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Discovery of membrane active quinolone-benzimidazoles based topoisomerase inhibitors as potential DNA-binding antimicrobial agents

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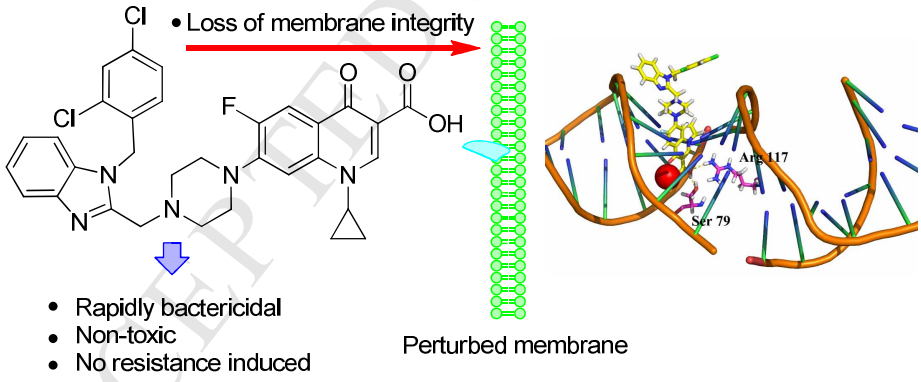
A novel series of membrane active quinolone benzimidazoles were synthesized and screened for their antimicrobial activities. Molecular modeling and experimental investigation with DNA suggested the possible antibacterial mechanism.



Title page

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Discovery of membrane active benzimidazole quinolones-based topoisomerase inhibitors as potential DNA-binding antimicrobial agents

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Abstract:

A series of novel benzimidazole quinolones as potential antimicrobial agents were designed and synthesized. Most of the prepared compounds exhibited good or even stronger antimicrobial activities in comparison with reference drugs. The most potent compound **15m** was membrane active and did not trigger the development of resistance in bacteria. It not only inhibited the formation of biofilms but also disrupted the established *Staphylococcus aureus* and *Escherichia coli* biofilms. It was able to inhibit the relaxation activity of *E. coli* topoisomerase IV at 10 μM concentration. Moreover, this compound also showed low toxicity against mammalian cells. Molecular modeling and experimental investigation of compound **15m** with DNA suggested that this compound could effectively bind with DNA to form a steady **15m**-DNA complex which might further block DNA replication to exert the powerful bioactivities.

***Keywords*:**

Quinolone; Benzimidazole; anti-MRSA; DNA; Topoisomerase

1. Introduction

Bacterial topoisomerases are able to catalyze changes in the topology of DNA and are clinically validated targets for antibacterial drug discovery. The complexity of reactions in DNA replication catalyzed by DNA topoisomerase offers multiple opportunities for therapeutic intervention. Fluoroquinolones, a clinically important kind of synthetic antibacterial agents, primarily target gyrase or topoisomerase in bacteria to stabilize the cleavage complex at specific sites on DNA, then block DNA replication and finally lead to bacterial cells death [1-3]. However, the emergence of resistance to quinolones has become one of the most paramount public health threats in recent years and much effort has been focusing on this topic [4]. Some researches [5] show that an important reason to induce the resistance to quinolones may be ascribed to the gene mutations in partial disruption of the water-metal ion bridge to hinder interactions of quinolone with the catalytic site of enzyme. Further structure activity relationship (SAR) reveals that the groups at C–7 position of quinolones are vicinity to the Arg456 residue in the topo IV–DNA complex whose mutation causes quinolone resistance. Meanwhile, the C–7 substituents in quinolone are capable to influence the cell permeability [6] which were also in relation with bacterial resistance [7]. Therefore, the structural modification of the C–7 position in quinolone nucleus is a promising strategy to extend the antibacterial spectrum and overcome the drug-resistance [8-10].

Benzimidazole with special benzene-fused imidazole ring prevalently present in a range of bioactive agents (e.g. albendazole, mebendazole, candesartan, astemizole) [11,12] has drawn much attention in medicinal chemistry especially antimicrobial aspect in very recent years [13,14]. Benzimidazole derivatives have been revealed to be specific topoisomerase inhibitors. It could bind to the minor groove of DNA, trap the reversible cleavable complex and produce highly specific single-strand DNA breakage [15]. Particularly, the C–2 alkylations of the benzimidazole nucleus have been actively investigated. Moreover, benzimidazoles as purine-like mimics are sometimes addressed as 1,3-dideaza-purines and are always used as model compounds to compete with purines [16]. These benzimidazole types of purine mimics have the potential to kill the bacterial strains or inhibit their growth through inhibition of the synthesis of nucleic acids and proteins inside the bacterial cell wall with distinct mechanism from that of common antibacterial drugs. All the above observations reveal that the development of benzimidazoles as novel antibacterial agents may be served as a good strategy to exploit new anti-infective agents with better activity and less bacterial resistance.

In view of this situation and as an extension of our previous work [17], it is of great interest for us to combine quinolones through their C–7 position with benzimidazole ring to generate a new structural type

of potentially antimicrobial hybrids and investigate their effect on antimicrobial activity (Figure 1). It is expected for these hybrids of two different type of topoisomerase inhibitors to have large potentiality in the treatment of disease-caused bacteria. Design of structures for target compounds was mainly from three aspects: (I) Structural modification on the benzimidazole nucleus; (II) Structural change on the quinolone ring; (III) The variation of linker between benzimidazole and quinolone nucleus. The related considerations were given as follows:

(1) Much literature evidenced that linkers could exert large effect on biological potency by regulating the lipid-water partition coefficient and binding affinity to target enzyme [18-21], thus various linkers between benzimidazole and quinolone nucleus in target compounds were incorporated, including no linker and flexible aliphatic chain such as different alkyl chains (e.g. one carbon -CH_2-, two carbon -CH_2–CH_2- groups);

(2) It is well known that amino moiety is an important structural fragment prevalently present in numerous clinical drugs, in which it could form hydrogen bonds, coordinate with metal ions and accept protons and/or perform quaternization to not only beneficially regulate physicochemical properties of desired molecules but also helpfully interact with various enzymes and receptors in biological system. Therefore, the -CH_2–NH- amino moiety as bioisostere of -CH_2–CH_2- linker was introduced into target compounds (Scheme 4). The *N*-Mannich reaction was successfully developed to achieve this goal in this paper. It is believed that this *N*-Mannich functional group could influence the bioactivities by the enhancement of cell permeability [22];

(3) Schiff bases (-C=N-) as important structural fragment in many antibacterial agents had the capacity to modulate the pharmacokinetic properties and enhance the antimicrobial efficacy [23]. As a comparison with the above -CH_2–NH- group, the rigid Schiff bases (-C=N-) was incorporated into target compounds (Scheme 4) to investigate its effect on antimicrobial activities;

(4) Numerous works revealed that substituents could significantly influence the bioactivities [24-26]. Especially, it was proposed that proper lipophilicity was a very important factor in the quinolones intestinal absorption. Rationally, various aliphatic chains with different lengths and substituted phenyl moieties including chloro, fluoro and nitro groups were further modified at the selected most active compounds based on the preliminary active screening (Scheme 3);

(5) The substituted groups on quinolone nucleus were also considered to investigate the effects of the substituents in the quinolone nucleus on antibacterial activities.

Figure 1

The designed structures of this series of novel benzimidazole quinolones are shown in Schemes 1–4. All the newly synthesized compounds were characterized by spectral analysis and evaluated for their antimicrobial activities *in vitro* against four Gram-positive bacteria, six Gram-negative bacteria and five fungi according to the National Committee for Clinical Laboratory Standards (NCCLS). Bactericidal kinetic assay, antibiofilm activity, bacterial membrane permeabilization, antibacterial resistance study and cytotoxicity as well as the preliminary antimicrobial mechanism with topoisomerase and DNA to highly active compound were also investigated.

2 Results and discussion

2.1. Chemistry

The synthetic route of target benzimidazole quinolones was outlined in Schemes 1–4. Initially, *N*-formyl quinolone **2** was selected as intermediate to construct the target benzimidazole-quinolone hybrid in which the 2-position of benzimidazole is directly linked with the piperazine ring in quinolone, and it could be conveniently obtained by the treatment of commercially available quinolone **1a** with formamide at 70 ºC. Its structure was confirmed by spectra and also supported by single crystal X-ray diffraction analysis (Figure 2, supporting information). However, the reaction of *N*-formyl derivative **2** with benzene-1,2-diamine was difficult *via* cyclization to produce benzimidazoles. This might be attributed to the undesired acid effect of carboxyl group to the acid-sensitive amino material. To confirm this hypothesis, quinolones **1a**–**b** were esterified to afford the corresponding esters **3a**–**b** in good yields, and further treated with formamide to give *N*-formyl intermediates **4a**–**b** in high yields. The following cyclization in 1,4-dioxane with anhydrous copper sulfate was able to occur smoothly to access the desired bezimidazoles **5a**–**b**, and then were further hydrolyzed at 100 ºC in the presence of sodium hydroxide solution (3%) to afford the target benzimidazole quinolones **6a**–**b** with yields of 85.2–87.8% (Scheme 1).

Scheme 1

The benzimidazole-quinolone hybrids **13**–**16** with different linkers and substituents were synthesized from haloalkyl benzimidazoles **8a**–**f**, **10a**–**h** and **12a**–**g** which were prepared from *o*-phenylene diamines and halogen-substituted carboxylic acids. The intermediates 2-chloromethyl benzimidazoles **8a**–**f** were easily obtained by the direct reaction of a series of *o*-phenylene diamine or its substituted benzene derivatives respectively with chloroacetic acid or chloropropionic acid. The alkylation of *o*-phenylene diamine with alkyl halides and then cyclization with chloroacetic acid produced the desired intermediates

N-alkyl benzimidazoles **10a**–**h** in high yields. *N*-Halobenzyl derivatives **12a**–**g** were provided in quantitative yields by the reaction of *o*-phenylene diamine with a series of halobenzyl halides, and then treatment with chloroacetic acid (Scheme 2). These prepared benzimidazole halides **8a**–**f**, **10a**–**h** and **12a**–**g** respectively were further coupled with the piperazine in quinolones **1a**–**b** to smoothly produce the target benzimidazole-quinolone hybrids **13**–**16** (Scheme 3).

Scheme2

Scheme 3

The -CH_2–NH- bridged benzimidazole quinolone hybrids **17a**–**b** were conveniently prepared in the yields of 29.4–31.5% *via* Mannich reaction starting from quinolones **1a**–**b** and paraformaldehyde (Scheme 4). Shiffbase-linked hybrids **18a**–**b** were smoothly obtained in 39.5–41.4% yields by condensation reaction of *N*-formyl quinolone esters **4a**–**b** with 2-aminobenzimidazole. However, these prepared esters **18a**–**b** could not be hydrolyzed to produce desired target carboxyl derivatives, which might be related with the base-sensitive shiffbases (Scheme 4). It was also noteworthy that the direct reaction of *N*-formyl quinolone **2** with 2-aminobenzimidazole *via* condensation was difficult to go.

All the newly synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR, MS and HRMS spectra. Their spectral analyses were consistent with the assigned structures and the spectral data were given in the experimental section.

Scheme 4

2.2. Biological activity

All the newly prepared compounds were evaluated for antimicrobial activities *in vitro* against four Gram-positive bacteria (*Staphylococcus aureus* ATCC25923, *Methicillin-resistant Staphylococcus aureus* N315, *Bacillus subtilis* ATCC6633 and *Micrococcus luteus* ATCC4698), six Gram-negative bacteria (*Escherichia coli* DH52, *Escherichia coli* JM109, *Shigella dysenteriae*, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC13315 and *Salmonella enterica* ATCC14028) and five fungi (*Candida albicans* ATCC10231, *Candida mycoderma* ATCC9888, *Candida utilis* ATCC9950, *Saccharomyces cerevisia* ATCC9763 and *Aspergillus flavus* ATCC204304) using two-fold broth dilution method in 96-well microtest plates recommended by NCCLS [27]. The biological tests were carried out in triplicate. Minimal inhibitory concentration (MIC, μg/mL) was defined as the lowest concentration of the tested new compounds that completely inhibited the growth of strains. Currently available antimicrobial drugs such as

chloromycin, norfloxacin, ciprofloxacin and fluconazole were used as standard drugs. Clinafloxacin, a newly synthesized fluoroquinolone antimicrobial agent that has been demonstrated to be more active than currently available fluoroquinolones with expanded activity against most bacteria was also selected as standard [10a].

The obtained results (depicted in Table 1 and supporting information: Table S1) revealed that most of the prepared benzimidazole quinolones could effectively inhibit the growth of all the tested strains *in vitro*. Compounds **13a** and **13f** with methylene linker between benzimidazole and the piperazine ring in quinolone gave low MIC values in the range of 0.125–2 μg/mL which exhibited obvious stronger antimicrobial efficacy in comparison with norfloxacin (MIC = 1–16 μg/mL) and ciprofloxacin (MIC = 0.25–1 μg/mL). In contrast to this, the directly linked ones **6a**–**b** (MIC = 0.5–8 μg/mL), and CH_2CH_2 (compounds **16a**–**b**: MIC = 0.5–128 μg/mL), CH_2NH (compounds **17a**–**b**: MIC = 0.25–16 μg/mL) or C=N (compounds **18a**–**b**: MIC = 2–128 μg/mL) bridged derivatives all gave generally bigger MIC values. This fact that either the decrease or increase of the linker length was unfavorable for the bioactivity, demonstrated the proper length of linker exerted an important influence in enhancing bioactivity, and the methylene linker was the most suitable one. Further, the replacement of one CH_2 moiety in the CH_2CH_2 linker (compounds **16a–b**) by NH group (compounds **17a–b**) was beneficial for the activity.

The introduction of chloro, fluoro, bromo or nitro group into the 5(6)-position of benzimidazole nucleus resulted in lower activity and they contributed to the antibacterial activities in the order of chloro ＞ fluoro ＞ nitro ＞ bromo group. Among them, chloro modified derivative **13h** showed obvious increase in inhibiting the growth of pathogenic bacterial strains (MIC = 0.25–0.5 μg/mL) in comparison with reference drug chloromycin (MIC = 8–32 μg/mL) and superior to norfloxacin (MIC = 1–16 μg/mL), ciprofloxacin (MIC = 1–2 μg/mL) and clinafloxacin (MIC = 0.25–1 μg/mL) respectively.

The further structure–activity relationship demonstrated that the significant effect of the substituents at the *N*-position of benzimidazoles on biological activity. Most of *N*-alkyl derivatives **14a**–**p** displayed moderate antibacterial activity against the tested bacteria. In comparison with other bacteria, they were relatively less sensitive to *E. coli* JM109 and *S. aureus*. Among all the alkyl derivatives, compounds **14e** and **14m** with octyl group gave the best bioactivity with MIC values in the range of 0.5–32 μg/mL. The replacement of octyl group by ethyl or propyl fragment resulted in low activity towards the tested strains. Moreover, when the alkyl substituents were extended to decyl, dodecyl and octadecyl groups, compounds showed relatively weaker inhibitory activity. The short alkyl chains with poor lipophilicity and long alkyl ones with high lipophilicity in these compounds might make them unfavorable to be delivered to the

binding sites.

In comparison with the alkyl derivatives **14a**–**p**, most of halobenzyl ones **15a**–**n** exerted relatively better activities in inhibiting the growth of the tested strains. Notably, in this series of halobenzyl derivatives, 2,4-dichlorobenzyl hybrid **15m** gave the best antibacterial efficiencies with MIC values of 0.0312 to 8 μg/mL towards the corresponding bacteria. Noticeably, this compound demonstrated effective bioactivity against *M. luteus* (MIC = 0.0625 μg/mL), *B. proteus* (MIC = 0.0312 μg/mL), and *S. enterica* (MIC = 0.0312 μg/mL) which were 128– and 1025–fold more potent than the reference drug chloromycin, and 32-fold more active than norfloxacin and ciprofloxacin. Particularly, it was even 8–, 16– and 8–fold more active than clinafloxacin. Besides, compound **15i** with 3-chlorophenyl group also displayed fairly good antibacterial activities with the MIC values in the range of 0.125–8 μg/mL in comparison with reference drug chloromycin (MIC = 8–32 μg/mL) and superior to norfloxacin (MIC = 1–16 μg/mL), ciprofloxacin (MIC = 1–2 μg/mL) and clinafloxacin (MIC = 0.25–1 μg/mL) respectively. The replacement of chlorobenzyl moiety by fluorobenzyl group (compounds **15j** and **15k**) resulted in unfavorable potency against all the tested strains at the concentrations of 0.25–256 μg/mL. The substitution of chlorine atom in compound **15m** by nitro group to give derivative **15n** was not beneficial for the antibacterial activities. These results manifested that 2,4-dichlorophenyl derivative **15m** should be worthy to be further investigated as potential antimicrobial agent.

Excitedly, the directly linked benzimidazole hybrid **6b**, methylene bridged ones **13f** and **13h**, halobenzyl derivatives **15a**, **15f**, **15i**–**j** and **15m**, NH_2CH_2 linked ones **17a**–**b** all exhibited good biological activity against MRSA with quite low MIC values of 0.125–0.5 μg/mL which were more active than chloromycin (MIC = 16 μg/mL), norfloxacin (MIC = 8 μg/mL), ciprofloxacin (MIC = 2 μg/mL) and clinafloxacin (MIC = 1 μg/mL). Especially, methylene bridged **13f** (MIC = 0.125 μg/mL), **15a** (MIC = 0.125 μg/mL), **15f** (MIC = 0.125 μg/mL) and **15m** (MIC = 0.125 μg/mL) exerted superior anti-MRSA activity to norfloxacin (MIC = 8 μg/mL), ciprofloxacin (MIC = 2 μg/mL) and clinafloxacin (MIC = 1 μg/mL). These implied that this new type of benzimidazole-quinolone hybrids should have the possibility to be developed as anti-MRSA agents.

In addition, the *in vitro* antifungal evaluation were also evaluated for these prepared benzimidazole quinolone hybrids and revealed that they exhibited moderate antifungal activities against most of the tested fungal strains (supporting information).

Table 1

2.3 Bactericidal kinetic assay

In order to understand the antibacterial potency of the prepared compounds, time−kill kinetics experiments of highly active benzimidazole quinolone hybrid **15m** against MRSA were performed. As shown in Figure 3, it revealed more than 3 log (CFU/mL) reduction in the number of viable bacteria within an hour at a concentration of 6 × MIC. The result manifested that this compound had rapid-bactericidal activity.

Figure 3

2.4 Antibiofilm activity

Bacteria with biofilms are inherently insensitive to antimicrobial agents and they are much more resistant to conventional antibiotic treatment [7]. Moreover, biofilms account for more than 80% of microbial infections in humans. Compound with the ability not only to inhibit bacterial biofilm formation but also to disperse established biofilms would be an ideal agent to tackle infections caused by bacteria. Both Gram-positive MRSA and Gram-negative *E. coli* with biofilms are known to cause many infections in humans. Hence the ability of compound **15m** to inhibit both MRSA and *E. coli* DH52 biofilm formation was evaluated. Further, the ability of target compound to disperse established MRSA and *E. coli* DH52 biofilms was also investigated.

2.4.1 Biofilm inhibition

Compound **15m** was found to be an effective inhibitor for both MRSA and *E. coli* DH52 biofilm formation. The IC_{50} (the concentration of the compounds that inhibits 50% biofilm development) values of **15m** against MRSA and *E. coli* DH52 biofilm formation were found to be 0.125 and 0.25 μg/mL, respectively (Figure 4), which made it a promising antibiofilm agent.

Figure 4

2.4.2 Biofilm disruption

Compound **15m** was selected to evaluate the efficiency to eradicate the established MRSA and *E. coli* DH52 biofilms. Matured MRSA biofilm (developed for 24 h) having an initial count of 11.8 $\log_{10}$ CFU/mL of bacteria was treated at four different concentrations (0.125, 0.25, 1 and 2 μg/mL). After the treatment, the cell viabilities in biofims decreased to 10.5, 9.6, 5, and 0.1 $\log_{10}$ CFU/mL at 0.125, 0.25, 1 and 2 μg/mL, whereas cell viability in untreated biofilm increased to 13.6 $\log_{10}$ CFU/mL. Thus, the EC_{50} (the concentration of compound that reduces 50% bacterial titer of a preformed biofilm) value of the biocide was 0.76 μg/mL. More importantly, the biocide at the highest tested concentration (2 μg/mL) showed about

zero cell viability in 24 h MRSA biofilm indicating nearly complete eradication of established biofilm (Figure 5A). Matured *E. coli* biofilm (developed for 48 h) having an initial count of 16.2 $\log_{10}$ CFU/mL of bacteria was similarly treated at four different concentrations (0.25, 0.5, 2, and 4 μg/mL respectively). After the treatment, the cell viabilities in biofims for the different concentrations decreased to 12.2, 10.1, 7.2, and 3.6 $\log_{10}$ CFU/mL at 0.25, 0.5, 2, and 4 μg/mL respectively (Figure 5B). Thus, the EC_{50} value of compound **15m** was 1.5 μg/mL. Moreover, this compound was able to reduce viable bacteria even in 48 h grown matured *E. coli* biofilm. The above results clearly indicated the biocide ability to disperse the biofilms and hence to kill the microorganism present in the biofilm.

Figure 5

2.5 Bacterial membrane permeabilization

Natural and synthetic membrane active antibacterial agents offer a hope to solve the problem of bacterial resistance as the membrane-active nature imparts low propensity for the development of resistance. The ability of compound **15m** to permeabilize the bacterial cell membrane was studied using propidium iodide (PI) (supporting information). This dye can pass through the membrane of compromised bacterial cells and fluoresces upon binding to the DNA [28,29]. As can be seen from Figure 6A and Figure 6B, the tested compound was efficient in permeabilizing the membranes of both Gram-positive (MRSA) and Gram-negative (*E. coli* DH52) bacteria.

