μg/ml. Of the remaining compounds tested in *E. coli* moderate growth inhibition (80–90%) was found with L-3,3′-dithiobis(2-aminopropionamide) dihydrochloride (300 μg/ml) and L-2-amino-3-(diphenylmethylthio)-propionamide (600 μg/ml).

None of the 38 compounds tested showed any significant cysteine–cystine replacement activity for growth of *L. mesenteroides*. In summary, none of the 38 compounds tested showed significant growth inhibition of *L. mesenteroides* and *E. coli*. In most instances, this inhibition was readily reversed by the addition of cysteine.

The microbiological assay and testing procedures were similar to those previously described.[1]

Antiamebic, Antimalarial, and Anthelmintic Effects of Distal Hydrazine Analogs of Azacrine, Quinacrine, and 7-{[3-(Octylamino)propyl]amino}benz[c]acridine[1,2]

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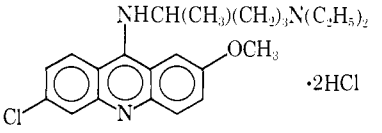
An array of basically substituted 9-aminoacridines,[3–11] 7-aminobenz[*c*]acridines,[3,4,9,10,12–14] and aminobenzonaphthyridines[4,9,15–18] exhibit noteworthy antiprotozoal, anthelmintic, antibacterial, and antitumor properties. Among them, quinacrine (I),[3–6] 3-chloro-9-{[4-(diethylamino)-1-methylbutyl]amino}acridine 10-oxide dihydrochloride (II),[7] 7-{[3-(octylamino)propyl]amino}benz[*c*]acridine dihydrochloride (III),[13] and azacrine (IV)[15] have been demonstrated to have appreciable antiprotozoal and anthelmintic activity in man. It was therefore of interest to synthesize representative {[3-(2,2-dialkylhydrazino)alkyl]amino}acridines, benz[*c*]acridines, and benzo[*b*][1,5]-naphthyridines to enable a determination of the effects of a distal hydrazine moiety on antiprotozoal and anthelmintic activity.



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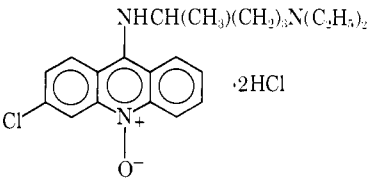

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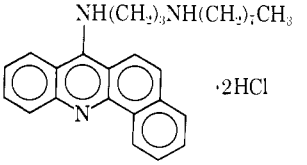

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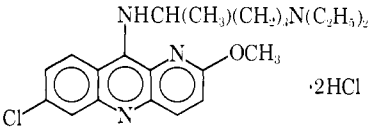
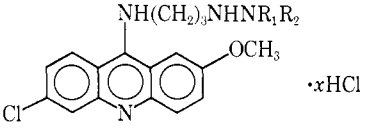


IV

The condensation of 6,9-dichloro-2-methoxyacridine with 2-(3-aminopropyl)-1,1-dimethylhydrazine,[19] 1-[(3-aminopropyl)amino]piperidine,[19] and 1-[(3-aminopropyl)amino]-4-methylpiperazine[19] in phenol afforded 6-chloro-9-{[3-(2,2-dimethylhydrazino)propyl]amino}-2-methoxyacridine dihydrochloride (Va) (55%), 6-chloro-2-methoxy-9-{[3-(piperidinoamino)-propyl]amino}acridine dihydrochloride (Vb) (38%), and 6-chloro-



Va, $NR_1R_2 = N(CH_3)_2$
b, $NR_1R_2 = N(CH_2)_5$
c, $NR_1R_2 = N[(CH_2)_2]_2NCH_3$

2-methoxy-9-({3-[(4-methyl-1-piperazinyl)amino]propyl}amino)acridine trihydrochloride (Vc) (40%), respectively (Table I, procedures I and II). 7-{[3-(Piperidinoamino)propyl]amino}benz[*c*]acridine dihydrochloride (VIa) and 7-({3-[(4-methyl-1-piperazinyl)amino]propyl}amino)benz[*c*]acridine hydrochloride (VIb) (68%) were obtained in a similar

(1) This is paper X of a series on synthetic amebicides and paper XVII of a series relating to antimalarial substances. For the previous paper, see L. M. Werbel, E. F. Elslager, A. A. Phillips, D. F. Worth, P. J. Islip, and M. C. Neville, *J. Med. Chem.*, **12**, 521 (1969).

