residue, in H₂O, was made basic with 12 M NH₄OH and extracted with Et₂O. Drying and evaporation of the extracts gave 17 mg of oil which was converted to 11 mg (58%) of 13-oxalate, mp 196–197°, identical with authentic material.²

Interconversion of 9 and 10. Compound 10 (10 mg, 0.034 mmol) and the MeOH-NaOMe prepared from 2 mg (0.087 mmol) of Na and 0.2 ml of MeOH were stirred overnight at room temperature. Removal of MeOH in vacuo, solution of the residue in CHCl₃, and evaporation of the H₂O-washed, dried CHCl₃ solution gave 8 mg of 9, identical (TLC, ir) with that described above.

Ac₂O (10 drops), 8 mg (0.032 mmol) of 9, and 1 drop of C_5H_5N were stirred at room temperature for 1 day, poured into ice-H₂O, basified with K₂CO₃, and extracted with CHCl₃. The dried, evaporated (in vacuo below 20°) extract gave 10 mg of solid which was recrystallized from ligroine (bp 30–60°) yielding 6 mg of 9, mp 94–96°, identical with that described above.

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Interferon Inducing Activities of Derivatives of 1,3-Dimethyl-4-(3-dimethylaminopropylamino)-1*H*-pyrazolo[3,4-*b*]quinoline and Related Compounds

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Syntheses and interferon inducing activities are reported for 137 relatives of 1,3-dimethyl-4-(3-dimethylaminopropylamino)-1*H*-pyrazolo[3,4-b]quinoline (1). Three different generalized synthetic schemes for the preparation of pyrazolo[3,4-b]quinolines are presented and limitations contrasted. Other heterocyclic nuclei containing the 3-dimethylaminopropylamino side chain include pyridine, quinoline, acridine, pyrazolo[3,4-b]pyridine, pyrazolo [3,4-b][1,8]naphthyridine, pyrazolo[4',3':5,6]pyrido[2,3-d]pyrimidine, dipyrazolo[3,4-b:4',3'-e]pyridine, pyrrolo-[2,3-b]quinoline, isothiazolo[5,4-b]quinoline, and pyrido[2,3-h]pyrazolo[3,4-b]quinoline. Structural requirements for interferon induction in this series are discussed and two of the more active compounds (172 and 196) are compared directly with tilorone.

The capacity of an organism to produce interferon in response to infection by viruses or certain protozoan parasites is thought to be an important nonspecific defense mechanism. The interferon system is the earliest component of the host defense to become operative following virus infection and may also play a role in the later stages of recovery.¹ It is now well established that, once evoked, interferon will inhibit the replication of a wide variety of both RNA- and DNA-containing cytopathic and oncogenic viruses.² An agent which stimulates release and/or in vivo synthesis of interferon thus has implications for the development of a clinically useful broad-spectrum antiviral drug. Since the discovery of the interferon system in 1957,³ a number of polymeric substances have been reported which stimulate interferon production, but all of these have limitations in clinical utility.⁴ Impetus for the search for a monomeric low-molecular-weight inducer of interferon was provided by reports that 2,7-bis[2-(diethylamino)ethoxy]fluoren-9-one (tilorone) and congeners induce interferon activity in mice.^{5,6} A preliminary report from our laboratories disclosed the interferon-eliciting activity of an entirely different structural type, viz. the pyrazolo[3,4-b]quinoline 1 (BL-20803).7 We now report results



of a research program designed to (i) define structural features of 1 responsible for the interferon-eliciting activity and (ii) increase potency relative to 1 with a concomitant improvement in therapeutic ratio.

Chemistry. Relatives of 1 which were prepared for biological evaluation are listed in Tables III and IV. Some of the simpler fragment moieties of 1 and related acridines were prepared by literature procedures as indicated in Table III. The amide 65, which may be viewed as a hydrolytically cleaved form of 1, was prepared from the corresponding acid chloride and 3-dimethylaminopropylamine. Treatment of 65 with diborane in THF gave the reduced derivative 66.

Scheme I



Most of the pyrazolo[3,4-b] quinolines were prepared as indicated in Scheme I. This route was first used by Wolfrum et al.^{8,9} for preparation of 4-halo-, alkoxy-, or aryloxy-substituted pyrazolo[3,4-b]quinolines useful as optical brightening agents. The procedure was subsequently used by Stein and coworkers for the preparation of 1 which was initially prepared as a potential antimalarial agent¹⁰ and later found to possess hypocholesterolemic activity.¹¹ Although neither group reported encountering the isomeric pyrazolylanthranilic acid V, we usually obtained V as a contaminant of the desired isomer IV in varying amounts depending on the substituents in the benzenoid ring and the reaction conditions used. We found that it generally was not necessary to isolate the intermediates II-VI in pure form prior to conversion to IX (cf. Experimental Section). Intermediate benzoxazinones (III) and acids (IV and V) which were isolated and characterized are listed in Tables I and II, respectively. Three pure isomers V (52, 53, and 54) were isolated for attempted conversion to a 2-methyl-substituted pyrazolo[3,4-b]quinoline derivative VII. However, treatment with POCl₃ produced the corresponding cyclic amide VIII rather than VII. As an incidental point, this experimental result confirms the isomeric assignments of the acids 52, 53, and 54 and also supports the assignment of pyrazolo[3,4-b]quinolines made from this route by us and by others⁸⁻¹¹ as the 1-alkyl rather than 2-alkyl isomers.

Scheme I permitted variation of R^1 and R^2 through choice of appropriately substituted anthranilic acids and hydrazines, respectively. This route could not be used for synthesis of 1-unsubstituted pyrazolo[3,4-b]quinolines (R^2 = H). When hydrazine was condensed with the benzoxazinone III (R^1 = H), the only product isolated was VIII

Table I.Substituted2-Acetonyl-4H-3,1-benzoxazin-4-ones

	R ¹ -		о Сн ₂ со	CH ₃
N.	ות	M- °04	Yield, ^t	Formerile ⁶
INO.	R-	Mp, U	70	r ormuta
2	6-C1	158-163	84	$C_{11}H_{8}CINO_{3}$
3	6-I	168-170	36	C ₁₁ H ₈ INO ₃
4	5-CH ₃	143-145	60	$C_{12}H_{11}NO_{3}$
5	6-CH ₃	138.5- 140	74	$C_{12}H_{11}NO_3$
6	7-CH ₃	142 - 144	48	C_{1}, H_{1}, NO_{3}
7	8-CH ₃	152.5-154.5	5	$C_{12}H_{11}NO_3$
8	6,7- (CH ₁),	126-129	53	C ₁₃ H ₁₃ NO ₃
9	6,8- (CH,),	156-159	63	$C_{13}H_{13}NO_{3}$
10	7,8- (CH,),	188.5- 192	58	$C_{13}H_{13}NO_{3}$
11	7-C, H,	93-97	54	$C_{1,1}H_{1,1}NO_{3}d$
12	7-CF	107-109	18	$\mathbf{C}_{1}, \mathbf{H}_{8}\mathbf{F}_{3}\mathbf{NO}_{3}^{e}$
13	5-CH ₃ O	158.5 - 161	49	$\mathbf{C}_{12}\mathbf{H}_{11}\mathbf{N}\mathbf{O}_{4}\mathbf{f}$
14	6-CH ₃ O	154.5- 157	8	$C_{12}H_{11}NO_{4}$
15	5,7- (CH ₂ O),	178-180	85	$C_{13}H_{13}NO_{5}$
16	5-NO, ''	150-153	65	$C_{11}H_{8}N_{2}O_{5}$
17	7-NO,	172 - 174	77	$C_1 H_8 N_2 O_5$
18	7-CO ₂ H	> 300	79	C ₁₂ H ₉ NO ₅ ^g

^a Broad melting ranges may reflect a mixture of tautomeric forms (cf. Experimental Section). ^b Yields of analytically pure products. All compounds were recrystallized from MeCN except 18 which was purified by trituration under warm AcOH. ^c All new compounds were analyzed for C, H, and N. Analytical results were within $\pm 0.4\%$ of the theoretical values unless otherwise indicated. ^d C: calcd, 67.52; found, 68.12. ^e C: calcd, 53.15; found, 53.58. ^f C: calcd, 61.80; found, 61.30. ^g C: calcd, 58.30; found, 55.98. Mass spectrum (70 eV) *m/e* 247.

Scheme II



 $(R^1, R^2 = H)$.¹² Additionally, the C-3 position could not be varied by Scheme I because the 3-methyl group in IX is derived from diketene.

Both of the aforementioned variations not permitted by Scheme I were possible by the alternative synthesis outlined in Scheme II. Synthesis of the acid 55 as indicated in Scheme II was reported by Bulka et al.¹³ We used the same procedure for the preparation of 56 starting with ethyl α -chloropropionylacetate and 4-phenylthiosemicarbazide. Acids 55 and 56 were converted to the

Scheme III



1-unsubstituted pyrazolo[3,4-b]quinolines 136 and 152, respectively. When the sodium salt of 136 in DMF was treated with 1 equiv of CH₃I, alkylation occurred exclusively at the 1 position to give a quantitative yield of 1. Scheme II thus provided an alternative to Scheme I for variation of \mathbb{R}^2 , as well as permitting variation of \mathbb{R}^3 .

Neither Scheme I nor Scheme II was applicable for preparation of a 3-unsubstituted pyrazolo[3,4-b]quinoline (IX, $\mathbb{R}^3 = \mathbb{H}$). This variation was made possible via Scheme III. In an Ullmann type reaction, 5-amino-1methylpyrazole¹⁴ was condensed with o-iodobenzoic acid to yield the acid 29 which then was converted through the usual sequence to 154. A similar reaction using 5amino-1,3-dimethylpyrazole¹⁵ provided an alternative synthesis of the acid 19 and, thus, an alternative to Scheme I for the synthesis of 1. A variation of Scheme III starting with 5-amino-3-methylisothiazole was used for the preparation of 75. The general approach of Scheme III was also used for the preparation of 70, 71, and 162 (cf. Experimental Section).

The 6-nitropyrazolo[3,4-b]quinoline 162 thus obtained via Scheme III was identical with the product obtained from direct nitration of 1. By inference, the product obtained from sulfonation of 1 was assigned as the 6-SO₃H derivative 181. The related sulfonamide derivatives 182 and 183 were prepared from 181.

Problems were encountered in initial attempts to obtain the 5- and 7-nitro derivatives 161 and 163. Treatment of the acid 38 with POCl₃ under even very mild conditions gave black tars from which none of the intermediate VI (R¹ = 5-NO₂; R² = CH₃) could be isolated. Treatment of 38 with H₂SO₄ gave the corresponding 4-hydroxy intermediate which condensed satisfactorily with POCl₃ to give VI (R¹ = 5-NO₂; R² = CH₃) which subsequently was converted to 161. Heating VI (R¹ = 7-NO₂; R² = CH₃) in excess 3-dimethylaminopropylamine gave none of the expected 7-nitro derivative 163, but rather a product which we have characterized as the tetracyclic derivative 77.¹⁶ Reaction of VI (R¹ = 7-NO₂; R² = CH₃) with an equimolar quantity of the amine gave 163.

