Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

In silico screening and design of N-substituted 7-piperazin-1-ylfluoroquinolone-derived DNA gyrase inhibitor antibiotics

Mokhenane Ahmed Mosetfa[1], Hadjadj-Aoul Fatima Zohra[2], Benabid Fatma Zohra[3*], Kraim Khaireddine[4], Zouai Foued[1] and Adouani Imene[1]

1: Laboratoire de Biopharmacie et pharmacotechnie, Faculty of Medicine, Sétif, Algeria
2: Laboratory of Medicinal Chemistry, Faculty of Medicine, Algiers, Algeria
3: LMPMP, Faculty of Technology, Faculty of Technology, Ferhat ABBAS University Sétif-1, Algeria
4: The Higher Normal School of Technological Education, Skikda, Algeria
* Corresponding author: fzbenabid@yahoo.fr

ABSTRACT

As multidrug-resistant pathogens have increasingly become a serious public health problem, especially in the last decade, the design of new active agents against resistant organisms is of great importance.
The design of new pharmacologically active molecules against an enzyme can be done by virtual screening, based either on the ligand or on the structure of the target.
Docking is the "structure based" method used to measure the affinity of ligands for the DNA Gyrase protein, it allows to generate the different poses, their positions, orientations and conformations in the active site.
The predictions provided by virtual screening allow to propose a good strategy for the development of a good pharmacophore, it can thus constitute an adequate support to validate or reject a hypothesis.
The protein data base used in our study is 2XCT: The 3.35A° paired structure of the S. aureus Gyrase complex with ciprofloxacin and DNA, this pdb was chosen according to the criteria mentioned below and was downloaded from the protein data base.
According to the first part of our results, the molecules that contain nitrogen rings in position 7 are the most active, most of the marketed drugs take this basic structure.
The 7-piperazin-1-ylfluoroquinolone series is not fully exploited, especially the N-acylated and N-chloroalkylated derivatives.
In this study, we performed a docking test on a series of molecules selected according to their structures close to the reference inhibitors and which are likely to interact with DNA gyrase in-silico.

Key-words
Virtual screening, in-silico activity, DNA gyrase, fluoroquinolones; activity prediction, pharamcophore.

Introduction

The phenomenon of multidrug-resistant pathogens continues to be a growing public human health problem especially in the last decade [1]; the complex clinical resistance involves interaction between the type of bacterium, its location and distribution in the body and its infection site concentration as well as the immune status of the patient [2]. Unfortunately, the emerging increase in antimicrobial resistance is threatening especially ill patients. Thereby the World Health Organization has named antibiotic resistance as one of the three most important public health threats of the 21st century [3].

For many years, researches continue to discover new potential targets and novel compounds with broad spectra of efficacy against bacterial antibiotic resistance, Matjaz Brvar et al. [4] identified a novel series of 4′-methyl-N2-phenyl-[4,5′-bithiazole]-2,2′-diamine inhibitors of gyrase B with a low micromolar inhibitory activity by implementing a two-step structure-based design procedure. They observed that the highest increase of thermal stability of the complex inhibitor 18−protein G24 matches the best in vitro activity of this compound. In addition, the significance of the inhibitor interaction with the protein loop residue Gly101 was revealed as significant factor in the binding of this compound class. On the other hand, the discovery and the development of new antibacterial drugs have been investigated by Nicole Jackson et al. [5]. Authors mentioned that in order to facilitate discovery activities researchers must be increased understanding of the scientific problems experienced by pharmaceutical companies. Due to the rapid emergence of antibiotic-resistant bacteria, Jonathan M. Stokes et al. [6] have trained a deep neural network capable of predicting molecules with antibacterial activity. They

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

performed predictions on multiple chemical libraries and discovered a molecule from the Drug Repurposing Hub-halicin which is structurally divergent from conventional antibiotics and displays bactericidal activity against a wide phylogenetic spectrum of pathogens including Mycobacterium tuberculosis and carbapenem-resistant Enterobacteriaceae.
DNA gyrase is an enzyme of the class II DNA topoisomerase family, which is capable of introducing a negative supercoil into DNA, its inhibition contributes to bactericidal effects [7]. The quinolone class of antibiotics inhibits the DNA synthesis of bacteria by disrupting the bacterial topoisomerase type II; inhibiting the catalytic activity of DNA gyrase and topoisomerase IV [8]. Quinolones kill bacteria by interfering with DNA synthesis and inhibiting their replication pathway [9].

