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Synthesis, antimycobacterial and antibacterial activity of ciprofloxacin derivatives containing a N-substituted benzyl moiety

Shuo Wang [a], Xue-Dong Jia [a], Ming-Liang Liu [a,*], Yu Lu [b,*], Hui-Yuan Guo [a]

[a] Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China
[b] Department of Pharmacology, Beijing Tuberculosis and Thoracic Tumor Research Institute, Beijing Chest Hospital, Beijing 101149, PR China

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ABSTRACT

We report herein the design and synthesis of a series of novel ciprofloxacin (CPFX) derivatives with remarkable improvement in lipophilicity by introducing a substituted benzyl moiety to the N atom on the C-7 piperazine ring of CPFX. Antimycobacterial and antibacterial activity of the newly synthesized compounds was evaluated. Results reveal that compound **4f** has good in vitro activity against all of the tested Gram-positive strains including MRSA and MRSE (MICs: 0.06–32 μg/mL) which is two to eightfold more potent than or comparable to the parent drug CPFX (MICs: 0.25–128 μg/mL), Gram-negative bacteria *P. aeruginosa* (MICs: 0.5–4 μg/mL) and *M. tuberculosis* H37Rv ATCC 27294 (MIC: 1 μg/mL).

Fluoroquinolones (FQs), one of the important classes of weapons in our antibacterial arsenal,[1] are used mainly for the treatment of respiratory tract infections (RTI), urinary tract infections (UTI), sexually transmitted diseases (STD), gastrointestinal and abdominal infections, skin and soft tissue infections, and infections of the bone and joints.[2] These antibacterial agents act by inhibiting two type II bacterial topoisomerase enzymes, DNA gyrase (subunits encoded by gyrA and gyrB) and topoisomerase IV (subunits encoded by parC and parE). Cell death is caused by trapping the topoisomerase protein–DNA complex thus disrupting normal DNA replication, inducing oxidative damage, and triggering cell-death mechanisms.[3,4]

On the other hand, DNA gyrase is considered to be the sole topoisomerase drug target of FQs in *Mycobacterium tuberculosis* (MTB) because of no evidence of the topoisomerase IV parC and parE gene homologs in the genome of MTB.[5] Resistance to FQs remains relatively low in clinical isolates of MTB currently, and there are no reports of cross-resistance or antagonism with other classes of anti-tuberculosis (anti-TB) drugs.[6,7] Ciprofloxacin (CPFX, Scheme 1), ofloxacin and sparfloxacin were recommended as second-line agents for the treatment of TB mainly in cases involving resistance or intolerance to first-line anti-TB therapy by WHO in 1996.[8] Moreover, moxifloxacin and gatifloxacin possessing a particularly strong in vitro and in vivo activity against MTB[9,10] are currently being further evaluated as anti-TB drugs.

However, increasing bacterial resistance to FQs due to the high level of use and to some degree of abuse, has put enormous pressure on the public health systems. For example, a significant increase in resistance of *Escherichia coli* and *Pseudomonas aeruginosa* as well as MTB to CPFX, the most consumed antibacterial agent worldwide,[11] was noted recently. It was reported that the overall resistance rate of Gram-negative and -positive isolates to CPFX increased from 40.8% (2000) to 51.7% (2004) at Xingshan County Hospital, China,[12] and overall resistance of MTB to CPFX increased from 6.0% (1989–1994) to 26.8% (1995–2000) at the Makati Medical Center, Makati City, Philippines.[13] Therefore, there is an urgent need for the discovery and development of effective novel FQs to confer desirable biological and pharmacological properties. Clearly, a more practical strategy is to modify the structures of existing FQs to increase potency and overcome resistance.

Structure–activity relationship (SAR) studies of FQs have indicated that the basic group at the C-7 position, the only an area that substitution of bulky functional group is permitted, greatly influences their antibacterial potency, spectrum and safety.[14,15] It is generally believed that the action of FQs increases with an increase in lipophilicity.[16] A large number of existing FQ derivatives were synthesized by introduction of an additional functional moiety on the N atom of the C-7 side chain to increase the lipophilicity, and some of which were found to have stronger antibacterial or anti-MTB activity than the corresponding parent FQs.[17,18] Moreover, several cephalosporin derivatives possessing a substituted benzyloxime moiety in the C-7 position, such as GR69153,[19]

* Corresponding authors. Tel./fax: +86 10 63036965 (M.-L.L.); tel.: +86 10 89509357; fax: +86 10 80505770 (Y.L.).
E-mail addresses: lmllyx@yahoo.com.cn (M.-L. Liu), luyu4876@hotmail.com (Y. Lu).