A particularly noteworthy result of our chemical investigation is illustrated in Scheme IV. Heating 98 in excess 3-dimethylaminopropylamine resulted in none of the expected triamine side-chain derivative shown but rather yielded 1 in essentially quantitative yield. Another example in which displacement of the entire 3-chloropropylamine side chain resulted involved reaction of 98 with cyclopropylamine to yield 86. On the other hand, reaction of 98 with excess pyrrolidine, methylamine, isopropylamine, *tert*-butylamine, N,N,N-trimethyl-1,3propanediamine, piperidine, and piperazine resulted in displacement of only the chloro group of 98 to yield 117, 103, 104, 105, 115, 119, and 122, respectively.

Synthesis of the symmetrical dipyrazolo[3,4-b:4',3'-e]pyridine derivative 72 (Scheme V) presented special problems. 5-Amino-1,3-dimethylpyrazole-4-carboxylic acid required for preparation of 72 analogously to Scheme I is Scheme IV





Scheme V



unknown. Reported attempts by Taylor and Hartke to isolate this acid were unsuccessful owing to its instability to decarboxylation.¹⁵ We thus carefully treated without isolation the presumed salt of the acid (obtained from basic hydrolysis of 5-amino-1,3-dimethylpyrazole-4-carbonitrile¹⁵) with diketene and obtained the N-acetoacetyl derivative 198. This product was converted to the oxazinone 199. Attempts to obtain the free acids of 200 and 201 by reaction of 199 with methylhydrazine in acetic acid

Table II. Substituted N-(Pyrazolyl)anthranilic Acids and Related Heterocyclic Acids

		R ¹ -					
			S N ∧ ∧	N N	Re-		
			н	R ²	crystn	Yield, ^b	
No.	Rʻ	R ²	R ³	Mp, °C	solvent ^a	%	Formula ^c
19	н	CH ₃	CH3	$208-210.5^d$	А	46	$C_{12}H_{13}N_{3}O_{2}$
20	H	C₂H,	CH ₃	185-187	A	45	$C_{13}H_{15}N_{3}O_{2}$
21 22	H H	$CH_2 CF_3$ $CH(CH_3)$	CH, CH	189-191.5 $212-215^{e}$	Б Д	62	$C_{13}H_{12}F_{3}N_{3}O_{2}$ CHN.O.
23	Ĥ	CH,CH,OH	ČH,	216.5-218 ^f	Ă	71	$C_{13}^{14}H_{15}^{17}N_{3}O_{3}^{2}$
24	H	CH ₂ C ₄ H,	CH,	193-194.5 ^g	A	69 0 5	$C_{18}H_{17}N_{3}O_{2}$
25	H H	C,H,	CH,	214-216.5	A A	85 66	$C_{17}H_{15}N_{3}O_{2}$
27	H	p-C ₄ H ₄ CO ₂ H	CH,	>300	ĉ	90	$C_{18}H_{15}N_{3}O_{4}$
28	Н	$(CH_2)_3N(CH_3)_2$	CH_3	194-196	D	62	$C_{16}H_{22}N_4O_2$
29	H 4 F	CH ₃	H	174-176.5	A	42	$C_{11}H_{11}N_{3}O_{2}$
30	4-F 5-F	CH, CH.	CH, CH.	228.5-230.5	A	33 44	$C_{12}H_{12}FN_{3}O_{2}$ $C_{12}H_{12}FN_{3}O_{2}$
32	3-C1	CH ₃	CH,	185-187.5	E	37	$C_{12}H_{12}CIN_{3}O_{2}$
33	4-Cl	CH,	CH_3	237-238'	A	92	$C_{12}H_{12}ClN_{3}O_{2}$
34 35	5-CI 5-I	CH ₃	CH ₃	241-242 239-240 5 dec	A C	79 67	$C_{12}H_{12}CIN_{3}O_{2}$ C.H.JN.O
36	4-NO,	CH ₃	CH ₃	255-257	Ă	67	$C_{12}H_{12}N_4O_4$
37	5-NO ₂	CH ₃	CH_3	278-280 dec	A	90	$C_{12}H_{12}N_4O_4$
38	6-NO ₂	CH,	CH,	236-248 dec	A	62 82	$C_{12}H_{12}N_4O_4$
40	4-CH ₃ 5-CH ₂	CH ₃ CH ₂	CH, CH,	194-196	A	69	$C_{13}H_{15}N_{3}O_{2}$ $C_{13}H_{12}N_{3}O_{3}$
41	6-CH ₃	CH ₃	CH,	171.5-173.5	A	47	$C_{13}H_{15}N_{3}O_{2}$
42	$4 - CF_3$	CH,	CH,	226~229	A	62 40	$C_{13}H_{12}F_{3}N_{3}O_{2}$
43	4-C₂H₅ 5-C.H.	CH ₃ CH ₂	CH ₃ CH ₃	195-197	B	40 37	$C_{14}H_{17}N_{3}O_{2}$ $C_{14}H_{17}N_{3}O_{3}$
45	3,5-(CH ₃) ₂	CH ₃	CH,	223-226 dec	Ā	60	$C_{14}H_{17}N_{3}O_{2}^{j}$
46	$4,5-(CH_3)_2$	CH ₃	CH ₃	220-221.5	A	32	$C_{14}H_{17}N_{3}O_{2}^{k}$
47	3-CH,0	CH, CH,	CH, CH.	225-226.5 dec	г А	20 46	$C_{13}H_{15}N_{3}O_{3}$ $C_{13}H_{15}N_{3}O_{3}$
49	3-CH,O, 6-Cl	ĊH,	ČH,	216-218.5 dec	A	42	$C_{13}H_{14}ClN_{3}O_{3}$
50	$4,5(CH_{3}O)_{2}$	CH ₃	CH,	211.5-213 dec	A	93	$C_{14}H_{17}N_{3}O_{4}$
51	4,5-benzo		CH3	245-247.5 dec	A	55	$O_{16}H_{15}N_{3}O_{2}$
52				195-196 dec	Α	14^l	C_1, H_1, ClN_3O_3
		N CH3					12 12 5 2
53				227-229	А	33 ¹	C ₁₃ H ₁₆ N ₃ O ₂
	H ₃ C	N CH3					13 13 5 2
		~CO2HCH3					
54				208.5-210	Α	25^l	$C_{13}H_{15}N_{3}O_{3}$
	Сн ₃ 0-	H N CH3					
	H ₃ C	CO ₂ H					
55	r			198 dec^m	Α	74	$C_{11}H_{11}N_{3}O_{2}$
	H5C2	CO2H					
56				199-200 dec	Α	24	$C_{12}H_{13}N_{3}O_{2}$
		ΗŇ					
		CO2H CH3					
57	k N − 1			263-265 dec	Α	64	$C_{11}H_{12}N_4O_2$
		Н СН ₃					
		CO2H CH3					
58				284-286 dec	G	58	$C_{10}H_{11}N_{5}O_{2}$
	N.	N N					~
		он от					
50				919-919 doo	п	17	СНИОС
00		[™] N [™] s [−] ^N		212-219 uec	D	11	$O_{11} II_{10} II_2 O_2 S$
		n					

<u>~</u>0 ⊔

^a A, EtOH; B, MeCN; C, AcOH; D, absolute EtOH; E, *i*-PrOH; F, MeNO₂; G, DMF. ^b For acids which were prepared via Scheme I, in which isomers were always encountered, yields are for crude products which were used directly in the next step except as noted in the Experimental Section. The melting points given for these acids are for chromatographically pure samples (GLC analysis after treatment with CH_2N_2) obtained by recrystallization of an aliquot of the crude acid from the indicated solvent. Yields for acids made by routes other than Scheme I (cf. Experimental Section) represent pure products. ^c See footnote c in Table I. ^d Lit.¹¹ mp 212-213°. ^e Lit.¹¹ mp 206-208°. ^f Lit.¹¹ mp 210-211°. ^g Lit.¹¹ mp 192-193°. ^h C: calcd, 66.86; found, 66.16. ⁱ Lit.¹⁰ mp 232-233°. ^j N: calcd, 16.21; found, 15.78. ^k N: calcd, 16.21; found, 15.61. ⁱ Percent of product relative to the corresponding 1-methyl isomer present in the crude product as determined by GLC assay (cf. Experimental Section), isolated analogous to the procedure described for **53**. ^m Lit.¹³ mp 195°.

led to the recovery of the symmetrical amine 202 as the only identifiable product. Experimental conditions then were developed which permitted preparation of the salts 200 and 201 and their subsequent elaboration without isolation into the tricyclic derivatives 72 and 203.

Biology. Details of oral or parenteral dosing of mice and the assay of serum interferon have been published.⁷ Briefly, compounds were dissolved or suspended in sterile distilled water and administered to 15-g female CD-1 mice. At least three mice were treated at each dose level of compound. Later (18–20 hr), mice were bled and their sera collected, pooled, and quantitatively assayed for interferon activity by a plaque reduction method employing mouse L cells and challenge by vesicular stomatitis virus. The reliability of the assay was verified by including a reference mouse interferon standard provided by the National Institute of Allergy and Infectious Diseases. In our hands, the standard assayed $10^{4.7}$ units/ml of interferon as compared to the assigned potency of $10^{4.5}$ units/ml.

For simplicity, the interferon data listed in Tables III and IV are expressed as the lowest dose of compound required to elicit a significant level of circulating interferon, i.e., ≥ 32 units/ml.⁷ The highest doses given to the animals were 400 mg/kg po or 300 mg/kg ip. In general, no major differences in activity were obtained between routes of administration with the exception that the parenteral route evinced toxicity more frequently than did the oral route.

For convenience in discussing structure-activity correlations, compounds assayed may be divided into the following groups: (i) fragment moieties of 1 (60, 61, 64a-c, 65-68, the acid 19, 1,3-dimethyl-5-aminopyrazole, and 3-dimethylaminopropylamine); (ii) side-chain variants (79-135); (iii) ring A (benzenoid) nuclear modifications (69-73); (iv) ring C (pyrazole) modifications (62, 63, 74, 75, 136-154); (v) ring A substituent variants (76, 77, 155-197); and (vi) bis compounds related to 1 (78).

Results obtained indicated that activity in this series is confined within rather narrow limits. Excepting the marginal activity shown by 70, fragment moieties of group i and ring A nuclear modifications of group iii were devoid of activity. Especially noteworthy is the inactivity seen with the progression 60, 61, 64a-c, and with the tetrahydro derivative 69. These results suggested that the nucleus requirement for activity is at least three appropriately fused aromatic or heteroaromatic rings. The one bis tricyclic compound prepared (78) in group vi was inactive or nearly so.

A number of compounds of group ii were active within certain structural limitations. Results seen among compounds 80–135 indicated that for activity the substitution at the 4 position of the pyrazolo[3,4-b]quinoline nucleus has to be NH attached to a side chain of at least three carbons in length terminating in a second amino function. Particularly noteworthy in this group are the inactive compounds 89 and 90 which differ from the parent 1 only by substitution for NH by O and S, respectively. For retention of activity, the side chain may be varied in length (cf. 92-94) and some variation of the terminal amine function is permissible although none of these changes resulted in an analog significantly more active than 1. Interestingly, even changing the terminal amine function to diethylamino (110) resulted in lower activity relative to 1. All derivatives of 1 having a tertiary rather than secondary amine function at the 4 position of the tricyclic nucleus were inactive (131-135).

