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# Synthesis and Antibacterial Activity of Novel 7-(3-Substituted-3 or 4-trifluoromethyl-1-pyrrolidinyl)-8-methoxyfluoroquinolones

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**Abstract**: The titled compounds were synthesized and evaluated for *in vitro* antibacterial activity. The (3*R*,4*S*)-3-aminomethyl-4-trifluoromethyl derivative (S-34109) was confirmed to be optimal because of its superior activity against quinolone and methicillin-resistant *Staphyrococcus aureus* and low side effect potential.

*Keywords* antibacterials; bacteria; chemotherapy

Since the introduction of antibacterial fluoroquinolones in the early 1980s, this class of compounds has become one of the most attractive agents in the antiinfective chemotherapy field [1]. Frequent clinical use of fluoroquinolones, however, has selected a quinolone-resistant *Staphylococcus aureus* (SA), in particular, most of which are methicillin-resistant. Prevalence of this organism is a serious problem in clinical settings. Therefore, a novel fluoroquinolone, which possesses potent antibacterial activity against methicillin as well as quinolone-resistant SA, is needed. Among the efforts to discover desirable fluoroquinolones, CI-960, which had the 3-aminopyrrolidine side chain at the 7-position and the chlorine atom at the 8-position of the quinolone nucleus, was reported to have a potent antibacterial activity against quinolone-resistant SA [2], but on the other hand showed a somewhat higher degree of cytotoxicity [3]. Domagala reviewed the structure-activity and structure-side effect relationships of the fluoroquinolones and suggested that the alkyl substitution of the pyrrolidine ring at the 7-position of the quinolone nucleus enhanced potency against Gram-positive bacteria and reduced cytotoxicity [4]. We chose the trifluoromethyl group as the alkyl substituent of the pyrrolidine ring and tried to introduce such a unique pyrrolidine into the quinolone nucleus in order to prepare novel fluoroquinolones which possess potent antibacterial activity against Gram-positive bacteria, especially quinolone-resistant SA with safety. Concerning the quinolone nucleus, we selected the 8-methoxy quinolone for our study considering Matsumotos' report about Q-35, in which they described that the introduction of a methoxy group at the 8-position of the quinolone nucleus made it stable under UV-irradiation [5]. In this paper, we report the synthesis and antibacterial activity of these fluoroquinolone derivatives.

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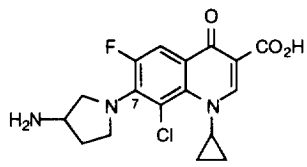
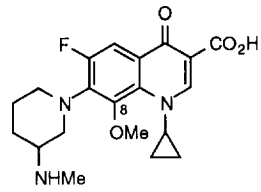
*PII:* S0960-894X(98)00514-9