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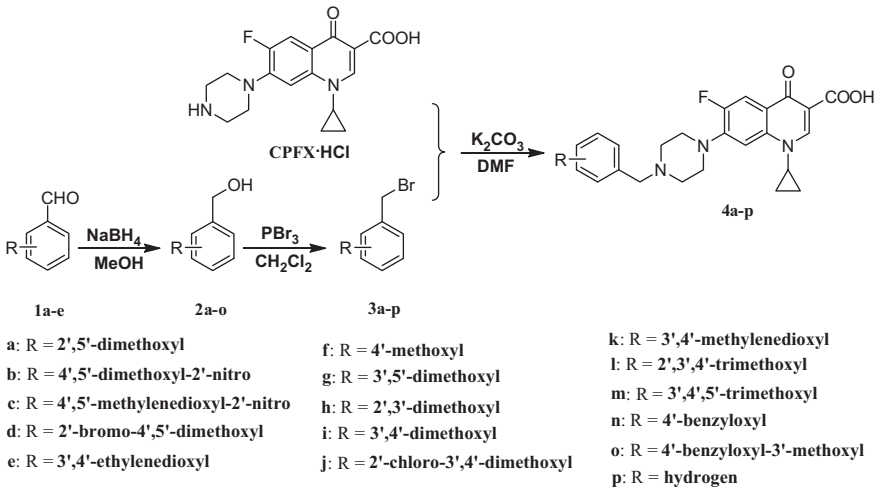


Scheme 1. Synthesis of novel ciprofloxacin derivatives **4a–p**.

LB10522[20] and RU-59863,[21] show superior antibacterial activity to the corresponding methyloxime analogs against some pathogens.

These research results intensified our interest, it was decided to design and synthesize a series of novel CPFX derivatives by introduction of a lipophilic substituted benzyl moiety to the N atom on the C-7 piperazine ring of CPFX in this study, and evaluate their anti-MTB and antibacterial activity. Our primary object was to optimize the potency of CPFX against MTB and clinically important pathogens including MRSA.

Detailed synthetic pathways to novel CPFX derivatives **4a–p** by one-pot method are depicted in Scheme 1. Reduction of various benzaldehydes **1a–e** with sodium borohydride in methanol gave the phenylmethanols **2a–e**, and then nucleophilic substitution of **2a–e** and commercially available compounds **2f–o** with phosphorus tribromide in dichloromethane yielded the corresponding (bromomethyl) benzenes **3a–o**. Finally, **3a–o** and commercially available **3p** were condensed directly with CPFX in the presence of potassium carbonate to produce the target compounds **4a–p** followed well-established literature procedures.[22,23] In addition, we were also successful in preparing X-ray quality single crystals of target compound **4k** obtained by slow evaporation of a dichloromethane-methanol solution. In the crystal structure, the piperazine ring and benzo[d][1,3]dioxole ring adopt chair and planar conformations, respectively. An intramolecular O–H...O hydrogen bond is observed between the carboxylic and carbonyl groups (Fig. 1).[24]

Lipophilicity of the new synthesized derivatives **4a–p** and the parent CPFX is expressed in the term of their *C*log*P* values which were calculated with Chem office 2010 software. As shown in Table 1, a remarkable improvement in the lipophilicity of the derivatives **4a–p** as evidenced by *C*log*P* values (0.87–3.26) which are much more than that of CPFX (−0.73) (statistically significant at $p < 0.001$ using t test) (Table 1).

The target compounds **4a–p** were initially evaluated for their in vitro activity against MTB H37Rv ATCC 27294 using the Microplate Alamar Blue Assay (MABA).[25,26] The minimum inhibitory concentration (MIC) is defined as the lowest concentration effecting a reduction in fluorescence of ⩾90% relative to the mean of replicate bacterium-only controls and MICs of the compounds along with CPFX for comparison are presented in Table 1.

The data reveal that the target compounds **4a–p** have potential activity against MTB H37Rv ATCC 27294 (MICs: 1→32 μg/mL), although they are less active than the parent drug CPFX (MIC: 0.5 μg/mL). Among of them, compounds **4f** and **4p** bearing the simplest substituent (*R* = methoxyl) and without substituents (*R* = hydrogen) on the benzene ring, respectively, display the highest activity (MICs: 1 μg/mL), and compounds **4c, 4e, 4g, 4h, 4j, 4k** have also useful activity (MICs: 2 μg/mL) against this strain.

