

pounds),<sup>23</sup> (b) no  $\alpha$ -hydrogens are available, or (c) the  $\alpha$ -hydrogens are inaccessible for abstraction (e.g., Bredt's rule).<sup>7</sup> 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,<sup>24</sup> can form *N*-oxides from many amines because it operates via a heterolytic mechanism involving a flavin 4a hydroperoxide,<sup>25</sup> 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.

(24) Ziegler, D. M. *Enzym. Basis Detoxication* 1980, 1, 201.

**Acknowledgment.** This work was supported by NIH Research Grants ES 01590 and ES 00267. Thanks are extended to J. E. Hill, T. M. Harris, and T. L. Macdonald for providing NMR spectra and helpful discussions.

(25) 1 proved not to be a substrate for this enzyme (Ziegler, D. M.; Guengerich, F. P., unpublished results).

F. Peter Guengerich\*

Department of Biochemistry and  
Center in Molecular Toxicology  
Vanderbilt University  
Nashville, Tennessee 37232

Received May 7, 1984

## Articles

### Novel Amino-Substituted 3-Quinolinecarboxylic Acid Antibacterial Agents: Synthesis and Structure-Activity Relationships<sup>1</sup>

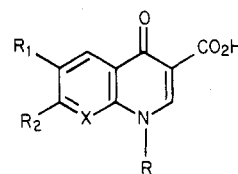
Mark P. Wentland,\* Denis M. Bailey, James B. Cornett, Richard A. Dobson, Ronald G. Powles, and Roland B. Wagner

Departments of Medicinal Chemistry and Microbiology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144.  
Received January 23, 1984

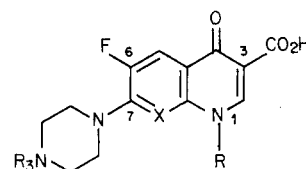
A series of novel 3-quinolinecarboxylic acid derivatives have been prepared and their antibacterial activity evaluated. These derivatives are characterized by fluorine attached to the 6-position and substituted amino groups appended to the 1- and 7-positions. Structure-activity relationship studies indicate 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 ( $\mu\text{g/mL}$ ) = 0.25; in vivo (mice) PD<sub>50</sub> (mg/kg) = 1.0 (po), 0.6 (sc).

Since the introduction in 1963 of nalidixic acid<sup>2</sup> as a systemic Gram-negative antibacterial agent, many related derivatives<sup>3</sup> have been made. The newest derivatives, which include pefloxacin,<sup>4</sup> norfloxacin,<sup>5</sup> AT-2266,<sup>6</sup> DL-8280,<sup>7</sup> and Bay o 9867<sup>8</sup> 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<sup>3</sup> 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<sub>2</sub>CH<sub>3</sub>; R<sub>1</sub> = H; R<sub>2</sub> = CH<sub>3</sub>; X = N  
oxolinic acid, R = CH<sub>2</sub>CH<sub>3</sub>; R<sub>1</sub>, R<sub>2</sub> = OCH<sub>2</sub>O; X = CH  
rosoxacin, R = CH<sub>2</sub>CH<sub>3</sub>; R<sub>1</sub> = H; R<sub>2</sub> = 4-pyridinyl, X = CH  
miloxacin, R = OCH<sub>3</sub>; R<sub>1</sub>, R<sub>2</sub> = OCH<sub>2</sub>O; X = CH

