prepared in our laboratory was cleaved by pepsin at a 50% level in 10 min by using this assay.

Rat Stomach Homogenate. A fresh rat stomach was homogenized in physiological saline (2 mL). The peptides (0.7 μmol) were dissolved in 30 μL of 0.1 N NaOH and mixed with 90 μL of 0.25% methyl Cellosolve in H_2O. The solution was partially neutralized with 27 μL of 0.1 N HCl. This solution was mixed with 150 mg of rat stomach homogenate and incubated at 37 °C. Degradation was monitored by TLC as described previously for trypsin.[2]

Rat Intestinal Homogenate. Rat small intestine was removed immediately after the rat was sacrificed and was minced in Krebs Ringer solution (10 mL/1 g of intestine). Peptide samples (0.2–0.5 μmol) were mixed with homogenate (1.0 mL of homogenate/1.0 μmol of peptide) and then shaken in a 37 °C bath. Degradations were monitored by TLC as described previously for trypsin.[2]

Angiotensin I Converting Enzyme Inhibition Assay in Normotensive Rats. The testing of compounds for ACE inhibition in rats was performed by Pharmakon Research Internation Inc., Waverly, PA. The testing methods that were employed are described below.

For testing of the inhibitors by the intravenous route, first, male Sprague–Dawley normotensive rats (290-540 g) were anesthetized with sodium pentobarbital at 50 mg/kg, ip, and supplemented as necessary. A patent airway was maintained by tracheal intubation for spontaneous respiration. The jugular vein was catherized with PE50 tubing for intravenous administration of angiotensin I and test compound. The carotid artery was catherized with PE50 tubing for measurement of pulsatile blood pressure. The blood pressure was measured with a Statham P23Db pressure transducer and Grass Model 7 polygraph. Angiotensin I at 175 ng/kg in distilled water was administered intravenously following iv injection of 1 mL/kg of the test compound vehicle to determine the control blood pressure response to angiotensin I. The test compound dissolved in distilled water or 5% sodium bicarbonate solution was then given iv at the desired dose. Blood pressure was then monitored while angiotensin I at 175 ng/kg in distilled water was given iv at 1.5, 5, 10, 15, 20, 30, 45, 60, and 75 min or until the blood pressure response to angiotensin had returned to normal. An ID_{50} dose was calculated as the inhibitor dose that decreased the rise in blood pressure expected for 175 ng/kg of iv angiotensin I by 50%.

For the testing of inhibitors by the oral route, male Sprague–Dawley normotensive rats (250–320 g) were cannulated for blood pressure and heart rate monitoring by the method of Weeks and Jones.[19] Prior to administration of the test compound, a control pressor response to angiotensin I at 350 ng/kg ia was determined. The test compound was dissolved in 5% sodium bicarbonate at the desired dose and administered orally. Angiotensin I was readministered to each rat at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, and 300 min and every 30 min for 3 more h, and blood pressure was monitored.

Peptide Penetration of Rat Intestine. Three 1.5-in. sections of freshly isolated rat intestine were rinsed inside and out with Krebs Ringer and then tied off at one end. Then, 0.2 mL of Krebs Ringer solution containing 1.0 mg of peptide was added to the inside of each of the intestinal sections through a blunt intubation tube. The second end of the intestinal sections were tied off, and they were incubated at 37 °C in 5 mL of Krebs Ringer for 0.5 h. The sections were then transferred to fresh 5-mL solutions of Krebs Ringer and incubated at 37 °C. Additional transfers and incubations were made at 1, 2, and 2.5 h. At the end of the incubation period, the intestinal sections were cut up and extracted with Krebs Ringer (5.0 mL). Concentrations of intact peptides in each incubation solution were measured by HPLC peak integration of a 20-λ injection compared to 20 λ of a peptide solution (1.0 mg in 5.2 mL of Krebs Ringer). A 4.6 mm × 25 cm Vydac TP21854 column was used and eluted with 35% CH_3CN in H_2O + 0.1% TFA.

The leakiness of tied intestinal sections like those used in these assays was assessed by pouring a nonpenetrating high molecular weight blue dye into them and tying off the ends and incubating them in Krebs Ringer (5 mL) for 3 h. There was no penetration of dye into the Krebs Ringer during this time period.

Assay of 19 in Rat Stomach and Intestine 2.5 h following Oral Dosing. Peptide **19** (5.3 mg) was mixed with 0.25% methyl Cellosolve in H_2O (2.5 mL). A 1.5-mL portion of this mixture was given to each rat (starved 72 h) by intubation into the stomach. Control rats (starved 72 h) were given 1.5 mL of 0.25% methyl Cellosolve in H_2O. After 2.5 h, the stomach and upper and lower small intestine were removed and homogenized first in 0.1 M NaH_2PO_4 buffer (pH 8.0, 5.0 mL) and then in EtOH (5.0 mL). After centrifugation, each supernatant was examined by HPLC (described in rat intestinal penetration assay) compared to a standard solution of **19** to obtain a percent recovery value.

Acknowledgment. This research was supported by NIH Grant HL19538.

