was separated on a paper strip chromatogram using the system butanol:phosphate buffer (pH 2.0) into 6-deoxy-7-nitrotetracycline and 6-deoxy-9-nitrotetracycline-7-^{3}H. A radio-scan[13] of this chromatogram showed very little radioactivity for the 7-nitro and a great deal of radioactivity for the 9-nitro-7-^{3}H derivative.

Acknowledgments.—We wish to express our appreciation to Mr. A. C. Dornbush for his coöperation with microbiological assays and to Dr. D. A. Buyske for obtaining the radioactivity determinations. We are also indebted to Mr. L. Brancone for microanalyses and to Mr. W. Fulmor for spectroscopic results.

Synthetic Amebicides. VI. Benzo[b][1,8]phenanthrolines, Benzo[b][1,10]phenanthrolines, Dibenzo[b,h][1,6]naphthyridines, and Benzo[h]quino[4,3-b]quinolines[1]

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Various 7-(mono- and dialkylaminoalkylamino)-benzo[b][1,8]phenanthrolines, -benzo[b][1,10]phenanthrolines and -dibenzo[b,h][1,6]naphthyridines have been prepared for antiamebic evaluation. These compounds were prepared by the condensation of the appropriate 7-chloroheterocyclic compound with a mono or dialkylaminoalkylamine in phenol. When tested against *Entamoeba histolytica in vitro* and against experimentally induced intestinal amebiasis in the rat, several of these compounds exhibited good antiamebic activity. Attempts to prepare 3-chloro-7-(3-diethylaminopropylamino)benzo[h]quino[4,3-b]quinoline were unsuccessful.

The preparation of various 7-(mono and dialkylaminoalkylamino)-benz[c]acridines as potential antiamebic agents has been reported previously.[2-4] Many of these compounds are more potent than 4-

(1) For previous paper in this series, see E. F. Elslager, and L. M. Werbel, *J. Org. Chem.*, **26**, 1337 (1961).

(2) E. F. Elslager, A. M. Moore, F. W. Short, M. J. Sullivan, and F. H. Tendick, *J. Am. Chem. Soc.*, **79**, 4699 (1957).

(3) F. W. Short, E. F. Elslager, A. M. Moore, M. J. Sullivan, and F. H. Tendick, *ibid.*, **80**, 223 (1958).

(4) E. F. Elslager, F. W. Short, M. J. Sullivan, and F. H. Tendick, *ibid.*, **80**, 451 (1958).

aminoquinolines and 9-aminoacridines such as chloroquine and quinacrine.[2-5] 7-(3-Octylaminopropylamino)benz[c]acridine dihydrochloride (I) emerged as one of the most promising members of the series and exhibits good antiamebic activity in experimental animals[5] and in man.[6]

·2 HCl

I

In order to study further the effects of nucleus variation on chemical structure-antiamebic activity relationships, we have investigated various mono- and dialkylaminoalkylamino heterocyclic compounds in which a second nitrogen atom has been introduced into the benz[c]acridine nucleus. Eleven of the sixty-six possible diazabenz[a]anthracene ring systems have been synthesized previously.[7]

The present paper describes the preparation of certain 7-(mono- and dialkylaminoalkylamino)-benzo[b][1,8]phenanthrolines, -benzo(b)-[1,10]phenanthrolines and -dibenzo[b,h][1,6]naphthyridines together with 3,7-dichlorobenzo[h]quino[4,3-b]quinoline. The benzo[b][1,8]-phenanthroline and benzo[h]quino[4,3-b]quinoline ring systems are not listed in *Chemical Abstracts* or the "Ring Index[8]" and appear to be new heterocyclic systems.

Condensation of the potassium salt of the appropriate *o*-chlorobenzoic acid (II) with 5-aminoisoquinoline (III) utilizing modifications of the procedures described by Ullmann[9] and by Bachman and Picha[10] gave the corresponding N-(5-isoquinolyl)anthranilic acid hydrochlorides (IVa and b). Ring-closure with phosphorus oxychloride afforded 7-chloro- and 7,10-dichlorobenzo[b][1,8]phenanthroline (Va and b), which were allowed to react with various mono- and dialkylaminoalkylamines in phenol to give the desired 7-(mono- and dialkylaminoalkylamino)benzo[b][1,8]phenanthrolines (VI) (Table I, methods I and II).

(5) P. E. Thompson, D. A. McCarthy, J. W. Reinertson, A. Bayles, and H. Najarian, *Antibiotics and Chemotherapy*, **8**, 37 (1958).

(6) R. A. Radke, *Gastroenterology*, **36**, 509 (1959).

(7) C. F. H. Allen, "The Chemistry of Heterocyclic Compounds. Six-membered Heterocyclic Nitrogen Compounds With Four Condensed Rings," A. Weissberger, ed., Interscience Publishers, New York, N. Y., 1951, p. 91.

(8) A. M. Patterson, L. T. Capell, and D. F. Walker, the "Ring Index," Second Edition, American Chemical Society, Washington, D. C. 1960.

(9) F. Ullmann, *Ann.*, **355**, 347 (1907).

(10) G. B. Bachman and G. M. Picha, *J. Am. Chem. Soc.*, **68**, 1959 (1946).