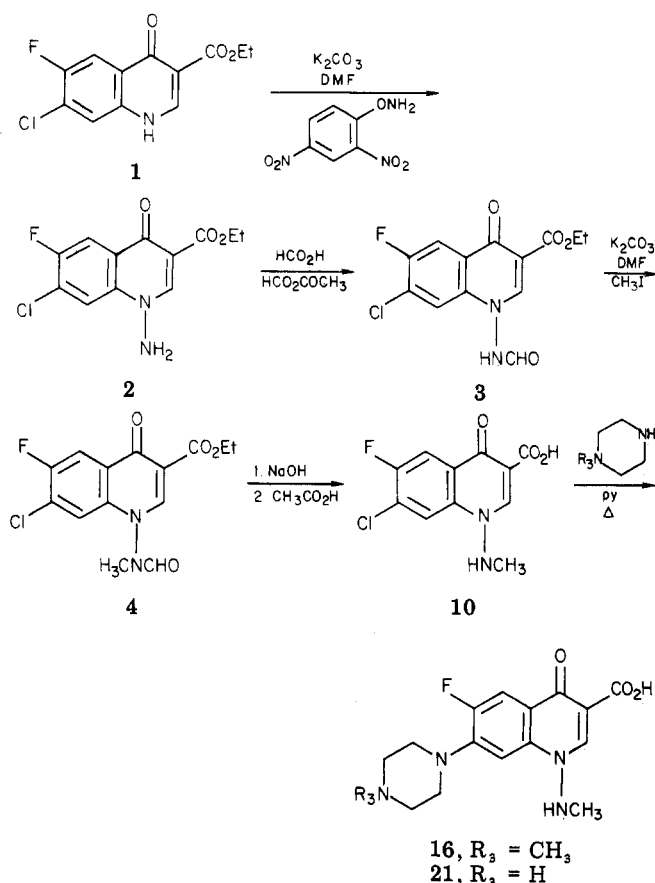


pefloxacin, R = CH<sub>2</sub>CH<sub>3</sub>; R<sub>3</sub> = CH<sub>3</sub>; X = CH  
norfloxacin, R = CH<sub>2</sub>CH<sub>3</sub>; R<sub>3</sub> = H; X = CH  
AT-2266, R = CH<sub>2</sub>CH<sub>3</sub>; R<sub>3</sub> = H; X = N  
DL-8280, X, R = COCH<sub>2</sub>CH(CH<sub>3</sub>); R<sub>3</sub> = CH<sub>3</sub>  
Bay o 9867, R = c-C<sub>6</sub>H<sub>5</sub>; R<sub>3</sub> = H; X = CH

Structure-activity relationship studies have indicated that antibacterial potency is closely related to the steric bulk of the 1-substituent.<sup>3</sup> 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) Leshner, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, P. R. *J. Med. Chem.* 1962, 5, 1063.
- (3) Albrecht, R., *Prog. Drug Res.* 1977, 21, 9.
- (4) Goueffon, Y.; Montay, G.; Roquet, F.; Pesson, M., *C. R. Hebd. Seances Acad. Sci.* 1981, 292, 37.
- (5) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* 1980, 23, 1358.
- (6) Nakamura, S.; Nakata, K.; Katae, H.; Minami, A.; Kashimoto, S.; Yamagishi, J.; Takase, Y.; Shimizu, M. *Antimicrob. Agents Chemother.* 1983, 23, 742.
- (7) Sato, K.; Matsuura, Y.; Inoue, M.; Une, T.; Osada, Y.; Ogawa, H.; Mitsuhashi, S. *Antimicrob. Agents Chemother.* 1982, 22, 548.
- (8) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* 1983, 23, 559.

