Synthesis of Potential Antimalarial Agents. V.[1] Pyrido[2,3-*b*]pyrazines

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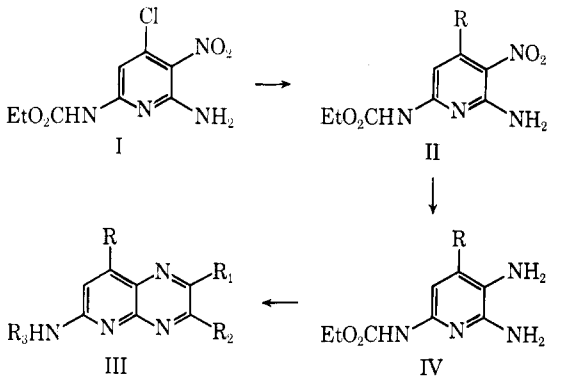
A series of 8-substituted 6-aminopyrido[2,3-*b*]pyrazines has been prepared for evaluation as antimalarial agents. Activity against the malaria caused by *Plasmodium berghei* in mice has been demonstrated for some compounds containing the 8-{[4-(diethylamino)-1-methylbutyl]amino} group.

The resistance of strains of *Plasmodium falciparum* to many of the known antimalarials prompted the preparation of new structure types that might be more effective agents. Previously we reported that 6-amino-3-(*p*-chlorophenyl)-8-{[4-(diethylamino)-1-methylbutyl]amino}pyrido[2,3-*b*]pyrazine (**47**) cured mice infected with the sensitive strain of *P. berghei*.[2] This paper reports a structure–activity study on a group of compounds containing the 6-aminopyrido[2,3-*b*]pyrazine moiety.

The 6-aminopyrido[2,3-*b*]pyrazines (**1–47**) were prepared by known procedures[2] (see Scheme I), or

SCHEME I



minor modifications thereof. In most of the condensation reactions of the 2,3-diaminopyridines (IV) with a phenylglyoxal, mixtures of the 2- and 3-phenyl isomers of III were obtained with the 3 isomer being the major product. Several mixtures were purified by column chromatography to give the pure 3 isomer, but usually the mixtures were submitted for testing. The isomer ratio in each mixture was determined by the pmr spectrum. The assignment of the 3 isomer as the major product is based on analogy with the major product obtained in the condensation of pyruvaldehyde with other 2,3-diaminopyridines.[3] In one case this assignment was confirmed by the synthesis of the 2-(*p*-chlorophenyl) isomer (**6**) of **5** by an unambiguous route. The properties of the new compounds are summarized in Table I and typical procedures are given in the Experimental Section.

Compounds were tested against lethal, blood-induced *P. berghei* infections in mice; test results are summarized in Table II.[4] These data showed that the 4-(diethylamino)-1-methylbutyl (noval) group in the 8 position was necessary for activity. The 8-chloro- (**1–4**), 8-(diphenylmethyl)amino- (**7–14**), 8-amino- (**15–19**), 8-(*p*-chloroanilino)- (**20**),[2] and 8-(*p*-aminomethyl)-benzenesulfonamide (**21**)[2] derivatives showed no significant activity. Note that the 6,8-diamino-3-*p*-chlorophenyl compound **16**, a potential antifol, is completely inactive. These observations suggested that the mechanism of action of **47**[2] containing the noval group might be similar to that of chloroquine. In fact the uv spectrum of **47** in citrate buffer is depressed and shifted to higher wavelength in the presence of calf thymus DNA. A similar observation with chloroquine is attributed to the complex formed between chloroquine and native DNA.[5]

