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ORIGINAL ARTICLE

Novel fluoroquinolones analogues bearing 4-(arylcarbamoyl)benzyl: design, synthesis, and antibacterial evaluation

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Abstract

Bacterial resistance to fluoroquinolone has been increasing at an alarming rate worldwide. In an attempt to find more potent anti-bacterial agents, an efficient, straightforward protocol was performed to obtain a large substrate scope of novel ciprofloxacin and sarafloxacin analogues conjugated with 4-(arylcarbamoyl)benzyl **7a–ab**. All prepared compounds were evaluated for their anti-bacterial activities against three gram-positive strains (Methicillin resistant *staphylococcus aureus* (MRSA), *Staphylococcus aureus*, and *Enterococcus faecalis*) as well as three gram-negative strains (*Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Escherichia coli*) through three standard methods including broth microdilution, agar-disc diffusion, and agar-well diffusion assays. Most of the compounds exhibited great to excellent anti-bacterial potencies against MRSA and *S. aureus*. Among the targeted compounds, derivative **7n** exhibited great antibacterial potency, which was noticeably more potent than parent ciprofloxacin. Subsequently, a molecular docking study was performed for this compound to find out its probable binding mode with the active site of *S. aureus* DNA gyrase (PDB ID: 2XCT).

Keywords Fluoroquinolones (FQs) · 4-(Arylcarbamoyl)benzyl · Antibacterial activity · Antibiotic resistance

Introduction

Nowadays, bacterial resistance originated from the excessive use of antibiotics has become a concerning issue all over the world, therefore, infectious diseases caused by multidrug resistant pathogens have been the noticeable threat leading to death over recent decades [1, 2]. Among them, the rate of fatalities owing to the methicillin-resistant *Staphylococcus aureus* (MRSA) has been remarkably more than other antibiotic-resistant pathogens [3]. Considering the difficulty of the treatment of MRSA infections, various antibiotics namely *β*-lactams [2], macrolides [4], glycopeptides [5], oxazolidinones [6], quinolones [5, 7, 8], and vancomycin have been frequently used. Quinolones are a group of antibiotics which tend to be prescribed for the treatment of a variety of bacterial infections such as hospital-acquired infections or other resistant pathogens including urinary tract infection, sexually transmitted disease, as well as gastrointestinal and abdominal infections [9, 10].

Since then, quinolones have become one of the most widely prescribed antibiotics. Their success might be contributed to their good bioavailability, low toxicity, favorable

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pharmacokinetics, enhanced tissue permeation, and good tolerability. However, the outbreak of quinolone-resistant and multi-drug-resistant bacteria have questioned the clinical use and efficacy of quinolone-based antibiotics [11, 12]. Therefore, discovery and development of new antimicrobial chemotherapeutic agents are highly demanding. Quinolones block DNA synthesis in bacteria through inhibition of two type-II bacterial topoisomerase enzymes named topoisomerase IV and DNA Gyrase, resulting into DNA synthesis and subsequently, the cell death. Topoisomerase-II plays a regulatory role on the topology and conformation of DNA during replication, transcription, and recombination of the strands of DNA in bacteria. Structurally, the role of quinolones to interfere with DNA synthesis in bacteria are attributed to their ability in formation of the hydrogen bonding interactions with single strand of DNA. As a result, this functionality is highly optimal and necessary for the antimicrobial activities of quinolones [13, 14].

In the quest for more effective quinolones, a fluorine atom has been introduced at the C-6 position, resulting into a remarkable breakthrough in the emergence and development of fluoroquinolones (FQ) which exhibit great effect on a wide spectrum of bacteria ranging from gram negative to positive bacteria. Structure-activity relationship studies of quinolones have demonstrated that the presence of fluorine atom improves metabolic characteristics through enhancing absorption and removing half-life of the quinolones [15]. Further ubiquitous and rational modifications leading to more effective fluoroquinolone analogues have proceeded through providing substituent at C-7 position, since studies have revealed that several properties including cell permeability, antibacterial spectrum, potency, safety and pharmacokinetics of fluoroquinolones are affected by C-7 side chains. Generally, 5 and 6-membered cyclic amines at the C-7 position are deemed as one of the most optimal substituents. Therefore, piperazin-1-yl moiety has been introduced at this position [16].

Overall, the presence of a fluorine atom at C-6 position and a piperazin-1-yl moiety at C-7 position of quinolone core structure have been remained, leading to find numerous potent drugs including pefloxacin, lomefloxacin, enrofloxacin, ofloxacin, levofloxacin, gatifloxacin, ciprofloxacin, and sarafloxacin, to name but a few [12, 17, 18]. The constructive role of piperazinyl are attributed to the transport of the fluoroquinolones into the bacteria and the inhibition of the target enzyme, type-II bacterial topoisomerase [19]. To afford more potent antimicrobial fluoroquinolone-based chemotherapeutic agents, numerous substituents conjugated with 7-piperazinyl moiety which were able to link with the special bulky substituents have been widely investigated [20–36].

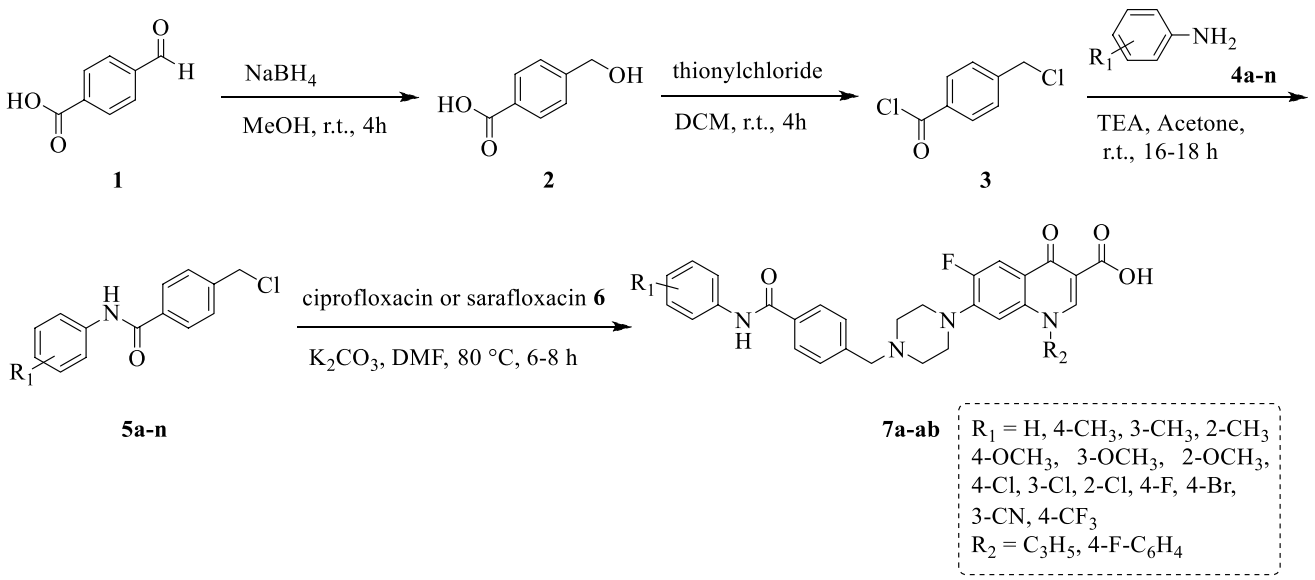
According to the literature, there are several reasons to provide substituents on the 7-piperazinyl moiety. For example, the alteration of the serine amino acid and disruption of formed ternary complex within the performance of DNA gyrase cause bacterial resistance to fluoroquinolones [18, 37]. Making extra interaction with the named enzyme is one strategy to remove this resistance. Introducing substituents on the 7-piperazinyl led to obtain more potent compounds with great activities against broader spectrum of bacteria due to their abilities to make this noticeable extra-interaction [25, 38]. Moreover, structure activity relationship studies of quinolones revealed that unsubstituted 7-piperazine moiety induces convulsion [39]. Additionally, the presence of a diamine group in the piperazinyl moiety predisposes fluorquinolones to bacterial GSH-mediated modification and activity reduction [40]. Finally, acetylation of the unsubstituted nitrogen from 7-piperazinyl moiety is another mechanism of bacterial resistance to fluorquinolones; therefore, introducing substituents on this functionality resulted in find compounds with better efficacy against resistant species [12].

As a part of our long-term effort to find new, potential *N*-substituted piperazinyl fluoroquinolones [20, 21, 41, 42], herein, we present an efficient and straightforward synthetic route, antibacterial activity evaluation, and docking study to afford novel ciprofloxacin and sarafloxacin bearing various substituted 4-(arylcarbamoyl)benzyl **7a–ab** moieties.

Results and discussion

Chemistry

The synthetic approach toward the desired fluoroquinolones including ciprofloxacin and sarafloxacin bearing different substituted 4-(arylcarbamoyl)benzyl **7a–ab** is outlined in Scheme 1. This efficient protocol was initiated through reducing 4-formylbenzoic acid **1** using sodium tetraborohydride (NaBH4) in MeOH at ambient temperature to give 4-(hydroxymethyl)benzoic acid 2. Subsequently, this moiety underwent the chlorination with thionyl chloride ($SOCl_2$) in DCM under the reflux conditions to afford 4-(chloromethyl) benzoyl chloride **3**. This adduct went through the amidation with various substituted anilines **4a–n** using TEA in acetone at the ambient temperature to afford corresponded 4-(chloromethyl)-*N*-arylbenzamide **5a–n**. The structures of the isolated products were deduced on the basis of their ^{1}H, and ^{13}C NMR spectroscopy. Finally, the chloromethyl moiety underwent through the nucleophilic substitution with ciprofloxacin or sarafloxacin **6a,b** in the presence of K_2CO_3 in DMF at 80 °C to obtain target compounds **7a–ab**. The structures of these products were characterized on the basis of their IR, ^{1}H and ^{13}C NMR, as well as their elemental analyses and MASS spectroscopy. Partial assignments of these resonances are given in the Experimental Part.



Scheme 1 Synthetic pathway for novel derivatives of ciprofloxacin and sarafloxacin bearing 4-(arylcarbamoyl)benzyl **7a–ab**

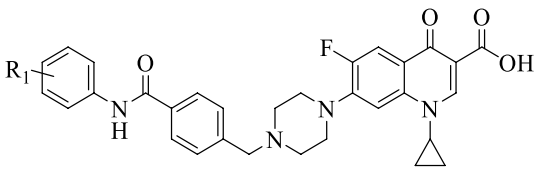
Antibacterial activity

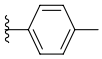
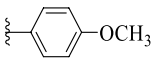
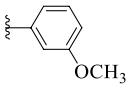
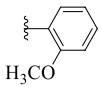
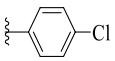
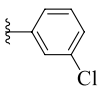
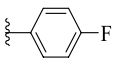
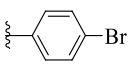
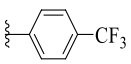
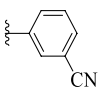
The figures in the antibacterial evaluation are reported as minimal inhibitory concentration (MIC, μM) values, showing the lowest concentration of each compound which inhibits visible growth of a bacterial culture under a defined set of experimental conditions. There are several routine methods used in many clinical microbiology laboratories for antimicrobial susceptibility testing to measure MIC values of antibiotic agents. In present study, our novel substituted ciprofloxacin and sarafloxacin derivatives **7a–ab** were evaluated for their *in vitro* antibacterial activities through three standard methods including broth microdilution, agar-disc diffusion, and agar-well diffusion assays. Three gram-positive strains (*Methicillin resistant staphylococcus aureus* (*MRSA*), *Staphylococcus aureus,* and *Enterococcus faecalis*) as well as three gram-negative strains (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*) were selected to evaluate the activities of our compounds against them.

Among various methods used to evaluate the antibacterial potencies, the most common and reliable one is "broth microdilution". Considering its accuracy and clear results, this approach is the most valuable method used in clinical microbiology laboratories worldwide. Through this method, wells are filled with broth containing different concentrations of the antibiotic. Afterwards, they are inoculated with bacteria and incubated overnight. The next day, the figures for MIC could be measured [43]. To determine the antibacterial potencies of our fluoroquinolone analogues conjugated with 4-(arylcarbamoyl)benzyl **7a–ab** through broth microdilution methods, various substituents either electron-donating group (EDG) or electron-withdrawing group (EWG) are provided on the 4-(arylcarbamoyl)benzyl in an effort to perform a comprehensive investigation about the role of this moiety on the anti-bacterial potencies of our fluoroquinolone analogues **7a–ab**. In an attempt to provide better description of results and compare with parent drugs, compounds are divided into two categories: ciprofloxacin-derivative series **7a–n** (Table 1) and sarafloxacin-derivative series **7o–ab** (Table 2).

