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Membrane active 7-thiazoxime quinolones as novel DNA binding agents to decrease the genes expression and exert potent anti-methicillin-resistant *Staphylococcus aureus* activity

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ABSTRACT

A novel class of 7-thiazoxime quinolones was developed as potential antimicrobial agents for the sake of bypassing resistance of quinolones. Biological assays revealed that some constructed 7-thiazoxime quinolones possessed effective antibacterial efficiency. Methyl acetate oxime derivative **6l** exhibited 32-fold more active than ciprofloxacin against MRSA, which also possessed rapidly bactericidal ability and low toxicity towards mammalian cells. The combination use of 7-thiazoxime quinolone **6l** and ciprofloxacin was able to improve antibacterial potency and effectively alleviate bacterial resistance. The preliminarily mechanism exploration revealed that compound **6l** could destroy the cell membrane and insert into MRSA DNA to bind with DNA gyrase, then decrease the expression of gyrB and femB genes. The above results strongly suggested that methyl acetate oxime derivative **6l** held a promise for combating MRSA infection.

1. Introduction

Five-membered heteroaromatic thiazole has enormously contributed to drug applications [1,2], especially in antimicrobial field [3,4]. As the continuous severity of antibiotic resistance [5,6], thiazole-based clinical drugs such as cefuzonam, cefoselis, cefmenoxime and abafungin have triggered extensive research to develop more bioactive molecules. Introducing thiazole into the existing drugs is one of the most widespread methods to overcome resistance [7]. For instance, the cephalosporins cefdinir and tigemonam possess excellent inhibitory efficacy to bacterial strains including resistant ones. Our previous work revealed that the thiazole-modified derivatives at the 3-carboxyl group of quinolone skeleton (Leads **A**, **B** and **C**, Fig. 1) not only exhibited broad antimicrobial spectrum, but also effectively retarded the development of resistance [8,9]. These aforesaid stimulating properties arouse the great enthusiasm in the further development of potential thiazolyl quinolone antibacterial agents.

Quinolones are one of the most commonly used prescription drugs to treat various infections [10,11]. In particular, the clinical levofloxacin, norfloxacin and ciprofloxacin have made great dedications for human health. These drugs access the bacteria *via* active transport of cell membrane and primarily target topoisomerase or gyrase to stabilize the cleavage complex, thus blocking DNA replication [12]. However, mutations in active sites of DNA gyrase such as gyrA and gyrB genes [13], changes in the membrane permeability caused by the adjustment of the pathogen defense system and shifts of the structure and quantity of the outer membrane proteins all make the evolution and widespread distribution of resistant strains [14]. The structural modification of quinolone skeleton at the C-7 position has been proved to be a promising strategy to circumvent antibiotic resistance mechanisms and retain inhibitory potency against multidrug-resistant bacteria [15]. Much work has shown that introducing azoles [16], such as imidazole [17], triazole [18] and tetrazole [19] at C-7 site of quinolone drugs afforded promising molecules. Nevertheless, few exemplifications on 7-thiazolylquinolones are described as potent antibacterial agents in the literature [20]. Therefore, we have overwhelming interest in incorporating thiazole into the C-7 position of quinolones with the expectance to discovery potential new antibacterial molecules.

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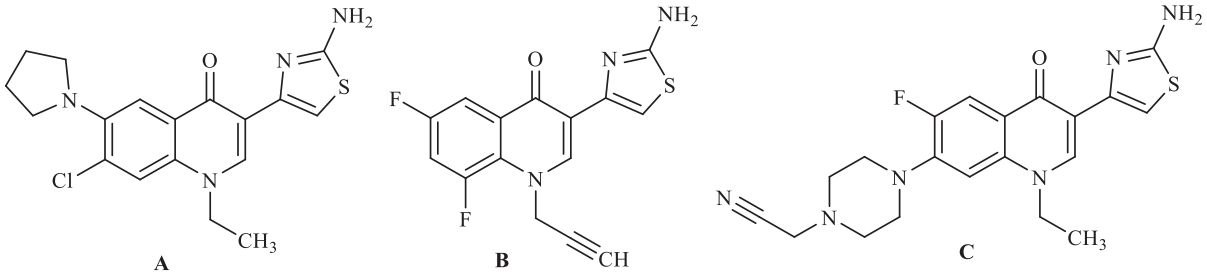


Fig. 1. Previously published biologically active 3-(thiazolyl) quinolones (Leads **A**, **B** and **C**).

Oxime characterized with electron accepting C═N group and donating -O- moiety is a favorable fragment in antimicrobial drugs [21]. For instance, the fourth-generation quinolone drug gemifloxacin and the fifth-generation cephalosporin ceftaroline fosamil with oxime modification exhibited excellent antibacterial potentiality to resistant bacteria [22], which further pointed the way to conduct new antibacterial agents with amendatory therapeutic indexes. Therefore, the introduction of oxime groups into thiazolyl quinolones is an attractive topic.

Considering all the aforementioned facts and continuing our prior works on the thiazolyl quinolones, herein thiazoxime was introduced into the 7-position of quinolone backbone to afford a class of novel 7-thiazoxime quinolones with large promise as antibacterial agents (Fig. 2). All the synthesized 7-thiazoxime quinolones were evaluated for their antibacterial ability. Further studies including drug resistance, time-killing kinetic, drug combination and cytotoxicity assays were implemented to assess the potential of high active molecule as new antibacterial drug. The possible antibacterial mechanism was explored through bacterial membrane disruption assay, interactions with MRSA DNA, molecular docking and the expression of resistance-related genes.

2. Results and discussion

2.1. Chemistry

The intermediate thiazolyl oximes and target 7-thiazoxime quinolones were prepared according to the synthetic process illustrated in Schemes 1 and 2. Commercially available starting material 2-acetothiazole **1** was reacted with bromine in glacial acetic acid system to get thiazole bromide **2** with satisfactory yield. Bromide **2** was selected to construct the 7-thiazolylquinolones **5a**–**b**, in which the norfloxacin **4a** and ciprofloxacin **4b** participated in the reaction. However, the reaction of 7-thiazolylquinolones **5a**–**b** with hydroxylamine hydrochlorides was difficult to produce 7-thiazoxime quinolones, in spite of using different catalysts such as anhydrous sodium acetate, sodium carbonate, piperidine, pyridine, and different solvents such as methanol and ethanol. This might be the consequence that the carboxyl group had undesirable effect on the active amine. After screening the reaction conditions, oximes **3a**–**f** were given by the condensation of compound **2** with various hydroxylamine hydrochlorides under the catalysis of anhydrous sodium acetate at room

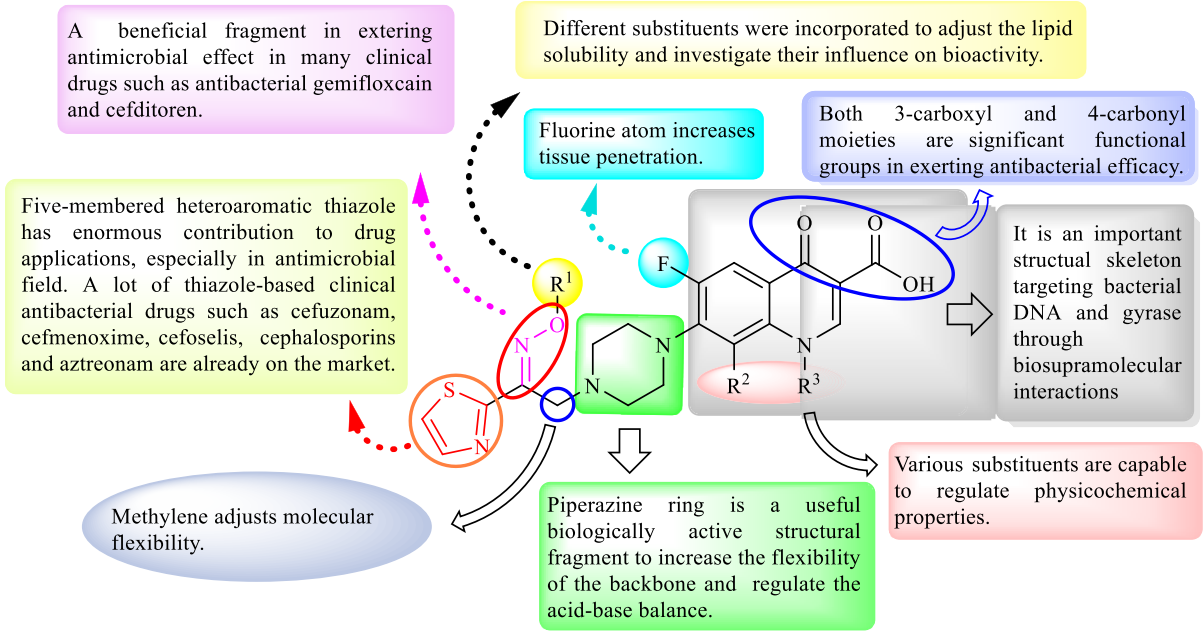
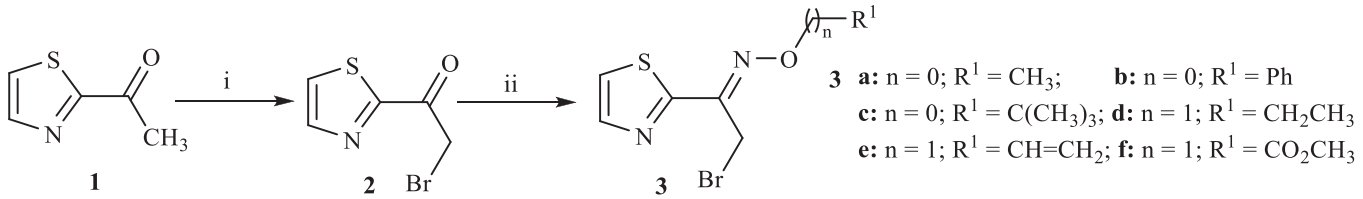
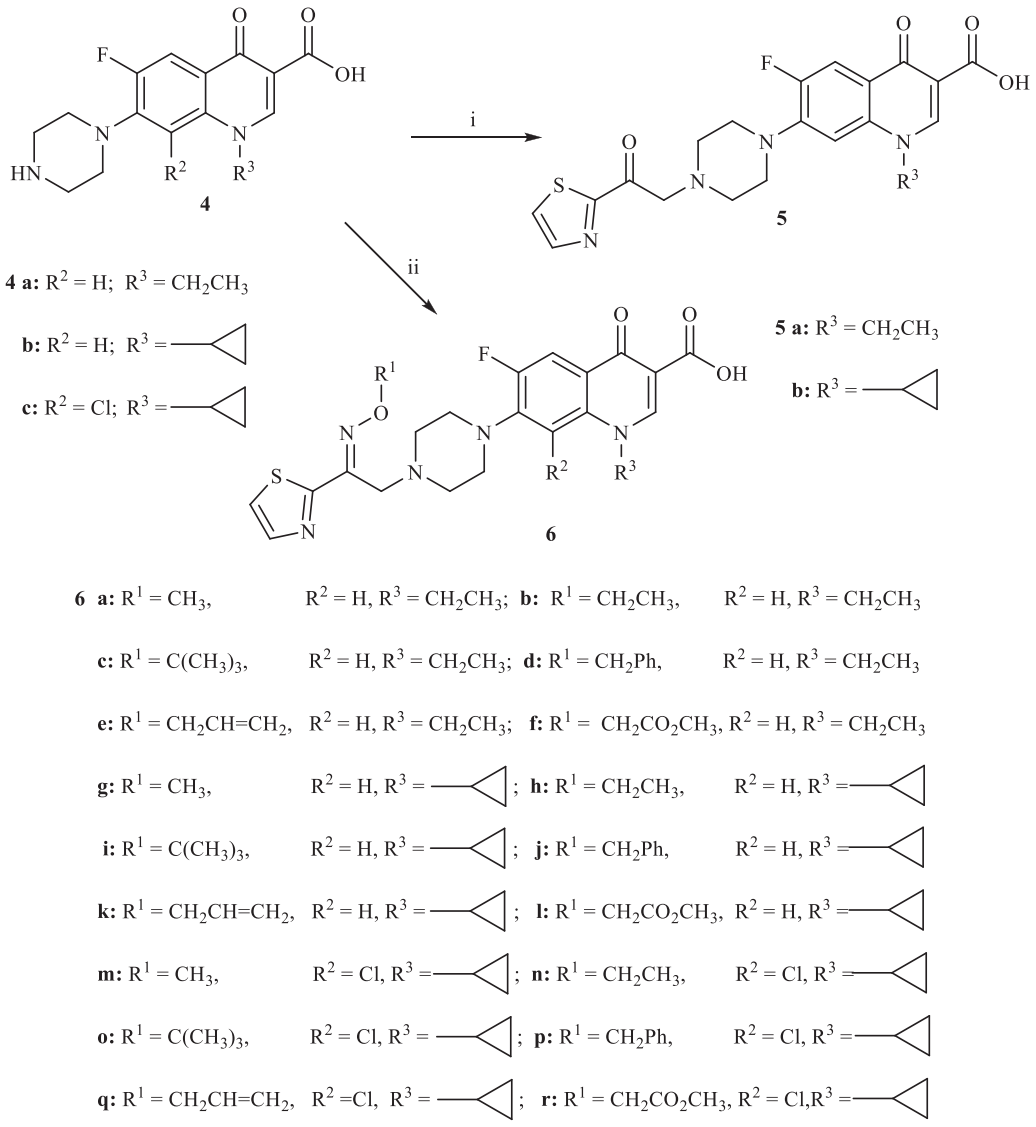


Fig. 2. Design of novel 7-thiazoxime quinolones.



