flash chromatography (20% $CH_3OH$-$CHCl_3$ with 1% $NH_4OH$) to give 420 mg (100%) **51** as a pale tan oil. The tan oil was dissolved in 15 mL of acetone and placed in a freezer for 4 days. The resulting crystals (170 mg) were collected: mp 232-234 °C; $^1H$ NMR ($CD_3OD$) $\delta$ 4.78 (d, 1 H, $J$ = 4.2 Hz), 5.14 (dd, 1 H, $J$ = 5.1 and 7.8 Hz), 6.30 (d, 1 H, $J$ = 7.8 Hz), 8.21 (s, 1 H), 8.34 (s, 1 H); $^{19}F$ NMR ($CD_3OD$, 282 MHz) $\delta$ -110.355 (s); $^{13}C$ NMR ($CD_3OD$) $\delta$ 74.33 (d, $J_{C-F}$ = 1.95 Hz); MS (CI/$CH_4$) $m/z$ 302 (M + H)$^+$. Anal. ($C_{10}H_9ClFN_5O_3 \cdot {}^1/_3C_3H_6O$) C, H, N.

**(*Z*)-4′,5′-Didehydro-5′-deoxy-5′-chloro-5′-fluoroadenosine (52).** Nucleoside **51** in acetone-$CH_3OH$ (2:1) was irradiated for 4 h. The $^{19}F$ NMR of the reaction indicated a 2:1 mixture of **51** and **52**. Nucleoside **52** was isolated by reverse phase HPLC (Zorbax $R_x$ 250 × 4.6 mm column, mobile phase methanol-water) and characterized by NMR and mass spectra.

The coupling constant of C-3′ to the fluorine was measured for **51** (2.3 Hz) and **52** (0). Comparison to the values recorded for **6** and **13** allowed assignment of olefin geometry: MS (CI/$CH_4$) $m/z$ 302 (M + H)$^+$; $^1H$ NMR ($CD_3OD$) $\delta$ 4.80 (d, 1 H, $J$ = 6 Hz), 5.18 (dd, 1 H, $J$ = 5.4 and 7.5 Hz), 6.28 (d, 1 H, $J$ = 7.5 Hz), 8.21 (s, 1 H), 8.34 (s, 1 H); $^{19}F$ NMR ($CD_3OD$) $\delta$ -126.63 (s).

**Acknowledgment.** The authors thank Dr. Edward Huber for obtaining NMR spectra and for assistance in interpretation.

**Supplementary Material Available:** Details of the preparation and assay of rat liver SAH hydrolase and details of the preparation of and spectra for compounds **22-27, 29, 31-35, 38-43, 47,** and **48** (13 pages). Ordering information is given on any current masthead page.

# Quinolone Antibacterials: Preparation and Activity of Bridged Bicyclic Analogues of the $C_7$-Piperazine

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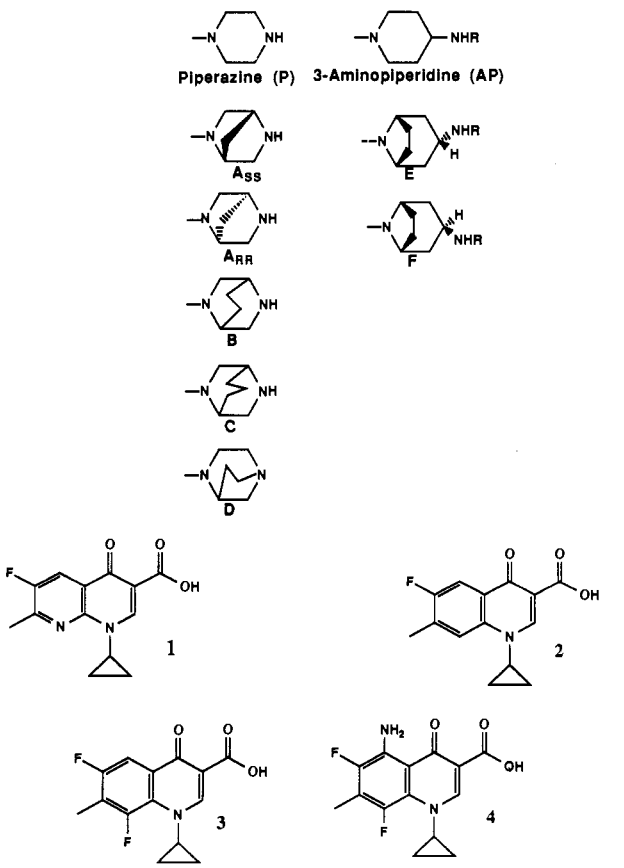
A series of quinolone and naphthyridine antibacterial agents possessing as the $C_7$-heterocycle bicyclic 2,5-diazabicyclo[*n*.2.*m*]alkanes, where $n$ = 2, 3 and $m$ = 1, 2, and a series including 4-aminopiperidine and 3-amino-8-azabicyclo[3.2.1]octanes have been prepared and evaluated in vitro and in vivo for antibacterial activity against a variety of Gram-negative and Gram-positive organisms. These compounds were also tested against the target enzyme bacterial DNA gyrase. All the examples investigated are nearly equipotent with the parent 7-piperazinyl analogues. Only *endo*-7-(3-amino-8-azabicyclo[3.2.1]oct-8-yl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid displays activity that surpasses that of the piperazine parent.

## Introduction

Quinolone antibacterial agents are a major class of antiinfectives with significant potential for continued development.[1] In virtually all modern examples of this antiinfectives class, a nitrogen heterocycle is attached at the $C_7$-position of the quinolone or naphthyridine nucleus.[2] This moiety plays a significant role in determining antibacterial spectrum and potency and represents a site amenable to significant modification. We report our results from a study replacing the frequently employed 7-piperazinyl substituent with various bicyclic analogues.[3] We were interested in determining the effect these bicyclic $C_7$-substituents would have on minimum inhibitory concentrations, inhibition of the gyrase enzyme, and in vivo protective doses in mice.

Ciprofloxacin, which contains a 7-piperazinyl moiety, served as the beginning point for our studies. The SAR of the $C_7$-side chain has been extensively explored[4,5] and the importance of proper side-chain length and ring size has been elucidated.[4] The optimum size for the $C_7$-substituent has been defined as a ring of five or six atoms.

**Chart I**

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These studies have described the requirements for optimal potency with regard to the length and width of the $C_7$-substituent, but the effect of having the $C_7$-substituent occupy volume in the third spatial dimension is essentially unexplored. In an attempt to further define the spatial requirement for the $C_7$-substituent, a number of quinolones and naphthyridines containing bridged bicyclic piperazines were synthesized. The bicyclic piperazines prepared and evaluated in this study were [1*R*,4*R*]- (1*R*-*cis*) and [1*S*,4*S*]- (1*S*-*cis*) 2,5-diazabicyclo[2.2.1]heptane[6,7] ($A_{RR}$, $A_{SS}$), 2,5-diazabicyclo[2.2.2]octane[8] (B), 6,8-diazabicyclo[3.2.2]nonane[9] (C), and 1,4-diazabicyclo[3.2.1]octane[10] (D) (Chart I). The work of Domagala and co-workers[5] has also shown that the second nitrogen of the $C_7$-substituent need not be part of the ring. Indeed, if this nitrogen is exocyclic to the ring, an increase in efficacy against Gram-positive organisms can be achieved. We also included as part of this study *exo*- and *endo*-3-amino-8-azabicyclo[3.2.1]octanes[11,12] (E and F, Chart I) to see if having the nitrogen exocyclic to the bicycle would have the same effect as in monocycles. To this end we included 4-aminopiperidine as the proper monocyclic comparison to structures E and F.