(2) This is communication III of a series on anthelmintic drugs. For paper II, see D. B. Capps, O. D. Bird, E. F. Elslager, Z. B. Gavrilis, J. A. Roush, P. E. Thompson, and J. W. Vaitkus, *J. Heterocyclic Chem.*, **5**, 355 (1968).

(3) A. Albert, "The Acridines," 2nd ed, Edward Arnold, London, 1966.

(4) For a review, see E. F. Elslager in "Medicinal Chemistry," A. Burger, Ed., 3rd ed, Interscience Division of John Wiley and Sons, Inc., New York, N. Y., 1969.

(5) For a review, see P. B. Russell in "Medicinal Chemistry," A. Burger, Ed., 2nd ed, Interscience Publishers, Inc., New York, N. Y., 1960, pp 814–850.

(6) For a review, see O. D. Standen in "Experimental Chemotherapy," Vol. 1, R. J. Schnitzer and F. Hawking, Ed., Academic Press, New York, N. Y., 1963, pp 701–892.

(7) E. F. Elslager, R. E. Bowman, F. H. Tendick, D. J. Tivey, and D. F. Worth, *J. Med. Pharm. Chem.*, **5**, 1159 (1962).

(8) E. F. Elslager and F. H. Tendick, *ibid.*, **5**, 1153 (1962).

(9) N. B. Ackerman, D. K. Haldorsen, F. H. Tendick, and E. F. Elslager, *ibid.*, **11**, 315 (1968).

(10) E. F. Elslager, F. W. Short, and F. H. Tendick, *J. Heterocyclic Chem.*, **5**, 599 (1968).

(11) E. F. Elslager, F. H. Tendick, and L. M. Werbel, *J. Med. Chem.*, **12**, 600 (1969).

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

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

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

(15) D. M. Besly and A. A. Goldberg, *J. Chem. Soc.*, 2448 (1958).

(16) E. F. Elslager and F. H. Tendick, *J. Med. Pharm. Chem.*, **5**, 546 (1962).

(17) P.-L. Chien and C. C. Cheng, *ibid.*, **11**, 164 (1968).

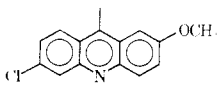
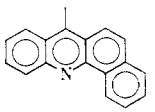
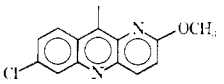
(18) E. F. Elslager, F. H. Tendick, and S. C. Perricone, 1969, unpublished data.

(19) E. F. Elslager, E. A. Weinstein, and D. F. Worth, *ibid.*, **7**, 493 (1964).

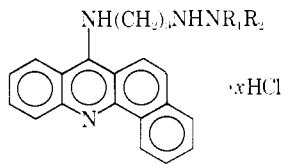
TABLE I

{[3-(2,2-DIALKYLHYDRAZINO)PROPYL]AMINO}ACRIDINES, BENZ[*c*]ACRIDINES, AND BENZO[*b*][1,5]NAPHTHYRIDINES[a]

HET-NH(CH$_2$)$_3$NHNR$_1$R$_2$

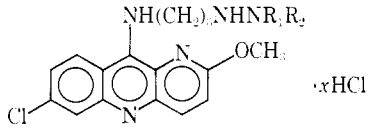
No.	–Het	NR$_1$R$_2$	Procedure	Purification solvent[b]	Yield, %	Mp, °C	Formula	Analyses[c]
Va		N(CH$_3$)$_2$	I	A	55	205–208	C$_{19}$H$_{23}$ClN$_4$O·2HCl·1.2H$_2$O	C, H, Cl; N[d]
Vb		N(CH$_3$)$_5$	II	B	38	132–135	C$_{22}$H$_{27}$ClN$_4$O·2HCl·1.5H$_2$O	C, H, N, H$_2$O
Vc		N[(CH$_2$)$_2$]$_2$NCH$_3$	I	C	40	257–258	C$_{22}$H$_{28}$ClN$_5$O·3HCl·2.5H$_2$O	C, H, N, H$_2$O
VIa		N(CH$_3$)$_5$	III	D	51	>190	C$_{25}$H$_{28}$N$_4$·2HCl·1.8H$_2$O	C, H, N, H$_2$O
VIb		N[(CH$_2$)$_2$]$_2$NCH$_3$	II	A	68	>170	C$_{28}$H$_{29}$N$_5$·2.7HCl	C, H, N, Cl
VIIa		N(CH$_3$)$_2$	I	C	41	228–230	C$_{18}$H$_{22}$ClN$_3$O·2HCl·1.1H$_2$O	C, H, N, H$_2$O
VIIb		N[(CH$_2$)$_2$]$_2$NCH$_3$	I	E	37	215–218	C$_{21}$H$_{27}$ClN$_5$O·3HCl·2.7H$_2$O	C, H, N, H$_2$O