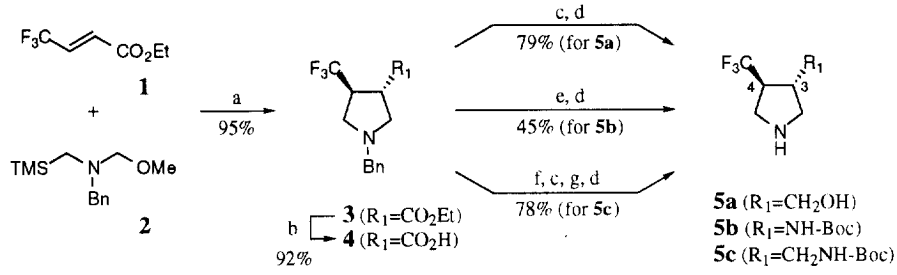



CI-960

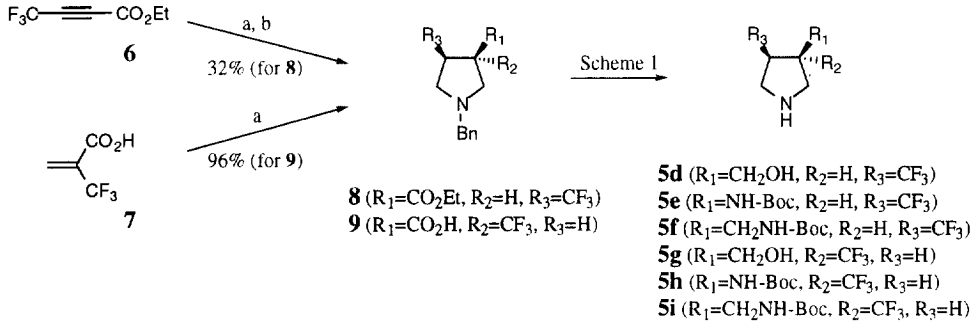



Q-35

**Chemistry**: The trifluoromethylated pyrrolidines (**5a-i**) were prepared as shown in Schemes 1 and 2. The 1,3-dipolar cycloaddition of α,β-unsaturated ester **1** with an azomethine ylide which was provided from amine **2** in the presence of trifluoroacetic acid (TFA) produced the *trans*-pyrrolidine derivative **3** in excellent yield [6]. The 3-hydroxymethyl derivative **5a** was obtained from **3** in two steps. The 3-amino and 3-aminomethyl derivatives (**5b** and **5c**) [7] were synthesized starting from carboxylic acid **4** which was obtained by hydrolysis of **3**. The *cis*-pyrrolidine derivatives (**5d-f**) and 3,3-disubstituted pyrrolidines (**5g-i**) were prepared in the same manner as the *trans*-analogs (**5a-c**) except using compounds **6** [8] and **7** as the starting material, respectively.



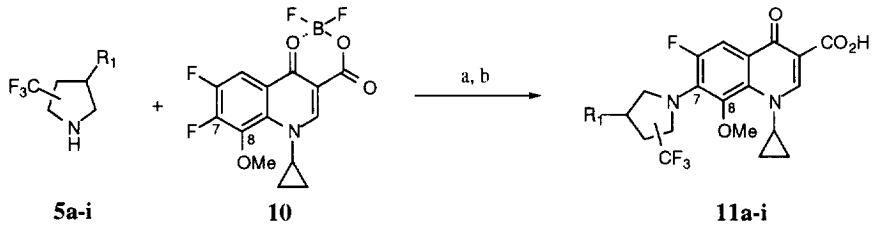

**Scheme 1.** (a) TFA, $CH_2Cl_2$; (b) NaOH, MeOH; (c) $LiAlH_4$, THF; (d) $H_2$, Pd-C, MeOH; (e) DPPA, $Et_3N$, *t*-BuOH; (f) $SOCl_2$, $CH_2Cl_2$; $NH_3$; (g) $Boc_2O$, $CH_2Cl_2$.




**Scheme 2.** (a) amine **2**, TFA, $CH_2Cl_2$; (b) $H_2$, $PtO_2$, AcOEt-benzene (1:1), AcOH.

As shown in Scheme 3, the pyrrolidines (**5a-i**) were condensed with the 8-methoxy quinolone (**10**) to give novel fluoroquinolones (**11a-i**), respectively. The 3-amino and 3-aminomethyl derivatives (**11b-c**, **11e-f** and **11h-i**) were obtained as salts after the removal of the *tert*-butoxycarbonyl (Boc) group by acid treatment.

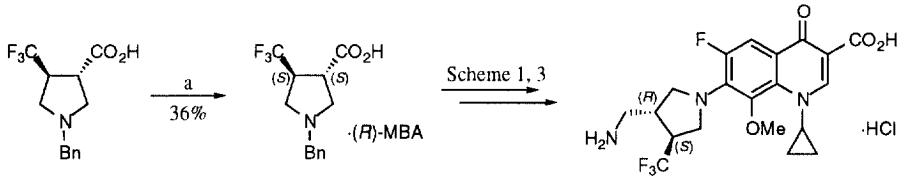
For comparison with **11c**, the *trans*-methyl substituted analog **12** [9] was prepared in the same way as **11c** except using ethyl *trans*-crotonate as the starting compound.




