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IN VITRO **ACTION OF THREE BENZO (de) ISOQUINOLINE-1,3-DIONE DERIVATIVES AGAINST** TRYPANOSOMA CRUZI

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SUMMARY

The "in vitro" action of three benzo(de) isoquinoline-1,3-dione derivatives, (5-nitro 2-(2-dimethylaminoethyl); 5-nitro 2-(2-(1-pyrrolidine)-ethyl) and 5 amino-2-(2-dimethylaminoethyl)), against **Trypanosoma cruzi** flagellate forms was tested. The results obtained show that all three products inhibit the normal development of culture forms of **T. cruzi.**

INTRODUCTION

There are previous reports showing that the three compounds M-4212 (5 nitro 2 (2 dimetylaminoethyl)-benzo (de) isoquinoline-1,3-dione), M-12210 (5-nitro-2-(2-(1-pyrrolidine)-ethyl)-benzo (de) isoquinoline-1,3-dione) and FA-142 (5-amino-2-(2-dimethylaminoethyl)-benzo (de) isoquinoline 1,3 dione), synthesized by ROLDAN et al.[8] in 1973, have cytostatic effects[3] and antiviral action[7] probably due to their intercalation on the DNA helix[4].

We have tested these compounds against culture forms of **T. cruzi**, protozoan responsible for the **Tripanosomiasis americana** or Chagas'Disease, at present considered to be the most wide-spread and dangerous disease on the American Continent and for which, so far, no effective treatment has been found.

MATERIAL AND METHODS

Figure 1 shows the formula of the three compounds used in this study. Stock solution of each of these compounds was 10 mg/ml in a solution containing 4 mg of anhydride sodium acetate, 5mg of acetic acid in 100 ml of deionized water.

The strain of **T. cruzi** used in this study was obtained from the Institute of Malariology in Caracas, where it was isolated from a human clinical case and has been maintained in our laboratory since 1973 in successive passages, in albino mice, on one hand, and in NNN medium with a liquid phase of Eagle's Minimum Essential Medium[6] supplemented with 20% of foetal calf serum, on the other. In order to avoid the presence of agar clots, which would have caused problems in the counting of the cultures, forms taken from cultures in NNN were cultivated in LIT medium[5], at 28°C.

Once the flagellate forms had been obtained in the LIT medium, they were pelleted at 450 g for 10 minutes. The flagellate forms were suspended in LIT medium and aliquots were distributed in culture flasks (2.10^5 flagellates/ml) which already contained the substances to be tested. The final concentration of the compounds was 0.05, 0.1, 0.2... to 1 μg/ml. Five culture tubes were used for each concentration and one control for each lot tested. To the control culture of the epimastigote forms an equivalent quantity of the solvent (4 mg of anhydride sodium acetate, 5 mg of acetic acid in 100 ml of deionized water) was added.

The forms were counted 36, 60, 84 and 108 hours after the starting of the cultures in each of the culture tubes, in a haemocytometric

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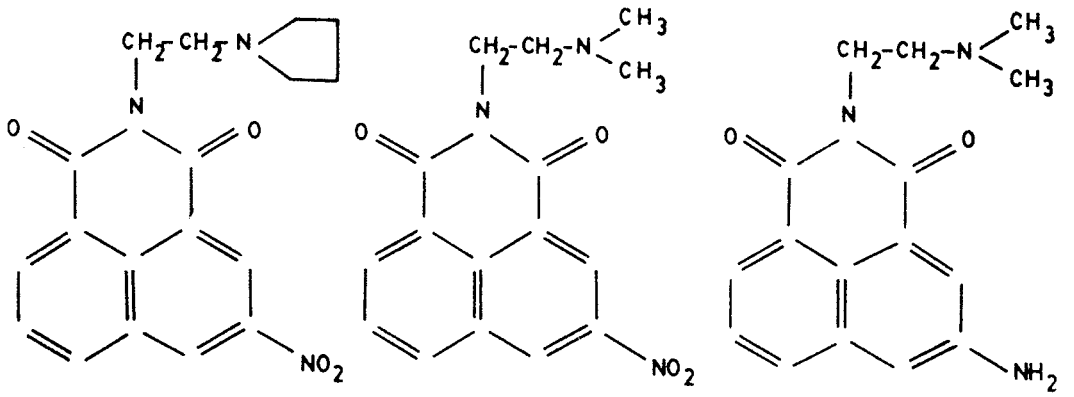


Fig. 1 — Formula of the three compounds used in this study

chamber; only the active flagellates were considered. The motility and morphological alterations were recorded and a drop of medium from each of the culture flasks counted was stained with Giemsa.