In addition to providing bulk in the third dimension, these bicycles impart rigidity to the piperazine ring system. From this, we hoped to learn if activity was dependent on the conformation of the piperazine portion of the bicycle. In bicycles A–C, the piperazine ring is restricted to the boat form as a consequence of the bridge. Bicycle D exists with the piperazine ring in a chair conformation. Within E and F the 4-aminopiperidine ring also exists in the chair form, but an additional variable must be considered in that the amine moiety exists in either the exo or endo orientation.

We selected as representative quinolone nuclei 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid[16] (1), 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[13] (2), 1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[14] (3), and 5-amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[15] (4) (Chart I). These were used to determine the effect each different bicycle had an overall antibacterial activity.

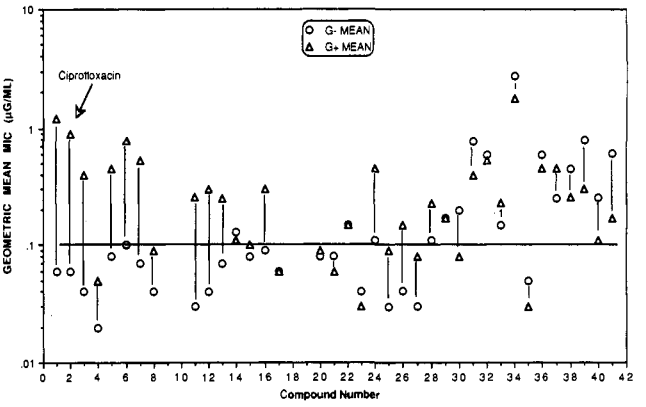


**Figure 1.** Comparison of geometric mean MICs for gram-negative and -positive organisms. Line indicates 0.1 μg/mL, an arbitrary target potency.

## Chemistry

The bicyclic side chains employed were prepared by literature procedures.[6-12] All attempts to synthesize or resolve the chiral forms of 2,5-diazabicyclo[3.2.2]nonane (C) were unsuccessful. Extension of the chemistry employed to prepare C was unsuccessful in an attempt to synthesize 2,5-diazabicyclo[4.2.2]decane, the next higher homologue. Coupling of the various bicylic amines to quinolone and naphthyridine substrates was performed as has been previously described.[13,15] Table I provides a compilation of the compounds prepared.

## Discussion

Table II summarizes the biological data gathered for the compounds prepared as part of this study. We have provided values for the geometric means for the minimum inhibitory concentrations (MIC) over five Gram-negative bacterial strains and over five Gram-positive strains.[17] This was done as a means of consolidating the large amount of data generated so that meaningful trends could be discerned. For assessing the effect of each bicycle relative to the others and to piperazine, it is useful to make comparisons employing a constant substrate. For our analysis of the data we have compared the potency of the bicycle versus the corresponding piperazine analogue within each substrate series and also relative to ciprofloxacin, the clinical standard.

From the measured DNA gyrase 50% inhibition ($I_{50}$) values, none of the compounds prepared show a significant variation in activity from that obtained with ciprofloxacin, excepting compound **1**, which is quite inactive ($I_{50}$ = 50), and **23** and **30**, which are 3–5 times more active ($I_{50}$ = 0.9) than average (Table II). For **23** and **30** this increased potency may reflect some synergistic effect of the 5-amino-6,8-difluoroquinolone nucleus and the bicycle. Overall these data would seem to indicate that the enzyme–DNA helix–drug complex readily accommodates the bulk of the various bridges employed here and that no lessening of drug potency occurs. The potency of the drug in the enzyme–DNA helix–drug complex is also unaffected

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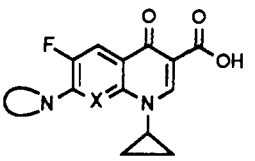
(17) The geometric mean was calculated with the following: geometric mean = antilog $[(\Sigma \log x_1 + \log x_2 + ...\log x_n)/n]$. The Gram-negative organisms employed were *E. coli* H560, *E. coli* Vogel, *Enterobacter cloacae* MA 2646, *Klebsiella pneumoniae* MGH-2, and *Proteus rettgeri* M1771. The Gram-positive organisms used included *Staphylococcus aureus* H-228 (resistant strain), *Staphylococcus aureus* UC-76 (sensitive strain), *Streptococcus faecalis* MGH-2, *S. pneumoniae* SV-1, and *Streptococcus pyogenes* C-203. The data for the individual MIC for each organism is available as supplementary data.