Figure 6

2.6 Resistance study

The high-level resistance of norfloxacin to MRSA strains was observed, so we investigated the ability of MRSA to develop drug resistance against the most potent compound **15m**. We exposed a standard strain of MRSA towards increasing concentrations of compound **15m** from sub-MIC for sustained passages and determined the MIC values of compound **15m** for each passage of MRSA. The freshly diluted MRSA strains (1.0×10^5 CFU) in the broth medium in the presence of 0.08 μg/mL (2/3 MIC) of compound **15m** were cultured at 37 ºC for 12 h on a shaker bed at 90 rpm, and tested its sensitivity to 0.125 μg/mL (the original MIC) of compound **15m** for each passage of MRSA. After 12 passages in the presence of two-thirds of the MIC of compound **15m**, bacterial resistance to the original MIC of compound **15m** (0.125 μg/mL) did not emerge, and the MIC values just changed between 0.125 μg/mL and 0.5 μg/mL. To make comparative analysis, parallel cultures were exposed to 2-fold dilutions of norfloxacin as a positive control. By contrast, MRSA quickly developed resistance to norfloxacin, showing resistance to the original MIC (8

μg/mL) after 6 passages. This assay indicated that bacteria MRSA did not develop resistance easily against compound **15m** as they did against norfloxacin [30].

2.7 Inhibition of DNA Relaxation Activity of EcTopo IV.

The DNA relaxation assay was done for the newly synthesized compound **15m**. In this experiment, superhelical plasmid DNA was incubated with EcTopo IV at increasing concentrations of the prepared compound (1, 5, 10, 25, and 50 μM) and DNA relaxation products were then resolved by gel electrophoresis on 1% agarose gel. Compound **15m** was able to inhibit the relaxation activity of EcTopo IV at 10 μM concentration (Figure S1, supporting information).

2.8 Cytotoxicity.

The cytotoxic activities of compound **15m** were tested by Cell Counting Kit-8 (CCK8) method. The *in vitro* cytotoxicity was evaluated in HEK293 (human embryonic kidney 293) cells. As shown in Figure 7, compound **15m** showed relative low toxicity to normal human embryonic kidney cells at high concentration, and the IC_{50} value was 106 μg/mL.

Figure 7

2.9 Molecular modeling

To rationalize the observed antibacterial activity and understand the possible mechanism of the target benzimidazole quinolones, a flexible ligand receptor docking investigation was undertaken. The crystal structure data (topoisomerase IV–DNA complex) were obtained from the protein data bank (PDB code: NA1059), which was representative target to investigate the antibacterial mechanism of quinolones [5,31]. Target compound **15m** and clinafloxacin were selected to dock with the topoisomerase IV–DNA complex.

According to the docking evaluation, hybrid **15m** possessed high total score (14.30) even superior to clinafloxacin (score: 12.45) against topoisomerase IV–DNA complex. Interactions of compound **15m** and clinafloxacin with topoisomerase IV–DNA receptor were shown in Figure 8(i-iii). The docking result of compound **15m** with topoisomerase IV–DNA complex might rationalize the possible antibacterial mechanism. The carboxyl group of this molecule was in close vicinity to the residue Arg117 of the topoisomerase IV–DNA complex. The prepared molecule **15m** could also form hydrogen bonds with Ser79 of topoisomerase IV–DNA complex through the hydrogen atom of carboxyl group. Furthermore, compound **15m** could intercalate into superhelical DNA of the enzyme–DNA complex (Figure 8 (iii) and supporting information: Figure S2). This cooperative binding might be beneficial to stabilize the

quinolone–DNA enzyme complex, which might be responsible for the strong inhibitory efficacy of compound **15m** against MRSA. Since clinafloxacin suffered the exposed severe side effects including phototoxicity and photohemolysis, these benzimidazole quinolones are worthy to be further investigated as potential candidates for infective chemotherapy.

Figure 8

2.10 Interactions with DNA

Based on the good activities of target compounds and the high score results of molecular modeling, the incorporation of benzimidazole might improve the interaction between target compounds and DNA of topoisomerase IV–DNA complexes. So the interaction between the most active compound **15m** and calf thymus DNA (CT DNA, a DNA model with medical importance, low cost and ready availability properties) was evaluated to further explore the preliminary antimicrobial mechanism. In contrast to this interaction between compound **15m** and DNA, the reference drug norfloxacin was employed to investigate the interaction with CT DNA.

Figure 9

With a fixed concentration of DNA, UV–vis absorption spectra were recorded with the increasing amount of compound **15m**. As shown in Figure 9, UV–vis spectra displayed that the maximum absorption peak of DNA at 260 nm exhibited proportional increase and slight red shift with the increasing concentration of compound **15m**. Meanwhile the phenomenon displayed that the absorption value of simply sum of free DNA and free compound **15m** was a little greater than the measured value of **15m**–DNA complex. These meant a weak hypochromic effect existed between DNA and compound **15m**. Furthermore, the intercalation of the aromatic chromophore of compound **15m** into the helix and the strong overlap of π-π* states of the large π-conjugated system with the electronic states of DNA bases were consistent with the observed spectral changes [32,33].

On the basis of variations in the absorption spectra of DNA upon binding to **15m**, equation (1) can be utilized to calculate the binding constant (K).

$$\frac{A^0}{A-A^0}=\frac{\xi_C}{\xi_{D-C}-\xi_C}+\frac{\xi_C}{\xi_{D-C}-\xi_C}\times\frac{1}{K[Q]} \qquad (1)$$

A^0 and A represent the absorbance of DNA in the absence and presence of compound **15m** at 260 nm, ξ_C and $\xi_{D\text{-}C}$ are the absorption coefficients of compound **15m** and **15m**–DNA complex respectively. The plot of $A^0/(A\text{-}A^0)$ versus 1/[compound **15m**] is constructed by using the absorption titration data and linear

fitting (Figure 10), yielding the binding constant, K = 4.30 ×10^4 L/mol, R = 0.999, SD = 0.24 (R is the correlation coefficient. SD is standard deviation).

Figure 10

As shown in Figure 11, there were some differences between norfloxacin and compound **15m**. The hyperchromism of DNA–norfloxacin complex was observed. With the gradually increasing concentration of compound **15m** or norfloxacin, the DNA–compound **15m** complex showed obviously stronger spectral change than DNA–norfloxacin complex, which suggested the new benzimidazole quinolone gave better interaction with DNA. Therefore, this type of interaction with DNA might improve binding ability between these compounds and topo–DNA complex.

Figure 11

3. Conclusion

In conclusion, a series of benzimidazole quinolones were successfully synthesized by a convenient and efficient procedure. Their structures were confirmed by ^{1}H NMR, ^{13}C NMR, MS, IR and HRMS spectra. The *in vitro* antimicrobial evaluation revealed that most of the synthesized compounds exhibited good bioactivities against the tested bacterial strains especially against MRSA even superior to reference drugs. Structure–activity relationship indicated that lengths of linker exerted obvious influence in enhancing bioactivity, and the methylene linker was the most active one. The prepared compound **15m** showed significant inhibition against all the tested bacterial strains with low inhibitory concentrations (MIC = 0.0312–8 μg/mL). Additionally, it not only inhibited the formation of biofilm but also dispersed the established bacterial biofilms. Further, it also displayed low toxicity against mammalian cells and did not trigger development of resistance in bacteria. Thus, this membrane-active antibacterial compound could be potentially used as therapeutic agent to treat bacterial infections caused by multidrug-resistant bacteria. Moreover, the most active compound **15m** was able to inhibit the relaxation activity of *E. coli* topoisomerase IV at 10 μM concentration. Further molecular modeling indicated the good antibacterial activity of the prepared compound **15m** against quinolone-resistant bacterial strains and the possible mechanism might be explained by the noncovalent interaction between compound **15m** and topo IV–DNA complex, especially these hydrogen bonds between molecule and Ser79 (resistance mutation region of topo IV–DNA complex). The preliminary interactive investigations of compound **15m** with CT DNA revealed that it gave better interaction with DNA than norfloxacin, which might improve binding ability between this compound with topo IV–DNA complex.

4. Experimental protocols

4.1 General methods

Melting points were recorded on X-6 melting point apparatus and uncorrected. TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm^{-1} range. NMR spectra were recorded on a Bruker AV 300 and 600 spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), as well as multiplet (m). The mass spectra were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with ESI resource. All fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan) equipped with 1.0 cm quartz cells, the widths of both the excitation and emission slit were set as 2.5 nm. The UV spectrum was recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. Tris(hydroxymethyl) aminomethane (Tris) and concentrated HCl were analytical purity. Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and were used without further purification.

*4.1.1. 1-Ethyl-6-fluoro-7-(4-formylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2**)*

Norfloxacin **1a** (2.000 g, 0.006 mol) was stirred in formamide (50 mL) at 70 ℃ for 2 h. After the mixture was kept in the cold overnight, the formed solid was filtered off and recrystallized with methanol to give the pure target compound **2** as pale solid (1.457 g). Yield: 70%; mp: > 250 °C (literature [21] mp: 292–293 °C). IR (KBr, cm^{-1}) ν: 3055 (aromatic C-H), 2937, 2813 (CH_3, CH_2), 1726, 1721 (C=O), 1615, 1559, 1538, 1517, 1447 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.96 (s, 1H, quinolone 2-*H*), 8.13 (s, 1H, C*H*O), 7.94 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.23 (d, *J* = 7.2 Hz, 1H, quinolone 8-*H*), 4.60 (q, *J* = 7.0 Hz, 2H, quinolone N-CH_2), 3.61 (dd, *J* = 10.5, 6.8 Hz, 4H, piperazine N-CH_2), 3.35 (d, *J* = 5.2 Hz, 2H, piperazine N-CH_2), 3.31–3.28 (m, 2H, piperazine N-CH_2), 1.43 (t, *J* = 7.1 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 165.4, 165.9, 159.1, 154.8, 145.2, 142.7, 138.3, 118.3, 106.0, 105.3, 99.7, 48.5, 46.1, 44.4, 42.0, 40.5, 34.8, 8.6 ppm; MS (m/z): 348 $[M+H]^+$; HRMS (TOF) calcd. for $C_{17}H_{18}FN_3O_4$: $[M+H]^+$, 348.1360; found, 348.1367.

*4.1.2. 1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid methyl ester (**3a**)*

Compound **3a** was prepared according to the literature procedure starting from norfloxacin (3.190 g,

0.010 mol), thionyl chloride (15 mL) and methanol (150 mL). Yield: 67%; mp: 190–191 °C. (literature [34] mp: 189–190 °C)

*4.1.3. 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid methyl ester (**3b**)*

Compound **3b** was prepared according to the literature procedure starting from ciprofloxacin (3.310 g, 0.010 mol), thionyl chloride (15 mL) and methanol (150 mL). Yield: 69%. (literature [35] mp: 272–274 °C)

*4.1.4. 1-Ethyl-6-fluoro-7-(4-formylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid methyl ester (**4a**)*

Compound **3a** (3.330 g, 0.010 mol) was stirred in formamide (50 mL) at 70 ℃ for 2 h. After the mixture was kept in the cold overnight, the formed solid was filtered off and recrystallized with methanol to give the pure target compound **4a** as pale solid (3.480 g). Yield: 96.4%; mp: 148–150 °C; IR (KBr, cm^{-1}) ν: 3055 (aromatic C-H), 2938, 2812 (CH_3, CH_2), 1725, 1720 (C=O), 1614, 1555, 1536, 1507, 1448 (aromatic frame); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.47 (s, 1H, quinolone 2-*H*), 8.13 (s, 1H, C*H*O), 8.11 (d, *J* = 13.0 Hz, 1H, quinolone 5-*H*), 6.78 (d, *J* = 6.7 Hz, 1H, quinolone 8-*H*), 4.23 (q, *J* = 7.2 Hz, 2H, quinolone N-CH_2), 3.93 (s, 3H, OCH_3), 3.81–3.77 (m, 2H, piperazine N-CH_2), 3.63–3.60 (m, 2H, piperazine N-CH_2), 3.30–3.26 (m, 2H, piperazine N-CH_2), 3.23–3.20 (m, 2H, piperazine N-CH_2), 1.55 (t, *J* = 7.2 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ: 176.8, 168.2, 161.6, 157.9, 155.9, 149.2, 143.7, 131.2, 119.5, 109.0, 105.5, 99.8, 47.2, 46.2, 44.7, 44.2, 40.7, 35.0, 9.6 ppm; MS (m/z): 362 $[M+H]^+$; HRMS (TOF) calcd. for $C_{18}H_{21}FN_3O_4$: $[M+H]^+$, 362.1516; found, 362.1518.

*4.1.5. 1-Cyclopropyl-6-fluoro-7-(4-formylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid methyl ester (**4b**)*

Compound **4b** was prepared according to the procedure described for compound **4a** starting from compound **3b** (3.450 g, 0.010 mol) and formamide (50 mL). The target compound **4b** (3.570 g) was obtained as yellow solid. Yield: 95.8%; mp: 149–150 °C; IR (KBr, cm^{-1}) ν: 3085 (aromatic C-H), 2958, 2818 (CH_3, CH_2), 1726, 1721 (C=O), 1611, 1557, 1536, 1505, 1445 (aromatic frame); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.45 (s, 1H, quinolone 2-*H*), 8.14 (s, 1H, C*H*O), 8.09 (d, *J* = 12.9 Hz, 1H, quinolone 5-*H*), 6.71 (d, *J* = 6.6 Hz, 1H, quinolone 8-*H*), 3.87 (m, 1H, cyclopropyl-C*H*), 3.90 (s, 3H, OCH_3), 3.79–3.75 (m, 2H, piperazine N-CH_2), 3.62–3.60 (m, 2H, piperazine N-CH_2), 3.30–3.25 (m, 2H, piperazine N-CH_2), 3.22–3.19 (m, 2H, piperazine N-CH_2), 1.36 (d, *J* = 6.2 Hz, 2H, cyclopropyl-CH_2), 1.23 (d, *J* = 6.8 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, $CDCl_3$) δ: 175.4, 168.8, 163.7, 158.1, 155.5, 149.1, 144.1,

132.5, 119.4, 109.1, 105.3, 99.7, 47.3, 46.1, 44.8, 44.3, 40.8, 35.1, 9.9 ppm; MS (m/z): 374 $[M+H]^+$; HRMS (TOF) calcd. for $C_{19}H_{21}FN_3O_4$: $[M+H]^+$, 374.1516; found, 374.1518.

*4.1.6. 7-(4-(1H-Benzimidazol-2-yl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid methyl ester (**5a**)*

To a stirred solution of benzene-1,2-diamine (0.300 g, 2.770 mmol) and copper sulfate (0.005 g, 0.030 mmol) in 1,4-dioxane (15 mL), a solution of compound **4a** (1.000 g, 2.770 mmol) in 1,4-dioxane (5 mL) was added dropwise during 15 min and heated at reflux for 24 h. After the reaction was completed (monitored by TLC, chloroform/methanol (30/1, V/V), the mixture was filtered. After the 1,4-dioxane was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with chloroform/methanol (60/1, V/V) to give the pure target compound **5a** as white power (0.520 g). Yield: 41.7%; mp: 155–157 °C; IR (KBr, cm^{-1}) ν: 3025 (aromatic C-H), 2945, 2825 (CH_3, CH_2), 1721 (C=O), 1622, 1563, 1553, 1545, 1435 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.09 (s, 1H, quinolone 2-*H*), 7.82–7.78 (m, 1H, quinolone 5-*H*), 7.46 (ddd, *J* = 8.8, 6.0, 3.2 Hz, 2H, benzimidazole 4,7-*H*), 7.13 (ddd, *J* = 38.6, 5.8, 3.1 Hz, 2H, benzimidazole 5,6-*H*), 6.38 (s, 1H, quinolone 8-*H*), 4.72 (m, 2H, quinolone N-CH_2), 3.58 (s, 3H, OCH_3), 3.34 (s, 4H, piperazine N-CH_2), 2.48 (s, 4H, piperazine N-CH_2), 1.51 (s, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.4, 154.2, 152.6, 151.7, 148.4, 145.6, 139.7, 122.1, 119.0, 111.5, 111.3, 107.3, 106.8, 79.7, 52.8, 49.8, 49.1, 36.4, 14.0 ppm; MS (m/z): 450 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{25}FN_5O_3$: $[M+H]^+$, 450.1941; found, 450.1942.

*4.1.7. 7-(4-(1H-Benzimidazol-2-yl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid methyl ester (**5b**)*

Compound **5b** was prepared according to the procedure described for compound **5a** starting from compound **4b** (1.000 g, 2.680 mmol) and formamide (50 mL). The target compound **5b** (0.530 g) was obtained as yellow solid. Yield: 42.8%; mp: 157–158 °C; IR (KBr, cm^{-1}) ν: 3085 (aromatic C-H), 2958, 2829 (CH_3, CH_2), 1721 (C=O), 1611, 1557, 1536, 1505, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.17 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.58–7.50 (m, 2H, benzimidazole 4,7-*H*), 7.15 (m, 2H, benzimidazole 5,6-*H*), 6.58 (s, 1H, quinolone 8-*H*), 3.80 (m, 1H, cyclopropyl-C*H*), 3.57 (s, 3H, OCH_3), 3.35 (s, 4H, piperazine N-CH_2), 2.49 (s, 4H, piperazine N-CH_2), 1.35 (d, *J* = 6.1 Hz, 2H, cyclopropyl-CH_2), 1.21 (d, *J* = 6.3 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.3, 154.1, 152.2, 151.7, 148.4, 145.6, 139.7, 122.1, 119.0, 111.5, 111.3, 107.3, 106.8, 52.7, 49.5, 49.2, 35.3, 8.0 ppm; MS (m/z): 462 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{25}FN_5O_3$: $[M+H]^+$, 462.1941; found, 462.1945.

*4.1.8. 7-(4-(1H-Benzimidazol-2-yl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6a**)*

To a stirred solution of aqueous solution sodium hydroxide (3%, 20 mL) was added compound **4a** (0.449 g, 0.001 mol). The mixture was stirred at 100 °C for 2 h. After the reaction was completed (monitored by TLC, chloroform/ methanol 30/1, V/V), the solution was treated with formic acid to approximately reach pH 7. The mixture was filtered and washed with water for three times to give compound **6a** (0.371 g) as white solid. Yield: 85.2%; mp: 157−158 °C; IR (KBr, cm^{-1}) ν: 3085 (aromatic C-H), 2948, 2831 (CH_3, CH_2), 2945, 1721 (C=O), 1611, 1557, 1536, 1505, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.28 (s, 1H, COO*H*), 12.25 (s, 1H, benzimidazole-N*H*), 8.99 (s, 1H, quinolone 2-*H*), 8.02 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.50 (s, 2H, benzimidazole 4,7-*H*), 7.22 (d, *J* = 6.6 Hz, 1H, quinolone 8-*H*), 7.12 (s, 2H, benzimidazole 5,6-*H*), 4.66–4.58 (m, 2H, quinolone N-CH_2), 3.61 (s, 4H, piperazine N-CH_2), 3.27 (s, 4H, piperazine N-CH_2), 1.46 (t, *J* = 6.9 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.5, 153.2, 152.7, 151.5, 148.2, 145.7, 140.1, 123.2, 118.3, 111.6, 111.2, 106.9, 106.5, 79.6, 49.8, 49.0, 36.3, 14.1 ppm; MS (m/z): 436 $[M+H]^+$; HRMS (TOF) calcd. for $C_{23}H_{23}FN_5O_3$: $[M+H]^+$, 436.1785; found, 436.1787.

*4.1.9. 7-(4-(1H-Benzimidazol-2-yl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro quinoline-3-carboxylic acid (**6b**)*

Compound **6b** was prepared according to the procedure described for compound **6a** starting from compound **5b** (0.461 g, 0.001 mol) and aqueous solution sodium hydroxide (3%, 20 mL). The target compound **6b** (0.390 g) was obtained as yellow solid. Yield: 87.8%; mp: 158−159 °C; IR (KBr, cm^{-1}) ν: 3018 (aromatic C-H), 2839 (CH_2), 1720 (C=O), 1625, 1542, 1478, 1467 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.26 (s, 1H, COO*H*), 12.15 (s, 1H, benzimidazole-N*H*), 8.58 (s, 1H, quinolone 2-*H*), 8.03 (d, *J* = 12.3 Hz, 1H, quinolone 5-*H*), 7.49 (s, 2H, benzimidazole 4,7-*H*), 7.22 (d, *J* = 6.5 Hz, 1H, quinolone 8-*H*), 7.11 (s, 2H, benzimidazole 5,6-*H*), 3.80 (m, 1H, cyclopropyl-C*H*), 3.62 (s, 4H, piperazine N-CH_2), 3.26 (s, 4H, piperazine N-CH_2), 1.32 (d, *J* = 6.2 Hz, 2H, cyclopropyl-CH_2), 1.09 (d, *J* = 6.1 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 174.9, 163.5, 153.4, 151.9, 151.1, 148.2, 144.9, 140.1, 123.2, 118.3, 111.6, 111.2, 106.9, 106.5, 79.6, 50.1, 49.0, 35.2, 8.1 ppm; MS (m/z): 448 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{24}FN_5O_3$: $[M+H]^+$, 448.1785; found, 448.1787.

*4.1.10. General procedures for the preparation of intermediates (**8–12**)*

The intermediates **8–12** were prepared according to the previously reported methods [36].

