Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 14, Issue 1, 2025, 3
https://doi.org/10.33263/LIANBS141.003

Trifluoroacetyl-Substituted Quinolones as New Antibacterials Against Antibiotic Resistant Strains

Diana Hodyna [1,*], Anton Klipkov [1], Yurii Shulha [1], Nadiia Shevchenko [1], Maryna Kachaeva [1], Vasyl Kovalishyn [1], Igor Gerus [1], Larysa Metelytsia [1]

[1] V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine, Kyiv, 02094, Ukraine; dianahodyna@gmail.com (D.H.); kostas176@gmail.com (A.K.); yuriishulha@ukr.net (Y.S.); nadina.sheva2105@gmail.com (N.S.), marinaka4aeva@gmail.com (M.K.); vkovalishyn@gmail.com (V.K.); igerus@hotmail.com (I.G.); larisametelitsa@gmail.com (L.M.);
* Correspondence: dianahodyna@gmail.com (D.H.);

Scopus Author ID 56897133500
Received: 14.11.2023; Accepted: 7.07.2024; Published: 21.09.2024

Abstract: This article describes the design of a series of new trifluoroacetyl-substituted quinolones, synthesis, and characterization by ^{1}H and ^{19}F NMR, mass spectroscopy, and their antimicrobial activity against a number of bacteria of medical interest. The online Chemical Modeling Environment (OCHEM) was used to perform a QSAR analysis on the new trifluoro acylated quinolones set that tested against *E. coli*. The six most promising compounds were identified, synthesized, and tested. Among these derivatives, compounds **2**, **3,** and **7** exhibited a wide spectrum of antibacterial properties against Gram-positive *S. aureus* and Gram-negative *E. coli* and *A. baumannii* bacteria, including antibiotic-resistant ones, while all compounds showed lower activity. The acute toxicity results of trifluoroacetyl-substituted quinolones to *D. magna* investigated in a series of 48-h immobilization assays in synthetic test solutions demonstrated the LC_{50} values in the range of 10.22-76.23 mg/L (slightly toxic compounds). Molecular docking results suggest that the antibacterial mechanisms of the compounds may be related to the inhibition of bacterial DNA gyrase or topoisomerase IV.

Keywords: QSAR; antibacterial activity; docking; trifluoroacetyl-substituted quinolones; toxicity.

1. Introduction

It is known that fluoroquinolones (FQs) are a group of synthetic antimicrobials derived from 1,4-dihydro-4-oxoquinoline-3-carboxylic acid. The quinolone scaffold is a privileged structure with a wide spectrum of bioactivity, and it is especially used to develop effective antimicrobials [1,2]. Based on the original bicyclic 4-quinolone molecule, more than 10,000 compounds have been developed with high therapeutic potential for numerous diseases [3-5].

As bactericidal agents, FQs are widely used to treat upper and lower respiratory tract infections such as tuberculosis [6], bronchitis, and pneumonia [7], as well as urinary tract infections [8], gastrointestinal infections, gonorrhea, skin infection, genitourinary cancer [9,10]. It should be noted that FQs have no analogs and, therefore, can provide efficacy against antibiotic-resistant strains that cause bacterial infections in humans and animals [11-13].

The first generation of quinolones (nalidixic acid, flumequin, oxolinic acid) was particularly effective against Gram-negative bacteria (*Salmonella spp.*, *Escherichia coli*, *Bordetella spp.* and *Yersinia spp.*). With the introduction of quinolones second generation (FQs) prototype enrofloxacin, the activity spectrum has been expanded to include Gram-

positive bacteria (*Staphylococcus*, *Streptococcus*, *Listeria monocytogenes*) also to include Campylobacter species, *Pseudomonas aeruginosa* and *Mycoplasma spp.*, as well as anaerobic Gram-positive and Gram-negative bacteria. Recently appearing FQs are even more effective because they are designed to meet the specific needs of human and veterinary therapy [14]. FQ is currently one of the most widely used antimicrobials with a wide range of indications, including prophylactic in immunocompromised patients [15]. These are chemically modified ciprofloxacin and norfloxacin [16], fluoroquinolone-1,2,4-triazoles [17], a number of fluoroquinolone-azole hybrids [18], various quinolone hybrids [19], dithienylethene-bridged fluoroquinolone derivatives [20], lipophilic fluoroquinolone derivatives [21], N-thioacylated ciprofloxacin derivatives [22], analogs of fluoroquinolones annulated with 6-substituted-2-aminobenzothiazoles [23] and others.

Our study presents *in silico* and *in vitro* results of studying trifluoro acylated quinolones as promising antibacterial agents against antibiotic-resistant strains of *E. coli*, *A. baumannii*, and *S. aureus*.

2. Materials and Methods

2.1. Chemistry.

The solvents were purified according to the standard procedures. Melting points were measured in open capillary tubes and were given uncorrected. ^{1} H, and ^{19} F{H} NMR spectra were recorded on a Bruker 170 Avance 500 spectrometer and a Varian Unity Plus 400 spectrometer. NMR chemical shifts were reported in ppm (δ scale) using TMS and CCl_3F as internal standards, respectively. LCMS experiments were carried out on an Agilent LC/MSD SL 1100 instrument (atmospheric pressure electrospray ionization (ES-API)).

2.1.1. The general procedure for the synthesis of enaminones **IIa-f**.

To a stirred solution of amino acid (5 mmol) in 1N NaOH (5ml), *β*-ethoxyvinyl trifluoromethyl ketone (5 mmol) was added, and at room temperature, the reaction mixture was stirred overnight. The solution was acidified using 6N HCl to pH = 3 and extracted with MTBE (2 x 10 ml). The organic layer was dried over Na_2SO_4 and evaporated under reduced pressure to give corresponding enaminones, which were used in the next stage without further purification.

2.1.2. (E)-5-fluoro-2-(methyl(4,4,4-trifluoro-3-oxobut-1-en-1-yl)amino)benzoic acid (**IIa**).

A yellow solid. Yield = 55 %. M.p. 146 - 148°C. ^{1}H NMR (400 MHz, DMSO-d6), δ, ppm: 8.23 (d, *J* = 11.5 Hz, 0.45H), 7.79 – 7.52 (m, 3,55H), 5.65 (d, *J* = 14.0 Hz, 0.55H), 4.66 (d, *J* = 14.1 Hz, 0.45H), 3.53 (s, 1.35H), 3.29 (s, 1.65H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -76.8 (s, CF_3), -113.1 (s, F).