Scheme I

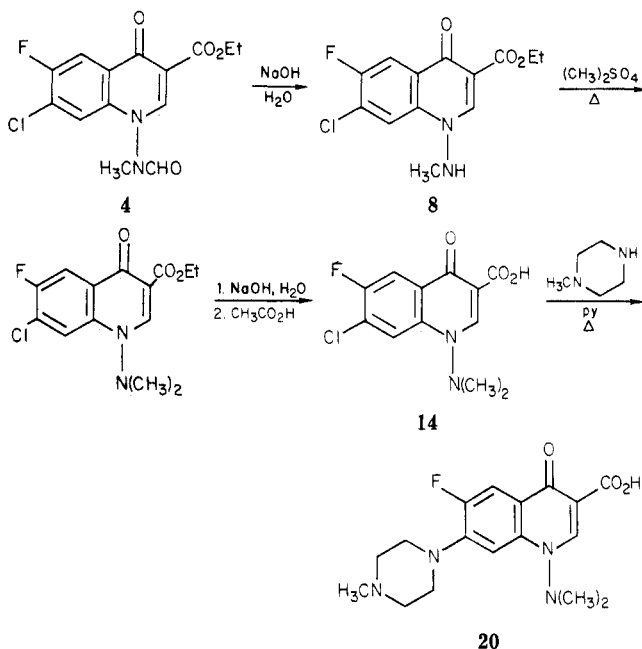


logues are generally more potent than those analogues having smaller or larger 1-alkyl substituents. In some instances the vinyl analogues, which have a similar steric bulk, showed potencies comparable to those of the ethyl derivatives. Two other variants are miloxacin<sup>9</sup> and Bay o 9867,<sup>8</sup> 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 ( $\text{NHCH}_3$ ) appendages. Methylamino and ethyl groups have similar steric bulk with the MR (bulk factor) being 10.33 and 10.30, respectively.<sup>10</sup> 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)<sup>5</sup> served as starting material for the synthesis of 16 and 21 (Scheme I). Amination of 1 in  $\text{K}_2\text{CO}_3/\text{DMF}$  with *O*-(2,4-dinitrophenyl)hydroxylamine<sup>11</sup> 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  $\text{CH}_3\text{I}/\text{K}_2\text{CO}_3/\text{DMF}$  at 25 °C gave 4. Alkaline hydrolysis of 4 ( $\text{NaOH}/\text{H}_2\text{O}/90^\circ\text{C}$ ) followed by acidification with excess acetic acid afforded 10. Compound 10 was con-

Scheme II



verted 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  $\text{K}_2\text{CO}_3/\text{DMF}$  with ethyl iodide, allyl bromide, or 1-bromopropane, (2) aqueous  $\text{NaOH}$  or  $\text{KOH}$  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  $\text{NaOH}$  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.<sup>12</sup> Compound 26, the *N*-oxide of 16, was made by treating the sodium salt of 16 with excess 30%  $\text{H}_2\text{O}_2$ . Compound 16 was esterified in refluxing  $\text{EtOH}$  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-

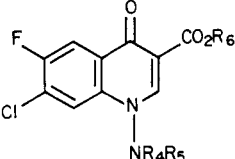
(9) Agui, H.; Mitani, T.; Izawa, A.; Komatsu, T.; Nakagome, T. *J. Med. Chem.* 1977, 20, 791.

(10) Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley-Interscience: New York, 1978; pp 49–51.

(11) Tamura, Y.; Minamikawa, J.; Sumoto, S.; Fujii, S.; Ikeda, M. *J. Org. Chem.* 1973, 38, 1239.

(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.

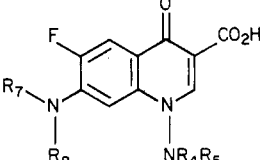
Table I. Physical Data for 1-Amino-7-chloro-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylates



compd	R <sub>4</sub>	R <sub>5</sub>	R <sub>6</sub>	mp, °C	yield, <sup>a</sup> %	formula <sup>b</sup>
2	H	H	CH <sub>2</sub> CH <sub>3</sub>	254–258	78	C <sub>12</sub> H <sub>10</sub> ClFN <sub>2</sub> O <sub>3</sub>
3	H	CHO	CH <sub>2</sub> CH <sub>3</sub>	263–264	91	C <sub>13</sub> H <sub>10</sub> ClFN <sub>2</sub> O <sub>4</sub>
4	CH <sub>3</sub>	CHO	CH <sub>2</sub> CH <sub>3</sub>	213–216	90	C <sub>14</sub> H <sub>12</sub> ClFN <sub>2</sub> O <sub>4</sub>
5	CH <sub>2</sub> CH <sub>3</sub>	CHO	CH <sub>2</sub> CH <sub>3</sub>	185–187	88	C <sub>15</sub> H <sub>14</sub> ClFN <sub>2</sub> O <sub>4</sub>
6	CH <sub>2</sub> CH=CH <sub>2</sub>	CHO	CH <sub>2</sub> CH <sub>3</sub>	178–182	83	C <sub>16</sub> H <sub>14</sub> ClFN <sub>2</sub> O <sub>4</sub>
7	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	CHO	CH <sub>2</sub> CH <sub>3</sub>	185–187	88	C <sub>16</sub> H <sub>16</sub> ClFN <sub>2</sub> O <sub>4</sub>
8	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>3</sub>	206–209	9	C <sub>13</sub> H <sub>12</sub> ClFN <sub>2</sub> O <sub>3</sub>
9	H	H	H	312–315	86	C <sub>10</sub> H <sub>8</sub> ClFN <sub>2</sub> O <sub>3</sub>
10	CH <sub>3</sub>	H	H	275–279	91	C <sub>11</sub> H <sub>9</sub> ClFN <sub>2</sub> O <sub>3</sub>
11	CH <sub>2</sub> CH <sub>3</sub>	H	K	258 dec	86	C <sub>12</sub> H <sub>9</sub> ClFN <sub>2</sub> O <sub>3</sub> ·0.5H <sub>2</sub> O <sup>d</sup>
12	CH <sub>2</sub> CH=CH <sub>2</sub>	H	H	240–242	97	C <sub>13</sub> H <sub>10</sub> ClFN <sub>2</sub> O <sub>3</sub> ·0.25H <sub>2</sub> O
13	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	H	K	267 dec	56	C <sub>13</sub> H <sub>11</sub> ClFN <sub>2</sub> O <sub>3</sub> ·0.5H <sub>2</sub> O
14	CH <sub>3</sub>	CH <sub>3</sub>	H	255–256	30 <sup>c</sup>	C <sub>12</sub> H <sub>10</sub> ClFN <sub>2</sub> O <sub>3</sub>

<sup>a</sup> Yields were not optimized. <sup>b</sup> Carbon, hydrogen, and nitrogen analyses were within ±0.4% of the theoretical values. <sup>c</sup> Overall yield from 8. <sup>d</sup> 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



compd	R <sub>4</sub>	R <sub>5</sub>	R <sub>7</sub> , R <sub>8</sub>	method <sup>a</sup>	mp, °C	yld, <sup>b</sup> %	formula <sup>c</sup>	antibacterial activity vs. <i>E. coli</i> Vogel <sup>e</sup>	
								in vitro MIC, µg/mL	in vivo PD <sub>50</sub> , mg/kg sc <sup>d</sup>
15	H	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	A	303–306	66	C <sub>15</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>3</sub>	1.95	2.9 (2.0–4.4)
16	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	A	299–301 <sup>f</sup>	65	C <sub>16</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>3</sub>	0.25	0.6 (0.4–0.7)
17	CH <sub>2</sub> CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	B	255–259	64	C <sub>17</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub>	0.5	4.1 (3.1–5.6)
18	CH <sub>2</sub> CH=CH <sub>2</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	B	220–222	40	C <sub>18</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub>	1.0	19 (14–26)
19	CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	B	210–212	61	C <sub>18</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>3</sub>	1.95	33 (20–44)
20	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	B	253–255	46	C <sub>17</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub>	0.5	1.6 <sup>g</sup>
21	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> NHCH <sub>2</sub> CH <sub>2</sub>	A	288–290	75	C <sub>16</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>3</sub>	1.0	0.3 (0.2–0.4)
22	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	B	260 dec	56	C <sub>17</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub>	0.5	1.4 <sup>g</sup>
23	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N[CH(CH <sub>3</sub> ) <sub>2</sub> ]CH <sub>2</sub> CH <sub>2</sub>	C	245–250	36	C <sub>18</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>3</sub>	0.5	1.5 (1.0–2.4)
24	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	C	272–277	72	C <sub>18</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>3</sub>	0.25	14.2 (9.9–20.0)
25	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(COCH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	B	295–300	51	C <sub>17</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>4</sub>	15.6	109 (80–146)
26	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(O)(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub>	a	231 dec	82	C <sub>16</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>4</sub>	15.6	9.4 <sup>g</sup>
27	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> OCH <sub>2</sub> CH <sub>2</sub>	B	288–289	63	C <sub>16</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>4</sub>	0.5	16.6 (12.2–22.2)
28	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> SCH <sub>2</sub> CH <sub>2</sub>	A	260–261	46	C <sub>16</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>3</sub> S	0.5	>200
29	CH <sub>3</sub>	H	(CH <sub>2</sub> ) <sub>5</sub>	A	208–211	53	C <sub>16</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>	3.9	>200
30	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> CH(OH)CH <sub>2</sub> CH <sub>2</sub>	A	230–236	42	C <sub>16</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>4</sub>	1.95	12.5 <sup>g</sup>
31	CH <sub>3</sub>	H	CH <sub>2</sub> CH(OH)CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	B	180 dec	29	C <sub>16</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>4</sub>	3.9	41 (28–56)
32	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> NHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	D	305 dec	16	C <sub>16</sub> H <sub>19</sub> FN <sub>4</sub> O <sub>3</sub> ·HCl	3.9	2.8 <sup>g</sup>
33	CH <sub>3</sub>	H	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub>	D	288 dec	23	C <sub>17</sub> H <sub>21</sub> FN <sub>4</sub> O <sub>3</sub> ·HCl·0.5H <sub>2</sub> O	1.95	5.5 (4.4–7.9)
34	CH <sub>3</sub>	H	(CH <sub>2</sub> ) <sub>4</sub>	A	314–318	41	C <sub>16</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>3</sub>	1.0	>200
35	CH <sub>3</sub>	H	CH <sub>2</sub> CH(OH)CH <sub>2</sub> CH <sub>2</sub>	A	319–321	51	C <sub>16</sub> H <sub>16</sub> FN <sub>3</sub> O <sub>4</sub>	1.0	10.4 <sup>g</sup>
36	ethyl ester of 16			a	255 dec	62	C <sub>18</sub> H <sub>23</sub> FN <sub>4</sub> O <sub>3</sub> ·CH <sub>3</sub> SO <sub>3</sub> H	62.5	4.4 (3.5–5.7)

