# Synthesis and Antibacterial Activity of Novel Pyridobenzoxazine Analogues

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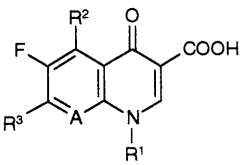
**A series of novel LVFX (7) analogues bearing 4,4-dialkyl-3-aminopyrrolidines at the C-10 position of pyridobenzoxazine was synthesized and their antibacterial activities, pharmacokinetics and acute toxicities in animals were evaluated. Non-alkylated pyrrolidine derivative 26a showed greater activity than LVFX (7) against gram-positive and gram-negative bacteria including *Pseudomonas aeruginosa*, but 26a possessed high acute toxicity in mice and unfavorable pharmacokinetics in rats. When compared with 26a, 4,4-dialkylated derivatives 26c, e, g showed more potent activity against gram-positive bacteria along with an improvement of pharmacokinetics and reduction of acute toxicity. Increases in lipophilicity by alkylation on the pyrrolidine ring resulted in a good influence on the above profiles.**

**Key words** pyridobenzoxazine; 4,4-dialkyl-3-aminopyrrolidine; antibacterial activity; pharmacokinetics; acute toxicty; lipophilicity

Since the discovery of nalidixic acid (NA, **1**),[1] a large number of 4-pyridone-3-carboxylic acid derivatives, so-called 4-quinolone, have been synthesized. In the process of searching for 4-quinolones with improved antibacterial activity, there have been extensive findings in their structure-activity relationships. Among them, the following knowledge serves as the base of current investigations of novel 4-quinolones: 1) introduction of a fluorine atom at the C-6 position of 4-quinolone nucleus enhances antibacterial activity,[2] 2) introduction of a basic substituent at the C-7 position forms a zwitter compound with the carboxyl group at the C-3 position, which improves oral absorption, tissue distribution and metabolic stability.[3] Basic substituents also contribute to improvement of antibacterial activity and spectrum. The typical basic substituents are piperazines [norfloxacin (NFLX, **2**),[4] ofloxacin (OFLX, **3**),[5] ciprofloxacin (CPFX, **4**),[6] sparfloxacin (SPFX, **5**)[7]] and 3-aminopyrrolidines [tosufloxacin (TFLX, **6**)[8]]. It is generally observed that piperazinyl quinolones are effective for gram-negative bacteria and that 3-aminopyrrolidinyl quinolones have well balanced activity against gram-negative and gram-positive bacteria.[9] In practice, however, such a tendency delicately changes with the combination of basic substituents and the 4-quinolone nucleus.

In our previous papers, we reported the optical resolution of OFLX (**3**) exhibiting potent antibacterial activity and low toxicity with good pharmacokinetics in humans and that levofloxacin (LVFX, **7**), the *S*-isomer of OFLX (**3**), was 8 to 128 times more active than its *R*-isomer (DR-3354, **8**) and almost two times more active than OFLX (**3**) against both gram-negative and gram-positive organisms.[10] Furthermore, we have recently reported that sitafloxacin (DU-6859, optically active N1-*cis*-fluorocyclopropyl-C7-4′-spirocyclopropyl-3′-aminopyrrolidinyl quinolone, **9**) shows great activity against gram-negative and gram-positive pathogens.[11] Introduction of an alkyl group at the adjacent position of the amino group on the pyrrolidine ring improved oral absorption in mice by increasing the lipophilicity, and enhanced antibacterial activity against gram-positive organisms compared with the corresponding non-alkylated 3-aminopyrrolidine derivative. These results prompted us to synthesize optically active pyridobenzoxazine derivatives by replacing the piperazine at the C-10 position with 4,4-dialkyl-3-aminopyrrolidine moieties to obtain novel quinolones bearing more potent antibacterial activity and a good pharmacokinetic profile.




| | A | R1 | R2 | R3 |
|---|---|---|---|---|
| 1: Nalidixic acid | N | Et | H | Me |
| 2: Norfloxacin | CH | Et | H | HN N |
| 3: Ofloxacin | C-OCH2CH-(Me) | | H | MeN N |
| 4: Ciprofloxacin | CH | cyclopropyl | H | HN N |
| 5: Sparfloxacin | CF | cyclopropyl | NH2 | Me HN N Me |
| 6: Tosufloxacin | N | 2,4-difluorophenyl | H | H2N N |
| 7: Levofloxacin | C-OCH2CH-(Me) | | H | MeN N |
| 8: DR-3354 | C-OCH2CH-(Me) | | H | MeN N |
| 9: Sitafloxacin | CCl | 1-cyclopropyl-2-F | H | H2N N |