2.1.3. (E)-3-fluoro-2-(methyl(4,4,4-trifluoro-3-oxobut-1-en-1-yl)amino)benzoic acid (**IIb**).

A yellow solid. Yield = 80 %. M.p. 144 - 145°C. 1H NMR (400 MHz, DMSO-d6), δ, ppm: 13.62 (br. s, 1H), 8.30 (d, *J* = 12.4 Hz, 0.45H), 7.88 – 7.53 (m, 3,55H), 5.70 (d, *J* = 12.5 Hz, 0.55H), 4.66 (d, *J* = 12.5 Hz, 0.45H), 3.52 (s, 1.35H), 3.27 (s, 1.65H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -76.9 (s, CF_3), -123.2 (s, F).

2.1.4. (E)-5-methyl-2-(methyl(4,4,4-trifluoro-3-oxobut-1-en-1-yl)amino)benzoic acid (**IIc**).

A yellow solid. Yield = 70 %. M.p. 157 - 159°C. ^{1}H NMR (400 MHz, DMSO-d6), δ, ppm: 13.26 (br. s, 1H), 8.21 (d, *J* = 15.3 Hz, 0.4H), 7.82 – 7.32 (m, 3,6H), 5.62 (d, *J* = 12.6 Hz, 0.6H), 4.66 (d, *J* = 12.8 Hz, 0.4H), 3.51 (s, 1.2H), 3.27 (s, 1.8H), 2.39 (s, 1.2H), 2.36 (s, 1.8H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -76.7 (s, CF_3), -77.4 (s, CF_3).

2.1.5. (E)-6-chloro-2-(methyl(4,4,4-trifluoro-3-oxobut-1-en-1-yl)amino)benzoic acid (**IId**).

An orange solid. Yield = 52 %. M.p. 151 - 153°C. ^{1}H NMR (400 MHz, DMSO-d6), δ, ppm: 11.96 (br. s, 1H), 8.23 (d, *J* = 12.8 Hz, 0.35H), 7.83 (d, *J* = 12.5 Hz, 0.65H) 7.71 – 7.42 (m, 3H), 5.71 (d, *J* = 12.5 Hz, 0.65H), 4.75 (d, *J* = 12.5 Hz, 0.35H), 3.49 (s, 1.05H), 3.31 (s, 1.95H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -76.9 (s, CF_3), -77.5 (s, CF_3).

2.1.6. (E)-3-methyl-2-(methyl(4,4,4-trifluoro-3-oxobut-1-en-1-yl)amino)benzoic acid (**IIe**).

A yellow solid. Yield = 81 %. M.p. 137 - 138°C. ^{1}H NMR (400 MHz, DMSO-d6), δ, ppm: 8.28 – 7.43 (m, 4H), 5.63 (d, *J* = 12.4 Hz, 0.35H), 4.49 (d, *J* = 12.2 Hz, 0.65H), 3.49 (s, 1.95H), 3.24 (s, 1.05H), 2.20 (s, 1.05H), 1.91 (s, 1.95H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -76.7 (s, CF_3), -77.2 (s, CF_3).

2.1.7. (E)-2-(ethyl(4,4,4-trifluoro-3-oxobut-1-en-1-yl)amino)-3-fluorobenzoic acid (**IIf**).

A yellow solid. Yield = 75 %. M.p. 127 - 128°C. ^{1}H NMR (400 MHz, DMSO-d6), δ, ppm: 13.62 (br. s, 1H), 8.31 (d, *J* = 12.3 Hz, 0.6H), 7.90 – 7.54 (m, 3.4H), 5.76 (d, *J* = 12.1 Hz, 0.4H), 4.68 (d, *J* = 12.2 Hz, 0.6H), 4.04 – 3.91 (m, 0.8H), 3.76 – 3.66 (m, 1.2H), 1.15 – 1.02 (m, 3H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -76.7 (br. s, CF_3), -120.8 (s, F), -122.1 (s, F).

To a stirred solution of enaminone (3.5 mmol) in chloroform (10 ml), 1 drop of DMF and thionyl chloride (10.5 mmol, 3 eq.) was added dropwise. The obtained solution was slowly heated to 60°C and stirred for 4 hours at this temperature. The solvent was evaporated under reduced pressure once it had cooled to room temperature, and there was antibacterial activity against Gram-positive. The residue was redissolved in MTBE and washed with Na2CO3 5% aqueous solution. The organic layer was dried over Na2SO4 and evaporated under reduced pressure to give a viscous oil that was recrystallized from a mixture of hexane and chloroform (10:1) to obtain corresponding quinolones **1-3** and **5-7**, respectively.

2.1.8. 6-Fluoro-1-methyl-3-(trifluoroacetyl)quinolin-4(1H)-one (**1**).

A brown solid. Yield - 60%. M.p. 150 - 152°C. ^{1}HNMR (400 MHz, DMSO-d6), δ, ppm: 8.48 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.92 – 7.84 (m, 1H), 7.78 – 7.73 (m, 1H), 3.99 (s, 3H). ^{19}FNMR (376 MHz, DMSO-d6), δ, ppm: -75.1 (s, CF_3), -116.4 (s, F). LC-MS (ES-API), m/z: 292.0 $[M+H_2O]^+$.

2.1.9. 8-Fluoro-1-methyl-3-(trifluoroacetyl)quinolin-4(1H)-one (**2**).

A grey solid. Yield - 85%.M.p. 170 - 172°C. ^{1}HNMR (500 MHz, DMSO-d6), δ, ppm: 8.37 (s, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 7.70 (ddd, *J* = 14.6, 7.9, 1.4 Hz, 1H), 7.52 – 7.45 (m, 1H), 4.14 (d, *J* = 8.7 Hz, 3H). ^{19}FNMR (376 MHz, DMSO-d6), δ, ppm: -74.4 (s, CF_3), -120.0 (s, F). LC-MS (ES-API), m/z: 292.0 $[M+H_2O]^+$.

2.1.10. 1,6-Dimethyl-3-(trifluoroacetyl)quinolin-4(1H)-one (**3**).