**5a-i** **10** **11a-i**

**Scheme 3.** (a) $Et_3N$, DMSO; (b) deprotection by TFA or HCl.

The optically active *trans*-3-aminomethyl-4-trifluoromethyl derivatives were synthesized *via* optical resolution of the carboxylic acid **4** as shown in Scheme 4. A mixture of diastereomeric salts prepared by the treatment of the racemic acid **4** with (*R*)-α-methylbenzylamine (MBA) was resolved to give the optically active salt **13** (95%ee) [10] which was converted into (3*R*,4*S*)-**11c** in the same manner as in the preparation of the racemate **11c**. The enantiomer (3*S*,4*R*)-**11c** was prepared in the same way except for using (*S*)-MBA as the resolving agent. The absolute configuration of **13** was determined by X-ray crystallographic analysis [11].

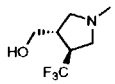
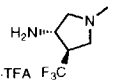
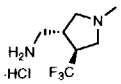
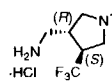
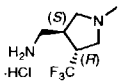
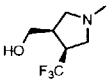
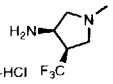
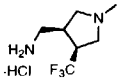



(±)-**4** **13** (3*R*,4*S*)-**11c** (S-34109)

**Scheme 4.** (a) (*R*)-α-methylbenzylamine (MBA), MeOH.

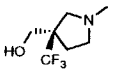
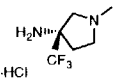
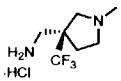
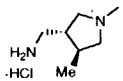
**Results and discussion**: The antibacterial activity of the novel fluoroquinolones (**11a-i**) was tested against 26 representative Gram-positive and Gram-negative strains. The minimum inhibitory concentrations (MICs) of the synthesized compounds, the *trans*-methyl substituted analog **12** and positive controls, ofloxacin (OFLX) and tosufloxacin (TFLX), are shown in Table 1 [12]. The antibacterial activity of the 3-hydroxymethyl-4-trifluoromethyl derivatives (**11a** and **11d**) was superior against quinolone-resistant SA but weak against Gram-negative strains. Among the tested compounds, the 3-amino-4-trifluoromethyl and 3,3-disubstituted derivatives (**11b**, **11e** and **11g-i**) showed a remarkable decrease in activity against quinolone-resistant SA. The antibacterial activity of the 3-aminomethyl-4-trifluoromethyl derivatives (**11c** and **11f**) was very potent against Gram-positive bacteria including quinolone-resistant SA. Concerning the activity against Gram-negative strains, compounds **11c** and **11f** were very similar to that of OFLX and TFLX, respectively. The antibacterial activity of **12** was about 2-fold more active than **11c**. On the other hand, the *in vitro* cytotoxicities ($IC_{50}$: μg/mL) of **11c**, **11f**, **12** and TFLX were 64, 9.7, 21 and 6.3, respectively [13, 14]. As for the mutagenesis to mice using the micronucleus test, compound **11c** was negative at a dose less than 200 mg/kg, whereas **11f** and **12** were

