Studies on Pyridonecarboxylic Acids. IV.[1a] Synthesis and Antibacterial Activity Evaluation of *S*-(−)- and *R*-(+)-6-Fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic Acids

Jun SEGAWA, Kenji KAZUNO, Masato MATSUOKA, Isao AMIMOTO, Masakuni OZAKI, Masato MATSUDA, Yoshifumi TOMII, Masahiko KITANO, and Masahiro KISE*

Chemistry Laboratories and Biology Laboratories, Nippon Shinyaku Company, Ltd., Nishioji Hachijo, Minami-ku, Kyoto 601, Japan. Received January 20, 1995; accepted February 24, 1995

Optically active isomers of 6-fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylic acid (NM394, 3) were prepared through optical resolution of their racemic intermediate (±)-1 by high-performance liquid chromatography (HPLC). The absolute configuration at the C-1 position in the thiazetoquinolone ring of (−)-3 was confirmed by X-ray analysis of (−)-4 to be *S*. The *in vitro* antibacterial activity of (−)-3 was 2—8 times that of (+)-3.

Key words thiazetoquinolone; optically active isomer; antibacterial activity; X-ray analysis; optical resolution; NM394

Quinolones are believed to have considerable potential as a source of new antibacterial agents.[2] In our search for new, potent quinolones, we have been studying tricyclic compounds characterized by an S-bridge between C-2 and the substituent at the N-1 of quinolones.[1] Among these derivatives, compound **3** (NM394) showed excellent *in vitro* antibacterial activity.[1b,c] Compound **3** has a methyl group at the C-1 position in the thiazetoquinolone ring, producing an asymmetric center at this position (Fig. 1). Recently, various optically active bicyclic quinolones[3,4] have been prepared, including a series of potent tricyclic compounds having a chiral center, such as S-25930 (**5**),[5a] ofloxacin (**6**)[5b] and T-3761 (**7**).[5c] In these compounds, the *S*-(−) isomers have been reported to be far more potent than the R-(+) isomers in terms of *in vitro* antibacterial activity against both gram-positive and -negative bacteria. Our interest was directed to the relationship between the two optical isomers of **3** and their antibacterial activity. In this paper, we report the synthesis and antibacterial activity of optically active isomers of **3**.

Chemistry and X-Ray Analysis The 6,7-difluoro-3-carboxylate (**1**)[1b] was resolved by HPLC to yield optically pure (−)-**1** and (+)-**1**. The optically active **3** was prepared by the same procedure as used for the preparation of the racemate[1b] by using (−)-**1** or (+)-**1**, as illustrated in Chart 1. Treatment of (−)-**1** with piperazine gave the (−)-7-(1-piperazinyl)-3-carboxylate (−)-**2**. Hydrolysis of (−)-**2** afforded the 3-carboxylic acid (−)-**3**. Similarly, (+)-**1** was converted through (+)-**2** to (+)-**3**. The enantiomeric purity of these compounds was determined by HPLC using a ligand exchange column.[6] Compound (−)-**4** was prepared by treatment of (−)-**1** with 1-methylpiperazine.

The absolute configuration of the enantiomers ((−)-**3**, (+)-**3**) was determined by a single-crystal X-ray analysis of (−)-**4**. The X-ray crystal structure of (−)-**4**, shown in Fig. 2, revealed that the absolute configuration at the C-1 position (C13, C32) is *S*.

Biology The *in vitro* antibacterial activities of the optical isomers of NM394 ((−)-**3**, (+)-**3**) together with the racemate (±)-**3** against gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) are shown in Table 1. The *in vitro* antibacterial activity of (−)-**3** was 2—8 times that of (+)-**3** against gram-positive and gram-negative bacteria. The same enantiomers (*S*-(−)-compounds) of **5**—**7** have been reported to be more active than the *R*-(+)-compounds to the extent of 8- to 128-fold against both gram-positive and gram-negative bacteria.[3]

Experimental

Chemistry Melting points are uncorrected. Elemental analyses were done with a Yanaco CHN Corder MT-3 element analyzer. ^{1}H-NMR spectra were recorded on a 200-MHz Varian XL-200 or a 60-MHz Hitachi R-24-B spectrometer with Me_4Si as the internal standard. IR spectra were recorded on a Shimadzu IR-453-U-03 spectrometer. Optical rotations were recorded on a Horiba SEPA-200 polarimeter. Column chromatographic separation was conducted on Wako Gel C-200.