A brown solid. Yield - 78%.M.p. 159 -161°C. ^{1}HNMR (400 MHz, DMSO-d6), δ, ppm: 8.47 (s, 1H), 8.06 (s, 1H), 7.71-7.59 (m, 2H), 3.98 (s, 3H), 2.46 (s, 3H). ^{19}FNMR (376 MHz, DMSO-d6), δ, ppm: -74.2 (s, CF_3).LC-MS (ES-API), m/z: 288.0 $[M+H_2O]^+$.

2.1.11. 5-Chloro-1-methyl-3-(trifluoroacetyl)quinolin-4(1H)-one (**5**).

A light orange solid. Yield - 62%.M.p. 176 - 178°C. ^{1}HNMR (400 MHz, DMSO-d6), δ, ppm:8.40 (s, 1H), 7.83 – 7.45 (m, 3H), 3.92 (s, 3H). ^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -73.9 (s, CF3). LC-MS (ES-API), m/z: 308.0 and 310.0 $[M+H_2O]^+$.

2.1.12. 1,8-Dimethyl-3-(trifluoroacetyl)quinolin-4(1H)-one (**6**).

A brown solid. Yield - 75%.M.p. 152 - 154°C. ^{1}HNMR (400 MHz, DMSO-d6), δ, ppm: 8.23 (s, 1H), 8.19 – 8.15 (m, 1H), 7.64 (d, *J* = 7.3 Hz, 1H), 7.47 – 7.36 (m, 1H), 4.21(s, 3H), 2.81 (s, 3H).^{19}F NMR (376 MHz, DMSO-d6), δ, ppm: -74.4 (s, CF3). LC-MS (ES-API), m/z: 288.0 $[M+H_2O]^+$.

2.1.13. 1-Ethyl-8-fluoro-3-(trifluoroacetyl)quinolin-4(1H)-one (**7**).

A dark brown solid. Yield - 78%.M.p.156 - 158°C. ^{1}HNMR (400 MHz, DMSO-d6), δ, ppm: 8.43 (s, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.74 (ddd, *J* = 15.0, 7.9, 1.5 Hz, 1H), 7.50 (td, *J* = 8.0, 4.3 Hz, 1H), 4.56 – 4.48 (m, 2H), 1.39 (t, *J* = 7.0 Hz, 3H). ^{19}FNMR (376 MHz, DMSO-d6), δ, ppm: -75.1 (s, CF_3), 122.5 (s, F). LC-MS (ES-API), m/z: 306.0 $[M+H_2O]^+$.

2.2. Biology.

2.2.1. Antibacterial activity assessment.

Synthesized trifluoroacetyl-substituted quinolones were evaluated for their *in vitro* antibacterial activity against Gram-negative *E. coli* ATCC 25922 and *E. coli* MDR isolate, *A. baumannii* MDR isolates 871, 1536 and 1355, and Gram-positive *S. aureus* ATCC 25923 and *S. aureus* MDR isolate strains by disc diffusion method [24] using Mueller-Hinton agar. All bacterial strains were obtained from the culture collection of the Shupyk National Healthcare University of Ukraine. Bacterial suspensions prepared from fresh oblique cultures in a sterile salt solution were adjusted to 0.5 McFarland (1×10^5 CFU/mL). Each compound dissolved in 1% dimethyl sulfoxide solution (DMSO) was tested at content on the disk of 5.0 µM. Using sterile standard paper disks (6 mm diameter), 0.02 mL of the tested compounds was performed in the agar plates. The diameter inhibition zone (mm) was measured after 24 h of incubation at 37°C. The antibiotics of different classes encompassing penicillins (oxacillin, 0.003 µM on disk), cephalosporins (ceftazidime, cefepime, 0.06 µM on disk), polymyxins (colistin, 0.01 µM on disk), and fluoroquinolones (ofloxacin, 0.02 µM on disk) were used to determine the sensitivity of the selected bacterial strains. DMSO was used as the negative control. The tests were performed three times, and the average activity values were calculated.

2.2.2. Acute toxicity assessment.

The acute toxicity of the promising trifluoroacetyl-substituted quinolones to *Daphnia magna* expressed as 48-h LC_{50} values were performed according to the OECD Guideline for

testing of chemicals 202 [25]. The experiments were carried out with *D. magna* neonates (age <24 h) at 16 h light / 8 h dark photoperiod and 23±1°C temperature in a climate chamber. *D. magna* was not fed during the trials. The 50-mL glass beaker with 30 mL of test solution (concentration ranging from 5 to 1000 µM of each 1% trifluoro acylated quinolones) contained six daphnids. The measured effect of LC_{50} was death, rated as immobilization for 15 s at 48 h of exposure. Before experiments, daphnia susceptibility (24-h LC_{50}) was tested with the known toxicant potassium dichromate ($K_2Cr_2O_7$). The LC_{50} values for *D. magna* and the respective 95% confidence intervals (CI) were determined using the Statistica 7 program.

2.3. Docking.

According to the mechanism of antibacterial action of the known quinolone derivatives, compounds **1-3**, **5-7** were studied using a molecular docking approach as possible ligands of bacterial complexes of DNA gyrase and topoisomerase (topo) IV with DNA. The available crystal structures of the enzymes-DNA complexes from *E. coli* (complex of DNA gyrase with DNA and Gepotidacin antibiotic; PDB code is 6RKS [26]), *S. aureus* (complex of DNA gyrase with DNA and imidazopyrazinone derivative; PDB code is 6FQM [27], and *A. baumannii* (complex of topo IV with DNA and moxifloxacin antibiotic; PDB codes are 2XKK [28]) were downloaded from Protein Data Bank (www.rcsb.org) [29]. The ligands, water molecules, and enzyme subunits, which are not used in the calculation, were removed from the files. Three-dimensional structures of compounds were prepared using Marvin Sketch software [30] and optimized in the Avogadro program [31] using MMFF94s force field. The addition of polar hydrogen atoms and Gasteiger charges to the enzyme-DNA complexes was performed by AutoDock Tools software version 1.5.6 [32]. The ligand charges were kept from the mol2 file. The PDBQT files of ligands and enzyme-DNA complexes, which were created by AutoDock Tools software, were used for molecular docking calculations by Autodock Vina software [33]. The grid box size of 20×20×20 points in dimension was applied for all calculations. The grid centers were 57.967, -48.94, 56.906 for the gyrase-DNA complex from *E. coli*; 158.558, 158.332, 147.614 for the gyrase-DNA complex from *S. aureus*; and -16.17, 31.35, -23.34 for enzyme-DNA complex from *A. baumannii*.