Very little change of the pyrazole moiety in 1 was permissible for retention of activity (group iv) and no member of this group proved more active than 1. Even the minimal change of the 1-normethyl (136) or 1-ethyl (137) or 3-ethyl (153) derivatives of 1 resulted in inactive or nearly inactive compounds. All other changes at the 1 position resulted in compounds inactive at nontoxic doses. Removal of the 3-methyl in 1 (154) resulted in a derivative of comparable activity to 1. Changing the pyrazole for benzenoid (62, 63) or isothiazole (75) resulted in inactivity. Interestingly, substitution of pyrazole by pyrrole (74) resulted in an active derivative, but one shown by quantitation of units of serum interferon to be less active than the pyrazole relative 154.

Success in enhancing potency of 1 was achieved with the preparation of certain ring A substituted derivatives of 1 in group v. Again, however, considerable structural specificity was seen within this group as was encountered in the other groups. With the exception of the 7-Cl derivative 158, substitution of halogen (155-160) resulted in considerable loss of activity. Nitro (161-163) and amine (164–169) derivatives were inactive with the exception of the $6-C_2H_5NH$ derivative 167 which was about four times as active as 1. The 6-CH₃ (171) and 7-CH₃ (172) derivatives proved to be among the most active compounds seen, yet the 5- (170) and 8- (173) CH₃ derivatives were devoid of activity. While the 8-CH3 derivative was inactive, the 7,8- (179) and 6,8- (180) di-CH₃ derivatives were active. Combination of the two active monomethyl derivatives (171 and 172) into the 6,7-disubstituted derivative 178 resulted in one of the more active compounds in the series. Additional examples of the structural specificity in this series are seen in the 7-CF₃ (174), 7-C₂H₅ (176), and 7-CH₂OH (177) relatives of 172, all of which are either inactive or considerably less active than 172. The only two tetracyclic compounds prepared (76 and 77) have been considered within this class of compounds comprising ring A substituted derivatives. Both compounds were active and again reflect, analogously to compounds 178 and 179, that the 6,7 and 7,8 positions may be disubstituted with retention of activity. Acid derivatives at positions 6 and 7 (181–187) were uniformly inactive. In contrast to the four monomethyl derivatives discussed above, each of the monomethoxy derivatives (188-191) was active. The 5,-7-dimethoxy derivative 196 was among the most active compounds prepared in the series. Surprisingly, the trimethoxy derivative 197 was inactive.

Of the compounds described herein, the most active appear to be 167, 171, 172, 178, 188, 190, 195, and 196. Two of the more active compounds, 172 and 196, were compared with tilorone in five separate dose-response studies (Figure Each compound received at least four separate 1). evaluations at each of the dose levels employed. There was considerable spread in the interferon response at dose levels required to elicit significant interferon activity in the circulation. It should also be noted that at 50 mg/kg, compound 172 failed to stimulate measurable amounts of interferon in three out of five trials. On the other hand, compound 196 and tilorone were active in one instance at 25 mg/kg and in all instances at 50 mg/kg. At the latter dose level, the response to tilorone tended to distribute at the higher end of the spread than did the response to 196. We conclude that an MED range of 25-50 mg/kg should be assigned to 196 and to tilorone while the MED of 172 is 50-100 mg/kg.

We have previously reported a good correlation between interferon-inducing activity and suppression of vaccinia virus infection by compound 1 in mice.⁷ Other compounds in this series have also shown this same close relationship. For example, the interferon-inducing and vaccinia virus-inhibiting MED's for compounds 171 and 172 were



Figure 1. The distribution of mouse serum interferon activities following oral dosing with various concentrations of 172 (\times), 196 (\circ), and tilorone (\triangle) in five separate experiments. The interrupted line represents the lower limit of assay sensitivity.

identical, i.e., 50 mg/kg given orally or intraperitoneally. Acute toxicity of compound 172 in mice is approximately equal to that reported for compound 1 (250 mg/kg ip and 1600 mg/kg po).⁷ Thus, based on MED's of 200 and 50 mg/kg for 1 and 172, respectively, a fourfold improvement in the therapeutic ratio has been achieved.

Discussion

At the time we started our research on the "lead" compound 1, tilorone was the only significant lowmolecular-weight inducer of interferon. Since the inception of our program, a few low-molecular-weight inducers other than the fluorenone type have been reported. Among these are a series of diamines,¹⁷ e.g., N,N-bis(2-hydroxyethyl)-N',N'-dioctadecyl-1,3-propanediamine,18-20 certain basic dyes, e.g., toluidine blue,²¹ and the antimalarial drugs quinacrine and acridine.²² Where the activity levels were indicated, the amounts of interferon produced were low relative to levels (Figure 1) produced in this series of compounds and with tilorone. The compounds of the present study thus add a new class of compounds to those known to elicit circulating interferon and some members of the series are among the most potent low-molecularweight inducers reported to date. Furthermore, compounds of the present series appear to afford an advantage over other reported interferon inducers in exhibiting a delay in onset of a hyporesponsive state after multiple dosing.7 Work is continuing in our laboratories and elsewhere to investigate the full therapeutic potential of this class of compounds.

Experimental Section

Melting points are capillary and are uncorrected. All compounds had consistent NMR and ir spectra. Where elemental analyses are indicated by symbols of the elements, analytical results were within $\pm 0.4\%$ of the theoretical values. All required anthranilic acids and hydrazines are previously described compounds. For brevity, detailed procedures are given for one representative example of a general type, and additional compounds synthesized from the general procedures are so indicated. Where reaction procedures were not straightforward and/or where compounds do not fit into generalized class reactions, the experimental details are given in full. "Alumina" refers to Merck & Co. reagent aluminum oxide (product no. 71707); "neutral alumina" and "basic alumina" refer to J. T. Baker Co. aluminum oxide, neutral (catalog no. 0540), and aluminum oxide, basic (catalog no. 0539), respectively.

2-Acetonyl-7-methyl-4H-3,1-benzoxazin-4-one (6). A mixture of 4-methylanthranilic acid (25.0 g, 0.17 mol) and diketene (13.95 g, 0.17 mol) in CCl₄ (250 ml) was heated at reflux for 2.5 hr. Acetic anhydride (18.3 g, 0.18 mol) was added and the mixture was stirred under reflux for 16 hr. The mixture then was cooled and the product isolated by filtration; recrystallization (MeCN) gave 6 (17.4 g, 48%), mp 142-144°. NMR analysis indicated that in solution (CDCl₃) the product is a mixture of the tautomeric forms A and B in a ratio of approximately 1:2, respectively: δ (A) 3.80 (s, CH₂), 2.50 (s, CH₃-aro), 2.32 ppm (s, CH₃CO); δ (B)



5.23 (s, CH=COH), 2.45 (s, CH₃-aro), 2.11 ppm (s, CH₃COH). All 3,1-benzoxazin-4-ones prepared were a mixture in solution of these two forms. Similarly prepared by this procedure starting with the appropriate anthranilic acids were 2–8, 11–14, and 17; for 3 and 13, THF was added to the CCl₄ to increase solubility. The benzoxazinone 15 was prepared by this procedure except the intermediate N-acetylacetamide was prepared and isolated (by the procedure which follows for 17) prior to cyclization in CCl₄.

2-Acetonyl-7-nitro-4H-3,1-benzoxazin-4-one (17). Diketene (17.30 g, 0.21 mol) was added during 30 min to a solution of 4-nitroanthranilic acid (37.60 g, 0.20 mol) in 1.33 N aqueous NaOH (150 ml, 0.20 mol) while maintaining the temperature at 28-30° with external cooling. The solution was stirred at 25° for 90 min and then was cooled and acidified with 1.5 equiv of 6 N aqueous HCl to yield N-(acetoacetyl)-4-nitroanthranilic acid (47.8 g, 90%), mp 172-175° dec. This product (47.5 g, 0.18 mol) was stirred with Ac₂O (25 ml, 0.27 mol) in AcOH (190 ml) at 80° for 2.5 hr. The solution was cooled and filtered to yield 17 (34.0 g, 77%): mp 172-174° (MeCN); NMR (Me₂SO) indicated an approximately 1:1 mixture of the tautomeric forms.

Similarly prepared by this procedure from the appropriate anthranilic acids were 9, 10, 16, and 18.

N-(1,3-Dimethylpyrazol-5-yl)-4-methylanthranilic Acid (39) and N-(1,5-Dimethylpyrazol-3-yl)-4-methylanthranilic Acid (53). The benzoxazinone 6 (15.31 g, 0.071 mol) was added to a solution of methylhydrazine (3.90 g, 0.085 mol) in acetic acid (150 ml) and the resultant solution was stirred at 80° for 2.5 hr. The solvent was removed and the product was triturated under MeCN at 0° to yield 14.50 g (83%) of crude 39 containing about 33% of the isomeric acid 53 (determined by GLC analysis after CH2N2 treatment). A 9.50-g aliquot was triturated under 300 ml of hot MeCN and the insoluble solid (2.23 g, about 85% of 53) isolated by filtration; additional recrystallization (EtOH) gave pure 53, mp 227-229°. The product obtained from the 300 ml of MeCN filtrate was recrystallized successively from MeCN and EtOH to yield pure 39, mp 188-192°.

Similarly prepared from the appropriate benzoxazinones and known hydrazines were acids 19-28, 30-36, and 38-54.

