*Rev. Inst. Med. trop. São Paulo*
*18(6):443-449, novembro/dezembro, 1976*

*UDC 616.995.122.22*

# EXPERIMENTAL CHEMOTHERAPY OF SCHISTOSOMIASIS MANSONI. XIV — ACTIVE DERIVATIVES OF AMINOETHANEPHOSPHOROTHIOATE, DISULFIDE, AND ISOTHIOUREA

David L. NELSON (1) and J. PELLEGRINO (2)

## SUMMARY

A group of N-substituted aminoethanephosphorothioates, disulfides, and isothioureas were selectively screened for activity on *Schistosoma mansoni* infection in mice by the oogram method. Compounds bearing small to medium-sized, apolar, alkyl substituents bound to the amino group were found to be active.

## INTRODUCTION

During the process of blind screening of more than 5,000 compounds obtained from the Walter Reed Army Institute of Research, adamantylaminoethanethiol was observed to be active in mice experimentally infected with *Schistosoma mansoni*.

Subsequent selective trials with aminoethanephosphorothioates, disulfides, isothioureas and other thiol derivatives showed that the activity was not limited to the thiols. A study of the chemical structure-activity relationship was made based on trials performed with 85 compounds.

## MATERIAL AND METHODS

*Drugs* — All compounds tested were received from the Walter Reed Army Institute of Research (WRAIR), Washington, D.C.

*Infection of animals* — The L.E. strain (Belo Horizonte, Brazil), isolated from a patient in the acute phase of schistosomiasis, was used throughout this study. Mice were infected, by the tail-immersion method (PELLEGRINO & KATZ [5]) with 100 ± 10 cercariae. The cheek-pouch route was used to infect hamsters with 80 ± 10 cercariae (PELLEGRINO, DE MARIA & FARIA [3]).

*Primary screening* — This was performed in groups of 5 mice per compound. When data of $LD_{50}$ were available, the schedule of treatment consisted in a daily oral dose corresponding to 1/5 of the $LD_{50}$, for 5 consecutive days. In all cases it was attempted to give the maximum tolerated dose.

*Secondary screening* — This was performed in groups of 10 mice treated with different dosage schedules. Some active compounds were tested in hamsters.

*Assessment of therapeutic activity* — For the primary screening in mice, the only parameter used was the oogram as described elsewhere (PELLEGRINO & FARIA [4]). In the secondary screening in mice and hamsters, the hepatic shift of schistosomes (STANDEN [6]) and the percentage of oogram changes were considered. In all cases, the animals were killed and examined 8 days after the beginning of dosing.

This study was supported, in part, by the "Conselho Nacional de Pesquisas", Brazil, and the U. S. Army (Walter Reed Army Institute of Research). Contribution number 81 from the Schistosomiasis Research Unit.

(1) Department of Biochemistry, Institute of Biological Sciences, Federal University of Minas Gerais
(2) Schistosomiasis Research Unit, Institute of Biological Sciences, Federal University of Minas Gerais, and "Centro de Pesquisas René Rachou", INERu-FIOCRUZ
Address for reprints: Caixa Postal 1404 — 30.000 Belo Horizonte, Brasil.

## RESULTS AND DISCUSSION

The most notable structural characteristic of the aminoethanephosphorothioates necessary for activity is that the substituent bound to the nitrogen must be apolar and neither very large nor small (Tables I and II). This characteristic is similar to that observed with the aminoethanethiols (NELSON & PELLEGRINO [1]) and with the Bunte salts (NELSON & PELLEGRINO [2]). In the present case, a number of analogs bearing alkyl substituents of intermediate length and volume are missing so it is difficult to identify exactly the size and structural characteristics of this substituent necessary for activity. Apparently, the activity depends to a large degree on the hydrophobicity of the compound; the *t*-butyl analog (3), which is more compact and thus should be more hydrophilic, is inactive whereas the *n*-butyl (2), adamantyl (4), adamantylmethyl (5), and norbornyl (7), derivatives are active. The decreasing activity of the series — adamantyl:adamantylmethyl:adamantyl-ethyl — indicates that there is a limit to the acceptable length of the alkyl substituent.

