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EVALUATION OF SOME ORGANIC COMPOUNDS ON BLOODSTREAM FORMS OF *TRYPANOSOMA CRUZI*

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Accidental transmission of Chagas' disease to man by blood transfusion is a serious problem in Latin-America. This paper describes the testing of several synthetic, semi-synthetic, and natural compounds for their activity against blood trypomastigotes in vitro *at 4 °C. The compounds embody several types of chemical structures: benzoquinone, naphthoquinone, anthracenequinone, phenanthrenequinone, imidazole, piperazine, quinoline, xanthene, and simple benzenic and naphthalenic derivatives. Some of them are for the first time tested against* Trypanosoma cruzi. *The toxic effect of these compounds on this parasite was done by two quite distinct sets of experiments. In one set, the compounds were added to infected blood as ethanolic solution. In this situation the most active one was a furan-1,2-naphthoquinone, in the same range as gentian violet, a new fact to be considered in the assessment of structure-activity relationships in this class of compounds. In other set, we tentatively evaluated the biological activity of water insoluble compounds by adding them in a pure form without solvent into infected blood. In this way some appear to be very active and it was postulated that the effectiveness of such compounds must result from interactions between them and specific blood components.*

Key words: *Trypanosoma cruzi* – chemical compounds – toxicity – naphthoquinones

Chagas' disease, which is caused by the protozoan parasite *Trypanosoma cruzi,* affects an estimated 16 to 18 million people in Latin America. Brazil, with over 6 million known cases and a third of its population at risk, has the greatest Chagas' disease problem, followed by Argentina, Chile and Venezuela (WHO, 1990).

Apart from the natural transmission through the triatomid bugs, blood transfusion is now recognized as having an increasingly important role in the transmission of the Chagas' disease (Dias & Brener, 1984). Even in countries where *T. cruzi* infections are not common, the risk of acquiring Chagas' disease through blood transfusion infection may exist. The annual incidence of transfusional Chagas' disease in Brazil is about 20.000 new cases (WHO, 1984). Thus an overall effective control of Chagas' disease requires not only tools for vector control in the field, but also complementary serological control and chemoprophylactic agents for the treatment of blood used in transfusion.

Chemoprophylaxis of Chagas' disease in blood began with Nussenzweig's attempt to eliminate the infectivity of trypomastigotes of *T. cruzi* in blood banks using gentian violet (Nussenzweig et al., 1953). Since that time, gentian violet was used by blood banks in endemic areas in an effort to eliminate transmission (Dias & Brener, 1984). Its main disadvantage is the coloring of the blood and the subsequent (reversible) staining of the patient's tissues. As a result many physicians do not use this drug routinely to treat blood (Docampo et al., 1987). Other side effects are microaglutination and "rouleaux" of erythrocytes (Resende et al., 1965) and it is not equally effective against all strains (Schlemper, 1978). These drawbacks have stimulated the search for new structural types with trypanosomicidal activ-

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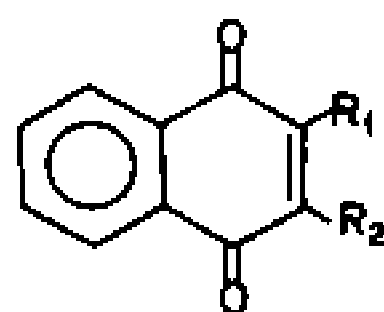
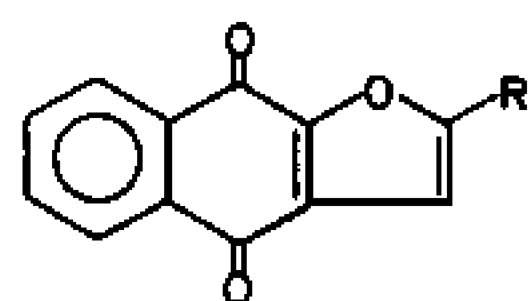
NAPHTHOQUINONE DERIVATIVES

