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Title: **WQ-3810 inhibits DNA gyrase activity in ofloxacin-resistant *Mycobacterium leprae***

Short title: WQ-3810 inhibits DNA gyrase in *M. leprae.*

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Abstract

Background

Mycobacterium leprae causes leprosy and ofloxacin is used to control this bacterium. However, specific amino acid substitutions in DNA gyrases of *M. leprae* interferes with the effect of ofloxacin.

Methodology/principal findings

Here we tested the inhibitory effect of WQ-3810 on DNA gyrases in *M. leprae*, using recombinant gyrases. We theorized that WQ-3810 and DNA gyrases interacted, which was tested *in silico*.

Compared with control drugs like ofloxacin, WQ-3810 showed a better inhibitory effect on ofloxacin-resistant DNA gyrases. The *in-silico* study showed that, unlike control drugs, a specific linkage between a R1 group in WQ-3810 and aspartic acid at position 464 in the subunit B of DNA gyrases existed, which would enhance the inhibitory effect of WQ-3810. This linkage was confirmed in a further experiment, using recombinant DNA gyrases with amino acid substitutions in subunits B instead.

Conclusions/significance

The inhibitory effect of WQ-3810 was likely enhanced by the specific linkage between a R1 group residue in its structure and DNA gyrases. Using interactions like the one found in the present work may help design new fluoroquinolones that contribute to halt the emergence of antibiotic-resistant pathogens.

Keywords: *Mycobacterium leprae*, DNA gyrase, WQ-3810, recombinant molecules

Introduction

Leprosy is a chronic, infectious disease caused by *Mycobacterium leprae*. The spread of leprosy has been kept under control using a multidrug therapy (MDT) recommended by the World Health Organization. However, in 2016 alone, more than 200,000 new cases were reported [1]. Fluoroquinolones are considered to be an important bactericidal drug for treating leprosy. As part of the above-mentioned MDT, ofloxacin (OFX) is the fluoroquinolone used for single skin lesion paucibacillary cases [2].

Fluoroquinolones block the activity of the enzyme DNA gyrase, which has a crucial role in DNA replication and transcription [3,4]. DNA gyrase is an isotetramer enzyme consisting of two subunits A (GyrA) and two subunits B (GyrB) [4]. Pathogenic bacteria can develop fluoroquinolone resistance by substituting amino acids in quinolone resistance-determining regions (QRDR) in both GyrA and GyrB [5]. In case of clinical OFX-resistant *M. leprae*, it replaces glycine with cysteine at position 89 (G89C) and A91V in GyrA [2,6]. In addition, it has been experimentally proved that D95G in GyrA, which is among the most frequently amino acid substitution in quinolone-resistant *M. tuberculosis*, also contributes to quinolone resistance in *M. leprae* [7]. By contrast, D461N and N499D in GyrB are found less frequently in clinical strains of *M. tuberculosis* [8,9]. However, experimentally but not clinically, it was shown that D464N and N502D in GyrB of DNA gyrases in *M. leprae*, which are similar to D461N and N499D in *M. tuberculosis*, cause quinolone resistance [10].

Recurrence is a major setback when trying to control leprosy because relapse cases have a higher possibility of being accompanied with resistance to MDT anti-leprosy drugs, and hence it limits the number of drugs available for leprosy MDT [2,11-14]. Thus, to make a more reliable

therapeutic regimen against quinolone-resistant leprosy, screening for drug candidates and developing new drugs are needed.

Moxifloxacin (MFX) is known to be a more effective fluoroquinolone against leprosy than OFX [7,10,15,16], and its bactericidal activity is estimated to be equivalent to that of rifampicin, which is one of the first-line drugs in MDT [15]. In multibacillary leprosy cases, MFX was proved to kill leprosy bacilli within days or weeks after a single dose [16]. In contrast, WQ-3810 is a newly developed fluoroquinolone with an innovative NH_2-based molecular structure at the R1 group (Fig 1A) [17, 18]. Although it has been reported that WQ-3810 has a strong bactericidal effect on several pathogenic bacteria [19], WQ-3810 is yet to be tested against *M. leprae*.

In the present study, we aimed to test WQ-3810 as a new drug candidate for *M. leprae.* Fluoroquinolones OFX (Fig 1B) and MFX (Fig 1C) were used as control drugs. To assess the potency of WQ-3810 as a therapeutic drug to treat leprosy and to facilitate comparison between WQ-3810 and control drugs, *in vitro* assays were conducted using recombinant *M. leprae* DNA gyrases including wild type (ML-GyrA^{WT}) and mutants bearing amino acid substitutions in GyrA (ML GyrA^{D91V} and ML-GyrA^{D95G}) and GyrB (ML-GyrB^{D464N} and ML-GyrB^{N502D}) instead of measuring minimum inhibitory concentration as *M. leprae* can not be cultured on artificial medium. In addition, an *in-silico* study was carried out to understand the molecular interaction between WQ-3810 and DNA gyrases.

Materials and Methods

Antibacterial agents

WQ-3810 was kindly provided by Wakunaga pharmaceutical Co., Ltd. (Osaka, Japan). Moxifloxacin and ofloxacin were purchased from LKT Laboratories, Inc. (St. Paul, MN) and FUJIFILM Wako Pure Chemical Industries Corp. (Osaka, Japan), respectively.

Bacterial strains and expression plasmids

The Thai-53 strain of *M. leprae* [20], maintained at the Leprosy Research Center, National Institute of Infectious Diseases (Tokyo, Japan), was used to prepare *M. leprae* DNA. *Escherichia coli* strain TOP-10 (Thermos Fisher Scientific Inc.; Waltham, MA) was used for cloning. *E. coli* strains Rosetta-gami^{TM} 2(DE3)pLysS and BL21(DE3)pLysS (Merck KGaA, Darmstadt, Germany) were used for protein expression. The plasmid vector pET20b(+) (Merck KGaA) was used to construct expression plasmids. Relaxed pBR322 DNA (Inspiralis Ltd.; Norwich, UK) was used for the DNA supercoiling assay.

Preparation of recombinant DNA gyrase subunits

DNA gyrase expression plasmids encoding ML-GyrA^{WT}, ML-GyrA^{G89C}, ML-GyrA^{A91V} and ML-GyrA^{D95G} and ML-GyrB^{WT}, ML-GyrB^{D464N} and ML-GyrB^{N502D} were constructed as previously described [7,10,21].

Expression and purification of recombinant DNA gyrase subunits were conducted as previously reported [7,21-23]. Briefly, expression plasmids bearing either *gyrA* or *gyrB* of *M. leprae* were transformed in *E. coli* Rosetta-gami2(DE3)pLysS or BL21(DE3)pLysS, respectively. Transformants were cultured in Luria-Bertani (LB) broth up to the log phase, under ampicillin selection (100 μg/ml).

