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Design, Synthesis and Evaluation of Antibacterial Effects of a New Class of Piperazinylquinolone Derivatives

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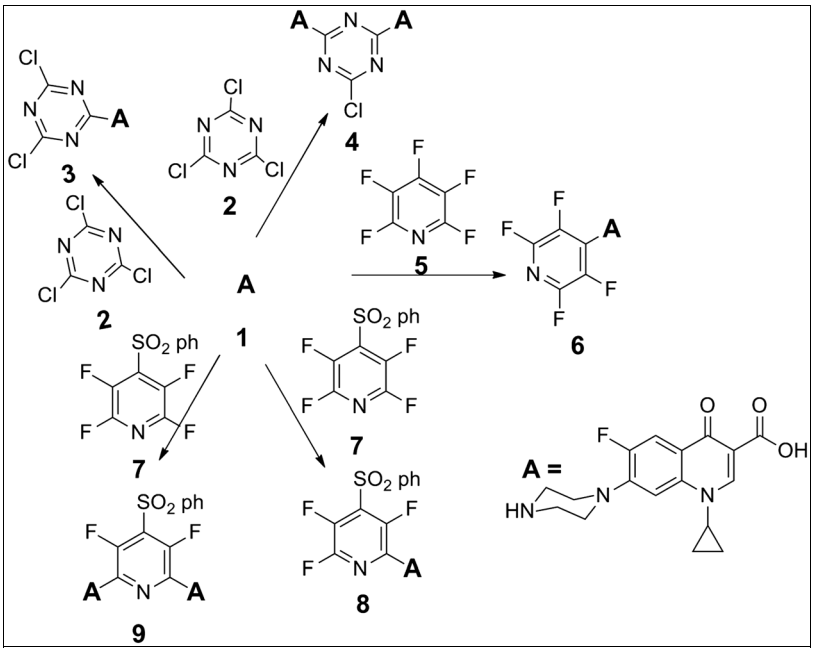


Pentafluoropyridine derivatives and cyanuric chloride were used for the synthesis of new piperazinylquinolone derivatives. These reactions provided *N*-fluoropyridiyl and *N*-cyanoryl chloride piperazinylquinolone derivatives in good yields. Synthesized compounds were evaluated for their antibacterial activities. These compounds displayed good to excellent antibacterial activities.

INTRODUCTION

The increasing incidence of infection caused by the rapid onset of bacterial resistance to available antibiotics is a serious health problem [1]. The drug discovery arena requires the availability of an ever-increasing, diverse range of polyfunctional heterocyclic core scaffolds based upon privileged structures with proven biological activity from which to synthesize arrays of structurally similar analogues that follow the Lipinski rule of 5 (RO5) parameters with the aim of reducing the resources used in hit-to-lead progression [2].

Perfluorinated heteroaromatic systems such as pentafluoropyridine and tetrafluoropyrimidine can be utilized as very effective core scaffolds for the synthesis of a diverse array of polysubstituted pyridine, pyrimidine, [5,6]-bicyclic and [6,6]-bicyclic fused-ring systems. Given the vast diversity of monofunctional and difunctional nucleophiles that are readily available from commercial suppliers, the core scaffolds and polyfunctional privileged structure systems that may be accessed from perfluorinated heteroaromatic starting materials offer, potentially, many great opportunities [3,4].

Perfluorinated diazines (pyrimidine, pyrazine and pyridazine) are typically 1000 times more reactive toward nucleophiles than is pentafluoropyridine, and application of the sequential nucleophilic substitution methodology to reactions involving various diazine systems with a range of nucleophiles would, in principle, lead to the synthesis of many novel polyfunctional diazine derivatives [5,6].

The reactivity profile established for pentafluoropyridine, where the 4, 2 and 6 positions are sequentially, regiospecifically substituted by a succession of oxygen-centered nucleophiles, has allowed medicinal chemists to use pentafluoropyridine as a core scaffold for the synthesis of small arrays of biologically active pyridine systems that fall within the Lipinski parameters.

The factor VIIa/TF (tissue factor) complex and Xa are proteins known to be involved in the blood coagulation cascade [7] and, as such, are validated targets in the search for novel antithrombotic drugs [8,9].