3. Results and Discussion

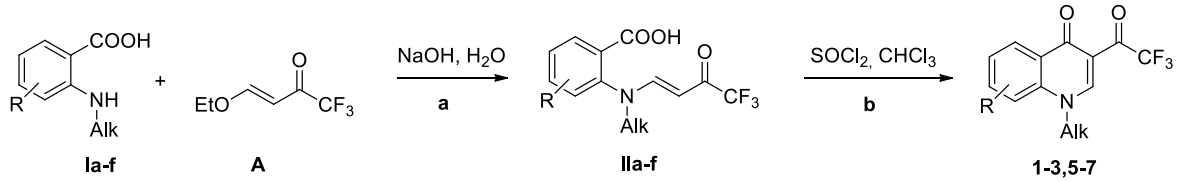
3.1. Prediction of the compound activities using OCHEM services.

In a research study, seven virtual trifluoroacetyl-substituted quinolones were subjected to a preliminary evaluation of their anti-Escherichia coli activity. The prediction was conducted using two consensus QSAR models, which had been previously published [34, 35]. The models were applied to determine the activity level of the quinolones in inhibiting the growth of *E. coli*. The study aimed to assess the potential of the virtual compounds as antibacterial agents and to provide insights into the structure-activity relationship of the quinolones. All compounds were predicted to be active by the classification consensus model [34] with high confidence levels (>90%) and were selected for further studies (Table S1 in Supplementary materials). Then, the activity level for new compounds against *E. coli* was evaluated using a published consensus regression model [35]. Six trifluoroacetyl-substituted quinolones were selected for synthesis and biological testing, as they were predicted to be active with high confidence levels (i.e., compounds with log(1/MIC) > 4.3) (refer to Table S2 in Supplementary materials). Even

though compound **7** was outside the applicability domain of the second model, we still included it in further studies based on predictions made by the consensus classification model.

3.2. Chemistry.

For the obtaining of new trifluoroacetyl-containing quinolones **1-3** and **5-7,** it was used available 2-(alkylamino)benzoic acids (**Ia-f)** and *β*-ethoxyvinyl trifluoromethyl ketone (**A)** at two steps proceeding (Scheme 1).



a: R = 5-F, Alk = Me; b: R = 3-F, Alk = Me;
c: R = 5-Me, Alk = Me; d: R = 6-Cl, Alk = Me;
e: R = 3-Me, Alk = Me; f: R = 3-F, Alk = Et

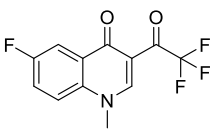
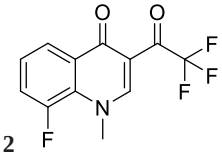
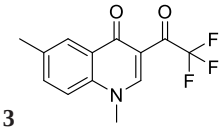
Scheme 1. Synthesis of substituted 1-alkyl-3-(trifluoroacetyl)quinolin-4(1*H*)-ones.

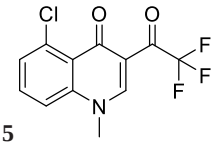
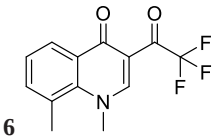
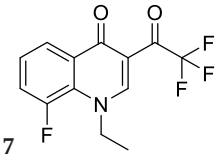
In the first step, aminobenzoic acids **Ia-f** react with enone **A** in water in the presence of sodium hydroxide, and corresponding enaminones **IIa-f** was obtained in yields 52-81% following previously reported procedure [36]. The NMR spectra of **IIa-f** were somewhat complex; at room temperature, in DMSO, the two conformers with *trans*-coupling constant can be observed [37]. Next, after activation of the carboxylic group with thionyl chloride in chloroform, enaminones **IIa-f** undergo intramolecular cyclization, giving target quinolone derivatives, which were purified by crystallization from a mixture of hexane and chloroform (10:1).

3.3. Antibacterial activity evaluation.

The antibacterial activity of synthesized trifluoroacetyl-substituted quinolones against Gram-positive and Gram-negative investigated by the inhibition zone (mm) are given in Table 1.

Table 1. Antibacterial data of trifluoroacetyl-substituted quinolones and referent antibiotics.

Compounds and referent antibiotics	**Gram-positive bacteria**		**Gram-negative bacteria**				
	***S. aureus* ATCC 25923**	***S. aureus* (isolate)**	***E. coli* ATCC 25922**	***E. coli* (isolate)**	***A. baumannii* (isolate 871)**	***A. baumannii* (isolate 1536)**	***A. baumannii* (isolate 725)**
1 	NA	NA	12	NA	10	13	14
 **2**	17	14	24	18	17	14	26
 **3**	10	13	21	17	17	13	22

Compounds and referent antibiotics	Gram-positive bacteria		Gram-negative bacteria				
	***S. aureus* ATCC 25923**	***S. aureus* (isolate)**	***E. coli* ATCC 25922**	***E. coli* (isolate)**	***A. baumannii* (isolate 871)**	***A. baumannii* (isolate 1536)**	***A. baumannii* (isolate 725)**
 **5**	12	NA	17	19	NA	NA	19
 **6**	12	14	13	15	NA	NA	20
 **7**	12	18	17	18	12	10	21
DMSO	NA	NA	NA	NA	NA	NA	NA
Oxacillin	12	NA	NA	NA	NA	NA	NA
Cefepime	23	10	NA	16	10	22	25
Ceftazidime	12	NA	10	NA	NA	15	11
Colistin	NA	NA	NA	NA	NA	NA	NA
Ofloxacin	32	31	33	33	NA	NA	NA

NA – no activity (diameter of inhibition zones < 10 mm).

