In silico evaluation of new fluoroquinolones as possible inhibitors of bacterial gyrases in resistant Gram-negative pathogens

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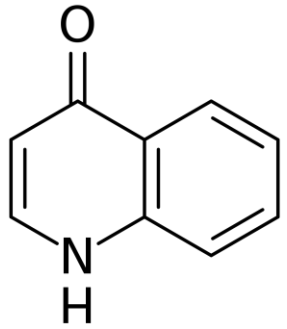
Abstract

The work seeks to identify molecules with inhibitory activity against the DNA gyrase of gram-negative microorganisms resistant to fluoroquinolones. Previously designed compounds were used to study antimicrobial potential *in silico*. Molecular docking was performed with 9 new ciprofloxacin analog molecules, optimized through the PM6/ZDO theory level, in GyrA Wild Type (WT) and Mutant Type (MT) of *C. jejuni*, *E. coli* (6RKU PDB ID), *N. gonorrhoeae*, *P. aeruginosa*, *S. enteritidis*, and *S. typhi*. The molecule with the highest affinity for GyrA was selected based on its binding free energy and inhibition constant. In addition, a retrospective docking was carried out, to guarantee the correct affinity of the ligand to the receptor at the defined binding site. The results show a molecule with greater affinity for GyrA of 5 microorganisms, showing a binding free energy of less than -7.0 kcal/mol, suggesting a good antibacterial activity *in silico*.

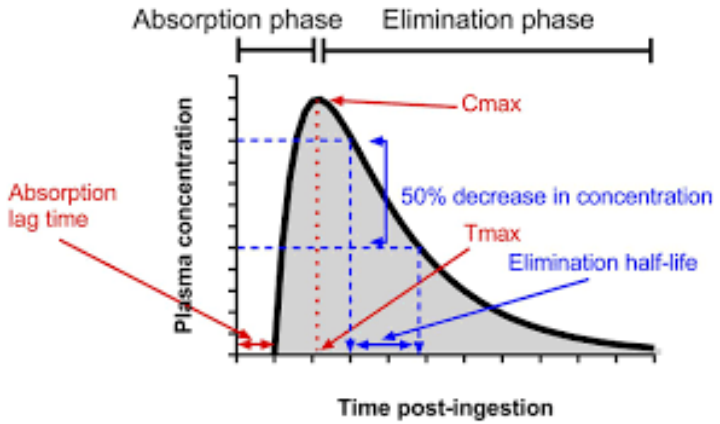
Keywords: Molecular Docking / Fluoroquinolones / DNA gyrase / Bacterial resistance

Introduction

Fluoroquinolones are synthetic fluorinated antibiotics that are born from a common basic chemical structure called 4-quinolone

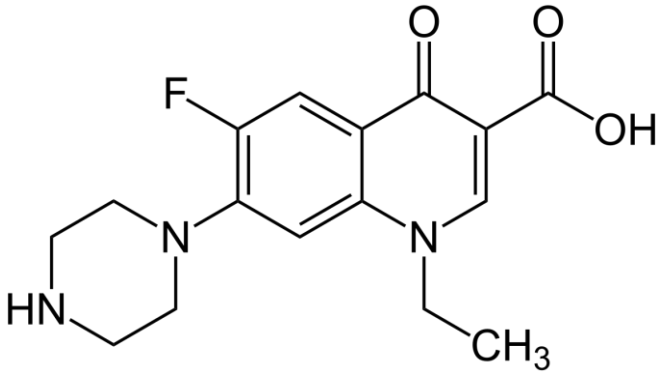


4-quinolone



Tom N. Lea-Henry, Jane E. Carland, Sophie L. Stocker, Jacob Sevastos, Darren M. Roberts. Clinical Pharmacokinetics in Kidney Disease, *Clin J Am Soc Nephrol* **2018**, *13*, p. 1086

Norfloxacin was the first fluoroquinolone obtained in 1980 by the addition of a fluorine atom at position 6 of the quinolone pharmacophore group



Norfloxacin

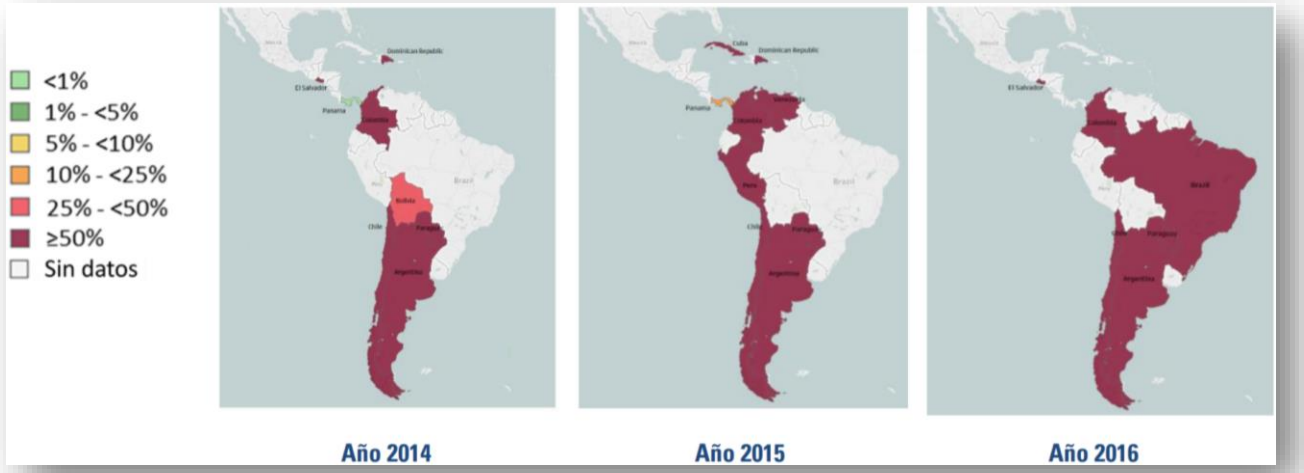
Fluoroquinolones Advantages
Quick absorption in the gastrointestinal tract
High serum concentrations in 1 - 2 h
High bioavailability
Large volume of distribution
Low affinity for proteins
Plasma half-life about 1,5 - 17 h

