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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 7-(2-AMINOALKYL)MORPHOLINOQUINOLONES AS ANTI-*HELICOBACTER PYLORI* AGENTS

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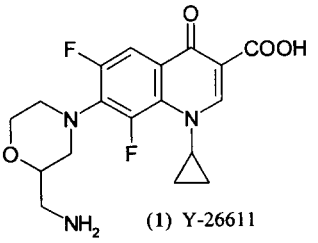
Abstract: A series of the titled compounds was synthesized and tested for anti- *Helicobacter pylori* activities. We discovered Y-34867 having the most potent activity against *Helicobacter pylori* among the quinolones tested along with high photostability. Furthermore, Y-34867 showed an excellent therapeutic effect in the experimental *Helicobacter pylori* infected Mongolian gerbil model.

Helicobacter pylori (*H. pylori*) is a primary cause of chronic gastritis[1] and is highly associated with peptic ulcer[2] and gastric cancer.[3] Eradication of the bacterium in patients with peptic ulcers dramatically decreases the recurrence rate.[4] Recently, new triple therapy regimens such as combination with omeplazole, amoxicillin and clarithromycin or metronidazole are widely used for the treatment of *H. pylori* infection to be clinically effective.[5] However, many problems such as side effects, acquired resistance and compliance of taking the drug correcting have been reported.[6] Therefore the development of novel class of anti-*H. pylori* agents is worthwhile.

Quinolone antimicrobial agents are useful for clinical treatment of various infectious diseases and have also been reported to exhibit anti-*H. pylori* activity *in vitro*.[7] Only a few studies on structural optimization of quinolone derivatives on the basis of the anti-*H. pylori* activities have been reported.[8]

In the course of our previous research on novel quinolone antimicrobial agents with morpholines at the 7-position,[9] we found compound **1** (Y-26611) possessed significant anti-*H. pylori* activity. However, compound **1** have poor oral potency in the experimental *H. pylori* infected Mongolian gerbil model.[10] Therefore we reinvestigated a series of 7-(2-aminoalkyl)morpholinoquinolones to optimize the anti-*H. pylori* activity.



(1) Y-26611

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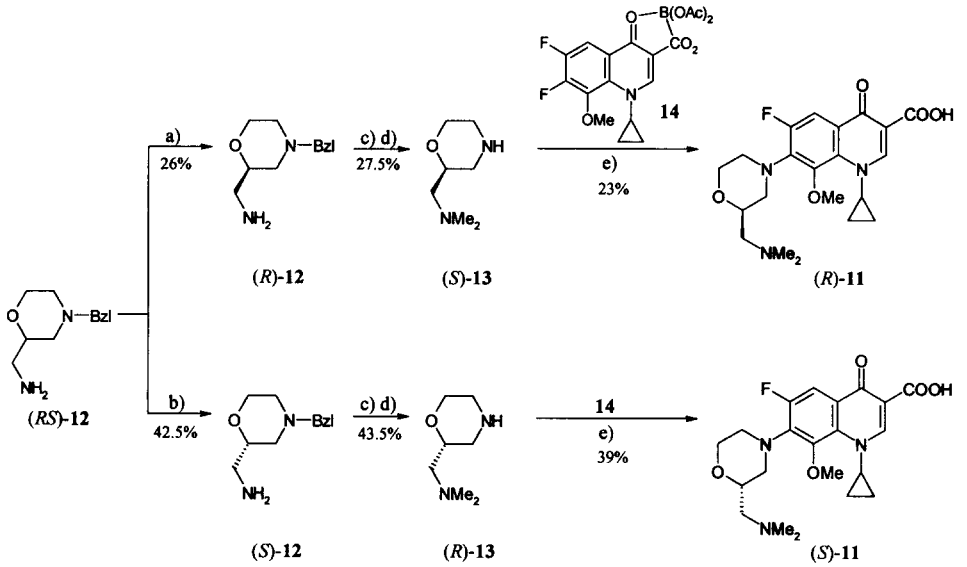
In this communication, we describe the synthesis of 7-(2-aminoalkyl)morpholinoquinolones and their structure-activity relationships on the basis of the anti-*H. pylori* activities.