4-(3-Dimethylaminopropylamino)-1,3,7-trimethyl-1Hpyrazolo[3,4-b]quinoline Dihydrochloride (172). A mixture of the above crude acid 39 (5.00 g, 0.02 mol) and POCl₃ (50 ml) was stirred at reflux for 3 hr. The cooled mixture was concentrated at 5 mm to a syrup which was poured onto ice. The mixture was basified with 4 N NaOH and then was extracted with chloroform. The chloroform extracts were washed, dried, and evaporated to yield crude 4-chloro-1,3,7-trimethyl-1H-pyrazolo[3,4-b]quinoline. The product was treated with 3-dimethylaminopropylamine (50 ml) and the mixture was stirred at 130° for 12 hr. The mixture

Table III. Heterocycle Derivatives Related to 1

			Re- crystn		Interfero	n assay, ^c (kg
No.	Structure	Mp, $^{\circ}C$	sol- vent ^a	Formula ^b	ip	po
60		188-191 ^d	А	C ₁₀ H ₁₇ N ₃ ·2HCl ^c	NA	
61	HN(CH ₂) ₃ N(CH ₃) ₂	58-60 ^f		$C_{14}H_{19}N_3$ ·2HCl	NA	
62	HN(CH ₂) ₃ N(CH ₃) ₂	250 ^g	E	$C_{18}H_{21}N_3$ ·2HCl	≥300	NA
63	$ = \bigcup_{n \in \mathbb{N}} $	241-243.5 ^h	С	$C_{19}H_{23}N_3$ ·2HCl		NA
	$\dot{C}H_3$ $HN[CH_2]_3N(CH_3)_2$ a, $\mathbf{R} = \mathbf{CO}_2\mathbf{C}_2\mathbf{H}_5$	196-200 dec	D	$C_{16}H_{25}N_5O_2 \cdot 2HCl \cdot 0.5H_2O^i$		NA (100)
64	$\bigcup_{\substack{N \\ C \in I_3}} \overset{\mathbb{I}^{-CH_3}}{\mathbf{b}}, \mathbf{R} = \mathbf{CO}_2 \mathbf{H}$	268-272 dec 272.5-276 dec	E D	$\begin{array}{c} C_{14}H_{21}N_5O_2 \cdot 2HCl \\ C_{13}H_{21}N_5 \cdot 2HCl \end{array}$		NA NA
65	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ C = C \\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ C H_{3} \\ \end{array}$	Foam		$\mathbf{C_{17}H_{25}N_{5}O\cdot HCl^{j}}$	NA	
66	$ \begin{array}{c} $	222-224	С	$\mathbf{C_{17}H_{27}N_{5}}\cdot\mathbf{2HCl}$	NA	
67		219-235	D	$C_{12}H_{13}N_3$ ·HCl ^k		NA
68	CH3 CH3	215-218.5 dec	В	$C_{12}H_{15}N_{3}$ ·HCl		NA
69	$(\begin{array}{c} HN(CH_2)_3 N(CH_3)_2 \\ HN(CH_2)_3 \\ $	269-271.5 dec	A	$C_{17}H_{27}N_s$ ·2HCl	NA (100)	NA
70	$ \begin{array}{c} HN(CH_2)_3 N(CH_3)_2 \\ \hline \\ N \\ \hline \\ \\ H_3 \\ \hline \\ \\ CH_3 \\ \hline CH_3 \\ \hline \\ CH_3 \\ \hline CH_3 \\ \hline \\ CH_3 \\ \hline CH_3 \\ CH$	298 dec	E	$C_{16}H_{22}N_6 \cdot 2HCl$	≥300	
71	$N = \frac{1}{2} $	279-281 dec	E	$C_{15}H_{21}N_7$ ·2HCl	NA	
72	$H_{3}C \xrightarrow{CH_{3}}_{N \\ (CH_{2})_{3}N(CH_{3})_{2}} CH_{3}$	272-275 dec	D	$C_{16}H_{25}N_{7}$ ·2HCl		NA
73	$ \begin{array}{c} $	265-270 dec	D	$C_{15}H_{23}N_{\gamma}\cdot 2HCl$		NA
74	HN(CH ₂) ₃ N(CH ₃) ₂	Gum		$C_{17}H_{22}N_4^{\ l}$		200

Table III (Continued)

			Re- crystn sol-	L	Interfer m	ron assay, ^c ng/kg
No.	Structure	Mp, °C	vent ^a	Formula ^b	ip	ро
75	$(1) + N(CH_2)_3 N(CH_3)_2$	>300	Е	C ₁₆ H ₂₀ N ₄ S·2HCl		NA
76	HN(CH ₂) ₃ N(CH ₃) ₂ CH ₃ CH ₃	>300	E	$C_{21}H_{25}N_{5}\cdot 2HCl$	100	
77	HN (CH ₂) ₃ N(CH ₃) ₂	293-296 dec	D	$C_{20}H_{24}N_6 \cdot 2HCl$	100	100
78	$(CH_2)_4 \xrightarrow{NH} CH_3 \xrightarrow{(CH_2)_4} CH_3$	194-195.5	D	$C_{28}H_{30}N_8$	≥300	

^a A, *i*-PrOH; B, MeCN; C, absolute EtOH; D, EtOH; E, aqueous EtOH. ^b See footnote c in Table I. ^c Minimal effective dose (mg/kg) required to elicit detectable serum interferon in mice by the indicated route of administration; cf. text for more complete description of assay. NA means not active at 300 mg/kg ip or 400 mg/kg po. NA qualified by a dose level shown in parentheses signifies that toxicity was encountered at higher doses and that the compound was not active at the indicated dose level. ^d Prepared from 4-chloropyridine using the procedure described for compound **172**. ^e N: calcd, 16.66, found, 17.13. ^f Lit. mp hydrate (base) 73°; the HCl salt was purified by trituration under Me₂CO; J. K. Landquist, J. Chem. Soc., 1038 (1951). ^g Lit. mp 249-250°: J. Peryt, E. Zylkiewicz, and A. Ledochowski, Rocz. Chem., **43**, 623 (1969), Chem. Abstr., **71**, 12990b (1969). ^h Lit. mp 243-244°: A. Ledochowski, B. Stefanska, and B. Kozinska, Rocz. Chem., **38**, 421 (1964); Chem. Abstr., **61**, 1830d (1964). ⁱ N: calcd, 17.45; found, 18.12. ^j C: calcd, 58.03; found, 59.10. ^k Analyzed as the free base. ^l Not analyzed, cf. Experimental Section.

was cooled and excess amine was evaporated at 5 mm. The residue was dissolved in chloroform and the resultant solution was washed in succession with aqueous K₂CO₃, water, and brine. Drying (Na₂SO₄) and evaporation gave a syrup which was dissolved in 1-propanol (330 ml) containing aqueous 1 N HCl (55 ml). The salt 172 was separated by filtration, washed with acetone, and recrystallized from aqueous EtOH: yield 3.78 g (48% based on weight of starting acid or 72% based on estimated content of pure 39 in the starting material); mp 290-293° dec.

Similarly prepared from the appropriate starting acids were **76**, 136–139, 143–147, 149 (from 23), 150 (4-Cl intermediate), 152, 154, 155–160, 162, 171–176, 178–180, 186, 188–191, and 194–197. Similar treatment of IV ($R^1 = H; R^2 = CH_2CO_2C_2H_5$) gave 148.

1,2,6-Trimethylpyrazolo[5,1-b]quinazolin-9(1*H*)-one (VIII, $\mathbf{R}^1 = 6$ -CH₃; $\mathbf{R}^2 = CH_3$) hydrochloride was prepared by refluxing the acid 53 for 3 hr in POCl₃: mp 245-248° dec after recrystallization (EtOH). Anal. (C₁₃H₁₃N₃O·HCl) H, Cl, N; C: calcd, 59.20; found, 58.73.

Similarly prepared from the acid 52 was the 7-chloro relative (VIII, $R^1 = 7$ -Cl; $R^2 = CH_3$): mp >300°. Anal. (C₁₂H₁₀ClN₃-O-HCl) C, H, N.

1,3-Dimethyl-4-(4-dimethylaminobutylamino)-1Hpyrazolo[3,4-b]quinoline Dihydrochloride (92). A mixture of 4-chloro-1,3-dimethyl-1H-pyrazolo[3,4-b]quinoline¹⁰ (81, 4.63 g, 0.02 mol), K₂CO₃ (2.76 g, 0.02 mol), and 4-dimethylaminobutylamine (2.32 g, 0.02 mol) in DMF (60 ml) was stirred at 130° for 16 hr. The solvent was removed at 5 mm and the residue was partitioned between water and chloroform. The organic phase was separated, washed with water, dried, and evaporated to leave an oil (6.02 g, 97%). The dihydrochloride salt was prepared as described above for 172; recrystallization (EtOH) gave mp 294° dec.

Similarly prepared from 81 and the appropriate amines were 87 (except heating was at 144° for 64 hr), 88, 91, 93, 94, 97, 100, 102, 106, 109–113, 118, 120, 121, 124, 126, 127, 129, 131, and 133–135; also 78 and 79 except 0.5 equiv of amine was used. Similarly prepared from 81 and the indicated nucleophiles were 82 (NaSH), 83 (PhCH₂SH), 89 [Me₂N(CH₂)₃ONa], and 90 [Me₂N(CH₂)₃SH]. Also prepared from 81 using excess of the appropriate amine as solvent and heating at 125–135° for 16 hr

were 101, 114, 116, 123, 125, and 128. For a number of the preceding products, standard chromatographic procedures were used for final purification before formation of salts. In some of the preceding examples, the 4-dimethylamino derivative 130 was encountered as a by-product, presumably arising from the DMF.

4-(3-Chloropropylamino)-1,3-dimethyl-1*H*-pyrazolo[3,4b]quinoline Hydrochloride (98). A solution of the hydroxy compound 97 (11.50 g, 0.038 mol) and thionyl chloride (110 ml) was heated at 60° for 2 hr. Evaporation gave 98, mp 210-214° after recrystallization (absolute EtOH).

1,3-Dimethyl-4-(3-pyrrolidinopropylamino)-1*H*pyrazolo[3,4-*b*]quinoline Dihydrochloride (117). A solution of 98 (2.00 g, 5.5 mmol) in pyrrolidine (20 ml) was heated at 80° for 2 hr. Usual work-up gave 117 free base (1.64 g, 92%) from which the salt was made as described above: mp>300° (aqueous EtOH). Similarly prepared from 98 and the appropriate amine except heating was at 80-100° for up to 16 hr in a steel bomb were 103 (absolute EtOH used as solvent), 104, 105, 115, 119, and 122. Also prepared from 98 and 1 equiv of appropriate amine and K₂CO₃ in *N*,*N*-dimethylacetamide (DMAC) at 135° were 107 and 108.

4-Cyclopropylamino-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]quinoline Hydrochloride (86). A mixture of 98 (3.25 g, 0.01 mol) and cyclopropylamine (9 ml) was heated in a bomb at 100° for 64 hr. The crude product was purified by chromatography on alumina (elution with 1:1 Et₂O-CH₃Ph), then converted to the HCl salt, and recrystallized (absolute EtOH) to yield 86 (1.57 g, 54%), mp 237-239° dec. Similarly, 98 (2.50 g) in 3-dimethylaminopropylamine (25 ml) was heated at 125° for 16 hr. Work-up gave pure 1 (2.68 g, 98%).

