pounds),[23] (b) no α-hydrogens are available, or (c) the α-hydrogens are inaccessible for abstraction (e.g., Bredt's rule).[7] Thus, not only does cytochrome P-450 not produce *N*-oxides as intermediates in overall dealkylation pathways but the enzyme *should not form these* at all except in the above three special cases. Another mammalian microsomal enzyme, the flavin-containing monooxygenase,[24] can form *N*-oxides from many amines because it operates via a heterolytic mechanism involving a flavin 4a hydroperoxide,[25] which clearly differs from the radicaloid cytochrome P-450 mechanism proposed here.

(23) Prough, R. A.; Brown, M. I.; Dannan, G. A.; Guengerich, F. P. *Cancer Res.* **1984**, *44*, 543.
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(25) 1 proved not to be a substrate for this enzyme (Ziegler, D. M.; Guengerich, F. P., unpublished results).

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Articles

Novel Amino-Substituted 3-Quinolinecarboxylic Acid Antibacterial Agents: Synthesis and Structure–Activity Relationships[1]

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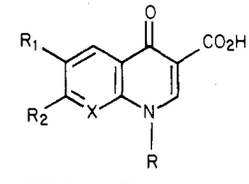
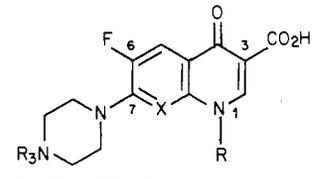
A series of novel 3-quinolinecarboxylic acid derivatives have been prepared and their antibacterial activity evaluated. These derivatives are characterized by fluorine attached to the 6-position and substituted amino groups appended to the 1- and 7-positions. Structure–activity relationship studies indicate that antibacterial potency is greatest when the 1-substituent is methylamino and the 7-substituent is either 4-methyl-1-piperazinyl, **16**, or 1-piperazinyl, **21**. Derivatives **16** and **21**, the 1-methylamino analogues of pefloxacin and norfloxacin, respectively, show comparable in vitro and in vivo antibacterial potency to these two known agents. The activity (vs. *Escherichia coli* Vogel) of **16** (amifloxacin) is the following: in vitro MIC (μg/mL) = 0.25; in vivo (mice) PD_{50} (mg/kg) = 1.0 (po), 0.6 (sc).

Since the introduction in 1963 of nalidixic acid[2] as a systemic Gram-negative antibacterial agent, many related derivatives[3] have been made. The newest derivatives, which include pefloxacin,[4] norfloxacin,[5] AT-2266,[6] DL-8280,[7] and Bay o 9867[8] are considerably more potent and have a broader spectrum of antimicrobial activity than their predecessors. These five agents share several common structural features among which are fluorine and piperazinyl groups attached to the quinoline or naphthyridine ring. Additionally, pefloxacin, norfloxacin, and AT-2266 along with most other therapeutically interesting antibacterials[3] in this class (e.g., nalidixic acid, cinoxacin, oxolinic acid, rosoxacin, and pipemidic acid) have an ethyl group appended to the ring nitrogen (commonly position 1).



nalidixic acid, R = CH_2CH_3; R_1 = H; R_2 = CH_3; X = N
oxolinic acid, R = CH_2CH_3; R_1, R_2 = OCH_2O; X = CH
rosoxacin, R = CH_2CH_3; R_1 = H; R_2 = 4-pyridinyl, X = CH
miloxacin, R = OCH_3; R_1, R_2 = OCH_2O; X = CH



pefloxacin, R = CH_2CH_3; R_3 = CH_3; X = CH
norfloxacin, R = CH_2CH_3; R_3 = H, X = CH
AT-2266, R = CH_2CH_3; R_3 = H; X = N
DL-8280, X, R = $COCH_2CH(CH_3)$, R_3 = CH_3
Bay o 9867, R = c-C_3H_5, R_3 = H; X = CH

Structure–activity relationship studies have indicated that antibacterial potency is closely related to the steric bulk of the 1-substituent.[3] In the case of 1-alkyl naphthyridine/quinoline antibacterial agents, the ethyl ana-