Introduction

Pan American Health Organization

Average ≥ 50% of non-sensitivity in gram-pathogens to fluoroquinolones in Brazil, Paraguay, Argentina, Chile, Colombia, and Peru.

Study of bacterial resistance carried out in 15 Latin American countries during 2014-2016



https://www.paho.org/es/documentos/magnitud-tendencias-resistencia-antimicrobianos-latinoamerica-relavra-2014-2015-2016

Priority level	Pathogens
Critical	***Acinetobacter baumannii***, carbapenem-resistant ***Pseudomonas aeruginosa***, carbapenem-resistant **Enterobacteriaceae**, carbapenem-resistant & third-generation cephalosporin-resistant
High	***Enterococcus faecium***, vancomycin-resistant ***Staphylococcus aureus***, methicillin-resistant, vancomycin intermediate and resistant ***Helicobacter pylori***, clarithromycin-resistant ***Campylobacter***, fluoroquinolone-resistant ***Salmonella* spp.**, fluoroquinolone-resistant ***Neisseria gonorrhoeae***, third-generation cephalosporin-resistant, fluoroquinolone-resistant
Medium	***Streptococcus pneumoniae***, penicillin-non-susceptible ***Haemophilus influenzae***, ampicillin-resistant ***Shigella* spp.**, fluoroquinolone-resistant

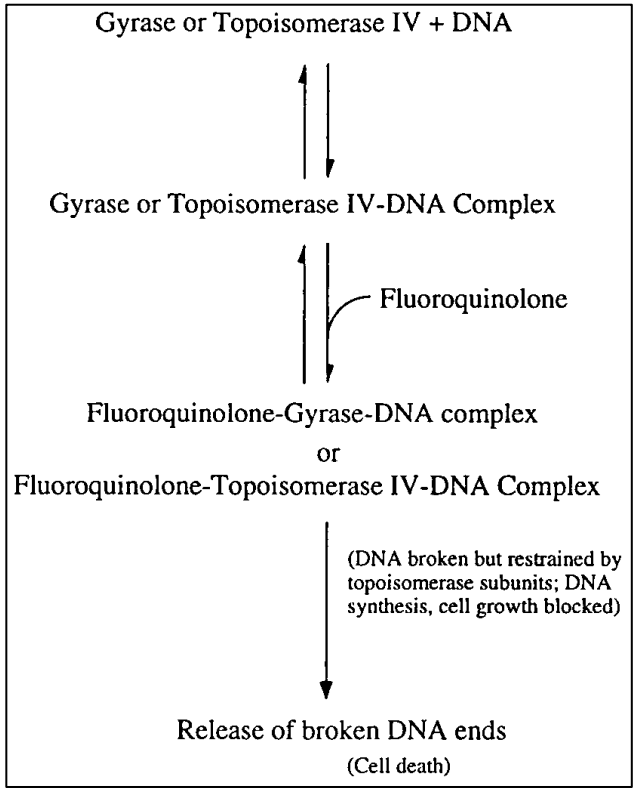
https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

In 2017 was published the first list of priority antibiotic-resistant pathogens, with the aim of encouraging R&D of new molecules