Although the noval group was necessary for activity in these pyrido[2,3-*b*]pyrazines, the degree of activity was dependent on other groups in the molecule. In the 6-amino compounds activity was observed in the 3-phenyl derivatives, but not in the unsubstituted (**43**),[1] alkyl (**44**),[2] or 2,3-bis(*p*-chlorophenyl) (**45**)[2] derivatives. The Ph compound (**27**) itself gave an increase in mean survival time (MST) of 6.4 days at the nontoxic dose of 320 mg/kg. A greater increase in MST at the 320 mg/kg dose was observed with the *p*-F- (**35**), *p*-Cl- (**47**), 3,4-Cl_2- (**31**), and *p*-CF_3- (**40**) phenyl derivatives. The latter cured all mice at the 640 mg/kg dose. The *p*-MeO- (**23**) and 3,4,5-$(MeO)_3$ (**25**) phenyl derivatives were less active. Thus, by comparison of these results with that of the unsubstituted Ph compound (**27**) at the 3 dosage levels, activity increases as the ability of the substituted Ph group to withdraw electrons increases. The latter is based on the σ constant for the Ph substituent, which accounts for both the inductive and resonance effects of the group (compare **23**, **27**, **35**, **47**, **40**.). Exceptions to this trend occur between **35** and **47** at the 160 mg/kg dose and between **24** and **27** at the 320 mg/kg dose. Also, at the 640 mg/kg dose toxic deaths were observed for **43**, **27**, and **47**, but not for **40**, which suggests that **40** containing the strongest electron-withdrawing group ($+CF_3C_6H_4$) has the greatest specificity for *P. berghei*.

In contrast to the *para*-substituted Ph compounds

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(2) C. Temple, Jr., J. D. Rose, R. D. Elliott, and J. A. Montgomery, *J. Med. Chem.*, **11**, 1216 (1968).

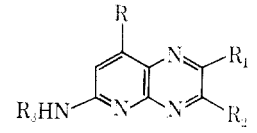
(3) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **33**, 2393 (1968).

(4) For a description of the test procedures, see T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967). NOTE ADDED IN PROOF: Compounds **33** and **42** were tested against the parent and chloroquine-resistant strains of *Plasmodium berghei* by P. Thompson and coworkers, University of Georgia, Athens, Ga. Compound **42** gave no significant activity, however, compound **33** gave 100% parasitemic suppression at 92 mg/kg per day against the parent strain and 94% parasitemic suppression at 95 mg/kg per day against the resistant strain.

(5) (a) F. E. Hahn, R. L. O'Brien, J. Ciak, J. L. Allison, and J. G. Olenick, *Mil. Med.*, **131**, *Suppl.*, 1071 (1966); (b) L. W. Blodgett and K. L. Yielding, *Biochim. Biophys. Acta*, **169**, 451 (1968).