4-Phenylsulfonylpyridine derivative and because the phenylsulfonyl group is strongly electron withdrawing, annelation by reaction with appropriate diamines proceeded readily to give pyrido[2,3-b]pyrazine scaffolds [10,11]. In addition, 4-bromopyridine and 4-cyanotetrafluoropyridine systems gave the corresponding pyrido[2,3-b]pyrazines, and, upon reaction with *N*,*N*′-dimethylethylene diamine [11].

However, not all tetrafluoropyridine derivatives were suitable substrates for the synthesis of pyrido[2,3-b] pyrazine scaffolds by analogous annelation reactions [11].

1,3,5-Triazine derivatives have been known for a long period of time. They have found widespread applications in the pharmaceutical, textile, plastic, and rubber industries and are used as pesticides, dyestuffs, optical bleaches, explosives, and surface active agents. The chemistry of this group of compounds has been studied intensively and has been the subject of many reviews [12,13].

Cyanuric chloride is an essential organic intermediate of which three chlorines can be replaced by $-NH_2$, $-OH$, $-SH$ or $-NHR$ step by step with high yield. Cyanuric chloride derivatives have been studied for decades, especially its amino derivatives [14].

Ciprofloxacin is one of the broad-spectrum fluoroquinolone antibiotics with low side effects [15]. Generally, fluoroquinolones are broad spectrum antibiotics used to treat a wide variety of both gram-negative and gram-positive bacterial infections [16,17], but the activity against clinical important gram-positive pathogens is relatively moderate [18]. Ciprofloxacin is a fluoroquinolone antibiotic that has demonstrated *in vitro* activity against *Staphylococcus* and *Bacillus* species and most gram-negative microorganisms including *Pseudomonas* species etc. and also has been shown to have antiproliferative and apoptotic activities in several cancer cell lines [19,20].

The most common substituents are cyclic amino groups, for example, piperazine and pyrrolidine rings and other groups have been less successful. Piperazine rings are particularly common (e.g. ciprofloxacin, norfloxacin or enoxacin) and confer potency against gram-negative bacteria [21,22].

We now report syntheses of diverse classes of antibacterial by coupling ciprofloxacin **1**, with pentafluoropyridine **5**, cyanuric chloride **2** and 2,3,5,6-tetrafluoro-4-(phenylsulfonyl)pyridine **7**.

The coupling of ciprofloxacin **1**, pentafluoropyridine **5**, cyanuric chloride **2** and 2,3,5,6-tetrafluoro-4-(phenylsulfonyl)pyridine **7** in DMF-water in the presence of K_2CO_3 for appropriate time, resulted in the formation a new class of piperazinylquinolone.

In this research, pentafluoropyridine, cyanuric chloride was selected as an N-link to quinolone derivatives in order to improve antibacterial properties. This type of pharmacophore combination could improve the antibacterial potential of quinolones.

RESULTS AND DISCUSSION

Many reports indicate that antitumor efficacy of fluoroquinolones can be augmented by increasing the lipophilicity of compounds. Introducing substituents at C-7 position of camptothecin improved the lipophilicity, and this led to the discovery of gimatecan, which is currently in phase II clinical trials. In bisquinolinium compounds, lipophilicity of the substituent enhanced their antiproliferative activity against HT-29 colon cancer cell line [15].

Because of broad spectrum of activates reported so far and in continuation of research on the synthesis of bioactive pipirazine quinolone derivatives [23], we described here the synthesis of new fluoro pipirazine quinolone compounds; also, their antibacterial effects were investigated. Synthesis of target molecules is outlined in Scheme 1.

