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1 Title: **Interaction of quinolones carrying new R1 group with *Mycobacterium leprae* DNA**
2 **gyrase**

3 Short title: Novel quinolones and *M. leprae* DNA gyrase

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28

29 **Abstract**

30 **Background**

31 Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and the treatment
32 of choice is ofloxacin. And specific amino acid substitutions in DNA gyrase of *M. leprae*
33 have been reported leading to resistance against the drug. In our previous study, WQ-3810, a
34 fluoroquinolone with a new R1 group (6-amino-3,5-difluoropyridin-2-yl) was shown to have
35 a strong inhibitory activity on ofloxacin-resistant DNA gyrases of *M. leprae*, and the
36 structural characteristics of its R1 group was predicted to enhance the inhibitory activity.

37 **Methodology/principal finding**

38 To further understand the contribution of the R1 group, WQ-3334 with the same R1 group
39 as WQ-3810, WQ-4064, and WQ-4065, but with slightly modified R1 group, were assessed
40 on their activities against recombinant DNA gyrase of *M. leprae*. An *in-silico* study was
41 conducted to understand the molecular interactions between DNA gyrase and WQ-
42 compounds. WQ-3334 and WQ-3810 were shown to have greater inhibitory activity against
43 *M. leprae* DNA gyrase than others. Further, analysis using quinolone-resistant *M. leprae*
44 DNA gyrases, showed that WQ-3334 had greater inhibitory activity than WQ-3810. The R8
45 group was shown to be a factor for the linkage of the R1 groups with GyrB by an *in-silico*
46 study.

47 **Conclusions/significance**

48 The inhibitory effect of WQ compounds that have a new R1 group against *M. leprae* DNA
49 gyrase, can be enhanced by improving the binding affinity with different R8 group
50 molecules. The information obtained by this work could be applied to design new
51 fluoroquinolones effective for quinolone-resistant *M. leprae* and other bacterial pathogens.

52

53 **Keywords:** *Mycobacterium leprae*, DNA gyrase, GyrB, WQ-3810, WQ-3334

55 **Introduction**

56 Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. The spread of
57 this disease has been controlled by a multidrug therapy (MDT) recommended by the World
58 Health Organization (WHO). Nonetheless, more than 200,000 new cases were still reported
59 in 2019 [1]. Relapse cases especially, are a serious concern because of a higher possibility of
60 being accompanied with resistance to anti-leprosy drugs, and hence, limiting the number of
61 drugs available for the treatment of leprosy [11-16]. To better treat MDT-resistant leprosy,
62 alternative drugs are needed. A fluoroquinolone, ofloxacin (OFX), is currently being used for
63 the treatment of MDT-resistant leprosy [2].

64 Fluoroquinolones bind to DNA gyrases and inhibit their enzymatic activity crucial for
65 DNA transcription and replication of bacteria [3, 4]. DNA gyrase consists of two subunits, A
66 (GyrA) and subunits B (GyrB) [4]. The quinolone resistance of DNA gyrase is developed by
67 substituting amino acids around quinolone binding sites, so-called quinolone resistance-
68 determining regions (QRDR), in either GyrA or GyrB [5]. In particular, amino
69 acid substitution from aspartic acid to glycine at the position of 94 (D94G) in GyrA is the
70 most frequently found substitution in quinolone-resistant *M. tuberculosis*. Homologous
71 amino acid substitution was experimentally confirmed to contribute to quinolone resistance in
72 *M. leprae* [2, 6]. By contrast, amino acid substitutions in QRDR of GyrB are less frequently
73 found in clinical strains; nonetheless, it is believed that mutations in GyrB may be
74 significantly related to DNA gyrase enzymatic activity. Therefore, D461N in GyrB of *M.*
75 *tuberculosis* and homologous amino acid substitutions in *M. leprae* have been proved to
76 confer quinolone resistance [8, 9].

77 To develop an efficient therapeutic regimen against OFX-resistant leprosy, there is an
78 urgent need for screening of new drugs and analyzing their individual structural
79 characteristics.

