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Synthesis, antimycobacterial and antibacterial activity of 1-(6-amino-3,5-difluoropyridin-2-yl)fluoroquinolone derivatives containing an oxime functional moiety

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Abstract: A series of novel 1-(6-amino-3,5-difluoropyridin-2-yl)fluoroquinolone derivatives containing an oxime functional moiety were synthesized and evaluated for their biological activity. Our results reveal that compounds **9a-9c** have considerable activity against both of MTB H37Rv ATCC 27294 (MICs: 3.81 - 7.13 μg/mL) and methicillin-sensitive *S. aureus* strains (MICs: < 0.008 - 0.5 μg/mL).

Key words: Fluoroquinolone derivatives; Synthesis**;** Antimycobacterial activity; Antibacterial activity

Since the discovery of norfloxacin in the early 1980s, fluoroquinolone (FQ) antibacterial agents which target two type II bacterial topoisomerase enzymes, DNA gyrase and / or topoisomerase IV, have been among the most attractive drugs in the anti-infective chemotherapy field.[1] Some of them, such as ciprofloxacin (CPFX), ofloxacin and levofloxacin (LVFX) are frequently used as second-line drugs for the treatment of tuberculosis (TB) including multi-drug resistant TB (MDR-TB).[2]

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However, FQ resistance increases in almost all Gram-negative and Gram-positive species as well as *M. tuberculosis* (MTB), due mainly to the high level of use and to some degree of abuse.[3] The ideal strategy to such challenges is to find novel agents that inhibit new targets in pathogens, but a more practical approach is to modify the structures of existing antibacterial agents to increase potency and to overcome resistance.[4]

FQs consist of a 4-quinolone / naphthyridone-3-carboxylic acid core and a secondary amino group attached to the C-7 position of the heterocyclic core. CPFX, LVFX, moxifloxacin and gemifloxacin (GMFX, Fig. 1) represent the most common cores of important FQs on the market. The basic substituent at C-7 position, playing an important role in the antibacterial potency, spectrum and safety of FQs,[1] is recognized as the most adaptable site for chemical change, and the presence of five- or six-membered nitrogen heterocycle including pyrrolidine, piperazine and piperidine at this position is particularly structural feature of FQs.[5]

Recently, some new FQs with novel cores different from the traditional ones, such as sitafloxacin, delafloxacin (DLFX, ABT-492) and AM-1954, have been reported. On the other hand, methyloxime-functionalized pyrrolidines as novel C-7 substituents have attracted great attention and led to the discovery of new FQs (GMFX, zabofloxacin and DW286).[6-8] In our previous works which have been published, some FQs containing oxime-functionalized azetidines, pyrrolidines or piperidines were found to have considerable biological activity.[9-15] These studies suggest the importance of the oxime functional group with respect to biological activity and pharmacokinetic profiles of FQs.

In our continuous program in the search of potent and safe FQ derivatives, we intended to make structural modifications on DLFX (Fig. 1) which is in phase III clinical trials currently,[16] a broad-spectrum FQ antibiotic possessing excellent activity against many MDR-Gram-positive organisms,[17-19] by introduction of a four-, five- or six-membered nitrogen heterocyclic amine moiety with various alkyloxime groups instead of the 3-hydroxyazetidin-1-yl one at the 7 position (Fig. 1). Thus, a series of novel 1-(6-amino-3,5-difluoropyridin-2-yl)FQ derivatives with an (*R*)

/(*S*)-3-alkoxyimino-2-aminomethylazetidyl,3-alkoxyimino-4-aminomethyl-(4-methyl) pyrrolidyl or 3-alkoxyimino-4-amino(hydroxyl)piperidyl group at the C-7 position were designed, synthesized and evaluated for their biological activity in this study. Our primary objective was to optimize the potency of these compounds against clinically important pathogens (especially Gram-positive ones) and MTB including MDR-MTB. A preliminary SAR study is also explored to facilitate the further development of FQs.

