European Journal of Medicinal Chemistry 261 (2023) 115798

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

Synthesis and biological evaluation of fluoroquinolones containing a pyridoxine derivatives moiety

Nikita V. Shtyrlin [a,**], Airat R. Kayumov [a], Maria N. Agafonova [a], Marsel R. Garipov [a], Alina E. Gatina [a], Mikhail V. Pugachev [a], Elena S. Bulatova [a], Denis Y. Grishaev [a], Alfiya G. Iksanova [a], Rail M. Khaziev [a], Ilnur M. Ganiev [a], Aleksandr M. Aimaletdinov [a], Oleg I. Gnezdilov [b], Yurii G. Shtyrlin [a,*]

[a] Kazan (Volga region) Federal University, Kremlyovskaya St. 18, Kazan, 420008, Russian Federation
[b] Kazan E. K. Zavoisky Physical-Technical Institute, Federal Research Center "Kazan Scientific Center of the Russian Academy of Sciences", 10/7 ul. Sibirskiy trakt, Kazan, 420029, Russian Federation

ARTICLE INFO

Handling Editor: Dr. Z Liu

Keywords:
Pyridoxine
Fluoroquinolone
Antibacterial activity
Toxicity
In vivo efficacy

ABSTRACT

We report herein the design, synthesis and biological evaluation of series of 7-substituted fluoroquinolones with pyridoxine derivatives. *In vitro* screening of antibacterial activity and toxicity of 39 synthesized fluoroquinolones defined compounds **7** and **28** as lead compounds for further investigations. On various clinical isolates lead compounds **7** and **28** exhibited antibacterial activity comparable with reference fluoroqinolones. Mutagenic effects haven't been observed for these compounds in SOS-chromotest. Compound **7** are non-toxic *in vivo* on mice (LD_{50} > 2000 mg/kg, oral) and rats (LD_{50} > 2000 mg/kg, oral). Compound **28** was more toxic (LD_{50} = 474 mg/kg, oral, mice). Moreover compound **7** showed greater *in vivo* efficacy compared to ciprofloxacin in a murine model of staphylococcal sepsis. Taken together the described active compound are promising candidate for preclinical trials.

1. Introduction

Nowadays, the increasing emergence of antibiotic resistance in pathogenic bacteria is one of the biggest problems of the healthcare worldwide [1,2]. Well-known bacteria from the ESKAPE group, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* remain the main pathogens leading to death driven with bacterial infection. Of particular danger to humans are methicillin-resistant strains of *S. aureus* (MRSA), which cause sepsis and severe infections of the skin and soft tissues [3].

One of the most effective classes of antimicrobial drugs used to the date are fluoroquinolones [4]. Most quinolones are bactericidal agents their mechanism of action is to disrupt the synthesis of bacterial cell DNA via inhibiting two crucial enzymes DNA gyrase and topoisomerase-IV [5]. Fluoroquinolones, approved for clinical use since the early 1980s (ciprofloxacin, norfloxacin, lomefloxacin, etc.), are distinguished by a wide spectrum of antimicrobial action against both gram-positive and gram-negative bacteria, as well as mycobacteria, and demonstrate good pharmacokinetic properties, which allows them to be used for the treatment of infections of various localization [6].

There are several synthetic approaches to obtain the new fluoroquinolone compounds with high antimicrobial activity. Many works in this area are based on the modification of the C-7 position of the quinolone ring, since numerous experimental studies of the structure-biological activity (SAR) relationship have shown that substituents in this position have the greatest effect on biological properties of compounds [4,6,7]. The most optimal substituents in this position are nitrogen-containing heterocycles piperazine and pyrrolidine. Thus, piperazine-containing fluoroquinolones have excellent pharmacokinetic properties and high activity against gram-negative microorganisms. In turn, pyrrolidine-containing fluoroquinolones are very active against gram-positive microorganisms, while have a number of disadvantages associated with low water solubility and low oral bioavailability.

One of the strategies for preparing of new fluoroquinolone compounds is the addition of various pharmacophore groups or natural compounds fragments to the molecule of a known quinolone drug. These

* Corresponding author.
** Corresponding author.
E-mail addresses: Nikita.Shtyrlin@kpfu.ru (N.V. Shtyrlin), Yurii.Shtyrlin@kpfu.ru (Y.G. Shtyrlin).

https://doi.org/10.1016/j.ejmech.2023.115798
Received 19 July 2023; Received in revised form 29 August 2023; Accepted 6 September 2023
Available online 7 September 2023

fluoroquinolone derivatives with flavonoids [8], aminoglycosides [9], 1-hydroxybisphosphonates [10], coumarins [11], 3-arylfuran-2 (5H)-ones [12], 1,2,4-triazole-5(4H)-thione [13], 1,2,3-triazoles [14], macrolides [15], benzothiazoles [16], oxazolidinones [17] and a number of others [18,19] have been described. In some cases, such modifications led to an increased antibacterial activity compared to the initial fluoroquinolone, a reduced toxicity, or the appearance of a new activity unusual for the initial quinolone.

One of the promising structures for the design of new physiologically active compounds is pyridoxine (vitamin B6) plays highly important roles in living cells as a key cofactor of many enzymes [20]. Its molecular scaffold is a valuable structural platform which has led to the development of several launched drugs (Pyritinol, Pirisudanol, Cycletanine, Mangafodipir) and a wide number of preclinical and clinical drug candidates. In our group, we have systematically studied chemistry and biological activity of pyridoxine derivatives [20]. Among them, a quaternary ammonium compounds, quaternary phosphonium compounds, sulfanilic acid derivatives have been described [21–24] (Fig. 2). Some of this compounds possess high antibacterial activity against various clinical Gram-positive and Gram-negative bacteria with minimal inhibitory concentration (MIC) of 0.5–16 μg/ml and low toxicity both *in vitro* and *in vivo*.

The present work describes the synthesis and biological evaluation a series of novel fluoroqinolones modified on the C-7 position of the quinolone ring by pyridoxine derivatives. The relationship between the structure of synthesized compounds and their antibacterial activity *in vitro* were investigated by variations of the quinolone and pyridoxine fragments, as well as substituents at the acetal carbon atom in the six-membered circle of pyridoxine. Identified lead compound inhibited growth of clinical Gram-positive and Gram-negative pathogens *in vitro* with MICs of 0.06–32 μg/ml, are non-toxic *in vivo* on mice (LD_{50} > 2000 mg/kg, oral) and rats (LD_{50} > 2000 mg/kg, oral) and showed greater then ciprofloxacin *in vivo* efficacy in a murine model of staphylococcal sepsis.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds was based on the alkylation of the piperazine nitrogen atom of fluoroquinolones by halo derivatives of pyridoxine. The synthesis of pyridoxine derivatives **2a-h**, **3**–**5** was carried out in 2–4 stages according to literature methods [22,23,25–27] (Fig. 1).

At first, compounds **6**, **8**, **10** and **12** were prepared via alkylation of commercial fluoroquinolones (ciprofloxacin, norfloxacin, lomefloxacin, moxifloxacin) by halo-derivatives of six-membered acetals of pyridoxine **2a-e** in DMF (Scheme 1). Further hydrolytic cleavage of the ketal protective group in compounds **6b**, **8b**, **10b**, **12b** under acidic conditions gave compounds **7, 9, 11, 13**.

Similarly, the reaction of bromo derivative **3** with fluoroquinolones were prepared compounds **14**, **16**, **18**, **20** (Scheme 2). Hydrolytic cleavage of the six-membered ketal cycles in acidic condition led to the derivatives **15**, **17**, **19** and **21** in 60–86% yields.

Also, by the reaction of chloro derivatives **3** and **4** with ciprofloxacin, norfloxacin, lomefloxacin, moxifloxacin in DMF at 60 °C, compounds **22**–**24** and **25**–**28** were synthesized (Schemes 3 and 4).

2.2. Primary screening of antibacterial activity in vitro

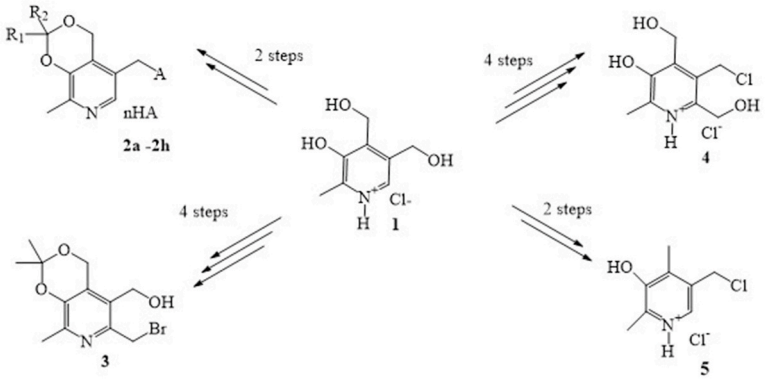
The antibacterial activity of synthesized compounds was evaluated on various strains of Gram-positive and Gram-negative bacteria. Table 1 shows the MIC values of compounds in comparison with ciprofloxacin, norfloxacin, lomefloxacin and moxifloxacin.

For six-membered pyridoxine acetals **6**, **8**, **10** and **12** containing fluoroquinolone fragments, the antibacterial activity were decreased with the increasing of alkyl chain length at acetal carbon atom (H,H > H, CH_3 ~ $2CH_3$ ~ H, C_3H_7 > H, C_8H_{17}). Compounds without acetal protection groups (**7**, **11, 9** and **13**) have an activity comparable to derivatives with hydrogen substituents at acetal carbon (**7a**, **11a, 9a** and **13a**).

In the case of pyridoxine derivatives containing fluoroquinolone fragments in the sixth position (**14**–**21**), there was no significant difference in the activity of compounds with a six-membered ketal ring and derivatives without protection of ketal groups. Pyridoxine derivatives containing ciprofloxacin fragments (**14** and **15**) or lomefloxacin fragments (**18** and **19**) in the sixth position were less active than derivatives at the fifth position **7** and **11**. In the case of norfloxacin, the activity of derivatives at the sixth position **16** and **17** exceeded the activity of the derivative at the fifth position **9**. In the case of moxifloxacin, the derivatives at the sixth position **20** and **21** had comparable activity with the derivatives at the fifth position.

Regioisomeric derivatives of 6-hydroxymethylpyridoxine (**22**–**24**), containing fluoroquinolone fragments in the fifth position, had activity comparable to derivatives at the sixth position (**15**, **17**, **19**, **21**) on gram-positive bacteria and higher activity on gram-negative ones.

Among all synthesized compounds, the series of compounds based on 4-deoxypyridoxine **25**–**28** showed the highest activity. Among them,



a) $R_1 = R_2 = H$, A=Cl, n=1; **b)** $R_1 = R_2 = CH_3$, A=Cl, n=1; **c)** $R_1 = CH_3$, $R_2 = H$, A=Br, n=0; **d)** $R_1 = n\text{-}C_3H_7$, $R_2 = H$, A=Br, n=0; **e)** $R_1 = n\text{-}C_8H_{17}$, $R_2 = H$, A=Br, n=0; **f)** $R_1 = CH_3$, $R_2 = H$, A=Cl, n=1; **g)** $R_1 = n\text{-}C_3H_7$, $R_2 = H$; A=Cl, n=1; **h)** $R_1 = n\text{-}C_8H_{17}$, $R_2 = H$, A=Cl, n=1.

Fig. 1. Synthesis of halo derivatives of pyridoxine **2**–**5**.

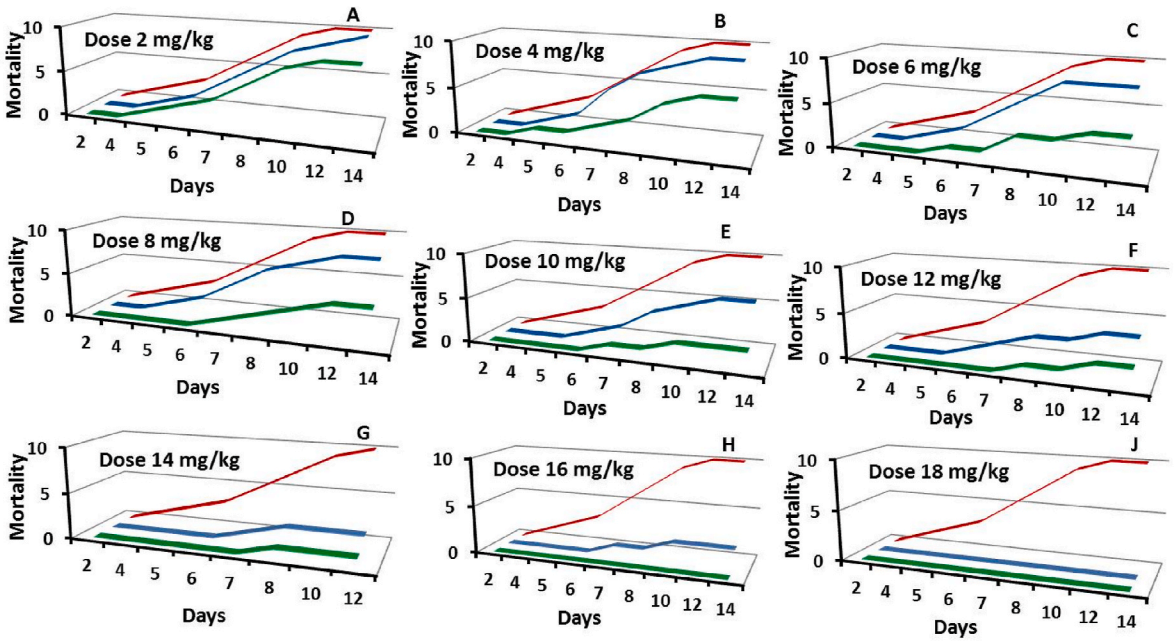
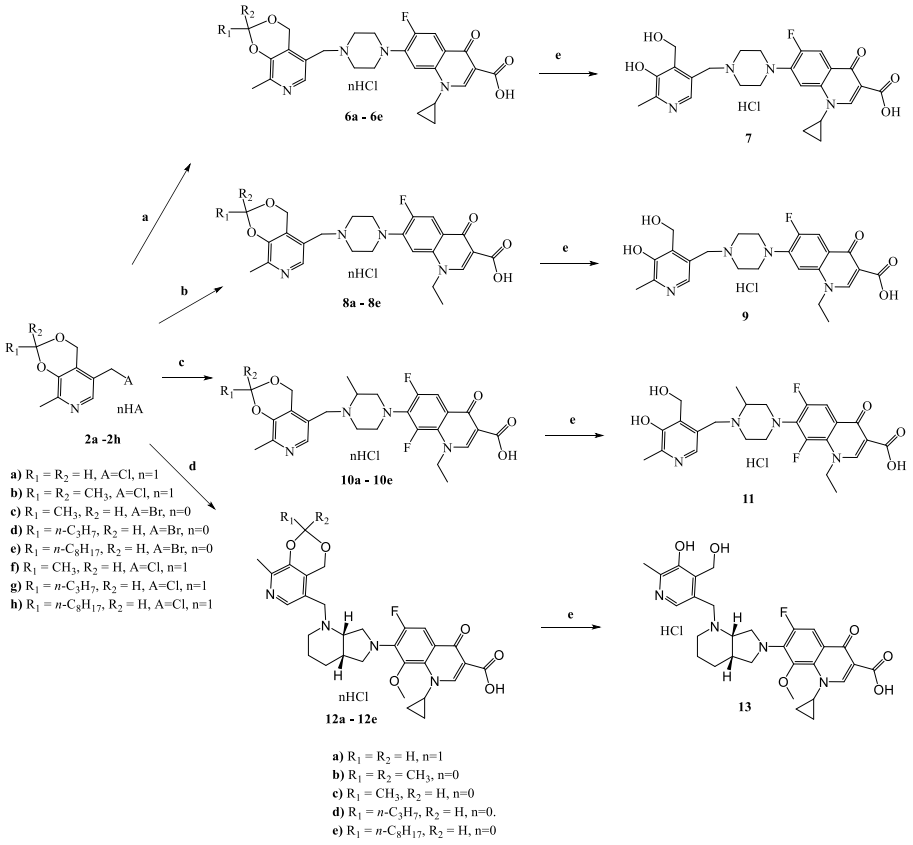
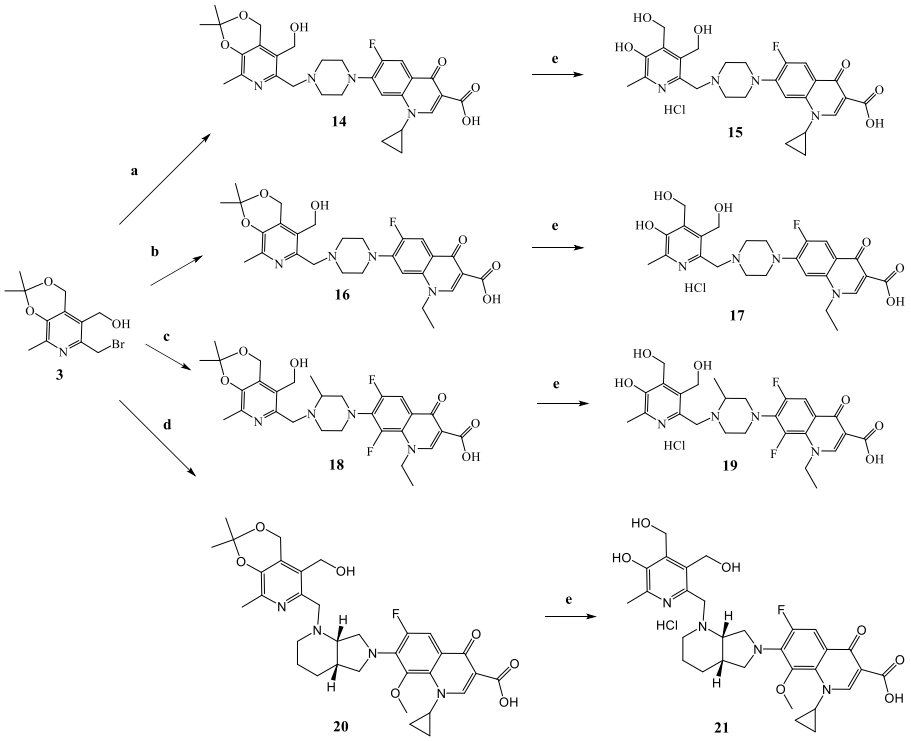


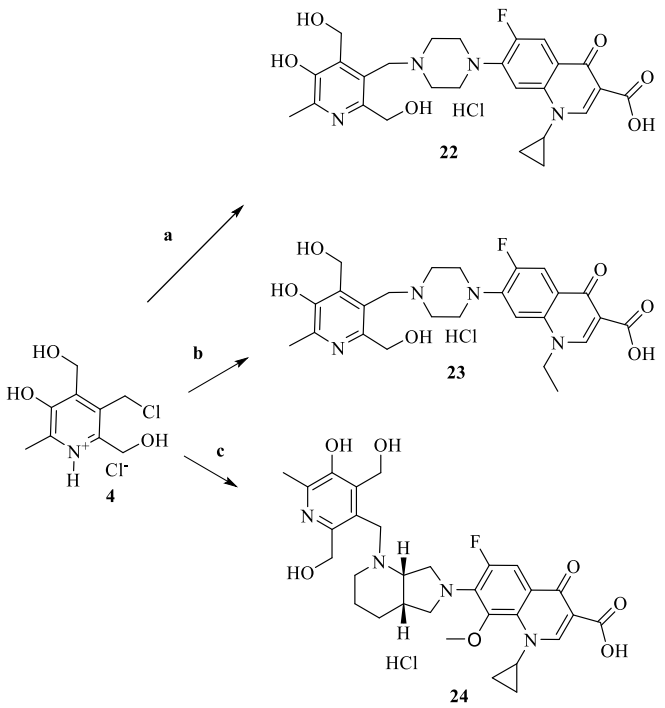
Fig. 2. *In vivo* compound **7** efficacy in comparison with ciprofloxacin in a staphylococcal sepsis (*S.aureus* №10) mouse model. X-axis of a plot – days; y-axis of a plot – mortality (number of dead animals); green line - compound **7**, blue line-ciprofloxacin, red line - control *S.aureus*.



Scheme 1. (a) DMF, ciprofloxacin hydrochloride, KI, $NaHCO_3$, 20 °C, 4 h; (b) DMF, norfloxacin hydrochloride, KI, $NaHCO_3$, 80 °C, 4 h; (c) DMF, lomefloxacin hydrochloride, KI, $NaHCO_3$, 80 °C, 4 h; (d) DMF, moxifloxacin hydrochloride, KI, $NaHCO_3$, 80 °C, 4 h; (e) H_2O, HCl, 25 °C, 24 h.



Scheme 2. (a) DMF, ciprofloxacin hydrochloride, KI, $NaHCO_3$, 20 °C, 4 h; (b) DMF, norfloxacin hydrochloride, KI, $NaHCO_3$, 80 °C, 4 h; (c) DMF, lomefloxacin hydrochloride, KI, $NaHCO_3$, 60 °C, 10 h; (d) DMF, moxifloxacin hydrochloride, KI, $NaHCO_3$, 80 °C, 4 h; (e) H_2O, HCl, 25 °C, 24 h.



Scheme 3. (a) DMF, ciprofloxacin hydrochloride, KI, $NaHCO_3$, 20 °C, 4 h; (b) DMF, norfloxacin hydrochloride, KI, $NaHCO_3$, 60 °C, 10 h; (c) DMF, moxifloxacin hydrochloride, KI, $NaHCO_3$, 60 °C, 10 h.

derivatives of ciprofloxacin **25** and moxifloxacin **28** turned out to be the most active, having activity comparable to that of moxifloxacin and ciprofloxacin.

Thus compounds **6a**, **7**, **8a**, **11**, **12a**, **12b**, **12d**, **13**, **16**, **20**, **21**, **22**, **24**, **25** and **28** with promising activities in the primary assay were selected for further in-depth investigation *in vitro*.

2.3. *In vitro antibacterial activity on clinical strains*

The antibacterial activity of **6a**, **7**, **8a**, **11**, **12a**, **12b**, **12d**, **13**, **16**, **20**, **21**, **22**, **24**, **25** and **28** was further studied on clinical isolates of various Gram-positive and Gram-negative bacteria. Ciprofloxacin, norfloxacin, lomefloxacin and moxifloxacin were used as reference drugs (Table 7).

