Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

7-(4-Alkylidenylpiperidinyl)-quinolone bacterial topoisomerase inhibitors ☆

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ARTICLE INFO

Article history:
Received 13 August 2014
Revised 26 September 2014
Accepted 1 October 2014
Available online xxxx

Keywords:
Antibacterial agent
Fluoroquinolone-resistant
Alkylidenylpiperidinyl fragment

ABSTRACT

Novel antibacterial fluoroquinolone agents bearing a 4-alkylidenylpiperidine 7-position substituent are active against quinolone-susceptible and quinolone-resistant gram-positive bacteria, including *Streptococcus pneumoniae* and MRSA. Analogs **22b**, **23c**, and **24** demonstrated superior in vitro and in vivo efficacy to ciprofloxacin against these cocci.

Since the discovery of nalidixic acid[1] over four decades ago, the quinolones have developed into a clinically useful class of antibacterial agents.[2] Several of the quinolones, including ciprofloxacin[3] and levofloxacin[4] are widely used in the clinic to treat a range of bacterial infections. The quinolones act against bacteria by selectively inhibiting the type II topoisomerases DNA gyrase and topoisomerase IV, enzymes that play a critical role in bacterial cell growth and division.[2c] However, extensive clinical use of these agents has led to increasing bacterial resistance to the available quinolones.[2d] The incidence of quinolone resistance in the important respiratory tract pathogen, *Streptococcus pneumoniae*, has generally remained low both in the United States[5] and globally.[6] However, recent reports of elevated resistance among pneumococcal isolates in regions with high quinolone usage signal the potential for further dissemination of resistant strains.[7,8] In contrast, the high rate of quinolone resistance among methicillin-resistant *Staphylococcus aureus* (MRSA) isolates has limited the empiric use of marketed quinolones in the treatment of skin and skin structure infections.[9,10] Thus a continuing challenge is to discover and develop newer quinolone antibacterial agents with activity against resistant organisms, including gram-positive bacteria associated with respiratory tract and skin infections.

Previous studies have demonstrated the importance of the quinolone C7 substituent for potent in vitro antibacterial activity, including against resistant gram-positive organisms.[11] In particular, the (3*S*)-amino-(4*R*)-ethylpiperidinyl fluoroquinolone **1** was shown to possess superior activity against MRSA and penicillin-resistant *S. pneumoniae* compared to marketed quinolones (Fig. 1).[11]

Modeling investigations of the C7 substituent of **1** revealed a low energy conformation that overlapped with the aminoethyl-pyrrolidine side chain of premafloxacin (**2**), another quinolone antibacterial agent with potent gram-positive activity.[12] We hypothesized that the 2-aminoethylidenylpiperidine substituent **5**, derived from fragment **4** by shifting the primary amine to the terminus of the ethyl group and restricting the side chain with an exocyclic double bond, would position the amino and alkyl groups in a similar region of three-dimensional space as the aminoethylpyrrolidine side chain (**6**) of premafloxacin (Fig. 1). To test this concept, we launched an investigation of the synthesis and antibacterial activity of quinolones and naphthyridones bearing this unprecedented 7-position substituent (**3**).

The general synthetic route to the protected 4-(2-aminoethylidenyl)piperidines is depicted in Scheme 1.[13] Ketone **7** was subjected to a Horner–Wadsworth–Emmons reaction with triethyl 2-substituted-2-phosphonoacetate **8** to afford substituted alkylidene ester **9**. Reduction to alcohol **10**, Mitsunobu reaction with phthalimide to give **11**, and acid deprotection provided secondary amine **12**. Allylic alcohol **13** was converted to the corresponding amine derivatives **15a**, **15b** and **15c** through a two-step sequence (Scheme 2).

Heterocyclic core structures related to marketed and clinical quinolones and naphthyridones were employed in the synthesis

☆ All authors were employed by Janssen Research & Development, L.L.C. at the time the work reported herein was conducted.