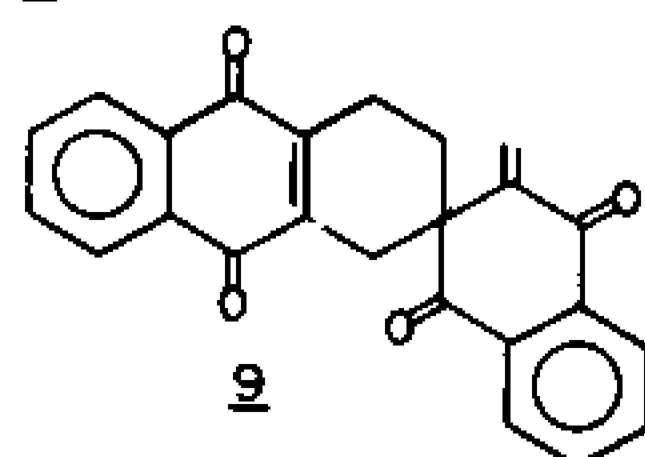
a. 1-4-Type



1 $R_1 = R_2 = Me$
2 $R_1 = Me$; $R_2 = CH_2Cl$
3 $R_1 = R_2 = CH_2Cl$
4 $R_1 = CH_2Cl$; $R_2 = CH_2Br$
5 $R_1 = R_2 = CH_2OAc$
6 $R_1 = OH$; $R_2 = Me$



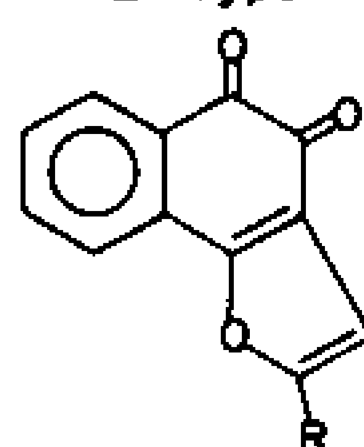
7 R = Et
8 R = COMe



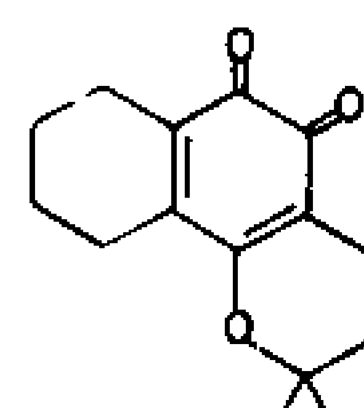
9

BENZOQUINONE DERIVATIVE

b. 1-2-Type



10 R = Et
11 $R = CH(Me)_2$



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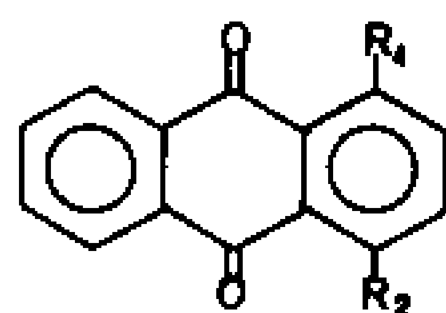
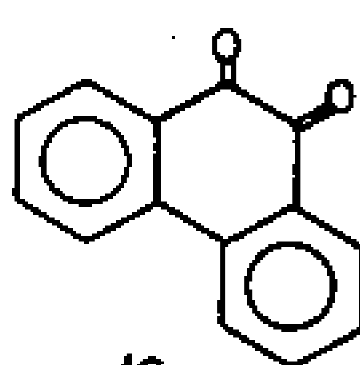
ANTHRAQUINONE DERIVATIVES



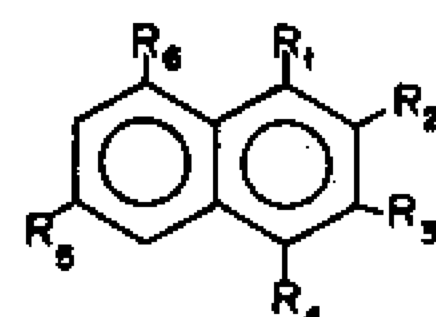
13 $R_1 = Cl$; $R_2 = H$
14 $R_1 = NH_2$; $R_2 = H$
15 $R_1 = R_2 = OH$

PHENANTHRENEQUINONE



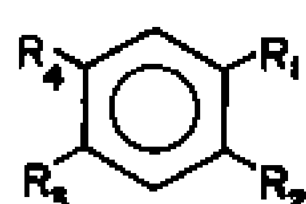
16

NAPHTHALENE DERIVATIVES



17 $R_1 = NO$; $R_2 = OH$; $R_3 = R_4 = R_5 = R_6 = H$
18 $R_1 = SO_3H$; $R_2 = NH_2$; $R_3 = R_4 = R_5 = R_6 = H$
19 $R_1 = NH_2$; $R_2 = OH$; $R_4 = SO_3H$; $R_3 = R_5 = R_6 = H$
20 $R_1 = R_6 = OH$; $R_3 = R_5 = SO_3H$; $R_2 = R_4 = H$

