5-Alkoxyimidazoquinolones as Potential Antibacterial Agents. Synthesis and Structure–Activity Relationships[1)]

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4-Substituted 6-cyclopropyl-6,9-dihydro-5-methoxy-9-oxo-1*H*-imidazo[4,5-*f*]quinoline-8-carboxylic acids (6) and 8-substituted 1,5,6,11-tetrahydro-5-methyl-1-oxo-imidazo[4,5-*g*]pyrido[1,2,3-*de*][1,4]benzoxazine-2-carboxylic acids (7) were prepared as potential antibacterial quinolone derivatives. The appendages at C-4 of -6 and at C-8 of -7 were selected from 1-piperazinyl, 4-methylpiperazinyl, 3-aminopyrrolidinyl, and 3-aminomethylpyrrolidinyl groups. The 5-methoxyimidazoquinolones 6 were superior to the corresponding ofloxacin type analogues 7 in *in vitro* antibacterial activity. The activity of 6 was equipotent against *S. aureus*, but 2 to 16 times less potent against *E. coli* and *P. aeruginosa* compared to that of the 5-fluoro analogue 3.

Key words quinolone; 1*H*-imidazo[4,5-*f*]quinoline; synthesis; imidazo[4,5-*g*]pyrido[1,2,3-*de*][1,4]benzoxazine; antibacterial activity; structure–activity relationship

Synthetic antibacterial quinolones are widely accepted as useful and indispensable antibacterial agents for the current chemotherapy against bacterial infectious disease, owing to their potent activity and broad spectrum.[2)] As represented by enoxacin (**1**)[3)] and sparfloxacin (**2**)[4)] (Chart 1), most of the prevailing quinolones possess a fluorine atom at C-6 and a cyclic amino group at C-7 of the 1-substituted 4-oxoquinoline- and 4-oxo-1,8-naphthyridine-3-carboxylic acids.

As part of our study of tricyclic quinolone derivatives without a fluorine atom at C-6 of the quinoline ring, we previously reported the synthesis and antibacterial activity of 4,5-disubstituted 6-cyclopropyl-6,9-dihydro-9-oxo-1*H*-imidazo[4,5-*f*]quinoline-8-carboxylic acids, including **3b** and **3c**.[5)] The quinolones **3b, c** exhibited a comparable *in vitro* antibacterial activity to enoxacin (**1**)[3)] and ofloxacin (**5**),[6)] whereas **3b** and **3c** were inefficacious in *in vivo* when orally administered in infected mice, probably owing to their limited absorption from animal digestive tracts. Among the quinolones currently utilized and under clinical trials, AM-1155 (**4**)[7)] and ofloxacin (**5**),[6)] which append an alkoxy group or its equivalent at C-8 of their quinolone rings, have been reported to have sufficient bioavailability.[8,9)] This prompted us to undertake the present study of 4-substituted 6-cyclopropyl-6,9-dihydro-5-methoxy-9-oxo-1*H*-imidazo[4,5-*f*]quinoline-8-carboxylic acids (**6**) and 8-substituted 1,5,6,11-tetrahydro-5-methyl-1-oxo-imidazo[4,5-*g*]pyrido[1,2,3-*de*][1,4]benzoxazine-2-carboxylic acids (**7**). A change of the C-5 fluorine of **3b, c** to a methoxy group (giving **6**) or replacement of the C-5 and C-6 substituents with a 3-methyloxazine ring (giving **7**) was expected to improve both the