World Health Organization

Introduction

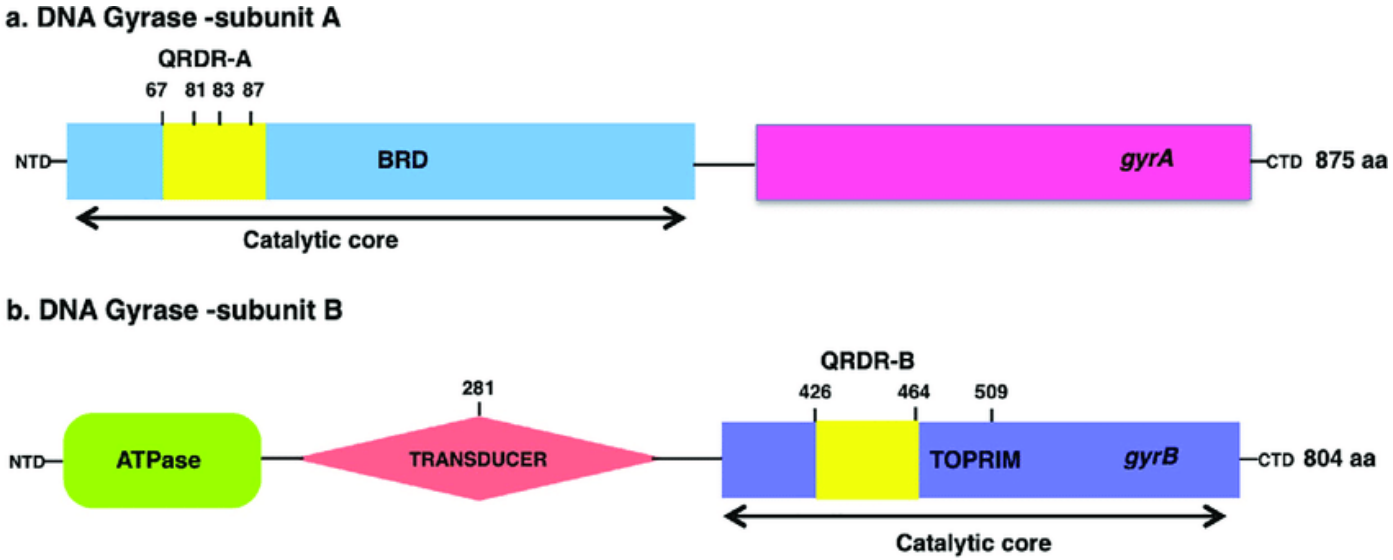
Mechanism of action of fluoroquinolones



Zhao, X., C. Xu, J. Domagala, and K. Drlica. DNA topoisomerase targets of the fluoroquinolones: a strategy for avoiding bacterial resistance. Proc. Natl. Acad. Sci. USA, **1997**, *94*, p. 13992

Fluoroquinolones bind to a region near Tyr-122 in the N-terminal domain of the GyrA subunits of the DNA gyrase

DNA girasa is a type II topoisomerase exclusive to prokaryotic organisms composed of two subunits A (GyrA) and two subunits B (GyrB) with ATPase activity



Bhatnagar, K.; Wong, A. The mutational landscape of quinolone resistance in *Escherichia coli*, **2019**, *PLoS ONE, 14*, p. 7

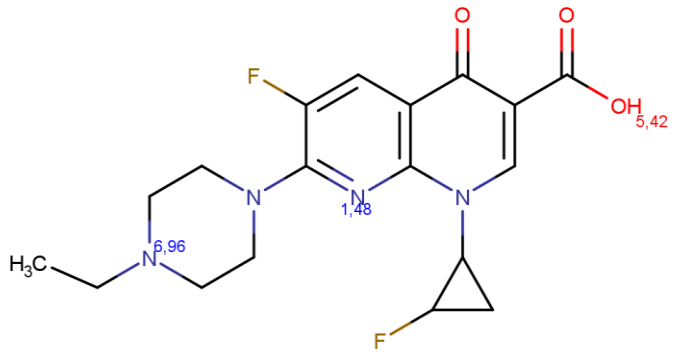
Some models based on observation and coupling calculations have established the Quinolone Resistance Determining Region (QRDR) in N-terminal domain of GyrA as the binding site for fluoroquinolones

Results

The study evaluated 9 new fluoroquinolones as possible inhibitors of DNA gyrase in resistant gram-negative pathogens using computational methods

