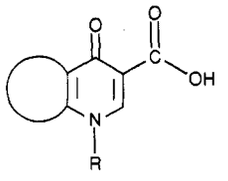
Synthesis, Absolute Configuration, and Antibacterial Activity of 6,7-Dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic Acid

John F. Gerster,* Steve R. Rohlfing, Sharon E. Pecore, Richard M. Winandy, Richard M. Stern, June E. Landmesser, Roger A. Olsen, and William B. Gleason

New Molecule Research Department, Riker Laboratories, Inc., 3M, 3M Center, St. Paul, Minnesota 55144.
Received September 5, 1986

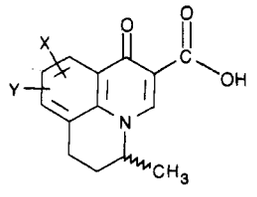
The tricyclic quinolone antibacterial agent 6,7-dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic acid has an asymmetric center at position 5 of the molecule. The *R* and *S* isomers of the compound have been prepared from the corresponding (*R*)- and (*S*)-2,5-dimethyl-6-fluoro-1,2,3,4-tetrahydroquinolines, which were separated via their diastereomeric amides of *N*-tosyl-(*S*)-proline. The absolute configuration was established by X-ray analysis of one of the diastereomeric amides. The 5-desmethyl analogue was prepared for antibacterial comparison with the isomers and the racemic mixture. It has now been established that the *S* isomer is much more active than the *R* isomer. The 5-desmethyl analogue was found to be more active than the *R* isomer but not as active as the *S* isomer or the racemic mixture. The importance of stereochemistry at position 5 in this system has been established.

In recent years numerous antibacterial agents have been prepared that contain the common 1-substituted 4-pyridone-3-carboxylic acid component, **1**. When R is alkyl,



1, R = CH_2CH_3, $CH{=}CH_2$, c-C_3H_5

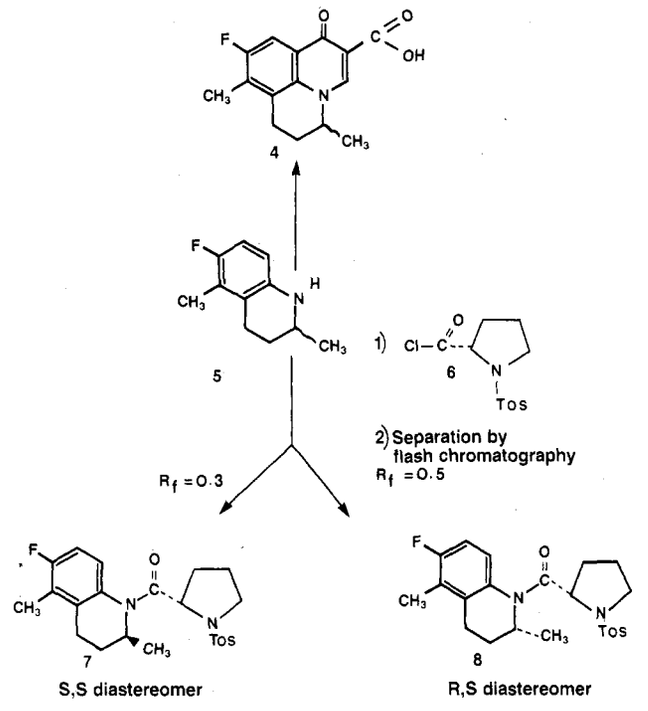
it has been found that maximum antibacterial activity is observed when the substituent is ethyl, vinyl,[1] or, more recently, cyclopropyl.[2] In general, a terminal methyl or methylene group two atoms removed from the nitrogen is important to achieve maximum antibacterial activity in systems with aliphatic N substitution. We have synthesized a series of 6,7-dihydro-5-methyl-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic acids as represented by **2**. The tricyclic structure was designed to see how anti-



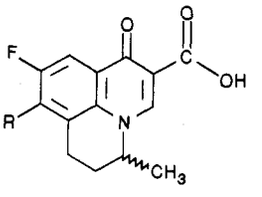
2

bacterial activity would be affected by restricting the free rotation of the *N*-ethyl group in the quinolone class of antibacterials. Since many members of the series show high antibacterial activity, it is evident that restricting the rotation of the terminal methyl group does not inhibit the antibacterial activity. When the methyl group is introduced into the 5-position of the system, an asymmetric center is formed. In earlier work we determined that the optical isomers of flumequine (**3**) show markedly different antibacterial potency.[3] In an extension of that work we

Scheme I



synthesized the optical isomers of a new, more potent analogue, **4**, more recently prepared.[4,5] The absolute