(1) This work was presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Astr 375, Oct 24–26, 1983, Las Vegas, NV.
(2) Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. *J. Med. Chem.* **1962**, *5*, 1063.
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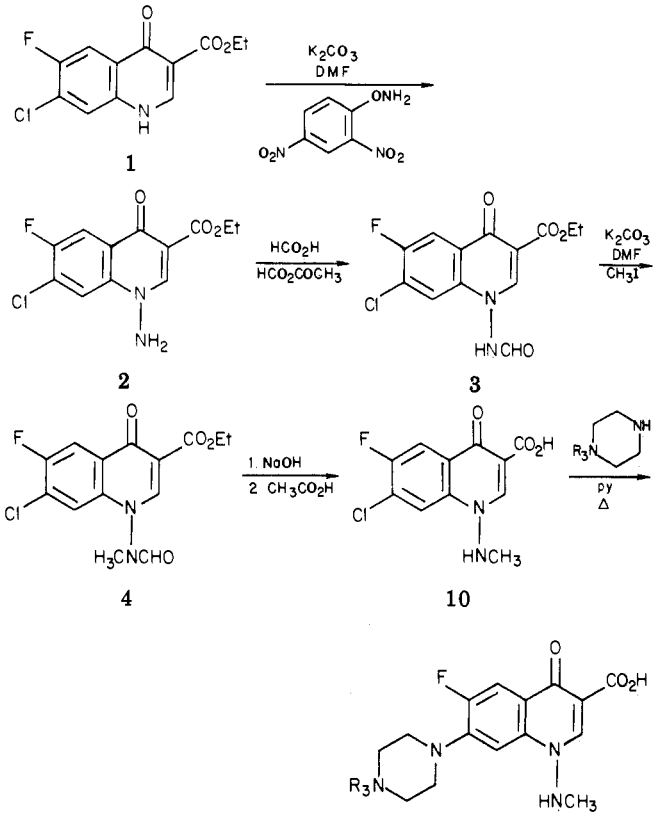
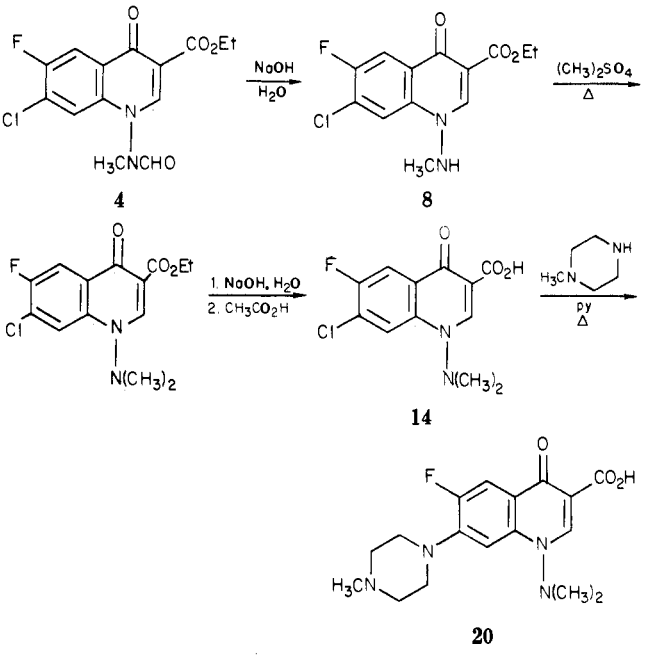
0022-2623/84/1827-1103$01.50/0

Scheme I



Scheme II



logues are generally more potent than those analogues having smaller or larger 1-alkyl substituents. In some instances the vinyl analogues, which have a similar steric bulk, showed potencies comparable to those of the ethyl derivatives. Two other variants are miloxacin[9] and Bay o 9867,[8] which have 1-methoxy and 1-cyclopropyl substitutions, respectively.

In this study we have made novel analogues, **16** and **21**, of pefloxacin and norfloxacin in which the ethyl groups of these antibacterials have been replaced by methylamino ($NHCH_3$) appendages. Methylamino and ethyl groups have similar steric bulk with the MR (bulk factor) being 10.33 and 10.30, respectively.[10] We then studied the antibacterial effects of various amine groups at positions 1 and 7.

Chemistry. Ethyl 7-chloro-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate (**1**)[5] served as starting material for the synthesis of **16** and **21** (Scheme I). Amination of **1** in K_2CO_3/DMF with *O*-(2,4-dinitrophenyl)hydroxylamine[11] gave **2** in good yield. The primary amine of **2** displayed weakly basic and nucleophilic properties and conversion to the formamide derivative was necessary for base-induced methylation to occur. Treatment of **2** with formic-acetic anhydride provided a surprisingly acidic and base-sensitive formamide **3**, methylation of which with CH_3I/K_2CO_3/DMF at 25 °C gave **4**. Alkaline hydrolysis of **4** ($NaOH/H_2O$/90 °C) followed by acidification with excess acetic acid afforded **10**. Compound **10** was converted to **16** or **21** by exposure to either excess 1-methylpiperazine or piperazine, respectively, in refluxing pyridine.

Compound **15**, the primary amino analogue of **16**, was made by the saponification of **2** followed by 1-methylpiperazine treatment of the resulting acid **9**. Alkylamino derivatives **17–19** were prepared via the following sequence: (1) alkylation of **3** in K_2CO_3/DMF with ethyl iodide, allyl bromide, or 1-bromopropane, (2) aqueous NaOH or KOH hydrolysis of the resulting formamides **5–7** followed by acetic acid acidification, and (3) treatment of **12** and the free acids of **11** and **13** with 1-methylpiperazine. Derivatives **11** and **13** were characterized as their potassium salts.

The synthesis of dimethylamino analogue **20** was complicated by the poor nucleophilicity of the 1-amino function. We were able to secure a small quantity of **20** using the following route (Scheme II). Partial hydrolysis of **4** in aqueous NaOH at 60 °C gave ester **8**. A mixture of **8** and excess dimethyl sulfate was stirred at 100 °C for a prolonged period to give after workup a poor yield of a mixture of ethyl and methyl esters of 7-chloro-1-(dimethylamino)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid. This crude ester mixture was converted to **20** by using the saponification/1-methylpiperazine sequence previously described.

