Bioorganic Chemistry 103 (2020) 104176

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Design and synthesis of novel desfluoroquinolone-aminopyrimidine hybrids as potent anti-MRSA agents with low hERG activity

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ARTICLE INFO

Keywords:
Desfluoroquinolone
Aminopyrimidine
Hybrids
Anti-MRSA
hERG activity

ABSTRACT

Despite the fact that the introduction of a fluorine atom at the C-6 position has resulted in the evolution of fluoroquinolones, fluoroquinolone-induced cardiac toxicity has drawn considerable attention. In this context, desfluoroquinolone-based hybrids with involvement of C-7 aminopyrimidine functional group were designed and synthesized. The biological results showed majority of these hybrids still demonstrated potent anti-MRSA activity with MIC values between 0.38 and 1.5 μg/mL, despite the lack of the typical C-6 fluorine atom. Particularly, the most active **B14** exhibited activities at submicromolar concentrations against a panel of MRSA strains including vancomycin-intermediate strains, levofloxacin-resistant isolates, and linezolid-resistant isolates, *etc*. As expected, it also displayed highly selective toxicity toward bacterial cells and low hERG inhibition. Further resistance development study indicated MRSA is unlikely to acquire resistance against **B14**. The docking study revealed that two hydrogen bonds were formed between the C-7 substituent and the surrounding DNA bases, which might contribute to overcome resistance by reducing the dependence on the magnesium-water bridge interactions with topoisomerase IV. These results indicate a promising strategy for developing new antibiotic quinolones to combat multidrug resistance and cardiotoxicity.

1. Introduction

The ever-increasing bacterial resistance to the existing antibiotics poses a global threat to public health. According to newly updated estimates reported by the Centers for Disease Control and Prevention (CDC), more than 2.8 million infections are caused by antibiotic-resistant pathogens annually in the United Sates, leading to at least 35,000 deaths [1]. Among them, the fatalities attributed to methicillin-resistant *Staphylococcus aureus* (MRSA) are particularly high as compared with other antibiotic-resistant pathogens. MRSA infections are difficult to treat because they have acquired resistance to a variety of classes of antibacterial drugs, including β-lactam antibiotics [2], macrolides [3], fluoroquinolones [4], glycopeptides [5], and oxazolidinones. [6] Vancomycin is currently used as an antibiotic of last resort for the treatment of MRSA infections. Unfortunately, since the first strain of MRSA with reduced susceptibility to vancomycin was reported in 1997 [7], there has been an increase in the number of cases with both vancomycin-intermediate MRSA (VISA) and vancomycin-resistant MRSA (VRSA) [8]. Therefore, the search for new anti-MRSA agents with improved resistance profiles continues to be a major challenge in antibacterial chemotherapy [9].

Quinolones are one of the most widely prescribed antibiotics used to treat various bacterial infections. They can be divided into four generations. The first-generation agents without a substituent at the C-6 position of the quinolone nucleus only exhibit weak to moderate activity against Gram-negative bacteria. A remarkable breakthrough in the development of quinolone antibiotics came with the introduction of a fluorine atom at the C-6 position, resulting in the evolution of

Abbreviations: CDC, Centers for Disease Control and Prevention; *E. Coli.*, *Escherichia coli*; MRSA, Methicillin-resistant *Staphylococcus aureus*; VISA, vancomycin-intermediate MRSA; VRSA, vancomycin-resistant MRSA; hEGR, human ether-a-go-go related gene; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide; DA5, deoxyadenosine monophosphate 5; DG1, deoxyguanosine monophosphate 1

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https://doi.org/10.1016/j.bioorg.2020.104176
Received 27 May 2020; Received in revised form 4 August 2020; Accepted 11 August 2020
Available online 26 August 2020
0045-2068/

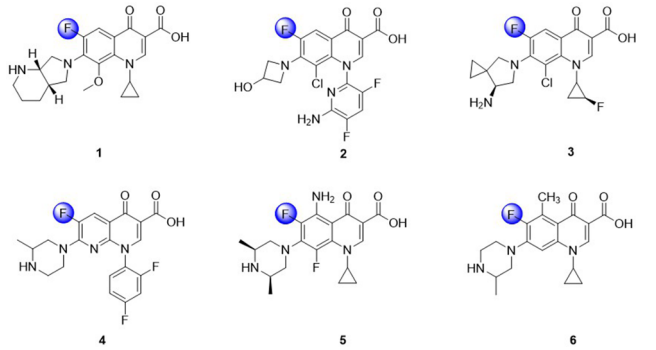


Fig. 1. Representative anti-MRSA fluoroquinolone antibiotics **1–3** and antibiotics **4–6** withdrawn from the market.

fluoroquinolones with expanding antibacterial spectrum from Gram-negative to -positive bacteria [10]. Consequently, the 6-fluoro substituent has been a fundamental structural feature of all the latter generations. In addition, some of the fourth-generation drugs such as moxifloxacin [11] (**1**, Fig. 1), delafloxacin [12] (**2**, Fig. 1), and sitafloxacin [13] (**3**, Fig. 1) can also demonstrate strong potency against MRSA. Despite their wide usage in clinical practice, fluoroquinolone-induced cardiac toxicity due to the blockage of the human Ether-a-go-go Related Gene (hEGR) potassium channel has drawn considerable attention. [14] This issue had been further stressed by the withdrawal of temafloxacin (**4**, Fig. 1), sparfloxacin (**5**, Fig. 1) and grepafloxacin (**6**, Fig. 1) from the market because of pro-arrhythmic side-effects. [15] Thus the development of this class of new antibiotics to overcome multidrug resistance and cardiotoxicity has become increasingly urgent.

1.1. Design and chemistry

Recently, ozenoxacin [16] (**7**, Fig. 2), garenoxacin [17] (**8**, Fig. 2) and nemonoxacin [18] (**9**, Fig. 2), the antibiotics lacking the typical 6-fluoro substituent on quinolone nucleus, have been approved in many regions for the treatment of bacterial infections including those caused by MRSA. Despite the particular interest generated by these antibiotics, few examples on desfluoroquinolones as potent antibacterial agents are described in the literature. Based on our understanding of quinolone-enzyme interactions, in this context, we exploited “merging” approach [19] by combining the structural features of antibiotic quinolones and another antibacterial aminopyrimidines [20] to generate a novel series of desfluoroquinolone-aminopyrimidine hybrids **A** (Fig. 3), with the aim to reduce their cardiotoxic potential while maintaining anti-MRSA properties.

Genetic evidence strongly suggests that the primary target of quinolones in *Staphylococcus aureus* is topoisomerase IV [21]. Quinolones act by stabilizing the topoisomerase IV-cleaved DNA complexes *via* a critical water-metal ion interaction, thus inhibiting DNA synthesis [22]. Target-mediated quinolone resistance is caused by specific mutations in topoisomerase IV that disrupt this water-metal ion bridge interaction [23]. Our newly designed hybrids **A** are characterized by the incorporation of an aminopyrimidine ring at the C-7 position of quinolones, which contains both hydrogen-accepting and -donating functionalities and is expected to be sufficiently close in space to the

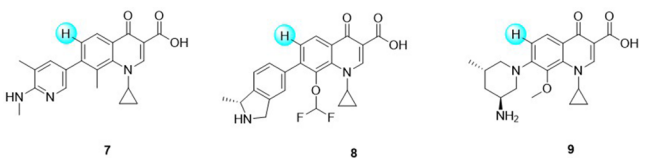


Fig. 2. Desfluoroquinolone antibiotics.

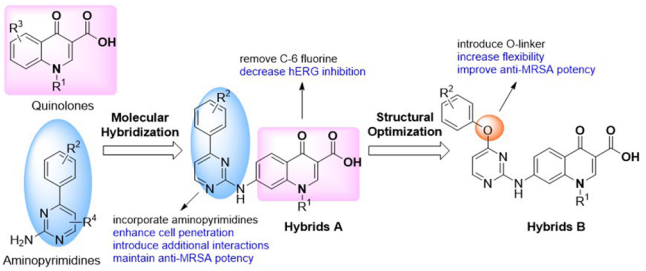
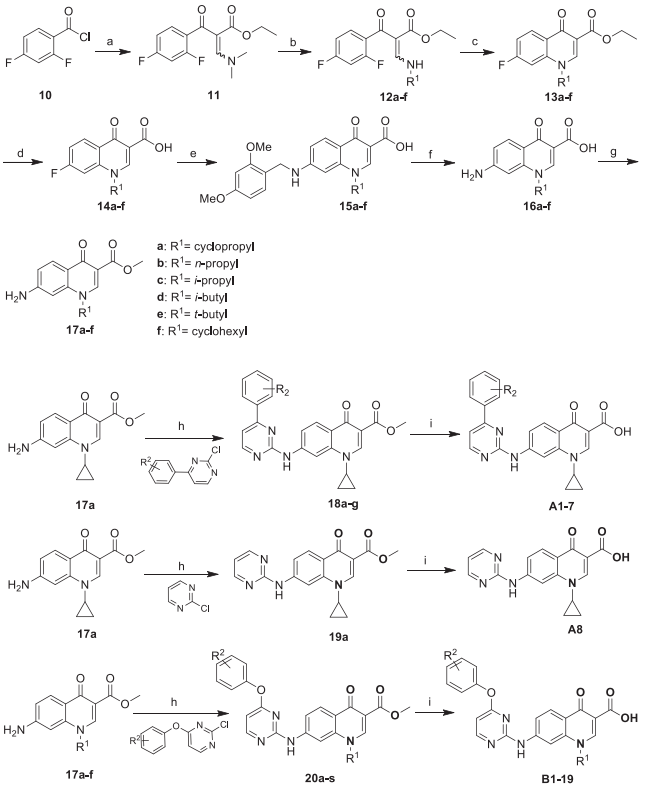


Fig. 3. Design of the desfluoroquinolone-aminopyrimidine hybrids.