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Clofazimine Analogues Active against a Clofazimine-Resistant Organism

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Clofazimine analogues active against a strain of *Mycobacterium smegmatis* 607 made resistant to the antileprosy agent have been synthesized. Activity (i.e., ≤2 μg/mL causing complete inhibition of growth) requires that there be a basic nitrogen in the "rimino" side chain and that the spacer distance between this nitrogen and the imino nitrogen be at least three carbon atoms. The nitrogen may be primary, secondary, or tertiary and may be part of an open chain or enclosed in a ring compound. Provided that the criteria of basicity and spacer distance are satisfied, all are active in vitro against both the sensitive and resistant strains. Substitution elsewhere in the molecule had little effect on the activity. The compounds have been shown to have growth inhibitory activity against human-derived *Mycobacterium leprae* in murine macrophages in culture.

Resistance to antileprosy agents such as rifampicin and dapsone has already been established,[1-5] and more recently, a case of clofazimine-resistant leprosy has been reported.[6] Clofazimine originated in the MRC Laboratories, and we have since 1977, under the aegis of WHO, been seeking clofazimine analogues that would be active against clof-

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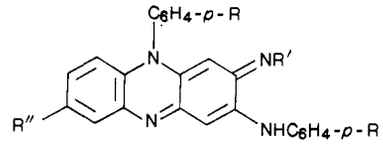
Table I. Activity in Vitro versus *M. smegmatis* 607 of Phenazine Compounds[a]