The syntheses of derivatives **22–25** and **27–35** were accomplished by refluxing **10** in pyridine or *N,N*-diisopropylethylamine with an excess of the appropriate cyclic amine.[12] Compound **26**, the *N*-oxide of **16**, was made by treating the sodium salt of **16** with excess 30% H_2O_2. Compound **16** was esterified in refluxing EtOH containing excess methanesulfonic acid to provide ethyl ester **36**, characterized as its methanesulfonate salt. The structures and physical properties of intermediates **2–14** are found in Table I.

Results and Discussion

A comparison of the antibacterial properties of methylamino analogues **16** and **21** with their ethylated coun-

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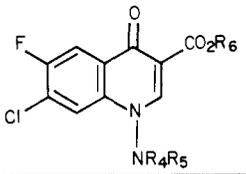
(12) All cyclic amines used in these conversions were commercially available except for 1-(1-methylethyl)piperazine dihydrochloride, which was prepared by the method of Mndzhoyan et al.: Mndzhoyan, A. L.; et al. *Arm. Khim. Zh.* **1968**, *21*, 603; *Chem. Abstr.* **1969**, *70*, 96754T.

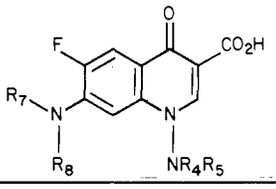
Table I. Physical Data for 1-Amino-7-chloro-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylates



compd	R_4	R_5	R_6	mp, °C	yield,[a] %	formula[b]
2	H	H	CH_2CH_3	254–258	78	$C_{12}H_{10}ClFN_2O_3$
3	H	CHO	CH_2CH_3	263–264	91	$C_{13}H_{10}ClFN_2O_4$
4	CH_3	CHO	CH_2CH_3	213–216	90	$C_{14}H_{12}ClFN_2O_4$
5	CH_2CH_3	CHO	CH_2CH_3	185–187	88	$C_{15}H_{14}ClFN_2O_4$
6	$CH_2CH{=}CH_2$	CHO	CH_2CH_3	178–182	83	$C_{16}H_{14}ClFN_2O_4$
7	$CH_2CH_2CH_3$	CHO	CH_2CH_3	185–187	88	$C_{16}H_{16}ClFN_2O_4$
8	CH_3	H	CH_2CH_3	206–209	9	$C_{13}H_{12}ClFN_2O_3$
9	H	H	H	312–315	86	$C_{10}H_6ClFN_2O_3$
10	CH_3	H	H	275–279	91	$C_{11}H_8ClFN_2O_3$
11	CH_2CH_3	H	K	258 dec	86	$C_{12}H_9ClFKN_2O_3{\cdot}0.5H_2O$[d]
12	$CH_2CH{=}CH_2$	H	H	240–242	97	$C_{13}H_{10}ClFN_2O_3{\cdot}0.25H_2O$
13	$CH_2CH_2CH_3$	H	K	267 dec	56	$C_{13}H_{11}ClFKN_2O_3{\cdot}0.5H_2O$
14	CH_3	CH_3	H	255–256	30[c]	$C_{12}H_{10}ClFN_2O_3$

[a] Yields were not optimized. [b] Carbon, hydrogen, and nitrogen analyses were within ±0.4% of the theortical values. [c] Overall yield from 8. [d] N: calcd, 43.44; found, 43.01.

Table II. Physical Data and Antibacterial Activity of 1,7-Diamino-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid Derivatives