**Table 1**. Antibacterial activity of novel synthesized fluoroquinolones.

| | Compound | **11a** | **11b** | (±)-**11c** | (3*R*,4*S*)-**11c** (S-34109) | (3*S*,4*R*)-**11c** | **11d** | **11e** | **11f** |
|---|---|---|---|---|---|---|---|---|---|
| | 7-amine |  |  |  |  |  |  |  |  |
| | Organism | Minimum inhibitory concentration (MIC, μg/mL) | | | | | | | |
| Gram (+) | *Staphylococcus aureus* FDA 209P JC-1 | 0.025 | 0.1 | 0.025 | 0.025 | 0.05 | 0.013 | 0.05 | 0.025 |
| Gram (+) | *Staphylococcus aureus* Smith | ≤0.006 | 0.05 | ≤0.006 | ≤0.006 | ≤0.006 | ≤0.006 | 0.025 | ≤0.006 |
| Gram (+) | *Staphylococcus aureus* JS-1[a] | 0.013 | 0.05 | 0.013 | ≤0.006 | 0.013 | ≤0.006 | 0.05 | ≤0.006 |
| Gram (+) | *Staphylococcus aureus* KP-90-3[b] | 0.78 | 3.13 | 0.39 | 0.39 | 0.78 | 0.39 | 1.56 | 0.2 |
| Gram (+) | *Streptococcus pyogenes* Cook | 0.2 | 0.39 | 0.025 | 0.013 | 0.025 | 0.05 | 0.39 | ≤0.006 |
| Gram (+) | *Enterococcus faecalis* 1373 | 0.2 | 0.39 | 0.05 | 0.025 | 0.05 | 0.1 | 0.39 | 0.05 |
| Gram (−) | *Escherichia coli* NIHJ JC-2 | 0.39 | 0.39 | 0.1 | 0.025 | 0.2 | 0.39 | 0.2 | 0.05 |
| Gram (−) | *Escherichia coli* ML4707 | 0.2 | 0.05 | 0.05 | 0.025 | 0.05 | 0.2 | 0.1 | 0.025 |
| Gram (−) | *Escherichia coli* CSH2/RK1 | 6.25 | 3.13 | 0.78 | 0.39 | 3.13 | 3.13 | 3.13 | 0.2 |
| Gram (−) | *Escherichia coli* CSH2/RE45 | 6.25 | 3.13 | 0.78 | 0.39 | 1.56 | 3.13 | 3.13 | 0.2 |
| Gram (−) | *Klebsiella pneumoniae* No.42 | 3.13 | 0.39 | 0.2 | 0.1 | 0.39 | 0.78 | 0.39 | 0.1 |
| Gram (−) | *Klebsiella pneumoniae* KC-1 | 1.56 | 0.39 | 0.05 | 0.05 | 0.1 | 0.39 | 0.2 | 0.05 |
| Gram (−) | *Klebsiella oxytoca* GN10650 | 1.56 | 0.39 | 0.1 | 0.1 | 0.2 | 0.78 | 0.39 | 0.05 |
| Gram (−) | *Proteus vulgaris* No.33 | 0.78 | 0.39 | 0.1 | 0.05 | 0.39 | 0.39 | 0.39 | 0.05 |
| Gram (−) | *Proteus vulgaris* KS-134 | 100 | 100 | 50 | 50 | 50 | 100 | 100 | 25 |
| Gram (−) | *Proteus mirabilis* JY-10 | 0.2 | 0.2 | 0.1 | 0.05 | 0.2 | 0.1 | 0.2 | 0.025 |
| Gram (−) | *Serratia marcescens* No.16-2 | 6.25 | 3.13 | 0.39 | 0.39 | 0.78 | 3.13 | 3.13 | 0.78 |
| Gram (−) | *Enterobacter cloacae* Nek39 | 3.13 | 3.13 | 0.39 | 0.39 | 1.56 | 1.56 | 3.13 | 0.39 |
| Gram (−) | *Citrobacter freundii* No.7 | 12.5 | 3.13 | 0.78 | 0.78 | 1.56 | 6.25 | 3.13 | 0.78 |
| Gram (−) | *Citrobacter freundii* KP-29 | >100 | 100 | 25 | 12.5 | 50 | >100 | 100 | 6.25 |
| Gram (−) | *Acinetobacter calcoaceticus* No.4 | 0.78 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 |
| Gram (−) | *Pseudomonas aeruginosa* PAO1 | 3.13 | 3.13 | 1.56 | 1.56 | 6.25 | 3.13 | 3.13 | 0.78 |
| Gram (−) | *Pseudomonas aeruginosa* AK109 | 6.25 | 6.25 | 1.56 | 1.56 | 6.25 | 3.13 | 6.25 | 0.78 |
| Gram (−) | *Pseudomonas aeruginosa* KP-254 | >100 | >100 | 100 | 100 | >100 | >100 | >100 | 50 |
| Gram (−) | *Pseudomonas aeruginosa* K-13 | 25 | 25 | 12.5 | 12.5 | 50 | 25 | 50 | 3.13 |
| Gram (−) | *Burkholderia cepacia* 23 | 3.13 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 | 1.56 |

[a] methicillin-resistant strain. [b] quinolone and methicillin-resistant strain.