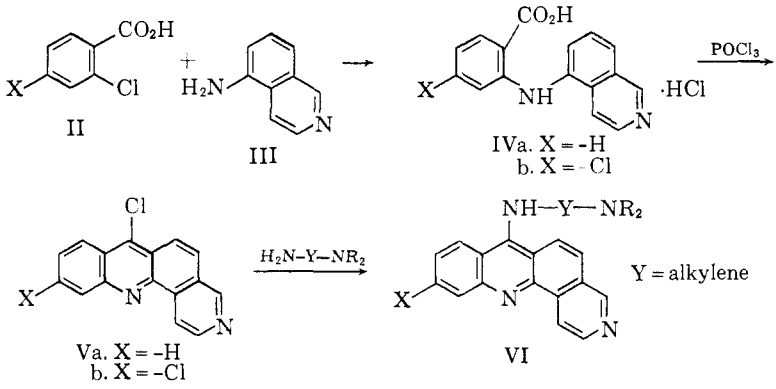
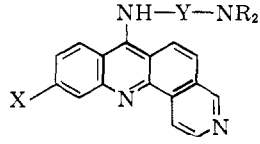


TABLE I

7-(MONO- AND DIALKYLAMINOALKYLAMINO)BENZO[b][1,8]PHENANTHROLINES[a,b]



X	Y	NR_2	M.p., °C.	Yield purified, %	Procedure	Purification solvent[c]
H	$-(CH_2)_3-$	$-N(CH_2)_4$	120 (eff.)	77	I	A
Cl	$-(CH_2)_5-$	$-N(CH_2)_5$	230 (dec.)	77	II	B
Cl	$-(CH_2)_3-$	$-NH(CH_2)_7CH_3$	245 (dec.)	48	II	B
Cl	$-CHCH_3(CH_2)_3-$	$-NC_2H_5CH_2CH_2OH$	200–205 (dec.)	28	II	C
Cl	$-(CH_2)_2-$	$-N[(CH_2)_2]_2NH$	250 (dec.)	59	II	B

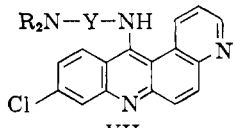
Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
$C_{23}H_{24}N_4 \cdot 3HCl \cdot 2.25H_2O$[d]	54.55	54.75	6.27	6.31	11.06	10.53
$C_{26}H_{29}ClN_4 \cdot 3HCl \cdot 1.5H_2O$	54.84	54.68	6.19	6.08	9.84	9.55
$C_{27}H_{33}ClN_4 \cdot 3HCl \cdot 0.5H_2O$	57.14	57.03	6.57	6.53	9.87	9.87
$C_{25}H_{29}ClN_4O \cdot 3HCl \cdot 2H_2O$	51.55	51.33	6.23	6.63	9.62	9.80
$C_{22}H_{22}ClN_5 \cdot 4HCl \cdot 3H_2O$	44.65	44.61	5.45	5.91	11.84	12.19

[a] All compounds are yellow or orange-yellow solids. [b] Allowed to equilibrate in air prior to analysis. [c] A, not recrystallized; B, ethanol–acetone; C, ethanol–ether. [d] Water (Karl Fischer), calcd., 8.00; found, 8.17.

The preparation of several 7-amino and 7-(dialkylaminoalkylamino)benzo[b][1,10]phenanthrolines previously has been reported.[11–14] Much of this early work was stimulated by the observa-

(11) J. Dobson and W. O. Kermack, *J. Chem. Soc.*, 150 (1946).
(12) H. R. Snyder and H. E. Freier, *J. Am. Chem. Soc.*, **69**, 1543 (1947).
(13) J. Dobson, W. C. Hutchison and W. O. Kermack, *J. Chem. Soc.*, 123 (1948).
(14) J. H. Wilkinson and I. L. Finar, *ibid.*, 288 (1948).

tion that various 9-chloro-12-(mono and dialkylaminoalkylamino)-benzo[b][4,7]phenanthrolines (VII) possessed marked antimalarial activity against *Plasmodium gallinaceum* in chicks.[11] Although the



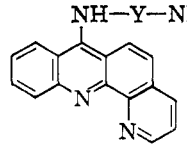
Y = alkylene

VII

7-dialkylaminoalkylaminobenzo[b][1,10]phenanthrolines of structure X were devoid of antimalarial activity even at high doses,[11-13] it was of interest to prepare representative 7-aminobenzo[b][1,10]phenanthrolines for antiamebic evaluation inasmuch as the apparent correlation of antimalarial and antiamebic properties among certain quinolines and acridines cannot be assumed to hold for other heterocyclic systems.[2] The 7-(mono- and dialkylaminoalkylamino)benzo[b][1,-10]phenanthrolines (X) (Table II, methods III and IV) were prepared by condensing 7-chlorobenzo[b][1,10]phenanthroline (IX) with the

TABLE II

7-(MONO- AND DIALKYLAMINOALKYLAMINO)BENZO[b][1,10]PHENANTHROLINES[a]

