[Chem. Pharm. Bull. 20(1) 206—208 (1972)] UDC 547.835.1.057.09 : 615.281.011.5.076.7

Synthesis of Some N,N'-Bis-(9-acridino)-α,ω-diaminoalkanes Dihydrochloride as Potential Antibacterial, Antitubercular and Antileprotics

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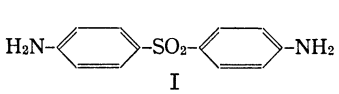
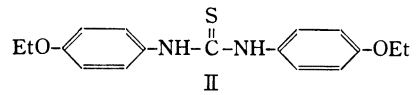
(Received January 1, 1971)

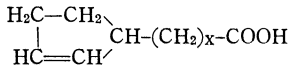
The drugs used in the treatment of tuberculosis are isoniazid,[2] streptomycin[3,4] and PAS[5] and regarding the treatment of leprosy, DDS(I) (diaminodiphenyl sulphone),[6] thiosemicarbazones,[7] 4,4'-diethoxydiphenyl thiourea[8] (II), chaulmoogra oil (III) and hydnocarpic oil (III) are widely popular. Since a variety of compounds (vide supra) show such activity and the last two particularly contain a polymethylene chain, it was considered of sufficient interest to investigate a new system of compounds with the general structure (IV), R-NH-$(CH_2)_n$-NH-R (R=9-acridino) incorporating both the polymethylene characteristic of III and basic characteristic of DDS. This forms the subject matter of the present communication.



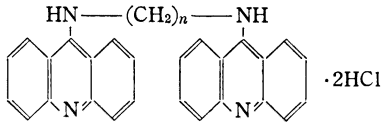
I
(DDS)



II
(4,4'-diethoxydiphenylthiourea)

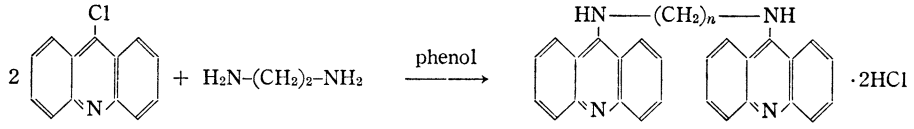


III
chaulmoogric acid, X=12
hydnocarpic acid, X=10



IV
where n=2 to 12

The preparation of this general type (IV) was accomplished according to the scheme:



9-Chloroacridine[9] required was obtained by reaction of $POCl_3$ with diphenylamine-2-carboxylic acid.[10] Reaction of 9-chloroacridine with an appropriate diamine at 130—135° in phenol[11] furnished (IV) directly as the dihydrochloride.

The compounds mentioned in Table I were studied for their antibacterial, antitubercular

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2) H. Meyer and J. Malley, *Mh. Chem.*, **33**, 393 (1912).
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10) A. Albert, "The Acridines," Edword Arnold & Co., London, 1951, p. 54.
11) A.R. Surrey and R.A. Cutler, *J. Am. Chem. Soc.*, **73**, 2623 (1951).

and antileprotic activities according to the procedures given in "Experimental."

The activity against *S. aureus* and *E. coli* is substantial with chain lengths having $n=3$ and 5 and is nil with all other values of n. The activity of these compounds against *M. tvberculosis* is appreciable only at 10^{-4} molar concentration (vide Table I). Compounds containing a chain length of $n=3,8,10$ and 12 are found active against *M. leprae* (Table I).

TABLE I

Compound No.	Structure $HN-(CH_2)_n-NH\cdot 2HCl$ (R, R) n	R	Antibacterial activity (Net values) 0.1 ml of 10^{-2} molar solution of the compound in water by Cup-plate method. Diameter of cup is 10 mm			
			strain *S. aureus*		strain *E. coli*	
			Compound	5% phenol control	Compound	5% phenol control
1	2	9-acridino	-ve	7 mm	-ve	8 mm
2	3	9-acridino	7 mm	7 mm	7 mm	8 mm
3	4	9-acridino	-ve	7 mm	-ve	8 mm
4	5	9-acridino	9 mm	13 mm	4 mm	10 mm
5	6	9-acridino	-ve	21 mm	-ve	12 mm
6	7	9-acridino	-ve	8 mm	-ve	6 mm
7	8	9-acridino	-ve	8 mm	-ve	6 mm
8	9	9-acridino	-ve	8 mm	-ve	6 mm
9	10	9-acridino	-ve	8 mm	-ve	6 mm
10	12	9-acridino	-ve	8 mm	-ve	6 mm
			inoculum: 25 ml of nutrient agar seeded with 0.1 ml of 18 hr old culture			

Compound No.	Antitubercular activity (On H37Rv strain) Growth in Youman's medium at the end of three weeks Concentration of drug (Molar)				Antileprotic activity Screening against *M. leprae* (I.C.R.C. strain)
	10^{-4}	10^{-5}	10^{-6}	Rv control	
1	+	##	###	###	no activity upto 400 μg/ml concentration
2	0	0	###	###	active at 100 μg/ml concentration
3	0	Sl	###	###	no activity upto 400 μg/ml concentration
4	0	#	###	###	no activity upto 400 μg/ml concentration
5	0	+	##	###	no activity upto 400 μg/ml concentration
6	0	###	###	###	no activity upto 400 μg/ml concentration
7	+	###	###	###	active at 100 μg/ml concentration
8	##	###	###	###	no activity upto 400 μg/ml concentration
9	##	###	###	###	active at 100 μg/ml concentration
10	0	###	###	###	active at 16 μg/ml concentration
	0=no growth + to ###=various stages of growth; Sl=Slight growth inculum: 1 loopful (4 mm diameter) from 12—14 days culture				A.J.L. Dapsone (standard drug) =active at 16 μg/ml concentration

Experimental[12]

N,N′-Bis-(9-acridino)-ethylenediamine Dihydrochloride (IV, $n=2$)——In a 50 ml round-bottomed flask fitted with a water condenser and a calcium chloride guard tube was placed a mixture of phenol 10 g, 9-chloro-

12) The melting points are uncorrected.