Regarding the anti-bacterial activities of the first series against gram-positive strains, there are almost similar trends about the MIC values against MRSA and *S. aureus*, while it seems different for *E. faecalis*. As it can be seen, all the synthesized compounds **7a–n** showed good to excellent inhibitory activities with MIC values of 0.2 nM to 0.976 μM against MRSA and *S. aureus* in comparison with the parent drug ($MIC_{MRSA} = 0.488$ μM and $MIC_{S.\ aureus} = 0.976$ μM). To explain the structure and observed activity correlations against MRSA and *S. aureus*, the un-substituted 4-(phenylcarbamoyl)benzyl **7a** showed the MIC values of 0.244 μM. Introducing a EDG including methyl and methoxy at C-4 position (compounds **7b** and **7e**) caused a decrease in antibacterial activity, whereas replacing these groups at C-3 position (compounds **7c** and **7f**) or C-2 position (compounds **7d** and **7g**) improved the potencies greatly. Moreover, it was found that introduction of an EWG like chlorine, fluorine, bromine, and trifluoromethyl at C-4 position (compounds **7h**, **7k**, **7l**, and **7m**, respectively) resulted into different antibacterial potencies. Although 4-Cl had a considerable deterioration in activity (**7h**, MIC values of 0.976 μM), other groups improved the potency remarkably. Providing a EWG at C-3 and C-2 positions (compounds **7i**, **7j**, and **7n**) led to the significant increase in anti-bacterial activity. Therefore,

Table 1 Broth microdilution assay results of compounds **7a–n** and ciprofloxacin: minimal inhibitory concentration (MIC, μM) and solubility

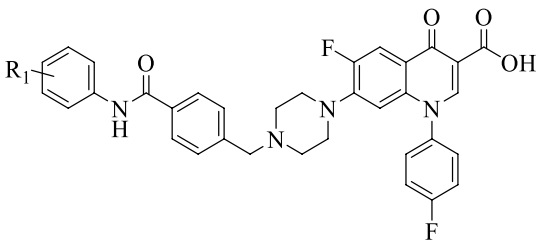


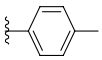
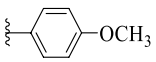
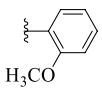
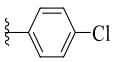
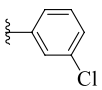
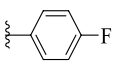
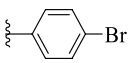
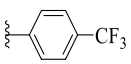
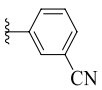
Compound	Ar	MRSA	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*	Solubility
7a		0.244	0.244	15.625	0.976	31.250	0.244	NCS [a]
7b		0.244	0.488	62.500	7.812	125	0.244	CS [b]
7c		0.122	0.122	0.488	1.953	125	0.122	NCS
7d		0.030	0.030	125	31.250	500	0.488	CS
7e		0.488	0.488	1.953	3.906	250	0.244	CS
7f		0.061	0.061	0.122	3.906	500	0.244	CS
7g		0.030	0.030	0.122	7.812	500	0.244	NCS
7h		0.976	0.976	125	62.500	500	3.906	CS
7i		0.030	0.030	62.500	125	125	0.488	CS
7j		0.061	0.061	62.500	1.953	62.500	0.122	CS
7k		0.061	0.122	0.976	31.250	500	1.953	CS
7l		0.030	0.030	62.500	3.906	250	0.122	CS
7m		0.030	0.030	0.976	15.625	250	0.244	CS
7n		0.0002	0.0002	0.244	3.906	250	0.122	CS
Ciprofloxacin	-	0.488	0.976	0.0038	0.030	3.906	0.0038	CS

[a]NCS: 80% soluble

[b]CS: completely soluble

Table 2 Broth microdilution assay results of compounds **7o–ab** and sarafloxacin: minimal inhibitory concentration (MIC, μM) and solubility



Compound	Ar	MRSA	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*	Solubility
7o		0.488	0.488	31.250	7.812	125	0.976	CS [a]
7p		0.488	0.488	15.625	31.250	250	1.953	NCS [b]
7q		0.030	0.030	1.953	62.500	500	3.906	CS
7r		0.030	0.030	3.906	62.500	500	7.812	CS
7s		15.625	15.625	15.625	31.250	500	31.250	CS
7t		0.061	0.122	1.953	15.625	500	0.976	CS
7u		0.244	0.244	1.953	125	500	7.812	CS
7v		0.122	0.244	62.500	62.500	500	7.812	CS
7w		0.122	0.122	31.250	15.625	125	1.953	CS
7x		0.122	0.122	31.250	31.250	500	3.906	NCS
7y		0.061	0.061	0.976	62.250	250	3.906	CS
7z		0.244	0.244	0.488	15.625	500	0.976	CS
7aa		0.244	0.244	0.488	15.625	500	0.976	NCS
7ab		0.122	0.122	7.812	31.250	500	1.953	CS
Sarafloxacin	-	0.244	0.244	2	0.061	2	0.031	CS

[a]NCS: 80% soluble

[b]CS: completely soluble

the most potent compound in this series, **7n**, was found as a probable, efficient antibiotic candidate with MIC values of 0.2 nM which was 2440 and 4880 times more potent than ciprofloxacin against MRSA and *S. aureus*, respectively.

None of the compounds **7a–n** showed higher anti-bacterial activity than parent drug against *E. faecalis*. To provide a brief SAR description, the un-substituted 4-(phenylcarbamoyl)benzyl **7a** demonstrated the moderate MIC value of 15.625 μM. The presence of methoxy group as EDG at C-3 or C-2 positions of 4-(arylcarbamoyl)benzyl moiety (compounds **7d**, and **7g**) as well as the cyano group as EWG at C-3 position (compound **7n**) resulted into very good improvement on the observed activities.

In an attempt to provide a conclusion about the anti-bacterial activities of our ciprofloxacin analogues conjugated with 4-(arylcarbamoyl)benzyl **7a–n** against gram-positive strains, all derivatives other than compound **7h** demonstrated the very effective anti-bacterial activity against MRSA and *S. aureus*, since they were more potent than the parent drug. Moreover, the presence of methyl and methoxy as EDGs at C-2 position (compounds **7d** and **7g**) as well as the presence of cyano as EWG at C-3 position (compound **7n**) could considerably improve the potency. In spite of excellent activities against MRSA and *S. aureus*, the synthesized compounds had low to moderate potencies against *E. faecalis*. To begin with the compound **7a**, it demonstrated MIC value of 15.625 μM. Introducing methyl group at C-4 position of the phenyl ring caused a detrimental effect (compound **7b**). Although moving this group to C-3 improved the potency noticeably (compound **7c** with MIC value of 0.488 μM), moving to C-2 caused a weaker agent (compound **7d**). The presence of methoxy group at any position resulted to more potent antibiotic agents. For example, compounds **7f** and **7g** showed the MIC values of 0.122 μM which were the most potent derivatives in this strain. On the other hand, the presence of chlorine at any position of the phenyl ring (compounds **7h–j**) had detrimental effects. Considering the role of other EWGs on the phenyl ring, the 3-cyano groups was the most efficient one (compound **7n** with MIC value of 0.244 μM).

About the anti-bacterial activities of the first series against the gram-negative strains, all of the prepared ciprofloxacin analogues **7a–n** demonstrated weaker potency than comparing with ciprofloxacin. About the strains of *P. aeruginosa* and *K. pneumonia*, compound **7a** showed the MIC values of 0.976 μM and 31.25 μM, respectively. Introducing any groups whether EDGs or EWGs at any position of the phenyl ring on the 4-(arylcarbamoyl)benzyl moiety caused detrimental effects on the anti-bacterial potencies. About *E. coli*, the un-substituted 4-(phenylcarbamoyl)benzyl **7a** had MIC value of 0.244 μM, and introducing methyl or methoxy at C-4 position of phenyl did not alter the anti-bacterial potency. Although the movement of this group from C-4 to whether C-2 or C-3 (compounds **7f** and **7g**) did not make any change on the activity, the movement of methyl led to noticeable change. The presence of 3-methyl enhanced the anti-bacterial activity (compound **7c** with MIC value of 0.122 μM), while the presence of 2-methyl deteriorated the anti-bacterial potency (compound **7d** with MIC value of 0.488 μM). To complete this study, various EWGs were introduced at different positions of the phenyl ring (compounds **7h–n**), among which compounds **7j** (bearing 2-Cl), **7l** (bearing 4-Br), and **7n** (bearing 3-CN) showed higher anti-bacterial potency (MIC values were 0.122 μM). Overall, against the gram-negative strains, the best results belonged to compounds **7j**, **7l**, and **7n** with MIC values of 0.122 μM against *E. coli*.

It could be obtained the similar SAR analysis results about the second series of our sarafloxacin-based derivatives **7o–ab** against both gram-positive and gram-negative strains. For example, comparing with parent drug, sarafloxacin, all of the derivatives except compound **7o**, **7p**, and **7s** showed better anti-bacterial activities against both MRSA and *S. aureus* (the MIC values were from 0.030 μM to 0.488 μM). To begin the description of SAR, the un-substituted 4-(phenylcarbamoyl)benzyl **7o** had MIC values of 0.488 μM. Introducing a methyl group at C-4 position of phenyl (compound **7p**) did not change the activity, whereas the presence of this moiety at C-3 or C-2 position (compound **7q** and **7r**, respectively) resulted to excellent anti-bacterial potency (the MIC values were 0.030 μM). Introducing methoxy at C-4 position of phenyl (compound **7s**) caused a highly detrimental effects on the activity and the MIC values increased to 15.625 μM. However, the presence of this EDG at C-3 and C-2 positions (compound **7t** and **7u**, respectively) improved the results significantly. Additionally, the presence of EWGs at C-4 position (compounds **7v**, **7y**, **7z**, and **7aa**) led to noticeable anti-bacterial activity. Among them, **7z** bearing 4-F showed the best potency with MIC values of 0.061 μM. Compounds **7w**, **7x**, and **7ab** bearing EWGs at other positions resulted into the great activity with the same MIC values of 0.122 μM.

It was found that all of the derivatives except compound **7o**, **7p**, and **7s** were more potent anti-biotic agents than sarafloxacin against MRSA and *S. aureus*. In particular, compounds **7q** and **7r** with MIC values of 0.030 μM were the most potent sarafloxacin analogues which were 16.3 and 32.5 times more potent than the parent drug against the strains of MRSA and *S. aureus*, respectively. However, some of the compounds including **7q**, **7t**, **7u**, **7y**, **7z**, and **7aa** showed better anti-bacterial activity against *E. faecalis* in comparison with parent drug. About this strain, the un-substituted phenyl derivative **7o** demonstrated MIC value of 31.250 μM. Introducing a EDGs at various positions of phenyl (compounds **7p–u**) led to better activities. It could be concluded that the presence of EDGs at C-2 or C-3 position was very helpful for the activity, for example, the MIC values for compounds **7q**, **7t**, and **7u** decreased remarkably

to 1.953 µM. Moreover, the presence of chlorine atom at any position of the phenyl ring (compounds **7v–x**) caused a detrimental effect on the anti-bacterial activities; however, the presence of other EWGs improved the potency noticeably, for example, compounds **7z** and **7aa** showed the MIC values of 0.488 µM against *E. faecalis*.

Same as the previous series, none of the sarafloxacin derivatives **7o–ab** were potent anti-biotic agents than the parent drug against the gram-negative strains. Among these strains, our flouroquinlones **7o–ab** showed the worst activities against *K. pneumonia*, while their activities were low to moderate agents against *P. aeruginosa* and *E. coli*. In an attempt to provide a brief SAR description, the un-substituted 4-(phenylcarbamoyl)benzyl **7o** exhibited the moderate MIC value of 7.812 µM against *P. aeruginosa*. Introducing any group whether EDGs or EWGs caused significant decrease for anti-bacterial activity. Moreover, the MIC value of compound **7o** was 0.976 M, among various modifications on the phenyl ring, only the presence of 3-OCH_3 (compound **7t**), 4-Br (compound **7z**), and 4-CF_3 (compound **7aa**) could retain the anti-bacterial activities against *E. coli*, while other attempts caused the potencies to decrease.

As it was described, in both ciprofloxacin-derivative series **7a–n** (Table 1) and sarafloxacin-derivative series **7o–ab** (Table 2), most of the derivatives other than **7h**, **7o**, **7p**, and **7s** were more potent than the corresponding standard drugs against MRSA and *S. aureus*, while none of them showed the better potencies comparing with positive controls against *P. aeruginosa*, *K. pneumonia*, and *E. coli*. Among the fluoroquinolone analogues conjugated with 4-(arylcarbamoyl)benzyl **7a–ab**, **7n** from first series as well as **7q** and **7r** from the second series exhibited the best anti-bacterial activities. Moreover, their aqueous solubility was high. Since, this is the most important pharmacokinetic property of fluoroquinolones, it might be possible to consider these compounds as the potential antibiotic candidates.

