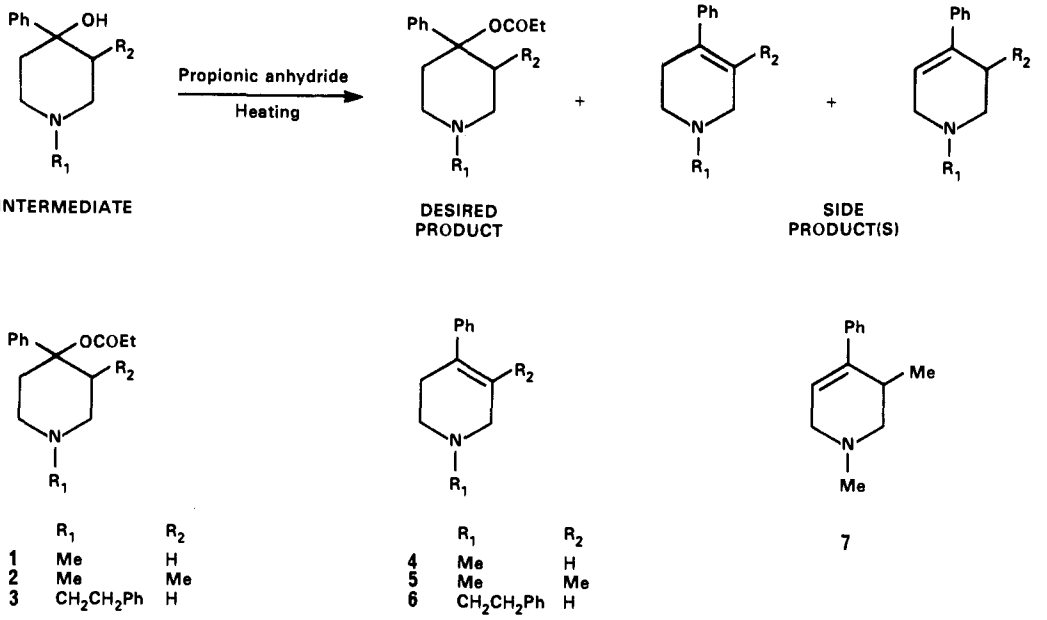
Scheme I. Synthesis of the Reverse Ester of Meperidine and Its Analogues



	R_1	R_2
1	Me	H
2	Me	Me
3	CH_2CH_2Ph	H

	R_1	R_2
4	Me	H
5	Me	Me
6	CH_2CH_2Ph	H

7

For inactivation studies, the concentration of PEPTP was 0.4 mM in 2 mM potassium phosphate buffer, pH 7.1, 10% glycerol, and 26.9 μg of MAO B:MAO B-1C2. At various times, 10-μL aliquots were removed and added to 0.5 mL of 25 mM sodium phosphate buffer, pH 7.4, 1 mM benzylamine, 1 mM 2,2′-azinobis(3-ethylbenzthiazoline 6-sulfonate) (ABTS) and 0.5 unit of peroxidase at 30 °C.[9] Initial rates were determined by monitoring the increase in absorption at 410 nm from 0 to 5 min. Benzylamine oxidation was also determined at 250 nm as described above.

In all assays, the analogues investigated were found to be inactive or much less active than MPTP (Table I). The results obtained, together with data obtained in vitro for several other analogues of MPTP[10,11] (with different *N*-alkyl groups and the phenyl substituent in the 2- or 5-position), indicate that the oxidation of MPTP by MAO B is highly structure specific in regard to the tetrahydropyridine moiety.

These studies reveal that PEPTP cannot be metabolized by MAO B in humans by a mechanism analogous to the activation of MPTP. This process seems to be necessary for neurotoxic activity of this group of compounds, but our results suggest that PEPTP probably is not toxic in vivo, unless it is activated by another mechanism.

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Registry No. 4, 28289-54-5; 5, 13515-63-4; 6, 13314-65-3; 7, 6672-51-1; MAO, 9001-66-5.

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1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid. New Quinolone Antibacterial with Potent Gram-Positive Activity

Sir:

In recent years, an old class of antibacterials known collectively as the quinolones[1] has aroused a great deal of new interest.[2] The original members of this class, oxolinic acid (**3**) and nalidixic acid (**4**), have been used for certain clinical indications for over 20 years but suffer from a rather limited antibacterial spectrum.[1] The current de-

(1) The generic class "quinolones" refers to any of the 4-pyridone-3-carboxylic acid antibacterials. These now include quinolines, naphthyridines, benzoxazines, etc., which share the common 4-pyridone-3-carboxylic acid. For a review, see: Albrect, R. *Prog. Drug Res.* **1977**, 21, 9.
(2) Many reports regarding the newer quinolones have been reported at the 23rd and the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct 24–26, 1983, Las Vegas, NV, and Oct 8–10, 1984, Washington, D.C. A portion of this work was presented at the Washington, DC, meeting; Abstr 80.

