

# Activity of 9-acridanone-hydrazone Drugs Detected at the Pre-postural Phase, in the Experimental Schistosomiasis *Mansoni*

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The compound Ro-15.5458/000, derivative in the class of 9-acridanone-hydrazones, was found to be effective against *Schistosoma mansoni* in mice, killing almost all the skin schistosomules (24 hr after infection), when administered at the dose of 100mg/kg. In experiments carried out with *Cebus* monkeys, the drug was shown to be fully effective at 25mg/kg, 7 days after infection. These data, associated with the good results obtained earlier at the post-postural phase of schistosomiasis, allow the inference that this promising compound may be important in the set of antischistosomal drugs, depending on further toxicological and clinical tests.

Key words: acridanone-hydrazones - *Schistosoma mansoni* - experimental schistosomiasis

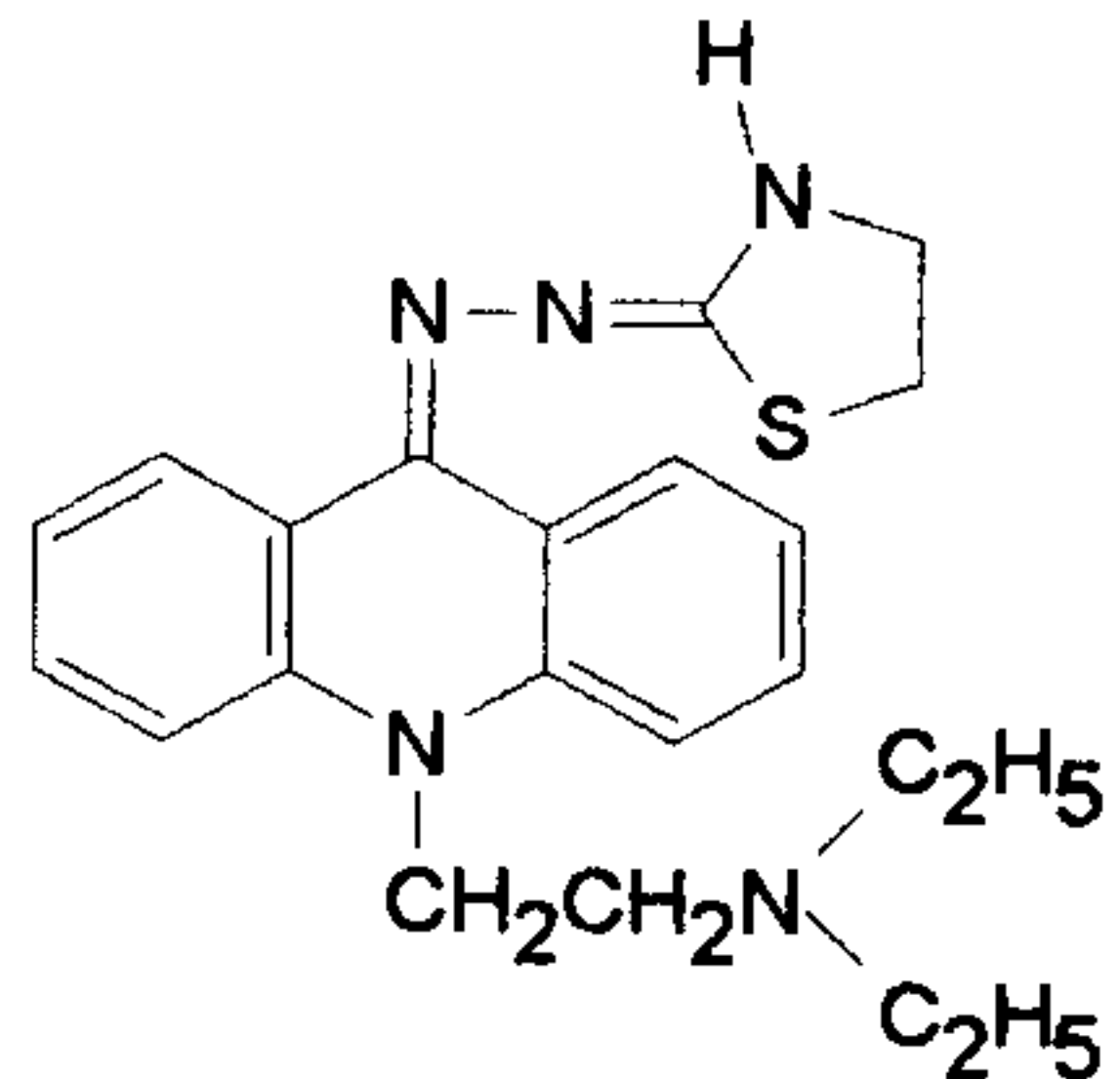
Derivatives in the chemical group of 9-acridanone-hydrazones showed high schistosomicidal activity at the post-postural phase of experimental schistosomiasis in primates (Sturrock et al. 1987, Coelho & Pereira 1991, Coelho et al. 1994 in press). Some compounds of this group, as Ro-15.5458/000, were found to be highly active against adult worms, at doses of 12.5mg/kg body weight, in *Cebus* monkeys infected with well defined Brazilian geographical strains of *Schistosoma mansoni* (Coelho & Pereira 1991, Coelho et al. 1994 in press).