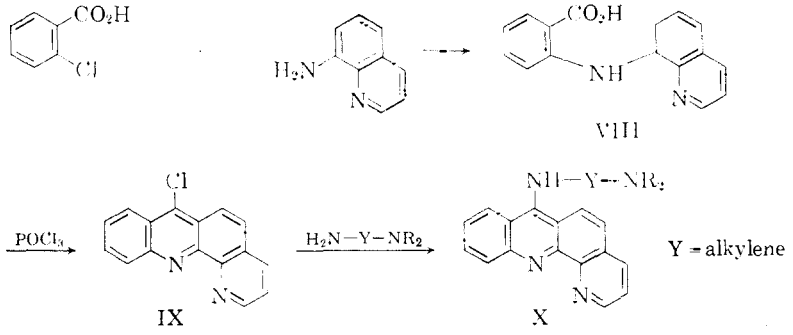


Y	NR_2	M.p., °C.	Yield purified, %	Procedure	Purification solvent[b]
$-(CH_2)_3-$	$-N(C_2H_5)_2$	244–245 (dec.)	81	III	A
$-(CH_2)_5-$	$-N(CH_2)_5$	s. 75	51	IV	D
$-(CH_2)_3-$	$-NH(CH_2)_7CH_3$	184–186 (dec.)	57	III	B, C
$-(CH_2)_3-$	$-N(CH_2CH_2OH)_2$	250–252	55	III	C

Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
$C_{23}H_{26}N_4 \cdot 2HCl$[c]	64.03	63.95	6.56	7.05	12.99	12.82
$C_{26}H_{30}N_4 \cdot 2C_{11}H_8O_3 \cdot 2.5H_2O$[d,e]	70.31	70.51	6.27	6.01	6.83	6.29
$C_{27}H_{34}N_4 \cdot 2HCl \cdot H_2O$[d]	64.15	63.94	7.58	7.88	11.08	11.36
$C_{23}H_{26}N_4O_2 \cdot 2HCl \cdot H_2O$[d]	57.38	57.75	6.28	6.50	11.64	11.95

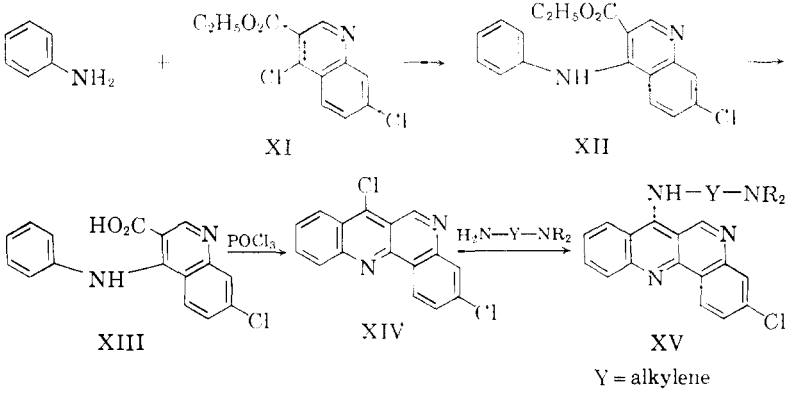
[a] All compounds are yellow solids. [b] A, 2-Propanol; B, acetone–water; C, ethanol; D, sample not recrystallized. [c] Dried *in vacuo* at 100° prior to analysis. [d] Allowed to equilibrate in air prior to analysis. [e] $C_{11}H_8O_3$ represents 3-hydroxy-2-naphthoic acid.

appropriate diamine in phenol as described previously.[11] The intermediate chloroheterocycle was obtained by phosphorus oxychloride



ring-closure of N-8-quinolylanthranilic acid (VIII), the product of the Ullmann reaction with 8-aminoquinoline and *o*-chlorobenzoic acid.[11]

The synthesis of several 7-(dialkylaminoalkylamino)dibenzo[b,h]-[1,6]naphthyridines (XV) also has been described.[15] Utilizing modifications of these procedures, ethyl 7-chloro-4-hydroxy-3-quinolinecarboxylate was chlorinated with a mixture of phosphorus oxychloride and phosphorus pentachloride to give ethyl 4,7-dichloro-3-quinolinecarboxylate (XI), which was condensed with aniline to give ethyl



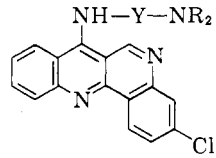
4-anilino-7-chloro-3-quinolinecarboxylate (XII). Hydrolysis with methanolic potassium hydroxide afforded the corresponding acid XIII, which was cyclized with phosphorus oxychloride to 3,7-dichlorodibenzo[b,h][1,6]naphthyridine (XIV). Compound XIV was allowed to react with various mono- and dialkylaminoalkylamines in

(15) W. O. Kermack and N. E. Storey, *J. Chem. Soc.*, 1389 (1951).

phenol (method V) to give the 3-chloro-7-(mono- and dialkylaminoalkylamino)dibenzo[b,h][1,6]naphthyridines (XV) (Table III). 7,7′-Ethylenebis(iminoethyleneimino)bis[3-chlorodibenzo[b,h][1,6]naphthyridine] (XVI) was prepared from two moles of XIV and one mole of triethylenetetramine.