TABLE I

PYRIDO[2,3-*b*]PRYAZINES



No.	Compd	Method	Reaction: Time, hr	Reaction: Temp, °C	Time, hr	Temp °C	Recrystn[a] solvent	Yield, %	Mp,[b] °C	Formula	Analyses
							R = Cl				
1	R_1 = H; R_2 = *p*-C_6H_4Cl; R_3 = CO_2Et	A	0.5	78	20	*c*	A[d]	47	259–261	$C_{16}H_{12}Cl_2N_4O_2$	C, H, Cl, N
2	R_1 = R_3 = H; R_2 = *p*-C_6H_4Cl	E	0.5	140			A[e,f]	80	>300[g]	$C_{13}H_8Cl_2N_4$	C, H, Cl, N
3	R_1 = R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = CO_2Et	B	60	78			A	33	206–208[g]	$C_{28}H_{28}ClN_4O_8$	C, H, Cl, N
4	R_1 = R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = H	C	6	78			A + B[h]	34	210–212[i]	$C_{25}H_{25}ClN_4O_6$	C, H, Cl, N
							R = $NHCH(C_6H_5)_2$				
5	R_1 = H; R_2 = *p*-C_6H_4Cl; R_3 = CO_2Et	B	18	*c*	2	50	A	58	215–217	$C_{29}H_{24}ClN_5O_2$	C, H, N
6	R_1 = *p*-C_6H_4Cl; R_2 = H; R_3 = CO_2Et	*j*					A	35	>300	$C_{29}H_{24}ClN_5O_2$	C, H, N
7	R_1 = H; R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = CO_2Et	A	0.5	*c*	0.5	78	A[d,h]	65	195–196	$C_{32}H_{31}N_5O_5$	C, H, N
8	R_1 = 3,4,5-$C_6H_2(OMe)_3$; R_2 = H; R_3 = CO_2Et	A	0.5	*c*	0.5	78	A[d]	10	210–212	$C_{32}H_{31}N_5O_5$	C, H, N
9	R_1 = R_3 = H; R_2 = 3,4,5-$C_6H_2(OMe)_3$	D	5	78			A + B	84	232–233[g]	$C_{29}H_{27}N_5O_3$	C, H, N
10	R_1 = R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = CO_2Et	A	23	78			A[d,f]	55	219–221[g]	$C_{41}H_{41}N_5O_8$	C, H, N
11	R_1 = R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = CO_2Bu	A	20	118			C[f]	38	219–220	$C_{43}H_{45}N_5O_8$	C, H, N
12	R_1 = R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = H	D	6	78			A[e,f]	87	238–240[g]	$C_{36}H_{37}N_5O_6{}^k$	C, H, N
13	R_1 = H; R_2 = OH; R_3 = CO_2Et	*j*					A	52	210–212	$C_{25}H_{25}N_5O_5$	C, H, N
14	R_1 = OH; R_2 = H; R_3 = CO_2Et	*j*					A[l]	10	275–277[g]	$C_{23}H_{21}N_5O_5$	C, H, N
							R = NH_2				
15	R_1 = H; R_2 = *p*-C_6H_4Cl; R_3 = CO_2Et	F	20	*c*			A[e,f]	88	>340	$C_{16}H_{14}ClN_5O_2$	C, H, Cl, N
16	R_1 = R_3 = H; R_2 = *p*-C_6H_4Cl	C	18	78			D[f]	83	>360	$C_{13}H_{10}ClN_5 \cdot HCl$	C, H, Cl, N
17	R_1 = H; R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = CO_2Et	F	4	*c*			*d*	30	295[g]	$C_{18}H_{21}N_5O_5$	C, H, N
18	R_1 = R_3 = H; R_2 = 3,4,5-$C_6H_2(OMe)_3$	G	48	*c*			A[e,h]	67	271–272[i]	$C_{16}H_{17}N_5O_3 \cdot HCl$	C, H, Cl, N
19	R_1 = R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = H	G	3	*c*			A[e,f]	81	*l*	$C_{25}H_{27}N_5O_6 \cdot HCl$	C, H, Cl, N
							CH_3 | R = $NHCH(CH_2)_3NEt_2$				
22	R_1 = H; R_2 = *p*-C_6H_4OMe; R_3 = CO_2Et	B	1	*c*	1	78	A + E[f]	76	210–211[g]	$C_{29}H_{39}N_6O_3 \cdot HCl$	C, H, Cl, N
23	R_1 = R_3 = H; R_2 = *p*-C_6H_4OMe	C	12	78			A + E[f]	94	241–242[g]	$C_{28}H_{32}N_6O \cdot 2HCl{}^m$	C, H, Cl, N
24[n]	R_1 = H; R_2 = 3,4,5-$C_6H_2(OMe)_3$; R_3 = CO_2Et	B	2	78			F + E[f]	92	*l*	$C_{28}H_{40}N_6O_5 \cdot 2HCl$	C, H, Cl, N
25[n]	R_1 = R_3 = H; R_2 = 3,4,5-$C_6H_2(OMe)_3$	C	8	78			F + E[f]	95	*l*	$C_{35}H_{36}N_6O_3 \cdot 2HCl$	C, H, Cl, N
26[o]	R_1 = H; R_2 = C_6H_5; R_3 = CO_2Et	B	1	*c*	1	78	F + E[f]	94	185–187[g]	$C_{32}H_{34}N_6O_2 \cdot 2HCl$	C, H, Cl, N
27[o]	R_1 = R_3 = H; R_2 = C_6H_5	C	7	78			F + E[f]	95	201–203[i]	$C_{32}H_{30}N_6 \cdot 2HCl$	C, H, Cl, N
28[o]	R_1 = H; R_2 = *o*-C_6H_4Cl; R_3 = CO_2Et	B	18	*c*	3	78	F[f,q]	78	*l*	$C_{25}H_{33}ClN_6O_2 \cdot 2HCl$	C, H, Cl, N

TABLE I (*Continued*)