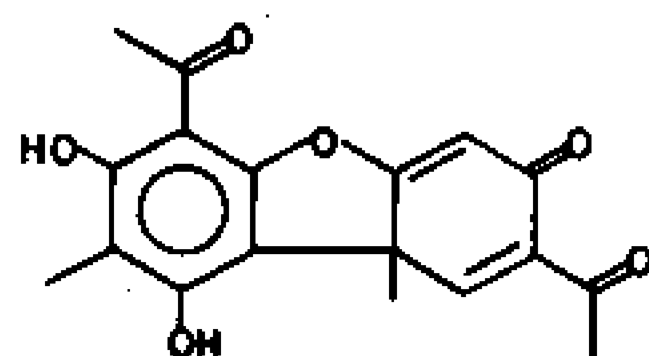
BENZENE DERIVATIVES



21 $R_1 = CONH(CH_2CO_2H)$; $R_2 = R_3 = R_4 = H$
22 $R_1 = NH_2$; $R_2 = SO_3H$; $R_4 = Me$; $R_3 = H$
23 $R_1 = NH$-Ribityl; $R_3 = R_4 = Me$; $R_2 = H$
24 $R_1 = NH$-Ribityl; $R_2 = N{=}N\phi$; $R_3 = R_4 = Me$

HETEROCYCLICS:

a. oxigem type



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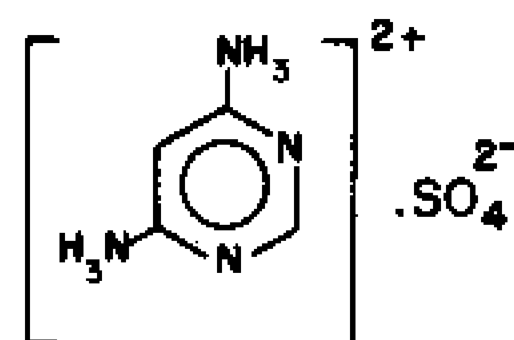
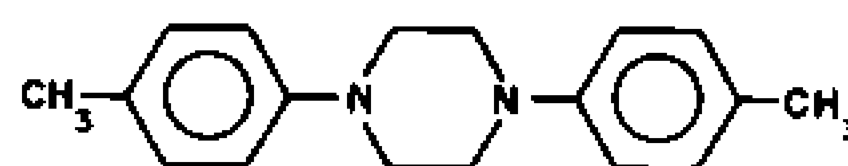
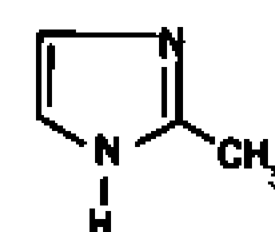


27

b. nitrogen type



28



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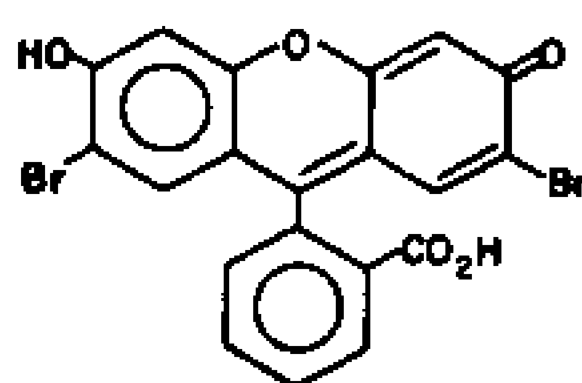

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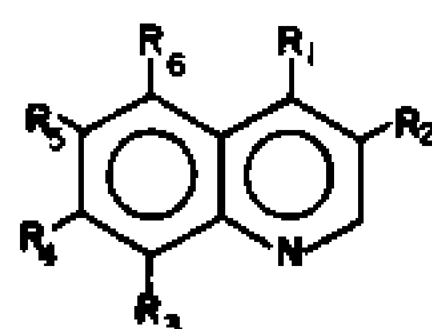
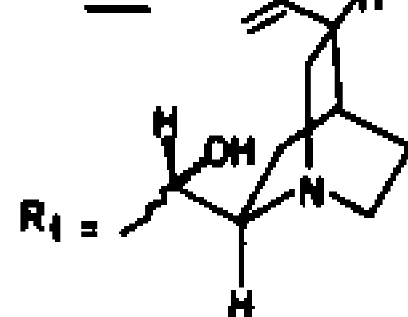


30: $R_1 = R_2 = R_5 = H$; $R_3 = OH$; $R_4 = I$; $R_6 = SO_3H$

31:



$R_2 = NH_2$; $R_5 = OMe$
$R_3 = R_4 = R_6 = H$

Chemical structures of tested compounds mentioned in the text.

ity. Several compounds have been tested against bloodstream forms of *T. cruzi* in experimentally infected mice, including amphotericin B (Cruz et al., 1980); 3-allyl-lapachone (Gonçalves et al., 1980); a quinone obtained from lapachol (Pinto & Casado, 1975); napththoquinones (Lopes et al., 1978); pyrazolopyrimidines (Avila, 1983); and amphilic cationic drugs (Hammond et al., 1984).