3, R = H
4, R = CH_3

configuration of the isomers was determined by X-ray analysis of **8**, a derivative of one of the key intermediates in the synthesis (Scheme I). Because the configuration of the proline moiety in **8** is known, this establishes the absolute configuration of the 2-methyl group on the molecule. In addition we prepared the 5-desmethyl ana-

(1) Albrecht, R. *Prog. Drug Res.* **1977**, *21*, 9-104.
(2) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.
(3) Gerster, J. F.; Rohlfing, S. R.; Winandy, R. M. *Synthesis and Antibacterial Activity of the Optical Isomers of 6,7-Dihydro-9-fluoro-5-methyl-1-oxo-1H,5H-benzo[ij]quinoline-2-carboxylic Acid* (*Flumequine*), North American Medicinal Chemistry Symposium, Toronto, 1982.
(4) Stern, R. M. U.S. Patent 4 472 405, 1984.
(5) Rohlfing, S. R.; Landmesser, J. E.; Gerster, J. F. *Antimicrob. Chemother.* **1985**, *15*, 539.

0022-2623/87/1830-0839$01.50/0

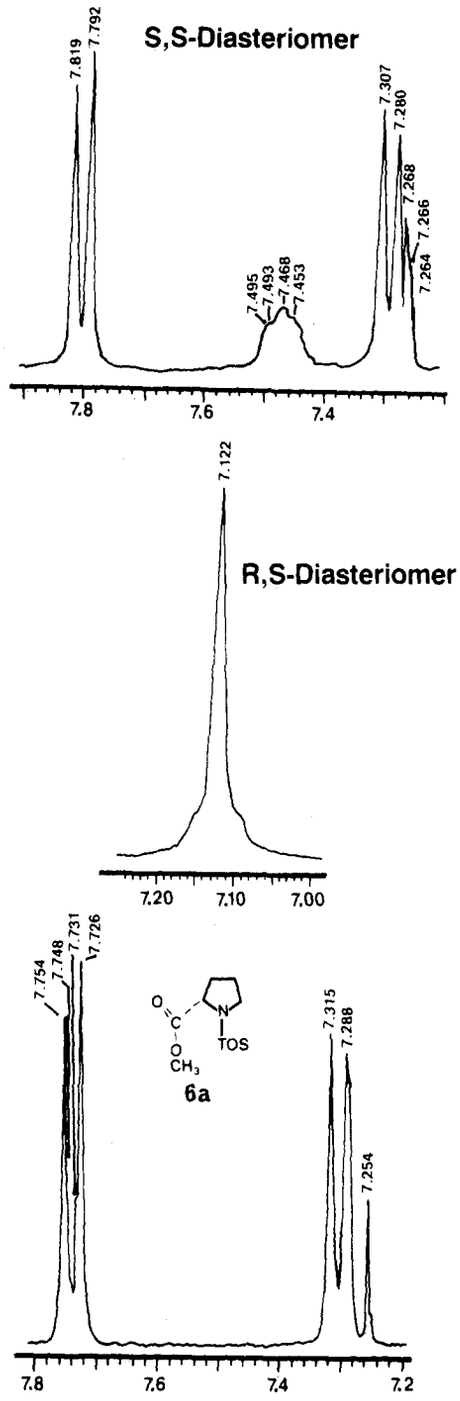


Figure 1.

logue of 4 to compare its in vitro antibacterial activity with that of the *R* and *S* isomers and racemic 4.[6]

Chemistry

Compound 4[4] and its desmethyl analogue 13 were prepared from the corresponding tetrahydroquinolines and diethyl (ethoxymethylene)malonate (DEEM) by standard methods.[4] The synthesis of the *R* and *S* isomers of 4 started with the resolution of 2,5-dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (DFTQ),[4] 5, into its enantiomers. A mixture of diastereomeric amides was prepared

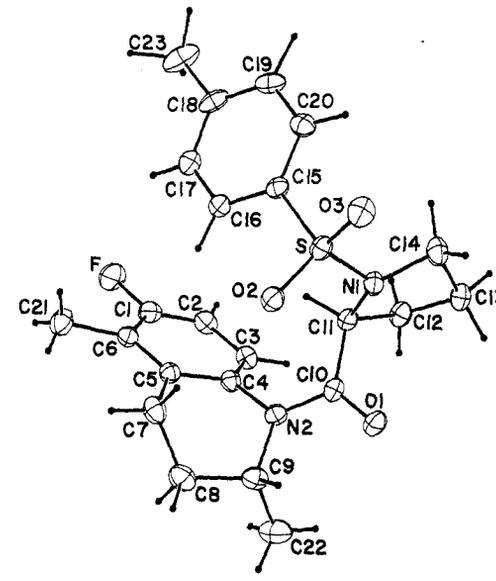


Figure 2.

from racemic DFTQ and *N*-tosyl-(*S*)-prolyl chloride,[7] 6. The mixture showed two spots on silica gel GF TLC plates (R_f 0.3 and 0.5) using 10% EtOAc in CH_2Cl_2. The mixture was then separated by flash chromatography on Merck 230–400-mesh silica gel and the above solvent mixture. The absolute configuration of the slower moving diastereomer was determined by X-ray analysis. Knowing the absolute configuration of the (*S*)-prolyl moiety established the absolute configuration of the 2-methyl substituent.