The design of new pharmacologically active molecules against an enzyme can be done by virtual screening, based either on the ligand or on the structure of the target. The predictions provided by virtual screening allow proposing a good strategy for the development of a good pharmacophore, it can thus constitute an adequate support to validate or reject a hypothesis [7]. Among the most used modeling methods, we can mention the QSAR method (quantitative structure-activity relatioships) [8]. In the case where the structure of the target is known, we speak of structure based virtual screening [9] and after screening, we move on to biological testing of molecules with significant theoretical activity [7].

1. Materials and methods

1.1. Materials

2.1.1. Database

A- The protein data base (PDB)

The used protein data base in this study is 2XCT: The 3.35A° paired structure of the S. aureus Gyrase complex with ciprofloxacin and DNA [10].This PDB which was chosen according to the criteria mentioned below was downloaded from the protein data base [11].

Choice criteria

- Ligand-protein complex whose ligand has been tested in vitro.
- Presence of a reference ligand.
- Low RMSD value after redocking
- PDB with no error after opening by the docking software.

The used PDB, ID and resolution results are shown in table 1.

Table 1: Used PDB, ID and resolution results

PDB	Ligand	Enzyme	RMSD average	Resolution
2XCS	GSK299423	*S. aureus* DNA gyrase	4.231	2.1A°
5NPK	Tiophene	*S. aureus* DNA gyrase	5.611	1.98A°

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

2XCT	Ciprofloxacin	*E. coli* DNA gyrase	1.716	3.35A°
3ILW	E60211	N-terminal subunit of *E. coli* DNA gyrase	5.728	1.60A°
4Z2C	Moxifloxacin	*S. pneumoneae* DNA gyrase	4.315	2.80A°

B- Ligands

The 1662 ligands were downloaded in SDF (structure data file) format from the PubChem chemical molecule database.

Selection criteria

- 90% similarity rate with the reference ligand (ciprofloxacin).
- The originality of the molecules was not taken into consideration when choosing the ligands to be docked.

1.1.2. Computers

Our molecular docking was run using a machine with an Intel Core i7 2630QM processor, equipped with an NVIDIA GeForce GT 540M Cuda 2GB graphics card and 8GB of ram.

1.1.3. Software

The docking was launched by the MOLEGRO VIRTUAL DOCKER software [12].The software that was used for the correction of the PDB is DeepView/Swiss-PdbViewer [13].

1.2. Methods

Molecular Docking

Molecular Docking is a topology of the active site by a three-dimensional representation, specifying the geometry of distances and energies of different types of bonds that significantly influence the stereochemistry on which the ligand-receptor interaction depends [14].

The drug development's goal against pathogenic organisms is the fully inhibition, leading to pathogen death. Antimicrobial drug targets should be necessary having one function and must be present only in the pathogen and be inhibited by a small molecule. The target must be known, as it is part of a crucial cycle in the cell, and its removal must lead to the death of the

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

pathogen. The target must be unique; no other pathway must be able to complement the function of the target and overcome the presence of the inhibitor. If the macromolecule meets all the criteria described for being a drug target, but functions equally well in healthy human cells as in a pathogen, specificity can often be built into the inhibitor, exploiting structural or biochemical differences between the pathogenic and human forms. Finally, enzymes are often excellent drug targets because compounds can be designed to fit into the active site pocket [14].

The first selection step consists of placing the ligand in the active site of the protein and sampling the possible conformations, positions and orientations (poses), retaining only those that represent the most favorable interaction modes [7].

The second step is the scoring, which consists in evaluating the ligand/protein affinity and giving a score to the poses obtained during the docking phase. This score will allow us to select the best pose among all those proposed [15].