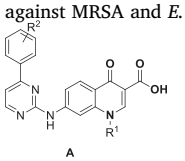
surrounding DNA bases in the binding sites to produce strong hydrogen and/or π-π interactions. The additional drug-enzyme contacts mediated by this C-7 substituent would offer the possibility to overcome quinolone resistance by reducing the dependence on the magnesium-water bridge interactions with topoisomerase IV. The phenyl moiety with lipophilic property directly linked on the aminopyrimidine ring would enhance the cell penetration and thus compensate the loss caused by the absence of the C-6 fluorine atom. Structural optimization resulted into hybrid compounds **B** (Fig. 3) in order to further enhance their potency.

The synthesis of the desfluoroquinolone-aminopyrimidine hybrids **A** and **B** is depicted in Scheme 1. The benzoyl chlorides **10** was coupled with ethyl 3-(dimethylamino)acrylate to give the acrylates **11**. Substitution with appropriate aliphatic amines followed by cyclization with potassium carbonate afforded quinolones **13**. Hydrolysis of **13** and then treatment with 2, 4-dimethoxybenzylamine furnished **15**. Removal of 2, 4-dimethoxybenzyl group and subsequent esterification with



Scheme 1. Synthesis of desfluoroquinolone-aminopyrimidine hybrids **A** and **B**.[a]
[a]Reagents and conditions: (a) ethyl 3-(dimethylamino)acrylate, Et_3N, toluene, 90 °C, 4 h; (b) aliphatic amines, THF, 50 °C, 3 h; (c) K_2CO_3, DMF, 60–90 °C, 1–6 h; (d) 5 M NaOH, THF, 50 °C, 3.5 h (e) 2,4-dimethoxybenzylamine; DMSO, 85 °C, 6 h; (f) CF_3COOH, DCM, rt, 5 h; (g) $TMSCH_2N_2$, THF, MeOH, rt, 24 h; (h) $Pd(OAc)_2$, K_2CO_3, BINAP, DMF, 90 °C, 12–18 h; (i) 5 M NaOH, THF, 50 °C, 3.5 h.

Table 1
Antibacterial activities of desfluoroquinolone-aminopyrimidine hybrids **A** against MRSA and *E. Coli.*[a]



A

Compd	R^1	R^2	MIC[b] (μg/mL)	
			MRSA (ATCC33591)	*E. coli* (ATCC25922)
A1	Cyclopropyl	H	> 50	> 50
A2	Cyclopropyl	2-Methyl	> 50	> 50
A3	Cyclopropyl	3-Methyl	6	> 50
A4	Cyclopropyl	4-Methyl	0.75	> 50
A5	Cyclopropyl	2,4-diMethyl	1.5	> 50
A6	Cyclopropyl	3,4-diMethyl	0.75	> 50
A7	Cyclopropyl	4-*n*-Propyl	12.5	> 50
A8			25	> 50
16a			> 50	> 50
Cip.			0.5	0.5
Van.			1.5	> 50

[a] Data provided by Prof. Jianfeng Cai. All experiments were performed in at least triplicates.
[b] MIC: minimum inhibitory concentration. Cip.: Ciprofloxacin. Van.: Vancomycin.

trimethylsilyldiazomethane yielded **17**. Buchwald-Hartwig amination with 2-chloro-4-arylpyrimidines and subsequent hydrolysis provided the target hybrids **A1-7**. For comparison, **A8** was also prepared. Buchwald-Hartwig amination with 2-chloro-4-aryloxypyrimidines and subsequent hydrolysis afforded the target hybrids **B1-22**.

2. Results and discussion

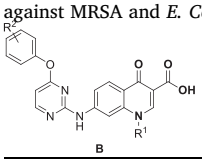
2.1. Inhibitory activities against MRSA ATCC33591

At the outset of our study, desfluoroquinolone-aminopyrimidine hybrids **A1-7** as well as parent compounds **A8** and **16a** were synthesized and evaluated for antibacterial activities against two bacterial cells (MRSA ATCC33591 and *E. coli* ATCC25922). FDA-approved drugs including commercial ciprofloxacin and vancomycin were used as reference according to the same procedure.

The summarized results are shown in Table 1. Hybrid **A1**, characterized by a cyclopropyl moiety at *N*-1 and a phenyl group directly linked to the pyrimidine ring, was devoid of anti-MRSA activity even at a concentration of 50 μg/mL. Although the introduction of a methyl group at the *ortho* position (**A2**) of the benzene ring still remained no inhibitory activity, the introduction of a methyl group at the *meta* or *para* position of the benzene ring resulted in **A3** and **A4**, exhibiting MIC values of 6 and 0.75 μg/mL, respectively. Particularly, **A4** was proved to be slightly less active than ciprofloxacin. We next introduced dimethyl groups into the benzene ring and found that both 2,4-dimethyl compound (**A5**) and 3,4-dimethyl compound (**A6**) showed good inhibitory activities, whereas the parent compound **16a** were completely inactive. Removal of the substituted benzene ring afforded **A8**, resulting in dramatically decreased activity. These observations highlight the importance of the hybrid scaffold for the anti-MRSA activity. All the synthesized compounds proved to be inactive in inhibiting Gram-negative *E. coli* at a concentration of 50 μg/mL, suggesting that these hybrids may exhibit Gram-positive specific inhibitory activity.

With this first set of compounds in our hands, we decided to make further modification in an attempt to obtain more potent anti-MRSA agents. Considering the rigidity of the structure of hybrids **A**, an oxygen-linker was introduced to connect the substituted benzene ring and the pyrimidine moiety with the aim to increase the structural flexibility, thus enhancing the interactions with DNA topoisomerase IV. A series of new hybrids **B** were prepared and assessed in parallel with ciprofloxacin and vancomycin against MRSA and *E. coli*.

Table 2
Antibacterial activities of desfluoroquinolone-aminopyrimidine hybrids **B** against MRSA and *E. Coli.*[a]



B

Compd	R^1	R^2	MIC[a] (μg/mL)	
			MRSA (ATCC33591)	*E. coli* (ATCC25922)
B1	Cyclopropyl	H	12.5	> 50
B2	Cyclopropyl	2-Methyl	1.5	> 50
B3	Cyclopropyl	3-Methyl	1.5	> 50
B4	Cyclopropyl	4-Methyl	0.38	> 50
B5	Cyclopropyl	4-Ethyl	1.5	> 50
B6	Cyclopropyl	4-*n*-Butyl	3	> 50
B7	Cyclopropyl	4-*i*-propyl	6	> 50
B8	Cyclopropyl	4-*t*-Butyl	6	> 50
B9	Cyclopropyl	4-Cyclohexyl	12.5	> 50
B10	Cyclopropyl	2-Methoxyl	6	> 50
B11	Cyclopropyl	3-Methoxyl	3	> 50
B12	Cyclopropyl	4-Methoxyl	3	> 50
B13	Cyclopropyl	2,4-diMethyl	1.5	> 50
B14	Cyclopropyl	3,4-diMethyl	0.38	> 50
B15	*n*-Propyl	4-Methyl	1.5	> 50
B16	*i*-Propyl	4-Methyl	1.5	> 50
B17	*i*-Butyl	4-Methyl	1.5	> 50
B18	*t*-Butyl	4-Methyl	1.5	> 50
B19	Cyclohexyl	4-Methyl	1.5	> 50
Cip.			0.5	0.5
Van.			1.5	> 50

[a] Data provided by Prof. Jianfeng Cai. All experiments were performed in at least triplicates.

As presented in Table 2, all the hybrids with an oxygen-linker exhibited moderate to excellent anti-MRSA potency with MIC values ranging from 0.38 to 12.5 μg/mL. Interestingly, a loss of activity in inhibiting *E. coli* with these new hybrids **B** was observed as well, showing Gram-positive specific inhibitory activity. The introduction of electron-donating groups into the benzene ring (**B2-15**) dramatically increased the anti-MRSA activity regardless of the features of the substituted groups, except for **B9**. The positive effect of introducing a substituent at the *para* position was greater than that at the *ortho* or *meta* position (**B4** vs **B2-3**, **B12** vs **B10**). Additionally, the introduction of a linear alkyl group at the *para* position of the benzene ring showed a decrease in activity with the length of the alkyl group (**B4-6**). The 4-dimethyl (**B4**) and 3,4-dimethyl (**B14**) compounds appeared to be the most active ones against MRSA with a MIC value of 0.38 μg/mL, being approximately 4-fold more potent than vancomycin and comparable to ciprofloxacin. *N*-substituent variation was extended to other more substituents (**B15-19**), and the *N*-cyclopropyl was confirmed to be optimal for anti-MRSA activity.

2.2. Inhibitory activities against clinical isolates of MRSA

The antibacterial activities of **B4** and **B14** were further evaluated against *S. aureus* Newman strain and a panel of clinical isolates of MRSA (NRS-1, NRS-70, NRS-100, NRS-108, NRS-271). As shown in Table 3, both **B4** and **B14** were more potent against *S. aureus* Newman strain than vancomycin. In particular, **B14** strongly exhibited antibacterial activity against all five clinical isolates of MRSA, with MIC values between < 0.17 and 0.69 μg/mL, whereas **B4** had comparable activity as vancomycin with a MIC value of 1.38 μg/mL.