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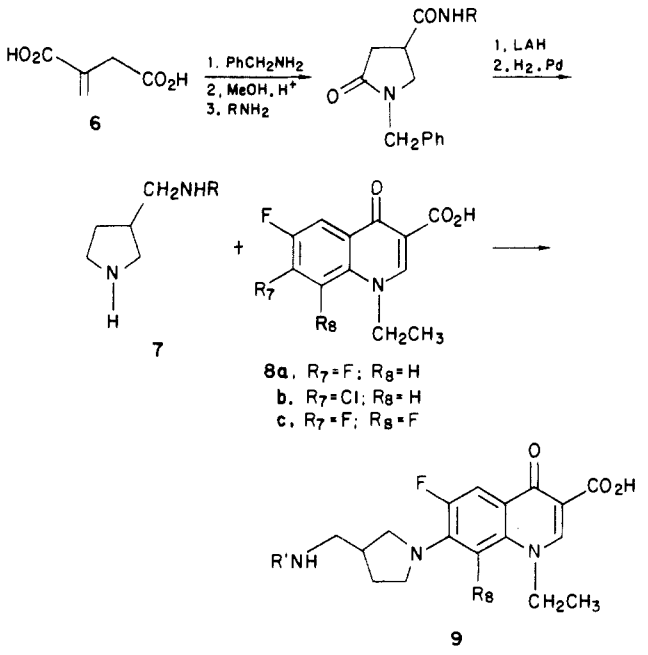
Scheme I



rivatives, shown in Figure 1, possess exceptional Gram-negative activity but still have weaknesses in their Gram-positive spectrum. Analysis of the compounds in Figure 1 reveals remarkable structural similarities. All possess a fluorine atom at the 6-position and a piperazine moiety at the 7-position. The only structural deviations observed in these drugs is found at the 8-position (X in 1) and the group appended to N_1.

During the course of our work we observed that the piperazine group, although beneficial, was not essential for displaying low minimum inhibitory concentrations (MICs) against bacteria or against the target enzyme DNA gyrase. The piperazine, possibly through the basic nitrogen, did confer proportionally good in vivo activity to those derivatives to which it was appended. In order to improve the spectrum of antibacterial activity without losing the obvious benefits of the piperazine moiety, we sought a new side chain that might satisfy both requirements. With the aid of molecular modelling and computer graphics it appeared to us that the amino group in the 3-(aminomethyl)pyrrolidines **5** might mimic the 4-piperazinyl nitrogen present in the known active drugs. Certainly the amino group in **5** would have several degrees of freedom relative to the piperazinyl nitrogen and might possess properties unique to this feature. In this communication we report our results from the synthesis and evaluation of a series of quinolones containing these 3-(aminomethyl)pyrrolidine heterocycles at the 7-position and, in particular, the discovery of 1-ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (CI-934), a new quinolone with excellent Gram-positive activity.

Chemistry. The desired pyrrolidines (**7**) were synthesized by using literature methods[3] from itaconic acid (**6**) and benzylamine as shown in Scheme I. The pyrrolidines obtained were coupled to the appropriate quinolines **8**[4] with use of standard conditions.[5] As expected,

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Table I. Biological Testing Results from the Drug-Induced DNA Cleavage Assay, Antibacterial Screening, and Mouse Protection Assay