Expression of DNA gyrases was induced by adding 1 mM isopropyl-beta-D-thiogalactopyranoside (FUJIFILM Wako Pure Chemical Industries Corp.), and further incubated for 16 to 24 h at 12 or 14 °C. Harvested *E. coli* were lysed by sonication (10 times for 40 s at output level 3 and 40% duty cycle with 40-s intervals) using Sonifier 250 (Branson, Danbury, CT). The supernatant was obtained by centrifugation (10,000× *g* for 30 min). Recombinant DNA gyrase subunits in the supernatant were purified by Ni-NTA Agarose column (Thermo Fisher Scientific Inc.) chromatography, as per the manufacturer's protocol. Next, the standard buffer was replaced by DNA gyrase dilution buffer (50 mM Tris-HCl pH 7.5, 100 mM KCl, 2 mM DTT, 1 mM EDTA), and recombinant DNA gyrase subunits were analyzed by PD-10 chromatography.

Assessment of supercoiling activity

ATP-dependent DNA supercoiling assays were carried out as previously described [21]. Briefly, the DNA supercoiling activity of purified DNA gyrases was assessed using a reaction mixture consisting of DNA gyrase reaction buffer [35 mM Tris-HCl pH 7.5, 6 mM $MgCl_2$, 1.8 mM spermidine, 24 mM KCl, 5 mM DTT, 0.36 mg/mL of BSA, 6.5% glycerol (w/v) and 1 mM ATP], relaxed pBR322 DNA (4 nM), ATP (1 mM), and DNA gyrase subunits; of ML-GyrA^{WT}, ML-GyrA^{G89C}, ML-GyrA^{A91V} or ML-GyrA^{D95G} (40 nM each) and ML-GyrB^{WT}, ML-GyrB^{D464N} or ML-GyrB^{N502D} (40 nM each) in a total volume of 30 µl. For DNA gyrases with ML-GyrA^{G89C}, and ML-GyrB^{WT} (160 nM each) were also used. The mixtures were incubated for 90 min at 30 °C. The reaction was stopped by adding 7.5 µL of 5× dye mix (5% SDS, 25% glycerol and 0.25 mg/mL of bromophenol blue). Next, 10 µL from each reaction mixture was subjected to electrophoresis on 1% agarose gel in 1× Tris-borate-EDTA buffer (TBE; 89 mM Tris, 89 mM borate, 2 mM EDTA, 8.3 pH). The gel was then stained with ethidium bromide (0.7 µg/mL).

Fluoroquinolone-inhibited DNA supercoiling assay

The concentration of fluoroquinolone necessary to inhibit the enzymatic activity of DNA gyrase by 50% was calculated as one IC_{50} resulting from the fluoroquinolone-inhibited DNA supercoiling assay, based on the method described by Fisher and Pan [24]. The assay was conducted with 30 µL of DNA gyrase reaction buffer, 4 nM relaxed pBR322 DNA, 40 nM GyrA (ML-GyrA^{WT}, ML-GyrA^{A91V} or ML-GyrA^{D95G}), 40 nM GyrB (ML-GyrB^{WT}, ML-GyrB^{D464N} or ML-Gyr

B^{N502D}) and fluoroquinolones. WQ-3810 and MFX were used in concentrations from 0.13 to 64 µg/mL for subunit combinations ML-GyrA^{WT}, ML-GyrA^{A91V}, and ML-GyrA^{D95G} with ML-GyrB^{WT}. Similarly, 0.13 to 320 µg/mL was used for subunit combinations ML-GyrB^{D464N}, and ML-GyrB^{N502D} with ML-GyrA^{WT}. OFX was used in concentrations from 1 to 512 µg/mL for every combination of subunits. Reactions were conducted for 90 min at 30 °C and stopped by adding 7.5 µL of dye mix. Next, 10 µL from each mixture was subjected to electrophoresis on 1% agarose gel in 1× TBE and stained with 0.7 µg/mL of ethidium bromide. The intensity of supercoiled DNA bands for electrophoresis was estimated with ImageJ software (https://imagej.nih.gov/ij/download.html).

Simulations for molecular interaction among DNA gyrase, DNA and fluoroquinolones.

Molecular docking and visualization studies were carried out using Molecular Operating Environment (MOE) (Chemical Computing Group ULC, Montreal, Quebec, Canada. https://www.chemcomp.com/index.htm) software and MolDesk Basic v1.1.54 (IMSBIO co., Ltd, Tokyo, Japan). The *M. leprae* DNA gyrase molecular structure has not been classified. Therefore, the coordinates of DNA gyrase for structure-based molecular modeling were retrieved from the Protein Data Bank (PDB, http://www.rcsb.org/pdb/) with the PDB ID #5BTA

(https://www.rcsb.org/structure/5BTA; Crystal structure model of Mtb-gyrase complex) as the amino acid sequence of both GyrA and B in Mtb-gyrase were highly homologous to *M. leprae*. The artificial amino acid substitution in GyrB of Mtb-D461N (D464N in ML) was introduced by WinCoot release 0.8.9.2 (http://bernhardcl.github.io/coot/). Ligand location and pocket size were set using the MFX coordinates, which were retrieved from the 5BTA structural information. Optional parameters in MOE software were used to create a topology file, which included the addition of hydrogen atoms, the calculation of a grid potential, and a docking simulation. The flexible docking method was used, and scores were calculated as the sum of five potentials: accessible surface area, coulomb potential, hydrogen bonds, hydrogen bond considering anisotropy, and van der Waals interactions. Protein-ligand binding free energy was estimated with MOE software using the Amber 10: EHT force-field and the default parameters of the MOE Dock application. The results of molecular docking were visualized with PyMOL v1.3 (http://www.pymol.org/). Distances between amino acids and the side chains of WQ-3810 were calculated using MOE software.

Results

ATP-dependent DNA supercoiling activity of DNA gyrases.

The enzymatic activity of purified DNA gyrase subunits was assessed with a DNA supercoiling assays using relaxed pBR322 DNA as the substrate. Relaxed DNA was supercoiled when GyrA, GyrB and ATP were all present and no DNA supercoiling activity was observed without any of these three (Fig 2). To detect the DNA supercoiling activity, 40 nM each was sufficient in DNA gyrases with ML-GyrA^{WT}, ML-GyrA^{A91V}, ML-GyrA^{D95G}, ML-GyrB^{WT}, ML-GyrB^{D464N} and ML-GyrB^{N502D}. In contrast, DNA gyrase with ML-GyrA^{G89C} needed 160 nM of both GyrA and GyrB to show sufficient activity.

Inhibitory activity of fluoroquinolones against *M. leprae* recombinant DNA gyrases.