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http://dx.doi.org/10.1016/j.bmcl.2014.10.014
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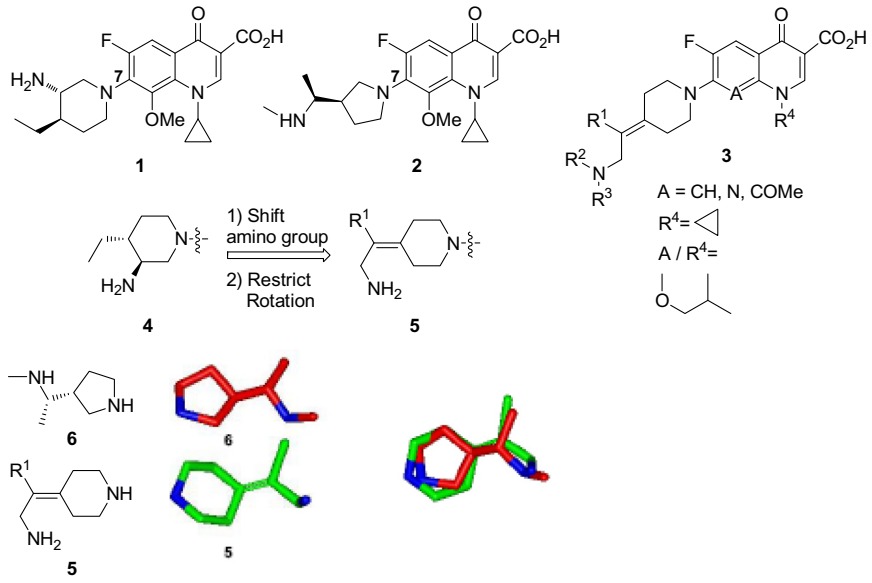
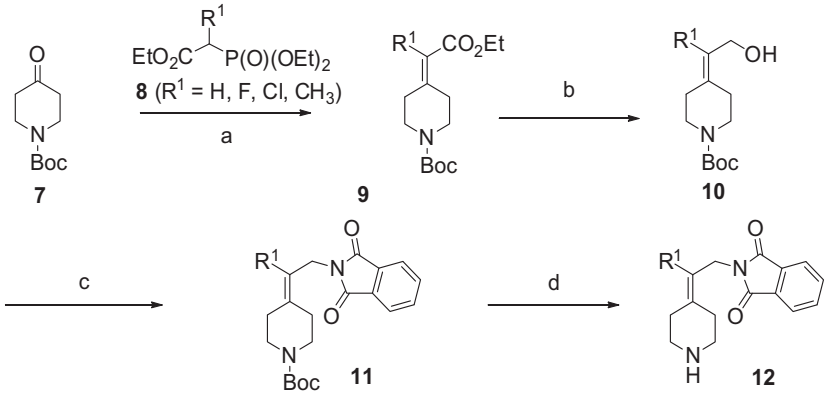


Figure 1. Chemical structures of quinolone antibacterial agents described in the present study.

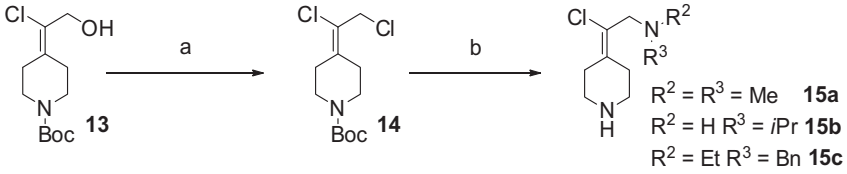


Scheme 1. Reagents and conditions: (a) **8** (R^1 = H, F, Cl, CH_3) NaH, THF or **8** (R^1 = F, Cl) K_2CO_3, EtOH, rt; (b) DIBAL, THF, −78 °C → 10 °C; (c) PPh_3, DIAD,THF, phthalimide; (d) (i) CF_3CO_2H, CH_2Cl_2 and (ii) 10% aq Na_2CO_3.