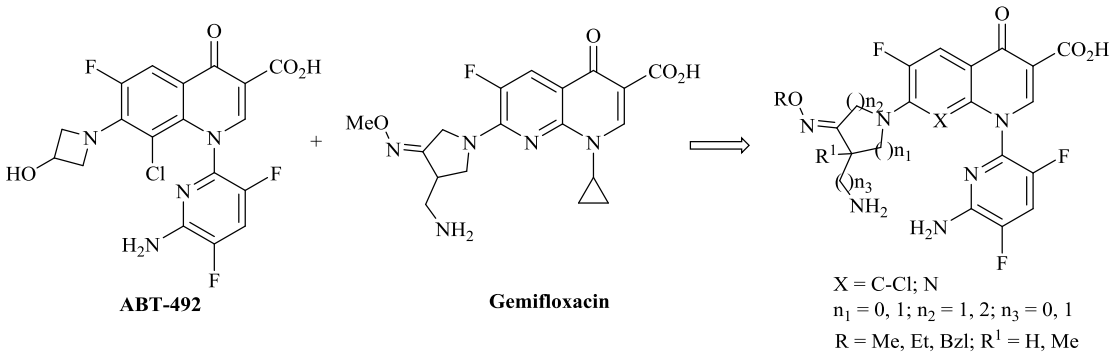
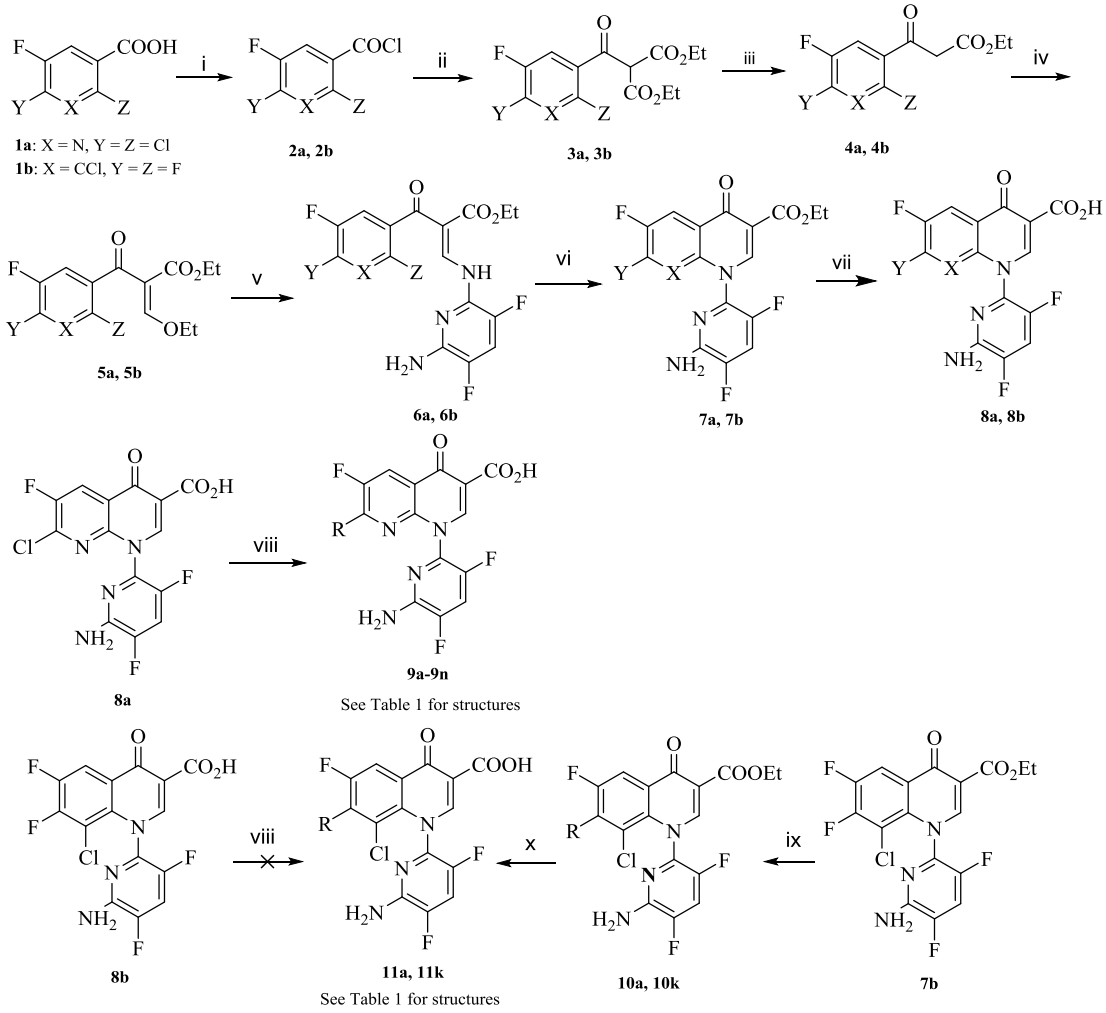


