Bioorganic & Medicinal Chemistry Letters 21 (2011) 3377–3380

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and in vitro antibacterial activity of 7-(4-alkoxyimino-3-aminomethylpiperidin-1-yl)fluoroquinolone derivatives

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ARTICLE INFO

Article history:
Received 18 January 2011
Revised 29 March 2011
Accepted 1 April 2011
Available online 7 April 2011

Keywords:
Fluoroquinolone
Synthesis
Antibacterial activity

ABSTRACT

We report herein the design and synthesis of novel 7-(4-alkoxyimino-3-aminomethylpiperidin-1-yl) fluoroquinolone derivatives. The antibacterial activity of the newly synthesized compounds was evaluated and compared with gemifloxacin, levofloxacin and ciprofloxacin. Results reveal that compounds **10**, **16**, and **17** have good activity against all of the tested Gram-positive organisms including drug-resistance strains (MICs: 0.125–4 μg/mL). In addition, compounds **16** and **17** (MICs: 4 μg/mL) were 2- to 8-fold more potent than the reference drugs against *Pseudomonas aeruginosa*.

Quinolones as chemotherapeutic drugs for the treatment of various bacterial infections in both community and hospital settings have attracted great attention because of their outstanding potency and steady safety.[1] These antibiotics inhibit two type-II topoisomerases, DNA gyrase and topoisomerase IV, by binding to the intermediate catalytic enzyme–DNA complex. The stabilization of the resulting quinolone–enzyme–DNA complex leads to the generation of double-strand DNA breaks that trigger a cascade of events leading to cell death.[2,3]

Since the discovery of norfloxacin by Koga et al. in the early 1980s,[4] most of the research concerning these drugs has been focused on the basic group at the C-7 position which greatly influences their potency, spectrum, and safety. As a result, ciprofloxacin (CPFX), levofloxacin (LVFX), gemifloxacin (GMFX), moxifloxacin (MXFX) and so on have been successfully introduced into the market. Most of them are generally characterized by a broad antimicrobial spectrum, but their activity against clinically important Gram-positive cocci including *Staphylococci, Streptococci* and *Enterococci* is relatively moderate, which has not only limited their use in infections caused by these organisms, but has also contributed to rapidly developing quinolone resistance. Thus, recent efforts have been directed toward the synthesis of new quinolones that can provide improved Gram-positive antibacterial activity, while retaining the good Gram-negative activity of early fluoroquinolones, such as CPFX.[5]

Five- and six-membered nitrogen heterocycles including piperazinyl, pyrrolidinyl and piperidinyl type side chains that contain peripheral nitrogens have proven to be the optimal substituents at the 7-position of fluoroquinolones. However, the piperidinyl-based quinolone antibacterial agents reported in the literature were significantly fewer than that of piperazinyl-, pyrrolidinyl-based analogs.[6–8]

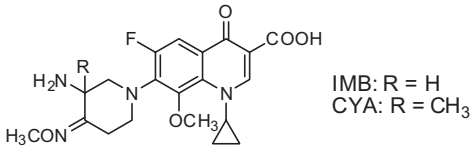

Figure 1. Structures of IMB and CYA.

Recently, in our continuous effects to develop new fluoroquinolones that display strong Gram-positive activity, we have focused on introducing new functional groups to the piperidine ring[9–14] and found IMB (R = H, Fig. 1), a 8-methoxyl fluoroquinolone containing an 3-amino-4-methoxyiminopiperidin-1-yl group at the C-7 position, have good in vitro and in vivo antibacterial activity which is comparable to MXFX and GMFX.[9] However, CYA (R = CH_3, Fig. 1), a methyl analog of IMB, shows far less antibacterial activity[15] and these data indicate that introduction of an additional methyl group in the 3-position of the piperidine ring causes reduced activity. We had previously reported that some of 7-(4-alkoxyimino-3-aminomethyl-3-methylpiperidin-1-yl)fluoroquinolones, were more active than or comparable to LVFX against Gram-positive organisms, such as *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA), *Staphylococcus epidermidis* and *Streptococcus pyogenes*.[16]