We have recently shown that certain simple quinones have *in vitro* activity against blood stream forms of *T. cruzi* (Pinto et al., 1987a). In the present work we describe the trypanocidal activity of several quinones and other structural compounds. Some of these compounds are from a programme for the chemical synthesis of antiparasitic drugs and have revealed activity as blocking cercarial skin

TABLE I

In vitro effect of compounds as an ethanolic solution of *Trypanosoma cruzi* trypomastigotes in blood[a]

Compound[b]	% of parasites after incubation time (hours)[c]			
	1	6	24	48
1	36[d]	33	20	9
2	6	7	8	7
3	8	7	2	0
4	44	44	33	7
5	11	8	5	5
6	26	25	20	12
7	41	35	13	8
8	45	40	45	14
9	33	35	22	18
10	35	16	0	0
11	9	0	0	0
12	45	45	18	9
13	15	14	7	9
14	58	53	14	6
15	41	27	25	11
16	7	13	2	0
17	25	22	0	0
18	37	44	15	0
19	72	54	56	47
20	33	24	8	0
21	3	6	5	5
22	62	62	56	59
23	52	52	17	13
24	22	21	0	0
25	44	34	34	14
26	52	45	46	42
27	66	73	74	72
28	45	27	25	10
29	57	58	22	22
30	21	22	8	8
31	18	23	14	12

a: solutions were added asseptically (20 μl of ethanol/ml of blood).
b: final concentration of each compound were 200 μg/ml.
c: see experimental conditions in Materials and Methods.
d: % of mobile parasite in the blood after the indicated time.

penetration (Gilbert et al., 1977), trypanocidal (Gonçalves et al., 1980) antiviral (Lagrota et al., 1988) or antitumoral (Oliveira et al., 1990). Other active compounds now described have not been previously reported.

MATERIALS AND METHODS

Parasites and animals – *Trypanosoma cruzi*, Y strain, was used in all the experiments (Silva & Nussenzweig, 1953). C3H/HeJ and BALB/c mice, 3-6 weeks old at the beginning of the experiments, were bled in our own animal facilities.

Assay of trypanosomicidal activity – Bloodstream forms of *T. cruzi* were harvested from BALB/c mice, 7-10 days after infection with 1 x 10^5 parasites. Infected blood collected was diluted to final concentration of 3,5 x 10^5 parasites/ml with phosphate buffer saline (PBS). The biological tests were performed by adding 100 μg of the compounds diluted in ethanol to 0.5 ml of blood. Test samples were then incubated (4 °C) at different times (1, 6, 24 and 48 h), followed by counting of live parasites under the light microscope. The results were represented as a percentage of life parasites in comparison with the control groups (parasites plus ethanol). We determined that the concentration of ethanol up to 30 μl/ml of blood did not affect the viability of trypomastigotes. Parallel assay was run without ethanol by adding pure compounds directly to infected blood to give a final concentration of 20 mg/ml of blood. Disappearance or immobility of trypomastigotes were judged to indicate drug action in both experiments.

Assay of infectivity in mice – Inoculum of 100 μl of blood incubated with active compounds for 24 h were applied intraperitoneally into 20 days old C3H mice. Four mice were used for each drug concentration. The course of parasitemia was determined 7, 10 and 15 days after infection by counting the number of parasites present in 5 μl tail vein blood as previously described (Melo & Brener, 1978).

Xenodiagnostic test – The tests were conducted with *Triatoma infestans* nymphs (5th stage) – six insects for each mouse. The insects intestinal tracts were examined 30 days after blood feeding for presence (+) or absence (–) of parasites.

Chemicals – The compounds are listed in Figure, broadly according to their chemical class group. Compounds 1-12, 23-26 were obtained from our programme aimed at the synthesis of potentially active compounds from abundant naturally occurring products. Several of these derivatives were quinones that have shown biodynamic activity as described above in the introduction.