Scheme 1. Synthetic scheme of thiazolyl oximes.



Scheme 2. Synthetic scheme of 7-thiazoxime quinolones.

temperature using methanol as solvent. The quinolones **4a**–**b** underwent nucleophilic substitution with thiazolyl oximes **3a**–**f** to afford norfloxacin and ciprofloxacin derivatives **6a**–**f**. The modification of ciprofloxacin analogue **4c** was conducted to produce the 8-chloroquinolones **6m**–**r** in 51.4–90.7% yield, with the purpose to investigate the effect of C-8 substitution on the growth inhibition of microorganisms.

In general, oxime has two configurations theoretically, *Z* and *E*. The prepared target products were cultured for single crystals to verify the configuration of the 7-thiazoxime quinolones. Fortunately, the single crystal of benzyl oxime **6p** was obtained and X-ray diffraction proved that the oxime geometry showed *E* configuration (Fig. 3). This might be the reason that the *Z* configuration in the 7-thiazoxime quinolones is usually unstable and can easily converted to the *E* configuration.

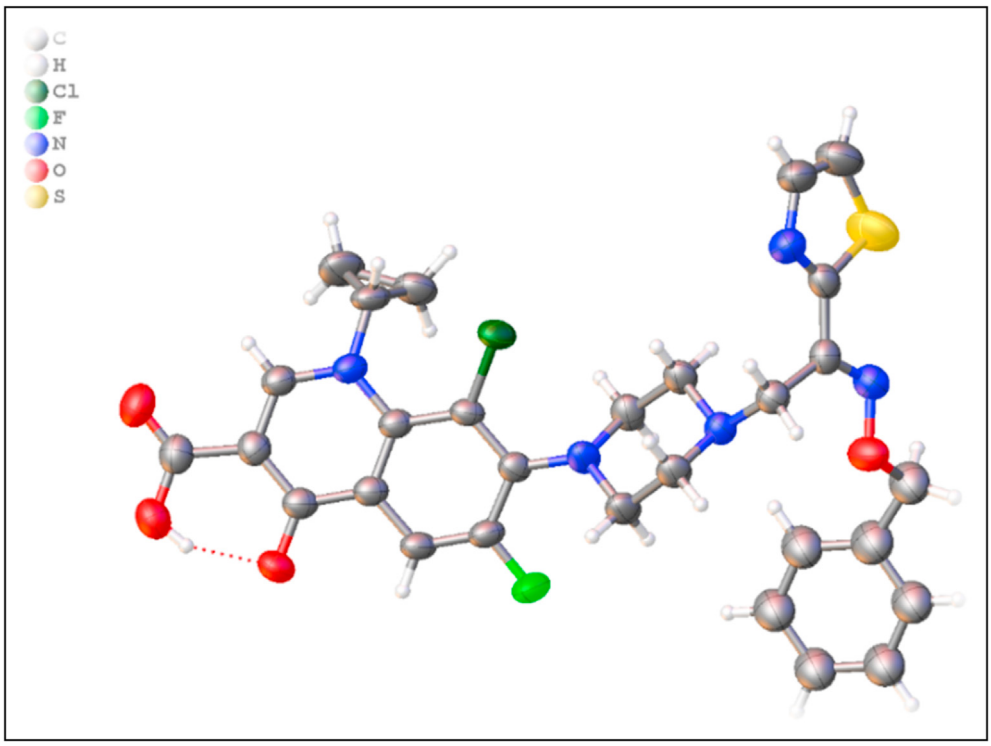


Fig. 3. Crystal structure of 7-thiazoxime ciprofloxacin **6p**.

2.2. Antibacterial evaluation

Antibacterial evaluation of the thiazolyl quinolones were carried out by the well-known two-fold micro-dilution standard assay [23]. Norfloxacin and ciprofloxacin were utilized as reference agents. Most of the newly developed 7-thiazoxime quinolones could suppress the growth of the tested bacteria effectively. The minimum inhibitory concentration (MIC) values were used to represent bioactivities depicted in Table 1–4.

Thiazolyl norfloxacin molecules **5a** and **6a**–**f** displayed good antibacterial activities against the tested stains in Tables 1 and 2. The acetylthiazolyl norfloxacin **5a** showed low MIC values of 1 and 4 μg/mL respectively against standard *Staphylococcus aureus* ATCC 29213 and drug-resistant MRSA, being 16 and 4 folds more active in comparison to the norfloxacin with MIC value of 16 μg/mL. Furthermore, the *Enterococcus faecalis* and *Pseudomonas aeruginosa* ATCC 27853 were also sensitive to acetylthiazolyl norfloxacin **5a**. The antibacterial behavior manifested that the thiazole modified norfloxacin derivatives gave satisfactory antibacterial potencies, especially towards the Gram-positive bacteria. To investigate the impact of oxime fragments on the biological activity of this class of compounds, the alkyl oximes **6a**–**b** were prepared. Methyl oxime **6a** and ethyl derivative **6b** with MIC values of 0.25 μg/mL efficiently suppressed the growth of MRSA, being 64-fold more potent in comparison to norfloxacin (MIC = 16 μg/mL). Towards *Staphylococcus aureus*, *Enterococcus faecalis* and *Acinetobacter baumannii*, the extension of alkyl chain was not conducive to improve the antibacterial potentiality. In order to investigate the influence of steric hindrance, *tert*-butyl oxime **6c** was prepared. The antibacterial activity of compound **6c** decreased in contrast to oximes **6a**–**b**. Especially, Gram-negative bacteria was more insensitive to molecule **6c** obviously (Table 2). These results verified that large steric hindrance substituents were unfavorable for antibacterial ability. To enrich the structure-activity relationship (SAR), unsaturated substituted oximes **6e**–**f** were developed. The antimicrobial activities of allyl oxime **6e** and methyl acetate oxime **6f** conspicuously strengthened in comparison with alkyl analogues **6a**–**c**. The results revealed that introducing unsaturated groups enhanced antimicrobial activities, which might be related to the increase in electron cloud density. With the aim to enrich the chemical diversity, benzyl oxime **6d** was designed and synthesized. The ability of compound **6d** to inhibit bacterial growth was stronger than alkyl oximes **6a**–**c**, suggesting that the electron cloud density had a more significant effect on the antibacterial efficacies. All 7-thiazoxime norfloxacin

Table 1
Antibacterial data of 7-thiazolyl norfloxacin derivatives **5a** and **6a**–**f** against Gram-positive bacteria.

Compds	MIC (μg/mL)				
	MRSA	*Staphylococcus aureus*	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis*
5a	4	4	2	1	2
6a	0.25	2	0.5	0.25	4
6b	0.25	8	0.5	4	16
6c	0.25	0.25	1	16	0.25
6d	0.25	4	0.25	256	0.25
6e	0.25	0.25	0.5	2	1
6f	0.25	4	0.25	2	0.25
Norfloxacin	16	4	2	2	4

Table 2
Antibacterial data of 7-thiazolyl norfloxacin derivatives **5a** and **6a**–**f** against Gram-negative bacteria.

Compds	MIC (μg/mL)					
	Klebsiella pneumonia	Acinetobacter baumannii	*Escherichia coli*	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* ATCC 27853
5a	32	16	32	64	4	2
6a	256	0.25	0.25	256	0.25	8
6b	256	2	0.25	256	0.25	8
6c	1	16	1	1	2	1
6d	128	1	0.5	64	0.5	1
6e	256	0.25	0.25	0.5	0.25	4
6f	256	1	32	0.25	0.25	0.25
Norfloxacin	4	2	16	16	2	8

molecules **6a**–**f** (MIC = 0.25 μg/mL) were 64-fold more active against MRSA in contrast with reference drug norfloxacin (MIC = 16 μg/mL), which suggested that the modified norfloxacin derivatives were advantageous to antibacterial potencies, especially for resistant strains.

With the aim to investigate the effectiveness of thiazoleoximes on the antibacterial activity of other quinolones, ciprofloxacin as a synthetic third-generation quinolone antibacterial drug was selected to optimize. Structural modifications showed that the SAR of thiazolyl ciprofloxacin compounds **6g**–**r** were similar to norfloxacin derivatives, and the overall bacteriostatic efficacies of this series of compounds were more potent than ciprofloxacin in Tables 3 and 4. It was worth noting that the replacement of the C-8 position by a chlorine atom resulted in 8-chlorociprofloxacin derivatives **6m**–**r** with a significant increase in biological activity against Gram-negative bacteria. These results showed that the 8-chloroquinolones **6m**–**r** exhibited strong efficacies (MIC = 0.25–2 μg/mL) against all the evaluated microbes except for *Klebsiella pneumonia*. In particular, all tested strains were sensitive to methyl acetate oxime **61**, especially against MRSA with a MIC value of 0.25 μg/mL. Preliminary analysis showed that the antibacterial activities of quinolone oximes tended to decrease with the increase of Clog P values. The methyl ester compounds **6f**, **6l** and **6r** had the lowest ClogP values and the best antibacterial activity in corresponding series.

Both series of 7-thiazoxime quinolones showed excellent activities against Gram-positive bacteria. Some promising molecules against *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* and MRSA were more clearly emerged in Fig. 4. Notably, the anti-MRSA activity of 7-thiazoxime quinolones remained outside the range of the basis of our previous researches conducive to yielding potent efficacy, which suggested that it might be an atypical mechanism to exert efficacy. All results demonstrated that the design of the 7-thiazoxime quinolones was reasonable.

2.3. Multistep resistance study

The prevalence and spread of MRSA is a huge challenge in addressing bacterial infections and drug development [24]. Therefore, it is necessary to evaluate the effect of 7-thiazoxime quinolones on MRSA resistance. The ciprofloxacin and norfloxacin were used as control to study the MRSA resistance of the highly active 7-thiazoxime quinolones **6a**, **6e**, **6g**, **6l**, **6m**, **6n**, **6o** and **6r**. The experimental results showed that the alkyl oximes **6a**, **6e**, **6g**, **6m**, **6n** and **6o** presented no obvious effect on the treatment of MRSA resistance (Fig. 5A), which indicated that alkyl derivatives and chlorine atom at C-8 were not conducive to overcoming resistance. But the methyl acetate oxime **6l** had a small chance in developing MRSA resistance after 14 passages in contrast to ciprofloxacin. Furthermore, when compound **6l** was combined with ciprofloxacin, no obvious drug resistance was generated after 16 passages in Fig. 5B. The obtained results all supported the difficulty of MRSA resistance development under the treatment of compound **6l** rather than norfloxacin and ciprofloxacin.

2.4. Bactericidal kinetic study

To further evaluate the effect of quinolone methyl acetate oxime **6l** on bacterial growth, the bactericidal kinetic was conducted. MRSA was treated by compound **6l** at different concentrations with clinical antibiotic ciprofloxacin as the positive control (Fig. 6A). The sterilization rate of ciprofloxacin was lower than the oxime derivative **6l** within 6 h at the same concentration (2 μg/mL). In addition, MRSA could be eradicated within 4 h treated by **6l** (4 μg/mL). The

Table 3
Antibacterial data of 7-thiazolyl ciprofloxacin derivatives **5b** and **6g**–**r** against Gram-positive bacteria.