Fig. 1

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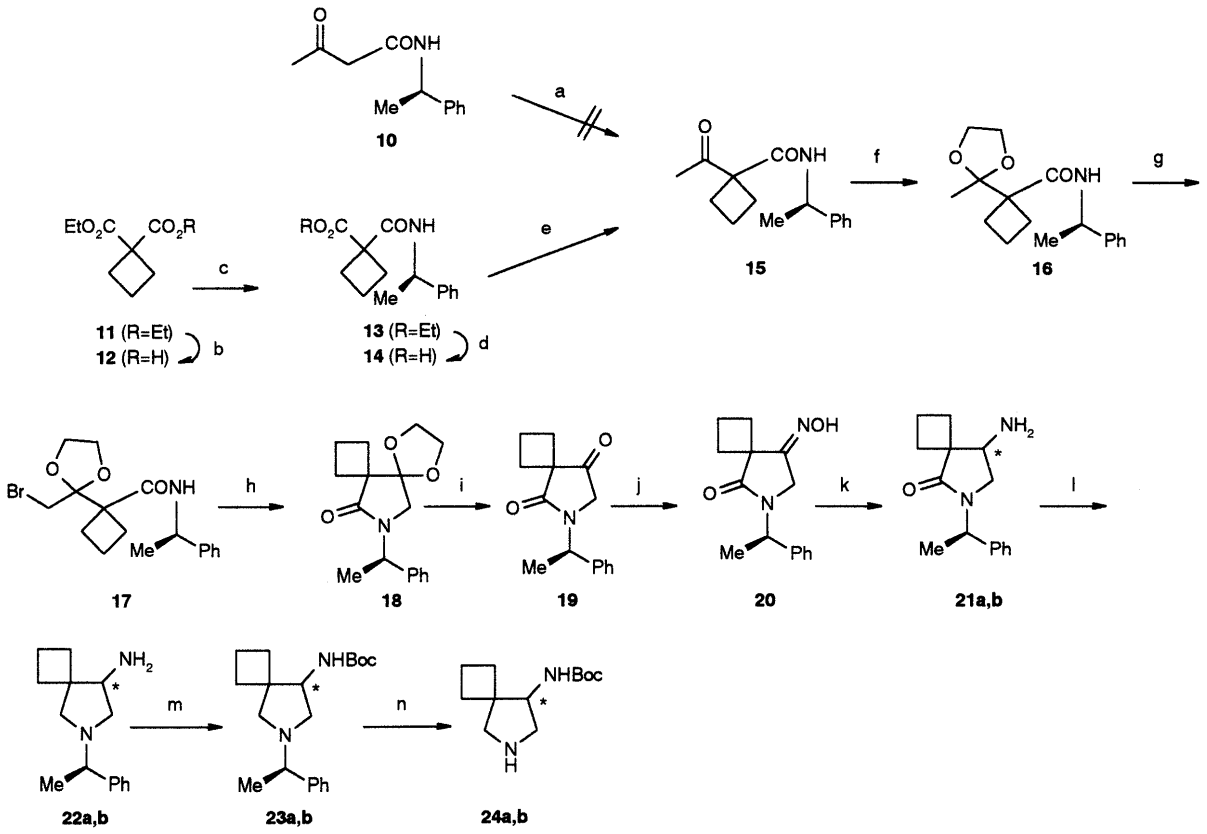


a) 1,3-Dibromopropane, $K_2CO_3$, DMF b) 10% aq. KOH, MeOH c) $ClCO_2Et$, $Et_3N$, $CHCl_3$, then (R)-(+)-Phenylethylamine

d) 20% aq. NaOH, EtOH e) 1) $SOCl_2$, benzene 2) MeLi, CuI, THF f) ethyleneglycol, *p*-TsOH, benzene g) $Br_2$, 1,4-dioxane

h) NaH, DMF i) *p*-TsOH, AcOH, acetone j) $NH_2OH$-HCl, $Et_3N$, EtOH k) $H_2$, Raney-Ni, EtOH l) $LiAlH_4$, THF m) Boc-ON, THF

n) $H_2$, 10% Pd-C, EtOH

Chart 1

In this paper, we report the synthesis and structure–activity relationships of a series of optically active pyridobenzoxazine analogues having a variety of 4,4-dialkyl-3-aminopyrrolidine substituents at the C-10 position from the view-point of avoiding the additional asymmetric carbon.

## Chemistry

The synthesis of the desired chiral 4-spirocyclobutyl-3-aminopyrrolidines **24a, b** is outlined in Chart 1. This synthetic method was based on our previous synthesis of chiral 3-aminopyrrolidine derivatives.[11,12] β-Ketoamido derivative **10** prepared by condensation of diketene and (*R*)-(+)-1-phenylethylamine was alkylated with alkyl halides in a mixture of *N*,*N*-dimethylformamide (DMF) and $K_2CO_3$ to give the cyclopropyl or dimethyl carboxamide derivatives, which were key intermediates for the synthesis of these series of chiral 4,4-dialkyl-3-aminopyrrolidines. Since alkylation of **10** with 1,3-dibromopropane in the aforementioned manner did not give cyclobutane derivative **15**, an alternative method was considered: after hydrolysis of commercially available 1,1-cyclobutane dicarboxylic acid diethylester (**11**) with one equimolar amount of 10% aqueous KOH, the resulting mono carboxylic acid **12** was condensed with (*R*)-(+)-1-phenylethylamine by using ethyl chloroformate in the presence of triethylamine ($Et_3N$) to give compound **13**. Hydrolysis of **13** with aqueous NaOH gave carboxylic acid **14**, which was converted to an acid chloride and successively treated with methyl lithium and copper iodide to give cyclobutylcarboxamide derivative **15.** After protection of the carbonyl group of **15** by ketalization, bromination of the resulting intermediate **16** in 1,4-dioxane afforded bromide **17**. Cyclization of **17** with sodium hydride in DMF, followed by deprotection of the ketal gave 2,4-dioxopyrrolidine **19**. Oxime formation of **19** with hydroxylamine, followed by catalytic hydrogenation yielded a diastereomeric mixture of amines **21**. The mixture was separated by silica gel column chromatography to afford **21a** and **21b**. After reduction of the diastereomers **21a** and **21b** with lithium aluminium hydride, the resulting diamines **22a, b** were treated with 2-[[(*tert*-butoxycarbonyl)oxy]-imino]-2-phenylacetonitrile (Boc-ON) to afford *tert*-butoxycarbonylamino derivatives **23a** and **23b**. Catalytic hydrogenation of **23a** and **23b** gave desired chiral 4-spirocyclobutyl-3-aminopyrrolidines **24a** and **24b**, respectively. Absolute configuration of the amino substituent on the pyrrolidine ring of **21a**, which was eluted first by silica gel column chromatography, was determined to be *S* based on the X-ray analysis of pyridobenzoxazine derivative **26g** (Fig. 2). The desired 10-substituted pyridobenzoxazine derivatives **26a—h** were obtained by treatment of $BF_2$-chelate **25**[10] with chiral 3-aminopyrrolidines in dimethyl sulfoxide (DMSO) followed

by dechelation with $Et_3N$ in aqueous ethanol (EtOH) and successive deprotection of *tert*-butoxycarbonyl group (Boc) of the terminal amino substituent (Chart 2). Their physical characteristics are shown in Table 1.