4,9-Dihydro-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]quinoline Hydrochloride (67) and 1,3-Dimethyl-1*H*-pyrazolo[3,4-*b*]quinoline Hydrochloride (80). A mixture of 81^{10} (6.00 g, 0.026 mol), NaOAc:3H₂O (3.54 g, 0.026 mol), and 5% Pd/C (0.5 g) in AcOH (200 ml) was shaken under H₂ at 50 psi of initial pressure for 2.5 hr. Usual work-up gave a product indicated by GLC to be a mixture of 80 (base), 81, and 67 (base) in an approximate ratio of 47:17:36, respectively. Chromatography on alumina gave initial fractions (elution with toluene) of mixtures of 81 and 80 (base) and later fractions (elution with 98:2 Et₂O-EtOH) of mainly

n assay, ^c ng/kg)	od	200 NA NA NA	NA NA NA		NA (200) 400	200 200	AN AN AN AN		200					400
Interferon MED (r	ip	100	NA NA (1001)	NA (UUU)	300 NA (50) NA (50)	150		NA NA ≥ 300	NA 150 ≥ 300	≥300 NA (100)	NA (100)	NA	NA	300
	Formula ^b	C ₁₇ H ₂₃ N ₅ ·2HCl C ₁₇ H ₂₃ N ₅ ·2HCl C ₁₂ H ₁₁ N ₃ ·2HCl C ₁₂ H ₁₁ N ₃ ·HCl C ₁₂ H ₁₀ ClN ₃	C _{1,} H ₁ ,N ₃ S C ₁₉ H ₁₈ CIN ₃ S C ₁₂ H ₁₂ N ₄ HCIN ₃ S C ₁₁ H ₁₂ N ₄ HCI-0.5H ₂ O	$C_{22}H_{26}N_4$ -HCl ^f	$C_{1,2}H_{2,N}$, e ² HCl·1.5H ₂ O $C_{1,3}H_{2,N}$, O·HCl·H ₂ O $C_{1,7}H_{2,N}$, S·HCl $C_{1,7}H_{2,N}$, S·HCl	C ₁₈ H ₂₅ N ₅ 2HCl C ₁₈ H ₂₅ N ₅ 2HCl C ₁₉ H ₂₇ N ₅ 2HCl C ₂₀ H ₂₉ N ₅ 2HCl	Ci ₆ H ₂₀ N ₄ S·HCI Ci ₆ H ₂₀ N ₄ OS·HCI-H ₂ O Ci ₅ H ₁₈ N ₄ O·HCI Ci ₅ H ₁₆ N ₄ O·HCI	Ci,Hi,N,NaO2 Ci,Hi,N4O2.HCl Ci,Hi,N52HCl	C(,H1,N,O.HC) C(,H1,N,·2HC) C(,H2,N,·2HC)	C ₁₉ H ₂₉ N ₅ ,2HCl C ₁₉ H ₂₉ N ₅ ,2HCl	C ₂₅ H ₃₃ N ₅ ·2HCl	$C_{20}H_{25}N_{\gamma}\cdot 2HCI$	C ₁₃ H ₁₈ N ₆ HCl·H ³ O ^h	$C_{19}H_{27}N_s$. 2HCl
Re- crystn sol·	vent ^a	C A A C	ACCH	<u>0</u>	AGFA	(D m m c	IJ Fr O W	ΒA	AUA	4 4	А	D	C	V
	Mp, °C	306-309 dec 264-272 dec 252-255 dec 118-120 ^d	$297-299$ $156-162$ $> 320^{e}$ > 300	>300	> 290 dec 235-245 202-204 > 300	294 dec 269-271 dec 215-219	145-148 190-192 dec 252.5-254.5 210-214	230-240 133-142 dec 311-314 dec	222-225 dec 307-309 dec >300	>305 307-309 dec	>300	288-290 dec	264 dec	297–299 dec ⁱ
⁴ ² ² ² ² ² ² ² ² ² ²	\mathbb{R}^4	HN(CH ₂),N(CH ₃), H CI	SH SCH ₂ Ph NH ₄ NHCH ₃		NH-c-N(CH, CH,), NCH, 0(CH,), N(CH,), S(CH,), N(CH,), HN(CH,), N(CH,),	$\frac{HN(CH_2)N(CH_3)}{HN(CH_2)^{4}N(CH_3)^{2}}$ $\frac{HN(CH_2)^{4}N(CH_3)}{HN(CH_2)^{6}N(CH_3)^{2}}$	HN(CH ₂),SCH ₃ HN(CH ₂),S(O)CH ₃ HN(CH ₂),OH HN(CH ₂),Cl	HN(CH ₂) ₃ CO ₂ ⁻ Na ⁺ HN(CH ₂) ₃ CO ₂ CH ₃ HN(CH ₂) ₃ NH ₂	HN(CH ₂) ₃ NHC(= O)H HN(CH ₂) ₃ NHCH ₃ HN(CH ₂) ₃ NHCH(CH ₃) ₂	$HN(CH_2)_{3}NHC(CH_{3})_{3}$ $HN(CH_2)_{3}NH-c-C_{6}H_{1}$	HN(CH ₂) ₃ NH	NIN(CH ₂) ₅ NH - N - N - N - N - CH ₃	HN HI N N HI CH 3	HN(CH ₂),N(C,H,),
	R	CH	ຍ້ອ້ອ້ອ້ອ	CH ₃	E CH CH	ยี้ยี่ยี่ยี่ยี่	ร์ย์ยี่ยี่ย	E E E E E	Ë E E	CH	CH ,	CH ,	cH ₃	CH,
	\mathbb{R}^2), CH3 CH3 CH3 CH3 CH3	ප්ප්ප්ප	CH ₃	CH CH CH CH CH CH CH CH CH CH CH CH CH C	CH CH CH CH CH CH CH CH CH CH CH CH CH C	ĊH ĊH ĊH ĊH Ċ	CH, CH, CH,	CH CH CH	CH, CH,	CH3	CH3	CH,	CH,
	R¹	H 5-HN(CH ₂) ₃ N(CH ₃ H H	нннн	H	ннин	H	ннн	H H	H H H H	Н	Н	Н	Н	Н
	No.	$\begin{array}{c}1\\79\\80\\81\end{array}$	8 2 7 7 7 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	87	88 90 91	92 94 93	95 96 98	99 100 101	102 103	105	107	108	109	110

	100	٨A		000		200 VA										ЧA	
NA	NA (100) 4	4	> 300	NA (100) 2	NA (100)	NA (100) 2 300 P	300	100	NA (100)	NA (100)	> 300	NA	NA (100)	NA (50)	NA (17)	NA ≥ 300 NA	NA
$C_{19}H_{27}N_{5}O_{2}\cdot 2HCI$	C ₂₃ H ₃₅ N ₅ .2HCV C ₁₉ H ₂₇ N ₅ O.2HCI	$C_{21}H_{31}N_5$ ·2HCl	$C_{21}H_{32}N_6.3HCl$	C ₁₈ H ₂₃ N ₅ .2HCl C ₁₉ H ₂₅ N ₅ .2HCl <i>k</i>	C ₁₉ H ₂₃ N ₅ O·HCl	C ₂₀ H ₂₇ N ₅ ·2HCl C ₁₉ H ₂₅ N ₅ O·2HCl	$C_{20}H_{28}N_6\cdot 2HCl$	C ₁₉ H ₂₆ N ₆ ·3HCl	C ₂₃ H ₃₃ N ₅ ·2HCl ¹	C ₁₉ H ₂₅ N ₅ ·2HCl	C ₁₉ H ₂₅ N ₅ ·2HCl	$C_{21}H_{23}N_5\cdot 2HCl$	C ₁₈ H ₁₇ N ₅ 2HCl	C ₁₉ H ₁₉ N ₅ ·2HCl	$C_{19}H_{19}N_5$ ·2HCl	C ₁₄ H ₁₆ N ₄ ·HCJ ^m C ₁₈ H ₂₅ N ₅ ·2HCl C ₁₉ H ₂₇ N ₅ ·2HCl C ₂₂ H ₃₄ N ₆ ·3HCl	C ₂₁ H ₂₉ N ₅ ·2HCl ⁿ
A	AC	Н	D	DA	в	AA	D	A	C	A	D	D	A	D	A	Q M I I	C
244-246.5 dec	100-105 dec 290 dec	236-239.5 dec	263-266	295–298 dec >300	234.5-237	> 300 > 300	266-270 dec	282-286 dec	180-210 dec	289-292 dec	298-305 dec	> 300	303-306 dec	>300	>300	235 220-224 dec 186-188.5 dec 227-230	140-150
HN(CH ₂),N(CH ₂ CH ₂ -	HN(CH ₂),N(n -C ₄ H ₂), HN(CH ₂ CH(OH)CH ₂ - HNCH ₂ CH(OH)CH ₂ -	$HNCH(CH_3)(CH_3)_3$ - NIC H)	$HN(CH_2)_{3}N(CH_3)(CH_3)(CH_2)_{3}$ -	N(CH ₃) ₂ HN(CH ₃) ₂ -e-NC ₄ H ₈ HN(CH ₂) ₃ -e-NC ₄ H ₈	Ne(2H)NH	$HN(CH_2)_3 - e - NC_5 H_{10}$ $HN(CH_2)_3 - e - N(CH_2 $	$CH_{_{2}})_{_{2}}O$ HN($CH_{_{2}})_{_{3}}$ -c-N($CH_{_{2}}$ -	UL12, 2, WCH3 HN(CH2,),-C-N(CH2- CH2,)2NH	HN (CH ₂) ₃ N C_{2H_5}	HN(CH ₂) ₂	HN CH ₃	HNCH ₂	NICH ₃ /2 HNCH ₂	HN(CH ₂)2	HN(CH ₂) ₂	CH ₃ NCH ₃ CH ₃ N(CH ₂),N(CH ₃), C ₂ H ₃ N(CH ₂),N(CH ₃), (CH ₃),N(CH ₂),N(CH ₃) ₂ M(CH ₃),N(CH ₂) ₃ .	(CH ₂)2 (CH ₂)2N(CH ₃)2
сH,	CH, CH,	CH3	сH,	CH, CH,	CH3	сн, СН	CH3	CH,	CH3	СН3	CH3	CH3	CH3	CH3	сH,	CH ³ CH ³ CH ³ CH ³	CH3
CH,	CH3 CH3	CH,	CH,	CH ₅ CH ₅	CH3	CH, CH,	CH,	CH3	CH,	CH,	CH,	CH3	CH3	CH3	CH3	CH ₅ CH ₅ CH ₅ CH ₅	CH3
Н	н	Н	Н	н	Н	нн	Н	Н	Н	Н	Н	Н	Н	Н	Н	ннн	Н
111	112 113	114	115	116 117	118	119 120	121	122	123	124	125	126	127	128	129	130 131 132 133	134