**Table I.** Physical Data



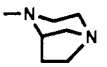
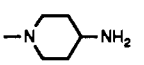
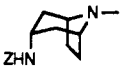
| no. | code | Z | X | synth method (% yield) | mp, °C | formula | analysis |
|---|---|---|---|---|---|---|---|
| | | | | | piperazines | | |
| 1 | 1P | H | N | | | ref. 11 | |
| 2 | 2P | H | OH | | | ref. 11 | Ciprofloxacin |
| 3 | 3P | H | CF | | | ref. 11 | |
| 4 | 4P | H | CF<br>5-$NH_2$ | | | ref. 13 | |
| | | | | |  | | |
| 5 | $1A_{SS}$ | H | N | A (66) | 257–9 | $C_{17}H_{17}FN_4O_3 \cdot H_2O$ | C, H, N |
| 6 | $2A_{SS}$ | H | CH | B (50) | 307 | $C_{18}H_{18}FN_3O_3 \cdot 1.8H_2O$ | C, H, N |
| 7 | $3A_{SS}$ | H | CF | A (83) | 295 | $C_{18}H_{17}F_2N_3O_3 \cdot 0.2H_2O$ | C, H, N |
| 8 | $4A_{SS}$ | H | CF<br>5-$NH_2$ | B (64) | 275–8 | $C_{18}H_{18}F_2N_4O_3 \cdot 2.85H_2O$ | C, H, N |
| 9 | $1A_{SS}$ | Me | N | E (66) | >300 | $C_{18}H_{19}FN_4O_3 \cdot HCl$ | C, H, N, Cl |
| 10 | $3A_{SS}$ | Me | CF | E (86) | 296.9 | $C_{19}H_{19}F_2N_3O_3 \cdot H_2O \cdot HCl$ | C, H, N |
| | | | | |  | | |
| 11 | $1A_{RR}$ | H | N | A (99) | 258.62 d | $C_{17}H_{17}FN_4O_3 \cdot 1.7H_2O \cdot 0.25HCl$ | C, H, N, Cl |
| 12 | $2A_{RR}$ | H | CH | A (79) | | $C_{18}H_{18}FN_3O_3 \cdot 0.6H_2O \cdot 0.4HBr$ | C, H, N, Cl |
| 13 | $4A_{RR}$ | H | CF | 7 (91) | >280 | $C_{18}H_{18}F_2N_4O_3 \cdot 0.3H_2O \cdot 0.7HBr$ | C, H, N, Br |
| | | | | |  | | |
| 14 | 1B | H | N | A (80) | 272.4 | $C_{18}H_{19}FN_4O_3 \cdot 1.5H_2O$ | C, H, N |
| 15 | 2B | H | CH | A (80) | 300.5 | $C_{19}H_{70}FN_3O_3 \cdot 0.25H_2O$ | C, H, N |
| 16 | 3B | H | CF | A (82) | 275.80 | $C_{19}H_{19}F_2N_3O_3 \cdot 0.25H_2O$ | C, H, N |
| 17 | 4B | H | CF<br>5-$NH_2$ | A (62) | 289.91 | $C_{19}H_{20}F_2N_4O_3$ | C, H, N |
| 18 | 1B | Me | N | E (83) | >310 | $C_{19}H_{21}FN_4O_3 \cdot HCl \cdot 0.75H_2O$ | C, H, N |
| 19 | 3B | Me | CF | E (40) | >300 | $C_{20}H_{21}F_2N_3O_3 \cdot 0.5H_2O$ | C, H, N |
| | | | | |  | | |
| 20 | 1C | H | N | A (52) | 255.8 | $C_{19}H_{21}FN_4O_3 \cdot HCl$ | C, H, N |
| 21 | 2C | H | CH | C (51) | 273.6 | $C_{20}H_{22}FN_3O_3 \cdot 0.67H_2O$ | C, H, N |
| 22 | 3C | H | CF | A (59) | 268.71 | $C_{20}H_{21}F_2N_3O_3 \cdot 0.25H_2O$ | C, H, N |
| 23 | 4C | H | CF<br>5-$NH_2$ | A (64) | 287.9 | $C_{20}H_{22}F_2N_4O_3$ | C, H, N |
| | | | | |  | | |
| 24 | 1D | H | N | A (37) | 276–8 | $C_{18}H_{19}FN_4O_3$ | C, H, N |
| 25 | 2D | H | CH | C (47) | >300 | $C_{19}H_{20}FN_3O_3 \cdot 1.05HCl \cdot 1.3H_2O$ | C, H, N, Cl |
| 26 | 3D | H | CF | A (37) | >280 | $C_{19}H_{19}F_2N_3O_3$ | C, H, N |
| 27 | 4D | H | CF<br>5-$NH_2$ | A (54) | 281–2 | $C_{19}H_{20}F_2N_4O_3 \cdot 0.5H_2O$ | C, H, N |
| | | | | |  | | |
| 28 | 1AP | H | N | D (78) | 243–5 d | $C_{17}H_{19}FN_4O_3 \cdot 0.2H_2O$ | C, H, N, F |
| 29 | 2AP | H | CH | D (17) | 265 d | $C_{18}H_{20}FN_3O_3 \cdot 1.8H_2O \cdot 1.0HCl$ | C, H, N, Cl, F |
| 30 | 3AP | H | CF | D (44) | >280 | $C_{18}H_{19}F_2N_3O_3 \cdot 2.1H_2O$ | C, H, N, F |
| | | | | |  | | |
| 31 | 1F | H | N | F (8) | 195 | $C_{19}H_{21}FN_4O_3 \cdot 0.33H_2O \cdot 0.1HCl$ | C, H, N, Cl |
| 32 | 1F | Et | N | A (31) | 265–8 | $C_{21}H_{25}FN_4O_3 \cdot 0.75H_2O$ | C, H, N |
| 33 | 2F | H | CH | F (28) | – | $C_{20}H_{22}FN_3O_3 \cdot 3.0H_2O$ | C, H,[a] N, F |
| 34 | 2F | Et | CH | C (47) | 276–8 | $C_{22}H_{26}FN_3O_3 \cdot 0.15H_2O \cdot 0.5HF$ | C, H, N |
| 35 | 3F | H | CF | F (28) | 214 | $C_{20}H_{21}F_2N_3O_3$ | C, H, N |
| 36 | 3F | Et | CF | A (20) | 242–5 | $C_{22}H_{25}F_2N_3O_3 \cdot 1.5H_2O$ | C, H, N |

**Table I** (Continued)

| no. | code | Z | X | synth method (% yield) | mp, °C | formula | analysis |
|---|---|---|---|---|---|---|---|
| | | | |  | | | |
| **37** | 1E | H | N | F (22) | 260 | $C_{19}H_{21}FN_4O_3 \cdot 0.5H_2O \cdot 1.2HCl$ | C, H, N, Cl |
| **38** | 1E | Et | N | A (75) | 236–8 | $C_{21}H_{25}FN_4O_3$ | C, H, N |
| **39** | 2E | Et | CH | C (43) | 276–8 | $C_{22}H_{26}FN_3O_3$ | C, H, N |
| **40** | 3E | H | CF | F (15) | 194 | $C_{20}H_{21}F_2N_3O_3 \cdot 1.0HCl \cdot 1.5H_2O$ | C, H, N, Cl |
| **41** | 3E | Et | CF | A (40) | 263–8 | $C_{22}H_{25}F_2N_3O_3 \cdot 0.2H_2O$ | C, H, N |

[a] H: calcd, 6.63; found, 5.98.

by conformation of the embedded piperazine ring within the bicycles.

Potency against whole bacterial as measured by the MIC is not controlled by enzyme inhibition alone. This is clearly demonstrated by comparing the MIC and $I_{50}$ values for **1** versus the same values for **2–4**. The ease with which the drug penetrates to the site of action is also an important factor.

As an initial observation concerning MICs (Figure 1), it is notable that the examples bearing piperazine in each substrate series show a significantly less potent Gram-positive mean MIC when compared to the corresponding Gram-negative mean MIC (Table II, **1–4**). For 2,5-diazabicyclo[2.2.2]octane (B) and 2,5-diazabicyclo[3.2.2]nonane (C), this trend is altered in all four substrate series (Table I and Figure 1; compare compounds **14–17** and **20–23** vs **1–4**). These two $C_7$-substituents (B and C) lower the Gram-positive mean MIC's significantly while slightly increasing the Gram-negative mean. The effect of these bicycles is that they lower the Gram-positive mean MIC to the point where it is equipotent with the Gram-negative mean MIC. Only compound **16** fails to display Gram-positive activity equal to the Gram-negative potency.

