Design, synthesis, molecular docking study, and antibacterial evaluation of some new fluoroquinolone analogues bearing a quinazolinone moiety

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Abstract

Background Increasing bacterial resistance to quinolones is concerning. Hence, the development of novel quinolones by chemical modifications to overcome quinolone resistance is an attractive perspective in this context.

Objective In this study, it is aimed to design and synthesize a novel series of functionalized fluoroquinolones using ciprofloxacin and sarafloxacin cores by hybridization of quinazolinone derivatives. This objective was tested by a comprehensive set of in vitro antibacterial assays in addition to SAR (structure–activity relationship) characterisation studies.

Methods A nucleophilic reaction of ciprofloxacin and sarafloxacin with 2-(chloromethyl)quinazolin-4(3H)-one in the presence of NaHCO₃ in dimethylformamide (DMF) was performed to obtain the desired compounds **5a–j**. Novel compounds were characterised by ¹H, ¹³C- NMR and IR spectroscopy, MS and elemental analysis. In silico pharmacokinetics prediction assays and molecular docking studies were performed to explore the binding characteristics and interactions. Antibacterial activities of the novel compounds were evaluated by Broth microdilution, well diffusion and disc diffusion assays against three gram-positive (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and *Enterococcus faecalis*) and three gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*).

Results The compounds exhibited moderate to good activities against gram-positive bacteria and weak to moderate activities against gram-negative bacteria. Amongst all ciprofloxacin-derivatives, compound **5d** was the most potent agent with high antibacterial activity against gram-positive bacteria, including MRSA and *S. aureus* ((minimum inhibitory concentration (MIC) = 16 nM for both), that is 60 times more potent than ciprofloxacin as parent drug. Compound **5i** from sarafloxacin-derivatives was the most potent compound against MRSA and *S. aureus* (MIC = 0.125 µM). Well diffusion and disk diffusion assay results demonstrated confirmatory outcomes for the quantitative broth microdilution assay. Molecular docking study results were in accordance with the results of antibacterial activity assays.

Conclusion The results of the current study demonstrated that the novel ciprofloxacin and sarafloxacin derivatives synthesized here have promising antibacterial activities. Particularly, compounds **5d** and **5i** have potential for wider antibacterial applications following further analysis.

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Introduction

In recent years, infectious diseases caused by multidrug resistant pathogens have received a significant attention. This is mainly owing to the fact that the spread of resistant bacteria is drastically rising [1–4] and it encourages medicinal chemists to tackle this problem with a strong tendency to design and develop novel antibacterial agents. Following the discovery of norfloxacin which was primarily utilized for the treatment of mild urinary tract infections [5], this kind of drugs classified among the most commonly prescribed antibacterial agents in the world [6, 7]. These synthetic antimicrobial agents possess broad-spectrum activity against gram-negative and gram-positive bacterial infections [6, 8–11] by targeting bacterial topoisomerase II and topoisomerase IV [12–15] which makes a substantial contribution to DNA replication and transcription. Topoisomerase II has been identified as a cellular target in gram-positive bacteria, as well as topoisomerase IV in gram-negative bacteria [16, 17]. Many studies have demonstrated that mutations in gyrase and/or topoisomerase IV lead to diminishing the drug-enzyme binding and is an underlying cause of quinolone resistance [18–21]. Hence, the development of novel quinolones to overcome quinolone resistance should be thought of indispensable. Over the years, a great deal of research into the screening of quinolone analogues with various structural modifications were done for their antibacterial activity in order to achieve more potent agents. One major factor relevant to enhance potency and strengthen interactions with the cellular targets (DNA gyrase and DNA topoisomerase IV) represents the identification of C₇ substituents as the most versatile position [22]. Some structural studies signified that C₇ substituents with aromatic rings lead to heighten antibacterial activity against gram-positive pathogens [15]. Following our previous works in the synthesis of novel quinolone analogues [23, 24], this work has helped to define how a quinazolinone moiety that has been attached to C₇ (piperazino) of quinolones can affect the antibacterial activity.

Many evidences suggest that quinazolinone moiety is an important structural motif that displays diverse classes of biological and pharmacological properties such as antibacterial [25], analgesic, anti-inflammatory, antifungal, antimalarial, antihypertensive, CNS depressant, anticonvulsant, antihistaminic, anti-parkinsonism and antiviral activities which partly arise from the stability of the quinazolinone nucleus [26–31]. It has been shown that quinazolinone derivatives exhibit excellent in vitro and in vivo activities against various fatal pathogens [25, 32] (Fig. 1). As mentioned, the side chain attached to the piperazine in fluoroquinolones is a determining factor in

antimicrobial activity and there has been tremendous research into the modification of this part of the molecule in order to achieve modified antibacterial agents with high potency [33–35]. For this purpose, we designed and synthesized a novel series of functionalized fluoroquinolones using ciprofloxacin and sarafloxacin cores by hybridization of quinazolinone derivatives to obtain a new class of antibacterial agents.

It is now widely accepted that altering the terminal nitrogen of the piperazine moiety of fluoroquinolones illustrates a high tolerance [9]. Given that the study is intended to investigate the SAR of antimicrobial fluoroquinolones bearing a quinazolinone moiety, we synthesized compounds **5a-j** through replacing the substituents at the N-piperazine part of the ciprofloxacin and sarafloxacin scaffold by a quinazolinone moiety (Fig. 2).