The ^1H NMR spectra of the *S,S* and *R,S* diastereomers 7 and 8, respectively, show distinctly different absorption patterns for the aromatic protons on the tosyl moiety. The tosyl aromatic protons in the *S,S* diastereomer 7 show a typical AB quartet (δ 7.81 and 7.29, J = 8.1 Hz), similar to that seen for the ortho coupling of the methyl ester of *N*-tosyl-(*S*)-proline (6a; δ 7.74 and 7.30, J = 8.1 Hz) (Figure 1). In the *R,S* diastereomer 8 the quartet collapses to a broad, shouldered singlet (δ 7.12). The protons ortho to the sulfonyl group directly above the aromatic protons of the tetrahydroquinoline ring are shifted upfield 0.6 ppm due to the ring current effect. The meta protons, being farther away, show a smaller shielding. The X-ray structure of the *R,S* diastereomer (Figure 2) shows this relationship. In related unpublished work we have observed the same shielding effect in the diastereomers prepared from *N*-tosyl-(*S*)-proline and 2-methyl-1,2,3,4-tetrahydroquinoline, 6-fluoro-2-methyl-1,2,3,4-tetrahydroquinoline, and several substituted 3,4-dihydro-3-methyl-1,4-benzoxazines. We believe that this may be a general method to determine the absolute configuration of 2-methyl- or perhaps 2-substituted-1,2,3,4-tetrahydroquinolines and the analogous 3,4-dihydro-3-substituted-1,4-benzoxazines.

Saponification of the amides gave pure (*S*)-9 and (*R*)-10, DFTQ, which were then used for the synthesis of (*S*)-11 and (*R*)-12, isomers of 4, as shown in Scheme II.

The 5-desmethyl analogue 17 was prepared from 6-fluoroquinoline[8] (13) by the method described in Scheme III. Bromination of 13 with Br_2 in the presence of $AlCl_3$ gave 5-bromo-6-fluoroquinoline (14). It has been shown that bromination of quinoline under similar conditions gave 5- and 8-bromoquinoline while bromination of 6-

(6) Gerster, J. F.; Rohlfing, S. R.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. *Stereochemical Aspects of the Antibacterial Activity of S-25930*, 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Oct 1985.

(7) Beecham, A. *J. Am. Chem. Soc.* **1957**, *79*, 3257.

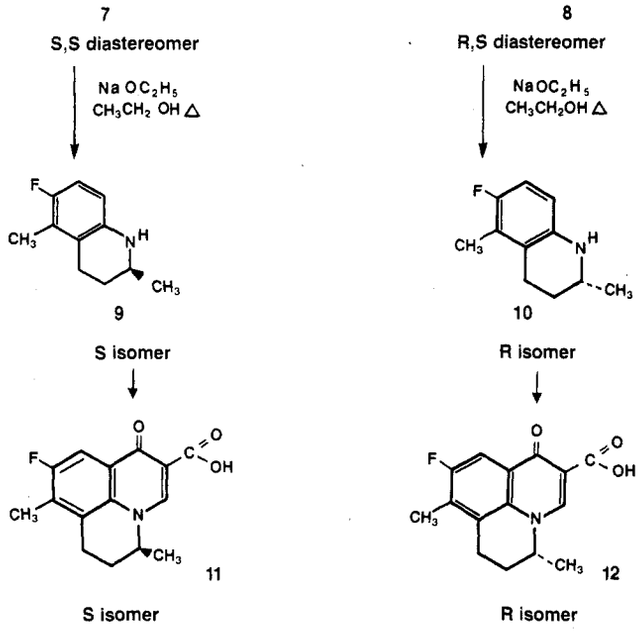
(8) Mirek, J. *Seszyty Nauk. Univ. Jugiel., Ser. Nauk. Chem.* **1962**, *7*, 173; *Chem. Abstr.* **1965**, *62*, 5252b.