Table II

Compound No. from Table I	Formula	Yield (%)	Crystallization solvent	mp (°C)	Analysis (%) Calcd. C	H	N	Found C	H	N
1	$C_{28}H_{22}N_4 \cdot 2HCl$	82.0	aqueous ethanol	245 (decomp.)	—	—	11.49	—	—	11.75
2	$C_{29}H_{24}N_4 \cdot 2HCl$	89.0	aqueous ethanol	170 (decomp.)	—	—	11.17	—	—	11.07
3	$C_{30}H_{26}N_4 \cdot 2HCl$	93.2	ethanol	255 (decomp.)	—	—	10.87	—	—	10.84
4	$C_{31}H_{28}N_4 \cdot 2HCl$	75.9	ethanol–ether	250 (decomp.)	—	—	10.58	—	—	10.32
5	$C_{32}H_{30}N_4 \cdot 2HCl$	86.5	ethanol–ether	146—147	—	—	10.31	—	—	10.67
6	$C_{33}H_{32}N_4 \cdot 2HCl \cdot H_2O$	68.2	ethanol–ether	135—136	68.86	6.26	9.73	68.63	6.70	10.07
7	$C_{34}H_{34}N_4 \cdot 2HCl \cdot H_2O$	91.0	ethanol–ether	154—155	69.26	6.45	9.50	68.79	6.88	9.07
8	$C_{35}H_{36}N_4 \cdot 2HCl$	85.4	ethanol–ether	126—127	71.96	6.49	9.57	71.58	6.21	9.78
9	$C_{36}H_{38}N_4 \cdot 2HCl \cdot H_2O$	86.7	ethanol–ether	130—131	70.01	6.80	9.07	69.86	6.49	8.94
10	$C_{38}H_{42}N_4 \cdot 2HCl \cdot 2H_2O$	92.5	ethanol–ether	100—101	68.77	7.23	8.44	69.08	6.91	8.61

acridine (0.02 mole) and ethylenediamine (0.01 mole). The flask was heated in an oil bath maintained at 130—135° for 14—16 hours. After cooling, the contents of the flask were diluted with 40 ml of ether. N,N′-Bis(9-acridino)-ethylenediamine dihydrochloride separated was filtered off and washed with ether. Recrystallisation from aqueous ethanol gave the pure product. Other compounds mentioned in Table I were prepared in a similar manner and their properties are given in Table II. C_3—C_5-Methylenediamines used herein were prepared according to the procedure described by Putokhin,[13] and other diamines were obtained from Fluka, Swiss.

Antibacterial Activity (*in Vitro* against *S. aureus* and *E. coli* by Agar-cup Method[14])——25 ml of nutrient agar in petridishes was seeded with 0.1 ml of 18 hours old culture. Holes were punched (10 mm diameter) and 0.1 ml of 10^{-2} molar solution of drug was added in each hole.

Antitubercular Activity (*in Vitro* against *M. tuberculosis*, H37Rv strain, by Youman's Method[15])——To a 5 ml of liquid (Youman's medium) 1 loopful (4 mm diameter) of 12—14 days culture was added. Cells were grown as stationary floating cultures. Growth of cells was followed at weekly intervals on Baush and Lomb spectrophotometer.

Antileprotic Activity (*in Vitro* against *M. leprae*, I.C.R.C. strain, by Dubo's Method[16])——Drug dilutions ranging from 400 μg per ml to 3.2 μg per ml were mixed with 4 ml of Dubo's medium and 1 ml of I.C.R.C. Bacilli (Bapat, *et al.*[17] 1961) was added to each dilution. Turbidimetric measurements were recorded on a Baush and Lomb spectrophotometer at 620 mμ for 12 days. The growth curve was plotted on graph and the rate of inhibition of bacilli was calculated. The results are recorded in Table I.

Acknowledgement We thank Prof. G.B. Singh, Head of the Department of Chemistry for the facilities and financial assistance to one of us (A.K.S.). We are deeply grateful to Dr. M.B. Bhide, Haffkine Institute, Bombay, and his staff for antileprotic screening. The authors are indebted to Dr. T. Ramakrishnan, Indian Institute of Science, Bangalore, and his staff for antibacterial and antitubercular screening. We wish to thank Mr. V.N. Muley and Mr. P.S. Gurjar for the microanalyses reported.

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15) G.P. Youman's, *Proc. Soc. Exp. Biol. New York*, **57**, 122 (1944).
16) Medical Research Council, *Lancet*, **2**, 862 (1948).
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