The results of supercoiling inhibitory assay against WT and mutant DNA gyrases under various concentrations of WQ-3810, MFX and OFX were shown in Fig. 3 and IC_{50}s calculated from the results are shown in Table. All fluoroquinolones examined in this study exhibited DNA supercoiling inhibitory activity against all DNA gyrases in dose-dependent manners. WQ-3810 and MFX showed significantly higher DNA supercoiling inhibitory activities against all DNA gyrases examined in this study than OFX. And the activities of all the three fluoroquinolones were diminished against mutant DNA gyrases with the prominent reduction against DNA gyrase harboring ML-GyrB^{N502D}. WQ-3810 showed superior activity comparing to MFX and OFX against all mutant DNA gyrases except for with ML-GyrB^{D464N}.

***In silico* study of the molecular interaction between DNA gyrases, the DNA molecular structure and fluoroquinolones.**

The fluoroquinolone binding site consists of subunits GyrA, GyrB and the DNA molecular structure. 5BTA, the *M. tuberculosis* DNA gyrase 3D structural model, has an intact fluoroquinolone binding site and the 3D structure has a ligand model of MFX positioned at the fluoroquinolone binding site. A computational simulation for WQ-3810 was carried out based on the binding site 5BTA in MFX. The docking simulation of WQ-3810 showed a docking score of -7.1960 (Fig. 4A) for the original model and -5.1241 (Fig. 4B) for the amino acid-substituted model of Mtb-GyrB -D461N (D464N in ML). In addition, the distances between the R1 group, R7 groups of each fluoroquinolone and the side chain of each amino acid were calculated. While the distance between the R1 group of WQ-3810 and the side chain of Mtb-GyrB-Asp461 was 1.96 Å, that of MFX was 9.36 Å (Fig. 5). Similarly, while the distance between the R7 group of WQ-3810 and the side chain of Mtb-GyrB-Asn499 (Asn502 in ML) was 7.21 Å, that of MFX

was 6.90 Å (Fig 5). In the ligand interaction simulation, the NH_2 molecule of the R1 (2,4-difluoro-5-aminopyridine substituent) group in WQ-3810 was able to interact with Mtb-GyrB-Asp461, but this interaction was not observed when Asp was replaced by Asn (Fig 5).

Discussion

The potential bactericidal effect of WQ-3810 on *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Neisseria gonorrhoeae* and *E. coli* has been proved [19]. Nonetheless, until now the effect of WQ-3810 on *M. leprae* was unknown. To elucidate this, instead of measuring minimum inhibitory concentration, we calculated the inhibitory activity of WQ-3810 against DNA gyrase of *M. leprae*, because *M. leprae* is yet to be cultured on an artificial medium. The result of DNA gyrase inhibitory assay, IC_{50}, have been used as a reliable criteria to analyze the potency of fluoroquinolone against *M. leprae* [4, 5, 8, 19, 20].

Recombinant DNA gyrases assessed in the current study showed a readily enzymatic activity, except for DNA gyrase with ML-$GyrA^{G89C}$, which needed a 4-fold concentration to express when compared with the other gyrases. Thus, as the amino acid substitution G89C in GyrA is rare when compared with A91V [2,6], we decided to focus instead on the inhibitory effect of WQ-3810 on DNA gyrase with ML-$GyrA^{A91V}$, which is considered a more representative amino acid substitution of the OFX-resistant DNA gyrase in *M. leprae*. DNA gyrase with ML-$GyrA^{D95G}$, showing a stronger fluoroquinolone resistance than that of A91V [7, 21], was also assessed in the present work. ML-Asp95 in GyrA may provide the most crucial linkage between fluoroquinolones and DNA. A linkage constructed by Asp at an equivalent position has been found in DNA gyrase *E. coli*, *S. aureus*, *S. pneumoniae* and *M. tuberculosis* [23, 24].

In the present study, IC_{50} of WQ-3810 against DNA gyrase with ML-$GyrA^{A91V}$ was similar to that against ML-$GyrA^{WT}$ where IC_{50}s of OFX was significantly higher (3.9-fold; $P < 0.005$) and that of MFX was slightly higher (1.5-fold; $P = 0.1061$) against DNA gyrase with ML-$GyrA^{A91V}$ comparing to that with ML-$GyrA^{WT}$. On the contrary, IC_{50} of WQ-3810 showed 4.3-fold increase ($P < 0.005$) against DNA gyrase with ML-$GyrA^{D95G}$ comparing to that with ML-$GyrA^{WT}$ while the increase of IC_{50}s of OFX and MFX were 12.8-fold ($P < 0.005$) and 9.9-fold ($P < 0.005$), respectively. Only OFX showed significantly increased IC_{50} against DNA gyrase with ML-$GyrA^{A91V}$ and suggested the decrease of OFX binding affinity because of the amino acid substitution A91V [25]. Distinct IC_{50}s among examined fluoroquinolones suggested the importance of interaction between GyrB and R1, R7 and R8 groups. Additional linkages of WQ-3810 with GyrB might provide better inhibitory effects against DNA gyrases with amino acid substitutions in GyrA.

To understand the detailed molecular interaction between DNA gyrases and WQ-3810, an *in-silico* study was carried out. Due to the molecular structure of *M. leprae* DNA gyrase is not listed in the protein databank (PDB) yet, we selected the DNA gyrase molecular structure of *M. tuberculosis* instead. 5BTA is the 3D molecular structural model of the *M. tuberculosis* DNA gyrase listed in PDB. This structural model shows intact isotetramers (two GyrAs and two GyrBs) with cleaved DNA and MFX. In addition, the information regarding the coordinates of MFX positioned at the fluoroquinolone binding site of 5BTA, was used for the docking simulation of WQ-3810. The *M. tuberculosis* DNA gyrase amino acid sequence around QRDR is identical to that of *M. leprae*, and amino acid substitutions at QRDR of *M. leprae* DNA gyrases associated with quinolone resistance have shown a similar activity as that of amino acid substitutions in *M. tuberculosis* DNA gyrases [7, 10, 21]. Thus, we speculated that an *in-silico* study using the

5BTA structure could provide a reliable simulation regarding the association of WQ-3810 with the QRDR of *M. leprae* DNA gyrases.