4.1.11. 7-(4-((1H-Benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro

*quinoline-3-carboxylic acid (**13a**)*

A mixture of norfloxacin (1.920 g, 0.006 mol), potassium carbonate (0.830 g, 0.006 mol) and potassium iodide (0.100 g, 0.006 mol) in acetonitrile (100 mL) was stirred at 50 °C for 1 h. After the mixture was cooled to room temperature, compound **8a** (1.000 g, 0.006 mol) was added. The reaction mixture was then heated at 50 °C for 3 h. After the reaction was completed (monitored by TLC, chloroform/methanol (50/1, V/V)), the reaction was cooled to room temperature and treated with formic acid to adjust the pH value to 5.5–6.5. After the acetonitrile was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with chloroform/methanol (60/1, V/V) to give the pure target compound **13a** as white power (1.120 g). Yield: 41.7%; mp: 155–157 °C; IR (KBr, cm^{-1}) ν: 3108 (aromatic C-H), 2939, 2870 (CH_3, CH_2), 1719 (C=O), 1635, 1588, 1478, 1457 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.34 (s, 1H, COO*H*), 12.35 (s, 1H, benzimidazole-N*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.50 (s, 2H, benzimidazole 4,7-*H*), 7.17 (s, 1H, quinolone 8-*H*), 7.16—7.12 (m, 2H, benzimidazole 5,6-*H*), 4.58 (q, *J* = 6.8 Hz, 2H, quinolone N-CH_2), 3.84 (s, 2H, benzimidazole-CH_2), 3.37 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 7.1 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.4, 154.2, 152.6, 151.7, 148.4, 145.6, 139.7, 122.1, 119.0, 111.5, 111.3, 107.3, 106.8, 79.7, 60.2, 55.7, 52.8, 49.8, 49.1, 14.0 ppm; MS (m/z): 450 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{25}FN_5O_3$: $[M+H]^+$, 450.1941; found, 450.1940.

*4.1.12. Ethyl-6-fluoro-7-(4-((6-fluoro-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13b**)*

Compound **13b** was prepared according to the procedure described for compound **13a** starting from compound **8b** (0.554 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.415 g, 0.003 mol) and potassium iodide (0.050 g, 0.003 mol). The target compound **13b** (0.558 g) was obtained as white solid. Yield: 39.8%; mp: 157–158 °C; IR (KBr, cm^{-1}) ν: 3078 (aromatic C-H), 2942, 2862 (CH_3, CH_2), 1728 (C=O), 1614, 1540, 1501, 1442 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.21 (s, 1H, COO*H*), 8.64 (s, 1H, quinolone 2-*H*), 7.87 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.55–7.49 (m, 1H, quinolone 8-*H*), 7.47 (dd, *J* = 8.1, 4.7 Hz, 1H, benzimidazole 7-*H*), 7.03 (dd, *J* = 18.8, 9.2 Hz, 2H, benzimidazole 4,5-*H*), 4.57 (q, *J* = 6.7 Hz, 2H, quinolone N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.33–3.30 (m, 4H, piperazine N-CH_2), 2.69 (s, 4H, piperazine N-CH_2), 1.40 (t, *J* = 7.0 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.4, 165.9, 156.1, 151.6, 151.5, 148.5, 145.6, 141.4, 137.6, 122.7, 118.1, 110.5, 108.3, 106.2, 79.6, 60.1, 52.6, 49.1, 48.0, 13.9 ppm; MS (m/z): 468 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{24}F_2N_5O_3$: $[M+H]^+$, 468.1847; found, 468.1846.

*4.1.13. 7-(4-((6-Chloro-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13c**)*

Compound **13c** was prepared according to the procedure described for compound **13a** starting from compound **8c** (0.603 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.415 g, 0.003 mol) and potassium iodide (0.050 g, 0.003 mol). The target compound **13c** (0.582 g) was obtained as white solid. Yield: 40.1%; mp: 157–159 °C; IR (KBr, cm^{-1}) ν: 3082 (aromatic C-H), 2955, 2861 (CH_3, CH_2), 1718 (C=O), 1615, 1545, 1525, 1493 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.33 (s, 1H, COO*H*), 12.54 (s, 1H, benzimidazole-N*H*), 8.93 (s, 1H, quinolone 2-*H*), 7.89 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.55 (d, *J* = 24.3 Hz, 2H, quinolone 8-*H*, benzimidazole 7-*H*), 7.20–7.14 (m, 2H, benzimidazole 4,5-*H*), 4.58 (d, *J* = 6.6 Hz, 2H, quinolone N-CH_2), 3.86 (s, 2H, benzimidazole-CH_2), 3.38 (s, 4H, piperazine N-CH_2), 2.72 (s, 4H, piperazine N-CH_2), 1.42 (t, *J* = 6.1 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.4, 166.9, 156.1, 152.4, 151.0, 147.5, 146.6, 143.4, 137.5, 123.7, 118.2, 110.4, 107.3, 105.7, 78.6, 61.1, 52.7, 49.9, 48.3, 14.0 ppm; MS (m/z): 485 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{24}ClFN_5O_3$: $[M+H]^+$, 484.1552; found, 484.1554.

*4.1.14. 7-(4-((6-Bromo-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13d**)*

Compound **13d** was prepared according to the procedure described for compound **13a** starting from compound **8d** (0.735 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.415 g, 0.003 mol) and potassium iodide (0.050 g, 0.003 mol). The target compound **13d** (0.558 g) was obtained as yellow solid. Yield: 35.2%; mp: 159–160 °C; IR (KBr, cm^{-1}) ν: 3079 (aromatic C-H), 2940, 2861 (CH_3, CH_2), 1729 (C=O), 1617, 1540, 1504, 1442 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.25 (s, 1H, COO*H*), 12.51 (s, 1H, benzimidazole-N*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.86 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.55 (d, *J* = 24.2 Hz, 2H, quinolone 8-*H*, benzimidazole 7-*H*), 7.21–7.13 (m, 2H, benzimidazole 4,5-*H*), 4.53 (d, *J* = 6.2 Hz, 2H, quinolone N-CH_2), 3.81 (s, 2H, benzimidazole-CH_2), 3.29 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.40 (t, *J* = 6.1 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 177.4, 167.8, 157.2, 153.4, 151.0, 148.3, 145.6, 143.2, 136.3, 124.7, 117.1, 111.4, 106.4, 105.5, 78.7, 61.2, 52.8, 49.8, 46.3, 14.1 ppm; MS (m/z): 529 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{24}BrFN_5O_3$: $[M+H]^+$, 528.1047; found, 528.1046.

*4.1.15. 1-Ethyl-6-fluoro-7-(4-((6-nitro-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13e**)*

Compound **13e** was prepared according to the procedure described for compound **13a** starting from

compound **8e** (0.635 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.415 g, 0.003 mol) and potassium iodide (0.050 g, 0.003 mol). The target compound **13e** (0.536 g) was obtained as yellow solid. Yield: 36.1%; mp: 158–159 °C; IR (KBr, cm^{-1}) ν: 3080 (aromatic C-H), 2949, 2862 (CH_3, CH_2), 1719 (C=O), 1613, 1542, 1522, 1492 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.24 (s, 1H, COO*H*), 12.52 (s, 1H, benzimidazole-N*H*), 8.93 (s, 1H, quinolone 2-*H*), 7.87 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.45 (d, *J* = 22.1 Hz, 2H, quinolone 8-*H*, benzimidazole 7-*H*), 7.20–7.14 (m, 2H, benzimidazole 4,5-*H*), 4.54 (d, *J* = 6.1 Hz, 2H, quinolone N-CH_2), 3.82 (s, 2H, benzimidazole-CH_2), 3.28 (s, 4H, piperazine N-CH_2), 2.70 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 5.9 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 177.5, 167.7, 157.4, 152.8, 151.0, 147.9, 145.6, 144.1, 142.3, 141.7, 133.1, 117.4, 116.4, 115.5, 110.4, 107.5, 103.5, 61.2, 53.8, 49.8, 48.5, 46.3, 14.1 ppm; MS (m/z): 495 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{24}FN_6O_5$: $[M+H]^+$, 495.1792; found, 495.1793.

*4.1.16. 7-(4-((1H-Benzimidazol-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13f**)*

Compound **13f** was prepared according to the procedure described for compound **13a** starting from compound **8a** (1.000 g, 0.006 mol), ciprofloxacin (1.986 g, 0.006 mol), potassium carbonate (0.830 g, 0.006 mol) and potassium iodide (0.100 g, 0.006 mol). The target compound **13f** (1.324 g) was obtained as yellow solid. Yield: 47.8%; mp: 157–158 °C; IR (KBr, cm^{-1}) ν: 3079 (aromatic C-H), 2862 (CH_2), 1727 (C=O), 1619, 1540, 1514, 1442 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.20 (s, 1H, COO*H*), 8.66 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.58–7.50 (m, 3H, benzimidazole 4,7-*H*, quinolone 8-*H*), 7.17 (s, 2H, benzimidazole 5,6-*H*), 3.88 (s, 2H, quinolone N-CH_2), 3.81 (s, 1H, cyclopropyl-C*H*), 3.39 (s, 4H, piperazine N-CH_2), 2.76 (s, 4H, piperazine N-CH_2), 1.31 (d, *J* = 5.9 Hz, 2H, cyclopropyl-CH_2), 1.18 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.4, 154.2, 152.6, 151.7, 148.4, 145.6, 139.7, 122.1, 119.0, 111.5, 111.3, 107.3, 106.8, 79.7, 60.2, 55.7, 52.8, 49.8, 49.1, 36.4, 8.0 ppm; MS (m/z): 462 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{25}FN_5O_3$: $[M+H]^+$, 462.1941; found, 462.1942.

*4.1.17. 1-Cyclopropyl-6-fluoro-7-(4-((6-fluoro-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13g**)*

Compound **13g** was prepared according to the procedure described for compound **13a** starting from compound **8b** (0.554 g, 0.003 mol), ciprofloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **13g** (0.515 g) was obtained as white solid. Yield: 35.8%; mp: 158–159 °C; IR (KBr, cm^{-1}) ν: 3042 (aromatic C-H), 2856 (CH_2), 1726

(C=O), 1618, 1546, 1525, 1491, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.32 (s, 1H, COO*H*), 8.63 (s, 1H, quinolone 2-*H*), 7.88 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.56–7.51 (m, 1H, quinolone 8-*H*), 7.48 (dd, *J* = 8.0, 4.6 Hz, 1H, benzimidazole 7-*H*), 7.03 (dd, *J* = 18.8, 9.2 Hz, 2H, benzimidazole 4,5-*H*), 3.88 (s, 2H, benzimidazole CH_2), 3.74 (dd, *J* = 8.3, 5.1 Hz, 1H, cyclopropyl-C*H*), 3.32–3.30 (m, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.30 (t, *J* = 6.7 Hz, 2H, cyclopropyl-CH_2), 1.16 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.5, 166.3, 156.2, 152.5, 151.5, 147.4, 145.5, 140.5, 138.2, 121.9, 117.1, 111.5, 111.3, 107.3, 106.8, 79.7, 60.2, 52.8, 49.8, 49.1, 35.4, 8.0 ppm; MS (m/z): 480 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{24}F_2N_5O_3$: $[M+H]^+$, 480.1847; found, 480.1848.

*4.1.18. 7-(4-((6-Chloro-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13h**)*

Compound **13h** was prepared according to the procedure described for compound **13a** starting from compound **8c** (0.603 g, 0.003 mol), ciprofloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **13h** (0.497 g) was obtained as white solid. Yield: 33.4%; mp: 158–160 °C; IR (KBr, cm^{-1}) ν: 3029 (aromatic C-H), 2876 (CH_2), 1718 (C=O), 1629, 1541, 1498, 1457 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.29 (s, 1H, COO*H*), 12.34 (s, 1H, benzimidazole-N*H*), 8.88 (s, 1H, quinolone 2-*H*), 7.85 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.71 (d, *J* = 6.2 Hz, 1H, quinolone 8-*H*), 7.55 (d, *J* = 24.3 Hz, 1H, benzimidazole 7-*H*), 7.28–7.13 (m, 2H, benzimidazole 4,5-*H*), 3.88 (s, 2H, benzimidazole-CH_2), 3.70 (s, 1H, cyclopropyl-C*H*), 3.36 (s, 4H, piperazine N-CH_2), 2.70 (s, 4H, piperazine N-CH_2), 1.29 (d, *J* = 6.4 Hz, 2H, cyclopropyl-CH_2), 1.12 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.4, 167.3, 155.3, 151.4, 150.3, 146.3, 145.6, 140.5, 138.1, 120.8, 118.0, 111.5, 110.4, 106.3, 105.3, 78.8, 61.2, 53.7, 49.8, 48.3, 35.3, 8.1 ppm; MS (m/z): 496 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{24}ClFN_5O_3$: $[M+H]^+$, 496.1552; found, 496.1553.

*4.1.19. 7-(4-((6-Bromo-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13i**)*

Compound **13i** was prepared according to the procedure described for compound **13a** starting from compound **8d** (0.737 g, 0.003 mol), ciprofloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **13i** (0.557 g) was obtained as white solid. Yield: 34.4%; mp: 157–158 °C; IR (KBr, cm^{-1}) ν: 3085 (aromatic C-H), 2886 (CH_2), 1721 (C=O), 1611, 1557, 1536, 1505, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.22 (s, 1H, COO*H*), 12.04 (s, 1H, benzimidazole-N*H*), 8.58 (s, 1H, quinolone 2-*H*), 7.65 (d, *J* = 13.2 Hz, 1H,

quinolone 5-*H*), 7.71 (d, *J* = 6.1 Hz, 1H, quinolone 8-*H*), 7.51 (d, *J* = 24.1 Hz, 1H, benzimidazole 7-*H*), 7.25–7.15 (m, 2H, benzimidazole 4,5-*H*), 3.86 (s, 2H, benzimidazole-CH_2), 3.71 (s, 1H, cyclopropyl-C*H*), 3.35 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.25 (d, *J* = 6.3 Hz, 2H, cyclopropyl-CH_2), 1.12 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.5, 167.4, 156.2, 152.5, 150.4, 146.7, 145.2, 142.5, 138.2, 121.8, 117.8, 111.6, 110.3, 107.3, 105.7, 78.7, 61.5, 52.5, 49.7, 48.5, 35.5, 8.1 ppm; MS (m/z): 541 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{24}BrFN_5O_3$: $[M+H]^+$, 540.1047; found, 540.1049.

*4.1.20. 1-Cyclopropyl-6-fluoro-7-(4-((6-nitro-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**13j**)*

Compound **13j** was prepared according to the procedure described for compound **13a** starting from compound **8e** (0.635 g, 0.003 mol), ciprofloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **13j** (0.492 g) was obtained as white solid. Yield: 32.4%; mp: 159–160 °C; IR (KBr, cm^{-1}) ν: 3027 (aromatic C-H), 2836 (CH_2), 1722 (C=O), 1624, 1564, 1555, 1542, 1436 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.27 (s, 1H, COO*H*), 13.16 (s, 1H, benzimidazole-N*H*), 9.06 (d, *J* = 4.9 Hz, 1H, quinolone 2-*H*), 8.71 (s, 1H, quinolone 5-*H*), 8.47 (d, *J* = 24.4 Hz, 1H, benzimidazole 7-*H*), 8.15 (s, 1H, benzimidazole 4,5-*H*), 7.95 (s, 1H, benzimidazole 4,5-*H*), 7.61 (d, *J* = 7.3 Hz, 1H, quinolone 8-*H*), 4.84 (s, 2H, quinolone N-CH_2), 3.87 (s, 1H, cyclopropyl-C*H*), 3.45 (s, 4H, piperazine N-CH_2), 2.81 (s, 4H, piperazine N-CH_2), 1.37 (d, *J* = 6.5 Hz, 2H, cyclopropyl-CH_2), 1.24 (d, *J* = 6.8 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.5, 167.5, 156.3, 152.6, 150.3, 147.5, 145.7, 144.2, 142.5, 141.6, 135.2, 118.8, 116.8, 115.4, 110.3, 107.3, 102.7, 61.6, 52.4, 49.6, 48.2, 35.2, 8.1 ppm; MS (m/z): 507 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{24}FN_6O_5$: $[M+H]^+$, 507.1792; found, 507.1790.

*4.1.21. 1-Ethyl-7-(4-((1-ethyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14a**)*

Compound **14a** was prepared according to the procedure described for compound **13a** starting from compound **10a** (0.558 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14a** (0.980 g) was obtained as white solid. Yield: 66.3%; mp: 150–152 °C; IR (KBr, cm^{-1}) ν: 3018 (aromatic C-H), 2961, 2862, 2849 (CH_3, CH_2), 1718 (C=O), 1621, 1542, 1463, 1454 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.37 (s, 1H, COO*H*), 8.98 (s, 1H, quinolone 2-*H*), 7.94 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.63 (dd, *J* = 24.8, 7.9 Hz, 2H, benzimidazole 4,7-*H*), 7.25 (ddd, *J* = 20.1, 13.5, 7.1 Hz, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*),

4.64–4.58 (m, 2H, benzimidazole N-CH_2), 4.42 (dd, *J* = 13.8, 6.7 Hz, 2H, benzimidazole CH_2), 3.95 (s, 2H, quinolone CH_2), 3.38 (s, 4H, piperazine N-CH_2), 2.75 (s, 4H, piperazine N-CH_2), 1.45 (dd, *J* = 14.5, 7.2 Hz, 6H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 171.8, 167.2, 160.4, 154.5, 151.1, 148.4, 145.9, 140.6, 137.6, 132.9, 129.0, 116.7, 112.2, 108.7, 106.6, 65.5, 60.3, 52.8, 49.8, 44.7, 30.5, 21.2, 19.1, 15.3, 14.5, 14.1 ppm; MS (m/z): 478 $[M+H]^+$; HRMS (TOF) calcd. for $C_{26}H_{29}FN_5O_3$: $[M+H]^+$, 478.2254; found, 478.2257.

*4.1.22. 1-Ethyl-6-fluoro-4-oxo-7-(4-((1-propyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**14b**)*

Compound **14b** was prepared according to the procedure described for compound **13a** starting from compound **10b** (0.647 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14b** (0.930 g) was obtained as white solid. Yield: 61.3%; mp: 152–153 °C; IR (KBr, cm^{-1}) ν: 3118 (aromatic C-H), 2960, 2873, 2852 (CH_3, CH_2), 1719 (C=O), 1655, 1578, 1488, 1449 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.16 (s, 1H, COO*H*), 8.97 (s, 1H), 7.96 (dd, *J* = 12.9, 6.3 Hz, 1H), 7.87 (dd, *J* = 45.1, 7.9 Hz, 2H), 7.55–7.47 (m, 2H), 7.27 (d, *J* = 6.5 Hz, 1H), 4.67 (s, 2H), 4.63 (d, *J* = 6.8 Hz, 2H), 4.44 (t, *J* = 7.3 Hz, 2H), 3.60 (s, 4H), 3.34 (s, 4H), 1.93–1.87 (m, 2H), 1.46 (t, *J* = 7.1 Hz, 3H), 1.00 (t, *J* = 7.3 Hz, 3H) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.6, 166.5, 154.1, 148.9, 144.9, 137.6, 133.9, 125.1, 120.2, 118.3, 116.4, 114.5, 112.6, 111.8, 107.7, 106.4, 52.5, 51.4, 49.5, 48.2, 46.1, 22.7, 14.6, 11.0 ppm; MS (m/z): 492 $[M+H]^+$; HRMS (TOF) calcd. for $C_{27}H_{31}FN_5O_3$: $[M+H]^+$, 492.2411; found, 492.2415.

*4.1.23. 1-Ethyl-6-fluoro-4-oxo-7-(4-((1-pentyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**14c**)*

Compound **14c** was prepared according to the procedure described for compound **13a** starting from compound **10c** (0.710 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14c** (0.940 g) was obtained as white solid. Yield: 58.6%; mp: 156–157 °C; IR (KBr, cm^{-1}) ν: 3029 (aromatic C-H), 2942, 2852, 2842 (CH_3, CH_2), 1717 (C=O), 1624, 1540, 1497, 1456 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.33 (s, 1H, COO*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.58 (dd, *J* = 29.8, 7.9 Hz, 2H, benzimidazole 4,7-*H*), 7.26–7.14 (m, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*), 4.57 (q, *J* = 7.0 Hz, 2H, benzimidazole N-CH_2), 4.33–4.29 (m, 2H, benzimidazole CH_2), 3.89 (d, *J* = 5.3 Hz, 2H, quinolone CH_2), 3.32 (s, 4H, piperazine N-CH_2), 2.70 (s, 4H, piperazine N-CH_2), 1.87–1.80 (m, 2H, CH_2(CH_2)$_2$$CH_3$), 1.44–1.33 (m, 7H, CH_2(CH_2)$_2$CH_3), 0.88 (t, *J* = 6.9 Hz, 3H, quinolone CH_3) ppm; ^{13}C NMR (151 MHz,

DMSO-d_6) δ: 176.6, 166.5, 154.2, 152.5, 151.1, 148.9, 145.9, 142.4, 137.7, 135.9, 122.7, 121.5, 119.4, 111.6, 110.5, 107.8, 106.2, 54.9, 52.8, 49.9, 49.6, 43.9, 29.6, 29.0, 22.3, 14.7, 14.4 ppm; MS (m/z): 520 $[M+H]^+$; HRMS (TOF) calcd. for $C_{29}H_{35}FN_5O_3$: $[M+H]^+$, 520.2724; found, 520.2722.