The other bridged piperazines, [1*S*,4*S*]- (1*S-cis*) or [1*R*,4*R*]- (1*R-cis*) 2,5-diazabicyclo[2.2.1]heptanes ($A_{SS}$, $A_{RR}$) and 1,4-diazabicyclo[3.2.1]octane (D), behave much like the parent piperazine in that the mean Gram-positive MICs are higher than the mean Gram-negative MICs. [1*R*,4*R*]- (1*R-cis*) 2,5-Diazabicyclo[2.2.1]heptane ($A_{RR}$) is slightly more potent than its 1*S*,4*S* diastereomer ($A_{SS}$) except when attached to the 5-amino-6,8-difluoroquinolone substrate. In this instance, the Gram-positive MIC is lower for **8** rather than for **13**. For compounds possessing 1,4-diazabicyclo[3.2.1]octane (D) the in vitro mean MICs are equal to or slightly more potent than that of the parent piperazine (compare **24–27** vs **1–4**; Table II and Figure 1).

These data suggest that B and C impart some physical property change that improves Gram-positive potency. Since our gyrase inhibition data is gathered from enzyme isolated from *Escherichia coli* H560 cells and the improvement observed is against Gram-positive cells, it is not possible to know if these compounds have an altered (improved) enzyme potency in Gram-positive cells or if this improvement reflects improved penetration into Gram-positive cells.

The gain in Gram-positive MIC for side chains B and C however does not provide an improvement in mouse protection ($PD_{50}$) values for Gram-positive infection (Table II). In fact, overall these two side chains are not as efficacious in vivo as the parent piperazine, as noted in the modest but consistent loss in vivo activity against *E. coli* Vogel (Table II). Compounds bearing 1,4-diazabicyclo-[3.2.1]octane (D) possess in vivo activity equal to the parent piprazines against *E. coli* Vogel and are somewhat more potent against *Streptococcus pneumonia* (Table II). Thus this tertiary amine analogue is the first to achieve the same overall potency as the parent piperazine.

The in vitro and in vivo results for the tertiary amine 1,4-diazabicyclo[3.2.1]octane (D) suggested that methylation of the other secondary amine bicycles to create a tertiary nitrogen might increase in vivo potency as has been seen in other analogues.[20] For [1*R*,4*R*]- (1*R-cis*) and [1*S*,4*S*]- (1*S-cis*) 5-methyl-2,5-diazabicyclo[2.2.1]heptane ($A_{RR}$, $A_{SS}$) this was indeed true as ca. 2–10 times increase in in vivo potency was observed (compounds **5** and **7** vs **9** and **10**, Table II). For *N*-methyl compounds **18** and **19**, in vitro potency was not increased over nonmethylated analogues **14** and **16** and no significant improvement in potency was achieved in vivo. From these data it seems that conversion of the secondary amine bicycles to tertiary amines via methylation does not consistently lead to improved activity.

We next turned our attention to the 4-aminopiperidine (AP) substituent and bicyclic analogues E and F as possible piperazine mimics and ones that could parallel the 3-aminopyrrolidine[4a] type of $C_7$-substituent. Although equipotent against the gyrase enzyme and improving the mean Gram-positive MIC relative to piperazine, we found that the 4-aminopiperidine modification significantly increased the mean Gram-negative MIC (**28–30** vs **1–4**, Table II). In vivo potency for **29–30** decreased 6–12 times. The related bicyclic analogues E and F showed no improvement over the 4-aminopiperidine or the piperazine series. In fact, as with 4-aminopiperidine, there is a major loss in activity against Gram-negative organisms for substituents E and F. A striking exception to this trend is compound **35** (substituent F). Compound **35** has significantly lower MICs and is the only compound found displaying excellent in vivo activity against a Gram-positive organism (Table II).

Within the E and F series, it was possible to monoethylate the primary nitrogen of the side chain. This was done in hopes of increasing activity through an increase in lipophilicity. Ethylation of the endo isomer F improved in vivo activity for naphthyridine (**31** vs **32**) but decreased activity for 6,8-difluoroquinolones (**35** vs **36**). For the exo series ethylation had a mixed effect. These data suggest that *N*-ethylation is not a useful modification.

From the data gathered, the bicyclic modifications undertaken in this study do not alter activity toward gyrase inhibition. The enzyme seems to well tolerate the ex-

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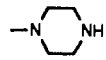
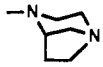
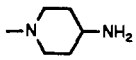
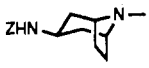
**Table II.** Microbiological Data

