Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 7274–7280
Bioorganic & Medicinal Chemistry

Synthesis and structure–activity relationship of 7-(substituted)-aminomethyl-4-quinolone-3-carboxylic acid derivatives

Zhenfa Zhang,[a,b,*] Aizhen Yu[b] and Weicheng Zhou[b]

[a]*College of Pharmacy, University of Kentucky, 725 Rose Street, Lexington, KY 40536, USA*
[b]*Center for Drug Development, Shanghai Institute of Pharmaceutical Industry, 1111 Zhongshanbeiyi Road, Shanghai 200437, PR China*

Received 16 July 2007; revised 14 August 2007; accepted 20 August 2007
Available online 24 August 2007

Abstract—Gram-positive organisms have re-emerged as the major hospital pathogens, which make the unmet medical needs for antibacterial therapy even worse. In searching for potent agents against Gram-positive pathogens, novel 7-(substituted)-aminomethyl-quinolone-3-carboxylic acids were designed, synthesized, and evaluated for their antibacterial activities in vitro. Many 7-monoarylaminomethyl derivatives exhibited high potency against Gram-positive organisms compared to reference agents: vancomycin and pazufloxacin. Additionally, a few 7-monoalkylaminomethyl derivatives exhibited good activities against both Gram-positive and Gram-negative organisms.

1. Introduction

Worldwide, Gram-positive organisms have re-emerged as the major hospital pathogens.[1,2] The increasing prevalence of multi-drug resistant Gram-positive isolates such as methicillin-resistant *Staphylococcus aureus* (MRSA),[3] vancomycin-resistant enterococci (VRE),[4] and penicillin-resistant *Streptococcus pneumococci* (PRSP) constitute serious threats to the treatment of nosocomial infection.[5] Recently the oxazolidinone linezolid, the streptogramins quinupristin–dalfopristin, and even more recently the lipopeptide daptomycin were introduced as alternative treatments of these life-threatening infection.[6,7] However, the limitations of these drugs have also been documented.[8] Clearly, the current antibiotic cupboard is rather bare for meeting the challenge of new outbreaks of such resistant bacteria. There is an urgent medical need for new antibacterial agent with improved profile against Gram-positive pathogens.

Fluoroquinolone antibacterials are among the most attractive agents in the treatment of bacterial infections since the discovery of Norfloxacin.[9] However, despite many advances achieved in fluoroquinolones, research during the last several decades; the current fluoroquinolones generally have low intrinsic activity against a number of clinically important Gram-positive pathogens.[10,11] Nevertheless, extensive clinical use of fluoroquinolones, especially ciprofloxacin, has resulted in increasing quinolone resistance among many pathogens.[12,13] Even many of the above-mentioned multidrug-resistant pathogens are resistant to fluoroquinolones as well. Thus, an effort on fluoroquinolone research has intensified to enhance their activity against Gram-positive organisms.[14–17]

From the chemical structural point of view, one of the most important features of the fluoroquinolone antibacterials is the presence of a five- or six-member nitrogen-containing heterocycles, particularly 1-piperazinyl or the 3-amino-1-pyrrolidinyl substituents, at the C-7 of the 4-quinolone-3-carboxylic acid nucleus (Fig. 1).[9] While almost all of the heterocycles evaluated at C-7 are linked to the 4-quinolone scaffold through the heterocyclic nitrogen, the exact role of this nitrogen atom has not been unequivocally defined. Relatively few reports are

Keywords: Fluoroquinolones; Antibacterial agent; Gram-positive bacteria.
* Corresponding author. Tel.: +1 859 257 2584; fax: +1 859 257 2787; e-mail: zzhan5@email.uky.edu

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.08.031

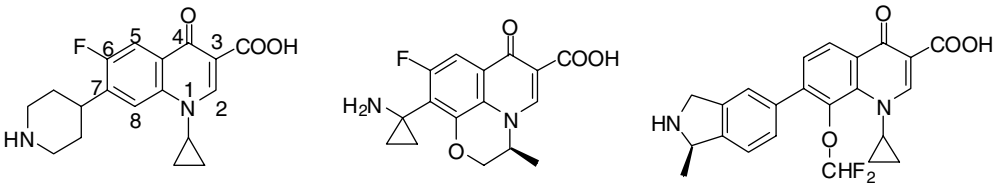


Figure 1. Structure of some quinolones with C–C linked side chain at the C-7-position.

present in the literature featuring the evaluation of the agents possessing a nitrogen-containing side chain attached to the C-7 of the 4-quinolone-3-carboxylic acid nucleus through a carbon–carbon bond rather than a nitrogen–carbon bond. Laborde et al. reported that the carbon isostere (**1**, Fig. 1) of ciprofloxacin exhibited good antibacterial activity.[18] Pazufloxacin (**2**)[19] is another example of such an agent, and recently garenoxacin (**3**),[20] a des-fluoro(6) quinolone (6-NFQ) bearing a carbon-linked aromatic ring at the C-7 of the 4-quinolone-3-carboxylic acid nucleus, has been reported to exhibit excellent potency, especially against Gram-positive organisms. The hypothesis of this study was that new fluoroquinolone agents with a carbon-linked nitrogen-containing side chain at the C-7 of the 4-quinolone-3-carboxylic acid nucleus may offer new insight into the structural–activity relation of the quinolone antibacterials, especially against Gram-positive organisms. The predominance of the C-7 nitrogen-linked substituent in the current fluoroquinolones may be due to their straightforward preparation rather than pharmacological necessity. Thus, series of 7-(substituted)-aminomethyl fluoroquinolones have been synthesized and evaluated for their antibacterial activities.

