and binding energy of -29.82 kJ/mol. ADME study shows that afzelin is not inhibitors of CYP450 IA2, 2C19, 2C9, 2D6, 3A4 isoenzymes which suggests a decrease in their plasma concentrations and a rapid elimination route. Molecular dynamics simulations were performed for 10 ns for afzelin using the gromacs package to assess the conformational stability of protein-ligand complexes during the simulation.

***Keywords:*** Binding energy, antibacterial activity, afzelin, molecular docking

## 1. Introduction

Nowadays, the resistance of pathogenic bacteria to therapeutic antibiotics is considered a major public health problem, this resistance of bacteria against multidrug-resistance is growing at a worrying percentage and it causes mortality [1,2]. As a result, there is an urgent need to develop new drugs with diverse mechanisms of action to fight the increasing danger of drug-resistant bacteria.

Afzelin is a very interesting compound and is reported for its pharmacological activities such as antibacterial [3,4], anti-inflammatory [5,6], antiepileptic [6], antidiabetic [7], and anti-cancer properties [6,8-10]. Moreover, afzelin is a natural product compound found in many plants and organisms. In addition, afzelin is a naturally occurring glycosyloxy flavone molecule that has been identified in all plant parts of more than 60 plants among which are known to be edible, for example, the specie Euphorbia falcata L that is widespread in Algeria [11,12]. Due to the well-known potential of afzelin to inhibit triple-negative breast cancer cell migration [13], further studies are needed to identify target proteins of afzelin, as part of developing targeted therapy.

Bacteria enzyme of pseudomonas putida and DNA gyrase complex of Staphylococcus aureus have long been known as an attractive target for antibacterial drugs [14], it is an essential enzyme across bacterial species, and inhibition results in a disruption of DNA synthesis and ultimately cell death [15]. It controls the topological state of DNA in cells by making a double-stranded break in one DNA duplex, transporting another DNA segment through this break, and then resealing it [16,17].

In our previous study we reported a combined molecular docking and dynamics simulations studies of natural compounds as potent inhibitors against SARS-CoV-2 main protease [18], this study aimed to identify the binding affinity of a potential target protein with afzelin that is associated with antibacterial activity. For this purpose, the ligand

[1] Corresponding Authors

e-mail: touhami-lanez@univ-eloued.dz

afzelin was docked to DNA gyrase complex of Staphylococcus aureus. Afzelin interaction with the target proteins was visualized and compared with experimental results based on its binding energy. Further, the ligand afzelin was scrutinized through toxicity analysis and ADMET study to predict the median lethal dose (LD50) and the drug-likeness properties.

## 2. Computational Method

The following software and online web server were used in the present study,

Software: AutoDockVina 1.1.2, MGLTools 1.5.7, Discovery Studio 3.5, PyMOL 2.5.2, gromacs. Online web server: ProTox-II and swissadme

### System information

Molecular docking simulations were conducted on a personal computer with the following characteristics: Processor: Intel CORE i3-7100U CPU @ 2.40 GHz processor, system memory: 4 GB RAM, system type: 64-bit operating system, Windows 11 as Operating System.

Molecular dynamics simulations were performed in HP z238 microtower workstation with i7 processor and system memory 8 GB RAM in Ubuntu 18 Operating System.

### Ligand preparation

The ligand afzelin structure was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) as 3D SDF file format. It was then converted to protein data bank format, and used for docking, figure 1.