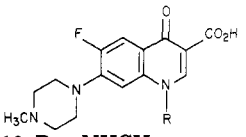
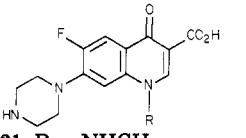
<sup>a</sup> See Experimental Section. <sup>b</sup> Yields were not optimized. <sup>c</sup> Carbon, hydrogen, and nitrogen analyses were within ±0.4% of the theoretical values. <sup>d</sup> (95% confidence limits). <sup>e</sup> Estimate – data insufficient for probit analysis. <sup>f</sup> With decomposition.

terparts, pefloxacin and norfloxacin, is shown in Table III. In vitro data<sup>13</sup> 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.<sup>13</sup> 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:  $\text{NHCH}_3$  vs.  $\text{CH}_2\text{CH}_3$  at Position 1 of 3-Quinolonecarboxylic Acids

compd	in vitro antibacterial MIC, $\mu\text{g/mL}^a$				in vivo vs. <i>E. coli</i> Vogel: <sup>a</sup> PD <sub>50</sub> , mg/kg <sup>b</sup>	
	<i>E. coli</i> Vogel	<i>K. pneum.</i> 39645	<i>P. aerug.</i> MGH-2	<i>S. aureus</i> Smith	sc	po
	0.25	0.25	1.0	1.0	0.6 (0.4-0.7)	1.0 (0.8-1.3)
pefloxacin, R = $\text{CH}_2\text{CH}_3$	0.25	0.5	1.0	1.0	0.5 <sup>c</sup>	1.1 (0.9-1.5)
	1.0	1.0	1.0	1.95	0.3 (0.2-0.4)	6.4 (4.9-9.1)
norfloxacin, R = $\text{CH}_2\text{CH}_3$	0.5	0.5	1.0	1.95	0.3 (0.2-0.4)	5.9 (4.3-8.2)

<sup>a</sup> See Experimental Section. <sup>b</sup> (95% confidence limits). <sup>c</sup> 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.<sup>14</sup> The bulk factors (MRs) for  $\text{NH}_2$ ,  $\text{NHCH}_3$ ,  $\text{NHCH}_2\text{CH}_3$ ,  $\text{NHCH}_2\text{C}\equiv\text{CH}$ ,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ , and  $\text{N}(\text{CH}_3)_2$  are 5.42, 10.33, 14.98, 19.12, 19.63, and 15.55 respectively.<sup>10,15</sup> This correlation between steric bulk of the 1-substituent and antibacterial potency is in general agreement with published SAR studies on 1-alkyl<sup>3</sup> and 1-alkoxy<sup>9</sup> 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.