Figure 1. Design of novel fluoroquinolone derivatives

Detailed synthetic pathways to novel naphthyridinone derivatives **9a**-**9n** and quinolone derivatives **11a**, **11k** are depicted in Scheme 1. Enol ethers (**5a**, **5b**) were conveniently obtained from the carboxylic acid **1a**, **1b** by chloroformylation (**2a**, **2b**), condensation with diethyl malonate (**3a**, **3b**), partial hydrolysis followed by decarboxylation (**4a**, **4b**) and condensation with triethylorthoformate in acetic anhydride successively. Nucleophilic substitution of **5a**, **5b** with 3,5-difluoropyridine-2,6-diamine in NMP and then base-assisted cyclization of the resulted enamino ethers **6a**, **6b** in butanone afforded core esters (**7a**, **7b**) according to well established procedures. [13, 20-25]

Naphthyridone derivatives **9a-9n** were easily prepared through direct condensation of the carboxylic acid **8a** which was obtained by hydrolysis of the corresponding core ester **7a**, with various side chain compounds (RH) in the presence of Et_3N.[14,15] However, direct condensation of the quinolone acid **8b** obtained from the ester **7b**, with RH turned out to be rather complicated and the products were difficult to purify. After various attempts, quinolone derivatives **11a,11k** were successfully obtained

from **7b** by condensation with RH in the presence of LiBr and DBU and then hydrolysis of the condensates **10a**, **10k** [24] (Scheme 1). All of the new synthetic compounds were well characterized by ^{1}H NMR, MS and HRMS.



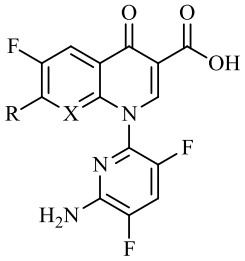
Reagents and conditions: (i): $SOCl_2$, DMF(cata.), reflux, 4 h; (ii): 1): Mg, $CH_2(COOEt)_2$, EtOH, reflux,2.5 h: 2): -10℃, 2 h: (iii): p-TsOH, H_2O, reflux, 5h: (iv): $CH(OEt)_3$, Ac_2O, reflux,3 h; (v): 3,5-difluoropyridine-2,6-diamine, NMP, CH_3CN, r,t,. 6 h, 37%-53%; (vi): K_2CO_3,butanone, 60 ℃,5 h, 67%-85%; (vii): HCl, HOAc, 95℃, 1.5 h: (viii): RH, Et_3N, CH_3CN, 50℃, 5-8 h, 31%-63%; (ix): RH, DBU, LiBr, DMSO, 100℃, 3-5 h; (x): 1): 5%NaOH(aq.), EtOH, 16 h; 2): 20%HOAc(aq.), pH 6.5-7.0, 17%-21%

Scheme 1. Synthesis of quinolone / naphthyridinone derivatives **9, 11**

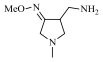
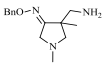
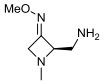
The target compounds **9**, **11** were initially evaluated for their *in vitro* activity against MTB H37Rv ATCC 27294 using the Microplate Alamar Blue Assay (MABA).[26, 27] The minimum inhibitory concentration (MIC) is defined as the lowest concentration effecting a reduction in fluorescence of ≥ 90% relative to the mean of replicate bacterium-only controls and MICs of these compounds along with CPFX, LVFX, isoniazid (INH) and rifampicin (RFP) for comparison are presented in Table 1.