Table	IV (Continued)								
						Re- crystn sol-		Interfero MED (r	n assay, ^c ng/kg)
No.	R¹	\mathbb{R}^2	R³	R4	Mp, °C	venta	Formula ^b	ip	bo
				(CH ₂) ₂ N(CH ₃) ₂					
135	Н	CH ₃	сH,	Z	219-223	D	C ₂₁ H ₂₉ N ₅ ·2HCl	NA (50)	
136	Н	Н	CH3	$HN(CH_2)_3N(CH_3)_2$	>270	A	C ₁₆ H ₂₁ N ₅ ·2HCl·0.5H ₂ O ⁰	NA	
137	H	C_2H_5	CH,	$HN(CH_2)_3N(CH_3)_2$	278-280 dec	٩ı	C ₁₈ H ₂₅ N ₅ ·2HCl	NA (100)	NA
138	н	CH,CF'	CH	$HN(CH_2)_3N(CH_3)_2$	285-288 dec		C ₁₈ H ₂₂ F ₃ N ₅ ·2HCl	NA (100)	
140	н	CH(CH ₃) ₂ CH CH OH	É E	$HN(CH_2)_3N(CH_3)_2$	275 dec 951-954	- ⊲		NA (50) NA	
141	H	CH,CO,CH,	CH,	HN(CH,),N(CH,),	211 - 212	¢ 🖂	CHN.O2HCP	NA (100)	
142	Н	CH ₂ CO ₂ H	CH	$HN(CH_2)_3N(CH_3)_2$	295 dec	V	CI,H,,N,O,2HCI2H,O'	NA	
143	H	CH ₂ Ph	CH	$HN(CH_1)_3N(CH_3)_2$	248 - 250	E I	C ₁₃ H ₂₇ N ⁵ 2HCl	NA (100)	
144 145	п		E E	HN(CH ₂) ₃ N(CH ₃) ₂	268-271		$C_{22}H_{25}N_{5}$ ·2HCl	NA	
140 146	H	-C,II,- <i>P</i> -UCII, -C,H,- <i>P</i> -CO,H	я́но Но	HN(CH ₂) ₃ N(CH ₃) ₂ HN(CH ₁) N(CH ₁)	220-223 aec 147-158	י ר	$C_{23}H_{27}N_{5}O$ ZHCI	AN AN	
147	H	$-(CH_2)_3N(CH_3)_2$	CH	$HN(CH_1), N(CH_1),$	225-227.5	, D	C.,H.,N., 3HCI	NA (100)	NA
148	Н	$-CH_2C(=O)NH(CH_2)_3N(CH_3)_2$	CH	$HN(CH_2)_3N(CH_3)_2$	283-286 dec	Ω	C ₂₃ H ₃₅ N,O.3HCl	NA	
149	Н	-CH ₂ CH ₂ NH(CH ₂),N(CH ₃) ₂	CH,	$HN(CH_2)_3N(CH_3)_2$	250-255 dec	Ω,	C ₂₃ H ₃₇ N ₇ .4HCl.0.5H ₂ O	NA (9)	
151	н	$-(CH_2)_{N}(CH_3)_{2}$	É E		240-242 166 176		C ₁ ,H ₁ ,CIN ₄ ·HCI	NA (17) NA (17)	
152	Н		C.H.C	HN(CH_)_N(CH_)	233-234		$C_{16}H_{20}N_{4}T_{1}$, $H_{10}C_{10}$	NA (100)	
153	H	ĊH,	C,H,	HN(CH,),N(CH,),	285-287 dec	D D	CHN. 2HCP0.5H.O	NA (100)	200
154	H	CHj	Ή	HN(CH ₂),N(CH ₃)	280-282 dec	Α	C ₁₆ H ₂₁ N ₅ ² 2HCi-H ₂ O		200
155	6-F	CH	CH	$HN(CH_2)_3N(CH_3)_2$	266-269 dec	Ωï	C ₁₇ H ₂₂ FN, 2HCI		≥400
150	-1-1. 5-01	CH,	ร์ฮ	$HN(CH_2)_3N(CH_3)_2$	268-276 dec	ы¢	$C_1, H_2, FN_5, 2HCl \cdot H_2O$		≥400
158	10-6 10-6	Сп. СН	<u></u>	$\frac{HN(CH_2)_3N(CH_3)_2}{HN(CH_1) N(CH_2)}$	288-291 dec 302-205 dec	ר -		(100) AN	0017
159	8-CI	CH,	ĞĤ	HN(CH,),N(CH ₁)	246-252	¢ D	CHCIN 2HCI	NA NA	§100
160	6-I	CH ₃	CH	HN(CH ₂) ₃ N(CH ₃) ₂	298 dec	A	$C_{17}^{1}H_{22}^{21}IN_{5}\cdot^{2}HCI$	NA (100)	≥400
161	5-NO, 6 NO	CH, CU	Ê	$HN(CH_2)_3N(CH_3)_2$	261-265 dec	Q 4	$\mathbf{C}_{1}, \mathbf{H}_{2}, \mathbf{N}_{s}, \mathbf{O}_{2}, 2$ HCI		NA
163	0-NO2 7-NO	СП, СН	É H	HN(CH ₂) ₃ N(CH ₃) ₂	262-264 dec 961-965 dec	4 <	C_1 H ₂₂ N ₆ O ₂ ZHCl ²	NA (100)	
164	5-NH ²	CH,	GH	HN(CH,),N(CH,)	>300	¢ D	CHN. 3HCl ^t	NAN	
165	6-NH,	CH,	CH	HN(CH ₂) ₃ N(CH ₃) ²	> 300	D	$\mathbf{C}_{\mathbf{i}7}^{1}\mathbf{H}_{24}^{24}\mathbf{N}_{6}^{2}2\mathbf{H}\mathbf{C}\mathbf{l}^{u}$	NA	
165	6-CH ₃ C(=U)NH-	CH, CH	Ē	$HN(CH_2)_{JN}(CH_3)_{2}$	65-76 910-914	×	CI,H2,N,O	NA	C L
168	0-CH ₃ CH ₂ NH- 7-NH	CH.	É E	HN(CH ₂) ₃ N(CH ₃) ₂ HN(CH ₂) N(CH ₃)	210-214 201 204 400	- <		25	50 V I V
169	7-CH, C(=0)NH-	CH.	GH.	HN(CH ₂), N(CH ₂),	301-304 uec 171-173 5 der	¢ ن	$O_{17}H_{24}H_{6}$, Z. 3HOI		NA NA
170	5-CH ₃	CH	GH	$HN(CH_2)_3N(CH_3)_2$	>300	A	C,"H, N, 2HCI	NA (100)	NA
171	6-CH,	CH,	CH,	$HN(CH_2)_3N(CH_3)_7$	274 dec	D	CisH25N32HCI-0.5H20	50	50
172	7-CH, ° CH	CH,	Ű.	$HN(CH_2)_3N(CH_3)_2$	290-293 dec	٩û	C ₁₈ H ₂₅ N ₅ ·2HCI	50	50
174	0-CH3	CH	E E	HN(CH ₂) ₃ N(CH ₃) ₂ HN(CH ²) N(CH ²)	272 dec		C ₁₈ H ₂ , N ₅ 2HCl	NA (100)	NA
175	6-C,H	CH.	CH	HN(CH,),N(CH,),	248-252	בר	$C_{18} M_{22} F_{3} M_{5} Z H C C$	(100) AN (100)	006
176	$7 \cdot C_2 H_{s}$	CH	CH	$HN(CH_2)_3N(CH_3)_2$	248-251 dec	, H	C ₁₀ H ₂₇ N ₄ 2HCl	NA (100)	200
178	7-CH ₂ OH 6 7-/CH)	CH. CH	CH CH	$HN(CH_2)_3N(CH_3)_2$	296-298 dec	٩ R	C ₁₈ H ₂₅ N ₅ O·2HCl	100	200
179	7,8-(CH ₃)	CH3	E	$HN(CH_2)_3N(CH_3)_2$	240-245 dec	a –	C ₁₉ H ₂₇ N ₅ ·2HCl·2H ₂ O C ₁₉ H ₂₇ N ₅ ·2HCl·2H ₂ O	200 200	50 200

Substituted 1H-Pyrazolo[3,4-b]quinoline

180	6 8-(CH)	Hu	CH	HN(CH_), N(CH_).	251-253 dec	D	CHN.·2HCI		400
181	6-SO.H	CH.	CH,	HN(CH,),N(CH,),	310-313 dec	Ω	Ċĺ,H,,N,O,S	NA	
182	6-SO,NH.	CH	CH,	HN(CH,),N(CH,),	194.5 - 196.5	Q	C,H,N,O,S'	NA	
183	6-(CH,), N(CH,), NHSO.	CH,	CH,	HN(CH,),N(CH,),	233-237 dec	Q	C.,H.N.O.S.3HCI	NA	
184	7-CO.H	CH	CH,	HN(CH,),N(CH,),	>300	A	C,H,NO,2HCI	NA	
185	7-CO,CH,	CH	CH,	HN(CH,),N(CH,),	206-210 dec	A	CluH2NO2HCI	NA	
186	7-(CH,),N(CH,),NH(C=0)	CH,	CH,	HN(CH,),N(CH,),	259-267 dec	A	C23HCI-2H2O-3HCI-2H2O	NA	
187	$7-\dot{C}(=\dot{O})\dot{N}\dot{H},$	CHČ	CH,	HN(CH,),N(CH,),	282-285 dec	A	C ₁₈ H ₂₄ N ₆ O·2HCI·2H ₂ O		NA
188	5-CH,O	ĊH	CH,	HN(CH,),N(CH,),	278 dec	A	C ₁ ,H ₂ ,N,O·2HCl	100	100
189	6-CH,O	CH	CH,	HN(CH,),N(CH,),	281–282 dec	D	C ₁₈ H ₂ ,N,O·2HCl	NA (100	200
190	7-CH.O	CH	CH,	HN(CH,),N(CH,),	261–263 dec	Ω	C ₁₈ H ₂ N ₂ O ₂ HCl	50	50
191	8-CH ₂ O	CH	CH	HN(CH,),N(CH,),	261.5-263 dec	Q	C, H, N, O 2HCI	50	100
192	6-HO ^w	CH	CH,	HN(CH,),N(CH,),	> 300	×	C, H, N, O 2HCl	NA	
193	mOH-1	CH	ĊH,	HN(CH,),N(CH,),	301-304 dec	A	C,H,N,O.2HCl ^z	NA	
194	5-Cl. 8-CH.O	CH	CH	HN(CH,),N(CH,),	178-180 dec	I	C.H.,CIN, O-2HCI		NA (100
195	6.7-(CH.O).	CH	CH,	HN(CH,),N(CH,),	259-261 dec	D	C."H."N.O.2HCH0.5H,O	50	50
196	5 7-(CH_O)	CH	CH	HN(CH ²),N(CH ²),	285 dec	¥	C,H,NO,2HCI	25	25
197	5.6.7-(CH.O),	CH	ĊĤ	HN(CH,),N(CH,),	235-239 dec	E	C ₂₀ H ₂₀ N ₅ O ₅ 2HCI		NA (200
a	V actionits BtOH: B absolute BtC	DH. C. M. CN. D. FIOH. F. "-P.O.	H.F. ah	scolute RtOH-Rt O. G. Me	OH-REO H MeOI	H-Me	CO: 1 i-PrOH: J. DMF: K.	H.O. b (f. footnote
, i	Table I ^c Cf footnote c in Table	111, d , 111 , d , 111 , u	Lit ¹⁰ ml	$n 350^\circ$ f C: calcd 69.0	1: found, 69.62.	0	alcd. 57.57: found. 56.87.	^h N: ca	lcd, 23.29;
four four	1 = 0.0376 i Lit ¹⁰ mm for free bas	c m j C: calcd 60.79; found	d 60.3	$\frac{1}{3}$ $\frac{k}{k}$ C: calcd. 57.58: for	and. 57.16. ¹ C: c	calcd.	61.06; found, 59.24. ^m C	: calcd, (9.97; found
69.0	1, n N: calcd 16.50 ; found 17	36. ^o H: calcd 6.62; found 7	7.13. p	⁷ H: calcd. 6.80: found. 6	.27. ^q N: calcd.	16.35	found, 15.89. ^r H: calc	id, 6.49; fc	und, 6.20.
ż	calcd. 15.55; found. 14.83. Cl:	caled. 15.74: found. 15.39. ^a C	: calcd	l. 49.17; found, 48.68.	C: calcd, 48.41; fo	ound,	52.29. N: calcd, 19.93;	found, 19.	30. ^u C:
calct	1, 52.99; found, 52.52. ^v N: ca	cd, 22.32; found, 21.83. ^w Prel	pared fr	om demethylation of the	corresponding meth	hyl etl	hers using BBr ₃ in CH ₂ Cl ₂ t	oy the prov	sedure of J.
F. V	'. McOmie, M. L. Watts, and D. E.	West, Tetrahedron, 24, 2289 (1:	968). '	^x Insufficient sample for r	ecrystallization. ^y	H:	alcd, 6.52; found, 5.88. A	Aass spectr	um, M ⁺ 313
z C:	calcd, 52.85; found, 50.40. N:	calcd, 18.13; found, 17.48. M	lass spec	ctrum, M ⁺ 313.					