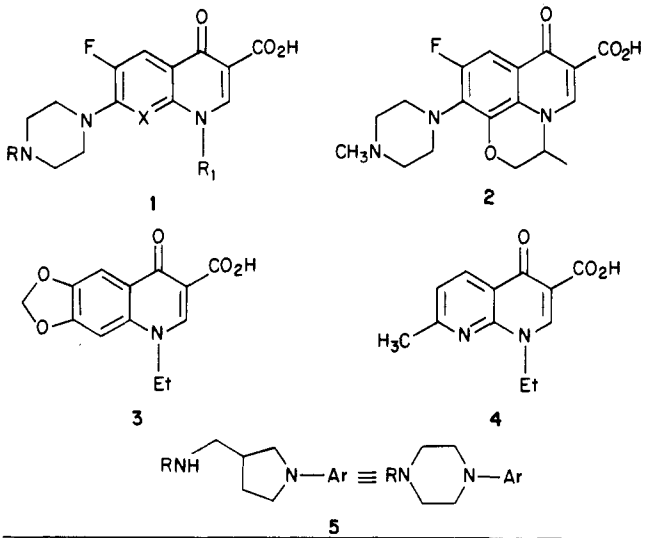
compd no.	minimum inhibitory concentrations (MIC),[a] μg/mL: Gram-negative organisms: *E. cloacae* HA 2646	*E. coli* Vogel	*E. coli* H560	*K. pneumoniae* MGH-2	*P. rettgeri* H1771	*P. aeruginosa* UI-18	Gram-positive organisms: *S. aureus* H228	*S. aureus* UC-76	*S. faecalis* MGH-2	*S. pneumoniae* SV-1	*S. pyogenes* C203	protective dose (PD_{50})[b] in mouse, mg/kg: *E. coli* Vogel po	*E. coli* Vogel sc	*S. aureus* UC-76 po	*S. aureus* UC-76 sc	*S. pneumoniae* SV-1 po	*S. pneumoniae* SV-1 sc	gyrase-drug induced cleavage,[c] μg/mL: *E. coli*
1a	0.1	0.025	0.1	0.05	0.025	0.2	0.8	0.05	1.6	1.6	0.8	4.0	1.0	23	15	150	>100	1.0
1b	0.05	0.025	0.1	0.05	0.05	0.4	0.2	0.1	0.8	0.8	0.8	2.8	1.6	9.5	3.7	200	96	1.0
1c	0.2	0.2	0.1	0.2	0.1	1.6	1.6	0.4	3.1	3.1	12.5	3	2	12	10	>200	>100	5.0
1d	0.05	0.05	0.025	0.1	0.1	0.4	3.1	0.2	0.8	1.6	0.8	1.2	0.25	9.5	3.0	>100	18	0.5
1e	0.1	0.1	0.025	0.2	0.2	0.8	1.6	0.4	3.1	12.5	12.5	4.5	1.0			>100	>25	2.5
2	0.1	0.1	0.1	0.1	0.2	0.4	0.4	0.1	0.8	0.8	0.8	2.0	0.8	11	5	68	38	5.0
3	0.4	0.2	0.2	0.2	0.1	6.3	1.6	0.8	12.5	100	>100	17	9	>100	63			10.0
4	6.3	3.1	6.3	6.3	6.3	>100	100	25	>100	>100	>100	45	26	>100	>100	>100	>100	50.0
9a	1.6	0.8	0.4	3.1	6.3	1.6	1.6	0.05	0.8	0.2	0.2	>100	25	>50	6			3.0
9b	1.6	0.2	0.2	1.6	6.3	1.6	0.8	0.013	1.6	0.2	0.1	>100	18	>50	8	>100	>25	
9c	0.2	0.1	0.1	0.4	1.6	1.6	0.2	0.05	0.2	0.2	0.1	>100	15	35	3.0			2.5
9d	0.2	0.1	0.1	0.4	0.4	0.8	0.1	0.1	0.1	0.2	0.1	90	1.2			100	5	0.5
9e	0.4	0.4	0.2	0.8	0.8	1.6	0.2	0.1	0.1	0.1	0.1	6.9	1.9			37	11	0.5
9f (CI-934)	0.2	0.1	0.1	0.2	0.4	1.6	0.1	0.05	0.1	0.1	0.1	12	1.7	2.8	1.6	20	4.4	2.5

[a] Standard microdilution techniques; see ref 6. [b] Dose required to protect 50% of mice from lethal infection. [c] Minimum concentration of drug needed to produce linear DNA at an intensity relative to oxolinic at 10 μg/mL.

Table II. Physical Properties of the [Substituted 3-(aminomethyl)-1-pyrrolidinyl]quinolines 9

compd no.	R′	R_8	starting quinoline[a]	yield, %	mp, °C	elemental formula analyzed for[c]	purification method[b]
9a	H	H	**8a**	78	234–236	$C_{17}H_{20}FN_3O_3 \cdot 0.3H_2O$ (C, H, N, H_2O)	trit (H_2O/EtOH)
9b	Me	H	**8a**	83	247–250	$C_{18}H_{22}FN_3O_3 \cdot 0.5H_2O$ (C, H, N, H_2O)	trit (Et_2O/EtOH)
9c	Et	H	**8b**	50	248–252	$C_{19}H_{24}FN_3O_3 \cdot 1.5H_2O$ (C, H, N, H_2O)	prec (H_2O (pH 7.0))
9d	H	F	**8c**	83	219–221	$C_{17}H_{19}F_2N_3O_3 \cdot 0.5H_2O$ (C, H, N)	trit (Et_2O)
9e	Me	F	**8c**	86	246–248	$C_{18}H_{21}F_2N_3O_3 \cdot 0.3H_2O$ (C, H, N)	trit (Et_2O)
9f (CI-934)	Et	F	**8c**	80	200–202	$C_{19}H_{23}F_2N_3O_3 \cdot 0.45H_2O$ (C, H, N, H_2O)	trit (H_2O/EtOH)