Additional confirmatory methods used to evaluate the anti-bacterial potency of our desired compounds against three gram-positive strains and three gram-negative strains were disk diffusion test or Kirby–Bauer test (which was performed on Mueller Hinton Agar (MHA)) and agar well diffusion. In the agar-disk diffusion test, also known as "Kirby-Bauer method", there is a zone of inhibition to measure the antibiotic potency of compounds. To determine this zone, initially, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, small filter paper disks containing antibiotic are placed on the agar surface, and the obtained plate is incubated. Finally, the zone of inhibition around each disk is determined. In the similar method, agar-well diffusion, the agar plate surface is inoculated by spreading a volume of the microbial inoculum. Subsequently, several holes with a diameter of 6 to 8 mm are punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent at different concentration are introduced into the well. Afterwards, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the tested microbial strain [43].

The inhibitory zone of the compounds **7a–ab** as diameter in mm were summarized in Table 3 and 4. There is a great agreement between the results of broth microdilution with disk diffusion and well diffusion assays, particularly against MRSA and *S. aureus*. For example, all compounds other than **7h** and **7s** showed comparable or even better activity in comparison with the parent drugs against MRSA and *S. aureus*.

Based upon the disc diffusion assay results (Table 3), compound **7n** from first series had a remarkable activity with inhibition zone of 32 and 28 mm. Additionally, the best results of second series were observed for compounds **7q** and **7r**, since the figures were 29 and 23 mm for **7q** as well as 31 and 25 mm for **7r**, which were better figures than those of the sarafloxacin.

According to the well diffusion assay results (Table 4), there is a similar trend, particularly against the strains of MRSA and *S. aureus*. Among the ciprofloxacin-based derivatives, compound **7n** exhibited the inhibitory zone of 38 and 33 against MRSA and *S. aureus*, respectively, which was more than that of ciprofloxacin. Among the sarafloxacin-based derivatives, the highest zone of inhibition belonged to compounds **7q** and **7r**, confirming their excellent potencies in comparison with sarafloxacin.

Molecular docking

In order to gain more insight into the binding mode of the most promising compound **7n** to the target protein, a molecular docking study was conducted using the crystal structure of a complex of DNA gyrase with ciprofloxacin (PDB ID: 2XCT). For validating the docking procedure, ciprofloxacin was redocked into the crystal structure of DNA gyrase. Ciprofloxacin's binding mode is similar to that of its co-crystallized ligand in X-ray structures, showing free binding energy of -8.29 kcal/mol. Ciprofloxacin's hydroxyl group participates in hydrogen bonding interactions with Ser1084 and the piperazine moiety interacts with Arg458 to stabilize the molecule. A metal carboxylate complex with manganese ion (Mn^{2+}) was also observed, which increased ligand binding affinity (Fig. 1A).

The docking results as shown in figure 1 and 2 revealed that there were similar binding modes for ciprofloxacin and compound **7n** in the active site and the same coordination toward Mn^{+2} metal ions (figure 2). Compound **7n** displayed important interactions with DNA gyrase with an affinity value of -7.96 kcal/mol (figure 1B). A hydrogen bond was

Table 3 Disc diffusion assay results for compounds **7a–ab**, ciprofloxacin, and sarafloxacin

Compound	MRSA	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*
7a	14	14	0	12	0	28
7b	16	14	0	0	0	20
7c	20	16	0	0	0	18
7d	18	15	0	0	0	19
7e	12	19	0	0	0	13
7f	18	16	0	0	0	14
7g	21	21	0	0	0	12
7h	10	9	0	0	0	7
7i	17	18	0	0	0	12
7j	19	15	0	0	0	20
7k	22	15	0	0	0	12
7l	18	18	0	0	0	13
7m	20	15	0	0	0	13
7n	32	28	0	0	0	18
Ciprofloxacin	22	22	10	26	12	33
7o	13	14	0	0	0	14
7p	13	15	0	0	0	12
7q	29	23	0	0	0	13
7r	31	25	0	0	0	12
7s	0	0	0	0	0	0
7t	14	14	0	0	0	9
7u	12	13	0	0	0	0
7v	12	13	0	0	0	0
7w	17	13	0	0	0	10
7x	14	12	0	0	0	11
7y	17	16	0	0	0	12
7z	14	16	0	0	0	13
7aa	18	14	0	0	0	17
7ab	17	17	0	0	0	16
Sarafloxacin	25	21	5	28	8	30

[a]The tested concentrations were 64 µg/ml.

[b]The reported zone of inhibition is in mm.

formed between the carboxyl moiety of this compound and Ser 1084. The two phenyl rings of C-7 piperazine substituent were stabilized by cation-π and alkyl-π interactions with Arg458. Moreover, additional hydrogen bond interaction was formed between the amide moiety of this ligand and Asp 437. Interestingly, in spite of the elongation of this structure, the flexible methylene bridge created a U-shaped conformation that allowed the scaffold to be correctly positioned within the active area.

Conclusion

In conclusion, we have represented a study about the novel fluoroquinolone analogues conjugated with 4-(arylcarbamoyl)benzyl **7a–ab** as potential anti-bacterial agents. Using an efficient, simple protocol from readily available starting materials and easy work-up without any need for chromatography purification processes led us to afford the targeted compounds in desired yields. Afterwards, the potencies of compounds were investigated through three reliable methods including broth microdilution, agar-disc diffusion, and agar-well diffusion assays against three gram-positive strains named MRSA, *S. aureus*, and *E. faecalis* as well as three gram-negative strains named *P. aeruginosa*, *K. pneumonia*, and *E. coli*. All derivatives demonstrated very good to excellent anti-bacterial activities against MRSA and *S. aureus* in comparison with the parent drugs, ciprofloxacin and sarafloxacin. However, they exhibited low to moderate potencies against other strains. It is more likely for these fluoroquinolones to have wider applications against other pathogens which could be investigated in further studies. Compound **7n** bearing 3-cyano on the phenyl ring

Table 4 Well diffusion assay results for compounds **7a–ab**, ciprofloxacin, and sarafloxacin

Compound	MRSA	*S. aureus*	*E. faecalis*	*P. aeruginosa*	*K. pneumonia*	*E. coli*
7a	20	20	0	17	0	33
7b	15	16	0	0	0	0
7c	25	25	0	0	0	27
7d	25	25	0	0	0	15
7e	17	17	0	0	0	19
7f	23	26	0	0	0	20
7g	24	24	0	0	0	20
7h	25	23	0	0	0	23
7i	24	22	0	0	0	18
7j	25	24	0	11	0	25
7k	27	24	0	0	0	19
7l	23	19	0	0	0	19
7m	28	23	0	0	0	20
7n	38	33	0	0	0	23
Ciprofloxacin	30	30	20	33	17	35
7o	20	19	0	0	0	22
7p	19	19	0	0	0	19
7q	35	39	0	0	0	16
7r	34	40	0	0	0	13
7s	0	0	0	0	0	15
7t	23	22	0	0	0	0
7u	24	20	0	0	0	0
7v	18	18	0	0	0	17
7w	20	20	0	0	0	19
7x	19	18	0	0	0	17
7y	26	21	0	0	0	13
7z	23	20	0	0	0	14
7aa	27	22	0	0	0	11
7ab	26	21	0	0	0	18
Sarafloxacin	35	33	18	35	16	38

[a]The tested concentrations were 64 µg/ml.

[b]The reported zone of inhibition is in mm.

from the ciprofloxacin-based series showed remarkable potencies with MIC values of 0.2 nM against MRSA and *S. aureus* which was 2440 and 4880 times more potent than ciprofloxacin on the related strains. Compounds **7q** and **7r** from the second series, which demonstrated MIC values of 0.030 µM, were the most potent sarafloxacin analogues with the potency of 16.3 and 32.5 times more than that of the parent drug the strains of MRSA and *S. aureus*, respectively. Molecular docking to the active site of *S. aureus* DNA gyrase co-crystalized with ciprofloxacin-based compound **7n** displayed appropriate fitting with relevant amino acids in the binding pocket with great score energy. Regarding the noticeable MIC value and solubility of these compounds, they could be considered as potential antibiotic candidates in further investigations.

Experimental

General procedures for the synthesis of compounds

All chemicals were purchased from Merck (Germany) and were used without further purification. Melting points were measured on an Electrothermal 9100 apparatus. Mass spectra were recorded on an Agilent Technologies (HP) 5973 mass spectrometer operating at an ionization potential of 20 eV. Elemental analyses for C, H and N were performed using a Heraeus CHN-O-Rapid analyzer. IR spectra were recorded on a Shimadzu IR-460 spectrometer. ^{1}H and ^{13}C NMR spectra were measured (DMSO-d_6 solution) with Bruker DRX-300 (at 300.1 and 75.5 MHz) instrument.

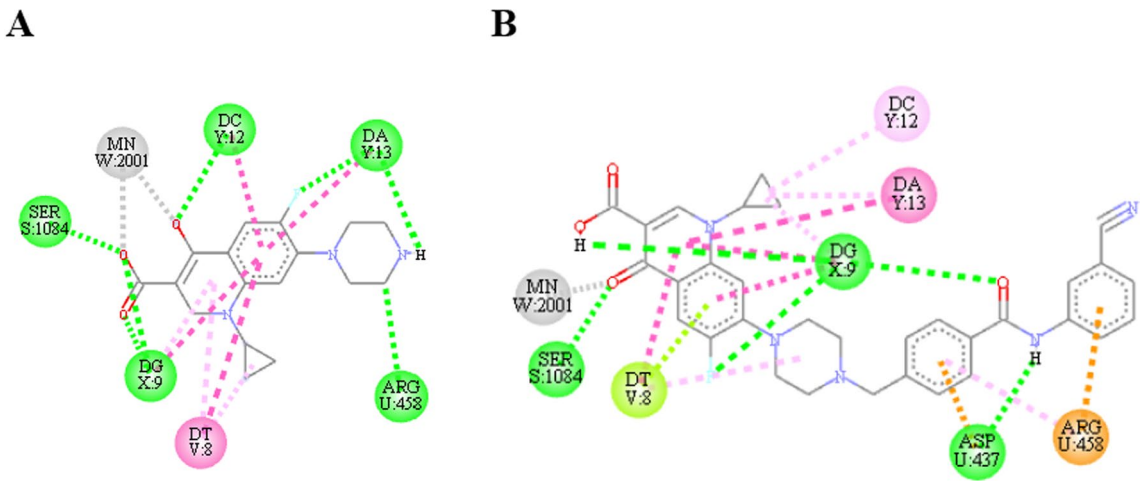


Fig. 1 Predicted binding mode at the binding site of crystal structure of *S. aureus* DNA gyrase for; **A** Ciprofloxacin using PDB ID: 2XCT [hydrogen bonds (green), hydrophobic interactions (pink), and metal bond (gray)], **B** Compound **7n** [hydrogen bonds (green), hydrophobic interactions (pink), anion or cation-π (orange), and metal bond (gray)]

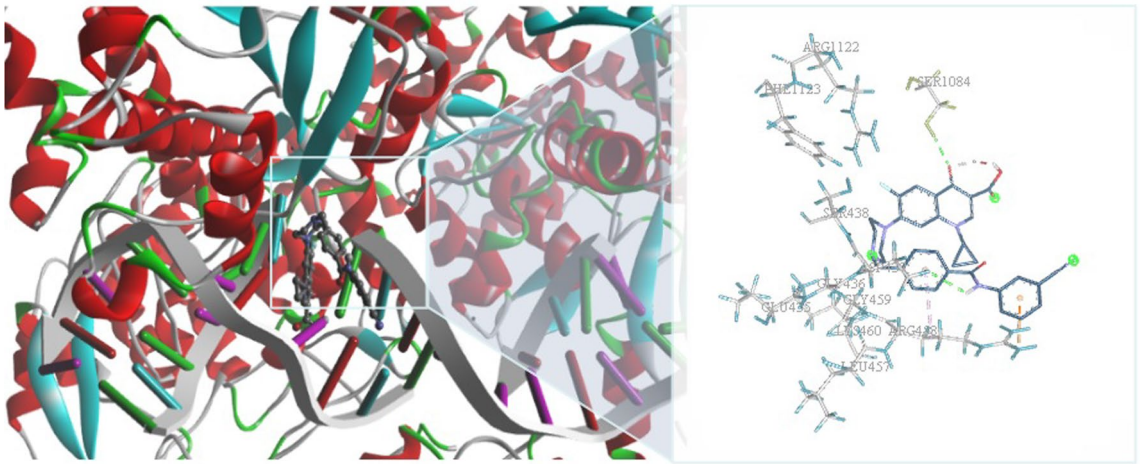


Fig. 2 Proposed binding mode of compound **7n.**

General procedure for the preparation of 4-(hydroxymethyl)benzoic acid 2

To the stirred solution of 4-formylbenzoic acid **1** (15 g, 100 mmol) in dry methanol (50 ml) under the ice-bath conditions, sodium tetraborohydride (11.4 g, 300 mmol) was added gradually within almost 1h. The reaction mixture continued stirring for further 4h at ambient temperature. After completion of the reaction according to the TLC analysis, the mixture was quenched with saturated solution of $NaHCO_3$ (60 ml) and extracted three times with EtOAc (3 × 120 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and then totally concentrated. The residue was recrystallized in ethanol to afford pure compound **2** (12.31 g, 81%) as a white solid [44]. mp: 176-179 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 14.30 (s, 1H, CO_2H), 7.88 (d, J = 7.9 Hz, 2H, 2CH), 7.48 (d, J = 7.9 Hz, 2H, 2CH), 5.23 (s, 1H, OH), 4.57 (s, 2H, CH_2). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 168.73 (C=O), 148.9, 130.69, 129.48, 129.26, 62.74 (CH_2). ESI-MS m/ z: 153.62 $[M + H]^+$.