Table I. In Vitro Antibacterial Comparison of Racemic Mixture 4, *R* and *S* Isomers 12 and 11, and Desmethyl Analogue 17[a]

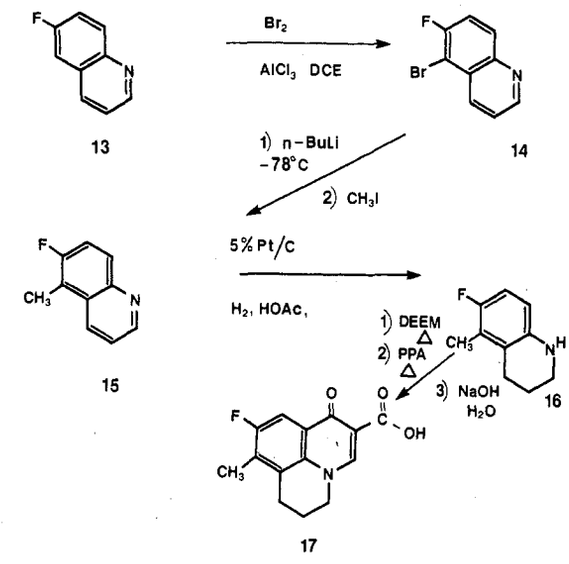
		M.I.C.: μg/ml			
Bacterium	ATCC	RS 4	R(12) Isomer	S(11) Isomer	Desmethyl Analog 17
Streptococcus pyogenes	1961-1	0.8	12.5	1.6	12.5
Streptococcus faecalis	10741	0.8	12.5	0.8	12.5
Staphylococcus aureus	6538-P	0.05	3.1	0.025	0.8
Staphylococcus epidermidis	12228	0.1	6.2	0.05	1.6
Haemophilus influenzae	9333	0.006	0.4	0.006	0.1
Bordetella pertussis	10380	0.025	1.6	0.012	0.8
Salmonella typhosa-CDC	(M4694)	0.1	3.1	0.05	0.4
Enterobacter cloacae	23355	0.2	3.1	0.1	0.4
Pseudomonas cepacia	25608	0.8	12.5	0.8	6.2
Pseudomonas aeruginosa	15422	6.2	12.5	3.1	12.5
Serratia marcescens	8100	0.4	3.1	0.2	0.8
Klebsiella pneumoniae	23357	0.2	6.2	0.1	0.8
Escherichia coli	15221	0.2	6.2	0.1	0.8
Proteus mirabilis-R*	(119)	0.8	12.5	0.4	1.6

[a] M.I.C., minimal inhibitory concentration. ATCC, American Type Culture Collection; CDC, Center for Disease Control; R*, Riker Laboratories.

Scheme II



Scheme III



chloroquinoline gives only 5-bromo-6-chloroquinoline.[9] The position of bromination of **13** was further substantiated by ^{13}C NMR analysis. Lithiation of **14** with *n*-butyllithium at −78 °C followed by methylation with methyl iodide gave 6-fluoro-5-methylquinoline (**15**). Compound **15** was used to prepare the 5-desmethyl analogue 17 following standard methods.

X-ray Crystallographic Study

A colorless, needle-shaped crystal of $C_{23}H_{27}FN_2O_3$ having approximate dimensions of 0.30 × 0.26 × 0.20 mm was grown from ethanol. The sample was mounted on a glass fiber, and preliminary examination and data collection were performed with Mo Kα radiation (λ = 0.71073 Å) on an Enraf-Nonius CAD4 diffractometer equipped with graphite monochromator. Cell constants and an orientation matrix for data collection were obtained from least-squares refinement with the setting angles of 25 reflections. The compound crystallized in the orthorhombic space group $P2_12_12_1$ with cell dimensions a = 8.673 (3) Å, b = 9.704 (1) Å, c = 26.407 (4) Å, V = 2222.2 Å^3. For Z = 4 and FW = 430.55, the calculated density was 1.29 g/cm^3. A total of 7986 reflections were collected of which, after averaging, 3918 were unique and not systematically absent. Intensities of equivalent reflections were averaged but not Friedel pairs. Since the linear absorption coefficient is 1.7 cm^{-1} for Mo Kα, no absorption correction was made. The structure was solved by direct methods. Hydrogen atoms were located and their positions were refined in least squares with isotropic displacement parameters held fixed at 5.0 Å^2. All non-hydrogen atoms were refined with anisotropic displacement parameters. Only the 2323 reflections having intensities greater than 3.0 times their standard deviations were used in the final refinement, which converged to a final R value of 0.034 and R_w of 0.01. A perspective view of the molecule, drawn with the aid of the ORTEP program, is shown in Figure 2.