Chemistry: According to the previously reported procedures,[9] compounds **2-9** and **11** were prepared by coupling of corresponding 2-aminoalkylmorpholine with 8-substituted-1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid or its diacetylborate. 8-Chloroquinolone derivative **10** was synthesized from 8-unsubstituted quinolone **9** by chlorination with sulfuryl chloride.[9]

The enantiomers of the most promising compound **11** were synthesized as shown in Scheme 1. The optically pure key intermediates (*R*) and (*S*)-**12** (>99% ee)[11] were obtained by optical resolution of (*RS*)-**12** using (-)-dibenzoyl-L-tartaric acid and (+)-dibenzoyl-D-tartaric acid, respectively. Subsequent dimethylation of **12** followed by hydrogenation gave **13**. Coupling of **13** with 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-4-oxo-3-quinolinecarboxylic acid diacetylborate (**14**) gave optically pure **11** (>99% ee).[12]

Scheme 1



a) 1) (-)-dibenzoyl-L-tartaric acid, 2) NaOH; b) 1) (+)-dibenzoyl-D-tartaric acid, 2) NaOH; c) HCOOH,HCHO; d) NH_2NH_2, Pd-C; e) Et_3N

Methods

Anti-*H. pylori* activity. *In vitro* antimicrobial activity against *H. pylori* was determined by twofold serial dilutions of the drugs in brucella broth plus 10% inactivated horse serum in the presence of approximately 10^5 CFU/mL.[13] After 72 h of incubation of *H. pylori* ATCC 43504 at 37 °C under an atmosphere of 8% CO_2, minimum inhibitory concentration (MIC) was defined as the lowest concentrations of the compound giving complete inhibiton of growth.

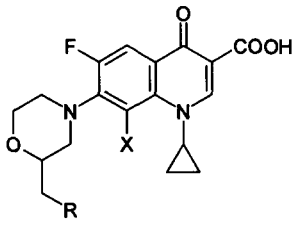
Therapy in Animal Model. The model used in this study was reported by Hirayama.[10] Briefly,

Mongolian gerbils (MGS/Sea, male, 7 weeks) were orally challenged with a highly motile broth culture of *H. pylori* ATCC 43504. Challenge was administered as a single 0.5 mL volume of culture (approximately 2×10^8 CFU). Gerbils were fasted 24 h before challenge. Therapy was initiated 6 weeks after challenge and continued twice a day for 7 days. Gerbils were sacrificed 3 days after final administration and each stomach was homogenized with 10 mL of PBS (pH 7.0) and diluted with the same solution. An aliquot (100 μ L) of this suspension was inoculated onto agar plates at 37 ℃. After 5-7 days of incubation at 37 ℃ under an atmosphere of 8% CO_2, the colonies of *H. pylori* were counted. The clearance rate was represented by the ratio of the number of gerbils in which *H. pylori* was not detected to that of gerbils tested (percent).

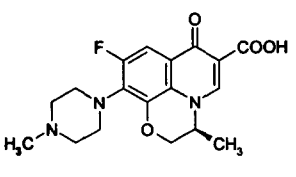
Photostability. The agents were dissolved in a small volume of 0.01 N NaOH solution and were then added to 1/15 M PBS (pH 7.0) to make a final concentration of 100 μg/mL. The solution was irradiated from a 15 cm distance with UV light.[14] Quantitation of the compounds was performed by HPLC on CAPCELL PAK SG120A column.