Acetypiperazine 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<sub>50</sub> 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 quinolones.

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  $\mu\text{g/mL}$ ) and other organisms in vitro. However, when tested in vivo, a PD<sub>50</sub> value of 4.4 mg/kg sc vs. *E. coli* Vogel was found. Similar findings have been observed in related systems<sup>8</sup> 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<sub>50</sub> 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 Intranal Laboratories, Rensselaer, NY, and Galbraith Laboratories, Knoxville, TN, were obtained for all new compounds reported. Carbon, hydrogen, and nitrogen analyses were within  $\pm 0.4\%$  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<sup>5</sup> (1; 76.9 g, 0.285 mol), 78.7 g (0.57 mol) of anhydrous  $\text{K}_2\text{CO}_3$ , and 1.5 L of DMF was stirred at room temperature for 3 h. To this was added *O*-(2,4-dinitrophenyl)hydroxylamine<sup>11</sup> (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  $\text{H}_2\text{O}$  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. ( $\text{C}_{12}\text{H}_{10}\text{ClFN}_2\text{O}_5$ ) 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  $\text{NHCH}_2\text{CH}=\text{CH}_2$  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<sub>2</sub>O, 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<sub>13</sub>H<sub>10</sub>ClFN<sub>2</sub>O<sub>4</sub>) 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<sub>2</sub>CO<sub>3</sub>, 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<sub>2</sub>O and CHCl<sub>3</sub>. The CHCl<sub>3</sub> layer was dried (MgSO<sub>4</sub>) and concentrated, giving 13.8 g (90%) of pure (by TLC) 4. A small sample was recrystallized from CHCl<sub>3</sub> and had mp 213–216 °C. Anal. (C<sub>14</sub>H<sub>12</sub>ClFN<sub>2</sub>O<sub>4</sub>) 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.<sup>16</sup>

**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<sub>2</sub>O 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<sub>13</sub>H<sub>12</sub>ClFN<sub>2</sub>O<sub>3</sub>) 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<sub>2</sub>O 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<sub>11</sub>H<sub>8</sub>ClFN<sub>2</sub>O<sub>3</sub>) C, H, N.

Carboxylic acid derivatives 9 and 12 were made in similar fashion from 2 and 6, respectively.<sup>16</sup>

Saponification of 5 and 7 in refluxing EtOH/H<sub>2</sub>O/KOH gave after cooling analogues 11 and 13 isolated and characterized as their potassium salts.<sup>16</sup> Both 11 and 13 were converted (CH<sub>3</sub>CO<sub>2</sub>H, H<sub>2</sub>O 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<sub>2</sub>O containing 91 g of K<sub>2</sub>CO<sub>3</sub> and was stirred for 2 h. A solid was collected and taken up in CHCl<sub>3</sub> (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<sub>2</sub>O at 100 °C followed by filtration and acidification with CH<sub>3</sub>CO<sub>2</sub>H (1 mL) gave 1.7 g (30% from 8) of 14, mp 255–256 °C. Anal. (C<sub>12</sub>H<sub>10</sub>ClFN<sub>2</sub>O<sub>3</sub>) 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<sub>2</sub> 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<sub>16</sub>H<sub>19</sub>FN<sub>4</sub>O<sub>3</sub>) 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.<sup>12,17</sup>

**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.<sup>12,17</sup>

**Method C.** Compounds 23 and 24 were made by treating intermediate 10 with a 4-fold excess of either 1-(1-methyl-ethyl)piperazine dihydrochloride<sup>12</sup> 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<sub>2</sub>O, and dried.<sup>17</sup>

**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<sub>2</sub>O. 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.<sup>17</sup>