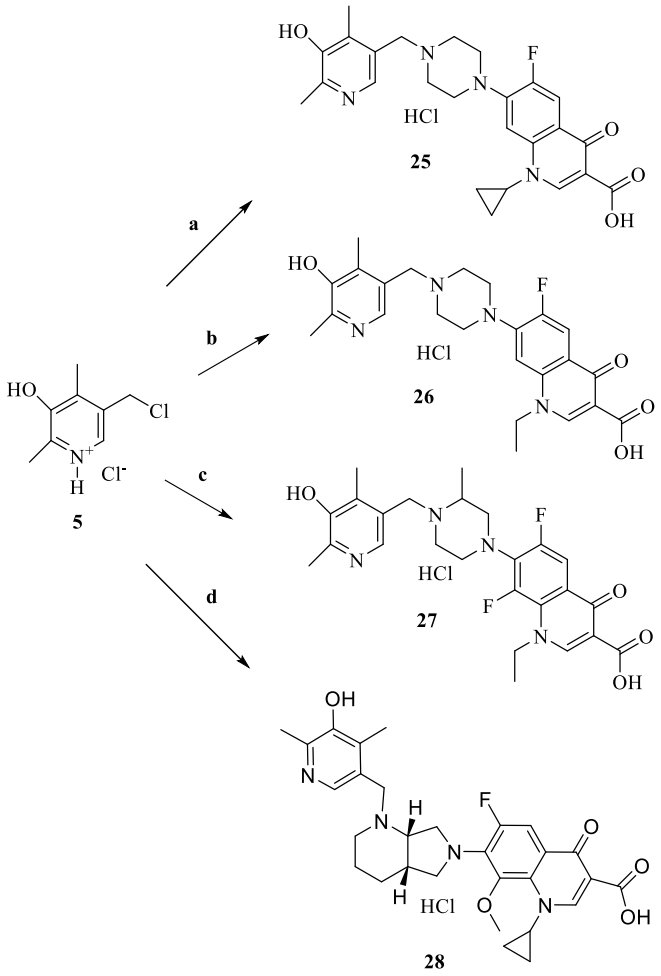
The most active compounds **6a**, **7**, **12a**, **13**, **21**, **24**, **25** and **28** exhibited antibacterial activity comparable with reference fluoroqinolones on Gram-positive bacteria, while being less active on Gram-negative ones.

2.4. *Cytotoxicity and genotoxicity*

Cytotoxicity of compounds **6a**, **7**, **8a**, **11**, **12a**, **12b**, **12d**, **13**, **16**, **20**, **21**, **22**, **24**, **25** and **28** was evaluated on human embryonic liver cells Chang-liver (CHL), human mesenchymal stem cells (MSC) and *primary human* skin *fibroblasts* (HSF*)* in comparison with fluoroquinolone antibacterials (Table 3). Compounds **6a**, **7**, **13**, **21**, **22**, **24** and **25** demonstrated the lowest cytotoxicity on all cell lines among the studied molecules. Their toxicity was comparable with references fluoroquinolones. All other compounds were more toxic.

Thus based on *in vitro* data of antibacterial activity and toxicity out of 39 synthesized fluoroquinolones containing a pyridoxine derivatives moiety compounds **7** and **28** were selected as lead compounds for further investigations.

Genotoxicity of compounds **7** and **28** was evaluated in Ames test. Since compounds demonstrated antibacterial activity on *S. typhymuriym* strains (data not shown), the spot-test modification of the Ames test has been used instead of classic technique. For that, the compound solution in water was dropped onto 5-mm whatmann disk placed onto agar surface. Neither **7** nor **28** led to the increase of revertants amount in the



Scheme 4. (a) DMF, ciprofloxacin hydrochloride, KI, $NaHCO_3$, 60 °C, 10 h; (b) DMF, norfloxacin hydrochloride, KI, $NaHCO_3$, 60 °C, 72 h; (c) DMF, lomefloxacin hydrochloride, KI, $NaHCO_3$, 60 °C, 72 h; (d) DMF, moxifloxacin hydrochloride, KI, $NaHCO_3$, 80 °C, 72 h.

region of non-lethal concentrations of compounds, allowing suggesting them as non-mutagenic in Ames test.

2.5. In vivo toxicity

Since *in vitro* tests revealed a relatively low toxicity of lead compounds **7** and **28**, acute oral toxicity on mice and rats of these compounds were performed. The study was carried out as described in section 4.5.

There was no death after single oral administration of compound **7** in dose 2000 mg/kg on mice and rats (LD_{50} > 2000 mg/kg, Tables 4 and 5). No remarkable pathological changes were found in the control and tests items groups during necropsy. All organs exhibited normal architecture similar to the control group.

Different picture was observed for compound **28**. Single oral administration of compound **28** at dose 2000 mg/kg to rats caused weakness with complete recovery by the 3rd day in males, and death of 2 females by the 2nd and 3rd day of observation (Table 6). Necropsy of compound **28** treated rat group revealed nephro and hepatotoxicity (darkening of liver and kidneys). All other organs exhibited normal architecture and color similar to the control group.

Compound **28** was more toxic on mice. After single oral administration of compound **28** in doses 2000 and 1000 mg/kg a high mortality (>80%) of animals was observed. Further the toxic effect decreased in the dose range of 500–125 mg/kg from 50% to 0 (Table 7). The autopsy of mice received compound **28** revealed pathology of liver and kidneys (change in consistency and color). No remarkable changes were noted in the control and test items (only at dose 125 mg/kg) groups at necropsy.

Thus, compound **7** was considerably less toxic for mice (LD_{50} >2000 mg/kg) than compound **28** (LD_{50} 406.3/540.8 mg/kg (male/female)). For rats, compound **7** was also to be safer. Therefore, compound **7** was chosen for the specific activity *in vivo* study.

2.6. In vivo antibacterial activity of compound 7

In vivo analysis of compound **7** efficacy in comparison with ciprofloxacin was carried out on a model of sepsis in SHK mice. Animals were infected by *S. aureus* № 10 strain intravenously (tail vain). Initially, the lowest load of *S. aureus* which is accompanied by 100% animals death (LD_{100}) was determined. The lowest value of LD_{100} was 750 million CFUs/mouse (Table 8).

To determine the comparative effectiveness of the test compound, mice (n = 10) were intravenously infected by LD_{100} of *S. aureus,* containing $7x10^8$ CFUs in a volume of 0.20 ml. Compound **7** and ciprofloxacin were administered orally at different doses (18; 16; 14; 12; 10; 8; 6; 4 and 2 mg/kg) 1 h after *S. aureus* infection. There was negative control group of untreated animals infected by *S. aureus* LD_{100}. From the obtained data (Fig. 2) it can be seen that the first cases of death were observed at doses 2 and 4 mg/kg on day 5 in the compound **7** group, the highest death were seen on days 8–14 after infection. Survival of 100% was observed at a doses 16 and 18 mg/kg. It should be noted that even at the minimum dose (2 mg/kg), 100% death of animals did not occur even by the 14th day of the experiment. ED_{50} for compound **7** was distinguished as 7.3 mg/kg.

The first cases of animal death in the group treated with ciprofloxacin were observed on day 4 at doses of 2–8 mg/kg, the highest death in this group occurred between 7 and 14 days after infection. Survival of 100% was observed at a dose of 18 mg/kg. The ED_{50} of ciprofloxacin determined as 11.2 mg/kg. Thus the ED_{50} of ciprofloxacin is 1.5 times higher than the ED_{50} of compound **7**, assuming 1.5-fold lower efficacy of ciprofloxacin. In the negative control group, the animal death was noted already from the 4th day and 100% death registered by the 10-12th day.

2.7. Unraveling the mechanism of antibacterial activity of 7 and 28

Since DNA gyrase and topoisomerase-IV are the known targets for quinolones, the ability of **7** and **28** to inhibit the activity of these enzymes has been evaluated *in vitro* on recombinant proteins (see Figs. S3–S8 in supplementary). The IC_{50} values for each compound in compare with reference drug are shown in Table 9.

Both compounds demonstrated the ability to inhibit both DNA gyrase and topoisomerase-IV and the mechanism of their antibacterial activity apparently is similar to other quinolones. As could be seen from Tables 9 and **7** seem to inhibit more efficiently the DNA cleavage activity DNA gyrase, and is less effective against topoisomerase IV. By contrast, **28** exhibited abilities to repress both enzymes similar to moxifloxacin. These data fit with *in vitro* activity data, see Tables 1 and 2.

3. Conclusion

In conclusion, a diverse library of 39 new 7-substituted fluoroquinolones with pyridoxine derivatives moieties was synthesized and *in vitro* primary screening against seven Gram-positive and Gram-negative bacterial strains was performed. 15 compounds exhibiting promising antibacterial activity were selected for further in-depth investigation. The evaluation of their antibacterial activity on various Gram-positive and Gram-negative clinical isolates and cytotoxicity studies on CHL, MSK and HSF cells demonstrated that two lead compounds **7** and **28** had comparable toxicity and activity with reference fluoroqinolones. Mutagenic effects have't been observed for these two lead compounds in Ames test. Compounds **7** and **28** demonstrated the

Table 1
Antibacterial activity of fluoroquinolones containing a pyridoxine derivatives moiety.

Compound	MICs (μg/ml)						
	Gram-positive bacteria				Gram-negative bacteria		
	M. luteus	*B. subtilis*	*S. aureus*	*S. epidermidis*	*P. aerugenosa*	*E. coli*	*S. typhimurium*
6a	16	2	1	0.5	32	8	0.5
6b	32	2	32	16	>64	64	4
6c	8	2	8	8	>64	>64	4
6d	64	2	64	64	>64	>64	4
6e	64	2	32	64	>64	>64	4
7	4	2	2	2	>64	2	2
8a	4	2	2	2	>64	4	2
8b	>64	64	>64	>64	>64	64	>64
8c	64	8	32	32	>64	>64	16
8d	64	2	16	32	>64	>64	2
8e	>64	64	>64	>64	>64	>64	>64
9	32	4	16	32	>64	64	4
10a	32	4	8	16	>64	16	4
10b	8	2	16	64	>64	>64	2
10c	>64	2	2	8	16	>64	2
10d	16	2	16	8	>64	>64	2
10e	>64	>64	>64	>64	>64	>64	>64
11	0.5	0.5	2	1	4	8	2
12a	8	2	2	16	64	2	2
12b	4	2	2	2	>64	>64	4
12c	4	1	1	4	>64	16	4
12d	2	2	2	2	>64	>64	4
12e	16	8	16	32	>64	>64	16
13	4	2	2	2	>64	>64	4
14	8	2	8	8	>64	64	4
15	32	2	64	32	>64	64	4
16	4	2	2	8	>64	>64	2
17	8	4	8	32	>64	>64	16
18	>64	4	64	16	>64	8	2
19	64	2	2	64	>64	2	2
20	2	2	2	4	>64	>64	2
21	2	2	2	2	>64	>64	2
22	2	4	0.5	8	>64	64	4
23	16	8	8	16	>64	8	4
24	2	2	2	2	>64	2	2
25	0.5	0.5	0.5	0.5	32	1	1
26	8	2	4	16	>64	2	64
27	32	1	2	>64	>64	2	2
28	0.5	0.5	0.5	0.5	1	2	1
Ciprofloxacin	0.5	0.5	0.5	0.5	1	1	1
Norfloxacin	8	1	2	1	4	1	1
Lomefloxacin	1	0.5	0.5	2	4	1	1
Moxifloxacin	0.5	2	0.5	0.5	4	1	1

ability to inhibit DNA gyrase and topoisomerase-IV and the mechanism of their antibacterial activity apparently is similar to other quinolones. *In vivo* toxicity studies on rats and mice showed that compound **7** are non-toxic on mice ($LD_{50} > 2000$ mg/kg, oral) and rats ($LD_{50} > 2000$ mg/kg, oral). Compound **28** was more toxic ($LD_{50} = 474$ mg/kg, oral, mice). Therefore, compound **7** was chosen for the *in vivo* study of specific activity. In this experiment compound **7** showed greater *in vivo* efficacy compared to ciprofloxacin in a murine model of staphylococcal sepsis. Taken together, our data allow to suggest the described active compounds are promising candidate for preclinical trials.

4. Experimental section

4.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a "Bruker AVANCE 400" at operating frequency 400 and 101.56 MHz, respectively. Chemical shifts were measured with reference to the residual protons of the solvent (DMSO-d_6, ^{1}H, 2.50 ppm, ^{13}C, 39.52 ppm; $CDCl_3$, ^{1}H, 7.26 ppm, ^{13}C, 77.16 ppm). Coupling constants (*J*) are given in Hertz (Hz). The following abbreviations are used to describe coupling: s = singlet; d = doublet; t = triplet; m = multiplet; br s = broad singlet, br m = broad multiplet, AB = AB system. Melting points were determined using a Stanford Research Systems MPA-100 OptiMelt melting point apparatus and are uncorrected. For thin layer chromatography analysis, silica gel plates from Sorbfil (Krasnodar, Russia) were used with UV light (254 nm/365 nm) or iron (III) chloride as developing agent. Column chromatography was performed on silica gel (60–200 mesh) from Acros or reversed-phase chromatography on PF-15C18HP column from Interchim.

High-resolution (HRMS) mass spectra were obtained on a quadrupole time-of-flight (qTOF) AB Sciex Triple TOF 5600 mass spectrometer using turbo-ion spray source (nebulizer gas nitrogen, a positive ionization polarity, needle voltage 5500 V). Recording of the spectra was performed in "TOF MS" mode with collision energy 10 eV, declustering potential 100 eV and with resolution more than 30 000 full-width half-maximum. Samples with the analyte concentration 5 μmol/l were prepared by dissolving the test compounds in a mixture of methanol (HPLC-UV Grade, LabScan) and water (LC-MS Grade, Panreac) in 1:1 ratio.

Analytical reversed-phase HPLC was used for determination of uncalibrated purity of the compounds **7** and **28** and conducted using a Atlantis T3 C18 column (5 μm, 150*4.6 mm); eluent A, 1.2% solution of triethylamine in water; eluent B CH_3CN; gradient elution (0 min A:B = 85:15; 15 min A:B = 65:35; 21 min A:B = 65:35) flow rate was 1.0 mL/min. HPLC analysis was performed at 40 °C during 21 min at 311 nm.

Table 2
In vitro antimicrobial activity of **6a**, **7**, **8a**, **11**, **12a**, **12b**, **12d**, **13**, **16**, **20**, **21**, **22**, **24**, **25**, **28** on an extended panel of clinical bacterial pathogens.

Strains	MICs (μg/ml)																
	6a	7	8a	11	12a	12b	12d	13	16	20	21	22	24	25	28	Cy	No	Lo	Mo
Gram-positive bacteria																			
Staphylococcus auricularis NKC 7	0.03	0.06	0.03	0.13	0.03	0.03	0.13	0.03	0.25	0.03	0.03	0.25	0.06	0.06	0.03	0.03	0.13	0.06	0.03
Staphylococcus haemolyticus NKC 31/2	8	8	8	16	1	32	64	8	32	32	8	16	8	16	8	4	8	8	1
Staphylococcus epidermidis NKC 37/1	0.25	0.5	2	1	0.03	1	4	0.06	4	2	0.25	1	0.25	0.5	0.06	1	4	2	0.13
Staphylococcus aureus 1812	0.25	1	2	1	0.06	1	4	0.25	8	2	0.5	4	0.5	1	0.25	1	2	2	0.13
Staphylococcus aureus 4721	0.13	0.5	1	1	0.03	0.5	2	0.13	4	1	0.5	2	0.25	0.5	0.06	1	1	1	0.06
Staphylococcus aureus MRSA 4771	2	1	1	1	0.03	4	4	0.13	4	1	0.25	2	8	0.5	0.06	0.5	1	1	4
Staphylococcus haemolyticus 1464	0.13	1	1	1	0.03	0.5	2	0.13	4	1	0.5	4	0.25	0.5	0.06	0.5	2	0.5	0.06
Enterococcus faecalis 1465	8	32	32	8	1	32	64	1	>64	16	4	32	4	16	4	32	>64	>64	4
Gram-negative bacteria																			
Escherichia coli NKC 1	2	0.5	0.13	2	4	4	4	4	16	8	2	0.5	4	0.13	2	0.06	0.06	0.06	0.06
Pseudomonas aeruginosa NKC 17	32	16	2	>64	>64	>64	>64	64	64	>64	32	16	32	16	64	0.13	0.25	1	1
Cedecea davisae 1813	0.03	1	0.25	2	0.03	0.5	2	0.13	2	0.5	0.25	1	0.5	0.5	0.03	0.25	1	0.25	0.03
Klebsiella pneumoniae 1678	16	8	4	>64	>64	32	>64	>64	>64	64	64	8	64	16	64	0.5	1	1	1
Enterobacter aerogenes NKC 8	16	2	1	8	>64	64	64	8	32	16	4	2	8	1	16	0.25	1	1	0.5

Table 3
Cytotoxicity (CC_{50}, μg/mL mean ± SD) of novel pyridoxine-based fluoroquinolones.

Compound	CC_{50}, μg/mL		
	CHL	HSF	MSC
6a	14.94 ± 1.22	40.26 ± 3.59	69.51 ± 4.74
7	30.95 ± 4.40	34.54 ± 2.31	40.33 ± 4.50
8a	9.78 ± 0.45	23.40 ± 2.08	21.17 ± 2.21
11	7.85 ± 1.72	22.31 ± 2.87	13.12 ± 1.79
12a	2.34 ± 0.29	5.80 ± 0.43	9.62 ± 1.45
12b	2.85 ± 0.34	3.44 ± 0.44	3.84 ± 0.42
12d	2.46 ± 0.42	2.34 ± 0.07	2.91 ± 0.67
13	18.59 ± 1.67	56.69 ± 4.74	48.39 ± 5.74
16	7.05 ± 0.09	24.86 ± 1.61	16.24 ± 2.19
20	6.41 ± 0.71	11.29 ± 1.12	7.26 ± 0.99
21	24.19 ± 2.24	33.85 ± 2.51	31.73 ± 4.12
22	31.84 ± 2.38	37.95 ± 3.10	52.59 ± 4.10
24	17.59 ± 2.67	35.19 ± 3.24	20.57 ± 2.67
25	14.24 ± 1.65	27.62 ± 0.90	25.12 ± 2.04
28	8.22 ± 1.07	17.65 ± 1.57	12.68 ± 1.14
Ciprofloxacin	29.34 ± 2.16	52.84 ± 4.05	41.62 ± 3.58
Norfloxacin	38.15 ± 4.11	38.15 ± 3.69	29.34 ± 2.16
Lomefloxacin	31.29 ± 3.34	46.16 ± 1.79	35.74 ± 2.11
Moxifloxacin	34.99 ± 3.52	36.46 ± 2.35	39.41 ± 3.20

Table 4
Data of compound **7** acute oral toxicity on mice.

Sex	Dose (mg/kg)	Dead/total animals	LD_{50}, mg/kg
Male	2000	0/6	>2000
Female	2000	0/6	

Table 5
Data of compound **7** acute oral toxicity on rats.

Sex	Dose (mg/kg)	Dead/total animals	LD_{50}, mg/kg
Male	2000	0/6	>2000
Female	2000	0/6	

Table 6
Data of compound **28** acute oral toxicity on rats.

Sex	Dose (mg/kg)	Dead/total animals	LD_{50}, mg/kg
Male	2000	0/6	>2000
Female	2000	2/6	

Table 7
Data of compound **28** acute oral toxicity on mice.

Sex	Dose (mg/kg)	Dead/total animals	LD_{50}, mg/kg
Male	2000	6/6	406.3
	1000	6/6	
	500	3/6	
	250	3/6	
	125	0/6	
Female	2000	5/6	540.8
	1000	6/6	
	500	2/6	
	250	1/6	
	125	0/6	

4.1.1. General procedure for synthesis of fluoroquinolones from halogenated pyridoxine derivatives

To a solution of pyridoxine derivative (1.1 equiv) in 30 ml of DMF was added sequentially fluoroquinolone (1.0 equiv), $NaHCO_3$ (2.0–3.1 equiv) and KI (0.2 equiv) at 20 °C.

Table 8
Determination of *S. aureus* № 10 LD_{100} on SHK mice[a].

Dose *S.aureus* (CFU[b]/mice)	Days (dead/surviving animals)										Dead (%)
	1	2	3	4	5	6	7	8	9	10	
$5x10^8$	0/10	0/10	0/10	2/8	4/6	5/5	5/5	5/5	5/5	5/5	50
$6x10^8$	0/10	0/10	1/9	2/8	3/7	5/5	6/4	7/3	8/2	8/2	80
$7x10^8$	0/10	0/10	2/8	3/7	5/5	6/4	7/3	9/1	10/0	10/0	100
$8x10^8$	0/10	1/9	2/8/	3/7	6/4	8/2	9/1	9/1	10/0	10/0	100
Intact mice	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0

[a] The study was carried out at the FSBI Gause Institute of New Antibiotics.
[b] CFU - colony-forming units.

Table 9
The impact of **7** and **28** on activities of DNA Gyrase and Topoisomerase IV. The IC_{50} values (μg/mL) are shown.

Compound	DNA Gyrase DNA cleavage activity	DNA Gyrase DNA supercoiling activity	Topoisomerase IV DNA decatenation activity
7	31 ± 2.3	760 ± 15.5	206 ± 6.6
Ciprofloxacin	80 ± 4.2	391 ± 14.4	41 ± 1.5
28	>400	52 ± 6.1	16 ± 2.2
Moxifloxacin	>400	40 ± 2.8	12 ± 1.8

The reaction mixture was stirred at 80 °C (for compounds **6a-6e**, **14**, **22**–20 °C; for compounds **18**, **23**, **24**–**27** – 60 °C) for 4 h (for compounds **18**, **23**, **25**–10 h; for compounds **26**–**28** – 72 h). Then the solvent was evaporated under reduced pressure. After several different workup procedures were used.