Compds	MIC (μg/mL)				
	MRSA	*Staphylococcus aureus*	*Staphylococcus aureus* ATCC 25,923	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis*
5b	16	1	4	2	8
6g	0.25	0.25	0.5	0.25	0.25
6h	0.25	0.25	0.25	4	0.25
6i	4	4	1	4	2
6j	0.25	0.25	1	0.5	2
6k	0.25	0.25	0.25	32	0.25
6l	0.25	0.25	0.25	0.25	0.25
6m	0.25	0.25	0.5	0.25	0.25
6n	0.25	0.25	0.5	0.25	0.25
6o	0.25	0.25	0.5	0.25	0.25
6p	0.25	2	1	2	0.25
6q	0.25	0.25	0.25	0.5	0.25
6r	0.25	0.25	0.25	0.25	0.25
Ciprofloxacin	8	2	2	0.5	2

Table 4
Antibacterial data of 7-thiazolyl ciprofloxacin derivatives **5b** and **6g**–**r** against Gram-negative bacteria.

Compds	MIC (μg/mL)					
	Klebsiella pneumonia	Acinetobacter baumannii	*Escherichia coli*	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa* ATCC 27853
5b	16	2	4	4	1	1
6g	256	1	0.25	256	0.25	2
6h	0.25	1	2	32	0.25	0.25
6i	64	4	128	1	0.25	0.25
6j	256	8	0.25	256	0.25	1
6k	0.25	0.25	1	0.5	0.25	0.25
6l	0.25	0.25	1	0.25	0.25	0.25
6m	0.25	0.25	0.25	0.25	0.5	0.25
6n	256	0.25	0.25	256	0.25	0.25
6o	256	0.25	4	0.5	0.25	0.25
6p	0.25	0.25	0.25	0.25	1	0.25
6q	256	0.25	0.25	0.25	0.25	0.25
6r	256	4	4	0.5	0.25	0.25
Ciprofloxacin	2	1	0.5	2	0.5	1

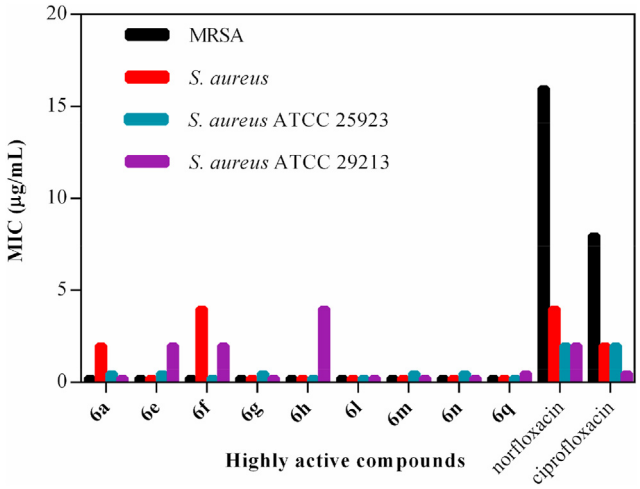


Fig. 4. The diagram for biological activity of the most potent compounds against MRSA, *S. aureus*, *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213.

experiment result showed that the methyl acetate oxime **6l** had a rapid killing effect against MRSA.

2.5. Drug combination study

The adjustments of bacterial defense system lead to the failure of single-target drugs. Combination therapy was applied as a promising way for efficiency improvement, side-effect avoidance and even the combat of drug resistance [25]. Therefore, the combination of active compound **6l** and ciprofloxacin against bacteria was carried out. The results exhibited that the strains treated by the combination drug were more sensitive than their individual use, and their combined action showed synergistic and additional effects (Table 5). From the facts above, the combination of ciprofloxacin and quinolone derivative **6l** hold a significant position in treating the microbial infective diseases.

2.6. Loss of membrane integrity

2.6.1. Membrane depolarization assay

The depolarization effect of the thiazolyl ciprofloxacin derivative **61** on the membrane was carried out with the fluorescent sensitive dye diSC35 [26]. The membrane depolarization was

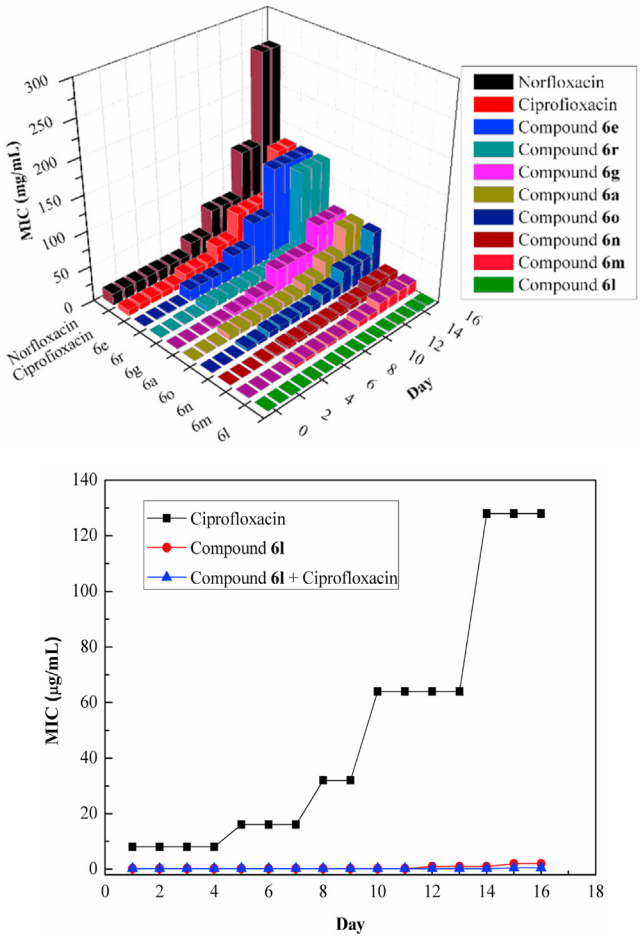


Fig. 5. The resistance development of 7-thiazoxime quinolones **6a**, **6e**, **6g**, **6l**, **6m**, **6n**, **6o** and **6r** against MRSA.

revealed by the use of scale-up of fluorescence. MRSA cells treated by methyl acetate oxime **6l** at MIC concentration showed the increase of fluorescence intensity in a time-dependent rate in comparison to untreated cells, which revealed that compound **6l** displayed obvious dissipation of the MRSA cell membrane potential (Fig. 7).

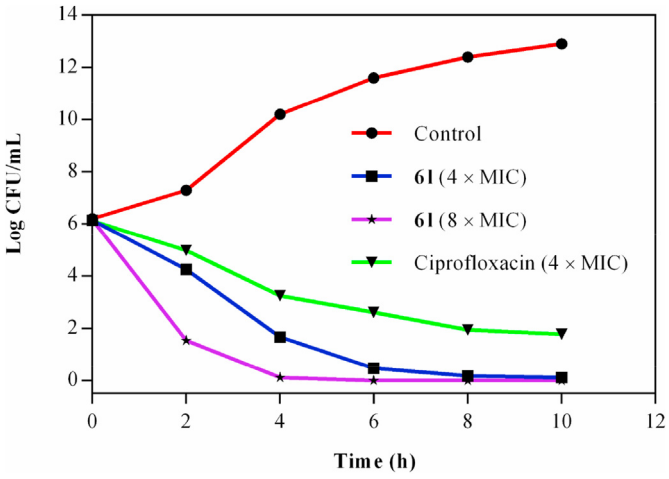


Fig. 6. Time-dependent killing of MRSA by methyl acetate oxime **6l**.

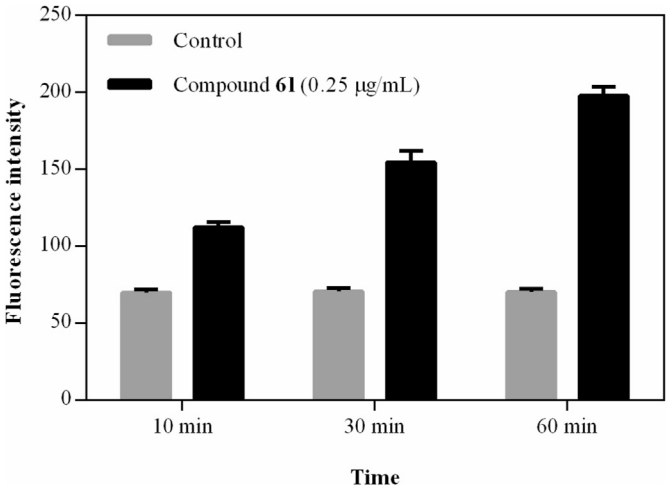


Fig. 7. Cytoplasmic membrane depolarization of MRSA treated by compound **6l**.

2.6.2. Inner membrane permeabilization

Destroying cell membranes could reduce the tendency of bacteria to develop resistance. In order to confirm whether these quinolone oximes acted by disrupting the bacterial cell membrane, the fluorescent probes calcein-AM and propidium iodide (PI) were used to study the destruction of membrane integrity by the methyl acetate oxime **6l** through fluorescence analysis. The PI staining experiments showed that a dose-dependent increase in fluorescence intensity appeared (Fig. 8A), which manifested molecule **6l** could cause extensive membrane damage. As the content of the methyl acetate oxime **61** increased, the fluorescence intensity of calcein-AM decreased significantly in Fig. 8B. These results indicated that the active molecule **6l** was capable of disrupting the membrane integrity.

2.6.3. Calcein-AM/PI dual stain fluorescence microscopy

Fluorescence micrographs (Fig. 8C and D) clearly demonstrated that the number of red fluorescent cells increased while the green fluorescence gradually decreased, demonstrating that compound **6l** could efficiently destroy the membrane and cause MRSA death. Generally, fluoroquinolones enter cells mainly through active transport of carriers to exert potent activity. The dual-targeting bactericidal effects of these two types of molecules might benefit their synergistic or additivity bactericidal effects. These findings provide an advisable approach to maximize antibacterial efficiency through different modes of action.

2.7. Interactions between the methyl acetate oxime *6l* and MRSA DNA

DNA is an important biological macromolecule considered to be an effective drug target [27]. The methyl acetate oxime **6l** contained hydrogen-donating and -accepting functionalities to deliver promising likelihood to construct favorable hydrogen bond and/or π-π interactions with the binding pocket of DNA in spatial environment. These might display different mechanisms from conventional quinolones to tackle the emergency of pathogenic resistance. Therefore, the exploration of the interaction mode between quinolone compound **61** and DNA isolated from MRSA was carried out by UV and fluorescence spectroscopy.

2.7.1. Influence of DNA configuration

The maximum absorption peak at 260 nm of DNA increased proportionally with the increase of the content of molecule **6l** in Fig. 9. At the same time, the absorption of the simple addition of the measured values of DNA and the methyl acetate oxime **6l** was slightly lesser than the **6l**-DNA complex. This phenomenon might be the result of electrostatic interaction between hybrid **6l** and DNA bases, providing strong evidence for changes in DNA duplexes and the formation of **6l**-DNA complexes. Supporting information (Fig. S1) displayed the equation and plot of binding constant.

2.7.2. Competitive interaction of compound *6l* and probes with MRSA DNA

Aiming to explore the binding mode of the compound and DNA, commercial 4′, 6-diamidino-2-phenylindole (DAPI) and marketable acridine orange (AO), whose action modes with DNA is characterized by intercalation, minor groove binding or electrostatic interactions, were used as spectral probes to explore the mechanism of action, respectively [28]. The fluorescence intensity at 537 nm was significantly weakened with the content of the methyl acetate oxime **6l** in Fig. 10, signifying that the molecule **6l** could interfere DNA-AO complex. On the contrary, the fluorescence intensity of DAPI hardly changed with the increase of compound concentration

Table 5
Combination effects of methyl acetate oxime **6l** with ciprofloxacin (MIC, μg/mL).

Bacteria	Alone		In combination		FIC Index[a]	Effect
	6l	Ciprofloxacin	6l	Ciprofloxacin		
Klebsiella pneumonia	0.25	2	0.0625	0.125	0.3125	Synergy
Escherichia coli	0.25	2	0.0625	0.125	0.3125	Synergy
MRSA	0.25	8	0.125	2	0.5	Synergy
Staphylococcus aureus ATCC 25923	0.25	2	0.25	1	1.5	Additivity

[a] The fractional inhibitory concentration (FIC) index = (MIC of compound A combined/MIC of compound A alone) + (MIC of compound B combined/MIC of compound B alone). FIC index ≤0.5, synergy; 0.5 < FIC index ≤2, additivity; FIC index >2, antagonism.