Results and Discussion

The importance of stereochemistry at position 5 on the antibacterial activity of 6,7-dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic acid (**4**) can be seen by the antibacterial comparison of the *S* and *R* isomers **11** and **12** and the desmethyl analogue **17**,

(9) Gordon, M.; Hamilton, H. J.; Adkins, C.; Hay, J.; Pearson, D. E. *J. Heterocyl. Chem.* **1967**, *4*, 410.

as shown in Table I. The *R* isomer **12** shows considerably weaker activity than the racemate **4**, while the *S* isomer **11** is approximately twice as active as **4**. As was seen earlier with flumequine,[1] and more recently with ofloxacin,[10] antibacterial activity is stereochemically dependent in these tricyclic systems. The desmethyl analogue is more active than the *R* isomer **12** but less active than the racemate **4**. In related tricyclic analogues, when the alkyl group at position 5 is lengthened to ethyl, a decrease in antibacterial activity is observed.[11] This is also found in going from ethyl to longer chain alkyl groups in the *N*-alkylquinoline antibacterials such as oxolinic acid.[12] This suggests a space limitation at the receptor site. Presumably the terminal methyl group fits into a specific site on the receptor. When the methyl group is in a favorable configuration, such as in the *S* isomer **11**, antibacterial activity is enhanced. It appears that in the *R* configuration the methyl may hinder fit at the receptor site and therefore antibacterial activity is diminished. The desmethyl analogue **17** would then fit the site better than the *R* isomer **12**, but does not allow for optimum fit since it lacks a methyl group at position 5. The envisioned receptor may be part of the enzyme DNA gyrase. Studies of the isomers with this enzyme are currently underway.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton and ^{13}C magnetic resonance (NMR) spectra were recorded on a Varian XL 300 NMR spectrometer operating at 300 MHz (^1H) and 75.4 MHz (^{13}C), and chemical shifts are reported in δ relative to tetramethylsilane. Carbon, hydrogen, and nitrogen analysis were performed by the 3M Central Research Analytical group and were within ±0.4% of the theoretical values. Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer, and optical rotations were taken on a Perkin-Elmer Model 241 polarimeter. The X-ray crystallography was determined on an Enraf-Nonius CAD-4 diffractometer. The structures of all compounds were consistent with their spectral data.

1-(*N*-Tosyl-(*S*)-prolyl)-(*R*- and -*S*)-2,5-dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (7, 8). 2,5-Dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (5) (30.5 g, 0.17 mol) was dissolved in a solution of diisopropylethylamine (25.9 g, 0.2 mol) in 300 mL of CH_2Cl_2. To the cooled solution was added *N*-tosyl-(*S*)-prolyl chloride[7] (51.5 g, 0.18 mol) in 300 mL of CH_2Cl_2. The resulting solution was refluxed for 30 min, cooled to room temperature, and diluted to a volume of 1000 mL with CH_2Cl_2. The CH_2Cl_2 solution was washed thoroughly with 0.1 N HCl, H_2O, and saturated aqueous $NaHCO_3$. After the CH_2Cl_2 solution was dried over $MgSO_4$, the CH_2Cl_2 was evaporated in vacuo to give 64.8 g of a stiff yellow syrup. The syrup was dissolved in hot EtOH and the solution diluted with approximately one-third the volume of petroleum ether (35–60 °C). The solid that crystallized from the cooled solution was filtered, washed with EtOH/petroleum ether (35–60 °C) (3:1), and dried. Second and third crops were obtained by evaporating the filtrate and repeating the above procedure. A combined yield of 42.5 g (58.1%) of solid product was obtained, which showed two spots on TLC, R_f 0.3 and 0.5, in 10% EtOAc in CH_2Cl_2. The residual syrup from the filtrates, (19.0 g, 26.0%), showed two major spots corresponding to those described above and two minor spots at R_f 0.4 and 0.8. The solid product was used for the chromatographic separation of the diastereomers.

Separation of Diastereomers 7 and 8. A 10.6-g sample of the diastereomeric mixture was dissolved in 10% EtOAc in CH_2Cl_2 and chromatographed on 300 g of silica gel (Merck, grade 60, 230–400 mesh, 60 A), with 10% EtOAc in CH_2Cl_2 as solvent. Fractions containing a mixture of **8** (R_f 0.3) and **7** (R_f 0.5) were chromatographed a second time. A combined yield of 17.8 g of the *S,S* diastereomer **7**, 10.7 g of the *R,S* diastereomer **8**, and 11.6 g of a mixture of **7** and **8** was obtained from the 42.5-g sample of the diastereomeric mixture.