Table 3
Antibacterial activity of **B4** and **B14** against *S. aureus* Newman strain and a panel of clinical isolates of MRSA.[a]

Compd	MIC[a] (μg/mL)					
	Newman	NRS-1	NRS-70	NRS-100	NRS-108	NRS-271
B4	0.8	1.38	1.38	1.38	1.38	1.38
B14	0.2	0.35	< 0.17	0.35	0.69	0.35
Van.	1.56	3.13	0.78	1.56	0.78	0.78

[a] Data provided by Prof. Caiguang Yang. All experiments were performed in at least triplicates. MRSA strains: NRS-1 (resistant to aminoglycosides and tetracycline), NRS-70 (resistant to erythromycin), NRS-100 (resistant to oxacillin and tetracycline), NRS-108 (resistant to gentamicin), NRS-271 (resistant to linezolid).

2.3. Inhibitory activities against fluoroquinolone-resistant MRSA and VISA

In addition to the five MRSA above, three clinical isolates of fluoroquinolone-resistant MRSA (19–25, 19–26, 19–27) and two VISA standard strains (ATCC700788, ATCC700699) were also selected to explore the antibacterial activities of **B4** and **B14**, as presented in Table 4. To our pleasure, both quinolone-based hybrids **B4** and **B14** were able to inhibit all three clinical isolates of fluoroquinolone-resistant MRSA. Notably, **B4** displayed comparable activity as vancomycin, whereas **B14** had approximately 16-fold more potent than this reference drug. Moreover, **B14** also exhibited good potency against VISA, while vancomycin showed reduced susceptibility.

2.4. Initial safety evaluation

As an initial safety evaluation, we assessed hERG inhibition and assayed for potential mammalian cytotoxicity with lung carcinoma A549, breast adenocarcinoma MDA-MB-231, prostate adenocarcinoma PC-3, as well as bonvine aorta endothelial ABAE. The results of the cytotoxicity assay showed that both **B4** and **B14** were unable to inhibit the growth of the three tested human cancer cell lines or ABAE cells at the highest tested concentration (100 μM), indicating its highly selective toxicity toward bacterial cells. Inhibition of cardiac potassium channels encoded by hERG is frequently associated with QT interval prolongation and life-threatening arrhythmia [14]. Hence, **B4** and **B14** were tested for their abilities to inhibit hERG potassium channel and cisapride was used as a positive control. The results showed that **B4** and **B14** demonstrated marginal inhibitory activities with an IC_{50} exceeding 40 μM, while the reference cisapride exhibited strong inhibitory activity with an IC_{50} value of 0.035 μM.

2.5. Propensity to induce bacterial resistance

A common concern for newly obtained antibiotics is the emergence of rapid resistance. Hence, **B14** was further subjected to the bacterial resistance study using MRSA ATCC43300 as the test pathogen due to its superior antibacterial profile. Vancomycin was used as a positive control. Starting MIC values for **B14** and vancomycin were found to be 0.5 μg/mL. ATCC43300 was exposed to **B14** and vancomycin from MIC for sustained passages and the new MIC values were determined every passage after propagation of MRSA cultures with fresh media, respectively. As shown in Fig. 4, the MIC value of **B14** toward MRSA did not change even after 15 passages, whereas MIC of vancomycin increased by 4-fold. It indicates that MRSA is less likely to acquire resistance against **B14** compared with vancomycin.

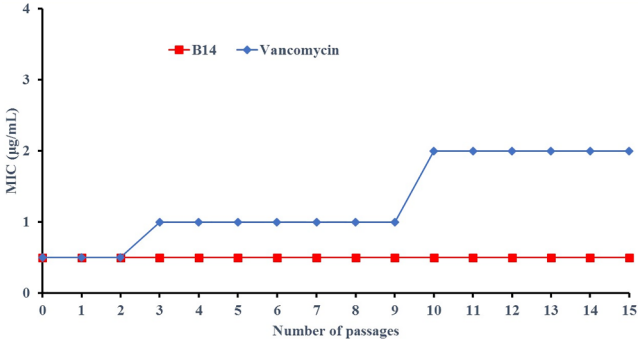


Fig. 4. Bacterial resistance study of **B14** against MRSA ATCC43300. Data provided by Sichuan Primed Shines Bio-tech Co., Ltd.

2.6. Docking study

In an attempt to investigate the potential binding mode of the newly synthesized hybrids, molecular simulation was conducted by using Sybyl-X 2.0. The model of *S. aureus* topoisomerase IV was homology constructed based on the original X-ray crystal structures of Topo IV from *S. pneumoniae* (pdb: 4KPF) [24].

Hybrids **A6** and **B14** were chosen as representatives to be docked into the homology constructed topoisomerase IV/DNA complex. As shown in Fig. 5a and b, **A6 and B14** established similar ligand-receptor interactions: (i) A Mg^{2+} chelation formed with the quinolone C3/C4 keto acid, (ii) Three hydrogen bonds between the nitrogen atom of the NH linker and DA5, the nitrogen atom of the pyrimidine ring and DG1, the quinolone 3-carboxylic acid and Arg118, (iii) Favorable π-π interaction of the quinolone ring with DG1 and DA5. The difference in the ligand-enzyme interactions is that 3,4-dimethylphenyl group of **B14** (Score 13.2917) is positioned deeply in a pocket, as defined by the side chains of LYS455, VAL456, LIE457, to engage hydrophobic interactions. Conversely, the 3,4-dimethylphenyl moiety of **A6** (Score 11.1128) is positioned toward the solvent accessible area (Fig. 5**c**).

3. Conclusions

Based on the understanding of quinolone-enzyme interactions, a series of desfluoroquinolone-aminopyrimidine hybrids was designed and synthesized with the aim to combat multidrug resistance and cardiotoxicity. Despite the lack of the typical C-6 fluorine atom on the

Table 4
Antibacterial activity of **B4** and **B14** against fluoroquinolone-resistant MRSA and VISA.[a]

Compd	MIC[a] (μg/mL)					
	Standard strain	Levofloxacin-resistant strain			Vancomycin-intermediate strain	
	ATCC29213	19–25	19–26	19–27	ATCC700788	ATCC700699
B4	1	2	2	2	2	2
B14	0.125	0.125	0.125	0.125	0.25	1
Van.	1	2	2	2	4	8
Lev.	0.125	> 64	> 64	> 64	64	16

[a] Data provided by Sichuan Primed Shines Bio-tech Co., Ltd. All experiments were performed in at least triplicates. Lev. = Levofloxacin.

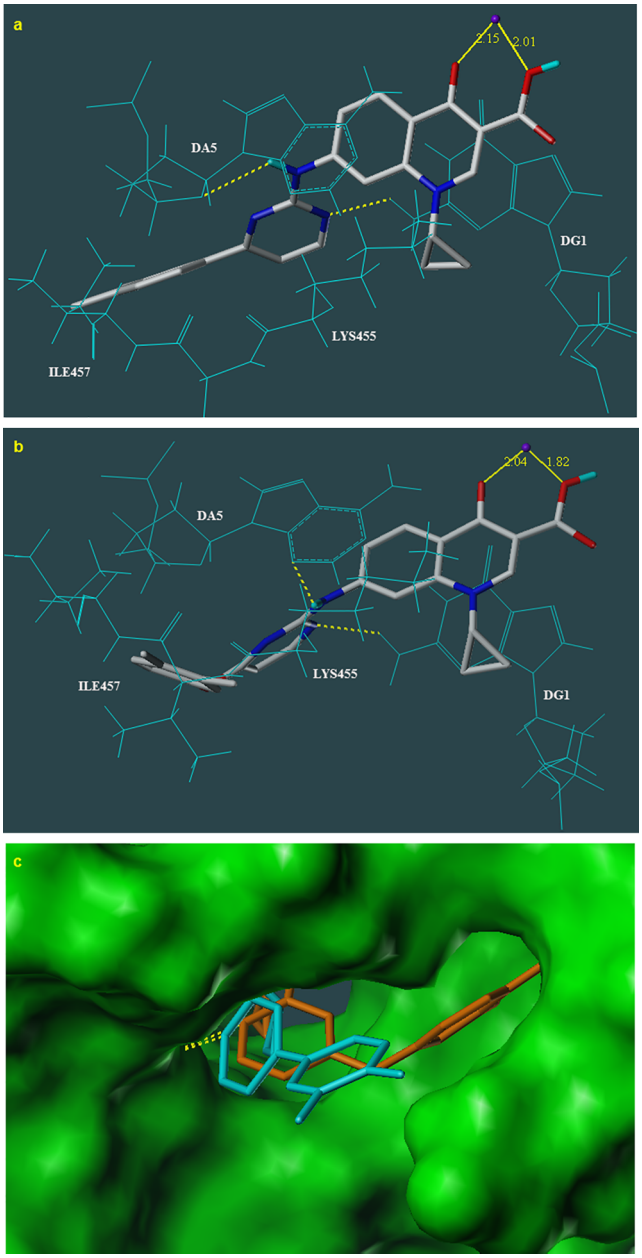


Fig. 5. (a) Predicted binding mode of hybrid **A6**, (b) predicted binding mode of hybrid **B14**, c) predicted binding mode of **A6** (cyan) and **B14** (orange) with topoisomerase IV/DNA complex (derived from PDB code 4KPF) for comparison. Hydrogen bonds are indicated with dashed lines in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

quinolone nucleus, majority of these hybrids still demonstrated potent anti-MRSA activity with MIC values between 0.38 and 1.5 μg/mL. Among them, **B14** exhibited powerful inhibitory activity against a panel of MRSA strains including vancomycin-intermediate strains, aminoglycosides and tetracycline-resistant isolates, linezolid-resistant isolates, *etc*. Notably, this quinolone-based agent even can inhibit levofloxacin-resistant isolates. As expected, **B14** also showed highly selective toxicity toward bacterial cells and no significant hERG toxicity. Further bacterial resistance study suggested that MRSA is unlikely to acquire resistance against **B14**. The docking study revealed that the introduced C-7 aminopyrimidine functional group established two hydrogen bonds with the surrounding DNA bases. The additional drug-enzyme contacts might contribute to overcome quinolone resistance by reducing the dependence on the magnesium-water bridge interactions with topoisomerase IV. These results suggested a promising strategy *via* enhancing functionality of C-7 substituent for developing new anti-MRSA quinolones with improved efficacy, resistance profile, and low cardiotoxicity.