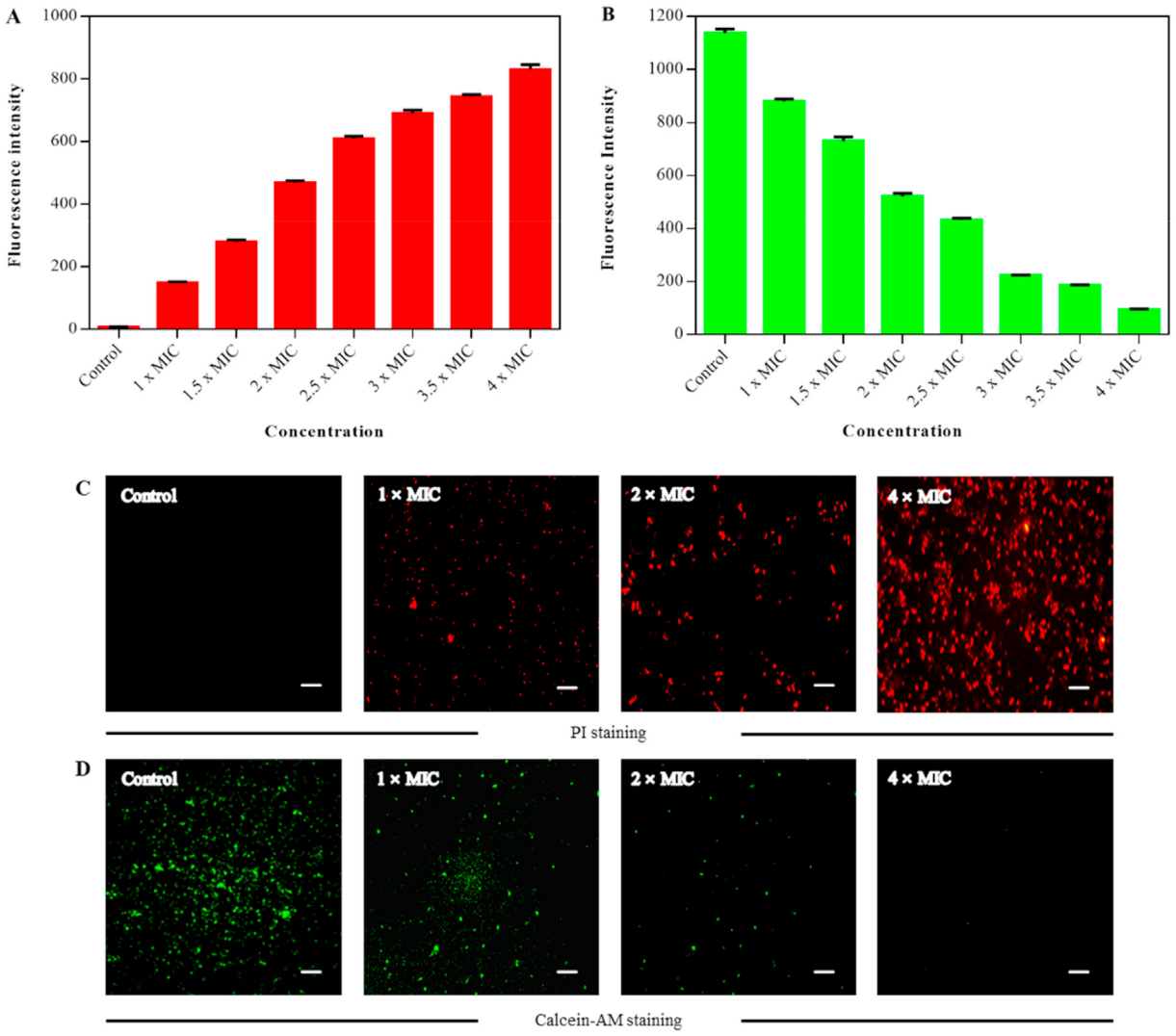


Fig. 8. Bacterial membrane permeabilization. (A, B) Estimation of membrane damage using PI and calcein-AM staining in MRSA treated with methyl acetate oxime **6l** (1–4 × MIC); (C, D) Fluorescence micrograph images of MRSA stained with calcein-AM and PI treated with compound **6l** (1–4 × MIC). Scale bar for all images is 100 μm.

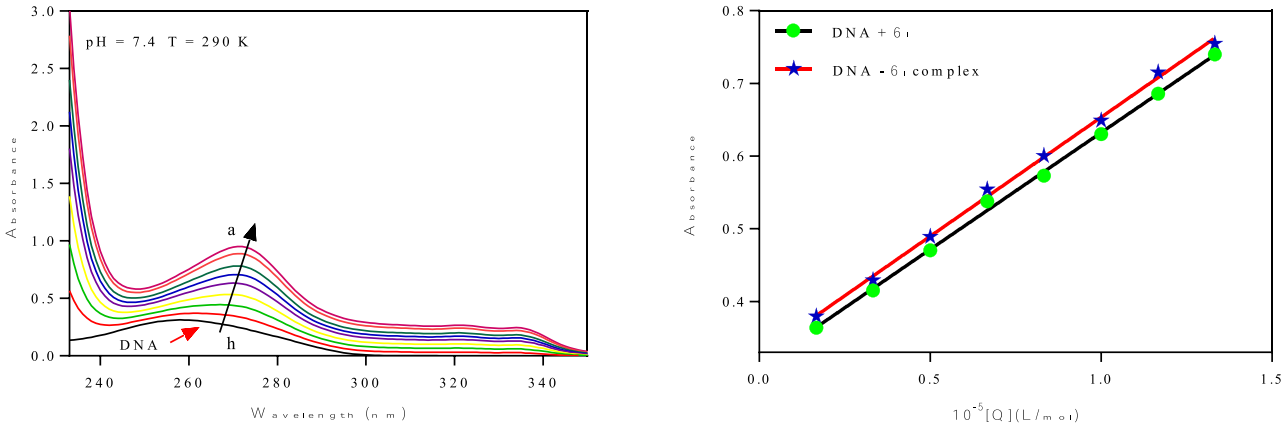


Fig. 9. Ultraviolet spectra of DNA with methyl acetate oxime **6l**.

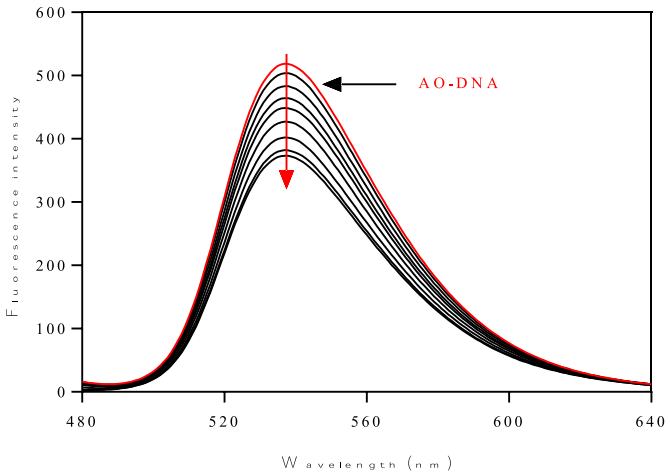


Fig. 10. Mode of action between methyl acetate oxime **6l** MRSA DNA.

in Fig. S2. Therefore, the above experimental data showed that the highly active compound **6l** could intercalate into MRSA DNA.

2.8. Molecular modeling study

Bacterial DNA gyrase as an important target of antibacterial quinolones plays a significant function in the process of DNA replication [29]. Considering that the active molecule **6l** could destroy the integrity of the membrane, we deduced that the methyl acetate oxime **6l** might destroy the bacterial cell membrane, and then interrupted the function of DNA gyrase, eventually leading to cell death. Hence, a proper ligand-receptor docking was done smoothly to investigate the possible antibacterial mechanism of quinolone oximes. These obtained results of ciprofloxacin thiazole hybrid **5b** and oxime **6l** binding to DNA gyrase B (PDB ID: 3U2K) were displayed in Fig. 11. The binding energies of compounds **5b** and **6l** were −8.75 and −6.72 kcal/mol, respectively. Compound **5b** could form three hydrogen bonds with the residue ARG-144 with distance of 1.6 Å, 1.8 Å and 2.1 Å. The active molecule **6l** could interact with four amino acid residues, namely ASP-81, ARG-84, GLY-85 and GLN-91. The supramolecular interaction by hydrogen bonds between DNA gyrase and thiazolyl ciprofloxacin **5b** or its oxime derivative **6l** might rationalize the antibacterial mechanism of quinolone oximes.

2.9. Effects of compound **6l** on MRSA genes expression

The resistance mechanism of MRSA is very complicated and involves many genes, such as mecA and femB [30]. Recently, studies shown that the modified molecules on the basis of existing drug fragments could alleviate the drug resistance of the globally apprehensive MRSA while guaranteeing the efficacy of drugs by regulating the expression of resistance-related genes [31]. Considering that the compound could form hydrogen bonds with DNA gyrase B, the expression levels of mecA, femB and gyrB genes in MRSA were observed by RT-PCR. As show in Fig. 12, the gyrB gene expression level decreased after compound **6l** was cultured with MRSA for 3 h, revealing that the quinolone oxime **6l** could interact with the gyrase to reduce the expression of related genes. Meanwhile, the relative expression of femB gene decreased rapidly, disclosing that the most active molecule **6l** might alleviate the resistance of MRSA by inhibiting the expression of different genes. However, compound **6l** cannot inhibit mecA gene expression. Recent studies suggested that the resistance level of MRSA has nothing to do with the output of the PBP2a protein encoded by the mecA gene, but is related to auxiliary genes, such as the femB gene [32]. The results opened up a new direction for us to explore its possiblity against MRSA mechanism in future research. All the primers in qRT-PCR are shown in Table S1.

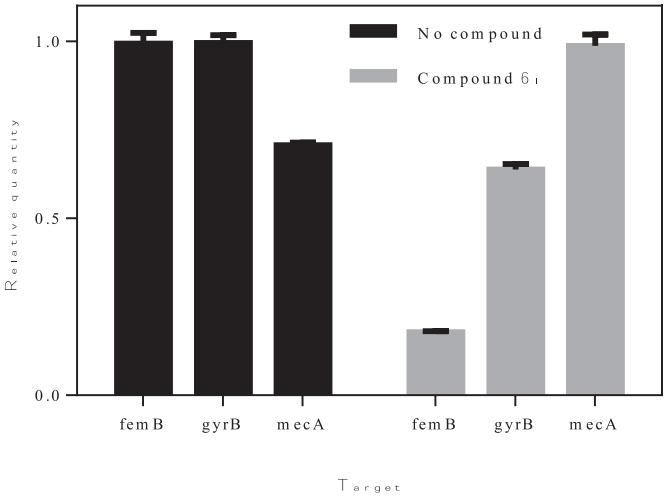


Fig. 12. Effects of methyl acetate oxime **6l** on MRSA genes expression.

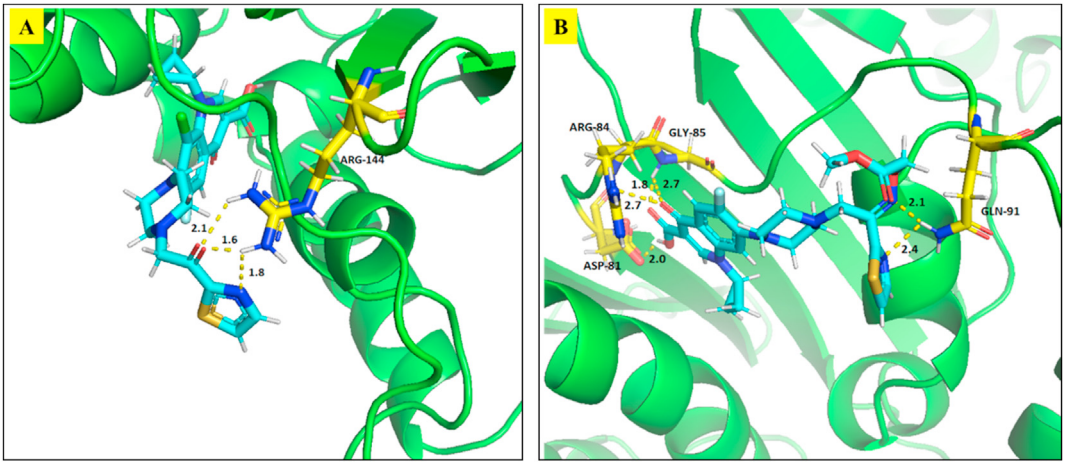


Fig. 11. The comparison of ciprofloxacin thiazole hybrid **5b** (A) and methyl acetate oxime **6l** (B) were predicted to multisite binding with GyrB ATPase domain.