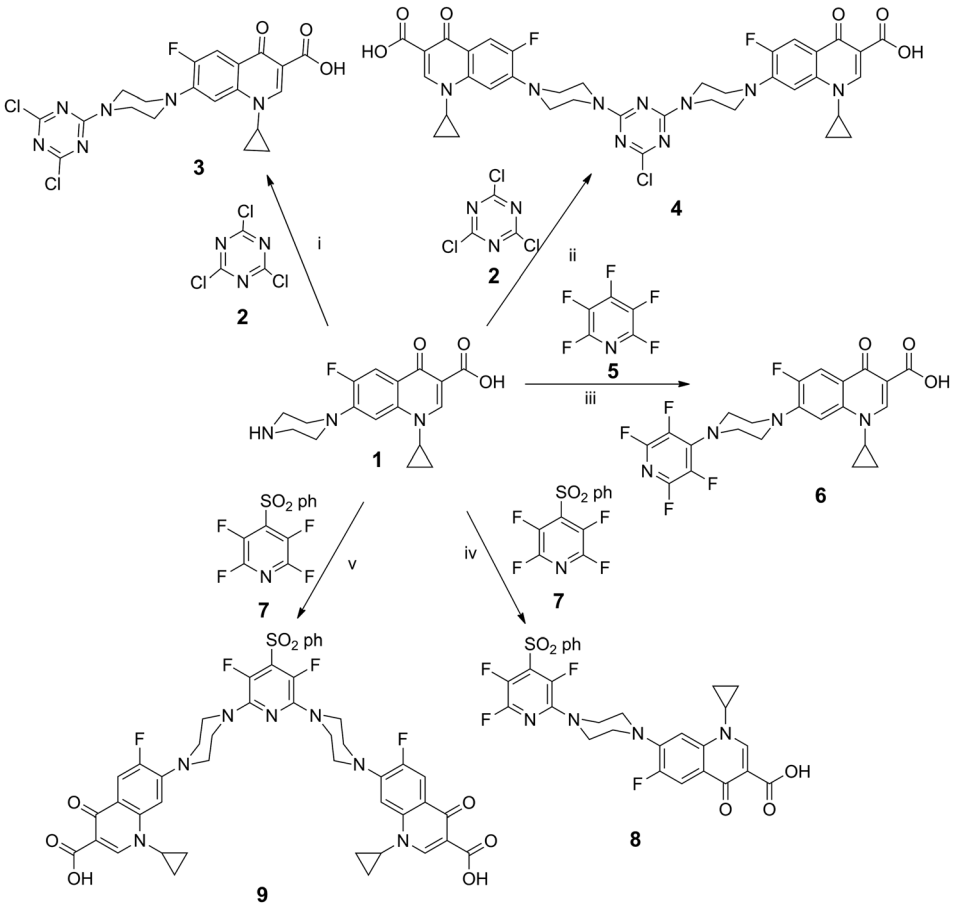
Reaction of **(2)**, **(5)**, **(7)**, with ciprofloxacine **(1)** was investigated (Scheme 1). First, different solvents such as chloroform, dichloromethane, acetone, THF, and water as the reaction medium were used. In these solvents, products were obtained low yield (25–40%). However, when we used DMF as a solvent, the propose products were produced in high yield. A reaction time of 18–48 h at reflux in chloroform, dichloromethane, acetone, THF, and water was maintained because a longer reaction time did not improve the yield. In order to decrease the reaction time, we used DMF-water (4:1) as solvent, the propose products were produced in high yield at shortly reaction time (12–16 h).

Cyanuric chloride (**2**) or pentafluoropyridine **(5)** or tetrafluoropyridine benzene sulfonate (**7**) were reacted with cipropfloxacine **(1)** to produce quinoline derivative. Nucleophilic aromatic substitution of chlorine or fluorine atom by ciprofloxacine **(1)** was carried out using K_2CO_3 as base and DMF-water as a solvent under reflux condition producing (**3**), (**4**), (**6**), (**8**), (**9**).

After selecting the solvent and optimizing the conditions' reaction, first, we synthesize the target molecule substituted cyanuric chloride **3** and **4**, using cyanuric chloride **(2)**, and ciprofloxacine **(1)** as starting materials. Piperazinylquinolone derivatives **3** and **4** show cyclopropyl ring protons at 1.17–1.52, 3.83–3.94 ppm, piperazine ring protons at 3.05–3.40 ppm in ^{1}H NMR spectrum. The latter was confirmed by the disappearing $-$N–H peak of piperazine ring and presence of $-$OH (COOH) peak at 15.16–15.34 ppm in proton NMR. Similarly, the presence of $-$OH steretching frequency at 3434–3450 cm^{-1}, C=O stretching frequency at 1720 cm^{-1} and C=N stretching frequency at 1627–1630 cm^{-1} in FT-IR spectrum.