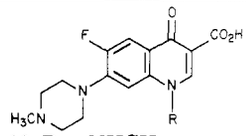
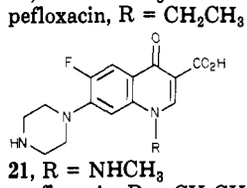
								antibacterial activity vs. *E. coli* Vogel[a]	
compd	R_4	R_5	R_7, R_8	method[a]	mp, °C	yld,[b] %	formula[c]	in vitro MIC, μg/mL	in vivo PD_{50}, mg/kg sc[d]
15	H	H	$CH_2CH_2N(CH_3)CH_2CH_2$	A	303–306	66	$C_{15}H_{17}FN_4O_3$	1.95	2.9 (2.0–4.4)
16	CH_3	H	$CH_2CH_2N(CH_3)CH_2CH_2$	A	299–301[f]	65	$C_{16}H_{19}FN_4O_3$	0.25	0.6 (0.4–0.7)
17	CH_2CH_3	H	$CH_2CH_2N(CH_3)CH_2CH_2$	B	255–259	64	$C_{17}H_{21}FN_4O_3$	0.5	4.1 (3.1–5.6)
18	$CH_2CH{=}CH_2$	H	$CH_2CH_2N(CH_3)CH_2CH_2$	B	220–222	40	$C_{18}H_{21}FN_4O_3$	1.0	19 (14–26)
19	$CH_2CH_2CH_3$	H	$CH_2CH_2N(CH_3)CH_2CH_2$	B	210–212	61	$C_{18}H_{23}FN_4O_3$	1.95	33 (20–44)
20	CH_3	CH_3	$CH_2CH_2N(CH_3)CH_2CH_2$	B	253–255	46	$C_{17}H_{21}FN_4O_3$	0.5	1.6[e]
21	CH_3	H	$CH_2CH_2NHCH_2CH_2$	A	288–290	75	$C_{15}H_{17}FN_4O_3$	1.0	0.3 (0.2–0.4)
22	CH_3	H	$CH_2CH_2N(CH_2CH_3)CH_2CH_2$	B	260 dec	56	$C_{17}H_{21}FN_4O_3$	0.5	1.4[e]
23	CH_3	H	$CH_2CH_2N[CH(CH_3)_2]CH_2CH_2$	C	245–250	36	$C_{18}H_{23}FN_4O_3$	0.5	1.5 (1.0–2.4)
24	CH_3	H	$CH_2CH_2N(CH_2CH_2CH_3)CH_2CH_2$	C	272–277	72	$C_{18}H_{23}FN_4O_3$	0.25	14.2 (9.9–20.0)
25	CH_3	H	$CH_2CH_2N(COCH_3)CH_2CH_2$	B	295–300	51	$C_{17}H_{19}FN_4O_4$	15.6	109 (80–146)
26	CH_3	H	$CH_2CH_2N(O)(CH_3)CH_2CH_2$	a	231 dec	82	$C_{16}H_{19}FN_4O_4$	15.6	9.4[e]
27	CH_3	H	$CH_2CH_2OCH_2CH_2$	B	288–289	63	$C_{15}H_{16}FN_3O_4$	0.5	16.6 (12.2–22.2)
28	CH_3	H	$CH_2CH_2SCH_2CH_2$	A	260–261	46	$C_{15}H_{16}FN_3O_3S$	0.5	>200
29	CH_3	H	$(CH_2)_5$	A	208–211	53	$C_{18}H_{18}FN_3O_3$	3.9	>200
30	CH_3	H	$CH_2CH_2CH(OH)CH_2CH_2$	A	230–236	42	$C_{18}H_{18}FN_3O_4$	1.95	12.5[e]
31	CH_3	H	$CH_2CH(OH)CH_2CH_2CH_2$	B	180 dec	29	$C_{16}H_{18}FN_3O_4$	3.9	41 (28–56)
32	CH_3	H	$CH_2CH_2NHCH_2CH_2CH_2$	D	305 dec	16	$C_{16}H_{19}FN_4O_3{\cdot}$ HCl	3.9	2.8[e]
33	CH_3	H	$CH_2CH_2N(CH_3)CH_2CH_2CH_2$	D	288 dec	23	$C_{17}H_{21}FN_4O_3{\cdot}$ $HCl{\cdot}0.5H_2O$	1.95	5.5 (4.4–7.9)
34	CH_3	H	$(CH_2)_4$	A	314–318	41	$C_{15}H_{16}FN_3O_3$	1.0	>200
35	CH_3	H	$CH_2CH(OH)CH_2CH_2$	A	319–321	51	$C_{15}H_{16}FN_3O_4$	1.0	10.4[e]
36	ethyl ester of 16			a	255 dec	62	$C_{16}H_{23}FN_4O_3{\cdot}$ CH_3SO_3H	62.5	4.4 (3.5–5.7)

[a] See Experimental Section. [b] Yields were not optimized. [c] Carbon, hydrogen, and nitrogen analyses were within ±0.4% of the theoretical values. [d] (95% confidence limits). [e] Estimate – data insufficient for probit analysis. [f] With decomposition.

terparts, pefloxacin and norfloxacin, is shown in Table III. In vitro data[13] represents testing vs. three Gram-negative bacteria, *Escherichia coli* Vogel, *Klebsiella pneumoniae* 39645, *Pseudomonas aeruginosa* MGH-2, and one Gram-positive organism, *Staphylococcus aureus* Smith. *E. coli* Vogel was used to evaluate the in vivo activity of the test compounds in experimental infections in mice.[13] The data indicate that **16** and **21** have similar in vitro and in vivo potencies compared to pefloxacin and norfloxacin, respectively. Of particular interest is the increased oral activity vs. *E. coli* of the two 7-(4-methyl-1-piperazinyl) derivatives, **16** and pefloxacin, relative to the two 7-(1-piperazinyl) analogues, **21** and norfloxacin. This enhanced

(13) See Experimental Section.

Table III. Structure–Activity Relationships: $NHCH_3$ vs. CH_2CH_3 at Position 1 of 3-Quinolinecarboxylic Acids

compd	in vitro antibacterial MIC, μg/mL[a]				in vivo vs. *E. coli* Vogel:[a] PD_{50}, mg/kg[b]	
	E. coli Vogel	*K. pneum.* 39645	*P. aerug.* MGH-2	*S. aureus* Smith	sc	po
						
16, R = $NHCH_3$	0.25	0.25	1.0	1.0	0.6 (0.4–0.7)	1.0 (0.8–1.3)
pefloxacin, R = CH_2CH_3	0.25	0.5	1.0	1.0	0.5[c]	1.1 (0.9–1.5)
						
21, R = $NHCH_3$	1.0	1.0	1.0	1.95	0.3 (0.2–0.4)	6.4 (4.9–9.1)
norfloxacin, R = CH_2CH_3	0.5	0.5	1.0	1.95	0.3 (0.2–0.4)	5.9 (4.3–8.2)

[a] See Experimental Section. [b] (95% confidence limits). [c] Estimate – data insufficient for probit analysis.

oral activity was the basis for choosing **16** rather than **21** as the reference compound for subsequent structure–activity relationship (SAR) studies.