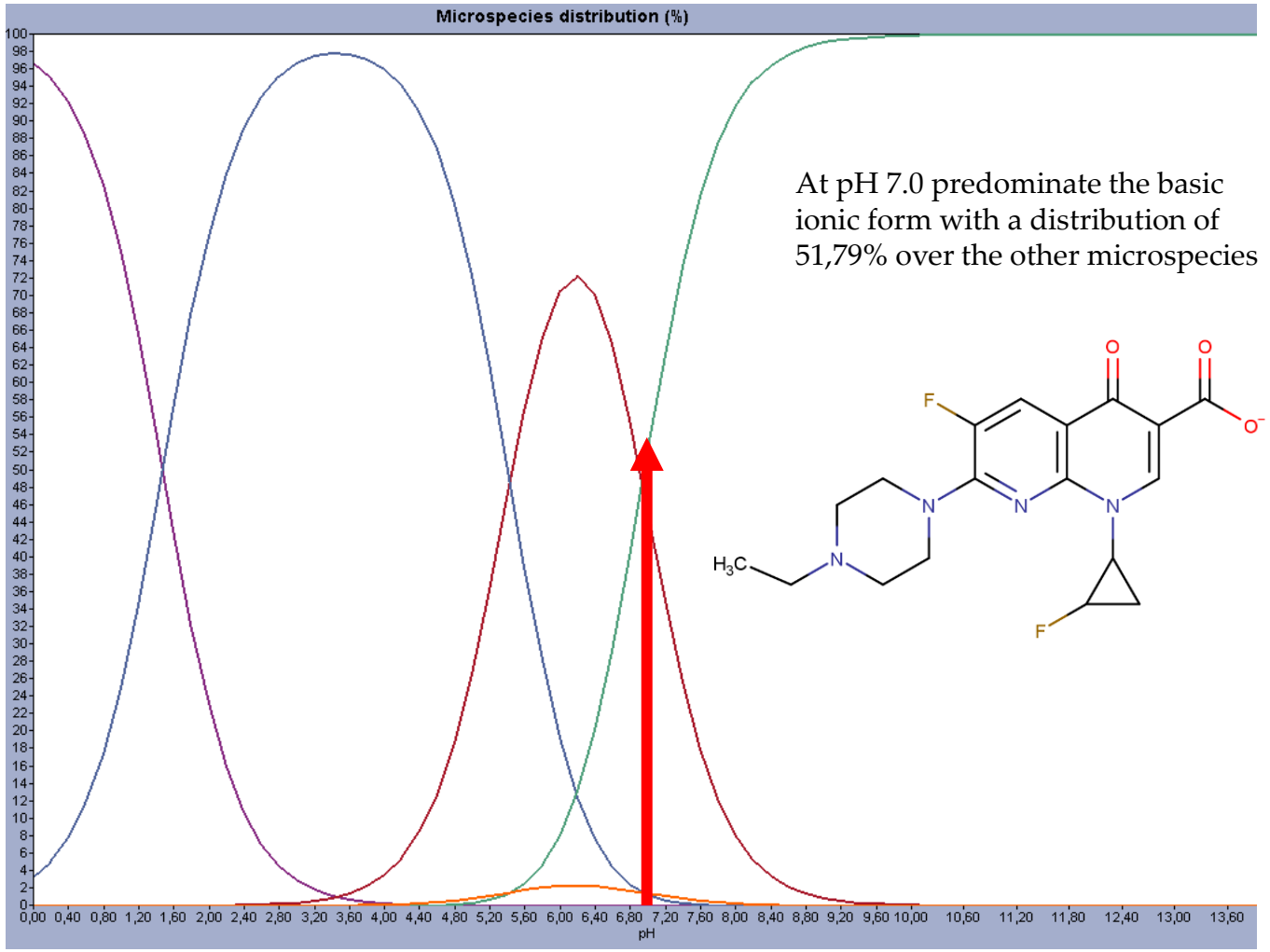
General characteristics of the ligands

The capacity with the fluoroquinolones penetrate the cell membrane is a complex process that depends on physical and chemical factors



pKa for Molecule 1

Fluoroquinolones cross the membranes by passive transport at physiological pH, being important to know the ionization state in which they are due to their amphoteric nature



At pH 7.0 predominate the basic ionic form with a distribution of 51,79% over the other microspecies

Results

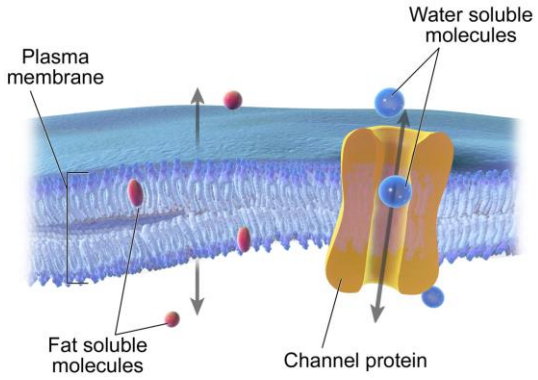
General characteristics of the ligands

At pH 7.0 basic ionic form → Molecule 1, 2, 5, 6, 8

At pH 7.9 zwitterionic form → Molecule 3, 4, 7, 9

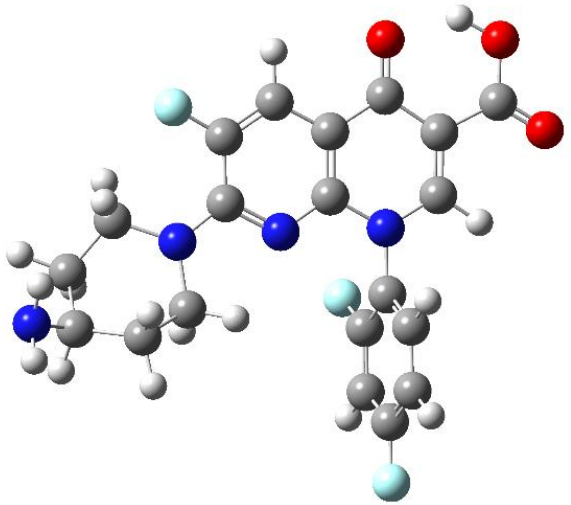
However, minimum energy structures of molecules 3 and 7 showed the neutral form how the most stable conformation, reaching energy values of -81,015 and -74,914 kcal/mol respectively

This is probably due to the spatial arrangement of their atoms to provide more stability to the molecule in the gas state.