The compund **6** was obtained by nucleophilic substitution reaction of pentafluoropyridine with ciprofloxacine. The presence of signals at δ 15.13 ppm reveals the presence of COOH group in the 1-cyclopropyl-6-fluoro-4-oxo-7-(4-(perfluoropyridin-4-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **6**. The multiplet signals at δ 3.07 and

Scheme 1. Reagents and conditions: (i) DMF-water, K_2CO_3, reflux, 14 h; (ii) DMF-water, K_2CO_3, reflux, 16 h; (iii) DMF-water, K_2CO_3, reflux, 12 h; (iv) DMF-water, K_2CO_3, reflux, 12 h; (v) DMF-water, K_2CO_3, reflux, 14 h.



δ 3.37 ppm depict the presence of pipirazine ring in the synthesized compound **6;** the presence of multiplets at δ 1.18–1.32 and 3.76 ppm depict the presence of cyclopropyl ring in the synthesized derivative. Two singlets at, δ 7.61 and 8.66 ppm and a doublet with coupling constant $J_{H\text{-}F} = 11.66$ Hz at, 7.93 ppm supports the presence of quinoline nucleus in the compound **6**. Also, the absence of signal at about δ 2 ppm confirms the absence of any residual ciprofloxacine in the synthesized compound, thus confirming the completion of reaction. Moreover, the presence of signals at δ −95.36, −154.26 ppm in the ^{19}F NMR confirms the attachment of ciprofloxacine with C4-pentafuoropprridine via pipirazine-nitrogen linkage. This spectral characterization supports the fact that amine group were attached to the pentafluoropyridine through a nitrogen linkage.

Compounds **8** and **9** were obtained by reaction of tetrafluoropyridine benzene sulfonate with ciprofloxacine using K_2CO_3 as base and DMF-water as a solvent under reflux condition. Compound synthesized **8** and **9** show cyclopropyl ring protons at 1.22–1.36, 3.56–4.31 ppm, piperazine ring protons at 2.72–2.99 ppm and carboxylic acid groups (COOH) at 15.34 ppm in ^{1}H NMR spectrum. All of the IR, NMR data corresponding to ciprofloxacine section and elemental analysis confirms the product structures **8** and **9**. Moreover, the presence of signals at δ −89.08, −126.14, −128.39 ppm and at δ −73.84, −139.23 ppm in the ^{19}F NMR confirms the attachment of ciprofloxacine with C2-pentafuoropprridine (for synthesized compound **8**) and C2, C5-pentafluoropprridine (for synthesized compound **9**) via pipirazine-nitrogen linkage. This spectral characterization supports the fact that amine group was attached to the tetrafluoropyridine benzene sulfonate through a nitrogen linkage.

Antibacterial activity. Hydrophobicity/lipophilicity plays a major role in determining where drugs are distributed within the body after adsorption and as a consequence, in how rapidly they are metabolized and excreted. In this context, the presence of a hydrophobic moiety is important for activity. Moreover, many of the proteins involved in drug disposition have hydrophobic binding sites thus adding to the importance of lipophilicity [1].

The newly synthesized compounds **3**, **4**, **6**, **8** and **9** were tested for their antibacterial *in vitro* growth inhibitory

activity against the standard strains of the Persian Type Culture Collection (PTCC) namely *Staphylococcus aureus* PTCC 1189, *Enterococcus* (gram-positive bacteria), *Escherichia coli* PTCC 1037, *Proteus*, *Shigella*, *Klebsiella*, *Pseudomonas*, *Klebsiella pneumonia* PTCC 1290 (gram-negative bacteria). The primary screening was carried out using the agar disc diffusion method using Müller–Hinton agar medium [24]. The results of the preliminary antimicrobial testing of compounds **3**, **4**, **6**,**8** and **9** (1 μg/disc), the antibacterial antibiotic vancomycin (1 μg/disc) and the ciprofloxacine drug (1 μg/disc) are shown in Table 1.