2.10. Hemolysis assay

The toxicity of methyl acetate oxime **6l** was further studied by hemolysis experiment. The processed red blood cells (RBCs) were incubated in 1% Triton X-100 (positive control) in PBS (blank) for 4 h at 37 °C and evaluated with various concentrations of compound **6l**. The results showed that the hemolysis caused by oxime **6l** was less than 3% at the effective bactericidal concentration (Fig. 13), and the hemolysis rate was still less than 5% even at a high concentration (64 × MIC). It was observed under the microscope that the erythrocytes remained intact by treating with compound **6l** (16 × MIC), which was similar to the saline control group.

2.11. Cytotoxicity assay

Cytotoxicity is an important aspect in defining reliable drug candidates [33]. The toxicity of oxime **6l** to LO2 cells (normal hepatocyte cell) and 293 T cells was evaluated by a colorimetric cell proliferation MTT assay. The survival rate at more than 90% displayed good tolerance of LO2 and 293 T cells to compound **6l**, even if the cells were incubated for 24 h with concentration of effective sterilization. Final result showed that molecule **6l** exhibited a significant amount of selectivity for bacterial membranes compared with mammalian membranes (Fig. 14).

2.12. ADMET study

The pharmacokinetic properties of the synthesized quinolone oximes, clinic drug cefditoren pivoxil and ciprofloxacin were predicted by using the online SwissADME and PreADMET software [34]. The methyl ester oxime **6l** and clinic drug cefditoren pivoxil were not completely satisfied Lipinski rule in Table S3. The predictive results pointed out that the oxime **6l** could not penetrate blood-brain barrier (BBB) and had low central nervous system toxicity. The methyl acetate oxime **6l** had the highest skin permeability and good human intestinal absorption. Moreover, the methyl ester oxime **6l** displayed no carcinogenicity in rat and mouse model, indicated that compound was non-carcinogenic in nature. These results demonstrated that methyl ester oxime **6l** could act as a promising molecule for further antibacterial development.

3. Conclusion

In conclusion, the present study explored a class of novel 7-thiazoxime quinolones as potentially antimicrobial agents. Bioactivity assay indicated that most of the developed compounds exhibited good inhibitory activity against the evaluated strains and SAR analysis was summarized in Fig. 15. Notably, methyl acetate oxime **6l** showed excellent inhibitory efficacy (MIC = 0.25–1 μg/mL) against the tested bacteria, especially against MRSA with low MIC value of 0.25 μg/mL. Furthermore, oxime **6l** exerted broad antimicrobial spectrum, rapidly bactericidal ability and low cytotoxicity to mammalian cell lines. Its combination with ciprofloxacin could enhance the antimicrobial efficiency and abate the tendency to induce bacterial resistance. Subsequent exploration of anti-MRSA action mechanism revealed that **6l** was able to insert into the tested DNA after effectively damaging cell membrane to interfere the DNA replication and result in femB and gryB genes to decrease expression. All above observations provided an important step forward for development of the 7-thiazoxime quinolones to access significant anti-MRSA agents.

4. Experimental protocols

4.1. General methods

Sichuan Provincial People's Hospital (Chengdu, China) provided the microbial strains. The PI, calcein-AM, AO and DAPI were purchased from Sigma-Aldrich. The high-resolution mass spectra (HRMS) were used the analyses of the prepared compounds on ESI-mass spectra were recorded on IonSpec FTeCR mass spectrometer with ESI resource and are reported in the form of (*m*/*z*). The reaction was monitored at 254 and 365 nm by TLC analysis using precoated silica gel plates. The synthesized compounds were analyzed by ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV 600 spectrometer (Bruker, Germany). Melting points were taken using basic melting point apparatus X-6 type (Focus, China) and were uncorrected. All fluorescence spectra were recorded on F-7000 spectrofluorimeter (Hitachi, Tokyo, Japan) and UV spectra were obtained through TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China), both of which were equipped with 1.0 cm quartz cells. Microscopic observations were made with a positive fluorescence microscope (LW300LFT, Cewei, China). All

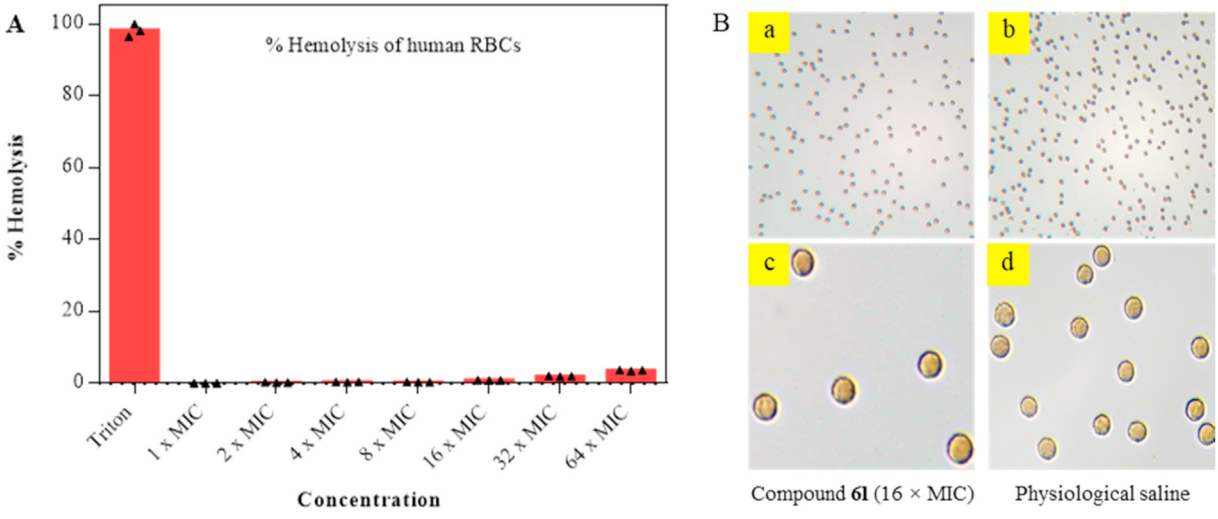


Fig. 13. Hemolysis assay. (A) Hemolysis rate of compound **6l** to red blood cells; (B) The microscope photos of RBCs treated by **6l** with 4 μg/mL (a and c) and physiological saline (b and d).

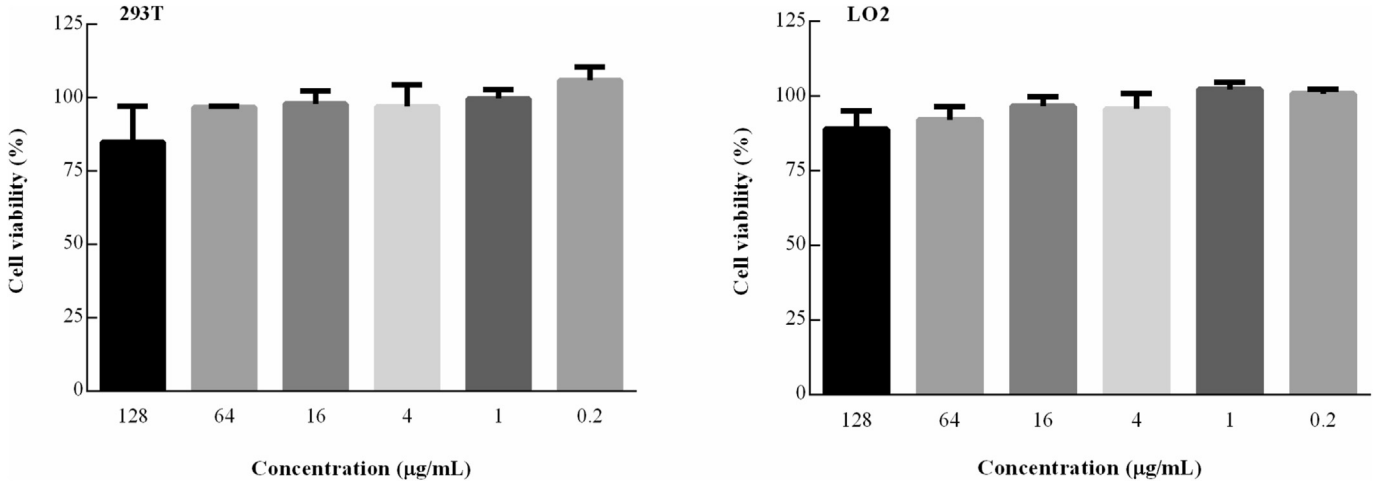


Fig. 14. Cytotoxicity of compound **6l** in the 293 T and LO2 cells by MTT methodology.

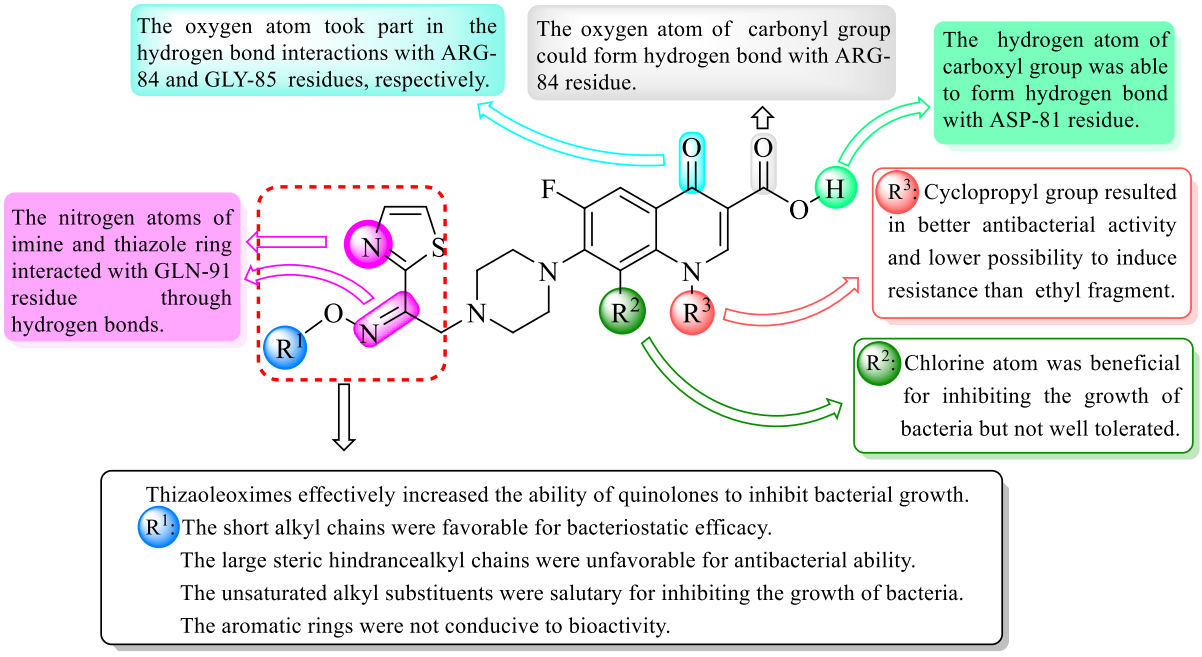


Fig. 15. The antibacterial behavior of 7-thiazoxime quinolones.

chemicals are commercially available and used without prior purification unless otherwise stated.

*4.2. Synthetic procedures and spectral data for compounds (**2**–**6**)*

*4.2.1. General experimental procedures for the synthesis of the desired intermediates (**2**)*

The desired intermediate **2** was prepared according to the previously reported procedures [35].

*4.2.2. General experimental procedures for the synthesis of the desired intermediates (**3a**–**f**)*

The desired intermediates **3a**–**f** were prepared according to the previously reported methods [36].