Presumably, the mechanism of action of these compounds should be similar to that of the Bunte salts and the cysteamines. Therefore, by analogy, one would expect the analog bearing a tertiary amino group beta to the phosphorothioate to be inactive, although none were tested in this series. Another similarity to cysteamines and Bunte salts is seen in the fact that compounds having phenyl substituents (71 and 72) alpha to the phosphorothioate group, while still bearing medium sized apolar substituents on the amino group, are active.

The mortality rate indicates that the dose utilized in these tests was close to he maximum tolerable. Especially interesting is the low toxicity of the more active compounds. For example, the adamantyl derivative is at least as active as its thiol analog (NELSON & PELLEGRINO [1]), while being much less toxic. The activity of the substances tested in hamsters (Table VI) paralleled the activity observed in mice.

In a similar way, the identical requirements for activity seem to hold for the disulfide and isothiourea derivatives of the aminoethanethiols (Tables III and IV). If anything, the disulfides seem to be even more active than their thiol counterparts. For example, the N-n-butyl-, N-isobutyl-, and N-sec-butylaminoethyl disulfides are active while their thiol (*) analogs are not. Although not shown in the Tables, cystine, cystine methylester and N-N'-diformylcysteine were found to be inactive. Again, in the case of the isothioureas and the thiobenzoates (Table V), the adamantyl and bornyl derivatives (8, 82, 83) continue to be active. The hemimercaptal (84) is an interesting case although probably not very useful.

## RESUMO

*Terapêutica experimental da esquistossomose mansoni. XIV — Derivados ativos de aminoetanofosforotioatos, disulfetos e isotiouréias.*

Foi feita uma triagem seletiva de derivados (N-substituídos) de aminoetanofosforotioatos, disulfetos e isotiouréas com a finalidade de estabelecer as relações entre estrutura e atividade. Os parâmetros básicos para a avaliação da atividade terapêutica foram constituídos pela porcentagem de alterações do oograma em camundongos infestados pelo *Schistosoma mansoni* e pelo grau de deslocamento dos esquistossomos para o fígado. Foi constatado que compostos dotados de substituintes alquila apolares (ligados ao grupo amino), com tamanho pequeno a médio, apresentaram atividade terapêutica sobre o *S. mansoni*.

## ACKNOWLEDGEMENTS

We are indebt to Drs. C. Davidson and Peter S. Loizeaux, Department of the Army, Walter Reed Army Institute of Research, Washington, U.S.A., for the assistance in the selection of drugs for screening, for providing the structural formulae, and for the financial support (Grants from the U.S. Army).

(*) Unpublished results

NELSON, D. L. & PELLEGRINO, J. — Experimental chemotherapy of schistosomiasis mansoni. XIV — Active derivatives of aminoethanephosphorothioate, disulfide, and isothiourea. *Rev. Inst. Med. trop. São Paulo* 18:443-449, 1976.