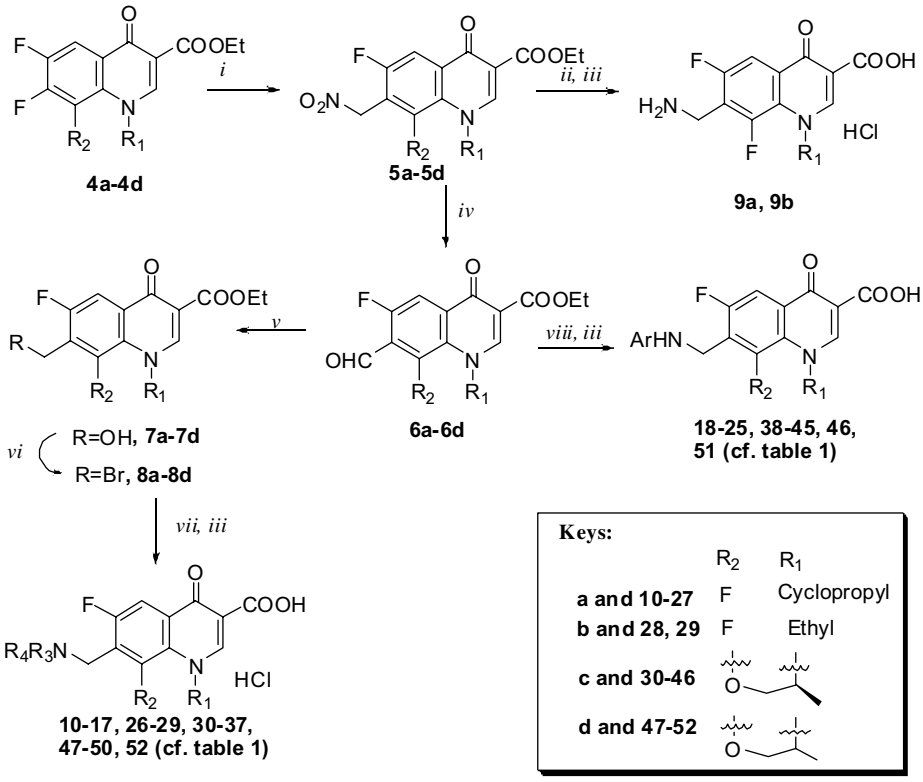
2. Chemistry

The introduction of a nitrogen-containing side chain to the C-7 of the 4-quinolone-3-carboxylic acid nucleus through a carbon–carbon bond rather than a carbon–nitrogen bond is not as straightforward as a simple S_NAR reaction of amines with 7-fluoro-4-quinolone-3-carboxylic acids, a method routinely employed in the synthesis of most of the fluoroquinolone derivatives bearing a nitrogen-linked substituent at C-7.[9] Considering the wide availability of intermediates **4a**–**4d** (Scheme 1) and versatile transformations that a nitro group can offer, the S_NAR reactions of nitromethane with **4a**–**4d** were designed to form the C-7 carbon–carbon bond of interest. Thus the resulting 7-nitromethyl-quinolones **5a**–**5d** could serve as the key intermediates for the synthesis of four series of target compounds featuring the core structure of 8-fluorociprofloxacin (**9a**, **10**–**27**, Table 1), lomefloxacin (**9b**, **28**, **29**), levofloxacin (**30**–**46**), and ofloxacin (**47**–**52**), respectively. The strategy for the synthesis of 7-(substituted)-aminomethyl quinolone-3-carboxylic acids starting from **4** is illustrated in Scheme 1.