TABLE III

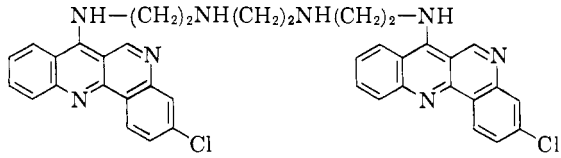
3-CHLORO-7-(MONO- AND DIALKYLAMINOALKYLAMINO)DIBENZO[b,h][1,6]-NAPHTHYRIDINES[a]



Y	NR_2	M.p., °C.	Yield purified, %	Procedure	Purification solvent[b]
$-(CH_2)_3-$	$-N(C_2H_5)_2$	122–123	74	V	A
$-(CH_2)_5-$	$-N(CH_2)_5$	148	78	V	B
$-(CH_2)_3-$	$-NH(CH_2)_7CH_3$	118–119	75	V	C, D

Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
$C_{23}H_{25}ClN_4$	70.30	70.37	6.41	6.63	14.26	14.47
$C_{26}H_{29}ClN_4$	72.12	71.96	6.75	6.92	12.94	13.09
$C_{27}H_{33}ClN_4$	72.22	72.59	7.41	7.76	12.48	12.72

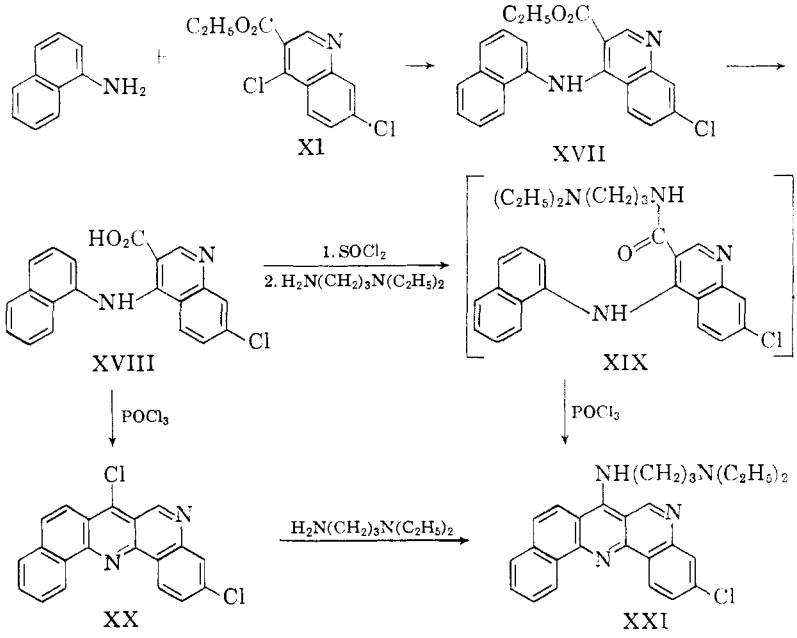
[a] All compounds are yellow solids. [b] A, Ethyl acetate–petroleum ether (b.p. 80–110°); B, ethyl acetate; C, 2-propanol; D, acetone.



XVI

In an analogous manner, ethyl 4,7-dichloro-3-quinolinecarboxylate (XI) was condensed with 1-naphthylamine and the intermediate ethyl 7-chloro-4-(1-naphthylamino)-3-quinolinecarboxylate (XVII) was hydrolyzed to the acid XVIII. Cyclization with phosphorus oxychloride gave 3,7-dichlorobenzo[h]quino[4,3-b]quinoline (XX), but attempts to prepare 3-chloro-7-(3-diethylaminopropylamino)benzo[h]quino[4,3-b]quinoline (XXI) either by the condensation of XX with N,N-diethyl-1,3-propanediamine or by ring closure via the amide XIX by known methods[2] were unsuccessful.

The absorption spectra of these derivatives are similar to those observed with the 7-(mono- and dialkylaminoalkylamino)benz[c]-acridines,[2-3] although the introduction of a second heterocyclic



nitrogen atom at different positions in the benz[c]acridine nucleus produces characteristic changes. Figure 1 gives the ultraviolet-visible absorption of representative compounds in 50% methanol-50% 0.1 *N* hydrochloric acid solution. Substitution of nitrogen at position 3 of the benz[c]acridine nucleus causes pronounced resolution of the intense band which occurs near 290 mμ with the 7-amino-benz[c]acridines and a bathochromic shift of the band at long wave length. Nitrogen at position 1 has comparatively little effect, although there is increased resolution at lower wave lengths. The principal effect of nitrogen at position 5 is a hypsochromic shift of the long wave length band.

The 7-(mono and dialkylaminoalkylamino)-benzo[b][1,8]phenanthrolines, -benzo[b][1,10]phenanthrolines and -dibenzo[b,h][1,6]naphthyridines described in the present communication were tested by Dr. Paul E. Thompson and co-workers of these laboratories against *Entamoeba histolytica in vitro*,[16] and against acute intestinal amebiasis

(16) For a description of test methods, see P. E. Thompson, J. W. Reinertson, D. A. McCarthy, A. Bayles and A. R. Cook, *Antibiotics and Chemotherapy*, **5**, 433 (1955).

in the rat.[17] In general, these compounds were much less active both *in vitro* and in rats than the corresponding 7-aminobenz[c]acridines. Amebicidal concentrations *in vitro* varied from 15 to 2000 μg./ml. In experimentally infected rats, most of the aminoheterocyclic com-