80 WQ-3810 is a quinolone compound which has an innovative NH₂-based molecular
81 structure, 6-amino-3,5-difluoropyridin-2-yl, at the R1 group (Fig 1B) [18, 19]. This
82 compound has been reported to have a strong bactericidal effect on several pathogenic
83 bacteria [18, 19, 21]. Furthermore, it was shown to have strong inhibitory activity against *M.*
84 *leprae* DNA gyrase with quinolone-resistant amino acid substitutions in GyrA [29]. This
85 superior inhibitory property may be enhanced by the structural characteristic of the R1 group
86 [29]. Thus, understanding the contribution of the R1 group to the inhibitory activity against
87 *M. leprae* DNA gyrase, seemed to be necessary.

88 WQ-3810 shares structural characteristics with the compounds WQ-3334, WQ-4064 and
89 WQ-4065 though with differences at R8 to WQ-3334, and R1 to WQ-4064 and WQ-4065.
90 Due to the distinct molecular structures, these compounds seem to exert different inhibitory
91 activities against wild-type (WT) and mutant DNA gyrases of *M. leprae*. In light of this, the
92 interaction of the molecular structures of the WQ compounds with DNA gyrases remains
93 unknown.

94 In the present study, WQ-3810, WQ-3334, WQ-4064, and WQ-4065 were assessed on the
95 ability of the R1 group to inhibit *M. leprae* DNA gyrase. To compare activities of these
96 compounds, *in vitro* assays were conducted using recombinant DNA gyrases, including WT
97 and mutants bearing amino acid substitutions, D95G and D464N in GyrA and GyrB,
98 respectively. In addition, an *in-silico* study was carried out to understand the molecular
99 interaction between WQ-compounds and DNA gyrases.

100

101 **Materials and Methods**

102 **Antibacterial agents**

103 Quinolones WQ-3810, WQ-3334, WQ-4064, and WQ-4065 were provided as 100 % pure
104 compounds by Wakunaga Pharmaceutical Co., Ltd. (Osaka, Japan).

105

106 **Bacterial strains and expression plasmids**

107 Thai-53 strain of *M. leprae* [20], maintained at the Leprosy Research Center, National
108 Institute of Infectious Diseases (Tokyo, Japan), was used for the preparation of *M. leprae*
109 DNA. *Escherichia coli* strain TOP-10 (Thermo Fisher Scientific, Waltham, MA) was used
110 for cloning. While *E. coli* strains Rosetta-gamiTM 2(DE3)pLysS and BL21(DE3)pLysS
111 (Merck KGaA, Darmstadt, Germany) were used for protein expression. Further, plasmid
112 vector pET20b(+) (Merck KGaA) was used to construct expression plasmids, and relaxed
113 pBR322 plasmid DNA (Inspiralis Ltd., Norwich, UK) was used for the assessment of
114 supercoiling activity.

115

116 **Preparation of recombinant DNA gyrase subunits**

117 DNA gyrase expression plasmids encoding ML-GyrA^{WT} and ML-GyrA^{D95G} and ML-
118 GyrB^{WT} and ML-GyrB^{D464N} were constructed as previously described [10, 21, 23].

119 Expression and purification of recombinant DNA gyrase subunits were conducted as
120 previously reported [10, 23-25]. Briefly, expression plasmids carrying either *gyrA* or *gyrB* of
121 *M. leprae* were introduced into *E. coli* Rosetta-gami2(DE3)pLysS or BL21(DE3)pLysS,
122 respectively. Transformants were cultured in Luria-Bertani (LB) broth up to the log phase,
123 under ampicillin selection (100 µg/ml). Expression of DNA gyrases was induced by adding 1
124 mM isopropyl-beta-D-thiogalactopyranoside (FUJIFILM Wako Pure Chemical Industries
125 Corp, Osaka, Japan) to the culture, and further incubated for 16 to 24 h at 12 or 14 °C. After

126 incubation, *E. coli* cells were harvested and lysed by sonication (10 times for 40 s at output
127 level 3 and 40% duty cycle with 40-s intervals) using a Sonifier 250 (Branson, Danbury, CT).
128 The supernatant was collected by centrifugation (10,000× *g* for 30 min) and recombinant
129 DNA gyrase subunits were purified by Ni-NTA Agarose (Thermo Fisher Scientific) column
130 chromatography, as per manufacturer's protocol, and dialyzed against DNA gyrase dilution
131 buffer (50 mM Tris-HCl pH 7.5, 100 mM KCl, 2 mM DTT, 1 mM EDTA).