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doi:10.1016/j.bmcl.2011.04.002

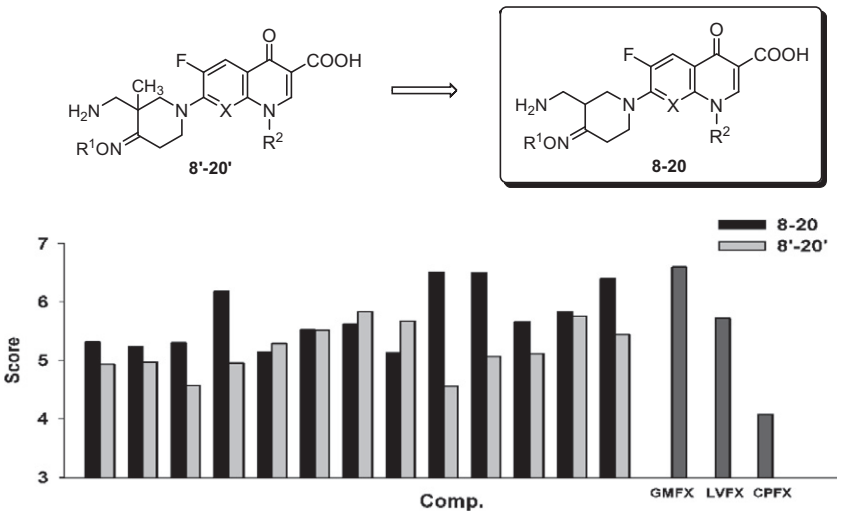


Figure 2. Scores for novel fluoroquinolones **8**–**20**.

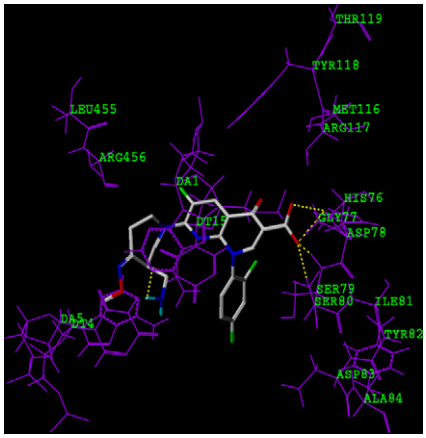
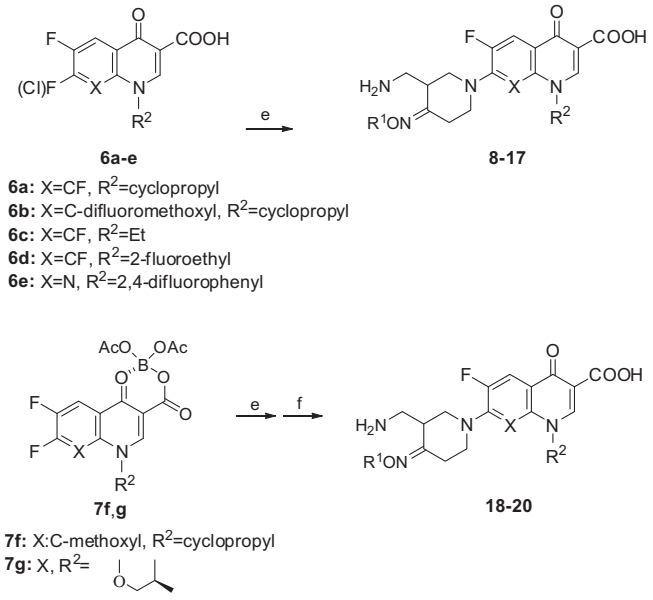


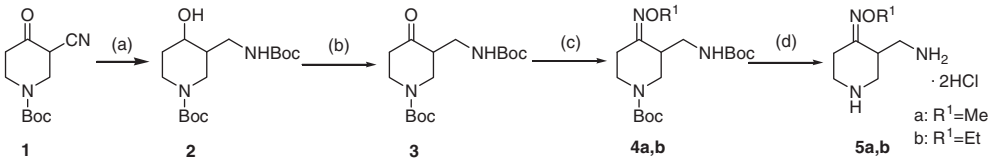
Figure 3. Predicted three-dimensional conformations of compound **16** docked in 3FOF.