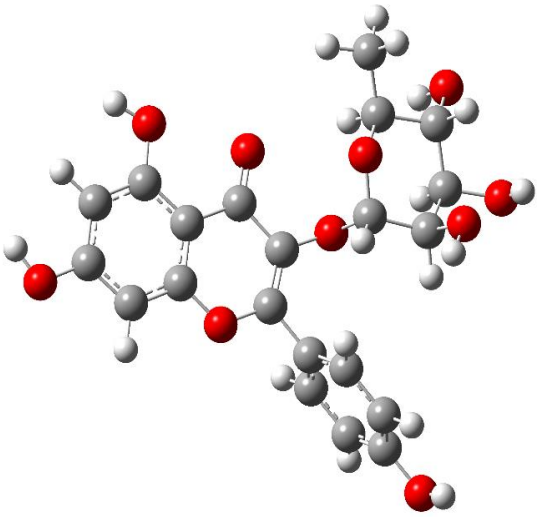


**Figure 1.** The 3D chemical structure of afzelin

### Protein preparation

The 3-dimensional structure of DNA gyrase, (PDB ID: 3TTZ) of staphylococcus aureus involved in this study was chosen and downloaded from a research collaboratory for structural bioinformatics protein data bank (RSCB PDB) (https://www.rcsb.org/pdb) [19] Figure 2, the protein was in a complex with co-crystal pyrrolamide inhibitor with a resolution of 1.63 Å. Water molecules, inhibitor, and other heteroatoms were removed from the protein structure using AutoDockTools and then saved as pdb format.

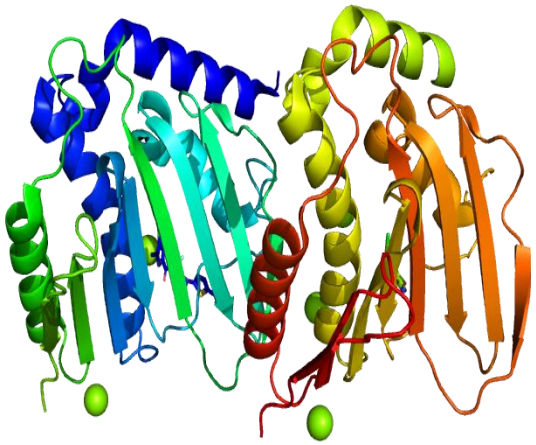


**Figure 2.** Three-dimensional structure of DNA gyrase (PDB ID: 3TTZ) of *staphylococcus aureus*

### Docking parameters

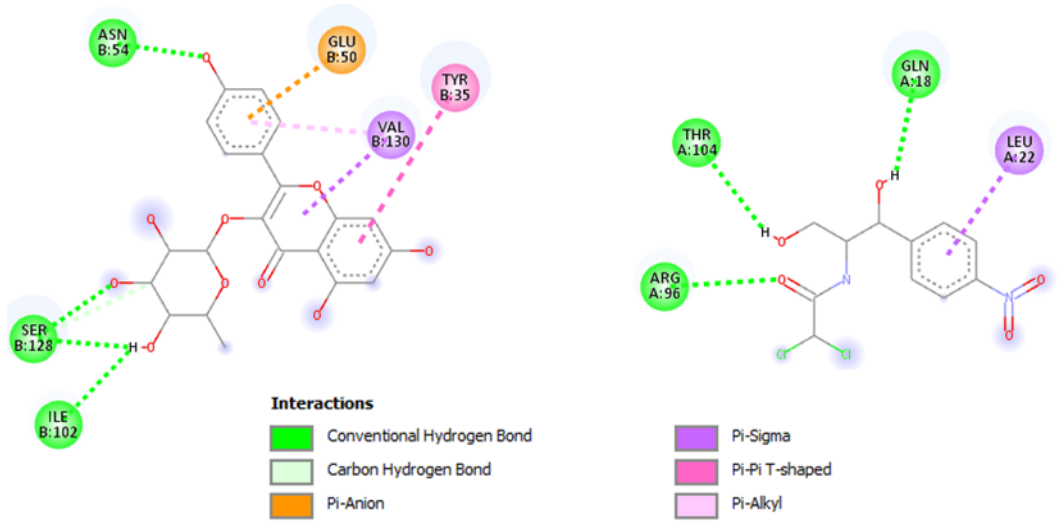
The grid parameters were set to cover the entire 3-dimensional active site of of DNA gyrase, grid spacing was set to its default value 0.375 Å. Center grid box values were set to x = 3.197, y = 1.623 and z = 20.052. The number of grid points along the x, y, and z dimensions was set as 40×40×40. The output was saved in the grid dimension file.