132

133 **Fluoroquinolone-inhibited DNA supercoiling assay**

134 A DNA supercoiling assay was carried out in 30 µL reaction mixture consisting of 1 x
135 DNA gyrase reaction buffer, 4 nM relaxed pBR322 DNA, 40 nM of each subunit GyrA (ML-
136 GyrA^{WT} or ML-GyrA^{D95G}), GyrB (ML-GyrB^{WT} or ML-GyrB^{D464N}) and fluoroquinolones
137 [26]. All WQ compounds were used at concentrations from 0.13 to 64 µg/mL for subunit
138 combination ML-GyrA^{WT} and ML-GyrB^{WT}. WQ-3810 and WQ-3334 were used for further
139 assays in concentrations from 0.13 to 64 µg/ml for subunit combinations ML-GyrA^{D95G} with
140 ML-GyrB^{WT} and ML-GyrA^{WT} with ML-GyrB^{D464N}. Reactions were conducted for 90 min at
141 30 °C and stopped by adding 7.5 µL of dye mix. Then, 10 µL from each reaction mixture was
142 analyzed by 1% agarose gel electrophoresis in 1× TBE buffer (Nacalai Tesque, Inc., Osaka,
143 Japan) stained with 0.7 µg/mL of ethidium bromide. The intensity of the supercoiled DNA
144 bands in agarose gel was calculated by the software, ImageJ
145 (<https://imagej.nih.gov/ij/download.html>) and the IC₅₀s were calculated with the AAT
146 Bioquest web tool (<https://www.aatbio.com>).

147

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

149 Molecular docking and visualization studies were carried out using Molecular Operating
150 Environment (MOE) (Chemical Computing Group ULC, Montreal, Quebec, Canada).

151 <https://www.chemcomp.com/index.htm>) and MolDesk Basic v1.1.54 (IMSBIO co., Ltd,
152 Tokyo, Japan). As the *M. leprae* DNA gyrase molecular structure is yet to be elucidated, the
153 coordinates of the DNA gyrase were retrieved from the Protein Data Bank (PDB,
154 <http://www.rcsb.org/pdb/>) for structure-based molecular modeling and the PDB ID #5BTA
155 (<https://www.rcsb.org/structure/5BTA>; Crystal structure model of Mtb-gyrase complex), the
156 *M. tuberculosis* DNA gyrase 3D structural model, highly homologous to *M. leprae*, was used.

157 Ligand location and pocket-size were set using the coordinates of moxifloxacin (MXF),
158 which is found in the PDB ID #5BTA model as a ligand component. Optional parameters in
159 software MOE were used to create a topology file, which included the addition of hydrogen
160 atoms, the calculation of a grid potential, and a docking simulation. The flexible docking
161 method was used and scores were calculated as the sum of five potentials: accessible surface
162 area, coulomb potential, hydrogen bonds, hydrogen bond considering anisotropy and van der
163 Waals interactions. Protein-ligand binding free energy was estimated by MOE using the
164 Amber 10: EHT force-field and the default parameters of the MOE Dock application. The
165 results of molecular docking were visualized with PyMOL v1.3 (<http://www.pymol.org/>).
166 Distances between amino acids and the side chains of WQ-3810 and WQ-3334 were
167 calculated using PyMOL v1.3.