[a] All compounds were yellow solids. [b] A, *i*-PrOH; B, EtOH; C, MeOH; D, not recrystallized; E, EtOH–*i*-PrOH. [c] N: calcd, 12.36; found, 11.76.



VIa, NR$_1$R$_2$ = N(CH$_3$)$_2$
b, NR$_1$R$_2$ = N[(CH$_2$)$_2$]$_2$NCH$_3$

manner from 7-chlorobenz[*c*]acridine[12–14] and the appropriate aminoalkylhydrazine[19] (Table I, procedures II and III). Treatment of 7,10-dichloro-2-methoxybenzo[*b*][1,5]naphthyridine[15] with 2-(3-aminopropyl)-1,1-dimethylhydrazine[19] and 1-[(3-aminopropyl)amino]-4-methylpiperazine[19] in phenol yielded 7-chloro-10-{[3-(2,2-dimethylhydrazino)propyl]amino}-2-methoxybenzo[*b*][1,5]naphthyridine dihydrochloride (VIIa) and 7-chloro-2-methoxy-10-({3-[(4-methyl-1-



VIIa, NR$_1$R$_2$ = N(CH$_3$)$_2$
b, NR$_1$R$_2$ = N[(CH$_2$)$_2$]$_2$NCH$_3$

piperazinyl)amino]propyl}amino)benzo[*b*][1,5]naphthyridine trihydrochloride (VIIb) in 44 and 37% yield, respectively (Table I, procedure I).

The {[3-(2,2-dialkylhydrazino)propyl]amino}acridines (Va–e), benz[*c*]acridines (VIa and b), and benzo[*b*][1,5]naphthyridines (VIIa and b) were tested against a variety of protozoa, helminths, and bacteria including *Plasmodium lophurae*, *Entamoeba histolytica*, *Trichomonas vaginalis*, *Leishmania donovani*, *Syphacia obvelata*, *Aspiculuris tetraptera*, *Nematospiroides dubius*, *Hymenolepis nana*, *Schistosoma mansoni*, *Litomosoides carinii*, *Streptococcus pyogenes* (C203), *Staphylococcus aureus* (UC-76), *Klebsiella pneumoniae* (AD), *Proteus mirabilis* (MGH-1), *Pseudomonas aeruginosa* (No 28), *Salmonella typhimurium* (V-31), and *Mycobacterium tuberculosis* (H$_{37}$Rv).[20–22]

The antimalarial effects of Va–c, VIb, and VIIa were studied in white Leghorn chicks infected with the 12A strain of *P. lophurae*.[20] Drugs were administered in a diet of mash for 5 days, starting the day before infection. Effects of therapy were measured in terms of suppression of the parasitemia by comparison of treated and untreated chicks on the fourth day after infection. Quinine equivalents were assigned by the ratio of the minimal effective diet concentration of quinine to that of the compound under test.[20] When administered at a dose of 0.05–0.1% in the diet for 5 days, Va and b, VIb, and VIIa lacked appreciable antimalarial effects and were <0.5 times as potent as quinine in the same test. By contrast, 6-chloro-2-methoxy-9-({3-[(4-methyl-1-piperazinyl)amino]propyl}amino)acridine dihydrochloride (Vc) exhibited significant antimalarial effects at 0.025–0.05% in the diet and was approximately as potent as quinine. For reference, quinacrine (I) and 3-chloro-9-{[4-(diethylamino)-1-methylbutyl]amino}acridine 10-oxide dihydrochloride (II)[7] were approximately 3 and 16 times as potent as quinine against *P. lophurae* under comparable test conditions.