no.	R′	formula	mp, °C	yield, %	concn causing complete inhibn of growth,[b] μg/mL A	B	C
R = Cl, R″ = H							
1	H	$C_{24}H_{16}N_4Cl_2$	c		3.0	3.0	40.0
2	CH_3	$C_{25}H_{18}N_4Cl_2$	*d*		2.0	2.0	100.0
3	C_2H_5	$C_{26}H_{20}N_4Cl_2$	*d*		2.0	2.0	100.0
4	$(CH_2)_2CH_3$	$C_{27}H_{22}N_4Cl_2$	*d*		3.0	3.0	100.0
5	$CH(CH_3)_2$	$C_{27}H_{22}N_4Cl_2$	*e*		1.5	0.5	30.0
6	$(CH_2)_3CH_3$	$C_{28}H_{24}N_4Cl_2$	*d*		3.0	3.0	100.0
7	$CH_2CH(CH_3)_2$	$C_{28}H_{24}N_4Cl_2$	*d*		4.0	4.0	100.0
8	$(CH_2)_2CH(CH_3)_2$	$C_{29}H_{26}N_4Cl_2$	168 dec	52	2.0	3.0	80.0
9	$CH(CH_3)CH(CH_3)_2$	$C_{29}H_{26}N_4Cl_2$	219 dec	51	3.0	4.0	30.0
10	$CH_2CH(OCH_3)_2$	$C_{28}H_{24}N_4Cl_2O_2$	170 dec	54	4.0	5.0	60.0
11	c-C_5H_9	$C_{29}H_{24}N_4Cl_2$	*g*		3.0	3.0	20.0
12	c-C_6H_{11}	$C_{30}H_{26}N_4Cl_2$	*e*		2.0	1.0	20.0
13	$CH(CH_2)_2CH(CH_3)CH_2CH_2$	$C_{31}H_{28}N_4Cl_2$	*d*		7.5	7.5	25.0
14	c-C_7H_{13}	$C_{31}H_{28}N_4Cl_2$	*d*		2.0	3.0	50.0
15	$CH_2C_6H_{11}$-c	$C_{31}H_{28}N_4Cl_2$	143 dec	70	3.0	3.0	100.0
16	$(CH_2)_2OH$	$C_{26}H_{20}N_4Cl_2O$	*d*		2.0	2.0	20.0
17	$(CH_2)_3OH$	$C_{27}H_{22}N_4Cl_2O$	*g*		2.0	2.0	10.0
18	$CH(CH_2)_2CH(OH)CH_2CH_2$	$C_{30}H_{26}N_4Cl_2O$	228 dec	54	2.0	4.0	30.0
19	$(CH_2)_2NH(CH_2)_2OH$	$C_{28}H_{25}N_5Cl_2O$	*g*		8.0	8.0	20.0
20	$CH(C_2H_5)CH_2OH$	$C_{28}H_{24}N_4Cl_2O$	234 dec	64	2.0	10.0	100.0
21	$(CH_2)_2N(C_2H_5)_2$	$C_{30}H_{29}N_5Cl_2$	*d*		0.6	0.6	12.0
22	$CH(CH_3)CH_2N(CH_3)_2$	$C_{29}H_{27}N_5Cl_2$	156 dec	51	0.8	1.2	16.0
23	$CH_2CH(CH_3)N(CH_3)_2$	$C_{29}H_{27}N_5Cl_2$	92 dec	45	1.2	6.0	16.0
24	$(CH_2)_2N(CH_2)_2OCH_2CH_2$	$C_{30}H_{27}N_5Cl_2O$	184 dec	58	1.0	1.0	20.0
25	$(CH_2)_2N(CH_2)_3CH_2$	$C_{30}H_{27}N_5Cl_2$	150 dec	56	0.5	0.5	10.0
26	$(CH_2)_2N(CH_2)_2NHCH_2CH_2$	$C_{30}H_{28}N_6Cl_2$	*g*		0.2	0.2	0.8
27	$CHCH_2N(C_2H_5)(CH_2)_2CH_2$	$C_{31}H_{29}N_5Cl_2$	111 dec	60	0.5	0.5	8.0
28	$(CH_2)_2N(CH_2)_4CH_2$	$C_{31}H_{29}N_5Cl_2$	147 dec	56	0.4	0.5	10.0
29	$CH_2CHN(C_2H_5)(CH_2)_2CH_2$	$C_{31}H_{29}N_5Cl_2$	149 dec	60	1.0	1.0	10.0
30	$(CH_2)_2N(CH_2)_3C{=}O$	$C_{30}H_{25}N_5Cl_2O$	183 dec	50	1.0	1.6	20.0
31	$(CH_2)_3NH_2$	$C_{27}H_{23}N_5Cl_2$	118 dec	62	0.3	0.4	1.0
32	$(CH_2)_3N(C_2H_5)_2$	$C_{31}H_{31}N_5Cl_2$	*g*		0.2	0.2	1.0
33	$(CH_2)_3N(CH_2)_3CH_2$	$C_{31}H_{29}N_5Cl_2$	156 dec	75	0.3	0.3	0.8
34	$(CH_2)_3N(CH_2)_3C{=}O$	$C_{31}H_{27}N_5Cl_2O$	180 dec	75	2.0	2.0	20.0
35	$(CH_2)_3N(CH_2)_4CH_2$	$C_{32}H_{31}N_5Cl_2$	172 dec	75	0.2	0.3	2.0
36	$(CH_2)_3NHCH_3$	$C_{28}H_{25}N_5Cl_2$	126 dec	75	0.2	0.3	0.6
37	$(CH_2)_3NHC_2H_5$	$C_{29}H_{27}N_5Cl_2$	151 dec	61	0.2	0.2	1.0
38	$(CH_2)_3NH(CH_2)_2CH_3$	$C_{30}H_{29}N_5Cl_2$	135 dec	53	0.2	0.2	2.0
39	$(CH_2)_3NHCH(CH_3)_2$	$C_{30}H_{29}N_5Cl_2$	141 dec	51	0.3	0.2	2.0
40	$(CH_2)_3NHCH(CH_2)_4CH_2$	$C_{33}H_{33}N_5Cl_2$	160 dec	53	0.2	0.2	1.0
41	$(CH_2)_3N(CH_2)_2OCH_2CH_2$	$C_{31}H_{29}N_5Cl_2O$	164 dec	68	0.5	0.4	12.0
42	$(CH_2)_3N(CH_2)_2N((CH_2)_3OH)CHCH_2$	$C_{34}H_{36}N_6Cl_2O$	95 dec	50	0.6	0.5	2.0
43	$(CH_2)_3N(CH_2)_2N(CH_3)CH_2CH_2$	$C_{32}H_{32}N_6Cl_2$	178 dec	64	0.3	0.3	1.0
44	$(CH_2)_3N(CH_2)_4CH(CH_3)$	$C_{33}H_{33}N_5Cl_2$	78 dec	50	0.2	0.2	2.0
45	$(CH_2)_3N((CH_2)_2OH)_2$	$C_{31}H_{31}N_5Cl_2O_2$	170 dec	53	0.6	1.0	3.0
46	$(CH_2)_3N((CH_2)_3CH_3)_2$	$C_{35}H_{39}N_5Cl_2$	*g*		0.4	0.8	12.0
47*	$CHCH_2C(CH_3)_2NHC(CH_3)_2CH_2$	$C_{33}H_{33}N_5Cl_2$	240 dec	65	0.2	0.5	2.0
48	$(CH_2)_4NH_2$	$C_{28}H_{25}N_5Cl_2$	145 dec	71	0.2	0.3	0.5
49	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	$C_{33}H_{35}N_5Cl_2$	107 dec	62	0.2	0.2	2.0
50	$CH_2CH(CH_2)_2NHCH_2CH_2$	$C_{30}H_{27}N_5Cl_2$	186 dec	70	0.2	0.2	0.6
51	$(CH_2)_5NH_2$	$C_{29}H_{27}N_5Cl_2$	153 dec	71	0.2	0.3	0.5
52	$(CH_2)_6NH_2$	$C_{30}H_{29}N_5Cl_2$	152	71	0.3	0.8	2.0
R = R″ = H							
53	H	$C_{24}H_{18}N_4$	*h*		3.0	8.0	12.0
54	C_2H_5	$C_{26}H_{22}N_4$	*d*		3.0	3.0	20.0
55	$(CH_2)_2CH_3$	$C_{27}H_{24}N_4$	*d*		2.0	3.0	20.0
56	$CH(CH_3)_2$	$C_{27}H_{24}N_4$	*e*		2.0	3.0	100 ppt[i]

Table I (Continued)