## 3. Results and discussion

Results from the molecular docking suggest that hydrogen bonding besides other bonding interactions are involved in the binding process. Figure 3 illustrates the interactions of afzelin and the commercial antibiotic chloramphenicol with the nearby residues in the active site of DNA gyrase complex of *Staphylococcus aureus*. The commercial antibiotic chloramphenicol is chosen as a control because it is an antibiotic drug that is prescribed for severe infections specially when there is a poor response to existing therapies. Since the 1950s, it has been used to treat a wide range of microbiological diseases, including typhoid fever

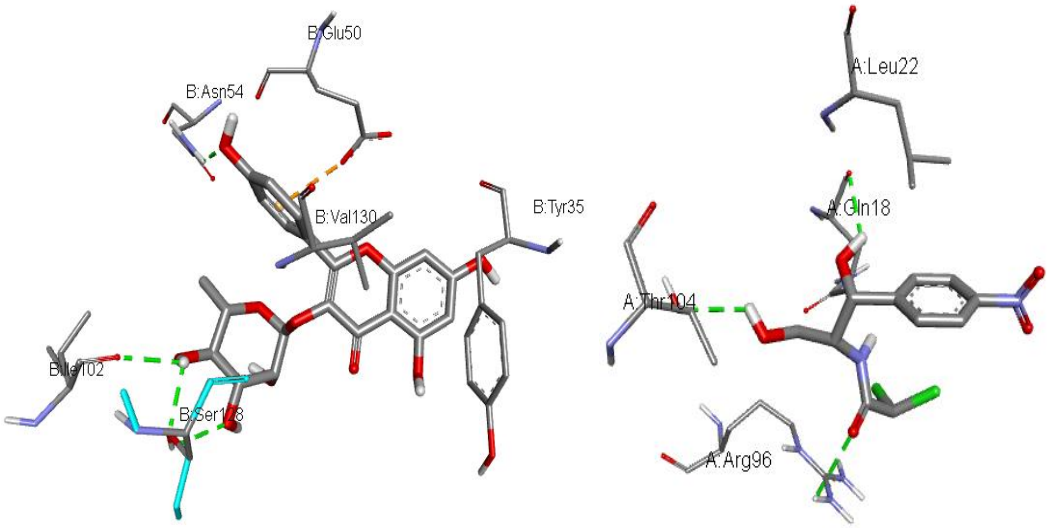
and various types of salmonellosis, as well as central nervous system, anaerobic, and ophthalmic infections [20].

To compare the affinity of afzelin with conventional drugs we simulated docking of afzelin with the target DNA gyrase (3TTZ) of *staphylococcus aureus*. The conventional drugs we chose is chloramphenicol, an antibiotic useful for the treatment of a number of bacterial infections.



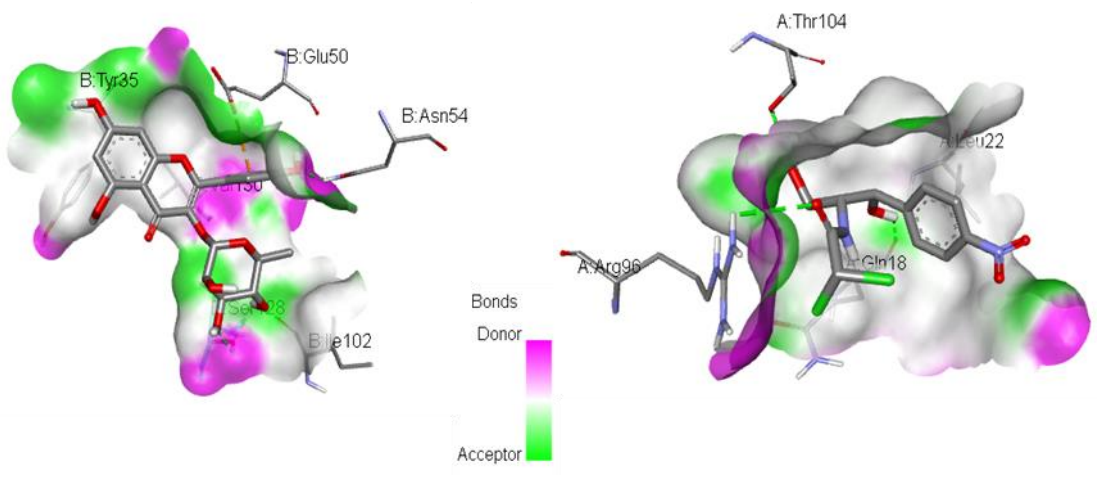

**(a)** **(b)**

**Figure 3.** 2D Molecular interactions DNA-gyrase complex with the ligand afzelin (a) and the drug chloramphenicol (b), the hydrogen bonds are presented in green dashed lines