Scheme 2. Reagents and conditions: (e) Compounds **5a**, **b**, Et_3N, MeCN, 25–50 °C, 2–10 h, 48–74%; (f) (1) 5% NaOH/H_2O, 40 °C, 0.5–2 h; (2) 2 N HCl, rt, 50–66% (two steps).

These research results intensified our interest, it was decided to design and synthesize some novel fluoroquinolone derivatives, having an alkoxyimino and an aminomethyl group at the 4- and 3-positions of the piperidine ring, respectively, and evaluate their antibacterial activity. In order to clarify rationale of the design idea, we initially conducted also molecular docking to predict the stabilization of the resulting target quinolone–enzyme–DNA complex.[17,18,20] The scores (Fig. 2) reflecting the fitting quality of ligand–receptor complex show that most of the designed fluoroquinolone derivatives (**8**–**20**) are more potent than the corresponding methyl analogs (**8′**–**20′**), and some are more potent than LVFX and even comparable to GMFX against *Strptococcus pneumoniae*. We report herein the synthesis of a series of novel 7-(4-alkoxyimino-3-aminomethylpiperidin-1-yl)fluoroquinolones and their antibacterial activity. A structure–activity relationship (SAR) study was also explored to facilitate the further development of the fluoroquinolones.

The synthetic routes of new piperidine derivatives **5a**, **b** and novel fluoroquinolones **8–20** are shown in Scheme 1 and 2, respectively. According to published procedures,[16] catalytic hydrogena-



Scheme 1. Reagents and conditions: (a) H_2, 5% Pd/C, Boc_2O, MeOH, rt, 10 h, 55%; (b) Jone's reagent, Me_2CO, 0 °C, 1 h, 85%; (c) R^1ONH_2·HCl, Et_3N, MeOH, 55–60 °C, 2 h, 88–91%; (d) HCl; (g), CH_2Cl_2, rt, 1 h, 97–99%.

Table 1
The structures, physical data and cytotoxicity of novel fluoroquinolones **8–20**

Compd	R^1	R^2	X	Yield (%)	mp[a] (°C)	CC_{50}[b] (μg/mL)
8	Me		CF	65	115–116	NT[c]
9	Et		CF	63	134–136	256.9
10	Me		$COCHF_2$	70	93–95	334.9
11	Et		$COCHF_2$	74	194–195	203.2
12	Me	CH_2CH_3	CF	67	184–186	>500
13	Et	CH_2CH_3	CF	62	96–98	409.5
14	Me	CH_2CH_2F	CF	64	186–188	NT
15	Et	CH_2CH_2F	CF	54	141–143	>500
16	Me	2,4-F_2-C_6H_3	N	53	131–133	96.0
17	Et	2,4-F_2-C_6H_3	N	48	179–181	72.6
18	Me		$COCH_3$	63	150–152	395.4
19	Et		$COCH_3$	66	183–185	173.2
20	Me			50	102–104	213.1

[a] Melting points are uncorrected.
[b] CC_{50}: The 50% cytotoxic concentration.
[c] NT: not tested.