of the desired final products (Fig. 2, **16a–f**). Naphthyridone derivatives (**17a–d**) were synthesized by direct condensation of amine **12** with chloronaphthyridine **16a** followed by deprotection with hydrazine (Scheme 3). In contrast, coupling of the amines to the quinolone core structures (**16b–f**) demanded activation of the heterocyclic carboxylic acids with boron trifluoride to yield the difluoroborate ester[14] or with boric acid, acetic anhydride, and zinc chloride to form the diacetylborate ester (**18b–f**).[13] After coupling, alcoholysis of the difluoroborate ester was achieved by refluxing in ethanolic triethylamine, whereas hydrolysis of the diacetylborate ester was accomplished with 10% hydrochloric acid in THF. Removal of the phthalimide protecting group under standard conditions provided the final products **19a–c**, **20a–b**, **21**, **22a–b**, **23a–c** after purification. Further elaboration of **23c** to the corresponding secondary amine **24** was achieved by protecting the primary amine of **23c** with di-*tert*-butyl dicarbonate, alkylation of the intermediate carbamate, and removal of the Boc group with trifluoroacetic acid.

Analogs **25a**, **25b** and **25c** were synthesized by direct condensation of amine **15a–c** with activated heterocycle **18f** followed by hydrolysis. Quinolone **26** was prepared by debenzylation of **25c** with 1-chloroethyl chloroformate (Scheme 4).

In vitro antibacterial activity was assessed initially against an abbreviated panel of gram-positive bacteria, which included two ciprofloxacin-susceptible strains (*Staphylococcus aureus* OC4172 and *Streptococcus pneumoniae* ATCC 49619) as well as three ciprofloxacin-resistant pneumococcal clinical isolates (*S. pneumoniae* OC6608, OC6578, and OC5465). Data for representative



Scheme 2. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2; (b) Et_3N, CH_3CN, R^2R^3NH (iii) CF_3CO_2H, CH_2Cl_2.

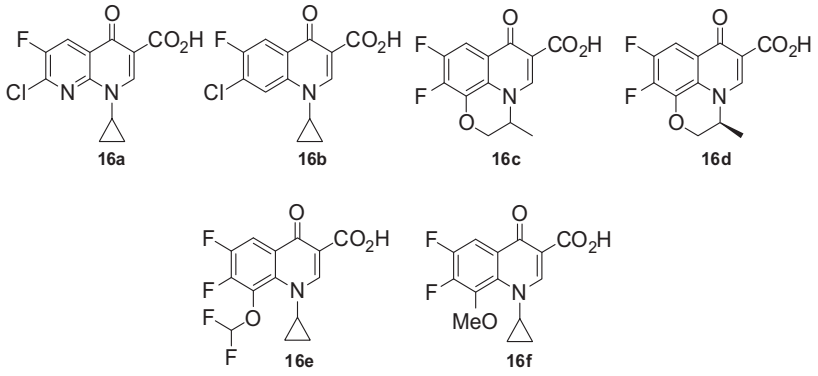
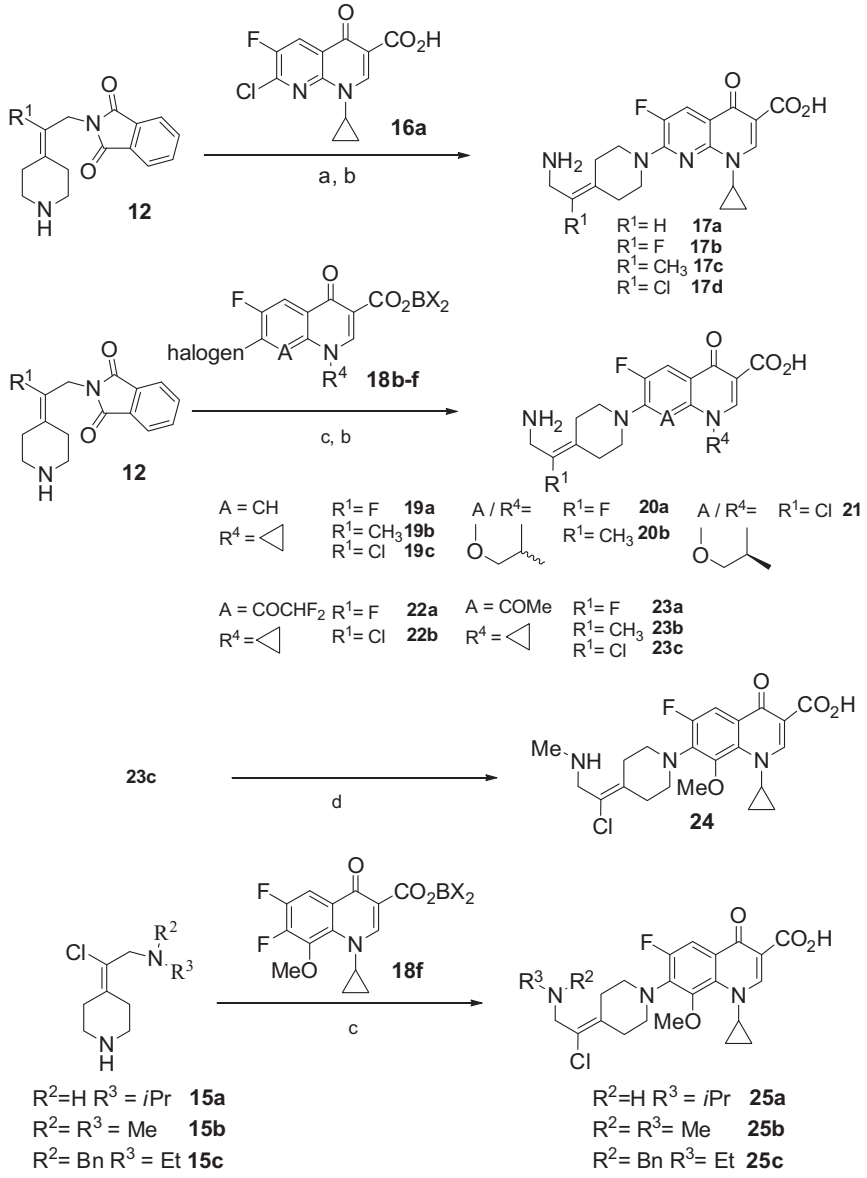