The starting materials for 1-6 were 1,4-naphthoquinone and menadione, modified according to procedures previously published (Marchalk et al., 1936; Wakselman et al., 1974; Pinto et al., 1980). The quinones 7, 8, 10, 11 and 12 were prepared as published in our ear-

TABLE II

Infectivity of mice inoculated with infected blood previously treated with compounds as an ethanolic solution

Compound[a]	Parasites (%) after incubation (h)				Infectivity[c]	
	1	6	24	48	Paras.	Xenod.
1	39[b]	30	20	9	+	+
2	9	9	8	7	+	+
3	12	8	2	0	+	+
4	35	38	33	7	+	+
5	20	12	5	5	–	–
8	40	40	40	14	+	+
10	31	16	0	0	–	–
11	7	0	0	0	–	–
14	58	53	14	6	+	+
16	7	13	2	0	+	+
17	25	22	0	0	–	–
18	25	44	15	0	+	+
21	3	6	5	5	+	+
24	22	21	0	0	+	+
26	52	45	46	42	+	+
28	45	27	25	10	+	+
29	57	58	22	22	+	+
31	18	23	14	12	+	+

a: as an ethanolic solution (20 μl of ethanol/ml of blood). The final concentration of the compounds were 200 μg/ml of blood.
b: % of mobile parasite in the blood after the indicated time.
c: inocule: 0,1 ml of treated blood/mouse incubated 24 h. Parasitemia and xenodiagnosis; presence (+) of absence (–) of parasites. See Materials and Methods for details.

TABLE III

Infectivity of mice inoculated with infected blood treated with compound *11* as an ethanolic solution[a]

Compound *11*	Parasites (x 10^6)/ml[b]		Infectivity[c]		% of mortality
	Before	After	Paras.	Xenod.	
+	1.1550	0.420	+	+	100
+	0.5775	0.084	–	–	0
+	0.2887	0	–	–	0
+	0.1443	0	–	–	0
+	0.0722	0	–	–	0
+	0.0361	0	–	–	0
+	0.0180	0	–	–	0
+	0.0090	0	–	–	0
+	0.0045	0	–	–	0
+	0.0023	0	–	–	0
–[d]	ND	0.084	+	+	100
–	1.1550	1.220	+	+	100

a: ethanolic solution: 20 μl/ml of blood. The final concentration of *11* in blood was 320 μg/ml.
b: number of parasites in the blood beore and 24 hours after incubation at 4 °C.
c: inocule: 0,1 ml of 24 h incubated blood/mouse. Parasitemia and xenodiagnosis; presence (+) or absence (–) of parasites. See Materials for details.
d: control with 20 μl/ml of pure ethanol; ND: not done.

lier studies (Pinto et al., 1987b, 1989). Compound 9 is a quinone dimer obtained via an unstable ortho-quinone dimethene that occurs in the reaction of 2,3-bis-(bromomethyl)-1,4-naphthoquinone with sodium iodide in dimethylformamide, following a procedure similar to that described by Lin & Sartorelli (1973). Compounds 23 and 24 are intermedi-

TABLE IV

Effect of pure compounds on trypomastigostes of *Trypanosoma cruzi* without ethanol

Compound[a]	% of parasites after incubation time (hours)			
	1	6	24	48
1	65[b]	1	0	0
2	10	10	9	0
3	6	2	1	0
4	19	19	10	0
5	18	10	13	0
6	39	10	0	0
7	32	27	12	11
8	41	35	30	0
9	15	13	26	8
10	25	20	12	0
11	36	17	3	0
12	7	1	0	0
13	78	75	64	56
14	35	32	19	3
15	27	31	7	1
16	95	29	0	0
17	6	2	0	0
18	0	0	0	0
19	10	31	13	4
20	45	36	5	2
21	0	0	0	0
22	51	59	42	40
23	49	50	35	19
24	46	35	17	4
25	36	22	24	20
26	0	0	0	0
27	86	73	72	70
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0

a: concentration: 20 mg/ml of blood.
b: % of mobile parasites after the indicated time.

ates in the synthesis of riboflavin and were obtained as described (Tischler & Carlson, 1944); compound 25 is d-usnic acid, isolated from an *Usnea* sp. (Mors, 1953), and compound 26 is from the bromination of fluorescein in acetic acid (Mano & Seabra, 1987). The other compounds 13, 22, 27-30 are dyestuff intermediates from local industries. Finally, the compound 31 is from Aldrich Chemical Company, Inc.