67 (base). The early fractions (4.30 g) were recrystallized from MeCN to remove the less soluble 81; the filtrate was evaporated and the residue was converted to the HCl salt which was recrystallized (absolute EtOH) to yield pure 80 (1.71 g, 28%), mp 252-255° dec. The latter fractions were recrystallized in succession from MeCN and PhH; the HCl salt then was prepared and recrystallized (EtOH) to yield 67 (1.65 g, 27%), mp 219-235°, indicated by GLC to be 97% pure.

1-(3-Dimethylaminopropyl)-3-methyl-1H-pyrazolo[3,4b]quinoline Dihydrochloride (151). A solution of 150 (3.03 g, 0.01 mol) in EtOAc (200 ml) and Et₃N (1.39 ml, 0.01 mol) containing 10% Pd/C (0.6 g) was shaken under H₂ at 50 psi until 1 equiv was absorbed (25 min). Usual work-up gave a residue which was triturated under Me₂CO to remove Et₃N-HCl. Evaporation of the Me₂CO gave the free amine of the title compound which was treated with 2 equiv of HCl. Recrystallization (*i*-PrOH) of the resultant di-HCl salt resulted in partial loss of HCl: yield 1.49 g (45%); mp 165–175°. Anal. Calcd for C₁₆H₂₀N₄·1.7HCl: C, 58.25; H, 6.59; Cl, 18.19; N, 16.98. Found: C, 58.54; H, 6.99; Cl, 18.23; N, 17.03; M⁺ 268.

1,3-Dimethyl-5,6,7,8-tetrahydro-1H-pyrazolo[3,4-b]quinoline Hydrochloride (68). The free amine of the 4benzylthio compound 83 (3.72 g, 0.0117 mol) in absolute EtOH (150 ml) containing Raney nickel (4 tsp) was stirred at reflux for 64 hr. Usual work-up gave a mixture of products from which the major component was separated by chromatography on alumina (elution with MeCN): yield 0.88 g (38%) from which the HCl salt was prepared; recrystallized (MeCN) to give 68, mp 215– 218.5°.

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-5,6,7,8tetrahydro-1*H*-pyrazolo[3,4-*b*]quinoline Dihydrochloride (69). A mixture of the di-HCl salt of 1^{10} (3.70 g, 0.01 mol), NaOAc·3H₂O (2.72 g, 0.02 mol), and PtO₂ in AcOH was shaken under H₂ (50 psi initially) for 16 hr. Usual work-up gave quantitatively the title free base (pure by GLC assay) which was converted to the di-HCl salt and recrystallized (*i*-PrOH) to yield 69, mp 269-271.5°.

4-(3-Dimethylaminopropylamino)-3-ethyl-1-methyl-1Hpyrazolo[3,4-b]quinoline Dihydrochloride (153). A mixture of 4-(3-dimethylaminopropylamino)-3-ethyl-1H-pyrazolo[3,4b]quinoline (136, 2.24 g, 0.0076 mol) and NaH (0.0076 mol) dispersion in oil was stirred under N₂ in DMF (32 ml) at 55° for 30 min. Methyl iodide (1.07 g, 0.0076 mol) was added and the mixture was heated at 42° for 18 hr. Usual work-up gave 1.99 g (85%) of 153 free amine containing a small amount of starting material; the latter was removed by chromatography on alumina; the product was converted to the salt and recrystallized to give 153, mp 285-287° dec.

Similarly prepared from 136 and the indicated alkylating agent were 140 $(BrCH_2CH_2OTHP)^{23}$ and 141 $(BrCH_2CO_2CH_3)$. Basic hydrolysis of 141 gave 142.

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-5-nitro-1H-pyrazolo[3,4-b]quinoline Dihydrochloride (161). A mixture of the 6-nitro acid 38 (5.00 g, 0.018 mol) and concentrated H₂SO₄ (30 ml) was stirred at 95° for 1.25 hr. The mixture was cooled, poured onto ice, and, after stirring 30 min, basified with concentrated NH₄OH. The precipitate (3.48 g, 75%) was separated by filtration, washed, dried, and then stirred in refluxing POCl₃ (40 ml) for 1 hr. Work-up as described above (cf. 172) gave the 4-chloropyrazolo[3,4-b]quinoline intermediate (2.32 g, 62%). Treatment of this product with equimolar quantities of 3-dimethylaminopropylamine and K₂CO₃ in DMAC at 90° for 4.5 hr gave, after usual work-up, 161: mp 261-265° dec after recrystallization (EtOH); yield 2.08 g (60%).

1,3-Dimethyl-5-(3-dimethylaminopropylamino)-1*H*pyrazolo[3,4-b]quinoline Dihydrochloride (79). A solution of 4-chloro-1,3-dimethyl-5-nitro-1*H*-pyrazolo[3,4-b]quinoline (7.95 g, 0.029 mol; cf. preceding example), Et₃N (4 ml, 0.029 mol), and 10% Pd/C (0.8 g) in EtOAc (200 ml) was shaken under H₂ at 50 psi for 16 hr. Work-up gave a product recrystallized from CH₃NO₂ to yield 5.08 g (75%) of 5-amino-1,3-dimethyl-1*H*-pyrazolo [3,4-b]quinoline, mp 227.5-231.5°. A 3.92-g (0.019 mol) sample of this product in DMAC (80 ml) containing NaH (0.019 mol) was heated at 50° for 1.5 hr. A solution of 3-dimethylaminopropyl chloride (2.31 g, 0.019 mol) in DMAC (60 ml) was added dropwise and the resultant mixture heated at 85° for 16 hr. After usual

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work-up, the product 79 was obtained by chromatography on basic alumina (elution with Et_2O): yield 1.40 g (20%).

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-6-nitro-1H-pyrazolo[3,4-b]quinoline Dihydrochloride (162). A mixture of 70% nitric acid (1.2 ml) and 96% sulfuric acid (13.6 ml) was added dropwise over 30 min to a solution at 5° of 1 (4.91 g, 0.017 mol) in nitromethane (30 ml). After the addition, the mixture was stirred at 10-20° for 2 hr and then was diluted with cold water and neutralized with ammonium hydroxide. The product was isolated by extraction into chloroform and worked up in the usual manner; yield 4.61 g (82%); mp 189-192°. The dihydrochloride salt had mp 262-264° dec (aqueous EtOH), identical with 162 prepared from the acid 37.

6-Amino-1,3-dimethyl-4-(3-dimethylaminopropylamino)-1H-pyrazolo[3,4-b]quinoline Dihydrochloride (165) and Derivatives. The above nitro compound 162 (4.00 g, 0.012 mol) suspended in EtOH (100 ml) containing 5% Pd/C (0.4 g) was shaken under hydrogen at an initial pressure of 50 psi for 2.5 hr. The catalyst was removed by filtration and the solvent was evaporated to leave the title compound. It was necessary to store the compound under nitrogen because it rapidly darkens upon exposure to air. The compound was converted to the salt in the usual manner and recrystallized (EtOH): mp 305° dec.

Similarly prepared from 161 and 163 were 164 and 168, respectively. Treatment of 165 or 168 with 1 equiv of Ac_2O in pyridine at 28° for 16 hr gave the 6- and 7-acetamido derivatives 166 and 169, respectively. The 6-ethylamino derivative 167 was prepared by reduction of 166 using BH₃ in THF.

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-7-nitro-1H-pyrazolo[3,4-b]quinoline Dihydrochloride (163). The 4-nitro acid 36 (5.00 g, 0.018 mol) was converted to the 4chloropyrazolo[3,4-b]quinoline derivative VI ($\mathbb{R}^1 = 7$ -NO₂; $\mathbb{R}^2 =$ CH₃) by the procedure described above for 172. This intermediate (2.76 g, 0.01 mol) was heated at 90° for 4.5 hr in DMAC (30 ml) containing 3-dimethylaminopropylamine (1.02 g, 0.01 mol) and K₂CO₃ (1.38 g, 0.01 mol). Usual work-up gave 3.33 g (97%) which was converted to the salt and recrystallized (aqueous *n*-PrOH) to 163, mp 261-265° dec.

10,8-Dimethyl-7-(3-dimethylaminopropylamino)-10*H*pyrido[2,3-*h*]pyrazolo[3,4-*b*]quinoline Dihydrochloride (77). A mixture of the 4-nitro acid 36 (15.00 g, 0.054 mol) and POCl₃ (150 ml) was heated at reflux for 2 hr. Excess POCl₃ was evaporated (5 mm) and the residue was treated (cautious addition with cooling) with 3-dimethylaminopropylamine (150 ml). The mixture was stirred at 130° for 16 hr and then was worked up as described for 172. The crude product was chromatographed on alumina (elution with 98:2 CH₂Cl₂-EtOH) to yield 5.3 g which was recrystallized (CH₃NO₂) to mp 137-138.5°; the structural assignment 77 was made on the basis of the spectral and analytical data and was proved by an independent synthesis.¹⁶

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-6-sulfo-1*H*-pyrazolo[3,4-*b*]quinoline (181) and Derivatives. The di-HCl salt of 1 (3.00 g, 0.008 mol) was added slowly to cold ClSO₃H (15 ml), and then the mixture was heated at 70° for 1 hr. Excess ClSO₃H was removed (5 mm) and the residue was cautiously treated with cold dilute NH₄OH. The resultant precipitate was collected by filtration, washed with water, and recrystallized (EtOH) to yield 181 (2.38 g, 78%), mp 310–313° dec. Similarly prepared were 182 (except the intermediate 6-SO₂Cl was treated with concentrated NH₄OH; yield 47%) and 183 (except treatment was with 3-dimethylaminopropylamine followed by refluxing for 8 hr; yield 64%).

7-Carbomethoxy-1,3-dimethyl-4-(3-dimethylaminopropylamino)-1*H*-pyrazolo[3,4-*b*]quinoline Dihydrochloride (184) and Derivatives. A mixture of *N*-(1,3-dimethylpyrazolo-5-yl)-4-carboxyanthranilic acid (20.0 g, 0.073 mol) and POCl₃ (200 ml) was stirred at reflux for 2 hr. Excess POCl₃ was evaporated and the residue was divided into two parts: (i) part treated with excess 3-dimethylaminopropylamine as described above for 172 to give 186 (79%); (ii) part treated with excess cold MeOH and then worked up to give VI ($R^1 = 7$ -CO₂CH₃; $R^2 = CH_3$) which was treated with 1 molar equiv of 3-dimethylaminopropylamine in DMF as described above (cf. 92) to give 184 (58%). Basic hydrolysis of 184 gave the acid 185. Treatment of 185 with SOCl₂ followed by NH4OH gave 187.