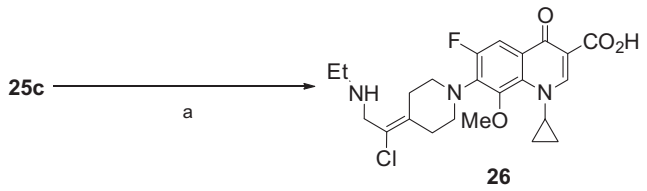
Figure 2. Chemical structures of the heterocyclic nuclei employed in the synthesis of 7-(4-alkylidenyl)piperidinyl quinolones and naphthyridones.



Scheme 3. Reagents and conditions: (a) **16a**, Et_3N, CH_3CN, reflux; (b) NH_2NH_2, MeOH, reflux; (c) (i) **18b–18f**, Et_3N, CH_3CN, reflux, (ii) X = F, Et_3N,EtOH, reflux or X = OAc, THF, 10%HCl; (d) (i) Boc_2O, Et_3N, CH_2Cl_2; (ii) MeI, NaH, DMF; (iii) NaOH, MeOH; (iv) CF_3CO_2H, CH_2Cl_2.

compounds and ciprofloxacin are presented in Table 1 as the minimal inhibitory concentration (MIC; lowest concentration of compound inhibiting visible growth).[15]

In the naphthyridone subseries, the analog with an unsubstituted alkylidenylpiperidine 7-position substituent (**17a**, R^1 = H) had comparable microbiological activity to ciprofloxacin against



Scheme 4. Reagents and conditions: (a) (i) 1-chloroethyl chloroformate, 1,2-dichloroethane, reflux; (ii) satd aq $NaHCO_3$.