As shown in Table 1, synthesized trifluoroacetyl-substituted quinolones exhibited a varying degree of antibacterial activity. Trifluoroacethyl-substituted quinolones **2**, **3**, and **7** were found to be effective against all bacterial strains. These compounds exhibited strong antibacterial activity against Gram-negative bacteria with inhibition zone values of 10-26 mm, while moderate antibacterial activity was recorded against Gram-positive bacteria with inhibition zone values of 10-18 mm. The largest inhibition zones were observed against *E. coli* ATCC 25922 (17-24 mm) and *E. coli* isolates (17-19 mm), demonstrated by compounds **2**, **3**, **5**, and **7**. It should also be noted that among Gram-negative bacteria, *A. baumannii* isolate 725 was the most sensitive to trifluoroacetyl-substituted quinolones; the diameters of inhibition zones were in the range of 19-26 mm indicated by compounds **2**, **3**, **5-7**. It can also be noted that trifluoro-acylated quinolones **1-3** and **7** showed appreciable inhibition zones against all *A. baumannii* isolates (ranging from 10-26 mm). DMSO, as a negative control, also displayed no signs of antibacterial activity against all tested bacteria. Figure 1 displayed the antibacterial activity comparative analysis of studied trifluoro acylated quinolones and known antibiotics. The results from Figure 1 showed that studied trifluoroacetyl-substituted quinolones **2**, **3**, and **7** demonstrated significant antibacterial activity compared to reference drugs except for ofloxacin, whose activity was high against *E. coli* and *S. aureus*. In contrast, no activity was registered against *A. baumannii* isolates. Compound **2** displayed the highest antibacterial activity towards the tested strains (Figure 1).

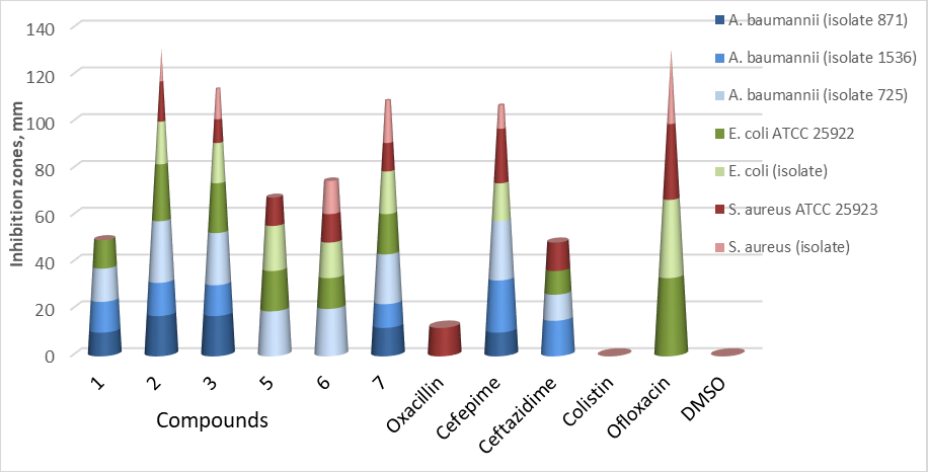


Figure 1. Comparative analysis of the antibacterial activity of trifluoroacetyl-substituted quinolones and referent antibiotics.

3.4. Acute toxicity evaluation.

The acute 48h LC_{50} toxicity concentrations for trifluoroacetyl-substituted quinolones to *D. magna* are presented in Table 2.

Table 2. The acute toxicity (LC_{50}) of five promising trifluoroacetyl-substituted quinolones to *D. magna*.

Compounds	LC_{50} (mg/L)	95% confidence intervals
2	59.28 ± 11.48	35.58-82.98
3	76.23 ± 17.43	39.60-112.85
5	32.29 ± 10.21	11.66-52.91
6	10.22 ± 2.18	6.58-13.84
7	33.39 ± 4.57	23.79-42.99

The acute toxicity results from Table 2 demonstrated that the LC_{50} values of the promising trifluoroacetyl-substituted quinolones towards *D. magna* were in the range of 10.22-76.23 mg/L. 1,6-dimethyl-3-(trifluoroacetyl)quinolin-4(1*H*)-one **3** turned out to be the least toxic of the tested trifluoroacylated quinolones, followed by compounds **2**, **5,** and **7**, and the 1,8-dimethyl-3-(trifluoroacetyl)quinolin-4(1*H*)-one **6** was the most toxic. Nevertheless, according to Passino and Smith's classification method, all tested compounds are categorized as slightly toxic compounds [38].

3.5. Docking.

Quinolones are a large group of widely used broad-spectrum synthetic antibiotics. The quinolone antibiotics are targeted on the bacterial DNA gyrase and topoisomerase (topo) IV. These enzymes, having a specific role in manipulating DNA topology, belong to the type II topoisomerases in bacteria and share considerable amino acid sequence similarity. The function of DNA gyrase is an introduction of the negative supercoils into DNA, while the function of topo IV is the decatenation of the multiply linked daughter chromosomes at the terminal stages of DNA replication. These processes occur through the breaking and rejoining of DNA strands. The quinolone antibiotics are intercalated to the complex of DNA gyrase or topo IV with cleaved DNA and stabilize the formed complex, leading to the accumulation of double-stranded DNA breaks, which are ultimately lethal to the bacterial cell.

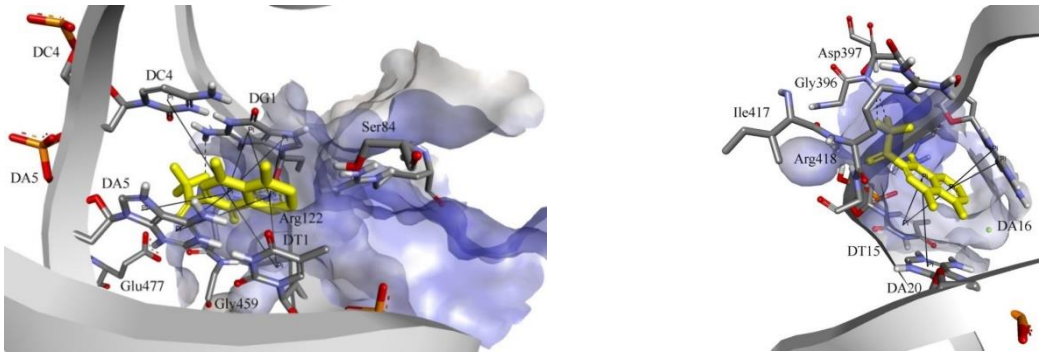
According to the action mechanism of the quinolone antibiotics, the compounds **1-3**, **5-7** were docked by AutoDock Vina software into the complexes of bacterial topoisomerases