TABLE I

Antischistosomal activity of aminoethanephosphorothioate derivatives in mice

R NH $CH_2$ $CH_2SPO_3H_2$

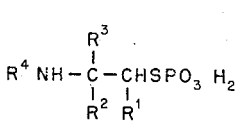
| Compound | R | Dose (mg/kg/day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|---|
| 1 | H | 500 | 5/4 | — | 0.0 |
| 2 | n-Bu | 400 | 10/1 | 59.5 | 55.6 |
| | | 200 | 10/1 | 31.4 | 25.0 |
| 3 | *t*-Bu | 50 | 5/1 | — | 0.0 |
| 4 | 1-adamantyl | 300 | 12/3 | 99.1 | 100.0 |
| | | 200 | 12/1 | 69.6 | 81.8 |
| | | 100 | 12/1 | 40.4 | 45.4 |
| | | 50 | 12/0 | 43.0 | 0.0 |
| 5 | 1-adamantyl$CH_2$(mono Na Salt) | 200 | 10/5 | 67.4 | 40.0 |
| 6 | 1-adamantyl$CH_2CH_2$(mono Na Salt) | 600 | 5/2 | — | 0.0 |
| 7 | 2-norbornyl (mono Na Salt) | 250 | 10/6 | 41.4 | 25.0 |
| 8 | $NCCH_2CH_2$ | 600 | 5/1 | — | 0.0 |
| 9 | 2-oxocyclohexylCH(Pr) | 500 | 5/2 | — | 0.0 |
| 10 | 5-Me-2-oxocyclohexyl$CMe_2$ | 300 | 5/4 | — | 0.0 |
| 11 | $EtC(=O)OCH_2$ | 600 | 5/1 | — | 0.0 |
| 12 | $MeOC(=O)CH_2CHPh$ | 500 | 5/0 | — | 0.0 |
| 13 | $EtOC(=O)CH_2CHPh$ | 600 | 5/2 | — | 0.0 |
| 14 | $MeOC(=O)CH_2CH(n\text{-}C_6H_{13})$ | 600 | 5/0 | — | 0.0 |
| 15 | $HC{\equiv}CCH_2OC(=O)CH_2CH_2$ | 600 | 5/1 | — | 0.0 |
| 16 | *i*-$BuOC(=O)CH_2CH_2$ | 600 | 5/0 | — | 0.0 |
| 17 | $C_{12}H_{25}OC(=O)CH_2CH_2$ | 600 | 5/0 | — | 0.0 |
| 18 | $MeOC(=O)CH_2CH_2$ | 600 | 5/1 | — | 0.0 |
| 19 | $EtOC(=O)CH_2CH_2$ | 600 | 5/1 | — | 0.0 |
| 20 | 5-norbornenyl$CH_2OC(=O)CH_2CH_2$ | 500 | 5/2 | — | 0.0 |
| 21 | $PhCH_2CH_2$ | 500 | 3/5 | — | 0.0 |
| 22 | $MeC(=O)CH_2$ | 600 | 5/1 | — | 0.0 |
| 23 | $MeOC(=O)(CH_2)_2NHCH_2CH_2$ | 600 | 5/2 | — | 0.0 |
| 24 | n-$C_{18}H_{37}OC(=O)CH_2CH_2$ | 600 | 5/1 | — | 0.0 |
| 25 | $PhNHC(=S)NH(CH_2)_3$ | 500 | 5/2 | — | 0.0 |
| 26 | *t*-$BuNHC(=O)OCH_2CH_2$ | 600 | 5/3 | — | 0.0 |
| 27 | $PhNHC(=O)NH(CH_2)_6$ | 500 | 5/1 | — | 0.0 |
| 28 | *o*-$MeC_6H_4NHC(=O)NHCH_2CH_2$ | 500 | 5/1 | — | 0.0 |
| 29 | 1-naphthylNHC(=O)$NH(CH_2)_3$ | 50 | 5/1 | — | 0.0 |
| 30 | *t*-$BuNHC(=O)NHN{=}CMeCH_2CMe_2$ | 400 | 5/4 | — | 0.0 |
| 31 | $C_6H_{11}NHC(=O)NHN{=}CMeCH_2CMe_2$ | 500 | 5/2 | — | 0.0 |
| 32 | $PhNHC(=O)NHN{=}CMeCH_2CH_2$ | 350 | 5/4 | — | 0.0 |
| 33 | 2-succinimidyl | 600 | 5/1 | — | 0.0 |
| 34 | $C_{18}H_{37}NHC(=O)NHCH_2CH_2$ | 600 | 5/3 | — | 0.0 |
| 35 | 1-naphthylNHC(=O)$NHN{=}CMeCH_2CH_2$ | 600 | 5/0 | — | 0.0 |
| 36 | ferrocenyl-$CH_2$ | 600 | 5/2 | — | 0.0 |
| 37 | *t*-$BuNHC(=O)NH(CH_2)_6$ | 600 | 5/2 | — | 0.0 |
| 38 | $C_6H_{11}NHC(=O)NH(CH_2)_6$ | 500 | 5/3 | — | 0.0 |
| 39 | 1-naphthylNHC(=O)$NHCH_2CH_2$ | 500 | 5/2 | — | 0.0 |
| 40 | $NH_2C(=O)NH(CH_2)_6$ | 400 | 5/3 | — | 0.0 |
| 41 | *p*-$EtOC_6H_4NHC(=O)NH(CH_2)_6$ | 600 | 5/1 | — | 0.0 |
| 42 | $NC(CH_2)_2NHCH_2CH_2$ | 600 | 5/1 | — | 0.0 |
| 43 | N-phthalimido-$(CH_2)_4$(mono Na Salt) | 500 | 5/3 | — | 0.0 |
| 44 | $PhNHN{=}CHCH_2CH_2$ | 500 | 5/3 | — | 0.0 |
| 45 | 1-naphthylNHC(=O)$NH(CH_2)_4$ | 400 | 10/0 | 17.3 | 25.0 |
| 46 | 2-tetrahydrofurylCH$_2$NH$(CH_2)_3$ | 600 | 5/4 | — | 0.0 |
| 47 | $C_6H_{11}NH(CH_2)_3$ | 60 | 5/0 | — | 0.0 |
| 48 | $C_6H_{13}NH(CH_2)_3$ | 60 | 5/2 | — | 0.0 |
| 49 | $PhCH_2NH(CH_2)_5$ | 500 | 5/1 | — | 0.0 |