In this study, undertaken to investigate the activity of the compound Ro-15.5458/000 at the pre-postural phase of infection, the authors used albino mice and *Cebus* monkeys as models.

## MATERIALS AND METHODS

The chemical structure of compound Ro-15.5458/000, which was developed at the laboratories of F. Hoffmann - La Roche & Co. Ltd. (Basel, Switzerland), is shown in the Figure.

*Test with mice* - Eighty adult albino mice (*Mus musculus*), all outbred females, reared at the Schistosomiasis Research Unit - Laboratory Prof.



Chemical structure of the 9-Acridanone-hydrazone compound used in this work.

José Pellegrino - Federal University of Minas Gerais (Brazil), were used.

The animals were transcutaneously infected with about 100 *S. mansoni* cercariae (LE strain), as described by Barbosa et al. (1978). The LE strain of *S. mansoni* has been maintained at the laboratories of the Schistosomiasis Research Unit for more than 30 years, through *Biomphalaria glabrata* passages, and using miracidia from the liver of hamsters (*Cricetus auratus*).

Twenty infected animals were kept as controls, and the other 60 were treated 24hr after infection. The compound Ro-15.5458/000 was

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given at a dose of 100mg/kg bodyweight, by oral route.

Thirty-five days after infection, the animals were killed by cervical fracture and their portal system was perfused for worms, in general terms, according to the technique described by Pellegrino and Siqueira (1956).

**Test with Cebus monkeys** - Five adult animals were transcutaneously infected with about 200 *S. mansoni* cercariae (LE strain). Two monkeys (M-16 and H-17) were maintained as controls, and three others (H-15, H-18 and M-19) received specific treatment (25mg/kg Ro-15.5458/000) by oral route, one day after infection (monkey H-15), 7 days after infection (monkey H-18), and 28 days after infection (monkey M-19). Rectal biopsies were carried out at 79 days after treatment, for collection of rectal snips, which were weighed and examined under optical microscope for counting and classification of *S. mansoni* eggs (quantitative oogram, as outlined by Katz et al. 1966). At 183 days after infection, the monkeys H-15, M-16 and H-18 were sacrificed by means of a lethal dose of pentobarbital sodium, and perfused for worms, as described by Pellegrino and Siqueira (1956). Fragments of the liver and intestinal mucosa were examined by using the quantitative oogram method (Katz et al. 1966).

**Statistical analysis** - The Student's t-test for unpaired samples was used for comparison of the mean numbers of worms recovered from mice.

## RESULTS

As can be seen in Table I, a marked difference ( $p < 0.001$ ) was observed between the average numbers of worms recovered from the control group and those from the treated group. Thus, the compound Ro-15.5458/000 was found to be fully effective at a rate of 100mg/kg, practically killing all the parasites in the treated group of mice, at 24hr after cercarial penetration.

The experiment performed with *Cebus* monkeys showed partial activity of the drug 24hr after infection, by means of periodic examinations of intestinal mucosal snips (Table II). Moreover, the same experiment clearly showed suppression of egg-laying, when the treatment was started at 7 days after infection.

At 183 days following infection, the monkeys H-18, H-15 and M-16 were sacrificed and perfused for worms.

Monkey H-18 (treated at 7 days after infection) presented no worms at perfusion. The absence of eggs in both hepatic tissue and rectal mucosa confirms the parasitological cure.

At perfusion, monkey H-15 (treated at 24hr after infection) showed the presence of 63 worms (26 females and 37 males), as well as viable eggs in the hepatic tissue and rectal mucosa.