168

169 **Results**

170 **Inhibitory effect of fluoroquinolones on *M. leprae* recombinant DNA gyrases.**

171 All fluoroquinolones inhibited DNA gyrases depending on the doses (Fig. 2). The trend of
172 inhibitory activity of each quinolone against WT and mutant DNA gyrase was as shown in
173 Fig 3. When compared against WT DNA gyrase, WQ-3334 showed the highest inhibitory
174 activity than compounds, WQ-3810, WQ-4064, and WQ-4065 as shown in Fig 3A. Further,
175 using mutant DNA gyrases, WQ-3334 showed better inhibitory activity than that of WQ-

176 3810 against both quinolone-resistant DNA gyrase with ML-GyrA^{D95G} and ML-GyrB^{D464N}
177 (Fig 3B and C). The IC₅₀s calculated by the assays are shown in Table 1. WQ-3334 showed
178 the highest inhibitory effect on WT DNA gyrase. In addition, the IC₅₀s of WQ-3810 and WQ-
179 3334 against quinolone-resistant DNA gyrases with ML-GyrA^{D95G} and ML-GyrB^{D464N} were
180 increased with the increase in IC₅₀s against DNA gyrase with ML-GyrB^{D464N} greater than that
181 against DNA gyrase with ML-GyrA^{D95G} (Table 1). Moreover, the increases in the IC₅₀s of
182 WQ-3334 against both mutant DNA gyrases were lower than those of WQ-3810.

183

184 ***In silico* study of the molecular interaction between DNA gyrase, DNA, and** 185 **fluoroquinolones.**

186 Fluoroquinolone binding site consists of subunits GyrA, GyrB, and the DNA molecule.
187 PDB ID #5BTA, which is the *M. tuberculosis* DNA gyrase 3D structural model, possesses
188 ~~the~~ an intact fluoroquinolone binding site and MFX positioned at the binding site as a ligand
189 model. The docking simulation of WQ-3334 showed an s-score of -28.4434 (Fig. 4). In
190 addition, the distance between the R1 group of WQ-3334 and the side chain of an amino acid
191 at position 461 (Asp461 in ML) was found to be 1.9 Å (Fig. 4.). In the simulation, the NH₂
192 molecule in the R1 group (2,4-difluoro-5-aminopyridine substituent) of WQ-3334 seemed to
193 polarly associate with Mtb-Asp461 (Asp464 in ML) (Fig 5).

194

195 **Discussion**

196 The R1 group, 6-amino-3,5-difluoropyridin-2-yl, is a new molecular structure for
197 fluoroquinolones. WQ-3810 with this R1 group showed a strong bactericidal effect on
198 *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Neisseria*
199 *gonorrhoeae*, *E. coli*, and *Salmonella Typhimurium* [19, 28].

200 In the previous study, to elucidate the potential of WQ-3810 as a drug for leprosy, its
201 inhibitory effect against DNA gyrases of *M. leprae* was assessed instead of measuring the
202 minimum inhibitory concentration [29]. This approach was used because *M. leprae* is yet to
203 be cultured in artificial media. The IC₅₀, calculated using a supercoiling inhibitory assay, was
204 used as a reliable criterion for the therapeutic potency of fluoroquinolones against *M. leprae*
205 [6, 7, 10, 21, 22]. Thus, WQ-3810 showed strong inhibitory activity against quinolone-
206 resistant DNA gyrases, which have amino acid substitutions in GyrA QRDR. In a subsequent
207 *in silico* study, WQ-3810 showed an additional association with GyrB that may enhance its
208 inhibitory effect on quinolone-resistant DNA gyrases bearing GyrA amino acid substitutions
209 [29]. For a better understanding of this assumption, an additional comparison with WQ-
210 compounds having different R1 and R8 groups was deemed necessary.