*4.1.1.1. 1-Cyclopropyl-6-fluoro-7-(4-((8-methyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(6a)**.* The reaction was carried out following the general procedure with compound **2a** (0.50 g, 2.11 mmol), ciprofloxacin hydrocloride (0.71 g, 1.93 mmol), $NaHCO_3$ (0.50 g, 5.95 mmol) and KI (0.06 g, 0.39 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and then was washed with acetone. The resulting precipitate was suspended in water then equimolar amount of 0.1 M HCl solution added and the solvent was removed in vacuo. Yield 34% (0.35 g); light yellow solid; mp 242–246 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.16–1.19 (m, 2H, CH_2 cyclopropyl), 1.29–1.33 (m, 2H, CH_2 cyclopropyl), 2.32 (s, 3H, CH_3), 2.53–2.63 (m, 4H, $2CH_2$ piperazinyl), 3.24–3.39 (m, 4H, $2CH_2$ piperazinyl), 3.47 (s, 2H, CH_2N), 3.77–3.83 (m, 1H, CH cyclopropyl), 5.06 (s, 2H, CH_2), 5.34 (s, 2H, CH_2), 7.55 (d, 1H, $J_{H\text{-}F}$ = 7.4 Hz, CH_{Ar}), 7.89 (d, 1H, $J_{H\text{-}F}$ = 13.2 Hz, CH_{Ar}), 7.92 (s, 1H, CH_{Ar}), 8.65 (s, 1H, CH_{Ar}), 15.19 (br s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 7.50 (s, $2CH_2$ cyclopropyl), 18.17 (s, CH_3), 35.79 (s, CH cyclopropyl), 49.43 (s, CH_2), 51.96 (s, CH_2), 56.18 (s, CH_2), 63.57 (s, CH_2), 90.79 (s, CH_2), 106.39 (s, C_{Ar}), 106.72 (s, C_{Ar}), 110.86 (d, $J_{C\text{-}F}$ = 23.1 Hz, C_{Ar}), 118.59 (d, $J_{C\text{-}F}$ = 7.5 Hz, C_{Ar}), 127.53 (s, C_{Ar}), 128.60 (s, C_{Ar}), 139.13 (s, C_{Ar}), 140.80 (s, C_{Ar}), 145.13 (d, $J_{C\text{-}F}$ = 9.6 Hz, C_{Ar}), 145.56 (s, C_{Ar}), 146.91 (s, C_{Ar}), 147.93 (s, C_{Ar}), 152.99 (d, $J_{C\text{-}F}$ = 249.4 Hz, C_{Ar}), 165.85 (s, C(O)OH), 176.31 (s, C═O). ESI-HRMS *m/z*: $[M\text{-}Cl]^+$ 495.2041 (calculated for $[C_{26}H_{28}FN_4O_5]^+$ - 495.2038).

*4.1.1.2. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-((2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **(6b)**.* The reaction was carried out following the general procedure with compound **2b** (1.38 g, 5.30 mmol), ciprofloxacin hydrocloride (1.77 g, 4.82 mmol), $NaHCO_3$ (1.25 g, 14.94 mmol) and KI (0.16 g, 0.96 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was recrystallized from the mixture acetone: water = 2:1. Yield 47% (1.27 g); light yellow solid; mp 243 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.14–1.18 (m, 2H, CH_2 cyclopropyl), 1.32–1.37 (m, 2H, CH_2 cyclopropyl), 1.53 (s, 6H, $2CH_3$), 2.36 (s, 3H, CH_3), 2.61 (br m, 4H, $2CH_2$ piperazinyl), 3.29 (br m, 4H, $2CH_2$ piperazinyl), 3.44 (s, 2H, CH_2N), 3.50–3.56 (m, 1H, CH cyclopropyl), 5.02 (s, 2H, CH_2), 7.32 (d, 1H, $J_{H\text{-}F}$ = 7.1 Hz, CH_{Ar}), 7.92 (s, 1H, CH_{Ar}), 7.96 (d, 1H, $J_{H\text{-}F}$ = 13.1 Hz, CH_{Ar}), 8.72 (s, 1H, CH_{Pyr}), 14.99 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 8.35 (s, $2CH_2$ cyclopropyl), 18.53 (s, CH_3), 24.86 (s, $2CH_3$), 35.40 (s, CH cyclopropyl), 49.95 (d, $J_{C\text{-}F}$ = 4.7 Hz, CH_2), 52.69 (s, CH_2), 57.55 (s, CH_2), 58.88 (s, CH_2), 99.79 (s, $\underline{C}(CH_3)_2$), 104.91 (d, $J_{C\text{-}F}$ = 3.0 Hz, C_{Ar}), 108.22 (s, C_{Ar}), 112.52 (d, $J_{C\text{-}F}$ = 23.5 Hz, C_{Ar}), 119.93 (d, $J_{C\text{-}F}$ = 7.8 Hz, C_{Ar}), 126.43 (C_{Ar}), 126.72 (s, C_{Ar}), 139.17 (s, C_{Ar}), 140.42 (s, C_{Ar}), 145.92 (d, $J_{C\text{-}F}$ = 10.2 Hz, C_{Ar}), 146.30 (s, C_{Ar}), 147.53 (s, C_{Ar}), 147.70 (s, C_{Ar}), 153.78 (d, $J_{C\text{-}F}$ = 251.6 Hz, C_{Ar}), 167.12 (s, C(O)OH), 177.21 (s, C═O). ESI-HRMS *m/z*: $[M+H]^+$ 523.2357 (calculated for $[C_{28}H_{32}FN_4O_5]^+$ - 523.2351).

*4.1.1.3. 1-Cyclopropyl-7-(4-((2,8-dimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6c)**.* The reaction was carried out following the general procedure with compound **2c** (0.38 g, 1.47 mmol), ciprofloxacin hydrocloride (0.49 g, 1.34 mmol), $NaHCO_3$ (0.24 g, 2.81 mmol) and KI (0.04 g, 0.27 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was recrystallized from the mixture acetone: water = 2:1. Yield 49% (0.33 g); light yellow solid; mp 233 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.17–1.20 (m, 2H, CH_2 cyclopropyl), 1.33–1.38 (m, 2H, CH_2 cyclopropyl), 1.58 (d, 3H, $J_{H\text{-}H}$ = 5.1 Hz, CH_3), 2.41 (s, 3H, CH_3), 2.61–2.63 (m, 4H, $2CH_2$ piperazinyl), 3.28–3.31 (m, 4H, $2CH_2$ piperazinyl), 3.43, 3.46 (AB, 2H, $J_{H\text{-}H}$ = 13.4 Hz, CH_2N), 3.49–3.53 (m, 1H, CH cyclopropyl), 5.02, 5.08 (AB, 2H, $J_{H\text{-}H}$ = 16.2 Hz, CH_2), 5.18 (q, 1H, $J_{H\text{-}H}$ = 5.1 Hz, CH), 7.30 (d, 1H, $J_{H\text{-}F}$ = 7.0 Hz, CH_{Ar}), 7.89 (d, 1H, $J_{H\text{-}F}$ = 13.1 Hz, CH_{Ar}), 7.92 (s, 1H, CH_{Ar}), 8.67 (s, 1H, CH_{Ar}), 14.95 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 8.30 (s, $2CH_2$ cyclopropyl), 18.43 (s, CH_3), 20.76 (s, CH_3), 35.39 (s, CH cyclopropyl), 49.91 (d, $J_{C\text{-}F}$ = 4.7 Hz, CH_2), 52.62 (s, CH_2), 57.59 (s, CH_2), 64.49 (s, CH_2), 97.21 (s, CH), 104.91 (d, $J_{C\text{-}F}$ = 3.0 Hz, C_{Ar}), 108.09 (s, C_{Ar}), 112.34 (d, $J_{C\text{-}F}$ = 23.5 Hz, C_{Ar}), 119.78 (d, $J_{C\text{-}F}$ = 7.8 Hz, C_{Ar}), 126.82 (s, C_{Ar}), 128.08 (s, C_{Ar}), 139.13 (s, C_{Ar}), 141.02 (s, C_{Ar}), 145.88 (d, $J_{C\text{-}F}$ = 10.4 Hz, C_{Ar}), 147.26 (s, C_{Ar}), 147.43 (s, C_{Ar}), 148.02 (s, C_{Ar}), 153.73 (d, $J_{C\text{-}F}$ = 251.6 Hz, C_{Ar}), 167.00 (s, C(O)OH), 177.08 (s, C═O). ESI-HRMS *m/z*: $[M+H]^+$ 509.2200 (calculated for $[C_{27}H_{30}FN_4O_5]^+$ - 509.2195).

*4.1.1.4. 1-Cyclopropyl-6-fluoro-7-(4-((8-methyl-2-propyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6d)**.* The reaction was carried out following the general procedure with compound **2d** (1.51 g, 5.27 mmol), ciprofloxacin hydrocloride (1.76 g, 4.79 mmol), $NaHCO_3$ (0.85 g, 10.1 mmol) and KI (0.16 g, 0.96 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was recrystallized from the mixture acetone: water = 2:1. Yield 26% (0.67 g); light yellow solid; mp 237–239 °C. ^{1}H NMR ($CDCl_3$) δ: 0.99 (t, 3H, J_{HH} = 7.4 Hz, CH_3), 1.13–1.19 (m, 2H, CH_2 cyclopropyl), 1.34–1.37 (m, 2H, CH_2 cyclopropyl), 1.49–1.60 (m, 2H, CH_2), 1.77–1.89

(m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.60–2.62 (m, 4H, $2CH_2$ piperazinyl), 3.27–3.29 (m, 4H, $2CH_2$ piperazinyl), 3.41, 3.45 (AB, 2H, $J_{H\text{-}H}$ = 13.2 Hz, CH_2N), 3.52 (br m, 1H, CH cyclopropyl), 5.00, 5.08 (AB, 2H, $J_{H\text{-}H}$ = 16.2 Hz, CH_2), 5.02 (q, 1H, $J_{H\text{-}H}$ = 5.2 Hz, CH), 7.29 (d, 1H, $J_{H\text{-}F}$ = 7.0 Hz, CH_{Ar}), 7.84 (d, 1H, $J_{H\text{-}F}$ = 13.1 Hz, CH_{Ar}), 7.90 (s, 1H, CH_{Ar}), 8.63 (s, 1H, CH_{Ar}), 14.94 (br s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 8.26 (s, $2CH_2$ cyclopropyl), 13.99 (s, CH_3), 17.07 (s, CH_2), 18.29 (s, CH_3), 35.39 (s CH, cyclopropyl), 36.43 (s, CH_2), 49.85 (d, $J_{C\text{-}F}$ = 4.7 Hz, CH_2), 52.59 (s, CH_2), 57.56 (s, CH_2), 64.54 (s, CH_2), 99.92 (s, CH), 104.90 (d, $J_{C\text{-}F}$ = 2.9 Hz, C_{Ar}), 107.96 (s, C_{Ar}), 112.20 (d, $J_{C\text{-}F}$ = 23.4 Hz, C_{Ar}), 119.63 (d, $J_{C\text{-}F}$ = 7.8 Hz, C_{Ar}), 126.89 (s, C_{Ar}), 128.37 (s, C_{Ar}), 139.09 (s, C_{Ar}), 140.80 (s, C_{Ar}), 145.84 (d, $J_{C\text{-}F}$ = 10.3 Hz, C_{Ar}), 147.19 (s, C_{Ar}), 147.37 (s, C_{Ar}), 148.07 (s, C_{Ar}), 153.68 (d, $J_{C\text{-}F}$ = 251.7 Hz, C_{Ar}), 166.95 (s, C(O)OH), 176.99 (d, $J_{C\text{-}F}$ = 2.0 Hz, C═O). ESI-HRMS *m/z*: $[M+H]^+$ 537.2513 (calculated for $[C_{29}H_{34}FN_4O_5]^+$ - 537.2508).

*4.1.1.5. 1-Cyclopropyl-6-fluoro-7-(4-((8-methyl-2-octyl-4H-[1,3]dioxino [4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6e)**.* The reaction was carried out following the general procedure with compound **2e** (1.20 g, 3.38 mmol), ciprofloxacin hydrocloride (1.13 g, 3.07 mmol), $NaHCO_3$ (0.54 g, 6.44 mmol) and KI (0.10 g, 0.61 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was recrystallized from the mixture acetone: water = 2:1. Yield 54% (1.01 g); yellow solid; mp 89–93 °C. ^{1}H NMR ($CDCl_3$) δ: 0.86 (t, 3H, J_{HH} = 6.8 Hz, CH_3), 1.15–1.38 (m, 14H, $7CH_2$), 1.48–1.54 (m, 2H, CH_2), 1.82–1.90 (m, 2H, CH_2), 2.41 (s, 3H, CH_3), 2.61–2.63 (m, 4H, $2CH_2$ piperazinyl), 3.29–3.33 (m, 4H, $2CH_2$ piperazinyl), 3.41, 3.45 (AB, 2H, $J_{H\text{-}H}$ = 13.2 Hz, CH_2N), 3.52 (br m, 1H, CH cyclopropyl), 5.01, 5.09 (AB, 2H, $J_{H\text{-}H}$ = 16.2 Hz, CH_2), 5.12 (q, 1H, $J_{H\text{-}H}$ = 5.2 Hz, CH), 7.30 (d, 1H, $J_{H\text{-}F}$ = 7.0 Hz, CH_{Ar}), 7.88 (d, 1H, $J_{H\text{-}F}$ = 13.1 Hz, CH_{Ar}), 7.91 (s, 1H, CH_{Ar}), 8.66 (s, 1H, CH_{Ar}), 14.95 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ: 8.29 (s, $2CH_2$ cyclopropyl), 14.18 (s, CH_3), 18.39 (s, CH_3), 22.73 (s, CH_2), 23.70 (s, CH_2), 29.28 (s, CH_2), 29.46 (s, CH_2), 29.55 (s, CH_2), 31.93 (s, CH_2), 34.44 (s, CH_2), 35.39 (s, CH cyclopropyl), 49.90 (d, $J_{C\text{-}F}$ = 4.6 Hz, CH_2), 52.62 (s, CH_2), 57.60 (s, CH_2), 64.58 (s, CH_2), 100.13 (s, CH), 104.89 (d, $J_{C\text{-}F}$ = 2.9 Hz, C_{Ar}), 108.06 (s, C_{Ar}), 112.32 (d, $J_{C\text{-}F}$ = 23.4 Hz, C_{Ar}), 119.75 (d, $J_{C\text{-}F}$ = 7.7 Hz, C_{Ar}), 126.82 (s, C_{Ar}), 128.28 (s, C_{Ar}), 139.12 (s, C_{Ar}), 140.93 (s, C_{Ar}), 145.88 (d, $J_{C\text{-}F}$ = 10.3 Hz, C_{Ar}), 147.30 (s, C_{Ar}), 147.42 (s, C_{Ar}), 148.08 (s, C_{Ar}), 153.72 (d, $J_{C\text{-}F}$ = 251.6 Hz, C_{Ar}), 167.00 (s, C(O)OH), 177.04 (d, $J_{C\text{-}F}$ = 2.5 Hz, C═O). ESI-HRMS *m/z*: $[M+H]^+$ 607.3296 (calculated for $[C_{34}H_{44}FN_4O_5]^+$ - 607.3290).

*4.1.1.6. 1-Ethyl-6-fluoro-7-(4-((8-methyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(8a)**.* The reaction was carried out following the general procedure with compound **2a** (0.50 g, 2.13 mmol), norfloxacin hydrocloride (0.64 g, 1.94 mmol), $NaHCO_3$ (0.50 g, 5.95 mmol) and KI (0.06 g, 0.39 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and then was washed with acetone. The resulting precipitate was suspended in water then equimolar amount of 0.1 M HCl solution added and the solvent was removed in vacuo. Yield 36% (0.36 g); gray solid; mp 178–182 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.41 (t, 3H, $J_{H\text{-}H}$ = 7.1 Hz, CH_3), 2.50 (s, 3H, CH_3), 3.21–3.97 (m, 8H, $4CH_2$), 4.36 (br s, 2H, CH_2), 4.62 (q, 2H, $J_{H\text{-}H}$ = 7.0 Hz, CH_2), 5.34 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 7.26 (d, 1H, $J_{H\text{-}F}$ = 7.3 Hz, CH_{Ar}), 7.98 (d, 1H, $J_{H\text{-}F}$ = 13.1 Hz, CH_{Ar}), 8.59 (s, 1H, CH_{Ar}), 8.97 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6) δ: 14.48 (s, CH_3), 42.41 (br s, CH_2), 46.41 (d, $J_{C\text{-}F}$ = 4.5 Hz, CH_2), 49.10 (s, CH_2), 50.53 (s, CH_2), 64.36 (s, CH_2), 91.38 (s, CH_2), 106.38 (s, C_{Ar}), 107.15 (s, C_{Ar}), 111.32 (d, $J_{C\text{-}F}$ = 22.9 Hz, C_{Ar}), 119.85 (d, $J_{C\text{-}F}$ = 6.8 Hz, C_{Ar}), 137.11 (s, C_{Ar}), 143.82 (br s, C_{Ar}), 144.41 (d, $J_{C\text{-}F}$ = 10.4 Hz, C_{Ar}), 148.63 (s, C_{Ar}), 148.65 (s, C_{Ar}),148.71 (s, C_{Ar}), 152.66 (d, $J_{C\text{-}F}$ = 249.2 Hz, C_{Ar}), 165.99 (s, C(O)OH), 176.07 (s, C═O). ESI-HRMS *m/z*: $[M\text{-}Cl]^+$ 483.2044 (calculated for $[C_{25}H_{28}FN_4O_5]^+$ - 483.2038).

*4.1.1.7. 1-Ethyl-6-fluoro-4-oxo-7-(4-((2,2,8-trimethyl-4H-[1,3]dioxino [4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid **(8b)**.* The reaction was carried out following the general procedure with compound **2b** (1.26 g, 4.78 mmol), norfloxacin hydrocloride (1.55 g, 4.35 mmol), $NaHCO_3$ (1.13 g, 13.48 mmol) and KI (0.14 g, 0.87 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 24% (0.57 g); light yellow solid; mp 218–221 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.56 (s, 6H, $2CH_3$), 1.57 (t, 3H, $J_{H\text{-}H}$ = 7.2 Hz, CH_3), 2.43 (s, 3H, CH_3), 2.64 (br m, 4H, $2CH_2$ piperazinyl), 3.29 (br m, 4H, $2CH_2$ piperazinyl), 3.48 (s, 2H, CH_2N), 4.30 (q, 2H, $J_{H\text{-}H}$ = 7.2 Hz, CH_2), 5.01 (s, 2H, CH_2), 6.80 (d, 1H, $J_{H\text{-}F}$ = 6.8 Hz, CH_{Ar}), 7.93 (s, 1H, CH_{Ar}), 8.00 (d, 1H, $J_{H\text{-}F}$ = 13.0 Hz, CH_{Ar}), 8.64 (s, 1H, CH_{Ar}), 15.06 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 14.58 (s, CH_3), 18.27 (s, CH_3), 24.86 (s, $2CH_3$), 49.88 (s, CH_2), 50.00 (d, $J_{C\text{-}F}$ = 4.8 Hz, CH_2), 52.67 (s, CH_2), 57.44 (s, CH_2), 58.87 (s, CH_2), 99.95 (s, C$(CH_3)_2$), 103.95 (d, $J_{C\text{-}F}$ = 3.1 Hz, C_{Ar}), 108.47 (s, C_{Ar}), 112.88 (d, $J_{C\text{-}F}$ = 23.3 Hz, C_{Ar}), 120.71 (d, $J_{C\text{-}F}$ = 7.8 Hz, C_{Ar}), 126.65 (C_{Ar}), 127.20 (s, C_{Ar}), 137.21 (s, C_{Ar}), 139.85 (s, C_{Ar}), 146.10 (d, $J_{C\text{-}F}$ = 10.6 Hz, C_{Ar}), 146.46 (s, C_{Ar}), 147.23 (s, C_{Ar}), 147.35 (s, C_{Ar}), 153.63 (d, $J_{C\text{-}F}$ = 251.7 Hz, C_{Ar}), 167.29 (s, C(O)OH), 177.08 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 511.2357 (calculated for $[C_{27}H_{32}FN_4O_5]^+$ - 511.2351).

*4.1.1.8. 7-(4-((2,8-Dimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(8c)**.* The reaction was carried out following the general procedure with compound **2f** (0.17 g, 0.66 mmol), norfloxacin hydrocloride (0.21 g, 0.60 mmol), $NaHCO_3$ (0.16 g, 1.86 mmol) and KI (0.02 g, 0.12 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 33% (0.10 g); gray solid; mp 188–192 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.57–1.61 (m, 6H, $2CH_3$), 2.47 (s, 3H, CH_3), 2.65 (br m, 4H, $2CH_2$ piperazinyl), 3.30 (br m, 4H, $2CH_2$ piperazinyl), 3.47 (br m, 2H, CH_2N), 4.31 (br m, 2H, CH_2), 5.04, 5.10 (AB, 2H, $J_{H\text{-}H}$ = 16.0 Hz, CH_2), 5.20 (br m, 1H, CH), 6.81 (br s, 1H, CH_{Ar}), 7.97 (br s, 1H, CH_{Ar}), 8.03 (d, 1H, $J_{H\text{-}F}$ = 13.0 Hz, CH_{Ar}), 8.65 (s, 1H, CH_{Ar}), 15.06 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 14.59 (s, CH_3), 18.01 (s, CH_3), 20.76 (s, CH_3), 49.94 (d, $J_{C\text{-}F}$ = 7.8 Hz, CH_2), 50.00 (s, CH_2), 52.66 (s, CH_2), 57.44 (s, CH_2), 64.50 (s, CH_2), 97.44 (s, CH), 104.01 (d, $J_{C\text{-}F}$ = 1.8 Hz, C_{Ar}), 108.56 (s, C_{Ar}), 112.96 (d, $J_{C\text{-}F}$ = 23.3 Hz, C_{Ar}), 120.84 (d, $J_{C\text{-}F}$ = 7.6 Hz, C_{Ar}), 127.15 (s, C_{Ar}), 128.98 (s, C_{Ar}), 137.22 (s, C_{Ar}), 140.10 (s, C_{Ar}), 146.09 (d, $J_{C\text{-}F}$ = 10.8 Hz, C_{Ar}), 146.95 (s, C_{Ar}), 147.26 (s, C_{Ar}), 148.32 (s, C_{Ar}), 153.66 (d, $J_{C\text{-}F}$ = 251.9 Hz, C_{Ar}), 167.27 (s, C(O)OH), 177.13 (d, $J_{C\text{-}F}$ = 1.8 Hz, C═O). ESI-HRMS *m/z*: $[M+H]^+$ 497.2200 (calculated for $[C_{26}H_{30}FN_4O_5]^+$ - 497.2195).