4. Experimental section

4.1. Chemistry

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance Neo 400 MHz spectrometer. Chemical shifts are reported in δ (ppm) units relative to the internal standard tetramethylsilane (TMS). HRMS were obtained on a Bruker micrOTOF II or Bruker Compact instrument using electrospray ionization (ESI) techniques. Melting points were measured with a SGW X-1 microscopic melting-point apparatus and are uncorrected. All chemicals and solvents used were of reagent grade and were purified and dried by standard methods before use. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel G plates at 254 nm under a UV lamp using dichloromethane/methanol or ethyl acetate/hexane as eluent. Column chromatography separations were obtained on silica gel (300–400 mesh) using dichloromethane and methanol as eluents. Analysis of sample purity was performed on a Shimadzu LC-20AD series HPLC system with C18 Inertsil ODS-3 (4.6 mm × 250 mm × 5 μm). HPLC conditions were the following: solvent A = water (0.03% TFA), solvent B = MeCN; Gradient: 5% B increase to 95% B within 10.5 min, 95% B decrease to 5% B within 5 min, 5% B for 5 min; flow rate = 1 mL/min. Purity was determined by the absorbance at 254 nm. All tested compounds have a purity of > 95%.

General procedure for the preparation of methyl 7-amino-1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**17**). To a mixture of **16** (2.0 mmol), which was prepared according to our previously reported protocol [25], in a mixture solution of dichloromethane (30 mL) and methanol (10 mL) was added $TMSCHN_2$ (3.0 mmol, 2 M in cyclohexane) at 0 °C. The resulting mixture was allowed to warm to room temperature. After stirring at room temperature for 24 h, 1% aqueous acetic acid (0.5 mL) was added slowly to the reaction solution. The solvents were removed under reduced pressure. The resulting precipitate was filtered, washed by water, and dried. The crude was purified by column chromatography on silica gel (dichloromethane/ methanol 25:1, v/v) to afford **17** as a solid.

Methyl 7-amino-1-cyclopropyl-4-oxo-1,4-dihydro-quinolone-3-carboxylate (17a). Yield 72% (from **16**); white solid; m.p. 274.7–275.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (s, 1H), 7.88 (d, J = 8.7 Hz, 1H), 7.03 (s, 1H), 6.72 (d, J = 8.7 Hz, 1H), 6.22 (s, 2H), 4.16–4.10 (m, 1H), 3.18 (s, 3H), 1.22–1.05 (m, 4H).

Methyl 7-amino-1-propyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (17b). Yield 72% (from **16**); white solid; m.p. 288.2–289.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 7.89 (d, J = 8.8 Hz, 1H), 6.67 (d, J = 9.2 Hz, 1H), 6.63 (s, 1H), 6.08 (s, 2H), 4.11 (t, J = 7.2 Hz, 2H), 3.76 (s, 3H), 1.81–1.72 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H).

Methyl 7-amino-1-isopropyl-4-oxo-1,4-dihydro-quinoline-3-carboxylate (17c). Yield 73% (from **16**); white solid; m.p. 308.3–309.1 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 7.95 (d, J = 8.7 Hz, 1H), 6.82 (s, 1H), 6.72 (d, J = 8.7 Hz, 1H), 6.10 (s, 2H), 4.78–4.72 (m, 1H), 3.73 (s, 3H), 1.49 (d, J = 6.5 Hz, 6H).

Methyl 7-amino-1-isobutyl-4-oxo-1,4-dihydroquino-line-3-carboxylate (17d). Yield 73% (from **16**); white solid; m.p. 243.8–244.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 7.89 (d, J = 8.8 Hz, 1H), 6.67 (d, J = 8.7 Hz, 1H), 6.62 (s, 1H), 6.08 (s, 2H), 3.97 (d, J = 7.4 Hz, 2H), 3.70 (s, 3H), 2.19–2.13 (m, 1H), 0.89 (d, J = 6.4 Hz, 6H).

Methyl 7-amino-1-(*tert*-butyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylate (17e). Yield 74% (from **16**); white solid; m.p.

221.8–222.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (s, 1H), 8.37 (d, J = 8.7 Hz, 1H), 7.00 (d, J = 1.5 Hz, 1H), 6.75 (dd, J = 8.7 Hz, 1.5 Hz, 1H), 3.89 (s, 3H), 1.85 (s, 9H).

Methyl 7-amino-1-cyclohexyl-4-oxo-1,4-dihydro-quinoline-3-carboxylate (17f). Yield 64% (from **16**); white solid; m.p. 226.0–226.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.04 (d, J = 8.8 Hz, 1H), 6.94(s, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.48 (s, 2H), 5.76–5.72 (m, 1H), 4.45–4.40 (m, 1H), 3.88 (s, 3H), 2.05–1.26 (m, 10*H*).

General procedure for the preparation of methyl 1-alkyl-4-oxo-7-((4-phenylpyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylate (18). A Schlenk vessel was charged with **17** (2 mmol), 2-chloro-4-phenylpyrimidine (3 mmol), $Pd(OAc)_2$ (0.2 mmol), $K2CO_3$ (3 mmol), BINAP (0.3 mmol) in DMF (20 mL). The reaction vessel was sealed and the contents were stirred and heated at 90 °C for 12–18 h under N2. After filtration, ethyl acetate (30 mL) was added and the organic layer was washed with brine (15 mL × 3) and water (15 mL), dried over $MgSO_4$, and then filtered. The filtrate was evaporated and the residue was purified by column chromatography on silica gel (dichloromethane/methanol 30:1, v/v) to give **18** as a solid.

Methyl 1-cyclopropyl-4-oxo-7-((4-phenylpyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylate (18a). Yield 38%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.71 (d, J = 5.3 Hz, 1H), 8.52 (d, J = 8.8 Hz, 1H), 8.06 (s, 1H), 7.95–7.98 (m, 3H), 7.49–7.41 (m, 5H), 3.90 (s, 3H), 3.47–3.40 (m, 1H), 1.11–1.07 (m, 4H).

Methyl 1-cyclopropyl-4-oxo-7-((4-(*o*-tolyl)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylate (18b). Yield 42%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 8.57 (d, J = 5.0 Hz, 1H), 8.54 (s, 1H), 8.38 (d, J = 8.7 Hz, 1H), 8.22 (s, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 6.8 Hz, 1H), 7.31–7.25 (m, 3H), 6.99 (d, J = 4.9 Hz, 1H), 3.90 (s, 3H), 3.37–3.31 (m, 1H), 2.44 (s, 3H), 1.02–0.97 (m, 4H).

Methyl 1-cyclopropyl-4-oxo-7-((4-(*m*-tolyl)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylate (18c). Yield 48%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.96 (d, J = 2.0 Hz, 1H), 8.68 (d, J = 5.2 Hz, 1H), 8.47 (s, 1H), 8.13 (d, J = 8.8 Hz, 1H), 8.04 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.80 (dd, J = 8.9, 2.0 Hz, 1H), 7.54 (d, J = 5.2 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.39 (d, J = 7.5 Hz, 1H), 3.74 (s, 3H), 3.69–3.65 (m, 1H), 2.41 (s, 3H), 1.17–1.14 (m, 2H), 1.12–1.10 (m, 2H).

Methyl 1-cyclopropyl-4-oxo-7-((4-(*p*-tolyl)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylate (18d). Yield 57%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.61 (s, 1H), 8.54 (d, J = 5.3 Hz, 1H), 8.44 (d, J = 8.8 Hz, 1H), 8.00 (d, J = 7.8 Hz, 2H), 7.42 (d, J = 8.9 Hz, 1H), 7.31 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 5.2 Hz, 1H), 3.94 (s, 3H), 3.54–3.49 (m, 1H), 2.46 (s, 3H), 1.30–1.26 (m, 2H), 1.15–1.13 (m, 2H).

Methyl 1-cyclopropyl-4-oxo-7-((4-(2, 4-dimethylphenyl) pyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylate (18e). Yield 55%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.55–8.53 (m, 2H), 8.37 (d, J = 8.8 Hz, 1H), 8.11 (s, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.25 (dd, J = 8.8, 2.0 Hz, 1H), 7.11–7.08 (m, 2H), 6.97 (d, J = 5.1 Hz, 1H), 3.90 (s, 3H), 3.38–3.33 (m, 1H), 2.42 (s, 3H), 2.37 (s, 3H), 1.03–1.02 (m, 4H).