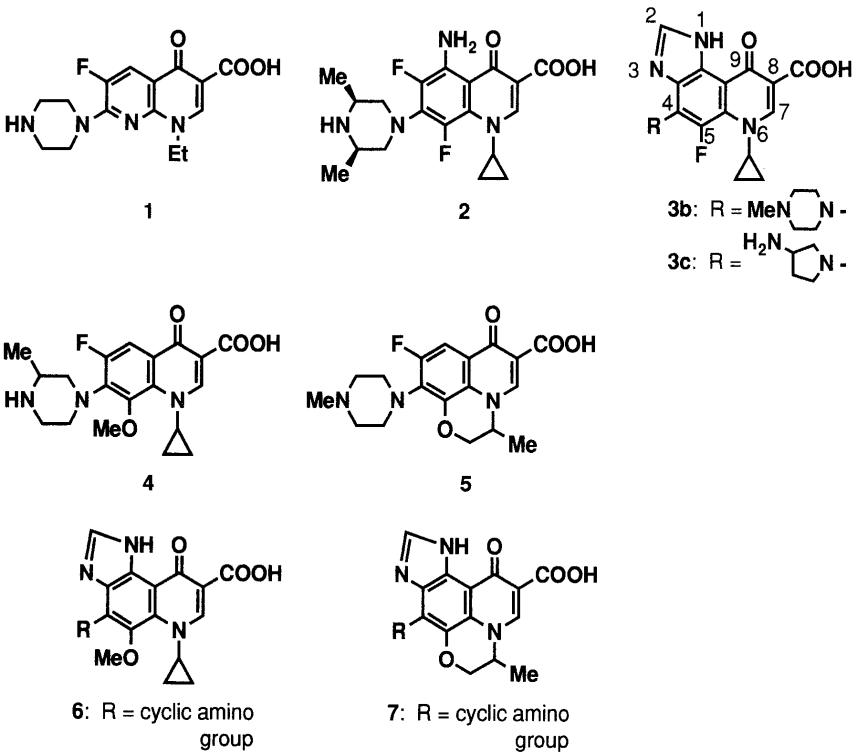

Chart 1

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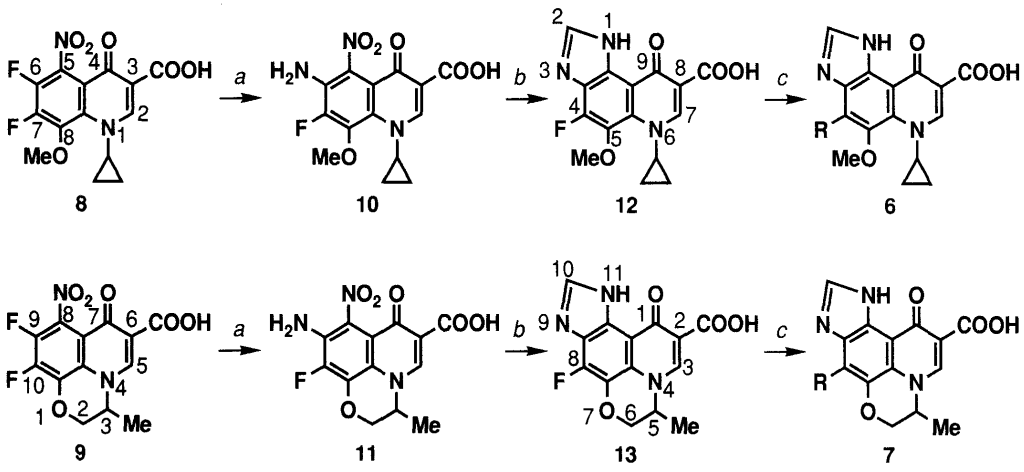


Chart 2

Table 1. Physical Data for the Substituted Imidazoquinolones

Compd.	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd				Found			
				C	H	Cl	N	C	H	Cl	N
6a	243—246 (aq. HCl)	10	$C_{19}H_{21}N_5O_4 \cdot 2HCl \cdot 3/4H_2O$	48.57	5.26	15.09	14.91	48.74	5.10	15.38	14.89
6b	283—286 (dec.) (aq. HCl)	38	$C_{20}H_{23}N_5O_4 \cdot 2HCl \cdot 1/2H_2O$	50.11	5.47	14.79	14.61	49.91	5.56	15.03	14.55
6c	236—238 (dec.) (aq. HCl–EtOH)	41	$C_{19}H_{21}N_5O_4 \cdot 2HCl \cdot H_2O$	48.11	5.31	14.95	14.76	48.05	5.34	14.84	14.49
6d	193—195 (aq. HCl)	11	$C_{20}H_{23}N_5O_4 \cdot 2HCl \cdot 9/4H_2O$	47.02	5.82	13.88	13.71	47.25	5.67	13.47	13.67
7a	290—294 (dec.) (aq. HCl–EtOH)	10	$C_{18}H_{19}N_5O_4 \cdot 2HCl \cdot 2H_2O$	45.20	5.27	14.82	14.64	45.26	5.22	14.55	14.56
7b	286—290 (dec.) (aq. HCl–EtOH)	43	$C_{19}H_{21}N_5O_4 \cdot 2HCl \cdot 2H_2O$	46.35	5.53	14.40	14.22	46.29	5.23	14.39	13.99
7c	275—278 (dec.) (aq. NH_4OH)	25	$C_{18}H_{19}N_5O_4$	58.53	5.18		18.96	58.53	5.24		18.94

in vitro and *in vivo* activities of **3b, c**, as would be reflected in the enhancement of their bioavailability. This paper describes the synthesis and the antibacterial activity of **6** and **7**.