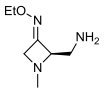
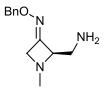
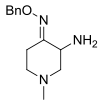
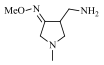
The data reveals that all of the new synthesized compounds **9**, **11** (MICs: 3.81 -> 32 μg/mL) are much less than the four reference drugs (MICs: 0.25 - 0.05 μg/mL), but the most active compounds **9a-9c** have promising activity with MICs of 7.13, 6.56 and 3.81 μg/mL, respectively, against this strain. In addition, our results suggest that simply increasing the lipophilicity which is expressed in the term of their Clog P values (Table 1) can improve the anti-MTB activity of FQs with similar structures (**9a** *vs* **9b** *vs* **9c**), which is consistent with the SAR in Sriram's study.[28]

Table 1. Structures, physical data and antimycobacterial activity of compounds **9**, **11**



9, **11**

Compd.	R	X	m.p. [°C][a]	Clog P[b]	MIC (μg/mL)
					MTB
9a		N	>250	1.52	7.13
9b		N	>250	1.86	6.56
9c		N	>250	3.26	3.81
9d		N	157-159	3.96	15.59
9e		N	>250	1.12	>32
9f		N	>250	1.45	>32
9g		N	220-222	2.85	>32
9h		N	>250	1.12	>32

9i		N	>250	1.45	>32
9j		N	>250	2.85	>32
9k		N	234-236	1.66	>32
9l		N	202-204	3.39	>32
9m		N	220-222	2.04	30.74
9n		N	138-140	3.77	>32
11a		C-Cl	179-181	1.99	30.52
11k		C-Cl	157-159	2.12	>32
CPFX				1.32	0.25
LVFX				1.35	0.25
INH					0.05
RFP					0.05

[a] Melting points are uncorrected ; [b] The Clog P is calculated by Chemoffice 2010 software; CPFX: Ciprofloxacin ; LVFX: Levofloxacin ; INH: Isoniazid; RFP: Rifampicin; MTB: MTB H37Rv ATCC 27294.

The target compounds **9**, **11** were also evaluated for their *in vitro* antibacterial activity against representative strains using standard techniques.[29] Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth, and MICs of **9**, **11** against Gram-negative and Gram-positive strains along with moxifloxacin (MXFX) and LVFX for comparison, are listed in Tables 2 and 3, respectively. These data suggest that some of the target compounds **9**, **11** have considerable potency against

Gram-positive strains, although they are generally less active than the reference drugs MXFX and LVFX against the Gram-negative strains with a few exceptions. For example, compounds **9a-9c** and **11a,11k** show excellent potency in inhibiting the growth of methicillin-sensitive *S. aureus* (MSSA) (MICs: < 0.008 - 0.5 μg/mL, four strains) (Table 3). It is noted that compound **11a** also has good activity against clinically important pathogens *Pseudomonas aeruginos* (MICs: 4 - 8 μg/mL, six strains) which is comparable to MXFX (MICs: 1 - 16 μg/mL) (Table 2).

Table 2. *In vitro* antibacterial activity of compounds **9, 11** against Gram-negative strains

Compd	Strains MIC (μg/mL)																	
	E.coli	E.co.1	E.co.2	E.co.3	E.co.4	K.p.1	K.p.2	K.p.3	K.p.4	K.p.5	K.p.6	K.p.7	P.a.	P.a.1	P.a.2	P.a.3	P.a.4	P.a.5
9a	2	16	>128	128	128	128	32	>128	>128	>128	>128	>128	4	16	32	32	32	64
9b	0.5	8	128	64	64	64	32	128	64	128	128	128	4	16	16	16	16	32
9c	0.5	8	128	128	128	64	32	128	128	128	128	128	8	32	32	32	32	32
9d	16	64	>128	>128	128	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	128	128
9e	2	64	>128	>128	>128	>128	64	>128	>128	>128	>128	>128	16	32	64	64	32	32
9f	2	64	>128	>128	>128	>128	64	>128	>128	>128	>128	128	16	32	64	64	64	64
9g	4	64	>128	>128	>128	>128	128	>128	>128	>128	>128	>128	64	128	128	128	128	28
9h	16	>128	>128	>128	>128	>128	64	>128	>128	>128	>128	>128	64	64	64	64	64	64
9i	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	128	128
9j	8	128	>128	>128	>128	>128	128	>128	>128	>128	>128	>128	128	128	128	128	128	128
9k	16	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	128	128
9l	64	128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	128	128
9m	64	64	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	128	128	128	128	128	128
9n	16	128	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	128	128	128	128	128	128
11a	0.125	2	64	64	64	32	16	128	64	>128	128	128	4	8	8	8	8	8
11k	1	64	>128	>128	>128	>128	128	>128	>128	>128	>128	>128	16	64	64	64	128	128
MXFX	0.06	2	16	8	16	8	1	64	8	16	32	16	2	4	16	16	8	1
LVFX	0.125	2	8	4	4	4	1	64	16	8	16	8	1	1	2	2	2	2

