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EXPERIMENTAL CHEMOTHERAPY OF SCHISTOSOMIASIS

XII — Active derivatives of aminoethanethiosulfuric acids

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SUMMARY

A series of mono, di and trisubstituted aminoethanethiosulfuric acids were selectively screened for activity in *Schistosoma mansoni* infection in mice by the oogram method. Compounds with secondary amino groups bearing small, apolar alkyl substituents were found to be active.

INTRODUCTION

In a previous article (NELSON & PELLEGRINO [7]) the results of tests performed on a series of aminoethanethiols in mice and hamsters experimentally infected with *Schistosoma mansoni* and a study of the structure-activity relationship were reported. In this paper, the results of a selective trial with a related class of compounds — the aminoethanethiosulfuric acids (Bunte salts) — are presented. A study of the chemical structure-activity relationship was based on trials performed with 70 compounds.

MATERIAL AND METHODS

Drugs — All compounds for the present selective screening were received from the Walter Reed Army Institute of Research (WRAIR), Washington, D.C.

Infection of animals — The L.E. strain of *S. mansoni* (Belo Horizonte, Brazil) was used in this study. Mice were exposed, by the tail immersion method (PELLEGRINO & KATZ [10]), to 100 ± 10 cercariae. Hamsters were infected via the cheek pouch with 80 ± 10 cercariae (PELLEGRINO, DE MARIA & FARIA [8]). All animals were dosed *per os*.

Primary screening — The initial screening was performed in groups of 5 mice per drug. When the LD_{50} was available, treatment consisted in a daily oral dose corresponding to 1/5 of the LD_{50}, for 5 consecutive days. In all instances it was attempted to administer the maximum tolerated dose.

Secondary screening — This was performed in groups of 10 mice treated with different schedules. As far as possible, active compounds were tested in hamsters.

Assessment of antischistosomal activity — For the primary screening in mice, the only criterion used was the oogram, performed 8 days after the beginning of dosing (PELLEGRINO & FARIA [9]). In the secondary screening in mice and in hamsters, the hepatic-shift of schistosomes (STANDEN [11]) and the

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TABLE I

Antischistosomal activity of compounds in mice experimentally infected with *Schistosoma mansoni*

$R\text{-}NHCH_2CH_2SSO_3H$

Compounds	R	Dose (mg/kg/day) × 5	Route	Animals/dead	Worms in the liver (%)	Oogram changes (%)
1	H	600	po	5/1		0.0
2	Et	600	po	5/2		0.0
3	*i*-Pr	700	po	10/3	31.0	71.4
		500	po	10/3	30.3	42.9
4	*n*-Bu	600	po	10/5	61.4	80.0
		500	po	10/0	98.0	100.0
5	*t*-Bu	250	po	10/0	77.8	80.0
		125	po	10/0	42.5	20.0
6	C_6H_{11}	600	po	5/3		0.0
7	$n\text{-}C_8H_{17}$	300	po	10/5	63.1	80.0
		150	po	10/3	29.3	0.0
8	$n\text{-}C_{12}H_{25}$	600	po	5/1		0.0
9	$n\text{-}C_{18}H_{37}$	600	po	5/1		0.0
10	cyclopentyl-$(CH_2)_6$	360	po	10/6	32.6	25.0
11	2-nobornyl	600	po	10/1	84.1	100.0
12	1-adamantyl	600	po	5/1		0.0
13	adamantyl-CH_2	200	po	10/2	64.8	50.0
14	$t\text{-}BuCH_2CHMeCH_2CH_2$	400	po	10/7	85.9	100.0
		200	po	10/6	22.2	0.0
15	Bz	600	po	5/2		0.0
16	$Ph(CH_2)_3$	550	po	5/4		100.0
17	$Ph(CH_2)_4$	600	po	5/3		0.0
18	$EtCHPhCH_2$	600	po	5/3		0.0
19	$n\text{-}C_5H_{11}CH(Pr)$	500	po	5/3		0.0
20	2-thienyl-$(CH_2)_4$	600	po	5/3		0.0
21	3-indolyl-CH_2	600	po	5/1		0.0
22	9-acridanyl	600	po	5/1		0.0
23	$NCCH_2CH_2$	600	po	5/2		0.0
24	$PhC(=O)CH_2$	600	po	5/3		0.0
25	$Et_2NC(=O)CH_2$	600	po	5/0		0.0
26	$MeOC(=O)CH_2$	600	po	5/1		0.0
27	$EtOC(=O)CH_2$	600	po	5/3		0.0
28	$EtOC(=O)CH_2CHMe$	600	po	5/3		0.0
29	$PhC(=O)CH_2CH(COPh)$	600	po	5/1		0.0
30	$n\text{-}BuOC(=O)CH_2CH_2$	600	po	5/0		0.0
31	$i\text{-}BuOC(=O)CH_2CH_2$	600	po	5/4		0.0
32	$n\text{-}C_5H_{11}OC(=O)CH_2CH_2$	600	po	5/4		0.0
33	$CH_2{=}CH\ CH_2OC(=O)CH_2CH_2$	600	po	5/1		0.0
34	$C_2F_5CH_2OC(=O)CH_2CH_2$	600	po	5/0		0.0
35	$EtOC(=O)CH_2CH_2$	600	po	5/0		0.0
36	$n\text{-}C_{12}H_{25}OC(=O)CH_2CH_2$	600	po	5/3		0.0
37	$PhCH_2CH_2(=O)CH_2CH_2$	600	po	5/0		0.0
38	$CHF_2(CF_2)_5CH_2OC(=O)CH_2CH_2$	600	po	5/4		0.0
39	$n\text{-}C_{18}H_{37}OC(=O)CH_2CH_2$	600	po	5/2		0.0
40	$t\text{-}BuNHCO_2CH_2CH_2$	600	po	5/1		0.0
41	$i\text{-}PrNHC(=N\text{-}i\text{-}Pr)NHCH_2CH_2$	600	po	5/2		0.0
42	$t\text{-}BuNHC(=O)NHN{=}CMeCH_2CMe_2$	600	po	5/3		0.0
43	$PhNHC(=O)NHN{=}CMeCH_2CH_2$	80	po	5/3		0.0
44	$PhNHC(=O)NHN{=}CMeCH_2CMe_2$	600	po	5/3		0.0
45	$Ph_2NC(=O)NHCH_2CH_2$	600	po	5/2		0.0
46	$t\text{-}BuNHC(=O)NHN{=}CMeCH_2CH_2$	600	po	5/2		0.0