no.	R′	formula	mp, °C	yield, %	concn, causing complete inhibn of growth,[b] μg/mL A	B	C
57	$(CH_2)_3CH_3$	$C_{28}H_{26}N_4$	*d*		3.0	3.0	20.0
58	$CH_2CH(CH_3)_2$	$C_{28}H_{26}N_4$	*d*		2.0	3.0	20.0
59	$(CH_2)_2CH(CH_3)_2$	$C_{29}H_{28}N_4$	136 dec	55	2.0	2.0	20.0
60	$CH_2CH(OCH_3)_2$	$C_{28}H_{26}N_4O_2$	145 dec	50	4.0	4.0	100 ppt
61	c-C_5H_9	$C_{29}H_{26}N_4$	201 dec	52	3.0	3.0	100 ppt
62	c-C_6H_{11}	$C_{30}H_{28}N_4$	*e*		3.0	2.0	10.0
63	$CH(CH_2)_2CH(CH_3)CH_2CH_2$	$C_{31}H_{30}N_4$	*d*		6.0	8.0	100 ppt
64	c-C_7H_{13}	$C_{31}H_{30}N_4$	*d*		2.0	2.0	20.0
65	$CH_2C_6H_{11}$-c	$C_{31}H_{30}N_4$	161 dec	80	2.0	2.0	100 ppt
66	$(CH_2)_2OH$	$C_{26}H_{22}N_4O$	*d*		3.0	5.0	20.0
67	$(CH_2)_3OH$	$C_{27}H_{24}N_4O$	*g*		4.0	8.0	20.0
68	$(CH_2)_2NH(CH_2)_2OH$	$C_{28}H_{27}N_5O$	*g*		5.0	6.0	10.0
69	$CH(C_2H_5)CH_2OH$	$C_{28}H_{26}N_4O$	232 dec	70	2.0	30 ppt	100 ppt
70	$(CH_2)_2N(C_2H_5)_2$	$C_{30}H_{31}N_5$	*d*		0.6	0.8	6.0
71	$(CH_2)_2N(CH_2)_2OCH_2CH_2$	$C_{30}H_{29}N_5O$	138 dec	54	2.0	2.0	10.0
72	$(CH_2)_2N(CH_2)_3CH_2$	$C_{30}H_{29}N_5$	161 dec	54	0.4	0.4	2.0
73	$CH(CH_2)_3N(C_2H_5)CH_2$	$C_{31}H_{31}N_5$	166 dec	63	0.4	0.6	6.0
74	$(CH_2)_2N(CH_2)_2NHCH_2CH_2$	$C_{30}H_{30}N_6$	*g*		0.4	0.4	1.0
75	$(CH_2)_2N(CH_2)_4CH_2$	$C_{31}H_{31}N_5$	127 dec	45	0.4	0.4	2.0
76	$CH_2CH(CH_2)_3NC_2H_5$	$C_{31}H_{31}N_5$	146 dec	56	0.5	0.4	6.0
77	$(CH_2)_3NHCH(CH_2)_4CH_2$	$C_{33}H_{35}N_5$	119 dec	63	0.2	0.2	0.6
78	$(CH_2)_3N(CH_2)_4CHCH_3$	$C_{33}H_{35}N_5$	126 dec	70	0.3	0.3	2.0
79	$(CH_2)_3N(CH_2)_2OCH_2CH_2$	$C_{31}H_{31}N_5O$	133 dec	51	1.0	0.8	6.0
80	$(CH_2)_3N(CH_2)_2N(CH_3)CH_2CH_2$	$C_{32}H_{34}N_6$	118 dec	50	0.5	0.5	1.0
81	$(CH_2)_3N(CH_2)_4CH_2$	$C_{32}H_{33}N_5$	118 dec	50	0.3	0.3	1.0
82	$CH_2CH(CH_2)_2NHCH_2CH_2$	$C_{30}H_{29}N_5$	156 dec	53	0.3	0.3	0.6
83	$CH(CH_3)(CH_2)_3N(C_2H_5)_2$	$C_{33}H_{37}N_5$	98 dec	25	0.3	0.4	2.0
84	$(CH_2)_3N(CH_2)_3CH_2$	$C_{31}H_{31}N_5$	122 dec	57	0.2	0.2	0.6
85	$CH_2CH(CH_3)N(CH_3)_2$	$C_{29}H_{29}N_5$	128 dec	45	0.8	1.0	4.0
R = H, R″ = Cl							
86	$(CH_2)_3CH_3$	$C_{28}H_{25}N_4Cl$	148 dec	56	2.0	3.0	100 ppt
87	$CH_2CH(CH_3)_2$	$C_{28}H_{25}N_4Cl$	143 dec	66	2.0	3.0	100 ppt
88	$(CH_2)_2CH(CH_3)_2$	$C_{29}H_{27}N_4Cl$	154 dec	50	3.0	3.0	100 ppt
89	$(CH_2)_2OH$	$C_{26}H_{21}N_4ClO$	194 dec	50	2.0	4.0	100 ppt
90	$(CH_2)_2NH(CH_2)_2OH$	$C_{28}H_{26}N_5ClO$	144 dec	51	5.0	10.0	20.0
91	$(CH_2)_2N(C_2H_5)_2$	$C_{30}H_{30}N_5Cl$	132 dec	50	1.0	2.0	30.0
92	c-C_6H_{11}	$C_{30}H_{27}N_4Cl$	*j*		3.0	0.6	20.0
93	$CH_2CH(CH_2)_2NHCH_2CH_2$	$C_{30}H_{23}N_5Cl$	168 dec	68	0.2	0.4	1.0
94	$(CH_2)_2N(CH_2)_2OCH_2CH_2$	$C_{30}H_{28}N_5ClO$	181 dec	50	1.0	1.5	50 ppt
R = CH_3, R″ = H							
95	$CH_2CH(CH_3)_2$	$C_{30}H_{30}N_4$	167	50	2.0	3.0	100 ppt
96	c-C_6H_{11}	$C_{32}H_{32}N_4$	235 dec	50	8.0	8.0	100 ppt
97	$(CH_2)_2N(C_2H_5)_2$	$C_{32}H_{35}N_5$	148 dec	60	0.5	0.5	3.0
98	$(CH_2)_2OH$	$C_{28}H_{26}N_4O$	204 dec	54	4.0	8.0	12.0
99	$CH_2CH(CH_2)_2NHCH_2CH_2$	$C_{32}H_{33}N_5$	168 dec	50	0.2	0.3	0.4
100	$(CH_2)_3NHCH(CH_2)_4CH_2$	$C_{35}H_{39}N_5$	128 dec	50	0.2	0.2	0.5
R = OC_2H_5, R″ = H							
101	H·HCl	$C_{28}H_{26}N_4O_2$·HCl	300 dec	90	6.0	50 ppt	100 ppt
102	$CH_2CH(CH_3)_2$	$C_{32}H_{34}N_4O_2$	177 dec	50	4.0	4.0	100 ppt
103	$(CH_2)_2OH$	$C_{30}H_{30}N_4O_3$	195 dec	53	5.0	10.0	20.0
104	$(CH_2)_2N(C_2H_5)_2$	$C_{34}H_{39}N_5O_2$	153 dec	49	1.0	1.0	5.0