General procedure for the preparation of 4-(chloromethyl) benzoyl chloride 3

A solution of 4-(hydroxymethyl)benzoic acid (12.31 g, 81 mmol) in dichloromethane (20 ml) was stirred under the ice-bath conditions at 0 °C for 30 min. Then, excess amount of thionyl chloride (8.71 ml, 120 mmol) was added dropwise over 5 min. The resulting mixture was refluxed under an argon atmosphere overnight. Afterwards, the reaction

mixture was concentrated in vacuo to remove the excess amount of excess thionyl chloride and dichloromethane. The obtained pale yellow oil was pure enough to use in next step (11.94 g, 78%) [45]. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 7.94 (d, J = 8.1 Hz, 2H, 2CH), 7.53 (d, J = 8.1 Hz, 2H, 2CH), 4.80 (s, 2H, CH_2). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 166.96 (C=O), 142.35, 130.64, 129.68, 128.92, 45.34 (CH_2). ESI-MS m/ z: 187.48 $[M]^+$.

General procedure for the preparation of 4-(chloromethyl)-N-arylbenzamides 5

A mixture of 4-(chloromethyl)benzoyl chloride **3** (1.19 g, 6.32 mmol), various substituted anilines **4a–n** (7.58 mmol), and triethyl amine (1.32 mL, 9.48 mmol) in acetone (15 mL) was stirred within 16 to 18 h. After completion of the reaction which was monitored by TLC, the mixture was quenched with water, and the obtained precipitate was filtered and washed with Et_2O to afford pure products **5** in great to excellent yields. Herein, the data of 4-(chloromethyl)-N-(4-methoxyphenyl)benzamide **5e** is provided:

White solid; mp: 123-125 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.99 (s, 1H, NH-amid), 7.94 (d, J = 8.1 Hz, 2H, 2CH), 7.65 (d, J = 7.6 Hz, 2H, 2CH), 7.34 (d, J = 8.1 Hz, 2H, 2CH), 6.91 (d, J = 7.6 Hz, 2H, 2CH), 4.58 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR (75.1 MHz, DMSO-d_6) δ 165.96 (C=O, amid), 155.36, 141.66, 135.89, 130.61, 129.66, 128.92, 122.13, 114.22, 55.37 (OCH_3), 45.32 (CH_2).

General procedure for the preparation of ciprofloxacin and sarafloxacin bearing 4-(arylcarbamoyl)benzyl 7

A mixture of ciprofloxacin hydrochloride **6a** (0.331 g, 1 mmol) or sarafloxacin hydrochloride **6b** (0.385 g, 1 mmol) and K_2CO_3 (0.207 g, 1.5 mmol) was stirred in DMF (5 mL) at 80 °C for 30 min. Then, 4-(chloromethyl)-N-arylbenzamides **5a–n** (1.2 mmol) was added portion by portion over 5 min. The resulting mixture was continued to heat under an argon atmosphere for 6 to 8 h. After completion of the reaction which was monitored by TLC, the mixture was cooled down to the ambient temperature. Afterwards, water (10 mL) was added to the mixture, and the precipitated product was filtered and washed with EtOH to afford pure desired products **7a–ab** in great to excellent yields.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(phenylcarbamoyl) benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7a**):

Milky solid; mp: 186-188 °C; IR (KBr) (ν_{max}/cm^{-1}): 3460–3000 (OH and NH), 1719, 1658, 1623 (3CO), 1598, 1496, 1411, 1356, 1288, 1169, 1023, 966, 847, 753, 706, 686, 633. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.92 (s, 1H, CO_2H), 10.23 (s, 1H, NH-amid), 8.63 (s, 1H, CH-quinolone), 8.08-7.70 (m, 5H, 5CH), 7.64-7.40 (m, 5H, 5CH), 7.33 (t, J = 7.4 Hz, 1H, CH), 3.85-3.70 (m, 1H, CH-cyclopropyl), 3.64 (s, 2H, CH_2), 3.32 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, $2CH_2$-N), 1.35-1.10 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.30 (C=O, ketone), 165.92 (CO_2H), 165.17 (C=O, amid), 152.99 (d, $^{1}J_{C\text{-}F}$ = 249.5 Hz, C-F), 147.74 (d, $^{2}J_{C\text{-}F}$ = 28.7 Hz, C), 145.17, 136.52, 134.64, 132.56, 129.54, 129.34, 128.58, 127.76, 127.43, 120.31, 115.47, 110.89 (d, $^{2}J_{C\text{-}F}$ = 22.5 Hz, CH), 106.76, 101.62, 65.47 (CH_2), 52.21 and 49.40 ($2CH_2$-N), 35.83 (CH-cyclopropyl), 7.64 and 7.55 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 540.36 $[M]^+$. Anal. Calcd. for $C_{31}H_{29}FN_4O_4$: C, 68.88; H, 5.41; N, 10.36.; found: C, 69.08; H, 5.72; N, 10.58 %.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(4-tolylcarbamoyl) benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7b**):

White solid; mp: 211-212 °C; IR (KBr) (ν_{max}/cm^{-1}): 3228 (OH), 3096 (NH), 1715, 1669, 1609 (3CO), 1588, 1499, 1412, 1372, 1288, 1209, 1189, 1123, 1096, 989, 963, 839, 754, 685, 648. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.73 (s, 1H, CO_2H), 10.15 (s, 1H, NH-amid), 8.64 (s, 1H, CH-quinolone), 7.97 (d, J = 8.6 Hz, 2H, 2CH), 7.87 (d, $^{3}J_{H\text{-}F}$ = 13.6 Hz, 1H, CH), 7.70-7.30 (m, 7H, 7CH), 3.89-3.70 (m, 1H, CH-cyclopropyl), 3.65 (s, 2H, CH_2), 3.33 (br. s, 4H, 2CH2-N), 2.60 (br. s, 4H, 2CH2-N), 2.26 (s, 3H, CH_3), 1.30-1.00 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.30 (C=O, ketone), 165.91 (CO_2H), 165.16 (C=O, amid), 152.97 (d, $^{1}J_{C\text{-}F}$ = 247.8 Hz, C-F), 147.72 (d, $^{2}J_{C\text{-}F}$ = 33.3 Hz, C), 145.07, 139.11, 134.68, 132.34, 129.53, 129.45, 129.32, 128.94, 127.81, 120.32, 118.51 (d, $^{3}J_{C\text{-}F}$ = 5.7 Hz, C), 111.18 (d, $^{2}J_{C\text{-}F}$ = 24.3 Hz, CH), 106.38, 101.51, 65.36 (CH_2), 52.19 and 49.37 ($2CH_2$-N), 35.82 (CH-cyclopropyl), 20.47 (CH_3), 7.53 and 7.50 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 555.89 $[M + H]^+$. Anal. Calcd. for $C_{32}H_{31}FN_4O_4$: C, 69.30; H, 5.63; N, 10.10.; found: C, 69.12; H, 5.88; N, 10.23 %.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(3-tolylcarbamoyl) benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7c**):

White solid; mp: 178-181 °C; IR (KBr) (ν_{max}/cm^{-1}): 3441-2850 (OH and NH), 1723, 1648, 1612 (3CO), 1583, 1502, 1423, 1383, 1286, 1232, 1145, 1080, 988, 923, 899, 805, 726, 685, 656, 633. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.85 (s, 1H, CO_2H), 10.39 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 8.10-7.87 (m, 3H, 3CH), 7.72 (d, J = 7.4 Hz, 1H, CH), 7.67-7.45 (m, 4H, 4CH), 7.12-7.00 (m, 2H, 2CH), 3.80-3.70 (m, 1H, CH-cyclopropyl), 3.64 (s, 2H, CH_2), 3.32 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 2.14 (s, 3H, CH_3), 1.35-1.00 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.26 (C=O, ketone), 165.91 (CO_2H), 165.16 (C=O, amid), 152.97 (d, $^{1}J_{C\text{-}F}$ = 247.8 Hz, C-F), 147.65 (d, $^{2}J_{C\text{-}F}$ = 35.0 Hz, C), 145.19, 139.09, 134.52, 132.75, 129.61,

129.53, 129.47, 129.33, 129.10, 128.90, 128.14, 127.79, 127.45, 118.57 (d, $^{3}J_{C\text{-}F}$ = 7.1 Hz, C), 110.86 (d, $^{2}J_{C\text{-}F}$ = 23.1 Hz, CH), 106.74, 101.33, 65.47 (CH_2), 52.20 and 49.39 ($2CH_2$-N), 35.77 (CH-cyclopropyl), 19.18 (CH_3), 7.61 and 7.54 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 554.26 $[M]^+$. Anal. Calcd. for $C_{32}H_{31}FN_4O_4$: C, 69.30; H, 5.63; N, 10.10.; found: C, 69.53; H, 5.43; N, 9.94 %.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-(2-tolylcarbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7d**):

Milky solid; mp: 168-169 °C; IR (KBr) (ν_{max}/cm^{-1}): 3432 (OH), 3348 (NH), 1718, 1652, 1634 (3CO), 1598, 1496, 1434, 1399, 1368, 1301, 1278, 1189, 1053, 1022, 994, 935, 899, 823, 752, 689, 643. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.13 (s, 1H, CO_2H), 10.64 (s, 1H, NH-amid), 8.60 (s, 1H, CH-quinolone), 7.98 (d, J = 8.6 Hz, 2H, 2CH), 7.91 (d, $^{3}J_{H\text{-}F}$ = 11.3 Hz, 1H, CH), 7.65-7.40 (m, 5H, 5CH), 7.30-7.10 (m, 2H, 2CH), 3.80-3.70 (m, 1H, CH-cyclopropyl), 3.61 (s, 2H, CH_2), 3.31 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 2.22 (s, 3H, CH_3), 1.35-1.00 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.24 (C=O, ketone), 167.47 (CO_2H), 165.92 (C=O, amid), 152.96 (d, $^{1}J_{C\text{-}F}$ = 248.6 Hz, C-F), 147.64 (d, $^{2}J_{C\text{-}F}$ = 27.6 Hz, C), 145.20, 139.07, 134.73, 132.50, 129.54, 129.50, 129.31, 129.11, 128.79, 128.20, 127.78, 127.50, 118.54 (d, $^{3}J_{C\text{-}F}$ = 7.4 Hz, C), 110.84 (d, $^{2}J_{C\text{-}F}$ = 22.3 Hz, CH), 106.26, 101.24, 65.50 (CH_2), 52.23 and 49.40 ($2CH_2$-N), 35.81 (CH-cyclopropyl), 19.54 (CH_3), 7.63 and 7.55 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 554.67 $[M]^+$. Anal. Calcd. for $C_{32}H_{31}FN_4O_4$: C, 69.30; H, 5.63; N, 10.10.; found: C, 69.18; H, 5.95; N, 10.36 %.

1-cyclopropyl-6-fluoro-7-(4-(4-((4-methoxyphenyl)carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7e**):

Pale yellow solid; mp: 221-224 °C; IR (KBr) (ν_{max}/cm^{-1}): 3320–2900 (OH and NH), 1726, 1640, 1618 (3CO), 1505, 1409, 1321, 1226, 1167, 1103, 1033, 898, 827, 749, 622. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.10 (s, 1H, CO_2H), 10.23 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 8.00-7.80 (m, 3H, 3CH), 7.68 (d, J = 7.8 Hz, 2H, 2CH), 7.45-7.20 (m, 3H, 3CH), 6.98 (d, J = 7.8 Hz, 2H, 2CH), 4.11 (s, 3H, OCH_3), 3.82-3.70 (m, 1H, CH-cyclopropyl), 3.63 (s, 2H, CH_2), 3.37 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.38-1.05 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 178.87 (C=O, ketone), 165.94 (CO_2H), 164.60 (C=O, amid), 155.55, 153.26 (d, $^{1}J_{C\text{-}F}$ = 203.7 Hz, C-F), 147.85 (d, $^{2}J_{C\text{-}F}$ = 36.1 Hz, C), 145.65, 136.34, 134.85, 132.12, 129.62, 129.49, 129.04, 121.96, 118.08, 113.71, 110.89 (d, $^{2}J_{C\text{-}F}$ = 18.2 Hz, CH), 106.55, 101.19, 65.78 (CH_2), 55.17 (OCH_3), 52.30 and 49.72 ($2CH_2$-N), 35.55 (CH-cyclopropyl), 7.71 and 7.54 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 570.38 $[M]^+$. Anal. Calcd. for $C_{32}H_{31}FN_4O_5$: C, 67.36; H, 5.48; N, 9.82.; found: C, 67.58; H, 5.24; N, 10.12 %.