(DNA gyrase or topo IV) with DNA. The studied trifluoroacetyl-substituted quinolones were placed in the binding site of co-crystallized ligands, which were intercalated into cleaved DNA strands. The docking energies of the compounds **1-3** and **5-7** are shown in Table 3. As can be seen from Figure S3, the positions of trifluoroacetyl-substituted quinolones **1-3** and **5-7** in their binding modes with complexes of *S. aureus* DNA gyrase with DNA and *A. baumannii* topo IV with DNA are similar. The obtained docking energy scores depend on the nature and position of the substituents at the quinolone scaffold. The binding models of compound **2** with both enzyme-DNA complexes were found to be more energetic preferable. Replacement of a fluorine atom in position 8 of the quinolone scaffold by the methyl group leads to a decrease in the binding affinity of compound **6**. Trifluoroacetyl-substituted quinolone **7** having ethyl group in position 1 of the quinolone scaffold showed worse docking energy by binding to *S. aureus* DNA gyrase with DNA than compound **2** bearing methyl group in this position. This agrees with antibacterial studies (Table 1) showing that compound **2** is a more effective antibacterial agent for *S. aureus* and *A. baumannii* than other studied trifluoroacylated quinolones.

Table 3. Docking energies (kcal/mol) of quinolone **1-3**, **5-7** into the complex of bacterial DNA gyrase and topo IV with DNA.

Compounds	*S. aureus*	*E. coli*	*A. baumannii*
	DNA gyrase with DNA	**DNA gyrase with DNA**	**Topo IV with DNA**
1	-8.9	-6.9	-8.1
2	-9.0	-6.8	-8.3
3	-8.6	-6.8	-8.2
5	-8.7	-6.8	-8.2
6	-8.6	-6.4	-8.0
7	-8.2	-6.7	-8.2

The binding mode of trifluoro acylated quinolone **2** in the ligand binding sites of the bacterial complexes of topoisomerases with DNA is shown in Figure 2. Both in the case of complex DNA gyrase with DNA from *S. aureus* and the case of complex topo IV with DNA from *A. baumannii*, the 4-quinolone scaffold of compound **2** is intercalated into an enzyme-cleaved DNA. Therefore, the position of trifluoro acylated many π-stacking interactions with DNA nucleotides stabilize quinolone 2. As can be seen from Figure 2, the trifluoroacetyl group of the ligand can have hydrogen and halogen bonds with nucleotides of DNA or amino acid residues of the enzyme.



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

Figure 2. Possible binding modes of trifluoro acylated quinolone **2** in the ligand binding sites of the bacterial complexes of (**a**) DNA gyrase with DNA; (b) topo IV with DNA.

Modern *in silico* methods of activity prediction and establishment of molecular mechanisms of action with biological cell targets make a key contribution to designing new bioactive compounds. In the current work, trifluoroacetyl-substituted quinolones were investigated using the previously published classification [34] and regression models [35] created with different machine-learning methods and descriptors sets integrated with the Online Chemical Database with Modeling Environment (OCHEM) [39]. On the base QSAR modeling, a series of trifluoroacetyl-substituted quinolones predicted to be the most active against *E. coli* were synthesized. The antibacterial activity of the studied compounds was assessed using *in vitro* assays. Experimental studies confirmed that compounds **2**, **3,** and **7** showed antibacterial activity against *S. aureus*, *E. coli*, and *A. baumannii* MDR clinical isolates. It should be mentioned that a series of quinoline derivatives displayed activity against *E. coli*, *S. aureus, P. aeruginosa,* and *K. pneumoniae* and antituberculosis activity [40] and also substituted 4-N-alkylated-2-trifluoromethyl-quinoline analogs were active against sensitive and resistant *M. tuberculosis* strains [41]. Some new trifluoromethyl quinoline-3-carbohydrazide derivatives screened for antibacterial activity against *M. smegmatis* and *P. aeruginosa* showed significant antimicrobial performance [42].

N1 position in the structure of synthesized quinolones is essential for its antimicrobial activity and some of its pharmacokinetic properties. That is why the presence of substituents in the adjacent benzene ring and their location can significantly affect the electronic properties of the nitrogen atom. The presence of substituents of different natures in the quinolone ring also affects the electronic properties of quinolones as π-systems, which determines the affinity to the target and other pharmacological properties—in contrast to compounds **1** and **5**, compounds **2** and **7**, where the fluorine atom is located in position 8, turned out to be significantly more active, which may be due to a greater electron-accepting effect of the fluorine atom on the nitrogen atom and the π-system of ligands.

It is known that substitution with a halogen (fluorine or chlorine) leads to decreased solubility, increased lipophilicity, and increased penetration of the drug through cell membranes. Introducing a halogen atom into the C8 position enhances antimicrobial activity by affecting the target DNA gyrase and topoisomerase IV. Chlorine located in position 8 increases the antibacterial activity against FQN-resistant mutants of *M. smegmatis* and *S. aureus* [43].

4. Conclusions

Based on the developed QSAR models, a series of new trifluoroacetyl-substituted quinolones with potential high predicted antibacterial activity were synthesized and tested *in vitro* and *in vivo*. Some newly synthesized compounds showed considerable broad-spectrum antibacterial activity against Gram-positive *S. aureus*, Gram-negative *A. baumannii* and *E. coli* bacteria, especially against MDR clinical isolates. In general, the results of the antibacterial evaluation of the trifluoroacetyl-substituted quinolones compared with the reference drugs indicated that compounds **2**, **3,** and **7** showed comparable or more potent antibacterial activity towards the reference drugs against all tested bacterial strains. According to Passino and Smith's classification, the in vivo acute toxicity results of the studied trifluoroacetyl-substituted quinolones to hydrobiont *D. magna* showed that all tested compounds are categorized as slightly toxic. The molecular docking studies suggest that trifluoroacetyl-substituted quinolones can target bacterial DNA gyrase or topoisomerase IV.

Funding

This research received no external funding.

Acknowledgments

This research was supported by the National Academy of Sciences of Ukraine under Grants of the NAS of Ukraine to research laboratories/groups of young scientists of the NAS of Ukraine to conduct research in the priority directions of the development of science and technology in 2022-2023 under Contract No. 24/02-2023(4).

Conflicts of Interest

The authors declare no conflict of interest.