The acridines (Va–c), benz[*c*]acridines (VIa and b), and benzo[*b*][1,5]naphthyridines (VIIa and b) were also evaluated against *E. histolytica in vitro* and against symptomatic intestinal amebiasis in rats.[21] Each compound was amebicidal *in vitro* at the lowest concentrations tested, namely 20–200 μg/ml. Six compounds (Va–c, VIb, and VIIa and b) were active against symptomatic intestinal amebiasis in rats,[21] and cured >50% of infected rats when administered by gavage for 4 days or by diet for 7 days at doses ranging from 62.5 to 250 mg/kg/day. Four compounds (Va–c, VIb) were

(20) For a description of antimalarial test methods, see P. E. Thompson, A. M. Moore, J. W. Reinertson, and A. Bayles, *Antibiot. Chemotherapy*, **3**, 399 (1953).

(21) For a description of antiamebic test methods, see P. E. Thompson, D. A. McCarthy, J. W. Reinertson, A. Bayles, and H. Najarian, *ibid.*, **8**, 37 (1958).

(22) For a description of antibacterial test methods, see M. W. Fisher and L. Doub, *Biochem. Pharmacol.*, **3**, 10 (1959).

effective in rats at daily doses ranging from 62.5 to 125 mg/kg, and thus showed activity comparable with or superior to 7-{[3-(octylamino)propyl]amino}benz[*c*]-acridine dihydrochloride (III).[13] Compounds Va and b and VIa and b killed *T. vaginalis in vitro* at concentrations of 25 μg/ml, but none was active against *L. donovani* in hamsters.

The effects of Va–c, VIb, and VIIa and b against intestinal helminths were assessed in mice infected with *S. obvelata*, *A. tetraptera*, *N. dubius*, and *H. nana*.[2] Four substances (Va–c and VIb) were active against the tapeworm *H. nana* in mice when given at doses of 62.5–125 mg/kg b.i.d. for 1 day and thus showed taeniacidal activity comparable with or superior to quinacrine.[6] Three compounds (Vc, VIb, and VIIb) were also effective against the mouse pinworms *S. obvelata* and *A. tetraptera*. None of the compounds tested was active against *S. mansoni* in mice or *L. carinii* in gerbils.

Each of the heterocyclic aminoalkylhydrazine derivatives caused complete inhibition of *S. pyogenes* (C203) and *S. aureus* (UC-76) *in vitro* at concentrations of 1.25–20 μg/ml.[22] Compounds Va, VIa and b, and VIIa also effected complete inhibition of *M. tuberculosis* ($H_{37}Rv$) at concentrations of 0.63–10 μg/ml, while VIa killed *K. pneumoniae* (AD) at 20 μg/ml. Under comparable experimental conditions, quinacrine was active against *S. pyogenes* (C203) and *M. tuberculosis* ($H_{37}Rv$) *in vitro* at 1.25 and 20 μg/ml, respectively. Three substances (Va, Vc, and VIa) were tested against streptococcus, staphylococcus, and tuberculosis infections in mice,[22] but none was active even at high dose levels.

The over-all results of the present study indicate that the substitution of a hydrazide moiety for an amine function at the distal position of quinacrine, azacrine, and the 7-[(aminoalkyl)amino]benz[*c*]acridines has a deleterious effect on antimalarial activity while potent antiamebic, anthelmintic, and antibacterial properties are maintained.

Experimental Section[23,24]

7-Chloro-2-methoxy-10-({3-[(4-methyl-1-piperazinyl)amino]-propyl}amino)benzo[*b*][1,5]naphthyridine Trihydrochloride (VIIb) (Procedure I).—A solution of 21.0 g (0.12 mole) of 1-[(3-aminopropyl)amino]-4-methylpiperazine[19] in 60 g of phenol was heated on a steam bath *in vacuo* for 20 min at 15 mm. 7,10-Dichloro-2-methoxybenzo[*b*][1,5]naphthyridine[15] (28.0 g, 0.10 mole) was then added and the mixture was stirred and heated on a steam bath for an additional 4 hr. The mixture was cooled and washed into a solution of 25 ml of concentrated HCl in 2 l. of Me_2CO with the aid of a small volume of EtOH. The resulting precipitate was collected by filtration, washed with fresh Me_2CO, and stirred into 1.5 l. of H_2O. A small amount of insoluble material was removed by filtration, and the filtrate was made alkaline with excess concentrated NH_4OH. The resulting mixture was extracted with $CHCl_3$, and the combined $CHCl_3$ extracts were washed with dilute NaOH and H_2O and concentrated on a rotary evaporator. The residue was dissolved in EtOH. The solution was filtered and then treated with excess HCl in *i*-PrOH. The yellow product slowly crystallized from the warm solvent, and was collected by filtration, washed well with *i*-PrOH and Me_2CO, and dried *in vacuo* at 60°. In order to avoid problems associated with hygroscopic, light-sensitive samples, the yellow solid was allowed to equilibrate with atmospheric H_2O in the dark for 24 hr prior to analysis. The hydrated hydrochloride melted at 215–218°.