The types of automated docking programs can be divided into:

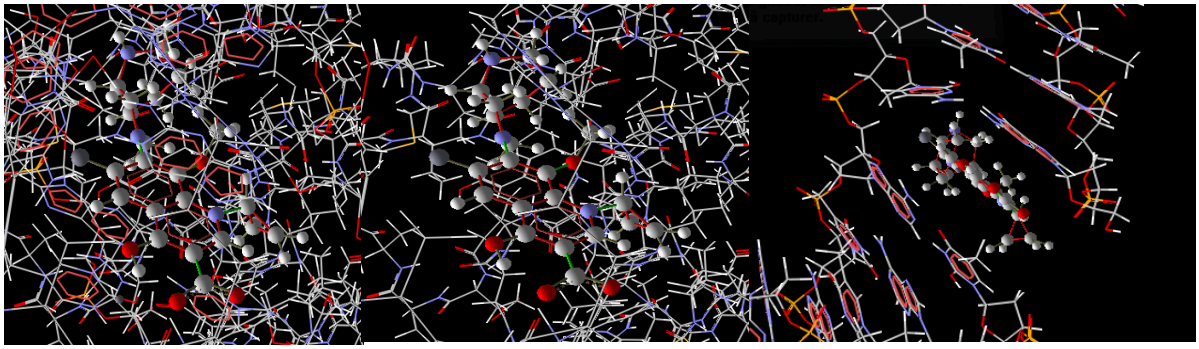
- Exhaustive: a large number of conformations are generated for each ligand, and all of them are evaluated (e.g. Fred);

- Stochastic: only a few solutions are evaluated; results may not be reproducible [16].

Docking is the "structure based" method used to measure the affinity of ligands for the DNA gyrase protein; it allows generating the different positions, orientations and conformations in the active site [17].

Reference ligand binding site as well as ligand-Protein interaction and ligand-DNA interaction are shown in figure 1.

The used search algorithm is the GPU screening CUDA and the score function: MolDock [12].



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

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

Figure 1: Reference ligand binding site (a), ligand-Protein interaction (b) and ligand-DNA interaction (c)

3. Results and discussion

3.1. Software validation

The RMSD (Root-mean-square deviation) values obtained during the redocking of PDBs (protein database) according to Table 1 vary from 1.716 to 5.728. The lowest value was chosen which corresponds to PDB 2XCT whose ligand is ciprofloxacin which is a reference antibiotic of the fluoroquinolone family [18].

3.2. Enzyme-ligand interaction energies

The 10 poses taken into consideration and represented in Table 2 are the molecules with the lowest binding energies, corresponding to the most stable ligand-enzyme complexes[19].

The selected molecules in our study are more active than the reference molecule. The binding energy of the reference ligand (ciprofloxacin) with the DNA of the enzyme complex has a value of -136.532 which is significantly lower than the value of its binding with the protein which is -17.741. The inhibitory activity of DNA gyrase, is therefore conditioned by a value of binding energy with the DNA lower than that of binding with the protein. Based on these data, the molecules with the lowest Ligand-DNA binding energies with a higher number of hydrogen bonds were considered, whose Ligand IDs are: 67941830, 56838103, 58180731.

Table 2: Enzyme-ligand binding energies of the database molecules

Ligand ID	Total energy	External energy		Internal energy	Number of H bonds with protein	Number of H bonds with DNA
		Prot-lig	DNA-lig			
Ciprofloxacin	-117,462	-17,741	-136,532	36	1	1
58344411	-227,36	-120,338	-130,173	23,152	5	4
67311808	-217,146	-63,992	-169,68	16,526	2	3
58180731	-212,910	-70,067	-180,520	40,677	2	5
42641770	210,317	-59,723	-168,259	17,666	5	4

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

59650039	207,700	-63,468	-160,613	16,381	6	2
24879259	206,421	-60,637	-163,113	17,328	2	1
67941830	206,193	-50,752	-172,302	16,861	2	4
68795618	205,889	-66,895	-170,371	31,377	3	2
14457298	205,604	-38,5	-182,521	15,417	1	2
56838103	-203,842-	-67,890	-175,343	39,391	1	4

3.3. Comparison of the interactions of the selected ligands with the reference one

By comparing the number of hydrogen bonds of the selected ligands, which increases from 4 to 5, with that of the reference one, which is equal to 1, we notice that the complexes formed with these molecules are more stable than that formed by the reference ligand (ciprofloxacin).