*4.1.24. 1-Ethyl-6-fluoro-7-(4-((1-hexyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14d**)*

Compound **14d** was prepared according to the procedure described for compound **13a** starting from compound **10d** (0.777 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14d** (0.936 g) was obtained as white solid. Yield: 56.6%; mp: 154–156 °C; IR (KBr, cm^{-1}) ν: 3080 (aromatic C-H), 2945, 2852, 2829 (CH_3, CH_2), 1719 (C=O), 1615, 1540, 1525, 1435 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.93 (s, 1H, quinolone 2-*H*), 7.87 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.58 (dd, *J* = 29.5, 7.7 Hz, 2H, benzimidazole 4,7-*H*), 7.25–7.11 (m, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*), 4.56 (q, *J* = 6.8 Hz, 2H, benzimidazole N-CH_2), 4.34–4.28 (m, 2H, benzimidazole CH_2), 3.89 (d, *J* = 5.3 Hz, 2H, quinolone CH_2), 3.33 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.86–1.81 (m, 2H, CH_2(CH_2)$_3$$CH_3$), 1.45–1.32 (m, 6H, CH_2(CH_2)$_3$$CH_3$), 0.92 (d, *J* = 7.4 Hz, 3H, CH_2(CH_2)$_3$CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.4, 152.8, 151.3, 148.3, 144.8, 141.7, 138.8, 135.8, 122.5, 121.7, 118.5, 111.9, 111.7, 107.5, 106.7, 56.1, 52.7, 49.8, 44.6, 36.3, 31.6, 29.4, 25.5, 22.5, 14.7, 13.5 ppm; MS (m/z): 534 $[M+H]^+$; HRMS (TOF) calcd. for $C_{30}H_{37}FN_5O_3$: $[M+H]^+$, 534.2880; found, 534.2884.

*4.1.25. 1-Ethyl-6-fluoro-7-(4-((1-octyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14e**)*

Compound **14e** was prepared according to the procedure described for compound **13a** starting from compound **10e** (0.864 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14e** (0.775 g) was obtained as white solid. Yield: 43.6%; mp: 156–157 °C; IR (KBr, cm^{-1}) ν: 3015 (aromatic C-H), 2952, 2869, 2840 (CH_3, CH_2), 1718 (C=O), 1626, 1545, 1498, 1457 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.17 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.89 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.57 (dt, *J* = 17.5, 8.5 Hz, 3H, benzimidazole 4,7-*H*, quinolone 8-*H*), 7.21 (dt, *J* = 33.0, 7.2 Hz, 2H, benzimidazole 5,6-*H*), 4.29 (dt, *J* = 25.8, 7.5 Hz, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.32 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.86–1.79 (m, 2H, CH_2(CH_2)$_5$$CH_3$), 1.40–1.17 (m, 10H, CH_2(CH_2)$_5$$CH_3$), 0.84 (t, *J* = 6.7 Hz, 3H, CH_2(CH_2)$_5$CH_3), 0.78 (t, *J* = 6.3 Hz, 3H, quinolone CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.3, 154.3, 154.0, 152.6, 151.1, 145.5, 142.4, 122.5, 121.8, 111.5,

110.7, 107.3, 106.7), 57.1, 54.9, 52.8, 49.9, 44.0, 43.8, 36.3, 31.7, 29.9, 26.9, 26.7, 14.4, 7.9 ppm; MS (m/z): 562 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{41}FN_5O_3$: $[M+H]^+$, 562.3193; found, 562.3195.

*4.1.26. 7-(4-((1-Decyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14f**)*

Compound **14f** was prepared according to the procedure described for compound **13a** starting from compound **10f** (0.951 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14f** (0.791 g) was obtained as white solid. Yield: 42.4%; mp: 163–164 °C; IR (KBr, cm^{-1}) ν: 3090 (aromatic C-H), 2948, 2867, 2850 (CH_3, CH_2), 1718 (C=O), 1630, 1540, 1500, 1459 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.0 Hz, 1H, quinolone 5-*H*), 7.57 (dd, *J* = 31.5, 7.9 Hz, 2H, benzimidazole 4,7-*H*), 7.20 (dt, *J* = 17.8, 7.0 Hz, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*), 4.57 (d, *J* = 6.8 Hz, 2H, benzimidazole N-CH_2), 4.30 (t, *J* = 7.3 Hz, 2H, quinolone N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.32 (s, 4H, piperazine N-CH_2), 2.70 (s, 4H, piperazine N-CH_2), 1.86–1.79 (m, 2H, CH_2(CH_2)$_7$$CH_3$), 1.42−1.09 (m, 17H, CH_2(CH_2)$_7$CH_3), 0.74 (d, *J* = 5.3 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.6, 166.5, 154.1, 151.1, 148.8, 145.8, 142.5, 137.6, 135.9, 122.5, 121.7, 119.4, 111.5, 110.6, 106.1, 54.9, 52.7, 49.9, 49.5, 49.0, 44.0, 31.6, 29.9, 29.4, 29.2, 26.9, 22.5, 14.7, 14.3 ppm; MS (m/z): 590 $[M+H]^+$; HRMS (TOF) calcd. for $C_{34}H_{45}FN_5O_3$: $[M+H]^+$, 590.3506; found, 590.3505.

*4.1.27. 7-(4-((1-Dodecyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14g**)*

Compound **14g** was prepared according to the procedure described for compound **13a** starting from compound **10g** (1.038 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14g** (0.867 g) was obtained as white solid. Yield: 44.4%; mp: 169–170 °C; IR (KBr, cm^{-1}) ν: 3092 (aromatic C-H), 2982, 2878, 2834 (CH_3, CH_2), 1727 (C=O), 1626, 1520, 1489, 1466, 1451 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.95 (s, 1H, quinolone 2-*H*), 7.91 (dd, *J* = 18.0, 12.4 Hz, 1H, quinolone 5-*H*), 7.57 (dd, *J* = 27.9, 7.2 Hz, 2H, benzimidazole 4,7-*H*), 7.19 (dd, *J* = 23.8, 15.7 Hz, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*), 4.57 (s, 2H, quinolone N-CH_2), 4.29 (s, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.31 (s, 4H, piperazine N-CH_2), 2.70 (s, 4H, piperazine N-CH_2), 1.83 (s, 2H, CH_2CH_2(CH_2)$_9$$CH_3$), 1.38–1.06 (m, 21H, $CH_2$$CH_2$(C$H_2$)$_9$$CH_3$, C$H_3$), 0.78 (d, *J* = 5.7 Hz, 3H, $CH_2$$CH_2$($CH_2$)$_9CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.6, 166.5, 154.2, 151.2, 148.9, 145.9, 142.5, 137.6, 136.1, 122.5, 121.7, 119.2, 111.8, 110.7, 107.6, 106.1, 55.0, 52.9, 50.0, 49.4, 43.9, 31.6, 29.8,

29.1, 26.7, 22.5, 14.6, 14.3 ppm; MS (m/z): 618 $[M+H]^+$; HRMS (TOF) calcd. for $C_{36}H_{49}FN_5O_3$: $[M+H]^+$, 618.3819; found, 618.3822.

*4.1.28. 1-Ethyl-6-fluoro-7-(4-((1-octadecyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14h**)*

Compound **14h** was prepared according to the procedure described for compound **13a** starting from compound **10h** (1.298 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **14h** (0.770 g) was obtained as white solid. Yield: 35.4%; mp: 191–193 °C; IR (KBr, cm^{-1}) ν: 3020 (aromatic C-H), 2964, 2848, 2825 (CH_3, CH_2), 1718 (C=O), 1625, 1544, 1494, 1451 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.82 (s, 1H, quinolone 2-*H*), 7.87 (s, 2H, quinolone 5-*H*), 7.80 (s, 1H, quinolone 8-*H*), 7.54 (s, 2H, benzimidazole 4,7-*H*), 7.13 (s, 2H, benzimidazole 5,6-*H*), 4.53 (s, 2H, quinolone N-CH_2), 4.33 (s, 2H, benzimidazole N-CH_2), 3.75 (s, 2H, benzimidazole CH_2), 3.29 (s, 4H, piperazine N-CH_2), 2.68 (s, 4H, piperazine N-CH_2), 1.83 (s, 2H, $CH_2CH_2(CH_2)_{15}CH_3$), 1.38–1.06 (m, 33H, $CH_2CH_2(CH_2)_{15}CH_3$, CH_3), 0.78 (d, *J* = 5.7 Hz, 3H, $CH_2CH_2(CH_2)_{15}CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.4, 152.5, 148.2, 145.3, 139.7, 136.6, 122.5, 121.6, 118.4, 117.1, 114.8, 113.2, 52.6, 49.8, 49.3, 46.3, 31.6, 29.1, 29.0, 27.0, 22.2, 14.1 ppm; MS (m/z): 702 $[M+H]^+$; HRMS (TOF) calcd. for $C_{42}H_{61}FN_5O_3$: $[M+H]^+$, 702.4758; found, 702.4759.

*4.1.29. 1-Cyclopropyl-7-(4-((1-ethyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14i**)*

Compound **14i** was prepared according to the procedure described for compound **13a** starting from compound **10a** (0.360 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14i** (0.617 g) was obtained as white solid. Yield: 63.0%; mp: 150–152 °C; IR (KBr, cm^{-1}) ν: 3090 (aromatic C-H), 2971, 2842, 2821 (CH_3, CH_2), 1725 (C=O), 1626, 1520, 1498, 1477, 1450 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.28 (s, 1H, COO*H*), 8.76 (s, 1H, quinolone 2-*H*), 8.01 (d, *J* = 11.1 Hz, 1H, quinolone 5-*H*), 7.69 (d, *J* = 24.6 Hz, 3H, benzimidazole 4,7-*H*, quinolone 8-*H*), 7.32 (d, *J* = 34.7 Hz, 2H, benzimidazole 5,6-*H*), 4.40 (s, 2H, benzimidazole N-CH_2), 4.01 (s, 2H, benzimidazole CH_2), 3.88 (s, 1H, quinolone N-C*H*), 3.39–3.34 (m, 4H, piperazine N-CH_2), 2.83 (s, 4H, piperazine N-CH_2), 0.90 (d, *J* = 47.8 Hz, 7H, CH_3, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 173.8, 167.3, 160.5, 154.6, 151.0, 147.4, 145.8, 140.5, 137.5, 133.8, 129.0, 116.5, 112.4, 108.6, 106.5, 65.6, 61.2, 53.1, 49.7, 44.6, 30.4, 21.5, 19.4, 15.5, 14.6, 8.1 ppm; MS (m/z): 490 $[M+H]^+$; HRMS (TOF) calcd. for $C_{27}H_{28}FN_5O_3$: $[M+H]^+$, 490.2254; found, 490.2256.

*4.1.30. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((1-propyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**14j**)*

Compound **14j** was prepared according to the procedure described for compound **13a** starting from compound **10b** (0.417 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14j** (0.591 g) was obtained as white solid. Yield: 58.7%; mp: 153–154 °C; IR (KBr, cm^{-1}) ν: 3039 (aromatic C-H), 2948, 2848 (CH_3, CH_2), 1721 (C=O), 1627, 1542, 1497, 1452 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.18 (s, 1H, COO*H*), 8.64 (s, 1H, quinolone 2-*H*), 7.88 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.58 (dd, *J* = 19.4, 8.0 Hz, 3H, quinolone 8-*H*, benzimidazole 4,7-*H*), 7.20 (dt, *J* = 15.0, 7.2 Hz, 2H, benzimidazole 5,6-*H*), 4.31–4.27 (m, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.80 (dt, *J* = 10.5, 3.6 Hz, 1H, cyclopropyl-C*H*), 3.33 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.86 (dd, *J* = 14.9, 7.4 Hz, 2H, $CH_2CH_2CH_3$), 1.30 (q, *J* = 6.6 Hz, 2H, cyclopropyl-CH_2), 1.16 (q, *J* = 6.8 Hz, 2H, cyclopropyl-CH_2), 0.94 (d, *J* = 7.4 Hz, 3H, $CH_2CH_2CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.5, 152.6, 148.4, 145.5, 139.8, 136.0, 122.4, 121.7, 119.5, 111.4, 110.8, 107.3, 106.8, 54.9, 52.6, 49.8, 45.3, 36.3, 23.2, 11.7, 8.1 ppm; MS (m/z): 504 $[M+H]^+$; HRMS (TOF) calcd. for $C_{28}H_{31}FN_5O_3$: $[M+H]^+$, 504.2411; found, 504.2413.

*4.1.31. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((1-pentyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**14k**)*

Compound **14k** was prepared according to the procedure described for compound **13a** starting from compound **10c** (0.473 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14k** (0.602 g) was obtained as white solid. Yield: 56.6%; mp: 154–155 °C; IR (KBr, cm^{-1}) ν: 3033 (aromatic C-H), 2932, 2853, 2820 (CH_3, CH_2), 1728 (C=O), 1604, 1513, 1476 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.18 (s, 1H, COO*H*), 8.64 (s, 1H, quinolone 2-*H*), 7.89 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.61–7.52 (m, 3H, quinolone 8-*H*, benzimidazole 4,7-*H*), 7.21 (dt, *J* = 34.3, 7.4 Hz, 2H, benzimidazole 5,6-*H*), 4.34–4.28 (m, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.79 (dd, *J* = 8.7, 5.2 Hz, 1H, quinolone N-C*H*), 3.32–3.30 (m, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.87–1.81 (m, 2H, C$H_2$$(CH_2)_2CH_3$), 1.36 (dd, *J* = 8.7, 5.3 Hz, 4H, CH_2(C$H_2$$)_2CH_3$), 1.30 (t, *J* = 6.7 Hz, 2H, cyclopropyl-CH_2), 1.16 (s, 2H, cyclopropyl-CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.9, 166.4, 151.2, 148.5, 142.5, 139.6, 135.9, 122.6, 121.8, 119.5, 111.5, 110.7, 107.3, 106.8, 54.9, 52.7, 50.0, 43.9, 36.4, 29.7, 29.0, 22.4, 14.4, 8.0 ppm; MS (m/z): 532 $[M+H]^+$; HRMS (TOF) calcd. for

$C_{30}H_{35}FN_5O_3$: $[M+H]^+$, 532.2724; found, 532.2726.

*4.1.32. 1-Cyclopropyl-6-fluoro-7-(4-((1-hexyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14l**)*

Compound **14l** was prepared according to the procedure described for compound **13a** starting from compound **10d** (0.501 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14l** (0.499 g) was obtained as white solid. Yield: 45.7%; mp: 154–155 °C; IR (KBr, cm^{-1}) ν: 3011 (aromatic C-H), 2935, 2843 (CH_3, CH_2), 1727 (C=O), 1600, 1515, 1478 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.18 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.61–7.53 (m, 3H, quinolone 8-*H*, benzimidazole 4,7-*H*), 7.21 (dt, *J* = 33.9, 7.4 Hz, 2H, benzimidazole 5,6-*H*), 4.34–4.28 (m, 2H, benzimidazole N-CH_2), 3.89 (s, 2H, benzimidazole CH_2), 3.79 (dd, *J* = 6.5, 3.1 Hz, 1H, quinolone N-C*H*), 3.26 (s, 4H, piperazine N-CH_2), 2.72 (s, 4H, piperazine N-CH_2), 1.83 (dt, *J* = 15.2, 7.7 Hz, 2H, CH_2(CH_2)$_3$$CH_3$), 1.38 (dd, *J* = 14.7, 6.9 Hz, 2H, CH_2CH_2(CH_2)$_2$$CH_3$), 1.34–1.25 (m, 4H, CH_2CH_2(CH_2)$_2$$CH_3$), 1.19–1.13 (m, 4H, cyclopropyl-CH_2), 0.84 (t, *J* = 7.0 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.3, 152.7, 151.1, 148.4, 145.5, 142.5, 139.6, 135.9, 122.6, 121.8, 119.4, 111.5, 110.7, 107.4, 106.8, 55.6, 52.8, 49.9, 44.5, 36.3, 31.5, 29.6, 26.5, 22.3, 14.3, 7.9 ppm; MS (m/z): 546 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{37}FN_5O_3$: $[M+H]^+$, 546.2880; found, 546.2881.

*4.1.33. 1-Cyclopropyl-6-fluoro-7-(4-((1-octyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14m**)*

Compound **14m** was prepared according to the procedure described for compound **13a** starting from compound **10e** (0.558 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14m** (0.514 g) was obtained as white solid. Yield: 44.8%; mp: 157–158 °C; IR (KBr, cm^{-1}) ν: 3012 (aromatic C-H), 2952, 2842 (CH_3, CH_2), 1720 (C=O), 1614, 1555, 1507, 1448 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.33 (s, 1H, COO*H*), 8.95 (s, 1H, quinolone 2-*H*), 7.92 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.56 (dt, *J* = 30.4, 9.5 Hz, 2H, benzimidazole 4,7-*H*), 7.25–7.15 (m, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*), 4.61–4.54 (m, 2H, benzimidazole N-CH_2), 4.28 (dt, *J* = 23.7, 7.5 Hz, 2H, benzimidazole CH_2), 3.88 (s, 1H, quinolone N-C*H*), 3.33 (s, 4H, piperazine N-CH_2), 2.72 (d, *J* = 27.4 Hz, 4H, piperazine N-CH_2), 1.86–1.79 (m, 2H, CH_2(CH_2)$_5$$CH_3$), 1.40–1.23 (m, 10H, CH_2(CH_2)$_5$$CH_3$), 0.77 (d, *J* = 6.4 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.5, 167.1, 151.2, 148.9, 145.9, 142.5, 138.2, 135.9, 122.5, 121.7, 119.5, 110.7, 107.6, 106.6, 72.8, 70.0, 60.6, 54.9, 52.8, 50.0, 49.5, 44.0, 31.5, 29.8, 29.2, 26.8, 22.5, 14.8, 14.3 ppm; MS

(m/z): 574 $[M+H]^+$; HRMS (TOF) calcd. for $C_{33}H_{40}FN_5O_3$: $[M+H]^+$, 574.3193; found, 574.3195.

*4.1.34. 1-Cyclopropyl-7-(4-((1-decyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14n**)*

Compound **14n** was prepared according to the procedure described for compound **13a** starting from compound **10f** (0.614 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14n** (0.513 g) was obtained as white solid. Yield: 42.6%; mp: 161–162 °C; IR (KBr, cm^{-1}) ν: 3034 (aromatic C-H), 2947, 2847 (CH_3, CH_2), 1723 (C=O), 1625, 1562, 1556, 1436 (aromatic frame); ^1H NMR (600 MHz, DMSO-d_6) δ: 15.18 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.61–7.52 (m, 3H, benzimidazole 4,7-*H*, quinolone 8-*H*), 7.25–7.16 (m, 2H, benzimidazole 5,6-*H*), 4.33–4.28 (m, 2H, benzimidazole N-CH_2), 3.89 (s, 2H, benzimidazole CH_2), 3.80–3.76 (m, 1H, quinolone N-C*H*), 3.33 (s, 4H, piperazine N-CH_2), 2.72 (s, 4H, piperazine N-CH_2), 1.82 (dd, *J* = 14.7, 7.5 Hz, 2H, C$H_2(CH_2)_7CH_3$), 1.39–1.11 (m, 18H, C$H_2(CH_2)_7CH_3$, cyclopropyl-CH_2), 0.76 (t, *J* = 6.7 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 177.9, 165.9, 153.6, 150.3, 143.4, 132.9, 122.4, 119.4, 113.7, 103.8, 79.2, 61.8, 54.6, 52.8, 50.1, 35.9, 33.3, 15.9, 7.9 ppm; MS (m/z): 602 $[M+H]^+$; HRMS (TOF) calcd. for $C_{35}H_{45}FN_5O_3$: $[M+H]^+$, 602.3506; found, 602.3507.

*4.1.35. 1-Cyclopropyl-7-(4-((1-dodecyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14o**)*

Compound **14o** was prepared according to the procedure described for compound **13a** starting from compound **10g** (0.670 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14o** (0.536 g) was obtained as white solid. Yield: 42.6%; mp: 170–171 °C; IR (KBr, cm^{-1}) ν: 3055 (aromatic C-H), 2960, 2845 (CH_3, CH_2), 1719 (C=O), 1622, 1541, 1463, 1454 (aromatic frame); ^1H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.96 (s, 1H, quinolone 2-*H*), 7.92 (dd, *J* = 18.1, 12.1 Hz, 1H, quinolone 5-*H*), 7.51 (dd, *J* = 27.5, 7.1 Hz, 2H, benzimidazole 4,7-*H*), 7.18 (dd, *J* = 23.6, 15.2 Hz, 3H, quinolone 8-*H*, benzimidazole 5,6-*H*), 3.78–3.71 (m, 1H, quinolone N-C*H*), 4.28 (s, 2H, benzimidazole N-CH_2), 3.87 (s, 2H, benzimidazole CH_2), 3.30 (s, 4H, piperazine N-CH_2), 2.71 (s, 4H, piperazine N-CH_2), 1.84 (s, 2H, $CH_2CH_2(CH_2)_9CH_3$), 1.38–1.04 (m, 22H, $CH_2CH_2(CH_2)_9CH_3$, cyclopropyl-CH_2), 0.77 (d, *J* = 5.2 Hz, 3H, $CH_2CH_2(CH_2)_9CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.6, 155.4, 151.3, 148.8, 145.7, 142.4, 136.7, 136.0, 122.3, 121.8, 119.3, 111.7, 110.5, 108.5, 105.1, 56.0, 52.8, 50.1, 49.5, 44.8, 34.6, 32.5, 29.9, 29.0, 26.6, 22.4, 14.7, 8.2 ppm; MS (m/z): 630 $[M+H]^+$; HRMS (TOF) calcd. for $C_{37}H_{49}FN_5O_3$:

$[M+H]^+$, 630.3819; found, 630.3821.