**(a)** **(b)**

**Figure 4.** 3D Binding mode of the ligand afzelin (a) and the drug chloramphenicol (b) with DNA-gyrase complex, the hydrogen bonds are presented in green dashed lines




**(a)** **(b)**

**Figure 5.** 3D Binding mode of the ligand afzelin (a) and the drug chloramphenicol (b) with DNA-gyrase complex

**Table 1**. Hydrogen bonding between ligands afzelin and the drug chloramphenicol, DNA gyrase complex of Staphylococcus aureus

| Molecule | Bond type | Amino acid | Distance (Å) | -ΔG (kJ/mol) |
|---|---|---|---|---|
| Afzelin | H-bonds | Ser128 | 2.49 | 29.82 |
| | | Ser128 | 2.26 | |
| | | Ile102 | 2.37 | |
| | | Asn54 | 2.02 | |
| Chloramphenicol | H-bonds | Arg96 | 2.87 | 24.36 |
| | | Thr104 | 2.75 | |
| | | Gln18 | 2.42 | |

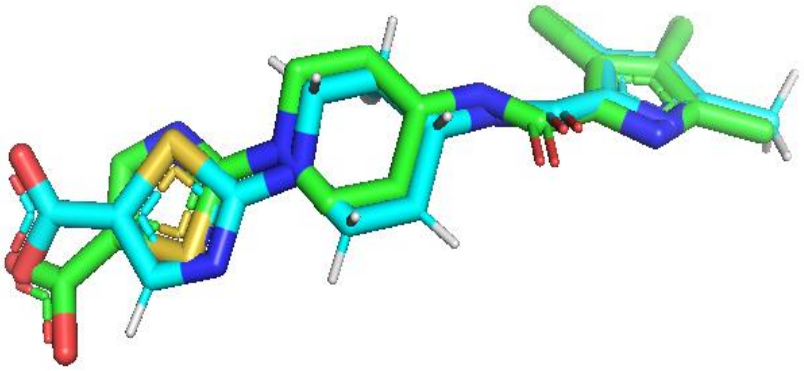


**Figure 6**. Comparison between the re-docked pose and original ligand; cyan: docked pose; green: original ligand) with an RMSD value of 0.513 Å

**Table 2.** Metabolism and excretion by the CYP450 isoenzymes inhibition of the ligand afzelin and the drug chloramphenicol

| Compound | IA2 | 2C19 | 2C9 | 2D6 | 3A4 |
|---|---|---|---|---|---|
| Afzelin | No | No | No | No | No |
| Chloramphenicol | No | No | No | No | No |

Afzelin had the highest negative interaction energy, it interacted with the target DNA gyrase (3TTZ) of staphylococcus aureus via four conventional hydrogen bonds with the residues Ser128, Ile102, and Asn54, however, the commercial antibiotic chloramphenicol interacted only via three conventional hydrogen bonds with the residues Arg96, Thr104, and Gln18. Table 1 summarised the interacting residues and their corresponding bond types and length with the binding energy.

**Docking validation**

The docking procedure was validated by redocking of the co-crystalized ligand (07 N) to the ATP binding site of DNA gyrase using the same methodology that was used previously in the docking process. The ligand (07 N) was separated from the protein structure and saved as a pdb file. Water molecules and co-factors, which did not affect the binding site, were removed. Hydrogen atoms were added. The ligand (07 N) was then re-docked into the active site using the same protocol including the grid parameters. The re-docked complex was then superimposed on to the reference co-crystallized complex, Figure 6 presents a superimposed view of the re-docked conformation (cyan color) and the original ligand (green color), and the RMSD value between them is 0.513 Å. The clear superimposed between both ligands and also the RMSD value less than 2 indicates the efficiency of the AutoDock Vina algorithms to perform molecular docking protocol with confidence.