3.4. Identification of biological activity with selected ligands

67941830; the molecule has promising antitubercular activity [20].

56838103; the molecule has antimicrobial activity; it is patented under patent US8329908B2

58180731; the molecule has antimicrobial activity against E. coli but its antitubercular activity is not yet proven.

Our screening allowed us to select 3 theoretically active molecules; the first two are already described in the literature (67941830 and 56838103).

Our study can be continued by a demonstration of the antitubercular activity of the recently discovered molecule 58180731.

3.5. Design of new molecules

According to the previous results, the molecules containing nitrogen rings in position 7 are the most active, most of the marketed drugs take this basic structure [18].

The series of 7-piperazine fluoroquinolones is not fully exploited, especially the N-acylated and N-chloroalkylated derivatives.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

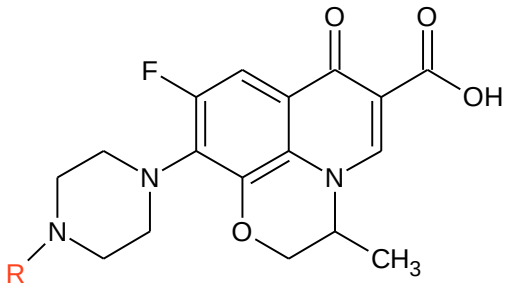


Figure 2: General structure of N-substituted 7-piperazine fluoroquinolones

In order to exploit this series, 10 molecules have been proposed of which 2 are already commercialized, 4 are already synthesized and their antibiotic activities are known, and 4 new molecules that have never been synthesized before, these molecules have been docked with the DNA gyrase PDB 2XCT with MOLEGRO VIRTUAL DOCKER via the cuda GPU scoring algorithm and the results are represented in Table 3.

Table 3: Enzyme-ligand binding energies of molecules from the design

R	Ligand-ID Dci/pubchem	Totale energy	External energy		Internal energy	Hydrogen bonds with the protein	Hydrogen bonds with DNA
			Prot-lig	DNA-Lig			
/	Ciprofloxacin	-118.638	-22.101	-128.481	31.944	Ser 1084 (B) Arg 1122 (B)	F, H, G
Methyl	Ofloxacine	-157.771	-68.846	-112.971	24.047	Gly 459 (B) Arg 1122 (D)	E
Propanoyl	Original molecule	-192.273	-67.982	-144.490	20.199	Gly 459 (B) Arg 1122 (B)	2xG, H, E
Chloromethyl	Original molecule	-178.398	-36.479	-167.615	25.696	Arg 1122 (G)	2xG, 2xH
Chloroethyl	Original molecule	-177.652	-32.372	-180.007	34.727	0	3xH, G, E
Acetyl	452725	-175.495	-68.971	-132.509	25.985	Arg 1122 (D) Gly 459 (B)	G, H, E
Ethyl	13060060	-174.166	-33.342	-173.772	32.947	0	3xH, G
Propyl	452724	-171.016	-69.501	-128.495	26.980	Gly 459 (B) Arg 1122 (D)	E
Isobutyl	Original molecule	-169.618	-29.911	-173.588	33.881	Ser 1084 (B) Arg 1122 (D)	G, H
Isopropyl	13400660	-166.719	-39.977	-160.967	34.224	Ser 1084 (B) x2 Arg 1122 (D)	G
Tertiobutyl	Original molecule	-165.901	-54.223	-150.634	38.955	Arg 1122(D) Gly 459 (B)	G, E

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

According to the results in Table 3, it is noted that:

The C=O groups of the quinolone ring, the OH of the acid and the oxygen of the oxazinan ring are common among all derivatives and are essential for DNA gyrase inhibitory activity, the type of binding varies from compound to compound, the C=O groups of the quinolone ring and the oxazinan oxygen form bonds with amino acids (see figure 3), while the OH of the acid function binds with the E fragment of DNA, which is consistent with the results of the study by Fedorowicz, J. 2019[21].