***In Vitro* Antibacterial Activity** Minimum inhibitory concentrations (MICs) were determined by the agar dilution method recommended by the Japan Society of Chemotherapy.[7] The bacterial inoculum contained approximately 10^6 colony-forming units/ml, and bacterial growth was monitored after 20 h of incubation at 37 °C.

Optical Resolution of (±)-Ethyl 6,7-Difluoro-1-methyl-4-oxo-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylate (±)-1 Compound (±)-**1**

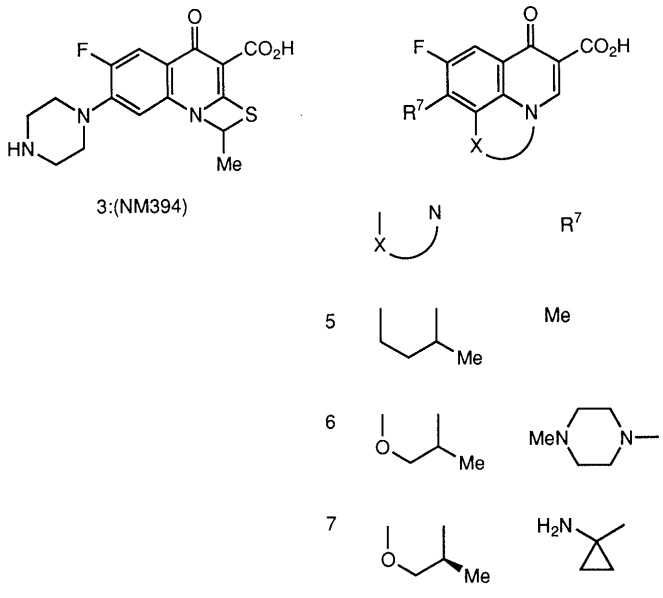
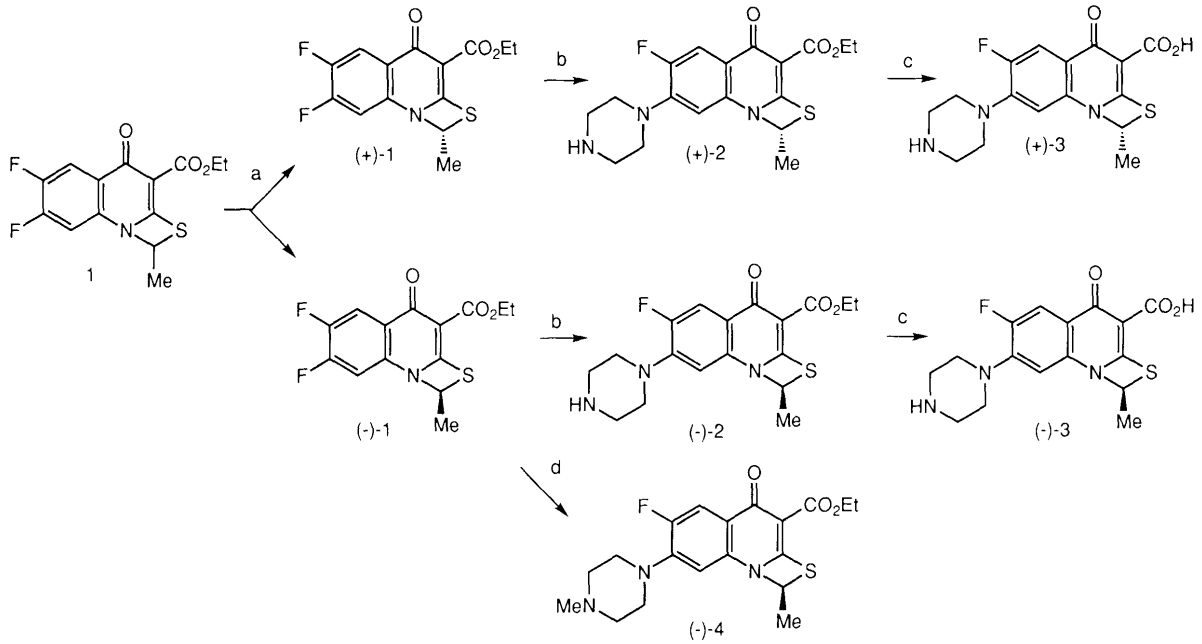


Fig. 1

* To whom correspondence should be addressed.