No.	Compd	Method	Reaction Time, hr	Reaction Temp, °C	Time, hr	Temp °C	Recrystn[a] solvent	Yield, %	Mp,[b] °C	Formula	Analyses
29[o]	$R_1 = R_3 = H$; $R_2 = o\text{-}C_6H_4Cl$	C	7	78			F + E[f,q]	86	*l*	$C_{22}H_{29}ClN_6 \cdot 2HCl$	C, H, Cl, N
30[n]	$R_1 = H$; $R_2 = 3,4\text{-}C_6H_3Cl_2$; $R_3 = CO_2Et$	B	1.5	78			F + E[f]	75	162–163[g]	$C_{25}H_{32}Cl_2N_6O_2 \cdot 2HCl$	C, H, Cl, N
31[n]	$R_1 = R_3 = H$; $R_2 = 3,4\text{-}C_6H_3Cl_2$	C	7	78			F + E[f]	99	*l*	$C_{22}H_{28}Cl_2N_6 \cdot 2HCl$	C, H, Cl, N
32[o]	$R_1 = H$; $R_2 = p\text{-}C_6H_4Br$; $R_3 = CO_2Et$	B	18	*c*	0.5	78	A + E[f]	86	151–153	$C_{25}H_{33}BrN_6O_2 \cdot HCl$[m]	C, H, Br, Cl, N
33	$R_1 = R_3 = H$; $R_2 = p\text{-}C_6H_4Br$	D	12	78			F + E[f]	80	232–235[g,i]	$C_{22}H_{29}BrN_6 \cdot 2HCl$	C, H, Br, Cl, N
34[o]	$R_1 = H$; $R_2 = p\text{-}C_6H_4F$; $R_3 = CO_2Et$	B	72	*c*	2	78	F + E[h]	79	168–169	$C_{25}H_{33}FN_6O_2 \cdot 2HCl$[m]	C, H, Cl, N
35[o]	$R_1 = R_3 = H$; $R_2 = p\text{-}C_6H_4F$	C	6	78			F + E[f]	91	263–265[g]	$C_{22}H_{29}FN_6 \cdot 2HCl$	C, H, Cl, N
36	$R_1 = o\text{-}C_6H_4CF_3$; $R_2 = H$; $R_3 = CO_2Et$	A	3	*c*	2	78	F + E[d,f]	3	122–124[i]	$C_{26}H_{33}F_3N_6O_2 \cdot 2HCl$	C, H, Cl, N
37[p]	$R_1 = H$; $R_2 = o\text{-}C_6H_4CF_3$; $R_3 = CO_2Et$	A	3	*c*	2	78	D + G[q]	85	*l*	$C_{26}H_{33}F_3N_6O_2$	C, H, N
38[p]	$R_1 = R_3 = H$; $R_2 = o\text{-}C_6H_4CF_3$	C	7	78			A[q]	75	*l*	$C_{23}H_{29}F_3N_6$	C, H, F, N
39	$R_1 = H$; $R_2 = p\text{-}C_6H_4CF_3$; $R_3 = CO_2Et$	B	72	*c*	2	78	A + E[f]	58	142–144	$C_{26}H_{33}F_3N_6O_2 \cdot HCl$[r]	C, H, Cl, N
40	$R_1 = R_3 = H$; $R_2 = p\text{-}C_6H_4CF_3$	C	6	78			F + E[q]	87	*l*	$C_{23}H_{29}F_3N_6 \cdot 2HCl$	C, H, Cl, N
41[n]	$R_1 = H$; $R_2 = p\text{-}C_6H_4NO_2$; $R_3 = CO_2Et$	B	48	*c*			F + E[f]	88	*l*	$C_{25}H_{33}N_7O_4 \cdot 2HCl$[m]	C, H, Cl, N
42	$R_1 = R_3 = H$; $R_2 = p\text{-}C_6H_4NO_2$	C	2	78			F + E[d,f]	30	*g*	$C_{22}H_{29}N_7O_2 \cdot 2HCl$	C, H, Cl, N