Table 2
In vitro antibacterial activity of the target compounds **8–20**

Strains	MIC (μg/ml)															
Compd	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	GMFX	LVFX	CPFX
S.a.	0.5	0.5	0.25	1	2	4	4	8	0.5	0.25	0.5	1	2	⩽0.03	0.06	0.125
MSSA	0.5	0.25	0.125	0.25	4	4	2	8	0.25	0.25	0.25	0.25	1	⩽0.03	0.06	0.25
MRSA	0.5	1	1	2	8	8	8	16	2	1	4	2	16	0.5	1	4
S.e.	1	0.25	0.5	2	8	8	8	16	0.5	0.25	1	2	4	0.06	0.125	0.25
MSSE	1	2	1	4	16	32	32	64	2	1	4	4	16	0.06	1	2
MRSE	4	0.25	0.5	2	4	8	8	8	0.5	0.25	4	2	4	⩽0.03	0.125	0.25
S.p.	8	8	4	8	32	64	32	64	1	2	4	8	8	0.5	2	1
PRSP	8	4	2	4	16	32	16	32	0.5	0.5	4	4	4	⩽0.03	0.5	1
E.fa.	4	2	1	2	8	16	8	16	2	1	4	4	4	0.06	0.25	0.5
VREF	4	2	4	4	32	32	16	64	4	4	8	8	8	0.5	2	1
S.py.	16	32	4	16	64	64	32	64	1	4	8	8	16	0.125	1	1
E.c.	1	1	1	4	4	8	8	8	1	2	4	4	4	⩽0.03	⩽0.03	⩽0.03
ESBL-Ec	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	32	32	128
K.p.	16	32	32	64	128	>128	128	>128	32	32	64	128	128	0.5	0.5	0.5
P.a.	8	16	8	16	32	128	32	64	4	4	8	32	8	32	8	16
S.s.	1	0.5	16	2	4	128	32	8	2	2	4	8	8	⩽0.03	⩽0.03	⩽0.03
B.t.	1	0.5	0.5	2	4	8	8	8	2	2	4	4	4	⩽0.03	⩽0.03	⩽0.03
E.cl.	32	>128	2	8	>128	>128	>128	16	2	2	>128	>128	16	⩽0.03	0.06	⩽0.03
A.c.	2	2	2	4	16	128	128	128	4	4	4	8	32	0.125	0.25	1
E.a.	1	2	2	8	8	16	32	16	2	2	4	16	8	⩽0.03	⩽0.03	⩽0.03
S.m.	4	4	8	32	8	16	16	16	8	16	8	32	8	0.125	0.25	0.25
M.m.	1	4	4	8	4	16	16	8	4	4	4	16	8	0.06	⩽0.03	⩽0.03
P.r.	8	8	4	16	8	32	16	32	8	8	8	32	16	0.06	0.25	⩽0.03
P.v.	4	4	4	8	8	16	16	16	8	4	8	32	4	⩽0.03	⩽0.03	⩽0.03

Abbreviations: S.a., Staphylococcus aureus ATCC13709; MSSA, methicillin-sensitive *Staphylococcus aureus* 08-49; MRSA, methicillin-resistant *Staphylococcus aureus* 08-52; *S.e., Staphylococcus epidermidis* ATCC12228; MSSE, methicillin-sensitive *Staphylococcus epidermidis* 08-17; MRSE, methicillin-resistant *Staphylococcus epidermidis* 08-18; *S.p., Streptococcus pneumoniae* ATCC6301; PRSP, penicillin-resistant *Streptococcus pneumoniae* 08-2; *E.fa., Enterococcus faecalis* ATCC51299; VREF, vancomycin-resistant *Enterococcus faecalis* EFL1004; *S.py., Streptococcus pyogenes* 556; *E.co., Escherichia coli* ATCC25922; ESBL-Ec, *Extended spectrum* β*-lactamase producing Escherichia coli* 08-5*; K.p., Klebsiella pneumoniae* ATCC700603; *P.a., Pseudomonas aeruginosa* 17; *S.s., Shigella sonnei* 51592; *B.t., Bacillus typhi* 50035; *E.cl., Enterobacter cloacae* 45301; *A.c., Acinetobacter calcoaceticus* 25001; *E.a., Enterobacter aerogenes* 45102; *S.m., Serratia marcescens* 41002; *M.m., Morganella morganii* 49086; *P.r., Proteus rettgeri* 49006; *P.v., Proteus vuigaris* 56.