*4.1.1.9. 1-Ethyl-6-fluoro-7-(4-((8-methyl-2-propyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(8d)**.* The reaction was carried out following the general procedure with compound **2g** (0.49 g, 1.76 mmol), norfloxacin hydrocloride (0.57 g, 1.60 mmol), $NaHCO_3$ (0.42 g, 4.96 mmol) and KI (0.05 g, 0.32 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 31% (0.26 g); gray solid; mp 221–224 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.02 (t, 3H, $J_{H\text{-}H}$ = 7.3 Hz, CH_3), 1.50–1.66 (m, 5H, CH_3 + CH_2), 1.82–1.96 (m, 2H, CH_2), 2.56 (s, 3H, CH_3), 2.69 (br m, 4H, $2CH_2$ piperazinyl), 3.33 (br m, 4H, $2CH_2$ piperazinyl), 3.44–3.59 (br m, 2H, CH_2N), 4.32 (q, 2H, $J_{H\text{-}H}$ = 7.1 Hz, CH_2), 5.09 (br m, 1H, CH), 5.07, 5.13 (AB, 2H, $J_{H\text{-}H}$ = 16.5 Hz, CH_2), 6.82 (d, 1H, $J_{H\text{-}F}$ = 6.5 Hz, CH_{Ar}), 8.05 (s, 1H, CH_{Ar}), 8.05 (d, 1H, $J_{H\text{-}F}$ = 12.4 Hz, CH_{Ar}), 8.67 (s, 1H, CH_{Ar}), 15.04 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 14.05 (s, CH_3), 14.61 (s, CH_3), 17.11, 17.76 (s, CH_2 + CH_3), 36.43 (s, CH_2), 49.90 (s, CH_2), 49.96 (d, $J_{C\text{-}F}$ = 5.0 Hz, CH_2), 52.68 (s, CH_2), 57.37 (s, CH_2), 64.58 (s, CH_2), 100.26 (s, CH), 104.02 (d, $J_{C\text{-}F}$ = 3.0 Hz, C_{Ar}), 108.57 (s, C_{Ar}), 112.99 (d, $J_{C\text{-}F}$ = 23.6 Hz, C_{Ar}), 120.88 (d,

$J_{C\text{-}F}$ = 7.9 Hz, C_{Ar}), 127.84 (s, C_{Ar}), 129.60 (s, C_{Ar}), 137.22 (s, C_{Ar}), 139.52 (s, C_{Ar}), 146.08 (d, $J_{C\text{-}F}$ = 10.4 Hz, C_{Ar}), 146.74 (s, C_{Ar}), 147.28 (s, CH_{Ar}), 148.54 (s, C_{Ar}), 153.67 (d, $J_{C\text{-}F}$ = 251.4 Hz, C_{Ar}), 167.31 (s, C(O)OH), 177.14 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 525.2513 (calculated for $[C_{28}H_{34}FN_4O_5]^+$ - 525.2508.

4.1.1.10. 1-Ethyl-6-fluoro-7-(4-((8-methyl-2-octyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(8e)***. The reaction was carried out following the general procedure with compound **2h** (0.48 g, 1.39 mmol), norfloxacin hydrocloride (0.45 g, 1.27 mmol), $NaHCO_3$ (0.33 g, 3.92 mmol) and KI (0.04 g, 0.25 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 20% (0.15 g); gray solid; mp 185–190 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 0.88 (t, 3H, $J_{H\text{-}H}$ = 6.4 Hz, CH_3), 1.17–1.45 (m, 10H, $5CH_2$), 1.46–1.65 (m, 2H, CH_2), 1.58 (t, 3H, $J_{H\text{-}H}$ = 7.2 Hz, CH_3), 1.82–1.96 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 2.64–2.68 (br m, 4H, $2CH_2$ piperazinyl), 3.20–3.39 (br m, 4H, $2CH_2$ piperazinyl), 3.46, 3.49 (AB, 2H, $J_{H\text{-}H}$ = 14.0 Hz, CH_2N), 4.31 (q, 2H, $J_{H\text{-}H}$ = 7.2 Hz, CH_2), 5.05 (t, 1H, $J_{H\text{-}H}$ = 5.4 Hz, CH), 5.04, 5.11 (AB, 2H, $J_{H\text{-}H}$ = 16.4 Hz, CH_2), 6.81 (d, 1H, $J_{H\text{-}F}$ = 6.6 Hz, CH_{Ar}), 7.98 (s, 1H, CH_{Ar}), 8.03 (d, 1H, $J_{H\text{-}F}$ = 13.0 Hz, CH_{Ar}), 8.66 (s, 1H, CH_{Ar}), 15.07 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 14.24 (s, CH_3), 14.61 (s, CH_3), 17.72 (s, CH_3), 22.79 (s, CH_2), 23.68 (s, CH_2), 29.33 (s, CH_2), 29.49 (s, CH_2), 29.59 (s, CH_2), 31.98 (s, CH_2), 34.41 (s, CH_2), 49.91 (s, CH_2), 49.94 (br s, CH_2), 52.68 (s, CH_2), 57.35 (s, CH_2), 64.59 (s, CH_2), 100.47 (s, CH), 104.03 (d, $J_{C\text{-}F}$ = 2.4 Hz, C_{Ar}), 108.54 (s, C_{Ar}), 112.96 (d, $J_{C\text{-}F}$ = 23.3 Hz, C_{Ar}), 120.85 (d, $J_{C\text{-}F}$ = 7.9 Hz, C_{Ar}), 127.53 (s, C_{Ar}), 129.79 (s, C_{Ar}), 137.22 (s, C_{Ar}), 139.38 (s, C_{Ar}), 146.06 (d, $J_{C\text{-}F}$ = 10.3 Hz, C_{Ar}), 146.69 (s, C_{Ar}), 147.28 (s, C_{Ar}), 148.56 (s, C_{Ar}), 153.65 (d, $J_{C\text{-}F}$ = 251.6 Hz, C_{Ar}), 167.31 (s, C(O)OH), 177.13 (d, $J_{C\text{-}F}$ = 1.8 Hz, C═O). ESI-HRMS m/z: $[M+H]^+$ 595.3290 (calculated for $[C_{33}H_{44}FN_4O_5]^+$ - 595.3290).

4.1.1.11. 1-Ethyl-6,8-difluoro-7-(3-methyl-4-((8-methyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(10a)***. The reaction was carried out following the general procedure with compound **2a** (0.54 g, 2.30 mmol), lomefloxacin hydrochloride (0.81 g, 2.09 mmol), $NaHCO_3$ (0.54 g, 6.47 mmol) and KI (0.07 g, 0.42 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and then was washed with acetone. The resulting precipitate was suspended in water then equimolar amount of 0.1 M HCl solution added and the solvent was removed in vacuo. Yield 27% (0.31 g); yellow solid; mp 167–173 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.45 (t, 3H, $J_{H\text{-}H}$ = 6.8 Hz, CH_3), 1.50–1.62 (br m, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.32–3.93 (m, 7H, $3CH_2$ + CH), 4.20–4.28 (br m, 1H, CH_2), 4.53–4.64 (m, 2H, CH_2), 4.79–4.91 (br m, 1H, CH_2), 5.33–5.53 (m, 3H, CH_2), 5.71–5.75 (br m, 1H, CH_2), 7.87 (d, 1H, $J_{H\text{-}F}$ = 11.1 Hz, CH_{Ar}), 8.78 (s, 1H, CH_{Ar}), 8.93 (s, 1H, CH_{Ar}), 12.14 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 13.87 (s, CH_3), 14.62 (s, CH_3), 15.98 (d, $J_{C\text{-}F}$ = 4.5 Hz, CH_3), 47.04 (br s, CH_2), 48.48 (br s, CH_2), 50.39 (br s), 53.18 (br s), 53.73 (d, $J_{C\text{-}F}$ = 15.5 Hz, CH_2), 60.62 (br s), 64.30 (s), 91.49 (s, CH_2), 107.02 (d, $J_{C\text{-}F}$ = 22.4 Hz, C_{Ar}), 107.12 (s, C_{Ar}), 121.34 (d, $J_{C\text{-}F}$ = 8.1 Hz, C_{Ar}), 123.05 (s, C_{Ar}), 127.12 (d, $J_{C\text{-}F}$ = 6.0 Hz, C_{Ar}), 132.16 (t, $J_{C\text{-}F}$ = 12.9 Hz, $J_{C\text{-}F}$ = 12.9 Hz, C_{Ar}), 137.10 (br s, C_{Ar}), 137.81 (s, C_{Ar}), 144.94 (s, C_{Ar}), 146.20 (dd, $J_{C\text{-}F}$ = 250.9 Hz, $J_{C\text{-}F}$ = 4.6 Hz, C_{Ar}), 149.07 (s, C_{Ar}), 151.34 (s, C_{Ar}), 154.40 (dd, $J_{C\text{-}F}$ = 248.2 Hz, $J_{C\text{-}F}$ = 4.6 Hz, C_{Ar}), 165.44 (s, C(O)OH), 175.48 (s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 515.2106 (calculated for $[C_{26}H_{29}F_2N_4O_5]^+$ - 515.2101).

4.1.1.12. 1-Ethyl-6,8-difluoro-7-(3-methyl-4-((2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(10b)***. The reaction was carried out following the general procedure with compound **2b** (1.18 g, 4.48 mmol), lomefloxacin hydrochloride (1.58 g, 4.07 mmol), $NaHCO_3$ (1.06 g, 12.6 mmol) and KI (0.14 g, 0.82 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed successively with acetone and water. Yield 66% (1.55 g); yellow solid; mp 184–186 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.19 (d, 3H, $J_{H\text{-}H}$ = 6.2 Hz, CH_3), 1.53 (s, 6H, $2CH_3$), 1.50–1.55 (br m, 3H, CH_3), 2.20–2.28 (m, 1H, CH), 2.36 (s, 3H, CH_3), 2.59–2.71 (m, 2H, CH_2), 3.10–3.13 (br m, 1H, CH_2), 3.23–3.30 (br m, 2H, CH_2), 3.33–3.36 (br m, 1H, CH_2), 3.08, 3.94 (AB, 2H, $J_{H\text{-}H}$ = 13.0 Hz, CH_2), 4.39–4.49 (m, 2H, $\underline{CH_2}$), 4.84, 5.16 (AB, 2H, $J_{H\text{-}H}$ = 16.7 Hz, CH_2), 7.83–7.88 (m, 2H, $2C_{Ar}$), 8.55 (s, 1H, C_{Ar}), 14.66 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 14.75 (s, CH_3), 16.37 (d, $J_{C\text{-}F}$ = 4.7 Hz, CH_3), 18.52 (s, CH_3), 24.57 (s, $C(\underline{CH_3})_2$), 24.95 (s, $C(\underline{CH_3})_2$), 50.31 (br s, CH_2), 51.21 (br s, CH_2), 53.27 (s, CH_2), 54.65 (d, $J_{C\text{-}F}$ = 16.5 Hz, CH_2), 56.02 (br s, CH), 57.61 (br s, CH_2), 59.03 (s, CH_2), 99.57 (s, $\underline{C}(CH_3)_2$), 107.96 (s, C_{Ar}), 108.21 (d, $J_{C\text{-}F}$ = 23.2 Hz, C_{Ar}), 121.15 (d, $J_{C\text{-}F}$ = 8.5 Hz, C_{Ar}), 126.33 (s, C_{Ar}), 127.03 (s, C_{Ar}), 127.16 (d, $J_{C\text{-}F}$ = 6.2 Hz, C_{Ar}), 134.39 (t, $J_{C\text{-}F}$ = 13.6 Hz, $J_{C\text{-}F}$ = 13.6 Hz, C_{Ar}), 140.61 (s, C_{Ar}), 145.85 (dd, $J_{C\text{-}F}$ = 248.6 Hz, $J_{C\text{-}F}$ = 6.4 Hz, C_{Ar}), 146.13 (s, C_{Ar}), 147.40 (s, C_{Ar}), 150.00 (s, C_{Ar}), 155.11 (dd, $J_{C\text{-}F}$ = 251.7 Hz, $J_{C\text{-}F}$ = 6.4 Hz, C_{Ar}), 166.59 (s, C(O)OH), 176.21 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 543. 2414 (calculated for $[C_{28}H_{33}F_2N_4O_5]^+$ - 543.2414).

4.1.1.13. 7-(4-((2,8-Dimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)-3-methylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(10c)***. The reaction was carried out following the general procedure with compound **2f** (0.48 g, 1.93 mmol), lomefloxacin hydrochloride (0.68 g, 1.75 mmol), $NaHCO_3$ (0.46 g, 5.44 mmol) and KI (0.06 g, 0.35 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed successively with acetone and water. Yield 21% (0.19 g); yellow solid; mp 195–202 °C (decomp). ^{1}H NMR ($CDCl_3$) δ (mixture of diastereomers): 1.19–1.25 (m, 3H, CH_3), 1.56 (t, 3H, $J_{H\text{-}H}$ = 6.9 Hz, CH_3), 1.61 (d, 3H, $J_{H\text{-}H}$ = 5.0 Hz, CH_3), 2.24–2.35 (m, 1H, CH), 2.47 (s, 3H, CH_3), 2.60–2.79 (m, 2H, CH_2), 3.06–3.22 (m, 2H, CH_2), 3.22–3.43 (m, 3H, CH_2), 3.91–4.03 (m, 1H, CH_2), 4.40–4.52 (m, 2H, CH_2), 4.97 (t, 1H, $J_{H\text{-}H}$ = 16.6 Hz, CH), 5.13–5.37 (m, 2H, CH_2), 7.92–8.01 (m, 2H, $2CH_{Ar}$), 8.60 (s, 1H, $1CH_{Ar}$), 14.64 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers): 14.51 (br s, CH_3), 15.03 (br s, CH_3), 16.47 (d, $J_{C\text{-}F}$ = 4.8 Hz, CH_3), 18.48 (s, CH_3), 18.49 (s, CH_3), 20.79 (s, CH_3), 50.08 (br s, CH_2), 50.50 (br s, CH_2), 51.30 (br s, CH_2), 53.34 (s, CH_2), 53.46 (s, CH_2), 54.74 (d, $J_{C\text{-}F}$ = 16.3 Hz, CH_2), 56.06 (s), 56.11 (s), 57.70 (br s, CH_2), 64.40 (s, CH_2), 65.01 (s, CH_2), 97.10 (s, CH), 97.23 (s, CH), 108.18 (s, C_{Ar}), 108.48 (d, $J_{C\text{-}F}$ = 23.4 Hz, C_{Ar}), 121.42 (d, $J_{C\text{-}F}$ = 8.1 Hz, C_{Ar}), 127.11–128.06 (m, C_{Ar}), 134.29–134.69 (m, C_{Ar}), 141.06 (s, C_{Ar}), 141.35 (s, C_{Ar}), 146.02 (dd, $J_{C\text{-}F}$ = 247.9 Hz, $J_{C\text{-}F}$ = 6.5 Hz, C_{Ar}), 147.08 (s, C_{Ar}), 147.16 (s, C_{Ar}), 148.02 (s, C_{Ar}), 150.09 (s, C_{Ar}), 155.24 (dd, $J_{C\text{-}F}$ = 251.7 Hz, $J_{C\text{-}F}$ = 6.5 Hz, C_{Ar}), 166.75 (s, C(O)OH), 176.37 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 529.2257 (calculated for $[C_{27}H_{31}F_2N_4O_5]^+$ - 529.2257).

4.1.1.14. 1-Ethyl-6,8-difluoro-7-(3-methyl-4-((8-methyl-2-propyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(10d)***. The reaction was carried out following the general procedure with compound **2g** (0.49 g, 1.76 mmol), lomefloxacin hydrochloride (0.62 g, 1.60 mmol), $NaHCO_3$ (0.42 g, 4.96 mmol) and KI (0.05 g, 0.32 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed successively with acetone and water. Yield 24% (0.22 g); yellow solid; mp 204–205°C. ^{1}H NMR ($CDCl_3$) δ (mixture of diastereomers): 1.02 (t, 3H, $J_{H\text{-}H}$ = 7.4 Hz, CH_3), 1.20 (t, 3H, $J_{H\text{-}H}$ = 6.5 Hz, CH_3), 1.48–1.63 (m, 5H, CH_3+CH_2), 1.80–1.94 (m, 2H, CH_2), 2.20–2.34 (m, 1H, CH), 2.42 (s, 3H, CH_3), 2.58–2.78 (m, 2H, CH_2), 3.01–3.20 (br m, 2H, CH_2), 3.29–3.44 (m, 3H, $2CH_2$), 3.90–4.04 (m, 1H, CH_2), 4.37–4.54 (m, 2H, CH_2), 4.91–5.37 (m, 3H, CH+CH_2), 7.91–7.94 (m, 2H, $2CH_{Ar}$), 8.58 (s, 1H, CH_{Ar}), 14.65, 14.66 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers): 14.08 (s, CH_3), 14.55 (s,

CH_3), 15.09 (s, CH_3), 16.47 (d, $J_{C\text{-}F}$ = 4.7 Hz, CH_3), 17.14 (s, CH_2), 17.16 (s, CH_2), 18.48 (s, CH_3), 18.50 (s, CH_3), 29.81 (s, CH_2), 50.10 (br s, CH_2), 50.52 (br s, CH_2), 51.30 (br s, CH_2), 53.37 (s, CH_2), 53.49 (s, CH), 54.73 (d, $J_{C\text{-}F}$ = 16.6 Hz, CH_2), 56.11 (br s, CH), 57.70 (br s, CH_2), 64.50 (s, CH_2), 65.10 (s, CH_2), 99.82 (s, CH), 99.94 (s, CH), 108.19 (s, C_{Ar}), 108.49 (d, $J_{C\text{-}F}$ = 23.3 Hz, C_{Ar}), 121.42 (d, $J_{C\text{-}F}$ = 8.6 Hz, C_{Ar}), 127.20–127.32 (m, C_{Ar}), 127.67 (s, C_{Ar}), 127.92 (s, C_{Ar}), 128.20 (s, C_{Ar}), 134.26–134.75 (m, C_{Ar}), 141.07 (s, C_{Ar}), 141.37 (s, C_{Ar}), 145.94 (dd, $J_{C\text{-}F}$ = 248.2 Hz, $J_{C\text{-}F}$ = 6.7 Hz, C_{Ar}), 147.17 (s, C_{Ar}), 147.25 (s, C_{Ar}), 148.05 (s, C_{Ar}),148.08 (s, C_{Ar}), 150.09 (s, C_{Ar}), 155.24 (dd, $J_{C\text{-}F}$ = 251.6 Hz, $J_{C\text{-}F}$ = 6.7 Hz, C_{Ar}), 166.75 (s, C(O)OH), 176.38 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 557.2570 (calculated for $[C_{29}H_{35}F_2N_4O_5]^+$ - 557.2570).

4.1.1.15. 1-Ethyl-6,8-difluoro-7-(3-methyl-4-((8-methyl-2-octyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(10e)***. The reaction was carried out following the general procedure with compound **2h** (0.78 g, 2.24 mmol), lomefloxacin hydrochloride (0.79 g, 2.04 mmol), $NaHCO_3$ (0.53 g, 6.31 mmol) and KI (0.07 g, 0.41 mmol). Yield 39% (0.50 g); mp 192–196 °C (decomp). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed successively with acetone and water. ^{1}H NMR ($CDCl_3$) δ (mixture of diastereomers): 0.79–0.98 (br m, 3H, CH_3), 1.12–1.45 (m, 13H, CH_3+5CH_2), 1.46–1.61 (m, 5H, CH_3+CH_2), 1.73–1.98 (m, 2H, CH_2), 2.19–2.34 (m, 1H, CH), 2.41 (s, 3H, CH_3), 2.55–2.79 (m, 2H, CH_2), 3.01–3.17 (br m, 2H, CH_2), 3.21–3.44 (m, 3H, 2CH_2), 3.90–3.99 (m, 1H, CH_2), 4.38–4.52 (m, 2H, CH_2), 4.91–5.38 (m, 3H, CH+CH_2), 7.91–7.94 (m, 2H, 2CH_{Ar}), 8.59 (s, 1H, CH_{Ar}), 14.68 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers): 14.20 (s, CH_3), 14.48 (br s, CH_3), 15.02 (br s, CH_3), 16.43 (d, $J_{C\text{-}F}$ = 4.5 Hz, CH_3), 18.46 (s, CH_3), 22.74 (s, CH_2), 23.69 (s, CH_2), 29.28 (s, CH_2), 29.46 (s, CH_2), 29.55 (s, CH_2), 31.93 (s, CH_2), 34.45 (s, CH_2), 50.07 (br s, CH_2), 50.49 (br s, CH_2), 51.27 (br s, CH_2), 53.32 (s, CH_2), 53.44 (s, CH_2), 54.72 (d, $J_{C\text{-}F}$ = 16.3 Hz, CH_2), 56.05 (br s, CH), 57.68 (br s, CH_2), 64.46 (s, CH_2), 65.07 (s, CH_2), 99.96 (s, CH), 100.08 (s, CH), 108.02 (s, C_{Ar}), 108.34 (d, $J_{C\text{-}F}$ = 23.2 Hz, C_{Ar}), 121.25 (d, $J_{C\text{-}F}$ = 8.7 Hz, C_{Ar}), 127.15–127.29 (m, C_{Ar}), 127.63 (s, C_{Ar}), 127.86 (s, C_{Ar}), 128.15 (s, C_{Ar}), 134.26–134.60 (m, C_{Ar}), 141.02 (s, C_{Ar}), 141.31 (s, C_{Ar}), 145.87 (dd, $J_{C\text{-}F}$ = 246.1 Hz, $J_{C\text{-}F}$ = 6.1 Hz, C_{Ar}), 147.08 (s, C_{Ar}), 147.16 (s, C_{Ar}), 147.99 (s, C_{Ar}),148.02 (s, C_{Ar}), 150.06 (s, C_{Ar}), 155.17 (dd, $J_{C\text{-}F}$ = 251.6 Hz, $J_{C\text{-}F}$ = 6.1 Hz, C_{Ar}), 166.71 (s, C(O)OH), 176.28 (s, C═O). ESI-HRMS *m/z*: $[M\text{-}Cl]^+$ 627.3348 (calculated for $[C_{25}H_{28}FN_4O_5]^+$ - 627.3353).