Results and discussion: The synthesized compounds, levofloxacin, tosufloxacin, sparfloxacin, amoxicillin and clarithromycin were evaluated for antimicrobial activity against *H. pylori*.[13] The results are summarized in Table 1. Modification of the primary amine of lead compound **1** with bulky groups decreased anti-*H. pylori* activities. For example, propylamino and acetylamino analogues **4** and **5** were weak in potency. Compound **6** having a dimethylaminomethyl group at the molpholino moiety exhibited enhanced anti-*H. pylori* activity over the nonmethylated and monomethylated analogues **1** and **2**. Compound **8** whose aminoalkyl side chain lengthened showed less activity than **1**. Compound **6** was found to have the most potent anti-*H. pylori* activity among 8-fluoroquinolone derivatives, but unfortunately it showed similar photolability to **1** (Fig. 1), which showed severe phototoxicity in clinical tests. The previous reports discribed that phototoxicity of some quinolones were caused by these photolability and that introduction of a methoxy group into the 8-position of quinolones improved their photostability.[14] Therefore, in order to improve the photostability of the compound **6**, we replaced its fluorine atom at the 8-position by other groups (hydrogen, chlorine and methoxy). The photostability[14] of the 8-substituted derivatives was shown in Fig. 1. Compound **11** having a methoxy group at the 8-position was the most stable among them. Furthermore, the methoxy derivative **11** displayed similar anti-*H. pylori* activity to **6**. Racemate (**11**) was optically separated to examine which is the eutomer between the two isomers ((*R*)-**11** and (*S*)-**11**). As for the activity against *H. pylori*, the *S*-isomer was about thirty times as potent as the *R*-isomer. Moreover, (*S*)-**11** (Y-34867) displayed much more potent anti-*H. pylori* activity than levofloxacin, tosufloxacin and sparfloxacin, and showed similar activity to amoxicillin and clarithromycin. Thus, we selected Y-34867 as a candidate for additional biological, physicochemical and pharmaceutical investigation. At the first step of such evaluations, the therapeutic efficacy of Y-34867 was estimated in the experimental *H. pylori* infected Mongolian gerbil model.[10] In this model, Y-34867 showed a much more highly therapeutic effect than levofloxacin, tosufloxacin, sparfloxacin, amoxicillin and clarithromycin (Table 2). From these results, Y-34867 would be a promising agent to eradicate *H. pylori* with short term administration at low doses.

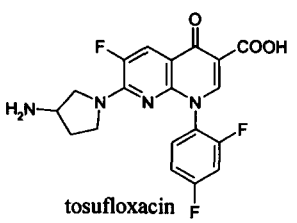
Table 1. Antimicrobial activities of 7-(2-aminoalkyl)morpholinoquinolones and other antimicrobial agents against *H. pylori* ATCC 43504



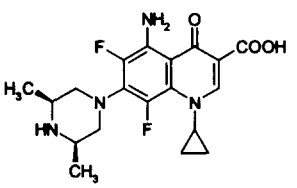
Compd. No.	R	X	mp (℃)	MIC (mg/mL)
1 (Y-26611)	NH_2	F	180-182	0.05
2	NHMe	F	251-254	0.05
3	NHEt	F	215-216	0.05
4	NHPr	F	184-186	0.39
5	NHAc	F	230-233	0.78
6	NMe_2	F	182-183	0.025
7	NEt_2	F	155-157	0.20
8	CH_2NH_2	F	261-263	0.20
9	NMe_2	H	225-227	0.10
10	NMe_2	Cl	115-117	0.05
11	NMe_2	OMe	165-167	0.025
(*R*)-11	NMe_2	OMe	144-145	0.39
(*S*)-11 (Y-34867)	NMe_2	OMe	144-146	0.012
levofloxacin				0.20
tosufloxacin				0.39
sparfloxacin				0.20
amoxicillin				0.025
clarithromycin				0.025



levofloxacin



tosufloxacin



sparfloxacin

Fig.1. The stability of 7-(2-aminoalkyl)morpholinoquinolones in an aqueous solution under UV-irradiation