*4.2.3. Synthesis of the desired intermediates (**5a**–**b**)*

To a stirred solution of different quinolones (0.30 mmol) and potassium carbonate (0.45 mmol) in acetonitrile (15 mL) stirred at 50 °C for 1 h, then added 2-bromo-1-(thiazol-2-yl) ethan-1-one (0.33 mmol). After the reaction was completed (monitored by TLC, dichloromethane/methanol (10/1, V/V). After the acetonitrile was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with dichloromethane/methanol (1/10–3/10, V/V) to give the pure target compound **5a**–**b**.

*4.2.4. Synthesis of (E)-1-ethyl-6-fluoro-7-(4-(2-(methoxyimino)-2-(thiazol-2-yl)ethyl)piperazin-1-yl) -4-oxo-1,4 -dihydroquinoline-3-carboxylic acid (**6a**)*

A mixture of norfloxacin (96 mg, 0.30 mmol) and potassium carbonate (62 mg, 0.45 mmol) in acetonitrile (25 mL) were stirred at 50 °C for 1 h, and then cooled to room temperature. Methyl oxime **3a** (78 mg, 0.33 mmol) was added, and the resulting mixture was stirred at 50 °C for 5 h. After the completion of reaction, the solvent was evaporated under reduced pressure. The crude product

was purified by silica gel column chromatography (eluent, dichloromethane/methanol (V/V) = 1/10–3/10) to afford target compound **6a** (81 mg) as yellow solid. Yield: 56.5%; Mp: 238.7–239.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.12 (bs, 1H, COO*H*), 8.65 (s, 1H, quinolone-2-*H*), 8.02 (d, *J* = 12.8 Hz, 1H, quinolone-5-*H*), 7.86 (d, *J* = 3.2 Hz, 1H, thiazole-5-*H*), 7.33 (d, *J* = 3.2 Hz, 1H, thiazole-4-*H*), 6.80 (d, *J* = 6.9 Hz, 1H, quinolone-8-*H*), 4.30 (dd, *J* = 7.1, 3.4 Hz, 2H, C*H*$_2$CH$_3$), 4.19 (s, 1H, OC*H*$_3$), 4.07 (s, 2H, OC*H*$_3$), 3.96 (s, 2H, piperazine-C*H*$_2$), 3.34 (s, 4H, piperazine-2,2-N-(C*H*$_2$)$_2$), 2.87 (s, 4H, piperazine-3,3-N-(C*H*$_2$)$_2$), 1.56 (t, *J* = 8.4, 5.9 Hz, 3H, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.97 (quinolone-4-*C*), 167.17 (*C*OOH), 164.25 (*C*=NO), (152.68, 147.04, 143.19, 142.17, 137.12, 123.16, 120.39, 112.77, 112.62, 108.35, 103.72, aromatic-*C*), 62.97, 57.99, 53.00, 52.68, 50.56, 14.38 ppm; HRMS (ESI, *m/z*) calcd for $C_{22}H_{24}FN_5O_4S$ $[M + H]^+$, 474.1611; found, 474.1594.

4.2.5. Synthesis of (E)-7-(4-(2-(ethoxyimino) -2-(thiazol-2-yl) ethyl)piperazin-1-yl) -1-ethyl-6-fluoro-4-oxo -1,4-dihydroquinoline-3-carboxylic acid (**6b**)

Compound **6b** was prepared according to the experimental procedure described for compound **6a** starting from intermediate **3b** (82 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and norfloxacin (96 mg, 0.30 mmol). The target compound **6b** (74 mg) was obtained as yellow solid. Yield: 50.6%; Mp: 214.8–215.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.10 (bs, 1H, COO*H*), 8.65 (s, 1H, quinolone-2-*H*), 8.00 (d, *J* = 3.1 Hz, 1H, quinolone-5-*H*), 7.86 (d, *J* = 3.2 Hz, 1H, thiazole-5-*H*), 7.31 (d, *J* = 3.0 Hz, 1H, thiazole-4-*H*), 6.80 (d, *J* = 6.7 Hz, 1H, quinolone-8-*H*), 4.46 (dd, *J* = 14.0, 7.0 Hz, 1H, OC*H*$_2$CH$_3$), 4.33 (dd, *J* = 8.7, 5.4 Hz, 1H, OC*H*$_2$CH$_3$), 4.30 (dd, *J* = 8.4, 6.0 Hz, 2H, NC*H*$_2$CH$_3$), 3.97 (s, 2H, piperazine-C*H*$_2$), 3.35 (s, 4H, piperazine-2,2-N-(C*H*$_2$)$_2$), 2.88 (s, 4H, piperazine-3,3-N-(C*H*$_2$)$_2$), 1.56 (t, *J* = 7.0 Hz, 3H, CH$_2$C*H*$_3$), 1.45 (t, *J* = 7.0 Hz, 1H, OCH$_2$C*H*$_3$), 1.36 (t, *J* = 7.0 Hz, 2H, OCH$_2$C*H*$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.94 (quinolone-4-*C*), 167.17 (*C*OOH), 164.62 (*C*=NO), (152.69, 147.05, 146.15, 143.14, 142.16, 137.12, 123.04, 120.23, 112.67, 108.33, 103.75, aromatic-*C*), 71.39, 71.01, 52.97, 52.63, 50.50, 49.90, 49.77, 14.68, 14.38 ppm; HRMS (ESI, *m/z*) calcd. for $C_{23}H_{26}FN_5O_4S$ $[M + H]^+$, 488.1762; found, 488.1757.

4.2.6. Synthesis of (E)-7-(4-(2-(tert-butoxyimino) -2-(thiazol-2-yl) ethyl) piperazin-1-yl) -1-ethyl-6-fluoro -4-oxo- 1,4-dihydroquinoline-3-carboxylic acid (**6c**)

Compound **6c** was prepared according to the experimental procedure described for compound **6a** starting from intermediate **3c** (92 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and norfloxacin (96 mg, 0.30 mmol). The target compound **6c** (95 mg) was obtained as white solid. Yield: 61.4%; Mp: 241.5–242.3 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.14 (bs, 1H, COO*H*), 8.65 (s, 1H, quinolone-2-*H*), 8.01 (d, 1H, quinolone-5-*H*), 7.99 (d, *J* = 2.8 Hz, 1H, thiazole-5-*H*), 7.54 (d, *J* = 3.1 Hz, 1H, thiazole-4-*H*), 6.81 (d, *J* = 6.1 Hz, 1H, quinolone-8-*H*), 4.31 (q, *J* = 7.1 Hz, 2H, C*H*$_2$CH$_3$), 4.01 (s, 1H, piperazine-C*H*$_2$), 3.97 (s, 1H, piperazine-C*H*$_2$), 3.33 (m, 4H, piperazine-2,2-N-(C*H*$_2$)$_2$), 2.87 (d, *J* = 32.0 Hz, 4H, piperazine-3,3-N-(C*H*$_2$)$_2$), 1.56 (t, *J* = 7.2 Hz, 3H, CH$_2$C*H*$_3$), 1.48 (s, 6H, OC(C*H*$_3$)$_3$), 1.39 (s, 3H, OC(C*H*$_3$)$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.98 (quinolone-4-*C*), 167.20 (*C*OOH), 165.92 (*C*=NO), (152.70, 147.04, 143.01, 142.04, 137.14, 122.52, 119.77, 112.74, 112.59, 108.33, 103.71, aromatic-*C*), 81.83, 80.95, 58.13, 52.93, 52.50, 49.69, 27.66, 14.40 ppm; HRMS (ESI, *m/z*) calcd. for $C_{25}H_{30}FN_5O_4S$ $[M + Na]^+$, 538.1895; found, 538.1884.

4.2.7. Synthesis of (E)-7-(4-(2-((benzyloxy) imino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl) -1-ethyl-6-fluoro-4-oxo -1,4-dihydroquinoline-3-carboxylic acid (**6d**)

Compound **6d** was prepared according to the experimental procedure described for compound **6a** starting from intermediate **3d** (93 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and norfloxacin (96 mg, 0.30 mmol). The target compound **6d** (66 mg) was obtained as yellow solid. Yield: 40.1%; Mp: 165.6–166.2 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.11 (bs, 1H, COO*H*), 8.64 (s, 1H, quinolone-2-*H*), 8.01 (d, *J* = 12.8 Hz, 1H, quinolone-5-*H*), 7.86 (d, *J* = 3.0 Hz, 1H, thiazole-5-*H*), 7.44 (dd, *J* = 14.1, 7.3 Hz, 2H, Ph-1, 5-2*H*), 7.38 (dd, *J* = 12.7, 5.9 Hz, 2H, Ph-2, 4-2*H*), 7.34 (d, *J* = 7.0 Hz, 1H, thiazole-4-*H*), 7.32 (d, *J* = 3.1 Hz, 1H, Ph-3-*H*), 6.78 (d, *J* = 6.7 Hz, 1H, quinolone-8-*H*), 5.44 (s, 1H, Ph-C*H*$_2$), 5.30 (s, 1H, Ph-C*H*$_2$), 4.29 (dd, *J* = 14.8, 7.4 Hz, 2H, C*H*$_2$CH$_3$), 3.97 (s, 2H, piperazine-C*H*$_2$), 3.30 (m, 4H, piperazine-2,2-N-(C*H*$_2$)$_2$), 2.80 (s, 4H, piperazine-3,3-N-(C*H*$_2$)$_2$), 1.55 (t, *J* = 7.2 Hz, 3H, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.98 (quinolone-4-*C*), 167.17 (*C*OOH), 164.31 (*C*=NO), (152.68, 147.05, 143.22, 142.23, 137.11, 136.87, 128.59–128.32 (m), 128.24, 123.42, 120.45, 112.79, 112.64, 108.38, 103.73, aromatic-*C*), 77.52, 52.98, 52.54, 50.65, 49.90, 49.78, 14.39 ppm; HRMS (ESI, *m/z*) calcd. for $C_{28}H_{28}FN_5O_4S$ $[M + H]^+$, 550.1919; found, 550.1920.

4.2.8. Synthesis of (E)-7-(4-(2-((allyloxy)imino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl) -1-ethyl-6-fluoro-4- oxo -1,4-dihydroquinoline-3-carboxylic acid (**6e**)

Norfloxacin (96 mg, 0.30 mmol) was dissolved in acetonitrile and stirred with potassium carbonate (62 mg, 0.45 mmol) for 1 h, followed by **3e** (86 mg, 0.33 mmol) for 5 h at 50 °C. The reaction mixture was concentrated under a reduced pressure, the solid was purified by column chromatography on silica gel (300–400 mesh) using dichloromethane/methanol (V/V = 1/10–3/10) as eluent to give 107 mg of the solid and got the yellow solid **6e**. yield: 71.5%; Mp: 185.5–186.2 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.10 (bs, 1H, COO*H*), 8.65 (s, 1H, quinolone-2-*H*), 8.01 (d, *J* = 12.7 Hz, 1H, quinolone-5-*H*), 7.86 (d, *J* = 3.0 Hz, 1H, thiazole-5-*H*), 7.32 (d, *J* = 3.0 Hz, 1H, thiazole-4-*H*), 6.80 (d, *J* = 7.0 Hz, 1H, quinolone-8-*H*), 6.08 (m, 1H, C*H*=CH$_2$), 5.40 (m, 1H, CH=C*H*$_2$), 5.30 (dd, *J* = 15.5, 10.8 Hz, 1H, CH=C*H*$_2$), 4.6l (d, *J* = 5.4 Hz, 1H, OC*H*$_2$), 4.77 (d, *J* = 5.5 Hz, 1H, OC*H*$_2$), 4.33–4.27 (m, 2H, C*H*$_2$CH$_3$), 3.98 (s, 2H, piperazine-C*H*$_2$), 3.34 (m, 4H, piperazine-2,2-N-(C*H*$_2$)$_2$), 2.88 (m, 4H, piperazine-3,3-N-(C*H*$_2$)$_2$), 1.56 (t, *J* = 6.9 Hz, 3H, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.97 (quinolone-4-*C*), 167.16 (*C*OOH), 164.33 (*C*=NO), (152.69, 147.04, 143.20, 142.23, 137.12, 133.38, 123.25, 120.39, 118.37, 112.90–112.83 (m), 112.72, 108.38, 103.72, aromatic-*C*), 76.19, 57.9, 53.01, 52.62, 50.64, 14.39 ppm; HRMS (ESI, *m/z*) calcd. for $C_{24}H_{26}FN_5O_4S$ $[M + H]^+$, 500.1762; found, 500.1752.