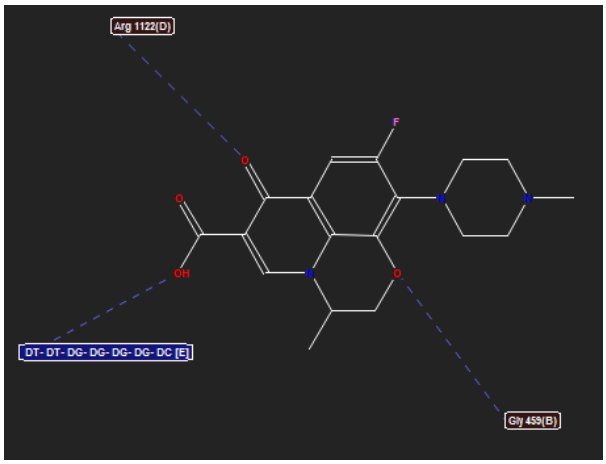


Figure 3: Ligand map of the interaction of the N-methyl derivative (ofloxacin) with DNA gyrase

The electron donating substituents including isopropyl, isobutyl, propyl, tertiary butyl favor the formation of a hydrogen bond between the DNA fragment G of the enzyme and the piperazine nitrogen; this may be due to the steric interaction between the isopropyl group and the Arg 458 residue (B) as shown in figure 4.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

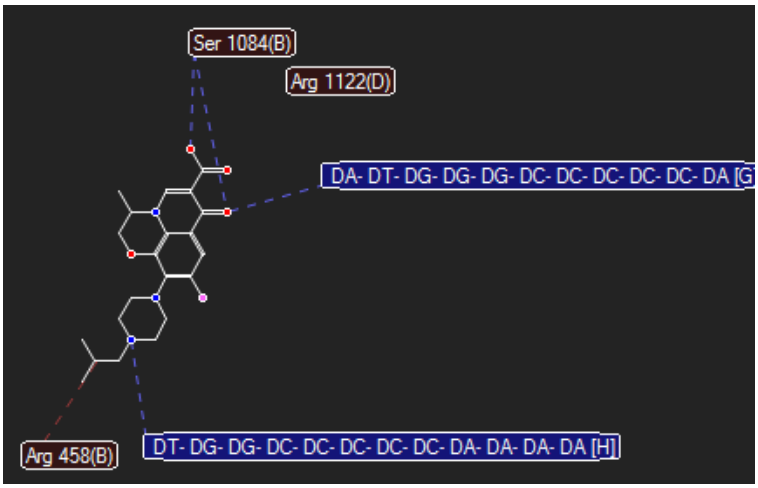


Figure 4: Ligand map of the interaction of the N-Isobutyl derivative with DNA gyrase

The C=O group of the N-acyl derivatives allows the establishment of additional bonds with DNA, which justifies the very low values of the binding energies between them and DNA gyrase (see table 3), and grants the formation of stable complexes (DNA, molecule and enzyme) thus favoring the inhibitory activity of the enzyme DNA gyrase.

Derivatives substituted on the N atom of the piperazine ring by electron-withdrawing groups such as the chloro groups in chloromethyl and chloroethyl promote the formation of steric bonds between the piperazine ring and the Arg 1122 residue (D).