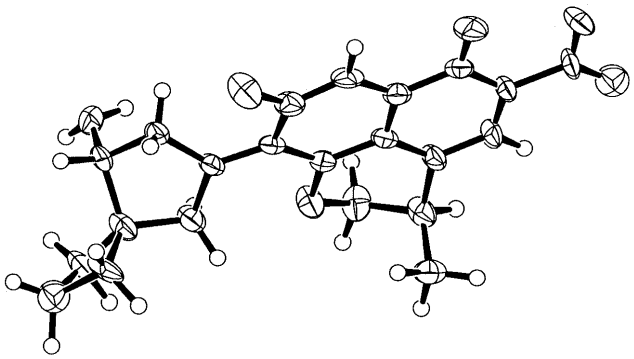

Fig. 2

## Biological Results and Discussion

The compounds **26a**—**h** prepared were tested against eight representative gram-negative and gram-positive organisms. The minimum inhibitory concentrations (MIC, $\mu$g/ml) of these compounds compared with those of SPFX (**5**), TFLX (**6**) and LVFX (**7**) are summarized in Table 2.

In this series of pyridobenzoxazines, the chirality of the amino group on the pyrrolidine ring affected the antibacterial activity. *S*-Isomers **26a**, **26c**, **26e** and **26g** were 2 to 8 times more active than *R*-isomers **26b**, **26d**, **26f** and **26h**. Compound **26a**, a non-alkylated 3-aminopyrrolidine derivative, had well balanced activity against both gram-negative and gram-positive bacteria, which was nearly equipotent to those of SPFX (**5**) and TFLX (**6**). Alkylated 3-aminopyrrolidine derivatives **26c**, **26e** and **26g** showed more potent activity

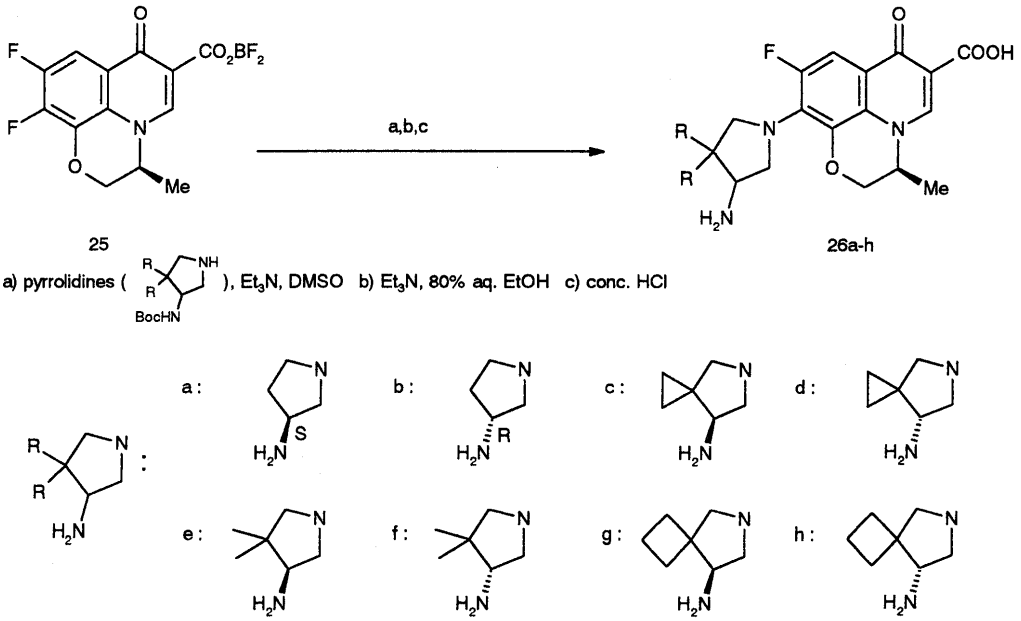


Chart 2

Table 1. Physical and Analytical Data of 10-(3′-Aminopyrrolidinyl)pyridobenzoxazine Analogues **26**

| Compd. No. | Yield (%) | mp (°C)[a] | $[\alpha]_D$[b] (conc.) | Formula | Analysis (%) Calcd (Found) | | |
|---|---|---|---|---|---|---|---|
| | | | | | C | H | N |
| **26a** | 66 | 240—244 (dec.) | −49.56 (0.690) | $C_{17}H_{18}FN_4 \cdot 1/2H_2O$ | 57.30 (57.26 | 5.37 5.60 | 11.79 11.58) |
| **26b** | 32 | 249—255 (dec.) | −79.60 (0.716) | $C_{17}H_{18}FN_3O_4 \cdot 1/4H_2O$ | 58.03 (57.71 | 5.30 5.69 | 11.94 11.79) |
| **26c** | 64 | 217—238 (dec.) | −109.22 (0.683) | $C_{19}H_{20}FN_3 \cdot 1/4H_2O$ | 60.39 (60.48 | 5.47 5.37 | 11.12 11.05) |
| **26d** | 46 | 241—248 (dec.) | +6.31 (0.665) | $C_{19}H_{20}FN_4 \cdot 1/4H_2O$ | 60.39 (60.05 | 5.47 5.71 | 11.12 11.93) |
| **26e** | 72 | 263—268 (dec.) | +147.60 (1.085) | $C_{19}H_{22}FN_3O_4$ | 60.79 (60.59 | 5.91 5.84 | 11.19 10.98) |
| **26f** | 88 | 248—275 (dec.) | −132.63 (0.950) | $C_{19}H_{22}FN_3O_4$ | 60.79 (60.81 | 5.91 5.97 | 11.19 11.09) |
| **26g** | 77 | 240—247 (dec.) | −99.42 (1.036) | $C_{20}H_{22}FN_3O_4 \cdot 1/2H_2O$ | 60.60 (60.37 | 5.85 5.64 | 10.60 10.26) |
| **26h** | 63 | 222—229 (dec.) | +15.24 (0.905) | $C_{20}H_{22}FN_3O_4 \cdot 1/2H_2O$ | 60.60 (60.75 | 5.85 5.82 | 10.60 10.36) |

*a*) All compounds were recrystallized from EtOH–28% $NH_4OH$. *b*) All compounds were measured in 0.1 N NaOH.