Results and discussion

Chemistry

The compounds **5a-j** were attained by a nucleophilic reaction of secondary amino (piperazino) function of ciprofloxacin with 2-(chloromethyl)quinazolin-4(3H)-one component, which is initially constructed by a cyclization reaction of commercially substituted O-aminobenzoic acids **2a-d** and chloroacetonitrile in the presence of sodium methoxide solution. The nucleophilic reaction of ciprofloxacin was performed in DFM in the presence of sodium bicarbonate, as summarized in Scheme 1, which were then stirred at 40 °C to afford a series of five different ciprofloxacin-derivatives **5a-e**. A similar synthetic pathway was used to obtain sarafloxacin analogues **5f-j**. The characterization data, including ¹H, ¹³C-NMR and IR spectroscopy spectra as well as Mass spectrometry and elemental analysis confirmed the chemical structure of the synthesized compounds **5a-j**.

Antibacterial activity

The synthesized compounds **5a-j** were evaluated for in vitro antibacterial activity against three gram-positive (Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and *Enterococcus faecalis*) and three gram-negative strains (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*) using 3 methods, including broth microdilution, well diffusion and disc diffusion assays. The results were presented with minimal inhibitory concentration

Fig. 1 Some 4(3H)-quinazolinone derivatives with antibacterial activity

(MIC) values using ciprofloxacin and sarafloxacin as controls, as shown in Table 1.

Broth microdilution is known as one of the most commonly used and as a clinically significant, golden standard technique to assess the MICs of antibacterial agents. In this screening method, most compounds showed moderate to good activities against gram-positive strains. All of the synthesized compounds from ciprofloxacin-derivatives series **5a-e** showed remarkable activities against MRSA and *S. aureus*. The most promising activity against these gram-positive bacteria were observed in **5c** and **5d** with MIC values ranging from 16 to 31 nM. It is noteworthy that compound **5d** was 60 times more active than the positive control against the mentioned strains. This implies that the presence of a methoxy group at R₁ position results in such favourable antibacterial activity. The broth microdilution results demonstrated that all of the synthesized sarafloxacin-derivatives series had approximately the same activity as parent drug against gram-positive

bacteria. Compound **5i**, with a methoxy group at R₁ position, revealed the most promising antibacterial activity against MRSA and *S. aureus* with MIC value of 0.125 μ M.

It is conspicuous that none of the synthesized compounds **5a-j** exhibited good activity against gram-negative strains compared to parent drugs. Thereby, the replacement of the hydrogen attached to the nitrogen of the piperazine moiety in ciprofloxacin and sarafloxacin by a 2-methylquinazolin-4(3H)-one group effectuated a remarkable raise in the antibacterial activity against gram-positive bacteria, especially MRSA and *S. aureus*. This activity was intensified by the presence of a methoxy group at R₁ position of the compounds. On the contrary, these modifications have no significant effect on the gram-negative bacteria. Since the aqueous solubility represents one of the most important pharmacokinetic properties of fluoroquinolones, we investigated compounds **5a-j** in terms of this parameter. The results indicated that the presence of methoxy group at R₁ position not only increases the

Fig. 2 Design strategy of final compounds **5a-j**, MIC = minimum inhibitory concentration

Scheme. 1 Synthetic pathway of target compounds **5a-j**. Reagents and conditions: a) NaOMe/ MeOH, 5 h, r.t; b) NaHCO₃, DMF, 60 °C, 12 h

potency of compound but improves the aqueous solubility compare to chlorine and bromine atoms in ciprofloxacin-derivatives. Moreover, replacement of the methoxy group with a hydrogen atom, yielded compound **5c** with the same solubility. On the contrary, among sarafloxacin-derivatives, the similar replacement achieved a less soluble compound **5h** in comparison with sarafloxacin. The substitution of chlorine at both R_1 and R_2 positions in ciprofloxacin-derivatives led to yield the soluble compounds **5a** and **5b**.

Agar well diffusion testing is performed as a confirmatory test to demonstrate the antimicrobial activity. In this study, the zones of inhibition were provided for the compounds **5a-j** as diameter in mm, as shown in Table 2. Among ciprofloxacin-derivatives series, **5c**, and **5d** had a remarkable activity against MRSA and *S. aureus* with the zones of inhibition ranging from 29 to 35 mm in comparison with positive control. Furthermore, compound **5i** from sarafloxacin-derivatives series showed comparable activity with positive control against the mentioned strains. The results confirmed that the well diffusion outcomes were in consistence with broth microdilution assay.

In order to conduct a comparative analysis of the study outcomes, agar disk diffusion assay (Disk diffusion test or Kirby–Bauer test) was also performed on Mueller Hinton Agar (MHA). This test is another efficient method to measure inhibition zones diameter which was first described by Bauer, Kirby et al. in the 1960s [36]. The most active agents among ciprofloxacin-derivatives series against gram-positive bacteria were **5c** and **5d** with the zones of inhibition ranging from 21 to 29 mm. Compounds **5h** and **5i** from sarafloxacin-derivatives series had antibacterial activity nearly as good as sarafloxacin (Table 3). We also observed that the zones of inhibition in this method and MICs in broth microdilution assay were concordant.