(a) HPLC resolution; (b) piperazine, CH_3CN-DMSO; (c) i) KOH, H_2O ii) H^+; (d) 1-methylpiperazine, DMSO

Chart 1

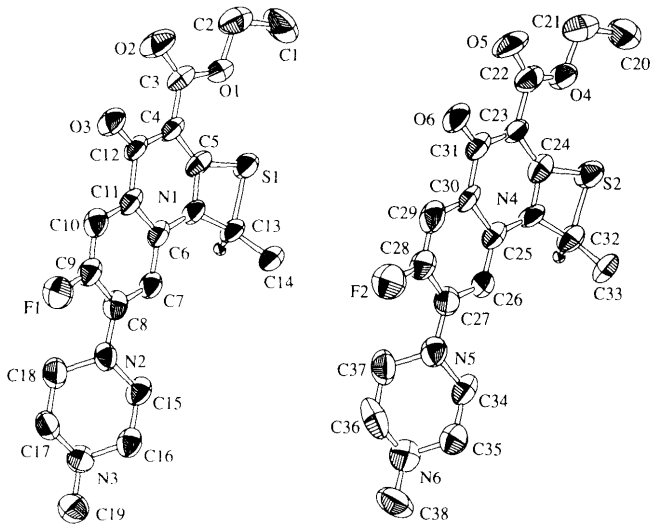


Fig. 2. Perspective ORTEP Drawing of Compound (−)-**4**

Table 1. *In Vitro* Antibacterial Activities[a)]

Organism	*MIC* (μg/ml)		
	(−)-**3**	(+)-**3**	(±)-**3**
Staphylococcus aureus FDA 209-P JC-1	0.025	0.20	0.025
Bacillus subtilis ATCC 6633	0.05	0.20	0.05
Escherichia coli NIHJ JC-2	0.012	0.05	0.012
Pseudomonas aeruginosa ATCC 27853	0.20	0.39	0.20

a) See Experimental.

(4.0 g, 12.8 mmol) was dissolved in 4.8 l of hexane–isopropanol–diethylamine (50 : 50 : 0.1) and the solution was subjected to HPLC to give the optically pure isomers (−)-**1** (1.9 g) and (+)-**1** (1.9 g). Each isomer (54.0 g) was obtained by repeating this operation. HPLC: Chiralcel OF column (500 × 200 mm i.d.) (Daicel Chemical Industries, Ltd.). Solvent, hexane : isopropanol : diethylamine = 50 : 50 : 0.1. Flow rate, 500 ml/min. Retention times, (+)-**1**, 225 min; (−)-**1**, 341 min.

Compound (−)-**1**: mp 206—207 °C; $[\alpha]_D^{20}$ −148.02° (c = 0.96, dimethylformamide (DMF)); IR 1720 cm^{-1}; ^{1}H-NMR ($CDCl_3$ + CF_3CO_2D) δ 1.48 (3H, t, J = 7 Hz), 2.31 (3H, d, J = 6 Hz), 4.61 (2H, q, J = 7 Hz), 6.60 (1H, q, J = 6 Hz), 7.60 (1H, dd, J = 6, 9 Hz), 8.31 (1H, dd, J = 8,9 Hz). *Anal.* Calcd for $C_{14}H_{11}F_2NO_3S$: C, 54.02; H, 3.56; N, 4.50. Found: C, 54.21; H, 3.58; N, 4.62.

Compound (+)-**1**: mp 206—207 °C; $[\alpha]_D^{20}$ +146.96° (c = 1.15, DMF); The IR and the ^{1}H-NMR spectra were identical with those of (−)-1. *Anal.* Calcd for $C_{14}H_{11}F_2NO_3S$: C, 54.02; H, 3.56; N, 4.50. Found: C, 54.01; H, 3.42; N, 4.65.