| no. | code | in vitro MIC's geometric mean[a] Gram neg | Gram pos | gyrase inhibition,[b] $I_{50}$, μg/mL | in vivo protection; dose $PD_{50}$,[c] mg/kg *E. coli* vogel oral | SC | *S. pneumonia* SV-1 oral | SC |
|---|---|---|---|---|---|---|---|---|
| | | | |  | | | | |
| 1 | 1P | 0.06 | 1.21 | 50 | 0.7 (0.2) | 0.4 (0.2) | | |
| 2 | 2P | 0.06 | 0.91 | 5.3 | 1.2 (N) | 0.3 (0.1) | >100 (N) | 29 (12) |
| 3 | 3P | 0.04 | 0.40 | 2.8 | 0.5 (0.2) | 0.3 (0.06) | 59 (29) | 29 (13) |
| 4 | 4P | 0.02 | 0.05 | 2.8 | 0.8 (0.3) | 0.2 (0.07) | 31 (11) | 23 (8) |
| | | | |  | | | | |
| 5 | $1A_{SS}$ | 0.08 | 0.46 | 6.3 | 53 (40) | 37 (10) | | |
| 6 | $2A_{SS}$ | 0.10 | 0.79 | 2.8 | 5.5 (2.5) | 0.6 (0.2) | >100 (N) | 51 (19) |
| 7 | $3A_{SS}$ | 0.07 | 0.53 | 2.8 | 1.7 (0.7) | 0.4 (0.1) | >100 (N) | 18.5 (6) |
| 8 | $4A_{SS}$ | 0.04 | 0.09 | 13.8 | 5.0 (1.7) | 1.0 (0.3) | >100 (N) | 13 (5) |
| 9 | $1A_{SS}$ | 0.08 | 0.35 | 3.8 | 0.5 (0.15) | 0.3 (0.08) | 23 (8) | 25 (8) |
| 10 | $3A_{SS}$ | 0.09 | 0.17 | 3.0 | 0.9 (0.2) | 0.5 (0.1) | 13 (5) | 14 (5) |
| | | | |  | | | | |
| 11 | $1A_{RR}$ | 0.03 | 0.26 | - | 2.2 (0.6) | 1.6 (0.5) | | |
| 12 | $2A_{RR}$ | 0.04 | 0.30 | 1.9 | 1.9 (0.6) | 0.2 (0.06) | >100 (N) | 12.5 (5.3) |
| 13 | $3A_{RR}$ | 0.07 | 0.25 | - | 6.1 (2.3) | 1.1 (0.4) | | |
| | | | |  | | | | |
| 14 | 1B | 0.13 | 0.11 | 6.3 | 1.7 (0.07) | 0.7 (0.2) | >100 (N) | 15.1 (7) |
| 15 | 2B | 0.08 | 0.10 | 1.5 | 5.5 (2) | 0.7 (0.2) | >100 (N) | 24 (7) |
| 16 | 3B | 0.09 | 0.30 | 6.3 | 2.9 (0.4) | 1.3 (1.1) | | |
| 17 | 4B | 0.06 | 0.06 | 5.5 | 3.6 (1) | 0.7 (0.2) | | |
| 18 | 1B | 0.1 | 0.26 | 5.5 | 1.4 (0.1) | 1.1 (N) | | |
| 19 | 3B | 0.13 | 0.15 | 6.3 | <1.6 (N) | <1.6 (N) | | |
| | | | |  | | | | |
| 20 | 1C | 0.08 | 0.09 | 3.0 | 2.3 (0.3) | 0.8 (0.8) | 25 (13) | 18 (11) |
| 21 | 2C | 0.08 | 0.06 | 1.8 | 5.7 (1.4) | 1.6 (0.4) | >100 (N) | 6.6 (2.5) |
| 22 | 3C | 0.15 | 0.15 | 2.9 | 2.9 (0.7) | 1.1 (N) | 48.5 (22) | 11.9 (6) |
| 23 | 4C | 0.04 | 0.03 | 0.9 | 4.8 (1.4) | 0.7 (0.5) | 35 (59)[d] | 6.3 (5.5)[d] |
| | | | |  | | | | |
| 24 | 1D | 0.11 | 0.46 | 13.8 | 0.5 (0.2) | 0.3 (0.1) | 32 (9) | 20 (8) |
| 25 | 2D | 0.03 | 0.09 | 3.0 | 1.0 (0.3) | 0.3 (0.09) | 35 (12) | 11 (3) |
| 26 | 3D | 0.04 | 0.15 | 2.9 | 0.5 (0.2) | 0.2 (0.08) | 32 (9) | 11 (2) |
| 27 | 4D | 0.03 | 0.08 | 5.5 | 0.6 (0.3) | 0.2 (0.05) | | |
| | | | |  | | | | |
| 28 | 1AP | 0.11 | 0.23 | 3.0 | 4.6 (2.6) | 0.8 (0.5) | | |
| 29 | 2AP | 0.17 | 0.17 | 0.8 | 15.0 (5) | 1.4 (0.5) | | |
| 30 | 3AP | 0.20 | 0.08 | <0.5 | 4.7 (2) | 1.4 (0.5) | | |
| | | | |  | | | | |
| 31 | 1F | 0.79 | 0.40 | 7.5 | 71 (N) | 6.0 (2) | | |
| 32 | 1F | 0.46 | 0.26 | 6.3 | 2.8 (1) | 2.8 (0.8) | 23 (7) | 16 (5) |
| 33 | 2F | 0.15 | 0.23 | 3.0 | 9.5 (3) | 2.0 (0.5) | | |
| 34 | 2F | 2.76 | 1.81 | 17.5 | | | | |
| 35 | 3F | 0.05 | 0.03 | 3.8 | 1.8 (0.6) | 0.75 (0.3) | 8.5 (3) | 3.1 (1) |
| 36 | 3F | 0.60 | 0.46 | 6.3 | >12.5 (N) | 6.5 (3) | | |
| | | | |  | | | | |
| 37 | 1E | 0.25 | 0.46 | 17.5 | 2.1 (0.5) | 1.1 (N) | 29 (24) | 21 (19) |
| 38 | 1E | 0.60 | 0.53 | 6.3 | 21 (1.0) | 9.1 (3) | | |
| 39 | 2E | 0.80 | 0.26 | 6.3 | 5.7 (3) | 2.3 (0.5) | 35 (6) | 23 (8) |
| 40 | 3E | 0.26 | 0.11 | 3.8 | 21 (6) | 4.0 (2) | | |
| 41 | 3E | 0.61 | 0.17 | 3.8 | 5.8 (2) | 3.0 (0.5) | | |

[a] See ref 18. [b] See ref 4. [c] See ref 18; number in parentheses is confidence limit for in vivo value and an "N" indicates no confidence limit determined. [d] Preliminary data.

**Table III.** Results from Free/Wilson Analyses[a]

| | Gram negative | Gram positive |
|---|---|---|
| $N$ | 41 | 41 |
| $R^2$ | 0.81 | 0.70 |
| $s$ | 0.25 | 0.29 |
| $F$ | 7.2 | 3.8 |
| $p$ | 0.0001 | 0.001 |
| intercept | 6.82 | 5.77 |
| $C_7$-Substituents (Figure 1) | | |
| $A_{SS}$ | −0.21* | 0.04* |
| $A_{SS}$ (Me) | −0.25* | 0.34* |
| $A_{RR}$ | −0.01* | 0.17* |
| B | −0.29 | 0.54 |
| B (Me) | −0.35* | 0.46* |
| C | −0.23* | 0.79 |
| D | −0.00* | 0.44 |
| AP | −0.50 | 0.57 |
| F | −0.54 | 0.62 |
| F (Et) | −1.25 | −0.08* |
| E | −0.70 | 0.43 |
| E (Et) | −1.04 | 0.43 |
| Quinolone Nuclei (Figure 1) | | |
| 1 | 0.00* | −0.07* |
| 3 | 0.10* | 0.20* |
| 4 | 0.28 | 0.62 |
| 2P (ciprofloxacin) | 6.74 | 5.58 |

[a] An asterisk indicates values not significantly different than 0 ($p > 0.05$).

panded molecular volume. The rigid conformations imparted by the bridge to the piperazine ring and to the bicyclic aminopiperidine analogues do not have a significant effect on the degree of inhibition of the gyrase enzyme.

At the bacterial level, the 2,5-diazabicyclo[2.2.2]octane (B) and 2,5-diazabicyclo[3.2.2]nonane (C) side chains significantly improved MICs against Gram-positive organisms and maintain potency against Gram-negative strains. This results in analogues B and C being superior to piperazine in their overall spectrum of activity. The 1,4-diazabicyclo[3.2.1]octane (D) maintains an overall improvement over the piperazine even though MICs against Gram-positive organisms have diminished relative to analogues bearing B and C. The 2,5-diazabicyclo[2.2.1]heptanes ($A_{RR}$ and $A_{SS}$) investigated displayed a reduced Gram-positive potency relative to Gram-negative MICs and are similar to piperazine. These results suggest that in whole bacteria the expanded volume of the bicycles is tolerated and that the piperazine ring conformation is not critical to potency. With the larger bicycles (B–D) we appear to be observing improved delivery into Gram-positive cells or possibly increased potency against gyrase in Gram-positive cells.

The 4-aminopiperidine and its bicyclic analogues E and F display Gram-positive MICs equal to or surpassing Gram-negative MICs. At first glance, this appears similar to the 3-aminopyrrolidine[4a] type of $C_7$-substituent, which maintains Gram-negative potency and achieves a significant increases in Gram-positive potency. In reality, relative to piperazine these compounds show greatly diminished Gram-negative activity while Gram-positive activity remains nearly constant.

Despite the variances in relative potencies noted above, it was of interest to quantitate the average impact of the $C_7$-substituent and also the impact of the four quinolone nuclei on the mean Gram-negative and -positive MICs. The data were analyzed by using the method of Free and Wilson[21] as modified by Fujita and Ban.[22] Compound **2**, ciprofloxacin, was designated as the parent. Initial analyses (Table III) included all 41 analogues. Subsequent analyses were run with compounds with substituent F removed since these compounds were the most significant nonadditive examples.