Methyl 1-cyclopropyl-4-oxo-7-((4-(3,4-dimethylphenyl) pyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylate (18f). Yield 46%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.60 (s, 1H), 8.52 (d, J = 5.2 Hz, 1H), 8.43 (d, J = 8.8 Hz, 1H), 7.86 (s, 1H), 7.82–7.80 (m, 2H), 7.38 (dd, J = 8.8, 1.9 Hz, 1H), 7.26 (s, 1H), 7.24 (d, J = 2.6 Hz, 1H), 3.93 (s, 3H), 3.78–3.74 (m, 1H), 2.35 (s, 6H), 1.28–1.23 (m, 2H), 1.16–1.10 (m, 2H).

Methyl 1-cyclopropyl-4-oxo-7-((4-(4-propylphenyl) pyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylate (18 g). Yield 40%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.61 (s, 1H), 8.53 (d, J = 5.2 Hz, 1H), 8.44 (d, J = 8.7 Hz, 1H), 8.01 (d, J = 8.1 Hz, 2H), 7.68 (s, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.35 (d, J = 8.1 Hz, 2H), 7.25 (s, 1H), 3.93 (s, 3H), 3.76–3.73 (m, 2H), 3.54–3.48 (m, 1H), 1.87–1.84 (m, 2H), 1.31–1.25 (m, 5H), 1.15–1.10 (m, 2H).

General procedure for the preparation of 1-alkyl-4-oxo-7-((phenylpyrimidin-2-yl)amino)-1,4-dihydro quinoline-3-carboxylic acid (A1-7). To a solution of 18 (1.0 mmol) in THF (8 mL) was added satd. aq. NaOH (5 mL, 5 M). After being stirred at 50 °C for 3 h, the mixture was cooled, poured into ice-water and acidified with 4 M HCl to pH ~ 2. The precipitate was filtered off, washed by water, and dried. The crude was purified by column chromatography on silica gel (dichloromethane/methanol 40:1 to 25:1, v/v) to afford the desired product as a solid.

1-Cyclopropyl-4-oxo-7-((4-phenylpyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (A1). Yield 83%; white solid; m.p. 207.8–208.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.23 (s, 1H), 8.82 (d, J = 5.3 Hz, 1H), 8.70 (s, 1H), 8.34 (d, J = 8.8 Hz, 1H), 8.07–8.05 (m, 4H), 7.95 (d, J = 5.3 Hz, 1H), 7.56–7.47 (m, 4H), 3.71–3.66 (m, 1H), 1.00–0.94 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.3, 164.8, 162.4, 160.1, 149.2, 148.4, 142.2, 136.0, 131.7, 129.4, 129.3, 127.6, 127.3, 126.7, 124.2, 117.2, 113.6, 113.4, 35.9, 7.8; HRMS m/z calcd for $C_{23}H_{18}N_4O_3$ ($[M + H]^+$): 399.1452, found 399.1455.

1-Cyclopropyl-4-oxo-7-((4-(*o*-tolyl)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (A2). Yield 85%; yellow solid; m.p. > 320 °C; ^{1}H NMR (400 MHz, DMSO- d_6) δ 15.50 (s, 1H), 10.65 (s, 1H), 9.25 (s, 1H), 8.73 (d, J = 5.0 Hz, 1H), 8.66 (s, 1H), 8.24 (d, J = 9.0 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 7.55 (d, J = 7.3 Hz, 1H), 7.42–7.33 (m, 3H), 7.21 (d, J = 5.0 Hz, 1H), 3.69–3.63 (m, 1H), 2.42 (s, 3H), 1.13–1.09 (m, 2H), 1.00–0.99 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.4, 167.7, 166.7, 159.8, 159.0, 148.8, 146.4, 143.1, 138.4, 136.0, 131.3, 129.9, 129.7, 126.8, 126.4, 119.0, 118.5, 114.3, 107.3, 104.9, 36.2, 20.6, 7.8; HRMS m/z calcd for $C_{24}H_{20}N_4O_3$ ($[M + H]^+$): 413.1608, found 413.1604.

1-Cyclopropyl-4-oxo-7-((4-(*m*-tolyl)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (A3). Yield 82%; white solid; m.p. 302.6–304.0 °C; 1H NMR (400 MHz, DMSO- d_6) δ 15.49 (s, 1H), 10.58 (s, 1H), 9.17 (d, J = 1.9 Hz, 1H), 8.72 (d, J = 5.2 Hz, 1H), 8.69 (s, 1H), 8.27 (d, J = 8.9 Hz, 1H), 8.05 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.95 (dd, J = 9.0, 1.9 Hz, 1H), 7.58 (d, J = 5.2 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 3.86–3.82 (m, 1H), 2.41 (s, 3H), 1.23–1.19 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 166.6, 164.6, 160.0, 159.4, 148.7, 146.4, 143.0, 138.6, 136.7, 132.2, 129.1, 127.8, 126.7, 124.7, 118.9, 118.4, 110.3, 107.2, 104.6, 36.0, 21.3, 8.0; HRMS m/z calcd for $C_{24}H_{20}N_4O_3$ ($[M + H]^+$): 413.1608, found 413.1598.

1-Cyclopropyl-4-oxo-7-((4-(*p*-tolyl)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (A4). Yield 91%; white solid; m.p. 314.6–315.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.50 (s, 1H), 10.57 (s, 1H), 9.15 (s, 1H), 8.71–8.70 (m, 2H), 8.28 (d, J = 8.9 Hz, 1H), 8.14 (d, J = 7.9 Hz, 2H), 7.98 (d, J = 9.2 Hz, 1H), 7.59 (d, J = 5.2 Hz, 1H), 7.38 (d, J = 7.9 Hz, 2H), 3.88–3.82 (m, 1H), 2.41 (s, 3H), 1.26–1.21 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 166.6, 164.3, 159.4, 148.7, 146.4, 143.0, 141.6, 130.0, 129.8, 127.4, 126.8, 126.7, 118.9, 118.4, 109.9, 107.2, 104.7, 36.1, 21.4, 8.0; HRMS m/z calcd for $C_{24}H_{20}N_4O_3$ ($[M + H]^+$): 413.1608, found 413.1617.

1-Cyclopropyl-7-((4-(2, 4-dimethylphenyl)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A5). Yield 79%; yellow solid; m.p. 288.6–289.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.60 (s, 1H), 9.23 (d, J = 1.9 Hz, 1H), 8.71 (d, J = 5.1 Hz, 1H), 8.67 (s, 1H), 8.25 (d, J = 8.9 Hz, 1H), 7.89 (dd, J = 8.9, 2.0 Hz, 1H), 7.47 (d, J = 7.7 Hz, 1H), 7.18 (d, J = 5.0 Hz, 2H), 7.16 (d, J = 7.9 Hz, 1H), 3.70–3.66 (m, 1H), 2.41 (s, 3H), 2.36 (s, 3H), 1.13–1.11 (m, 2H), 1.06–1.04 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 167.5, 166.6, 159.6, 158.7, 148.6, 146.3, 142.9, 139.3, 138.1, 135.8, 135.4, 131.8, 129.7, 126.9, 126.6, 118.3, 114.2, 107.1, 104.7, 36.1, 21.1, 20.5, 7.7; HRMS m/z calcd for $C_{25}H_{22}N_4O_3$ ($[M + H]^+$): 427.1765, found 427.1762.

1-Cyclopropyl-7-((4-(3,4-dimethylphenyl)pyrimidin-2-yl)

amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (A6). Yield 85%; yellow solid; m.p. 316.9–318.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 9.18 (d, J = 1.9 Hz, 1H), 8.70–8.68 (m, 2H), 8.27 (d, J = 9.0 Hz, 1H), 8.02 (s, 1H), 7.97–7.94 (m, 2H), 7.57 (d, J = 5.3 Hz, 1H), 7.32 (d, J = 7.9 Hz, 1H), 3.88–3.83 (m, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 1.23–1.20 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 166.6, 164.5, 160.0, 159.3, 148.6, 146.4, 143.0, 140.4, 137.3, 134.2, 130.3, 128.2, 126.7, 125.0, 118.8, 118.4, 109.9, 107.2, 104.6, 36.1, 29.3, 19.8, 8.0; HRMS m/z calcd for $C_{25}H_{22}N_4O_3$ ([M + H]$^+$): 427.1765, found 427.1766.

1-Cyclopropyl-4-oxo-7-((4-(4-propylphenyl) pyrimidin-2-yl) amino)-1,4-dihydro quinoline-3-carboxylic acid (A7). Yield 72%; yellow solid; m.p. 299.6–300.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.50 (s, 1H), 10.57 (s, 1H), 9.15 (d, J = 2.0 Hz, 1H), 8.71 (s, 1H), 8.70 (s, 1H), 8.29 (d, J = 8.9 Hz, 1H), 8.16 (d, J = 8.3 Hz, 2H), 8.00 (dd, J = 9.0, 1.9 Hz, 1H), 7.58 (d, J = 5.3 Hz, 1H), 7.44 (d, J = 8.3 Hz, 2H), 3.86–3.82 (m, 1H), 3.03–2.96 (m, 2H), 2.03–1.97 (m, 2H), 1.27–1.20 (m, 7H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 166.6, 164.4, 160.0, 159.4, 152.3, 148.6, 146.4, 142.9, 134.4, 127.6, 127.2, 126.7, 118.9, 118.4, 110.0, 107.2, 104.7, 36.1, 33.7, 26.9, 24.0, 8.0; HRMS m/z calcd for $C_{26}H_{24}N_4O_3$ ([M + H]$^+$): 441.1921, found 441.1926.