Interestingly, the S_NAR reaction of nitromethane with the fluoroquinolone intermediates turned out to be elusive, presumably because of the ambient and weak nucleophilicity of the nitronate anion. After an in-depth investigation, the scope and limitation of this methodology was established,[21] and the preparation of the desired nitromethyl-quinolones **5a**–**5d** was achieved. Starting from the key intermediate, different strategies were utilized to build a nitrogen-containing side chain from the nitromethyl moiety. Firstly, simple catalytic hydrogenation of **5a** and **5b** followed by hydrolysis afforded **9a** and **9b** with a bare aminomethyl side chain. For this transformation, Rany-Ni was the catalyst of choice while hydrogenation with Pd–C resulted in over-reduction and left only a bare methyl behind at the C-7. Furthermore, Nef reactions of **5a**–**5d** were envisioned as decisive transformations to furnish the common intermediate **6a**–**6d** for the synthesis of the derivatives bearing a substituted aminomethyl side chain. However, initial experiments following either a classic procedure[22] or an alternative methodology employing a reductive reagent[23] failed to give the desired aldehydes, presumably because of the poor solubility of **5** and/or their nitronates in the aqueous reaction medium. From these trials, the starting **5** either remained unchanged or hydrolyzed. Eventually **6a**–**6d** was prepared employing an oxidative Nef reaction process.[24] Thus treatment of the alkali alcoholic solution of **5a**–**5d** with dilute aqueous solution of $KMnO_4$ while buffered with boric acid afforded **6a**–**6d**. These precursors expedited the introduction of various amines of interest onto the C-7 of the 4-quinolone scaffold. Firstly, reductive amination of **6a**–**6d** with the appropriate primary aromatic amine afforded the 7-mono-arylaminomethyl 4-oxo-1,4-dihydro-quinoline-3-carboxylic acid derivatives after hydrolysis. In the cases of **46**, where *N*-methyl-4-fluorophenylaminyl methyl was the side chain, consecutive reductive aminations were utilized to meet this end. Other than these primary aromatic amines, the introduction of various other substituted-amines onto the methylene spacer was then accomplished through a different method employing bromides **8a**–**8d** as the key intermediates. Thus the reduction of **6a**–**6d** with sodium boronhydride followed by the bromination of the resulting alcohol **7a**–**7d** provided the bromides **8a**–**8d**. Subsequently **8a**–**8d** were treated with different secondary amines or an excess of volatile primary alkylamines followed by hydrolysis to furnish the derivatives bearing a di-substituted-amine side chain or mono-alkylamine side chain on the C-7-methylene spacer accordingly.

3. Results and discussion

The in vitro antibacterial activity of four series of 7-(substituted)-aminomethyl 4-oxo-1,4-dihydro-quinoline-3-carboxylic acids against drug-sensitive bacteria,



Scheme 1. Synthesis of the 7-(substituted)-aminomethyl quinolones. Reagents and conditions: (i) CH_3NO_2, NaH, DMSO; (ii) Raney-Ni, EtOH; (iii) HOAc/HCl; (iv) $KMnO_4$, NaB_4O_7, MeOH/H_2O; (v) $NaBH_4$, MeOH; (vi) PBr_3, DCM; (vii) R_1R_2NH, CH_3CN; (viii) (substituted)phenylamine, $NaCNBH_3$, (for **46**, and then HCHO).

three Gram-positive strains (*Staphylococcus aureus* 26003, *Streptococcus epidermidis* 26069, and *Streptococcus pneumoniae* 31002) and three Gram-negative strains (*Shigella boydii* 51313, *Klebsiella pneumoniae* 46101 and *Salmonella citrobacter* 48107), are summarized in Table 1 along with lomefloxacin, pazufloxzcin, and vancomycin for comparison. Overall, considerable relationships between their structures and antibacterial activities in vitro are observed. The derivatives with 1-cyclopropyl substituent exhibited higher antibacterial activity in vitro than the corresponding 1-ethyl derivatives as seen from **9a**, and **10**, versus **9b**, and **29**. The comparison between these two series and the series from levofloxacin or oflaxcin scaffold also confirm that rigidification of the 1-substituent resulted in significant enhancement in their activity against Gram-positive organisms.[9] In addition, compounds derived from the racemic ofloxacin nucleus are less potent than those from levofloxacin nucleus (**34**, **35**, and **39** vs **49**, **50**, and **51**). These results are in agreement with previous general observations.[9]

As the starting point of this study, aminomethyl analogs **9a** and **9b** demonstrated good antibacterial activities compared to references lomefloxacin and pazufloxacin against Gram-negative organisms tested. Mono-alkylation of this primary amine with small size alkyl groups such as methyl or ethyl retained this potency (**10**–**14** vs **9a**, **28** and **29** vs **9b**). Among these compounds, compound **13**, bearing a cyclopropyl on the C-7 side chain, exhibited the highest potency against Gram-negative organisms and considerable potency against Gram-positive as well. However, di-alkylation of this primary amine was detrimental to their antibacterial activities (**16** and **15**, **34**–**37**, **49** and **50**). These results indicate the presence of only one substitution on the nitrogen of the 7-aminomethyl side chain is important in maintaining the antibacterial activity of these 7-aminomethylquinolone derivatives. Independent QSAR study based on the data from our previous publication on the preliminary study has recently been published and indicated good relationships between the electronic characters of these compounds and their activity against Gram-positive organisms.[25]