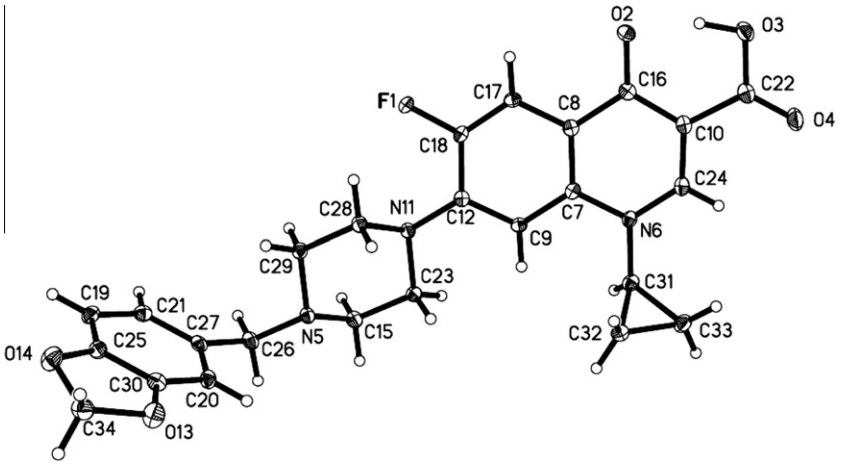
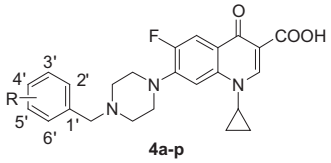


Figure 1. X-ray structure of compound **4k**.

Table 1
Structures, lipophilicity and antimycobacterial activity of compounds **4a**–**p**



4a-p

Compd.	R	*C*log*P*[a]	mp[b] (°C)	MIC (μg/mL) MTB[c]
4a	2′,5′-Dimethoxyl	1.58	232	>32
4b	4′,5′-Dimethoxyl-2′-nitro	1.17	>280	>32
4c	4′,5′-Methylenedioxyl-2′-nitro	1.48	215	2
4d	2′-Bromo-4′,5′-dimethoxyl	2.16	254–255	>32
4e	3′,4′-Ethylenedioxyl	1.50	242	2
4f	4′-Methoxyl	1.49	248–249	1
4g	3′,5′-Dimethoxyl	1.58	199	2
4h	2′,3′-Dimethoxyl	1.23	177–178	2
4i	3′,4′-Dimethoxyl	1.23	172–173	16
4j	2′-Chloro-3′,4′-dimethoxyl	1.78	187–188	2
4k	3′,4′-Methylenedioxyl	1.54	238	2
4l	2′,3′,4′-Trimethoxyl	0.87	197	16
4m	3′,4′,5′-Trimethoxyl	0.87	185	32
4n	4′-Benzyloxyl	3.26	213	32
4o	4′-Benzyloxyl-3′-methoxyl	3.00	105	16
4p	hydrogen	1.57	237–238	1
CPFX		−0.73		0.5

CPFX, ciprofloxacin.
[a] The *C*log*P* is calculated by Chem office 2010 software.
[b] Melting points are uncorrected.
[c] MTB: MTB H37Rv ATCC 27294.

The target compounds **4a**–**p** were evaluated for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques.[27] Minimum inhibitory concentration (MIC)[28] is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with CPFX for comparison are reported in Tables 2 and 3.

Generally, the target compounds **4a**–**p**[29] have considerable antibacterial activity against the tested Gram-positive strains. For example, they show good potency in inhibiting the growth of methicillin-sensitive *S. aureus* (MSSA) (MICs:0.03–4 μg/mL). Among of them, the activity of compounds **4a**, **4e**, **4f**, **4h**, **4i**, **4k**, **4l** against methicillin-resistant *S. aureus* (MRSA) (MICs: 4–64 μg/mL), and compounds **4e** and **4f** against *S. epidermidis* including

Table 2
In vitro antibacterial activity of CPFX derivatives **4a–p** against Gram-positive strains