1-Cyclopropyl-4-oxo-7-(pyrimidin-2-ylamino)-1,4-dihydroquinoline-3-carboxylic acid (A8)

The preparation for **A8** was similar to **A1-7**. Yield 44%; white solid; m.p. 325.7–327.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (s, 1H), 9.14 (s, 1H), 8.68–8.66 (m, 3H), 8.24 (d, J = 9.5 Hz, 1H), 7.90 (dd, J = 8.9, 2.0 Hz, 1H), 7.05 (td, J = 4.7, 1.6 Hz, 1H), 3.75–3.73 (m, 1H), 1.36–1.35 (m, 2H), 1.29–1.22 (m, 2H); 13C NMR (100 MHz, DMSO-d_6) δ 177.3, 166.6, 159.8, 158.6, 148.7, 146.2, 142.9, 130.0, 126.6, 118.2, 114.5, 107.2, 104.8, 36.0, 7.9; HRMS m/z calcd for $C_{17}H_{13}N_4O_3$ ([M−H]$^-$): 321.0993, found 321.0981.

General procedure for the preparation of 1-alkyl-4-oxo-7-((4-phenylpyrimidin-2-yl)amino)-1,4-dihydro-quinoline-3-carboxylic acid (B1-24). The preparation for **B1-22** was similar to **A1-7**.

1-Cyclopropyl-4-oxo-7-((4-phenoxypyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (B1). Yield 70%; yellow solid; m.p. 295.5–296.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.75 (s, 1H), 8.65 (s, 1H), 8.54 (d, J = 5.4 Hz, 1H), 8.08 (d, J = 9.0 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 7.51 (t, J = 7.2 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 7.30 (d, J = 7.8 Hz, 2H), 6.61 (d, J = 5.4 Hz, 1H), 3.50–3.45 (m, 1H), 1.25–1.21 (m, 2H), 1.17–1.13 (m, 2H); 13C NMR (100 MHz, DMSO-d_6) δ 177.5, 169.9, 166.6, 160.7, 159.7, 152.9, 148.8, 146.2, 142.8, 130.5, 126.6, 126.1, 122.1, 119.2, 118.3, 107.3, 105.4, 100.9, 36.0, 8.0; HRMS m/z calcd for $C_{23}H_{18}N_4O_4$ ([M + H]$^+$): 415.1401, found 415.1408.

1-Cyclopropyl-4-oxo-7-((4-(*o*-tolyloxy)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (B2). Yield 83%; white solid; m.p. 266.7–268.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.72 (s, 1H), 8.64 (s, 1H), 8.51 (d, J = 5.5 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.39 (d, J = 7.3 Hz, 1H), 7.32 (t, J = 6.9 Hz, 1H), 7.26 (t, J = 6.8 Hz, 1H), 7.20 (d, J = 7.7 Hz, 1H), 6.57 (d, J = 5.6 Hz, 1H), 3.57–3.50 (m, 1H), 2.12 (s, 3H), 1.26–1.22 (m, 2H), 1.17–1.12 (m, 2H); 13C NMR (100 MHz, DMSO-d_6) δ 177.4, 169.6, 166.6, 160.6, 159.7, 151.2, 148.8, 146.1, 142.7, 131.9, 130.6, 127.9, 126.5, 126.4, 122.5, 119.1, 118.1, 107.2, 105.4, 100.2, 36.0, 16.3, 8.0; HRMS m/z calcd for $C_{24}H_{20}N_4O_4$ ([M + H]$^+$): 429.1557, found 429.1541.

1-Cyclopropyl-4-oxo-7-((4-(*m*-tolyloxy)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (B3). white solid; m.p. 285.1–286.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.41 (s, 1H), 10.43 (s, 1H), 8.79 (s, 1H), 8.66 (s, 1H), 8.53 (d, J = 5.6 Hz, 1H), 8.10 (d, J = 9.0 Hz, 1H), 7.88 (dd, J = 9.0, 1.9 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.17 (d, J = 7.5 Hz, 1H), 7.12 (s, 1H), 7.09 (d, J = 8.0 Hz, 1H), 6.59 (d, J = 5.6 Hz, 1H), 3.56–3.51 (m, 1H), 2.36 (s, 3H), 1.26–1.24 (m, 2H), 1.18–1.16 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.5, 169.9, 166.6, 160.6, 159.7, 152.8, 148.8, 146.2, 142.8, 140.3, 130.2, 126.8, 126.6, 122.5, 119.2, 119.1, 118.3, 107.3, 105.4, 100.8, 36.0, 21.3, 8.1; HRMS m/z calcd for $C_{24}H_{20}N_4O_4$ ([M + H]$^+$): 429.1557, found 429.1540.

1-Cyclopropyl-4-oxo-7-((4-(*p*-tolyloxy)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (B4). Yield 82%; white solid; m.p. 301.8–302.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.19 (s, 1H), 8.85 (s, 1H), 8.80 (s, 1H), 8.37 (d, J = 5.2 Hz, 1H), 8.33 (d, J = 9.0 Hz, 1H), 7.53 (s, 1H), 7.33 (d, J = 9.4 Hz, 1H), 7.23 (d, J = 7.9 Hz, 2H), 7.07 (d, J = 7.9 Hz, 2H), 6.46 (d, J = 5.3 Hz, 1H), 3.32–3.20 (m, 1H), 2.42 (s, 3H), 1.30–1.24 (m, 2H), 1.18–1.07 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.1, 167.3, 159.4, 159.0, 150.2, 147.8, 144.4, 142.7, 135.6, 130.2, 127.7, 121.2, 120.2, 117.5, 108.4, 104.2, 100.9, 35.1, 20.9, 8.1; HRMS m/z calcd for $C_{24}H_{20}N_4O_4$ ([M + H]$^+$): 429.1557, found 429.1565.

1-Cyclopropyl-7-((4-(4-ethylphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B5). Yield 71%; white solid; m.p. 289.0–290.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.75 (s, 1H), 8.63 (s, 1H), 8.50 (d, J = 5.6 Hz, 1H), 8.08 (d, J = 9.0 Hz, 1H), 7.83 (dd, J = 9.0, 1.7 Hz, 1H), 7.32 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H), 6.58 (d, J = 5.6 Hz, 1H), 3.46–3.41 (m, 1H), 2.66 (q, J = 7.6 Hz, 2H), 1.25–1.21 (m, 5H), 1.15–1.11 (m, 2H); 13C NMR (100 MHz, DMSO-d_6) δ 177.4, 169.9, 166.6, 160.5, 159.6, 150.7, 148.7, 146.1, 142.7, 141.6, 129.6, 126.5, 121.9, 119.1, 118.3, 107.2, 105.3, 100.8, 35.9, 28.0, 16.1, 8.0; HRMS m/z calcd for $C_{25}H_{22}N_4O_4$ ([M + H]$^+$): 443.1714, found 443.1702.

7-((4-(4-Butylphenoxy)pyrimidin-2-yl)amino)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B6). Yield 72%; white solid; m.p. 237.6–238.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.71 (s, 1H), 8.62 (s, 1H), 8.49 (d, J = 5.6 Hz, 1H), 8.07 (d, J = 9.2 Hz, 1H), 7.82 (d, J = 9.1 Hz, 1H), 7.29 (d, J = 7.5 Hz, 2H), 7.15 (d, J = 7.6 Hz, 2H), 6.57 (d, J = 5.2 Hz, 1H), 3.44–3.39 (m, 1H), 2.62 (t, J = 7.5 Hz, 2H), 1.63–1.55 (m, 2H), 1.38–1.29 (m, 2H), 1.24–1.20 (m, 2H), 1.15–1.10 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.5, 170.0, 166.6, 160.6, 159.7, 150.8, 148.8, 146.2, 142.7, 140.3, 130.1, 126.6, 121.8, 119.1, 118.3, 107.3, 105.4, 100.8, 36.0, 34.7, 33.7, 22.3, 14.2, 8.0; HRMS m/z calcd for $C_{27}H_{26}N_4O_4$ ([M + H]$^+$): 471.2027, found 471.2036.

1-Cyclopropyl-7-((4-(4-isopropylphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B7). Yield 72%; white solid; m.p. 286.9–288.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 8.72 (s, 1H), 8.62 (s, 1H), 8.50 (d, J = 5.3 Hz, 1H), 8.07 (d, J = 8.9 Hz, 1H), 7.83 (d, J = 9.0 Hz, 1H), 7.35 (d, J = 7.8 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 6.58 (d, J = 5.7 Hz, 1H), 3.46–3.39 (m, 1H), 2.99–2.92 (m, 1H), 1.24 (d, J = 6.9 Hz, 6H), 1.22–1.20 (m, 3H), 1.15–1.10 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.5, 170.0, 166.7, 160.6, 159.7, 150.8, 148.7, 146.2, 146.2, 142.7, 128.1, 126.6, 121.8, 119.2, 118.3, 107.3, 105.4, 100.8, 36.0, 33.4, 24.5, 8.1; HRMS m/z calcd for $C_{26}H_{24}N_4O_4$ ([M + H]$^+$): 457.1870, found 457.1851.