Interestingly, when an aromatic ring was introduced to the N of the 7-aminomethyl side chain, significant enhancements of potency against Gram-positive organisms are achieved. The 7-arylaminomethyl compounds **18**–**25** demonstrated high antibacterial activity against Gram-positive organisms tested. Even better potencies were achieved from the levofloxacin nucleus through the same strategy. Thus, **38**–**45** exhibited high potency against Gram-positive organisms, which is comparable to that of vancomycin, the last-resort treatment for infections caused by Gram-positive resistant pathogens. It is also worthwhile to point out that **39** and **42** exhibited the highest activity against all the Gram-positive strains tested, more potent than all three reference agents. Compared with 6-NFQ, where the 6-F is absent from the 4-quinolone nucleus and a 7-aromatic ring replaces the commonly-used saturated nitrogen-containing heterocycles, and which are demonstrated to possess

Table 1. Antibacterial activities of compounds **9a**, **10**–**27**, **9b**, **28**, **29**, **30**–**46** and **47**–**52** (for core structures refer to Scheme 1)

Compounds		MIC (μg/mL)					
		Gram-positive organisms			Gram-negative organisms		
No.	R_3R_4N/ArNH (cf. Scheme 1)	*S. aureus* 26003	*S. epidermidis* 26069	*S. pneumoniae* 31002	*S. boydii* 51313	*K. pneumoniae* 46101	*S. citrobacter* 48107
	Lemofloxacin	0.78	0.78	0.195	0.098	0.098	0.098
	Pazufloxacin	0.195	0.39	0.049	0.049	0.049	0.049
	Vancomycin	0.39	0.78	0.098	>6.25	>6.25	>6.25
9a	NH_2	12.5	12.5	0.78	0.098	0.098	0.098
10	EtNH	>6.25	>6.25	0.78	⩽0.049	⩽0.049	⩽0.049
11	*n*-PrNH	>6.25	>6.25	3.13	0.195	0.39	0.195
12	*i*-PrNH	>6.25	>6.25	1.56	0.195	0.098	0.098
13	Cyclopropylamino	3.13	6.25	0.195	0.098	⩽0.049	⩽0.049
14	*n*-BuNH	>6.25	>6.25	3.13	0.39	⩽0.049	0.098
15	Cyclohexanylamino	>6.25	6.25	1.56	0.78	0.195	0.195
16	Piperazinyl	6.25	6.25	6.25	0.78	0.195	0.195
17	4-Methylpiperazinyl	12.5	12.5	1.56	1.56	1.56	1.56
18	Phenylaminyl	0.78	1.56	0.049	0.39	0.78	0.39
19	4-Chlorophenylamino	0.39	1.56	⩽0.049	1.56	1.56	1.56
20	3-Chlorophenylaminol	0.195	1.56	⩽0.049	0.78	1.56	1.56
21	4-Fluorophenylamino	0.78	1.56	0.049	0.195	0.78	0.39
22	3-Chloro-4-fluorophenylamino	0.39	1.56	0.39	1.56	1.56	0.78
23	4-Methylphenylamino	0.39	1.56	0.049	0.39	1.56	0.78
24	3,4-Dimethylphenylamino	0.39	0.78	0.098	1.56	1.56	1.56
25	(4,6-Dimethyl-pyridin-2-yl)-amino	0.39	1.56	0.098	0.39	0.78	0.78
26	1H-imidazol-1-yl	6.25	>6.25	0.78	0.39	0.195	0.195
27	N-ethylanilinyl	3.13	6.25	3.13	3.13	6.25	3.13
9b	NH_2	>25	>25	6.25	0.78	0.78	0.39
28	MeNH	>6.25	>6.25	3.13	0.39	0.195	0.39
29	EtNH	>6.25	>6.25	3.13	0.39	0.195	0.39
30	EtNH	>6.25	>6.05	1.56	0.195	0.049	0.195
31	*n*-PrNH	>6.25	>6.25	3.13	0.39	0.195	0.39
32	*i*-PrNH	>6.25	>6.25	3.13	0.39	0.195	0.39
33	Cyclopropylamino	3.13	>6.25	0.39	0.049	0.049	0.049
34	Pyrrolidinyl	6.25	>6.25	0.78	0.39	0.049	0.098
35	Piperidinyl	>6.25	>6.25	1.56	0.78	0.195	0.39
36	Piperazinyl	>6.25	>6.25	>6.25	>6.25	3.13	>6.25
37	4-Methylpiperazinyl	>6.25	>6.25	1.56	1.56	0.78	1.56
38	Phenylamino	1.56	1.56	0.098	0.39	0.78	0.78
39	4-chlorophenylamino	0.098	0.098	⩽0.049	0.195	0.195	0.098
40	3-Chlorophenylamino	1.56	3.13	0.195	1.56	6.25	6.25
41	4-Fluorophenylamino	0.78	0.78	0.049	0.78	0.78	0.78
42	3-Chloro-4-fluorophenylamino	0.098	0.195	⩽0.049	0.195	0.195	0.39
43	4-Methylphenylamino	0.78	3.13	0.098	0.78	3.13	1.56
44	3,4-Dimethylphenylamino	3.13	3.13	0.195	1.56	6.25	3.13
45	(4,6-Dimethyl-pyridin-2-yl)-amino	0.78	0.78	0.195	0.195	0.39	0.78
46	*N*-Methyl-4-fluorophenylamino	3.13	3.13	0.39	1.56	1.56	1.56
47	MeNH	3.13	>6.25	3.13	0.098	0.098	⩽0.049
48	Cyclopropylamino	3.13	3.13	0.39	⩽0.049	⩽0.049	⩽0.049
49	Pyrrolidinyl	>6.25	>6.25	3.13	0.39	0.195	0.39
50	Piperidinyl	>6.25	>6.25	3.13	0.78	0.39	0.78
51	4-Chlorophenylamino	0.195	0.78	⩽0.049	1.56	>6.25	1.56
52	1H-imidazol-1-yl	3.13	3.13	0.78	0.78	0.195	0.39