Strains	MIC(μg/mL)																
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	**4m**	**4n**	**4o**	**4p**	**CPFX**
S.a.	0.5	1	0.5	2	0.125	0.06	1	0.06	0.125	0.06	0.25	0.5	0.25	4	2	0.25	0.25
MSSA .1	0.5	1	0.5	4	0.25	0.125	1	0.03	0.125	0.5	0.5	0.125	0.25	0.25	0.5	0.25	0.5
MSSA .2	0.5	2	1	4	0.25	0.25	0.5	0.03	0.125	0.5	1	0.25	0.5	0.5	2	0.25	0.5
MSSA .3	1	1	1	4	0.5	0.25	1	0.125	0.25	0.5	1	0.125	0.25	0.5	1	0.25	0.5
MRSA.1	32	64	32	32	16	16	128	16	32	64	32	16	64	32	>128	64	32
MRSA.2	32	64	32	32	16	16	>128	32	64	128	64	64	128	128	>128	64	128
MRSA.3	16	16	32	32	4	4	64	16	16	64	16	16	32	128	>128	16	16
MSSE.1	1	2	1	8	0.5	0.5	4	0.25	4	1	2	0.25	0.5	1	1	32	0.5
MSSE.2	16	16	16	>128	8	8	128	8	8	32	16	16	32	32	32	16	8
MRSE.1	16	16	16	>128	8	8	>128	8	8	32	16	64	32	16	128	16	8
MRSE .2	16	16	32	32	16	8	128	32	32	32	16	64	32	32	64	0.5	16
E.f.1	16	16	32	>128	4	4	128	8	8	8	16	16	8	8	32	16	8
E.f.2	128	128	64	>128	32	32	>128	>128	>128	64	32	>128	64	64	64	16	64
E.f.3	16	16	32	>128	32	8	>128	32	64	32	16	64	64	32	32	16	16
E.fa.1	16	8	2	8	2	1	16	4	4	4	8	8	4	8	16	16	1
E.fa.2	16	64	16	>128	32	16	>128	32	64	32	8	32	32	32	32	16	16
S.p.1	8	8	2	8	1	1	8	2	2	2	8	8	4	8	16	16	2
S.p.2	64	128	16	>128	32	16	>128	32	32	64	16	64	64	32	32	16	32

Abbreviations: S.a., *Staphylococcus aureus* ATCC 2592; MSSA.1, methicillin-sensitive *Staphylococcus aureus* 10-11; MSSA.2, methicillin-sensitive *Staphylococcus aureus* 10-13; MSSA.3, methicillin-sensitive *Staphylococcus aureus* 10-14; MRSA.1, methicillin-resistant *Staphylococcus aureus* 10-11; MRSA.2, methicillin-resistant *Staphylococcus aureus* 10-13; MRSA.3, methicillin-resistant *Staphylococcus aureus* 10-15; MSSE.1, methicillin-sensitive *Staphylococcus epidermidis* 10-11; MSSE.2, methicillin-sensitive *Staphylococcus epidermidis* 10-15; MRSE.1, methicillin-resistant *Staphylococcus epidermidis* 10-10; MRSE.2, methicillin-resistant *Staphylococcus epidermidis* 10-13; E.f.1, *Enterococcus faecalis* 10-5; E.f.2, *Enterococcus faecalis* 10-6; E.f.3, *Enterococcus faecalis* 10-7; E.fa.1, *Enterococcus faecium* 10-6; E.fa.2, *Enterococcus faecium* 10-9; S.p.1, *Streptococcus pneumonia* 10-1; S.p.2, *Streptococcus pneumonia* 10-6: CPFX, ciprofloxacin.

Table 3
In vitro antibacterial activity of CPFX derivatives **4a–p** against Gram-negative strains

Strains	MIC(μg/mL)																
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	**4m**	**4m**	**4o**	**4p**	**CPFX**
E.co.	1	2	0.125	0.25	0.125	0.125	1	0.5	0.5	1	0.25	0.5	1	1	0.5	0.25	0.008
E.co.1	>128	>128	32	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
E.co. 2	64	8	1	>128	128	8	>128	32	16	32	16	16	32	32	32	16	4
E.co. 3	16	8	8	>128	16	8	>128	>128	64	>128	16	64	>128	>128	>128	16	4
E.co.1*	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
E.co. 2*	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
E.co. 3*	>128	>128	8	>128	128	8	>128	128	64	>128	16	64	>128	>128	>128	16	16
K.p.1	>128	>128	64	>128	>128	>128	>128	>128	>128	>128	64	>128	>128	>128	>128	>128	32
K.p.2	16	8	2	8	8	4	64	16	16	32	8	16	32	32	32	16	0.25
K.p.3	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
K.p.1*	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
K.p.2*	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
K.p.3*	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32
P.a.1	16	8	1	8	16	4	64	32	32	64	16	32	32	16	32	16	0.25
P.a.2	16	8	1	8	64	4	128	64	64	128	16	32	32	32	64	16	0.25
P.a.3	8	16	1	8	16	2	64	32	32	64	32	32	64	32	32	16	0.25
P.a.4	16	8	0.5	16	32	2	64	32	32	64	16	32	64	128	64	32	0.5
P.a.5	16	8	2	8	16	4	128	64	64	128	8	64	128	64	32	8	1
P.a.6	32	4	4	16	32	4	64	128	128	64	16	32	64	32	32	16	2