Chemistry In the previous paper,[5] we dealt with two methods for construction of the imidazole ring fused to quinolones. For easy synthesis of the target compounds **6** and **7**, we planned to develop another type of imidazole ring formation (Chart 2).

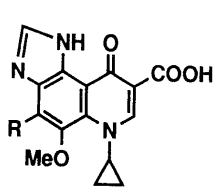
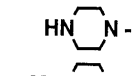
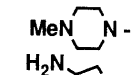
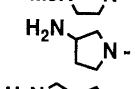
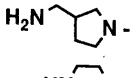
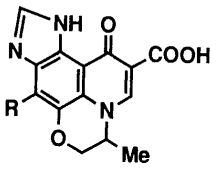
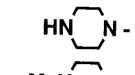
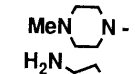
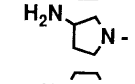
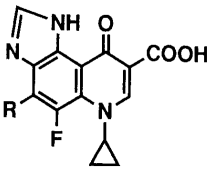
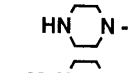
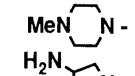
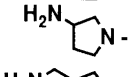
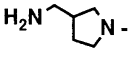
Compounds **6** and **7** were assumed to us to be favorably derived from nitro quinolones **8**[10] and **9**[11] with appropriate functional groups, respectively. Both of the C-6 and C-7 positions of **8** could react with a nucleophile, owing to the electron-withdrawing properties of the C-5 nitro and C-4 oxo groups. The electron-donating effect of the C-8 alkoxy groups of **8** might decrease the reactivity at C-7 of **8**. Hence nucleophilic substitution of **8** would proceed predominantly at its C-6 position. For a similar reason, nucleophilic substitution of **9** would proceed predominantly at C-9. The reaction of **8** and **9** with ammonium carbonate in *N,N*-dimethylformamide (DMF) actually occurred at C-6 and C-9, and gave 6-amino-5-nitroquinolones **10** and **11**, respectively, in high yields.

The nitro groups at C-5 of **10** and at C-8 of **11** were hydrogenated to amino groups, and the resulting intermediate diamines, without isolation, were subsequently treated with triethyl orthoformate and formic acid at 80 °C for 1 h to give the imidazole-fused compounds **12** and **13** in 49 and 67% yields, respectively. Compounds **12** and **13** were allowed to react with a cyclic amine such as piperazine, *N*-methylpiperazine, 3-aminopyrrolidine, or 3-aminomethylpyrrolidine in hot dimethyl sulfoxide (DMSO), thus giving the corresponding displacement products **6a—d** and **7a—c** in 10% to 43% yields after purification.

Antibacterial Activity The *in vitro* antibacterial activity of compounds **6a—d** and **7a—c** was tested against gram-positive (*Staphylococcus aureus* 209P JC-1) and gram-negative bacteria (*Escherichia coli* NIHJ JC-2 and *Pseudomonas aeruginosa* 12). The results are summarized in Table 2, in which the data for enoxacin (**1**), ofloxacin (**5**) and **3a—d** are included for comparison.

Among the 5-methoxy compounds **6a—d**, the activity against gram-positive bacterium (*S. aureus*) was arranged in the decreasing order of 3-aminomethylpyrrolidinyl (**6d**) > 3-aminopyrrolidinyl (**6c**) > piperazinyl (**6a**) > 4-methylpiperazinyl (**6b**) derivatives. The tendency observed in the activity of **6a—d** against *S. aureus* holds true for the ofloxacin type compounds **7a—c** and 5-fluoro compounds **3a—d**. The piperazinyl derivative **6a** among **6a—d** was the most active against gram-negative bacteria (*E. coli* and *P. aeruginosa*). The activity of the 3-aminomethylpyrrolidinyl derivative **6d** against gram-negative bacteria was noticeably weak.