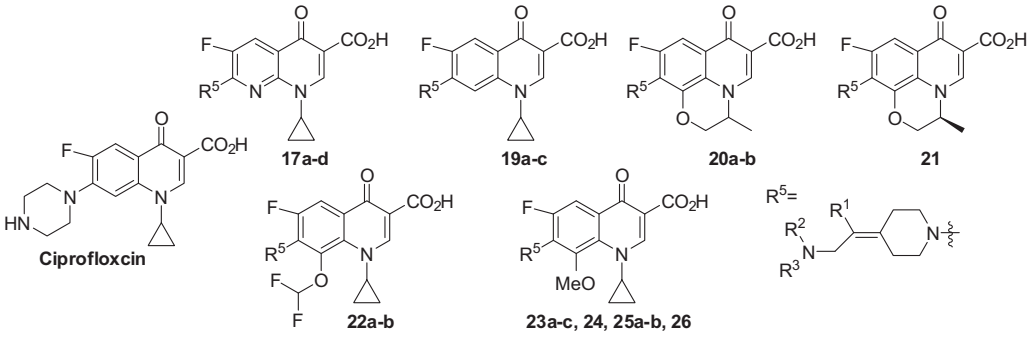
susceptible strains and had lower MICs against the resistant pneumococcal isolates. The incorporation of a fluoro (**17b**), methyl (**17c**), or chloro (**17d**) R^1 substituent improved microbiological activity against *S. pneumoniae*, particularly the resistant isolates. In view of the superior activity of the substituted alkylidene analogs in the naphthyridone subseries, the corresponding 7-position substituents were incorporated into other heterocyclic nuclei. In the 8-H quinolone subseries, the MIC values of the fluoro (**19a**) and chloro (**19c**) analogs were uniformly lower than the methyl analog (**19b**), a trend that was maintained in the tricyclic quinolone subseries based on the core structure of levofloxacin (**20a–b**, **21**). It should be noted that the fluoro (**20a**) and methyl (**20b**) analogs were racemic, whereas and the chloro analog (**21**) had the (*S*)-stereochemistry of levofloxacin. Nevertheless, the significantly lower MIC values of **21** were clear evidence of the superiority of the chloroalkylidene side chain in this subseries. Incorporation of fluoro (**22a**) and chloro (**22b**) R^1 substituents in the 8-$OCHF_2$ subseries provided analogs with similar to each other in vitro antibacterial profiles, including against ciprofloxacin-resistant *S. pnuemoniae*. Consistent with observations from earlier studies,[5,12] the 8-methoxyquinolone core structure conferred potent gram-positive activity (**23a–c**). Specifically, the combination of an 8-methoxy group and a chloroalkylidenylpiperidine 7-position substituent provided an analog (**23c**) with some of the lowest MIC values against the ciprofloxacin-susceptible and -resistant bacteria in the primary testing panel (Table 1).

Modification of the primary amino group of the 7-position substituent was investigated using compound **23c** as a template (Table 1). Addition of a methyl group was well-tolerated and afforded an analog (**24**) with comparable antibacterial activity to **23c**. Increasing steric bulk as in analogs **25a** and **26**, or introduction of another methyl group to give the tertiary amine (**25b**) increased MIC values, particularly against the resistant isolates.

The attractive in vitro profile of quinolones **22b**, **23c**, and **24** (Fig. 3) prompted further in vitro susceptibility testing against an expanded panel of organisms including methicillin-resistant ciprofloxacin-resistant staphylococci and the gram-negative pathogen, *Escherichia coli* (Table 2). All three compounds had MIC values 16 to 64-fold lower than ciprofloxacin against the resistant staphylococci. In contrast, the alkylidenylpiperidinyl quinolone analogs were less potent than ciprofloxacin against *E. coli*.

Quinolones **22b**, **23c**, and **24** were tested for efficacy in a murine lethal systemic infection model, in which Swiss Webster mice were infected with methicillin-susceptible *S. aureus* (Smith) and oral ED_{50} values (50% effective dose) determined.[16] Compound **24**