Monkey M-16 (untreated, kept as control) showed a total of 135 worms (86 males and 49 females) at perfusion. This worm recovery, clearly higher than that obtained from monkey H-15, suggests a partial activity of the drug, which was able to eliminate some but not all schistosomes at skin level.

## DISCUSSION

The results from the present study indicate the efficacy of Ro-15.5458/000 against *S. mansoni*, at the skin phase, in mice treated with 100mg/kg bodyweight. However, the results obtained from *Cebus* monkeys treated with this drug, at the dose of 25mg/kg, 24hr after infection (skin phase), were not as satisfactory as those seen in mice, due to the survival of a significant number of worms. On the other hand, at the pulmonary phase of the parasites cycle (7 days after infection), a complete eradication of the parasitism was achieved in *Cebus* monkeys. These latest results showed the full effectiveness of the drug Ro-15.5458/000 given at a relatively lower dose (25mg/kg), at the pulmonary phase. Possibly, us-

TABLE I

Recovery of worms from mice, by means of perfusion of the portal system, 35 days after transcutaneous infection with about 200 *Schistosoma mansoni* cercariae, from the group treated with Ro-15.5458/000 (100mg/kg), at 24hr after infection, and the respective control group

	Male	Female	Total worms
Treated mice with Ro-15.5458/000 <sup>a</sup> (100mg/kg) No. of mice = 60	Total = 3 M ± SD = 0.05 ± 0.22	Total = 7 M ± SD = 0.12 ± 0.32	Total = 10 M ± SD = 0.17 ± 0.37
Control No. of mice = 16	Total = 393 M ± SD = 23.94 ± 7.87	Total = 158 M ± SD = 9.87 ± 4.27	Total = 551 M ± SD = 34.44 ± 9.63

<sup>a</sup>: 10 out of 60 treated mice were found to be positive (1 worm/per animal)

The Student's t-test showed highly significant differences for all the values from the treated group in relation to the respective values from the control group ( $p < 0.001$ ).

TABLE II

Activity of Ro-15.5458/000 (25mg/kg, *per os*, single dose) in prepostural periods of infection by *Schistosoma mansoni* (LE strain) in *Cebus* monkeys. Each primate was previously exposed to about 200 cercariae

Monkey	Time of treatment (days after infection)	Days after treatment	Oogram (viable eggs)					Dead eggs	No. of viable eggs per gram of rectal snips
			1st	2nd	3rd	4th	mature		
H-15	1 day	79	14	0	0	1	3	47	269
		100	0	0	0	0	0	10	0
		130	0	0	4	0	1	5	147
		142	8	3	8	3	7	7	617
		163	0	0	0	2	6	13	267
		186	0	0	0	0	2	5	55
H-18	7 days	79	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0
		130	0	0	0	0	0	0	0
		142	0	0	0	0	0	0	0
		163	0	0	0	0	0	0	0
		186	0	0	0	0	0	0	0
M-19	28 days	59	46	56	135	71	156	336	9098
		72	26	4	31	11	45	335	2127
		87	1	0	0	13	3	17	370
		117	0	0	5	2	18	68	781
		126	8	5	12	15	59	84	2813
		147	0	1	2	68	175	28	6482
		158	19	1	4	1	11	14	1091
M-16 control monkey	no drug used	87	0	0	0	0	0	83	0
		101	9	5	2	0	0	22	400
		131	1	6	30	10	1	22	1379
		143	17	31	2	0	18	116	2267
		164	9	29	50	15	30	48	3325
		178	0	0	0	0	4	8	103
H-17 control monkey	no drug used	87	0	5	5	0	30	28	755
		101	5	20	31	16	25	47	2694
		131	3	0	2	0	51	77	1041
		143	0	3	0	5	80	85	2009
		164	25	12	5	28	36	31	3365
		178	8	9	5	20	47	45	2713

ing higher doses in *Cebus* monkeys, the parasite's population could be markedly reduced at the skin phase (24hr after infection). Another experiment was devised to investigate this point of interest, but difficulties in getting *Cebus* monkeys prevent us to carry out a subsequent study.

Earlier results obtained with 9-acridanone-hydrazones showed the efficacy of these drugs at the post-postural phase of schistosomiasis mansoni (Sturrock et al. 1987, Coelho & Pereira 1991, Coelho et al. 1994 in press). The results recorded so far, added to the ones presented in this study, indicate that these extremely promising

compounds may be considered as an important reserve in the set of schistosomicide drugs. The 9-acridanone-hydrazone compounds, if approved by means of toxicological and clinical tests, may play an important role in the treatment for human schistosomiasis.

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