enhanced activity against Gram-positive organisms, the same mechanism that accounts for the preferential enhancement of the antibacterial spectrum of 6-NFQ may also contribute to the selectivity of 7-arylamino-methyl quinolones in this study. Additionally, **39** also demonstrated the balanced activity against both Gram-positive and Gram-negative organisms.

Again, as in the 7-alkylaminomethyl derivatives, when the N of the 7-arylaminomethyl side chain is alkylated further, the resulting derivatives **27** and **46** showed dramatic decrease in their activity against all the bacterial tested.

4. Conclusion

As detailed above, four series of 7-(substituted)-amino-methyl 4-oxo-1,4-dihydro-quinoline-3-carboxylic acids have been designed, synthesized, and evaluated for their antibacterial activity in vitro in order to discover potent agents against Gram-positive bacteria. Compounds

bearing 7-(monoalkyl)aminomethyl side chains exhibited high activity against Gram-negative organism comparable to that of lomefloxacin and pazufloxacin, while 7-arylaminomethyl counterparts showed significant enhancements for their potency against Gram-positive organisms. Compared to vancomycin, most of the 7-arylaminomethyl quinolones exhibited high antibacterial activity against Gram-positive strains tested. Among them, **39** exhibited excellent and balanced activity against both Gram-positive and Gram-negative organisms. In the light of the increasing needs for treatment against infections caused by Gram-positive pathogens, the 7-substituted-aminomethyl 4-oxo-1,4-dihydro-quinoline-3-carboxylic acids would be a potential scaffold for the exploration of novel quinolone antibacterials.

5. Experimental

Melting points were obtained manually by capillary methods and are uncorrected. NMR spectra were recorded on Bruke AM-400 (400 MHz) spectrometer. Chemical shifts are reported in ppm related to tetramethylsilane as the internal standard. Significant ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet); coupling constance(s) in hertz and number of proton(s). The mass spectra were recorded on Finnign-MAT212 under electron impact (EI) ionization condition or otherwise specified. Elemental analyses were performed on Carlo Erba1106 automatic elemental analyzer and the analytical results were within 0.4% of the theoretical values. Optical rotations were recorded on Perkin Elmer P-341 polarimeter. Column chromatography refers to flash column chromatography on silicon gel (200–300 mesh). Thin layer chromatography (TLC) was performed on HSF-254 TLC plate and compounds visualized under UV lamp.

5.1. In vitro antibacterial activity

The minimum inhibitory concentrations (MICs μg/mL) of the compounds tested in this study were determined by the agar dilution method with the concentration range of 0.049–6.25 μg/mL. The MIC was defined as the tested lowest concentration of an antibacterial agent that inhibited visible growth after incubation at 37 °C for 24 h.

5.2. General procedure for the preparation of 5a–5d

Nitromethane (0.9 g, 15 mmol) in dry DMSO (5 mL) was dropped into the suspension of NaH (60% suspension in mineral oil, 0.6 g, 15 mmol) in dry DMSO (15 mL) while stirring. After the bubbling subsided, **4** (5 mmol) was added and the resulting mixture was stirred at 65 °C for 4 h. The reaction mixture was then cooled to rt and poured into ice-water, acidified with 6 N HCl, and then extracted with ethyl acetate (3 × 150 mL). The combined organic extraction was washed with water and brine consecutively, and dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuum gave a pale yellow residue, which was recrystallized from alcohol to afford **5**.

5.2.1. 1-Cyclopropyl-6,8-difluoro-7-nitromethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (5a). Yield 92%; mp: 188–189 °C;^{1}H NMR ($CDCl_3$ δ ppm): 8.52 (s, 1H), 7.85 (dd, J_1 = 9.4 Hz, J_2 = 1.2 Hz, 1H), 6.11 (s, 2H), 4.24 (q, J = 6.8 Hz, 2H), 4.04–4.14 (m, 1H), 1.26 (t, J = 6.8 Hz, 3H), 1.12–1.15 (m, 4H).