In silico pharmacokinetics prediction

As an important aspect of identification of the newly synthesized compounds and their potential liabilities in the early stage of drug development, we utilized the predicted human pharmacokinetics (PK) using the SwissADME web-based tool (<http://www.swissadme.ch/>) [37]. In this investigation, some of the most important PK parameters were determined as presented in Table 4. Bioavailability which refers to the fraction of a chemically unchanged form of a medicine that gets to the systemic circulation was measured based on Abbott bioavailability score. The results revealed that all compounds except **5j** possess a remarkable bioavailability score as well as gastrointestinal (GI) absorption; which demonstrated that these compounds are worthwhile in terms of oral bioavailability and achieved approved drug-likeness values. As presented in Table 4, log P and TPSA (topological polar surface area) are two most important descriptors carrying information about the lipophilicity. Accord with the definition of log P , the concentration of compound between the organic and aqueous partition, all compounds exhibited favourable amounts of log P based on the average amount of different methods, including iLOGP, WLOGP, MLOGP, and SILICOS-IT. Furthermore, TPSA amounts indicated that all the compounds **5a-j** tend to be moderate at permeating cell membranes.

Molecular docking

To explore the binding mode and interactions of the most potent compound **5d** with the active site of topoisomerase II DNA gyrase, docking studies were employed. The X-ray structure of DNA gyrase (PDB ID: 2XCT) with ciprofloxacin

Table 1 Broth Microdilution assay results (MIC, $\mu\text{g/ml}$) and the solubility for compounds **5a-j**, ciprofloxacin, and sarafloxacin **Minimal inhibitory concentrations (μM)**

Compound	R ₁	R ₂	MRSA	S. <i>aureus</i>	E. <i>faecalis</i>	P. <i>aeruginosa</i>	K. <i>pneumoniae</i>	E. <i>coli</i>	Solubility
5a	Cl	H	0.25	0.25	>64	16	>64	4	NCS*
5b	H	Cl	0.031	0.125	8	8	>64	0.25	NCS
5c	H	H	0.031	0.031	0.25	16	>64	0.25	CS**
5d	OMe	H	0.016	0.016	0.5	1	64	1	CS
5e	Br	H	0.29	0.29	>64	16	>64	8	NCS
Ciprofloxacin	-	-	0.49	0.98	0.0038	0.03	3.91	0.00	CS
								38	
5f	Cl	H	0.25	0.25	4	>64	>64	8	CS
5g	H	Cl	0.25	0.25	8	64	>64	4	CS
5h	H	H	0.25	0.25	64	32	>64	4	NCS
5i	OMe	H	0.125	0.125	2	32	>64	4	CS
5j	Br	H	0.25	0.25	8	>64	>64	8	NCS
Sarafloxacin	-	-	0.25	0.25	2	0.062	2	0.03	CS
								1	

MRSA: Methicillin-resistant *Staphylococcus aureus*; S. aureus: *Staphylococcus aureus*; E. faecalis: *Enterococcus faecalis*; P. aeruginosa: *Pseudomonas aeruginosa*; K. pneumonia: *Klebsiella pneumoniae*; E. coli: *Escherichia coli*; **CS: Completely soluble; *NCS: 75% Soluble; μM : micromolar

as its inhibitor was retrieved from RCSB website (<https://www.rcsb.org/>).

Docking studies of compound **5d** were performed using Autodock 4.2.1 package [38, 39] and the obtained binding energy and interactions were compared to ciprofloxacin. The docking results as shown in Figs. 3 and 4 revealed the similar binding modes for **5d** and ciprofloxacin in the active site and also the same coordination toward Mn^{+2} metal ions was observed for these compounds. However, compound **5d** provided better binding energy (-17.73 kcal/mol) in comparison to ciprofloxacin (-12.83 kcal/mol). The carboxyl moiety of compound **5d** interacted by its oxygen atom as hydrogen bond acceptor with Ser 1084. The piperazine group

as hydrogen bond donors formed three nonclassical hydrogen bonds with Arg 458, DC 112, and DC 113. The bicyclic quinoline core formed π - π stacked interactions with DG 86 and DG 88. Moreover, additional van der Waals and hydrophobic interactions were formed between the quinazolinone moiety of this ligand and some residues such as DA 111. Despite the elongation of this structure, the quinazolinone warhead bent through the flexible methylene bridge and made U-shaped scaffold that could be properly placed into the active site. Overall, these favourable interactions in addition to excellent binding score which were in accordance with biological results, can explain the better activity of this ligand.

Table 2 Well diffusion assay results for compounds **5a–j**, ciprofloxacin, and sarafloxacin (the tested concentration = 64 µg/ml). The zone of inhibition was given in mm

Comp.	MRSA	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
5a	26	24	0	0	0	18
5b	25	25	0	15	0	22
5c	30	31	0	15	0	23
5d	35	29	0	20	0	25
5e	25	22	0	0	0	17
Ciprofloxacin	30	30	20	33	17	35
5f	23	21	0	0	0	14
5 g	27	23	0	0	0	15
5 h	22	25	0	0	0	20
5i	26	25	0	0	0	17
5j	22	22	0	0	0	15
Sarafloxacin	35	33	18	35	16	38

MRSA: Methicillin-resistant *Staphylococcus aureus*; *S. aureus*: *Staphylococcus aureus*; *E. faecalis*: *Enterococcus faecalis*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *K. pneumonia*: *Klebsiella pneumonia*; *E. coli*: *Escherichia coli*; µM: microMolar.

Conclusion

Here, ciprofloxacin and sarafloxacin derivatives were synthesized and evaluated for their antibacterial activities against three gram-positive (MRSA, *S. aureus* and *E. faecalis*) and three gram-negative pathogens (*P. aeruginosa*, *K. pneumoniae*, *E. coli*). Antibacterial activities of the novel compounds were demonstrated by performing comprehensive and comparative analysis by conducting three state of the art bioassay methods in vitro.