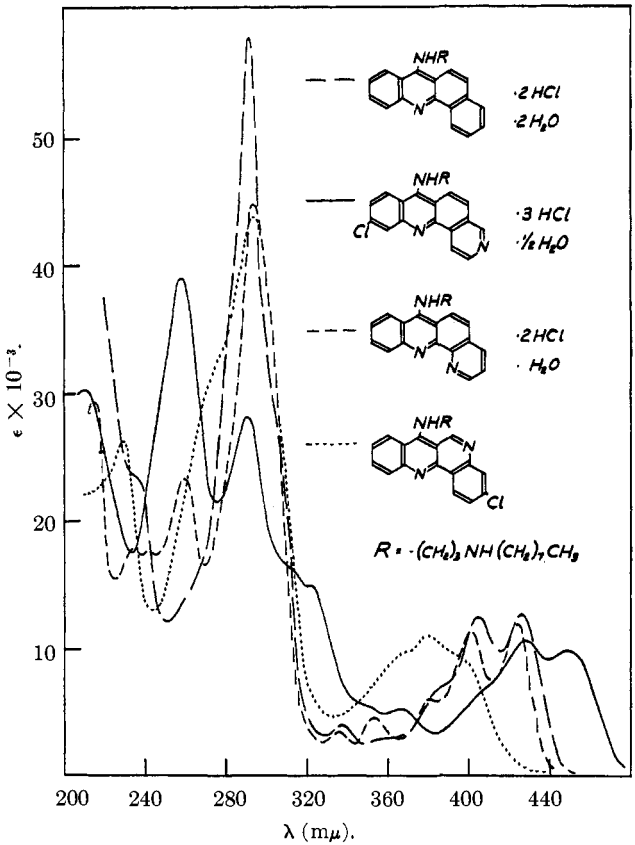


Fig. 1.—Absorption in 50% MeOH–50% 0.1 *N* HCl solution taken with a Cary 11 spectrophotometer.

pounds exhibited little or no antiamebic activity at maximum tolerated doses, although the 7-(5-piperidinopentylamino)benzo[b][1,10]-phenanthroline salt with 2 equivalents of 3-hydroxy-2-naphthoic acid, 10 - chloro - 7 - (5 - piperidinopentylamino)benzo[b][1,8]-phenanthroline trihydrochloride, and 10-chloro-7-[2-(1-piperazinyl)-ethylamino]benzo[b][1,8]phenanthroline tetrahydrochloride reduced

(17) For a description of test methods, see P. E. Thompson, M. C. Dunn, A. Bayles and J. W. Reinertson, *Am. J. Trop. Med.*, **30**, 203 (1950).

the average degree of infection >50% at diet concentrations varying from 0.0625 to 0.2% for a period of seven days.[18]

Acknowledgment.—The authors take this opportunity to thank Dr. Loren M. Long for encouragement in this investigation, Dr. Paul E. Thompson, Miss Anita Bayles, Mrs. Sheila Herbst, Dr. D. A. McCarthy, Dr. Haig Najarian, and Miss Bronislawa Olszewski for the antiamebic evaluation of these compounds, and Dr. Franklin W. Short and Miss Helen E. Svarath for synthesizing several of the compounds described herein. The authors are also indebted to Mr. Charles E. Childs and associates for the microanalyses, and to Dr. J. M. Vandenbelt and associates for determination and interpretation of the infrared and ultraviolet absorption spectra.

Experimental[19]

N-(5-Isoquinolyl)anthranilic Acid Hydrochloride (IVa).—A mixture of 57.6 g. (0.40 mole) of 5-aminoisoquinoline, 62.4 g. (0.40 mole) of *o*-chlorobenzoic acid, 58.0 g. (0.42 mole) of anhydrous potassium carbonate, 1 g. of copper powder and 800 ml. of dry 1-pentanol was stirred and boiled under reflux for 5 hr. Water was continuously removed through a Barrett distilling receiver. The reaction mixture was cooled and the solid collected by filtration, washed thoroughly with acetone, and dried. The grey powder was added to 250 ml. of 2 *N* hydrochloric acid and the suspension was stirred and warmed on the steam-bath to give a homogeneous thick paste. The crude yellow salt was collected by filtration, washed with 10 ml. of 2 *N* hydrochloric acid, and dried *in vacuo;* yield 26.5 g. (22%). Crystallization from methanol (decolorizing charcoal) containing a few drops of ethanolic hydrogen chloride yielded bright yellow crystals, m.p. 262–264° (dec.).

Anal. Calcd. for $C_{16}H_{12}N_2O_2 \cdot HCl \cdot 1.5H_2O$: C, 58.62; H, 4.92; N, 8.55. Found: C, 58.98; H, 5.13; N, 8.38.

4-Chloro-N-(5-isoquinolyl)anthranilic Acid Hydrochloride (IVb).—A mixture of 43.2 g. (0.30 mole) of 5-aminoisoquinoline, 57.3 g. (0.30 mole) of 2,4-dichlorobenzoic acid, 41.5 g. (0.30 mole) of anhydrous potassium carbonate, 0.5 g. of copper powder and 400 ml. of 1-pentanol was stirred and boiled under reflux for 5 hr. The water formed was removed continuously through a Barrett distilling receiver During the last hour of heating, approximately 100 ml. of 1-pentanol was removed. Upon cooling, the reaction mixture was diluted with an equal volume of acetone and the solid collected by filtration, washed with acetone and dried. The filter cake was added to 1 l. of boiling 2 *N* hydrochloric acid, cooled and filtered. The residue was crystallized from a methanol-acetone mixture (decolorizing charcoal) to give 15.0 g. (15%) of yellow crystals, m.p. 291° (dec.).