Upon completion of the *in-silico* study, an additional linkage and specific amino acids were observed (Fig 5). It was detected that the NH_2 molecule of the R1 group in WQ-3810 interacted with Mtb-GyrB-Asp461, but that this interaction disappeared when Asn was substituted for Asp (Fig 5). These results were in agreement with the decreased score of WQ-3810 with mutant DNA gyrase, when compared with that of WT (Fig 4). Thus, we theorized that a specific R1 group in WQ-3810 was the source of the additional linkage that contributed to a stronger relationship with Mtb-GyrB-Asp461, which MFX did not have. To confirm this theory, additional DNA gyrase inhibitory assays using recombinant *M. leprae* DNA gyrase subunits ML-GyrA^{WT}, ML-GyrB^{WT}, ML-GyrB^{D464N} and ML-GyrB^{N502D} were conducted (Fig. 3B)[10]. WQ-3810 had an almost 2-fold higher IC_{50} ($P < 0.005$) for DNA gyrase with ML-GyrB^{D464N} than MFX (Table). This result seemed to explain the reason WQ-3810, but not MFX, had a stronger interaction with GyrB-Asp464 of *M. leprae* DNA gyrase. Amino acid substitutions in GyrB that cause quinolone resistance has not been reported in clinical *M. leprae* yet, although admittedly, the number of reports related to quinolone-resistant leprosy is still limited. Even as data of the association of *M. tuberculosis* with quinolone resistance caused by amino acid substitutions is vast, information of quinolone resistance in clinical isolates with mutation in GyrB is rare [8, 9]. Amino acid substitutions in QRDR of GyrB at position of 461 and 499 may cause significant changes in DNA gyrase activity. Moreover, interaction of WQ-3810 with the QRDR of GyrB can potentially inhibit the activity of DNA gyrase with amino acid substitutions in QRDR of GyrA. Therefore, WQ-3810 may be a good candidate compound for treating OFX-resistant leprosy.

In conclusion, WQ-3810 showed a better inhibitory effect on DNA gyrases of *M. leprae* than OFX did. WQ-3810 also showed a better effect on DNA gyrase with ML-GyrA^{D95G} than MFX. Upon an *in-silico* study, it was theorized that an interaction of the R1-group in WQ-3810 with Asp at position of 464 existed and that it may have enhanced its inhibitory effect on DNA gyrase with ML-GyrA^{D95G}. A subsequent experiment using recombinant DNA gyrase subunits confirmed the inhibitory effect of WQ-3810 on DNA gyrase with ML-GyrA^{D95G}. A possible treatment application of WQ-3810 against OFX-resistant leprosy was demonstrated in the present work. The results deriving from the present study showing the interaction between the R1 group in WQ-3810 and gyrases may contribute to design innovative fluoroquinolones that could help lower the possibility of further emergence of antibiotic-resistant *M. leprae* and other pathogens.

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COI statement: There is no conflict of interest.

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Figure legends

Fig 1. Structures of the quinolones tested in the present study

(A) WQ-3810 (B) Moxifloxacin (C) Ofloxacin

Fig 2. ATP-dependent DNA supercoiling assay

(A) The supercoiling activity of DNA gyrases consisting of ML-GyrA^{WT}, ML-GyrA^{G89C}, ML-GyrA^{A91V} or ML-GyrA^{D95G} and ML-GyrB^{WT} was confirmed. Relaxed DNA (pBR322) was incubated with GyrA, GyrB, or both, of the subunits in the presence or absence of ATP. Lane 1: relaxed pBR322 DNA only; lanes 2–6: relaxed pBR322 DNA, ATP, GyrA (40 nM), and GyrB (40 nM); lanes 7–11: relaxed pBR322 DNA, ATP, and GyrA (40 nM); lane 12: relaxed pBR322 DNA, ATP, and GyrB (40 nM); lane 13–17: relaxed pBR322 DNA, GyrA (40 nM), and GyrB (40 nM). *Lane 4: The amount of DNA gyrase subunit was 4-fold (160 nM). (B) The supercoiling activity of DNA gyrases consisting of ML-GyrB^{WT}, ML-GyrB^{D464N} or ML-GyrB^{N502D} and ML-GyrA^{WT} was confirmed. Relaxed pBR322 DNA was incubated with GyrA, GyrB, or both, of the subunits in the presence or absence of ATP. Lane 1: relaxed pBR322 DNA only; lanes 2–4: relaxed pBR322 DNA, ATP, GyrA (40 nM), and GyrB (40 nM); lanes 5–7: relaxed pBR322 DNA, ATP, and GyrB (40 nM); lane 8: relaxed pBR322 DNA, ATP, and GyrA (40 nM); lane 9–11: relaxed pBR322 DNA, GyrA (40 nM), and GyrB (40 nM). R and S at the left side of agarose gel indicates the position of relaxed DNA and supercoiled DNA, respectively.

Fig 3. Fluoroquinolone-inhibited DNA supercoiling assay

(A) Relaxed DNA (pBR322) was mixed and incubated with GyrA, GyrB, ATP and quinolones at the indicated concentrations. Each quinolone was screened for its inhibitory effect on WT DNA

gyrases and mutant DNA gyrases with ML-GyrA^{A91V} and ML-GyrA^{D95G} substitutions. Lanes labeled as R indicate relaxed pBR322 DNA. (B) Relaxed pBR322 DNA was mixed and incubated with GyrA, GyrB, ATP and quinolones at the indicated concentrations. Each quinolone was screened for its inhibitory effect on WT DNA gyrases and mutant DNA gyrases with ML-GyrB^{D464N} and ML-GyrB^{N502D} substitutions. Lanes labeled as R indicate relaxed pBR322 DNA. R and S at the left side of agarose gel indicates the position of relaxed DNA and supercoiled DNA, respectively.

Fig 4. Molecular interaction of WQ-3810 with DNA gyrases.

(A) indicates the docking simulation result of WQ-3810 (shown in purple) and 5BTA without MFX. Asp at the amino acid position 461 of GyrB is circled in red and the Mg ion is shown as a blue circle. The specific linkage between NH_2 of the R1 group in WQ-3810 and Asp at position 461 of GyrB are circled in red. (B) indicates the docking simulation result of WQ-3810 and the 5BTA without MFX, in which Asp is replaced by Asn at position 461 in GyrB. Asn at 461 and NH_2 of the R1 group in WQ-3810 are circled in red. (C) indicates the molecular interaction of (A). (D) indicates the molecular interaction of (B). WQ-3810 docking scores are shown in this figure.

Fig 5. Molecular interaction between DNA gyrase, the DNA structure and WQ-3810.