Abbreviations: E.co., *Escherichia coli* ATCC 25922; E.co.1, *Escherichia coli* 10-1; E.co.2, *Escherichia coli* 10-2; E.co.3, *Escherichia coli* 10-3; E.co.1*, *Escherichia coli* ESBLs$^+$ 10-2 ; E.co.2*, *Escherichia coli* ESBLs$^+$ 10-3 ; E.co.3*, *Escherichia coli* ESBLs$^+$ 10-4 ; K.p.1, *Klebsiella pneumonia* 10-2; K.p.2, *Klebsiella pneumonia* 10-3; K.p.3, *Klebsiella pneumonia* 10-4; K.p.1*, *Klebsiella pneumonia* ESBLs$^+$ 10-1; K.p.2*, *Klebsiella pneumonia* ESBLs$^+$ 10-2; K.p.3*, *Klebsiella pneumonia* ESBLs$^+$ 10-3; P.a.1, *Pseudomonas aeruginosa* 10-5; P.a.2, *Pseudomonas aeruginosa* 10-9; P.a.3, *Pseudomonas aeruginosa* 10-12; P.a.4, *Pseudomonas aeruginosa* 10-15; P.a.5, *Pseudomonas aeruginosa* 10-18; P.a.6, *Pseudomonas aeruginosa* 10-20; ESBLs$^+$, extended spectrum beta-lactamases (ESBLs)-producing; CPFX, ciprofloxacin.

methicillin-resistant *S. epidermidis* (MRSE) (MICs: 0.5–16 μg/mL) is better than or comparable to CPFX. In particular, the most active compound **4f** (MICs: 0.06–32 μg/mL) in this study was found to be two to eightfold more potent than or comparable to CPFX (MICs: 0.25–128 μg/mL) against all of the tested Gram-positive strains.

On the other hand, the target compounds **4a–p** are generally less active than the parent CPFX against the tested Gram-negative strains with few exceptions. It is noted that compounds **4c** and **4f** possess good potency against all of the six clinical strains of P. aeruginosa (MICs: 0.5–4 μg/mL). However, all of **4a–p**, like CPFX, have no virtual activity against the extended-spectrum β-lactamase (ESBLs)-producing *E. coli* and Klebsiella pneumonia due partly to resistance of these ESBLs-producing strains inherently to FQs.

Variations (R) on the benzene ring of the benzyl moiety in this study include mono-/bi-/tri-methoxyl, benzyloxyl, 3′,4′-methylenedioxyl/ethylenedioxyl, nitro, chlorine, bromine and hydrogen substitution (Table 1). The activity imparted to CPFX derivatives by R groups against Gram-positive strains was in the order: methoxyl (**4f**) > H (**4p**) > benzyloxyl (**4n**) for mono-substitution; 2′,3′-dimethoxyl (**4h**) > 3′,4′-dimethoxyl (**4i**) > 2′,5′-dimethoxyl (**4a**) > 3′,5′-dimethoxyl (**4g**) for dimethoxyl substitution. It is also interesting to note that introduction of an additional group on the 3′,4′-dimethoxylbenzene ring of CPFX derivative (**4i**) seems to be detrimental to the activity and decreased activity caused by the introduction of an electron-withdrawing group is more remarkable than an electron-donating one. It can be exemplified by the relative contribution of related R groups to activity as follows: 3′,4′-dimethoxyl > 2′,3′,4′-trimethoxyl ≈ 3′,4′,5′-trimethoxyl > 2′-chloro-3′,4′-dimethoxyl > 2′-bromo-4′,5′-dimethoxyl > 4′,5′-dimethoxyl-2′-nitro.

In summary, a series of novel CPFX derivatives with remarkable improvement in lipophilicity, as compared to the parent drug CPFX, were designed, synthesized and evaluated for their in vitro anti-MTB and antibacterial activity. Our results reveal that the most active compound **4f** has good Gram-positive activity (MICs: 0.06–32 μg/mL) which is two to eightfold more potent than or comparable to the parent CPFX (MICs:0.25–128 μg/mL), useful activity against Gram-negative bacteria *P. aeruginosa* (MICs: 0.5–4 μg/mL) and MTB H37Rv ATCC 27294 (MIC: 1 μg/mL). However, the target compounds **4a–p** are generally less active than CPFX against MTB H37Rv ATCC 27294 and Gram-negative strains. It suggests that merely an increase in lipophilicity of the tested compounds does not result in enhanced antimycobacterial and antibacterial activity.