7-((4-(4-(*tert*-Butyl)phenoxy)pyrimidin-2-yl)amino)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B8). Yield 69%; white solid; m.p. 302.1–303.2 °C; 1H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.71 (s, 1H), 8.62 (s, 1H), 8.51 (d, J = 5.5 Hz, 1H), 8.06 (d, J = 8.9 Hz, 1H), 7.83 (d, J = 9.0 Hz, 1H), 7.49 (d, J = 8.3 Hz, 2H), 7.18 (d, J = 8.1 Hz, 2H), 6.59 (d, J = 5.5 Hz, 1H), 3.46–3.39 (m, 1H), 1.33 (s, 9H), 1.23–1.22 (m, 2H), 1.16–1.11 (m, 2H); 13C NMR (100 MHz, DMSO-d_6) δ 177.4, 169.9, 166.6, 160.6, 159.6, 150.5, 148.7, 148.4, 146.1, 142.6, 127.0, 126.5, 121.4, 119.1, 118.2, 107.2, 105.4, 100.8, 36.9, 34.7, 31.7, 8.0; HRMS m/z calcd for $C_{27}H_{26}N_4O_4$ ([M + H]$^+$): 471.2027, found 471.2016.

7-((4-(4-Cyclohexylphenoxy)pyrimidin-2-yl)amino)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B9). Yield 74%; white solid; m.p. 297.5–299.0 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.72 (s, 1H), 8.62 (s, 1H), 8.50 (d, J = 5.6 Hz, 1H), 8.07 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 8.9 Hz, 1H), 7.32 (d, J = 7.9 Hz, 2H), 7.16 (d, J = 7.7 Hz, 2H), 6.58 (d, J = 5.7 Hz, 1H), 3.46–3.41 (m, 1H), 2.59–2.53 (m, 1H), 1.82–1.70 (m, 5H), 1.48–1.29 (m, 5H), 1.24–1.20

(m, 2H), 1.17–1.10 (m, 2H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 177.4, 169.9, 166.7, 160.6, 159.6, 150.8, 148.7, 146.1, 145.4, 142.6, 128.4, 126.6, 121.8, 119.1, 118.3, 107.2, 105.4, 100.8, 43.6, 36.0, 34.5, 26.8, 26.0, 8.0; HRMS *m/z* calcd for $C_{29}H_{28}N_4O_4$ ([M + H]$^+$): 497.2183, found 497.2186.

1-Cyclopropyl-7-((4-(2-methoxyphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B10). Yield 75%; white solid; m.p. 265.5–267.2 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.40 (s, 1H), 10.38 (s, 1H), 8.69 (s, 1H), 8.66 (s, 1H), 8.50 (d, J = 5.6 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.85 (dd, J = 9.0, 1.8 Hz, 1H), 7.36 (td, J = 7.7, 1.3 Hz, 1H), 7.29–7.25 (m, 2H), 7.07 (td, J = 7.7, 1.3 Hz, 1H), 6.58 (d, J = 5.6 Hz, 1H), 3.74 (s, 3H), 3.60–3.54 (m, 1H), 1.30–1.25 (m, 2H), 1.19–1.15 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.4, 169.7, 166.6, 160.3, 159.6, 151.7, 148.8, 146.2, 142.7, 141.2, 127.4, 126.5, 123.4, 121.5, 119.0, 118.0, 113.8, 107.2, 105.4, 100.2, 56.2, 36.0, 8.0; HRMS *m/z* calcd for $C_{24}H_{20}N_4O_5$ ([M + H]$^+$): 445.1506, found 445.1502.

1-Cyclopropyl-7-((4-(3-methoxyphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B11). Yield 76%; white solid; m.p. 270.8–272.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.78 (s, 1H), 8.65 (s, 1H), 8.52 (d, J = 5.6 Hz, 1H), 8.09 (d, J = 9.0 Hz, 1H), 7.87 (dd, J = 9.0, 1.5 Hz, 1H), 7.39 (t, J = 8.1 Hz, 1H), 6.92–6.89 (m, 2H), 6.84 (d, J = 8.3 Hz, 1H), 6.59 (d, J = 5.6 Hz, 1H), 3.77 (s, 3H), 3.54–3.48 (m, 1H), 1.24–1.22 (m, 2H), 1.16–1.12 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.4, 169.8,166.6, 161.0, 160.6, 159.7, 153.8, 148.8, 146.1, 142.7, 130.9, 126.5, 119.1, 118.3, 114.1, 111.9, 108.0, 107.2, 105.3, 100.8, 55.9, 36.0, 8.0; HRMS *m/z* calcd for $C_{24}H_{20}N_4O_5$ ([M + H]$^+$): 445.1506, found 445.1502.

1-Cyclopropyl-7-((4-(4-methoxyphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B12). Yield 73%; white solid; m.p. 282.8–284.5 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.79 (s, 1H), 8.66 (s, 1H), 8.51 (d, J = 5.6 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H), 7.88 (dd, J = 9.0, 1.9 Hz, 1H), 7.22 (d, J = 9.1 Hz, 2H), 7.05 (d, J = 9.1 Hz, 2H), 6.56 (d, J = 5.6 Hz, 1H), 3.82 (s, 3H), 3.57–3.52 (m, 1H), 1.27–1.24 (m, 2H), 1.18–1.14 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 170.1, 166.5, 160.3, 159.6, 157.2, 148.7, 146.0, 142.6, 130.0, 126.5, 123.0, 119.0, 118.2, 115.2, 107.1, 105.2, 100.5, 55.9, 35.9, 7.9; HRMS *m/z* calcd for $C_{24}H_{20}N_4O_5$ ([M + H]$^+$): 445.1506, found 445.1498.

1-Cyclopropyl-7-((4-(2, 4-dimethylphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B13). Yield 86%; white solid; m.p. 290.4–291.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.76 (s, 1H), 8.64 (s, 1H), 8.49 (d, J = 5.6 Hz, 1H), 8.06 (d, J = 9.0 Hz, 1H), 7.81 (dd, J = 9.0, 1.6 Hz, 1H), 7.18 (s, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.06 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 5.6 Hz, 1H), 3.54–3.50 (m, 1H), 2.33 (s, 3H), 2.07 (s, 3H), 1.26–1.22 (m, 2H), 1.16–1.14 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.4, 169.7, 166.6, 160.5, 159.7, 148.9, 148.8, 146.1, 142.7, 135.5, 132.3, 130.1, 128.3, 126.5, 122.2, 119.1, 118.2, 107.2, 105.4, 100.2, 36.0. 20.8, 16.3, 8.0; HRMS *m/z* calcd for $C_{25}H_{22}N_4O_4$ ([M + H]$^+$): 443.1714, found 443.1708.

1-Cyclopropyl-7-((4-(3,4-dimethylphenoxy)pyrimidin-2-yl) amino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (B14). Yield 82%; white solid; m.p. 287.7–289.7 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.40 (s, 1H), 8.83 (s, 1H), 8.66 (s, 1H), 8.51 (d, J = 5.6 Hz, 1H), 8.11 (d, J = 9.0 Hz, 1H), 7.87 (dd, J = 9.0, 1.7 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 7.08 (d, J = 2.3 Hz, 1H), 6.99 (dd, J = 8.1, 2.4 Hz, 1H), 6.56 (d, J = 5.6 Hz, 1H), 3.52–3.47 (m, 1H), 2.28 (s, 3H), 2.25 (s, 3H), 1.26–1.23 (m, 2H), 1.17–1.14 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.3, 170.0, 166.5, 160.3, 159.6, 150.6, 148.7, 146.1, 142.6, 138.5, 134.0, 130.9, 126.4, 122.7, 119.0, 118.2, 107.1, 105.2, 100.6, 35.9, 19.8, 19.1, 7.9; HRMS *m/z* calcd for $C_{25}H_{22}N_4O_4$ ([M + H]$^+$): 443.1714, found 443.1704.

4-Oxo-1-propyl-7-((4-(*p*-tolyloxy)pyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylic acid (B15). Yield 84%; light yellow solid; m.p. 274.0–274.4 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 8.94 (s, 1H), 8.47 (d, J = 5.5 Hz, 1H), 8.32 (s, 1H), 8.10 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 8.9 Hz, 1H), 7.29 (d, J = 7.9 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 6.55 (d, J = 5.5 Hz, 1H), 4.24 (t, J = 6.9 Hz, 2H), 2.36 (s, 3H), 1.84–1.79 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.2, 170.0, 166.8, 160.5, 159.6, 150.5, 149.6, 146.2, 140.7, 135.4, 130.8, 126.9, 121.9, 119.6, 118.2, 107.3, 104.8, 100.7, 55.4, 21.9, 20.9, 11.1; HRMS *m/z* calcd for $C_{24}H_{22}N_4O_4$ ([M + H]$^+$): 431.1714, found 431.1710.

1-Isopropyl-4-oxo-7-((4-(*p*-tolyloxy)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (B16). Yield 76%; white solid; m.p. 279.4–280.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 15.54 (s, 1H), 10.27 (s, 1H), 8.78 (s, 1H), 8.50 (d, J = 5.6 Hz, 1H), 8.46 (s, 1H), 8.14 (d, J = 9.0 Hz, 1H), 7.92 (d, J = 8.2 Hz, 1H), 7.31 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 6.59 (d, J = 5.6 Hz, 1H), 4.83–4.79 (m, 1H), 2.37 (s, 3H), 1.58 (d, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.9, 170.0, 166.8, 160.4, 159.6, 150.6, 146.3, 141.0, 135.3, 130.8, 130.1, 126.9, 121.8, 119.7, 118.2, 107.5, 104.5, 100.8, 29.4, 21.6, 20.9; HRMS *m/z* calcd for $C_{24}H_{22}N_4O_4$ ([M + H]$^+$): 431.1714, found 431.1701.