1-cyclopropyl-6-fluoro-7-(4-(4-((3-methoxyphenyl)carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7f**):

Milky solid; mp: 197-201 °C; IR (KBr) (ν_{max}/cm^{-1}): 3420-3161 (OH and NH), 1717, 1663, 1620 (3CO), 1593, 1499, 1445, 1413, 1354, 1278, 1229, 1156, 1044, 991, 936, 899, 833, 753, 737, 685, 660. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.12 (s, 1H, CO_2H), 10.20 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 7.95 (d, J = 8.6 Hz, 2H, 2CH), 7.81 (d, $^{3}J_{H\text{-}F}$ = 12.1 Hz, 1H, CH), 7.42-7.16 (m, 6H, 6CH), 6.66 (d, J = 7.6 Hz, 1H, CH), 4.06 (s, 3H, OCH_3), 3.84-3.72 (m, 1H, CH-cyclopropyl), 3.63 (s, 2H, CH_2), 3.32 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.37-0.98 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.27 (C=O, ketone), 165.91 (CO_2H), 165.18 (C=O, amid), 156.22, 152.96 (d, $^{1}J_{C\text{-}F}$ = 247.1 Hz, C-F), 147.67 (d, $^{2}J_{C\text{-}F}$ = 28.8 Hz, C), 145.21, 136.25, 134.92, 132.70, 129.54, 129.34, 129.10, 127.78, 127.48, 118.55 (d, $^{3}J_{C\text{-}F}$ = 7.2 Hz, C), 112.49, 110.85 (d, $^{2}J_{C\text{-}F}$ = 22.5 Hz, CH), 106.74, 106.36, 101.50, 65.49 (CH_2), 54.98 (OCH_3), 52.22 and 49.44 ($2CH_2$-N), 35.84 (CH-cyclopropyl), 7.87 and 7.56 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 571.64 $[M + H]^+$. Anal. Calcd. for $C_{32}H_{31}FN_4O_5$: C, 67.36; H, 5.48; N, 9.82.; found: C, 67.14; H, 5.65; N, 9.66 %.

1-cyclopropyl-6-fluoro-7-(4-(4-((2-methoxyphenyl)carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7g**):

Milky solid; mp: 174-176 °C; IR (KBr) (ν_{max}/cm^{-1}): 3400-3050 (OH and NH), 1733, 1676, 1618 (3CO), 1596, 1508, 1422, 1367, 1296, 1244, 1182, 1133, 1066, 996, 825, 755, 685, 639. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.19 (s, 1H, CO_2H), 11.23 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 8.02 (d, J = 8.1 Hz, 2H, 2CH), 7.82 (d, $^{3}J_{H\text{-}F}$ = 12.9 Hz, 1H, CH), 7.68-7.28 (m, 4H, 4CH), 7.22 (d, J = 7.4 Hz, 1H, CH), 7.12-6.90 (m, 2H, 2CH), 3.98 (s, 3H, OCH_3), 3.85-3.70 (m, 1H, CH-cyclopropyl), 3.63 (s, 2H, CH_2), 3.31 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.40-1.05 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.27 (C=O, ketone), 165.91 (CO_2H), 165.19 (C=O, amid), 155.60, 152.97 (d, $^{1}J_{C\text{-}F}$ = 247.0 Hz, C-F), 147.75 (d, $^{2}J_{C\text{-}F}$ = 19.2 Hz, C), 145.08, 136.83, 134.20, 132.73, 129.55, 129.34, 129.12, 127.79, 127.49, 122.32, 118.57 (d, $^{3}J_{C\text{-}F}$ = 9.0 Hz, C), 113.23, 110.86 (d, $^{2}J_{C\text{-}F}$ = 17.3 Hz, CH), 106.76, 101.75, 65.49 (CH_2), 54.53 (OCH_3), 52.23 and 49.44 ($2CH_2$-N), 35.80 (CH-cyclopropyl), 7.81 and 7.56 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 570.38 $[M]^+$. Anal. Calcd. for $C_{32}H_{31}FN_4O_5$: C, 67.36; H, 5.48; N, 9.82.; found: C, 67.23; H, 5.73; N, 9.68 %.

7-(4-(4-((4-chlorophenyl)carbamoyl)benzyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7h**):

Milky solid; mp: 235-236 °C; IR (KBr) (νmax/cm–1): 3389 (OH), 2946 (NH), 1714, 1640, 1618 (3CO), 1497,

1455, 1266, 1176, 1098, 1014, 955, 843, 804, 756, 708, 630. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.19 (s, 1H, CO_2H), 10.38 (s, 1H, NH-amid), 8.64 (s, 1H, CH-quinolone), 8.01 (d, J = 8.2 Hz, 2H, 2CH), 7.86 (d, $^{3}J_{H\text{-}F}$ = 13.4 Hz, 1H, CH), 7.74 (d, J = 8.6 Hz, 2H, 2CH), 7.68-7.30 (m, 5H, 5CH), 3.80-3.70 (m, 1H, CH-cyclopropyl), 3.64 (s, 2H, CH_2), 3.33 (br. s, 4H, 2CH2-N), 2.60 (br. s, 4H, 2CH2-N), 1.40-1.00 (m, 4H, 2CH_2-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.32 (C=O, ketone), 166.87 (CO_2H), 165.89 (C=O, amid), 152.97 (d, $^{1}J_{C\text{-}F}$ = 248.9 Hz, C-F), 147.89 (d, $^{2}J_{C\text{-}F}$ = 13.3 Hz, C), 144.91, 139.12, 134.49, 133.84, 132.56, 129.61, 129.52, 129.35, 128.90, 121.80, 118.77, 110.89 (d, $^{2}J_{C\text{-}F}$ = 22.1 Hz, CH), 106.72, 101.45, 66.22 (CH_2), 52.00 and 45.29 (2CH_2-N), 35.87 (CH-cyclopropyl), 7.70 and 7.57 (2CH_2-cyclopropyl). ESI-MS m/ z: 576.46 $[M + H]^+$. Anal. Calcd. for $C_{31}H_{28}ClFN_4O_4$: C, 64.75; H, 4.91; N, 9.74.; found: C, 65.03; H, 5.18; N, 9.49 %.

7-(4-(4-((3-chlorophenyl)carbamoyl)benzyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7i**):

White solid; mp: 218-221 °C; IR (KBr) (ν_{max}/cm^{-1}): 3450-3100 (OH and NH), 1709, 1635, 1609 (3CO), 1595, 1495, 1396, 1373, 1297, 1254, 1186, 1071, 1010, 954, 877, 766, 729, 684, 649. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.13 (s, 1H, CO_2H), 10.39 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 8.09-7.80 (m, 4H, 4CH), 7.81 (d, $^{3}J_{H\text{-}F}$ = 12.8 Hz, 1H, CH), 7.65-7.35 (m, 4H, 4CH), 7.13 (d, J = 7.8 Hz, 1H, CH), 3.85-3.71 (m, 1H, CH-cyclopropyl), 3.62 (s, 2H, CH_2), 3.31 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.36-1.06 (m, 4H, 2CH_2-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.23 (C=O, ketone), 165.90 (CO_2H), 165.19 (C=O, amid), 152.95 (d, $^{1}J_{C\text{-}F}$ = 248.0 Hz, C-F), 147.70 (d, $^{2}J_{C\text{-}F}$ = 21.6 Hz, C), 145.05, 139.06, 134.94, 133.79, 132.72, 129.54, 129.48, 129.29, 129.10, 128.77, 127.77, 127.49, 118.54 (d, $^{3}J_{C\text{-}F}$ = 7.0 Hz, C), 110.84 (d, $^{2}J_{C\text{-}F}$ = 23.3 Hz, CH), 106.28, 101.47, 65.49 (CH_2), 52.21 and 49.45 (2CH_2-N), 35.80 (CH-cyclopropyl), 7.77 and 7.54 (2CH_2-cyclopropyl). ESI-MS m/ z: 575.35 $[M]^+$. Anal. Calcd. for $C_{31}H_{28}ClFN_4O_4$: C, 64.75; H, 4.91; N, 9.74.; found: C, 64.99; H, 5.23; N, 9.96 %.

7-(4-(4-((2-chlorophenyl)carbamoyl)benzyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7j**):

White solid; mp: 196-198 °C; IR (KBr) (ν_{max}/cm^{-1}): 3439 (OH), 3166 (NH), 1712, 1639, 1612 (3CO), 1594, 1496, 1378, 1298, 1234, 1180, 1122, 1038, 994, 939, 926, 803, 755, 686, 658, 634. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.72 (s, 1H, CO_2H), 10.09 (s, 1H, NH-amid), 8.65 (s, 1H, CH-quinolone), 8.10-7.90 (m, 3H, 3CH), 7.65-7.35 (m, 6H, 6CH), 7.22 (d, J = 7.2 Hz, 1H, CH), 3.86-3.80 (m, 1H, CH-cyclopropyl), 3.77 (s, 2H, CH_2), 3.36 (br. s, 4H, 2CH2-N), 2.61 (br. s, 4H, 2CH2-N), 1.36-0.90 (m, 4H, 2CH_2-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.38 (C=O, ketone), 165.97 (CO_2H), 165.07 (C=O, amid), 152.94 (d, $^{1}J_{C\text{-}F}$ = 251.3 Hz, C-F), 147.88 (d, $^{2}J_{C\text{-}F}$ = 16.5 Hz, C), 145.68, 139.81, 134.59, 133.62, 132.58, 129.92, 129.57, 129.39, 129.21, 127.84, 127.74, 127.70, 118.52, 110.98 (d, $^{2}J_{C\text{-}F}$ = 16.9 Hz, CH), 106.81, 101.13, 65.51 (CH_2), 52.20 and 49.57 (2CH_2-N), 34.83 (CH-cyclopropyl), 7.69 and 7.51 (2CH_2-cyclopropyl). ESI-MS m/ z: 575.84 $[M]^+$. Anal. Calcd. for $C_{31}H_{28}ClFN_4O_4$: C, 64.75; H, 4.91; N, 9.74.; found: C, 64.49; H, 4.76; N, 9.98 %.

1-cyclopropyl-6-fluoro-7-(4-(4-((4-fluorophenyl)carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7k**):

Pale yellow solid; mp: 186-188 °C; IR (KBr) (ν_{max}/cm^{-1}): 3385-2900 (OH and NH), 1712, 1637, 1608 (3CO), 1599, 1504, 1437, 1410, 1370, 1294, 1245, 1174, 1144, 1078, 1029, 991, 944, 908, 836, 756, 729, 687, 642. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.15 (s, 1H, CO_2H), 10.32 (s, 1H, NH-amid), 8.60 (s, 1H, CH-quinolone), 8.01 (d, J = 8.2 Hz, 2H, 2CH), 7.81 (d, $^{3}J_{H\text{-}F}$ = 13.4 Hz, 1H, CH), 7.68-7.30 (m, 5H, 5CH), 7.17 (t, J = 7.6 Hz, 2H, 2CH), 3.84-3.70 (m, 1H, CH-cyclopropyl), 3.63 (s, 2H, CH_2), 3.31 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.29-1.02 (m, 4H, 2CH_2-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.25 (C=O, ketone), 165.91 (CO_2H), 165.20 (C=O, amid), 162.72 (d, $^{1}J_{C\text{-}F}$ = 281.5 Hz, C-F), 152.97 (d, $^{1}J_{C\text{-}F}$ = 249.3 Hz, C-F), 147.75 (d, $^{2}J_{C\text{-}F}$ = 16.1 Hz, C), 145.22, 134.43, 134.31, 132.67, 129.54, 129.47, 128.75, 122.12 (d, $^{3}J_{C\text{-}F}$ = 8.1 Hz, 2CH), 118.54 (d, $^{3}J_{C\text{-}F}$ = 6.3 Hz, C), 115.15 (d, $^{2}J_{C\text{-}F}$ = 21.7 Hz, 2CH), 110.85 (d, $^{2}J_{C\text{-}F}$ = 23.9 Hz, CH), 106.74, 101.61, 65.53 (CH_2), 52.22 and 49.46 (2CH_2-N), 35.81 (CH-cyclopropyl), 7.74 and 7.56 (2CH_2-cyclopropyl). ESI-MS m/ z: 559.28 $[M + H]^+$. Anal. Calcd. for $C_{31}H_{28}F_2N_4O_4$: C, 66.66; H, 5.05; N, 10.03.; found: C, 66.82; H, 4.96; N, 9.79 %.