211 In the present study, compounds WQ-3810, WQ-3334, WQ-4064, and WQ-4065 were
212 compared with each other. When compared with WQ-3810 (Fig 1B), WQ-3334 had only one
213 different atom of bromine at the R8 group (Fig 1C), which may have caused a change in the
214 angle of the R1 group. Furthermore, unlike WQ-3810, WQ-4064 and 4065 have 6-
215 methylamino-3,5-difluoropyridin-2-yl and 6-ethylamino-3,5-difluoropyridin-2-yl,
216 respectively, at the R1 group (Fig 1D and 1E). In an inhibitory assay, WQ-4064 and WQ-
217 4065 showed weaker inhibitory effects on DNA gyrase with ML-GyrA^{WT} than WQ-3334 and
218 WQ-3810 (Fig 3A). The IC₅₀ notably increased by substituting 6-amino-3,5-difluoropyridin-
219 2-yl group in WQ-3810 to 6-methylamino-3,5-difluoropyridin-2-yl or 6-ethylamino-3,5-
220 difluoropyridin-2-yl at R1 group in WQ-4064 and 4065, respectively (Table 1). It seemed that
221 at the R1 group, 6-amino-3,5-difluoropyridin-2-yl (WQ-3810 and WQ-3334) associated with
222 GyrB better than those in WQ-4064 and WQ-4065. The IC₅₀ of WQ-3334 against DNA
223 gyrase with ML-GyrA^{WT} was almost 4-fold and 39-fold lower than those of WQ-4064

224 ($P < 0.005$) and WQ-4065 ($P < 0.005$), respectively. In light of this, WQ-3810 and WQ-3334
225 were further compared using DNA gyrases bearing ML-GyrA^{D95G} and ML-GyrB^{D464N}.

226 Aspartic acid at position 95 in GyrA of *M. leprae* DNA gyrase may provide a metal ion
227 bridge effect, which seems to be a crucial linkage between fluoroquinolones and DNA
228 gyrases. An equivalent effect has been found at the same position in quinolone-resistant DNA
229 gyrase in *E. coli*, *S. aureus*, *S. pneumoniae*, and *M. tuberculosis* [25, 26]. In the present study,
230 an increase in the IC₅₀s of WQ-3810 and WQ-3334 was observed with DNA gyrases bearing
231 ML-GyrA^{D95G} (Table 1). Interestingly, WQ-3334 showed a greater inhibitory effect on DNA
232 gyrase with ML-GyrA^{D95G} than did WQ-3810 ($P < 0.005$) (Fig 3B and Table 1).

233 Aspartic acid at-position 464 in GyrB of *M. leprae* DNA gyrase has been considered an
234 important amino acid for the inhibitory effect of WQ-3810, probably due to a greater
235 association of the R1 group in WQ-3810 with GyrB [29]. This association may contribute to
236 enhanced the inhibitory effect on quinolone-resistant DNA gyrase with GyrA amino acid
237 substitutions such as D95G [29]. In this study, substantial increases in IC₅₀s against DNA
238 gyrase with ML-GyrB^{D464N} were found in both WQ-3810 and WQ-3334, and the rates of
239 increase for these two quinolones were almost the same (WQ-3810: 7-fold; WQ-3334: 6-
240 fold) (Table 1). These results may indicate that the R1 group enhances the binding affinity of
241 WQ-3334 in a similar manner to that of WQ-3810. However, the trend of inhibitory activity
242 (Fig 3C) and IC₅₀ of WQ-3334 was better than that of WQ-3810 ($P < 0.005$) (Table 1). This
243 may be due to differences between the R8 groups, which may affect the R1 group and hence,
244 enhance the binding affinity of WQ-3334 to wild and mutant type DNA gyrases.

245 To further understand the molecular interaction between DNA gyrases with WQ-3810 and
246 WQ-3334, an *in-silico* study was carried out. The molecular structure of *M. leprae* DNA
247 gyrase is not yet listed in the protein databank (PDB), hence, the DNA gyrase of *M.*
248 *tuberculosis* was used. The amino acid sequence of QRDR and the surrounding region of