6-Chloro-2-methoxy-9-{[3-(piperidinoamino)propyl]amino}-acridine Dihydrochloride (Vb) (Procedure II).—A mixture of 10.0 g (0.064 mole) of 1-[(3-aminopropyl)amino]piperidine[19] and 18.0 g (0.064 mole) of 6,9-dichloro-2-methoxyacridine in 50 g of phenol was stirred and heated on a steam bath for 3 hr. A red-brown solid slowly precipitated from the deep red solution. This hot mixture was then poured into a well-stirred solution of 27 ml of concentrated HCl in 1.5 l. of Me_2CO to give 27.0 g of a yellow solid which was collected by filtration and washed well with Et_2O. Recrystallization from MeOH gave 20.0 g of a bright yellow powder which did not analyze correctly. The crude product was stirred into hot H_2O, and a small amount of insoluble material was removed by filtration. The filtrate was poured into cold H_2O containing excess NH_4OH, and the liberated free base was extracted into $CHCl_3$. The $CHCl_3$ solution was washed with H_2O and dried (K_2CO_3). Dilution with Et_2O followed by the addition of excess HCl gave a precipitate which upon crystallization from EtOH yielded 12.1 g of pure product as bright yellow crystals, mp 132–135°.

7-{[3-(Piperidinoamino)propyl]amino}benz[*c*]acridine Dihydrochloride (VIa) (Procedure III).—A solution of 10.0 g (0.064 mole) of 1-[(3-aminopropyl)amino]piperidine[19] and 16.0 g (0.064 mole) of 7-chlorobenz[*c*]acridine[12–14] in 50 g of phenol was heated on a steam bath for 3 hr. The hot solution was poured into a cooled solution of 5 ml of concentrated HCl in 500 ml of Me_2CO. The Me_2CO was decanted, and the tarry residue was triturated twice with Et_2O and then dissolved in EtOH. This solution was poured into Et_2O, and the yellow deliquescent precipitate was collected and dried in an evacuated desiccator. This material was stirred into warm H_2O and treated with decolorizing charcoal, and the filtrate was chilled and treated with excess dilute NaOH. The sticky base was collected by filtration and washed with H_2O. An Et_2O solution of this base was dried over anhydrous K_2CO_3 and treated with excess HCl. The hygroscopic solid thus formed was collected by filtration and dried in an evacuated desiccator to obtain 16.0 g of yellow powder, mp >100°.

Acknowledgments.—The authors are indebted to Dr. P. E. Thompson, Dr. M. W. Fisher, and coworkers of these laboratories for the antiparasitic and antibacterial studies, Miss Joan D. Multhaup for chemical assistance, Dr. J. M. Vandenbelt and associates for the spectral determinations, and Mr. Charles E. Childs and coworkers for the microanalyses.

(23) Melting points (corrected) were taken on a Thomas–Hoover capillary melting point apparatus.

(24) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4 % of the theoretical values. Water determinations were by the Karl Fischer method.

Trifluoromethylbenzanilides as Anticoccidial Agents

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As part of a program directed toward the use of trifluoromethyl compounds as antiprotozoal agents, we wish to report our investigation of a series of trifluoromethylbenzanilides. Our interest in trifluoromethyl compounds was prompted by the discovery that, in anticoccidial benzamides bearing a nitro group, the nitro group and a trifluoromethyl group could be exchanged without loss of activity.[1] This work was underway when other reports appeared relating to anti-

(1) D. E. Welch, R. R. Baron, and B. A. Burton, *J. Med. Chem.*, **12**, 299 (1969).