7-(4-(4-((4-bromophenyl)carbamoyl)benzyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7l**):

Yellow solid; mp: 234-237 °C; IR (KBr) (ν_{max}/cm^{-1}): 3520-3100 (OH and NH), 1722, 1665, 1613 (3CO), 1595, 1499, 1437, 1415, 1366, 1295, 1232, 1178, 1144, 1085, 1041, 992, 936, 833, 756, 740, 685, 655, 622. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.47 (s, 1H, CO_2H), 10.36 (s, 1H, NH-amid), 8.60 (s, 1H, CH-quinolone), 7.99 (d, J = 7.6 Hz, 2H, 2CH), 7.80 (d, $^{3}J_{H\text{-}F}$ = 10.2 Hz, 1H, CH), 7.68-7.40 (m, 4H, 4CH), 7.35-7.28 (m, 3H, 3CH), 3.80-3.70 (m, 1H, CH-cyclopropyl), 3.62 (s, 2H, CH_2), 3.31 (br. s, 4H, 2CH2-N), 2.58 (br. s, 4H, 2CH2-N), 1.30-1.00 (m, 4H, 2CH_2-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.23 (C=O, ketone), 165.90 (CO_2H), 165.16 (C=O, amid), 152.94 (d, $^{1}J_{C\text{-}F}$ = 247.2 Hz, C-F), 147.64 (d, $^{2}J_{C\text{-}F}$ = 27.2 Hz, C), 145.15, 136.82, 134.32, 132.60, 131.39, 129.64, 129.52, 129.11, 122.13, 120.76, 118.56, 110.85 (d, $^{2}J_{C\text{-}F}$ = 23.8 Hz, CH), 106.25, 101.46, 65.49 (CH_2), 52.21

and 49.41 ($2CH_2$-N), 35.79 (CH-cyclopropyl), 7.75 and 7.55 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 620.38 $[M + H]^+$. Anal. Calcd. for $C_{31}H_{28}BrFN_4O_4$: C, 60.10; H, 4.56; N, 9.04.; found: C, 60.32; H, 4.38; N, 8.79 %.

1-cyclopropyl-6-fluoro-4-oxo-7-(4-(4-((4-(trifluoromethyl)phenyl)carbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7m**):

Yellow solid; mp: 189-201 °C; IR (KBr) (ν_{max}/cm^{-1}): 3450-2900 (OH and NH), 1715, 1648, 1612 (3CO), 1588, 1512, 1463, 1388, 1289, 1233, 1184, 1123, 1087, 1010, 936, 901, 821, 755, 736, 686, 639. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.15 (s, 1H, CO_2H), 10.58 (s, 1H, NH-amid), 8.60 (s, 1H, CH-quinolone), 7.91 (d, J = 7.8 Hz, 2H, 2CH), 7.80 (d, $^3J_{H\text{-}F}$ = 13.2 Hz, 1H, CH), 7.69 (d, J = 8.2 Hz, 2H, 2CH), 7.58-7.30 (m, 5H, 5CH), 3.80-3.70 (m, 1H, CH-cyclopropyl), 3.62 (s, 2H, CH_2), 3.32 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.40-0.98 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.26 (C=O, ketone), 165.89 (CO_2H), 165.16 (C=O, amid), 152.96 (d, $^1J_{C\text{-}F}$ = 248.1 Hz, C-F), 147.63 (d, $^2J_{C\text{-}F}$ = 28.9 Hz, C), 145.21, 140.75, 134.10, 133.24, 132.02, 129.49, 129.30, 128.78, 125.85, 124.37, 120.05, 118.52 (d, $^3J_{C\text{-}F}$ = 7.8 Hz, C), 110.84 (d, $^2J_{C\text{-}F}$ = 23.2 Hz, CH), 106.73, 101.46, 65.49 (CH_2), 52.23 and 49.45 ($2CH_2$-N), 35.83 (CH-cyclopropyl), 7.79 and 7.56 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 608.49 $[M]^+$. Anal. Calcd. for $C_{32}H_{28}F_4N_4O_4$: C, 63.15; H, 4.64; N, 9.21.; found: C, 63.38; H, 4.88; N, 9.43 %.

7-(4-(4-((3-cyanophenyl)carbamoyl)benzyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7n**):

White solid; mp: 211-214 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3000 (OH and NH), 2258 (CN), 1719, 1633, 1615 (3CO), 1596, 1492, 1475, 1399, 1313, 1294, 1246, 1149, 1072, 1046, 1011, 913, 832, 760, 655. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.12 (s, 1H, CO_2H), 10.55 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 8.08-7.86 (m, 4H, 4CH), 7.81 (d, $^3J_{H\text{-}F}$ = 13.0 Hz, 1H, CH), 7.75-7.30 (m, 5H, 5CH), 3.86-3.70 (m, 1H, CH-cyclopropyl), 3.62 (s, 2H, CH_2), 3.31 (br. s, 4H, 2CH2-N), 2.59 (br. s, 4H, 2CH2-N), 1.38-1.02 (m, 4H, $2CH_2$-cyclopropyl). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.25 (C=O, ketone), 165.90 (CO_2H), 165.15 (C=O, amid), 152.95 (d, $^1J_{C\text{-}F}$ = 248.2 Hz, C-F), 147.65 (d, $^2J_{C\text{-}F}$ = 25.5 Hz, C), 145.20, 141.88, 134.82, 133.29, 132.16, 130.02, 129.49, 129.32, 128.78, 125.51, 122.20, 119.19, 118.54 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C), 117.34, 110.83 (d, $^2J_{C\text{-}F}$ = 22.9 Hz, CH), 106.74, 101.68, 65.46 (CH_2), 52.21 and 49.39 ($2CH_2$-N), 35.80 (CH-cyclopropyl), 7.91 and 7.53 ($2CH_2$-cyclopropyl). ESI-MS m/ z: 566.38 $[M]^+$. Anal. Calcd. for $C_{32}H_{28}FN_5O_4$: C, 67.95; H, 4.99; N, 12.38.; found: C, 67.78; H, 5.12; N, 12.64 %.

6-fluoro-1-(4-fluorophenyl)-4-oxo-7-(4-(4-(phenylcarbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7o**):

Milky solid; mp: 184-187 °C; IR (KBr) (ν_{max}/cm^{-1}): 3350-2900 (OH and NH), 1718, 1656, 1612 (3CO), 1597, 1462, 1378, 1299, 1216, 1199, 1156, 1093, 984, 899, 797, 746, 668, 624. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.27 (s, 1H, CO_2H), 10.23 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 8.02 (d, J = 8.0 Hz, 2H, 2CH), 7.97 (d, $^3J_{H\text{-}F}$ = 12.4 Hz, 1H, CH), 7.75 (d, J = 8.0 Hz, 2H, 2CH), 7.70-7.10 (m, 10H, 10CH), 3.58 (s, 2H, CH_2), 3.05 (br. s, 4H, 2CH2-N), 2.72 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.26 (C=O, ketone), 168.53 (CO_2H), 165.70 (C=O, amid), 162.21 (d, $^1J_{C\text{-}F}$ = 265.7 Hz, C-F), 153.19 (d, $^1J_{C\text{-}F}$ = 202.6 Hz, C-F), 148.51 (d, $^2J_{C\text{-}F}$ = 23.4 Hz, C), 146.31, 136.64, 135.67, 133.36, 131.56 (d, $^4J_{C\text{-}F}$ = 1.9 Hz, C), 130.46 (d, $^3J_{C\text{-}F}$ = 6.7 Hz, 2CH), 129.83, 129.52, 128.63, 127.76, 127.40, 120.36, 118.14 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C), 117.21 (d, $^2J_{C\text{-}F}$ = 24.4 Hz, C-F), 110.95 (d, $^3J_{C\text{-}F}$ = 26.7 Hz, CH), 107.84, 102.33, 66.65 (CH_2), 52.13 and 49.05 ($2CH_2$-N). 0ESI-MS m/ z: 595.34 $[M + 1]^+$. Anal. Calcd. for $C_{34}H_{28}F_2N_4O_4$: C, 68.68; H, 4.75; N, 9.42.; found: C, 68.44; H, 4.99; N, 9.21 %.

6-fluoro-1-(4-fluorophenyl)-4-oxo-7-(4-(4-(4-tolylcarbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7p**):

White solid; mp: 208-207 °C; IR (KBr) (νmax/cm–1): 3500–3100 (OH), 3066 (NH), 1721, 1640, 1628 (3CO), 1510, 1467, 1385, 1336, 1301, 1262, 1178, 1102, 1018, 946, 891, 834, 763, 706, 634, 588, 550, 514, 467. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.81 (s, 1H, CO_2H), 10.16 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 8.03 (d, J = 7.6 Hz, 2H, 2CH), 7.94 (d, $^3J_{H\text{-}F}$ = 13.1 Hz, 1H, CH), 7.70-7.20 (m, 9H, 9CH), 7.14 (t, J = 7.8 Hz, 2H, 2CH), 3.56 (s, 2H, CH_2), 3.03 (br. s, 4H, 2CH2-N), 2.72 (br. s, 4H, 2CH2-N), 2.26 (s, 3H, CH_3). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.87 (C=O, ketone), 168.62 (CO_2H), 165.73 (C=O, amid), 162.45 (d, $^1J_{C\text{-}F}$ = 246.7 Hz, C-F), 153.55 (d, $^1J_{C\text{-}F}$ = 147.6 Hz, C-F), 148.69 (d, $^2J_{C\text{-}F}$ = 15.4 Hz, C), 146.39, 139.11, 134.68, 133.79, 131.58, 130.54 (d, $^3J_{C\text{-}F}$ = 9.9 Hz, 2CH), 129.84, 129.52, 129.29, 128.95, 127.75, 120.32, 118.50, 117.24 (d, $^2J_{C\text{-}F}$ = 22.6 Hz, 2CH), 110.99 (d, $^2J_{C\text{-}F}$ = 23.9 Hz, CH), 107.51, 102.11, 65.47 (CH_2), 52.05 and 49.07 ($2CH_2$-N), 20.47 (CH_3). ESI-MS m/ z: 609.74 $[M + H]^+$. Anal. Calcd. for $C_{35}H_{30}F_2N_4O_4$: C, 69.07; H, 4.97; N, 9.21.; found: C, 68.88; H, 5.14; N, 8.98 %.

6-fluoro-1-(4-fluorophenyl)-4-oxo-7-(4-(4-(3-tolylcarbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7q**):

Milky solid; mp: 189-192 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3200 (OH), 3098 (NH), 1723, 1648, 1613 (3CO), 1598, 1474, 1399, 1323, 1276, 1191, 1095, 994, 839, 754, 685, 648. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.94 (s, 1H, CO_2H), 10.61 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 7.95 (d, J = 7.2 Hz, 2H, 2CH), 7.89 (d, $^3J_{H\text{-}F}$ = 10.8 Hz, 1H, CH), 7.65-7.26 (m, 8H, 8CH), 7.20-7.00 (m, 3H,

3CH), 3.55 (s, 2H, CH_2), 3.04 (br. s, 4H, 2CH2-N), 2.72 (br. s, 4H, 2CH2-N), 2.15 (s, 3H, CH_3). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.88 (C=O, ketone), 168.23 (CO_2H), 165.73 (C=O, amid), 162.47 (d, $^1J_{C\text{-}F}$ = 247.3 Hz, C-F), 152.97 (d, $^1J_{C\text{-}F}$ = 246.2 Hz, C-F), 148.42 (d, $^2J_{C\text{-}F}$ = 25.7 Hz, C), 146.45, 139.13, 135.86, 133.72, 131.59 (d, $^4J_{C\text{-}F}$ = 2.3 Hz, C), 130.40 (d, $^3J_{C\text{-}F}$ = 6.4 Hz, 2CH), 129.84, 129.53, 129.48, 129.27, 129.01, 128.71, 127.76, 127.48, 118.52, 117.25 (d, $^2J_{C\text{-}F}$ = 23.1 Hz, 2CH), 110.99 (d, $^2J_{C\text{-}F}$ = 24.1 Hz, CH), 107.48, 102.14, 65.46 (CH_2), 52.06 and 49.12 ($2CH_2$-N), 21.11 (CH_3). ESI-MS m/ z: 608.32 $[M]^+$. Anal. Calcd. for $C_{35}H_{30}F_2N_4O_4$: C, 69.07; H, 4.97; N, 9.21.; found: C, 69.26; H, 4.68; N, 9.46 %.