Table 2. *In Vitro* Antibacterial Activity of 10-(3′-Aminopyrrolidinyl)pyridobenzoxazines **26**

| Compd. No. | MIC (μg/ml)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *S. aureus* FDA 209P | *S. epidermidis* 56556 | *S. pyogenes* G36 | *E. faecalis* ATCC19433 | *E. coli* NIHJ | *K. Pneumoniae* Type 1 | *S. marcescens* 10104 | *P. aeruginosa* 32104 |
| **26a** | 0.05 | 0.2 | 0.78 | 0.78 | 0.025 | 0.1 | 0.1 | 0.2 |
| **26b** | 0.05 | 0.39 | 1.56 | 1.56 | 0.05 | 0.39 | 0.1 | 0.39 |
| **26c** | 0.025 | 0.1 | 0.2 | 0.39 | 0.013 | 0.2 | 0.1 | 0.1 |
| **26d** | 0.05 | 0.39 | 1.56 | 1.56 | 0.05 | 0.39 | 0.2 | 0.78 |
| **26e** | 0.05 | 0.05 | 0.39 | 0.39 | 0.025 | 0.1 | 0.1 | 0.39 |
| **26f** | 0.05 | 0.1 | 0.78 | 0.39 | 0.025 | 0.2 | 0.1 | 0.39 |
| **26g** | 0.025 | 0.05 | 0.2 | 0.2 | 0.025 | 0.1 | 0.1 | 0.2 |
| **27h** | 0.05 | 0.05 | 0.2 | 0.2 | 0.05 | 0.2 | 0.1 | 0.39 |
| SPFX (**5**) | 0.05 | 0.1 | 0.78 | 0.39 | 0.013 | 0.05 | 0.2 | 0.2 |
| TFLX (**6**) | 0.05 | 0.1 | 0.39 | 0.39 | 0.006 | 0.05 | 0.1 | 0.1 |
| LVFX (**7**) | 0.1 | 0.2 | 1.56 | 1.56 | 0.013 | 0.1 | 0.1 | 0.39 |

*a*) See Experimental section.

Table 3. Physicochemical Properties and Pharmacokinetic Parameters of Selected Compounds after Oral Administration to Rats[a] (20 mg/kg)

| Compd. No. | $P'$[b] | $C_{max}$ (μg/ml) | $T_{1/2}$ (min) | Urinary recovery (%) | |
|---|---|---|---|---|---|
| | | | | Unchanged | Conjugated |
| **26a** | 0.3 | 0.3 | N.D.[c] | N.D. | N.D. |
| **26c** | 1.8 | 1.1 | 51 | 7.0 | 5.3 |
| **26e** | 10.7 | 3.0 | 57 | 16.8 | 0.6 |
| **26g** | 16.6 | 1.4 | 106 | 11.4 | 0.3 |

*a*) See Experimental section. *b*) Apparent partition coefficient, $CHCl_3$/0.1 M phospate buffer (pH 7.4). N.D.: Not determined.

Table 4. Mortality in Mice Treated Intravenously with Selected Compounds[a]

| Dose (mg/kg) | Compd. No. | | | |
|---|---|---|---|---|
| | **26a** | **26c** | **26e** | **26g** |
| 200 | 5/5 | 3/3 | 2/5 | 4/5 |
| 150 | 1/1 | 0/3 | 1/5 | 0/3 |
| 100 | 1/1 | | | |

*a*) See Experimental section. The mortality rate is expressed as number of animals dead/number of animals treated.

than **26a** against gram-positive bacteria, while the activity against gram-negative bacteria was almost equal to that of **26a**. These results indicate that alkylation on the C-4 position of the pyrrolidine ring increases the activity against gram-positive bacteria.

In the next study, we tested the effect of the alkyl group on the pyrrolidine ring on pharmacokinetics in rats. Table 3 shows the parameters of pharmacokinetics in rats and the apparent partition coefficients ($P'$) of **26a**, **26c**, **26e** and **26g**. Compound **26a** showed the lowest peak plasma concentration ($C_{max}$) in the compounds tested. Increases in $P'$ values of **26c**, **26e** and **26g** by alkylation on the pyrrolidine ring showed higher $C_{max}$ values than that of **26a**. This result indicates that **26a** shows poor absorption after oral administration due to its low lipophilicity and that alkylation on the pyrrolidine ring contributes to improvement of oral absorption.

Furthermore, the lipophilicity of these compounds had a considerable effect on their acute toxicity in mice (Table 4). Non-alkylated compound **26a** showed the highest toxicity among the compounds tested, while alkylated derivatives with higher lipophilicity had lower acute toxicity. Their toxic order (from highest to lowest) was **26a**>**26c**>**26e**≒**26g**. This result indicates that alkylation on the pyrrolidine ring also contributes to reduced toxicity.

In conclusion, we have synthesized optically active 4,4-dialkyl-3-aminopyrrolidines successfully, and then introduced them to an optically active pyridobenzoxazine skeleton to prepare novel LVFX analogues. Alkylation on the pyrrolidine ring resulted in desirable effects on their antibacterial activity, pharmacokinetic profile and acute toxicity. We found that **26e** and **26g** exhibited enhanced antibacterial activity against both gram-negative and gram-positive bacteria compared with LVFX (**7**). Compound **26g** (DV-7751) showed the most potent activity against gram-positive organisms among the compounds synthesized and was finally selected for clinical evaluation.

## Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra ($^1$H-NMR) were recorded on 90 MHz with a JEOL FX-90 spectrometer and 400 MHz with a JEOL JNM-EX400 spectrometer. Chemical shifts are expressed in ppm ($\delta$) with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Elemental analyses are indicated only by the symbols of the elements; analytical results were within ±0.4% of the theoretical values unless otherwise noted. Optical rotations were measured at 589 nm with a Horiba SEPA-200 polarimeter. All organic solvent extracts were dried over anhydrous sodium sulfate. Column chromatography was carried out with Merck Silica gel 60 (230—400 mesh). Thin layer chromatography (TLC) was performed on Merck Silica gel 60 F254 TLC plates.