Table 2. *In Vitro* Antibacterial Activity of the Imidazoquinolones and Related Compounds

Compound	R	Minimum inhibitory conc.,[a] (μg/ml)		
		S. aureus 209P JC-1	*E. coli* NIHJ JC-2	*P. aeruginosa* 12
6a	 	0.1	0.1	0.78
6b		0.2	0.1	3.13
6c		0.05	0.2	1.56
6d		0.025	1.56	12.5
7a	 	0.78	25	>100
7b		0.78	0.78	3.13
7c		0.2	1.56	12.5
3a	 	0.2	0.05	0.39
3b		0.2	0.05	0.2
3c		0.05	0.025	0.2
3d		0.025	0.1	0.78
1	Enoxacin	0.39	0.1	0.78
2	Ofloxacin	0.2	0.05	0.78

a) See Experimental.

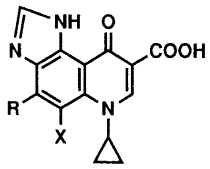
In comparison of the 5-methoxy compounds **6a**—**c** with the corresponding ofloxacin type compounds **7a**—**c** (*i.e.*, **6a** *vs.* **7a**, **6b** *vs.* **7b**, and **6c** *vs.* **7c**), the former, **6a**—**c**, were 4 times or more active than the latter, **7a**—**c**, with the exception of **6b** *vs.* **7b**, which were equipotent against *P. aeruginosa*. The replacement of the C-5 fluorine of **3a**—**d** with a methoxy group (giving **6a**—**d**) caused no change in activity against gram-positive bacterium but diminished the activity against gram-negative bacteria in a range from one-half (the piperazinyl derivative **6a** *vs.* **3a**) to one-sixteenth (the 3-aminomethylpyrrolidinyl derivative **6d** *vs.* **3d**).

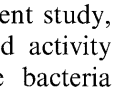
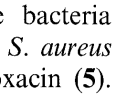
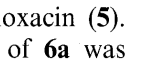
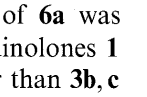
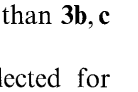
Among the compounds prepared in the present study, **6a** showed the most potent and well-balanced activity toward the gram-positive and gram-negative bacteria tested. The antibacterial activity of **6a** against *S. aureus* was superior to that of enoxacin (**1**) and ofloxacin (**5**). Against gram-negative bacteria, the activity of **6a** was almost comparable to that of the reference quinolones **1** and **5**. Compound **6a**, however, was not better than **3b**, **c** in *in vitro* activity, contrary to our expectation.

The 5-methoxy compounds **6a**—**c** were selected for testing their efficacy on systemic infection due to *P. aeruginosa* 12 in mice. It follows from the data listed in Table 3 that **6a**—**c** are less efficacious than **3a**,**b**, enoxacin (**1**), and ofloxacin (**5**). A remarkable decrease in oral efficacy (ED_{50}, *p.o.*) of **6a**—**c** was observed as compared to their intravenous efficacy (ED_{50}, i.v.).

In summary, we developed an easy method for the construction of the imidazo[4,5-*f*]quinolone ring and synthesized 4-substituted 6-cyclopropyl-6,9-dihydro-5-methoxy-9-oxo-1*H*-imidazo[4,5-*f*]quinoline-8-carboxylic acids (**6a**—**d**). Compounds **6a**—**d** were found to have considerable potency in terms of antibacterial activity; in particular, the 4-piperazinyl compound **6a** was more active against *S. aureus* than enoxacin (**1**) and ofloxacin

Table 3. *In Vivo* Efficacy of Selected and Reference Compounds on Systemic Infections



Compd.	X	R	*P. aeruginosa* 12		
			MIC[a]	ED_{50} (*p.o.*)[b]	ED_{50} (i.v.)[b]
6a	OMe		0.78	>25	4.04
6b	OMe		3.13	34.7	6.25
6c	OMe		1.56	>100	3.13
3b	F		0.2	8.42	0.982
3c	F		0.2	36.0	0.443
1	Enoxacin		0.78	8.41	—
5	Ofloxacin		0.78	6.62	2.82

a) Minimum inhibitory concentration (μg/ml). *b*) Shown in milligrams per kilogram. See Experimental.

(**5**). The activity of **6a**—**d** against gram-negative bacteria, however, was less than that of the corresponding 5-fluoro compounds **3a**—**d** which had been reported in the previous paper.[5] The oral protective efficacy of **6a**—**c** did not reflect their *in vitro* activity.