The results revealed that replacing the hydrogen atom of the HN-piperazine part of the ciprofloxacin scaffold by a 2-methylquinazolin-4(3H)-one group has a significantly improved antibacterial effect against gram-positive bacteria, especially MRSA and *S. aureus*. Compound **5d**, from ciprofloxacin-derivatives series, with a methoxy group at R₁ position was the most potent agent in broth microdilution assay which was 60 times more potent than ciprofloxacin. Especially, compound **5d** may have a great potential for its future applications as an antibacterial

Table 3 Disc Diffusion assay results for compounds **5a–j**, ciprofloxacin, and sarafloxacin (the tested concentration = 64 µg/ml). The zone of inhibition was given in mm

Comp.	MRSA	<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
5a	20	15	0	0	0	10
5b	21	15	0	0	0	12
5c	29	22	0	0	0	15
5d	27	21	0	0	0	18
5e	19	14	0	0	0	10
Ciprofloxacin	22	22	10	26	12	33
5f	19	13	0	0	0	8
5 g	19	15	0	0	0	11
5 h	22	18	0	0	0	10
5i	22	17	0	0	0	9
5j	17	16	0	0	0	8
Sarafloxacin	25	21	5	28	8	30

MRSA: Methicillin-resistant *Staphylococcus aureus*; *S. aureus*: *Staphylococcus aureus*; *E. faecalis*: *Enterococcus faecalis*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *K. pneumonia*: *Klebsiella pneumonia*; *E. coli*: *Escherichia coli*; µM: microMolar.

Table 4 SwissADME pharmacokinetics prediction for the compounds

Compound	Bioavailability score	log P	GI absorption	TPSA ^a (Å ²)	Drug likeness
5a	0.55	3.47	high	107.24	Yes
5b	0.55	2.91	high	107.24	Yes
5c	0.55	2.92	high	107.24	Yes
5d	0.55	3.32	high	116.47	Yes
5e	0.55	3.33	high	107.24	Yes
5f	0.55	3.26	high	108.71	Yes
5 g	0.55	3.48	high	111.53	Yes
5 h	0.55	3.17	high	111.53	Yes
5i	0.55	3.89	high	116.47	Yes
5j	0.55	3.65	high	111.53	No

log P: log (partition coefficient); GI: gastrointestinal; TPSA: topological polar surface area;

agent after further investigations. The well diffusion and disc diffusion assays results confirmed the findings. Even though compound **5i** from sarafloxacin-derivatives series showed a moderate activity against gram-positive strains, the modifications in the sarafloxacin scaffold seem trivial convenience next to those in the ciprofloxacin scaffold. Finally, the novel compounds described here may have wider applications against many other pathogens which can be tested in future studies.

Fig. 3 Proposed binding mode for compound **5d**

Methods

All required chemical compounds and solvents were purchased from Merck and Aldrich or synthesized. IR spectra were recorded on a Nicolet FT-IR Magna 550 spectrometer (potassium bromide disks). ¹H NMR spectra were obtained from a Bruker Avance 300 spectrometer using Me₄Si ($\delta = 0.0$) as the internal standard and DMSO-*d*₆ as the solvent. Furthermore, the ¹³C NMR was performed with a Bruker Avance 300 spectrometer at 75 MHz and all chemical shifts were reported in PPM. All characterization data along with spectra were presented in the [supporting file](#) in detail. The PerkinElmer 240-C apparatus (PerkinElmer, Beaconsfield, UK) was used to measure the elemental analyses. The melting points were recorded by a Kofler hot stage apparatus. Also, an HP Agilent Technologies 5937 apparatus at ionization potential of 70 eV was used to report the Mass spectra of the synthesized compounds.

General procedure for the synthesis of compounds 5a-e

A mixture of ciprofloxacin (1.0 mmol) in DMF (5 mL) in the presence of NaHCO₃ (1.0 mmol) was stirred at room temperature. After 30 min, the corresponding 2-(chloromethyl)quinazolin-4(3H)-one (1 mmol) was added and the mixture was stirred at 50 °C for 12 h. Consumption of the ciprofloxacin was monitored by TLC (thin-layer chromatography). After completion of the reaction, the mixture was poured into about 20 mL ice/water and the harvest precipitate was filtered. Some precipitates required further purification by column chromatography (1:9 methanol/chloroform) to obtain purified compounds.

Fig. 4 a 2D binding conformation of compound **5d**; b Superimposition of compound **5d** in gray color with ciprofloxacin in red color

General procedure for the synthesis of compounds 2a-e

In order to synthesis of 2-(chloromethyl)quinazolin-4(3H)-one, chloroacetonitrile (15 mmol) was added to a solution of sodium methoxide (1 M, 5 mL) and the resulting mixture was stirred under an argon atmosphere at room temperature. After 30 min, the corresponding O-aminobenzoic acid (10 mmol) was added dropwise and the resultant suspension was stirred again for another 6 h at room temperature. Then the precipitate was filtered, washed with methanol, and used for the next step without further purification.