The data for the first six entries (compounds **15–20**) in Table II summarizes the effect on antibacterial potency of altering the 1-alkylamino group of **16**. Optimal in vitro and in vivo activity vs. *E. coli* occurred with the methylamino substituent (compound **16**). Smaller (**15**) or larger (**17–20**) amino groups decreased potency.[14] The bulk factors (MRs) for NH_2, $NHCH_3$, $NHCH_2CH_3$, $NHCH_2CH{=}CH_2$, $NHCH_2CH_2CH_3$, and $N(CH_3)_2$ are 5.42, 10.33, 14.98, 19.12, 19.63, and 15.55 respectively.[10,15] This correlation between steric bulk of the 1-substituent and antibacterial potency is in general agreement with published SAR studies on 1-alkyl[3] and 1-alkoxy[9] substituted naphthyridine/quinoline antibacterial agents.

The results of varying the cyclic amine attached to the 7-position of **16** are also shown in Table II. For those analogues (**16** and **21–24**) having either piperazine or 4-alkylpiperazine at the 7-position, comparable in vitro potencies vs. *E. coli* were observed. In vivo potencies (sc) vs. *E. coli*, however, decreased as size of the 4-substituent (on piperazine) increased.

Acetylpiperazine analogue **25** exhibited poor activity in vitro and in vivo. Compound **26**, the *N*-oxide of **16**, had weak in vitro activity but was moderately active in vivo. In vitro activity was noted at reasonable levels for morpholine, thiomorpholine, and piperidine analogues **27–31**. In vivo activity of **27** and **30**, however, was substantially diminished relative to **16**. Compounds **28** and **29** were essentially inactive in vivo. The 3-hydroxypiperidine analogue **31** was less potent than the 4-hydroxy isomer **30**.

In an attempt to relate antimicrobial activity to the ring size of the 7-substituent, derivatives **32–35** were made. The homopiperazine analogues **32** and **33** showed good in vitro and in vivo activity but were less potent than **16**. The pyrrolidine derivatives **34** and **35** showed good in vitro potency; however, **35** had moderate in vivo potency while **34** was essentially inactive in vivo.

It is apparent that the in vivo antibacterial potencies of these 1-methylamino derivatives are greater (PD_{50} values < 6 mg/kg sc) when the cyclic amine at position 7 has an additional basic nitrogen incorporated in it, examples being piperazine and homopiperazine analogues **16**, **21–23**, **32**, and **33**. One exception is **24**, the 4-propylpiperazine analogue, which has moderate in vivo activity. Given the available data, it cannot be determined which parameters (steric bulk, electronic, and/or hydrophobicity) best account for the observed activity of these 7-substituted quinolines.

Comparisons of the antibacterial properties of these compounds reveal qualitatively similar trends in the in vitro and in vivo SARs. The quantitative differences are much greater in vivo, however, which suggest significant differences in bioavailability.

Compound **36**, the ethyl ester of **16**, was shown to be relatively inactive vs. *E. coli* Vogel (MIC = 62.5 μg/mL) and other organisms in vitro. However, when tested in vivo, a PD_{50} value of 4.4 mg/kg sc vs. *E. coli* Vogel was found. Similar findings have been observed in related systems[3] and the unexpectedly high in vivo potency of **36** is probably a result of its metabolism to **16**.

The in vitro antibacterial potency of **16** is similar for the Gram-negative and Gram-positive bacteria shown in Table III. The in vivo data, however, indicate that **16** is more potent vs. Gram-negative than Gram-positive organisms. For example, the PD_{50} values (in mg/kg sc) of **16** vs. *E. coli*, *P. aeruginosa*, and *S. aureus* are 0.6, 2.5, and 6.8, respectively.

Compound **16** (amifloxacin) has emerged from this study as a very potent antibacterial agent and is presently undergoing preclinical evaluation. An expanded description of the microbiological, toxicological, and pharmacokinetic profile of amifloxacin (**16**) will be reported elsewhere.

Experimental Section

Elemental analyses, performed by Instranal Laboratories, Rensselaer, NY, and Galbraith Laboratories, Knoxville, TN, were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analyses were within ±0.4% of the theoretical values. Melting points were taken in capillary tubes and are not corrected. All compounds were routinely checked by proton NMR (Varian HA-100), IR (Perkin-Elmer 21), TLC (silica gel), and in most instances mass spectrometry (JEOLCO JMS-1-OCS).

Ethyl 1-Amino-7-chloro-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate (2). A mixture of ethyl 7-chloro-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylate[6] (**1**; 76.9 g, 0.285 mol), 78.7 g (0.57 mol) of anhydrous K_2CO_3, and 1.5 L of DMF was stirred at room temperature for 3 h. To this was added *O*-(2,4-dinitrophenyl)hydroxylamine[11] (57.8 g, 0.291 mol) and the reaction mixture was stirred at room temperature for 3 h. Most of the solvent was removed in vacuo at 50 °C and the residue was slurried in 2 L of H_2O and then stirred for 2 h. The solid was collected and recrystallized from boiling DMF to give after drying 63.5 g (78%) of **2**, mp 254–258 °C. Anal. ($C_{12}H_{10}ClFN_2O_3$) C, H, N.