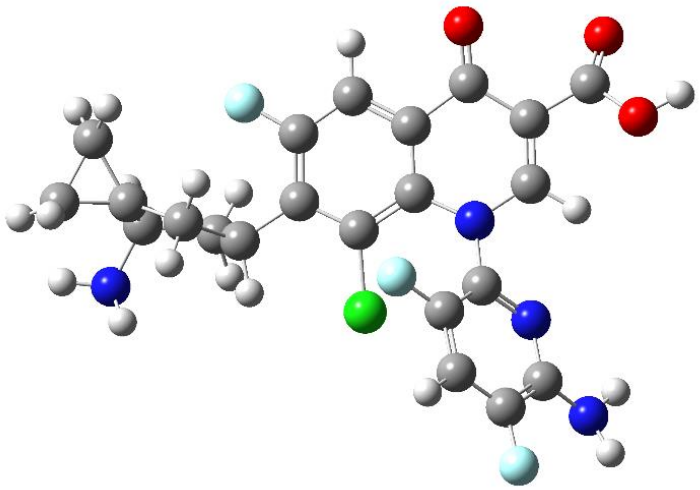


Diffusion Across the Plasma Membrane

https://en.wikipedia.org/wiki/Passive_transport



Molecule 3



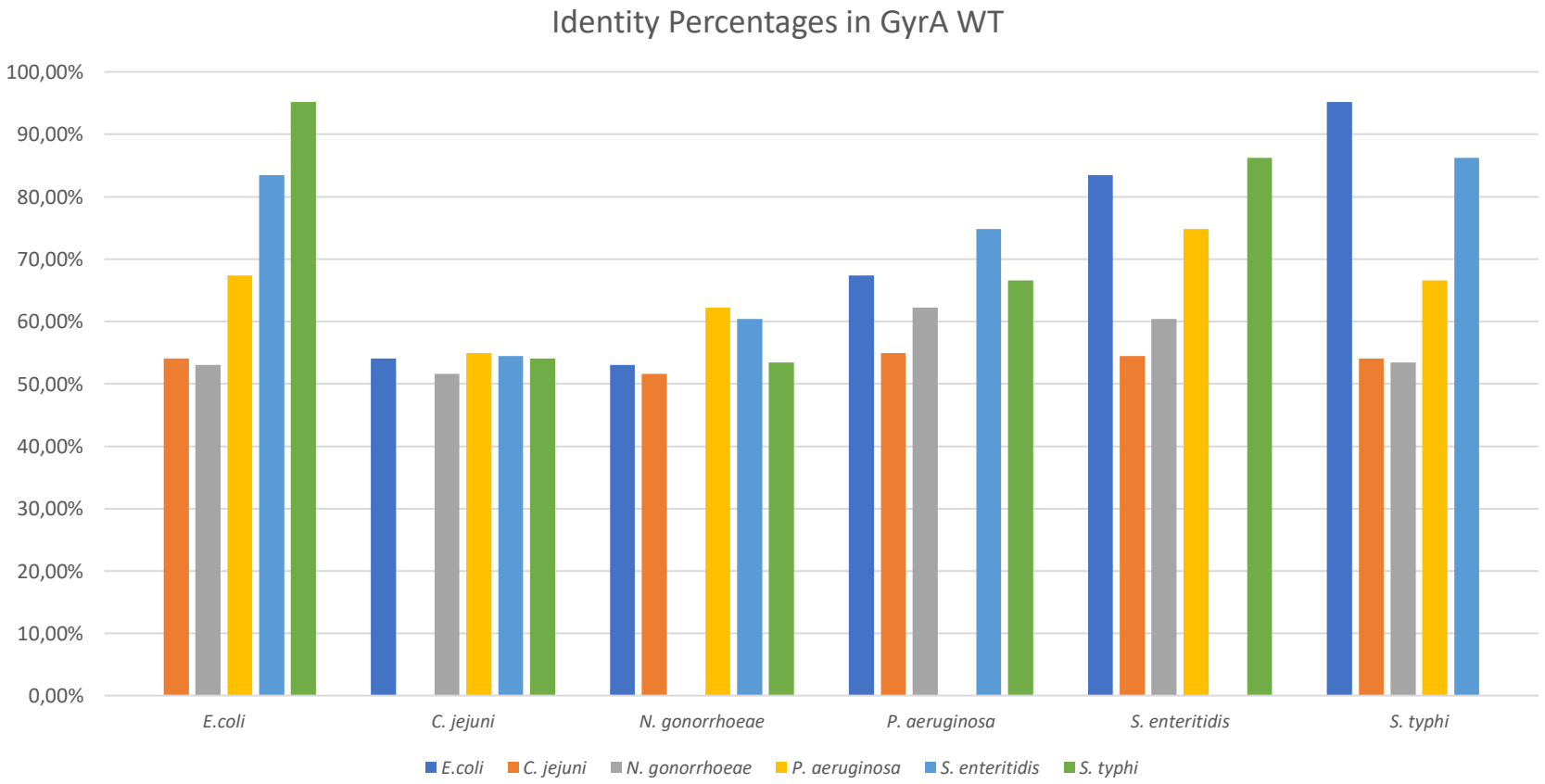
Molecule 7

These molecules cross the lipid bilayer is usually done only with non-ionized forms

Results

General characteristics of the ligands

The identity percentages obtained from the multiple alignment of sequences in the GyrA WT and GyrA MT is more than 50% in all comparisons, this indicate a structural homology between all subunit A of DNA gyrase



The sequences were aligned based on the *E. coli* GyrA (6RKU PDB ID) as a reference

The alignment on QRDR shows a high conservation of amino acids, which suggests a high affinity of the same molecule for the gyrase of different microorganisms

Results

Molecular Docking

Molecular Docking Results in GyrA MT of *Campylobacter jejuni*

From values of free binding energy, inhibition constant, total intermolecular energy and electrostatic energy, the lowest quintile was calculated for each parameter and a threshold value was obtained from this preliminary result, with it a score was established that allowed the identification of a group of better molecules