E.coli: *E. coli* ATCC 25922. E.co.1: Extended-spectrum β-lactamase-producing (ESBL$^+$) *E. coli* 14-1. E.co.2: ESBL$^+$ *E. coli* 14-2. E.co.3: *E. coli* 14-1. E.co.4: *E. coli* 14-2. K.p.1: ESBL$^+$ *K. pneumoniae* 14-17. K.p.2: ESBL$^+$ *K. pneumoniae* 14-18. K.p.3: ESBL$^+$ *K. pneumoniae* 14-19. K.p.4: *K. pneumoniae* 14-1. K.p.5: *K. pneumoniae* 14-2. K.p.6: *K. pneumoniae* 14-3. K.p.7: *K. pneumoniae* 14-4. P.a.: *P. aeruginosa* ATCC 27853. P.a.1: *P. aeruginosa* 14-9. P.a.2: *P. aeruginosa* 14-14. P.a.3: *P. aeruginosa* 14-15. P.a.4: *P. aeruginosa* 14-16. P.a.5: *P. aeruginosa* 14-19. MXFX: Moxifloxacin. LVFX: Levofloxacin.

Table 3. *In vitro* antibacterial activity of compounds **9**, **11** against Gram-positive strains

Compd	Strains MIC (μg/mL)																	
	S.a.	MSSA1	MSSA2	MSSA3	MRSA1	MRSA2	MSSE1	MSSE2	MSSE3	MRSE1	MRSE2	MRSE3	S.p.	E.fm.1	E.fm.2	E.fs. 1	E.fs. 2	E.fs. 3
9a	0.5	0.5	0.5	0.25	>128	64	>128	64	64	64	128	64	64	>128	64	16	16	32
9b	0.06	0.25	0.5	0.5	64	64	64	32	32	32	64	32	16	128	8	16	16	32
9c	<0.008	0.015	0.25	0.25	64	128	64	32	32	16	32	32	16	128	16	8	16	16
9d	0.25	0.25	8	4	64	64	>128	>128	>128	64	>128	>128	32	>128	8	>128	>128	>128
9e	4	8	8	4	>128	>128	>128	64	64	>128	>128	>128	64	>128	128	>128	>128	>128
9f	2	4	8	4	>128	>128	>128	64	128	>128	>128	>128	32	>128	32	>128	>128	>128
9g	2	2	4	4	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	32	>128	>128	>128
9h	16	32	32	16	>128	>128	>128	>128	>128	>128	>128	>128	128	>128	64	>128	>128	>128
9i	16	32	32	32	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	128	>128	>128	>128
9j	4	4	8	4	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	32	>128	>128	>128
9k	8	8	8	16	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	64	>128	>128	>128
9l	8	8	4	8	>128	>128	>128	>128	>128	>128	>128	>128	64	>128	64	128	128	>128
9m	2	2	4	4	>128	128	>128	>128	>128	>128	>128	>128	64	>128	64	>128	>128	>128
9n	2	4	4	4	>128	>128	>128	>128	>128	>128	>128	>128	32	>128	32	>128	>128	>128
11a	0.03	0.06	0.125	0.25	128	128	64	32	64	64	64	32	16	>128	8	16	64	32
11k	0.5	0.5	0.25	0.5	>128	>128	>128	128	128	>128	>128	>128	64	>128	16	>128	>128	>128
MXFX	0.06	0.06	<0.008	<0.008	12	8	32	2	2	8	8	8	0.015	32	0.5	8	16	8
LVFX	0.125	0.25	0.125	0.5	64	8	128	1	2	32	64	64	0.5	128	1	16	32	64