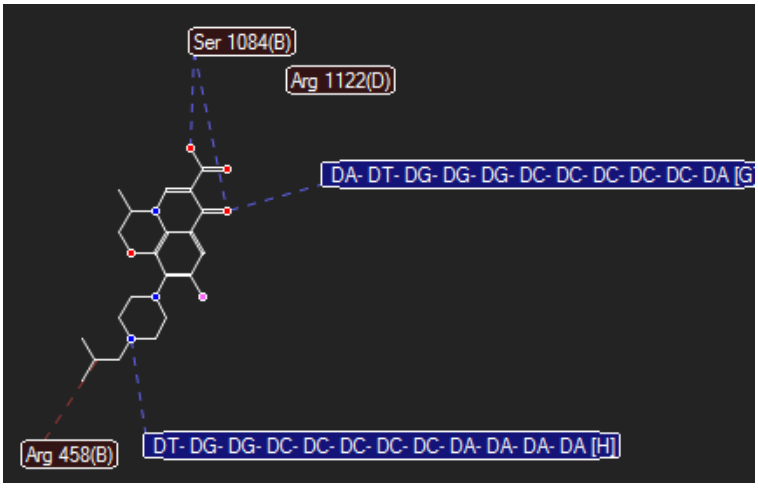


Figure 5: Ligand map of the interaction of the N-Isobutyl derivative with DNA gyrase

In the other hand, Figures 5 and 6 show that derivatives substituted on the N atom of the piperazine ring by electron-withdrawing groups such as the chloro groups in chloromethyl and chloroethyl promote steric bonding between the piperazine ring and Arg 1122 (D).

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20510 – 20520
Received 05 August 2021; Accepted 15 September 2021.

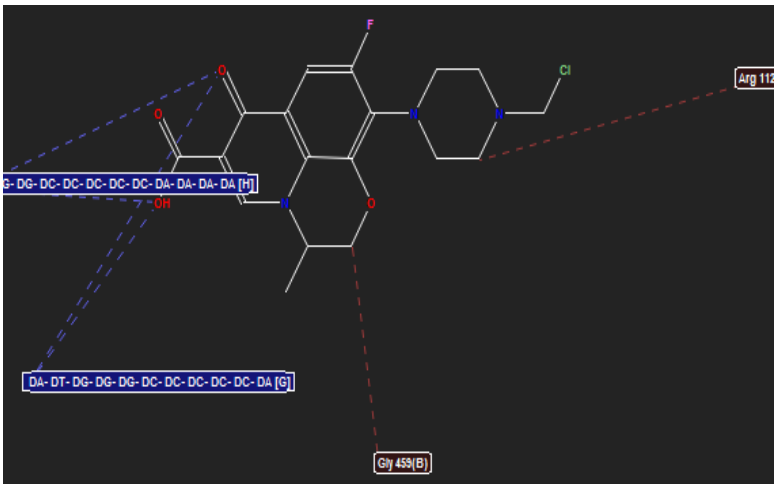


Figure 6: Ligand map of the interaction of the N-chloromethyl derivative with DNA gyrase

Conclusion

In this study, we performed a docking test on a series of molecules selected according to their structures close to the reference inhibitors and which are likely to interact with DNA gyrase in-silico. The analysis of the different structures of the first 10 molecules with the lowest binding energy scores directed us towards the derivatives of 7-piperazine quinolone. The results of the interaction energy score measurements after docking of these molecules showed that the N-propanoylated and N-chloromethylated derivatives of 7-piperazine quinolone are promising and proved to be active in-silico with a higher predicted activity than that of the reference molecules, in particular ciprofloxacin and ofloxacin.

References

[1] Serweci ´nska, L. (2020). Antimicrobials and Antibiotic-Resistant Bacteria: A Risk to the Environment and to Public Health, *Water*, 12, 3313.

[2] Dekaboruah, E., Vasant Suryavanshi, M., Chettri, D., & Kumar Verma,A., (2020). Human microbiome: an academic update on human body site specifc surveillance and its possible role, *Archives of Microbiology*, 202, 2147–2167.

[3] Munita1, JM., & Arias, CA. (2016). Mechanisms of Antibiotic Resistance, *Microbiol Spectr*. **4**(2), doi:10.1128/microbiolspec.VMBF-0016-2015.

[4] Brvar, M., Perdih, A., Renko, M., Anderluh, G., Turk, D., & Solmajer, T. (2012). Structure-Based Discovery of Substituted 4,5-Bithiazoles as Novel DNA Gyrase Inhibitors, *J. Med. Chem.* 55, 6413−6426.