[a] All new compounds were analyzed for C, H, N, and, where present, Cl. Satisfactory analyses were obtained for all the new agents. Note, however, (**31**) N calcd 14.3, found 13.7; (**36**) N calcd 13.1, found 13.6; (**42**) H calcd 5.9, found 5.3; (**44**) C calcd 69.5, found 69.0; and (81) C calcd 78.9, found 78.3. [b] A, dapsone sensitive; B, rifampicin resistant; C, clofazimine resistant. [c] Reference 11. [d] Reference 8. [e] Reference 12. [f] Clofazimine. [g] Reference 9. [h] Reference 13. [i] ppt = precipitation. [j] Reference 15. [k] * = DMF used as solvent.

azimine-resistant organisms. We have used a *Mycobacterium smegmatis* 607 strain made resistant to the anti-leprosy agent for in vitro screening tests.

The compounds prepared and tested have been combined into a single table (Table I). The method of preparation is shown in Scheme I. In general, the imi-

(3) Pettit, J. H. S.; Rees, R. J. W. *Lancet* **1964**, 673.
(4) Pettit, J. H. S.; Rees, R. J. W.; Ridley, D. S. *Int. J. Lepr. Other Microbact. Dis.* **1966**, *34*, 375.
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(6) Warndorff-van Diepen, T. *Int. J. Lepr. Other Microbact. Dis.* **1982**, *50*, 139.

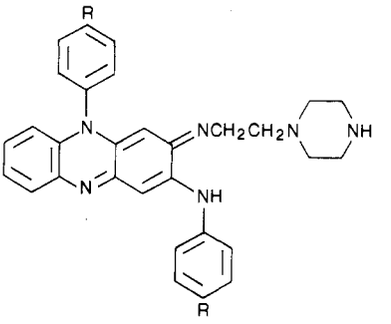
nophenazines (III) were obtained by the ferric chloride oxidation of the appropriate 2-aminodiphenylamine hydrochloride as recently described.[7] Subsequent treatment with the required amine gave the "rimino" compounds (IV)[8,9] (i.e., a substituent other than hydrogen on the imino nitrogen). Dioxane was the solvent of choice used in the condensation, but in the case of the 2-amino-5-(diethylamino)pentane and the 3-amino-*N*-ethylpiperidine, no solvent was used, the mixture being heated at the boiling point for 4–5 min. Thin-layer chromatography was used to verify the purity of each compound. No attempt was made to optimize yields which ranged from 25% to 80% of theory.

The crystal and molecular structures of two modifications of clofazimine and those of the inactive isomeric compound (i.e., the groups on the nitrogens in the 2- and 3-positions of the phenazine nucleus are interchanged) have been determined by Hodgson and his co-workers,[10] using samples prepared by us during the period of this study. The results confirm earlier conclusions regarding the structures.[11-13]

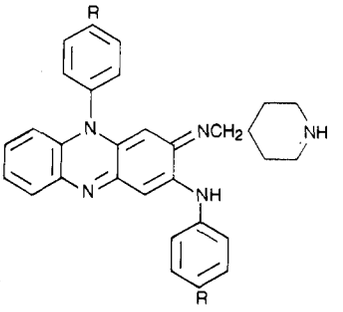
The isomers of the analogues are not obtained by the synthetic procedures described here. The isomer of III is formed during the oxidation of II to III and comprises about 5% of the products. Chromatography on silica gel readily separates the two bases. Isoclofazimine is then prepared by the reduction of the isomer of III in acetone. Clofazimine and isoclofazimine when mixed are readily separated by chromatography.

Clofazimine (**5**) completely inhibited growth of the sensitive strain at 1.5 μg/mL while the inhibition of the resistant strain required 30 μg/mL. Therefore, compounds completely inhibiting growth of the resistant strain at 2 μg/mL or less are considered active.