**1,1-Cyclobutanedicarboxylic Acid Monoethyl Ester (12)** To a solution of 1,1-cyclobutanedicarboxylic acid diethyl ester **11** (105 g, 0.53 mol) in methanol (MeOH) (110 ml) was added dropwise 10% aqueous KOH (290 ml, 0.52 mol) at 0 °C over a period of 1 h, and then the mixture was stirred at room temperature for 18 h. After evaporation of MeOH the residue was washed with $CHCl_3$ and the aqueous layer was acidified with concentrated HCl, and then was extracted with ethyl acetate (AcOEt). The extract was washed with brine, dried and concentrated to give **12** (85.0 g, 93%) as a colorless oil. NMR ($CDCl_3$) $\delta$: 1.30 (3H, t, $J$=7.20 Hz), 1.90—2.20 (2H, m), 2.50—2.80 (4H, m), 4.25 (2H, q, $J$=7.20 Hz).

**Ethyl 1-[*N*-1(*R*)-Phenylethyl]carbamoyl-1-cyclobutane Carboxylate (13)** Ethyl chloroformate (38.6 g, 0.36 mol) was added dropwise at 0 °C to a mixture of **12** (55.5 g, 0.32 mol) and $Et_3N$ (40.2 g, 0.40 mol) in $CHCl_3$ (300 ml). The mixture was stirred at room temperature for 20 min. After addition of a solution of (*R*)-(+)-1-phenylethylamine (40.2 g, 0.33 mmol) in $CHCl_3$ (100 ml) with ice-water cooling, the reaction mixture was stirred for an additional 30 min at room temperature. The mixture was washed with 10% aque-

ous citric acid and brine. The organic layer was dried and concentrated to give **13** (88.0 g, quant.) as a colorless oil. NMR ($CDCl_3$) $\delta$: 1.24 (3H, t, $J$=7.20 Hz), 1.48 (3H, d, $J$=7.20 Hz), 1.70—2.20 (2H, m), 2.30—2.80 (4H, m), 4.22 (2H, q, $J$=7.20 Hz), 5.15 (1H, q, $J$=7.20 Hz), 7.36 (5H, s).

**1-[*N*-1(*R*)-Phenylethyl]carbamoyl-1-cyclobutane Carboxylic Acid (14)** To a solution of **13** (88.0 g, 0.32 mol) in EtOH (200 ml) was added dropwise 20% aqueous NaOH (90 ml, 0.45 mol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with water and washed with $CHCl_3$. The aqueous layer was acidified with concentrated HCl with ice-water cooling, and then was extracted with AcOEt. The extract was washed with brine, dried and concentrated to give **14** (73.0 g, 94%) as a colorless solid. mp 103—106 °C. NMR ($CDCl_3$) $\delta$: 1.50 (3H, d, $J$=7.20 Hz), 1.70—2.20 (2H, m), 2.40—2.80 (4H, m), 5.18 (1H, q, $J$=7.20 Hz), 6.90 (1H, br d, $J$=7.20 Hz), 7.36 (5H, s), 9.80 (1H, br s).

**1-Acetyl-1-[*N*-1(*R*)-phenylethyl]carbamoyl]cyclobutane (15)** A mixture of **14** (20.0 g, 0.08 mol) and thionyl chloride (100 ml) in dry benzene (200 ml) was refluxed for 3 h. After cooling, the solution was evaporated to dryness under reduced pressure to give acid chloride as a colorless crystal. Methyl lithium [1.6 M solution in diethyl ether ($Et_2O$), 100 ml, 0.16 mol] was added dropwise to a suspension of copper iodide (33.5 g, 0.18 mol) in dry tetrahydrofuran (THF) (600 ml) at −20 °C under a nitrogen atmosphere over a period of 30 min, and the mixture was stirred at the same temperature for 20 min. A solution of the above acid chloride in dry THF (100 ml) was added dropwise to the mixture at the same temperature over a period of 20 min, and was allowed to stand for 30 min at room temperature. After addition of 1 N HCl (30 ml) to the reaction mixture, the solvents were evaporated under reduced pressure. AcOEt was added to the residue, and then the precipitate was removed by filtration. The filtrate was washed with 5% sodium thiosulfate and water. The organic layer was dried and concentrated to give slightly yellow crystals. The residue was washed with diisopropyl ether (IPE) and colorless crystals were collected by filtration to give **15** (9.70 g, 50%). The filtrate was concentrated and the resulting oil was chromatographed on silica gel using *n*-hexane–AcOEt (2 : 1) as an eluent to afford **15** (2.80 g, 14%) as a colorless solid. mp 57—60 °C. NMR ($CDCl_3$) $\delta$: 1.45 (3H, d, $J$=7.20 Hz), 1.70—2.20 (2H, m), 2.10 (3H, s), 2.30—2.70 (4H, m), 5.10 (1H, q, $J$=7.20 Hz), 5.80—6.99 (1H, m), 7.30 (5H, m).

**1-[1,1-(Ethylenedioxy)ethyl]-[*N*-1(*R*)-phenylethyl]-1-cyclobutane Carboxamide (16)** A mixture of **15** (13.9 g, 56.7 mmol), ethylene glycol (15 ml) and *p*-toluenesulfonic acid monohydrate (500 mg) in benzene was refluxed for 3 h under azeotropic conditions, and then cooled. The reaction mixture was poured into saturated aqueous $NaHCO_3$. The organic layer was separated, washed with brine, dried and concentrated to give **16** (16.5 g, quant.) as a colorless oil. NMR ($CDCl_3$) $\delta$: 1.14 (3H, s), 1.48 (3H, d, $J$=7.20 Hz), 1.70—2.10 (2H, m), 2.10—2.70 (4H, m), 4.10 (4H, s), 5.16 (1H, q, $J$=7.20 Hz), 6.90—7.10 (1H, m), 7.40 (5H, s).