(14) A similar trend in SARs was noted both in vitro and in vivo against other test organisms; thus for the sake of clarity only the data vs. *E. coli* is shown.

(15) The MR (19.12) of $NHCH_2CH{=}CH_2$ is an estimated value.

Ethyl 7-Chloro-6-fluoro-1-(formylamino)-1,4-dihydro-4-oxo-3-quinolinecarboxylate (3). Formic acid (24.5 mL, 0.625 mol) was added dropwise to 59 mL (0.625 mol) of acetic anhydride with stirring at 0 °C. After addition was complete, the mixture was stirred for 15 min at 0 °C and 15 min at 50 °C and was then cooled to 0 °C again. To this solution was added dropwise a solution of 17.8 g (0.0625 mol) of **2** in 130 mL of formic acid. The reaction mixture was stirred for 3.5 days at 25 °C at which time TLC showed some **2** present; therefore, an additional 48 mL of mixed anhydride was added and the resulting mixture was stirred 6 h at room temperature. The solid product was collected by filtration, washed with H_2O, and recrystallized from DMF–EtOH to give 17.8 g (91%) of pure (by TLC) **3**. A portion was recrystallized from DMF–EtOH to give analytically pure **3**, mp 263–264 °C. Anal. ($C_{13}H_{10}ClFN_2O_4$) C, H, N.

Ethyl 7-Chloro-6-fluoro-1,4-dihydro-1-(formylmethylamino)-4-oxo-3-quinolinecarboxylate (4). A mixture of 14.75 g (0.047 mol) of **3**, 12.9 g (0.094 mol) of anhydrous K_2CO_3, and 200 mL of DMF was stirred at room temperature for 90 min. Methyl iodide (20.0 g, 0.141 mol) was added and stirring was continued for 90 min. The DMF was removed in vacuo at 50 °C and the residue was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was dried ($MgSO_4$) and concentrated, giving 13.8 g (90%) of pure (by TLC) **4**. A small sample was recrystallized from $CHCl_3$ and had mp 213–216 °C. Anal. ($C_{14}H_{12}ClFN_2O_4$) C, H, N.

Analogues **5–7** were prepared in similar fashion from **3**, using ethyl iodide, allyl bromide, and *n*-propyl bromide, respectively, as alkylating agents.[16]

Ethyl 7-Chloro-6-fluoro-1,4-dihydro-1-(methylamino)-4-oxo-3-quinolinecarboxylate (8). This partially hydrolyzed adduct of **4** was obtained from a reaction attempting the conversion **4** → **10**. A mixture of 148 g (.45 mol) of **4**, 70 g (1.75 mol) of NaOH, and 3 L of H_2O was stirred at 60 °C for 3.5 h. A solid was collected and slurried in boiling EtOH. After filtration, the filtrate was chilled and 12.4 g (9%) of **8** was obtained. A further recrystallization from EtOH gave a sample of **8** having mp 206–209 °C. Anal. ($C_{13}H_{12}ClFN_2O_3$) C, H, N.

7-Chloro-6-fluoro-1,4-dihydro-1-(methylamino)-4-oxo-3-quinolinecarboxylic Acid (10). A mixture of 11.8 g of **4** (0.036 mol), 5.8 g (0.144 mol) of NaOH, and 300 mL of H_2O was stirred on a steam bath for 2 h. The solution was decolorized (charcoal), filtered, and acidified with 9.5 g (0.158 mol) of acetic acid. A solid was collected and recrystallized from boiling DMF to give 8.7 g (91%) of **10**, mp 275–279 °C dec. Anal. ($C_{11}H_8ClFN_2O_3$) C, H, N.

Carboxylic acid derivatives **9** and **12** were made in similar fashion from **2** and **6**, respectively.[16]

Saponification of **5** and **7** in refluxing EtOH/H_2O/KOH gave after cooling analogues **11** and **13** isolated and characterized as their potassium salts.[16] Both **11** and **13** were converted (CH_3CO_2H, H_2O treatment) to the free acids prior to use in the next step.

7-Chloro-1-(dimethylamino)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (14). A mixture of 6.0 g (0.02 mol) of **8** and 60 mL of dimethyl sulfate was stirred at 100 °C for 9 h. This mixture was poured into 600 mL of H_2O containing 91 g of K_2CO_3 and was stirred for 2 h. A solid was collected and taken up in $CHCl_3$ (100 mL), filtered to remove insoluble materials, and concentrated to give 2.6 g of crude product. Subsequent treatment with 0.6 g (0.015 mol) of NaOH in 60 mL of H_2O at 100 °C followed by filtration and acidification with CH_3CO_2H (1 mL) gave 1.7 g (30% from **8**) of **14**, mp 255–256 °C. Anal. ($C_{12}H_{10}ClFN_2O_3$) C, H, N.

6-Fluoro-1,4-dihydro-1-(methylamino)-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid (16). Method A. A pyridine (30 mL) solution of **10** (5.0 g, 0.0185 mol) and 1-methylpiperazine (7.4 g, 0.0739 mol) was refluxed under N_2 for 15 h and was cooled. A solid was filtered, washed with ether, and recrystallized from boiling DMF (125 mL) to give 4.0 g (65%) of **16**, mp 299–301 °C dec. Anal. ($C_{16}H_{19}FN_4O_3$) C, H, N.