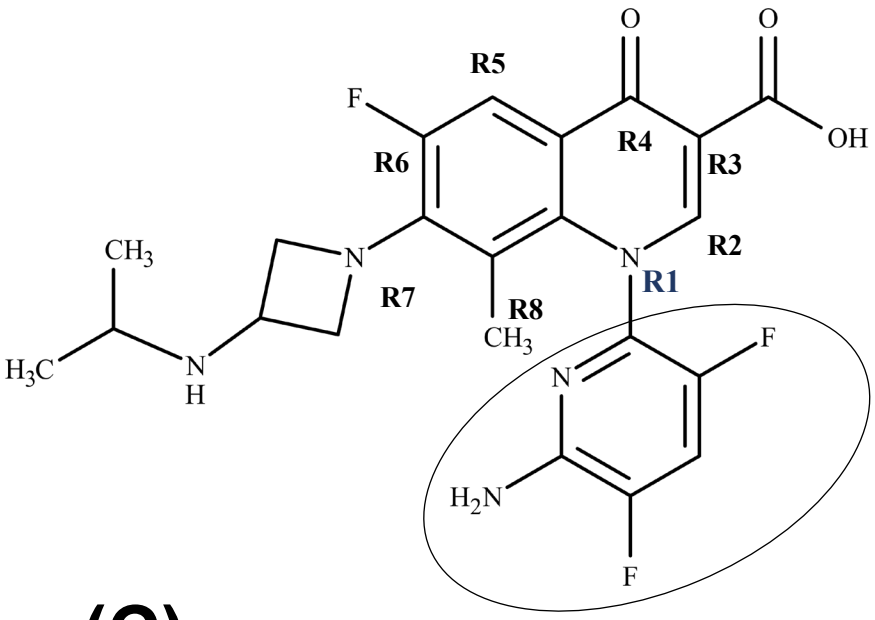
MFX is shown in yellow and WQ-3810 in purple. The spatial distance information is shown in this figure.

Table. IC_{50}s of quinolones for ML DNA gyrases in WT and mutants

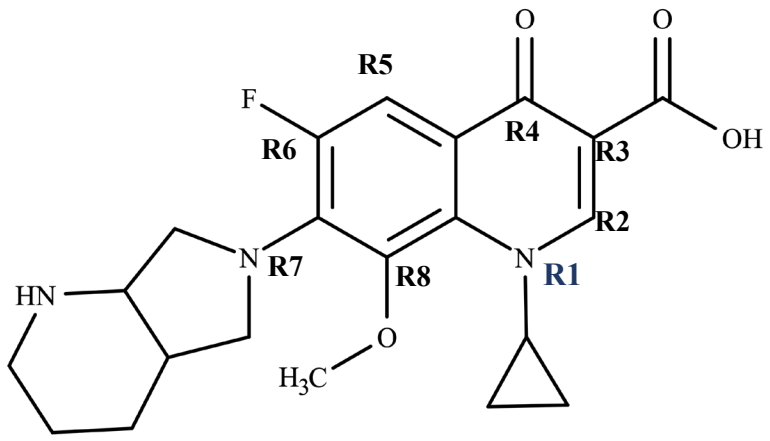
Drug	IC_{50} ± SD (μg/mL)				
	WT (n=6)	A91V (n=3)	D95G (n=3)	D464N (n=3)	N502D (n=3)
WQ-3810	1.6 ± 0.4	1.7 ± 0.5	6.9 ± 1.8	10.5 ± 0.4	22.0 ± 3.0
MFX	1.4 ± 0.5	2.1 ± 0.3	13.9 ± 1.9	5.1 ± 0.2	45.1 ± 10.3
OFX	3.7 ± 0.2	14.3 ± 0.4	47.3 ± 6.9	79.1 ± 9.1	124.2 ± 3.6

IC_{50}: Quinolone concentration for 50% inhibitory activity against DNA gyrase
WT: Wild type
SD: Standard deviation

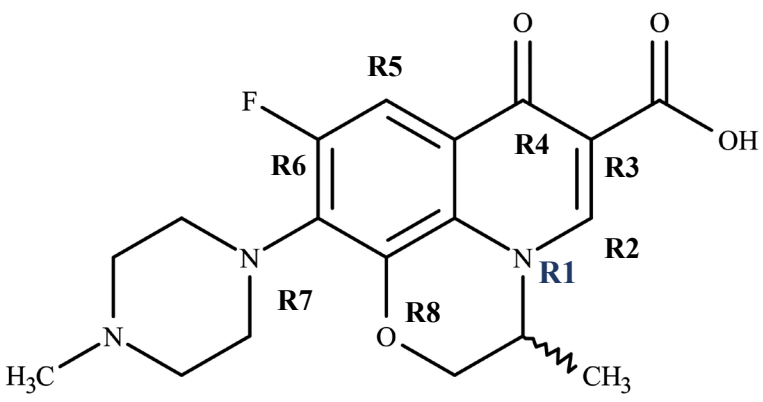
(A)



(B)

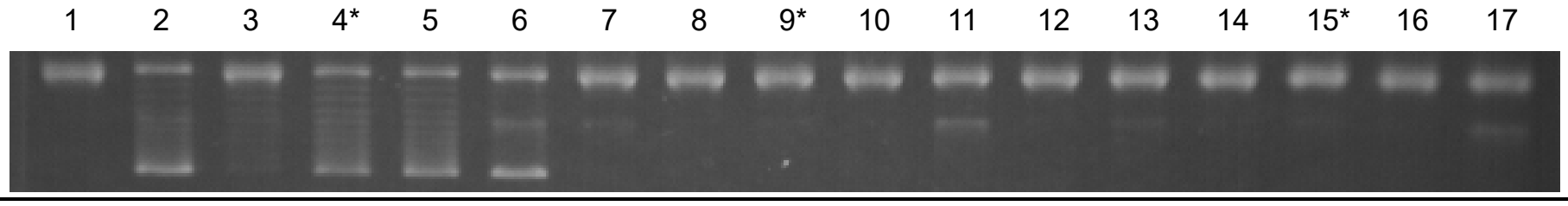


(C)



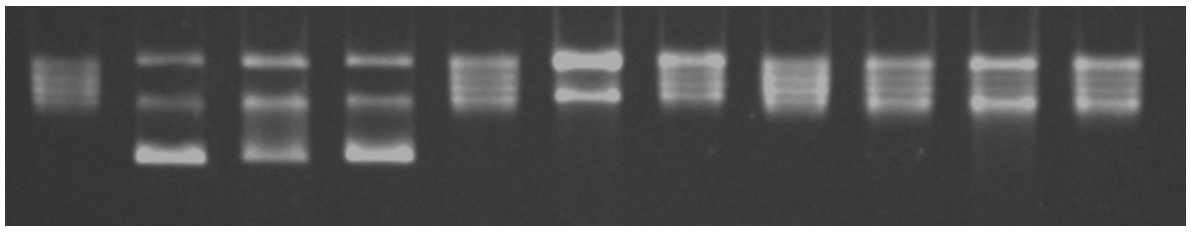
Park et al. Fig 1

(A)



	1	2	3	4*	5	6	7	8	9*	10	11	12	13	14	15*	16	17
GyrA	-	WT	G89C	G89C	A91V	D95G	WT	G89C	G89C	A91V	D95G	-	WT	G89C	G89C	A91V	D95G
WT GyrB	-	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
ATP	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Relaxed DNA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