RESULTS AND DISCUSSION

Nearly all compounds listed in Figure are water insoluble compounds an were tested by two quite distinct sets of experiments. In one set, the compounds were added to infected blood as ethanolic solutions (Table I). In the other set, the compounds were added in a pure form into infected blood without ethanol as solvent (Table IV). The results indicated that the most active compounds are 10, 11, 17, and 24. The first two are quinonoid structures and the last two naphthalene and benzene derivatives, respectively. Among several types of quinones, the most active were naphthoquinones of the 1,2-type (ortho-quinone), 10 and 11. These results are in agreement with the known fact that 1,2-naphthoquinones are more active than the corresponding 1,4-type in the toxic effect on blood trypomastigotes. Also, the 1,2-naphthoquinone β-lapachone is more active than the 1,4-isomer α-lapachone against bacteria and fungi (Lopes et al., 1978; Gonçalves de Lima et al., 1962). It is worth pointing out that these two quinones are heterocyclic compounds of the furan type. These results represent a new fact to be considered in the assessment of structure-activity relationships in this class of compounds (Pinto et al., 1987a). The assay of infectivity in mice (Tables II, and III) indicates that only 11, a furano-1,2-naphthoquinone, is in fact a trypanocidal in this set, using ethanol as solvent. Although 11 is active in less than 6 h of incubation and in the same concentration (83×10^{-5} M) range as gential violet (61×10^{-5} M), it is a water insoluble compound and, therefore, not suitable for direct use in banked blood, unless properly formulated. Further chemical studies can lead to water soluble structural derivatives of potential interest. It was previously shown that furanaphthoquinones inhibited the parasite epimastigote form growth equally or more effectively than nifurtimox and benzmidazole (Ribeiro-Rodrigues et al., 1989).

Table IV shows the results of the other set of experiments, where we tentatively evaluate the biological activity of these insoluble compounds by adding them, in a pure form withouth solvent, into infected blood. In this way, some compounds appear to be very active against trypomastigote blood forms. One group of compounds, 1, 6, 12, 16 and 17, showed activity in less than 24 hours of incubation. Another group, 18, 21, 26, 28, 29, 30 and 31 are active in less than 1 h of incubation. The assays of invectivity in mice (Table V) indicate that most are in fact trypanocidal and growth inhibitors. Unfortunately, these compounds cause partial hemolysis of the red cells in the range concentration indicated in Table IV. It is interesting to observe that between two set of experimental conditions (Table I, IV) there is little correlation of results. In fact, compounds 10, 11 and 24, that are active when added as ethanolic

TABLE V

Infectivity of mice inoculated with infected blood treated with pure compounds without ethanol

Compound[a]	Parasites (%) after incubation (h)				Infectivity[c]	
	1	6	24	48	Paras.	Xenod.
1	5[b]	1	0	0	–	–
2	10	10	9	0	–	–
3	6	6	1	0	–	–
4	19	19	10	0	–	–
6	39	10	0	0	+	+
8	41	35	30	0	+	+
10	25	20	12	0	–	–
11	36	17	3	0	–	–
12	7	1	0	0	–	–
16	95	29	0	0	–	–
17	6	2	0	0	–	–
18	0	0	0	0	–	–
21	0	0	0	0	–	–
26	0	0	0	0	–	–
28	0	0	0	0	–	–
29	0	0	0	0	–	–
30	0	0	0	0	–	–
31	0	0	0	0	–	–

a: concentration in blood: 20 mg/ml.
b: % of mobile parasites in the blood after indicated time.
c: C3H mice received 100 μl of 24 hours-incubated blood per animal. The presence (+) or absence (–) of circulating parasites was examined by parasitemia and xenodiagnosis. See Materials and Methods for details.

solution into infected blood, are not active when added, without ethanol as solvent, in a pure form. In contrast, some are only very active when added in a pure form. The only exception is 17, active in both cases.

The group of compounds includes several types of chemical structures. The structural types 1,4-naphthoquinonic derivatives (1 and 6), the imidazolic derivative (29), the quinolinic derivative (30 and 31), and the benzoquinonic derivative (12) are already known as trypanocides (Lopes et al., 1978; Avila, 1983; Chiari et al., 1991). However, the phenanthrenic type quinone (16), the nitrosophenolic and the aminosulphonic acid derivatives of the naphthalene ring (17 and 18 respectively), the benzamidic derivative (21, hippuric acid), the isobenzofuranxanthenic derivative (26, 2,7-dibromfluorescein) and the piperazinic derivative (28) are new compounds active against blood trypomastigotes of *T. cruzi in vitro* at 4 °C and might therefore warrant further biological and chemical studies for preventing transmission of Chagas' disease by blood transfusion.