	Binding Free Energy (kcal/mol)	**Inhibition Constant (μM)**	**Total Intermolecular Energy (kcal/mol)**	**Electrostatic Energy (kcal/mol)**
Min.	-7,14	5,82	-8,93	-2,2
Max.	-5,06	194,14	-6,26	-0,32
R	2,08	188,32	2,67	1,88
R/5	0,416	37,664	0,534	0,376
Threshold Value	-6,724	43,484	-8,396	-1,824

Min.: The minimum value obtained among all the data
Max The maximum value obtained among all the data
R: Difference between the maximum value and the minimum value
R/5: Lowest quintile
Threshold Value: Min. – (R/5)

Results

Molecular Docking

Molecular Docking Results in GyrA MT of *Campylobacter jejuni*

Molecule	Microorganism	Binding Free Energy (kcal/mol)	Inhibition Constant (μM)	Total Intermolecular Energy (kcal/mol)	Electrostatic Energy (kcal/mol)
7	***C. jejuni* MT**	-7,14	5,82	-8,93	-1,22
7	***C. jejuni* WT**	-7,64	2,51	-9,43	-0,83
7	***E. coli* MT**	-7,45	3,44	-9,24	-1,3
7	***E. coli* WT**	-7,44	3,54	-9,23	-1,24
3	***N. gonorrhoeae* MT**	-6,81	10,21	-8,3	-2,53
7	***N. gonorrhoeae* WT**	-7,11	6,14	-8,9	-1,74
7	***P. aeruginosa* MT**	-8,13	1,1	-9,92	-1,51
7	***P. aeruginosa* WT**	-7,95	1,48	-9,74	-1,43
5	***S. enteritidis* MT**	-7,91	1,58	-9,11	-0,99
5	***S. enteritidis* WT**	-7,75	2,1	-8,94	-0,87
7	***S. typhi* MT**	-7,38	3,92	-9,17	-1,2
7	***S. typhi* WT**	-7,38	3,89	-9,17	-1,27

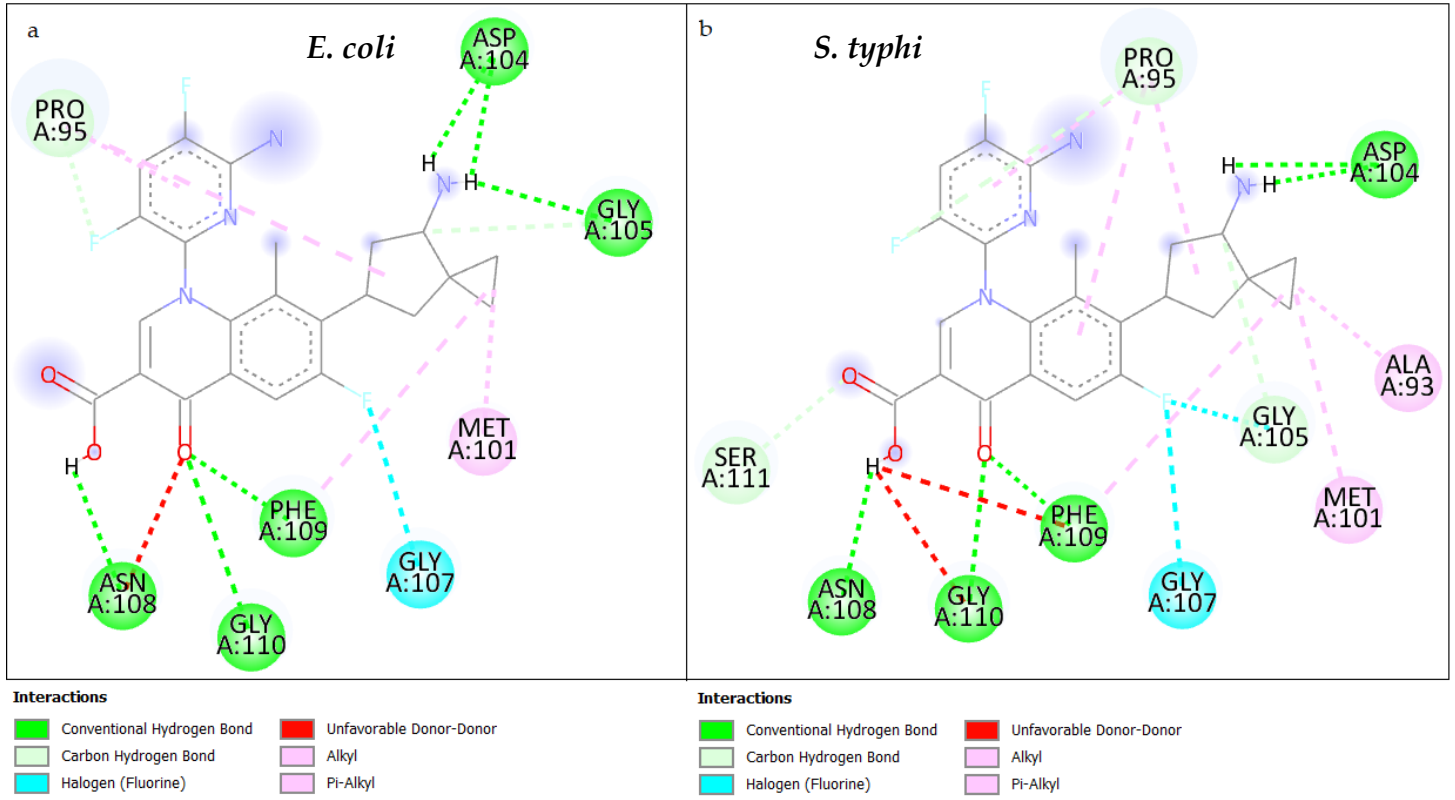
Here summarized the best values of the parameters and shows molecule 7 as the one with the highest affinity against GyrA of each microorganism