Ethyl (−)-6-Fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylate (−)-2 A mixture of (−)-**1** (63.6 g, 0.20 mol) and piperazine (35.2 g, 0.41 mol) in dimethylsulfoxide (DMSO) (318 ml) and acetonitrile (318 ml) was stirred at 85 °C for 1 h. The precipitate was collected by filtration and washed with acetonitrile to give 71.2 g (92%) of (−)-**2** as a colorless powder. mp 226—227 °C (dec.); $[\alpha]_D^{20}$ −121.08° (c = 0.74, DMF); IR 1725 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 1.48 (3H, t, J = 7 Hz), 2.28 (3H, d, J = 6.5 Hz), 3.6—4.0 (8H, m), 4.60 (2H, q, J = 7 Hz), 6.51 (1H, q, J = 6.5 Hz), 6.97 (1H, d, J = 7 Hz), 8.01 (1H, d, J = 12 Hz). *Anal.* Calcd for $C_{18}H_{20}FN_3O_3S \cdot 1/5H_2O$: C, 56.74; H, 5.39; N, 11.02. Found: C, 56.73; H, 5.27; N, 10.72. Compound (+)-**2** was obtained from (+)-**1** in 85% yield by using the same procedure as above. mp 226—227 °C (dec.); $[\alpha]_D^{20}$ +119.08° (c = 0.79, DMF); The IR and the ^{1}H-NMR spectra were identical with those of (−)-**2**. *Anal.* Calcd for $C_{18}H_{20}FN_3O_3S \cdot 1/5H_2O$: C, 56.74; H, 5.39; N, 11.02. Found: C, 56.83; H, 5.26; N, 10.79.

(−)-6-Fluoro-1-methyl-4-oxo-7-(1-piperazinyl)-4*H*-[1,3]-thiazeto-[3,2-*a*]quinoline-3-carboxylic Acid (−)-3 A mixture of (−)-**2** (70.5 g, 0.19 mol) and KOH (44.5 g, 0.68 mol) in H_2O (400 ml) was stirred at 60 °C for 3 h. After filtration, the filtrate was acidified with aqueous HCl. After filtration, the filtrate was concentrated under reduced pressure until the volume reached 80 ml, then acetonitrile (300 ml) was added to the solution at 60 °C. The precipitate was collected by filtration and washed with 20% acetonitrile in H_2O, affording the hydrochloride of (−)-**3** (49.4 g), which was dissolved in H_2O (600 ml) and neutralized with aqueous NaOH. The precipitate was collected by filtration and washed with H_2O to afford (−)-**3** (33.5 g, 51%) as a colorless powder. mp > 300 °C (dec.); $[\alpha]_D^{20}$ −149.65° (c = 0.86, 0.1% NaOH); IR 1700 cm^{-1}; ^{1}H-NMR (CF_3CO_2D) δ 2.38 (3H, d, J = 6 Hz), 3.7—4.1 (8H, m), 6.59 (1H, q, J = 6 Hz), 7.10 (1H, d, J = 6 Hz), 8.22 (1H, d, J = 12 Hz). *Anal.* Calcd for $C_{16}H_{16}FN_3O_3S \cdot 2H_2O$: C, 49.86; H, 5.23; N, 10.90. Found: C, 50.18; H, 5.02; N, 10.85. Compound (+)-**3** was obtained from (+)-**2** in 66% yield by the same procedure as above. mp > 300 °C (dec.); $[\alpha]_D^{20}$ +140.19° (c = 0.93, 0.1% NaOH); The IR and the ^{1}H-NMR spectra were identical with those of (−)-**3**. *Anal.* Calcd for $C_{16}H_{16}FN_3O_3S \cdot 2H_2O$: C, 49.86; H, 5.23; N, 10.90. Found: C, 49.53; H, 5.30; N, 10.54.

HPLC: YMC AM312 ODS-AM (15 cm × 4.6 mm i.d.) S-5 120 A. Eluent: 20% MeOH in H_2O containing 6 mM L-phenylalanine and 3 mM copper sulfate (pH was not adjusted). Flow rate: 1.0 ml/min. Detection: UV 350 nm. Retention times: (−)-**3**, 12.1 min; (+)-**3**, 15.3 min.

(−)-Ethyl 6-Fluoro-1-methyl-7-(4-methyl-1-piperazinyl)-4*H*-[1,3]thiazeto[3,2-*a*]quinoline-3-carboxylate (−)-4 A mixture of (−)-**1** (501 mg, 1.61 mmol) and 1-methylpiperazine (484 mg, 4.83 mmol) in 3.5 ml of DMSO was stirred at 60 °C for 6 h. The mixture was poured into ice-water and extracted with $CHCl_3$. The organic layer was washed with water, dried and concentrated under reduced pressure. The residue was chromatographed on silica gel with 5% MeOH in $CHCl_3$ to give 527 mg (84%) of (−)-**4** as a colorless powder. This was recrystallized from acetonitrile, and a plate crystal thus obtained was submitted to X-ray analysis. mp 227—228 °C (dec.); $[\alpha]_D^{20}$ −110.54° (c=0.99, DMF); IR 1715 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.39 (3H, t, J=7 Hz), 2.10 (3H, d, J=7 Hz), 2.37 (3H, s), 2.4—3.5 (8H, m), 4.36 (2H, q, J=7 Hz), 5.92 (1H, q, J=7 Hz), 6.31 (1H, d, J=7 Hz), 7.87 (1H, d, J=14 Hz). *Anal.* Calcd for $C_{19}H_{22}FN_3O_3S$: C, 58.30; H, 5.66; N, 10.73. Found: C, 58.23; H, 5.60; N, 10.64.