**ADME study**

In silico ADME study was carried out to predict the adverse metabolic effects of oral administration of afzelin as drug candidate, as well as their half-life in the organism and excretion route [21]. Cytochrome P450 isoenzymes (CYP450) are oxidases that interact with drugs in order to decrease their plasma concentration and reduce the risks of toxicity by metabolic activation, as well as making them more water soluble for elimination [22-25]. Thus, a drug candidate should not inhibit cytochrome CYP450 isoenzymes because inhibition may increase the plasma concentration.

Table 2 shows that both afzelin and chloramphenicol are not inhibitors of CYP450 IA2, 2C19, 2C9, 2D6, 3A4 isoenzymes which suggests a decrease in their plasma concentrations and a rapid elimination route.

**Table3.** Toxicity end points of the ligand afzelin and the drug chloramphenicol

| Compound | $LD_{50}$ | Hepato | Carcino | Immuno | Mutagen | Cito | TC |
|---|---|---|---|---|---|---|---|
| Afzelin | 5000 | -0.73 | 0.5 | 0.92 | -0.71 | -0.93 | 5 |
| Chloramphenicol | 1500 | -0.70 | -0.6 | -0.99 | 0.63 | -0.64 | 4 |

LD50 (mg/kg), - (Inactive toxic class (probability score)), + (Active toxic class (probability score))

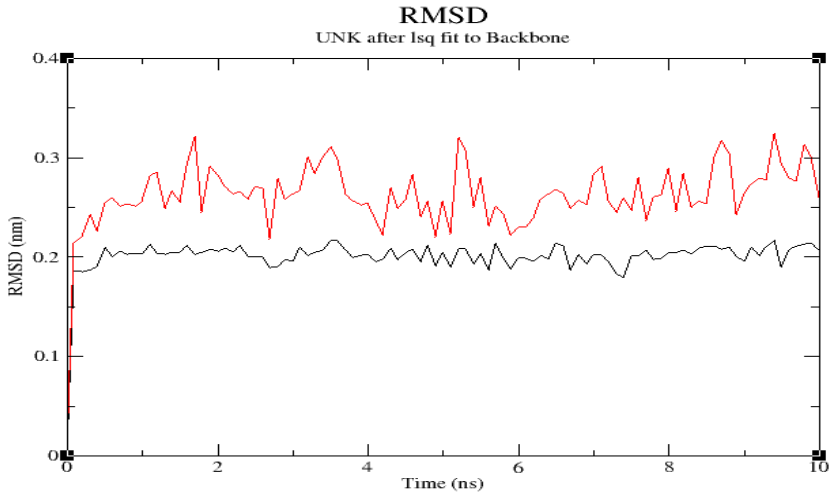



**Figure 7.** The RMSD analysis of molecular dynamics trajectories for of afzelin/ DNA gyrase complex

**Toxicity study**

Toxicity study aims to determinate the toxicity proprieties of the ligand afzelin and the drug chloramphenicol. The study was performed using the ProTox-II web server [26]. It aims to predict hepatotoxicity (hepato), carcinogenicity (carcino), immunotoxicity (immuno), mutagenicity (mutagen), cytotoxicity (cyto), median lethal dose (LD50), and toxicity class (TC). According to in silico toxicity profiles presented in Table 3, the toxicity class was detected to be equal to 5 for afzelin and 4 for chloramphenicol, afzelin was

predicted to be toxic in carcinogenicity and immunotoxicity, but chloramphenicol was predicted to be toxic in mutagenicity.

**Molecular dynamics simulations**

The molecular dynamics simulation was carried out to investigate the stability of the examined docked complex afzelin/DNA gyrase complex in physiological conditions using gromacs software. The molecular dynamics simulations were performed for 10 ns. The root mean square deviation (RMSD) value were key indexes to evaluate the stability of afzelin/DNA gyrase complex. The RMSD values during the MD simulation were provided in Figure 7. The RMSD values tended to relative equilibrium states with an average value of 0.15-0.30 nm during the 10 ns. Therefore, the examined docked complex had good stability.