For Gram-negative organisms assayed, substituents $A_{SS}$, $A_{RR}$, B–D did not give significant coefficients. However, substituents AP, F, and E reduced potency 0.5–0.7 units (3–5-fold) compared to piperazine (P). The ethylated derivatives of E and F reduced potency even more severely (10–16-fold).

For the Gram-positive organisms investigated, substituents $A_{SS}$ and $A_{RR}$ were, again, equivalent to piperazine along with the ethylated derivative of F. In sharp contrast to the results for Gram-negative bacteria, all the remaining variations of the $C_7$-substituent provided increased potency (0.4–0.8 units, 2–6-fold) compared to piperazine.

For both Gram-negative and -positive bacteria, the quinolone nuclei 1 and 3 (Chart 1) were equivalent to the parent **2**. The 5-amino-6,8-difluoroquinolone nucleus, **4**, provided a 2–4-fold increase in potency.

For these analyses compounds bearing the F substituent gave the greatest residuals. However, repeating the analyses without these analogues provided only a marginal statistical improvement and did not alter the above conclusions.

In mouse protection studies, none of these analogues surpassed the simple piperazine in potency, although D is equipotent to piperazine. It is not possible to discern why the bicycle D is the variation achieving in vivo equality with the simple piperazine. In the whole animal the reason may be the rigid chair conformation of the piperazine portion of bicycle, the fact the basic nitrogen is tertiary, the combination of these two factors, or some additional unrecognized factor that positively effects the pharmacokinetic parameters.

Overall, when considering MICs and in vivo potency together within a substrate series, substituting a bicyclic analogue for the $C_7$-piperazine does not lead to an improvement in antibacterial activity. However, evaluating the compounds from this study for analogues that viewed from the data generated are overall more potent than ciprofloxacin, compounds **9**, **10**, **20**, **23**, **25**, **26**, **27** and **35** display equal or superior activity. This is primarily due to the fact these compounds possess greater activity against Gram-positive organisms. The one exceptional compound is **35**, *endo*-7-(3-amino-8-azabicyclo[3.2.1]oct-8-yl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid, which possesses excellent MICs and in vivo potency for both Gram-negative and Gram-positive organisms and is measurably more potent against Gram-positive organisms. The reason for this increased efficacy for **35** relative to the other analogues prepared is unknown at this time but appears to be some peculiar synergy between the bicycle and the substrate.

## Experimental Section

Melting points were taken on a Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were determined on a Nicolet FT IR SX-20 with 2 $cm^{-1}$ resolution. Proton magnetic resonance ($^1H$ NMR) spectra were recorded on a Varian XL-200 spectrometer. Chemical shifts are reported in $\delta$ units relative to tetramethylsilane. Mass spectra were recorded on either a Finnigan 4500 GCMS or a VG Analytical 7070E/HF with a 11/250 data system. Column chromatography was performed with W.R. Grace silica gel 60, 230–400. Solutions were dried over magnesium sulfate. Solutions were concentrated under reduced pressure on a Büchi rotary evaporator. C, H, N elemental analyses were performed on either a Control Equipment Corp. Model 240XA or a Carlo-Erba Model 1106 elemental analyzer and

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(22) Fujita, T.; Ban, T. J. *J. Med. Chem.* **1971**, *14*, 148.

halogen determinations were performed by the closed flask combustion method, employing a titrimetric determination. HPLC purity of the final products was performed on reverse-phase C18 columns using 20%:80% THF–0.05 M ammonium phosphate buffer (pH = 3.0) mobile phase at 1.0 mL/min with product detection by absorbance at 287 nm.

**Data Processing.** Regression analyses were run on a VAX 6430 using the SAS program package.[23] MICs were converted to -log (molar concentration) units. Complete regression output is provided as supplementary material.

**Synthesis of Bicyclic Side Chains. 6,8-Diazabicyclo[3.2.2]nonane (C).** The intermediate 6,8-dibenzyl-6,8-diazabicyclo[3.2.2]nonane-7,9-dione (mp 201–2 °C, lit.[9] mp 199–200 °C) was prepared by the method of Eastwood[9] and used as follows: Lithium aluminum hydride (15.18 g, 0.40 mol) was suspended in THF (250 mL) and to this was added over 10 min the bicyclic dione (22.3 g, 66.7 mmol) as a suspension in THF (1 L). The reaction mixture was heated to reflux for 19 h and cooled to room temperature. Cautiously, water (23.2 mL) was added to the reaction, and after gas evolution had ceased, the inorganic salts were removed by filtration. The filtrate was evaporated to give an oil. This oil was dissolved in ether and 6 N HCl in 2-propanol (23 mL) was added and the solid formed was collected to give 6,8-dibenzyl-6,8-diazabicyclo[3.2.2]nonane dihydrochloride (20.3 g, 80%, mp 160–5 °C dec). A solution of the dihydrochloride in methanol–water (400 mL, 2:1 v/v) was hydrogenated using 20% Pd/C (2.5 g). After the uptake of hydrogen was complete, the reaction mixture was filtered and evaporated to yield the crude product. This product was triturated with 2-propanol to give, as a solid, C (9.84 g, 92%): mp 300–10 °C dec; $^1$H NMR ($D_2O$): $\delta$ 4.15–3.95 (m, 2 H), 3.55 (app s, 4 H), 2.40–2.00 (m, 4 H), 1.95–1.80 (5-line m, 2 H); MS (EI) $m/z$ 126 ($M^+$), 97, 82, 68, 56, 43. Anal. Calcd for $C_7H_{14}N_2 \cdot 2HCl$: C, 42.23; H, 8.10; N, 14.07; Cl, 35.61. Found: C, 42.03; H, 7.88; N, 13.83; Cl, 35.69.

**4-[(Trifluoroacetyl)amino]piperidine.** In $CHCl_3$ (100 mL) was dissolved trifluoroacetic anhydride (50 mL) and the resulting solution was cooled to 0 °C under argon. Portionwise to this solution was aded 4-aminopyridine (20.0 g, 0.21 mol) which caused a precipitate to form. After stirring overnight some unreacted pyridine remained and an additional 15 mL of trifluoroacetic anhydride was added. The mixture was refluxed for 2 h, a second 15-mL portion of trifluoroacetic anhydride was added, and reflux continued for three additional hours. The reaction was cooled, and the precipitated solids were collected by filtration, washed successively with $CHCl_3$ and ether, then dried under vacuum at 55 °C overnight to give 61.5 g of crude product. This product was dissolved in acetic acid (100 mL) and to this was added 10% palladium on carbon (10.0 g). The reaction was pressurized with hydrogen to 50 psig and the reaction shaken until the required hydrogen uptake had been recorded. The reaction was depressurized, filtered through Celite, and evaporated to an oil which crystallized as colorless needles upon standing. These crystals were isolated by addition of acetic acid (20 mL) followed by filtration and air-drying to give the title compound as the trifluoroacetate salt (19.1 g, 29%): mp 195–6 °C; $^1$H NMR ($CF_3COOD$) $\delta$ 7.60–6.80 (br s, 1 H), 4.40–4.31 (m, 1 H), 3.83–3.77 (m 2H), 3.43–3.38 (m, 2 H), 2.42–2.23 (m, 23 H), 2.10–2.08 (m, 2 H); MS (EI) $m/z$ 197 (M + 1), 83, 69, 56 (base); IR (KBr) 3258, 3104, 3035, 2843, 1735, 1693, 1566, 1236, 1202, 1172, 1141, 842, 726. Anal. Calcd for $C_7H_{11}N_2F_3O \cdot CF_3COOH$: C, 34.84; H, 3.90; N, 9.03; F, 36.75. Found: C, 34.69; H, 3.58; N, 8.79; F, 37.17.