249 DNA gyrase in *M. leprae* is identical to that of *M. tuberculosis*. Besides, several amino acid
250 substitutions at QRDR in both GyrA and GyrB of *M. leprae* have been shown to confer
251 resistance similar to those of *M. tuberculosis* [10, 21, 23]. Thus, it was theorized that an *in-*
252 *silico* study using the 5BTA model could provide a reliable simulation to elucidate the
253 association of target compounds with *M. leprae* DNA gyrases. PDB ID: 5BTA is the 3D
254 molecular structural model of the intact heterotetramers consisting of two GyrAs and GyrBs
255 of *M. tuberculosis*, cleaved DNA, and MFX. Hence, the information regarding the
256 coordinates of MFX positioned at the quinolone binding site of 5BTA was used for the
257 docking simulation of WQ-3334 following a previous study conducted at these premises [29].

258 A better binding affinity to DNA gyrase of WQ-3334 than that of WQ-3810 was estimated
259 by the computational simulation (Fig 4). The distance between R1 group of WQ-3334 and
260 461st Asp in GyrB was slightly longer than that of WQ-3810 and the angle between R7 and
261 R1 group in WQ-3334 was shown to be distinct from that in WQ-3810 (Fig 4). This might
262 cause the different binding affinity (S-score) of WQ-3334 to DNA gyrase from WQ-3810 and
263 associate with the distinct inhibitory activities of these compounds against *M. leprae* DNA
264 gyrase. This was supported by the predicted molecular interaction shown in Fig 5A and B as
265 2D and 3D graphics, respectively. This theoretical approach was in good agreement with the
266 report by Kuramoto et al. [17].

267 Amino acid substitutions in GyrB associated with quinolone resistance have not yet been
268 reported in clinical *M. leprae*, perhaps due to the limited number of reports related to
269 quinolone-resistant leprosy. Though numerous reports on quinolone-resistance associated
270 amino acid substitutions in GyrA have been published, information on clinical isolates
271 showing quinolone resistance due to amino acid substitutions in GyrB is rare [11, 12]. This
272 fact may indicate that amino acid substitutions in QRDR of GyrB associate with a significant
273 reduction of DNA gyrase activity. Moreover, the interaction of WQ-3810 and WQ-3334 with

274 the QRDR of GyrB can potentially inhibit the activity of DNA gyrase with amino acid
275 substitutions in QRDR of GyrA. Therefore, acquiring more information on the molecular
276 structural characteristics of WQ-3810 and WQ-3334 may be the first steps to designing better
277 drugs that target quinolone-resistant leprosy.

278 In conclusion, WQ-3810 and WQ-3334 with 6-amino-3,5-difluoropyridin-2-yl at the R1
279 group showed better inhibitory activity against DNA gyrase of *M. leprae* than WQ-4064 and
280 WQ-4065 with 6-methylamino-3,5-difluoropyridin-2-yl and 6-ethylamino-3,5-
281 difluoropyridin-2-yl, respectively, at the R1 group. WQ-3334 showed greater inhibitory
282 activity against both DNA gyrase with ML-GyrA^{D95G} and ML-GyrB^{D464N} than WQ-3810.
283 Additionally, the *in-silico* study suggested the impact of R8 group on the affinity of WQ-
284 3810 and WQ-3334 to DNA gyrases. The information obtained by the present work could be
285 applied to design new fluoroquinolones effective for several quinolone-resistant *M. leprae*
286 and other bacterial pathogens.

287

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296

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298

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399

400 **Table. IC₅₀s of quinolones for ML DNA gyrases in WT and mutants**

Drug	IC ₅₀ ± SD (µg/mL)			
	WQ-3810	WQ-3334	WQ-4064	WQ-4065
WT (n=3)	1.4 ± 0.1	0.8 ± 0.0	4.4 ± 0.2	31.2 ± 1.0
D95G (n=3)	7.3 ± 0.7	3.5 ± 0.1	ND	ND
D464N (n=3)	9.9 ± 0.1	4.9 ± 0.1	ND	ND

401 IC₅₀: Quinolone concentration for 50% inhibitory activity against DNA gyrase

402 WT: Wild type

403 SD: Standard deviation

404 ND: Not determined

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421 Figure legends

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

423 (A) Positions of each R group in the basic quinolone structure. (B) WQ-3810, (C) WQ-3334,
424 (D) WQ-4064 and (E) WQ-4065

