Synthesis of Antimicrobial Agents. IV.

Synthesis of 1-Hydroxypiperazine Dihydrochloride and its Applications to Pyridone Carboxylic Acid Antibacterial Agents

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1-Hydroxypiperazine dihydrochloride **7** was prepared and it was applied to the syntheses of new pyridone carboxylic acid antibacterial agents (PCA-antibacterial agents). 1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(4-hydroxypiperazin-1-yl)-4-oxoquinoline-3-carboxylic acid **13** showed the most potent antibacterial activity.

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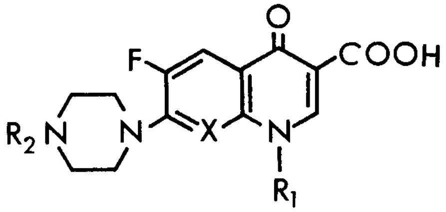
We recently found [1] that the microbiological and toxicological profile of the 6-fluoro-7-(4-hydroxypiperazin-1-yl) quinolone derivatives **1**, **2** and **3** were superior than the corresponding deoxy derivatives, norfloxacin (**NFLX**), ciprofloxacin (**CPFX**) and enoxacin (**ENX**). In order to apply the 4-hydroxypiperazin-1-yl group to other PCA-antibacterial agents such as 6,8-difluoroquinolone derivatives **16** [2], **17** and **18** [3], which have been reported to be more potent than the corresponding 6-fluoro derivatives, we attempted the preparation of 1-hydroxypiperazine diiodide according to Rida *et al.* [4], but in spite of our efforts, piperazine diiodide was only obtained.



	R_1	R_2	X
1	Et	OH	CH
NFLX	Et	H	CH
2		OH	CH
CPFX		H	CH
3	Et	OH	N
ENX	Et	H	N

This paper describes the preparation of 1-hydroxypiperazine dihydrochloride (**7**) and its applications to the syntheses of PCA-antibacterial agents containing the 4-hydroxypiperazin-1-yl group. Firstly, 4-(2-ethoxycarbonylethyl)-1-formylpiperazine (**5**) was prepared by the reaction of 1-formylpiperazine (**4**) with ethyl acrylate, and then **5** was allowed to react with hydrogen peroxide [5] in the presence of sodium tungstate to give 4-hydroxy-1-formylpiperazine **6**. Finally, **6** was hydrolized with hydrochloric acid to afford 1-hydroxypiperazine dihydrochloride (**7**), which was converted to the free base **8** by treatment with alkali (Scheme 1). The structure of **8** was confirmed by its mass and infrared (potassium bromide) spectral data: m/e 102 (M^+), 85 (M^+-OH); 3178 cm^{-1} (ν O-H). The dipicrate **9** was also obtained, and its physical data (melting point and infrared spectral data) were found to be in disagreement with the data of so-called "1-hydroxypiperazine dipicrate" reported by Rida *et al.* [4]. We consider that Rida *et al.* obtained another compound rather than **9**.