The results revealed that the compounds showed varying degrees of inhibition against the tested microorganisms. In general, strong activity was displayed by the compounds **3**, **8** and **9**, which produced growth inhibition zones ≥26 mm against one or more of the tested microorganisms. Meanwhile, compounds **4** and **6** showed moderate activity (growth inhibition zones ≥9–28 mm). The tested compounds were generally active against both the gram-positive and the gram-negative bacteria; the compounds **3**, **8** and **9** showed strong activity against *S. aureus*, *E. coli* and *K. pneumonia*. The results showed that all compounds have effects on bacteria. The minimal inhibitory concentrations (MICs) [24] for the most active compounds **3**, **4**, **6**, **8**, **9**, which are shown in Table 2, were in accordance with the results obtained in the primary screening.

The results showed (Table 2) that compounds **6** and **8** have potential bacteriostatic than other compounds. Also, the compound **6** (Table 3) has a greater potential bactericidal than other compounds. Results showed that these compounds have more potential bacteriostatic than antibacterial drugs vancomycin and Ciprofloxacin.

Structure activity relationship (SAR) observations. According to the results, we can conclude that replacement of one chlorine atom of cyanuric chloride by ciprofloxacine increases the antibacterial activity of compound **3,** but when the two chlorine atoms in cyanuric chloride were replaced by two ciprofloxacine molecules, antibacterial activity of compound **4** decreases (Scheme 1 and Table 1).

Also, we selected pentafluoropyridine derivatives as a link to ciprofloxacine in order to improve of biological activity. Presence of the pentafluoro benzene sulfonate nucleus increased antibacterial activity of compound **8**. Also, in these reaction, replacement of one fluorine atom of tetrafluoropyridine benzene sulfonte by ciprofloxacine increases the antibacterial activity (compound **8**), but when the two fluorine atoms in tetrafluoropyridine benzene sulfonte were replaced by two ciprofloxacine molecules, antibacterial activity (compound **9**) decreases (Scheme 1 and Table 1).

From the previous summary, it can be concluded that the pentafluoropyridine derivative and cyanuric chloride N-linked to quinolone derivative increase their antibacterial properties.

Table 1

Antibacterial activity of compounds **3**, **4**, **6**, **8** and **9** (1 μg/disc), the broad spectrum antibacterial antibiotics vancomycin (1 μg/disc), ciprofloxacine drug (1 μg/disc) against *Proteus* (P^1), *Shigella* (S), *Klebsiela* (K), *Pseudomonas* (P^2), *Enterococcus* (E), *Staphylococcus aureus* PTCC 1189 (SA), *Escherichia coli* PTCC 1037 (EC) and *Klebsiella pneumonia* PTCC 1290 (KP).

Comp. no.	Diameter of growth inhibition zone (mm)							
	P^1	S	K	P^2	E	SA	EC	KP
3	34	40	33	40	37	50	36	32
4	18	25	14	24	0	27	28	27
6	–	–	–	–	–	9	13	12
8	26	27	28	28	0	32	28	27
9	–	–	–	–	–	30	32	30
Ciprofloxacin	25	–	22	0	0	–	26	22
Vancomycin	0	–	0	0	16	–	0	0

(−): Inactive (inhibition zone <10 mm).

Table 2

The minimal inhibitory concentrations (MICs, μg/mL) of compounds **3**, **4**, **6**, **8**, **9** (2, 1, 0.5, 0.25, 0.125 μg/mL) and the broad spectrum antibacterial drugs vancomycin (2, 1, 0.5, 0.25, 0.125 μg/mL), and drug ciprofloxacin (2, 1, 0.5, 0.25, 0.125 μg/mL) against *Bacillus cereus* accession number (JX843766.1) (BC), *Bacillus thuringiensis* accession number (JX941572.1) (BT), *Escherichia coli* PTCC 1037 (EC) and *Staphylococcus aureus* PTCC 1189 (SA).

Comp. No.	Minimal inhibitory concentration (MIC, μg/mL)			
	BC	BT	EC	SA
3	1	1	0.5	ND
4	2	1	2	2
6	1	1	1	1
8	1	1	0.5	1
9	2	1	1	2
Ciprofloxacin	1	1	0.125	ND
Vancomycin	ND	ND	ND	ND

ND: not determinate.