*4.1.36. 1-Cyclopropyl-6-fluoro-7-(4-((1-octadecyl-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14p**)*

Compound **14p** was prepared according to the procedure described for compound **13a** starting from compound **10h** (0.838 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **14p** (0.536 g) was obtained as white solid. Yield: 37.6%; mp: 194–196 °C; IR (KBr, cm^{-1}) ν: 3044 (aromatic C-H), 2952, 2843 (CH_3, CH_2), 1721 (C=O), 1614, 1555, 1536, 1507, 1448 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.82 (s, 1H, quinolone 2-*H*), 7.87 (s, 2H, quinolone 5-*H*), 7.80 (s, 1H, quinolone 8-*H*), 7.54 (s, 2H, benzimidazole 4,7-*H*), 7.13 (s, 2H, benzimidazole 5,6-*H*), 4.53 (s, 2H, quinolone N-CH_2), 4.33 (s, 2H, benzimidazole N-CH_2), 3.75 (s, 2H, benzimidazole CH_2), 3.29 (s, 4H, piperazine N-CH_2), 2.68 (s, 4H, piperazine N-CH_2), 1.83 (s, 2H, $CH_2CH_2(CH_2)_{15}CH_3$), 1.38–1.06 (m, 33H, $CH_2CH_2(CH_2)_{15}CH_3$, CH_3), 0.78 (d, *J* = 5.7 Hz, 3H, $CH_2CH_2(CH_2)_{15}CH_3$) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.5, 166.5, 152.5, 148.3, 146.8, 142.8, 138.7, 135.5, 122.4, 121.5, 118.5, 117.2, 114.7, 113.3, 52.7, 49.7, 49.0, 46.2, 33.5, 31.7, 29.8, 29.0, 27.0, 22.2, 14.1, 8.1 ppm; MS (m/z): 714 $[M+H]^+$; HRMS (TOF) calcd. for $C_{43}H_{61}FN_5O_3$: $[M+H]^+$, 714.4758; found, 714.4759.

*4.1.37. 7-(4-((1-(2-Chlorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15a**)*

Compound **15a** was prepared according to the procedure described for compound **13a** starting from compound **12a** (0.903 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15a** (0.756 g) was obtained as white solid. Yield: 42.5%; mp: 208–209 °C; IR (KBr, cm^{-1}) ν: 3022 (aromatic C-H), 2943, 2847 (CH_3, CH_2), 1725 (C=O), 1625, 1563, 1545, 1455 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6): 15.16 (s, 1H, COO*H*), 8.64 (s, 1H, quinolone 2-*H*), 7.86 (d, *J* = 12.6 Hz, 1H, quinolone 5-*H*), 7.71 (s, 1H, ClPh-3-*H*), 7.66 (d, *J* = 3.7 Hz, 1H, benzimidazole 4,7-*H*), 7.48 (d, *J* = 2.1 Hz, 1H, benzimidazole 4,7-*H*), 7.42 (d, *J* = 6.4 Hz, 1H, benzimidazole 5,6-*H*), 7.31 (d, *J* = 8.2 Hz, 1H, benzimidazole 5,6-*H*), 7.21 (d, *J* = 2.7 Hz, 2H, ClPh-5,6-*H*), 6.54 (d, *J* = 8.1 Hz, 1H, quinolone 8-*H*), 5.67 (s, 2H, benzimidazole N-CH_2), 4.55–4.51 (m, 2H, quinolone N-CH_2), 3.86 (s, 2H, benzimidazole CH_2), 2.93 (s, 4H, piperazine N-CH_2), 2.53 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 7.1 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.5, 164.3, 154.3, 152.3, 147.2, 146.5, 144.6, 142.4, 140.8, 139.5, 134.5, 132.3, 123.8, 123.0, 122.3, 119.7, 119.0, 115.6, 114.7, 113.6, 111.5, 110.5, 109.9, 106.7, 105.6, 57.6, 54.8, 52.5, 48.8, 46.7, 14.2 ppm; MS

(m/z): 575 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}ClFN_5O_3$: $[M+H]^+$, 574.2021; found, 574.2022.

*4.1.38. 7-(4-((1-(3-Chlorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15b**)*

Compound **15b** was prepared according to the procedure described for compound **13a** starting from compound **12b** (0.903 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15b** (0.685 g) was obtained as white solid. Yield: 38.5%; mp: 209–210 °C; IR (KBr, cm^{-1}) ν: 3085 (aromatic C-H), 2958, 2847 (CH_3, CH_2), 1721 (C=O), 1611, 1557, 1536, 1505, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.69–7.63 (m, 1H, benzimidazole 4,7-*H*), 7.47–7.42 (m, 1H, benzimidazole 4,7-*H*), 7.36 (t, *J* = 7.6 Hz, 2H, ClPh-2,4-*H*), 7.31 (d, *J* = 7.8 Hz, 1H, benzimidazole 5,6-*H*), 7.24–7.20 (m, 2H, ClPh-5,6-*H*), 7.19 (d, *J* = 7.8 Hz, 1H, benzimidazole 5,6-*H*), 7.08 (d, *J* = 6.8 Hz, 1H, quinolone 8-*H*), 5.64 (s, 2H, benzimidazole N-CH_2), 4.59–4.53 (m, 2H, quinolone N-CH_2), 3.90 (s, 2H, benzimidazole CH_2), 3.16 (s, 4H, piperazine N-CH_2), 2.68 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 7.0 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.5, 166.5, 154.1, 152.6, 151.6, 148.9, 145.8, 142.4, 140.5, 137.6, 136.1, 133.7, 130.9, 127.7, 127.3, 126.0, 123.0, 122.2, 119.6, 111.7, 111.6, 110.8, 107.6, 106.1, 54.7, 52.6, 49.7, 49.5, 46.8, 14.7 ppm; MS (m/z): 575 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}ClFN_5O_3$: $[M+H]^+$, 574.2021; found, 574.2022.

*4.1.39. 1-Ethyl-6-fluoro-7-(4-((1-(2-fluorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15c**)*

Compound **15c** was prepared according to the procedure described for compound **13a** starting from compound **12c** (0.851 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15c** (0.701 g) was obtained as white solid. Yield: 39.7%; mp: 203–204 °C; IR (KBr, cm^{-1}) ν: 3067 (aromatic C-H), 2958, 2832 (CH_3, CH_2), 1721 (C=O), 1611, 1535, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.33 (s, 1H, COO*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.88 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.66 (d, *J* = 6.6 Hz, 1H, quinolone 8-*H*), 7.47 (d, *J* = 6.4 Hz, 1H, benzimidazole 4,7-*H*), 7.31 (d, *J* = 5.8 Hz, 1H, benzimidazole 4,7-*H*), 7.28–7.20 (m, 3H, FPh-3,4,5-*H*), 7.10 (t, *J* = 7.2 Hz, 1H, FPh-6-*H*), 7.06 (d, *J* = 6.5 Hz, 1H, benzimidazole 5,6-*H*), 6.86 (t, *J* = 6.9 Hz, 1H, benzimidazole 5,6-*H*), 5.70 (s, 2H, benzimidazole N-CH_2), 4.56 (d, *J* = 6.7 Hz, 2H, quinolone N-CH_2), 3.89 (s, 2H, benzimidazole CH_2), 3.09 (s, 4H, piperazine N-CH_2), 2.61 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 6.6 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.6, 166.6, 162.6, 154.2, 151.5, 148.8, 142.6, 138.2, 136.1, 129.2, 122.6, 119.2, 115.6, 111.3, 106.6, 105.1, 55.1, 52.6,

49.7, 49.3, 49.0, 46.4, 14.6 ppm; MS (m/z): 558 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}F_2N_5O_3$: $[M+H]^+$, 558.2317; found, 558.2317.

*4.1.40. 1-Ethyl-6-fluoro-7-(4-((1-(3-fluorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15d**)*

Compound **15d** was prepared according to the procedure described for compound **13a** starting from compound **12d** (0.851 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15d** (0.703 g) was obtained as white solid. Yield: 40.7%; mp: 207–208 °C; IR (KBr, cm^{-1}) ν: 3047 (aromatic C-H), 2942, 2831 (CH_3, CH_2), 1728 (C=O), 1611, 1514, 1478 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.32 (s, 1H, COO*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.91 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.66–7.64 (m, 1H, benzimidazole 4,7-*H*), 7.45–7.42 (m, 1H, benzimidazole 4,7-*H*), 7.39–7.35 (m, 1H, FPh-5-*H*), 7.22–7.20 (m, 2H, FPh-4,6-*H*), 7.11–7.06 (m, 3H, FPh-2-*H*, benzimidazole 5,6-*H*), 7.03 (d, *J* = 7.8 Hz, 1H, quinolone 8-*H*), 5.65 (s, 2H, benzimidazole N-CH_2), 4.60–4.55 (m, 2H, quinolone N-CH_2), 3.89 (s, 2H, benzimidazole CH_2), 3.17 (s, 4H, piperazine N-CH_2), 2.67 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 7.1 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.5, 163.6, 161.9, 154.2, 152.5, 151.5, 149.2, 145.9, 137.6, 131.0, 123.3, 123.0, 122.2, 119.8, 119.6, 114.6, 114.5, 114.3, 114.1, 111.8, 111.6, 110.9, 107.6, 106.2, 79.8, 57.1, 54.9, 52.6, 49.8, 49.5, 48.9, 46.9, 14.6 ppm; MS (m/z): 558 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}F_2N_5O_3$: $[M+H]^+$, 558.2317; found, 558.2316.

*4.1.41. 1-Ethyl-6-fluoro-7-(4-((1-(4-fluorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15e**)*

Compound **15e** was prepared according to the procedure described for compound **13a** starting from compound **12e** (0.851 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15e** (0.646 g) was obtained as white solid. Yield: 37.4%; mp: 204–205 °C; IR (KBr, cm^{-1}) ν: 3089 (aromatic C-H), 2936, 2816 (CH_3, CH_2), 1728 (C=O), 1604, 1513, 1476 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.33 (s, 1H, COO*H*), 8.94 (s, 1H, quinolone 2-*H*), 7.90 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.54 (dd, *J* = 122.3, 4.5 Hz, 2H, benzimidazole 4,7-*H*), 7.30–7.25 (m, 2H, benzimidazole 5,6-*H*), 7.16 (ddd, *J* = 35.7, 17.2, 5.3 Hz, 5H, quinolone 8-*H*, FPh-2,3,5,6-*H*), 5.62 (s, 2H, benzimidazole N-CH_2), 4.57 (d, *J* = 6.9 Hz, 2H, quinolone N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.20 (s, 4H, piperazine N-CH_2), 2.67 (s, 4H, piperazine N-CH_2), 1.41 (t, *J* = 6.8 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.5, 162.7, 154.1, 151.4, 148.9, 142.5, 137.6, 136.2, 129.4, 122.9, 119.5, 115.8, 111.7, 107.6, 106.2, 54.9, 52.7, 49.8, 49.5, 49.1, 46.6,

14.7 ppm; MS (m/z): 558 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}F_2N_5O_3$: $[M+H]^+$, 558.2317; found, 558.2316.

*4.1.42. 7-(4-((1-(2,4-Dichlorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15f**)*

Compound **15f** was prepared according to the procedure described for compound **13a** starting from compound **12f** (1.009 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15f** (0.756 g) was obtained as white solid. Yield: 40.1%; mp: 205–207 °C; IR (KBr, cm^{-1}) ν: 3085 (aromatic C-H), 2958, 2840 (CH_3, CH_2), 1721 (C=O), 1611, 1557, 1536, 1505, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.31 (s, 1H, COO*H*), 8.94 (d, *J* = 11.4 Hz, 1H, quinolone 2-*H*), 7.87 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.70 (d, *J* = 13.1 Hz, 2H, ClPh-3-*H*, benzimidazole 4,7-*H*), 7.44 (s, 1H, benzimidazole 4,7-*H*), 7.31 (d, *J* = 7.3 Hz, 1H, benzimidazole 5,6-*H*), 7.25 (s, 2H, ClPh-5,6-*H*), 7.03 (d, *J* = 5.3 Hz, 1H, benzimidazole 5,6-*H*), 6.55 (d, *J* = 7.8 Hz, 1H, quinolone 8-*H*), 5.69 (s, 2H, benzimidazole N-CH_2), 4.56 (d, *J* = 6.0 Hz, 2H, quinolone N-CH_2), 3.90 (s, 2H, benzimidazole CH_2), 3.04 (s, 4H, piperazine N-CH_2), 2.61 (s, 4H, piperazine N-CH_2), 1.41 (s, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.4, 163.5, 161.8, 154.5, 152.4, 151.3, 149.1, 145.8, 135.6, 132.3, 123.4, 123.2, 122.1, 119.7, 119.2, 114.5, 114.2, 114.1, 114.0, 111.8, 111.5, 110.8, 107.5, 106.3, 79.7, 57.2, 54.9, 52.6, 49.8, 49.4, 48.8, 46.6, 14.5 ppm; MS (m/z): 608 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}Cl_2FN_5O_3$: $[M+H]^+$, 608.1631; found, 608.1632.

*4.1.43. 1-Ethyl-6-fluoro-7-(4-((1-(4-nitrobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15g**)*

Compound **15g** was prepared according to the procedure described for compound **13a** starting from compound **12g** (0.903 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.433 g, 0.003 mol) and potassium iodide (0.433 g, 0.003 mol). The target compound **15g** (0.794 g) was obtained as white solid. Yield: 45.3%; mp: 205–206 °C; IR (KBr, cm^{-1}) ν: 3086 (aromatic C-H), 2947, 2858 (CH_3, CH_2), 1725 (C=O), 1621, 1547, 1533, 1515, 1449 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.32 (s, 1H, COO*H*), 8.95 (s, 1H, quinolone 2-*H*), 7.89 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.56 (dd, *J* = 120.8, 4.6 Hz, 2H, benzimidazole 4,7-*H*), 7.31–7.27 (m, 2H, benzimidazole 5,6-*H*), 7.17 (ddd, *J* = 36.6, 17.5, 5.5 Hz, 5H, quinolone 8-*H*, NO_2Ph-2,3,5,6-*H*), 5.62 (s, 2H, benzimidazole N-CH_2), 4.57 (d, *J* = 7.0 Hz, 2H, quinolone N-CH_2), 3.89 (s, 2H, benzimidazole CH_2), 3.22 (s, 4H, piperazine N-CH_2), 2.68 (s, 4H, piperazine N-CH_2), 1.40 (t, *J* = 6.7 Hz, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.4, 163.2, 153.8, 151.6, 149.1, 142.6, 138.5, 136.4, 129.5, 123.8, 119.6, 116.4, 112.6, 107.5, 106.5, 55.8, 52.6,

49.7, 48.9, 48.1, 45.4, 14.8 ppm; MS (m/z): 585 $[M+H]^+$; HRMS (TOF) calcd. for $C_{31}H_{30}FN_6O_5$: $[M+H]^+$, 585.2262; found, 585.2263.

*4.1.44. 7-(4-((1-(2-Chlorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15h**)*

Compound **15h** was prepared according to the procedure described for compound **13a** starting from compound **12a** (0.582 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15h** (0.470 g) was obtained as white solid. Yield: 40.1%; mp: 208–209 °C; IR (KBr, cm^{-1}) ν: 3005 (aromatic C-H), 2858 (CH_2), 1721 (C=O), 1626, 1544, 1479, 1460 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.17 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.87 (d, *J* = 12.8 Hz, 1H, quinolone 5-*H*), 7.72 (s, 1H, ClPh-3-*H*), 7.69 (d, *J* = 3.9 Hz, 1H, benzimidazole 4,7-*H*), 7.45 (d, *J* = 2.2 Hz, 1H, benzimidazole 4,7-*H*), 7.40 (d, *J* = 6.5 Hz, 1H, benzimidazole 5,6-*H*), 7.31 (d, *J* = 8.3 Hz, 1H, benzimidazole 5,6-*H*), 7.24 (d, *J* = 2.8 Hz, 2H, ClPh-5,6-*H*), 6.53 (d, *J* = 8.2 Hz, 1H, quinolone 8-*H*), 5.68 (s, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.78 (s, 1H, cyclopropyl-C*H*), 2.97 (s, 4H, piperazine N-CH_2), 2.58 (s, 4H, piperazine N-CH_2), 1.31 (d, *J* = 5.9 Hz, 2H, cyclopropyl-CH_2), 1.18 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.4, 163.2, 154.3, 152.4, 148.2, 147.3, 144.5, 142.3, 140.7, 139.4, 134.5, 132.2, 123.4, 123.0, 122.2, 119.6, 119.0, 114.6, 114.5, 113.5, 111.4, 110.4, 109.8, 106.5, 105.5, 57.8, 54.9, 52.6, 49.8, 46.8, 36.5, 7.9 ppm; MS (m/z): 587 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{30}ClFN_5O_3$: $[M+H]^+$, 586.2021; found, 586.2020.

*4.1.45. 7-(4-((1-(3-Chlorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15i**)*

Compound **15i** was prepared according to the procedure described for compound **13a** starting from compound **12b** (0.582 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15i** (0.491 g) was obtained as white solid. Yield: 41.9%; mp: 207–208 °C; IR (KBr, cm^{-1}) ν: 3035 (aromatic C-H), 2861 (CH_2), 1727 (C=O), 1615, 1542, 1511, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.17 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.88 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.67–7.64 (m, 1H, benzimidazole 4,7-*H*), 7.47 (d, *J* = 7.4 Hz, 1H, benzimidazole 4,7-*H*), 7.46–7.43 (m, 1H, ClPh-2-*H*), 7.38–7.30 (m, 3H, benzimidazole 5,6-*H*, ClPh-4-*H*), 7.24–7.17 (m, 3H, ClPh-5,6-*H*, quinolone 8-*H*), 5.63 (s, 2H, benzimidazole N-CH_2), 3.90 (s, 2H, benzimidazole CH_2), 3.81–3.75 (m, 1H, cyclopropyl-C*H*), 3.14 (s, 4H, piperazine N-CH_2), 2.68 (s, 4H, piperazine N-CH_2), 1.30 (q, *J* = 6.6 Hz, 2H, cyclopropyl-CH_2), 1.17 (d, *J* =

6.9 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.3, 152.7, 151.7, 148.5, 145.5, 142.4, 140.6, 136.2, 130.9, 127.8, 127.3, 126.1, 122.9, 122.3, 119.7, 119.2, 111.6, 111.4, 110.7, 107.3, 106.7, 79.6, 54.7, 52.5, 49.5, 46.7, 36.5, 8.2 ppm; MS (m/z): 587 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{29}ClFN_5O_3$: $[M+H]^+$, 586.2021; found, 586.2022.

*4.1.46. 1-Cyclopropyl-6-fluoro-7-(4-((1-(2-fluorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15j**)*

Compound **15j** was prepared according to the procedure described for compound **13a** starting from compound **12c** (0.549 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15j** (0.420 g) was obtained as white solid. Yield: 36.9%; mp: 204–205 ℃; IR (KBr, cm^{-1}) ν: 3078 (aromatic C-H), 2883 (CH_2), 1721 (C=O), 1613, 1557, 1535, 1451 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.18 (s, 1H, COO*H*), 8.66 (s, 1H, quinolone 2-*H*), 7.89 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.64 (d, *J* = 14.6 Hz, 1H, quinolone 8-*H*), 7.46 (dd, *J* = 15.5, 7.3 Hz, 2H, benzimidazole 4,7-*H*), 7.26–7.16 (m, 4H, FPh-3,4,5,7-*H*), 7.10 (dd, *J* = 6.1, 5.1 Hz, 1H, benzimidazole 5,6-*H*), 6.87–6.81 (m, 1H, benzimidazole 5,6-*H*), 5.70 (s, 2H, benzimidazole N-CH_2), 4.75–4.71 (m, 1H, cyclopropyl-C*H*), 3.90 (s, 2H, benzimidazole CH_2), 3.06 (s, 4H, piperazine N-CH_2), 2.62 (s, 4H, piperazine N-CH_2), 1.33–1.27 (m, 2H, cyclopropyl-CH_2), 1.17 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.4, 165.5, 156.2, 144.6, 143.2, 135.7, 129.4, 122.8, 121.6, 119.7, 116.4, 115.8, 112.2, 107.7, 79.8, 54.9, 53.2, 49.8, 49.0, 46.7, 36.5, 8.1 ppm; MS (m/z): 570 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{30}F_2N_5O_3$: $[M+H]^+$, 570.2317; found, 570.2318.

*4.1.47. 1-Cyclopropyl-6-fluoro-7-(4-((1-(3-fluorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15k**)*

Compound **15k** was prepared according to the procedure described for compound **13a** starting from compound **12d** (0.549 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15k** (0.454 g) was obtained as white solid. Yield: 39.9%; mp: 205–206 °C; IR (KBr, cm^{-1}) ν: 3018 (aromatic C-H), 2892 (CH_2), 1718 (C=O), 1636, 1588, 1478, 1455 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.18 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.88 (d, *J* = 13.2 Hz, 1H, quinolone 5-*H*), 7.67–7.64 (m, 1H, benzimidazole 4,7-*H*), 7.48 (d, *J* = 7.4 Hz, 1H, benzimidazole 4,7-*H*), 7.45–7.42 (m, 1H, FPh-5-*H*), 7.37 (dd, *J* = 14.0, 7.7 Hz, 1H, FPh-2-*H*), 7.22–7.20 (m, 2H, FPh-4,6-*H*), 7.08 (dd, *J* = 14.7, 6.2 Hz, 2H, benzimidazole 5,6-*H*), 7.03 (d, *J* = 7.7 Hz, 1H, quinolone 8-*H*), 5.65 (s, 2H, benzimidazole N-CH_2), 3.90 (s, 2H, benzimidazole CH_2), 3.79 (ddd, *J* = 11.0, 7.2, 4.1 Hz, 1H, cyclopropyl-C*H*), 3.14 (s, 4H, piperazine N-CH_2), 2.67 (s, 4H,

piperazine N-CH_2), 1.30 (q, *J* = 6.7 Hz, 2H, cyclopropyl-CH_2), 1.18 (t, *J* = 7.8 Hz, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.3, 163.4, 161.9, 154.3, 152.6, 148.4, 145.6, 142.4, 140.9, 139.6, 136.2, 131.0, 123.3, 123.0, 122.2, 119.6, 119.2, 114.6, 114.5, 114.2, 114.1, 111.5, 111.3, 110.9, 107.3, 106.7, 57.1, 54.8, 52.6, 49.7, 46.9, 36.3, 7.9 ppm; MS (m/z): 570 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{30}F_2N_5O_3$: $[M+H]^+$, 570.2317; found, 570.2318.