7-(4-((6-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5a**)

White solid; yield: 67%; mp: 300 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 14.91 (s, 1H, OH), 12.26 (s, 1H, NH), 8.62 (s, 1H, CH-N), 8.02 (d, $J=2.5$ Hz, 1H, Ar), 7.86–7.79 (m, 2H, Ar), 7.67 (d, $J=8.7$ Hz, 1H, Ar), 7.53 (d, $J=7.3$ Hz, 1H, Ar), 3.78 (s, 1H, CH-cyclopropyl), 3.56 (s, 2H, CH_2), 3.36 (t, $J=4.6$ Hz, s, 4H, 2 $\text{CH}_2\text{-N}$), 2.75 (t, $J=4.6$ Hz, 4H, $\text{CH}_2\text{-N}$), 1.30 (d, $J=6.6$ Hz, 2H, $\text{CH}_2\text{-cyclopropyl}$), 1.16 (s, 2H, $\text{CH}_2\text{-cyclopropyl}$). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 176.24, 165.85(COOH), 160.62, 154.58, 152.97(d, $J_{\text{C-F}}=254.5$ Hz), 147.47(d, $J_{\text{C-F}}=58.5$), 145.17, 145.01, 139.08, 134.39, 130.71, 129.24, 124.74, 122.60, 118.53(d, $J_{\text{C-F}}=8.3$ Hz), 111.85 (d, $J_{\text{C-F}}=23.2$ Hz), 106.70, 106.31(d, $J_{\text{C-F}}=4.5$ Hz), 60.24 (CH_2), 52.14 (2C), 49.30(2C), 35.78(CH-cyclopropyl), 7.51(2 $\text{CH}_2\text{-cyclopropyl}$) Anal. Calcd. For $\text{C}_{26}\text{H}_{23}\text{ClFN}_5\text{O}_4$: C, 59.60; H, 4.42; N, 13.37; Found: C, 59.76; H, 4.45; N, 13.29; ESI-MS m/z : 524.15 $[\text{M} + \text{H}]^+$.

7-(4-((7-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**5b**)

White solid; yield: 72%; mp: 293 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 15.07 (s, 1H, OH), 12.15 (s, 1H, NH), 8.64 (s, 1H, CH-N), 8.09 (d, $J=8.4$ Hz, 1H, Ar), 7.87 (d, $J=13.2$ Hz, 1H, Ar), 7.71 (d, $J=2.1$ Hz, 1H, Ar), 7.53 (td, $J=6.2, 3.0$ Hz, 2H, Ar), 3.79 (s, 1H, CH-cyclopropyl), 3.55 (s, 2H, CH_2), 3.35 (m, 4H, 2 $\text{CH}_2\text{-N}$), 2.74 (m, 4H, 2 $\text{CH}_2\text{-N}$), 1.29 (d, $J=6.8$ Hz, 2H, $\text{CH}_2\text{-cyclopropyl}$), 1.16 (d, $J=4.2$ Hz, 2H, $\text{CH}_2\text{-cyclopropyl}$). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 176.34, 165.89, 160.99, 155.73, 152.84 (d, $J_{\text{C-F}}=218.5$ Hz), 148.40 (d, $J_{\text{C-F}}=70.5$), 145.20, 144.98, 139.13, 138.94, 127.85, 126.77, 126.19, 120.18, 118.57(d, $J_{\text{C-F}}=9.7$ Hz), 111.17(d, $J_{\text{C-F}}=36.04$ Hz), 106.71, 106.54 (d, $J_{\text{C-F}}=4.3$ Hz), 60.21 (CH_2), 52.14 (2C), 49.31 (2C), 35.82 (CH-cyclopropyl), 7.53 (2 $\text{CH}_2\text{-cyclopropyl}$). Anal. Calcd. For $\text{C}_{26}\text{H}_{23}\text{ClFN}_5\text{O}_4$: C, 59.60; H, 4.42; N, 13.37; Found: C, 59.49; H, 4.39; N, 13.34; ESI-MS m/z : 524.15 $[\text{M} + \text{H}]^+$.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-((4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**5c**)

White solid; yield: 52%; mp: 251 °C; ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 15.13 (s, 1H, OH), 11.98 (s, 1H, NH), 8.59 (s, 1H, CH-N), 8.09 (d, $J=7.9$ Hz, 1H, Ar), 7.81–7.75 (m, 3H, Ar), 7.64 (d, $J=8.1$ Hz, 1H, Ar), 7.52–7.45 (m, 6.2 Hz, 2H, Ar), 3.77 (s, 1H, CH-cyclopropyl), 3.55 (s, 2H, CH_2), 2.76–2.71 (m, 4H, 2 $\text{CH}_2\text{-N}$), 3.35–3.38 (m, 4H, 2 $\text{CH}_2\text{-N}$), 1.30 (d, $J=6.3$ Hz, 2H, $\text{CH}_2\text{-cyclopropyl}$), 1.15 (s, 2H, $\text{CH}_2\text{-cyclopropyl}$). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$) δ : 176.20, 165.86 (COOH), 161.57, 153.99, 152.92 (d, $J_{\text{C-F}}=247.8$ Hz), 148.08 (d, $J_{\text{C-F}}=43.5$), 145.16, 145.02, 139.05, 134.32, 127.00, 126.46, 125.74, 121.31, 118.49 (d, $J_{\text{C-F}}=7.5$ Hz), 110.85 (d, $J_{\text{C-F}}=23.2$ Hz), 106.69, 106.22 (d, $J_{\text{C-F}}$

$F = 5.2$ Hz), 60.30 (CH₂), 52.54 (2C), 49.02 (2C), 36.50 (CH-cyclopropyl), 7.53 (2 H₂-cyclopropyl). Anal. Calcd. For C₂₆H₂₄FN₅O₄: C, 63.80; H, 4.94; N, 14.31; Found: C, 63.96; H, 4.91; N, 14.26; ESI-MS m/z : 490.18 [M + H]⁺.