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Supplementary Material

Parameters of the Classification models

The previously published classification models [34] were created with five machine-learning methods, including k-Nearest Neighbors (kNNs) [44], WEKA-RF (Random Forest) [45], Associative Neural Networks (ASNNs) [46], Extreme Gradient Boosting (XGBoost) [47] and Least Squares Support Vector Machines (LS-SVM) [48]. The QSAR models were created using several descriptors sets such as E-state indices [49], AlogPS [50] and ChemAxon [51] integrated in the Online Chemical Database and Modeling Environment [39]. A consensus model was built as a simple average of the five models (http://ochem.eu/article/112525 , Figure S1).

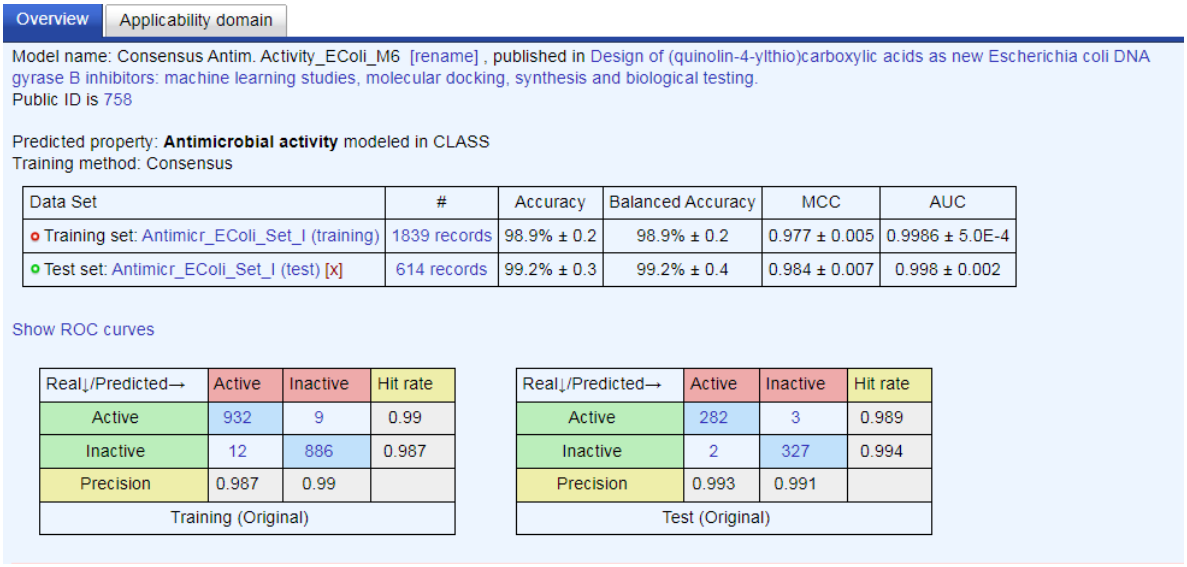


Figure S1. Consensus classification machine learning model built by the OCHEM server. The training and test sets included 1839 and 614 molecules, respectively.

Parameters of the Regression models

The previously published regression models [35] were created with two machine-learning methods, including Message Passing Neural Networks (MPNN) [52] and Random Forest (RFR) [45]. The best RFR model was built by the use of the, E-state indices [49], AlogPS [50], MOLD2 [53] and Inductive descriptors [54]. A consensus model was built as a simple average of the two models (http://ochem.eu/article/159260, Figure S2).

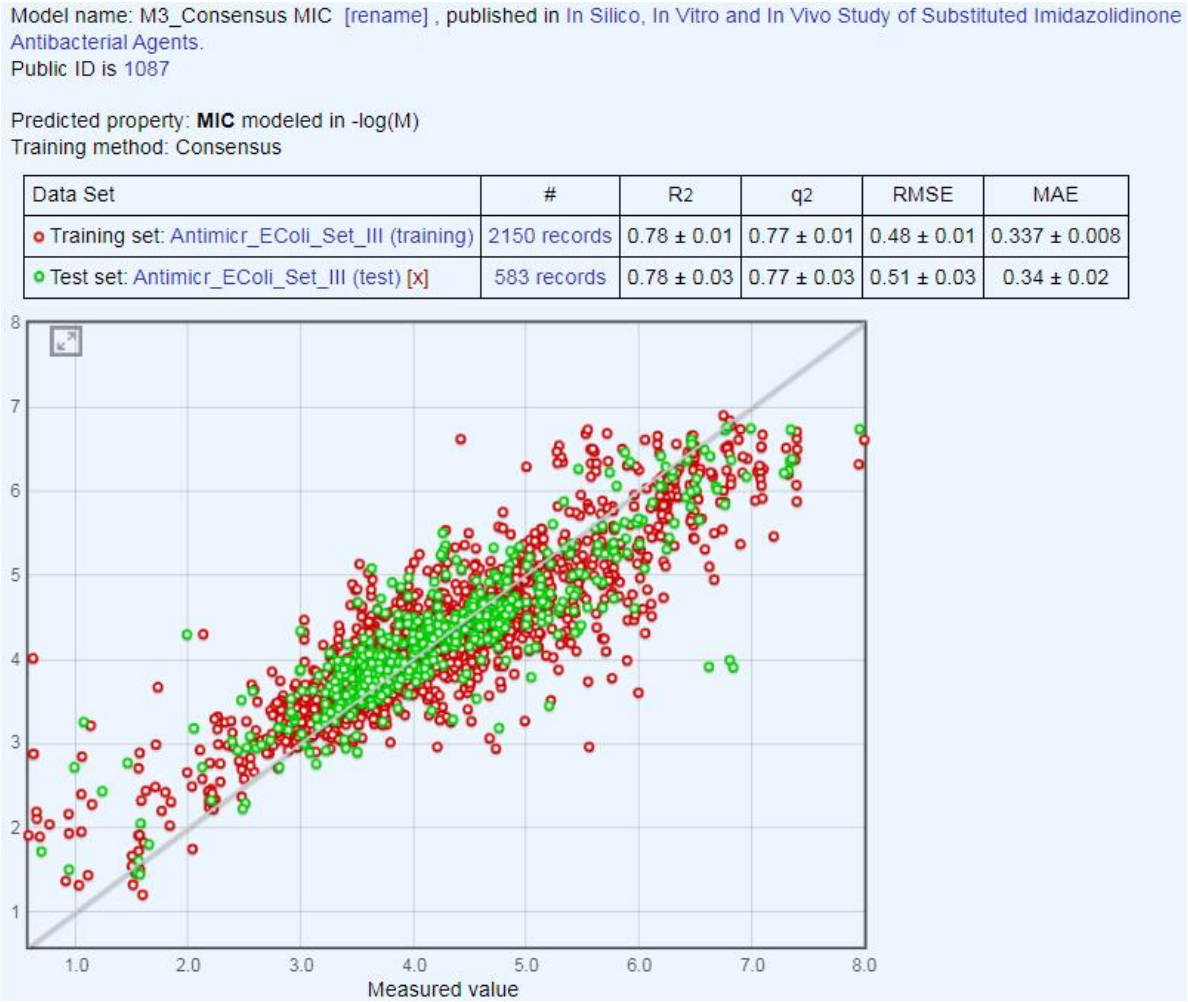
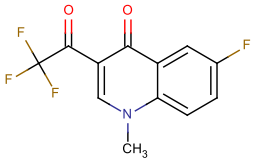
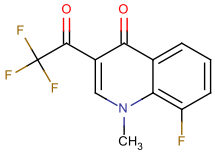
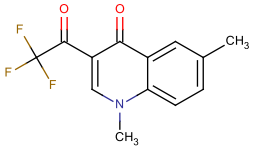
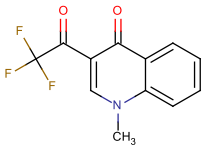
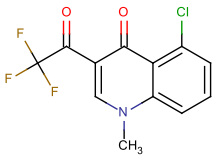
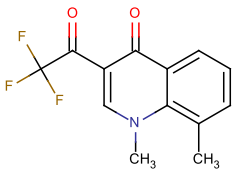
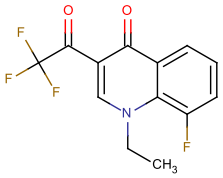