Straightforward alkyl derivatives, whether consisting of straight-chain, branched-chain, or ring compounds, were fully cross resistant with clofazimine when tested against the resistant strain. The introduction of a nitrogen into the side chain as in compounds **21** and **70**, the (diethylamino)ethyl derivatives with and without a chlorine substituent on the para position of the peripheral phenyl groups, showed some movement in the required direction, inhibiting the test strain at 12 and 6 μg/mL, respectively. Ring closure of one of the ethyl groups through an additional carbon atom to one of the spacer carbons as in agents **27**, **29**, and **73**, **76** did not result in compounds with any greater activity against the resistant strain, nor was there any reduction in the activity noted. When the two ethyl groups were ring closed to give the pyrrolidine compounds **25** and **72**, or with inclusion of an additional carbon to give the piperidine derivatives **28** and **75**, the unchlorinated pair **72** and **75** were active (i.e., caused total inhibition at 2 μg/mL). Ring closure of the two ethyl groups through an additional nitrogen to give the piperazine compounds **26** and **74** yielded agents active at 0.8 and 1.0 μg/mL, respectively. Ring closure through an oxygen



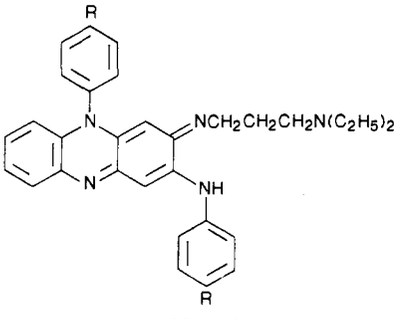
26: R=Cl
74: R=H



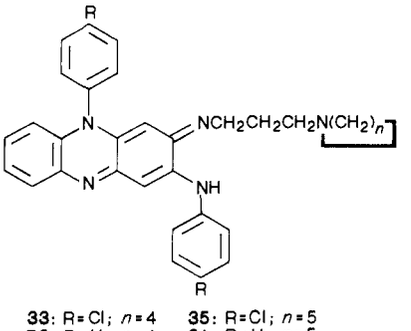
50: R=Cl
82: R=H

atom to yield the morpholino derivatives **24** and **71** proved disadvantageous.

The piperazine compounds **26** and **74** have one nitrogen at a spacer distance of two from the imino nitrogen while the second nitrogen is at a spacer distance of five from the reference atom. accordingly it was of interest to synthesize the piperidine derivatives **50** and **82** by condensing 4-(aminomethyl)piperidine with the parent phenazines (**1** and **53**). The result was most satisfactory, both compounds being active at 0.6 μg/mL. The (diethylamino)propyl derivative **32**, which has a three-carbon spacer, also proved active at 1.0 μg/mL.



32: R=Cl



33: R=Cl; *n*=4 **35**: R=Cl; *n*=5
72: R=H; *n*=4 **81**: R=H; *n*=5

Ring closure of the ethyl groups on the last-mentioned compound to give a five-membered ring or including an additional carbon to give a six-membered ring also yielded active derivatives. The five-membered ring agents **33** and

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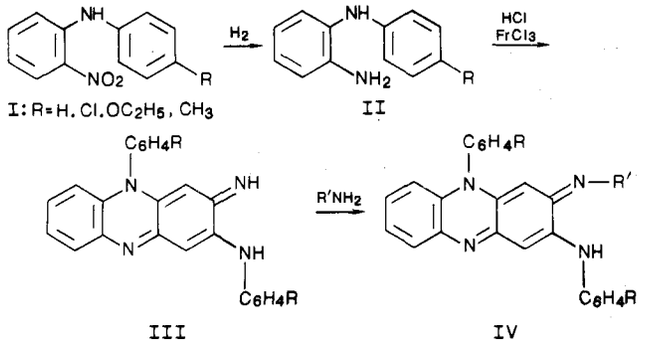
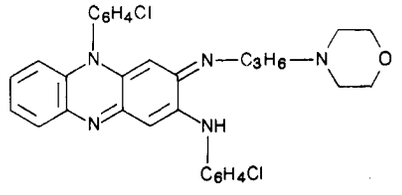
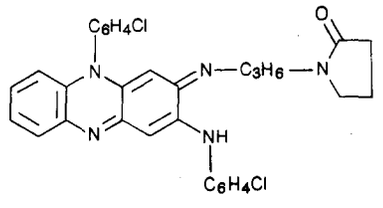
Scheme I



72 were active at 0.8 and 2.0 μg/mL, respectively, and the six-membered ones 35 and 81 were active at 2.0 and 1.0 μg/mL, respectively. Once more, ring closure through an oxygen atom (41 and 71) reduced the activities. As the



41



34

oxygen would reduce the basicity of the nitrogen, we next looked at a compound (34) where the basicity is nullified, the nitrogen being bound in an amide link. The result was a derivative inactive against the resistant strain.

Ring closure of the two ethyl groups on the nitrogen through an additional nitrogen atom did not greatly effect the activity, with the ring compounds 43 and 80 showing activity at 1.0 μg/mL. Spacer distances of four (i.e., 48, 49, 83), five (i.e., 51), and six (i.e., 52) also gave active derivatives.

The results show that the desired activity requires that a basic nitrogen be present in the side chain on the imino nitrogen. Additionally, it is important that the spacer distance between this nitrogen and the imino nitrogen be at least three carbon atoms. Compounds with spacer distances of four, five, and six carbons are also active. The nitrogen may be primary, secondary, or tertiary and may be part of an open chain or enclosed in a ring compound. Provided that the other criteria of basicity and spacer distance are satisfied, all are active in vitro against both the sensitive and the resistant strain. Substitution on the para position of the peripheral phenyl groups had little effect on the in vitro activity.

When tested against *Mycobacterium leprae* in murine macrophages in culture, the materials showed growth inhibiting activity.[14] In general, the analogues exerted levels of inhibition similar to that of clofazimine at 10 and 100 ng/mL. The compounds, therefore, can reach phagocytosed *M. leprae*. In order to determine which compounds we should submit to the mouse footpad test, we are currently investigating the absorption of a number of them when given to mice by gavage.