Table 3

Minimum Bactericidal Concentration (MBC, μg/ml) of compounds **3**, **4**, **6**, **8**, and **9** (2, 1, 0.5, 0.25, and 0.125 μg/ml) and the broad spectrum antibacterial drugs vancomycin (2, 1, 0.5, 0.25, and 0.125 μg/ml), and drug ciprofloxacin (2, 1, 0.5, 0.25, and 0.125 μg/ml) against *Bacillus cereus* (BC), *Bacillus thuringiensis* (BT), *Escherichia coli* PTCC 1037 (EC), and *Staphylococcus aureus* PTCC 1189 (SA).

Comp. No.	Minimum bactericidal concentration (MBC, μg/mL)			
	BC	BT	EC	SA
3	0.25	0.5	0.5	0.25
4	0.25	0.5	0.25	0.25
6	0.125	0.5	<0.125	0.125
8	ND	0.5	0.25	0.5
9	0.5	0.25	0.25	0.5
Ciprofloxacin	0.25	0.5	<0.125	ND
Vancomycin	ND	ND	ND	ND

ND: not determinate.

These compounds would have promising antibacterial effects.

CONCLUSION

In conclusion, a series of piperazinylquinolone derivatives were designed and synthesized by the reaction of pentafluoropyridine derivatives and cyanuric chloride with ciprofloxacin under mild conditions. We selected pentafluoropyridine derivatives or cyanuric chloride as an N-link to quinolone derivatives in order to improve their antibacterial properties. Synthesized compounds were evaluated for their antibacterial activities. Many antibacterial conjugate shows promising preliminary results and may be equivalent or more effective than the original parent drug. These modifications may provide options for treatment of bacterial-resistant strains, with the benefit of enhanced drug uptake and/or diminished adverse side effects. According to the finding of present study, it would be concluded that reagent **6** is a more potent bactericide for bacteria and the potency of reagents **3** and **8** is less than other reagent **6**, while they are more potent bacteriostatic properties rather than bactericide. Further studies on biological activities of these compounds are in progress.

EXPERIMENTAL

All reactions were performed with magnetic stirring in flame-dried glassware with dry and distilled solvents.

Chemicals and solvents were purchased from Merck AG and Aldrich Chemical companies. Melting points were determined with a Barnstead electrothermal. IR spectra (KBr) were obtained on a Matson-1000 FT-IR spectrometer. The proton and carbon-13 NMR spectra were recorded by a BRUKER DRX-500 AVANCE spectrometer at 500 and 125.7 MHz, respectively, using Me_4Si as an internal standard (chemical shifts in δ, ppm). ^{19}F NMR spectra were taken on Brucher AM-300 (282 MHz) spectrometer using $CFCl_3$ as external standard. Element analyses (C, H, N and S) were performed with a EUROVECTOR EuroEA3000 CHNSO analyzer. The mass spectra were run on a Finnigan TSQ-70 spectrometer (Finnigan, USA) at 70 eV. Merck silica gel 60F254 plates were used for analytical TLC.

General experimental procedures. ***General procedure for synthesis of piperazinylquinolone derivatives 3 and 4.*** A mixture of ciprofloxacin **1** (1 mmol for **3** and 2 mmol for **4**) and K_2CO_3 (138 mg, 1 mmol for **3** and 276 mg, 2 mmol for **4**) in DMF (10 mL) and water (2.5 mL) was stirred at room temperature for 10 min, and then, a solution of cyanuric chloride **2** (1 mmol) in DMF (8 mL) and water (2.5 mL) were added successively. The mixture was refluxed for 14 h for **3** and 16 h for **4**. After consumption of piperazinylquinolone (monitored by TLC), $NaHCO_3$ (20 mL, 10%) was added and the resulting solids were washed with water and recrystallized from DMF to give piperazinylquinolone compounds.