*4.1.48. 1-Cyclopropyl-6-fluoro-7-(4-((1-(4-fluorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15l**)*

Compound **15l** was prepared according to the procedure described for compound **13a** starting from compound **12e** (0.549 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15l** (0.452 g) was obtained as white solid. Yield: 39.7%; mp: 206–207 °C; IR (KBr, cm^{-1}) ν: 3024 (aromatic C-H), 2858 (CH_2), 1718 (C=O), 1615, 1545, 1520, 1494 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.19 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.88 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.64 (d, *J* = 6.4 Hz, 1H, quinolone 8-*H*), 7.46 (dd, *J* = 33.4, 5.4 Hz, 2H, benzimidazole 4,7-*H*), 7.26 (s, 2H, benzimidazole 5,6-*H*), 7.17 (dd, *J* = 18.4, 9.6 Hz, 4H, FPh-2,3,5,6-*H*), 5.62 (s, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole CH_2), 3.79 (s, 1H, cyclopropyl-C*H*), 3.17 (s, 4H, piperazine N-CH_2), 2.67 (s, 4H, piperazine N-CH_2), 1.30 (d, *J* = 5.6 Hz, 2H, cyclopropyl-CH_2), 1.17 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 180.5, 175.1, 165.6, 156.1, 144.5, 143.3, 136.9, 129.5, 122.9, 122.2, 119.6, 116.5, 115.9, 111.2, 106.8, 79.7, 54.9, 52.7, 49.8, 49.1, 46.8, 36.2, 8.0 ppm; MS (m/z): 570 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{30}F_2N_5O_3$: $[M+H]^+$, 570.2317; found, 570.2317.

*4.1.49. 1-Cyclopropyl-7-(4-((1-(2,4-dichlorobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15m**)*

Compound **15m** was prepared according to the procedure described for compound **13a** starting from compound **12f** (0.549 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15m** (0.507 g) was obtained as white solid. Yield: 40.9%; mp: 206–208 °C; IR (KBr, cm^{-1}) ν: 3025 (aromatic C-H), 2838 (CH_2), 1728 (C=O), 1619, 1540, 1501, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.17 (s, 1H, COO*H*), 8.65 (s, 1H, quinolone 2-*H*), 7.87 (d, *J* = 12.8 Hz, 1H, quinolone 5-*H*), 7.72 (s, 1H, ClPh-3-*H*), 7.69 (d, *J* = 3.9 Hz, 1H, benzimidazole 4,7-*H*), 7.45 (d, *J* = 2.2 Hz, 1H, benzimidazole 4,7-*H*), 7.40 (d, *J* = 6.5 Hz, 1H, benzimidazole 5,6-*H*), 7.31 (d, *J* = 8.3 Hz, 1H, benzimidazole 5,6-*H*), 7.24 (d, *J* = 2.8 Hz, 2H, ClPh-5,6-*H*), 6.53 (d, *J* = 8.2 Hz, 1H, quinolone 8-*H*), 5.68 (s, 2H, benzimidazole N-CH_2), 3.88 (s, 2H, benzimidazole

CH_2), 3.78 (s, 1H, cyclopropyl-C*H*), 2.97 (s, 4H, piperazine N-CH_2), 2.58 (s, 4H, piperazine N-CH_2), 1.31 (d, *J* = 5.9 Hz, 2H, cyclopropyl-CH_2), 1.18 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.7, 166.4, 163.2, 161.8, 154.4, 152.5, 148.3, 144.6, 142.5, 140.8, 139.5, 135.5, 132.3, 123.4, 123.0, 122.3, 119.5, 119.1, 114.6, 114.5, 114.1, 113.1, 111.4, 110.3, 109.9, 106.3, 105.6, 57.1, 54.8, 52.6, 49.7, 46.9, 36.3, 7.9 ppm; MS (m/z): 620 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{30}Cl_2FN_5O_3$: $[M+H]^+$, 620.1631; found, 620.1632.

*4.1.50. 1-Cyclopropyl-6-fluoro-7-(4-((1-(4-nitrobenzyl)-1H-benzimidazol-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15n**)*

Compound **15n** was prepared according to the procedure described for compound **13a** starting from compound **12g** (0.602 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **15n** (0.481 g) was obtained as white solid. Yield: 40.3%; mp: 207–208 °C; IR (KBr, cm^{-1}) ν: 3039 (aromatic C-H), 2829 (CH_2), 1719 (C=O), 1613, 1542, 1522, 1492 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.21 (s, 1H, COO*H*), 8.63 (s, 1H, quinolone 2-*H*), 7.79 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.63 (d, *J* = 6.5 Hz, 1H, quinolone 8-*H*), 7.47 (dd, *J* = 33.2, 5.5 Hz, 2H, benzimidazole 4,7-*H*), 7.27 (s, 2H, benzimidazole 5,6-*H*), 7.19 (dd, *J* = 18.2, 9.7 Hz, 4H, NO_2Ph-2,3,5,6-*H*), 5.66 (s, 2H, benzimidazole N-CH_2), 3.89 (s, 2H, benzimidazole CH_2), 3.78 (s, 1H, cyclopropyl-C*H*), 3.18 (s, 4H, piperazine N-CH_2), 2.68 (s, 4H, piperazine N-CH_2), 1.31 (d, *J* = 5.7 Hz, 2H, cyclopropyl-CH_2), 1.15 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 181.3, 176.2, 166.3, 157.3, 145.5, 144.3, 137.9, 129.4, 123.8, 122.8, 118.9, 116.7, 114.8, 112.2, 105.7, 79.5, 55.5, 52.6, 49.8, 48.5, 46.7, 36.4, 8.1 ppm; MS (m/z): 597 $[M+H]^+$; HRMS (TOF) calcd. for $C_{32}H_{30}FN_6O_5$: $[M+H]^+$, 597.2262; found, 597.2263.

*4.1.51. 7-(4-(2-(1H-Benzimidazol-2-yl)ethyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**16a**)*

Compound **16a** was prepared according to the procedure described for compound **13a** starting from compound **8f** (0.540 g, 0.003 mol), norfloxacin (1.000 g, 0.003 mol), potassium carbonate (0.414 g, 0.003 mol) and potassium iodide (0.498 g, 0.003 mol). The target compound **16a** (0.545 g) was obtained as white solid. Yield: 39.2%; mp: 156–158 °C; IR (KBr, cm^{-1}) ν: 3029 (aromatic C-H), 2903, 2840 (CH_3, CH_2), 1720 (C=O), 1615, 1545, 1523, 1495 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.36 (s, 1H, COO*H*), 12.16 (s, 1H, benzimidazole-N*H*), 8.95 (s, 1H, quinolone 2-*H*), 7.91 (d, *J* = 13.3 Hz, 1H, quinolone 5-*H*), 7.47 (s, 2H, benzimidazole 4,7-*H*), 7.17 (d, *J* = 7.1 Hz, 1H, quinolone 8-*H*), 7.13–7.10 (m, 2H, benzimidazole 5,6-*H*), 4.58 (dd, *J* = 13.7, 6.6 Hz, 2H, quinolone N-CH_2), 3.04 (t, *J* = 7.4 Hz, 2H,

benzimidazole-CH_2), 2.89 (t, J = 7.4 Hz, 2H, benzimidazole-CH_2CH_2), 2.69 (s, 4H, piperazine N-CH_2), 2.52–2.49 (m, 4H, piperazine N-CH_2), 1.41 (t, J = 7.1 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.6, 166.5, 154.1, 152.5, 148.9, 145.8, 137.5, 121.7, 119.7, 111.7, 111.5, 107.5, 66.9, 56.2, 52.6, 49.8, 49.6, 26.6, 14.6 ppm; MS (m/z): 464 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{26}FN_5O_3$: $[M+H]^+$, 464.2098; found, 464.2099.

*4.1.52. 7-(4-(2-(1H-Benzimidazol-2-yl)ethyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**16b**)*

Compound **16b** was prepared according to the procedure described for compound **13a** starting from compound **8f** (0.360 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol), potassium carbonate (0.299 g, 0.002 mol) and potassium iodide (0.332 g, 0.002 mol). The target compound **16b** (0.338 g) was obtained as white solid. Yield: 35.5%; mp: 156–158 °C; IR (KBr, cm^{-1}) ν: 3026 (aromatic C-H), 2829 (CH_2), 1718 (C=O), 1617, 1555, 1527, 1465 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.21 (s, 1H, COO*H*), 8.66 (s, 1H, quinolone 2-*H*), 7.90 (d, J = 13.2 Hz, 1H, quinolone 5-*H*), 7.56 (d, J = 7.4 Hz, 1H, quinolone 8-*H*), 7.48 (dd, J = 5.8, 3.2 Hz, 2H, benzimidazole 4,7-*H*), 7.12 (dd, J = 5.9, 3.1 Hz, 2H, benzimidazole 5,6-*H*), 3.81 (td, J = 7.0, 3.7 Hz, 1H, cyclopropyl-C*H*), 3.06 (t, J = 7.4 Hz, 2H, benzimidazole-CH_2), 2.93 (t, J = 7.4 Hz, 2H, benzimidazole-CH_2CH_2), 2.74 (s, 4H, piperazine N-CH_2), 2.51–2.50 (m, 4H, piperazine N-CH_2), 1.31 (q, J = 6.8 Hz, 2H, cyclopropyl-CH_2), 1.21–1.14 (m, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.9, 166.3, 154.3, 153.9, 152.6, 148.4, 145.6, 145.6, 145.5, 139.7, 121.6, 119.0, 111.5, 111.4, 107.2, 106.8, 56.1, 52.6, 49.0, 36.3, 26.8, 8.1 ppm; MS (m/z): 476 $[M+H]^+$; HRMS (TOF) calcd. for $C_{26}H_{27}FN_5O_3$: $[M+H]^+$, 476.2098; found, 476.2097.

*4.1.53. 7-(4-((1H-Benzimidazol-2-ylamino)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**17a**)*

To a stirred solution of 1H-benzimidazol-2-amine (0.399 g, 0.003 mol) and norfloxacin (1.000 g, 0.003 mol) in acetic acid (40 mL), appropriate amount of paraformaldehyde was added and heated at reflux for 12 h. After the reaction was completed (monitored by TLC, chloroform/methanol (50/1, V/V), the acetic acid was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with chloroform/methanol (60/1, V/V) to give the pure target compound **17a** as white power (0.410 g). Yield: 29.4%; mp: 155–157 °C; IR (KBr, cm^{-1}) ν: 3015 (aromatic C-H), 2906, 2808 (CH_3, CH_2), 1739 (C=O), 1595, 1524, 1480, 1443 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.34 (s, 1H, COO*H*), 8.95 (s, 1H, quinolone 2-*H*), 7.93 (t, J = 13.6 Hz, 1H, quinolone 5-*H*), 7.30 (dd, J = 56.9, 20.6 Hz, 2H, benzimidazole 4,7-*H*), 7.18 (d, J = 5.6 Hz, 1H, benzimidazole 4,7-*H*), 7.00 (s, 1H, benzimidazole 4,7-*H*),

5.95 (d, *J* = 27.7 Hz, 1H, quinolone 8-*H*), 4.60 (d, *J* = 5.9 Hz, 2H, quinolone N-CH_2), 3.35 (s, 4H, piperazine N-CH_2), 2.59 (s, 2H, benzimidazole-CH_2), 2.31 (s, 2H, piperazine N-CH_2), 1.92 (s, 2H, piperazine N-CH_2), 1.43 (s, 3H, CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.6, 172.2, 166.6, 154.2, 152.5, 148.8, 145.9, 137.7, 121.4, 119.7, 119.0, 115.7, 111.6, 108.0, 107.6, 106.7, 106.3, 54.6, 49.3, 45.9, 21.5, 14.9 ppm; MS (m/z): 465 $[M+H]^+$; HRMS (TOF) calcd. for $C_{24}H_{26}FN_6O_3$: $[M+H]^+$, 465.2050; found, 465.2053.

*4.1.54. 7-(4-((1H-Benzimidazol-2-ylamino)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**17b**)*

Compound **17b** was prepared according to the procedure described for compound **17a** starting from 2-amino-benzimidazole (0.266 g, 0.002 mol), ciprofloxacin (1.000 g, 0.002 mol) and paraformaldehyde. The target compound **17b** (0.300 g) was obtained as white solid. Yield: 31.5%; mp: 158–160 °C; IR (KBr, cm^{-1}) ν: 3029 (aromatic C-H), 2807 (CH_2), 1738 (C=O), 1600, 1528, 1485, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 15.34 (s, 1H, COO*H*), 8.74 (s, 1H, quinolone 2-*H*), 7.98 (d, *J* = 12.9 Hz, 1H, quinolone 5-*H*), 7.64 (d, *J* = 6.9 Hz, 1H, quinolone 8-*H*), 7.43–6.95 (m, 4H, benzimidazole 4,5,6,7-*H*), 5.77 (s, 1H, cyclopropyl-C*H*), 3.25 (s, 4H, piperazine N-CH_2), 2.62 (s, 2H, benzimidazole-CH_2), 2.34 (s, 2H, piperazine N-CH_2), 1.98 (s, 2H, piperazine N-CH_2), 1.39 (d, *J* = 5.9 Hz, 2H, cyclopropyl-CH_2), 1.26 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.8, 166.1, 153.9, 152.5, 148.4, 143.9, 139.4, 118.2, 116.3, 114.4, 112.5, 111.5, 107.4, 75.5, 71.2, 70.9, 52.5, 48.6, 46.8, 42.5, 36.0, 7.8 ppm; MS (m/z): 477 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{26}FN_6O_3$: $[M+H]^+$, 477.2050; found, 477.2053.

*4.1.55. 1-Methyl-7-(4-((1H-benzimidazol-2-ylimino)methyl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**18a**)*

To a stirred solution of 2-aminobenzimidazole (0.368 g, 0.003 mol) and copper sulfate (0.075 g, 0.0003 mol) in 1, 4-dioxane (40 mL), compound **4a** (1.000 g, 0.003 mol) was added and heated at reflux for 24 h. After the reaction was completed (monitored by TLC, chloroform/methanol (50/1, V/V), the 1, 4-dioxane was removed under reduced pressure, the mixture was purified by flash silica gel column eluting with chloroform/methanol (60/1, V/V) to give the pure target compound **18a** as white power (0.592 g). Yield: 41.4%. mp: 170–171 °C; IR (KBr, cm^{-1}) ν: 3027 (aromatic C-H), 2952, 2861 (CH_3, CH_2), 1718 (C=O), 1629, 1553, 1537, 1508, 1445 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 13.36 (s, 1H, benzimidazole-N*H*), 8.99 (s, 1H, N=C*H*), 8.13 (s, 1H, quinolone 2-*H*), 8.02 (d, *J* = 13.1 Hz, 1H, quinolone 5-*H*), 7.50 (s, 2H, benzimidazole 4,7-*H*), 7.22 (d, *J* = 6.6 Hz, 1H, quinolone 8-*H*), 7.12 (s, 2H, benzimidazole 5,6-*H*), 4.62 (d, *J* = 6.9 Hz, 2H, quinolone N-CH_2), 3.61 (s, 3H, OCH_3), 3.34 (s, 4H,

piperazine N-CH_2), 3.27 (s, 4H, piperazine N-CH_2), 1.46 (t, J = 6.9 Hz, 3H, CH_2CH_3) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 175.7, 163.2, 161.2, 154.5, 152.7, 149.2, 145.9, 143.6, 136.7, 129.3, 124.9, 121.1, 113.4, 112.1, 111.9, 107.2, 50.4, 49.6, 49.2, 45.0, 14.1 ppm; MS (m/z): 477 $[M+H]^+$; HRMS (TOF) calcd. for $C_{25}H_{26}FN_6O_3$: $[M+H]^+$, 477.2050; found, 477.2050.

*4.1.56. 1-Methyl-7-(4-((1H-benzimidazol-2-ylimino)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**18b**)*

Compound **18b** was prepared according to the procedure described for compound **18a** starting from 2-aminobenzimidazole (0.368 g, 0.003 mol), compound **4b** (1.000 g, 0.003 mol) and copper sulfate (0.075 g, 0.0003 mol). The target compound **18b** (0.578 g) was obtained as white solid. Yield: 39.5%; mp: 171–172 °C; IR (KBr, cm^{-1}) ν: 3028 (aromatic C-H), 2953, 2890 (CH_3, CH_2), 1715 (C=O), 1631, 1556, 1536, 1505, 1440 (aromatic frame); ^{1}H NMR (600 MHz, DMSO-d_6) δ: 13.32 (s, 1H, benzimidazole-N*H*), 9.01 (s, 1H, N=C*H*), 8.12 (s, 1H, quinolone 2-*H*), 8.01 (d, J = 12.9 Hz, 1H, quinolone 5-*H*), 7.51 (s, 2H, benzimidazole 4,7-*H*), 7.23 (d, J = 6.4 Hz, 1H, quinolone 8-*H*), 7.11 (s, 2H, benzimidazole 5,6-*H*), 5.77 (s, 1H, cyclopropyl-C*H*), 3.63 (s, 3H, OCH_3), 3.35 (s, 4H, piperazine N-CH_2), 3.28 (s, 4H, piperazine N-CH_2), 1.39 (d, J = 5.9 Hz, 2H, cyclopropyl-CH_2), 1.26 (s, 2H, cyclopropyl-CH_2) ppm; ^{13}C NMR (151 MHz, DMSO-d_6) δ: 176.2, 163.3, 162.4, 155.3, 152.4, 148.6, 146.9, 144.1, 137.4, 128.4, 125.4, 122.3, 113.5, 112.4, 110.9, 106.1, 50.5, 49.4, 49.0, 45.4, 14.5 ppm; MS (m/z): 489 $[M+H]^+$; HRMS (TOF) calcd. for $C_{26}H_{26}FN_6O_3$: $[M+H]^+$, 489.2050; found, 489.2053.

4.2. Antibacterial and antifungal assays

Minimal inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of target compounds that completely inhibited the growth of bacteria, by means of standard two-fold serial dilution method in 96-well microtest plates according to the NCCLS. The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloromycin, norfloxacin, ciprofloxacin, clinafloxacin and fluconazole were used as standard drugs. To ensure that the solvent had no effect on bacterial growth, a control test was performed with tested medium supplemented with DMSO at the same dilutions used in the experiment. All the bacteria and fungi growth were monitored visually and spectrophotometrically, and all the experiments were performed in triplicate.

4.3. Bactericidal kinetic assay.

The rate of bactericidal activity, that is, the rate at which the compounds killed bacteria was evaluated by performing time-kill kinetics. Briefly, MRSA bacterium was grown in suitable growth medium at 37 ºC for 6 h and diluted in respective media. Compound **15m** was added to the bacterial solution (MRSA of approximately 1.8×10^5 CFU/mL) at concentrations of 6 × MIC in a 96-well plate. The plate was then incubated at 37 ºC. At different time intervals (0, 60, 90, 120, 150, 180, 210 and 240 min), 20 μL of aliquots from the solution were taken out and serially diluted (10-fold serial dilution) in 0.9% saline. Then 20 μL of the dilutions was plated on respective agar plates and incubated at 37 ºC for 24 h. The bacterial colonies were counted, and results are represented in logarithmic scale: $\log_{10}$ (CFU/mL) vs time (in min).

4.4. Antibiofilm activity.

4.4.1. Biofilm inhibition assay.

Midlog phase bacteria (MRSA and *E. coli* DH52, 6 h grown culture) were diluted to a concentration of 10^5 CFU/mL in suitable broths supplemented with 1% glucose and NaCl. Compound **15m** was serially diluted (2-fold), and 25 μL of these serial dilutions was added to the wells of a 96-well plate. Then 75 μL of bacterial suspension (~10^5 CFU/mL) was added into the wells containing biocide solutions. A similar experiment was performed by taking 75 μL of the bacterial suspension (~10^5 CFU/mL) and 25 μL of sterile water as control. The plates were then incubated under stationary conditions for 24 h. After incubation, the medium was removed and washed a single time with 1 × PBS (phosphate buffer saline). Then 0.1% crystal violet (CV) solution (100 μL) was added into the wells and allowed to incubate for 30 min. Crystal violet solution was then discarded, and the plates were washed with 1× PBS. The residual was solubilized with 200 μL of 95% ethanol solution and diluted 10-fold. The OD at 540 nm was then recorded using a plate reader. Biofilm inhibition was quantified by considering 100% biofilm formation in the case of nontreated control.

4.4.2. Biofilm disruption assay.

Bacteria (MRSA and *E. coli* DH52) (6 h grown, midlog phase) were suspended to 10^5 CFU/mL into suitable broths (nutrient media supplemented with 1% glucose and NaCl for MRSA and M9 media supplemented with 0.02% casamino acid and 0.5% glycerol for *E. coli*). The well plates containing 100 μL of these suspensions were then incubated under stationary conditions (for about 24 h for MRSA and 48 h for *E. coli*). After incubation, the medium was removed and washed with 1× PBS single time. Compound **15m** (100 μL at various concentrations) was then added to the wells containing established bacterial

biofilms and allowed to incubate for 24 h. A control was made where 100 μL of medium was added instead of test compound. After 24 h, medium was discarded and planktonic cells were removed by washing with 1× PBS. Then 100 μL of trypsin−EDTA solution was added to the treated biofilm to dissolve. Cell suspension of biofilms was then assessed by plating serial 10-fold dilutions on suitable agar plates. After 24 h of incubation, bacterial colonies were counted, and cell viability was expressed as $\log_{10}$ (CFU/mL) along with the control.