[a] Typical reaction is carried out in CH_3CN, at 50-80 °C, with 1–3 equiv of the desired pyrrolidine. The products were filtered and purified accordingly. For **8b** β-picoline was employed as solvent. [b] Trituration (trit), precipitation (prec). [c] All analyses were ±0.4% of theoretical values.



name	no.	X	R_1	R	ref
norfloxacin	**1a**	CH	Et	H	5
pefloxacin	**1b**	CH	Et	Me	5
enoxacin	**1c**	N	Et	H	9
ciprofloxacin	**1d**	CH	c-Pr	H	10
amifloxacin	**1e**	CH	$NHCH_3$	Me	11

Figure 1. Clinically significant quinolone antibacterials.

a fluorine leaving group at the 7-position in **8a** or **8c** permitted pyrrolidine displacement under mild conditions (CH_3CN, 80 °C). The substrate **8b** required more forcing conditions (β-picoline, 130 °C). The 3-aminomethyl group did not require protection as displacement occurred exclusively at the pyrrolidinyl nitrogen. The properties of these derivatives **9a–f** are listed in Table II.

Biology. The pyrrolidinyl quinolones **9** were tested against an assortment of 10 organisms by using standard microtitration techniques,[6a] and their minimum inhibitory concentrations (MICs) were compared to the standard drugs in Figure 1. The compounds were also tested for their inhibition of the target enzyme DNA gyrase,[7] which was obtained from *Escherichia coli* H560 cells.[7a,8] This bacterial enzyme maintains the topology of the bacterial DNA and can be assayed in many ways. The assay employed for this study measured the concentration of drug (micrograms/milliliter) required to produce linear DNA from closed circular DNA by the denaturation of the drug–gyrase–DNA complex.[8,12] The linear DNA was resolved and visualized by gel electrophoresis and ethidium bromide stain.[8b] Aqueous stock solutions were prepared with use of 0.1 N sodium or potassium hydroxide. The in vivo potency, expressed as the median protective dose (PD_{50}, milligrams/kilogram), of these compounds was determined in acute, lethal systemic infections in 18–22-g female Charles River CD-1 mice. Challenges were accomplished by the intraperitoneal injection of an estimated 100 median lethal dose in 0.5-mL volumes of 5% hog gastric mucin (*E. coli*, *Staphylococcus aureus*) or tryptic soy broth (*Streptococcus pneumoniae*). Single doses of compound, in twofold rising incremental series, were administered concurrently with challenge in 0.5-mL volumes: subcutaneously as aqueous solutions and orally by gavage in 5% gum acacia. Survival percentages among groups of eight mice at each dose interval were used to estimate the median protective doses by the log probit method.[6b] The combined results from all assays are given in Table I.

Discussion

To be effective therapeutically an antiinfective agent should have MIC's several times below its effective blood level. By these criteria, the currently significant quinolines would require a minimum MIC of 0.8–1.6 μg/mL for any effectiveness in vivo. Examination of the standard drugs (**1a–3**) in Table I shows that all the currently significant analogues have potent activity against Gram-negative organisms. All of these compounds also possessed good gyrase activity, displaying enzyme inhibition at concentrations 2–20 times lower than that for oxolinic or nalidixic acids. The "gaps" in the Gram-positive activity of these standards are clear. For example, ciprofloxacin (**1d**) has a 3.1 μg/mL MIC vs. *Straphylococcus aureus* H228 and a 1.6 μg/mL vs. *Streptococcus pneumoniae*. Ofloxacin (**2**)[13] and pefloxacin (**1b**), as seen in Table I, represent the best quinolines known against Gram-positive organisms. The same trends hold when comparing the in vivo PD_{50}s. Here however, even ofloxacin (**2**) and pefloxacin (**1b**) show unexciting performance, especially vs. *Streptococcus*