425

426 **Fig 2. Fluoroquinolone-inhibited DNA supercoiling assay**

427 Relaxed DNA (pBR322) was mixed and incubated with GyrA, GyrB, ATP, and
428 quinolones at the indicated concentrations. Each quinolone was screened for its inhibitory
429 effect on WT DNA gyrases and mutant DNA gyrases with ML-GyrA^{D95G} and ML-GyrB^{D464N}
430 substitutions. Lanes labeled as R indicate relaxed pBR322 DNA.

431

432 **Fig 3. Sigmoidal graph for DNA gyrase activity of fluoroquinolones in a dose-dependent**
433 **manner**

434 (A) The inhibitory activity of compounds WQ-3810, WQ-3334, WQ-4064, and WQ-4065
435 against WT DNA gyrase is shown as sigmoidal graphs. (B) The inhibitory activity of WQ-
436 3810 and WQ-3334 against mutant DNA gyrase with ML-GyrA^{D95G} is shown as sigmoidal
437 graphs. (C) The inhibitory activity of WQ-3810 and WQ-3334 against mutant DNA gyrase
438 with ML-GyrB^{D464N} is shown as sigmoidal graphs.

439

440 **Fig 4. Molecular interaction of WQ-3334 with DNA gyrases**

441 The docking simulation result of quinolone WQ-3334 is shown in yellow. The 3D
442 coordinates are on the left and the s-score on the right top. The docking simulation result
443 (analysis from a previous study) of quinolone WQ-3810 is shown in green. For comparison,
444 the results are overlapped. The calculated spatial distances between the R1 group of each
445 quinolone and the 461st amino acid of 5BTA are shown on the right bottom. The DNA

446 gyrase molecular structure is shown in purple and the Mg ion is shown as a cyan-colored
447 sphere. Amino acid positions related to the present study are shown in orange (94th amino
448 acid) and gray (461st amino acid).

449

450 **Fig 5. Molecular interaction between DNA gyrase, the DNA structure, and WQ-3334**

451 (A) Molecular interaction of WQ-3334 with 5BTA. (B) Visualization of the interaction as 3D
452 coordinates. Amino acid positions related to the present study are shown in orange (94th
453 amino acid) and gray (461st amino acid).

454

WQ-3810 ($\mu\text{g/ml}$)

WT

D95G

D464N

0.13 0.25 0.5 1 2 4 8 16 32 64 R 0.13 0.25 0.5 1 2 4 8 16 32 64 R 0.13 0.25 0.5 1 2 4 8 16 32 64 R

L
SC



WQ-3334 ($\mu\text{g/ml}$)

WT

D95G

D464N

L

0.13 0.25 0.5 1 2 4 8 16 32 64 R 0.13 0.25 0.5 1 2 4 8 16 32 64 R 0.13 0.25 0.5 1 2 4 8 16 32 64 R

L
SC

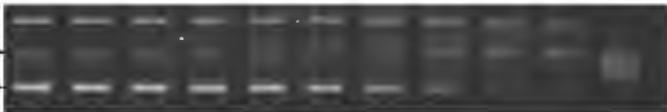


WQ-4064 ($\mu\text{g/ml}$)