tion of both functional groups of readily available cyano ketone **1** and subsequent protection of the resulting primary amine by *tert*-butoxycarbonyl (Boc) group yielded the amino alcohol **2**. Oxidation of the alcohol **2** by Jone's reagent afforded the corresponding ketone **3**, on which the oxime functional group was introduced via condensation with methoxylamine/ethoxylamine to give alkyloximes **4a, b**. The bis-Boc-protecting groups of compounds **4a, b** were removed by pumping hydrogen chloride gas in methylene chloride to afford the new piperidine derivative dihydrochlorides **5a, b** in good yield.

Finally, the target compounds **8–20** were obtained by coupling the new piperidine derivatives **5a**, **b** with various compounds containing quinolone and naphthyridone cores according to well-established literature procedures (Scheme 2)[19]. In the case of quinolones **8–17**, condensation of **5a**, **b** with **6a–e** was performed in the presence of triethylamine. However for

18–**20**, boric chelates **7f** and **g** were required to increase reactivity. All of the synthetic compounds were well characterized through the spectral characteristics and the results were summarized in Table 1.

The novel fluoroquinolones **8–20** were evaluated for their in vitro antibacterial activity against representative Gram-positive and Gram-negative strains using standard techniques. Minimum inhibitory concentration (MIC) is defined as the concentration of the compound required to give complete inhibition of bacterial growth and MICs of the synthesized compounds along with the standard drugs GMFX, CPFX and LVFX for comparison are reported in Table 2.

Since the oxime group can exist in the *E* or *Z* configuration, it was necessary to determine the geometries of all the oxime target compounds **8–20**. However, it was a pity that we were not successful in preparing X-ray quality single crystals of any oxime intermediate or product, but the oxime geometry would be expected to have the *E*-configuration according to the data in published papers.[8,16] It is also obvious that the target compounds **8–20** and intermediates **5a, b** are all racemates.

All of the target compounds **8–20** have generally potent antibacterial activity against the tested strains. For Gram-positive organisms, compounds **8–11** and **16–19** show good potency in inhibiting the growth of *S. aureus* including MRSA and *S. epidermidis* including methicillin-resistant *S. epidermidis* (MRSE) (MICs: 0.125–4 μg/mL). Among them, compounds **10**, **16** and **17** had useful activity against *S. pneumoniae* including penicillin-resistant *S. pneumoniae*, *Enterococcus faecalis including* vancomycin-resistant *E. faecalis*, *and S. pyogenes* (MICs: 0.5–4 μg/mL). But for Gram-negative organisms, all the target compounds are far less active than GMFX, LVFX and CPFX, with the only exception that compounds **16** and **17** (MICs: 4 μg/mL) are 2- to 8-fold more potent than the reference drugs against *Pseudomonas aeruginosa.*

Overall the piperidinyl analogs exhibit less active than the reference drugs against the tested Gram-positive and Gram-negative organisms. Our results indicate that des-methylation at the 3-position of the 4-alkoxyimino-3-aminomethyl-3-methyl piperidine ring does not increase the antibacterial activity which could be also involved with other factors, such as the substituent (aminomethyl) at the 3-position of the piperidine ring and regiochemical effects. In addition, the fact that the tested activity against *S. pneumoniae* is not in agreement with what we predicted based only on inhibition of topoisomerase IV suggests that both of the topoisomerase IV and DNA gyrase in Gram-positive bacteria could be the targets of the fluoroquinolones.

Generally, the activity of the quinolone nuclei in this study are in the order 1-(2,4-difluorophenyl)-1,8-naphthyridone > 1-cyclopropyl-8-difluoromethoxylquinolone > 1-cyclopropyl-8-fluoroquinolone≈1-cyclopropyl-8-methoxylquinolone > levofloxacin > 1-ethyl-8-fluoroquinolone≈1-(2-fluoroethyl)-8-fluoroquinolone. In addition, fluoroquinolones featuring methyloxime-incorporated piperidino-substitution at C-7 position are at least as potent as the analogs containing ethyloxime.