[a] A, EtOH; B, H_2O; C, BuOH; D, MeOH–HCl; E, Et_2O; F, EtOH–HCl; G, $CHCl_3$. [b] Melting points were determined with a Mel-Temp apparatus. [c] Room temperature. [d] This compound was obtained homogenous by elution from a silica gel H column followed by recrystallization. [e] Soxhlet extraction. [f] Dried *in vacuo* over P_2O_5 at 78°. [g] Decomposition. [h] Dried *in vacuo* over P_2O_5 at 110°. [i] Presoftening. [j] See Experimental Section. [k] Monohydrate. [l] Indefinite. [m] Hemihydrate. [n] The pmr spectrum in DMSO-d_6 indicated that this product was a 17:3 mixture of the 3- and 2-phenyl isomers. [o] The pmr spectrum in DMSO-d_6 indicated that this product was a 9:1 mixture of the 3- and 2-phenyl isomers. [p] The pmr spectrum in DMSO-d_6 indicated that this product was a 3:1 mixture of the 3- and 2-phenyl isomers. [q] Evaporated to dryness *in vacuo* to give a brittle glass. [r] Sesquihydrate.

the corresponding *o*-Cl (**29**) and *o*-$F_3CC_6H_4$ (**38**) derivatives were completely inactive, which might be due to the steric effects encountered in *ortho*-substituted benzenes. Similarly the inactivity of the bis-*p*-ClC_6H_4 derivative (**45**) might be due to steric interaction between the Ph groups in the *ortho*-substituted pyrazine ring. These results suggest but do not prove the surmise that coplanarity between the phenyl and pyrazine rings is necessary for activity in this type compound. Substitution on the 6-NH_2 of the active compounds with the ethoxycarbonyl group gave inactive compounds. The corresponding position in chloroquine is unsubstituted. and the lack of activity in these pyridopyrazines may be due to steric hindrance in the binding site.

Experimental Section[6]

Typical procedures are given for the preparation of the compounds listed in Table I.

Method A. Ethyl 8-Chloro-3-(*p*-chlorophenyl)pyrido[2,3-*b*]-pyrazine-6-carbamate (1).—A suspension of I[2] (10.0 g, 38.4 mmoles) in EtOH (150 ml) was hydrogenated over Raney Ni (~20 g) at an initial H_2 pressure of 3.5 kg/cm². The catalyst was removed by filtration under N_2, and the colorless filtrate was treated with *p*-chlorophenylglyoxal monohydrate (7.50 g, 40.0 mmoles). The resulting solution deposited a heavy precipitate of cream-colored solid after about 15 min of stirring at room temperature, and after about 30 min the reaction mixture was heated just to boiling and allowed to stand for 20 hr; yield 13.0 g (93%). This material was shown by tlc to be a mixture of the 2- and 3-(*p*-ClC_6H_4) isomers. The solid was dissolved in $CHCl_3$, poured onto a silica gel H column (300 g), and eluted with $CHCl_3$–C_6H_6 (1:1). After separation into 2 major zones, the compounds crystallized in the column, preventing effective separation. Separate column chromatography of each of these crude fractions gave, in each case, a faster-traveling major zone which was essentially pure 3 isomer and a slower-traveling minor zone which was a mixture of both isomers. The white solids obtained from the 2 major zones were combined and recrystd 4 times from EtOH to give a chromatographically homogeneous sample of the 3-(*p*-ClC_6H_4) isomer; yield 6.5 g. In the preparation of **11**, BuOH was used as the solvent.

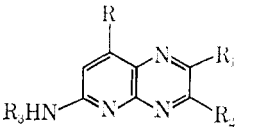
Method B. Ethyl 8-{[4-(Diethylamino)-1-methylbutyl]-amino}-3-(α,α,α-trifluoro-*p*-tolyl)pyrido[2,3-*b*]pyrazine-6-carbamate Monohydrochloride Sesquihydrate (39).—A suspension of ethyl 6-amino-4-{[4-(diethylamino)-1-methylbutyl]amino}-5-nitro-2-pyridinecarbamate monohydrochloride (II)[2] (7.13 g, 17.0 mmol) in EtOH (200 ml) was hydrogenated over Raney Ni catalyst (~15 g) at an initial H_2 pressure of 3.5 kg/cm². The catalyst was removed by filtration under N_2 and the colorless filtrate was treated with solid α,α,α-trifluoro-*p*-tolylglyoxal monohydrate (4.00 g, 18.2 mmoles). The resulting yellow solution was stirred under N_2 at room temperature for 72 hr, then refluxed for 2 hr. The partially cooled solution was treated with charcoal, filtered through Celite, and evapd to dryness *in vacuo*. The residue was dissolved in abs EtOH (200 ml) and the solution was diluted with Et_2O (300 ml). The yellow solid that deposited after about 10 min of vigorous magnetic stirring was collected by filtration under N_2 and washed with Et_2O. A second recrystallization under the same conditions gave bright yellow crystals; yield 5.7 g.