S.a.: *S. aureus* CMCC 26003. MSSA1: Methicillin-sensitive *S. aureus* 14-2. MSSA2: Methicillin-sensitive *S. aureus* 14-3. MSSA3: Methicillin-sensitive *S. aureus* 14-4. MRSA1: Methicillin-resistant *S. aureus* 14-4. MRSA2: Methicillin-resistant *S. aureus* 14-5. MSSE1: Methicillin-sensitive *S. epidermidis* 14-2. MSSE2: Methicillin-sensitive *S.epidermidis* 14-4. MSSE3: Methicillin-sensitive *S. epidermidis* 14-6. MRSE1: Methicillin-resistant *S.epidermidis* 14-22. MRSE2: Methicillin-resistant *S. epidermidis* 14-37. MRSE3: Methicillin-resistant *S.epidermidis* 14-39. S.p.: *S. pneumoniae* ATCC 19615. E. fm. 1: *E. faecium* 14-1. E. fm. 2: *E. faecium* 14-2. E. fs. 1: *E. faecalis* 14-1. E. fs. 2: *E. faecalis* 14-2. E. fs. 3: *E. faecalis* 14-3. MXFX: Moxifloxacin. LVFX: Levofloxacin.

In the case of Gram-positive MSSA (four strains), the activity of the 1-(6-amino-3,5-difluoropyridin-2-yl) FQs in this study is closely related to both of the groups at the 7- and 8-positions. In the series of naphthyridinone derivatives **9a-9n**, the sizes of the heterocycles and alkyl groups of the oxime moiety at the 7-position are especially important for the activity. Pyrrolidyl-based derivatives (**9a-9c**) are generally more active than azetidyl- and piperidinyl-based ones with the same alkyl group. The contribution of the alkyl groups is as follows: benzyl > ethyl > methyl for pyrrolidyl-based ones (**9a-9c**), which suggest that simply increasing the lipophilicity could improve the antibacterial activity. However, introduction of an additional methyl group at C-3 of the pyrrolidyl moiety significantly reduces the activity (**9c** *vs* **9d**). It is interesting that piperidinyl-based derivatives are much less active than the corresponding pyrrolidyl-based ones (**9a** *vs* **9k**, **9c** *vs* **9l**), but replacement of the amino group of the piperidinyl moiety leads to improved activity (**9k** *vs* **9m**, **9l** *vs* **9n**). Moreover, the chirality at C-2 of the azetidinyl group of the target compounds influences the activity, and the *R* enantiomers are more active than the corresponding *S* ones (**9e** *vs* **9h**, **9f** *vs* **9i**, **9g** *vs* **9j**). On the other hand, the activity of the group at the 8-position is in the order: C-Cl ≥ N (**9a** *vs* **11a**) when a pyrrolidine served as the C-7 side chain, and C-Cl >> N (**9k** *vs* **11k**) for the piperidinyl-based derivatives.

In summary, a series of novel 1-(6-amino-3,5-difluoropyridin-2-yl) FQ derivatives containing a four-, five- or six-membered nitrogen heterocyclic amine moiety with various alkyloxime groups at the C-7 position were designed, synthesized and evaluated for their biological activity. Our results reveal that compounds **9a-9c** have promising activity against MTB H37Rv ATCC 27294 (MICs: 3.81 - 7.13 μg/mL). On the other hand, compounds **9a-9c** and **11a**, **11k** possess excellent *in vitro* activity against all of the MSSAs (MICs: < 0.008 - 0.5 μg/mL, four strains). In addition, our results suggest that the lipophilicity seems to be an important parameter affecting both the anti-MTB and antibacterial activity.