***S,S* Diastereomer 7**: recrystallized from EtOH to give colorless crystals, mp 167–169 °C; $[\alpha]_D$ +144.2° (*c* 0.2005 g/10 mL, dichloroethane); NMR ($CDCl_3$) δ 7.81 (d, J = 8.1 Hz, 2 H, Tos Ar H), 7.47 (m, 1 H, C7-H), 7.29 (d, J = 8.1 Hz, 2 H, Tos Ar H), 6.98 (t, 1 H, C8-H), 2.41 (s, 3 H, Tos CH_3), 2.22 (d, J = 1.5 Hz, 3 H, C5-CH_3), 1.08 (d, J = 6.3, C2-CH_3). Anal. ($C_{23}H_{27}FN_2O_3S$) C, H, N.

***R,S* Diastereomer 8**: recrystallized from EtOH to give colorless crystals, mp 154–156 °C; $[\alpha]_D$ −373.4° (*c* 0.2003 g/10 mL, dichloroethane); NMR ($CDCl_3$) δ 7.12 (t, 4 H, Tos Ar H), 6.87 (t, 1 H, C7-H), 2.37 (s, 3 H, Tos CH_3), 2.30 (s, 3 H, C5-CH_3), 1.08 (d, J = 6.9, 3 H, C2-CH_3). Anal. ($C_{23}H_{27}FN_2O_3S$) C, H, N.

(*S*)-2,5-Dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (9). A solution of **7** (15.4 g, 0.036 mol) in 200 mL of 2 N NaOEt was refluxed for 4 h. TLC of the reaction mixture showed the absence of **7**. The EtOH was evaporated in vacuo, the residue mixed with H_2O, and the product extracted into petroleum ether (35–60 °C). The combined extracts were dried over NaCl and evaporated in vacuo to yield 6.4 g (100%) of **9** as a pale yellow oil. The crude product was used as such for the preparation of **11**. A sample of the crude **9** was benzoylated as described below to give a solid product for further characterization.

(*S*)-*N*-Benzoyl-2,5-dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (9a). A solution of 50% NaOH (1.0 mL) was added dropwide to a vigorously stirred mixture of **9** (0.5 g, 2.8 mmol) and benzoyl chloride (0.43 g, 3.1 mmol) in 10 mL of H_2O. The product crystallized from the mixture within minutes and was filtered, washed with water, and dried. The crude product was recrystallized from hexane to give 0.4 g (50.6%) of pure product as colorless crystals, mp 99–100 °C; $[\alpha]_D$ +298.7° (*c* 0.129] g/10 mL, dichloroethane). Anal. ($C_{18}H_{18}FNO$) C, H, N.

(*S*)-6,7-Dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[*ij*]quinoline-2-carboxylic Acid (11). A mixture of **9** (5.8 g, 0.032 mol) and diethyl (ethoxymethylene)malonate (7.6 g, 0.035 mol) was heated in a 200-mL round-bottom flask at 135–140 °C for 1 h. The syrup was cooled to room temperature and polyphosphoric acid (PPA) (50.0 g) was added to the flask. The mixture was heated on the steam bath and stirred manually until foaming began. Heating was continued for 30 min with occasional stirring. A small amount of H_2O was added to the mixture to begin decomposition of the PPA followed by an additional 150 mL of H_2O. The pH of the cooled solution was adjusted to 3–4 by the addition of 50% NaOH to precipitate the ethyl ester of **11**. The ester was filtered from the mixture, washed with H_2O, and saponified in 100 mL of 5% NaOH by heating on the steam bath for 1 h after all solid had dissolved. The free acid was precipitated from solution by the addition of 6 N HCl to pH 3–4. The solid was filtered from the mixture, washed with H_2O, and dried to yield 5.8 g (65.2%) of crude product. Recrystallization of the solid from DMF gave 3.9 g (43.8%) of pure product as colorless crystals: mp 274–276 °C; $[\alpha]_D$ −107.4° (*c* 0.1109 g/10 mL, dichloroethane); NMR ($CDCl_3$) δ 8.71 (s, 1 H, C3-H), 8.00 (d, J = 9.3 Hz, 1 H, C10-H), 4.6 (m, 1 H, C5-H), 3.04 (m, 2 H, C6-H), 2.4 (d, J = 1.8 Hz, 3 H, C8-CH_3), 2.28 (m, 2 H, C7-H), 1.51 (d, J = 6.9 Hz, 3 H, C5-CH_3). Anal. ($C_{15}H_{14}FNO_3$) C, H, N.

(*R*)-2,5-Dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (10). The *R,S* diastereomer **8** was saponified as described for the *S,S* diastereomer **7** to give a 92.7% crude yield of **10** as a pale yellow oil. The crude product was used for the preparation of **12** and for the preparation of the *N*-benzoyl derivative for further characterization.