5.2.2. 1-Ethyl-6,8-difluoro-7-nitromethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (5b). Yield 82%; mp: 172–174 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 8.41 (s, 1H), 8.01 (d, J = 9.2 Hz, 1H), 6.17 (s, 2H), 4.43–4.83 (m, 4H), 1.53 (t, J = 7.6 Hz, 3H), 1.41 (t, J = 7.2 Hz, 3H).

5.2.3. (*R*)9-Fluro-3-methyl-10-nitromethyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-caboxylic acid ethyl ester (5c). Yield 89%; mp: 204–206 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 8.71 (s, 1H), 7.53 (d, J = 9.6 Hz, 1H), 5.88–5.98 (m, 2H), 4.78–4.81 (m, 1H), 4.60 (d, J = 11.6 Hz, 1H), 4.42 (d, J = 11.6 Hz, 1H), 4.19–4.26 (m, 2H), 1.39 (d, J = 6.8 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H).

5.2.4. (*R*,*S*)9-Fluro-3-methyl-10-nitromethyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-caboxylic acid ethyl ester (5d). Yield 91%; mp: 214–216 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 8.36 (s, 1H), 7.68 (d, J = 9.6 Hz, 1H), 5.87–5.90 (m, 2H), 4.47–4.49 (m, 3H), 4.35 (q, J = 7.2 Hz, 2H), 1.57 (d, J = 6.8 Hz, 3H), 1.38 (t, J = 7.2 Hz, 3H).

5.2.5. 7-Aminomethyl-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (9a). Compound **5a** (11.8 g, 33.5 mmol) was dissolved in ethanol (500 mL) and hydrogenated under 50 atm overnight in the presence of newly prepared Raney-Ni (10 g). The reaction mixture was filtered and acidified with alcoholic HCl. After the solvent was removed in vacuum, the resulting residue was partitioned between water and ethyl acetate. The aqueous layer was separated and basified with aqueous Na_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 extraction was washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent in vacuum, the residue was recrystallized from alcohol to afford **9a** (5.1 g, 48% yield); mp: 186–188 °C; ^{1}H NMR (DMSO-d_6, δ ppm), 8.52 (s, 1 H), 7.82 (d, J = 9.2 Hz, 1H), 6.88 (br, 2H), 4.20–4.31 (m, 4H), 3.98–4.01 (m, 1H), 1.28 (t, J = 7.2 Hz, 3H), 1.17 (br, 4H); EIMS (m/z) 322 (M^+).

5.3. General procedure of the preparation of 6a–6d

The suspension of compound **5** (20 mmol) in methanol (140 mL) was cooled to −10 to −5 °C and then the freshly prepared solution of KOH (60 mmol) in methanol (200 mL) was added drop wise. After stirring for an additional 30 min, the solution of $KMnO_4$ (2.2 g, 13.4 mmol) and $MgSO_4$ (60 mmol) in water (600 mL) was added drop wise with vigorous stirring. After the addition was complete, the reaction mixture was stirred further at the same

temperature. When the reaction was complete, the mixture was filtered over a thin layer of Celite. The filtrate was saturated with NaCl and extracted with CH_2Cl_2. The combined extract was washed with saturated Na_2CO_3 and brine successively and then dried over $MgSO_4$. After removal of the solvent in vacuum, the residue was recrystallized from acetone to afford **6**.

5.3.1. 1-Cyclopropyl-6,8-difluoro-7-formyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (6a). Yield 68%; mp: 184–186 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 10.41 (s, 1H), 8.57 (s, 1H), 8.01 (dd, J_1 = 11 Hz, J_2 = 2.2 Hz, 1H); 4.33 (q, J = 7.2 Hz, 2H); 3.87–3.89 (m, 1H), 1.32 (t, J = 7.2 Hz, 3H); 1.25–1.27 (m, 2H), 1.07–1.09 (m, 2H); EIMS: 321 (M^+).

5.3.2. 1-Ethyl-6,8-difluoro-7-formyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (6b). Yield 67%; mp: 168–170 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 10.41 (s, 1H); 8.42 (s, 1H), 8.10 (dd, J_1 = 10.4 Hz, J_2 = 1.6 Hz, 1H), 4.36–4.44 (m, 4H), 1.56 (t, J = 6.8 Hz, 3H), 1.39 (t, J = 7.2 Hz, 3H); EIMS: 309 (M^+).

5.3.3. (*R*)9-Fluro-3-methyl-10-formyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-caboxylic acid ethyl ester (6c). Yield 68%; mp: 192–194 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 10.49 (s, 1H), 8.40 (s, 1H), 7.75 (d, J = 10.8 Hz, 1H), 4.37–4.54 (m, 5H), 1.63 (d, J = 6.4 Hz, 3H), 1.41(t, J = 7.2 Hz, 3H); EIMS: 319 (M^+); $[\alpha]_D$ −68.79° (*c* 1, DMSO).