6-fluoro-1-(4-fluorophenyl)-4-oxo-7-(4-(4-(2-tolylcarbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7r**):

White solid; mp: 168-169 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3100 (OH and NH), 1719, 1664, 1622 (3CO), 1594, 1444, 1377, 1283, 1189, 1123, 1068, 991, 957, 843, 790, 753, 645, 623. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.00 (s, 1H, CO_2H), 10.90 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 8.02 (d, J = 7.7 Hz, 2H, 2CH), 7.90 (d, $^3J_{H\text{-}F}$ = 12.5 Hz, 1H, CH), 7.65-7.25 (m, 6H, 6CH), 7.20-7.00 (m, 5H, 5CH), 3.54 (s, 2H, CH_2), 3.02 (br. s, 4H, 2CH2-N), 2.72 (br. s, 4H, 2CH2-N), 2.17 (s, 3H, CH_3). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.41 (C=O, ketone), 167.32 (CO_2H), 165.77 (C=O, amid), 162.48 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C-F), 152.88 (d, $^1J_{C\text{-}F}$ = 247.2 Hz, C-F), 148.45 (d, $^2J_{C\text{-}F}$ = 18.8 Hz, C), 146.67, 139.11, 135.83, 133.70, 131.05 (d, $^4J_{C\text{-}F}$ = 2.9 Hz, C), 130.62 (d, $^3J_{C\text{-}F}$ = 4.2 Hz, 2CH), 129.85, 129.55, 129.30, 129.02, 128.72, 127.76, 127.48, 118.53 (d, $^3J_{C\text{-}F}$ = 5.3 Hz, C), 117.29 (d, $^2J_{C\text{-}F}$ = 23.3 Hz, 2CH), 110.99 (d, $^2J_{C\text{-}F}$ = 25.4 Hz, CH), 107.51, 102.21, 65.39 (CH_2), 52.07 and 49.11 ($2CH_2$-N), 20.57 (CH_3). ESI-MS m/ z: 608.48 $[M]^+$. Anal. Calcd. for $C_{35}H_{30}F_2N_4O_4$: C, 69.07; H, 4.97; N, 9.21.; found: C, 69.16; H, 5.23; N, 9.57 %.

6-fluoro-1-(4-fluorophenyl)-7-(4-(4-((4-methoxyphenyl) carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7s**):

Yellow solid; mp: 236-239 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3150 (OH), 3068 (NH), 1722, 1635, 1612 (3CO), 1598, 1548, 1391, 1368, 1297, 1188, 1079, 997, 935, 897, 752, 668, 623. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.36 (s, 1H, CO_2H), 10.12 (s, 1H, NH-amid), 8.43 (s, 1H, CH-quinolone), 7.93 (d, J = 7.8 Hz, 2H, 2CH), 7.89 (d, $^3J_{H\text{-}F}$ = 10.4 Hz, 1H, CH), 7.84-7.28 (m, 7H, 7CH), 7.13 (t, J = 7.8 Hz, 2H, 2CH), 6.91 (d, J = 8.4 Hz, 2H, 2CH), 3.73 (s, 3H, OCH_3), 3.56 (s, 2H, CH_2), 2.98 (br. s, 4H, $2CH_2$-N), 2.77 (br. s, 4H, $2CH_2$-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.77 (C=O, ketone), 168.42 (CO_2H), 166.42 (C=O, amid), 162.43 (d, $^1J_{C\text{-}F}$ = 273.7 Hz, C-F), 155.51, 152.78 (d, $^1J_{C\text{-}F}$ = 210.8 Hz, C-F), 148.58 (d, $^2J_{C\text{-}F}$ = 22.3 Hz, C), 146.90, 136.55, 134.38, 133.79, 132.21 (d, $^4J_{C\text{-}F}$ = 5.2 Hz, C), 129.98 (d, $^3J_{C\text{-}F}$ = 8.9 Hz, C), 129.58, 129.23, 128.62, 121.92, 118.86, 117.22 (d, $^2J_{C\text{-}F}$ = 23.1 Hz, 2CH), 113.69, 111.41 (d, $^2J_{C\text{-}F}$ = 12.3 Hz, CH), 108.35, 102.55, 64.89 (CH_2), 55.15 (OCH_3), 52.11 and 49.27 ($2CH_2$-N). ESI-MS m/ z: 625.36 $[M + H]^+$. Anal. Calcd. for $C_{35}H_{30}F_2N_4O_5$: C, 67.30; H, 4.84; N, 8.97.; found: C, 67.14; H, 5.06; N, 9.12 %.

6-fluoro-1-(4-fluorophenyl)-7-(4-(4-((3-methoxyphenyl) carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7t**):

Pale yellow solid; mp: 212-215 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-2900 (OH and NH), 1718, 1648, 1614 (3CO), 1596, 1511, 1497, 1346, 1287, 1192, 1153, 1084, 997, 835, 776, 658, 635. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.59 (s, 1H, CO_2H), 10.21 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 8.12-7.80 (m, 3H, 3CH), 7.70-7.00 (m, 10H, 10CH), 6.67 (d, J = 7.4 Hz, 1H, CH), 3.74 (s, 3H, OCH_3), 3.62 (s, 2H, CH_2), 3.03 (br. s, 4H, $2CH_2$-N), 2.78 (br. s, 4H, $2CH_2$-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 178.84 (C=O, ketone), 168.33 (CO_2H), 165.17 (C=O, amid), 162.46 (d, $^1J_{C\text{-}F}$ = 244.7 Hz, C-F), 155.43, 152.87 (d, $^1J_{C\text{-}F}$ = 249.3 Hz, C-F), 148.44 (d, $^2J_{C\text{-}F}$ = 19.2 Hz, C), 146.63, 136.16, 134.59, 133.68, 131.16 (d, $^4J_{C\text{-}F}$ = 1.9 Hz, C-F), 129.87, 129.53, 129.51 (d, $^3J_{C\text{-}F}$ = 5.1 Hz, 2CH), 129.28, 129.00, 127.75, 127.46, 118.63, 117.26 (d, $^2J_{C\text{-}F}$ = 23.6 Hz, 2CH), 112.49, 110.99 (d, $^2J_{C\text{-}F}$ = 22.7 Hz, CH), 107.49, 106.36, 102.39, 65.48 (CH_2), 54.96 (OCH_3), 52.05 and 49.08 ($2CH_2$-N). ESI-MS m/ z: 624.58 $[M]^+$. Anal. Calcd. for $C_{35}H_{30}F_2N_4O_5$: C, 67.30; H, 4.84; N, 8.97.; found: C, 67.56; H, 4.54; N, 8.78 %.

6-fluoro-1-(4-fluorophenyl)-7-(4-(4-((2-methoxyphenyl) carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7u**):

Pale yellow solid; mp: 192-195 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3000 (OH), 2968 (NH), 1728, 1656, 1618 (3CO), 1578, 1532, 1432, 1397, 1292, 1188, 1132, 1095, 989, 933, 844, 732, 684, 625. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.34 (s, 1H, CO_2H), 10.10 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 7.98 (d, J = 8.0 Hz, 2H, 2CH), 7.90 (d, $^3J_{HF}$ = 12.1 Hz, 1H, CH), 7.65-7.28 (m, 8H, 8CH), 7.20-6.80 (m, 3H, 3CH), 3.87 (s, 3H, OCH_3), 3.60 (s, 2H, CH_2), 3.03 (br. s, 4H, $2CH_2$-N), 2.86 (br. s, 4H, $2CH_2$-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 178.97 (C=O, ketone), 167.65 (CO_2H), 165.89 (C=O, amid), 162.43 (d, $^1J_{C\text{-}F}$ = 263.3 Hz, C-F), 155.13, 152.99 (d, $^1J_{C\text{-}F}$ = 249.0 Hz, C-F), 148.53 (d, $^2J_{C\text{-}F}$ = 20.4 Hz, C), 146.18, 136.15, 134.18, 133.11, 132.41, 131.47 (d, $^4J_{C\text{-}F}$ = 1.3 Hz, C), 129.94, 129.59 (d, $^3J_{C\text{-}F}$ = 4.3 Hz, 2CH), 128.83, 127.85, 127.55, 122.63, 118.59, 117.33 (d, $^2J_{C\text{-}F}$ = 22.2 Hz, 2CH), 113.15, 111.08 (d, $^2J_{C\text{-}F}$ = 20.6 Hz, CH), 107.52, 102.42, 65.45 (CH_2), 54.40 (OCH_3), 52.10 and 49.16 ($2CH_2$-N). ESI-MS m/ z: 625.56 $[M + H]^+$. Anal. Calcd. for $C_{35}H_{30}F_2N_4O_5$: C, 67.30; H, 4.84; N, 8.97.; found: C, 67.68; H, 5.12; N, 9.23 %.

7-(4-(4-((4-chlorophenyl)carbamoyl)benzyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7v**):

White solid; mp: 268-270 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-2900 (OH and NH), 1719, 1652, 1618 (3CO), 1602, 1445, 1422, 1398, 1277, 1252, 1129, 1098, 1032, 988, 928, 848, 776, 729, 687, 635. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.09 (s, 1H, CO_2H), 10.40 (s, 1H, NH-amid), 8.63 (s, 1H, CH-quinolone), 7.98 (d, J = 7.4 Hz, 2H, 2CH), 7.89 (d, $^3J_{HF}$ = 11.8 Hz, 1H, CH), 7.78 (d, J = 8.5 Hz, 2H, 2CH), 7.68-7.00 (m, 9H, 9CH), 3.55 (s, 2H, CH_2), 3.08 (br. s, 4H, 2CH2-N), 2.88 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.43 (C=O, ketone), 168.23 (CO_2H), 165.63 (C=O, amid), 162.47 (d, $^1J_{C\text{-}F}$ = 246.8 Hz, C-F), 152.89 (d, $^1J_{C\text{-}F}$ = 238.6 Hz, C-F), 148.26 (d, $^2J_{C\text{-}F}$ = 18.4 Hz, C), 146.18, 136.15, 135.08, 133.68, 132.98, 131.74, 129.87, 129.53 (d, $^3J_{C\text{-}F}$ = 3.6 Hz, 2CH), 128.89, 128.50, 127.98, 121.98, 118.41, 117.25 (d, $^2J_{C\text{-}F}$ = 21.7 Hz, 2CH), 110.99 (d, $^2J_{C\text{-}F}$ = 23.6 Hz, CH), 107.36, 102.48, 65.57 (CH_2), 52.47 and 49.53 (2CH_2-N). ESI-MS m/ z: 630.43 [M + H]$^+$. Anal. Calcd. for $C_{34}H_{27}ClF_2N_4O_4$: C, 64.92; H, 4.33; N, 8.91.; found: C, 65.13; H, 4.58; N, 9.18 %.

7-(4-(4-((3-chlorophenyl)carbamoyl)benzyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7w**):

White solid; mp: 236-239 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3200 (OH), 3062 (NH), 1724, 1647, 1629 (3CO), 1595, 1498, 1472, 1349, 1258, 1263, 1149, 1085, 1035, 972, 948, 885, 784, 738, 625. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.36 (s, 1H, CO_2H), 10.41 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 8.10-7.80 (m, 5H, 5CH), 7.70-7.30 (m, 6H, 6CH), 7.25-7.00 (m, 3H, 3CH), 3.55 (s, 2H, CH_2), 3.04 (br. s, 4H, 2CH2-N), 2.88 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.37 (C=O, ketone), 168.17 (CO_2H), 165.73 (C=O, amid), 162.28 (d, $^1J_{C\text{-}F}$ = 219.7 Hz, C-F), 153.03 (d, $^1J_{C\text{-}F}$ = 231.1 Hz, C-F), 148.72 (d, $^2J_{C\text{-}F}$ = 16.3 Hz, C), 146.54, 139.15, 135.15, 133.86, 133.51, 131.40 (d, $^4J_{C\text{-}F}$ = 1.2 Hz, C), 129.87, 129.51 (d, $^3J_{C\text{-}F}$ = 5.2 Hz, 2CH), 129.25, 128.70, 128.55, 127.78, 127.76, 127.48, 119.44, 117.25 (d, $^2J_{C\text{-}F}$ = 31.1 Hz, 2CH), 111.02 (d, $^2J_{C\text{-}F}$ = 27.2 Hz, CH), 107.49, 102.34, 65.48 (CH_2), 52.07 and 49.15 (2CH_2-N). ESI-MS m/ z: 629.48 [M]$^+$. Anal. Calcd. for $C_{34}H_{27}ClF_2N_4O_4$: C, 64.92; H, 4.33; N, 8.91.; found: C, 64.78; H, 4.14; N, 9.14 %.