4.5. Inner membrane permeabilization assay.

The 6 h grown culture (mid log phase) of MRSA and *E. coli* were harvested (3500 rpm, 5 min), washed, and resuspended in 5 mM glucose and 5 mM HEPES buffer (pH 7.2) in 1:1 ratio. Then an amount of 10 μL of test compound **15m** (12 × MIC) was added to a cuvette containing 2 mL of bacterial suspension and 10 μM propidium iodide (PI). Fluorescence was monitored at excitation wavelength of 535 nm (slit width of 10 nm) and emission wavelength of 617 nm (slit width of 5 nm). As a measure of inner membrane permeabilization, the uptake of PI was monitored by the increase in fluorescence for 15 min. A control experiment was performed by treating the preincubated bacterial and dye solution only with water (10 μL).

4.6. Cytotoxicity.

Toxicity of compound **15m** toward HEK293 cells was determined by using Cell Counting Kit-8 (CCK8, Yeasen Company) based on WST-8 (4-(3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-tetraz-ol-3-ium-5-yl)benzene-1,3-disulfonate) reduction assay following literature procedures [37]. The HEK293 cells (5000 cells per well) were seeded into 96-well plates. The cells were then incubated in a culture medium containing compound **15m** with a particular concentration for 24 h. After that, 10 mL of CCK8 was added to each well. After 4 h, the unreacted dye was removed by aspiration. The OD values were spectrophotometrically measured in an ELISA plate reader (model 550, Bio-Rad) at a wavelength of 450 nm. The cell survival was expressed as follows: cell viability = (OD treated/OD control) × 100%.

4.7. Relaxation assay of EcTopo IV.

EcTopo IV was diluted with a buffer of 10 mM Tris-Ac, pH 7.5, 50 mM KCl, 35 mM $(NH_4)_2SO_4$, 1 mM DTT, 0.1 mM EDTA and 50% glycerin. Compound **15m** was added to 1 μL of the diluted enzyme present in 20 μL volume before addition of 12 μL of the same buffer containing 6 μL of supercoiled PUC19-T DNA. The mixture was incubated at 37 ℃ for 2 h before termination of the reaction and analyzed by agarose gel electrophoresis as described previously. The ethidium bromide stained gel was photographed

over UV light for densitometry analysis.

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Lists of tables, figures and schemes captions

Table 1. Antibacterial data as MIC (μg/mL)[a,b] for target compounds **6**, **13–18**.

Compds	Gram-positive bacteria				Gram-negative bacteria					
	MRSA	*S. aureus*	*B. subtilis*	*M. luteus*	*E. coli DH52*	*E. coli JM109*	*S. dysenteriae*	*P. aeruginosa*	*B. proteus*	*S. enterica*
6a	4 ± 0.58	2 ± 0.23	2 ± 0.23	4 ± 0.58	2 ± 0.23	8 ± 1.15	4 ± 0.58	2 ± 0.23	1 ± 0.12	2 ± 0.23
6b	0.5 ± 0.05	1 ± 0.12	2 ± 0.23	1 ± 0.12	1 ± 0.12	2 ± 0.23	0.5 ± 0.05	0.5 ± 0.05	2 ± 0.23	1 ± 0.12
13a	1 ± 0.12	2 ± 0.23	1 ± 0.12	1 ± 0.12	0.5 ± 0.05	4 ± 0.58	2 ± 0.23	1 ± 0.12	0.5 ± 0.05	0.5 ± 0.05
13b	16 ± 1.73	16 ± 1.73	8 ± 1.15	0.5 ± 0.05	16 ± 1.73	32 ± 3.46	8 ± 1.15	4 ± 0.58	2 ± 0.23	8 ± 1.15
13c	1 ± 0.12	2 ± 0.23	16 ± 1.73	1 ± 0.12	0.5 ± 0.05	2 ± 0.23	1 ± 0.12	1 ± 0.12	0.5 ± 0.05	0.5 ± 0.05
13d	32 ± 3.46	64 ± 6.35	8 ± 1.15	2 ± 0.23	16 ± 1.73	16 ± 1.73	16 ± 1.73	2 ± 0.23	4 ± 0.58	8 ± 1.15
13e	8 ± 1.15	16 ± 1.73	8 ± 1.15	1 ± 0.12	8 ± 1.15	32 ± 3.46	4 ± 0.58	2 ± 0.23	2 ± 0.23	4 ± 0.58
13f	0.125 ± 0.01	0.25 ± 0.02	0.125 ± 0.01	0.125 ± 0.01	0.125 ± 0.01	0.5 ± 0.05	1 ± 0.12	0.5 ± 0.05	0.125 ± 0.01	0.125 ± 0.01
13g	2 ± 0.23	4 ± 0.58	0.5 ± 0.05	2 ± 0.23	1 ± 0.12	4 ± 0.58	2 ± 0.23	2 ± 0.23	1 ± 0.12	1 ± 0.12
13h	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.25 ± 0.02	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05
13i	4 ± 0.58	64 ± 6.35	16 ± 1.73	16 ± 1.73	8 ± 1.15	32 ± 3.46	16 ± 1.73	1 ± 0.12	8 ± 1.15	4 ± 0.58
13j	2 ± 0.23	16 ± 1.73	8 ± 1.15	8 ± 1.15	4 ± 0.58	8 ± 1.15	4 ± 0.58	0.5 ± 0.05	4 ± 0.58	4 ± 0.58
14a	8 ± 1.15	128 ± 68.65	2 ± 0.23	4 ± 0.58	4 ±0.58	32 ± 3.46	128 ± 68.65	32 ± 3.46	1 ± 0.12	0.5 ± 0.05
14b	16 ± 1.73	256 ± 137.29	2 ± 0.23	8 ± 1.15	32 ± 3.46	128 ± 68.65	32 ± 3.46	32 ± 3.46	128 ± 68.65	16 ± 1.73
14c	64 ± 6.35	512 ± 49.65	32 ± 3.46	16 ± 1.73	8 ± 1.15	128 ± 68.65	128 ± 68.65	64 ± 6.35	8 ± 1.15	256 ± 137.29
14d	32 ± 3.46	256 ± 137.29	16 ± 1.73	4 ± 0.58	4 ± 0.58	64 ± 6.35	32 ± 3.46	16 ± 1.73	4 ± 0.58	8 ± 1.15
14e	2 ± 0.23	32 ± 3.46	32 ± 3.46	4 ± 0.58	8 ± 1.15	32 ± 3.46	16 ± 1.73	2 ± 0.23	4 ± 0.58	4 ± 0.58
14f	8 ± 1.15	32 ± 3.46	4 ± 0.58	16 ± 1.73	0.5 ± 0.05	32 ± 3.46	8 ± 1.15	4 ± 0.58	8 ± 1.15	8 ± 1.15
14g	256 ± 137.29	128 ± 68.65	128 ± 68.65	256 ± 137.29	256 ± 137.29	512 ± 49.65	256 ± 137.29	256 ± 137.29	256 ± 137.29	256 ± 137.29
14h	128 ± 68.65	256 ± 137.29	128 ± 68.65	128 ± 68.65	256 ± 137.29	256 ± 137.29	256 ± 137.29	256 ± 137.29	256 ± 137.29	256 ± 137.29
14i	128 ± 68.65	256 ± 137.29	0.5 ± 0.05	2 ± 0.23	2 ± 0.23	128 ± 68.65	8 ± 1.15	2 ± 0.23	0.5 ± 0.05	0.5 ± 0.05

14j	2 ± 0.23	32 ± 3.46	32 ± 3.46	1 ± 0.12	1 ± 0.12	4 ± 0.58	4 ± 0.58	1 ± 0.12	32 ± 3.46	4 ± 0.58
14k	64 ± 6.35	128 ± 68.65	4 ± 0.58	4 ± 0.58	1 ± 0.12	128 ± 68.65	8 ± 1.15	2 ± 0.23	2 ± 0.23	4 ± 0.58
14l	128 ± 68.65	256 ± 137.29	16 ± 1.73	32 ± 3.46	16 ± 1.73	64 ± 6.35	64 ± 6.35	16 ± 1.73	16 ± 1.73	16 ± 1.73
14m	1 ± 0.12	16 ± 1.73	32 ± 3.46	1 ± 0.12	0.5 ± 0.05	2 ± 0.23	4 ± 0.58	1 ± 0.12	0.5 ± 0.05	4 ± 0.58
14n	64 ± 6.35	64 ± 6.35	64 ± 6.35	128 ± 68.65	128 ± 68.65	128 ± 68.65	64 ± 6.35	32 ± 3.46	32 ± 3.46	32 ± 3.46
14o	64 ± 6.35	32 ± 3.46	4 ± 0.58	4 ± 0.58	8 ± 1.15	16 ± 1.73	2 ± 0.23	4 ± 0.58	4 ± 0.58	16 ± 1.73
14p	64 ± 6.35	64 ± 6.35	64 ± 6.35	128 ± 68.65	128 ± 68.65	128 ± 68.65	64 ± 6.35	32 ± 3.46	64 ± 6.35	16 ± 1.73
15a	0.125 ± 0.01	8 ± 1.15	16 ± 1.73	0.5 ± 0.05	2 ± 0.23	16 ± 1.73	16 ± 1.73	1 ± 0.12	4 ± 0.58	0.5 ± 0.05
15b	2 ± 0.23	64 ± 6.35	16 ± 1.73	2 ± 0.23	8 ± 1.15	64 ± 6.35	64 ± 6.35	2 ± 0.23	32 ± 3.46	4 ± 0.58
15c	256 ± 137.29	512 ± 49.65	128 ± 68.65	512 ± 49.65	512 ± 49.65	512 ± 49.65	512 ± 49.65	512 ± 49.65	512 ± 49.65	512 ± 49.65
15d	32 ± 3.46	128 ± 68.65	128 ± 68.65	64 ± 6.35	128 ± 68.65	128 ± 68.65	1 ± 0.12	8 ± 1.15	4 ± 0.58	8 ± 1.15
15e	16 ± 1.73	8 ± 1.15	8 ± 1.15	8 ± 1.15	32 ± 3.46	32 ± 3.46	16 ± 1.73	8 ± 1.15	32 ± 3.46	8 ± 1.15
15f	0.125 ± 0.01	4 ± 0.58	8 ± 1.15	0.25 ± 0.02	1 ± 0.12	4 ± 0.58	2 ± 0.23	1 ± 0.12	1 ± 0.12	0.25 ±0.02
15g	8 ± 1.15	4 ± 0.58	2 ± 0.23	4 ± 0.58	16 ± 1.73	8 ± 1.15	4 ± 0.58	4 ± 0.58	16 ± 1.73	4 ± 0.58
15h	4 ± 0.58	16 ± 1.73	0.5 ± 0.05	4 ± 0.58	2 ± 0.23	16 ± 1.73	16 ± 1.73	2 ± 0.23	1 ± 0.12	2 ± 0.23
15i	0.5 ± 0.05	8 ± 1.15	4 ± 0.58	0.125 ± 0.01	0.5 ± 0.05	4 ± 0.58	4 ± 0.58	0.125 ± 0.01	0.5 ± 0.05	0.25 ± 0.02
15j	0.25 ± 0.02	16 ± 1.73	8 ± 1.15	0.25 ± 0.02	1 ± 0.12	2 ± 0.23	4 ± 0.58	2 ± 0.23	1 ± 0.12	0.25 ± 0.02
15k	8 ± 1.15	256 ± 137.29	32 ± 3.46	64 ± 6.35	256 ± 137.29	256 ± 137.29	256 ± 137.29	16 ± 1.73	128 ± 68.65	4 ± 0.58
15l	4 ± 0.58	16 ± 1.73	8 ± 1.15	16 ± 1.73	32 ± 3.46	16 ± 1.73	16 ± 1.73	8 ± 1.15	16 ± 1.73	16 ± 1.73
15m	0.125 ± 0.01	8 ± 1.15	4 ± 0.58	0.0625 ± 0.007	0.125 ± 0.01	1 ± 0.12	1 ± 0.12	0.5 ± 0.05	0.0312 ± 0.005	0.0312 ± 0.005
15n	2 ± 0.23	4 ± 0.58	4 ± 0.58	8 ± 1.15	16 ± 1.73	8 ± 1.15	8 ± 1.15	4 ± 0.58	8 ± 1.15	16 ± 1.73
16a	16 ± 1.73	64 ± 6.35	2 ± 0.23	64 ± 6.35	32 ± 3.46	32 ± 3.46	128 ± 68.65	32 ± 3.46	16 ± 1.73	32 ± 3.46
16b	4 ± 0.58	4 ± 0.58	128 ± 68.65	1 ± 0.12	4 ± 0.58	2 ± 0.23	0.5 ± 0.05	0.5 ± 0.05	2 ± 0.23	1 ± 0.12

17a	0.5 ± 0.05	2 ± 0.23	8 ± 1.15	4 ± 0.58	1 ± 0.12	16 ±	0.5 ± 0.05	1 ± 0.12	8 ± 1.15	2 ± 0.23
17b	0.25 ± 0.01	1 ± 0.12	1 ± 0.12	1 ± 0.12	1 ± 0.12	4 ± 0.58	0.25 ± 0.01	0.5 ± 0.05	1 ± 0.12	1 ± 0.12
18a	128 ± 68.65	64 ± 6.35	4 ± 0.58	32 ± 3.46	32 ± 3.46	64 ± 6.35	64 ± 6.35	8 ± 1.15	32 ± 3.46	16 ± 1.73
18b	64 ± 6.35	32 ± 3.46	2 ± 0.23	4 ± 0.58	8 ± 1.15	8 ± 1.15	8 ± 1.15	2 ± 0.23	4 ± 0.58	4 ± 0.58
Chloromycin	16 ± 1.73	16 ± 1.73	32 ± 3.46	8 ± 1.15	32 ± 3.46	32 ± 3.46	16 ± 1.73	32 ± 3.46	32 ± 3.46	32 ± 3.46
Norfloxacin	8 ± 1.15	2 ± 0.23	4 ± 0.58	2 ± 0.23	1 ± 0.12	1 ± 0.12	16 ± 1.73	16 ± 1.73	8 ± 1.15	4 ± 0.58
Ciprofloxacin	2 ± 0.23	0.5 ± 0.05	2 ± 0.23	2 ± 0.23	2 ± 0.23	2 ± 0.23	2 ± 0.23	1 ± 0.12	2 ± 0.23	1 ± 0.12
Clinafloxacin	1 ± 0.12	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.5 ± 0.05	0.25 ± 0.01

[a] Minimum inhibitory concentrations were determined by micro broth dilution method for microdilution plates.
[b] MRSA, Methicillin-Resistant *Staphylococcus aureus* N315; *S. aureus*, *Staphylococcus aureus* ATCC25923; *B. subtilis*, *Bacillus subtilis* ATCC6633; *M. luteus*, *Micrococcus luteus* ATCC4698; *E. coli* DH52, *Escherichia coli* DH52; *E. coli* JM109, *Escherichia coli* JM109; *S. dysenteriae*, *Shigella dysenteriae*; *P. aeruginosa*, *Pseudomonas aeruginosa* ATCC27853; *B. proteus*, *Bacillus proteus* ATCC13315; *S. enterica*, *Salmonella enterica* ATCC14028.

Fig. 1. Design of novel benzimidazole quinolones.

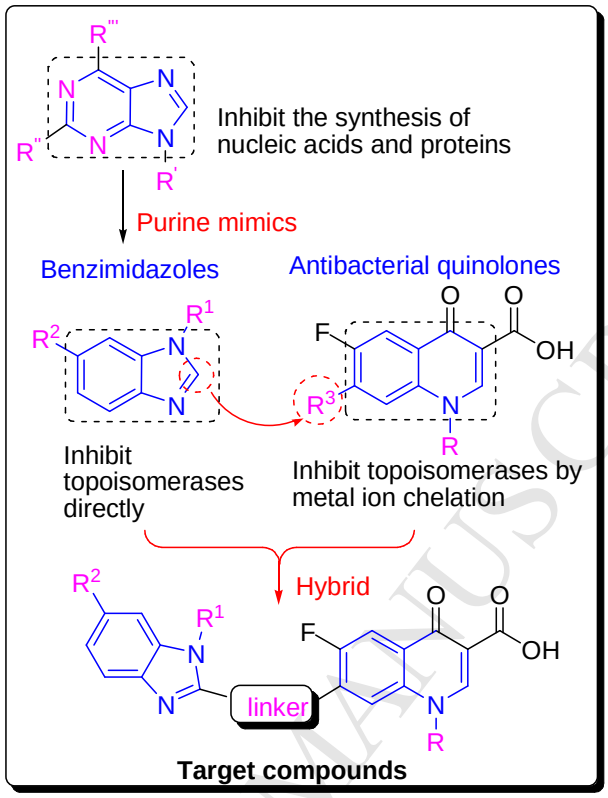


Fig. 2. X-ray single-crystal structure of compound **2**.

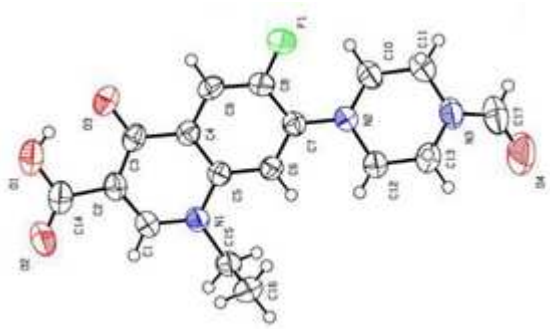


Fig. 3. Time−kill kinetics of compound **15m** (6 × MIC) against MRSA. The data obtained are from two independent experiments performed in triplicate.

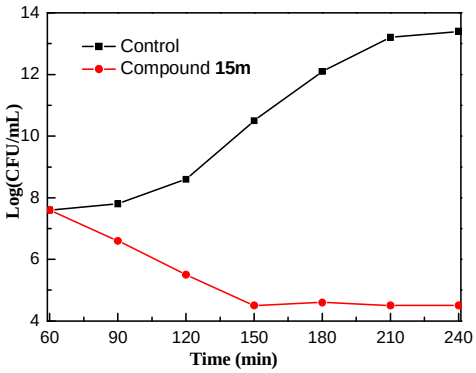
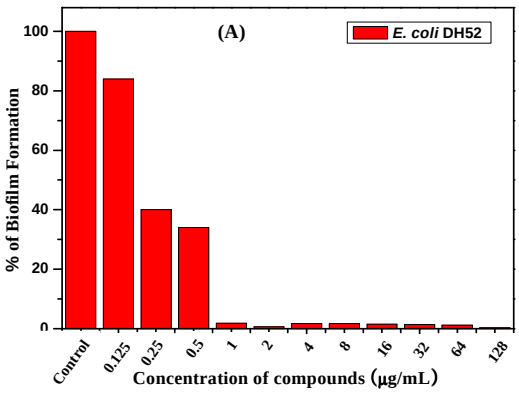
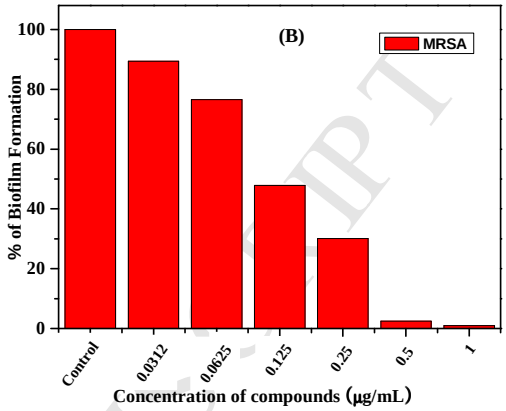


Fig. 4. Antibiofilm activity of compound **15m**: (A) *E. coli* DH52 cell viability in presence of compound **15m** at different concentrations and (B) MRSA cell viability in presence of compound **15m** at different concentrations.

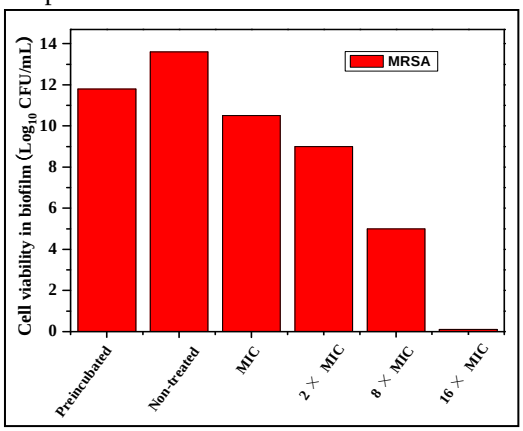


(A)

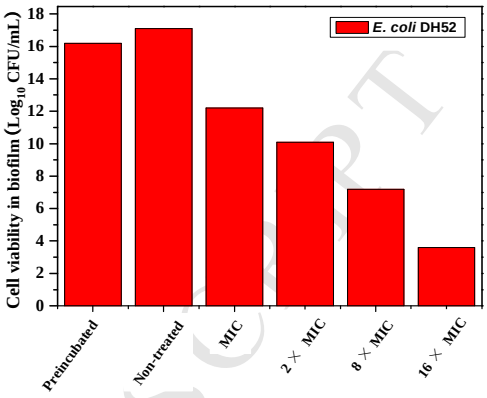


(B)

Fig. 5. Biofilm disruption by compound **15m**: (A, B) cell viability in biofilms of MRSA and *E. coli* DH52, respectively, obtained by plating and counting the viable bacteria after treating with different concentrations of compound **15m**.