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-5-eth-

oxycarbonyl-1*H*-pyrazolo[3,4-*b*]pyridine Dihydrochloride (64a). A mixture of 4-chloro-1,3-dimethyl-5-ethoxycarbonyl-1*H*-pyrazolo[3,4-*b*]pyridine²⁴ (10.0 g, 0.04 mol), 3-dimethylaminopropylamine (4.24 g, 0.04 mol), and K₂CO₃ (5.45 g, 0.04 mol) in DMAC (100 ml) was stirred at 100° for 10 hr. Usual work-up followed by treatment with aqueous HCl gave 64a (10.9 g, 70%) after recrystallization (EtOH).

The acid **64b** was prepared by refluxing crude **64a** base in aqueous ethanolic NaOH for 15 hr, followed by usual work-up; the yield of **64b** after recrystallization (aqueous EtOH) was 30%.

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-1*H*pyrazolo[3,4-*b*]pyridine Dihydrochloride (64c). 1,3-Dimethyl-4-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acid (3.70 g, 0.018 mol; prepared from the corresponding ethyl ester²⁴) was kept at 230° for 20 min. The crude decarboxylated product was heated at reflux in POCl₃ (30 ml) for 4 hr. Usual work-up gave the 4-chloro derivative which was dissolved in 3-dimethylaminopropylamine (40 ml) and the resultant solution heated at 115° for 15 hr. The product was chromatographed on basic alumina (elution with 99:1 Et₂O-absolute EtOH) to yield 64c base (1.02 g, 23%) which was converted to 64c by treatment with 2 equiv of HCl.

2-(1,3-Dimethylpyrazol-5-ylamino)nicotinic Acid (57). A mixture under nitrogen of 5-amino-1,3-dimethylpyrazole (4.85 g, 0.05 mol) and NaH (0.05 mol) in hexamethylphosphoramide (HMPA, 50 ml) was stirred at 45° for 2 hr. Similarly, 2-chloronicotinic acid (7.85 g, 0.05 mol) and NaH (0.05 mol) were stirred in HMPA (60 ml) at 25° for 2 hr. The two salt solutions were mixed and stirred at 80° for 16 hr. The mixture was poured onto ice water, and the resultant solution was extracted twice with CHCl₃ and then was acidified with 1 N HCl to precipitate 57 (7.38 g 64%): mp 263-265° dec after recrystallization (EtOH).

N-(1,3-Dimethylpyrazol-5-yl)-5-nitroanthranilic acid (37) was prepared from the sodium salts of 5-amino-1,3-dimethylpyrazole and 2-chloro-5-nitrobenzoic acid in HMPA by the preceding procedure used for 57. The yield of 37 of mp 275–278° dec was 90%. Recrystallization of an aliquot from a large volume of EtOH gave the analytical sample having mp 278–280° dec.

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-1*H*pyrazolo[3,4-b][1,8]naphthyridine Dihydrochloride (70). A mixture of the acid 57 (5.30 g, 0.023 mol) and POCl₃ (60 ml) was heated at reflux for 3 hr. Excess POCl₃ was evaporated (5 mm) and the residue was cooled and treated with 3-dimethylaminopropylamine (75 ml). The mixture was heated at 130° for 16 hr and then worked up as described above for 172: yield 5.10 g (60%); mp 298° dec (aqueous EtOH).

4-(1,3-Dimethyl-5-pyrazolylamino)pyrimidine-5-carboxylic Acid (58). A mixture of 5-carbethoxy-4-chloropyrimidine²⁵ (18.6 g, 0.1 mol), 5-amino-1,3-dimethylpyrazole (9.70 g, 0.1 mol), and Et₃N (10.1 g, 0.1 mol) in benzene (100 ml) was heated at reflux for 16 hr. Usual work-up and recrystallization (MeCN) gave 58 ethyl ester (7.48 g, 29%) which was hydrolyzed in refluxing aqueous ethanolic NaOH to yield 58 (3.88 g, 58%), mp 284–286° dec (DMF).

1,3-Dimethyl-4-(3-dimethylaminopropylamino)-1*H*pyrazolo[4',3':5,6]pyrido[2,3-*d*]pyrimidine dihydrochloride (71) was prepared from the acid 58 by the procedure described above for 163 with the exception that in the last step heating was at 80° for 4 hr: yield 74%; mp 276-280° dec.

5-Acetoacetylamino-1,3-dimethyl-4-pyrazolecarboxylic Acid (198). A mixture of 5-amino-1,3-dimethyl-4-pyrazolecarbonitrile¹⁵ (16.32 g, 0.12 mol) and NaOH (30.12 g, 0.754 mol) in H₂O (120 ml) was stirred at reflux for 15 hr. The solution was cooled to -5° and then treated with aqueous HCl (200 ml of 1 N; 100 ml of 4 N; 25 ml of 1 N; added in succession by dropwise addition). Diketene (11.10 g, 0.13 mol) was added dropwise at 15-30°. The mixture was stirred 2 hr at 25° (evolution of CO₂ was detected during addition of the last portions of the diketene at 30°) and then was cooled and acidified. Filtration yielded 198 (10.9 g, 38%), mp 194° dec. An aliquot dissolved in DMF (60°) was filtered and the filtrate diluted with Et₂O to give an analytical sample, mp 198° dec. Anal. (C₁₀H₁₃N₃O₄) H, N; C: calcd, 50.21; found, 49.75.

2-Acetonyl-5,7-dimethyl-4,7-dihydropyrazolo[4,3-d]-3,1oxazin-4-one (199). A mixture of the acid 198 (4.94 g, 0.021 mol) and Ac₂O (2.28 g, 0.022 mol) in DMF (50 ml) was stirred at 85°

Substituted 1H-Pyrazolo[3,4-b]quinoline

for 16 hr. The solvent was evaporated (5 mm) and the residue was extracted with CH₂Cl₂ to yield crude **199** (4.24 g) which was recrystallized (MeCN) to yield **199** as white needles (0.76 g, 17%): mp ~130-165° (NMR indicates that in CDCl₃ about 75% is in the enol form). Anal. (C₁₀H₁₁N₃O₃) C, H, N; M⁺ 221.

Bis(1,3-dimethylpyrazol-5-yl)amine (202). In an attempt to obtain the acid 200, the crude oxazinone 199 was treated with methylhydrazine in AcOH as described above for 39. The product was chromatographed on neutral alumina (elution with CH₂Cl₂) to give 202 (yield, 39%): mp 156.5-158.5° (MeCN); NMR (CDCl₃) δ 2.18 (s, 6, CH₃C), 3.60 (s, 6, CH₃N), 5.50 (s, 2, pyrazole ring CH), 6.14 ppm (s, 1, NH; exchangeable). The sharp single absorbances for the two methyl groups and the ring proton are considered as evidence to support assignment as the symmetrical structure 199 as opposed to the unsymmetrical isomer which would result from decarboxylation of 201. Anal. (C10H15N5) C, H, N; M⁺ 205.

1,7-Dihydro-4-(3-dimethylaminopropylamino)-1,3,5,7tetramethyldipyrazolo[3,4-b:4',3'-e]pyridine Dihydrochloride (72) and 1,2,5,7-Tetramethyl-1H,5H-dipyrazolo[1,5-a:5',4'd]pyrimidin-8-one (203). A mixture of the crude oxazinone 199 (2.24 g, 0.01 mol) and methylhydrazine (0.93 g, 0.02 mol) was stirred in DMF (25 ml) at 80° for 2 hr. The DMF was removed and the residue was partitioned between water containing NaOH (0.01 mol) and CHCl₃. Evaporation (4 mm) of the water left a foam (2.7 g). POCl₃ (60 ml) was cautiously added with cooling and the mixture was heated at reflux for 2.5 hr. Excess POCl₃ was removed and the residue was treated with 3-dimethylaminopropylamine (30 ml). The mixture was heated at reflux for 15 hr and then was worked up as described above (cf. 172) to yield 1.04 g; chromatography on neutral alumina (elution with CH₂Cl₂) gave in initial fractions 203 (326 mg, 14%), mp 178.5-180.5 (MeCN). Anal. (C11H13N5O) C, H, N; M+ 231. Later fractions gave 72 free base (265 mg, 8%), M⁺ 315, which was converted to the di-HCl salt and recrystallized (EtOH) to 72, mp 272-275° dec.

The 3-normethyl relative 73 was similarly prepared.

N-(3-Methylisothiazol-5-yl)anthranilic Acid (59). A mixture of o-iodobenzoic acid (45.0 g, 0.18 mol), 5-amino-3-methylisothiazole hydrochloride (30.0 g, 0.20 mol), K₂CO₃ (54.8 g, 0.4 mol), and Cu (4.5 g, 1 μ) in water (250 ml) was stirred at reflux for 5 hr. Standard work-up and recrystallization (absolute EtOH) gave 59 (7.38 g, 17%), mp 212-213° dec.

4-(3-Dimethylaminopropylamino)-3-methylisothiazolo-[5,4-b]quinoline dihydrochloride (75) was prepared from the acid 59 by the general procedure described above for 172; purified by chromatography on alumina prior to conversion to the salt, mp >300°.

4-(3-Dimethylaminopropylamino)-1-methylpyrrolo[2,3b]quinoline (74). A mixture of 4-chloro-1-methylpyrrolo-[2,3-b]quinoline^{26,27} (134 mg, 0.62 mmol) and KI (100 mg, 0.6 mmol) in 3-dimethylaminopropylamine (25 ml) was heated at reflux for 15 hr. The mixture was worked up as described for 172. Chromatography (neutral alumina; elution with Et20 plus 0.5% EtOH) gave 74 (86 mg, 49%) as a gum; elemental analyses were not obtained owing to scarcity of material; NMR (CDCl₃) δ 1.8 (m, 2, CH₂CH₂CH₂), 2.24 [s, 6, (CH₃)₂N], 2.45 (t, 2, CH₂N), 3.74 (s, 3, NCH₃), 3.86 (t, 3, HNCH₂), 6.59 (d, 1, NCH=CH), 6.87 (d, 1, NCH=CH), 7.0-8.0 (m, 4, aro); M⁺ 282.

N-(1-Methylpyrazol-5-yl)anthranilic Acid (29). A mixture of o-iodobenzoic acid (20.6 g, 0.08 mol), 5-amino-1-methylpyrazole hydrochloride¹⁴ (12.0 g, 0.09 mol), K₂CO₃ (24.8 g, 0.18 mol), and Cu (2.25 g, 0.035 g-atom; 1- μ mesh) in water (47 ml) was stirred at reflux for 16 hr. Additional water (135 ml) was added; the mixture was refluxed 30 min, then filtered, and worked up in the usual manner; recrystallization (EtOH) gave 29 (7.62 g, 42%), mp 174–176.5°.

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