1-cyclopropyl-6-fluoro-7-(4-((6-methoxy-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5d)

White solid; yield: 36%; mp: 215 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 14.88 (s, 1H, OH), 11.96 (s, 1H, NH), 8.61 (s, 1H, CH-N), 7.82 (d, $J = 13.3$ Hz, 1H, Ar), 7.59 (d, $J = 9.0$ Hz, 1H, Ar), 7.52–7.47 (m, 2H, Ar), 7.40–7.36 (m, 1H, Ar), 3.85 (s, 3H, OCH₃), 3.79 (s, 1H, CH-cyclopropyl), 3.61 (s, 2H, CH₂), 3.52 (s, 4H, 2 CH₂-N), 2.73 (s, 4H, 2 CH₂-N), 1.29 (d, $J = 6.7$ Hz, 2H, CH₂-cyclopropyl), 1.15 (s, 2H, CH₂-cyclopropyl). ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 176.25, 165.91 (COOH), 161.39, 157.59, 152.92 (d, $J_{C-F} = 253.1$ Hz), 151.60, 148.14 (d, $J_{C-F} = 29.3$), 145.13, 142.82, 139.06, 128.69, 123.97, 123.70, 122.11, 118.54 (d, $J_{C-F} = 4.5$ Hz), 110.87 (d, $J_{C-F} = 23.3$ Hz), 106.29, 105.82 (d, $J_{C-F} = 4.2$ Hz), 60.23 (CH₂), 55.58 (OCH₃), 52.17 (2C), 49.36 (2C), 36.78 (CH-cyclopropyl), 7.53 (2 CH₂-cyclopropyl). Anal. Calcd. For C₂₇H₂₆FN₅O₅: C, 62.42; H, 5.04; N, 13.48; Found: C, 62.25; H, 4.99; N, 13.51; ESI-MS m/z : 520.20 [M + H]⁺.

7-(4-((6-bromo-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5e)

White solid; yield: 48%; mp: 297 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 15.16 (s, 1H, OH), 12.20 (s, 1H, NH), 8.62 (s, 1H, CH-N), 8.16 (s, 1H, Ar), 7.89 (dd, $J = 24.9, 10.8$ Hz, 2H, Ar), 7.58 (t, $J = 12.5$ Hz, 2H, Ar), 3.77 (s, 1H, CH-cyclopropyl), 3.55 (s, 2H, CH₂), 2.72 (s, 4H, 2 CH₂-N), 2.46 (s, 4H, 2 CH₂-N), 1.30 (s, 2H, CH₂-cyclopropyl), 1.16 (s, 2H, CH₂-cyclopropyl). ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 176.27, 165.89 (COOH), 160.50, 154.81, 152.71 (d, $J_{C-F} = 209.53$ Hz), 147.64 (d, $J_{C-F} = 41.3$), 145.18, 144.95, 139.12, 137.17, 129.44, 127.89, 123.00, 121.98, 118.79 (d, $J_{C-F} = 14.3$ Hz), 110.89 (d, $J_{C-F} = 22.5$ Hz), 106.70, 106.34 (d, $J_{C-F} = 5.1$ Hz), 60.28 (CH₂), 52.16 (2C), 49.31 (2C), 35.44 (CH-cyclopropyl), 7.54 (2CH₂-cyclopropyl). Anal. Calcd. For C₂₆H₂₃BrFN₅O₄: C, 54.94; H, 4.08; N, 12.32; Found: C, 55.02; H, 4.07; N, 12.29; ESI-MS m/z : 568.10 [M + H]⁺.

General procedure for the synthesis of compounds 5f-j

All the compounds **5f-j** were prepared according to the procedure described for the synthesis of compounds **5a-e**, with the exception of using sarafloxacin instead of ciprofloxacin.

7-(4-((6-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5f)

White solid; yield: 60%; mp: 275 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 15.10 (s, 1H, OH), 12.08 (s, 1H, NH), 8.63 (s, 1H, CH-N), 8.05–7.91 (m, 2H, Ar), 7.79 (dd, $J = 12.0, 8.7$ Hz, 3H, Ar), 7.65 (d, $J = 8.6$ Hz, 1H, Ar), 7.51 (t, $J = 8.5$ Hz, 2H, Ar), 6.37 (d, $J = 7.2$ Hz, 1H, Ar), 3.48 (s, 2H, CH₂), 3.11–3.02 (m, 4H, 2CH₂-N), 2.64 (d, $J = 4.8$ Hz, 4H, 2CH₂-N). ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 176.64, 165.65 (COOH), 162.48 (d, $J_{C-F} = 249.33$ Hz), 160.57, 154.63, 152.92 (d, $J_{C-F} = 247.8$ Hz), 147.51 (d, $J_{C-F} = 67.5$), 145.22, 145.14, 139.19, 136.13, 134.40, 130.69, 129.89, 129.51 (d, $J_{C-F} = 31.5, 2C$), 124.73, 122.64, 118.66 (d, $J_{C-F} = 9.3$ Hz), 117.22 (d, $J_{C-F} = 23.2$ Hz), 110.98 (d, $J_{C-F} = 23.2$ Hz), 107.33, 106.47 (d, $J_{C-F} = 5.3$ Hz), 60.13 (CH₂), 51.98 (2C), 49.03 (2C). Anal. Calcd. For C₂₉H₂₂ClF₂N₅O₄: C, 60.26; H, 3.84; N, 12.12; Found: C, 60.18; H, 3.86; N, 12.17; ESI-MS m/z : 578.14 [M + H]⁺.