7-(4-(4-((2-chlorophenyl)carbamoyl)benzyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7x**):

Milky solid; mp: 198-201 °C; IR (KBr) (ν_{max}/cm^{-1}): 3450-3000 (OH and NH), 1724, 1635, 1612 (3CO), 1599, 1506, 1436, 1348, 1294, 1233, 1172, 1061, 990, 898, 831, 786, 686, 635. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.08 (s, 1H, CO_2H), 10.06 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 7.95 (d, J = 7.6 Hz, 2H, 2CH), 7.89 (d, $^3J_{HF}$ = 12.8 Hz, 1H, CH), 7.80-7.20 (m, 9H, 9CH), 7.15 (t, J = 7.8 Hz, 2H, 2CH), 3.57 (s, 2H, CH_2), 3.04 (br. s, 4H, 2CH2-N), 2.72 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 176.65 (C=O, ketone), 167.31 (CO_2H), 165.76 (C=O, amid), 162.49 (d, $^1J_{C\text{-}F}$ = 245.9 Hz, C-F), 152.83 (d, $^1J_{C\text{-}F}$ = 238.8 Hz, C-F), 148.59 (d, $^2J_{C\text{-}F}$ = 7.9 Hz, C), 146.30, 139.17, 135.02, 133.61, 132.70, 131.22 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, C), 129.88, 129.53 (d, $^3J_{C\text{-}F}$ = 4.3 Hz, 2CH), 129.33, 128.92, 127.96, 127.80, 127.72, 127.49, 118.64, 117.27 (d, $^2J_{C\text{-}F}$ = 23.7 Hz, 2CH), 111.02 (d, $^1J_{C\text{-}F}$ = 23.2 Hz, CH), 107.43, 102.26, 65.52 (CH_2), 52.07 and 49.13 (2CH_2-N). ESI-MS m/ z: 628.63 [M]$^+$. Anal. Calcd. for $C_{34}H_{27}ClF_2N_4O_4$: C, 64.92; H, 4.33; N, 8.91.; found: C, 64.68; H, 4.09; N, 8.76 %.

6-fluoro-1-(4-fluorophenyl)-7-(4-(4-((4-fluorophenyl)carbamoyl)benzyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7y**):

White solid; mp: 228-231 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3300 (OH), 2987 (NH), 1717, 1652, 1620 (3CO), 1575, 1511, 1485, 1327, 1254, 1082, 1046, 945, 823, 766, 628. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.11 (s, 1H, CO_2H), 10.26 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 7.96 (d, J = 7.8 Hz, 2H, 2CH), 7.87 (d, $^3J_{HF}$ = 12.1 Hz, 1H, CH), 7.68-7.20 (m, 7H, 7CH), 7.18 (t, J = 7.6 Hz, 2H, 2CH), 7.07 (t, J = 7.4 Hz, 2H, 2CH), 3.59 (s, 2H, CH_2), 3.05 (br. s, 4H, 2CH2-N), 2.86 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.01 (C=O, ketone), 167.43 (CO_2H), 165.78 (C=O, amid), 162.35 (d, $^1J_{C\text{-}F}$ = 258.3 Hz, C-F), 162.02 (d, $^1J_{C\text{-}F}$ = 261.0 Hz, C-F), 152.83 (d, $^1J_{C\text{-}F}$ = 243.6 Hz, C-F), 148.44 (d, $^2J_{C\text{-}F}$ = 23.6 Hz, C), 146.79, 135.27, 134.75, 133.60, 131.17, 129.89, 129.47 (d, $^3J_{C\text{-}F}$ = 1.8 Hz, 2CH), 128.65, 127.89, 122.18 (d, $^3J_{C\text{-}F}$ = 3.4 Hz, 2CH), 118.65, 117.54 (d, $^2J_{C\text{-}F}$ = 19.3 Hz, 2CH), 115.20 (d, $^2J_{C\text{-}F}$ = 18.7 Hz, 2CH), 110.83 (d, $^2J_{C\text{-}F}$ = 21.8 Hz, CH), 107.63, 102.53, 65.56 (CH_2), 52.08 and 48.32 (2CH_2-N). ESI-MS m/ z: 612.49 [M]$^+$. Anal. Calcd. for $C_{34}H_{27}F_3N_4O_4$: C, 66.66; H, 4.44; N, 9.15.; found: C, 66.94; H, 4.69; N, 8.89 %.

7-(4-(4-((4-bromophenyl)carbamoyl)benzyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7z**):

Pale yellow solid; mp: 262-266 °C; IR (KBr) (ν_{max}/cm^{-1}): 3400-2900 (OH and NH), 1718, 1636, 1613 (3CO), 1595, 1434, 1345, 1293, 1233, 1145, 1061, 995, 846, 789, 748, 647, 623. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.91 (s, 1H, CO_2H), 10.37 (s, 1H, NH-amid), 8.61 (s, 1H, CH-quinolone), 7.95 (d, J = 7.6 Hz, 2H, 2CH), 7.89 (d, $^3J_{HF}$ = 12.8 Hz, 1H, CH), 7.76 (d, J = 8.4 Hz, 2H, 2CH), 7.70-7.10 (m, 9H, 9CH), 3.56 (s, 2H, CH_2), 3.04 (br. s, 4H, 2CH2-N), 2.88 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.75 (C=O, ketone), 167.37 (CO_2H), 165.74 (C=O, amid), 162.44 (d, $^1J_{C\text{-}F}$ = 248.3 Hz, C-F), 152.88 (d, $^1J_{C\text{-}F}$ = 252.4 Hz, C-F), 148.51 (d, $^2J_{C\text{-}F}$ = 13.8 Hz, C), 146.34, 136.15, 134.30, 133.41, 131.38, 129.83, 129.50 (d, $^3J_{C\text{-}F}$

= 3.3 Hz, 2CH), 129.29, 128.69, 127.75, 122.13, 121.45, 118.62, 117.28 (d, $^{2}J_{C\text{-}F}$ = 22.9 Hz, 2CH), 110.98 (d, $^{2}J_{C\text{-}F}$ = 23.2 Hz, CH), 107.59, 102.38, 65.47 (CH_2), 52.04 and 49.06 ($2CH_2$-N). ESI-MS m/ z: 672.58 $[M]^+$. Anal. Calcd. for $C_{34}H_{27}BrF_2N_4O_4$: C, 60.63; H, 4.04; N, 8.32.; found: C, 60.88; H, 3.76; N, 8.66 %.

6-fluoro-1-(4-fluorophenyl)-4-oxo-7-(4-(4-((4-(trifluoromethyl)phenyl)carbamoyl)benzyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**7aa**):

White solid; mp: 278-280 °C; IR (KBr) (ν_{max}/cm^{-1}): 3500-3000 (OH and NH), 1722, 1646, 1612 (3CO), 1602, 1482, 1453, 1371, 1285, 1253, 1198, 1045, 939, 879, 755, 695, 644, 623. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 15.13 (s, 1H, CO_2H), 10.54 (s, 1H, NH-amid), 8.63 (s, 1H, CH-quinolone), 7.97 (d, J = 7.8 Hz, 2H, 2CH), 7.89 (d, $^{3}J_{HF}$ = 12.1 Hz, 1H, CH), 7.78 (d, J = 8.0 Hz, 2H, 2CH), 7.68-7.40 (m, 5H, 5CH), 7.38 (d, J = 8.0 Hz, 2H, 2CH), 7.15 (t, J = 7.6 Hz, 2H, 2CH), 3.58 (s, 2H, CH_2), 3.05 (br. s, 4H, 2CH2-N), 2.72 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.40 (C=O, ketone), 168.34 (CO_2H), 166.56 (C=O, amid), 162.52 (d, $^{1}J_{C\text{-}F}$ = 270.0 Hz, C-F), 153.26 (d, $^{1}J_{C\text{-}F}$ = 234.1 Hz, C-F), 148.54 (d, $^{2}J_{C\text{-}F}$ = 22.3 Hz, C), 146.90, 140.69, 133.77, 133.40, 131.58, 129.88, 129.50 (d, $^{3}J_{C\text{-}F}$ = 5.8 Hz, 2CH), 128.75, 127.82, 125.89, 124.55, 121.10, 118.50, 117.50 (d, $^{2}J_{C\text{-}F}$ = 13.8 Hz, 2CH), 110.68 (d, $^{2}J_{C\text{-}F}$ = 27.9 Hz, CH), 107.82, 102.88, 64.28 (CH_2), 52.08 and 49.78 ($2CH_2$-N). ESI-MS m/ z: 663.24 $[M + H]^+$. Anal. Calcd. for $C_{35}H_{27}F_5N_4O_4$: C, 63.44; H, 4.11; N, 8.46.; found: C, 63.89; H, 3.93; N, 8.23 %.

7-(4-(4-((3-cyanophenyl)carbamoyl)benzyl)piperazin-1-yl)-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**7ab**):

Milky solid; mp: 221-224 °C; IR (KBr) (ν_{max}/cm^{-1}): 3450-2900 (OH and NH), 2249 (CN), 1721, 1648, 1622 (3CO), 1598, 1494, 1455, 1399, 1343, 1298, 1242, 1176, 1128, 1096, 1038, 915, 826, 795, 759, 736, 689, 655. ^{1}H NMR (300.1 MHz, DMSO-d_6): δ 14.14 (s, 1H, CO_2H), 10.54 (s, 1H, NH-amid), 8.62 (s, 1H, CH-quinolone), 8.03 (d, J = 7.5 Hz, 2H, 2CH), 7.87 (d, $^{3}J_{HF}$ = 11.2 Hz, 1H, CH), 7.76 (s, IH, CH), 7.70-7.30 (m, 8H, 8CH), 7.12 (t, J = 7.6 Hz, 2H, 2CH), 3.57 (s, 2H, CH_2), 3.04 (br. s, 4H, 2CH2-N), 2.88 (br. s, 4H, 2CH2-N). ^{13}C NMR (75.1 MHz, DMSO-d_6): δ 177.39 (C=O, ketone), 167.25 (CO_2H), 165.72 (C=O, amid), 162.45 (d, $^{1}J_{C\text{-}F}$ = 243.8 Hz, C-F), 153.23 (d, $^{1}J_{C\text{-}F}$ = 202.1 Hz, C-F), 148.44 (d, $^{2}J_{C\text{-}F}$ = 26.6 Hz, C), 146.34, 141.82, 135.37, 133.83, 133.03, 131.47, 130.65, 130.06, 129.50 (d, $^{3}J_{C\text{-}F}$ = 4.8 Hz, 2CH), 128.69, 127.77, 125.70, 122.62, 119.98, 118.56, 117.96, 117.24 (d, $^{3}J_{C\text{-}F}$ = 22.7 Hz, 2CH), 110.99 (d, $^{3}J_{C\text{-}F}$ = 27.4 Hz, CH), 107.37, 102.75, 65.48 (CH_2), 52.03 and 49.10 ($2CH_2$-N). ESI-MS m/ z: 620.58 $[M + H]^+$. Anal. Calcd. for $C_{35}H_{27}F_2N_5O_4$: C, 67.84; H, 4.39; N, 11.30.; found: C, 68.12; H, 4.64; N, 11.18 %.

Antibacterial assays

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 pneumonia* ATCC 11706, *Escherichia coli* ATCC 25922) were utilized to evaluate the antibacterial activities of synthesized compounds **7a–ab** using three methods, including broth microdilution, well diffusion and disc diffusion assays.

Broth microdilution

In order to determine the MIC of compounds **7a–ab**, broth microdilution assay was performed as described previously [19]. 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 **7a–ab** were assessed using well diffusion assay on MHA as described previously [46]. 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 millimeters (mm).

Disk diffusion assay

Agar disk-diffusion assay was performed on MHA, as explained previously [46]. 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 **7a–ab** 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

Based on the crystal structure of topoisomerase II DNA gyrase cocrystallized with ciprofloxacin (PDB ID: 2XCT, https://www.rcsb.org), docking study was employed to explore the binding mode of **7n** in comparison with ciprofloxacin in the active site of the enzyme. Using AutoDock 4.2.1, the protein-DNA complex was prepared as a pdbqt file. In the 2XCT structure, the chains 'S, U, V, W, X, and Y' were choosen, CPF 1020 bound to Mn2001 was deleted, and the grid was created at coordinates x = 41.533, y = 45.643, z= -18.864, and 40 × 40 × 40 A. Redocking of cocrystallized ligand ciprofloxacin within the active site of topoisomerase II DNA-gyrase was performed to assess the docking accuracy. With a RMSD of 0.21 A, AutoDock reproduced ciprofloxacin's binding position successfully. The 3D structure of **7n** was generated by MarvineSketch 5.8.3, 2012, ChemAxon (www.chemaxon.com) and converted to pdbqt format by AutoDock Tools. Each docked system was carried out by 100 runs by the Lamarckian genetic algorithm. The results were displayed using Discovery Studio 4.0 Client [47].

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Author contributions Prof. AF designed the study and conducted the experiments. Dr. FP, TS, and MN synthesized the targeted compounds. Dr. FP, Dr. MGD, and Dr. HRB wrote the manuscript and analyzed the characterization data. Dr. LF and Dr. ZE carried out the docking studies. Dr. MN, Dr. MG, and GŞ performed the biological evaluations. Dr. MBT revised the manuscript. All authors read and approved the final manuscript.

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Data availability The authors confirm that the data supporting the finding of this study are available within the manuscript.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval This study did not involve human subjects and/or animals.

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