Acknowledgments

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29. MIC values 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; NCCLS: Wayne, PA, USA, 2001, M100-S11). MIC was defined as the lowest concentration of each compound that inhibits visible growth of bacteria after incubation at 35°C for 18-24 h.
30. Compound **9a**, off-white solid (23.1%), m.p. >250℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (1H, s, C_2-H), 8.05 (1H, d, J = 12.0 Hz, C_5-H), 7.93 - 7.89 (1H, m), 4.29 (2H, s), 3.90 (2H, s), 3.78 (3H, s, O-CH_3), 2.97 (1H, brs), 2.79 - 2.64 (2H, m). MS-ESI (*m/z*): 478.28$(M+H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{24}H_{23}O_4N_4F_2(M+H)^+$: 478.14451; Found: 478.14398. Compound **9b**, off-white solid (18.9%), m.p. >250℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.89 (1H, s, C_2-H), 8.10 (1H, d, J = 12.0 Hz, C_5-H), 7.95 - 7.89 (1H, m), 4.24 (2H, s), 4.06 (2H, q, J = 8.0 Hz), 3.90 (2H, s), 3.08 (1H, s), 2.79 - 2.64 (2H, m), 1.20 (3H, t, J = 8.0 Hz). MS-ESI (*m/z*): 492.25$(M+H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{24}H_{23}O_4N_4F_2(M+H)^+$: 492.16016; Found: 492.15945. Compound **9c**, off-white solid (22.4%), m.p. >250℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.91 (1H, s, C_2-H), 8.16 (1H, d, J = 12.0 Hz, C_5-H), 7.82-7.75 (1H, m), 7.43 - 7.36 (5H, m, OCH_2-Ar), 5.15 (2H, s, O-CH_2Ar), 4.28 (4H, m), 3.13 - 3.07 (3H, m). MS-ESI (*m/z*): 554.33$(M+H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{24}H_{23}O_4N_4F_2(M+H)^+$: 554.17581; Found: 554.17515. Compound **11a**, off-white solid (17.3%), m.p. 179 - 181℃. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.74 (1H, s, C_2-H), 8.02 (1H, d, J = 12.0 Hz, C_5-H), 7.97 - 7.92 (1H, m), 3.93 (2H, s), 3.80 (1H, brs), 3.76 (3H, s, O-CH_3), 3.66 (1H, brs), 3.53 (1H, brs), 3.27 - 3.20 (2H, m). MS-ESI (*m/z*): 511.34 $(M+H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{21}H_{19}O_4N_6ClF_3$ $(M+H)^+$: 511.11011; Found: 511.11029. Compound **11k**, off-white solid (21.2%), m.p. 157 - 159℃. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (1H, s, C_2-H), 8.07 (1H, d, J = 12.0 Hz, C_5-H), 7.99 (1H, brs), 5.12 - 5.06 (1H, m), 3.71 (3H, s), 3.54 (1H, brs), 3.37 (2H, brs), 3.30 - 3.21 (1H, m), 3.14 - 2.95 (2H, m). MS-ESI (*m/z*): 511.02$(M+H)^+$. HRMS-ESI (*m/z*): Calcd. for $C_{24}H_{23}O_4N_4F_2(M+H)^+$: 511.11029; Found: 511.11236.

Synthesis, antimycobacterial and antibacterial activity of 1-(6-amino-3,5-difluoropyridin-2-yl)fluoroquinolone derivatives containing an oxime functional moiety

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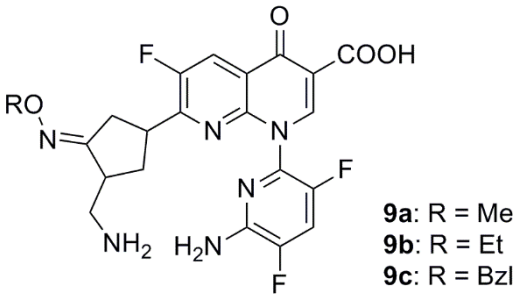
A series of novel 1-(6-amino-3,5-difluoropyridin-2-yl)fluoroquinolone derivatives containing an oxime functional moiety were synthesized and evaluated for their biological activity. Our results reveal that compounds **9a-9c** have considerable activity against MTB H37Rv ATCC 27294 (MICs: 3.81 - 7.13 μg/mL) and methicillin-sensitive *S. aureus* strains (MICs: < 0.008 - 0.5 μg/mL).



9a: R = Me
9b: R = Et
9c: R = Bzl

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