Anal. Calcd. for $C_{16}H_{11}ClN_2O_2 \cdot HCl$: C, 57.33; H, 3.61; N, 8.36. Found: C, 57.48; H, 3.72; N, 8.18.

7-Chlorobenzo[b][1,8]phenanthroline (Va).—A mixture of 23.1 g. (0.0874 mole) of N-(5-isoquinolyl)anthranilic acid hydrochloride (IVa) and 100 ml. of phosphorus oxychloride was stirred and boiled under reflux for 3 hr. Upon cooling, the melt was poured slowly with stirring into an excess of ice and concentrated

(18) P. E. Thompson, unpublished results.

(19) Melting points are uncorrected.

ammonium hydroxide, and the mixture was stirred for 2 hr. The pale yellow solid was collected by filtration, washed with water and dried. The crude product was crystallized twice from a benzene–petroleum ether (b.p. 80–110°) mixture (decolorizing charcoal) to give 9.0 g. (39%) of pale yellow needles, m.p. 201–202°.

Anal. Calcd. for $C_{16}H_9ClN_2$: C, 72.59; H, 3.42; N, 10.58. Found: C, 72.24; H, 3.90; N, 10.61.

7,10-Dichlorobenzo[b][1,8]phenanthroline (Vb).—A mixture of 51.0 g. (0.152 mole) of 4-chloro-N-(5-isoquinolyl)anthranilic acid hydrochloride (IVb) and 200 ml. of phosphorus oxychloride was stirred and slowly heated to the reflux temperature. A gel formed, and 50 ml. of phosphorus oxychloride was added to form a clear solution. After boiling under reflux for 2.5 hr., a portion of the phosphorus oxychloride was removed *in vacuo* and the clear residue was poured slowly with vigorous stirring into an excess of ammonium hydroxide and ice. The mixture was stirred for 2 hr. and allowed to stand at room temperature for 18 hr. The tan solid was collected by filtration and dried at 60°. Crystallization from chlorobenzene (decolorizing charcoal) yielded 39.1 g. (85%) of pale yellow needles, m.p. 260°.

Anal. Calcd. for $C_{16}H_8Cl_2N_2$: C, 64.23; H, 2.69; N, 9.36. Found: C, 64.27; H, 2.92; N, 9.11.

Ethyl 4,7-Dichloro-3-quinolinecarboxylate (XI).—A mixture of 25.1 g. (0.10 mole) of ethyl 7-chloro-4-hydroxy-3-quinolinecarboxylate, 40 ml. of phosphorus oxychloride and 21.0 g. (0.10 mole) of phosphorus pentachloride was boiled under reflux for 2 hr. and cooled. This mixture was poured slowly with stirring into 1 l. of concentrated ammonium hydroxide and 1 kg. of ice, keeping the mixture alkaline to phenolphthalein at all times. Stirring was continued for 2 hr., and the precipitate was collected by filtration and air-dried. The solid was leached with several portions of boiling petroleum ether (b.p. 80–110°), the residue was discarded, and the petroleum ether extracts were combined, treated with decolorizing charcoal, evaporated until crystallization began, and cooled. The colorless rods which separated were collected by filtration and dried; yield, 21.5 g. (80%), m.p. 80–82°.

Anal. Calcd. for $C_{12}H_9Cl_2NO_2$: C, 53.36; H, 3.36. Found: C, 53.50; H, 3.62.

When the above procedure was repeated on a 0.20 and 0.50 mole scale, the yields were 40 and 45%, respectively.

Ethyl 4-Anilino-7-chloro-3-quinolinecarboxylate (XII).—Ethyl 4,7-dichloro-3-quinolinecarboxylate (XI) (55.5 g., 0.205 mole) was stirred with 20.0 g. (0.215 mole) of aniline in a beaker. An exothermic reaction occurred, the mixture became pasty, and the slurry turned orange, then yellow in color. The mixture was allowed to cool and was digested with warm dilute hydrochloric acid to free it from the beaker. The acid suspension was stirred into an excess of dilute ammonium hydroxide and extracted with ether. The combined ether extracts were dried over anhydrous potassium carbonate, the drying agent was collected by filtration, and the ether removed *in vacuo*. The oily residue was crystallized from ethanol to give 55.5 g. (82%) of pale yellow needles, m.p. 114–115°.

Anal. Calcd. for $C_{18}H_{15}ClN_2O_2$: C, 66.15; H, 4.62; N, 8.57. Found: C, 65.79; H, 4.56; N, 8.56.