TABLE I — Continuation

Compounds	R	Dose (mg/kg/day) × 5	Route	Animals/dead	Worms in the liver (%)	Oogram changes (%)
47	n-$C_{18}H_{37}NHC(=O)NH(CH_2)_6$	600	po	5/1		0.0
48	ferrocenyl-CH_2	400	po	5/0		0.0
49	2-succinimidyl	600	po	5/0		0.0
50	$(EtO)_2P(=O)CH_2CH_2$	600	po	5/3		0.0
51	10, 13-dimethyl-3-hidroxy-5-cyclopentanoperhydro-phenanthryl-17-$C(=O)CH_2CH_2$	600	po	5/1		0.0
52	2-hydroxy-1-indanyl	600	po	5/1		0.0
53	1-naphthyl-$NHC(=O)NH(CH_2)_4$	600	po	5/2		0.0
54	$HO(CH_2)_3$	600	po	5/2		0.0
55	$Me_2CHOHCHOHCH_2$	600	po	5/3		0.0
56	$HO_3SSCH_2CH_2NH(CH_2)_4$	600	po	5/4		0.0
57	$HO_2C(CH_2)_3$	600	po	5/3		0.0
58	$HO_3SSCH_2CH_2NH(CH_2)_7$	600	po	5/4		0.0
59	$C_6H_{11}CH_2O(CH_2)_5$	500	po	10/5	62.1	66.7
		250	po	10/4	23.3	20.0
60	*p*-$MeOC_6H_4CHEtCH_2$	600	po	5/1	41.2	25.0

percentage of oogram changes were taken into consideration. In all cases the animals were killed and examined 8 days after the beginning of treatment.

RESULTS AND DISCUSSION

It may be seen that the present group of compounds is similar to the aminoethanethiols in many respects. First of all, the activity seems to depend, in part, on the hydrophobicity of the alkyl group bound to the nitrogen, substances with polar groups being inactive. Although data are lacking for a number of intermediate linear compounds, within the series C_2 to C_{12}, the activity increases up to the *t*-butyl analog (*5*), while compounds bearing substituents

TABLE II

Antischistosomal activity of compounds in hamsters experimentally infected with *Schistosoma mansoni*

R-$NHCH_2CH_2SSO_3H$

Compounds	R	Dose (mg/kg/day) × 5	Route	Animals/dead	Worms in the liver (%)	Oogram changes (%)
3	*i*-Pr	500	po	5/2	51.2	66.7
4	*n*-Bu	600	po	5/1	97.0	100.0
5	*t*-Bu	400	po	4/0	84.9	50.0
		200	po	4/0	37.3	25.0
7	n-C_8H_{17}	300	po	5/2	69.4	100.0
		150	po	5/2	47.0	100.0
10	cyclopentyl-$(CH_2)_6$	360	po	5/2	50.5	67.7
59	$C_6H_{11}CH_2O(CH_2)_5$	200	po	5/2	74.8	0.0

TABLE III

Antischistosomal activity of compounds in mice and hamsters experimentally infected with *Schistosoma mansoni*