1-Cyclopropyl-7-(4-(4,6-dichloro-1,3,5-triazin-2-yl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (3) [25]. Brown solid; yield: 75%; mp 178–180°C; IR (KBr) 3450 (−COOH), 1720 (C=O), 1627,1464 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d6, 500 MHz); δ 1.17–1.32 (m, 4H, CH_2 cyclopropyl), 3.05 (m, 4H, piperazine), 3.40 (m, 4H, piperazine), 3.83 (m, 1H, cyclopropyl), 7.59 (s, 1H, H_8 quinoline), 7.92 (d, 1H, H_5 quinoline, $J_{H\text{-}F} = 13.80$ Hz), 8.64 (s, 1H, H_2 quinoline), 15.16 (s, 1H, COOH) ppm. ^{13}C NMR (DMSO-d6 300 MHz); δ 7.85 (CH_2 cyclopropyl), 35.82 (CH cycloprppyl), 49.32, 50.13 (CH_2 piperazine), 106.60, 110.91, 119.38, 124.91, 147.91, 151.95, 153.93, 165.51, 176.29 ppm. *Anal.* Calcd for $C_{20}H_{17}Cl_2FN_6O_3$ (479.29): C, 50.12; H, 3.58; N, 17.53%. Found: C, 50.52; H, 3.64; N, 17.26%.

7,7′-(4,4′-(6-chloro-1,3,5-triazine-2,4-diyl)bis(piperazine-4,1-diyl))bis(1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) (4). White solid; yield: 45%; mp 324–327°C; IR (KBr) 3469 (−COOH), 1719 (C=O), 1650,1466 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d6, 500 MHz); δ 1.22–1.54 (m, 8H, CH_2 cyclopropyl), 3.5 (m, 8H, piperazine), 3.33 (m,8H, piperazine), 3.94 (m, 2H, cyclopropyl), 7.22–7.24 (s, 2H, H_8 quinoline), 7.96 (d, 2H, H_5 quinoline, $J_{H\text{-}F} = 13.50$ Hz), 8.96 (s, 2H, H_2 quinoline), 15.34 (s, 1H, COOH) ppm. *Anal.* Calcd for $C_{37}H_{34}ClF_2N_9O_6$ (774.17): C, 57.40; H, 4.43; N, 16.28. Found: C, 57.73, H, 4.13; N, 16.42%.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(perfluoropyridin-4-yl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (6). A mixture of piperazinylquinolone **1** (1 mmol) and K_2CO_3 (138 mg, 1 mmol) in DMF (8 mL) and water (2 mL) was stirred at room temperature for 10 min, and then, a solution of pentafluoropyridine **5** (1 mmol) in DMF (5 mL) were added successively. The mixture was refluxed for 12 h. After consumption of piperazinylquinolone (monitored by TLC), $NaHCO_3$ (20 mL, 10%) was added and the resulting solids were washed with water and recrystallized from DMF to give piperazinylquinolone compounds.

Yellowish solid; yield: 80%; mp >300°C; ^1H NMR (DMSO-d6, 500 MHz) δ 1.18–1.32 (m, 4H, CH_2 cyclopropyl), 3.07 (m,4H, piperazine), 3.37 (m, 4H, piperazine), 3.76 (m, 1H, cyclopropyl), 7.61 (s, 1H, H_8 quinoline), 7.93 (d, 1H, H_5 quinoline, $J_{H\text{-}F} = 11.65$ Hz), 8.66 (s, 1H, H_2 quinoline), 15.13 (s, 1H, COOH) ppm. ^{19}F NMR (DMSO-d6, 282 MHz) δ −95.36 (2F, F-2,6-pyridine ring), −122.18 (s, 1F, F-6, quinoline ring), −154.26 (2F, F-3,5-pyridine ring) ppm. *Anal.* Calcd for $C_{22}H_{17}F_5N_4O_3$ (480.39): C, 55.00; H, 3.57; N, 11.66%. Found: C, 55.21; H, 3.69; N, 11.42%.

General procedure for synthesis of piperazinylquinolone derivatives 8 and 9. A mixture of ciprofloxacin **1** (1 mmol for **8** and 2 mmol for **9**) and K_2CO_3 (138 mg, 1 mmol for **8** and 276 mg, 2 mmol for **9**) in DMF (10 mL) and water (2.5 mL) was stirred at room temperature for 10 min, and then, a solution of 2,3,5,6-tetrafluoro-4-(phenylsulfonyl)pyridine **7** (1 mmol) in DMF (8 mL) and water (2 mL) were added successively. The mixture was refluxed for 12 h for **8** and 14 h for **9**. After consumption of piperazinylquinolone (monitored by TLC), $NaHCO_3$ (20 mL, 10%) was added, and the resulting solids were washed with water and recrystallized from DMF to give piperazinylquinolone compounds.