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27. MICs were determined as described by the NCCLS (see: National Committee for Clinical Laboratory Standards, Performance Standards for Antimicrobial Susceptibility Testing: 11th Informational Supplement, vol. 21, National Committee for Clinical Laboratory Standards, Wayne, PA, 2001, M100-S11. The MIC was defined as the lowest concentration of each compound resulting in inhibition of visible growth of bacteria after incubation at 35 °C for 18–24 h.
28. To a stirring solution of 2,5-dimethoxyl benzaldehyde **1a** (0.67 g, 4 mmol) dissolved in anhydrous methanol (30 mL) was added $NaBH_4$ (0.84 g, 22 mmol) in batches at 0 °C over a period of 0.5 h. The reaction mixture was stirred at the same temperature for 2 h. After removal of the methanol under reduced pressure, the residue was diluted with methylene chloride (30 mL), washed with distilled water (3 × 10 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give crude product **2a** which was then dissolved in anhydrous methylene chloride (50 mL) and cooled to 0–5 °C by ice bath. To this solution was added dropwise phosphorus tribromide (0.5 mL, 5 mmol) over a period of 15 min and stirred for 0.5 h at the same temperature, and then washed with saturated brine (3 × 15 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to give crude compound **3a**. To a solution of **3a** dissolved in *N*,*N*-dimethyl formamide (20 mL) was added anhydrous potassium carbonate (1.10 g, 8 mmol) and 1-cyclopropyl-6-fluoro-7-(piperazin-1-yl)-1,4-dihydroquinoline-4-oxo-3-carboxylic acid hydrochloride (CPFX·HCl, 0.73 g, 2 mmol). The reaction mixture was stirred at 40 °C for 12 h, and then diluted with methylene chloride (50 mL), washed with distilled water (3 × 50 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue obtained was purified by column chromatography (silica gel) eluted with petroleum ether and ethyl acetate (*v*:*v* = 10:1) to afford the target compound **4a** . Yield: 62.38 % (from **1a**), off-white solid.^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.17–1.19 (2H, m),1.30–1.31 (2H, m) , 2.62–2.66 (4H, m), 3.34–3.35 (4H, m,), 3.54 (2H, s), 3.70 (3H, s), 3.73 (3H, s), 3.80–3.81 (1H, m), 6.78–6.81 (1H, m), 6.90–6.95 (2H, m), 7.56–7.58 (1H, d, *J* = 8 Hz), 7.88–7.90 (1H, d, *J* = 8 Hz), 8.65 (1H, s), 15.22 (1H, s). ESI–MS: *m/z* 482 ($M+H^+$). HRMS–ESI: *m/z* $C_{26}H_{28}FN_3O_5$ Calcd: 482.2085; Found: 482.2085.
29. The other target compounds **4b–p** were obtained as off-white solids in a similar manner as for the preparation of **4a**. Compound **4b**, yield: 23.74% (from **2b**), yellow solid. ^{1}H NMR(400 MHz, $CDCl_3$ + D_2O) δ: 1.17 (2H, m), 1.30–1.31 (2H, m), 2.54–2.65 (4H, m), 3.28–3.34 (4H, m), 3.57 (2H, s), 3.76–3.81 (7H, m), 7.08–7.13 (2H, d, *J* = 20 Hz), 7.56–7.58 (1H, d, *J* = 8 Hz), 7.90 (1H, s), 8.65 (1H, s), 15.20 (1H, s). ESI–MS: *m/z* 527 ($M+H^+$). HRMS (ESI, *m/z*): $C_{26}H_{27}FN_4O_7$ ($M+H^+$). Calcd: 527.1956; Found: 527.1936. Compound **4c**, yield: 26.47 % (from **1c**), yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.17–1.18 (2H, m), 