7-(4-((7-chloro-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5g)

White solid; yield: 69%; mp: 274 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 15.07 (s, 1H, OH), 12.07 (s, 1H, NH), 8.62 (s, 1H, Ar), 8.07 (d, $J = 8.5$ Hz, 1H, Ar), 7.95 (d, $J = 13.1$ Hz, 1H, Ar), 7.77 (dd, $J = 8.8, 4.8$ Hz, 2H, Ar), 7.67 (d, $J = 2.0$ Hz, 1H, Ar), 7.51 (dd, $J = 8.7, 5.4$ Hz, 3H, Ar), 6.36 (d, $J = 7.2$ Hz, 1H, Ar), 3.48 (s, 2H, CH₂), 3.06 (d, $J = 5.0$ Hz, 4H, 2CH₂-N), 2.64 (d, $J = 4.3$ Hz, 4H, 2CH₂-N). ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 176.69, 165.71 (COOH), 162.315 (d, $J_{C-F} = 269.6$ Hz), 160.96, 155.70, 152.76 (d, $J_{C-F} = 232.8$ Hz), 149.06 (d, $J_{C-F} = 63.8$ Hz), 145.45, 145.28, 139.16, 138.88, 136.25, 129.82 (d, $J_{C-F} = 8.2$), 127.82, 126.71, 126.14, 120.20, 118.63 (d, $J_{C-F} = 7.3$ Hz), 117.23 (d, $J_{C-F} = 22.53$ Hz, 2C), 111.14 (d, $J_{C-F} = 27.8$ Hz), 107.13, 106.47 (d, $J_{C-F} = 6.2$ Hz), 60.10 (CH₂), 51.97 (2C), 48.99 (2C). Anal. Calcd. For C₂₉H₂₂ClF₂N₅O₄: C, 60.26; H, 3.84; N, 12.12; Found: C, 60.19; H, 3.82; N, 12.15; ESI-MS m/z : 578.14 [M + H]⁺.

6-fluoro-1-(4-fluorophenyl)-4-oxo-7-(4-((4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (5h)

White solid; yield: 43%; mp: 290 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ : 11.88 (s, 1H, OH), 8.63 (s, 1H, NH), 8.08 (dd, $J = 7.9, 1.5$ Hz, 1H, Ar), 7.96 (d, $J = 13.2$ Hz, 1H, Ar), 7.81–7.75 (m, 3H, Ar), 7.62 (d, $J = 8.1$ Hz, 1H, Ar), 7.50 (q, $J = 7.9, 7.2$ Hz, 4H, Ar), 6.37 (d, $J = 7.2$ Hz, 1H, Ar), 3.48 (s, 2H, CH₂), 3.06 (d, $J = 5.0$ Hz, 4H, 2CH₂-N), 2.64 (d, $J = 4.9$ Hz, 4H, 2CH₂-N). ¹³C NMR (75 MHz, DMSO-*d*₆) δ : 176.70,

165.73 (COOH), 162.98 (d, J_{C-F} = 174.9 Hz), 160.82, 153.97, 152.77 (d, J_{C-F} = 252.3 Hz), 148.10 (d, J_{C-F} = 85.6), 145.35, 145.04, 139.22, 136.14, 134.32, 129.84 (d, J_{C-F} = 9.1, 2C), 126.98, 126.46, 125.74, 121.34, 118.53 (d, J_{C-F} = 5.6 Hz), 117.24 (d, J_{C-F} = 23.2 Hz, 2C), 111.05 (d, J_{C-F} = 21.7 Hz), 107.36, 106.52 (d, J_{C-F} = 9.1 Hz), 60.20 (CH₂), 52.01 (2C), 49.08 (2C). Anal. Calcd. For C₂₉H₂₃F₂N₅O₄: C, 64.08; H, 4.27; N, 12.89; Found: C, 64.15; H, 4.29; N, 12.91; ESI-MS m/z : 544.18 [M + H]⁺.

6-fluoro-1-(4-fluorophenyl)-7-(4-((6-methoxy-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5i)

White solid; yield: 41%; mp: 262 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 15.08 (s, 1H, OH), 11.84 (s, 1H, NH), 8.62 (s, 1H, Ar), 7.96 (d, J = 13.2 Hz, 1H, Ar), 7.77 (dd, J = 8.8, 4.8 Hz, 2H, Ar), 7.59–7.46 (m, 4H, Ar), 7.38 (dd, J = 8.9, 3.0 Hz, 1H, Ar), 6.37 (d, J = 7.3 Hz, 1H, Ar), 3.85 (s, 3H, OCH₃), 3.45 (s, 2H, CH₂), 3.07 (t, J = 5.0 Hz, 4H, 2CH₂-N), 2.62 (t, J = 4.9 Hz, 4H, 2CH₂-N); ¹³C NMR (75 MHz, DMSO-*d*₆) δ: 176.66, 165.71 (COOH), 162.71 (d, J_{C-F} = 208.7 Hz), 160.82, 157.57, 153.00 (d, J_{C-F} = 262.1 Hz), 151.55, 148.31 (d, J_{C-F} = 51.1), 145.30, 142.79, 139.18, 136.17, 129.82 (d, J_{C-F} = 9.1 Hz, 2C), 128.67, 124.06, 123.67, 122.12, 118.61 (d, J_{C-F} = 6.3 Hz), 117.22 (d, J_{C-F} = 23.3 Hz, 2C), 110.98 (d, J_{C-F} = 23.2 Hz), 107.35, 105.81 (d, J_{C-F} = 5.5 Hz), 60.10 (CH₂), 55.57 (OCH₃), 51.98 (2C), 49.05 (2C). Anal. Calcd. For C₃₀H₂₅F₂N₅O₅: C, 62.82; H, 4.39; N, 12.21; Found: C, 62.76; H, 4.38; N, 12.19; ESI-MS m/z : 574.19 [M + H]⁺.