Experimental Section

Chemistry. Melting points were determined on a Gallenkamp capillary melting point apparatus and are uncorrected. TLC was carried out with Merck silica gel (Art. 7733) and developed with 5–10% methanol in chloroform. Microanalyses were performed by the microanalysis laboratories of May and Baker Ltd., Essex, England.

3-(4-Chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-[(4-piperidinylmethyl)imino]phenazine (50). The hydrochloride of 1[7] (5 g, 11 mmol) was suspended in dioxane (50 mL), and 4-(aminomethyl)piperidine (8 mL, 70 mmol) was added. The mixture was heated under reflux for 5 h. The solution was filtered, and the flask was washed out with ethanol (30 mL). The filtrate was diluted with water until just cloudy and kept at room temperature overnight. The product was filtered off, washed with 50% aqueous ethanol, and dried, mp 184 °C dec (yield 70%–80%). Following recrystallization from methanol/chloroform, it melted at 186–188 °C dec. Anal. ($C_{30}H_{27}Cl_2$) C, H, N, Cl.

3-(4-Chloroanilino)-10-(4-chlorophenyl)-2-[[4-(diethylamino)-1-methylbutyl]imino]-2,10-dihydrophenazine (49). The hydrochloride of (21 g, 4.2 mmol) and 2-amino-5-(diethylamino)pentane (8 mL, 80 mmol) were heated at the boiling point for 3–4 min. When the mixture was cool, alcohol (40 mL) was added, and the mixture was heated to the boiling point and filtered. The filtrate was diluted with 50% aqueous ethanol until cloudy. The product gradually separated as glistening bronze crystals, which were washed with 50% aqueous ethanol to yield 2.4 g (58%), mp 104 °C dec. Following recrystallization from dioxane/ethanol/water, it melted at 107 °C dec. Anal. ($C_{33}H_{35}N_5Cl_2$) C, H, N, Cl.

Growth Inhibition Assay. Growth inhibition assays were carried out with a 20× step up clofazimine-resistant mutant isolated from *M. smegmatis* ATCC607. This strain is sensitive to dapsone.

Assays were carried out in modified Kirchners medium (5 mL) containing 0.5% Tween 80 inoculated with 10^5 viable cells. Drugs were added in dimethyl sulfoxide (DMSO), and the final concentration of DMSO in all tubes was 2%. The cells were incubated in roller tubes at 37 °C for 72 h. Transmission measurements were made in a Coleman Junior Model spectrophotometer at 550 μm. Three strains were used in the test: A, dapsone sensitive; B, rifampicin resistant; and C, clofazimine resistant. Duplicate determinations were made.

Acknowledgment. We thank S. Bance, May and Baker Ltd., for microanalyses, and Deirdre Conlon for production of the formulas. This investigation received financial support from the UNDP/World Bank/WHO Special program for Research and Training in Tropical Diseases.

Registry No. 1, 102262-55-5; 1·HCl, 90690-85-0; 2, 102884-69-5; 3, 103051-26-9; 4, 23395-94-0; 5, 2030-63-9; 6, 103280-89-3; 7, 103280-91-7; 8, 103839-61-8; 9, 111435-84-8; 10, 111435-85-9; 11, 96870-19-8; 12, 14395-92-7; 13, 111554-24-6; 14, 15012-26-7; 15, 111435-86-0; 16, 108247-75-2; 17, 102263-10-5; 18, 111435-87-1; 19, 103369-40-0; 20, 103839-60-7; 21, 78182-93-1; 22, 111435-88-2; 23, 111435-89-3; 24, 111435-90-6; 25, 111435-91-7; 26, 111435-93-9; 28, 111435-94-0; 29, 111468-54-3; 30, 111435-95-1; 31, 111435-96-2; 32, 98270-76-9; 33, 111435-97-3; 34, 111468-27-0; 35, 111435-98-4; 36, 111435-99-5; 37, 111436-00-1; 38, 111436-01-2; 39, 111436-02-3; 40, 111436-03-4; 41, 111436-04-5; 42, 111436-05-6; 43, 111436-06-7; 44, 111436-07-8; 45, 111436-08-9; 46, 96675-95-5; 47, 111436-09-0; 48, 111436-10-3; 49, 111436-11-4; 50, 111436-12-5; 51, 111436-13-6; 52, 111436-14-7; 53, 4733-21-5; 54, 102876-06-2; 55, 111436-15-8; 56, 90690-82-7; 57, 103098-35-7; 58, 78182-94-2; 59, 111436-16-9; 60, 111436-17-0; 61, 98332-53-7; 62, 78182-92-0; 63, 111436-18-1; 64, 103278-58-6; 65, 111436-19-2; 66, 111436-20-5; 67, 102345-87-9; 68, 103514-07-4; 69, 111436-21-6; 70, 103276-37-5; 71, 111436-22-7; 72, 111436-23-8; 73, 111436-24-9; 74, 111436-25-0; 75, 111436-26-1; 76, 111436-27-2; 77, 111468-55-4; 78, 111436-28-3; 79, 111436-29-4; 80, 111436-30-7; 81, 111436-31-8; 82, 111436-32-9; 83, 96676-75-4;

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84, 111436-33-0; **85**, 111436-34-1; **86**, 111436-35-2; **87**, 111436-36-3; **88**, 111436-37-4; **89**, 111436-38-5; **90**, 111436-39-6; **91**, 111436-40-9; **92**, 24028-59-9; **93**, 111436-41-0; **94**, 111436-42-1; **95**, 111436-43-2; **96**, 111436-44-3; **97**, 111436-45-4; **98**, 111436-46-5; **99**, 111436-47-6; **100**, 111436-48-7; **101**, 111436-49-8; **101**·HCl, 111436-53-4; **102**, 111436-50-1; **103**, 111436-51-2; **104**, 111436-52-3; 4-(aminomethyl)piperidine, 7144-05-0; 2-amino-5-(diethylamino)pentane, 140-80-7.