5.3.4. (*R*,*S*)9-Fluro-3-methyl-10-formyl-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-caboxylic acid ethyl ester (6d). Yield 66%; mp: 207–209 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 10.49 (s, 1H), 8.39 (s, 1H), 7.75 (d, J = 11.0 Hz, 1H), 4.36–4.53 (m, 5H), 1.57 (d, J = 6.8 Hz, 3H), 1.41(t, J = 7.2 Hz, 3H). ^{1}H NMR (DMSO, δ ppm): 10.51 (s, 1H), 8.71 (s, 1H), 7.54 (d, J = 9.6 Hz, 1H), 4.79–4.81 (m, 1H), 4.40–4.61 (ddd, 2H), 4.19–4.26 (m, 2H), 1.39 (d, J = 6.8 Hz, 3H), 1.29 (t, J = 7.2 Hz, 3H); EIMS: 319 (M^+).

5.4. General procedure for the synthesis of 7a–7d

Compound **6a** (6.0 g, 18.7 mmol) was dissolved in methanol (500 mL) and cooled over ice-water. $NaBH_4$ (0.35 g, 9.3 mmol) was added potion-wise over 20 min. The resulting mixture was stirred at the same temperature for additional 1 h. Acetone was added to quench the reaction and the reaction mixture was stirred for another 10 min. The methanol was removed in vacuum and the resulting residue was partitioned between water and ethyl acetate. The extract was washed with water and brine and dried over $MgSO_4$. After the solvent was removed, the residue was recrystallized from alcohol to afford **7a**.

5.4.1. 1-Cyclopropyl-6,8-difluoro-7-hydroxymethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (7a). Mp: 168–170 °C (4.7 g, 78% yield); ^{1}H NMR ($CDCl_3$, δ ppm): 8.57(s, 1H), 7.91 (dd, J = 9.2 Hz, 1H), 4.89 (s, 2H), 4.39 (q, J = 7.2 Hz, 2H); 3.92–3.93 (m, 1H), 1.40 (t, J = 7.2 Hz, 3H); 1.25–1.27 (m, 2H), 1.14–1.19 (m, 2H).

5.4.2. 1-Ethyl-6,8-difluoro-7-hydroxymethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (7b). Yield 72%; mp: 157–159 °C; ^{1}H NMR ($CDCl_3$, δ ppm): 8.41 (s, 1H), 8.01 (d, J = 11.2 Hz, 1H), 4.90 (s, 2H), 4.38–4.43 (m, 4H), 1.54 (t, J = 6.8 Hz, 3H), 1.41 (t, J = 7.2 Hz, 3H).

5.5. General procedure for the preparation of compounds 8a–8d

Compound **7a** (4.7 g, 14.6 mmol) was dissolved methylene chloride (150 mL) and cooled over ice bath. Phosphorus bromide (1.37 mL, 14.6 mmol) was added drop-wise. The resulting mixture was stirred at the same temperature for 4 h and then diluted with methylene chloride, washed with dilute sodium bicarbonate, water, and brine successively, and dried over $MgSO_4$. Removal of the methylene chloride afforded **8a** as a pale yellow solid (5.0 g, 89% yield), which was used in the next step without further purification.

5.6. Synthesis of 10 as the example of the general procedure for the preparation of 10–17, 26–37, 47–50 and 52

In the cases of the synthesis of **10**–**16**, **28**–**36**, and **47**–**50**, over 5 equiv excess of starting amines were employed. In the cases of **17**, **26**, **27**, **37**, and **52**, slight excess (1.2 equiv) of amine was employed.

1-Cyclopropyl-6,8-difluoro-7-ethylaminomethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid hydrochloride (**10**). The solution of **8a** (1 mmol) in acetonitrile was added dropwise to the mixture of ethylamine (over 5 equiv excess) and potassium carbonate (3 equvalent) in acetonitrile. The mixture was stirred at rt for 4 h. Excess ethylamine and the solvent were removed in vacuum. The resulting residue was titrated with water and ethyl acetate and the aqueous layer was extracted two more times with ethyl acetate. The combined extraction was washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was refluxed in the mixture of hydrochloric acid and acetic acid (1:3 v/v, 10 mL) for 3 h. After the solvent was removed, the residue was recrystallized from 95% ethanol to give **10** (46% yield). Mp: 278–280 °C; ^{1}H NMR (DMSO-d_6 + D_2O), δ ppm. 8.77 (s, 1H), 7.98 (d, J = 8.8 Hz, 1H), 4.37 (s 2H), 4.13–4.14 (m, 1H), 3.09 (q, J = 6.8 Hz, 2H), 1.21–1.26 (m, 7H); Anal. for $C_{16}H_{17}F_2N_2O_3$·HCl. C, H, N.