Some compounds were further examined for toxicity (CC_{50}) in a mammalian Vero cell line from 1000 to 7.81 μg/mL concentrations. After 48 h of exposure, viability was assessed and the results are reported in Table 1. Thirteen compounds when tested showed CC_{50} values ranging from >500 to 72.6 μg/mL. A comparison of the substitution pattern at C-7 position demonstrates that ethyloxime-incorporated piperidino-substitutions are generally more cytotoxic than the analogues containing methyloxime.

In summary, we report herein the synthesis of some novel 7-(4-alkoxyimino aminomethyl-3-methylpiperidin-1-yl)fluoroquinolone derivatives. The antibacterial activity of the newly synthesized compounds were evaluated and correlated with their physicochemical properties. Results reveal that compounds **10**, **16**, and **17** have good activity against the tested Gram-positive strains (MICs: 0.125–4 μg/mL). It was worth noting that compounds **16** and **17** (MICs: 4 μg/mL) were 2- to 8-fold more potent than CPFX, LVFX and GMFX against *P. aeruginosa.* However, these piperidinyl analogs are generally less activity than the reference drugs against Gram-positive and Gram-negative strains.

Acknowledgment

The work was supported by the National S&T Major Special Project on Major New Drug Innovation. Item Number: 2009ZX09301-003.