Figure S2. Consensus regression machine learning model built by the OCHEM server. The training and test sets included 2150 and 583 molecules, respectively. The cross-validation results are reported for the training set.

Prediction activity of new compounds

Table S1. Anti-*Escherichia coli* activity calculated by using the consensus classification model for seven virtual compounds.

Compounds	Chemical Structure	Predicted activity	CONSENSUS -STD	Estimated accuracy	AD
1		Active	0.3	0.92	FALSE
2		Active	0.31	0.92	FALSE
3		Active	0.29	0.92	TRUE
4		Active	0.11	0.99	TRUE
5		Active	0.27	0.92	TRUE
6		Active	0.29	0.92	TRUE
7		Active	0.28	0.92	TRUE

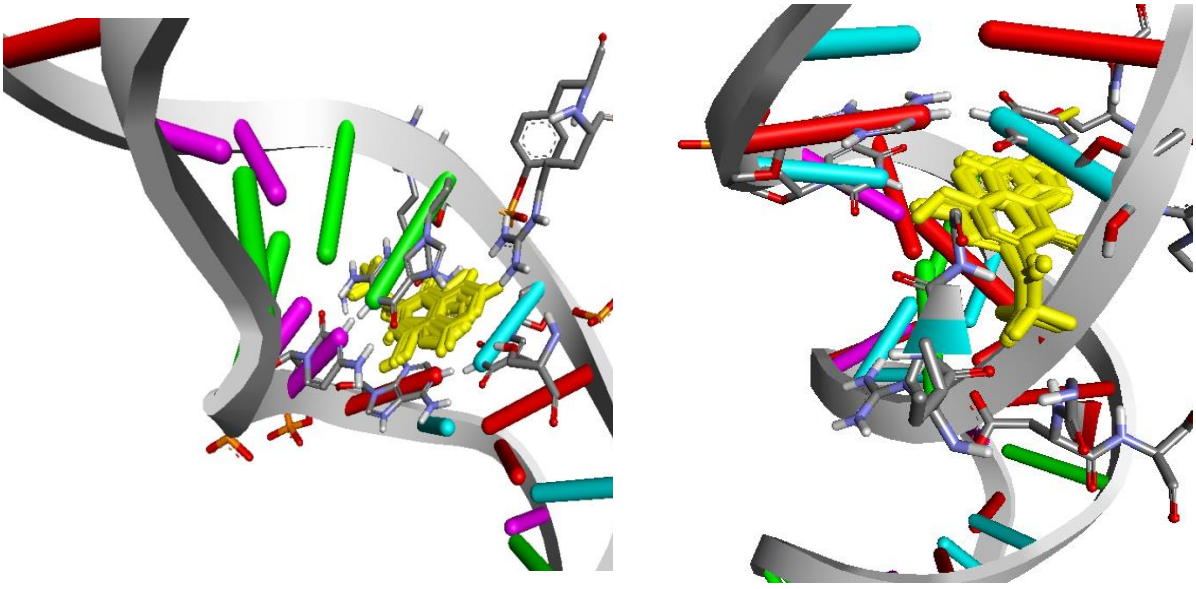
CONSENSUS-STD – the standard deviation of the predictions, obtained from an ensemble of models. AD – applicability domain

Table S2. The activity against *E. coli* calculated by the consensus regression model for the compounds under study.

Compounds	log(1/MIC), M	CONSENSUS STD	Estimated RMSE	AD
1[a]	4.46	0.53	0.53	TRUE
2[a]	4.47	0.54	0.53	TRUE
3[a]	4.39	0.24	0.53	TRUE
4	4.23	0.2	0.53	TRUE
5[a]	4.36	0.38	0.53	TRUE
6[a]	4.46	0.38	0.53	TRUE
7[a]	4.74	0.79	0.53	FALSE

[a]Final set compounds are represented in bold. MIC – minimal inhibitory concentration; CONSENSUS-STD – the standard deviation of the predictions, obtained from an ensemble of models; RMSE is the root mean square error; AD – applicability domain.

Superposition of trifluoroacylated quinolones



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

Figure S3. Possible binding modes of trifluoroacylated quinolones **1**-**3** and **5**-**7** in the ligand binding sites of the bacterial complexes of DNA gyrase with DNA (**a**) and topo IV with DNA (**b**).

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