Table 1
Minimum inhibitory concentrations



Compd	R^1	R^2	R^3	MIC(μg/ml)[15]				
				A	B	C	D	E
Cipro				0.12	1	>16	>16	>16
17a	H	H	H	0.12	0.5	8	8	ND
17b	F	H	H	0.03	0.12	2	0.5	1
17c	CH_3	H	H	0.06	0.12	0.25	1	1
17d	Cl	H	H	0.06	0.12	1	0.25	1
19a	F	H	H	0.03	0.06	ND	1	ND
19b	CH_3	H	H	0.12	1	8	2	8
19c	Cl	H	H	0.03	0.12	ND	0.5	1
20a	F	H	H	0.25	0.5	2	8	8
20b	CH_3	H	H	0.5	1	4	>16	>16
21	Cl	H	H	⩽0.015	0.03	0.25	0.25	0.5
22a	F	H	H	0.06	0.25	0.25	2	0.25
22b	Cl	H	H	0.06	0.12	0.5	1	0.12
23a	F	H	H	0.03	0.12	0.5	1	0.5
23b	CH_3	H	H	0.03	0.12	ND	0.25	0.25
23c	Cl	H	H	⩽0.015	0.06	0.25	0.25	0.25
24	Cl	H	CH_3	⩽0.015	0.06	0.25	0.5	0.25
25a	Cl	H	*i*Pr	0.12	0.5	ND	4	2
25b	Cl	CH_3	CH_3	0.25	0.5	8	8	2
26	Cl	H	Et	0.12	0.25	1	1	1

(A) *Staphylococcus aureus* OC4172; (B) *S. pneumoniae* ATCC49619; (C) *Streptococcus pneumoniae* OC6608; (D) *S. pneumoniae* OC6578; (E) *S. pneumoniae* OC5465; ND: not determined.

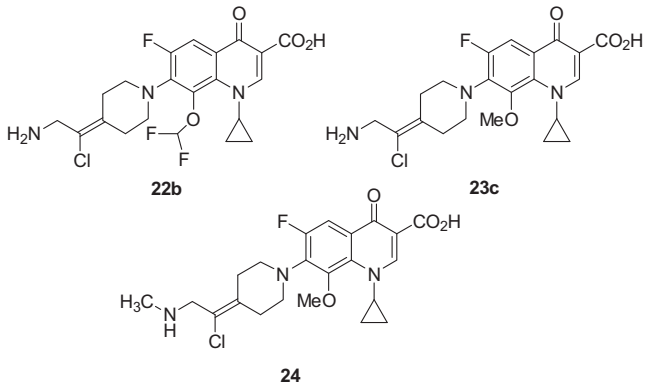


Figure 3. Chemical structures of the lead compounds from the 7-(4-alkylidenyl)piperidinyl quinolone series.

Table 2
Minimum inhibitory concentrations against methicillin-resistant staphylococci and *E. coli*

Compd	MIC (μg/ml)[15]		
	A	B	C
Cipro	16	8	0.015
22b	1	0.5	0.5
23c	0.25	0.25	0.25
24	0.5	0.5	0.5

(A) *MRSA* OC2805; (B) methicillin-resistant *Staphylococcus epidermidis* OC5340; (C) *E. coli* ATCC25922.

demonstrated the lowest ED_{50} value at 3.6 mg/kg with compounds **23c** (4.1 mg/kg), and **22b** (6.4 mg/kg) being superior to ciprofloxacin (ED_{50} = 11 mg/kg).

In summary, we developed and synthesized a series of 7-(4-alkylidenyl)piperidinyl fluoroquinolone and naphthyridone antibacterial agents by capitalizing on a previously reported conformational analysis approach for design of a novel 7-position substituent. The spectrum of activity included ciprofloxacin-susceptible and ciprofloxacin-resistant gram-positive cocci, such as *S. pneumoniae* and *S. aureus*. The best analogs of this series (compounds **22b**, **23c**, and **24**) also demonstrated superior in vivo efficacy to the clinically approved quinolone ciprofloxacin. Further studies of this series of quinolone antibacterial agents will be reported in the future.

Acknowledgment

The authors wish to thank Ellyn Wira for contributions to in vitro testing.

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15. The minimal inhibitory concentrations (MICs) were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI M07-A9, (CLSI, formerly NCCLS) guidelines. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*-9th ed. Modal MIC values are reported in cases where multiple MIC values were determined for a compound, including multiple test dates and multiple batches.
16. The in vivo efficacy of compounds **22b**, **23c**, and **24** was tested in female Swiss-Webster mice infected ip with the Smith strain of *S. aureus* (OC4172). Mice were dosed orally with test compound one hour following infection. Mortality was observed over a period of three days. A group of infected mice not treated with drug served as controls.