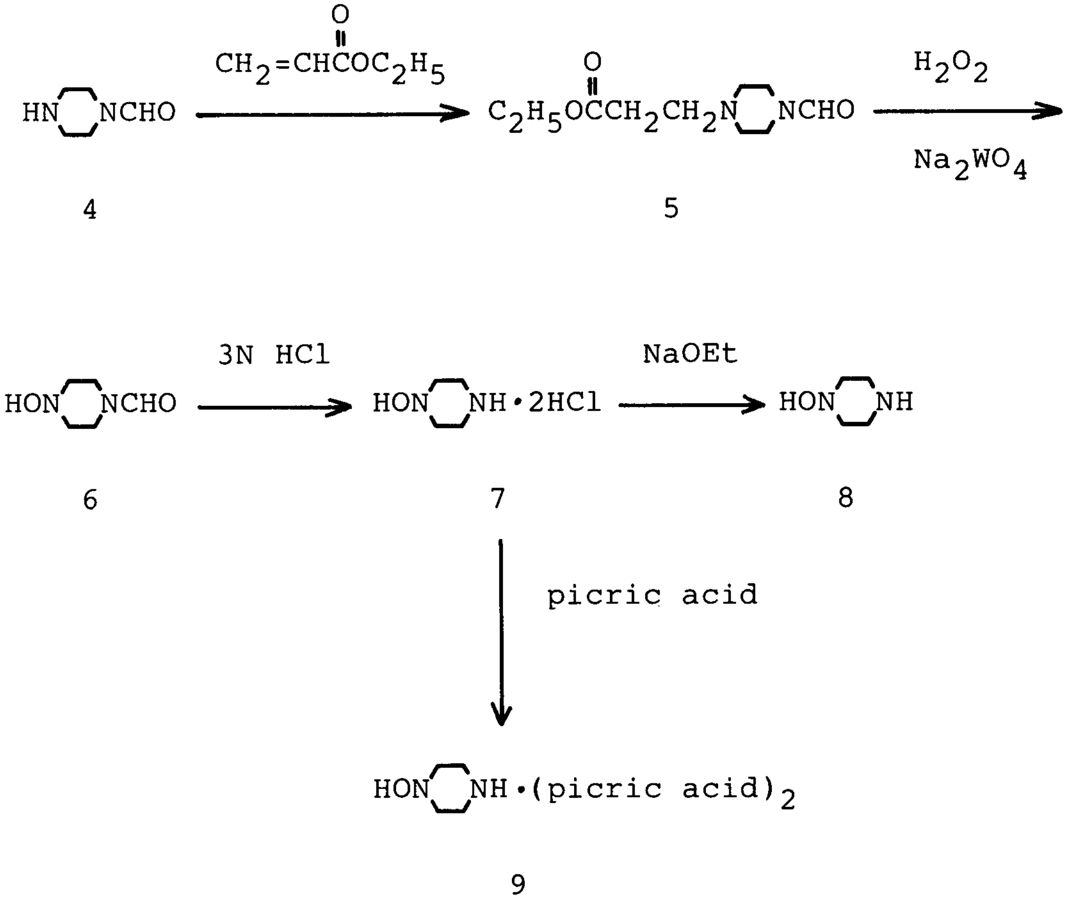
Table I

Antibacterial Activities and Acute Toxicities

	S. aureus IID 803		*E. coli* KC-14		LD_{50}
Compound	MIC [a] (μg/ml)	ED_{50} [b] (mg/kg)	MIC (μg/ml)	ED_{50} (mg/kg)	(p.o.) (mg/kg)
13	0.39	3.84 (2.91-5.07) [c]	0.05	0.19 (0.13-0.26)	>4000
16	0.20	5.07 (3.84-6.69)	0.0125	0.28 (0.19-0.42)	1414
14	0.78	26.8 (19.1-37.6)	0.20	0.84 (0.60-1.17)	>4000
17	0.78	40.6 (26.2-63.0)	0.10	1.46 (0.94-2.26)	1072
15	1.56	107 (76.3-151)	0.39	1.46 (1.04-2.04)	>4000
18	1.56	123 (93.3-162)	0.20	5.82 (4.15-8.18)	NT [d]
NFLX	1.56	93.3 (66.4-131)	0.10	5.82 (3.76-9.03)	>4000
CPFX	0.39	27.3 (17.2-43.2)	0.05	1.46 (1.04-2.04)	>4000
ENX	0.78	13.4 (8.64-20.8)	0.20	2.53 (1.63-3.93)	NT

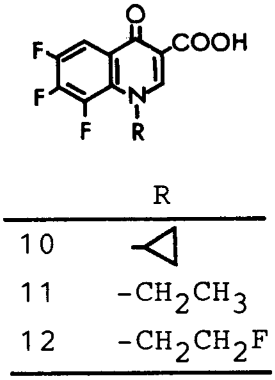
[a,b] See the Experimental. [c] 95% Confidence linits. [d] Not tested.

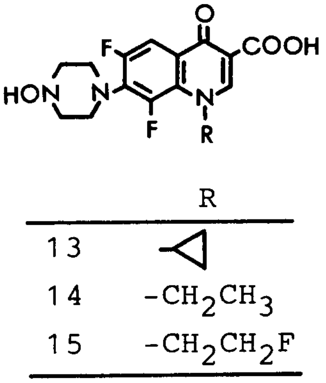
Scheme 1

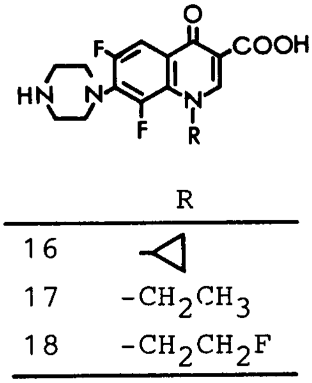


For applications of **7** to the syntheses of new PCA-antibacterial agents, **7** was allowed to react with the trifluoro-PCAs **10** [2], **11** and **12** [3]. As a result, 6,8-difluoro-7-(4-hydroxypiperazin-1-yl) derivatives **13**, **14** and **15** were obtained in good yields. Against experimental infections in mice with *S. aureus* IID 803 and *E. coli* KC-14, compounds **13**, **14** and **15** showed higher efficacy than the corresponding deoxy derivatives **16**, **17** and **18**. The results are summarized in Table I together with the minimal inhibitory concentrations (MIC) against the infection organisms and the 50% lethal doses (LD_{50}) by oral admistration in mice. The data for clinically used PCA-antibacterial agents, **NFLX**, **CPFX** and **ENX** are also included for comparison.