Method C. 6-Amino-3-(3,4-dichlorophenyl)-8-{[4-(diethylamino)-1-methylbutyl]amino}pyrido[2,3-*b*]pyrazine Dihydrochloride (31), Mixture with 6-Amino-2-(3,4-dichlorophenyl)-8-{[4-(diethylamino)-1-methylbutyl]amino}pyrido[2,3-*b*]pyrazine Dihydrochloride (17:3).—A mixture (17:3) of **30** and the correspond

(6) Silica gel H was obtained from Brinkmann Instruments, Inc., and Raney Active Catalyst No. 28 from W. R. Grace & Co. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

TABLE II

ANTIMALARIAL ACTIVITY[a]



Compd[c]	Increase in MST, days[b] — Dosage, mg/kg 160	320	640
		R = Cl	
1	0.2	0.6	0.8
2	0.3	0.3	0.3
3	0.3		0.7
4	1.9		2.1
		R = $NHCH(C_6H_5)_2$	
7	0.2		0.2
8	0.3		0.3
9	0.7		0.7
10	0.1		0.5
11	0.1		0.1
12	0.3		0.3
13	0.1	0.1	0.1
14		0.9	
		R = NH_2	
15	0.2	0.2	0.4
16	0		0.2
17	0.1		0.1
18	0.3		0.3
19	0.1		0.7
		R = NHR_4	
20, $R_1 = R_3 = H$; $R_2 = R_4 = p$-C_6H_4Cl	0.7		0.7
21, $R_1 = R_3 = H$; $R_2 = p$-C_6H_4Cl; $R_4 = p$-$CH_2C_6H_4SO_2NH_2$	0.1		0.1
		CH_3	
		R = $NHCH(CH_2)_3NEt_2$	
22	0.3		0.9
23	2.7	7.3A	7.5A
24	0.1		0.1
25		1.1	
26	0.5		1.4 (3T)
27	4.3	6.4A	12.4A (1T)
28	0.1		0.5
29	0.4	0.4	1.8 (2T)
30	0.3		0.5
31	5.3	12.9A	
32	0.5	0.7	3.3
33[d]			
34	0.4	2.0	5.9
35	7.9A	10.2A	11.7 (1C)
37	0.5		0.5
38		0.1	
39	0.5	0.5	3.9
40	16.6 (3C)	25.9 (4C)	(5C)
41	0.3	2.5	5.3
42[d]			
43, $R_1 = R_2 = R_3 = H$	0.5		0.9 (2T)
44, $R_1 = R_3 = H$; $R_2 = CH_3$	0.3		0.5
45, $R_1 = R_2 = p$-C_6H_4Cl; $R_3 = H$	0.3		0.3
46, $R_1 = H$; $R_2 = p$-C_6H_4Cl; $R_3 = CO_2Et$			0.4
47, $R_1 = R_3 = H$; $R_2 = p$-C_6H_4Cl	6.0	16.1A (2C)	(3C)(2T)
Chloroquine	10.0		(5T)

[a] Tests were carried out in five mice infected with a lethal dose of *P. berghei* by L. Rane and coworkers, Malaria Screening Laboratory, University of Miami, Miami, Florida.[4] Results were supplied by the Walter Reed Army Institute of Research, Washington, D. C. [b] MST, mean survival time over controls (6.2 ± 0.49 days); A, active (MST of treated animals is greater than twice the MST of control group); C, number of cures (mice surviving to 60 days); T, number of toxic deaths (mice dying before 6.2 ± 0.49 days). [c] The preparation of **20, 21,** and **43–47** have been reported in ref 2. [d] See footnote 4.