References and notes

1. Hooper, D. C.; Wolfson, J. S. *N. Eng. J. Med.* **1991**, *32*, 384.
2. de Souza, M. V. N. *Mini Rev. Med. Chem.* **2005**, *5*, 1009.
3. Hooper, D. C. *Clin. Infect. Dis.* **2001**, *32*, S9.
4. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Iridura, T. *J. Med. Chem.* **1980**, *23*, 1358.
5. Srivastava, B. K.; Solanki, M.; Mishra, B.; Soni, R.; Jayadev, S.; Valani, D.; Jain, M.; Patel, P. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1924.
6. Bryskier, A.; Chantot, J. F. *Drugs* **1995**, *49*, S16.
7. Dang, Z.; Yang, Y. S.; Ji, R. Y.; Zhang, S. H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4523.
8. Hong, C. Y.; Kim, Y. K.; Chang, J. H.; Kim, S. H.; Choi, H.; Nam, D. H.; Kim, Y. Z.; Kwak, J. H. *J. Med. Chem.* **1997**, *40*, 3584.
9. Wang, X. Y.; Guo, Q.; Wang, Y. C.; Liu, B. Q.; Liu, M. L.; Sun, L. Y.; Guo, H. Y. *Acta Pharmacol. Sin.* **2008**, *43*, 819.
10. Zhang, Y. B.; Feng, L. S.; You, X. F.; Guo, Q.; Guo, H. Y.; Liu, M. L. *Arch. Pharm. Chem. Life Sci.* **2010**, *343*, 143.
11. Wang, J. X.; Guo, Q.; Chai, Y.; Feng, L. S.; Guo, H. Y.; Liu, M. L. *Chin. Chem. Lett.* **2010**, *21*, 55.
12. Huang, X. G.; Zhang, A. Q.; Chen, D. L.; Jia, Z. H.; Li, X. S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2859.
13. Srinivasan, S.; Shafreen, R. M. B.; Nithyanand, P.; Manisankar, P.; Pandian, S. K. *Eur. J. Med. Chem.* **2010**, *45*, 6101.
14. Zhang, Y. B.; Li, G. Q.; Liu, M.; You, X. L.; Feng, L. S.; Lv, K.; Cao, J.; Guo, H. Y. *Bioorg. Med. Chem. Lett.* **2010**. doi:10.1016/j.bmcl.2010.12.073.
15. Chai, Y.; Wan, Z. L.; Wang, B.; Liu, M. L.; Guo, H. Y. *Eur. J. Med. Chem.* **2009**, *44*, 4063.
16. Chai, Y.; Liu, M. L.; Wang, B.; You, X. F.; Feng, L. S.; Zhang, Y. B.; Cao, J.; Guo, H. Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5195.
17. Laponogov, I.; Sohi, M. K.; Veselkov, D. A.; Pan, X. S.; Sawhney, R.; Thompson, A. W.; McAuley, K. E.; Fisher, L. M.; Sanderson, M. R. *Nat. Struct. Mol. Biol.* **2009**, *16*, 667.
18. An, J. H.; Lee, D. C. W.; Law, A. H. Y.; Yang, C. L. H.; Poon, L. L. M.; Lau, A. S. Y.; Jones, S. J. M. *J. Med. Chem.* **2009**, *52*, 2667.
19. A mixture of **6a** (0.28 g, 1 mmol), **5a** (0.30 g, 1.3 mmol), triethylamine (0.70 mL, 5 mmol) and dry acetonitrile (10 mL) was stirred at 30 °C for 3 h and then filtered. The resulting solid was purified via silica gel column chromatography (chloroform/methanol, 10:1, V/V) to give **8** (0.27 g, 65%) as a white solid, mp: 115–116 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.17–1.28 (4H, m, 2×cyclopropylCH_2), 2.29–2.36 (1H, m), 2.57–2.60 (1H, m), 2.81–2.90 (3H, m), 2.99–3.03 (1H, m), 3.18–3.22 (1H, m), 3.75–3.78 (2H, m), 3.88 (3H, s, OCH_3), 3.95–3.97 (1H, m), 7.85 (1H, d, *J* 12.0, C_5-H), 8.72 (1H, s, C_2-H). ESI-MS: *m*/*z* 421 $(M+H)^+$. HR-MS-ESI: *m*/*z* Calcd for $C_{20}H_{23}F_2N_4O_4$ $(M+H)^+$: 421.16874; found 421.16906. A mixture of **7f** (0.42 g, 1 mmol), **5a** (0.30 g, 1.3 mmol), triethylamine (0.70 mL, 5 mmol) and dry acetonitrile (10 mL) was stirred at 60 °C for 6 h, and then concentrated under reduced pressure. The residue was dissolved in 5% sodium hydroxide solution (6.0 mL), heated to 45 °C and stirred for 2 h at the same temperature. The reaction mixture was cooled to room temperature and adjusted to pH 7–7.5 with 2 N HCl. The solid product was collected by suction, purified via silica gel column chromatography (chloroform/methanol, 10:1, V/V) to give **18** (0.27 g, 63%) as a pale yellow solid, mp: 150–152 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 0.99–1.04 (2H, m, cyclopropylCH_2), 1.19–1.22 (2H, m, cyclopropylCH_2), 2.33–2.39 (1H, m), 2.61–2.64 (1H, m), 2.81–2.92 (3H, m), 3.02–3.05 (1H, m), 3.19–3.24 (1H, m), 3.72 (3H, s, $COCH_3$), 3.75–3.78 (2H, m), 3.89 (3H, s, $NOCH_3$), 3.95–3.98 (1H, m), 7.84 (1H, d, *J* 12.8, C_5-H), 8.75 (1H, s, C_2-H), ESI-MS: *m*/*z* 433 $(M+H)^+$. HR-MS-ESI: *m*/*z* Calcd for $C_{21}H_{26}FN_4O_5$ $(M+H)^+$: 433.18872; found 433.18992.
20. Molecular docking was executed by Sybyl (version 7.3. Tripos. Inc.). The crystal structure data is the quinolone–DNA cleavage complex of *S. pneumoniae* type IV (PDB accessions 3FOF). The binding pocket of the protein (receptor) is represented by a set of grid maps, and each compound (ligand) of the target compounds was docked to the pocket. It is a flexible-ligand/rigid receptor docking. Receptor conformations determined by crystallography. The predicted three-dimensional conformation of compound **16** was shown in Figure 3.