1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(3,5,6-trifluoro-4-(phenylsulfonyl)pyridin-2-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (8). Yellow-brownish solid; yield: 73%; mp 360°C (dec); IR (KBr) 3424 (−COOH), 1625, 1585 (C=N, C=C) cm^{-1}. ^1H NMR (DMSO-d6, 500 MHz) δ 1.22–1.36 (m, 4H, CH_2 cyclopropyl), 2.72 (m,4H, piperazine), 2.88 (m, 4H, piperazine),

3.56 (m, 1H, cyclopropyl), 7.07 (s, 1H, H_8 quinoline), 7.43 (m, 2H, Are), 7.72 (m, 1H, Are), 7.94 (d, 1H, H_5 quinoline, $J = 12.9$ Hz), 8.05 (m, 2H, Are), 8.07 (s, 1H, H_2 quinoline), 15.34 (s, 1H, COOH) ppm. ^{19}F-NMR (DMSO-d6, 282 MHz) δ −89.08 (1F, F-6, pyridine ring), −126.14 (s, 1F, 6-F, quinoline ring), −128.39 (1F, F-3, pyridine ring), −154.88 (1F, F-5, pyridine ring) ppm. *Anal.* Calcd for $C_{28}H_{22}F_4N_4O_5S$ (461.39): C, 55.81; H, 3.68; N, 9.30; S, 5.32%. Found: C, 55.39; H, 3.81; N, 9.56; S, 5.14%.

7,7′-(4,4′-(3,5-difluoro-4-(phenylsulfonyl)pyridine-2,6-diyl) bis(piperazine-4,1-diyl))bis(1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid) (9). Yellow-brownish solid; yield: 43%; mp 280°C (dec); IR (KBr) 3450 (−COOH), 1625, 1494 (C=N, C=C) cm^{-1}. ^{1}H NMR (DMSO-d6, 500 MHz) δ 1.32–1.35 (m, 8H, CH_2 cyclopropyl), 2.87 (m, 8H, piperazine), 2.99 (m, 8H, piperazine), 4.31 (m, 2H cyclopropyl), 7.74 (m, 1H, Ar), 7.60 (s, 1H, H_8 quinoline), 7.67 (m, 2H, Ar), 7.93 (d, 1H, H_5-quinoline, $J_{H,F} = 13.3$ Hz), 8.10 (m, 2H, Are), 8.55 (s, 2H, H_2 quinoline), 15.34 (s, 1H, COOH) ppm. ^{19}F NMR (DMSO-d6, 282 MHz) δ −73.84 (2F, F-3 and F-5, pyridine ring), −139.23 (2F, F-6, quinoline rings) ppm. *Anal.* Calcd for $C_{45}H_{39}F_4N_7O_8S$ (772.72): C, 59.14; H, 4.30; N, 10.73; S, 3.51%. Found: C, 60.17; H, 4.03; N, 11.89; S, 3.76%.

Antibacterial activity evaluation. The 0.01 g of synthesized powder (antibacterial compounds tested) was dissolved in 200 μL DMSO, and then, blank discs were soaked with 40 μL of the previous solution containing different compounds. Discs were put at 60°C for 30 min to get dried. *E. coli* and *K. pneumonia* as gram-negative bacteria and *S. aureus* as gram-positive were obtained from PTCC with 1037, 1290 and 1189 codes respectively and cultured. Antibiogram test performed according to Kirby–Bauer method on Mueller–Hinton Agar (Merck, Germany). We used ciprofloxacin and vancomycin as positive controls (Padtan Teb, Iran). Five synthesized compounds (**3**, **4**, **6**, **8**, and **9**, as mentioned) discs with positive controls tested. After 18 h, the diameter of inhibition zones was measured (mm) and reported as shown in Table 1. These compounds would have promising well to excellent antibacterial effects, although more experiments to examine reproducibility and effective dose are required.

Broth dilution MIC method were determined by microdilution as described by the NCCLS dilution standard M7-T [26]. Following an overnight incubation at 37°C, the MIC is determined by observing the lowest concentration of the drug that will inhibit visible growth of the test bacteria [27] (Table 2).

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