## 4. Conclusions

The *in silico* antibacterial activity of afzelin was accessed through molecular docking followed by molecular dynamic of the interaction of afzelin with DNA gyrase complex of staphylococcus. The results of the analysis shows that the afzelin possesses the highest negative interaction energy and interacted with the target DNA gyrase of staphylococcus aureus via four hydrogen bonds. However, the commercial antibiotic chloramphenicol possesses the lowest negative interaction energy and interacted only via three hydrogen bonds, furtermore the results of the toxicity study indicate that afzelin displayed a median lethal dose of 5000 mg/Kg, and a toxicity class equal to 5. Finaly, Molecular dynamics simulations of the docked complex confirme its stability.

## References

[1] V.M. D'Costa, C.E. King, L. Kalan, M. Morar, W.W.L. Sung, C. Schwarz, D. Froese, G. Zazula, F. Calmels, R. Debruyne, Antibiotic resistance is ancient, Nature 477 (2011) 457–461.

[2] K. Bhullar, N. Waglechner, A. Pawlowski, K. Koteva, E.D. Banks, M.D. Johnston, H.A. Barton, G.D. Wright, Antibiotic resistance is prevalent in an isolated cave microbiome, PLoS One 7 (2012) e34953-e34953.

[3] S.Y. Lee, Y.J. So, M.S. Shin, J.Y. Cho, J. Lee, Antibacterial Effects of Afzelin Isolated from Cornus macrophylla on Pseudomonas aeruginosa, A Leading Cause of Illness in Immunocompromised Individuals, Molecules 19 (2014) 3173-3180.

[4] J. Wang, A.M. Sci, M. Cao, B. Fan, T. Zhen, A pre-clinical trial study on afzelin: anti-human lung cancer, anti-cholinesterase, and anti-glucosidase properties, Archives of Medical Science (2021) 1-9.

[5] K. Shingnaisui, T. Dey, P. Manna, J. Kalita, Therapeutic potentials of Houttuynia cordata Thunb. against inflammation and oxidative stress: A review, J Ethnopharmacology 220 (2018) 35–43.

[6] B.M. Radu, F.B. Epureanu, M. Radu, P.F. Fabene, G. Bertini, Nonsteroidal anti-inflammatory drugs in clinical and experimental epilepsy, Epilepsy Research 131 (2017) 15–27.

[7] D.G. Lee, J.S. Lee, N.G. Quilantang, S.D. Jacinto, S. Lee, Determination of Afzelin and Astragalin from Lespedeza cuneata on Aldose Reductase Inhibition, Journal of chromatographic science 59 (2021) 381–387.

[8] K.C. Zhu, J.M. Sun, J.G. Shen, J.Z. Jin, F. Liu, X.L. Xu, L. Chen, L.T. Liu, J.J. Lv, Afzelin exhibits anti-cancer activity against androgen-sensitive LNCaP and androgen-independent PC-3 prostate cancer cells through the inhibition of LIM domain kinase 1, Oncology Letters 10 (2015) 2359–2365.

[9] I. Radziejewska, K. Supruniuk, R. Czarnomysy, K. Buzun, A. Bielawska, Anti-Cancer Potential of Afzelin towards AGS Gastric Cancer Cells, Pharmaceuticals (Basel), 14 (2021) 1-16.

[10] D. Utepbergenov, U. Derewenda, N. Olekhnovich, G. Szukalska, B. Banerjee, M.K. Hilinski, D.A. Lannigan, P.T. Stukenberg, Z.S. Derewenda, Insights into the inhibition of the p90 ribosomal S6 kinase (RSK) by the flavonol glycoside SL0101 from the 1.5 Å crystal structure of the N-terminal domain of RSK2 with bound inhibitor, Biochemistry 51 (2012) 6499–6510.

[11] S. Kumar, A.K. Pandey, Chemistry and biological activities of flavonoids: An overview, The Scientific World Journal 2013 (2013) 1-16.

[12] F.M. Afendi, T. Okada, M. Yamazaki, A. Hirai-Morita, Y. Nakamura, K. Nakamura,

S. Ikeda, H. Takahashi, M. Altaf-Ul-Amin, L.K. Darusman, K. Saito, S. Kanaya, KNApSAcK family databases: integrated metabolite-plant species databases for multifaceted plant research, Plant and Cell Physiology 53 (2012) 1-12.

[13] E. Rachmi, B.B. Purnomo, A.T. Endharti, L.E. Fitri, Identification of afzelin potential targets in inhibiting triple-negative breast cancer cell migration using reverse docking, Porto Biomedical Journal 5 (2020) 1-8.