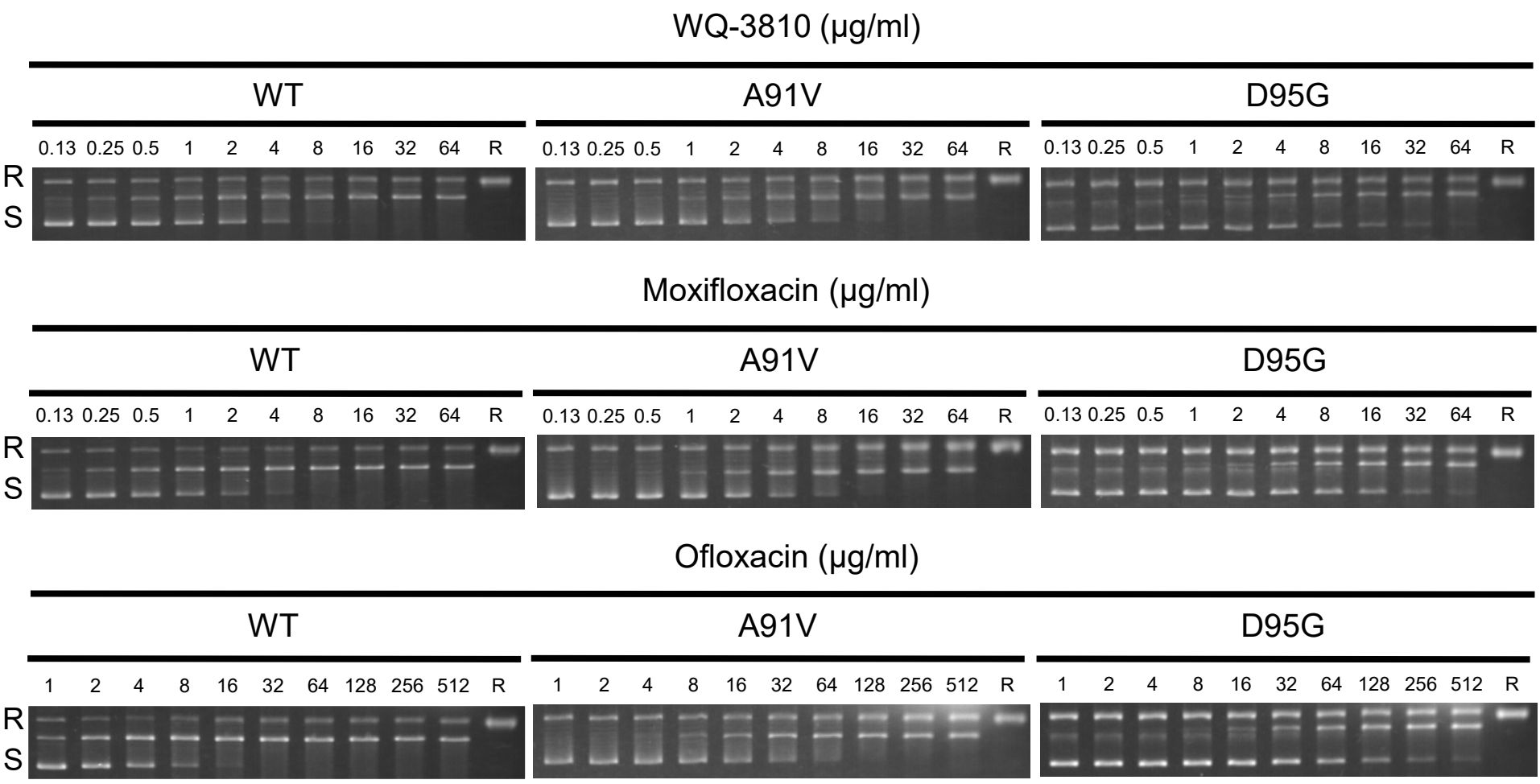
(B)



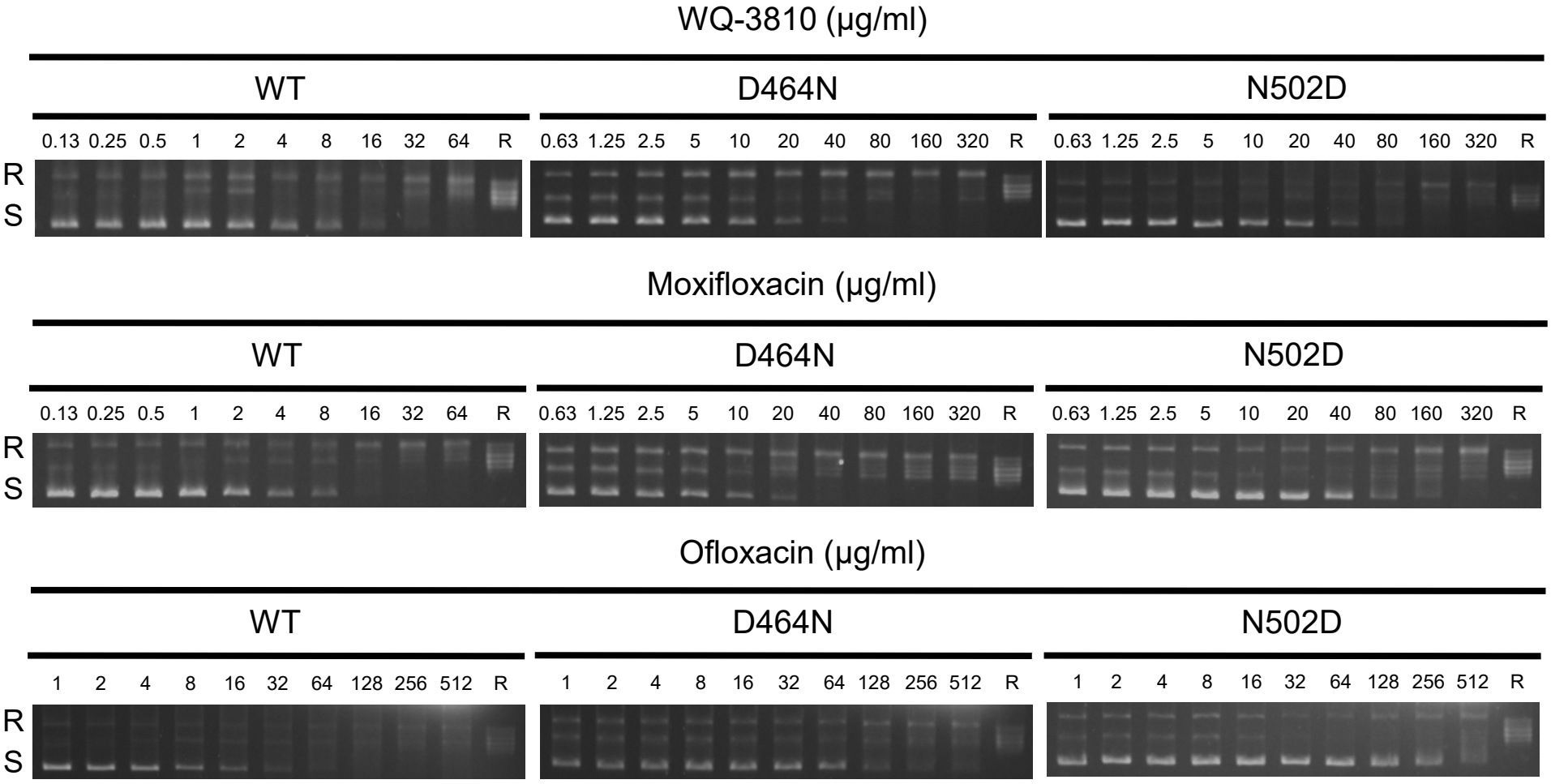
	1	2	3	4	5	6	7	8	9	10	11
GyrB	-	WT	D464N	N502D	WT	D464N	N502D	-	WT	D464N	N502D
WT GyrA	-	+	+	+	-	-	-	+	+	+	+
ATP	-	+	+	+	+	+	+	+	+	+	+
Relaxed DNA	+	+	+	+	+	+	+	+	+	+	+