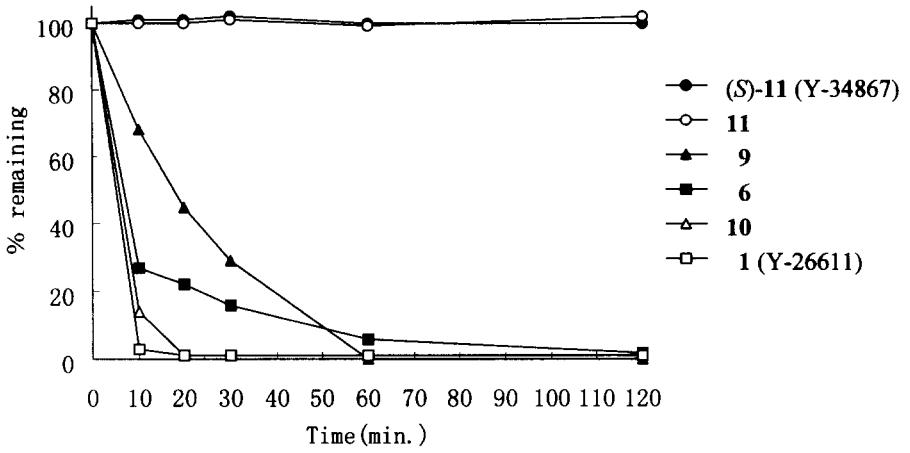


Table 2. Therapeutic efficacy of Y-26611, Y-34867 and other antimicrobial agents against the experimental *H. pylori* infection in Mongolian gerbils

Compound	Dose[1] (mg/kg)	Clearance rate[2] (%)
Y-26611	10	80
Y-34867	1	100
	0.3	0
levofloxacin	10	60
	3	0
tosufloxacin	10	0
sparfloxacin	10	0
amoxicillin	10	100
	3	0
clarithromycin	30	100
	10	0

(N = 5)

1) Administration ; p.o., b.i.d. × 7days
drugs were administrated to the gerbils 6 weeks after infection
2) determined 3 days after final administration

Conclusion: A series of new 7-(2-aminoalkyl)morpholinoquinolone derivatives was synthesized and evaluated for *in vitro* and *in vivo* anti-*H. pylori* activities. From these results, Y-34867 ((*S*)-**11**, (*S*)-(-)-1-cyclopropyl-7-[2-(dimethylaminomethyl)morpholino]-6-fluoro-1,4-dihydro-8-methoxy-4-oxo-quinoline-3-carboxylic acid) has been selected as a candidate for further studies.

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11. The enantiomeric purity was determined by HPLC on CROWNPAK CR-(+) column. Data of (*R*)-**12**: $[\alpha]^{24}_D = +29.4^\circ$ (c = 1, MeOH). ^{1}H-NMR ($CDCl_3$) δ 1.42 (s, 2H), 1.88 (t, 2H, *J* = 5Hz), 2.11-2.21 (m, 1H), 2.65-2.72 (m, 4H), 3.45-3.64 (m, 3H), 3.67-3.72 (m, 1H), 3.83-3.88 (m, 1H), 7.24-7.32 (m, 5H). (*S*)-**12**: $[\alpha]^{25}_D = -27.45^\circ$ (c = 1, MeOH).
12. The enantiomeric purity was determined by HPLC on STR-ODS II column. Data of (*R*)-**11**: $[\alpha]^{24}_D = +7.95^\circ$ (c = 1, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 1.01-1.05 (m, 2H), 1.21-1.24 (m, 2H), 1.29-1.35 (m, 1H), 2.32 (s, 6H), 2.53 (dd, 1H, *J* = 13.2, 7.3Hz), 3.11-3.16 (m, 1H), 3.37-3.48 (m, 3H), 3.81-3.90 (m, 1H), 3.83 (s, 3H), 4.00-4.07 (m, 2H), 7.83 (d, 1H, *J* = 11.9Hz), 8.79 (s, 1H). (*S*)-**11**: $[\alpha]^{24}_D = -7.8^\circ$ (c = 1, $CHCl_3$).
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