ing 2-($Cl_2C_6H_3$) isomer (10.6 g, 17.9 mmoles) was refluxed under N_2 for 7 hr in a solution of KOH (5.6 g, 100 mmoles) in EtOH (400 ml). The pptd KCl was removed by filtration and the filtrate was evapd to dryness *in vacuo*. The residue was stirred for several minutes in H_2O (200 ml) containing concd HCl (12 ml), then the solution was adjusted to pH 11 with 50% NaOH and extracted with $CHCl_3$ (3 × 150 ml). The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4), and evapd to dryness. The residue was dissolved in EtOH (100 ml), the resulting solution was acidified with excess 3.3 *N* ethanolic HCl (15 ml) and diluted with Et_2O to deposit a yellow semisolid, which solidified after refrigeration. The solid was collected by filtration and dissolved in warm EtOH (150 ml). The resulting solution was treated with charcoal and diluted by the slow addition of Et_2O (500 ml) to give a light yellow ppt, which was collected under N_2; yield 9.2 g.

Method D. 6-Amino-8-[(diphenylmethyl)amino]-3-(3,4,5-trimethoxyphenyl)pyrido[2,3-*b*]pyrazine (9).—A solution of **7** (9.32 g, 16.5 mmoles) and $NaOCH_3$ (0.91 g, 17.0 mmoles) in EtOH (700 ml) was refluxed under N_2 for 5 hr, then cooled to room temperature. The reaction mixture was acidified with 3.4 *M* ethanolic HCl (10 ml), stirred for 5 min, and then readjusted to pH 8 with a solution of $NaOCH_3$ in EtOH. The pptd NaCl was filtered off and the filtrate was set aside for 24 hr to crystallize. Two crops of large yellow crystals were obtained on long standing. This material was ground in a mortar with a few milliliters of H_2O, collected by filtration, and recrystd from (4:3) EtOH–H_2O (700 ml) to give long yellow needles; yield 6.87 g.

Method E. 6-Amino-8-chloro-3-(*p*-chlorophenyl)pyrido[2,3-*b*]pyrazine (2).—A magnetically stirred suspension of **1** (3.40 g, 9.4 mmoles) in 1,3-propanediol (275) ml) was heated in an oil bath at 140° for 30 min during which time a yellow crystalline solid deposited. After cooling the solid was collected by filtration, washed with EtOH, and recrystd by extraction into EtOH (500 ml) in a Soxhlet apparatus. The yellow solid that deposited was collected by filtration; yield 2.16 g.

Method F. Ethyl 8-Amino-3-(*p*-chlorophenyl)pyrido[2,3-*b*]-pyrazine-6-carbamate (15).—A suspension of **5** (7.20 g, 14.1 mmoles) and PhOH (~50 mg) in 10% HBr in glacial HOAc (200 ml) was stirred at room temperature for 20 hr. EtOH (300 ml) was added and the slurry was evapd to dryness *in vacuo*. Trituration of the residue with EtOH (100 ml) gave the hydrobromide as a white solid which was collected by filtration. A stirred suspension of the finely powdered solid in warm H_2O (250 ml) was treated with 10 *M* NaOH (10 ml). The bright yellow solid was collected by filtration, washed thoroughly with H_2O, and air-dried. Extraction of this material into EtOH (300 ml) in a Soxhlet apparatus followed by cooling gave yellow crystals, which were collected by filtration; yield 4.30 g.

Method G.—Similar to method F except that 5% HBr in glacial HOAc was used.

Ethyl 6-[(*p*-Chlorophenacyl)amino]-4-[(diphenylmethyl)-amino]-5-nitro-2-pyridinecarbamate Oxime.—A mixture of 2-amino-4′-chloroacetophenone oxime·HCl (2.66 g, 12.0 mmoles),[7] ethyl 6-chloro-4-[(diphenylmethyl)amino]-5-nitro-2-pyridinecarbamate (5.14 g, 12.0 mmoles),[8] Et_3N (2.44 g, 24.1 mmoles), and MeOH (60 ml) was stirred at reflux temperature under N_2 for 30 min. The mixture was refrigerated and the yellow product was collected by filtration, washed with EtOH, and dried *in vacuo* over P_2O_5: yield 2.54 g (37%); mp 204–205°. Additional product, 1.74 g (25%), mp 186–187°, was obtained by concentration of the mother liquors. Tlc indicated that both crops were mixtures of the *syn* and *anti* isomers of the oxime. *Anal.* ($C_{29}H_{27}ClN_6O_5$) C, H, N.