TABLE I — Continuation

| Compound | R | Dose (mg/kg/ day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|---|
| 50 | $Me_2C{=}CH(CH_2)_2CMe{=}CHCH_2NHCH_2CH_2$ | 600 | 5/2 | — | 0.0 |
| 51 | $Me_2C{=}CH(CH_2)CHMe(CH_2)_2NHCH_2CH_2$ | 300 | 5/4 | — | 0.0 |
| 52 | cyclo-$C_{12}H_{23}$-$NH(CH_2)_4$ | 60 | 5/0 | — | 0.0 |
| 53 | ferrocenyl$CH_2NHCH_2CH_2$ | 60 | 5/0 | — | 0.0 |
| 54 | N-phthalimido$(CH_2)_4NH(CH_2)_3$ | 500 | 5/0 | — | 0.0 |
| 55 | cyclo-$C_8H_{15}NH(CH_2)_4$ | 60 | 5/0 | — | 0.0 |
| 56 | $Me_2C{=}CH(CH_2)_2CMe{=}CHCH_2NH(CH_2)_4$ | 60 | 5/0 | — | 0.0 |
| 57 | cyclo-$C_{12}H_{23}$-$NH(CH_2)_6$ | 360 | 5/4 | — | 0.0 |
| 58 | piperazinyl$(CH_2)_2NH(CH_2)_3$ | 600 | 5/3 | — | 0.0 |
| 59 | N-cyclohexane-1,2-dicarboximido | 500 | 5/1 | — | 0.0 |
| 60 | $PhCHOHCH_2$(mono Na Salt) | 500 | 5/4 | — | 0.0 |
| 61 | *p*-$MeOC_6H_4SiMe_2CH_2NHCH_2CH_2$ | 350 | 5/3 | — | 0.0 |
| 62 | *p*-$MeOC_6H_4SiMe_2CH_2$ | 400 | 5/3 | — | 0.0 |
| 63 | *p*-$MeC_6H_4SiMe_2CH_2$ | 50 | 5/1 | — | 0.0 |
| 64 | *t*-$BuCH_2CHMeCHOHCH_2$ | 60 | 5/3 | — | 0.0 |
| 65 | $NH_2(CH_2)_4$ | 300 | 5/4 | — | 0.0 |
| 66 | $NH_2(CH_2)_5$ | 600 | 5/3 | — | 0.0 |
| 67 | $NH_2CH_2CH_2CMe_2CH_2$ | 600 | 5/3 | — | 0.0 |
| 68 | n-$C_8H_{17}NH(CH_2)_3$ | 300 | 5/4 | — | 0.0 |

TABLE II

Antischistosomal activity of aminoethanephosphorothioate derivatives in mice



| Compound | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Dose (mg/kg/ day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|---|---|---|---|
| 69 | H | Me | Me | H | 600 | 5/0 | — | 0.0 |
| 70 | H | Et | H | H | 600 | 5/2 | — | 0.0 |
| 71 | Ph | H | H | $C_6H_{11}$ | 150 | 12/6 | 28.5 | 33.3 |
| 72 | Ph | H | H | *t*-$BuCH_2CMe_2$ | 400 | 12/5 | 70.0 | 100.0 |