Analogues of [(Triethylsilyl)ethynyl]estradiol as Potential Antifertility Agents

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Various 17α-ethynylsteroids were prepared and derivatized as the corresponding triethylsilyl compounds **2–35**, which were examined for a ratio of antifertility to estrogenic activity that would be more beneficial than that of the presently used agent. Among the triethylsilyl compounds evaluated, only **23** displayed this desired ratio, although two other compounds without the triethylsilyl moiety, **18** and **26**, shared similar characteristics.

We recently described a series of ethynylestradiol (EE) derivatives that featured a systematic variation of trialkylsilyl groups pendant to the ethynyl side chain.[1] The mere presence of silicon was proven to be surprisingly beneficial. Other investigators have assumed the existence of a direct relationship between antifertility and estrogenic activity,[2] but our silyl derivatives displayed a marked reduction in estrogenic activity, with retention of the level of oral antifertility activity. This latter trend is attractive in light of the endocrine disorders and other undesired side effects attributed to the estrogenic activity of prescribed contraceptives.[3] Although we had shown that some remarkable separation of estrogenic from antifertility activities as described above could be achieved by structural variations of the EE side chain, we had heretofore not examined modifications of the EE steroidal nucleus. We have now examined the effects of structural changes in the A, B, C, and D rings of EE while incorporating the C-21 triethylsilyl moiety (for example, see structures **2–35**, Table I).

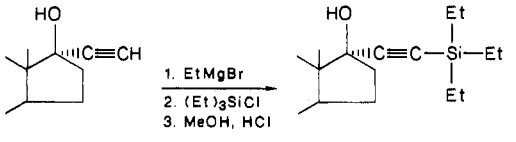
Chemistry Results

The desired analogues **2–35** were synthesized in a straightforward fashion: nuclear-modified ethynylestradiols **3–35** were synthesized from their respective ketones by standard ethynylation procedures. Upon treatment with ethylmagnesium bromide, each was converted to the corresponding acetylide anion, which was capped with chlorotriethylsilane (Scheme I, see the Experimental Section for synthetic details on particular compounds).

Biological Results

Table II shows the compounds, their corresponding antifertility (*A*) and estrogenic (*E*) potencies in rats relative to EE or at maximum dosage levels given, and the ratio of antifertility to estrogenic activity (*A*/*E*). For comparison, ethynylestradiol (EE, 1) and the unsilylated terminal

Scheme I



17α-acetylenes were examined and included in Table II.

In general, introduction of the triethylsilyl group onto the ethynylestrane derivatives resulted in a decrease of estrogenic activity when the initial estrogenicity of the parent ethynylestranes was greater than 2%. The only exceptions occurred in the 6α- and 11β-hydroxy derivatives (**13** and **27**, respectively), where the introduction of the triethylsilyl group showed an actual increase of 10%–20% in estrogenicity over that of the parent ethynylestrane. However, no parallels could be drawn concerning the antifertility activity.

Ring A substitution at position C-2 with a methoxyl or (di-*n*-propylamino)methyl functionality (**3** and **7**, respectively) drastically reduced both antifertility and estrogenic activity. A lipophilic methyl group retained significant antifertility activity and lost substantial estrogenicity. The presence of a C-4 allyl substituent (as in **8** and **9**) led to an essential void of activity. Antifertility and estrogenicity for the unsubstituted 3-desoxy EE analogue **10** were simultaneously reduced in magnitude. In contrast, the corresponding silylated 3-desoxy EE analogue **11** retained antifertility potency equal to that of EE, with only 13% of the estrogenicity of EE.

Examples with polar hydroxyl substituents on ring B did not exhibit enhanced antifertility activity unless the C-6α hydroxyl was accompanied by a C-17α (triethylsilyl)ethynyl moiety (for example, **12a** compared to **13**). Other types of ring B substituents were also examined—for example, the presence of a C-6β methyl group led to a substantial reduction in overall activity. Unfortunately, a similar lack of activity was observed for ring C and D substituted, triethylsilyl derivatives.

In summary, the best triethylsilyl derivative in Table I was the ring B substituted Δ^6 analogue **23**. Compound **23** had twice the potency of EE as an antifertility agent but only 2% of the estrogenic activity of EE. The 100-fold separation of estrogenic activity and antifertility activity for **23** relative to EE is the greatest that we have observed for any silylethynyl EE derivative.

Surprisingly, two of the intermediate ethynyl compounds, 11β-hydroxy EE (**26**) and 7α-hydroxy EE (**18**),

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(3) (a) Bengel, A. S.; Benoit, P. S. *J. Pharm. Sci.* **1973**, *62*, 179. (b) Population Reports, Series A, No. 2, March 1975. Published by Department of Medical and Public Affairs & George Washington University Medical Center, Washington, DC.

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