4.1.1.16. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(1-((8-methyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(12a)***. The reaction was carried out following the general procedure with compound **2a** (0.50 g, 2.11 mmol), moxifloxacin hydrochloride (0.84 g, 1.92 mmol), $NaHCO_3$ (0.50 g, 5.95 mmol) and KI (0.06 g, 0.38 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and then was washed with acetone. The resulting precipitate was suspended in water then equimolar amount of 0.1 M HCl solution added and the solvent was removed in vacuo. Yield 26% (0.30 g); yellow solid; mp 121–127 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 0.92–1.23 (m, 4H, 2CH_2), 1.40–1.52 (m, 1H), 1.57–1.72 (m, 3H), 2.02–2.13 (m, 1H), 2.21 (s, 3H, CH_3), 2.35–2.46 (m, 1H), 2.57–2.65 (m, 1H), 2.95–3.02 (m, 1H), 3.12, 3.80 (AB, 2H, $J_{H\text{-}H}$ = 13.7 Hz, CH_2), 3.46–3.53 (m, 1H), 3.56 (s, 3H, CH_3), 3.66–3.73 (m, 1H), 3.76–3.91 (m, 2H), 4.07–4.16 (m, 1H), 4.83, 4.97 (AB, 2H, $J_{H\text{-}H}$ = 16.0 Hz, CH_2), 5.19, 5.23 (AB, 2H, $J_{H\text{-}H}$ = 5.7 Hz, CH_2), 7.63 (d, 1H, $J_{H\text{-}F}$ = 14.1 Hz, CH_{Ar}), 7.85 (s, 1H, CH_{Ar}), 8.64 (s, 1H, CH_{Ar}), 15.20 (s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 8.54 (s, CH_2), 9.39 (s, CH_2), 18.12 (s, CH_3), 21.74 (s, CH_2), 23.03 (s, CH_2), 37.03 (s), 40.62 (s), 49.79 (s), 52.87 (d, $J_{C\text{-}F}$ = 4.1 Hz), 53.29 (d, $J_{C\text{-}F}$ = 5.9 Hz), 54.13 (s), 61.22 (s), 62.24 (s), 63.45 (s), 90.61 (s, CH_2), 106.25 (s, C_{Ar}), 106.41 (d, $J_{C\text{-}F}$ = 24.3 Hz, C_{Ar}), 116.74 (d, $J_{C\text{-}F}$ = 8.8 Hz, C_{Ar}), 128.26 (s, C_{Ar}), 128.65 (s, C_{Ar}), 134.52 (s, C_{Ar}), 137.18 (d, $J_{C\text{-}F}$ = 10.7 Hz, C_{Ar}), 140.51 (s, C_{Ar}), 145.22 (s, C_{Ar}), 146.81 (s, C_{Ar}), 150.11 (s, C_{Ar}), 152.82 (d, $J_{C\text{-}F}$ = 249.3 Hz, C_{Ar}), 165.89 (s, C(O)OH), 175.92 (s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 565.2457 (calculated for $[C_{30}H_{34}FN_4NO_6]^+$ - 565.2462).

4.1.1.17. 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(1-((2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-1,4-dihydroquinoline-3-carboxylic acid ***(12b)***. The reaction was carried out following the general procedure with compound **2b** (1.30 g, 4.92 mmol), moxifloxacin hydrochloride (1.96 g, 4.48 mmol), $NaHCO_3$ (1.17 g, 13.88 mmol) and KI (0.15 g, 0.90 mmol). The dry residue is extracted with ethyl acetate, the insoluble matter is filtered off, the filtrate is dried to dryness and purified by column chromatography (eluent $CHCl_3$:C_2H_5OH = 7:1). Yield 25% (0.70 g); light yellow solid; mp 110–112 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 0.90–1.05 (m, 2H, CH_2), 1.11–1.25 (m, 2H, CH_2), 1.45 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.50–1.58 (m, 1H), 1.61–1.75 (m, 3H), 2.12–2.22 (m, 1H), 2.35 (s, 3H, CH_3), 2.32–2.45 (m, 1H), 2.62–2.75 (m, 1H), 3.05–3.08 (m, 1H), 3.19, 3.72 (AB, 2H, $J_{H\text{-}H}$ = 13.6 Hz, CH_2), 3.55–3.59 (m, 1H), 3.56 (s, 3H, CH_3), 3.65–3.77 (m, 2H), 3.85–4.05 (m, 2H), 4.72, 4.97 (AB, 2H, $J_{H\text{-}H}$ = 16.6 Hz, CH_2), 7.76 (d, 1H, $J_{H\text{-}F}$ = 14.1 Hz, CH_{Ar}), 7.86 (s, 1H, CH_{Ar}), 8.75 (s, 1H, CH_{Ar}), 15.07 (s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 9.36 (s, CH_2), 9.83 (s, CH_2), 18.47 (s, CH_3), 22.57 (s, CH_2), 23.91 (s, CH_2), 24.62 (s, CH_2), 24.85 (s, CH_3), 37.58 (s), 40.53 (s), 49.68 (s), 52.10 (d, $J_{C\text{-}F}$ = 6.1 Hz), 54.39 (d, $J_{C\text{-}F}$ = 6.5 Hz), 55.16 (s), 58.79 (s), 61.24 (s), 62.24 (s), 99.65 (s, $C(CH_3)_2$), 107.63 (s, C_{Ar}), 108.04 (d, $J_{C\text{-}F}$ = 24.0 Hz, C_{Ar}), 118.18 (d, $J_{C\text{-}F}$ = 9.0 Hz, C_{Ar}), 126.37 (s, C_{Ar}), 127.29 (s, C_{Ar}), 134.63 (s, C_{Ar}), 137.64 (d, $J_{C\text{-}F}$ = 10.9 Hz, C_{Ar}), 140.00 (s, C_{Ar}), 146.19 (s, C_{Ar}), 147.33 (s, C_{Ar}), 149.65 (s, C_{Ar}), 153.51 (d, $J_{C\text{-}F}$ = 250.6 Hz, C_{Ar}), 167.22 (s, C(O)OH), 176.80 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 593.2770 (calculated for $[C_{32}H_{38}FN_4O_6]^+$ - 593.2775).

4.1.1.18. 1-Cyclopropyl-7-(1-((2,8-dimethyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(12c)***. The reaction was carried out following the general procedure with compound **2f** (0.48 g, 1.93 mmol), moxifloxacin hydrochloride (0.77 g, 1.76 mmol), $NaHCO_3$ (0.46 g, 5.45 mmol) and KI (0.06 g, 0.35 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 38% (0.39 g); yellow solid; mp 120–130 °C (decomp). ^{1}H NMR ($CDCl_3$) δ (mixture of diastereomers): 0.91–1.07 (m, 2H, CH_2), 1.11–1.28 (m, 2H, CH_2), 1.52–1.59 (m, 3H), 1.62–1.80 (m, 3H), 2.15–2.27 (m, 1H), 2.38, 2.40 (s, 3H, CH_3), 2.62–2.75 (m, 1H), 3.01–3.15 (m, 1H), 3.17–3.25 (m, 1H), 3.57, 3.57 (s, 3H, CH_3), 3.57–3.78 (m, 4H), 3.84–3.93 (m, 1H), 3.95–4.03 (m, 1H), 4.77–5.15 (m, 3H), 7.79 (d, 1H, $J_{H\text{-}F}$ = 14.1 Hz, CH_{Ar}), 7.90, 7.91 (s, 1H, CH_{Ar}), 8.76 (s, 1H, CH_{Ar}), 15.04 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers): 9.40 (s, CH_2), 9.45 (s, CH_2), 9.80 (s, CH_2), 9.83 (s, CH_2), 18.40 (s, CH_3), 20.73 (s, CH_3), 20.76 (s, CH_3), 22.62 (s, CH_2), 22.68 (s, CH_2), 23.93 (s, CH_2), 23.96 (s, CH_2), 37.56 (s), 37.64 (s), 40.53 (s), 49.59 (s), 49.71 (s), 51.72 (d, $J_{C\text{-}F}$ = 4.6 Hz), 52.05 (d, $J_{C\text{-}F}$ = 3.9 Hz), 54.32 (d, $J_{C\text{-}F}$ = 7.3 Hz), 54.55 (d, $J_{C\text{-}F}$ = 5.9 Hz), 55.13 (s), 55.46 (s), 61.21 (s), 61.32 (s), 61.98 (s), 62.36 (s), 64.20 (s), 64.63 (s), 97.12 (s, CH), 97.17 (s, CH), 107.70 (s, C_{Ar}), 107.72 (s, C_{Ar}), 108.08 (d, $J_{C\text{-}F}$ = 24.1 Hz, C_{Ar}), 108.14 (d, $J_{C\text{-}F}$ = 24.1 Hz, C_{Ar}), 118.22 (d, $J_{C\text{-}F}$ = 8.9 Hz, C_{Ar}), 118.37 (d, $J_{C\text{-}F}$ = 8.9 Hz, C_{Ar}), 127.70 (s, C_{Ar}), 127.79 (s, C_{Ar}), 127.93 (s, C_{Ar}), 134.63 (s, C_{Ar}), 137.59 (d, $J_{C\text{-}F}$ = 10.8 Hz, C_{Ar}), 137.63 (d, $J_{C\text{-}F}$ = 10.9 Hz, C_{Ar}), 140.43–140.67 (m, C_{Ar}), 146.93 (s, C_{Ar}), 147.00 (s, C_{Ar}), 147.97 (s, C_{Ar}), 147.98 (s, C_{Ar}), 149.71 (s, C_{Ar}), 153.51 (d, $J_{C\text{-}F}$ = 250.7 Hz, C_{Ar}), 153.61 (d, $J_{C\text{-}F}$ = 250.6 Hz, C_{Ar}), 167.24 (s, C(O)OH), 176.84 (s, C═O), 176.86 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 579.2610 (calculated for $[C_{31}H_{36}FN_4O_6]^+$ - 579.2613).

4.1.1.19. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(1-((8-methyl-2-propyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(12d)***. The reaction was carried out following the general procedure with compound **2g** (0.49 g, 1.76 mmol), moxifloxacin hydrochloride (0.70 g, 1.60 mmol), $NaHCO_3$ (0.42 g, 4.96 mmol) and KI (0.05 g, 0.32 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 30% (0.29 g); yellow solid; mp 118–120°C. ^{1}H NMR ($CDCl_3$) δ (mixture of diastereomers): 0.89–1.09 (m, 5H, CH_2+CH_3), 1.09–1.28 (m, 2H, CH_2), 1.43–1.89 (m, 7H), 2.15–2.28 (m, 1H), 2.36–2.51 (m, 3H), 2.63–2.75 (m, 1H), 3.01–3.27 (m, 2H), 3.57, 3.58 (s, 3H, CH_3), 3.57–3.93 (m, 5H), 3.95–4.04 (m, 1H), 4.76–5.15 (m, 3H), 7.79 (d, 1H, $J_{H\text{-}F}$ = 14.0 Hz, CH_{Ar}), 7.92, 7.93 (s, 1H, CH_{Ar}), 8.77 (s, 1H, CH_{Ar}), 15.05 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers): 9.31 (s, CH_2), 9.45 (s, CH_2), 9.82 (s, CH_2), 9.85 (s, CH_2), 13.99 (s, CH_3), 17.05 (s), 17.46 (s), 17.52 (s), 22.43 (s, CH_2), 22.59 (s, CH_2), 23.76 (s, CH_2), 23.88 (s, CH_2), 36.30 (s), 37.54 (s), 37.69 (s), 40.53 (s), 49.77 (s), 50.04 (s), 51.94 (d, $J_{C\text{-}F}$ = 6.0 Hz), 52.56 (br s), 54.16 (d, $J_{C\text{-}F}$ = 7.5 Hz), 54.49 (d, $J_{C\text{-}F}$ = 5.4 Hz), 54.90 (s), 55.26 (s), 61.33 (s), 61.41 (s), 62.16 (s), 62.43 (s), 64.29 (s), 64.73 (s), 100.17 (s, CH), 100.28 (s, CH), 107.75 (s, C_{Ar}), 107.77 (s, C_{Ar}), 108.13 (d, $J_{C\text{-}F}$ = 24.1 Hz, C_{Ar}), 108.18 (d, $J_{C\text{-}F}$ = 24.1 Hz, C_{Ar}), 118.36 (d, $J_{C\text{-}F}$ = 8.6 Hz, C_{Ar}), 118.50 (d, $J_{C\text{-}F}$ = 8.5 Hz, C_{Ar}), 128.65–129.80 (m, C_{Ar}), 134.65 (s, C_{Ar}), 137.53 (d, $J_{C\text{-}F}$ = 10.8 Hz, C_{Ar}), 137.57 (d, $J_{C\text{-}F}$ = 11.0 Hz, C_{Ar}), 140.54–140.67 (m, C_{Ar}), 146.14 (s, C_{Ar}), 146.25(s, C_{Ar}), 148.49 (s, C_{Ar}), 148.56 (s, C_{Ar}), 149.74 (s, C_{Ar}), 153.53 (d, $J_{C\text{-}F}$ = 250.3 Hz, C_{Ar}), 153.61 (d, $J_{C\text{-}F}$ = 250.6 Hz, C_{Ar}), 167.21 (s, C(O)OH), 176.85 (s, C═O), 176.87 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 607.2926 (calculated for $[C_{33}H_{40}FN_4O_6]^+$ - 607.2926).

4.1.1.20. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(1-((8-methyl-2-octyl-4H-[1,3]dioxino[4,5-c]pyridin-5-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(12e)***. The reaction was carried out following the general procedure with compound **2h** (0.49 g, 1.41 mmol), moxifloxacin hydrochloride (0.56 g, 1.28 mmol), $NaHCO_3$ (0.33 g, 3.96 mmol) and KI (0.04 g, 0.26 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 30% (0.26 g); yellow solid; mp 87–90 °C (decomp). ^{1}H NMR ($CDCl_3$) δ (mixture of diastereomers): 0.85–0.90 (br m, 3H, CH_3), 0.91–1.08 (m, 2H, CH_2), 1.10–1.91 (m, 20H), 2.15–2.31 (m, 1H), 2.44–2.47 (m, 4H), 2.63–2.75 (m, 1H), 3.02–3.29 (m, 2H), 3.58, 3.59 (s, 3H, CH_3), 3.59–3.93 (m, 5H), 3.95–4.04 (m, 1H), 4.78–5.15 (m, 3H), 7.79 (d, 1H, $J_{H\text{-}F}$ = 14.0 Hz, CH_{Ar}), 7.93, 7.95 (s, 1H, CH_{Ar}), 8.77 (s, 1H, CH_{Ar}), 15.03 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ (mixture of diastereomers): 9.35 (s, CH_2), 9.48 (s, CH_2), 9.78 (s, CH_2), 9.90 (s, CH_2), 14.24 (s, CH_3), 17.87 (br s, CH_3), 22.52 (s, CH_2), 22.68 (s, CH_2), 22.79 (s, CH_2), 23.65 (s, CH_2), 23.84 (s, CH_2), 23.95 (s, CH_2), 29.34 (s, CH_2), 29.44 (s, CH_2), 29.55 (s, CH_2), 31.97 (s), 34.33 (s), 37.53 (s), 37.66 (s), 40.53 (s), 49.64 (s), 49.87 (s), 51.67 (d, $J_{C\text{-}F}$ = 5.2 Hz), 52.28 (br s), 54.27 (d, $J_{C\text{-}F}$ = 7.2 Hz), 54.57 (d, $J_{C\text{-}F}$ = 4.8 Hz), 54.98 (s), 55.37 (s), 61.28 (s), 61.36 (s), 62.06 (s), 62.35 (s), 64.32 (s), 64.75 (s), 100.23 (s, CH), 100.33 (s, CH), 107.73 (s, C_{Ar}), 107.75 (s, C_{Ar}), 108.10 (d, $J_{C\text{-}F}$ = 23.9 Hz, C_{Ar}), 108.17 (d, $J_{C\text{-}F}$ = 24.2 Hz, C_{Ar}), 118.31 (d, $J_{C\text{-}F}$ = 8.7 Hz, C_{Ar}), 118.45 (d, $J_{C\text{-}F}$ = 8.8 Hz, C_{Ar}), 128.27–129.17 (m, C_{Ar}), 134.64 (s, C_{Ar}), 137.55 (d, $J_{C\text{-}F}$ = 11.0 Hz, C_{Ar}), 137.59 (d, $J_{C\text{-}F}$ = 10.5 Hz, C_{Ar}), 140.51–140.64 (m, C_{Ar}), 146.49 (s, C_{Ar}), 146.58 (s, C_{Ar}), 148.30 (s, C_{Ar}), 148.36 (s, C_{Ar}), 149.72 (s, C_{Ar}), 153.53 (d, $J_{C\text{-}F}$ = 250.6 Hz, C_{Ar}), 153.61 (d, $J_{C\text{-}F}$ = 250.4 Hz, C_{Ar}), 167.22 (s, C(O)OH), 176.84 (s, C═O), 176.86 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 677.3709 (calculated for $[C_{38}H_{50}FN_4O_6]^+$ - 677.3709).

4.1.1.21. 1-Cyclopropyl-6-fluoro-7-(4-((5-(hydroxymethyl)-2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-6-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(14)***. The reaction was carried out following the general procedure with compound **3** (1.30 g, 4.30 mmol), ciprofloxacin hydrocloride (1.44 g, 3.91 mmol), $NaHCO_3$ (0.66 g, 7.82 mmol) and KI (0.13 g, 0.78 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was recrystallized from the mixture acetone: water = 2:1. Yield 44% (0.95 g); light yellow solid; mp 225 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.13 (br m, 2H, CH_2 cyclopropyl), 1.30 (br m, 2H, CH_2 cyclopropyl), 1.51 (s, 6H, $2CH_3$), 2.35 (s, 3H, CH_3), 2.76 (br m, 4H, $2CH_2$ piperazinyl), 3.26 (br m, 4H, $2CH_2$ piperazinyl), 3.46 (br m, 1H, CH cyclopropyl), 3.81 (s, 2H, CH_2N), 4.41 (s, 2H, CH_2), 4.91 (s, 2H, CH_2), 7.26 (br s, 1H, CH_{Ar}), 7.87 (d, 1H, $J_{H\text{-}F}$ = 12.3 Hz, CH_{Ar}), 8.64 (s, 1H, CH_{Ar}), 14.90 (br s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 8.30 (s, $2CH_2$ cyclopropyl), 18.39 (s, CH_3), 24.79 (s, $2CH_3$), 35.43 (s, CH cyclopropyl), 49.62 (s, $J_{C\text{-}F}$ = 2.9 Hz, CH_2), 52.27 (s, $2CH_2$), 57.71 (s, CH_2), 58.99 (s, CH_2), 63.07 (s, CH_2), 99.65 (s, $\underline{C}(CH_3)_2$), 105.20 (s, C_{Ar}), 108.09 (s, C_{Ar}), 112.39 (d, $J_{C\text{-}F}$ = 23.4 Hz, C_{Ar}), 120.08 (d, $J_{C\text{-}F}$ = 7.7 Hz, C_{Ar}), 125.65 (s, C_{Ar}), 130.11 (s, C_{Ar}), 139.05 (s, C_{Ar}), 145.61 (d, $J_{C\text{-}F}$ = 10.2 Hz, C_{Ar}), 145.92 (s, C_{Ar}), 146.08 (s, C_{Ar}), 146.20 (s, C_{Ar}), 147.54 (s, C_{Ar}), 153.73 (d, $J_{C\text{-}F}$ = 251.5 Hz, C_{Ar}), 167.02 (s, C(O)OH), 177.07 (s, C═O). ESI-HRMS *m/z*: $[M+H]^+$ 553.2462 (calculated for $[C_{29}H_{34}FN_4O_6]^+$ - 553.2457).

4.1.1.22. 1-Ethyl-6-fluoro-7-(4-((5-(hydroxymethyl)-2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-6-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(16)***. The reaction was carried out following the general procedure with compound **3** (0.55 g, 1.82 mmol), norfloxacin hydrocloride (0.59 g, 1.65 mmol), $NaHCO_3$ (0.28 g, 3.30 mmol) and KI (0.06 g, 0.33 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed with acetone. Yield 77% (0.69 g); white solid; mp 210–213 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.51 (t, 3H, $J_{H\text{-}H}$ = 7.2 Hz, CH_3), 1.53 (s, 6H, $2CH_3$), 2.36 (s, 3H, CH_3), 2.77 (br m, 4H, $2CH_2$ piperazinyl), 3.27 (br m, 4H, $2CH_2$ piperazinyl), 3.83 (s, 2H, CH_2N), 4.27 (q, 2H, $J_{H\text{-}H}$ = 7.2 Hz, CH_2), 4.43 (s, 2H, CH_2), 4.93 (s, 2H, CH_2), 6.77 (d, 1H, $J_{H\text{-}F}$ = 6.8 Hz, CH_{Ar}), 7.93 (d, 1H, $J_{H\text{-}F}$ = 12.9 Hz, CH_{Ar}), 8.60 (s, 1H, CH_{Ar}), 15.02 (br s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 14.56 (s, CH_3), 18.49 (s, CH_3), 24.81 (s, $2CH_3$), 49.65 (br s, CH_2), 49.87 (s, CH_2), 52.27 (s, CH_2), 57.76 (s, CH_2), 59.02 (s, CH_2), 63.06 (s, CH_2), 99.66 (s, $\underline{C}(CH_3)_2$), 104.29 (d, $J_{C\text{-}F}$ = 1.9 Hz, C_{Ar}), 108.46 (s, C_{Ar}), 112.87 (d, $J_{C\text{-}F}$ = 23.2 Hz, C_{Ar}), 121.02 (d, $J_{C\text{-}F}$ = 7.8 Hz, C_{Ar}), 125.64 (s, C_{Ar}), 130.19 (s, C_{Ar}), 137.09 (s, C_{Ar}), 145.76 (s, C_{Ar}), 145.92 (d, $J_{C\text{-}F}$ = 10.5 Hz, C_{Ar}), 146.14 (s, C_{Ar}), 147.31 (s, C_{Ar}), 153.62 (d, $J_{C\text{-}F}$ = 251.8 Hz, C_{Ar}), 167.21 (s, C(O)OH), 177.05 (d, $J_{C\text{-}F}$ = 2.0 Hz, C═O). ESI-HRMS m/z: $[M+H]^+$ 541.2457 (calculated for $[C_{28}H_{34}FN_4O_6]^+$ - 541.2457).