TABLE III

Antischistosomal activity of bis-(aminoethyl) disulfides in mice

$(RNHCH_2CH_2S)_2$

| Compound | R | Dose (mg/kg/ day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|---|
| 73 | n-Bu | 300 | 10/4 | 49.6 | 16.7 |
| 74 | iso-Bu [a] | 480 | 5/3 | — | 100.0 |
| 75 | *sec*-Bu [a] | 360 | 5/3 | — | 100.0 |
| 76 | $n\text{-}C_8H_{17}$ | 300 | 10/5 | 73.9 | 100.0 |
| | | 150 | 10/6 | 26.9 | 25.0 |
| 77 | $n\text{-}C_{10}H_{21}$ | 600 | 10/6 | 25.6 | 25.0 |
| 78 | Bz [a] | 600 | 5/1 | — | 0.0 |
| 79 | piperonyl [a] | 600 | 5/1 | — | 0.0 |
| 80 | 3-Me-2-thenyl [a] | 600 | 5/0 | — | 0.0 |

[a] Produced in this laboratory

TABLE IV

Antischistosomal activity of aminoethylisothiourea derivatives in mice

$$RNHCH_2CH_2S-\underset{\underset{NH}{\|}}{C}-NH_2$$

| Compound | R | Dose (mg/kg/ day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|---|
| 81 | bornyl | 250 | 5/4 | — | 100.0 |
| 82 | adamantyl | 360 | 5/4 | — | 100.0 |

TABLE V

Antischistosomal activity of other thiol derivatives in mice

| Compound | Structure | Dose (mg/kg/ day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|---|
| 83 | adamantylNHCH$_2$CH$_2$SC(=O)Ph | 400 | 10/1 | 93.9 | 100.0 |
| | | 200 | 10/0 | 81.0 | 80.0 |
| | | 100 | 10/0 | 62.9 | 60.0 |
| 84 | NH$_2$CH$_2$CH$_2$SCHOHCCl$_3$ | 250 | 10/6 | 41.4 | 25.0 |
| 85 | morpholino-S-S-C(=S)NH$_2$ | 500 | 10/7 | 30.4 | 33.3 |
| | | 350 | 10/1 | 14.6 | 27.3 |

TABLE VI

Antischistosomal activity of aminoethanephosphorothioates and disulfides in hamsters

| Number of compound | Dose (mg/kg/ day) x 5, p.o. | Animals /dead | % worms in the liver | % oogram changes |
|---|---|---|---|---|
| 2 | 800 | 4/2 | 94.4 | 100.0 |
| | 600 | 4/2 | 71.9 | 50.0 |
| | 300 | 4/1 | 32.8 | 0.0 |
| 4 | 200 | 4/1 | 27.5 | 100.0 |
| 5 | 200 | 5/3 | 66.7 | 50.0 |
| 45 | 400 | 5/2 | 30.9 | 0.0 |
| 72 | 150 | 5/1 | 47.2 | 0.0 |
| 76 | 150 | 5/2 | 53.9 | 100.0 |
| | 75 | 5/2 | 58.5 | 50.0 |

NELSON, D. L. & PELLEGRINO, J. — Experimental chemotherapy of schistosomiasis mansoni. XIV — Active derivatives of : aminoethanephosphorothioate, disulfide, and isothiourea. *Rev. Inst. Med. trop. São Paulo* **18**:443-449, 1976.

## REFERENCES

1. NELSON, D. L. & PELLEGRINO, J. — Experimental chemotherapy of schistosomiasis. XI — Active derivatives of aminoethanethiols. *Rev. Inst. Med. trop. São Paulo* **18**:264-267, 1976.

2. NELSON, D. L. & PELLEGRINO, J. — Experimental chemotherapy of schistosomiasis. XII — Active derivatives of aminoethanethiosulfuric acids. *Rev. Inst. Med. trop. São Paulo* **18**:365-370, 1976.

3. PELLEGRINO, J.; DE MARIA, M. & FARIA, J. — Infection of the golden hamster with *Schistosoma mansoni* cercariae through the cheek pouch. *J. Parasit.* **51**:1015, 1965.

4. PELLEGRINO, J. & FARIA, J. — The oogram method for the screening of drugs in schistosomiasis mansoni. *Amer. J. Trop. Med. Hyg.* **14**:363-369, 1965.

5. PELLEGRINO, J. & KATZ, N. — Experimental chemotherapy of schistosomiasis mansoni. Ed. Ben Dawes. *Advances in Parasitology* **6**:233-290, 1968.

6. STANDEN, O. D. — Experimental schistosomiasis. III — Chemotherapy and mode of drug action. *Ann. Trop. Med. Parasit.* **47**: 26-43, 1953.

Recebido para publicação em 21/10/1975.