1.28–1.33 (2H, m), 2.49–2.60 (4H, m), 3.28–3.03 (6H, m), 3.78–3.83 (3H, m), 7.25 (2H, s), 7.55–7.57 (2H, m), 7.88–7.92 (1H, d, *J* = 13.2 Hz), 8.65 (1H, s), 15.20 (1H, s). ESI–MS: *m/z* 511 ($M+H^+$). HRMS (ESI, *m/z*): $C_{25}H_{23}FN_4O_7$ ($M+H^+$). Calcd: 511.1620; Found: 511.1623. Compound **4d**, yield: 27.72% (from **1d**), yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$+D_2O) δ: 0.83–0.84 (2H, m), 1.67–1.31 (4H, m), 2.49–2.66 (4H, m), 3.28–3.30 (5H, m), 3.77–3.87 (6H,m), 7.27 (1H, s), 7.56 (2H, s), 7.88–7.92 (1H, m), 8.65 (1H, s), 15.20 (1H, s). ESI-MS: *m/z* 560 ($M+H^+$). Compound **4e**, yield: 51.15% (from **1e**), light yellow solid.^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.16 (2H, s), 1.29–1.31 (2H, m), 2.55–2.66 (4H, m), 3.25–3.30 (4H, m), 3.35–3.44 (2H, m), 3.80–3.82 (1H, m), 4.21 (4H, s), 6.78–6.81 (3H, m), 7.55–7.57 (1H, s), 7.88–7.91 (1H, m), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 480 ($M+H^+$). HRMS (ESI, *m/z*): $C_{26}H_{26}FN_3O_5$ ($M+H^+$). Calcd: 480.1926; Found: 480.1926. Compound **4f**, yield: 31.04% (from **2f**), light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.16–1.17 (2H, m), 1.27–1.31 (2H, m), 2.55–2.57 (4H, m), 3.30–3.31 (4H, m), 3.49 (2H, s), 3.73 (3H, s), 3.78–3.82 (1H, m), 3.79–3.82 (1H, m), 6.88–6.91 (2H, d, *J* = 8 Hz), 7.23–7.25 (2H, d, *J* = 8 Hz), 7.54–7.56 (1H, d, *J* = 8 Hz), 7.88–7.91 (1H, d, *J* = 12 Hz), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 452 ($M+H^+$). HRMS (ESI, *m/z*): $C_{25}H_{26}FN_3O_4$ ($M+H^+$). Calcd: 452.2085; Found: 452.1980. Compound **4g**, yield: 37.42% (from **2g**), light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$+D_2O) δ: 1.16–1.18 (2H, m), 1.30–1.31 (2H, m), 2.58–2.60 (4H, m), 3.32–3.35 (4H, m), 3.49 (2H, s), 3.73–3.74 (6H, m), 3.78–3.81 (1H, m), 6.39–6.40 (1H, m), 6.50–6.51 (2H, m), 7.56–7.57 (1H, d, *J* = 8 Hz), 7.88–7.91 (1H, d, *J* = 12 Hz), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 482 ($M+H^+$). HRMS (ESI, *m/z*): $C_{26}H_{28}FN_3O_5$ ($M+H^+$). Calcd: 482.2082; Found: 482.2085. Compound **4h**, yield: 23.91% (from **2h**), off white solid. ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.16–1.17 (2H, m), 1.29–1.31 (2H, m), 2.60–2.62 (4H, m), 3.30–3.32 (4H, m), 3.55–3.56 (3H, m), 3.74 (3H, s), 3.79 (3H, s), 6.64–7.06 (3H, m), 7.55–7.56 (1H, d, *J* = 4 Hz), 7.88–7.91 (1H, d, *J* = 12 Hz), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 482 ($M+H^+$). HRMS (ESI, *m/z*): $C_{26}H_{28}FN_3O_5$ ($M+H^+$). Calcd: 482.2083; Found: 482.2085. Compound **4i**, yield: 43.66% (from **2i**), light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.16–1.18 (2H, m), 1.28–1.31 (2H, m), 2.58–2.59 (4H, m), 3.28–3.33 (4H, m), 3.49 (2H, s), 3.73 (6H, s), 3.79–3.97 (1H, m), 6.79–6.91 (3H, m), 7.55–7.57 (1H, d, *J* = 8 Hz), 7.88–7.9 1(1H, m), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 482 ($M+H^+$). HRMS (ESI, *m/z*): $C_{26}H_{28}FN_3O_5$ ($M+H^+$). Calcd: 482.2080; Found: 482.2085. Compound **4j**, yield: 33.98% (from **2j**), light yellow solid. ^{1}H NMR (400 Hz, DMSO-/d_6 + D_2O) δ: 1.17–1.19 (2H, m), 1.29–1.31 (2H, m), 