Results

Molecular Docking

Results of Interactions in Molecular Docking

The residues involved in the interaction with the molecule 7 within QRDR allowed to establish similarities



For *E. coli* and *S. thypi*, share interactions in residues Pro95, Met101, Asp104, Gly105, through hydrogen bridges

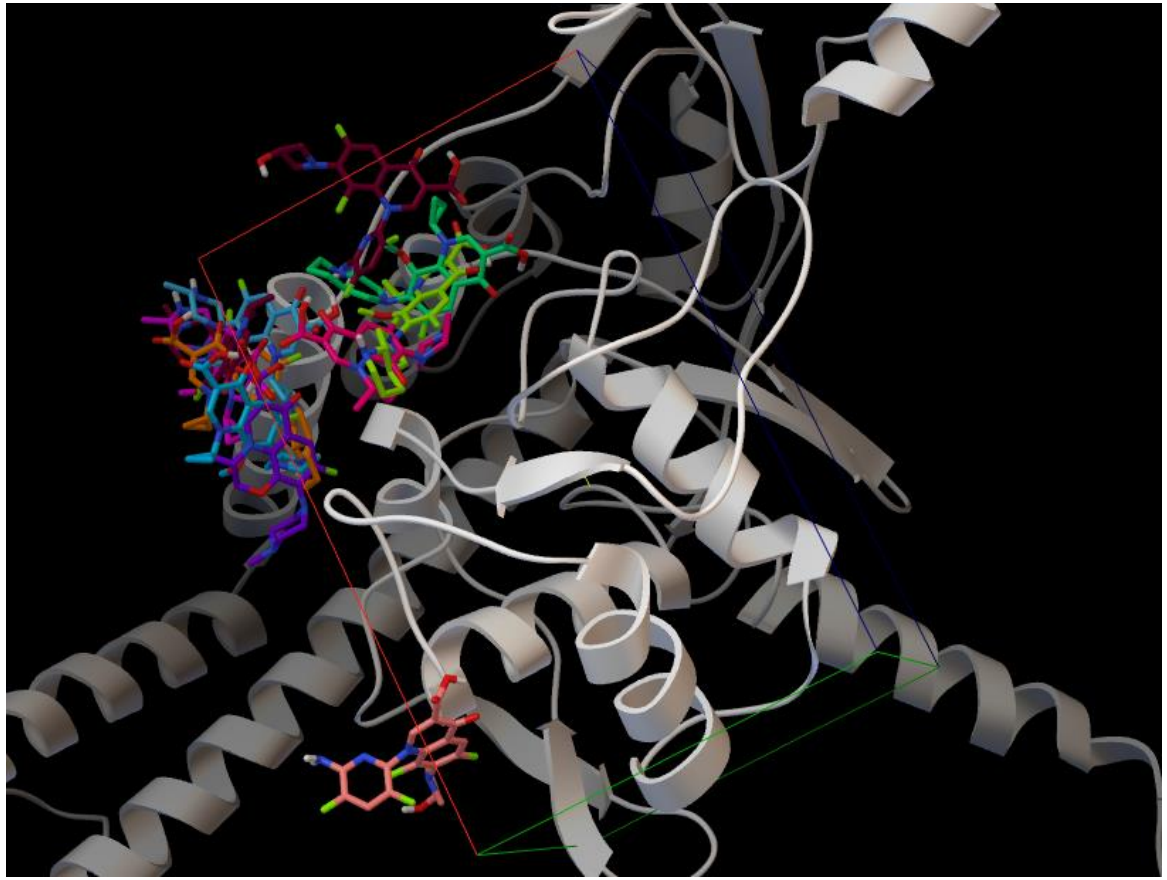
The interactions are strong in the binding of fluoroquinolone to the enzyme

The interactions occurs with the carboxyl group of position 3, the carbonyl group in C_4, and the amino group of the side chain on C_7

Results

Retrospective Docking

Carried out from a library of ligands and decoy molecules observing the binding sites in which the coupling of the molecules with activity takes place primarily in GyrA through *blind docking*