7-(4-((6-bromo-4-oxo-3,4-dihydroquinazolin-2-yl)methyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (5j)

White solid; yield: 42%; mp: 298 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 15.07 (s, 1H, OH), 12.08 (s, 1H, NH), 8.60 (s, 1H, Ar), 8.13 (s, 1H, Ar), 7.94–7.87 (m, 2H, Ar), 7.75 (s, 2H, Ar), 7.56–7.47 (m, 3H, Ar), 6.34 (d, J = 6.9 Hz, 1H, Ar), 3.47 (s, 2H, CH₂), 3.05 (s, 4H, 2CH₂-N), 2.63 (s, 4H, 2CH₂-N). ¹³C NMR (75 MHz, DMSO-*d*₆) δ: 176.55, 165.82 (COOH), 162.78 (d, J_{C-F} = 199.7 Hz), 160.52, 154.84, 152.78 (d, J_{C-F} = 243.3 Hz), 147.94 (d, J_{C-F} = 91.6 Hz), 145.66, 145.21, 139.15, 137.06, 136.43, 129.74 (d, J_{C-F} = 10.1, 2C), 129.36, 127.85, 122.99, 121.15, 118.79 (d, J_{C-F} = 5.3 Hz), 117.25 (d, J_{C-F} = 22.5 Hz, 2C), 111.5 (d, J_{C-F} = 24.7 Hz), 106.71, 106.41 (d, J_{C-F} = 8.2 Hz), 60.17 (CH₂), 52.00 (2C), 49.04 (2C). Anal. Calcd. For C₂₉H₂₂BrF₂N₅O₄: C, 55.96; H, 3.56; N, 11.25; Found: C, 55.89; H, 3.57; N, 11.28; ESI-MS m/z : 622.09 [M + H]⁺.

Antibacterial assays

A panel of selected standard bacterial strains, including gram-positive (MRSA ATCC 12493, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) and gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 11706, *Escherichia coli* ATCC 25922) were utilized to evaluate the antibacterial activities of synthesized compounds **5a-j** using three methods, including broth microdilution, well diffusion and disc diffusion assays.

Broth microdilution

In order to determine the MIC of compounds **5a-j**, broth microdilution assay was performed as described previously [22]. Briefly, the starting concentrations of compounds were 100 µg/mL and they were prepared by dissolving the compounds in DMSO (1 mL), dilution with water (9 mL). The turbidity of the bacterial solution was adjusted by using McFarland as 0.5 which corresponded 10⁷ CFU/mL and then, two-fold dilution was made. After adding bacterial solutions (1–5 × 10⁵ CFU/mL) into the 96-well plate, the plate incubated at 35–37 °C aerobically. After 18 h, MICs were characterized as the lowest concentration of an agent which can prevent the visible growth on the plate.

Well diffusion assay

Antibacterial activities of compounds **5a-j** were assessed using well diffusion assay on MHA as described previously [40]. Preparing the bacterial suspension was done by adjusting the turbidity of the solution as 0.5 McFarland. After the inoculation of bacterial strains on MHA agar plates, 50 µl of the targeted compound solutions poured into the wells (diameter = 6 mm) and incubated at 37 °C. After 24 h, the growth inhibition zones were determined in diameter. The zones of inhibition were given in millimetres (mm).

Disk diffusion assay

Agar disk-diffusion assay was performed on MHA, as explained previously [40]. In summary, each bacterium was cultured in MHA and incubated at 37 °C. After 24 h, in order to prepare a suspension of 10⁵ CFU/mL, the bacteria were suspended in saline solution, in accordance with the McFarland protocol (0.5 McFarland). Fresh stock solutions of compounds **5a-j** were prepared in DMSO. Then, the different concentrations were produced by dilution of the stock solution of each test compound. The discs (6.0 mm diameter) were injected with the different concentrations of the test compound and inoculated on the MHA. After incubation of the plates at 37 °C for 18 h, the antibacterial activity of each

compound was characterized by the formation of an inhibitory zone, which reported in mm.

Molecular docking study

The crystal structure of topoisomerase II DNA gyrase (PDB ID: 2XCT) was taken from PDB database and the compounds (**5d** and ciprofloxacin) were docked into the active site by Autodock 4.2.1 software. Further optimizations were carried out for ligands and protein. MarvinSketch 15.8.1 software was used for ligand preparation, including hydrogen addition and energy minimization. Following, the protein was prepared through the addition of partial charges and removal of extra ligands in Autodock software. The grid box was defined at a distance 10 Å from the original ligand centroid and 30 conformations were generated for ligands. Finally, the predicted binding modes were analyzed by Discovery Studio visualizer 4.5.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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