By use of this procedure compound **15** was made from **9**, while derivatives **21**, **28–30**, **34**, and **35** were prepared from **10** and an excess of the appropriate cyclic amines.[12,17]

Method B. Treatment of **12**, **14**, and the free acids of **11** and **13** with an excess of 1-methylpiperazine in refluxing pyridine for 14–18 h provided **18**, **20**, **17**, and **19**, respectively. The workup consisted of dilution of the cooled pyridine solutions with ether, collection of the resulting solids, washing with water, and drying. By use of this procedure compound **10** was converted to **22**, **25**, **27**, or **31** by treatment with a 3- to 4-fold excess of the appropriate cyclic amine.[12,17]

Method C. Compounds **23** and **24** were made by treating intermediate **10** with a 4-fold excess of either 1-(1-methylethyl)piperazine dihydrochloride[12] or 1-propylpiperazine dihydrobromide, respectively, in refluxing *N,N*-diisopropylethylamine for 16 h. The mixtures were diluted with ether, and the resulting solids were collected, washed with H_2O, and dried.[17]

Method D. Analogues **32** and **33** were prepared by refluxing **10** for 48 h in pyridine containing either a 10-fold excess of hexahydro-1*H*-1,4-diazapine or a 4-fold excess of hexahydro-4-methyl-1*H*-1,4-diazapine, respectively. The mixtures were concentrated, and the concentrate was slurried in H_2O. The resulting solids were collected, dissolved in dilute NaOH, filtered, and acidified with a mixture of dilute acetic acid and HCl. The HCl salts that precipitated were collected and dried.[17]

6-Fluoro-1,4-dihydro-1-(methylamino)-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic Acid 4′-Oxide (26). A solution of 5.0 g (0.015 mol) of **16** in 150 mL (0.015 mol) of 0.1 N NaOH was heated to 45 °C. After addition of 3.1 mL (0.03 mol) of 30% H_2O_2, the solution was stirred for 20 h at 45 °C. The solution was cooled to 25 °C and 50 mg of 10% Pd/C was added and stirring was continued for 1 h. The mixture was filtered and acidified with 150 mL (0.015 mol) of 0.1 N HCl. The product that crystallized was collected and dried, giving 4.3 g (82%) of **26**, mp 231 °C dec. Anal. ($C_{16}H_{19}FN_4O_4$) C, H, N.

Ethyl 6-Fluoro-1,4-dihydro-1-(methylamino)-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylate Monomethanesulfonate (36). Methanesulfonic acid (98%, 3.1 g, 0.031 mol) was added to a suspension of **16** (3.5 g, 0.011 mol) in ethanol (500 mL). After a 3-day reflux the clear yellow solution was concentrated under reduced pressure and the residue was taken up in 50 mL of H_2O. $NaHCO_3$ (6.0 g, 0.071 mol) was added portionwise and the solid that separated was collected, washed with water, and suspended in 30 mL of H_2O. After the addition of 1.2 g (0.013 mol) of 98% CH_3SO_3H, the clear yellow solution was filtered and diluted with 200 mL of acetone. The resulting solid was collected, giving 3.0 g (62%) of **36**, mp 255 °C dec. Anal. ($C_{18}H_{23}FN_4O_3 \cdot CH_3SO_3H$) C, H, N.

In Vitro Antibacterial Activity. The in vitro antibacterial activity of the test compounds in a side by side comparison was determined by conventional serial dilution procedures. Bacterial cultures were grown in tryptose phosphate broth or brain heart infusion broth overnight at 37 °C. Stock solutions of the test compound containing 1000 μg/mL (based on free acid) were prepared by dissolution in a minimum amount of 0.5 N NaOH and subsequent dilution with distilled H_2O. Serial dilution of the stock solutions was achieved in distilled water and 0.5 mL of each dilution was transferred to tubes. Each tube was inoculated with 0.5 mL of the appropriate culture resulting in a final bacterial concentration of 1×10^5 cell/mL. The tubes were then incubated at 37 °C for 18–20 h. The lowest concentration of test substance that inhibited visible growth was considered to be the minimal inhibitory concentration (MIC).

In Vivo Antibacterial Activity. The in vivo antibacterial activity of the test compounds was determined in female ICR mice weighing 18–20 g each. Aqueous solutions of the test compounds were made by dissolving the free acid in dilute NaOH and diluting with distilled H_2O to the desired volume. Cultures of *Escherichia coli* Vogel prepared in brain heart infusion broth and *Pseudomonas aeruginosa* MGH-2 grown on brain heart infusion agar were suspended in physiological saline for use as test inoculum. A stock frozen pool of *Staphylococcus aureus* Smith was diluted in 5% gastric mucin.

Mice were inoculated intraperitoneally with 0.5 mL of bacterial inoculum containing 1.9×10^7, 5×10^6, and 1.9×10^4 cells/mL of *E. coli*, *P. aerugonisa*, and *S. aureus*, respectively.

(16) See Table I for physical data.

(17) See Table II for physical data.