***exo*-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-8-azabicyclo[3.2.1]octane.** *exo*-3-Amino-8-benzyl-8-azabicyclo[3.2.1]octane[11,12] (11.8 g, 54.5 mmol) was dissolved in *tert*-butyl alcohol (80 mL) and 1 N NaOH solution (52 mL) and to this was added di-*tert*-butyl dicarbonate (12.6 g, 57.5 mmol). After stirring for 3 h, the pH of the reaction mixture was adjusted to pH = 8 with 1 N HCl solution and evaporated to a colorless solid. This solid was partitioned between $CH_2Cl_2$ and water. The water layer reextracted with fresh $CH_2Cl_2$. The combined $CH_2Cl_2$ layers were dried, filtered, and evaporated to a solid. This solid was triturated with ether to give a colorless solid (6.16 g). Anal. Calcd for $C_{19}H_{28}N_2O_2$: C, 72.11; H, 8.92; N, 8.85; Found: C, 71.92; H, 9.03; N, 8.67. This solid (5.6 g, 17.7 mmol) was dissolved in methanol (100 mL) and to this was added 20% palladium on carbon (0.5 g). The mixture was shaken under hydrogen (50 psig) until the required pressure drop had been recorded. The reaction was depressurized, filtered through Celite, and evaporated to give a colorless solid (4.19 g, 34% overall): mp 118–121 °C; $^1$H NMR ($CDCl_3$) 4.50–4.20 (m, 1 H), 3.95–3.70 (m, 1 H), 3.60–3.50 (br s, 2 H), 2.35–2.25 (br s, 2 H), 2.00–1.70 (m, 6 H), 1.45–1.15 (m, 10 H); MS (CI, $CH_4$ 0.6 Torr) $m/z$ 227 (M + 1), 211, 199, 171 (base). Anal. Calcd for $C_{12}H_{22}N_2O_2 \cdot 0.15CH_3OH$: C, 63.14; H, 9.86; N, 12.12. Found: C, 63.16; H, 9.64; N, 12.15.

***endo*-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-8-azabicyclo[3.2.1]octane.** The procedure described for the preparation of *exo*-3-[[(1,1-dimethylethoxy)carbonyl]amino]-8-azabicyclo[3.2.1]octane was followed to give the title compound: mp 118–121 °C; $^1$H NMR ($CDCl_3$) $\delta$ 4.50–4.20 (m, 1 H), 3.95–3.70 (m, 1 H), 3.60–3.50 (br s, 2 H), 2.35–2.25 (br s, 2 H), 2.00–1.70 (m, 6 H), 1.45–1.15 (m, 10 H), MS (CI, $CH_4$ 0.6 Torr) $m/z$ 227 (M + 1), 211, 199, 171 (base). Anal. Calcd for $C_{12}H_{22}N_2O_2 \cdot 0.15CH_3OH$: C, 63.14; H, 9.86; N, 12.12. Found: C, 63.16; H, 9.64; N, 12.15. The intermediate *exo*-3-[[(1,1-dimethylethoxy)carbonyl]amino]-8-benzyl-8-azabicyclo[3.2.1]octane had the following analysis calculated for $C_{19}H_{28}N_2O_2 \cdot 0.13H_2O$: C, 71.59; H, 8.94; N, 8.79. Found: C, 71.58; H, 8.94, N, 8.75.

***exo*-3-(Ethylamino)-8-azabicyclo[3.2.1]octane Dihydrochloride.** *exo*-3-(Acetylamino)-8-benzyl-8-azabicyclo[3.2.1]octane acetic acid salt (8.2 g, 25.8 mmol) was added slowly to a suspension of lithium aluminum hydride (5.9 g, 155 mmol) in THF (100 mL) and this mixture was heated at reflux for 18 h. The reaction was cooled to room temperature and quenched by the slow addition of $H_2O$ (9 mL). After allowing the reaction to cool to room temperature, again the salts were removed by filtration, and the filtrate was evaporated to an oil. This oil was dissolved in ether and HCl gas was bubbled into the solution. The precipitated HCl salt was collected by filtration to give 7.1 g of the *exo*-3-(ethylamino)-8-benzyl-8-azabicyclo[3.2.1]octane dihydrochloride (86%). Anal. Calcd for $C_{16}H_{24}N_2 \cdot 2HCl \cdot 0.33H_2O$: C, 59.44; H, 8.31; N, 8.66. Found: C, 59.43; H, 8.28; N, 8.58. This dihydrochloride salt was dissolved in methanol (100 mL) and water (20 mL), and 1.0 g of 20% palladium on carbon was added. This slurry was shaken under hydrogen (50 psig) until the required hydrogen uptake had been observed. The reaction was filtered through Celite and the filtrate evaporated to an oil. To this oil was added 2-propanol to give a solid. This solid was collected and washed with 2-propanol and ether and dried to give 3.6 g of *exo*-3-(ethylamino)-8-azabicyclo[3.2.1]octane dihydrochloride. This material proved to be very hygroscopic and satisfactory analysis could not be obtained. The material was used as is in subsequent coupling reactions: $^1$H NMR ($D_2O$) $\delta$ 1.21 (t, 3 H, $J$ = 7.3 Hz), 1.90–2.25 (m, 6 H), 2.45–2.60 (m, 2 H), 3.11 (q, 2 H, $J$ = 7.3 Hz), 3.49–3.55 (m, 1 H), 3.95–4.10 (br m, no fine structure, 2 H); $^{13}$C NMR (50 MHz, $D_2O$) $\delta$ 55.1, 50.0, 45.6, 32.7, 28.5, 13.1.