4.2.9. Synthesis of (E)-1-ethyl-6-fluoro-7-(4-(2-((2-methoxy-2-oxoethoxy)imino) -2-(thiazol-2-yl)ethyl) piperazin -1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6f**)

Compound **6f** was prepared according to the experimental procedure described for compound **6a** starting from intermediate **3f** (97 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and norfloxacin (96 mg, 0.30 mmol). The target compound **6f** (122 mg) was obtained as yellow solid. Yield: 76.2%; Mp: 167.3–168.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.08 (bs, 1H, COO*H*), 8.65 (s, 1H, quinolone-2-*H*), 8.01 (d, *J* = 13.5 Hz, 1H, quinolone-5-*H*), 7.88 (d, *J* = 3.1 Hz, 1H, thiazole-5-*H*), 7.35 (d, *J* = 3.1 Hz, 1H, thiazole-4-*H*), 6.81 (d, *J* = 6.2 Hz, 1H, quinolone-8-*H*), 4.94 (s, 1H, OC*H*$_2$), 4.83 (s, 1H, OC*H*$_2$), 4.30 (dd, *J* = 13.9, 6.8 Hz, 2H, C*H*$_2$CH$_3$), 4.01 (s, 2H, piperazine-C*H*$_2$), 3.79 (s, 3H, OC*H*$_3$), 3.34 (s, 4H, piperazine-2,2-N-(C*H*$_2$)$_2$), 2.90 (s, 4H, piperazine-3,3-N-(C*H*$_2$)$_2$), 1.56 (t, *J* = 6.6 Hz, 3H, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.99 (quinolone-4-*C*), 169.61, 169.43 (s), 167.17 (*C*OOH), 163.30 (*C*=NO), (152.68, 147.05, 143.34, 142.31, 137.13, 120.87, 113.37–113.19 (m), 112.71, 108.37, 103.74, aromatic-*C*), 71.40, 53.01, 52.52, 52.01, 50.85, 14.41 ppm; HRMS (ESI, *m/z*) calcd. for $C_{24}H_{26}FN_5O_6S$ $[M + H]^+$, 532.1661; found, 532.1669.

*4.2.10. Synthesis of (E)-1-cyclopropyl-6-fluoro-7-(4-(2-(methoxyimino)-2-(thiazol-2-yl)ethyl) piperazin-1-yl) -4- oxo-1,4-dihydroquinoline-3-carboxylic acid (**6g**)*

To a stirred solution of ciprofloxacin (100 mg, 0.30 mmol) and potassium carbonate (62 mg, 0.45 mmol) in acetonitrile (15 mL) stirred at 50 °C for 1 h, then added compound **3a** (78 mg, 0.33 mmol). After the reaction was completed (monitored by TLC, dichloromethane/methanol (10/1, V/V). After the acetonitrile was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with dichloromethane/methanol (1/10–3/10, V/V) to give the pure target compound **6g** as white power (99 mg). Yield: 68.0%; Mp: > 250 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.02 (bs, 1H, COO*H*), 8.73 (s, 1H, quinolone-2-*H*), 7.98 (d, J = 4.2 Hz, 1H, quinolone-5-*H*), 7.87 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 7.34 (d, J = 8.2 Hz, 1H, thiazole-4-*H*), 7.32 (d, J = 6.9 Hz, 1H, quinolone-8-*H*), 4.20 (s, 1H, OCH_3), 4.07 (s, 2H, OCH_3), 3.97 (s, 2H, piperazine-CH_2), 3.52 (d, J = 3.9 Hz, 1H, cyclopropane-C*H*), 3.37 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.88 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.39–1.34 (m, 2H, cyclopropane-CH_2), 1.18 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.11 (quinolone-4-*C*), 166.99 (*C*OOH), 164.27 (*C*=NO), (152.85, 147.35, 143.20, 142.19, 139.08, 123.19, 120.40, 112.46, 112.31, 108.15, 104.75 aromatic-*C*), 62.99, 53.02, 52.69, 50.54, 35.24, 8.20 ppm; HRMS (ESI, *m/z*) calcd. for $C_{23}H_{24}FN_5O_4S$ $[M + H]^+$, 486.1606; found, 486.1601.

*4.2.11. Synthesis of (E)-1-cyclopropyl-7-(4-(2-(ethoxyimino)-2-(thiazol-2-yl)ethyl) piperazin-1-yl) -6-fluoro -4-oxo -1,4-dihydroquinoline-3-carboxylic acid (**6h**)*

Compound **6h** was prepared according to the experimental procedure described for compound **6h** starting from intermediate **3b** (82 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and ciprofloxacin (100 mg, 0.30 mmol). The target compound **6h** (74 mg) was obtained as white solid. Yield: 50.6%; Mp: > 250 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.01 (bs, 1H, COO*H*), 8.72 (s, 1H, quinolone-2-*H*), 7.95 (d, J = 13.0 Hz, 1H, quinolone-5-*H*), 7.86 (d, J = 2.6 Hz, 1H, thiazole-5-*H*), 7.33 (d, J = 8.0 Hz, 1H, thiazole-4-*H*), 7.32 (s, 1H, quinolone-8-*H*), 4.46 (dd, J = 13.9, 6.9 Hz, 1H, OCH_2CH_3), 4.33 (dd, J = 13.8, 6.8 Hz, 1H, OCH_2CH_3), 3.98 (s, 2H, piperazine-CH_2), 3.52 (s, 1H, cyclopropane-C*H*), 3.36 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.88 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.45 (t, J = 7.0 Hz, 1H, OCH_2CH_3), 1.37 (d, J = 6.5 Hz, 2H, OCH_2CH_3), 1.36 (s, 2H, cyclopropane-CH_2), 1.18 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.08 (quinolone-4-*C*), 166.97 (*C*OOH), 164.67 (*C*=NO), (152.85, 147.31, 143.15, 142.16, 139.08, 123.00, 120.20, 112.32 (d, J = 23.5 Hz), 112.22–112.05 (m), 108.10, 104.76 aromatic-*C*), 71.36, 71.00, 53.00, 52.67, 50.54, 35.25, 14.68, 8.19 ppm; HRMS (ESI, *m/z*) calcd. for $C_{24}H_{26}FN_5O_4S$ $[M + H]^+$, 500.1762; found, 500.1765.

*4.2.12. Synthesis of (E)-7-(4-(2-(tert-butoxyimino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl)-1-cyclopropyl -6-fluoro -4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6i**)*

Compound **6i** was prepared according to the experimental procedure described for compound **6h** starting from intermediate **3c** (92 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and ciprofloxacin (100 mg, 0.30 mmol). The target compound **6i** (97 mg) was obtained as white solid. Yield: 61.2%; Mp: > 250 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 15.04 (bs, 1H, COO*H*), 8.75 (s, 1H, quinolone-2-*H*), 8.01 (d, J = 3.1 Hz, 1H, quinolone-5-*H*), 7.99 (d, J = 13.0 Hz, 1H, thiazole-5-*H*), 7.56 (d, J = 3.0 Hz, 1H, thiazole-4-*H*), 7.35 (d, J = 6.5 Hz, 1H, quinolone-8-*H*), 4.00 (s, 2H, piperazine-CH_2), 3.53 (d, J = 3.2 Hz, 1H, cyclopropane-C*H*), 3.37 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.89 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.50 (s, 6H, OC(CH_3)$_3$), 1.41 (s, 3H, OC(CH_3)$_3$), 1.38 (d, J = 6.8 Hz, 2H, cyclopropane-CH_2), 1.19 (d, J = 2.6 Hz, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.10 (quinolone-4-*C*), 166.99 (*C*OOH), 165.93 (*C*=NO), (152.86, (147.31, 143.00, 142.03, 139.09, 122.50, 119.75, 112.42, 112.26, 108.12, 104.72 aromatic-*C*), 81.82, 58.15, 52.95, 52.52, 35.24, 27.66, 8.20 ppm; HRMS (ESI, *m/z*) calcd. for $C_{26}H_{30}FN_5O_4S$ $[M + Na]^+$, 550.1990; found, 550.1886.

*4.2.13. Synthesis of (E)-7-(4-(2-((benzyloxy)imino)-2-(thiazol-2-yl) ethyl) piperazin-1-yl)-1-cyclopropyl -6-fluoro -4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6j**)*

Compound **6j** was prepared according to the experimental procedure described for compound **6h** starting from intermediate **3d** (93 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and ciprofloxacin (100 mg, 0.30 mmol). The target compound **6j** (79 mg) was obtained as yellow solid. Yield: 46.9%; Mp: 145.3–146.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.98 (bs, 1H, COO*H*), 8.71 (s, 1H, quinolone-2-*H*), 7.97 (d, J = 5.7 Hz, 1H, quinolone-5-*H*), 7.85 (d, J = 2.9 Hz, 1H, thiazole-5-*H*), 7.44 (dd, J = 13.0, 7.4 Hz, 2H, Ph-1, 5-2*H*), 7.37 (dd, J = 12.9, 6.5 Hz, 2H, Ph-2, 4-2*H*), 7.34 (d, J = 7.1 Hz, 1H, thiazole-4-*H*), 7.32 (d, J = 3.1 Hz, 1H, Ph-3-*H*), 7.29 (d, J = 6.9 Hz, 1H, quinolone-8-*H*), 5.30 (s, 2H, Ph-CH_2), 3.97 (s, 2H, piperazine-CH_2), 3.50 (s, 1H, cyclopropane-C*H*), 3.32 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.80 (s, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.35 (d, J = 6.8 Hz, 2H, cyclopropane-CH_2), 1.17 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.09 (quinolone-4-*C*), 166.92 (*C*OOH), 164.36 (*C*=NO), (152.84, 147.31, 143.20, 142.20, 139.08, 136.90, 128.43 (d, J = 10.3 Hz), 128.21, 123.34, 120.40, 112.44, 112.28, 108.18, 104.72 aromatic-*C*), 77.51, 53.00, 52.55, 50.69, 35.22, 8.17 ppm; HRMS (ESI, *m/z*) calcd. for $C_{29}H_{28}FN_5O_4S$ $[M + H]^+$, 562.1619; found, 562.1615.

*4.2.14. Synthesis of (E)-7-(4-(2-((allyloxy)imino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl)-1-cyclopropyl -6-fluoro -4-oxo- 1,4-dihydroquinoline-3-carboxylic acid (**6k**)*

Compound **6k** was prepared according to the experimental procedure described for compound **6h** starting from intermediate **3e** (86 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and ciprofloxacin (100 mg, 0.30 mmol). The target compound **6k** (82 mg) was obtained as yellow solid. Yield: 53.4%; Mp: 203.5–204.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.98 (bs, 1H, COO*H*), 8.71 (s, 1H, quinolone-2-*H*), 7.95 (d, J = 13.1 Hz, 1H, quinolone-5-*H*), 7.86 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 7.33 (d, J = 9.2 Hz, 1H, thiazole-4-*H*), 7.31 (d, J = 4.2 Hz, 1H, quinolone-8-*H*), 6.08 (m, 1H, C*H*=CH_2), 5.40 (m, 1H, CH=CH_2), 5.29 (dd, J = 14.3, 11.1 Hz, 1H, CH=CH_2), 4.77 (d, J = 5.5 Hz, 2H, OCH_2), 3.98 (s, 2H, piperazine-CH_2), 3.52 (d, J = 3.8 Hz, 1H, cyclopropane-C*H*), 3.36 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.88 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.36 (d, J = 6.3 Hz, 2H, cyclopropane-CH_2), 1.18 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.08 (quinolone-4-*C*), 166.96 (*C*OOH), 164.34 (*C*=NO), (154.51, 152.84, 147.32, 143.21, 142.24, 139.08, 133.43, 120.39, 118.34, 112.41, 112.26, 108.12, 104.76 aromatic-*C*), 76.20, 53.04, 52.64, 50.66, 35.24, 8.20 ppm; HRMS (ESI, *m/z*) calcd. for $C_{25}H_{26}FN_5O_4S$ $[M + H]^+$, 512.1762; found, 512.1753.