[14] H. Lade, J.S. Kim, Bacterial targets of antibiotics in methicillin-resistant staphylococcus aureus, Antibiotics 10 (2021) 1-29.

[15] D.J. Dwyer, M.A. Kohanski, B. Hayete, J.J. Collins, Gyrase inhibitors induce an oxidative damage cellular death pathway in Escherichia coli, Molecular Systems Biology 3 (2007) 1-15.

[16] A.Y.C. Saiki, L.L. Shen, C.-M. Chen, J. Baranowski, C.G. Lerner, DNA Cleavage Activities of Staphylococcus aureus Gyrase and Topoisomerase IV Stimulated by Quinolones and 2-Pyridones, Antimicrobial Agents and Chemotherapy 43 (1999) 1574-1577.

[17] S. Gottschalk, D. Ifrah, S. Lerche, C.T. Gottlieb, M.T. Cohn, H. Hiasa, P.R. Hansen, L. Gram, H. Ingmer, L.E. Thomsen, the antimicrobial lysine-peptoid hybrid LP5 inhibits DNA replication and induces the SOS response in Staphylococcus aureus, BMC Microbiology 13 (2013) 1-8.

[18] M. Ouassaf, S. Belaidi, S. Chtita, T. Lanez, F. Abul Qais, H. Md Amiruddin. Combined molecular docking and dynamics simulations studies of natural compounds as potent inhibitors against SARS-CoV-2 main protease, Journal of Biomolecular Structure and Dynamics 40 (2022**)** 11264-11273.

[19] B.A. Sherer, K. Hull, O. Green, G. Basarab, S. Hauck, P. Hill, J.T. Loch, G. Mullen, S. Bist, J. Bryant, A. Boriack-Sjodin, J. Read, N. Degrace, M. Uria-Nickelsen, R.N. Illingworth, A.E. Eakin, Pyrrolamide DNA gyrase inhibitors: Optimization of antibacterial activity and efficacy, Bioorganic Medicinal Chemistry Letters 21 (2011) 7416–7420.

[20] L. Drago, Chloramphenicol resurrected: A journey from antibiotic resistance in eye infections to biofilm and ocular microbiota, Microorganisms 7 (2019) 1-12.

[21] A.C.R. Silvino, G.L. Costa, F.C.F. De Araújo, D.B. Ascher, D.E.V. Pires, C.J.F. Fontes, L.H. Carvalho, C.F.A. De Brito, T.N. Sousa, Variation in Human Cytochrome P-450 Drug-Metabolism Genes: A Gateway to the Understanding of Plasmodium vivax Relapses, PLoS One 11 (2016) 1-14.

[22] T. Eitrich, A. Kless, C. Druska, W. Meyer, J. Grotendorst, Classification of highly unbalanced CYP450 data of drugs using cost sensitive machine learning techniques, J of Chemical Information and Modeling 47 (2007) 92–103.

[23] M. Louet, C.M. Labbé, C. Fagnen, C.M. Aono, P. Homem-de-Mello, B.O. Villoutreix, M.A. Miteva, Insights into molecular mechanisms of drug metabolism dysfunction of human CYP2C9*30, PLoS One 13 (2018) 1-21.

[24] R.A. Terkeltaub, D.E. Furst, J.L. Digiacinto, K.A. Kook, M.W. Davis, Novel evidence-based colchicine dose-reduction algorithm to predict and prevent colchicine toxicity in the presence of cytochrome P450 3A4/P-glycoprotein inhibitors, Arthritis and Rheumatology 63 (2011) 2226–2237.

[25] A. Zahno, K. Brecht, R. Morand, S. Maseneni, M. Török, P.W. Lindinger, S. Krähenbühl, The role of CYP3A4 in amiodarone-associated toxicity on HepG2 cells, Biochemical Pharmacology 81 (2011) 432–441.

[26] M.N. Drwal, P. Banerjee, M. Dunkel, M.R. Wettig, R. Preissner, ProTox: a web server for the *in-silico* prediction of rodent oral toxicity, Nucleic Acids Research 42 (2014) W53–W58.