Water insoluble drugs are not considered suitable as trypanocidal additives to banked blood. However, these compounds can be very effective as demonstrated by the infectivity assay in mice (Table V). In our opinion these results deserve some attention. Although the dose was one hundred times higher than that of the active compounds tested as ethanolic solutions, the remarkable effectiveness of such insoluble water compounds must result from some interaction between them and specific blood components. Their activity may reflect the formation of a strong host-guest complex between these water insoluble organic compounds and blood macromolecules resulting in sufficient solubility that they can be evaluated in a hydrophilic medium. Whether such complexion actually occurs and can be further used in transfusional-transmitted Chagas' disease chemotherapy has to be evaluated. We are now measuring the minimal inhibitory dose of the active compound and observing the effects on red cell hemolysis, first step in this evaluation.

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REFERENCES

AVILA, J. L., 1983. New rational approaches to Chagas' disease chemotherapy. *Interciência, 8:* 405-416.
CHIARI, E.; OLIVEIRA, A. B.; RASLAN, D. S.; MESQUITA, A. A. L. & TAVARES, K. G., 1991. Screen-

ing *in vitro* of natural products against blood forms of *Trypanosoma cruzi. Trans. R. Soc. Trop. Med. Hyg., 85:* 372-374.

CRUZ, F. G.; MARR, J. J. & BERENS, R. L., 1980. Prevention of transfusion-induced Chagas' disease by amphotericin B. *Am. J. Trop. Med. Hyg., 29:* 761-765.

DIAS, J. C. P. & BRENER, S., 1984. Chagas' disease and blood transfusion. *Mem. Inst. Oswaldo Cruz, 79:* 139-141.

DOCAMPO, R.; MORENO, S. N. J. & CRUZ, S. F., 1987. Enhancement of the cytotoxity of crystal violet against *Trypanosoma cruzi* in the blood by ascorbate. *Mol. Bioch. Parasitol., 27:* 241-248.

GILBERT, B.; PELLEGRINO, J.; PINTO, M. C. F. R.; MELO, R. T. & PINTO, A. V., 1977 Schistosomiasis mansoni: blockage of cercarial skin penetration by chemical agents: I. Naphthoquinones and derivatives. *Trans. R. Soc. Trop. Med. Hyg., 71:* 1331-1335.

GONÇALVES DE LIMA, O.; D'ALBUQUERQUE, I. L. & MAIA, M. H. D., 1962. Atividade antimicrobiana de alguns derivados do lapachol em comparação com a xiloidona, nova ortonaftoquinona natural isolada do extrato do cerne de pau d'arco, *Tabebuia avellanedae* Lor. ex Griseb. *Rev. Inst. Antibióticos de Recife, 4:* 3-10.

GONÇALVES, A. M.; VASCONCELLOS, M. E.; DOCAMPO, R.; CRUZ, F. S.; DE SOUZA, W. & LEON, W., 1980. Evaluation of the toxicity of 3-allyl-β-lapachone against *Trypanosoma cruzi* bloodstream forms. *Mol. Bioch. Parasitol., 1:* 167-176.

HAMMOND, D. J.; COVER, B. & GUTTERIDGE, W. E., 1984. A novel series of chemical structures active in vitro against the trypomastigote forms of *Trypanosoma cruzi. Trans. R. Soc. Trop. Med. Hyg., 78:* 91-95.

LAGROTA, M. H. C.; WIGG, M. D.; SANTOS, M. G. M.; PINTO, M. C. F. R. & PINTO, A. V., 1988. Atividade antivirótica de naftoquinonas, II. Derivados 1,4-naftoquinônicos frente a enterovirus. *Rev. Microbiol.* (São Paulo), *19:* 338-342.

LIN, A. J. & SARTORELLI, A. C., 1973. 2,3-Dimethyl-5,6-bis (methylene)-1,4-benzoquinone. The active intermediate of bioreductive alkylating agents. *J. Org. Chem., 88:* 813-817.

LOPES, J. N.; CRUZ, P. S.; DOCAMPO, R.; VASCONCELLOS, M. E.; PINTO, M. C. F. R.; GILBERT, B. & PINTO, A. V., 1978. In vitro and in vivo evaluation of the toxicity of 1,4-naphthoquinone and 1,2-naphthoquinone derivatives against *Trypanosoma cruzi. Ann. Trop. Med. Parasitol., 72:* 1-9.