(*R*)-*N*-Benzoyl-2,5-dimethyl-6-fluoro-1,2,3,4-tetrahydroquinoline (10a). A sample of crude **10** was benzoylated as described for the *S* isomer. Recrystallization of the crude product from EtOH gave colorless crystals, mp 99–100 °C; $[\alpha]_D$ −300.6° (*c* 0.1286 g/10 mL, dichloroethane). Anal. ($C_{18}H_{18}FNO$) C, H, N.

(*R*)-6,7-Dihydro-5,8-dimethyl-9-fluoro-1-oxo-1*H*,5*H*-benzo[*ij*]quinolizine-2-carboxylic Acid (12). An analytically pure sample of **12** was prepared in 42.9% yield by the method used for the *S* isomer **11**. The product was recrystallized from DMF to give colorless crystals, mp 274–276 °C; $[\alpha]_D$ +106.8 °C (*c* 0.1165 g/10 mL, dichloroethane); NMR spectra identical with

(10) Hayakawa, I.; et al. *Antimicrob. Agents Chemother.* **1986**, *29*, 163.
(11) Unpublished results.
(12) Kaminsky, D.; Meltzer, R. I. *J. Med. Chem.* **1968**, *11*, 160.

that of the S isomer 11. Anal. ($C_{15}H_{14}FNO_3$) C, H, N.

5-Bromo-6-fluoroquinoline (14). To a solution of 13[7] (40.0 g, 0.27 mol) in 190 mL of dichloroethane was added $AlCl_3$ (66.5 g, 0.50 mol) in small portions with stirring. The temperature of the mixture rose spontneously to approximately 55 °C and a tan precipitate formed. Bromine (78.2 g, 0.49 mol) in 20 mL of dichloroethane was added dropwise to the stirred mixture over a period of 2 h at 45–50 °C. The dark red solution was then refluxed for 3 h and poured over ice and the ice mixture made strongly basic with 50% NaOH. The mixture was extracted with 2 × 250 mL of CH_2Cl_2, and the combined extracts were washed with water. The organic phase was washed with dilute aqueous HCl and the acidic extract made basic with concentrated NH_4OH. A solid precipitated, which was filtered, washed with water, and dried to give 16.0 g (26.1%) of crude product. The crude product was recrystallized from hexane to give colorless crystals, mp 102–104 °C. The ^{13}C and proton NMR data are available as supplementary material. Anal. (C_9H_5BrFN) C, H, N.

6-Fluoro-5-methylquinoline (15). A solution of 14 (5.6 g, 0.025 mol) in THF/ether (250 mL, 1:1) in a three-neck round-bottom flask equipped with a rubber septum, thermometer, and mechanical stirrer was cooled to −78 °C in a dry ice bath. *n*-Butyllithium (22.5 mL of 2.2 M hexane solution, 0.05 mol) was added dropwise to the stirred solution at −78 °C. When addition was complete, the solution was stirred for 15 min and CH_3I (7.2 g, 0.05 mol) was added to the reaction mixture. After the mixture was stirred for 15 min, EtOH was added cautiously followed by a saturated solution of NH_4Cl. The mixture was warmed to room temperature and the organic phase evaporated to dryness in vacuo. The residue was dissolved in dilute HCl, the solution washed with CH_2Cl_2, and the aqueous phase made basic with 50% NaOH. The crude product was flash chromatographed on silica gel (Merck, grade 60, 230–400 mesh, 60 A) with 5% EtOAc/CH_2Cl_2 to give 1.1 g of product as a clear oil. This product was taken on without further purification.

6,7-Dihydro-9-fluoro-8-methyl-1-oxo-1*H*,5*H*-benzo[*ij*]-quinolizine-2-carboxylic Acid (17). Compound 15 (1.0 g, 6.2 mmol) was hydrogenated on a Parr apparatus in glacial HOAc eith 5% Pt/C as catalyst. The catalyst was filtered from the mixture and the filtrate evaporated in vacuo. The residue was mixed with H_2O and made strongly basic with 50% NaOH and the intermediate tetrahydroquinoline 16 was extracted into petroleum ether (35–60 °C). The petroleum ether was dried over $MgSO_4$ and evaporated in vacuo to give 1.0 g of crude 16. Crude 16 was converted to 17 in 37.5% yield by the same method described for 11 and 12. Several recrystallizations from DMF gave analytically pure product as colorless needles: mp 292–296 °C; NMR ($CDCl_3$) δ 8.70 (s, 1 H, C3-H), 8.00 (d, *J* = 9.3 Hz, 1 H, C10-H), 4.27 (t, *J* = 5.7 Hz, 2 H, C5-H), 3.03 (m, 2 H, C6-H), 2.38 (d, *J* = 1.5 Hz, 3 H, C8-CH_3), 2.36 (m, 2 H, C7-H). Anal. ($C_{14}H_{12}FNO_3$) C, H, N.