**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<sub>2</sub>O<sub>2</sub>, 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<sub>16</sub>H<sub>18</sub>FN<sub>4</sub>O<sub>4</sub>) 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<sub>2</sub>O. NaHCO<sub>3</sub> (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<sub>2</sub>O. After the addition of 1.2 g (0.013 mol) of 98% CH<sub>3</sub>SO<sub>3</sub>H, 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<sub>18</sub>H<sub>23</sub>FN<sub>4</sub>O<sub>3</sub>·CH<sub>3</sub>SO<sub>3</sub>H) 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<sub>2</sub>O. 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<sup>5</sup> 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<sub>2</sub>O 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<sup>7</sup>, 5 × 10<sup>6</sup>, and 1.9 × 10<sup>4</sup> cells/mL of *E. coli*, *P. aeruginosa*, 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. aeruginosa* 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<sub>50</sub>) were calculated by using probit analysis.<sup>18</sup>

**Acknowledgment.** We thank the Analytical Chemistry

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

Department of this Institute for spectral measurements.

**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-1H-1,4-diazepine, 505-66-8; hexahydro-4-methyl-1H-1,4-diazepine, 4318-37-0.

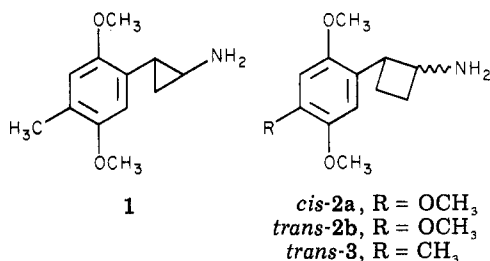
## Synthesis and Evaluation of Substituted 2-Phenylcyclobutylamines as Analogues of Hallucinogenic Phenethylamines: Lack of LSD-like Biological Activity

David E. Nichols,\*† Kiran P. Jadhav,† Robert A. Oberlender,† Joseph E. Zabik,‡ Josef F. Bossart,§ Akihiko Hamada,§ and Duane D. Miller§

Departments of Medicinal Chemistry and Pharmacognosy and Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Division of Medicinal Chemistry, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210. Received December 27, 1983

*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<sub>50</sub> 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.<sup>1,2</sup> Furthermore, it has been subsequently found that



racemic 1 is hallucinogenic in man, with an effective dose of the hydrochloride in the 16–20-mg range.<sup>3</sup> 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).<sup>4,5</sup> Since the pK<sub>a</sub> of 1 is 8.11,<sup>5</sup> while that of substituted amphetamines is about 9.6,<sup>6</sup> 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  $\alpha$  side chain carbon of the substituted amphetamine type hallucinogens abolishes biological activity.<sup>4,7,8</sup> 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

\*Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

†Department of Pharmacology and Toxicology, Purdue University.

§Division of Medicinal Chemistry, Ohio State University.

- (1) Nichols, D. E.; Woodard, R.; Hathaway, B. A.; Lowy, M. T.; Yim, G. K. W. *J. Med. Chem.* 1979, 22, 458.
- (2) Nichols, D. E.; Pfister, W. R.; Yim, G. K. W. *Life Sci.* 1978, 22, 2165.
- (3) (a) Human activity for the hydrochloride of compound 1 was determined by Shulgin (personal communication) following procedures described in ref 3b. An active range (orally, in normal, adult volunteers) was 16–20 mg (with  $n = 7$  and in 17 experimental trials). (b) Jacob, P., III; Shulgin, A. T. *J. Med. Chem.* 1983, 26, 746.
- (4) Jacob, J. N.; Nichols, D. E. *J. Med. Chem.* 1982, 25, 526.
- (5) Kothari, P. J.; Hathaway, B. A.; Nichols, D. E.; Yim, G. K. W. *J. Med. Chem.* 1981, 24, 882.
- (6) Liffler, E. B.; Spencer, H. M.; Burger, A. *J. Am. Chem. Soc.* 1951, 73, 2611.
- (7) Standridge, R. T.; Howell, H. G.; Gylys, J. A.; Partyka, R. A.; Shulgin, A. T. *J. Med. Chem.* 1976, 19, 1400.
- (8) Shulgin, A. T. *Experientia* 1963, 19, 127.