4-Anilino-7-chloro-3-quinolinecarboxylic Acid (XIII).—A mixture of 55.5 g. (0.17 mole) of ethyl 4-anilino-7-chloro-3-quinolinecarboxylate (XII) and 200 ml.

of 10% methanolic potassium hydroxide was boiled under reflux for 1 hr. and poured into 2 l. of water. The mixture was acidified with dilute acetic acid and the acid was collected by filtration, washed with water and dried. The pale yellow powder was digested with boiling ethanol, cooled and filtered; yield, 50.2 g. (98%), m.p. 278° (dec.).

Anal. Calcd. for $C_{16}H_{11}ClN_2O_2$: C, 64.32; H, 3.71; N, 9.38. Found: C, 64.19; H, 3.74; N, 9.33.

3,7-Dichlorodibenzo[b,h][1,6]naphthyridine (XIV).—A mixture of 20.0 g. (0.067 mole) of 4-anilino-7-chloro-3-quinolinecarboxylic acid (XIII) and 100 ml. of phosphorus oxychloride was boiled under reflux for 3 hr. The cooled melt was poured slowly with stirring into an excess of ice and concentrated ammonium hydroxide, taking precautions to maintain the mixture alkaline to phenolphthalein at all times. The solid was collected by filtration, washed with water and dried *in vacuo* at 50° for 18 hr. Crystallization from chloroform gave 12.4 g. (62%) of cream-colored fibrous needles, m.p. 242°.

Anal. Calcd. for $C_{16}H_8Cl_2N_2$: C, 64.23; H, 2.69; N, 9.36. Found: C, 64.36; H, 2.55; N, 9.41.

Ethyl 7-Chloro-4-(1-naphthylamino)-3-quinolinecarboxylate (XVII).—A mixture of 10.8 g. (0.04 mole) of ethyl 4,7-dichloro-3-quinolinecarboxylate (XI) and 5.7 g. (0.04 mole) of 1-naphthylamine was placed in a beaker and stirred and heated on the steam bath for 1 hr. A clear melt formed initially but crystallization soon began and the thick mass became difficult to stir. The mush was removed from the beaker with a mixture of ammonium hydroxide and chloroform and sufficient ether was added to float the chloroform layer. The organic layer was separated and dried over anhydrous potassium carbonate, and the solvents were removed *in vacuo*. Crystallization of the residue from ethanol gave 11.1 g. (74%) of fibrous yellow needles, m.p. 171°.

Anal. Calcd. for $C_{22}H_{17}ClN_2O_2$: C, 70.11; H, 4.55; N, 7.43. Found: C, 69.92; H, 4.41; N, 7.34.

7-Chloro-4-(1-naphthylamino)-3-quinolinecarboxylic Acid (XVIII).—Ethyl 7-chloro-4-(1-naphthylamino)-3-quinolinecarboxylate (XVII) (10.0 g., 0.027 mole) was hydrolyzed with potassium hydroxide in methanol according to the method outlined above for the preparation of 4-anilino-7-chloro-3-quinolinecarboxylic acid; yield, 9.2 g. (99%) of yellow crystals, m.p. 278° (dec.).

Anal. Calcd. for $C_{20}H_{13}ClN_2O_2$: C, 68.86; H, 3.75; N, 8.03. Found: C, 68.57; H, 3.98; N, 7.72.

3,7-Dichlorobenzo[h]quino[4,3-b]quinoline (XX).—A mixture of 20.0 g. (0.057 mole) of 7-chloro-4-(1-naphthylamino)-3-quinolinecarboxylic acid (XVIII) and 120 ml. of phosphorus oxychloride was boiled under reflux for 3 hr. The reaction mixture was cooled and poured into a mixture of ice and excess ammonium hydroxide with vigorous mechanical stirring. The crude product was collected by filtration, washed with water, and dried; weight, 19.4 g. (98%). Crystallization from chloroform (decolorizing charcoal) gave yellow crystals, m.p. 279°.

Anal. Calcd. for $C_{20}H_{10}Cl_2N_2$: C, 68.78; H, 2.88; N, 8.02. Found: C, 68.05; H, 3.00; N, 7.77.

Methods for Preparing 7-(mono- and dialkylaminoalkylamino)-benzo[b][1,8]-phenanthrolines, -benzo[b][1,10]phenanthrolines and -dibenzo[b,h][1,6]naphthyridines (Tables I–III).—Method I.—A mixture of 0.034 mole of the chloro heterocyclic compound, 0.030 mole of the appropriate amine and 40 g. of phenol was stirred and heated at 100° for 3 hr. The reaction mixture was cooled, poured

into a large excess of 10% sodium hydroxide solution and the base extracted with ether. The combined ether extracts were washed successively with dilute sodium hydroxide solution and water and dried over anhydrous potassium carbonate. The drying agent was collected by filtration, and the filtrate treated with anhydrous hydrogen chloride. The crude hydrochloride salt was collected by filtration, washed with acetone and ether and dried *in vacuo* at 35–50°. Crystallization from the solvent indicated gave the desired amino heterocyclic compound.