**1-[2-Bromo-1,1-(ethylenedioxy)ethyl]-[*N*-1(*R*)-phenylethyl]-1-cyclobutane Carboxamide (17)** Bromine (13.0 g, 81 mmol) was added dropwise to 1,4-dioxane (40 ml) and the mixture was stirred at room temperature for 20 min. A solution of **16** in 1,4-dioxane (50 ml) was added dropwise to the mixture, and the mixture was stirred at room temperature for 20 h. The reaction mixture was diluted with AcOEt and washed with 5% aqueous sodium thiosulfate, saturated aqueous $NaHCO_3$ and brine. The organic layer was separated, dried and concentrated to give **17** as a yellow oil (23.0 g, 99%), which was used without further purification. NMR ($CDCl_3$) $\delta$: 1.48 (3H, d, $J$=7.20 Hz), 1.60—2.10 (2H, m), 2.10—2.70 (4H, m), 3.34 (2H, AB-q, $J$=11.00 Hz), 4.00—4.60 (4H, m), 5.10 (1H, q, $J$=7.20 Hz), 6.70 (1H, br s), 7.40 (5H, s).

**5,8-Dioxo-6-[1(*R*)-phenylethyl]-6-azaspiro[3.4]octane 8-Ethylene Acetal (18)** Sodium hydride in oil (60%, 4.20 g, 0.10 mol) was added portionwise with ice-water cooling to a solution of **17** (32.4 g, 88.0 mmol) in DMF (150 ml). The mixture was stirred at the same temperature for 30 min and was allowed to stand for 1 h at room temperature. The reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with 10% aqueous citric acid and brine, dried and concentrated. The residue was chromatographed on silica gel using *n*-hexane–AcOEt (2 : 1) as an eluent to afford **18** (23.0 g, 91%) as a slightly yellow oil. NMR ($CDCl_3$) $\delta$: 1.48 (3H, d, $J$=7.20 Hz), 1.60—2.60 (6H, m), 2.80 (1H, d, $J$=10.5 Hz), 3.08 (1H, d, $J$=10.5 Hz), 3.96 (4H, s), 5.52 (1H, q, $J$=7.20 Hz), 7.30 (5H, s).

**5,8-Dioxo-6-[1(*R*)-phenylethyl]-6-azaspiro[3.4]octane (19)** A mixture of **18** (23.0 g, 80.0 mmol), *p*-toluenesulfonic acid monohydrate (2.00 g), acetic acid (200 ml) and $H_2O$ (100 ml) was refluxed for 7 h. After concentration of the reaction mixture, AcOEt was added to the residue. The organic layer was separated and washed with 5% aqueous NaOH and brine, and dried. The solution was concentrated to give slightly red crystals, which were washed with IPE and colorless crystals were collected by filtration to give **19** (15.0 g, 77%). mp 68—69 °C. NMR ($CDCl_3$) $\delta$: 1.56 (3H, d, $J$=7.20 Hz), 1.80—2.80 (6H, m), 3.50 (2H, AB-q, $J$=17.50 Hz), 5.78 (1H, q, $J$=7.20 Hz), 7.32 (5H, s).

**8-Hydroxyimino-6-[1(*R*)-phenylethyl]-5-oxo-6-azaspiro[3.4]octane (20)** A mixture of **19** (15.0 g, 62.0 mmol), hydroxylamine hydrochloride (12.0 g, 0.173 mol), $Et_3N$ (18.0 g, 0.178 mol) in EtOH (100 ml) was stirred at room temperature for 30 min, then was heated at 70 °C for 1 h. After evaporation of EtOH, $CHCl_3$ was added to the residue, which was washed with $H_2O$, 10% aqueous citric acid and brine, dried and then concentrated under reduced pressure. The resulting residue was washed with IPE and the colorless crystals were collected by filtration to give **20** (12.0 g, 75%). mp 203—208 °C. NMR ($CDCl_3$) $\delta$: 1.56 (3H, d, $J$=7.20 Hz), 1.90—2.80 (6H, m), 3.68 (1H, d, $J$=17.0 Hz), 4.08 (1H, d, $J$=17.5 Hz), 5.64 (1H, q, $J$=7.20 Hz), 7.34 (5H, s).

**8-Amino-6-[1(*R*)-phenylethyl]-5-oxo-6-azaspiro[3.4]octane (21a, 21b)** A mixture of **20** (8.75 g, 33.9 mmol), Raney nickel (26 ml) in MeOH (300 ml) was stirred under a hydrogen atmosphere for 15 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give a mixture of **21a** and **21b**, which showed two spots on silica gel TLC [**21a**: $Rf$=0.56, **21b**: $Rf$=0.49] using $CHCl_3$–MeOH (95 : 5). The mixture was chromatographed on silica gel using $CHCl_3$–MeOH (99 : 1) as an eluent to afford **21a** (3.20 g, 39%), **21b** (1.80 g, 22%), each as a slightly yellow oil, and a mixture of **21a** and **21b** (3.0 g, 36%). **21a**: NMR ($CDCl_3$) $\delta$: 1.48 (3H, d, $J$=7.20 Hz), 1.90—2.40 (6H, m), 2.50—2.53 (1H, m), 3.20—3.52 (2H, m), 5.50 (1H, q, $J$=7.20 Hz), 7.32 (5H, s). **21b**: NMR ($CDCl_3$) $\delta$: 1.50 (3H, d, $J$=7.33 Hz), 1.85—1.95 (2H, m), 2.10—2.33 (4H, m), 2.80 (1H, dd, $J$=4.88, 9.77 Hz), 3.03 (1H, dd, $J$=5.96, 9.77 Hz), 3.22 (1H, dd, $J$=4.88, 5.96 Hz), 5.50 (1H, q, $J$=7.33 Hz), 7.20—7.47 (5H, m).