2.64–2.65 (4H, m), 3.30–3.32 (4H, m), 3.59 (2H, s), 3.74–3.82 (7H, m), 7.03–7.05 (1H, d, *J* = 8 Hz), 7.20–7.22 (1H, d, *J* = 8 Hz), 7.56–7.58 (1H, d, *J* = 8 Hz), 7.88–7.92 (1H, d, *J* = 12 Hz), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 516 ($M+H^+$). HRMS (ESI, *m/z*): $C_{26}H_{27}ClFN_3O_5$ ($M+H^+$). Calcd: 516.1695; Found: 516.1696. Compound **4k**, yield: 34.41% (from **2k**), light yellow solid. ^{1}H NMR(400 MHz, DMSO-d_6 + D_2O) δ: 1.16–1.18 (2H, m), 1.28–1.31 (2H, m), 2.57–2.58 (4H, m), 3.30–3.33 (4H, m), 3.47 (2H, s), 3.78–3.82 (1H, m), 5.99 (2H, s), 6.78–6.90 (3H, m), 7.55–7.57 (1H, d, *J* = 8 Hz), 7.88–7.91 (1H, d, *J* = 12 Hz), 8.649 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 466 ($M+H^+$). HRMS (ESI, *m/z*): $C_{25}H_{24}FN_3O_5$ ($M+H^+$). Calcd: 466.1769; Found: 466.1772. Compound **4l**, yield: 31.31% (from **2l**), light yellow solid. ^{1}H NMR(400 MHz, $CDCl_3$ + D_2O) δ: 1.16–1.18 (2H, m), 1.29–1.31 (2H, m), 2.59–2.60 (4H, m), 3.30–3.31 (5H, m), 3.48 (2H, s), 3.74 (3H, s), 3.78 (3H, s), 3.80(3H, m), 6.77–6.79 (1H, d, *J* = 8 Hz), 7.00–7.02 (1H, d, *J* = 8 Hz), 7.54–7.56 (1H, d, *J* = 8 Hz), 7.87–7.91 (1H, d, *J* = 12 Hz), 8.65(1H, s), 15.21 (1H, s). ESI–MS: *m/z* 512 ($M+H^+$). HRMS (ESI, *m/z*): $C_{27}H_{30}FN_3O_6$ ($M+H^+$). Calcd: 512.2100; Found: 512.2191. Compound **4m**, yield: 41.10% (from **2m**), light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.17–1.19 (2H, m), 1.30–1.31 (2H, m), 2.60 (4H, m), 3.26–3.36 (5H, m), 3.49 (2H, s), 3.64 (3H, s), 3.77 (6H, s), 6.64 (2H, s), 7.56–7.58 (1H, d, *J* = 8 Hz), 7.88–7.92 (1*H*, d, *J* = 16 Hz), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 512 ($M+H^+$). HRMS (ESI, *m/z*): $C_{27}H_{30}FN_3O_6$ ($M+H^+$). Calcd: 512.2190; Found: 512.2191. Compound **4n**, yield: 48.39% (from **2n**), light yellow solid. ^{1}H NMR(400 MHz, $CDCl_3$ + D_2O) δ: 1.19–1.21 (2H, m), 1.34–1.36 (2H, m), 2.67 (4H, s), 3.34 (4H, s), 3.50–3.54 (3H, m), 5.07 (2H, s), 6.97 (1H, s), 7.26–7.28 (4H, m), 7.33–7.45 (5H, m), 8.00–8.03 (1H, d, *J* = 12 Hz), 8.76 (1H, s), 15.02 (1H, s). ESI–MS: *m/z* 512 ($M+H^+$). HRMS (ESI, *m/z*): $C_{31}H_{30}FN_3O_4$ ($M+H^+$). Calcd: 528.2313; Found: 528.2293. Compound **4o**, yield: 52.06% (from **2o**), yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6 + D_2O) δ: 1.16–1.17 (2H, m), 1.29–1.31 (2H, m), 2.58 (4H, s), 3.15–3.16 (1H, m), 3.30–3.33 (3H, m), 3.48 (2H, s), 3.77–3.80 (4H, m), 5.05 (2H, s), 6.81–6.83 (1H, m), 6.95–7.00 (2H, m), 7.32–7.44 (5H, m), 7.55–7.57 (1H, d, *J* = 8 Hz), 7.88–7.91 (1H, d, *J* = 12 Hz), 8.65 (1H, s), 15.21 (1H, s). ESI–MS: *m/z* 558 ($M+H^+$). HRMS (ESI, *m/z*): $C_{32}H_{32}FN_3O_5$ ($M+H^+$). Calcd: 558.2395; Found: 558.2398. Compound **4p**, yield: 52.26% (from **2p**), yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$ + D_2O) δ: 1.16–1.21 (2H, m), 1.34–1.38 (2H, m), 2.73 (4H, m), 3.39–3.52 (4H, m), 3.53–3.55 (1H, m), 3.65 (2H, s), 7.26–7.37 (6H, m), 7.96–8.00 (1H, d, *J* = 16 Hz), 8.74 (1H, s), 15.00 (1H, s). ESI–MS: *m/z* 558 ($M+H^+$). HRMS (ESI, *m/z*): $C_{24}H_{24}FN_3O_3$ ($M+H^+$). Calcd: 422.1877; Found: 422.1874.