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pneumoniae. The initial pyrrolidinylquinolines prepared and tested were **9a–c**, which represent the primary amino, methylamino, and 3-[(ethylamino)methyl]-1-pyrrolidinyl analogues of norfloxacin. The [3-[(ethylamino)methyl]-1-pyrrolidinyl]quinoline **9c** showed excellent MICs with very impressive coverage (≤0.2 μg/mL) against Gram-positive organisms. Even the less potent compounds **9a,b** still possessed better Gram-positive activity than the standard drugs (≤1.6 μg/mL). Furthermore, replacement of the piperazine moiety with the substituted 3-(aminomethyl)-1-pyrrolidinyl moiety did not compromise the gyrase inhibition, which is further proof that the piperazine group is not essential for antibacterial activity. The gyrase cleavage value for **9c** of 2.5 μg/mL is equal to that of amifloxacin (**1e**) and superior to that of enoxacin (**1c**) and ofloxacin (**2**). The in vivo activity of **9c**, however, was very poor. In order to increase the in vivo potency of **9c** without sacrificing the MICs and gyrase activity already in hand, small molecular changes to increase solubility and possibly absorption were pursued. The result of this search led to the synthesis of the 6,8-difluoro analogues **9d–f**. The primary amino pyrrolidinyl-6,8-difluoroquinoline **9d** displayed a 5-fold improvement in gyrase activity with improved MICs and in vivo efficacy as well when compared to **9a**. 1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (**9f**) showed no enhancement of gyrase inhibition over **9c** but displayed a marked 8-fold improvement in the mouse protection assay. Especially noteworthy are the PD_{50}s vs. *S. aureus* and *S. pneumoniae* when compared to ofloxacin and pefloxacin. This new extended spectrum quinoline **9f** (CI-934) shows the best Gram-positive activity in vitro and in vivo of any quinoline tested in this study.

Registry No. 7 (R = H), 67318-88-1; 7 (R = CH_3), 91187-81-4; 7 (R = CH_3CH_2), 91187-83-6; **8a**, 70032-25-6; **8b**, 68077-26-9; **8c**, 75338-42-0; **9a**, 91187-93-8; **9b**, 91187-94-9; **9c**, 91187-95-0; **9d**, 91187-96-1; **9e**, 99947-82-7; **95**, 91188-00-0.

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Articles

Tranexamic Acid Derivatives with Enhanced Absorption

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Derivatives of the antifibrinolytic drug tranexamic acid [*trans*-4-(aminomethyl)cyclohexanecarboxylic acid] containing one or two tranexamic acid moieties were synthesized. Most of the derivatives have good stability in acidic and neutral solutions but are easily hydrolyzed in plasma. By measuring the amount of tranexamic acid excreted in the urine after an oral dose, relative absorptions of a number of derivatives in the rat were estimated. Most of the derivatives showed greater absorption than tranexamic acid itself. 1-[(Ethoxycarbonyl)oxy]ethyl *trans*-4-(aminomethyl)cyclohexanecarboxylate hydrochloride (1) was chosen for studies in man.

Tranexamic acid [*trans*-4-(aminomethyl)cyclohexanecarboxylic acid, Cyclokapron] is a clinically used antifibrinolytic drug. The haemostatic properties of this drug mainly relate to its ability to inhibit the activation of plasminogen to plasmin,[1,2] thereby preventing excessive loss of blood in hyperfibrinolytic conditions.

Tranexamic acid is incompletely absorbed from the gastrointestinal tract, possibly due to its amphoteric nature. In man, after administration of an oral dose of 10–15 mg/kg of body weight, about 40% was recovered in the urine within 24 h.[3] After a single intravenous injection to two volunteers of 1 g (about 15 mg/kg) of tranexamic acid, 88 and 94%, respectively, of the unchanged drug was excreted in the urine within 24 h.[4] In an attempt to increase the gastrointestinal absorption of the drug, derivatives lacking the amphoteric nature of tranexamic acid were synthesized. As tranexamic acid is used clinically in rather large doses (2–6 g/day), it was desirable to keep the molecular weight of the synthesized compounds low. With this in mind, potential prodrug containing two moieties of tranexamic acid were also synthesized.

Chemistry

The potential prodrugs of tranexamic acid that are described in this paper can be divided into two groups; mono derivatives containing one moiety of tranexamic acid (Table I) and bis derivatives containing two moieties of tranexamic acid per mole of potential prodrug (Table II). All of the potential prodrugs except compounds **9**, **10**, and **20** contain the (acyloxy)methyl ester moiety. They can also be considered bis esters of geminal diols (hydrate forms of aldehydes).

The key intermediate for the synthesis of the mono derivates **1–4** and **6–8** was an α-chloro- or α-bromoalkyl ester of a carboxylic[5] or carbonic acid,[6] as illustrated for **1** (method A, Chart I). This intermediate was reacted with

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