**8(*S*)-Amino-6-[1(*R*)-phenylethyl]-6-azaspiro[3.4]octane (22a)** Lithium aluminium hydride (1.00 g) was added portionwise to a solution of **21a** (3.20 g, 13.1 mmol) in dry THF (130 ml) with ice-water cooling. The mixture was stirred at room temperature for 30 min, and then was refluxed for 2.5 h. Water (1 ml), 15% aqueous NaOH (1 ml) and water (3 ml) was added to the mixture with ice-water cooling, and the mixture was stirred for 30 min at room temperature. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure to give **22a** (2.90 g, 96%) as a colorless oil. NMR ($CDCl_3$) $\delta$: 1.32 (3H, d, $J$=6.84 Hz), 1.64—1.95 (5H, m), 2.09—2.19 (1H, m), 2.37—2.42 (1H, m), 2.60—2.80 (3H, m), 3.00—3.09 (1H, m), 3.27 (1H, q, $J$=6.84 Hz), 7.19—7.38 (5H, m).

An analogous procedure was used to obtain compound **22b** as a colorless oil. NMR ($CDCl_3$) $\delta$: 1.33 (3H, d, $J$=6.34 Hz), 1.42—1.55 (2H, m), 1.63—1.98 (5H, m), 2.02—2.18 (2H, m), 2.57—2.68 (2H, m), 2.98—3.10 (2H, m), 3.20—3.30 (1H, m), 7.17—7.40 (5H, m).

**8(*S*)-(*tert*-Butoxycarbonylamino)-6-[1(*R*)-phenylethyl]-6-azaspiro-[3.4]octane (23a)** Boc-ON (3.30 g, 13.4 mmol) was added to a solution of **22a** (2.90 g, 12.6 mmol) in THF (30 ml), and the mixture was stirred for 30 min at room temperature. After evaporation of THF the residue was dissolved in AcOEt. This solution was washed twice with 5% aqueous NaOH, dried and concentrated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (99 : 1) as an eluent to afford **23a** (4.10 g, quant.) as a slightly yellow oil. NMR ($CDCl_3$) $\delta$: 1.32 (3H, d, $J$=7.20 Hz), 1.47 (9H, s), 1.60—2.20 (6H, m), 2.30—2.90 (4H, m), 3.60 (1H, q, $J$=7.20 Hz), 3.80—4.15 (1H, m), 4.90 (1H, br s), 7.28 (5H, s).

An analogous procedure was used to obtain compound **23b** as a slightly yellow oil. NMR ($CDCl_3$) $\delta$: 1.33 (3H, d, $J$=7.20 Hz), 1.44 (9H, s), 1.71—2.15 (6H, m), 2.19—2.28 (1H, m), 2.50—2.58 (1H, m), 2.73—2.85 (2H, m), 3.18—3.28 (1H, m), 3.90—3.97 (1H, m), 4.78 (1H, br s), 7.20—7.36 (5H, m).

**8(*S*)-(*tert*-Butoxycarbonylamino)-6-azaspiro[3.4]octane (24a)** A mixture of **23a** (4.10 g, 12.4 mmol), 50% aqueous palladium on carbon (4.50 g) in EtOH (60 ml) was shaken under a hydrogen atmosphere at 4 kg/cm$^2$ for 6 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure to give **24a** (2.88 g, quant.) as an amorphous foam. NMR ($CDCl_3$) $\delta$: 1.46 (9H, s), 1.77—2.03 (5H, m), 2.09—2.18 (1H, m), 2.70—2.75 (1H, m), 2.89 (1H, d, $J$=11.2 Hz), 3.23 (1H, d, $J$=11.2 Hz), 3.20—3.30 (1H, m), 3.93—4.05 (1H, m), 4.75 (1H, br s).

An analogous procedure was used to obtain compound **24b** as an amorphous foam.

**10-[8(*S*)-Amino-6-azaspiro[3.4]octan-6-yl]-9-fluoro-2,3-dihydro-3(*S*)-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic Acid (26g)** A solution of 9,10-difluoro-2,3-dihydro-3(*S*)-methyl-7-oxo-7*H*-pyrido[1,2,3-*de*][1,4]benzoxazine-6-carboxylic acid $BF_2$-chelete (**25**, 10.0 g, 30.4 mmol), **24a** (10.9 g, 48.2 mmol) and $Et_3N$ (3.80 g, 37.6 mmol) in DMSO (40 ml) was

Table 5. $^1$H-NMR Spectral Data of 10-(3′-Aminopyrrolidinyl)pyridobenzoxazine Analogues **26**