Mice infected with *E. coli* or *S. aureus* were medicated by the subcutaneous (sc) or oral (po) routes once (0.5 mL) at 0.5-h postinfection. Deaths were recorded daily for 7 days. Mice infected with *P. aerugonisa* were medicated sc (0.2 mL) at 0.5-, 4-, and 7-h postinfection. Deaths were recorded daily for 7 days.

Groups of 10 animals each for four or five dose levels were thus treated and the number of survivors in each group recorded. Nonmedicated control animals were included in each test. Fifty percent protective dose values (PD_{50}) were calculated by using probit analysis.[18]

Acknowledgment. We thank the Analytical Chemistry Department of this Institute for spectral measurements.

(18) Proc Probit Subroutine in "SAS User's Guide: Statistics", 1982 Ed.; SAS Institute: Cary, NC, 1982.

Registry No. **1**, 75073-15-3; **2**, 88569-30-6; **3**, 88569-33-9; **4**, 88569-34-0; **5**, 88569-35-1; **6**, 88569-38-4; **7**, 88569-36-2; **8**, 88569-45-3; **9**, 88569-31-7; **10**, 88569-39-5; **11**, 88569-41-9; **12**, 88569-44-2; **13**, 88569-43-1; **14**, 88569-47-5; **15**, 88569-56-6; **16**, 86393-37-5; **17**, 88569-59-9; **18**, 88569-68-0; **19**, 88569-60-2; **20**, 88569-61-3; **21**, 88569-57-7; **22**, 88569-62-4; **23**, 88569-65-7; **24**, 88569-67-9; **25**, 88569-70-4; **26**, 88569-53-3; **27**, 88569-69-1; **28**, 88569-77-1; **29**, 88569-71-5; **30**, 88569-73-7; **31**, 88569-74-8; **32**, 90584-37-5; **32** (free base), 89990-93-2; **33**, 90584-38-6; **33** (free base), 89990-44-3; **34**, 88569-72-6; **35**, 88569-78-2; **36**, 89990-96-5; **36** (free base), 88569-63-5; 1-(1-methylethyl)piperazine dihydrochloride, 88569-66-8; 1-propylpiperazine dihydrobromide, 64262-23-3; hexahydro-1*H*-1,4-diazepine, 505-66-8; hexahydro-4-methyl-1*H*-1,4-diazepine, 4318-37-0.

Synthesis and Evaluation of Substituted 2-Phenylcyclobutylamines as Analogues of Hallucinogenic Phenethylamines: Lack of LSD-like Biological Activity

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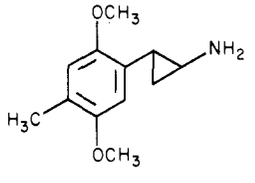
cis- and *trans*-2-(2,4,5-trimethoxyphenyl)cyclobutylamine and *trans*-2-(2,5-dimethoxy-4-methylphenyl)cyclobutylamine were synthesized as conformationally restricted analogues of hallucinogenic phenylisopropylamines. In rats trained to discriminate saline from LSD (0.08 mg/kg, ip) in a two-lever drug discrimination paradigm, no generalization of the LSD stimulus to the cis trimethoxy compound occurred at doses up to 20 mg/kg. For both of the trans compounds, partial generalization of the LSD cue occurred at doses of 5 mg/kg or greater. In contrast, complete generalization occurred with *trans*-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine. The ED_{50} for this compound and the doses of the trans cyclobutyl homologues at which significant drug-appropriate responding occurred indicate that the latter are on the order of 50–75 times less potent than the cyclopropylamine analogue. The lack of generalization to the cyclobutylamines indicates either that their discriminative stimulus properties differ from LSD or that they lack discriminative effects.

In our continuing studies to elucidate the structure–activity requirements of phenethylamine type hallucinogens, we reported that the cyclopropyl analogue **1** has potent hallucinogen-like activity in several animal models.[1,2] Furthermore, it has been subsequently found that



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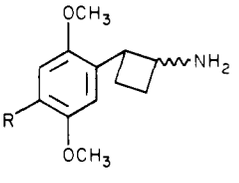

cis-**2a**, R = OCH_3
trans-**2b**, R = OCH_3
trans-**3**, R = CH_3

racemic **1** is hallucinogenic in man, with an effective dose of the hydrochloride in the 16–20-mg range.[3] The compound is therefore some 15–20 times more potent than mescaline. In contrast, **1** has relatively weak agonist activity in the rat fundus assay, when compared with its phenethylamine counterpart, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM, STP).[4,5] Since the pK_a of **1** is 8.11,[5] while that of substituted amphetamines is about 9.6,[6] it seems likely that the high in vivo activity for **1** may be at least partially attributed to more favorable partitioning into the CNS, as a result of a higher fraction of the unionized form of the compound at physiological pH. Thus, higher concentrations of **1** in the brain could compensate for decreased efficacy at the receptor.

It is now fairly evident that addition of alkyl groups larger than methyl to the α side chain carbon of the substituted amphetamine type hallucinogens abolishes biological activity.[4,7,8] It was therefore of interest to examine the cyclobutane homologues **2** and **3** for hallucinogen-like activity. In these compounds the ring of **1** has been expanded by one methylene unit. This would further test

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