Ethyl 6-[(*p*-Chlorophenacyl)amino]-4-[(diphenylmethyl)-amino]-5-nitro-2-pyridinecarbamate.—A stirred mixture of ethyl 6-[(*p*-chlorophenacyl)amino]-4-[(diphenylmethyl)amino]-5-nitro-2-pyridinecarbamate oxime (3.89 g, 6.77 mmoles) and EtOH (780 ml) was refluxed until most of the solid was in solution. Aqueous 1 *N* HCl (78 ml) was added under N_2 to the refluxing mixture over a period of 2 min. The solution was refluxed an additional 5 min and cooled in ice–salt mixture to −2°. The crystalline product was collected by filtration, washed with cold 1:1 EtOH–H_2O (30 ml), and dried *in vacuo* over P_2O_5: yield 1.59 g (42%); mp 215° dec. *Anal.* ($C_{29}H_{26}ClN_5O_5$) C, H, N.

Ethyl 2-(*p*-Chlorophenyl)-8-[(diphenylmethyl)amino]pyrido-[2,3-*b*]pyrazine-6-carbamate (6).—A suspension of ethyl 6-[(*p*-chlorophenacyl)amino]-4-[(diphenylmethyl)amino]-5-nitro-2-pyridinecarbamate (280 mg, 0.500 mmole) in EtOH (25 ml) and DMAC (10 ml) was hydrogenated at room temperature and atmospheric pressure in the presence of Raney Ni (900 mg, weighed wet with EtOH). After 3 days, additional DMAC (10 ml) and Raney Ni (200 mg) were added. The hydrogenation was continued an additional 24 hr until a theoretical quantity of H_2 was taken up. The resulting mixture was charcoaled, filtered, and evapd *in vacuo* at 100°. The resulting gum was dissolved in Me_2CO (10 ml), treated dropwise with an 0.27% solution of $KMnO_4$ in Me_2CO (15 ml, 0.256 mmole), and stirred for 1 hr. The oxidation mixture was filtered and evapd to dryness *in vacuo*. Crystallization of the orange residue from EtOH (8 ml) gave a yellow product: yield 88 mg (35%); mp >300°.

Ethyl 2,3-Dihydro-8-[(diphenylmethyl)amino]-2-oxopyrido-[2,3-*b*]pyrazine-6-carbamate (14) and Ethyl 3,4-Dihydro-8-[(diphenylmethyl)amino]-3-oxopyrido[2,3-*b*]pyrazine-6-carbamate (13).—A suspension of ethyl 6-amino-4-[(diphenylmethyl)-amino]-5-nitro-2-pyridinecarbamate (12.0 g, 29.5 mmoles) in EtOH (300 ml was hydrogenated over Raney Ni catalyst (~20 g) at an initial H_2 pressure of 3.5 kg/cm². The catalyst was removed by filtration under N_2, and the colorless filtrate was evapd to dryness *in vacuo*. The resulting solid was dissolved in PrOH (300 ml) and treated dropwise with freshly distilled ethyl glyoxylate (3.51 g, 34.4 mmoles). This solution was stirred at room temperature for 1 hr, heated to boiling, and then cooled slowly. The heavy cake of yellow solid that deposited was collected by filtration and dried *in vacuo* over P_2O_5: yield 11.0 g (90%); mp 228–232° dec with presoftening. This solid was extracted with boiling EtOH (1 l.) The insoluble material from the EtOH extraction and the 2 crops obtained by cooling the EtOH extract and concentration of the extract to half-volume were combined (1.29 g) and recrystd by extraction into EtOH (300 ml) in a Soxhlet apparatus. The white crystals of **14** that deposited were collected by filtration; yield 1.16 g.

The EtOH extract described above was concd further to 300 ml and refrigerated for 18 hr. The fluorescent yellow solid that deposited was collected by filtration, dissolved in $CHCl_3$, and this solution was poured onto a silica gel H column (250 g) which had been washed with $CHCl_3$. The $CHCl_3$ eluate was collected in seven fractions; the last 5 were pooled and evapd to dryness, and the residue was recrystd from EtOH to give 13; yield 6.33 g.

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