Method II.—A mixture of 0.033 to 0.040 mole of the chloro heterocyclic compound, 0.050 mole of the amine and 25 to 50 g. of phenol was stirred and heated at 125–135° for 3 hr. The cooled reaction mixture was poured into 1 l. of acetone containing an excess of ethanolic hydrogen chloride. The crude salt which separated was collected by filtration, washed thoroughly with acetone and dried *in vacuo*. The dry salt was suspended in 25 ml. of ethanol, an excess of ammonium hydroxide was added, and the mixture was shaken with 1 l. of chloroform. The chloroform solution was washed successively with several portions of dilute sodium hydroxide solution and water, filtered and the chloroform removed *in vacuo*. The residue was dissolved in methanol or ethanol (decolorizing charcoal) and the alcohol solution made strongly acid to congo red with ethanolic hydrogen chloride and diluted with several volumes of acetone or ether. The yellow hydrochloride which separated was collected by filtration, washed with acetone or ether, and dried *in vacuo*. Purification from the solvents indicated gave the desired salts.

Method III.—A mixture of 0.030 mole of the chloro heterocyclic compound, 0.032 mole of the appropriate amine and 25 g. of phenol was stirred and heated at 100° for 3 hr. Upon cooling, the reaction mixture was poured slowly into a beaker containing a stirred solution of 10 ml. of concentrated hydrochloric acid in 200 ml. of anhydrous acetone. The mixture was diluted with an equal volume of acetone and chilled. The crude hydrochloride salt was collected by filtration, washed throughly with acetone and dried *in vacuo* at 40°. Crystallization from the solvent indicated (decolorizing charcoal) yielded the pure salts.

Method IV.—A mixture of 0.038 mole of the chloro heterocyclic compound, 0.042 mole of the amine and 40 g. of phenol was stirred and heated at 100° for 3 hr. The cooled reaction melt was poured into a solution of 10 ml. of concentrated hydrochloric acid in 125 ml. of acetone. The orange-yellow oil which formed could not be induced to solidify upon trituration with a variety of organic solvents. The oil was shaken with a solution of 10 g. of sodium hydroxide in 100 ml. of water and the alkaline mixture extracted with three 150-ml. portions of chloroform. The combined chloroform extracts were washed with three 150 ml. portions of water and dried over anhydrous sodium carbonate. To the chloroform solution of the base was subsequently added a solution of 14.3 g. (0.076 mole) of 3-hydroxy-2-naphthoic acid in 250 ml. of anhydrous ether, and the solvents were decanted from the sticky solid which separated. The solid was triturated with several portions of anhydrous ether, collected by filtration, washed thoroughly with anhydrous ether and dried *in vacuo* at room temperature for 18 hr.

Method V.—A mixture of 0.07 mole of the appropriate amine and 50 g. of phenol was heated *in vacuo* on the steam-bath for 3 hr. to remove traces of moisture. The chloro heterocyclic compound (0.04 mole) was then added, and the mixture stirred and heated at 110–120° for 4 hr. The melt was cooled and poured slowly with stirring into a large excess of aqueous sodium hydroxide. The base

which separated was extracted thoroughly with ether, and the combined ether extracts were washed successively with several portions of dilute sodium hydroxide and water. The base was thoroughly extracted from the ether with *N* acetic acid solution and the extract was filtered and made strongly alkaline with sodium hydroxide. The base was again extracted with ether, the combined ether extracts were dried over anhydrous potassium carbonate, and the ether was removed. Crystallization of the residues from the solvents indicated gave the desired bases as bright yellow solids.

7,7′-Ethylenebis(iminoethyleneimino)bis[3-chlorodibenzo[b,h][1,6]naphthyridine] (XVI).—A mixture of 2.92 g. (0.02 mole) of triethylenetetramine (Eastman Kodak Co.) and 40 g. of phenol was heated *in vacuo* on the steam-bath for 3 hr. to remove traces of moisture; 11.96 g. (0.04 mole) of 3,7-dichlorobenzo-[b,h][1,6]naphthyridine (XIV) subsequently was added, and the mixture was stirred and heated at 110–125° for 4 hr. The warm reaction mixture was diluted with ethanol and stirred into a solution of 25 ml. of concentrated hydrochloric acid in 250 ml. of acetone. The mixture was further diluted with 500 ml. of acetone, and allowed to stand for 18 hr. The crude yellow hydrochloride salt was collected by filtration, dried, suspended in ethanol, and made strongly alkaline with concentrated ammonium hydroxide. The mixture was shaken with chloroform, filtered, and the aqueous layer discarded. The residue and chloroform layer were combined and evaporated to dryness. The residue was triturated with several portions of boiling ethanol and filtered. The bright yellow residue weighed 6.0 g. (45%), m.p. 248–252°. Recrystallization from a mixture of dimethylformamide, 2-propanol and petroleum ether (b.p. 80–110°) (decolorizing charcoal) raised the melting point to 256–257°.

Anal. Calcd. for $C_{38}H_{32}Cl_2N_8$: C, 67.95; H, 4.80; N, 16.69. Found: C, 68.14; H, 4.59; N, 16.92.

Potential Purine Antagonists. XXXI. The Preparation of Certain 9-Alkyl-2-amino-6-purinethiols and Related Derivatives as Antitumor Agents[1]

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A new, convenient preparation has been achieved for certain 9-alkyl-2-amino-6-purinethiols and their 6-alkylthio derivatives. A number of these compounds exhibit complete tumor inhibition of Adenocarcinoma 755 *in vivo* at varied dosage levels. These data are presented and discussed.

Studies of the antitumor activity of 2-amino-9-methyl-6-purine-