Experimental

Chemistry All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Jasco A-102 or Perkin Elmer 1600 Series FTIR spectrophotometer. ^{1}H-NMR spectra were taken at 80 MHz on a Varian FT-80A spectrometer or at 200 MHz on a Varian Gemini-200 spectrometer. Chemical shifts are expressed in ppm (δ) with tetramethylsilane as an internal standard. Mass spectra were obtained on a JEOL JMS D-300 mass spectrometer for electron impact-mass spectrum (EI-MS), Hitachi M-80B mass spectrometer for secondary ion mass spectrum (SI-MS), or Hitachi M-1000 LC API mass spectrometer for atmospheric pressure chemical ionization mass spectrum (APCI-MS). The spectral data for all compounds were obtained and were consistent with the assigned structures. All solid compounds were analyzed for C, H, F, and N.

6-Amino-1-cyclopropyl-7-fluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic Acid (10) A mixture of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-8-methoxy-5-nitro-4-oxoquinoline-3-carboxylic Acid (**8**)[10] (16.7 g, 48.8 mmol) and $(NH_4)_2CO_3$ (14.1 g, 147 mmol) in DMF (50 ml) was heated at 90 °C for 2 h. To the suspended mixture was added a mixture of ice and diluted HCl. The resulting solid was collected by filtration, washed successively with water, EtOH, and iso-Pr_2O, and then dried to give 16.2 g (98%) of **10**, mp 242—245 °C (dec., DMF–EtOH). IR (KBr) cm^{-1}: 3330, 3222, 1727, 1644. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.00—1.25 (4H, m, cyclopropyl C$\underline{H}_2$C$\underline{H}_2$), 4.04 (3H, d, J=2.0 Hz, Me), 4.10—4.25 (1H, m, cyclopropyl CH), 6.50 (2H, br s, NH_2), 8.63 (1H, s, 2-H), 14.40 (1H, br s, COOH). SI-MS m/z: 338 (M^++1), 320. *Anal.* Calcd for $C_{14}H_{12}FN_3O_6$: C, 49.86; H, 3.59; F, 5.63; N, 12.46. Found: C, 50.00; H, 3.53; F, 5.56; N, 12.34.

9-Amino-10-fluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (11) According to the same method for the preparation of **10**, 9,10-difluoro-2,3-dihydro-3-methyl-8-nitro-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (**9**)[11] (7.50 g, 23.0 mmol) was treated and gave 6.76 g (91%) of **11**, mp>300 °C (aqueous ammonia). IR (KBr) cm^{-1}: 3460, 1710, 1645, 1610. ^{1}H-NMR (80 MHz, NaOD–D_2O) δ: 1.50 (3H, d, J=6.5 Hz, Me), 4.3—4.9 (3H, m, OC$\underline{H}_2$C$\underline{H}$N), 8.30 (1H, s, 5-H). EI-MS m/z: 323 (M^+), 279. *Anal.* Calcd for $C_{13}H_{10}FN_3O_6$: C, 48.31; H, 3.12; F, 5.88; N, 13.00. Found: C, 48.60; H, 3.18; F, 5.66; N, 13.18.

6-Cyclopropyl-4-fluoro-6,9-dihydro-5-methoxy-9-oxo-1*H*-imidazo[4,5-*f*]quinoline-8-carboxylic Acid (12) A mixture of **10** (15.50 g, 46.0 mmol) and DMF (300 ml) was hydrogenated over 5% Pd–C (1.55 g) under atmospheric pressure at 50 °C for 8 h. The mixture was filtered to remove the catalyst. The filtrate was allowed to react with $HC(OEt)_3$ (150 ml) and HCOOH (7.5 ml) at 80 °C for 1 h. The mixture was concentrated *in vacuo* to leave a solid residue, which was triturated with EtOH. The resultant solid was collected by filtration, washed successively with EtOH and iso-Pr_2O, and then dried to give 6.91 g (49%) of **12**, mp 281—282 °C (dec., $CHCl_3$–EtOH). IR (KBr) cm^{-1}: 1728, 1614. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.05—1.30 (4H, m, cyclopropyl C$\underline{H}_2$C$\underline{H}_2$), 4.00 (3H, s, Me), 4.25—4.40 (1H, m, cyclopropyl CH), 8.42 (1H, s, 2-H), 8.82 (1H, s, 7-H), 13.38 (1H, br s, NH), 14.94 (1H, br s, COOH). SI-MS m/z: 318 (M^++1), 300, 273. *Anal.* Calcd for $C_{15}H_{12}FN_3O_4$: C, 56.78; H, 3.81; F, 5.99; N, 13.24. Found: C, 56.72; H, 3.63; F, 5.95; N, 13.21.