4.1.1.23. 1-Ethyl-6,8-difluoro-7-(4-((5-(hydroxymethyl)-2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-6-yl)methyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(18)***. The reaction was carried out following the general procedure with compound **3** (0.65 g, 2.15 mmol), lomefloxacin hydrochloride (0.76 g, 1.96 mmol), $NaHCO_3$ (0.33 g, 3.91 mmol) and KI (0.07 g, 0.39 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was washed successively with acetone, ethanol and water. . Yield 72% (0.81 g); white solid; mp 210–213 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.21 (d, 3H, $J_{H\text{-}H}$ = 5.9 Hz, CH_3), 1.42 (t, 3H, $J_{H\text{-}H}$ = 6.5 Hz, CH_3), 1.48 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 2.26–2.33 (m, 4H, CH_3+CH), 2.60–2.69 (br m, 2H, CH_2), 3.05 (br m, 1H, CH_2), 3.16 (br m, 1H, CH_2), 3.25–3.34 (br m, 2H, CH_2), 3.39, 4.25 (AB,

$J_{H\text{-}H}$ = 11.8 Hz, CH_2), 4.46, 4.54 (AB, $J_{H\text{-}H}$ = 12.8 Hz, CH_2), 4.52–4.61 (br m, 2H, CH_2), 4.93, 4.99 (AB, $J_{H\text{-}H}$ = 16.5 Hz, CH_2), 7.82 (d, 1H, $J_{H\text{-}F}$ = 11.8 Hz, CH_{Ar}), 8.91 (s, 1H, CH_{Ar}), 14.81 (br s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 14.34 (s, CH_3), 15.90 (d, $J_{C\text{-}F}$ = 4.9 Hz, CH_3), 18.23 (s, CH_3), 24.24 (s, C($\underline{C}H_3$)$_2$), 24.76 (s, C($\underline{C}H_3$)$_2$), 50.06 (br s, CH_2), 50.50 (br s, CH_2), 53.70 (d, $J_{C\text{-}F}$ = 15.9 Hz, CH_2), 55.74 (br s, CH_2), 56.08 (s, CH), 57.00 (br s, CH_2), 57.92 (s, CH_2), 58.42 (s, CH_2), 99.14 (s, $\underline{C}(CH_3)_2$), 106.83 (d, $J_{C\text{-}F}$ = 23.3 Hz, C_{Ar}), 106.97 (s, C_{Ar}), 120.43 (d, $J_{C\text{-}F}$ = 8.2 Hz, C_{Ar}), 126.31 (s, C_{Ar}), 127.21 (d, $J_{C\text{-}F}$ = 7.5 Hz, C_{Ar}), 129.90 (s, C_{Ar}), 133.57 (t, $J_{C\text{-}F}$ = 14.0 Hz, $J_{C\text{-}F}$ = 14.0 Hz, C_{Ar}), 143.81 (s, C_{Ar}), 144.67 (s, C_{Ar}), 145.92 (d, $J_{C\text{-}F}$ = 250.1 Hz, $J_{C\text{-}F}$ = 6.2 Hz, C_{Ar}),146.53 (s, C_{Ar}), 151.15 (s, C_{Ar}), 154.52 (dd, $J_{C\text{-}F}$ = 249.3 Hz, $J_{C\text{-}F}$ = 6.2 Hz, C_{Ar}), 165.45 (s, C(O)OH), 175.49 (s, C═O). ESI-HRMS m/z: $[M+H]^+$ 573.2519 (calculated for $[C_{29}H_{35}F_2N_4O_6]^+$ - 573.2519).

4.1.1.24. 1-Cyclopropyl-6-fluoro-7-(1-((5-(hydroxymethyl)-2,2,8-trimethyl-4H-[1,3]dioxino[4,5-c]pyridin-6-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(20)***. The reaction was carried out following the general procedure with compound **3** (0.45 g, 1.49 mmol), moxifloxacin hydrochloride (0.59 g, 1.35 mmol), $NaHCO_3$ (0.23 g, 2.71 mmol) and KI (0.05 g, 0.27 mmol). The dry residue is dissolved in chloroform and purified by column chromatography (eluent ethyl alcohol/chloroform = 1:3).Yield 42% (0.35 g); light yellow solid; mp 138–140 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 0.88–1.04 (m, 2H, CH_2), 1.12–1.25 (m, 2H, CH_2), 1.46–1.57 (br m, 1H), 1.50 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.64–1.76 (br m, 2H), 2.34 (s, 3H, CH_3), 2.30–2.45 (br m, 2H), 2.60–2.71 (br m, 1H), 3.37–3.49 (m, 2H), 3.56 (s, 3H, CH_3), 3.59–3.74 (br m, 2H), 3.79–3.84 (m, 1H), 3.97–3.99 (m, 2H), 4.08–4.12 (br m, 1H), 4.31, 4.36 (AB, 2H, $J_{H\text{-}H}$ = 13.2 Hz, CH_2), 4.87, 4.92 (AB, 2H, $J_{H\text{-}H}$ = 16.0 Hz, CH_2), 7.72 (d, 1H, $J_{H\text{-}F}$ = 14.0 Hz, CH_{Ar}), 8.73 (s, 1H, CH_{Ar}), 15.02 (br s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$) δ: 9.28 (s, CH_2), 9.89 (s, CH_2), 18.38 (s, CH_3), 22.95 (s, CH_2), 24.03 (s, CH_2), 24.68 (s, C($\underline{C}H_3$)$_2$), 24.85 (s, C($\underline{C}H_3$)$_2$), 36.92 (s), 40.47 (s), 47.43 (s), 49.25 (br s), 55.28 (d, $J_{C\text{-}F}$ = 7.5 Hz), 57.63 (s), 58.95 (s), 61.19 (s), 61.33 (s), 61.41 (s), 99.57 (s, $\underline{C}(CH_3)_2$), 107.60 (s, C_{Ar}), 107.97 (d, $J_{C\text{-}F}$ = 24.2 Hz, C_{Ar}), 118.34 (d, $J_{C\text{-}F}$ = 8.7 Hz, C_{Ar}), 125.61 (s, C_{Ar}), 128.57 (s, C_{Ar}), 128.63 (s, C_{Ar}), 130.05 (s, C_{Ar}), 132.14 (d, $J_{C\text{-}F}$ = 9.9 Hz, C_{Ar}), 134.58 (s, C_{Ar}), 137.63 (d, $J_{C\text{-}F}$ = 10.6 Hz, C_{Ar}), 140.54 (d, $J_{C\text{-}F}$ = 7.0 Hz, C_{Ar}), 145.82 (s, C_{Ar}), 149.69 (s, C_{Ar}), 153.52 (s, $J_{C\text{-}F}$ = 251.0 Hz, C_{Ar}), 167.14 (s, C(O)OH), 176.74 (d, $J_{C\text{-}F}$ = 2.7 Hz, C═O). ESI-HRMS m/z: $[M+H]^+$ 623.2876, (calculated for $[C_{33}H_{40}FN_4O_7]^+$ - 623.2876).

4.1.1.25. 1-Cyclopropyl-6-fluoro-7-(4-((5-hydroxy-2,4-bis(hydroxymethyl)-6-methylpyridin-3-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(22)***. The reaction was carried out following the general procedure with compound **4** (0.71 g, 2.79 mmol), ciprofloxacin hydrocloride (0.93 g, 2.54 mmol), $NaHCO_3$ (0.66 g, 7.87 mmol) and KI (0.08 g, 0.51 mmol). The dry residue was extracted with ethyl acetate and the insoluble part was filtered. The filtrate was dried in vacuo and the product was recrystallized from the mixture acetone: water = 2:1. After recrystallization to the resulting precipitate was suspended in water then equimolar amount of 0.1 M HCl solution added and the solvent was removed in vacuo. Yield 65% (0.90 g); light yellow solid; mp 190–195 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.16–1.20 (br m, 2H, CH_2 cyclopropyl), 1.28–1.34 (m, 2H, CH_2 cyclopropyl), 2.66 (s, 3H, CH_3), 3.48 (br m, 4H, 2CH_2 piperazinyl), 3.63 (br m, 4H, 2CH_2 piperazinyl), 3.84 (br m, 1H, CH cyclopropyl), 4.69 (s, 2H, CH_2), 4.95 (s, 2H, CH_2), 4.97 (s, 2H, CH_2), 7.59 (d, 1H, $J_{H\text{-}F}$ = 7.4 Hz, CH_{Ar}), 7.91 (d, 1H, $J_{H\text{-}F}$ = 13.3 Hz, CH_{Ar}), 8.66 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6) δ: 7.48 (s, 2CH_2 cyclopropyl), 19.28 (s, CH_3), 35.80 (s, CH cyclopropyl), 49.44 (d, $J_{C\text{-}F}$ = 4.6 Hz, CH_2), 51.60 (s, CH_2), 53.41 (s, CH_2), 56.17 (s, CH_2), 63.26 (s, CH_2), 106.49 (d, $J_{C\text{-}F}$ = 3.1 Hz, C_{Ar}), 106.72 (s, C_{Ar}), 110.85 (d, $J_{C\text{-}F}$ = 23.0 Hz, C_{Ar}), 118.68 (d, $J_{C\text{-}F}$ = 7.7 Hz, C_{Ar}), 127.72 (s, C_{Ar}), 135.03 (s, C_{Ar}), 139.08 (s, C_{Ar}), 144.39 (s, C_{Ar}), 144.95 (d, $J_{C\text{-}F}$ = 10.2 Hz, C_{Ar}), 147.91 (s, C_{Ar}), 148.79 (s, C_{Ar}), 149.44 (s, C_{Ar}), 152.97 (d, $J_{C\text{-}F}$ = 249.6 Hz, C_{Ar}), 165.82 (s, C(O)OH), 176.31 (s, C═O). ESI-HRMS *m/z*: $[M\text{-}Cl]^+$ 513.2149 (calculated for $[C_{26}H_{30}FN_4O_6]^+$ - 513.2144).

4.1.1.26. 1-Ethyl-6-fluoro-7-(4-((5-hydroxy-2,4-bis(hydroxymethyl)-6-methylpyridin-3-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(23)***. The reaction was carried out following the general procedure with compound **4** (0.80 g, 3.15 mmol), norfloxacin hydrocloride (1.02 g, 2.86 mmol), $NaHCO_3$ (0.75 g, 8.87 mmol) and KI (0.10 g, 0.57 mmol). The dry residue is suspended in water and refluxed. The insoluble part is filtered off and dried in vacuo. To the residue water and concentrated hydrochloric acid were added until the solution is homogenized. The resulting solution is dried in vacuo and to the residue DMF is added. The undissolved part is separated, dissolved in water, and an equimolar amount of sodium bicarbonate is added. The formed precipitate is filtered off, suspended in water, and equimolar 0.1 M hydrochloric acid is added until the solution is homogenized. Then the solvent was removed in vacuo. Yield 85% (1.31 g); beige solid; mp 215–218 °C (decomp). ^{1}H NMR ($CDCl_3$) δ: 1.41 (t, 3H, $J_{H\text{-}H}$ = 7.1 Hz, CH_3), 2.69 (s, 3H, CH_3), 3.50–3.90 (br m, 8H, 4CH_2 piperazinyl), 4.61 (q, 2H, $J_{H\text{-}H}$ = 7.1 Hz, CH_2), 4.77 (br s, 2H, CH_2), 4.98 (s, 4H, 2CH_2), 7.25 (d, 1H, $J_{H\text{-}F}$ = 7.2 Hz, CH_{Ar}), 7.96 (d, 1H, $J_{H\text{-}F}$ = 13.0 Hz, CH_{Ar}), 8.96 (s, 1H, CH_{Ar}). ^{13}C NMR ($CDCl_3$) δ: 14.51 (s, CH_3), 15.50 (s, CH_3), 46.88 (br s, CH_2), 49.23 (s, CH_2), 50.97 (s, CH_2), 51.41 (s, CH_2), 55.83 (s, CH_2), 58.56 (s, CH_2), 106.69 (br s, C_{Ar}), 107.20 (s, C_{Ar}), 111.49 (d, $J_{C\text{-}F}$ = 23.2 Hz, C_{Ar}), 120.01 (d, $J_{C\text{-}F}$ = 7.6 Hz, C_{Ar}), 125.09 (C_{Ar}), 137.16 (s, C_{Ar}), 143.94 (d, $J_{C\text{-}F}$ = 10.3 Hz, C_{Ar}), 144.10 (s, C_{Ar}), 146.91 (s, C_{Ar}), 148.81 (s, C_{Ar}), 151.46 (s, C_{Ar}), 152.75 (s, $J_{C\text{-}F}$ = 249.3 Hz, C_{Ar}), 166.11 (s, C(O)OH), 176.23 (s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 501.2144 (calculated for $[C_{25}H_{30}FN_4O_6]^+$ - 501.2144).

4.1.1.27. 1-Cyclopropyl-6-fluoro-7-(1-((5-hydroxy-2,4-bis(hydroxymethyl)-6-methylpyridin-3-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(24)***. The reaction was carried out following the general procedure with compound **4** (1.24 g, 4.88 mmol), moxifloxacin hydrochloride (1.94 g, 4.44 mmol), $NaHCO_3$ (1.16 g, 13.8 mmol) and KI (0.15 g, 0.89 mmol). The dry residue is suspended in water and refluxed. The insoluble part is filtered off, suspended in acetone and refluxed. The residue is filled with water and concentrated hydrochloric acid is added until the solution is homogenized. The resulting solution was dried to dryness and purified by reverse phase column chromatography (eluent water-acetonitrile 5:1). Yield 10% (0.28 g); light yellow solid; mp 195–200 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 0.88–1.24 (br m, 4H, 2CH_2), 1.57–1.87 (br m, 4H), 2.62 (s, 3H, CH_3), 2.71–2.85 (br m, 1H), 2.93–3.22 (br m, 2H), 3.62 (s, 3H, CH_3), 3.66–3.84 (br m, 2H), 3.94–4.30 (br m, 5H), 4.45–5.00 (br m, 6H, 3CH_2), 7.71 (d, 1H, $J_{H\text{-}F}$ = 13.8 Hz, CH_{Ar}), 8.68 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6) δ: 8.78 (s, CH_2), 9.11 (s, CH_2), 16.17 (s, CH_3), 21.43 (CH_2), 35.73 (s), 40.56 (s), 50.80 (s), 55.16 (s), 59.42 (br s), 61.89 (s), 106.44 (s, C_{Ar}), 106.49 (d, $J_{C\text{-}F}$ = 23.2 Hz, C_{Ar}), 117.80 (d, $J_{C\text{-}F}$ = 7.8 Hz, C_{Ar}), 134.45 (s, C_{Ar}), 136.49 (d, $J_{C\text{-}F}$ = 10.6 Hz, C_{Ar}), 141.28 (br s, C_{Ar}), 147.14 (m, C_{Ar}), 150.37 (s, C_{Ar}), 150.75 (br s, C_{Ar}), 152.94 (d, $J_{C\text{-}F}$ = 249.3 Hz, C_{Ar}), 165.79 (s, C(O)OH), 176.05 (br s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 583.2563 (calculated for $[C_{30}H_{36}FN_4O_7]^+$ - 583.2563).

4.1.1.28. 1-Cyclopropyl-6-fluoro-7-(4-((5-hydroxy-4,6-dimethylpyridin-3-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(25)***. The reaction was carried out following the general procedure with compound **5** (1.10 g, 5.29 mmol), ciprofloxacin hydrocloride (1.77 g, 4.81 mmol), $NaHCO_3$ (1.25 g, 14.91 mmol) and KI (0.16 g, 0.96 mmol). The dry residue is suspended in water and refluxed. The insoluble part is filtered off and dried in vacuo. To the residue water and concentrated hydrochloric acid were added until the solution is

homogenized. The resulting solution is dried in vacuo and to the residue DMF is added. The undissolved part is separated, dissolved in water, and an equimolar amount of sodium bicarbonate is added. The formed precipitate is filtered off, suspended in water, and equimolar 0.1 M hydrochloric acid is added until the solution is homogenized. Then the solvent was removed in vacuo. The residue is refluxed with ethanol. The undissolved part is separated and dried to dryness. Yield 46% (1.12 g); beige solid; mp > 190 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.13–1.17 (br m, 2H, CH_2 cyclopropyl), 1.28–1.33 (m, 2H, CH_2 cyclopropyl), 2.50 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.37–3.50 (br m, 4H, $2CH_2$ piperazinyl), 3.55–3.66 (br m, 4H, $2CH_2$ piperazinyl), 3.77–3.82 (br m, 1H, CH cyclopropyl), 4.51 (s, 2H, CH_2N), 7.58 (d, 1H, $J_{H\text{-}F}$ = 7.3 Hz, CH_{Ar}), 7.93 (d, 1H, $J_{H\text{-}F}$ = 12.9 Hz, CH_{Ar}), 8.49 (s, 1H, CH_{Ar}), 8.68 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6) δ: 7.99 (s, $2CH_2$ cyclopropyl), 14.32 (s, CH_3), 15.86 (s, CH_3), 36.33 (s, CH cyclopropyl), 46.99 (s, CH_2), 51.23 (s, CH_2), 53.84 (s, CH_2), 107.07 (s, C_{Ar}), 107.38 (s, C_{Ar}), 111.68 (d, $J_{C\text{-}F}$ = 22.7 Hz, C_{Ar}), 119.93 (d, $J_{C\text{-}F}$ = 7.5 Hz, C_{Ar}), 127.24 (s, C_{Ar}), 135.21 (s, C_{Ar}), 139.44 (s, C_{Ar}), 142.24 (s, C_{Ar}), 144.16 (d, $J_{C\text{-}F}$ = 10.3 Hz, C_{Ar}), 145.30 (s, C_{Ar}), 148.77 (s, C_{Ar}), 152.62 (s, C_{Ar}), 153.31 (d, $J_{C\text{-}F}$ = 249.6 Hz, C_{Ar}), 166.50 (s, C(O)OH), 176.79 (s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 467.2089 (calculated for $[C_{25}H_{28}FN_4O_4]^+$ - 467.2089).

4.1.1.29. 1-Ethyl-6-fluoro-7-(4-((5-hydroxy-4,6-dimethylpyridin-3-yl) methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(26)***. The reaction was carried out following the general procedure with compound **5** (1.00 g, 4.81 mmol), norfloxacin hydrocloride (1.55 g, 4.37 mmol), $NaHCO_3$ (1.14 g, 13.55 mmol) and KI (0.15 g, 0.87 mmol). The dry residue is suspended in water and refluxed. The insoluble part is filtered off and dried in vacuo. To the residue water and concentrated hydrochloric acid were added until the solution is homogenized. The resulting solution is dried in vacuo and to the residue DMF is added. The undissolved part is separated, dissolved in water, and an equimolar amount of sodium bicarbonate is added. The formed precipitate is filtered off, suspended in water, and equimolar 0.1 M hydrochloric acid is added until the solution is homogenized. Then the solvent was removed in vacuo. Yield 47% (1.01 g); beige solid; mp 295–300 °C (decomp). ^{1}H NMR (DMSO-d_6+D_2O) δ: 1.37 (br m, 3H, CH_3), 2.46 (s, 3H, CH_3), 2.55 (s, 3H, CH_3), 3.40–3.63 (m, 8H, $4CH_2$ piperazinyl), 4.39 (br m, 2H, CH_2), 4.54 (s, 2H, CH_2N), 7.07 (d, 1H, $J_{H\text{-}F}$ = 3.8 Hz, CH_{Ar}), 7.73 (d, 1H, $J_{H\text{-}F}$ = 12.8 Hz, CH_{Ar}), 8.35 (s, 1H, CH_{Ar}), 8.68 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6+D_2O) δ: 15.02 (s, CH_3), 15.12 (s, CH_3), 16.28 (s, CH_3), 47.39 (s, CH_2), 50.74 (s, CH_2), 52.18 (s, CH_2), 54.66 (s, CH_2), 107.22 (s, C_{Ar}), 107.55 (s, C_{Ar}), 112.45 (d, $J_{C\text{-}F}$ = 23.0 Hz, C_{Ar}), 120.94 (d, $J_{C\text{-}F}$ = 7.7 Hz, C_{Ar}), 126.96 (s, C_{Ar}), 135.44 (s, C_{Ar}), 138.02 (s, C_{Ar}), 143.40 (s, C_{Ar}),145.00 (d, $J_{C\text{-}F}$ = 10.2 Hz, C_{Ar}), 146.64 (s, C_{Ar}), 149.20 (s, C_{Ar}), 153.59 (s, C_{Ar}), 153.84 (d, $J_{C\text{-}F}$ = 250.3 Hz, C_{Ar}), 168.16 (s, C(O)OH), 177.03 (s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 455.2089 (calculated for $[C_{24}H_{28}FN_4O_4]^+$ - 455.2089).