Park et al. Fig 2

(A)

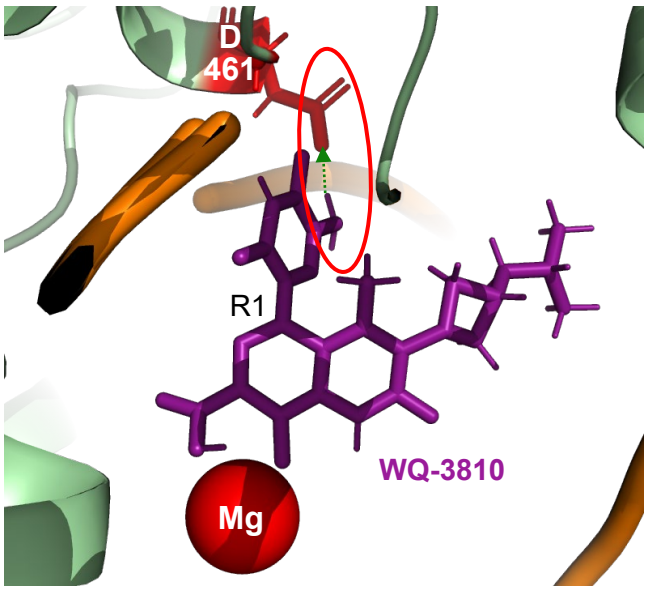


(B)



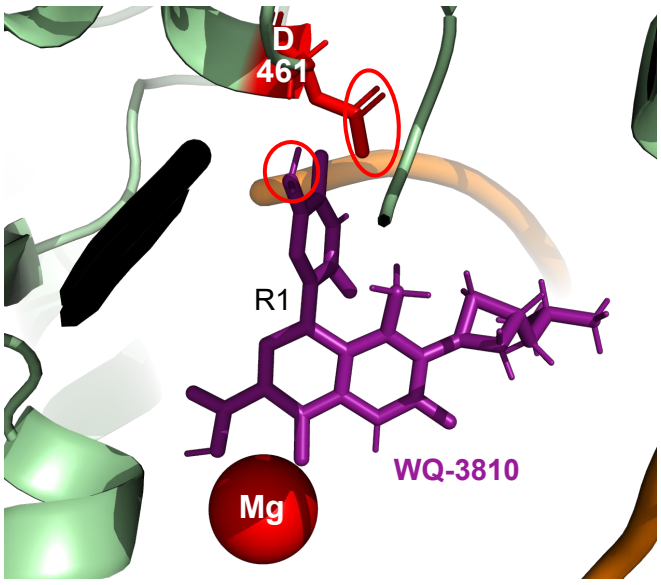
Park et al. Fig 3

(A)



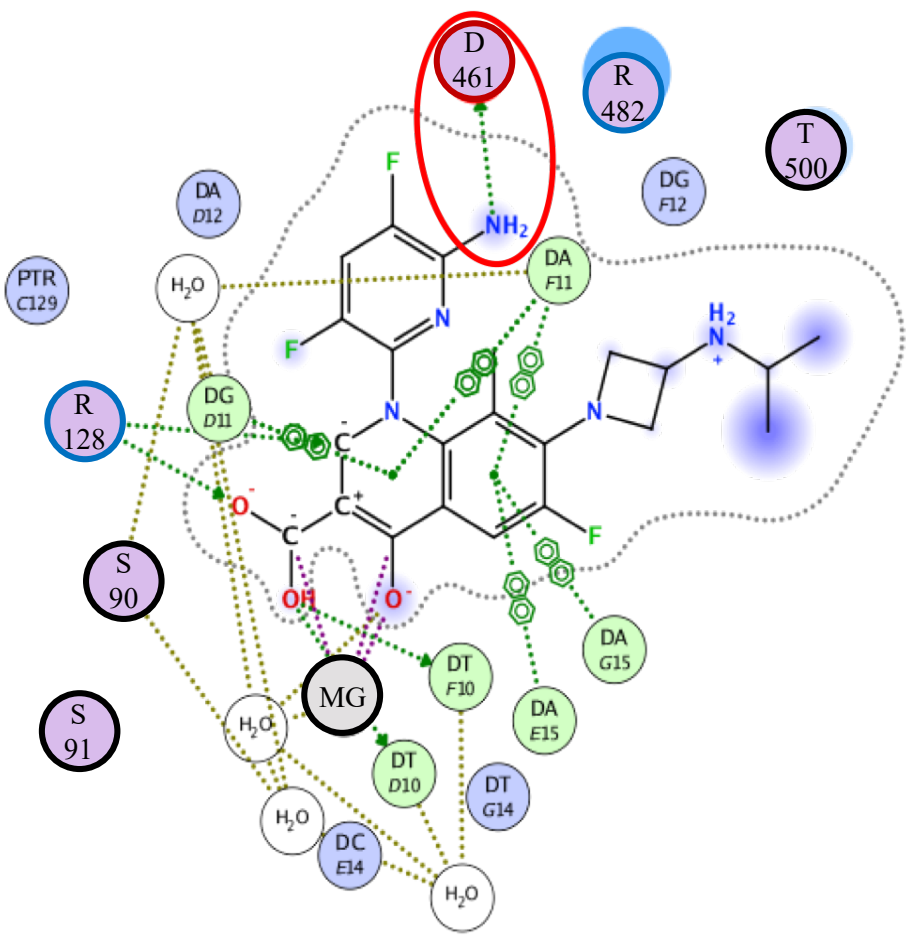
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(B)