$R^3\text{-NH-CH(}R^2\text{)-CH(}R^1\text{)-SSO}_3\text{H}$

Compounds	R^1	R^2	R^3	Mice					Hamsters				
				Dose (mg/kg/day) × 5	Route	Animals/dead	Worms in the liver (%)	Oogram changes (%)	Dose (mg/kg/day) × 5	Route	Animals/dead	Worms in the liver (%)	Oogram changes (%)
61	H	Et	H	600	po	5/1	—	0.0					
62	Ph	H	H	600	po	5/3	—	0.0					
				600	po	5/1	—	0.0					
63	Ph	H	n-Pr	400	po	10/0	89.1	100.0					
				200	po	10/0	67.9	90.0	150	po	5/2	—	100.0
				100	po	10/1	61.1	66.7	75	po	5/1	—	50.0
64	Ph	H	*t*-BuCH[2]	540	po	5/4	—	0.0					
65	Ph	H	*t*-$BuCH_2CMe_2$	600	po	5/2	—	0.0					
66	H	*p*-HOBz	H	350	po	5/2	—	0.0					
67	Ph	H	n-Bu	300	po	10/3	88.3	100.0	150	po	5/2	—	100.0
				150	po	10/2	71.5	87.5	75	po	5/1	—	100.0
68	Ph	H	*i*-Bu	500	po	10/0	53.6	50.0	400	po	5/0	—	20.0
69	Ph(L-isomer)	Me	Me	300	po	10/3	63.8	85.7	200	po	5/0	—	100.0
				100	po	10/2	37.0	0.0	100	po	5/1	—	50.0
70	Ph	Me	Me	600	po	10/4	59.1	67.7	600	po	5/4	—	100.0
				400	po	10/4	56.9	0.0					

longer than C_{10} are usually inactive. However, compounds such as *13* and *14*, which have a larger number of carbons but with a more spherical structure, are active. The substituents on these two compounds occupy approximately the same volume. Also, although the N-(6-cyclopentylhexil) derivative (*10*), which has an eleven carbon substituent, shows some activity, the length of this substituent is only slightly greater than that of the n-octyl chain. Likewise, the length of the N-(5-cyclohexylmethoxypentyl) derivative (*59*) is similar to that of an n-decyl-group.

An interesting difference from the aminoethanethiols is seen in the fact that the 2-(n-butylamino) ethanethiosulfuric acid (*4*) is active whereas the thiol analog is not. However, since the Bunte salts seem, in general, to be slightly more active than the analogous mercaptans, this difference may be simply one of degree of activity. Other differences which may be noted are that the cyclohexyl derivative (*6*) did not show activity and that the relative activities of the norbornyl (*11*) and adamantyl (*12*) derivatives are the reverse of those observed with the corresponding mercaptans.

As can be seen in Tables I and II, the activity in hamsters was roughly parallel (*3, 4, 5, 7, 10, 59*) to that observed in mice. The most notable difference was the greater activity shown by the n-butyl and n-octyl derivatives as compared to the *t*-butyl, although the difference was small. Apparently, in this case the sphericity of the substituent is not so important for activity.

Since no analogs bearing a beta tertiary amino group were tested, one can not conclude that the amino group must be a secondary amine, as appears to be the case with the aminoethanethiols. However, the derivatives containing a primary amino group (*1, 61, 62, 66*) were inactive.

In the only analogs of the above mentioned active alkyl-aminoethanethiolsulfuric acids in which the position alpha to the thiosulfate group is substituted (*63, 67, 68*), the presence of a phenyl group seemed to increase the activity. Compounds *69* and *70* illustrate an apparent stereospecificity in the activity.

The mortality rate of the animals indicates that the schedule of treatment used was close to he maximum which could be tolerated. Of the active compounds, the lowest toxicity was shown by compounds *5, 63,* and *68*. The most indicated for further studies because of their combination of high degree of activity, low toxicity, and simplicity of structure are the N-*t*-butyl-(*5*) and 2-(n-propylamino)-1-phenylethanethiosulfuric acids (*63*).

It is noteworthy that many aminoethanethiols and Bunte salts are also active as protectors against the effects of radiation (KLAYMAN & SHINE [5]; MAURES & SCHREIBER [6]). It has been shown that *in vivo* the Bunte salts react with sensitive protein sulphydryl groups to form mixed disulfides as well as reacting to liberate sulfite and cysteamines which may protect against radiation by capturing free radicals (HART et al. [2]; KELLEY et al. [3, 4]). It may be that these compounds act against *S. mansoni* by blocking necessary sulphydryl groups, by the reduction of disulfide bridges or by interfering with oxidation-reduction processes. The cysteamines may also react with the coenzyme pyridoxal phosphate (DE MARCO & BOGNOLO [1]) to form thiazolidines, thus exerting an inhibitory action on pyridoxal phosphate dependant enzymes. This could explain the lack of activity of the tertiary aminoethanethiols, since these compounds would be unable to form thiazolidines. The mode of action of these substances remains to be studied.

RESUMO

Terapêutica experimental da esquistossomose. XII — Derivados ativos de ácidos aminoetanotiosulfúricos

Uma série de ácidos aminoetanotiosulfúricos mono, di e tri-substituídos foram testados seletivamente para detecção de atividade sobre o *Schistosoma mansoni*, em camundongos, pelo método do oograma. Compostos com grupos amino secundários, possuindo substituintes alquilas pequenos e apolares mostraram atividade esquistossomicida.

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