4.1.1.30. 1-Ethyl-6,8-difluoro-7-(4-((5-hydroxy-4,6-dimethylpyridin-3-yl) methyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(27)***. The reaction was carried out following the general procedure with compound **5** (0.50 g, 2.40 mmol), lomefloxacin hydrochloride (0.85 g, 2.18 mmol), $NaHCO_3$ (0.57 g, 6.77 mmol) and KI (0.07 g, 0.44 mmol). The dry residue is suspended in water and refluxed. The insoluble part is filtered off and dried in vacuo. To the residue water and concentrated hydrochloric acid were added until the solution is homogenized. The resulting solution is dried in vacuo and to the residue DMF is added. The undissolved part is separated, dissolved in water, and an equimolar amount of sodium bicarbonate is added. The formed precipitate is filtered off, suspended in water, and equimolar 0.1 M hydrochloric acid is added until the solution is homogenized. Then the solvent was removed in vacuo. The residue is refluxed with ethanol. The undissolved part is separated and dried to dryness. The residue is recrystallized from a mixture of methanol-water (10:1). Yield 14% (0.13 g); beige solid; mp 230–250 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.45 (t, 3H, $J_{H\text{-}H}$ = 6.9 Hz, CH_3), 1.60 (br s, 3H, CH_3), 2.59 (s, 3H, CH_3), 2.66 (s, 3H, CH_3), 3.16–3.89 (m, 7H, CH piperazinyl + $3CH_2$ piperazinyl), 4.32–4.45 (br m, 1H, CH_2), 4.53–4.65 (br m, 2H, CH_2), 4.98 (br m, 1H, CH_2), 7.89 (d, 1H, $J_{H\text{-}F}$ = 11.6 Hz, CH_{Ar}), 8.74 (br s, 1H, CH_{Ar}), 8.95 (s, 1H, CH_{Ar}), 10.93 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 13.89 (s, CH_3), 14.24 (s, CH_3), 15.22 (s, CH_3), 15.92 (d, $J_{C\text{-}F}$ = 4.6 Hz, CH_3), 46.99 (br s), 49.87 (br s), 50.13 (br s), 53.16 (s), 53.69 (d, $J_{C\text{-}F}$ = 15.5 Hz, CH_2), 60.55 (s), 107.02 (d, $J_{C\text{-}F}$ = 22.4 Hz, C_{Ar}), 107.13 (s, C_{Ar}), 121.35 (br s, C_{Ar}), 126.45 (s, C_{Ar}), 127.12 (d, $J_{C\text{-}F}$ = 6.5 Hz, C_{Ar}), 132.13 (br m, C_{Ar}), 134.89 (br s, C_{Ar}), 141.64 (s, C_{Ar}), 145.41 (s, C_{Ar}), 146.19 (d, $J_{C\text{-}F}$ = 254.4 Hz, C_{Ar}), 151.32 (s, C_{Ar}), 152.43 (s, C_{Ar}), 154.40 (d, $J_{C\text{-}F}$ = 249.5 Hz, C_{Ar}), 165.40 (s, C(O)OH), 175.48 (s, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 487.2151 (calculated for $[C_{25}H_{29}F_2N_4O_4]^+$ - 487.2151).

4.1.1.31. 1-Cyclopropyl-6-fluoro-7-(1-((5-hydroxy-4,6-dimethylpyridin-3-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(28)***. The reaction was carried out following the general procedure with compound **5** (0.50 g, 2.40 mmol), moxifloxacin hydrochloride (0.96 g, 2.18 mmol), $NaHCO_3$ (0.57 g, 6.77 mmol) and KI (0.07 g, 0.44 mmol). The dry residue is suspended in water and refluxed. The insoluble part is filtered off and dried in vacuo. To the residue water and concentrated hydrochloric acid were added until the solution is homogenized. The resulting solution is dried in vacuo and the residue was washed with ethanol. Yield 50% (0.63 g); yellow solid; mp 230–250 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 0.80–1.30 (m, 4H, $2CH_2$), 1.40–1.61 (m, 1H), 1.63–1.87 (m, 2H), 1.89–2.14 (m, 1H), 2.50 (s, 3H, CH_3), 2.64 (s, 3H, CH_3), 2.74–2.90 (m, 1H), 3.00–3.29 (m, 2H), 3.63 (s, 3H, CH_3), 3.71–4.86 (m, 7H), 7.68 (br s, 1H, CH_{Ar}), 8.66 (s, 1H, CH_{Ar}), 8.75 (br s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6+D_2O) δ: 10.46 (br s, CH_2), 15.50 (s, CH_3), 16.59 (s, CH_3), 22.58 (CH_2), 25.43 (CH_2), 37.17 (s), 42.83 (s), 55.05 (s), 63.33 (s), 106.57 (s, C_{Ar}), 107.60 (d, $J_{C\text{-}F}$ = 23.8 Hz, C_{Ar}), 118.69 (d, $J_{C\text{-}F}$ = 9.3 Hz, C_{Ar}), 127.46 (s, C_{Ar}), 135.88 (br m, C_{Ar}), 138.37 (d, $J_{C\text{-}F}$ = 10.8 Hz, C_{Ar}), 142.99 (d, $J_{C\text{-}F}$ = 7.2 Hz, C_{Ar}), 144.72 (s, C_{Ar}), 148.01 (s, C_{Ar}), 151.80 (br s, C_{Ar}), 154.47 (s, C_{Ar}), 154.97 (d, $J_{C\text{-}F}$ = 251.6 Hz, C_{Ar}), 170.12 (s, C(O) OH), 176.70 (d, $J_{C\text{-}F}$ = 3.5 Hz, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 537.2508 (calculated for $[C_{29}H_{34}FN_4O_5]^+$ - 537.2508). HPLC analysis: retention time 16.88 min; purity 98.57%.

4.1.2. General procedure for removal of ketal protection from pyridoxine-containing fluoroquinolones

The solution of pyridoxine-containing fluoroquinolones (1.0 equiv) in 20 ml of water was added 1 ml of concentrated HCl and stirred for 24 h at 25 °C. The solution was neutralized to pH = 6 with $NaHCO_3$. The precipitate was filtered and washed with 10 ml of water and 10 ml of acetone (for compounds **7**, **9** and **15**) or ethanol (for compound **13**). After washing the precipitate was added an equimolar amount of 0.1 N HCl solution and then the solvent was removed in vacuo.

4.1.2.1. 1-Cyclopropyl-6-fluoro-7-(4-((5-hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride ***(7)***. The reaction was carried out following the general procedure with compound **6b** (0.15 g, 0.28 mmol). Yield 86% (0.13 g); beige solid; mp > 190 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.19 (br m, 2H, CH_2 cyclopropyl), 1.31 (br m, 2H, CH_2 cyclopropyl), 2.67 (s, 3H, CH_3), 3.54–3.90 (m, 9H, $4CH_2$ piperazinyl + CH cyclopropyl), 4.77 (br s, 2H, CH_2), 4.96 (s, 2H, CH_2), 7.60 (br s, 1H, CH_{Ar}), 7.95 (d, 1H, $J_{H\text{-}F}$ = 13.0 Hz, CH_{Ar}), 8.67 (s, 1H, CH_{Ar}), 8.80 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6) δ: 7.59 (s, $2CH_2$ cyclopropyl), 15.09 (s, CH_3), 35.96 (s, CH cyclopropyl), 46.10 (s, CH_2), 50.58 (s, CH_2), 52.40 (s, CH_2), 55.41 (s, CH_2), 106.79 (s, C_{Ar}), 106.91 (s, C_{Ar}), 111.16 (d, $J_{C\text{-}F}$ = 23.2 Hz, C_{Ar}), 119.31 (d, $J_{C\text{-}F}$ = 8.1 Hz, C_{Ar}), 126.36 (s, C_{Ar}), 134.62 (s, C_{Ar}), 139.01 (s, C_{Ar}), 143.52 (d, $J_{C\text{-}F}$ = 10.4 Hz, C_{Ar}), 145.10 (s, C_{Ar}), 148.18 (s, C_{Ar}), 152.38 (s, C_{Ar}), 152.76 (d, $J_{C\text{-}F}$ = 248.9 Hz, C_{Ar}), 165.77 (s, C(O)OH),

176.32 (s, C═O). ESI-HRMS *m/z*: $[M-Cl]^+$ 483.2044 (calculated for $[C_{25}H_{28}FN_4O_5]^+$ - 483.2038). HPLC analysis: retention time 8.45 min; purity 98.24%.

*4.1.2.2. 1-Ethyl-6-fluoro-7-(4-((5-hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(9)**.* The reaction was carried out following the general procedure with compound **8b** (0.20 g, 0.39 mmol). Yield 70% (0.14 g); light yellow solid; mp 230–232 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.41 (t, 3H, J_{H-H} = 7.1 Hz, CH_3), 2.64 (s, 3H, CH_3), 3.32–3.92 (m, 8H, 4CH_2 piperazinyl), 4.62 (q, 2H, J_{H-H} = 7.1 Hz, CH_2), 4.69 (s, 2H, CH_2), 4.95 (s, 2H, CH_2), 7.27 (d, 1H, J_{H-F} = 7.2 Hz, CH_{Ar}), 7.97 (d, 1H, J_{H-F} = 13.1 Hz, CH_{Ar}), 8.73 (s, 1H, CH_{Ar}), 8.97 (s, 1H, CH_{Ar}). ^{13}C NMR (DMSO-d_6) δ: 14.45 (s, CH_3), 15.77 (s, CH_3), 46.48 (s, CH_2), 49.13 (s, CH_2), 50.71 (s, CH_2), 52.87 (s, CH_2), 55.50 (s, CH_2), 106.51 (s, C_{Ar}), 107.19 (s, C_{Ar}), 111.45 (d, J_{C-F} = 22.7 Hz, C_{Ar}), 119.94 (d, J_{C-F} = 7.5 Hz, C_{Ar}), 126.33 (s, C_{Ar}), 135.64 (br s, C_{Ar}), 137.14 (s, C_{Ar}), 143.61 (s, C_{Ar}), 143.93 (d, J_{C-F} = 10.1 Hz, C_{Ar}), 144.16 (s, C_{Ar}), 148.76 (s, C_{Ar}), 152.00 (s, C_{Ar}), 152.69 (d, J_{C-F} = 249.1 Hz, C_{Ar}), 166.02 (s, C(O)OH), 176.18 (s, C═O). ESI-HRMS m/z: $[M-Cl]^+$ 471.2044 (calculated for $[C_{24}H_{28}FN_4O_5]^+$ - 471.2038).

*4.1.2.3. 1-Ethyl-6,8-difluoro-7-(4-((5-hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl)methyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(11)**.* The reaction was carried out following the general procedure with compound **10b** (0.50 g, 0.91 mmol). Yield 60% (0.29 g); yellow solid; mp 201–216 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.45 (t, 3H, J_{H-H} = 6.3 Hz, CH_3), 1.46 (d, 3H, J_{H-H} = 6.5 Hz, CH_3), 2.50 (s, 3H, CH_3), 2.90–3.74 (m, 7H, CH + 3CH_2), 4.11 (br s, 1H, CH_2), 4.52–4.78 (m, 3H, 2CH_2), 4.87, 4.91 (AB, 2H, J_{H-H} = 13.7 Hz, CH_2), 7.89 (d, 1H, J_{H-F} = 11.5 Hz, CH_{Ar}), 8.31 (s, 1H, CH_{Ar}), 8.95 (s, 1H, CH_{Ar}), 9.92 (br s, 1H, OH), 14.82 (s, 1H, C(O)OH). ^{13}C NMR ($CDCl_3$+DMSO-d_6) δ: 11.53 (s, CH_3), 14.11 (d, J_{C-F} = 5.0 Hz, CH_3), 15.95 (s, CH_3), 46.21 (br s), 47.42 (br s), 49.14 (s), 51.86 (d, J_{C-F} = 15.4 Hz, CH_2), 52.38 (br s), 53.80 (s), 56.39 (br s), 105.19 (d, J_{C-F} = 19.7 Hz, C_{Ar}), 105.30 (s, C_{Ar}), 119.33 (d, J_{C-F} = 8.7 Hz, C_{Ar}), 125.30 (d, J_{C-F} = 6.7 Hz, C_{Ar}), 130.81 (t, J_{C-F} = 15.0 Hz, J_{C-F} = 15.0 Hz, C_{Ar}), 135.98–138.02 (m, C_{Ar}), 144.36 (dd, J_{C-F} = 251.3 Hz, J_{C-F} = 5.5 Hz, C_{Ar}), 144.39 (br s, C_{Ar}), 148.77 (br s, C_{Ar}), 149.45 (s, C_{Ar}), 152.63 (dd, $^1J_{C-F}$ = 249.6 Hz, $^3J_{C-F}$ = 5.5 Hz, C_{Ar}), 163.61 (s, C(O)OH), 173.70 (s, C═O). ESI-HRMS m/z: $[M-Cl]^+$ 503.2101 (calculated for $[C_{25}H_{29}F_2N_4O_5]^+$ - 503.2101).

*4.1.2.4. 1-Cyclopropyl-6-fluoro-7-(1-((5-hydroxy-4-(hydroxymethyl)-6-methylpyridin-3-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(13)**.* The reaction was carried out following the general procedure with compound **12b** (0.65 g, 1.08 mmol). Yield 62% (0.40 g), light green solid, mp > 175 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.01–1.14 (m, 4H, 2CH_2), 1.47–1.49 (m, 1H), 1.63–1.77 (m, 3H), 2.51 (s, 3H, CH_3), 2.65–2.75 (m, 2H), 3.02 (m, 1H), 3.61 (s, 3H, CH_3), 3.61–3.84 (m, 3H), 4.07–4.16 (m, 3H), 4.07 (br s, 1H), 4.77, 4.84 (AB, 2H, J_{H-H} = 14.0 Hz, CH_2), 7.66 (d, 1H, J_{H-F} = 13.9 Hz, CH_{Ar}), 8.26 (s, 1H, CH_{Ar}), 8.66 (s, 1H, CH_{Ar}), 15.11 (br s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 8.99 (s, CH_2), 35.48 (s), 40.59 (s), 53.00 (s), 55.46 (br s), 61.70 (br s), 106.38 (s, C_{Ar}), 106.46 (d, J_{C-F} = 23.3 Hz, C_{Ar}), 117.65 (br s, C_{Ar}), 134.42 (s, C_{Ar}), 136.55 (d, J_{C-F} = 10.5 Hz, C_{Ar}), 141.24 (s, C_{Ar}), 150.30 (s, C_{Ar}), 151.17 (br s, C_{Ar}), 152.94 (s, J_{C-F} = 249.3 Hz, C_{Ar}), 165.76 (s, C(O)OH), 175.99 (d, J_{C-F} = 2.5 Hz, C═O). ESI-HRMS m/z: $[M-Cl]^+$ 553.2457 (calculated for $[C_{29}H_{34}FN_4O_6]^+$ - 553.2457).

*4.1.2.5. 1-Cyclopropyl-6-fluoro-7-(4-((5-hydroxy-3,4-bis(hydroxymethyl)-6-methylpyridin-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(15)**.* The reaction was carried out following the general procedure with compound **14** (0.91 g, 1.65 mmol). Yield 76% (0.69 g); light yellow solid; mp 212–215 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.18 (br m, 2H, CH_2 cyclopropyl), 1.30–1.34 (m, 2H, CH_2 cyclopropyl), 2.44 (s, 3H, CH_3), 3.44 (br m, 4H, 2CH_2 piperazinyl), 3.66 (br m, 4H, 2CH_2 piperazinyl), 3.84 (br m, 1H, CH cyclopropyl), 4.48 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.80 (s, 2H, CH_2), 5.90 (br s, 1H, OH), 7.60 (br s, 1H, CH_{Ar}), 7.91 (d, 1H, J_{H-F} = 12.9 Hz, CH_{Ar}), 8.65 (s, 1H, CH_{Ar}), 9.69 (br s, 1H, OH), 15.10 (br s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 7.62 (s, 2CH_2 cyclopropyl), 19.26 (s, CH_3), 35.97 (s, CH cyclopropyl), 46.60 (s, CH_2), 51.15 (s, CH_2), 55.92 (s, CH_2), 56.03 (s, CH_2), 106.80 (s, C_{Ar}), 111.13 (d, J_{C-F} = 23.6 Hz, C_{Ar}), 119.18 (d, J_{C-F} = 7.7 Hz, C_{Ar}), 133.50 (s, C_{Ar}), 134.00 (s, C_{Ar}), 139.05 (s, C_{Ar}), 143.85 (d, J_{C-F} = 10.1 Hz, C_{Ar}), 145.67 (s, C_{Ar}), 148.08 (s, C_{Ar}), 150.07 (s, C_{Ar}), 152.81 (d, J_{C-F} = 249.5 Hz, C_{Ar}), 165.81 (s, C(O)OH), 176.32 (s, C═O). ESI-HRMS *m/z*: $[M-Cl]^+$ 513.2149 (calculated for $[C_{26}H_{30}FN_4O_6]^+$ - 513.2144).

*4.1.2.6. 1-Ethyl-6-fluoro-7-(4-((5-hydroxy-3,4-bis(hydroxymethyl)-6-methylpyridin-2-yl)methyl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(17)**.* The reaction was carried out following the general procedure with compound **16** (0.39 g, 0.72 mmol). Yield 97% (0.38 g); white solid; mp 227–230 °C (decomp). ^{1}H NMR (DMSO-d_6) δ: 1.41 (t, 3H, J_{H-H} = 7.0 Hz, CH_3), 2.45 (s, 3H, CH_3), 3.51 (br m, 4H, 2CH_2 piperazinyl), 3.70 (br m, 4H, 2CH_2 piperazinyl), 4.57 (s, 2H, CH_2), 4.62 (br m, 4H, 2CH_2), 4.80 (s, 2H, CH_2), 7.26 (d, 1H, J_{H-F} = 7.1 Hz, CH_{Ar}), 7.94 (d, 1H, J_{H-F} = 13.1 Hz, CH_{Ar}), 8.96 (s, 1H, CH_{Ar}), 9.75 (br s, 1H, OH), 15.27 (br s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 14.52 (s, CH_3), 19.23 (s, CH_3), 46.42 (d, J_{C-F} = 3.7 Hz, CH_2), 49.15 (s, CH_2), 51.07 (s, 2CH_2), 55.80 (s, CH_2), 55.96 (s, CH_2), 56.58 (s, CH_2), 106.44 (br s, C_{Ar}), 107.17 (s, C_{Ar}), 111.41 (d, J_{C-F} = 22.7 Hz, C_{Ar}), 119.87 (d, J_{C-F} = 7.5 Hz, C_{Ar}), 133.61 (C_{Ar}), 134.26 (s, C_{Ar}), 137.13 (s, C_{Ar}), 138.43 (s, C_{Ar}), 144.01 (d, J_{C-F} = 10.4 Hz, C_{Ar}), 145.79 (s, C_{Ar}), 148.70 (s, C_{Ar}), 150.19 (s, C_{Ar}), 152.67 (s, J_{C-F} = 249.4 Hz, C_{Ar}), 166.05 (s, C(O)OH), 176.15 (d, J_{C-F} = 2.0 Hz, C═O). ESI-HRMS m/z: $[M-Cl]^+$ 501.2144 (calculated for $[C_{25}H_{30}FN_4O_6]^+$ - 501.2144).

*4.1.2.7. 1-Ethyl-6,8-difluoro-7-(4-((5-hydroxy-3,4-bis(hydroxymethyl)-6-methylpyridin-2-yl)methyl)-3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(19)**.* The reaction was carried out following the general procedure with compound **18** (0.33 g, 0.58 mmol). Yield 88% (0.27 g); yellow solid; mp 185–187 °C (decomp).^{1}H NMR (DMSO-d_6) δ: 1.45 (t, 3H, J_{H-H} = 6.9 Hz, CH_3), 1.49 (d, 3H, J_{H-H} = 6.4 Hz, CH_3), 2.44 (s, 3H, CH_3), 3.33–3.79 (br m, 7H, 3CH_2+CH), 4.44, 4.72 (AB, 2H, J_{H-H} = 14.4 Hz, CH_2), 4.56–4.64 (br m, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.80 (s, 2H, CH_2), 7.91 (d, 1H, J_{H-F} = 11.5 Hz, CH_{Ar}), 8.95 (s, 1H, CH_{Ar}), 9.72 (br s, 1H, OH), 14.80 (br s, 1H, C(O)OH). ^{13}C NMR (DMSO-d_6) δ: 13.09 (s, CH_3), 15.90 (d, J_{C-F} = 4.6 Hz, CH_3), 19.20 (s, CH_3), 47.03 (br s), 50.11 (br s), 53.07 (d, J_{C-F} = 11.5 Hz, CH_2), 53.70 (d, J_{C-F} = 15.5 Hz, CH_2), 55.81 (s), 55.96 (s), 57.85 (s), 107.04 (d, J_{C-F} = 23.0 Hz, C_{Ar}), 107.15 (s, C_{Ar}), 121.35 (d, J_{C-F} = 8.5 Hz, C_{Ar}), 127.13 (d, J_{C-F} = 6.3 Hz, C_{Ar}), 132.40 (t, J_{C-F} = 14.2 Hz, J_{C-F} = 14.2 Hz, C_{Ar}), 133.58 (s, C_{Ar}), 134.18 (s, C_{Ar}), 138.53 (s, C_{Ar}), 145.77 (s, C_{Ar}), 146.30 (dd, J_{C-F} = 251.4 Hz, J_{C-F} = 5.6 Hz, C_{Ar}), 150.15 (s, C_{Ar}), 151.34 (s, C_{Ar}), 154.46 (dd, J_{C-F} = 249.3 Hz, J_{C-F} = 5.3 Hz, C_{Ar}), 165.41 (s, C(O)OH), 175.50 (s, C═O). ESI-HRMS m/z: $[M-Cl]^+$ 533.2206 (calculated for $[C_{26}H_{31}F_2N_4O_6]^+$ - 533.2206).