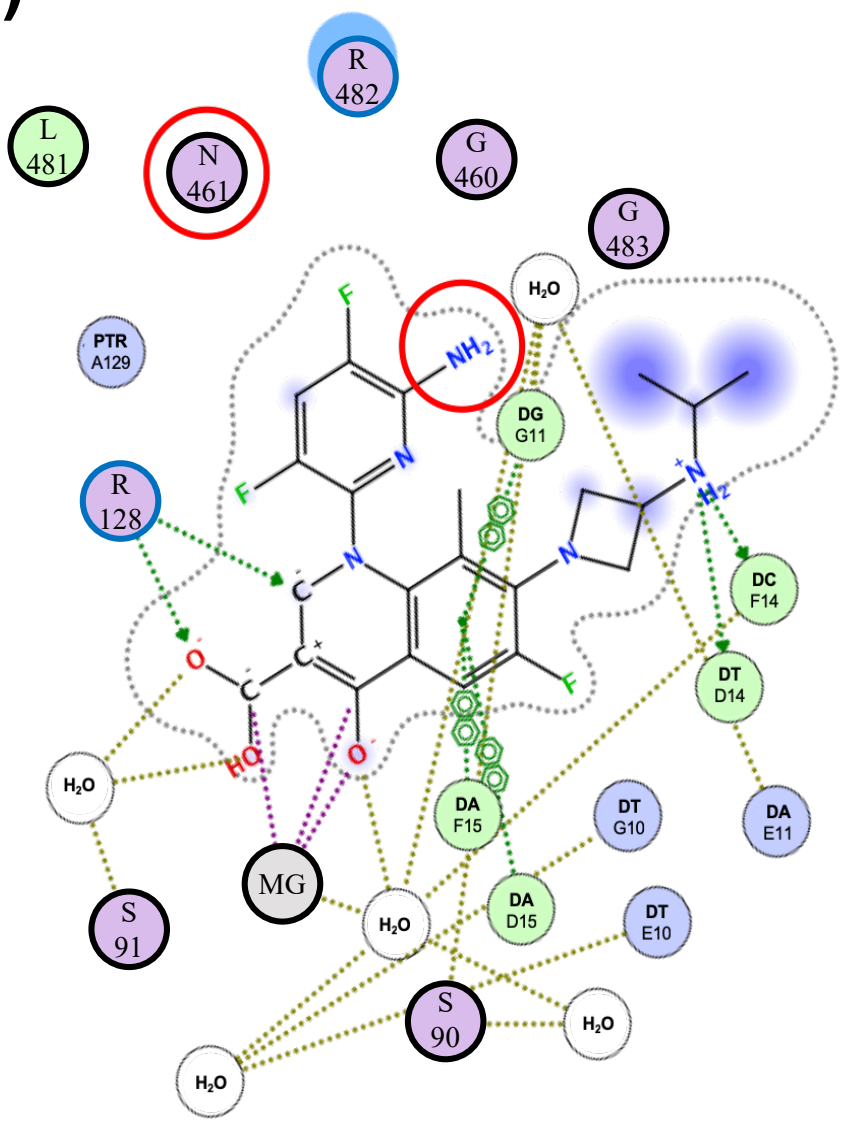


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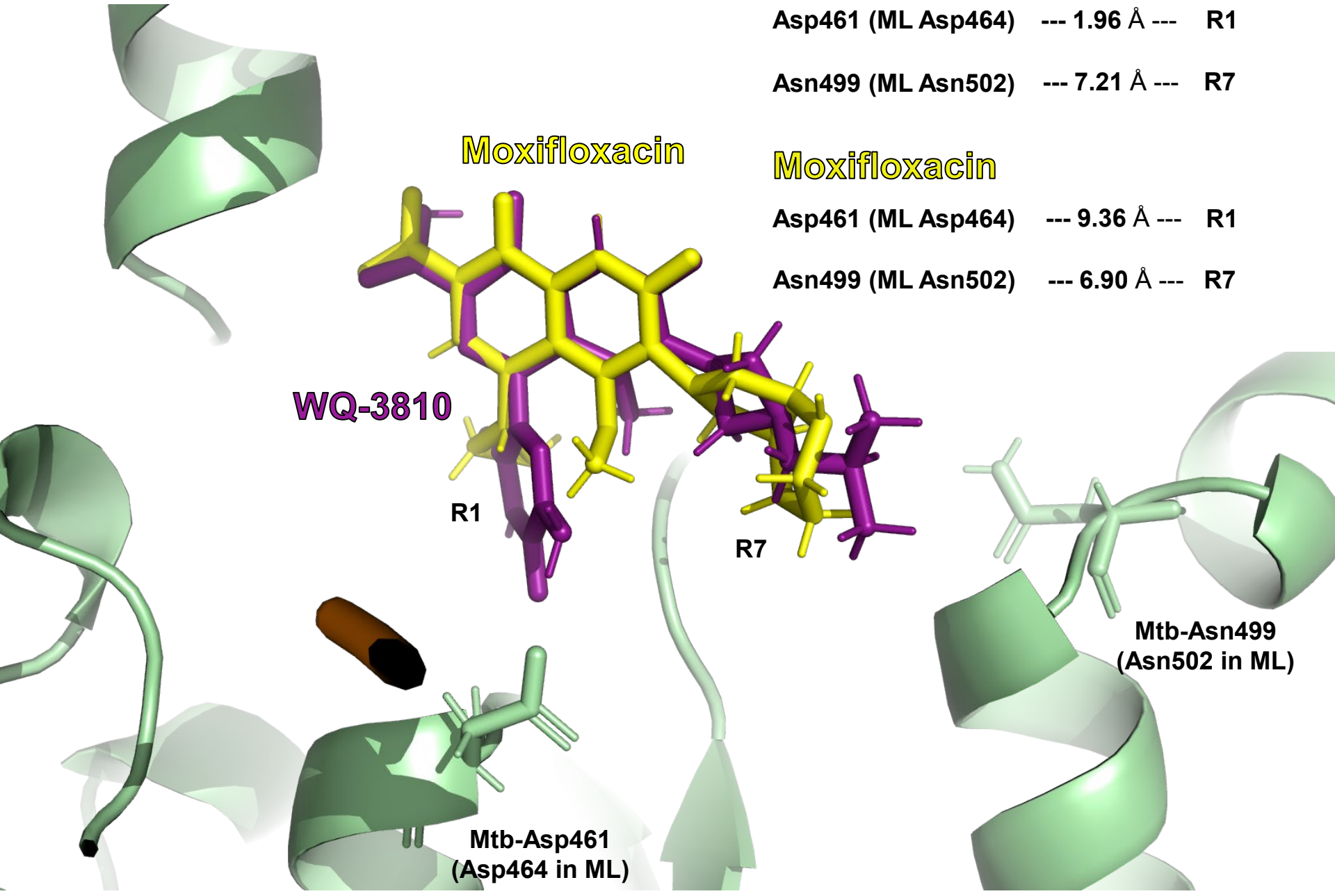
(C)



(D)



Park et al. Fig 4



Park et al. Fig 5