WT

0.13 0.25 0.5 1 2 4 8 16 32 64 R

L
SC

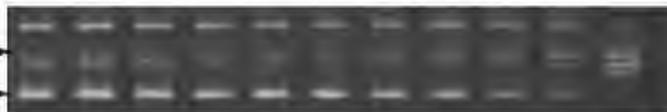


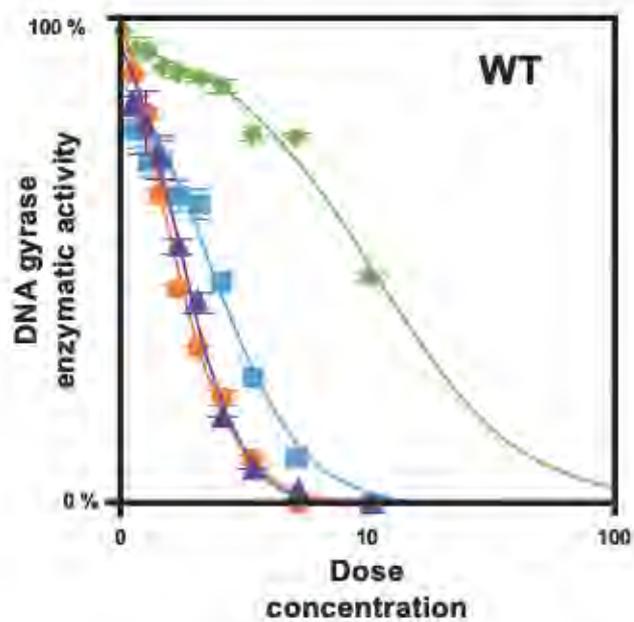
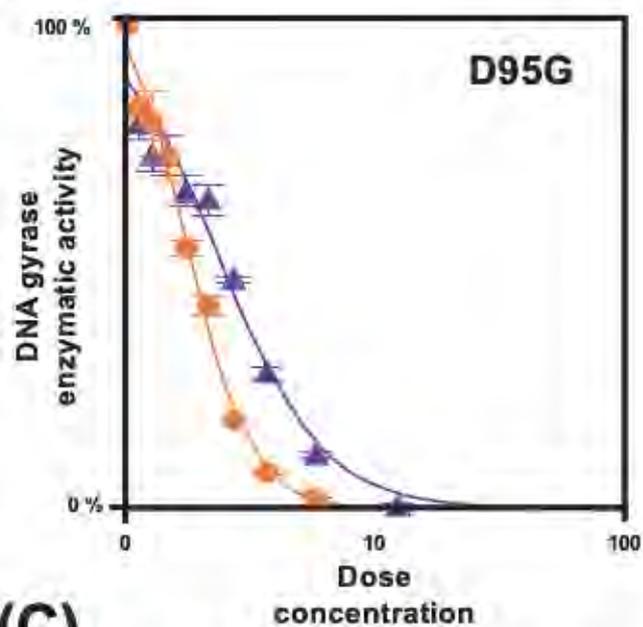
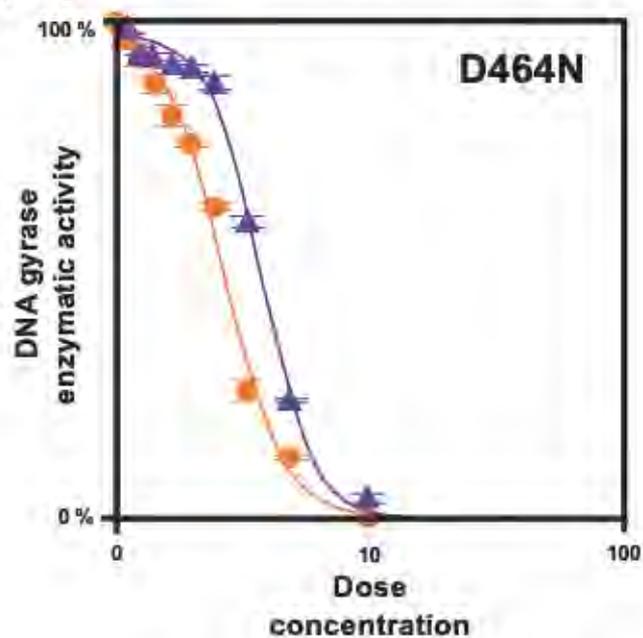
WQ-4065 ($\mu\text{g/ml}$)

WT

0.13 0.25 0.5 1 2 4 8 16 32 64 R

L
SC



(A)**(B)****(C)**

Compound	S-score	Compound	S-score
 WQ-3810	-7.1960	 WQ-3334	-28.4434