*4.1.2.8. 1-Cyclopropyl-6-fluoro-7-(1-((5-hydroxy-3,4-bis(hydroxymethyl)-6-methylpyridin-2-yl)methyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride **(21)**.* The reaction was carried out following the general procedure with compound **20** (0.20 g, 0.32 mmol). Yield 55% (0.11 g); white solid; mp 172–174 °C (decomp).^{1}H NMR (DMSO-d_6) δ: 0.94–1.05 (m, 2H, CH_2), 1.06–1.18 (m, 2H, CH_2), 1.56–1.68 (m, 1H), 1.74–1.94 (m, 3H), 2.41 (s, 3H, CH_3), 2.73–2.83 (m, 1H), 3.21–3.46 (m, 2H), 3.61 (s, 3H, CH_3), 3.78–3.92 (m, 2H), 4.08–4.19 (m, 3H), 4.50, 4.66 (AB, 2H, J_{H-H} = 14.0 Hz, CH_2), 4.59, 4.62 (AB, 2H, J_{H-H} = 14.5 Hz, CH_2), 4.79 (s,

2H, CH_2), 7.68 (d, 1H, $J_{H\text{-}F} = 13.8$ Hz, CH_{Ar}), 8.67 (s, 1H, CH_{Ar}), 9.96 (br s, 1H, OH). ^{13}C NMR (DMSO-d_6) δ: 8.87 (s, CH_2), 9.16 (s, CH_2), 18.52 (s, CH_3), 19.53 (s, CH_2), 21.70 (s, CH_2), 34.93 (s), 40.66 (s), 48.82 (s), 49.54 (br s), 53.96 (d, $J_{C\text{-}F} = 6.6$ Hz), 55.65 (br s), 55.88 (s), 60.77 (s), 61.83 (s), 106.44 (s, C_{Ar}), 106.51 (d, $J_{C\text{-}F} = 23.4$ Hz, C_{Ar}), 117.95 (d, $J_{C\text{-}F} = 8.8$ Hz, C_{Ar}), 134.36 (br s, C_{Ar}), 134.43 (s, C_{Ar}), 135.40 (br s, C_{Ar}), 136.39 (d, $J_{C\text{-}F} = 10.7$ Hz, C_{Ar}), 137.78 (br s, C_{Ar}), 141.49 (d, $J_{C\text{-}F} = 6.9$ Hz, C_{Ar}), 145.41 (s, C_{Ar}), 150.39 (s, C_{Ar}), 150.74 (s, C_{Ar}), 153.03 (s, $J_{C\text{-}F} = 249.9$ Hz, C_{Ar}), 165.83 (s, C(O)OH), 176.05 (d, $J_{C\text{-}F} = 2.6$ Hz, C═O). ESI-HRMS m/z: $[M\text{-}Cl]^+$ 583.2563 (calculated for $[C_{30}H_{36}FN_4O_7]^+$ - 583.2563).

4.2. Antibacterial activity

The antibacterial activity of all obtained compounds was carried out againts the following Gram-positive bacteria: methicillin sensitive *Staphylococcus aureus* ATCC 29213, *Micrococcus luteus* (clinical isolate), *Staphylococcus epidermidis* (clinical isolate), *Bacillus subtilis 168* and Gram-negative bacteria: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium TA100*. Additionally antimicrobial susceptibility of compounds **6a**, **7**, **8a**, **11**, **12a**, **12b**, **12d**, **13**, **16**, **20**, **21**, **22**, **24**, **25** and **28** were tested on a number of various clinical isolates of methicillin-resistant *Staphylococcus aureus, Staphylococcus auricularis, Staphylococcus haemolyticus, Staphylococcus epidermidis* and *Staphylococcus intermedius, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Cedecea davisae* and *Enterobacter aerogenes,* which were obtained from bacteriology laboratory of Republic Clinical Hospital (Kazan, Russia)

The bacterial strains were stored in 10% (V/V) glycerol stocks at −80 °C and freshly streaked on full Muller-Hinton (MH) agar plates (Sigma aldrich) and grown overnight at 37 °C before use. Fresh colony was grown overnight in MH-broth and then used to adjust an optical density of 0.5 McFarland (equivalent to 10^8 cells/mL) in 0.9% NaCl solution that was used as a working suspension. *Salmonella typhimurium* TA1535/pSK1002 was used for SOS-chromotest. *S. typhimurium* TA1535, TA1537, TA98, TA100, TA102 were used in Ames test. Bacteria were maintained and grown on the LB medium containing ampicillin at final concentration of 100 μg/mL. For the biofilm assay the previously developed BM broth (glucose 5 g, peptone 7 g, $MgSO_4 \times 7H_2O$ 2.0 g and $CaCl_2 \times 2H_2O$ 0.05 g in 1.0 L tap water) where all strains formed rigid biofilms in 2 days was used. For differential CFUs count of *E.coli, P. aeruginos* and *S.aureus*, the Endo-agar (Sigma aldrich), Cetrimide agar (Sigma aldrich) and Salt-mannitol agar (peptones 10 g, meat extract 1 g, NaCl 75 g, D-mannitol 10 g, agar-agar 12 g in 1.0 L tap water) were used, respectively.

The MIC of compounds was determined by the broth microdilution method in 96-well microtiter plates (Eppendorf) according to the EUCAST rules for antimicrobial susceptibility testing [28]. The bacterial culture adjusted to 3–9 × 10^5 cells/ml in the MH broth was seeded into 96-well polystyrol culture plates (Eppendorf). The concentrations of substances to be tested ranged from 0.25 to 64 μg/ml for. ATCC and 0.03–64 μg/ml for clinical isolate. The minimal inhibitory concentration was determined as the lowest concentration of compound for which no visible bacterial growth could be observed after 24 h of incubation.

4.3. Cytotoxicity and genotoxicity

The cytotoxicity of compounds **6a**, **7**, **8a**, **11**, **12a**, **12b**, **12d**, **13**, **16**, **20**, **21**, **22**, **24**, **25** and **28** was evaluated on CHL (human liver cells), MSC (human mesenchymal stem cells) and HSF (*primary human* skin *fibroblasts)* by MTT assay. Cells were cultured in a-MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 μg/mL penicillin and 100 μg/mL streptomycin. Cells were seeded in 96-well plates at the density of 20 000 cells per well and grown overnight at 37 °C and 5% CO_2 in humidified atmosphere. Then the medium was changed to the fresh one containing compounds to be tested in concentration of 0.2–100 μg/mL. After 72 h of cultivation the cultural fluid was discarded and MTT solution (in Dulbecco's phosphate-buffered saline) was added to the fresh media until final concentration of 0.5 mg/mL. In 2 h The liquid was replaced by dimethyl sulfoxide (Sigma-Aldrich, St.Louis, MO) to dissolve formazan crystals, and absorption was measured on Tecan Infinite 200Pro at 557 nm with reference 700 nm. Based on data obtained, the CC_{50} values (concentrations decreasing the proliferative activity by 2-fold) were calculated.

The Ames test was carried out using *S. typhimurium* TA98, TA100, TA102 strains as described in Ref. [29] as the spot test modification due to compounds toxicity to *S. typhimurium*. For that, 5 μl of sample (10 mg/mL solution in water) was dropped onto 5-mm filter disk placed on the top agar surface. The amount of revertants was then calculated by using the in-house developed software [30]. The tested compound was considered to be mutagenic if the count of revertants in the experiment was increased at higher concentrations of compound [31]. As a negative control, a pure water was used.

4.4. In vivo toxicity

Male and female F1 C57BL/6*CBA mice (weighing 18–22 g) for acute oral toxicity study were purchased from the Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. CD-1 female mice, weighing about 34.30 ± 3.20 g between 2 and 3 months of age obtained from Nursery for laboratory animals "Pushchino"(Russia), were used for acute cutaneous toxicity study. All animals acclimatized for 2 weeks before the experiment. Animals were kept in polypropylene cages (6 animals per cage), maintained under standard conditions (12 h light and 12 h dark cycle; 22 ± 3 °C and 30–70% relative humidity). They were given standard pellet diet and water *ad libitum* throughout the course of the study. Randomly, they were assigned into groups comprising 6 animals per group. Prior to treatment, animals were weighed, marked. Animals were observed two times once daily. All surviving animals were euthanized with CO_2 inhalation at the end of the study on day 14, and their vital organs were individually observed for overt pathology by necropsy. All experimental procedures were performed in accordance with the Ethical Principles in Animal Research and were approved by the Local Ethics Committee of the Kazan Federal University.

For acute oral toxicity study prior to treatment, animals were fasted for 3 h with free access to water. Compounds **7** and **28** were dissolved in distilled water and administered to the mice by oral gavage. An equal number of animals (mice) served as a control group, and the control animals received an equal volume of distilled water. Dosage precision was regulated by the variable volume of the drug solution administered (normalized to the animal's body weight) at a constant concentration of the solution.At the end of the exposure period, residual test substances were removed by water.

Clinical signs related with a drug-toxicity and the changes in the general behaviors of the animals were monitored and recorded every 1 h for the first 6 h after treatment, and then once daily over 14 days. Attention was given to changes in skin, fur, mucous membrane, eyes, respiration, behavior patterns, body weight, food and water consumption. Individual body weights were checked immediately before drug treatment and then at day 1, 3, 7, and 14 thereafter.

4.5. In vivo antibacterial activity

SHK mice between 2 and 3 months of age obtained from "Central Nursery of the Russian Academy of Medical Sciences "Kryukovo".

All animals were given standard pellet diet and water *ad libitum* throughout the course of the study. Randomly, they were assigned into groups comprising twelve animals per group. Prior to treatment, animals were weighed, marked, and allowed for overnight fasting with free access to water. Animals were randomly assigned to two groups of each sex (n = 10) on the basis of their individual body weights. After quarantine

period (14 days), healthy animals were used for this study. All experimental procedures were performed in accordance with the Ethical Principles in Animal Research and were approved by the Local Ethics Committee of the FSBI Gause Institute of New Antibiotics.

The strain of *S. aureus* N°10 sensitive to fluoroquinolones was used as an infectious agent (this strain was adapted to mice and obtained from "FSBI Gause Institute of New Antibiotics" collection). Mice were infected intravenously. Initially, for this line of mice, lethal doses (LD_{100}) of *S. aureus* were encountered intravenously. Accounting for the death of mice was observed daily for 10 days.

To determine the comparative effectiveness of the test compounds mice (n = 10) were infected intravenously at LD_{100} in a volume of 0.25 ml. Compound **7** and ciprofloxacin were administered orally at doses (18; 16; 14; 12; 10; 8; 6; 4 and 2 mg/kg) 1 h after infection. As a control dose in the experiment, there was a group of untreated animals infected with *S. aureus* at LD_{100}. The animals were observed for 14 days the death of experimental animals was recorded daily.

4.6. Investigation of the mechanism of antibacterial activity

The impact of **7** and **28** on the *S. aureus* topoisomerase IV and DNA gyrase was assessed by using decatenation kit (Cat. SAD4001), *S. aureus* DNA gyrase supercoiling assay kit (Cat. SAS4001) and *S. aureus* DNA gyrase cleavage assay kit (Cat. SAGC001) (Inspiralis, Norwich, UK) according to the protocols recommended by manufacturer. The final concentrations of compounds were 8–800 μM for **7** and ciprofloxacin as reference, and 0.4–400 μM for **28** and moxifloxacin.

4.7. Statistics and data analysis

All experiments were performed in biological triplicates (i.e. newly prepared cultures and medium) with three repeats in each run. The data were analyzed and graphically visualized using GraphPad Prism version 6.00 for Windows (GraphPad Software, USA, www.graphpad.com). Comparison against negative control using the non-parametric Kruskal–Wallis one-way analysis of variance test has been performed in each experiment. Significant differences against respective controls were considered at $p < 0.05$ and are specified in the corresponding figure captions.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

The chemical part, *in vitro* primary screening of antibacterial activity and *in vivo* experiments was funded by the subsidy allocated to Kazan Federal University by Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014–2020 (Project N°14.575.21.0037, the unique identifier of the agreement RFMEF157514X0037). Gnezdilov O.I. performed NMR measurements within the framework of the state task of the Federal research center "Kazan Scientific Center of the Russian Academy of Sciences. All other *in vitro* experiments was performed by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities (project number FZSM-2022-0018). We also thank Mirchink E.P., Isakova E.B. and Treshchalin M.I. from FSBI Gause Institute of New Antibiotics for studies of antibacterial activity *in vivo*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2023.115798.

References

[1] H. Nikaido, Multidrug resistance in bacteria, Annu. Rev. Biochem. 78 (2009) 119–146, https://doi.org/10.1146/annurev.biochem.78.082907.145923.
[2] M. Frieri, K. Kumar, A. Boutin, Antibiotic resistance, J. Infect. Public Health. 10 (2017) 369–378, https://doi.org/10.1016/j.jiph.2016.08.007.
[3] N.A. Turner, B.K. Sharma-Kuinkel, S.A. Maskarinec, E.M. Eichenberger, P.P. Shah, M. Carugati, T.L. Holland, V.G. Fowler Jr., Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research, Nat. Rev. Microbiol. 17 (2019) 203–218, https://doi.org/10.1038/s41579-018-0147-4.
[4] L.A. Mitscher, Bacterial topoisomerase inhibitors: quinolone and pyridone antibacterial agents, Chem. Rev. 105 (2005) 559–592, https://doi.org/10.1021/cr030101q.
[5] T. Khan, K. Sankhe, V. Suvarna, A. Sherje, K. Patel, B. Dravyakar, DNA gyrase inhibitors: progress and synthesis of potent compounds as antibacterial agents, Biomed. Pharmacother. 103 (2018) 923–938, https://doi.org/10.1016/j.biopha.2018.04.021.
[6] H.A.A. Ezelarab, S.H. Abbas, H.A. Hassan, G.E.-D.A. Abuo-Rahma, Recentupdates of fluoroquinolones as antibacterial agents, Arch. Pharm. 351 (9) (2018), 1800141, https://doi.org/10.1002/ardp.201800141.
[7] P. Lucia, Quinolones:Synthesis and antibacterial activity, in: V. Bobbarala (Ed.), Antimicrobial Agents, in Tech, Rijeka (Croatia), 2012, pp. 255–272.
[8] V.H. Tran, D. Marks, R.K. Duke, M. Bebawy, C.C. Duke, B.D. Roufogalis, Modulation of P-glycoprotein-Mediated anticancer drug accumulation, cytotoxicity, and ATPase activity by flavonoid interactions, Nutr. Cancer 63 (3) (2011) 435–443, https://doi.org/10.1080/01635581.2011.535959.
[9] V. Pokrovskaya, V. Belakhov, M. Hainrichson, S. Yaron, T. Baasov, Design, synthesis, and evaluation of novel fluoroquinolone-aminoglycoside hybrid antibiotics, J. Med. Chem. 52 (8) (2009) 2243–2254, https://doi.org/10.1021/jm900028n.
[10] J.C. McPherson III, R. Runner, T.B. Buxton, J.F. Hartmann, D. Farcasiu, I. Bereczki, E. Roth, S. Tollas, E. Ostorházi, F. Rozgonyi, P. Herczegh, Synthesis of osteotropic hydroxybisphosphonate derivatives of fluoroquinolone antibacterials, Eur. J. Med. Chem. 47 (2012) 615–618, https://doi.org/10.1016/j.ejmech.2011.10.049.
[11] Q. Guo, M.-L. Liu, L.-S. Feng, K. Lv, Y. Guan, H.-Y. Guo, C.-L. Xiao, Synthesis and in-vitro antimycobacterial activity of fluoroquinolone derivatives containing a coumarin moiety, Arch. Pharm. Chem. Life Sci. 344 (2011) 802–809, https://doi.org/10.1002/ardp.201000256.
[12] L. Racané, L. Ptiček, M. Sedić, P. Grbčić, S. Kraljević Pavelić, B. Bertoša, I. Sović, G. Karminski-Zamola, Eco-friendly synthesis, in vitro anti-proliferative evaluation, and 3D-QSAR analysis of a novel series of monocationic 2-aryl/heteroaryl-substituted 6-(2-imidazolinyl)benzothiazole mesylates, Mol. Divers. 22 (3) (2018) 723–741, https://doi.org/10.1007/s11030-018-9827-2.
[13] A. Mermer, S. Demirci, S.B. Ozdemir, A. Demirbas, S. Ulker, F.A. Ayaz, F. Aksakal, N. Demirbas, Conventional and microwave irradiated synthesis, biological activity evaluation and molecular docking studies of highly substituted piperazine-azole hybrids, Chin. Chem. Lett. 28 (5) (2017) 995–1005, https://doi.org/10.1016/j.cclet.2016.12.012.
[14] P. López-Rojas, M. Janeczko, K. Kubiński, Á. Amesty, M. Masłyk, A. Estévez-Braun, Synthesis and antimicrobial activity of 4-substituted 1,2,3-triazole-coumarin derivatives, Molecules 23 (1) (2018) 199, https://doi.org/10.3390/molecules23010199.
[15] D. Pavlović, S. Kimmins, S. Mutak, Synthesis of novel 15-membered 8a-azahomoerythromycin A acylides: consequences of structural modification at the C-3 and C-6 position on antibacterial activity, Eur. J. Med. Chem. 125 (2016) 210–224, https://doi.org/10.1016/j.ejmech.2016.09.022.
[16] P.C. Sharma, R. Kumar, M. Chaudhary, A. Sharma, H. Rajak, Synthesis and biological evaluation of novel benzothiazole clubbed fluoroquinolone derivatives, J. Enzym. Inhib. Med. Chem. 28 (1) (2013) 1–10, https://doi.org/10.3109/14756366.2011.611943.
[17] M.F. Gordeev, C. Hackbarth, M.R. Barbachyn, L.S. Banitt, J.R. Gage, G.W. Luehr, M. Gomez, J. Trias, S.E. Morin, G.E. Zurenko, C.N. Parker, J.M. Evans, R.J. White, D.V. Patel, Novel oxazolidinone-quinolone hybrid antimicrobials, Bioorg. Med. Chem. Lett. 13 (2003) 4213–4216, https://doi.org/10.1016/j.bmcl.2003.07.021.
[18] H.A.A. Ezelarab, S.H. Abbas, H.A. Hassan, G. El-Din, A. Abuo-Rahma, Recent updates of fluoroquinolones as antibacterial agents, Arch Pharm Chem Life Sci 351 (9) (2018), 1800141, https://doi.org/10.1002/ardp.201800141.
[19] G.A.R.Y. Suaifan, A.A.M. Mohammed, Fluoroquinolones structural and medicinal developments (2013–2018): where are we now, Bioorg. Med. Chem. 27 (14) (2019) 3005–3060, https://doi.org/10.1016/j.bmc.2019.05.038.
[20] Y.G. Shtyrlin, A.S. Petukhov, A.D. Strelnik, N.V. Shtyrlin, A.G. Iksanova, M. V. Pugachev, R.S. Pavelyev, M.S. Dzyurkevich, M.R. Garipov, K.V. Balakin, Chemistry of pyridoxine in drug design, Russ. Chem. Bull. 68 (2019) 911–945, https://doi.org/10.1007/s11172-019-2504-5.
[21] N.V. Shtyrlin, S.V. Sapozhnikov, S.A. Koshkin, A.G. Iksanova, A.H. Sabirov, A. R. Kayumov, A.A. Nureeva, M.I. Zeldi, Y.G. Shtyrlin, Synthesis and antibacterial activity of novel quaternary ammonium pyridoxine derivatives, Med. Chem. 11 (2015) 656–665, https://doi.org/10.2174/1573406411666150504122930.

[22] S.V. Sapozhnikov, N.V. Shtyrlin, A.R. Kayumov, A.E. Zamaldinova, A.G. Iksanova, E.V. Nikitina, E.S. Krylova, D.Y. Grishaev, K.V. Balakin, Y.G. Shtyrlin, New quaternary ammonium pyridoxine derivatives: synthesis and antibacterial activity, Med. Chem. Res. 26 (2017) 3188–3202, https://doi.org/10.1007/s00044-017-2012-9.

[23] M.V. Pugachev, N.V. Shtyrlin, E.V. Nikitina, L.P. Sysoeva, T.I. Abdullin, A. G. Iksanova, A.A. Ilaeva, E.A. Berdnikov, R.Z. Musin, YuG. Shtyrlin, Synthesis and antibacterial activity of novel phosphonium salts on the basis of pyridoxine, Bioorg. Med. Chem. 21 (14) (2013) 4388–4395, https://doi.org/10.1016/j.bmc.2013.04.051.

[24] S.V. Sapozhnikov, A.E. Sabirova, N.V. Shtyrlin, A.Y. Druk, M.N. Agafonova, M. N. Chirkova, R.R. Kazakova, D.Y. Grishaev, T.V. Nikishova, E.S. Krylova, E. V. Nikitina, A.R. Kayumov, Y.G. Shtyrlin, Design, synthesis, antibacterial activity and toxicity of novel quaternary ammonium compounds based on pyridoxine and fatty acids, Eur. J. Med. Chem. 211 (2021), 113100, https://doi.org/10.1016/j.ejmech.2020.113100.

[25] Y.G. Shtyrlin, A.S. Petukhov, A.D. Strelnik, E.V. Nikitina, M.R. Garipov, Antibacterial compounds based on sulphanilic acid and pyridoxine, Patent RU2480471 (2012). https://patents.google.com/patent/RU2480471C1/ru?oq=RU+2480471.

[26] M.V. Pugachev, N.V. Shtyrlin, S.V. Sapozhnikov, L.P. Sysoeva, A.G. Iksanova, E. V. Nikitina, R.Z. Musin, O.A. Lodochnikova, E.A. Berdnikov, Y.G. Shtyrlin, Bis-phosphonium salts of pyridoxine: the relationship between structure and antibacterial activity, Bioorg. Med. Chem. 21 (2013) 7330–7342, https://doi.org/10.1016/j.bmc.2013.09.056.

[27]] R. Serwa, T. Nam, L. Valgimigli, S. Culbertson, C.L. Rector, B.-S. Jeong, D.A. Pratt, N.A. Porter, Preparation and investigation of vitamin B6-derived aminopyridinol antioxidants, Chem. Eur J. 16 (2010) 14106–14114, https://doi.org/10.1002/chem.201001382.

[28] R. Cantón Leclercq, D.F.J. Brown, C.G. Giske, P. Heisig, A.P. MacGowan, J. W. Mouton, P. Nordmann, A.C. Rodloff, G.M. Rossolini, C.-J. Soussy, M. Steinbakk, T.G. Winstanley, G. Kahlmeter, EUCAST expert rules in antimicrobial susceptibility testing, Clin. Microbiol. Infect. 19 (2013) 141–160, https://doi.org/10.1111/j.1469-0691.2011.03703.x.

[29] Y. Oda, S. Nakamura, I. Oki, T. Kato, H. Shinagawa, Evaluation of the new system (umu-test) for the detectiion of enviromental mutagens and carcinogens, Mutat. Res. 147 (1985) 219–229, https://doi.org/10.1016/0165-1161(85)90098-6.

[30] OECD, Bacterial Reverse Mutation Test, 1997. Test Guideline N° . 471.

[31] K. Fedorova, A. Kayumov, K. Woyda, O. Ilinskaja, K. Forchhammer, Transcription factor TnrA inhibits the biosynthetic activity of glutamine synthetase in Bacillus subtilis, FEBS Lett. 587 (2013) 1293–1298, https://doi.org/10.1016/j.febslet.2013.03.015.