MANO, E. B. & SEABRA, A. P., 1987. *Práticas de Química Orgânica,* 3ª ed., Edgard Blücher Ltda., São Paulo.

MARSCHALK, C.; KOENIG, F. & OUROUSSOFF, N., 1936. New method of introducing side chains into the anthraquinone nucleus. *Bull. Soc. Chim. France, 3:* 1545-1575.

MELO, R. C. & BRENER, Z., 1978. Tissue tropism of different *Trypanosoma cruzi* strains. *J. Parasitol., 64:* 475-478.

MORS, W. B., 1953. Investigações químicas sobre líquens brasileiros: Estudo da *Usneae* da Serra dos Órgãos. *Anais da Associação Brasileira de Química, 13:* 61-66.

NUSSENZWEIG, V.; SONNTAG, R.; BIANCALANA, A.; FREITAS, J. L. P.; AMATO NETO, V. & KLOETZEL, J., 1953. Ação de corantes tri-fenilmetânicos sobre *Trypanosoma cruzi in vitro.* Emprego do violeta de genciana na profilaxia da transmissão da moléstia de Chagas por transfusão de sangue. *O Hospital, 44:* 731-744.

OLIVEIRA, M. M.; FÁVARO, O. C. N.; ROSSINI, M. A. A.; KAMAKURA, C. R.; PINTO, M. C. F. R. & PINTO, A. V., 1990. Seleção por meio de células KB de substâncias e extratos potencialmente ativos em quimioterapia do câncer. *An. Acad. bras. Ciênc., 62:* 217-224.

PINTO, A. V. & CASADO, M. T. F., 1975. Alquilação do lapachol. *Ciência e Cultura, 27:* (suplemento), 165.

PINTO, A. V.; FERREIRA, V. F.; CAPPELLA, R. S.; GILBERT, B.; PINTO, M. C. R. & SILVA, J. S., 1987a. Activity of some naphthoquinones on blood stream forms of *Trypanosoma cruzi. Trans. R. Soc. Trop. Med. Hyg., 81:* 609-610.

PINTO, A. V.; FERREIRA, V. F.; PINTO, M. C. F. R. & SILVA, M. M., 1987b. Selective aromatic reduction in pyranonaphthoquinone systems. *J. Chem. Res.* (S): 26.

PINTO, A. V.; PINTO, M. C. F. R. & FERREIRA, V. F., 1989. An easy approach to prepare napththo[2,3-b]furan-4,9-diones. *Synth. Comm., 19:* 1061-1069.

PINTO, A. V.; PINTO, M. C. F. R. & TORRES DE OLIVEIRA, C. S., 1980. Síntese de Naftoquinonas naturais a partir do lapachol. *An. Acad. bras. Ciênc., 52:* 481.

RESENDE, J. M; ZUPELLI, W. & BAFUSTO, M. G., 1965. O problema da transmissão da doença de Chagas por transfusão de sangue. Emprego da violeta de genciana como medida profilática. *Rev. Goiana de Med., 11:* 35-47.

RIBEIRO-RODRIGUES, R.; ZAWNI, C. L.; OLIVEIRA, A. B. & ROMANHA, A. J., 1989. Naphthofuran and naphthofuranquinone derivatives as inhibitors of *Trypanosoma cruzi* epimastigotes growth in culture. *Brazilian-Sino Symposium on Chemistry and Pharmacology of Natural Products,* 209. Fundação Oswaldo Cruz – Rio de Janeiro, Brasil.

SCHLEMPER JR., B. R., 1978. *Estudos experimentais de quimioprofilaxia da transmissão da doença de Chagas por transfusão sanguínea.* Thesis, UFMG, *Rev. Patol. Trop., 7:* 55-111.

SILVA, L. H. P. & NUSSENZWEIG, V., 1953. Sobre uma cepa altamente virulenta para o camundongo branco. *Fol. Clin. Biol., 20:* 191-208.

TISHTER, M. & CARLSON, G. H., 1944. U. S. Patent no. 2,350,376. June, 6th, (to Merck & Co.).

WAKSELMAN, M.; HAMON, J. F. & VILKAS, M., 1974. Lactones phenoliques halomethylées, inhibiteurs bifonctionnels. *Tetrahedron, 30:* 4069-4071.

WHO, World Health Organization, 1984. Meeting on the Development of Trypanocidal Compounds for the Sterilization of Blood, TDR/CHA/BS/84.3.

WHO, World Health Organization, 1990. Programme for Research and Training in Tropical disease (TDR), No. 34.