**Table 1** (Continued)

| | Compound | **11g** | **11h** | **11i** | **12** | ofloxacin (OFLX) | tosufloxacin (TFLX) |
|---|---|---|---|---|---|---|---|
| | 7-amine |  |  |  |  | | |
| | Organism | Minimum inhibitory concentration (MIC, μg/mL) | | | | | |
| Gram (+) | *Staphylococcus aureus* FDA 209P JC-1 | 0.1 | 0.05 | 0.2 | 0.013 | 0.39 | 0.05 |
| Gram (+) | *Staphylococcus aureus* Smith | 0.025 | ≤0.006 | 0.05 | ≤0.006 | 0.2 | 0.025 |
| Gram (+) | *Staphylococcus aureus* JS-1[a] | 0.05 | 0.025 | 0.1 | ≤0.006 | 0.39 | 0.05 |
| Gram (+) | *Staphylococcus aureus* KP-90-3[b] | 3.13 | 1.56 | 6.25 | 0.2 | 25 | 12.5 |
| Gram (+) | *Streptococcus pyogenes* Cook | 0.39 | 0.1 | 0.78 | ≤0.006 | 0.78 | 0.1 |
| Gram (+) | *Enterococcus faecalis* 1373 | 0.39 | 0.2 | 0.78 | 0.05 | 1.56 | 0.2 |
| Gram (−) | *Escherichia coli* NIHJ JC-2 | 1.56 | 0.39 | 3.13 | 0.05 | 0.1 | 0.05 |
| Gram (−) | *Escherichia coli* ML4707 | 0.05 | ≤0.006 | 0.05 | 0.025 | 0.025 | 0.013 |
| Gram (−) | *Escherichia coli* CSH2/RK1 | 25 | 3.13 | >12.5 | 0.39 | 0.78 | 0.39 |
| Gram (−) | *Escherichia coli* CSH2/RE45 | 25 | 3.13 | >12.5 | 0.39 | 0.78 | 0.39 |
| Gram (−) | *Klebsiella pneumoniae* No.42 | 3.13 | 0.39 | 6.25 | 0.1 | 0.2 | 0.025 |
| Gram (−) | *Klebsiella pneumoniae* KC-1 | 1.56 | 0.2 | 1.56 | 0.1 | 0.05 | 0.025 |
| Gram (−) | *Klebsiella oxytoca* GN10650 | 3.13 | 0.39 | 6.25 | 0.1 | 0.1 | 0.05 |
| Gram (−) | *Proteus vulgaris* No.33 | 1.56 | 0.39 | 3.13 | 0.1 | 0.05 | 0.05 |
| Gram (−) | *Proteus vulgaris* KS-134 | 100 | 100 | >12.5 | 50 | 50 | >12.5 |
| Gram (−) | *Proteus mirabilis* JY-10 | 0.39 | 0.2 | 0.78 | 0.05 | 0.05 | 0.05 |
| Gram (−) | *Serratia marcescens* No.16-2 | 12.5 | 3.13 | >12.5 | 0.39 | 0.78 | 0.39 |
| Gram (−) | *Enterobacter cloacae* Nek39 | 6.25 | 1.56 | 12.5 | 0.39 | 0.39 | 0.2 |
| Gram (−) | *Citrobacter freundii* No.7 | 25 | 3.13 | >12.5 | 0.39 | 0.39 | 0.39 |
| Gram (−) | *Citrobacter freundii* KP-29 | >100 | 100 | >12.5 | 12.5 | 50 | >12.5 |
| Gram (−) | *Acinetobacter calcoaceticus* No.4 | 1.56 | 0.2 | 1.56 | 0.2 | 0.78 | 0.025 |
| Gram (−) | *Pseudomonas aeruginosa* PAO1 | 12.5 | 6.25 | >12.5 | 0.78 | 1.56 | 0.39 |
| Gram (−) | *Pseudomonas aeruginosa* AK109 | 12.5 | 6.25 | >12.5 | 0.78 | 1.56 | 0.39 |
| Gram (−) | *Pseudomonas aeruginosa* KP-254 | >100 | >100 | >12.5 | 100 | >100 | >12.5 |
| Gram (−) | *Pseudomonas aeruginosa* K-13 | 50 | 25 | >12.5 | 6.25 | 12.5 | 6.25 |
| Gram (−) | *Burkholderia cepacia* 23 | 3.13 | 1.56 | 3.13 | 1.56 | 3.13 | 0.78 |