1-Isobutyl-4-oxo-7-((4-(*p*-tolyloxy)pyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylic acid (B17). Yield 78%; white solid; m.p. 279.4–279.9 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.26 (s, 1H), 8.73 (s, 1H), 8.64 (s, 1H), 8.39–8.34 (m, 3H), 7.57–7.51 (m, 1H), 7.35–7.29 (m, 2H), 7.06 (d, J = 8.4 Hz, 2H), 6.41 (d, J = 5.6 Hz, 1H), 4.01 (d, J = 8.0 Hz, 2H), 2.49–2.44 (m, 2H), 2.42 (s, 3H), 1.34–1.23 (m, 3H), 1.03 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 170.2, 167.4, 159.2, 159.0, 150.0, 148.5, 144.5, 141.0, 135.8, 130.4, 127.8, 121.2, 120.8, 117.4, 108.1, 104.0, 100.7, 62.3, 27.5, 20.9, 19.9; HRMS *m/z* calcd for $C_{25}H_{24}N_4O_4$ ([M + H]$^+$): 445.1870, found 445.1860.

1-(*tert*-Butyl)-4-oxo-7-((4-(*p*-tolyloxy)pyrimidin-2-yl)amino)-1,4-dihydroquinoline-3-carboxylic acid (B18). Yield 90%; white solid; m.p. 188.9–189.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.38 (s, 1H), 9.46 (s, 1H), 9.09 (s, 1H), 8.43–8.36 (m, 2H), 7.21–7.18 (m, 2H), 7.27 (s, 1H), 7.05–7.06 (m, 3H), 6.38 (d, J = 4.4 Hz, 1H), 2.41 (s, 3H), 1.98 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 170.3, 167.8, 159.1, 150.0, 145.4, 143.1, 140.8, 135.8, 130.4, 127.9, 122.0, 121.2, 116.9, 108.1, 107.4, 100.5, 64.6, 30.5, 20.9; HRMS *m/z* calcd for $C_{25}H_{24}N_4O_4$ ([M + H]$^+$): 445.1870, found 445.1860.

1-Cyclohexyl-4-oxo-7-((4-(*p*-tolyloxy)pyrimidin-2-yl) amino)-1,4-dihydroquinoline-3-carboxylic acid (B19). Yield 81%; white solid; m.p. 157.8–158.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.42 (s, 1H), 8.87 (s, 2H), 8.41–8.37 (m, 2H), 7.32–7.29 (m, 4H), 7.08 (d, J = 7.8 Hz, 2H), 6.42 (d, J = 5.1 Hz, 1H), 4.69–4.38 (m, 1H), 2.44 (s, 3H), 2.31–2.28 (m, 2H), 2.10–2.07 (m, 2H), 1.86–1.80 (m, 4H), 1.37–1.28 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 170.2, 167.8, 159.2, 150.6, 150.0, 144.6, 143.9, 141.2, 135.8, 130.4, 127.9, 121.2, 120.9, 117.3, 108.4, 103.3, 100.6, 77.2, 32.7, 25.9, 25.3, 20.9; HRMS *m/z* calcd for $C_{27}H_{26}N_4O_4$ ([M + H]$^+$): 471.2027, found 471.2029.

4.2. In vitro antibacterial assay [26]

Prior to the experiment, a single colony was picked from TSA plate. All strains were grown at 37 °C overnight in TSB without the antibiotic. 1000-fold dilution of overnight cultures were grown at 37 °C for 2–3 h until A_{600} = 0.6. Then bacteria were diluted 1:400 into fresh TSB medium, compounds were dissolved in DMSO to a concentration of to a concentration of 10 mg/mL, and the solutions were diluted with medium before antibacterial activity test. Then, the stock solution was stocked at −20 °C. The commercial ciprofloxacin and vancomycin were used as the reference drugs. Equal volume of bacteria and compounds were added to 96 well plates and mixed well by shaking. Plates were incubated at 37 °C for 16–18 h followed by observations of MIC values by the absence or presence of visible growth. All experiments were done in duplicates and repeated for at least three times. MIC was determined as the lowest concentration needed to inhibit bacterial growth.

4.3. Cytotoxicity assay [27]

The cell lines were maintained at 37 °C in 5% CO_2 in culture flasks in RPMI-1640 medium, supplemented with 10% fetal calf serum, 100 U/mL penicillin, and 0.1 mg/mL streptomycin. Upon reaching confluence, the cells were trypsinized with 0.25% trypsin containing 0.01% EDTA for 5 min at 37 °C and then stopped by the addition of complete medium. About 5×10^5 of the viable cells were then re-suspended in complete medium.

The cell lines were seeded in 96-well plates and incubated for 24 h to allow the cells to attach. A stock solution of 400 mM xanthone derivatives was prepared in DMSO and stored at −20 °C. The stock solution was diluted to the appropriate concentrations with culture medium. The final concentration of DMSO was less than 0.1% (v/v). The same amount of DMSO was used as the vehicle control throughout this study. Desfluoroquinolone-aminopyrimidine hybrids were serial diluted (final concentration 0–400 μM) and added to the microtiter plate. Commercial 5-FU was added as positive control. The cells were incubated with compounds for 48 h. MTT assay was conducted following the protocol described previously. The cell viability was determined at 560 nm absorbance using a Spectra Max M2 plate reader. Experiment was performed in triplicates.

4.4. hERG assay

hERG-CHO cells (constructed in-house) were cultured in T75 flasks to maximum 70–80% confluence at 37 °C in 5% CO2 incubator. The culture media ((F-12 medium (Invitrogen 11765062, ThermoFisher, USA) supplemented with 10% fetal bovine serum (Invitrogen 10099141, ThermoFisher, USA), 100 g/mL G418 (Invitrogen 11811023, ThermoFisher, USA) and 100 g/mL Hygromycin B (Invitrogen 10687010, ThermoFisher, USA) was removed and the hERG-CHO cells were washed with 7 mL PBS. Then the cells were dissociated with 3 mL Detachine reagent in 37 °C incubator for 3 min, and 7 mL medium was added to gently suspend cells by pipetting up and down several times. Finally, the cells were harvested by 800 rpm/min centrifuge and adjusted to 2–5 million cells/mL for automated Qpatch 16× (Qpatch 16x, Sophion, Denmark) experiments. Before assay, all the tested compounds stock solutions (20 mM in DMSO) were diluted with external solution to the desired concentrations and final concentration of DMSO is 0.2%, which has no effects on hERG currents. External solution was prepared as follows (pH 7.4): 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1.25 mM $MgCl_2$, 10 mM Glucose, 10 mM HEPES.

The hERG current was recorded in the whole-cell patch clamp configuration on QPatch 16× using single-hole QPlate. The cells were voltage-clamped at a holding potential of −80 mV. Then the hERG current was activated by depolarizing at +20 mV for 5 s, after which the current was taken back to −50 mV for 5 s to remove the inactivation. Finally, the deactivating tail current was observed. The peak size of tail current was used to quantify hERG current amplitude. Raw data included membrane resistance Rm > 100 MΩ and tail current amplitude > 300 pA. Internal solution was prepared as follows (pH 7.2): 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA.

4.5. Propensity to induce bacterial resistance [28]

At beginning, the MIC value of compound **B14** and vancomycin was determined against MRSA ATCC43300 in a 96-well plate. Here, the visual end point where there is no bacterial growth was considered to be the MIC of the tested compound. For the next-day MIC experiment, the bacterial dilution was made by using the bacteria from sub-MIC concentration of the compounds (at MIC/2). Then, the concentration of this bacterium was adjusted to $\sim 10^5$ CFU/mL on the basis of OD_{600} and subjected to the next MIC assay. After a 24 h incubation period, again bacterial dilution was prepared by using the bacterial suspension from sub-MIC concentration of the compound (at MIC/2) and assayed for the other MIC experiment. The process was repeated for 15 passages, and the fold increase in MIC was determined. The results indicate the fold of increase in MIC every day.

4.6. Homology model of topoisomerase IV/DNA complex

The protein sequence of *S. aureus* topoisomerase IV was obtained from UniProt Knowledgebase. The protein consists of four subunits in the form of AABB. The entry of the subunit A and subunit B in UniProt Knowledgebase were Q6GH50 and Q6GH51 [29]. Only the following portions of subunit A and B which contained both the DNA- and ligand-binding sites were modelled, respectively: 1–497 in subunit A and 412–637 in subunit B. Sequence identities between S. aureus topoisomerase IV and the template in subunit A and B were 64.1% and 77.9%, respectively. Sequence alignment was performed in ORCHESTRAR panel. Structurally conserved regions (SCRs) were built by using one chain of 4KPF as model.

The loop regions comprised between residues 1–4, 483–497 in subunit A and 540–553, 564–574, 636–637 in subunit B were constructed by using default settings. Then the sidechains were added by using Borrow Chin1 + 2 + 3/Restrict Chin1 + 2 as borrow option. The model was energy minimized using MMFF94 as force filed. The other three chains were modeled by the same method and energy optimized by means of staged minimization to homology construct the overall model of S. aureus topoisomerase IV.

The co-crystallized ligand was extracted and the protein was prepared by adding hydrogens. Gasteiger-Hückel charges were assigned to the ligand atoms. The structures of A6 and B14 were individually energy minimized using the conjugate gradient method.

Declaration of Competing Interest

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

Acknowledgment

This work was supported by the National Natural Science Foundation of China (21971043, 81861138046) and the Science and Technology Commission of Shanghai Municipality (17XD1404400, 18XD1400800, 19ZR1403400). The technical assistance of Dr. Chaomei Liu and Mrs. Mei Zhang from University of Florida for the cytotocixity assay, Dr. Zhaobing Gao from Shanghai Institute of Materia Medica for hERG assay is gratefully acknowledged.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104176.

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