*4.2.15. Synthesis of (E)-1-cyclopropyl-6-fluoro-7-(4-(2-((2-methoxy-2-oxoethoxy)imino) -2-(thiazol-2-yl) ethyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6l**)*

Compound **6l** was prepared according to the experimental procedure described for compound **6h** starting from intermediate **3f** (97 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and ciprofloxacin (100 mg, 0.30 mmol). The target hybrid **6l** (56 mg) was obtained as white solid. Yield: 34.3%; Mp: 178.5–180.1 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.99 (bs, 1H, COO*H*), 8.72 (s, 1H, quinolone-2-*H*), 7.96 (d, J = 13.0 Hz, 1H, quinolone-5-*H*), 7.88 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 7.35 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 7.33 (d, J = 7.0 Hz, 1H, quinolone-8-*H*), 4.83 (s, 2H, OCH_2), 4.02 (s, 2H, piperazine-CH_2), 3.79 (s, 3H, OCH_3), 3.54–3.50 (m, 1H,

cyclopropane-C*H*), 3.36 (s, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.90 (s, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.37 (q, J = 6.4 Hz, 2H, cyclopropane-CH_2), 1.18 (d, J = 3.1 Hz, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 177.10 (quinolone-4-*C*), 169.61, 169.42, 166.98 (*C*OOH), 163.31 (*C*=NO), (152.84, 147.33, 143.34, 142.32, 139.09, 120.87, 112.43, 112.27, 108.12, 104.77 aromatic-*C*), 71.40, 53.03, 52.55, 52.01, 50.89, 35.25, 8.20 ppm; HRMS (ESI, *m/z*) calcd. for $C_{25}H_{26}FN_5O_6S$ [M + H]$^+$, 544.1661; found, 544.1649.

4.2.16. Synthesis of (E)-1-cyclopropyl-6-fluoro-7-(4-(2-(methoxyimino)-2-(thiazol-2-yl)ethyl) piperazin-1-yl) -4- oxo-1,4-dihydroquinoline-3-carboxylic acid (**6 m**)

A solution of compound **4c** (110 mg, 0.30 mmol) and potassium carbonate (62 mg, 0.45 mmol) in acetonitrile (20 mL) was stirred at 50 °C for 1 h. Then intermediate **3a** was added (78 mg, 0.33 mmol). The mixture was stirred 50 °C until TLC showed that the reaction was completed. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent, dichloromethane/methanol (V/V) = 1/10−3/10) to afford target hybrid **6m** (105 mg) as yellow solid. Yield: 67.4%; Mp: 228.2−229.4 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.45 (bs, 1H, COO*H*), 8.89 (s, 1H, quinolone-2-*H*), 7.99 (d, J = 11.6 Hz, 1H, quinolone-5-*H*), 7.87 (s, 1H, thiazole-5-*H*), 7.34 (s, 1H, thiazole-4-*H*), 4.34 (m, 1H, cyclopropane-C*H*), 4.07 (s, 3H, OCH_3), 3.93 (s, 2H, piperazine-CH_2), 3.42 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.82 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.29 (d, J = 6.6 Hz, 2H, cyclopropane-CH_2), 0.95 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.81 (quinolone-4-*C*), 166.05 (*C*OOH), 164.30 (*C*=NO), (155.38, 151.85, 143.21, 138.09, 137.01, 128.45, 128.16, 120.44, 111.71, 111.56, 108.66 aromatic-*C*), 77.59, 77.44, 53.86, 53.49, 41.20, 11.36 ppm; HRMS (ESI, *m/z*) calcd. for $C_{23}H_{23}ClFN_5O_4S$ [M + H]$^+$, 520.1216; found, 520.1234.

4.2.17. Synthesis of (E)-8-chloro-1-cyclopropyl-7-(4-(2-(ethoxyimino)-2-(thiazol-2-yl)ethyl) piperazin-1-yl) -6- fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6n**)

A solution of compound **4c** (110 mg, 0.30 mmol) and potassium carbonate (62 mg, 0.45 mmol) in acetonitrile (20 mL) was stirred at 50 °C for 1 h. Ethyl oxime **3b** (82 mg, 0.33 mmol) was added, and the resulting mixture was stirred at 50 °C for 5 h. The mixture was stirred until TLC showed that the reaction was completed. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (eluent, dichloromethane/methanol (V/V) = 1/10−3/10) to afford target hybrid **6n** (145 mg) as yellow solid. Yield: 90.7%; Mp: 176.1−177.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.53 (bs, 1H, COO*H*), 8.82 (s, 1H, quinolone-2-*H*), 7.95 (d, J = 3.1 Hz, 1H, quinolone-5-*H*), 7.89 (d, J = 11.8 Hz, 1H, thiazole-5-*H*), 7.78 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 4.38 (dd, J = 9.6, 5.7 Hz, 1H, cyclopropane-C*H*), 4.26 (q, J = 7.0 Hz, 2H, OCH_2), 3.86 (s, 2H, piperazine-CH_2), 3.30 (s, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.68 (s, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.39−1.28 (m, 3H, OCH_2CH_3), 1.18 (d, J = 6.2 Hz, 2H, cyclopropane-CH_2), 0.97 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ 176.59 (quinolone-4-*C*), 165.54 (*C*OOH), 164.16 (*C*=NO), (156.83, 153.16, 144.12, 143.74, 138.49, 123.14, 122.07, 119.76, 111.05, 110.89, 108.14 aromatic-*C*), 70.84, 54.03, 53.52, 50.89, 41.9, 14.96, 11.25 ppm; HRMS (ESI, *m/z*) calcd. for $C_{24}H_{25}ClFN_5O_4S$ [M + H]$^+$, 534.1373; found, 534.1379.

4.2.18. Synthesis of (E)-7-(4-(2-(tert-butoxyimino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl) -8-chloro-1- cyclopropyl -6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6o**)

Compound **6o** was prepared according to the experimental procedure described for compound **6m** starting from intermediate **3c** (92 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and compound **4c** (110 mg, 0.30 mmol). The target hybrid **6o** (98 mg) was obtained as yellow solid. Yield: 58.1%; Mp: 143.8−144.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.49 (bs, 1H, COO*H*), 8.6l (s, 1H, quinolone-2-*H*), 8.02 (d, J = 2.5 Hz, 1H, quinolone-5-*H*), 8.01 (d, J = 5.7 Hz, 1H, thiazole-5-*H*), 7.56 (d, J = 3.0 Hz, 1H, thiazole-4-*H*), 4.35 (dt, J = 10.3, 3.4 Hz, 1H, cyclopropane-C*H*), 3.99 (s, 2H, piperazine-CH_2), 3.43 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.84 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.50 (s, 6H, OC(CH_3)$_3$), 1.41 (s, 3H, OC(CH_3)$_3$), 1.31 (d, J = 6.9 Hz, 2H, cyclopropane-CH_2), 0.96 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.81 (quinolone-4-*C*), 166.09 (*C*OOH), 155.24, 151.81, 143.01, 142.03, 138.12, 123.24, 122.42, 119.70, 119.12, 111.72, 111.63, 108.67, 81.71, 53.80, 53.34, 51.32, 41.17, 27.66 (d, J = 8.8 Hz), 11.34 ppm; HRMS (ESI, *m/z*) calcd. for $C_{26}H_{29}ClFN_5O_4S$ [M + H]$^+$, 562.1686; found, 562.1687.

4.2.19. Synthesis of (E)-7-(4-(2-((benzyloxy)imino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl)-8-chloro-1- cyclopropyl -6 -fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6p**)

Compound **6p** was prepared according to the experimental procedure described for compound **6m** starting from intermediate **3d** (93 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and compound **4c** (110 mg, 0.30 mmol). The target hybrid **6p** (115 mg) was obtained as yellow solid. Yield: 64.4%; Mp: 162.9−163.8 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.46 (bs, 1H, COO*H*), 8.88 (s, 1H, quinolone-2-*H*), 7.96 (d, 1H, quinolone-5-*H*), 7.86 (d, J = 3.0 Hz, 1H, thiazole-5-*H*), 7.44 (d, J = 7.7 Hz, 2H, Ph-1, 5-2*H*), 7.38 (d, J = 6.9 Hz, 2H, Ph-2, 4-2*H*), 7.33 (d, J = 3.0 Hz, 1H, thiazole-4-*H*), 7.27 (s, 1H, Ph-3-*H*), 5.31 (s, 2H, OCH_2), 4.33 (dd, J = 6.6, 3.2 Hz, 1H, cyclopropane-C*H*), 3.95 (s, 2H, piperazine-CH_2), 3.36 (s, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.75 (s, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.29 (d, J = 6.7 Hz, 2H, cyclopropane-CH_2), 0.95 (s, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.81 (quinolone-4-*C*), 166.05 (*C*OOH), 164.30 (*C*=NO), (157.06, 155.38, 151.85, 144.63, 143.21, 142.21, 138.09, 137.01, 128.43, 128.16, 123.31, 120.44, 111.71, 111.56, 108.66 aromatic-*C*), 77.44, 53.86, 53.49, 51.15, 41.20, 11.36 ppm; HRMS (ESI, *m/z*) calcd. for $C_{29}H_{27}ClFN_5O_4S$ [M + H]$^+$, 596.1529; found, 596.1538.

4.2.20. Synthesis of (E)-7-(4-(2-((allyloxy)imino)-2-(thiazol-2-yl) ethyl)piperazin-1-yl)-8-chloro-1- cyclopropyl -6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6q**)

Compound **6q** was prepared according to the experimental procedure described for compound **6m** starting from intermediate **3e** (86 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and compound **4c** (110 mg, 0.30 mmol). The target hybrid **6q** (84 mg) was obtained as yellow solid. Yield: 51.4%; Mp: 183.1−184.6 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.44 (bs, 1H, COO*H*), 8.88 (s, 1H, quinolone-2-*H*), 7.98 (d, J = 11.7 Hz, 1H, quinolone-5-*H*), 7.87 (d, J = 3.0 Hz, 1H, thiazole-5-*H*), 7.33 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 6.14−6.02 (m, 1H, C*H*=CH_2), 5.41−5.27 (m, 1H, CH=CH_2), 4.78 (s, 2H, OCH_2), 4.33 (m, 1H, cyclopropane-C*H*), 3.97 (s, 2H, piperazine-CH_2), 3.42 (m, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.83 (m, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.29 (q, J = 6.6 Hz, 2H, cyclopropane-CH_2), 0.95 (d, J = 3.3 Hz, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.79 (quinolone-4-*C*), 166.04 (*C*OOH), 164.27 (*C*=NO), 157.06, 155.38, 151.84, 151.39, 143.18, 142.21, 138.08, 133.52, 120.39, 118.21, 111.70, 111.54, 108.64, 76.14, 53.87, 53.52, 51.11, 41.20, 11.34 ppm; HRMS (ESI, *m/z*) calcd. for $C_{25}H_{25}ClFN_5O_4S$ [M + H]$^+$, 546.1373; found, 546.1383.

4.2.21. Synthesis of (E)-8-chloro-1-cyclopropyl-6-fluoro-7-(4-(2-((2-methoxy-2-oxoethoxy) imino)-2- (thiazol -2 -yl)ethyl) piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6r**)

Compound **6r** was prepared according to the experimental procedure described for compound **6m** starting from intermediate

3f (97 mg, 0.33 mmol), potassium carbonate (62 mg, 0.45 mmol) and compound **4c** (110 mg, 0.30 mmol). The target hybrid **6r** (135 mg) was obtained as yellow solid. Yield: 78.0%; Mp: 205.4–206.2 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 14.42 (bs, 1H, COO*H*), 8.88 (s, 1H, quinolone-2-*H*), 7.99 (d, J = 11.6 Hz, 1H, quinolone-5-*H*), 7.89 (d, J = 3.1 Hz, 1H, thiazole-5-*H*), 7.36 (d, J = 3.1 Hz, 1H, thiazole-4-*H*), 4.84 (s, 2H, OCH_2), 4.35–4.31 (m, 1H, cyclopropane-C*H*), 4.01 (s, 2H, piperazine-CH_2), 3.79 (s, 3H, OCH_3), 3.41 (s, 4H, piperazine-2,2-*N*-(CH_2)$_2$), 2.85 (s, 4H, piperazine-3,3-*N*-(CH_2)$_2$), 1.29 (q, J = 6.9 Hz, 2H, cyclopropane-CH_2), 0.94 (q, J = 6.5 Hz, 2H, cyclopropane-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.80 (quinolone-4-*C*), 169.64 (*C*OOH), 166.06 (*C*=NO), 163.20, 157.06, 155.38, 151.86, 144.70, 143.33, 138.08, 120.89, 119.31, 111.73, 111.57, 108.67, 71.40, 53.88, 51.96, 51.37, 51.19, 41.19, 11.35 ppm; HRMS (ESI, m/z) calcd. for $C_{25}H_{25}ClFN_5O_6S$ $[M + H]^+$, 578.1271; found, 578.1302.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113340.

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