As a conclusion, the 4-hydroxypiperazin-1-yl group was shown to be a more beneficial group than the piperazine-1-yl group in PCA-antibacterial agents, and 1-hydroxypiperazine dihydrochloride **7** is a useful reagent for the development of better antibacterial agents.







EXPERIMENTAL

Melting points were measured with a Yanagimoto micro melting point apparatus, and are uncorrected. Infrared (ir) spectra were measured on a Hitachi 270-50 infrared spectrometer. Proton nuclear magnetic resonance (pmr) spectra were recorded on a JEOL PS-100 spectrometer with tetramethylsilane as an internal reference. Mass spectra (ms) were measureed with a Hitachi M-60 mass spectrometer.

In Vitro Antibacterial Activity.

All MIC values of PCAs were measured by the serial dilution method [6].

In Vivo Antibacterial Activity.

The *in vivo* antibacterial activity of the test compounds were determined in ddY-strain male mice (20-25 g body weight, five per group). The test compounds were dispersed in distilled water or in 5% sodium carboxymethylcellulose solution (5% CMC) to the desired concentration. *S. aureus* IID 803 and *E. coli* KC-14 were incubated in tripticase-soy broth at 37° for 18 hours, and the culture was diluted in 5% (w/v) mucin, then 0.5 ml was injected intraperitoneally into mice. The mice were treated orally with the test compounds at 1 hour after infection. Fifty percent effective doses (ED_{50}) were calculated from the cumulative mortalities on the seventh day after infection by using the trimmed version of the Weil method.

Acute Toxicity.

A suspension of each of the test compounds in a 0.5% CMC was administrated orally to ddY-strain male mice (20-25 g body weight, five per group). Seven days later, LD_{50} values were determined by using the Weil method.

4-(2-Ethoxycarbonylethyl)-1-formylpiperazine (**5**).

Ethyl acrylate (326 g) was added to a solution of **4** (320 g) in chloroform (2 l), and the reaction mixture was stirred at room temperature for 3 days. The solvent was evaporated under reduced pressure to yield **5** (580 g) as an orange oil; pmr (deuteriochloroform): δ 1.25 (3H, t, J = 8 Hz, CH_2CH_3), 2.25-2.90 (8H, m), 3.20-3.70 (4H, m), 4.03 (2H, q, J = 8 Hz, CH_2CH_3), 8.01 (1H, s, CHO).

1-Formyl-4-hydroxypiperazine (**6**).

To a solution of the mixture of **5** (114 g) and sodium tungstate dihydrate (7.25 g) in water (500 ml), 31% hydrogen peroxide (82 ml) was added dropwise with stirring at 30-35°. The mixture was stirred for 4 hours at room temperature and for 5 hours at 50-55°, then was shaken with ethyl acetate (300 ml). The aqueous layer was evaporated under reduced pressure to afford a dark brown oil. The oil was applied to silica-gel column chromatography (10:1 chloroform-methanol) and was recrystallized from ethyl acetate to afford **6** (38.2 g) as pale yellow prisms, mp 118-124°; pmr (deuteriochloroform): δ 2.20-4.50 (8H, m, piperazine ring), 6.72 (1H, broad s, OH), 8.00 (1H, s, CHO); ms: m/e 130 (M^+).

Anal. Calcd. for $C_5H_{10}N_2O_2$: C, 46.14; H, 7.75; N, 21.52. Found: C, 46.12; H, 7.57; N, 21.55.

1-Hydroxypiperazine Dihydrochloride (**7**).

A solution of **6** (5.0 g) in 3*N* hydrochloric acid (50 ml) was stirred at 77-87° for 20 minutes. Water was removed under reduced pressure to afford a pale yellow residue. After washing with ethanol, the residue was recrystallized from a mixture of water and ethanol to give **7** (5.0 g) as light brown prisms, mp 164-175° dec; pmr (DMSO-d_6): δ 3.10-3.80 (8H, m, piperazine ring), 9.4-10.1 (1H, m); ms: m/e 102 (M^+).

Anal. Calcd. for $C_4H_{10}N_2O \cdot 2HCl$: C, 27.44; H, 6.91; N, 16.00. Found: C, 27.18; H, 6.96; N, 15.97.

1-Hydroxypiperazine (**8**).

To a sodium ethoxide-ethanol solution, which was prepared by addition of sodium (0.69 g) to ethanol (80 ml), **7** (2.63 g) was added. After being stirred at room temperature for 15 minutes, acetone (80 ml) was added, then insoluble materials were filtered off. The filtrate was evaporated under reduced pressure, and the resulting residue was sublimated (70-75°, 0.15 mm Hg) to afford **8** (1.44 g) as colorless prisms, mp 130.0-131.5°; ir (potassium bromide): 3178, 1511, 852 cm^{-1}; pmr (deuteriochloroform): δ 2.36-3.40 (m); ms: m/e (relative intensity) 102 (M^+, 20), 85 (100), 56 (81).