***N*-Tosyl-(*S*)-proline Methyl Ester (6a).** The methyl ester of *N*-tosyl-(*S*)-proline was prepared from the acid chloride[7] and methanol. Recrystallization of the product from ether/petroleum ether (35–60 °C) gave colorless crystals: mp 73–74.5 °C; $[\alpha]_D$ −98.1° (*c* 0.1449 g/10 mL, dichloroethane); NMR ($CDCl_3$) δ 7.74 (d, *J* = 0.1 Hz, 2 H, Tos Ar H), 7.3 (d, *J* = 8.1 Hz, 2 H, Tos Ar H), 4.3 (m, 1 H, C2-H), 3.7 (s, 3 H, OCH_3), 3.3 (m, 1 H C5-H), 2.42 (s, 3 H, Tos CH_3), 1.98 (m, 3 H, C3-H, C4-H_2), 1.75 (m, 1 H, C3-H). Anal. ($C_{13}H_{17}FNO_3$) C, H, N.

Microbiology. Solutions of tricyclic quinolone antibacterials were prepared in dimethyl sulfoxide at a concentration of 5 mg/mL. Serial 2-fold dilutions in nutrient broth were than carried out for 11 tubes of 0.5 mL final volume. The contents were further diluted to 50.0 mL with Mueller–Hinton (M–H) agar (55 °C), mixed by repeated inversion, and poured into square 100 × 15 mm dishes to harden, M–H agar was supplemented with 5% defibrinated rabbit blood to promote growth of hemophilic bacteria. The drug dilution series contained the following concentrations: 12.5, 6.2, 3.1, 1.6, 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.012, 0.006 μg/mL. Inocula were prepared by harvesting the growth from an overnight culture (except *Bordetella pertussis*) in sterile saline and resuspending turbidimetrically to McFarland standard 6 (2 × 10^6/mL). A uniform inoculum was deposited on the surface of prepared plates with the Steers replicator. The test conditions of incubation were 37 °C for 30 h (except *B. pertussis*). The lowest drug concentration preventing visible growth (MIC) was recorded.

Acknowledgment. We thank Thomas S. Robison of the Riker Chemical and Analytical Development group for ^{13}C and ^1H NMR verification of the structure of 5-bromo-6-fluoroquinoline.

Supplementary Material Available: Tables listing fractional coordinates, anisotropic thermal parameters, hydrogen atom coordinates, bond lengths, and bond angles (7 pages); table of structure factors for diastereomer 8 (15 pages). Ordering information is given on any current masthead page.

Potential Antitumor Agents. 51. Synthesis and Antitumor Activity of Substituted Phenazine-1-carboxamides

Gordon W. Rewcastle,* William A. Denny, and Bruce C. Baguley

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.
Received September 3, 1986

In a further investigation of electron-deficient DNA-intercalating ligands as antitumor drugs, a series of substituted *N*-[2-(dimethylamino)ethyl]phenazine-1-carboxamides have been synthesized and evaluated. Fluorine-directed ring closure of *N*-phenyl-3-nitroanthranilic acids provided a new, unequivocal synthesis of several of the required phenazine-1-carboxylic acids, and the corresponding carboxamides were prepared and evaluated against L1210 leukemia in vitro and against P388 leukemia and Lewis lung carcinoma in vivo. Substitution on the phenazine ring was broadly tolerated, and the cytotoxicity of the resulting compounds correlated positively with the electron-withdrawing power of the substituent group. The positional effects of substituents were even more evident, with 9-substituted compounds being the most active. One derivative, *N*-[2-(dimethylamino)ethyl]-9-methoxyphenazine-1-carboxamide, had activity against Lewis lung carcinoma in mice equal to that of the best DNA-intercalating agents yet described, being capable of effecting a high-proportion cure of the advanced disease.

We have recently described[1] the synthesis and evaluation of a series of acridine-4-carboxamides (1), a new class of DNA-intercalating antitumor drugs with broad-spectrum in vivo activity against both leukemia and solid-tumor models. In this series, biological activity depends critically not only on intercalation of the acridine chromophore but also on positioning of the carboxamide side chain peri to the acridine nitrogen. Thus the acridine-1-carboxamide (3) and the anthracene-1-carboxamide (4) derivatives, which lack the nitrogen in this position, are inactive although both intercalate DNA efficiently.[2] A

(1) Atwell, G. J.; Rewcastle, G. W.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.*, in press.

(2) Unpublished work, this laboratory.

0022-2623/87/1830-0843$01.50/0