***endo*-3-(Ethylamino)-8-azabicyclo[3.2.1]octane Dihydrochloride.** The procedure described for the exo isomer was repeated to give the endo isomer as a hygroscopic glassy solid. The title compound was used as is in the subsequent coupling reactions: $^1$H NMR ($D_2O$) $\delta$ 1.20 (t, 3 H, $J$ = 7.3 Hz), 1.80–2.35 (m, 8 H), 3.04 (q, 2 H, $J$ = 7.3), 3.50–3.70 (m, 1 H), 4.15–4.25 (br m, no fine structure, 2 H). $^{13}$C NMR (50 MHz, $D_2O$) $\delta$ 57.1, 50.6, 43.3, 33.9, 27.9, 13.5; MS (EI, $m/z$) 155 ($M^+$), 111, 82, 68 (base), 56. The intermediate *endo*-3-(ethylamino)-8-benzyl-8-azabicyclo[3.2.1]-octane was obtained as the dioxalate salt. Anal. Calcd for $C_{16}H_{24}N_2 \cdot 2.0C_2H_2O_4 \cdot 0.33H_2O$: C, 55.81; H, 6.71; N, 6.51. Found: C, 55.75; H, 6.51; N, 6.45.

**General Methods for Coupling and Purification of $C_7$-Bicycles to Quinolone Substrates. General Method A. (1*S*-*cis*)-5-Amino-1-cyclopropyl-7-(2,5-diazabicyclo[2.2.2]-oct-2-yl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (17).** 5-Amino-1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[15] (4; 1.19 g, 4.0 mmol), 2,5-diazabicyclo[2.2.2]octane dihydrochloride[8] (B; 0.81 g, 4.4 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.80 mL, 12.0 mmol) were added to acetonitrile (50 mL), and the mixture was heated to reflux for 3 h and then cooled to room temperature. The precipitated solid was collected by filtration and washed with ethanol

(23) *SAS User's Guide: Statistics, Version 5 Edition*; SAS Institute, Inc.: Cary, NC, 1985.

to give 17 (0.97 g, 62%).

**General Method B. (1*S-cis*)-5-Amino-1-cyclopropyl-6,8-difluoro-7-(2,5-diazabicyclo[2.2.1]hept-2-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (8).** 5-Amino-1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[15] (4; 2.98 g, 10.0 mmol), (1*S*,4*S*) (1*S*,-*cis*)-2,5-diazabicyclo[2.2.1]heptane dihydrochloride[7] ($A_{SS}$; 3.36 g, 12.9 mmol), and triethylamine (6.0 g, 60.0 mmol) were added to acetonitrile (75 mL), and the mixture was heated to reflux for 7.5 h and then cooled to room temperature. The precipitated solid was collected by filtration and washed successively with ethanol, acetonitrile, and ether to give the crude product. This solid was dissolved in water, made basic (pH = 11), and filtered, and the pH slowly adjusted to pH = 7.2 with an HCl solution. The precipitated solid was collected and washed successively with water, 2-propanol, and ether to give 8 (2.40 g, 64%).

**General Method C. *exo*-1-Cyclopropyl-6-fluoro-7-[3-(ethylamino)-8-azabicyclo[3.2.1]oct-8-yl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (39).** 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[13] (2; 0.66 g, 2.5 mmol), *exo*-3-(ethylamino)-8-azabicyclo[3.2.1]octane dihydrochloride[11,12] (E; 0.62 g, 2.75 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.12 mL, 7.5 mmol) were added to pyridine (10 mL), and the mixture was heated to reflux for 4 h and then cooled to room temperature. The precipitated solid was collected by filtration and washed with ethanol to give 39 (0.43 g, 43%).

**General Method D. 7-(4-Amino-1-piperidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (29).** 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[13] (2; 1.20 g, 4.6 mmol), 4-[(trifluoroacetyl)amino]piperidine trifluoroacetic acid salt (1.84 g, 6.0 mmol), and triethylamine (3.8 mL, 27.3 mmol) were added to acetonitrile (40 mL), and the mixture was heated overnight at reflux, then cooled to room temperature and diluted with diethyl ether (50 mL). The precipitate formed was collected by filtration and dissolved in a solution of ethanol (150 mL), 2 N HCl (150 mL), and acetic acid (200 mL) and heated at reflux for 36 h. The reaction was cooled and concentrated to a solid. The solid was crystallized from methanol-diethyl ether to give a solid. This solid was recrystallized from methanol to give 0.30 g (17%) of the title compound; 29.

**General Method E. (1*S-cis*)-1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl)-4-oxo-3-quinolinecarboxylic Acid (10).** A solution of (1*S-cis*)-1-cyclopropyl-6,8-difluoro-7-(2,5-diazabicyclo[2.2.1]hept-2-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (7; 3.60 g, 9.5 mmol), 85% formic acid (50 mL), and 37% formaldehyde solution (50 mL) was heated to reflux for 4.25 h. After cooling, the reaction mixture was evaporated and the residue was dissolved in hot ethanol and 6 N HCl in 2-propanol (3 mL) added. Upon cooling, a solid precipitated from the solution and was collected and dried to give 10 (3.05 g, 86%).

**General Method F. *exo*-1-Cyclopropyl-7-[3-(ethylamino)-8-azabicyclo[3.2.1]oct-8-yl]-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (33).** The difluoroborate ester of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid[19] (1.50 g, 4.8 mmol) and *endo*-3-[[(1,1-dimethylethoxy)carbonyl]amino]-8-azabicyclo[3.2.1]octane (E) (2.71 g, 12 mmol) were added to acetonitrile (10 mL), and the mixture was heated to reflux for 4 h and then cooled to room temperature and allowed to stand for 48 h. The precipitated solid was collected by filtration to give the protected intermediate (1.17 g, 47%). This intermediate was dissolved in ethanol (95%, 80 mL) containing triethylamine (0.5 mL) and heated to reflux for 24 h. The reaction was cooled and evaporated to a solid residue. This residue was dissolved in trifluoroacetic acid (10 mL) and stirred for 1.5 h, then evaporated to a solid. This crude product was dissolved in 5 N HCl (200 mL) and the pH adjusted to 7 with 50% solution of NaOH. The precipitated solid was collected by filtration and washed with water to give 33 (0.59 g, 61% for the deprotection steps).

**Supplementary Material Available:** MIC data, regression data, and analytical analysis for all compounds (18 pages). Ordering information is given on any current masthead page.

# Configuration and Preferential Solid-State Conformations of Perindoprilat (S-9780). Comparison with the Crystal Structures of Other ACE Inhibitors and Conclusions Related to Structure-Activity Relationships

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The conformation of perindoprilat, an antihypertensive drug, is studied in the solid state by X-ray analysis. The resolution of its structure reveals important analogies between its observed conformation and that of several ACE inhibitors of the same family. This comparison points out a constant relative orientation of the functional groups, regardless of the molecular environment. This angular constancy appears to us as not being accidental and is a good argument for the spatial design of the ACE binding site. Although ACE is a carboxydipeptidase, the binding site may not contain two but one unique hydrophobic pocket receiving the C-terminal end of the inhibitors.

## Introduction

Perindopril (S-9490) (1) (Figure 1) belongs to the class of antihypertensive drugs, acting through the inhibition of angiotensin converting enzyme (EC 31.15.1, ACE), a zinc metalloenzyme involved in the control of blood pressure. Perindopril is an acid-ester prodrug. It is well absorbed through the oral route and desesterified in the liver by esterases, resulting in perindoprilat (2), its active diacid form.

1 was synthesized through a stereospecific method,[1,2] and 2 by saponification of 1. Nevertheless, it was desirable to

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