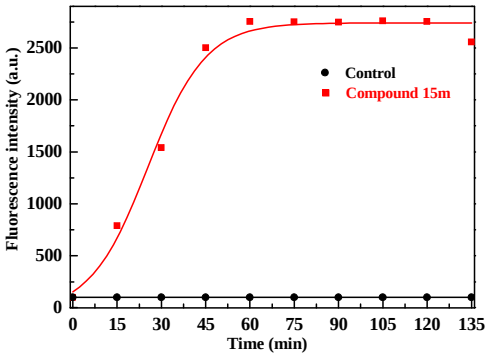


(A)

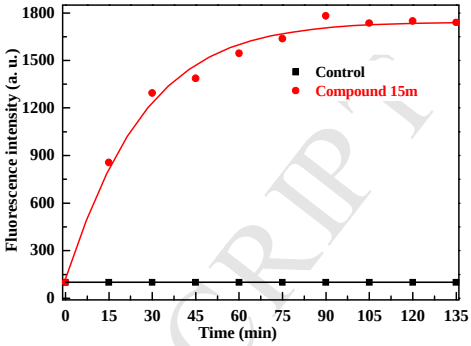


(B)

Fig. 6. Mechanism of antibacterial action of compound **15m** at concentrations of 12 × MIC: (A) membrane permeabilization of *E. coli* DH52; (B) membrane permeabilization of MRSA.



(A)



(B)

Fig. 7. Relative cell viabilities of compound **15m** in HEK293 cells.

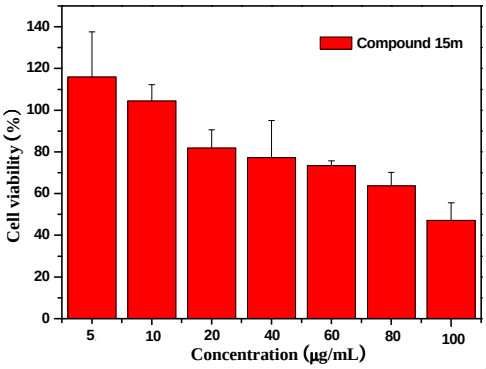


Fig. 8(i) Three-dimensional conformation of compound **15m** docked in topoisomerase IV–DNA complex (i)

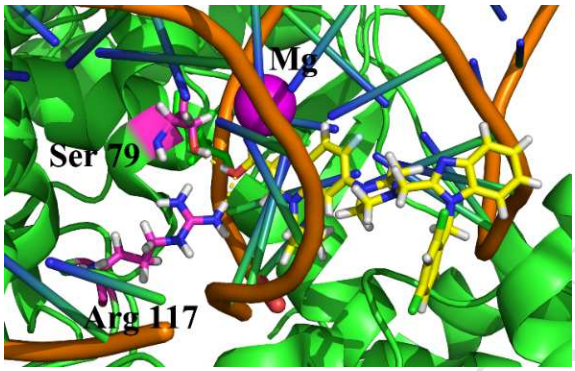


Fig. 8(ii) Three-dimensional conformation of clinafloxacin docked in topoisomerase IV–DNA complex (ii)

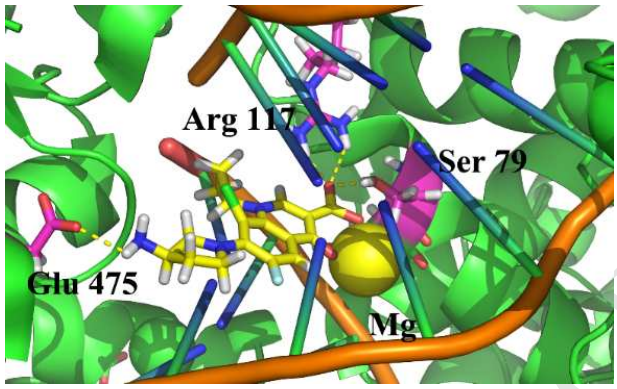


Fig. 8(iii) Three-dimensional conformation of compound **15m** docked in topoisomerase IV–DNA complex (iii)

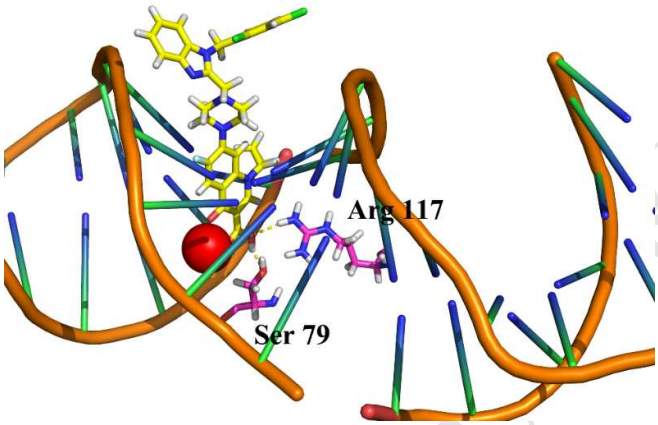


Fig. 9 UV absorption spectra of DNA with different concentrations of compound **15m** (pH = 7.4, T = 298 K). Inset: comparison of absorption at 260 nm between the **15m**–DNA complex and the sum values of free DNA and free compound **15m**. *c*(DNA) = 8.32 × 10^{-5} mol/L, and *c*(compound **15m**) = 0–1.2 × 10^{-5} mol/L for curves a–g respectively at increment of 0.2 × 10^{-5}.

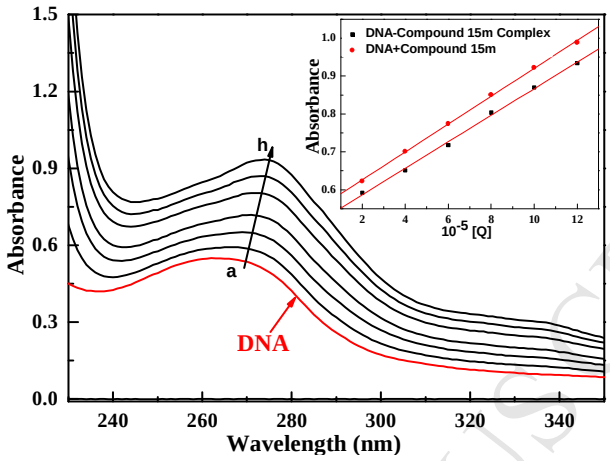


Fig. 10 The plot of $A^0/(A-A^0)$ versus 1/[compound **15m**].

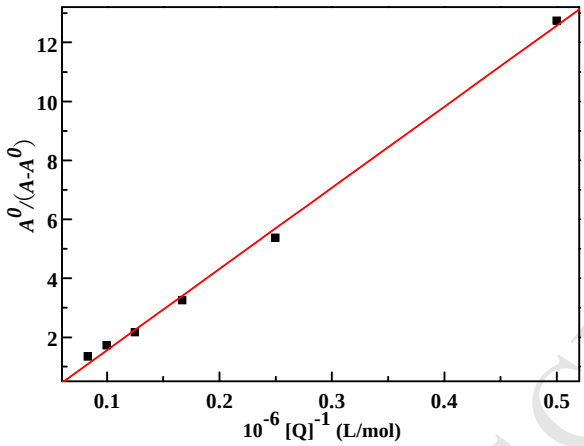
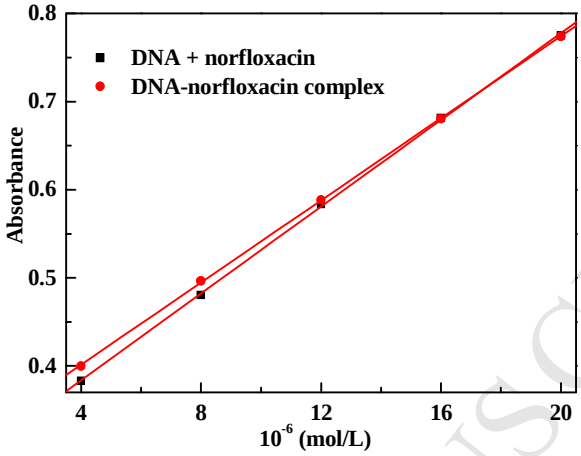
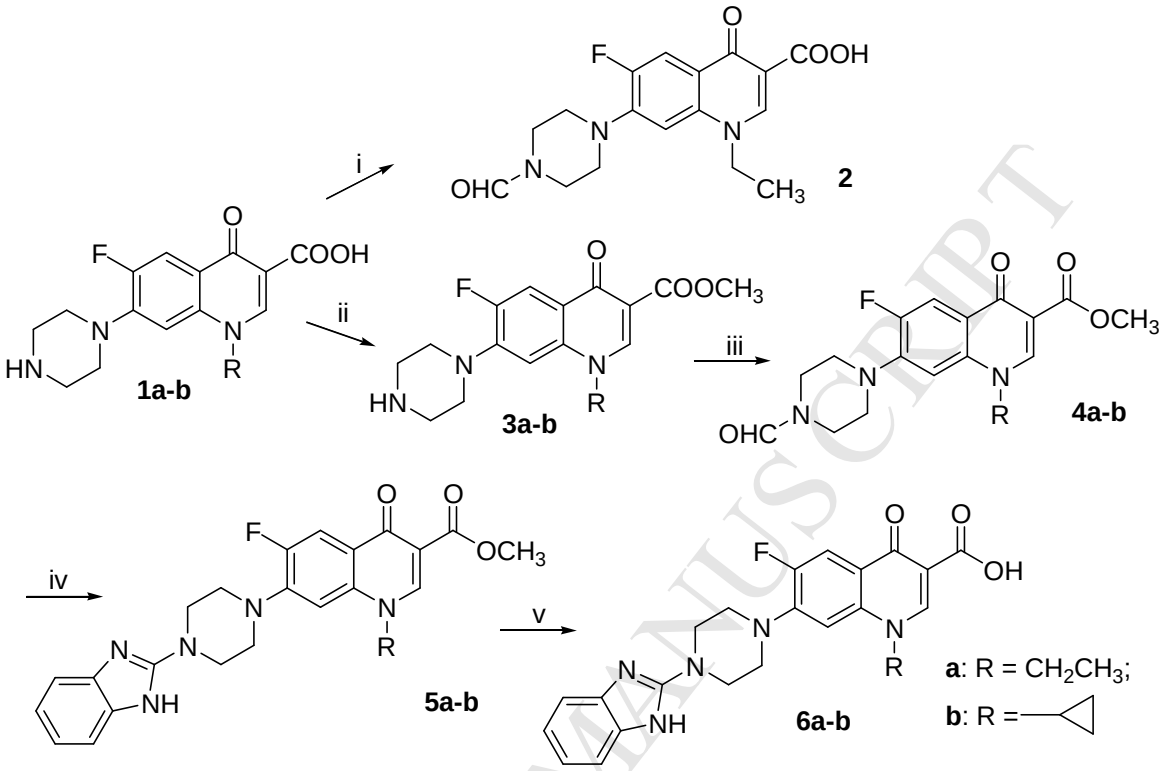


Fig. 11 Comparison of absorption at 260 nm between the DNA–norfloxacin complex and the sum values of free DNA and free norfloxacin. c(DNA) = 7.61 × 10^{-5} mol/L, and c (norfloxacin) = 0–2 × 10^{-5} mol/L at increment of 0.4 × 10^{-5}.

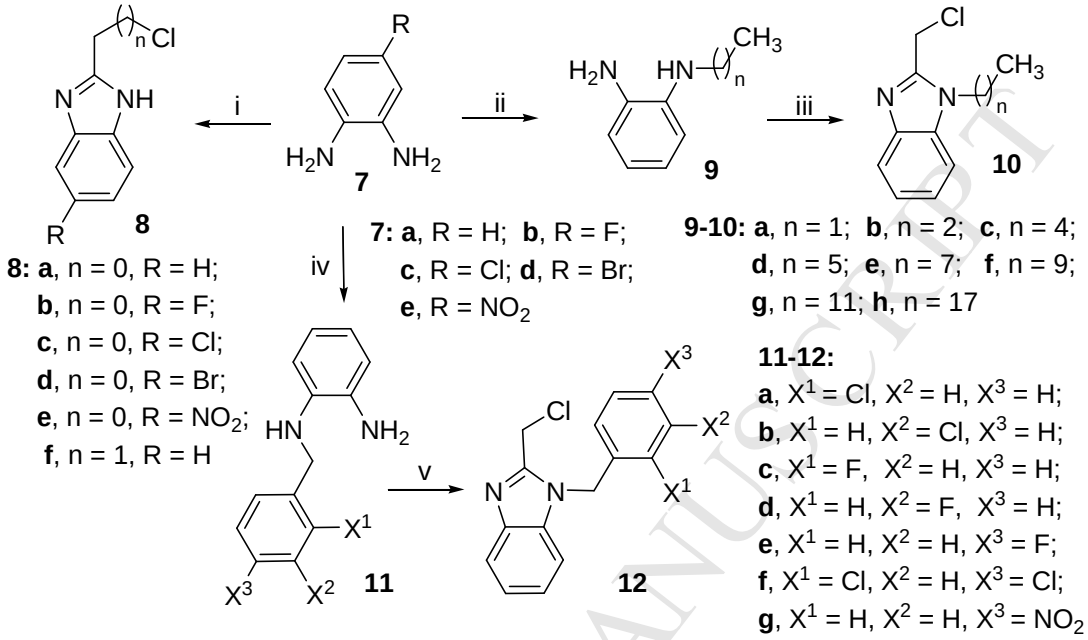


Scheme 1. Synthesis of benzimidazole quinolones **6a**–**b**.



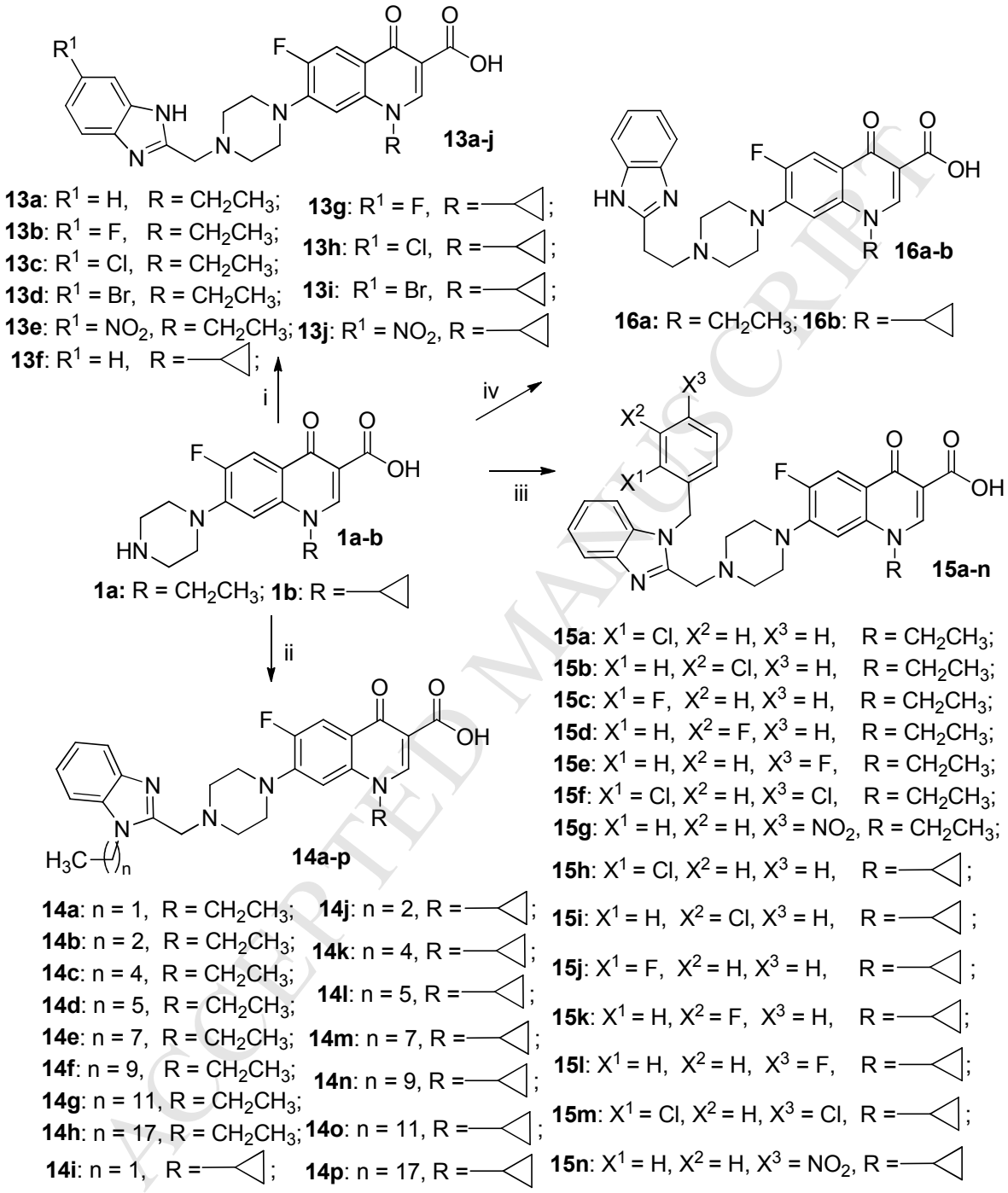
Reagents and conditions: (i) formamide, 70 ºC; (ii) methanol, thionyl chloride, reflux; (iii) formamide, 70 ºC; (iv) *o*-phenylene diamine, copper sulfate, 1,4-dioxane, reflux; (v) 3% sodium hydroxide solution, 100 ºC.

Scheme 2. Synthesis of chloroalkyl benzimidazoles **8**, **10** and **12**.



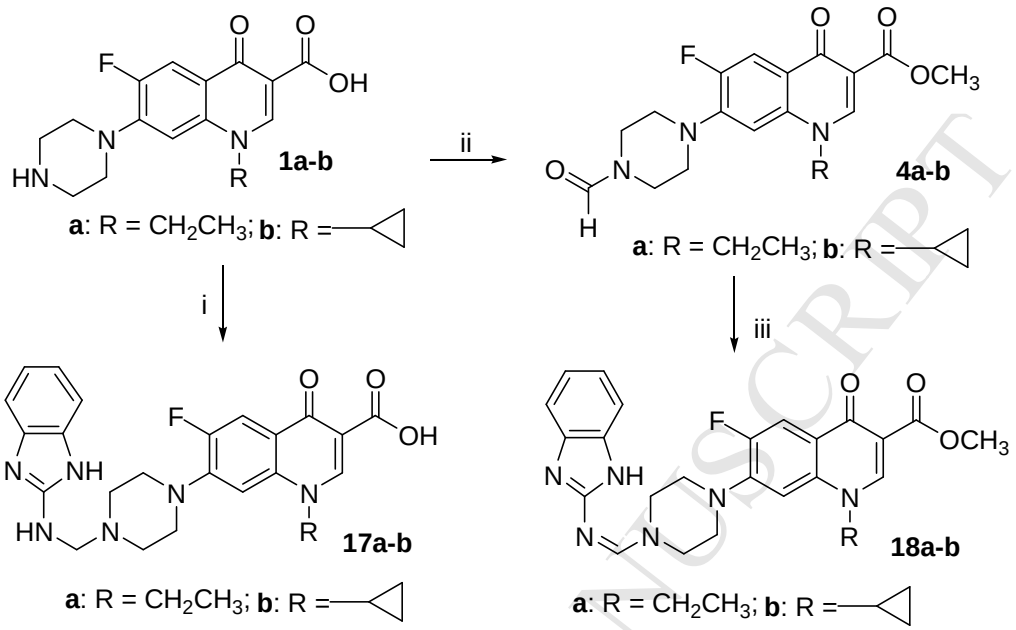
[a]Reagents and conditions: (i) chloroacetic acid or chloropropionic acid, 5 mol/L hydrochloric acid, reflux; (ii) alkyl bromide, dimethylformamide, potassium carbonate, rt; (iii) chloroacetic acid, 5 mol/L hydrochloric acid, reflux; (iv) halobenzyl halide, potassium carbonate, acetonitrile, 60 ºC; (v) chloroacetic acid, 5 mol/L hydrochloric acid, reflux.

Scheme 3 Synthesis of benzimidazole quinolones **13**–**16**.



Reagents and conditions: (i) 2-chloromethyl benzimidazoles **8a–e**, potassium carbonate, acetonitrile, 0–50 ºC; (ii) *N*-alkyl benzimidazoles **10a–h**, potassium carbonate, acetonitrile, 0–50 ºC; (iii) *N*-halobenzyl derivatives **12a–g**, potassium carbonate, acetonitrile, 0–50 ºC; (iv) 2-chloroethyl benzimidazole **8f**, potassium carbonate, acetonitrile, 0–50 ºC.

Scheme 4 Synthesis of benzimidazole quinolones **17**–**18**.



Reagents and conditions: (i) 2-aminobenzimidazole, paraformaldehyde, acetic acid, reflux; (ii) (1) methanol, thionyl chloride, reflux; (2) formamide, 70 ºC; (iii) 2-aminobenzimidazole, copper sulfate, 1,4-dioxane, reflux.

- ► Prepared quinolones gave strong antimicrobial activity and broad spectrum.
- ► Some compounds exhibited strong anti-MRSA activity.
- ► Inducing bacterial resistance by target compound was much slower than clinical drugs.
- ► Molecular modeling with DNA rationalized the high antibacterial activity.
- ► Interactions of compound and DNA indicated a possible interaction mechanism.