Anal. Calcd. for $C_4H_{10}N_2O$: C, 47.04; H, 9.87; N 27.43. Found: C, 46.91; H, 10.10; N, 27.38.

1-Hydroxpiperazine Dipicrate (**9**).

To a solution of **7** (1 g) in water (2 ml), ethanol saturated with picric acid was added. The resulting precipitate was collected by filtration, washed with water and ethanol, and recrystallized from a mixture of acetone and water to afford **9** (783 mg) as pale yellow prisms, mp 210-221° dec; ir (potassium bromide): 2800, 2770, 2530, 1630, 1608, 1565, 1556, 1528, 936, 907 cm^{-1}; pmr (DMSO-d_6-deuterium oxide): δ 2.70-3.60 (8H, m, piperazine ring), 8.61 (4H, s, phenyl); ms: m/e 102 (M^+, 1-hydroxypiperazine), 229 (M^+, picric acid).

Anal. Calcd. for $C_{16}H_{16}N_8O_{15}$: C, 34.30; H, 2.88; N, 20.00. Found: C, 34.45; H, 2.89; N, 20.28.

1-Cyclopropyl-6.8-difluoro-1,4-dihydro-7-(4-hydroxypiperazin-1-yl)-4-oxoquinoline-3-carboxylic Acid (**13**) and It's Hydrochloride Salt.

A mixture of **10** (5.0 g), which was synthesized in accordance with the literature [2], **7** (4.64 g) and triethylamine (8.9 g) in DMSO (25 ml) was stirred at 130° for 45 minutes. After cooling the resulting solid was collected by filtration, washed with water, and recrystallized from a mixture of DMF and water to afford **13** (4.6 g) as pale yellow prisms, mp 223-227° dec; pmr (DMSO-d_6): δ 1.10-1.30 (4H, m, cyclopropyl), 2.42-3.60 (8H, piperazine ring), 4.10-4.20 (1H, m, cyclopropyl), 7.64 (1H, dd, J = 14 and 2 Hz, 5-H), 8.08 (1H, s, OH), 8.50 (1H, s, 2-H); ms: m/e 365 (M^+).

Anal. Calcd. for $C_{17}H_{17}F_2N_3O_4$: C, 55.89; H, 4.69; N, 11.50. Found: C, 55.90; H, 4.69; N, 11.56.

The hydrochloride salt of **13** was prepared by the crystallization from a mixture of DMF and 3*N* hydrochloric acid, mp 250-258° dec.

Anal. Calcd. for $C_{17}H_{17}F_2N_3O_4 \cdot HCl$: C, 50.82; H, 4.51; N, 10.46. Found: C, 50.81; H, 4.52; N, 10.38.

Compounds **14**, **15** and their hydrochloride salts were prepared from **11** [3] and **12** [3], respectively, by the similar method described for the preparation of **13** and its hydrochloride salt. Physical data are as follows; **14**, mp 253-260° dec; pmr (DMSO-d_6): δ 1.48 (3H, t, J = 6 Hz, ethyl), 2.40-3.80 (8H, m, piperazine ring), 4.40-4.80 (2H, m, ethyl), 7.80 (1H, dd, J = 12 and 2 Hz, 5-H), 8.22 (1H, s, OH), 8.86 (1H, s, 2-H), 14.61 (1H, broad s, COOH).

Anal. Calcd. for $C_{16}H_{17}F_2N_3O_4$: C, 54.39; H, 4.85; N, 11.85. Found: C, 54.29; H, 4.77; N, 11.93.

The hydrochloride salt of **14** had mp 252-265° dec.

Anal. Calcd. for $C_{16}H_{17}F_2N_3O_4 \cdot HCl$: C, 49.30; H, 4.65; N, 10.78. Found: C, 49.26; H, 4.66; N, 10.74.

Compound **15** had mp 220-229° dec; pmr (DMSO-d_6): δ 2.40-3.80 (8H, m, piperazine ring), 4.50-5.30 (4H, m, 2-fluoroethyl), 7.86 (1H, dd, J = 12 and 2 Hz, 5-H), 8.21 (1H, s, OH), 8.83 (1H, s, 2-H), 14.40 (1H, broad s, COOH).

The hydrochloride salt of **15** had mp 210-222° dec.

Anal. Calcd. for $C_{16}H_{16}F_3N_3O_4 \cdot HCl \cdot H_2O$: C, 45.13; H, 4.50; N, 9.87. Found: C, 45.28; H, 4.48; N, 9.76.

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