8-Fluoro-1,5,6,11-tetrahydro-5-methyl-1-oxo-imidazo[4,5-*g*]pyrido[1,2,3-*de*][1,4]benzoxazine-2-carboxylic Acid (13) According to the same method for the preparation of **12**, **11** (2.00 g, 6.19 mmol) was treated and gave 1.26 g (67%) of **13**, mp >300 °C (aqueous ammonia). IR (KBr) cm^{-1}: 3362, 1712, 1645, 1622. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.51 (3H, d, J=7.0 Hz, Me), 4.40 (1H, dd, J=2.0, 11.5 Hz, OCH), 4.63 (1H, dd, J=1.5, 11.5 Hz, OCH), 5.07 (1H, br q, J=7.0 Hz, 5-H), 8.40 (1H, s, 10-H), 9.13 (1H, s, 3-H), 13.4 (1H, br s, NH), 15.22 (1H, br s, COOH). EI-MS m/z: 303 (M^+), 259. *Anal.* Calcd for $C_{14}H_{10}FN_3O_4$: C, 55.45; H, 3.32; F, 6.26; N, 13.86. Found: C, 55.10; H, 3.64; F, 6.17; N, 13.70.

4-Substituted 6-Cyclopropyl-6,9-dihydro-5-methoxy-9-oxo-1*H*-imidazo[4,5-*f*]quinoline-8-carboxylic Acids (6a—d) and 8-Substituted 1,5,6,11-Tetrahydro-5-methyl-1-oxo-imidazo[4,5-*g*]pyrido[1,2,3-*de*][1,4]benzoxazine-2-carboxylic Acids (7a—c) A mixture of **12** (500 mg, 1.577 mmol) and piperazine (678 mg, 7.88 mmol) in DMSO (5.0 ml) was heated at 130—140 °C for 2 h. The solvent was distilled *in vacuo*. The residue was triturated with EtOH. The resultant solid was collected by filtration, washed successively with EtOH and iso-Pr_2O, and then dried to give 567 mg of a solid. Recrystallization from aqueous HCl gave 75 mg (10%) of **6a**·2HCl·3/4H_2O. IR (KBr) cm^{-1}: 3360. 1702, 1617. ^{1}H-NMR (200 MHz, D_2O) δ: 0.95—1.1, 1.2—1.4 (both 2H, m, total cyclopropyl C$\underline{H}_2$C$\underline{H}_2$), 3.55—3.7, 3.9—4.1 (both 4H, m, total 2×HNC$\underline{H}_2$C$\underline{H}_2$N), 3.84 (3H, s, OMe), 4.25—4.4 (1H, m, cyclopropyl CH), 8.90 (1H, s, 7-H), 8.92 (1H, s, 2-H). APCI-MS m/z: 384 (M^++1).

In a similar manner, compounds **6b**—**d** and **7a**—**c** were prepared from **12** and **13**, respectively (see Table 1).

***In Vitro* Antibacterial Activity** According to the assay method recommended by the MIC Committee of the Japan Society of Chemotherapy,[12] the MIC (in microgram per milliliter) was determined by the two-fold agar dilution method using Mueller-Hinton agar (pH 7.4, Difco); the bacterial inocula contained approximately 10^6 colony-forming units and the bacterial growth was observed after 20 h of incubation at 37 °C.

***In Vivo* Efficacy on Systemic Infections** An *in vivo* activity assay was carried out according to the method of Nakamura, *et al.*[13] Groups of 8 or more male mice (Std-ddY, 20±2 g) were infected with *P. aeruginosa* 12 (i.p., 4×10^3 cells). For evaluation of ED_{50} (*p.o.*), the test compounds were suspended in 0.4% carboxymethyl cellulose sodium salt and administered orally at 0 and 6 h post-infection. For the determination of ED_{50} (i.v.), the test compounds were dissolved in water with equimolar NaOH and injected intravenously at 0 and 6 h post-infection. Survival rates were evaluated after 1 week, and ED_{50} (*p.o.*) and ED_{50} (i.v.) were calculated from the rates.

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References and Notes

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