5.7. Synthesis of 22 as the example of the general procedure for the synthesis of 18–25, 38–45 and 51

1-Cyclopropyl-6,8-difluoro-7-(3-chloro-4-fluoro-phenylaminomethyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid hydrochloride (**22**). Compound **6a** (0.35 g, 1.1 mmol) was dissolved in ethanol (20 mL). 3-Chloro-4-fluoro-phenylamine (160 mg, 1.1 mmol) was added and the mixture was refluxed for 1 h and cooled to rt. Small amount of methyl purple was added followed by $NaBH_3CN$ (80 mg, 1.29 mmol). The reaction mixture turned purple and faded away when alcoholic hydrochloric acid was added. During the reaction the addition

of HCl was repeated periodically to keep the reaction mixture from turning back to purple. After 4 h, the solvent was removed and **22** was obtained following the same work-up procedure as stated previously for **10**. (280 mg, 60% yield); mp: 207–209 °C; ^{1}H NMR (DMSO-d_6), δ ppm. 14.4 (br), 8.73 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.12 (t, J = 9.0 Hz, 1H), 6.79–6.82 (m, 1H), 6.63–6.67 (m, 1H), 6.38 (br), 4.47 (s, 2H), 4.15–4.19 (m, 1H), 1.21 (d, J = 6.0 Hz, 4H). Anal. for $C_{20}H_{14}ClF_3N_2O_3$, C, H, N.

Acknowledgments

This work was supported by the SIPI Drug Discovery Foundation. We thank the Research and Development Center of Xinchang Pharmaceutical Ltd for the generosity in providing some intermediates for this study. Thanks to John Culver and Dr. Aarron Haubner for their help in the preparation of this manuscript.

Supplementary data

The mp, NMR, and elemental analysis data of compounds **9a**, **10**–**27**, **9b**, **28**, **29**, **30**–**46**, and **47**–**52**; the optical rotation data of compounds **30**–**46** are included in the Supplementary Data file. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.031.

References and notes

1. Stefani, S. *Curr. Med. Chem. Anti-Infect. Agents* **2005**, *4*, 235.
2. Rice, L. B. *Biochem. Pharmacol.* **2006**, *71*, 991.
3. Barrett, J. F. *Expert Opin. Ther. Targets* **2005**, *9*, 253.
4. Boneca, I. G.; Chiosis, G. *Expert Opin. Ther. Targets* **2003**, *7*, 311.
5. Wise, R. *J. Antimicrob. Chemother.* **2003**, *51*, ii5.
6. Appelbaum, P. C.; Jacobs, M. R. *Curr. Opin. Microbiol.* **2005**, *8*, 510.
7. Abbanat, D.; Macielag, M.; Bush, K. *Expert Opin. Invest. Drugs* **2003**, *12*, 379.
8. Gillman, P. K. *J. Antimicrob. Chemother.* **2004**, *54*, 844.
9. Mitscher, L. A. *Chem. Rev.* **2005**, *105*, 559.
10. Andersson, M. I.; MacGowan, A. P. *J. Antimicrob. Chemother.* **2003**, *51*, 1.
11. Emmerson, A. M.; Jones, A. M. *J. Antimicrob. Chemother.* **2003**, *51*, 13.
12. Thomson, C. J. *J. Antimicrob. Chemother.* **1999**, *43*(Suppl. A), 31.
13. Vila, J. *Front. Antimicrob. Resist.* **2005**, 41.
14. Schmitz, F. J.; Higgins, P. G.; Mayer, S.; Fluit, A. C.; Dalhoff, A. *Eur. J. Clin. Microbiol. Infect. Diseases* **2002**, *21*, 647.
15. Hooper, David C. *Lancet Infect. Diseases* **2002**, *2*, 530.
16. van Bambeke, F.; Michot, J. M.; Van Eldere, J.; Tulkens, P. M. *Clin. Microbiol. Infect.* **2005**, *11*, 256.
17. Zhang, Z.; Zhou, W.; Yu, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 393.
18. Laborde, E.; Kiely, J. S.; Culbertson, T. P.; Lesheski, L. E. *J. Med. Chem.* **1993**, *36*, 1964.
19. Todo, Y.; Takagi, H.; Iino, F.; Fukuoka, Y.; Takahata, M.; Okamoto, S.; Saikawa, I.; Narita, H. *Chem. Pharm. Bull.* **1994**, *42*, 2569.
20. Fung-Tomc, J. C.; Minassian, B.; Kolek, B.; Huczko, E.; Aleksunes, L.; Stickle, T.; Washo, T.; Gradelski, E.; Valera, L.; Bonner, D. P. *Antimicrob. Agents. Chemother.* **2000**, *44*, 3351.
21. Zhang, Z.; Zhou, W. *Tetrahedron Lett.* **2005**, *46*, 3855.
22. van Tamelen, E. E.; Theide, R. J. *J. Am. Chem. Soc.* **1952**, *74*, 2615.
23. McMurry, J. E.; Melton, J. *J. Org. Chem.* **1973**, *38*, 4367.
24. Steliou, K.; Poupart, M. A. *J. Org. Chem.* **1985**, *50*, 4971.
25. Xu, J.; Gao, S.; Zhang, X.; Xie, Y.; Huang, X.; Xie, X. *Chinese J. Struct. Chem. (Jiegou Huaxue)* **2007**, *26*, 91.