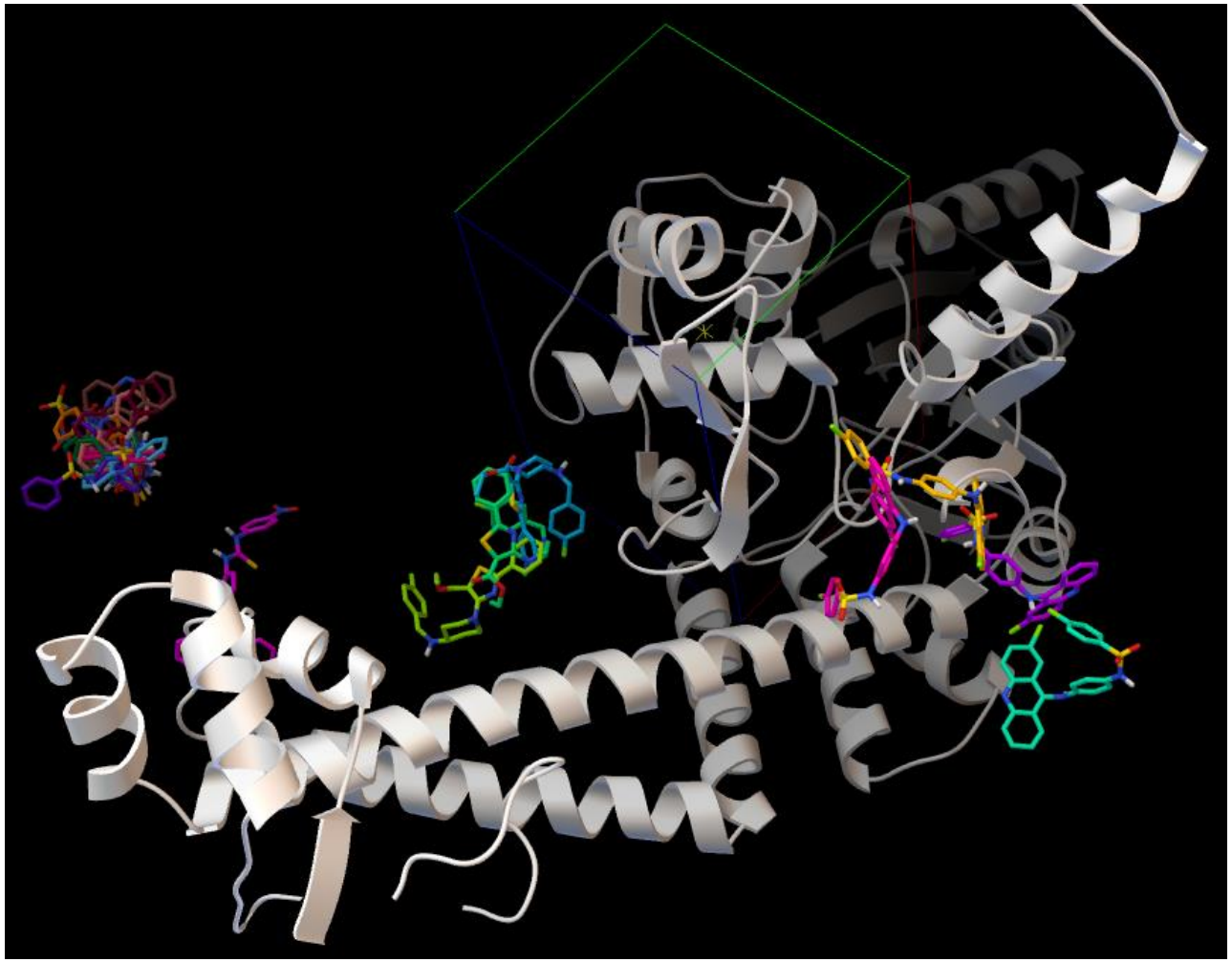


The image shows some modes in which the ligand binds to the receptor (GyrA of *E. coli*) after observational analysis of a total of 15 conformations

The positions where occurs the docking with four fluroquinolones are in an area too close to the region covered by the grid defined as the binding site of the new molecules.

Results

Retrospective Docking



Exist a diverse affinity of inactive molecules by different sites throughout the structure of Gyr A of *E. coli*

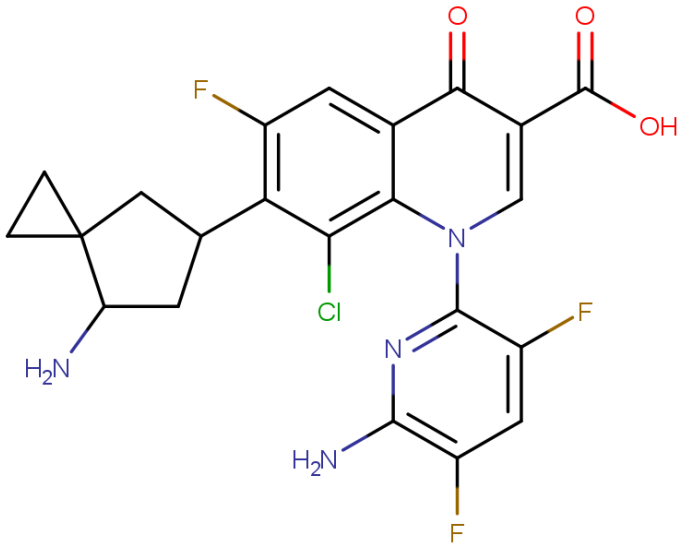
The positions of the different couplings generated in the *blind docking* with inactive compounds in relation to the position covered by the grid, it was observed that several conformations are far from the QRDR.

This assure the existence of a degree of affinity between the new molecules and the GyrA with reliable molecular docking results

Conclusions

The results of molecular docking point to molecule 7 as the largest affinity against the subunit A of the DNA gyrase wild type and mutant type of *Campylobacter jejuni*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica* serovar *typhi*

Retrospective docking ensures that the affinity shown by new molecules to GyrA has a good degree of reliability, as several conformations of active compounds bind to an area very close to QRDR



The molecule 7 showed better affinity parameters with respect to its analog ciprofloxacin.

Thank You!