| Compd. No. | NMR (0.1 N NaOD) $\delta$ |
|---|---|
| **26a** | 1.48 (3H, d, $J$=6.84 Hz), 1.62—1.74 (1H, m), 2.12—2.24 (1H, m), 3.24—3.29 (1H, m), 3.50—3.78 (4H, m), 4.29, 4.44 (each 1H, d, $J$=9.77 Hz), 4.50—4.63 (1H, m), 7.43 (1H, d, $J$=14.16 Hz), 8.31 (1H, s) |
| **26b** | 1.47 (3H, d, $J$=6.73 Hz), 1.63—1.72 (1H, m), 2.09—2.17 (1H, m), 3.21—3.27 (1H, m), 3.48—3.76 (4H, m), 4.28, 4.46 (each 1H, d, $J$=11.72 Hz), 4.52—4.60 (1H, m), 7.42 (1H, d, $J$=14.16 Hz), 8.30 (1H, s) |
| **26c** | 0.38—0.42 (1H, m), 0.43—0.51 (2H, m), 0.62—0.68 (1H, m), 1.32 (1H, d, $J$=7.14 Hz), 2.90—2.94 (1H, m), 3.16 (1H, d, $J$=9.52 Hz), 3.22—3.26 (1H, m), 3.64 (1H, d, $J$=9.52 Hz), 3.84—3.89 (1H, m), 4.09, 4.27 (each 1H, d, $J$=10.32 Hz), 4.37—4.40 (1H, m), 7.29 (1H, d, $J$=14.29 Hz), 8.17 (1H, s) |
| **26d** | 0.54—0.60 (1H, m), 0.61—0.70 (2H, m), 0.79—0.88 (1H, m), 1.47 (1H, d, $J$=6.26 Hz), 3.04—3.07 (1H, m), 3.27 (1H, d, $J$=9.76 Hz), 3.38—3.46 (1H, m), 3.84 (1H, d, $J$=9.52 Hz), 4.00—4.09 (1H, m), 4.28, 4.44 (each 1H, d, $J$=11.23 Hz), 4.52—4.58 (1H, m), 7.43 (1H, d, $J$=14.16 Hz), 8.29 (1H, s) |
| **26e** | 0.98, 1.06 (each 3H, s), 1.45 (3H, d $J$=6.84 Hz), 2.99—3.03 (1H, m), 3.33—3.37 (1H, m), 3.48—3.55 (2H, m), 3.65—3.72 (1H, m), 4.23, 4.39 (each 1H, d, $J$=10.25 Hz), 4.45—4.53 (1H, m), 7.37 (1H, d, $J$=14.65 Hz), 8.25 (1H, s) |
| **26f** | 0.87, 0.94 (each 3H, s), 1.34 (3H, d, $J$=7.15 Hz), 2.86—2.91 (1H, m), 3.26—3.30 (1H, m), 3.35—3.40 (2H, m), 3.60—3.66 (1H, m), 4.07, 4.26 (each 1H, d, $J$=11.11 Hz), 4.36—4.41 (1H, m), 7.25 (1H, d, $J$=15.08 Hz), 8.19 (1H, s) |
| **26g** | 1.39 (3H, d, $J$=6.35 Hz), 1.70—1.91 (5H, m), 2.02—2.10 (1H, m), 3.09—3.12 (1H, m), 3.19—3.25 (1H, m), 3.50—3.56 (1H, m), 3.70—3.79 (2H, m), 4.14, 4.33 (each 1H, d, $J$=11.11 Hz), 4.40—4.48 (1H, m), 7.34 (1H, d, $J$=14.28 Hz), 8.21 (1H, s) |
| **26h** | 1.26 (3H, d, $J$=6.35 Hz), 1.60—1.79 (5H, m), 1.95—2.02 (1H, m), 2.96—3.01 (1H, m), 3.07—3.13 (1H, m), 3.34—3.38 (1H, m), 3.58—3.70 (2H, m), 4.03, 4.20 (each 1H, d, $J$=11.11 Hz), 4.28—4.36 (1H, m), 7.18 (1H, d, $J$=14.28 Hz), 8.12 (1H, s) |

stirred at room temperature for 72 h. After evaporation of $Et_3N$, $H_2O$ was added to the residue with ice-water cooling, and then the mixture was stirred at room temperature for 30 min. The precipitate was washed with $H_2O$ and collected by filtration, then was dissolved in 80% aqueous MeOH (500 ml). $Et_3N$ (100 ml) was added to the solution and the mixture was refluxed for 6 h. After concentration, the residue was dissolved in $CHCl_3$, which was washed with 10% aqueous citric acid and brine, dried and then evaporated to dryness. The residue was dissolved in concentrated HCl (20 ml) with ice-water cooling and stirred for 5 min at room temperature. The mixture was adjusted to pH 11 with 20% aqueous NaOH with ice-water cooling, and then was neutralized with 10% aqueous HCl to pH 7.4, which was extracted with $CHCl_3$. The extract was dried and evaporated to dryness to afford a crude **26g** (9.00 g, 77%), which was recrystallized from EtOH–28% $NH_4OH$ to give **26g** (6.40 g, 55%) as slightly yellow needles.

An analogous procedure was used to obtain compounds **26a**—**f** and **26h**. Physical, analytical and $^1$H-NMR spectral data of compounds **26a**—**h** are listed in Tables 1 and 5.

***In Vitro* Antibacterial Activity** The MICs of the compounds tested in this study were measured according to the two-fold micro broth dilution method using Muller–Hinton broth (Difco Laboratories, Detroit, MI) with an inoculum size of approximately $10^5$ cfu/ml. The MIC was defined as the lowest concentration which prevented visible bacterial growth after incubation at 37 °C for 18 h.

**Determination of Apparent Partition Coefficients** The apparent partition coefficients of the compounds tested in this study were measured according to the method reported previously.[13]

**Pharmacokinetic Studies** The test compounds were administered in solution by oral gavage to groups of five animals each. Blood samples were obtained 0.5, 1, 3, 4, 5, 6 and 24 h after dosing. Urine samples were collected 0—4, 4—8, 8—24 h after dosing. Plasma levels and urinary excretion of the test compounds were determined by a microbiological assay. *Bacillus subtilis* ATCC 6051 was used as a test organism.

**Acute Toxicity Test** The test compounds were dissolved in 0.1 N NaOH in saline at different concentrations. The solution was administered intravenously to five week-old male Slc : ddY mice at the speed of 0.1 ml/30 s. The total volume of administration was adjusted to 10 ml/kg of body weight. The number of dead mice was counted 7 d after the administration.

**X-Ray Crystallographic Analysis of 26g** **26g** was crystallized from ethanol to give yellow, needle-shaped crystal of $C_{20}H_{22}FN_3O_4$ having approximate dimension of 0.10×0.10×0.30 mm. The lattice parameters and intensities were measured on a Mac Science MXC18 diffractometer with graphite monochromated Cu$K_\alpha$ radiation using $\omega$–2$\theta$ scan technique. The compound crystallized in monoclinic space group P21 with cell dimensions $a$=13.557(4) Å, $b$=18.110(6)Å, $c$=7.359(3) Å, $V$=1782.0 Å$^3$. For $Z$=4 and F.W.=387.41, the calculated density was 1.44 g/cm$^3$. The structure was solved by the direct method with the program CRYSTAN. The final $R$ value was 0.041. A perspective view of the molecule is shown in Fig. 2.

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