[5] Jackson, N., Czaplewski, L., & Piddock,L.(2018). Discovery and development of new antibacterial drugs: learning from experience? *J Antimicrob Chemother*.73,1452–1459.

[6] Stokes, J.,Yang, K., Swanson, K., Jin, W., Cubillos-Ruiz, A., Donghia, N., MacNair, C., French, S., Carfrae, L., Bloom-Ackermann, Z.,Tran, V., Chiappino-Pepe, A., Badran, A.,

Andrews, I., Chory, E.,Church, G., Brown, E., Jaakkola, T., Barzilay, R., & Collins, J. (2020). A Deep Learning Approach to Antibiotic Discovery, *Cell*.180, 688–702.

[7] Bush, N., Diez-Santos, I., Abbott, L., & Maxwell, A.(2020).Quinolones: Mechanism, Lethality and Their Contributions to Antibiotic Resistance, *Molecules*. 25, 5662.

[8] Pham, T., Ziora, Z., & Blaskovich, M. (2019). Quinolone antibiotics, *Med. Chem. Commun*.10, 1719–1739.

[9] Aldred, K., Kerns, R., & Osheroff, N. (2014). Mechanism of Quinolone Action and Resistance, *Biochemistry*.53, 1565−1574.

[10] Bank RPD. RCSB PDB - 2XCT: The twinned 3.35A structure of S. aureus Gyrase complex with Ciprofloxacin and DNA [Internet]. [cité 6 mars 2021]. Disponible sur: https://www.rcsb.org/structure/2XCT

[11] Bank RPD. RCSB PDB: Homepage [Internet]. [cité 9 avr 2021]. Disponible sur: https://www.rcsb.org/

[12] CLC bio company. MOLEGRO VIRTUAL DOCKER. FERKOUS,F. University of BADJI MOKHTAR Annaba; Algeria,2013.

[13] Swiss Institute of Bioinformatics. DeepViewSwiss-PdbViewer.

[14] Magda, A-A., Abdel-Aziz,NI., Alaa, A-M., El-Azab,AS., Asiri, YA., ElTahir,K.(2011). Design, synthesis, and biological evaluation of substituted hydrazone and pyrazole derivatives as selective cox-2 inhibitors: molecular docking study. Bioorganic & medicinal chemistry. 19(11), 3416-24.

[15] Chaudhury, S., & Gray, JJ. (2008).Conformer selection and induced fit in flexible backbone protein-protein docking Mol. Biol.1068-87.

[16] Chaudhary ,KK., Mishra,N. (2016).A Review on Molecular Docking: Novel Tool for Drug Discovery. JSM Chem. 4(3): 1029.

[17] Agarwal, S., & Mehrotra, R. (2016). An overview of molecular docking. JSM Chem.4, 1024.

[18] Bryskier , A. E. Fluoroquinolones: Classification, propriétés physicochimiques, activités antibactériennes et pharmacocinétiques [Internet]. EM-Consulte.

[cité14mars2021]. Disponible sur: https://www.emconsulte.com/article/11761/fluoroquinolones-i-classification-proprietes-physi

[19] Cole, J., Davis, E., Jones, G., Sage, CR. Molecular Docking—A Solved Problem? In: Comprehensive Medicinal Chemistry III [Internet]. Elsevier; 2017 [cité 6 mars 2021]. p. 297-318. Disponible at: https://linkinghub.elsevier.com/retrieve/pii/B9780124095472123522

[20] Guo et al. (2011). Arch. Pharm. Chem. Life sci. 344,802-809.

[21] Fedorowicz ,J., Sączewski ,J., Drażba ,Z., Wiśniewska,P., Gdaniec,M., Wicher, B., et al. (2019). Synthesis and fluorescence of dihydro-[1,2,4]triazolo[4,3-a]pyridin-2-ium-carboxylates: An experimental and TD-DFT comparative study. Dyes and Pigments. 1 févr 2019;**161**:347-59.