X-Ray Analysis of (−)-4 Crystal Data: $C_{19}H_{22}FN_3O_3S$, triclinic, space group $P1$; a=9.900(2) Å, b=12.141(4) Å, c=8.068(1) Å, α=90.39(2) Å, β=93.98(2) Å, γ=97.40(2) Å, V=959.2(4) Å^3, Z=2, D_c=1.355 g/cm^3 and μ(CuK_α)=8.16 cm^{-1}. Data collection: A crystal was mounted on a Rigaku AFC5R diffractometer with graphite-monochromated CuK_α radiation. The cell dimensions were refined by the least-squares method using 24 reflections. The intensity data were collected at a temperature of 23±1 °C using the ω–2θ scan technique to a maximum 2θ value of 120.3 °C. Of 2862 independent reflections collected, 2674 reflections with $I > 3.00\sigma(I)$ were used for the structure determination and refinement. Data were corrected for Lorentz and polarization effects. The structure was solved by the direct method using the TEXSAN program.[8] The positional coordinates were refined by the full-matrix least-squares method using anisotropic temperature factors for all the non-hydrogen atoms and isotropic ones for hydrogen atoms. The final refinement converged to R=0.085 and R_w=0.097. The atomic scattering factors were taken from Ref. 9. Considering the Bijvoet differences in observed and calculated structure factors, the absolute configuration was determined to be *S* at the 1-position (C13, C32), as shown in Fig. 2.

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References and Notes

1) *a*) Segawa J., Kazuno K., Matsuoka M., Shirahase I., Ozaki M., Matsuda M., Tomii Y., Kitano M., Kise M., *Chem. Pharm. Bull.*, **43**, 63 (1995); *b*) Segawa J., Kitano M., Kazuno K., Matsuoka M., Shirahase I., Ozaki M., Matsuda M., Tomii Y., Kise M., *J. Med. Chem.*, **35**, 4727 (1992); *c*) Ozaki M., Matsuda M., Tomii Y., Kimura K., Kazuno, K., Kitano M., Kise M., Shibata K., Otsuki, M., Nishino T., *Antimicrob. Agents Chemother.*, **35**, 2490 (1991).
2) Chu D. T. W., Fernandes P. B., *Antimicrob. Agents Chemother.*, **33**, 131 (1989).
3) Cesare P. D., Bouzard D., Essiz M., Jacquet J. P., Ledoussal B., Kiechel J. R., Remuzon P., Kessler R. E., Fung-Tomc J., Desiderio J., *J. Med. Chem.*, **35**, 4205 (1992).
4) Kimura Y., Atarashi S., Kawakami K., Sato K., Hayakawa I., *J. Med. Chem.*, **37**, 3344 (1994).
5) *a*) Gerster J. F., Rohlfing S. R., Pecore S. E., Winandy R. M., Stern R. M., Landmesser J. E., Olsen R. A., Gleason W. B., *J. Med. Chem.*, **30**, 839 (1987); *b*) Tanaka M., Otsuki M., Une T., Nishino T., *J. Antimicrob. Chemother.*, **26**, 659 (1990); *c*) Todo Y., Takagi H., Iino F., Fukuoka Y., Takahata M., Okamoto S., Saikawa I., Narita H., *Chem. Pharm. Bull.*, **42**, 2569 (1994).
6) Arai T., Koike H., Hirata K., Oizumi H., *J. Chromatogr.*, **448**, 439 (1988).
7) MIC Committee of the Japan Society of Chemotherapy, *Chemotherapy* (Tokyo), **29**, 76 (1981).
8) TEXSAN: Crystal Structure Analysis Package, Molecular Structure Corporation (1985, 1992).
9) Cromer D. T., Waber J. T., "International Tables for X-Ray Crystallography," Vol. IV, The Kynoch Press, Birmingham, England, 1974, Table 2.2A.