[a] methicillin-resistant strain. [b] quinolone and methicillin-resistant strain.

considered as positive at a dose of 50 mg/kg [15]. Based on these results, it was shown that the *trans*-analog **11c** was the best compound posessing superior activity against quinolone-resistant SA with sufficient safety [16]. The chirality on the pyrrolidine ring slightly affected the antibacterial activity. Thus, (3*R*,4*S*)-**11c** [17] had antibacterial activity similar to that of the racemate **11c** and was about 2–8-fold more active than its enantiomer.

In conclusion, we demonstrated the efficacy of the introduction of the trifluoromethyl group to the pyrrolidine ring at the 7-position of the quinolone nucleus and selected (3*R*,4*S*)-7-(3-aminomethyl-4-trifluoromethyl-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-4-oxoquinoline-3-carboxylic acid hydrochloride ((3*R*,4*S*)-**11c**, S-34109) as the most promising candidate for further studies.

**Acknowledgment**: The authers wish to thank Ms. Yayoi Watanuki for her technical assistance and Mr. Junichi Yoshida and Mr. Yuji Ito for the micronucleus testing.

**References and notes**

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[13] Determination of $IC_{50}$; Confluent monolayer of HeLa cells (human cervical cancer cell line) was cultured in the presence of a serial 2-fold dilution of each drug for 3 days. The viability was determined by MTT method [14]. The drug concentration whose absorbance at 540 nm indicates 50% of the drug-free control was designated as $IC_{50}$.
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[15] Micronucleus test: Male Crj:CD-1 mice (8weeks old) were intraperitoneally given test drugs (suspension in olive oil) twice. The peripheral blood was collected from a tail blood vessel at 24-h intervals from 0 to 72 h after the first administration. Blood smear preparations were made by staining with acridine orange. Micronucleated reticulocytes (MNRETs) were observed with fluorescent microscopy. The group which showed the higher MNRET frequency than that before the administration was considered as positive.
[16] Maejima T, Senda H, Iwatani W, Tatsumi Y, Arika T, Fukui H, Shibata T, Nakano J, Naito T. 35th ICAAC 1995;Abstract No.F189.
[17] (3*R*,4*S*)-**11c**: $[\alpha]_D^{34} = +9.2°$ (*c* 0.50, MeOH). $^1$H-NMR (270 MHz, DMSO-$d_6$) δ (ppm): 1.04-1.15 (m, 4H), 2.77-2.82 (m, 1H), 2.94-3.12 (m, 2H), 3.37-3.40 (m, 1H), 3.50-3.56 (m, 1H), 3.64 (s, 3H), 3.68 (dd, *J* = 11.1, 4.5 Hz, 1H), 3.86-3.95 (m, 2H), 4.16-4.17 (m, 1H), 7.75 (d, *J* = 13.2 Hz, 1H), 8.33 (s, 3H), 8.70 (s, 1H), 14.94 (s, 1H).