The percentage of growth inhibition produced in the cultures of **T. cruzi** by the three products was calculated as follows:

$$PI = \frac{TC - TP}{TC} \times 100$$

PI. — Inhibition growth percentage.

TC. — Average number per ml of flagellate forms in the control tubes.

TP. — Average number per ml of flagellate forms in the tubes with the differents drugs.

After 108 hours all the culture flasks were centrifuged at 450 g for 10 min. to eliminate the culture medium together with the drugs and after three successive washings in the centrifuge with BSS Hanks solution they were cultivated in LIT medium. Counts were made 72 and 96 hours after this subcultivation.

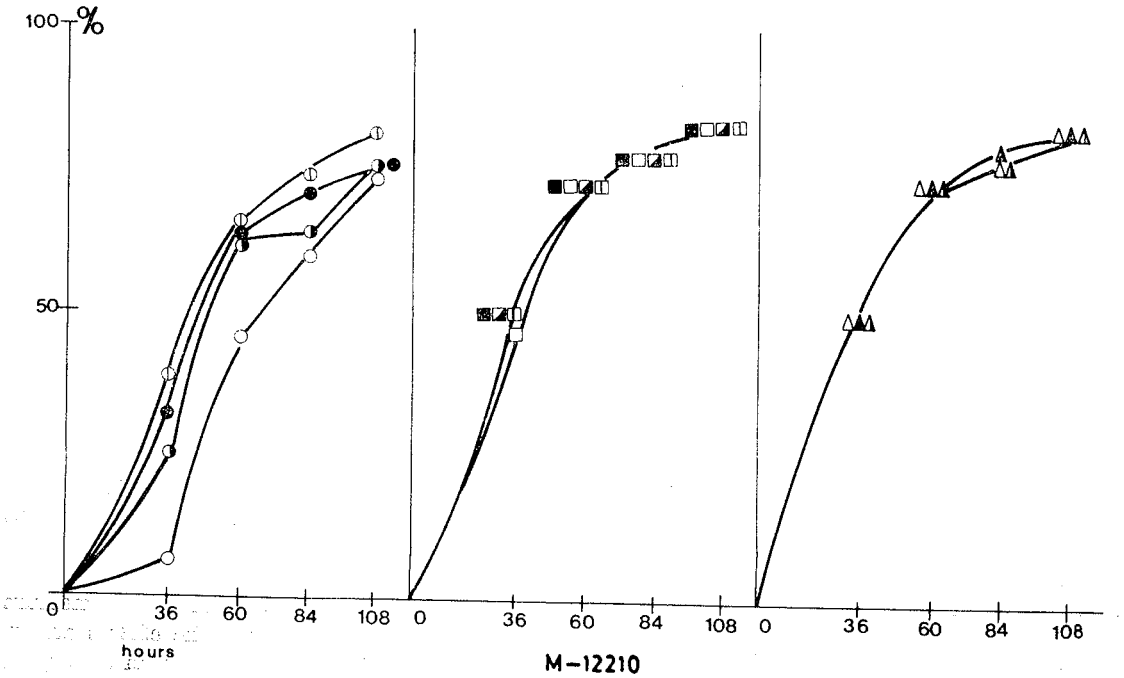
RESULTS AND DISCUSSION

The results obtained show that all three products tested have some degree of action on the culture forms of **T. cruzi** although the one with the most rapid action was M-12210, as can be seen in Figs. 2, 3, 4. This figure presents the percentage of growth inhibition of the three products tested.

The subcultures made in fresh medium after 108 hours showed that the effects are irreversible for M-12210 starting from 0.3 μg/ml. No growth whatever was obtained after 96 hours of culture in fresh medium using the concentrations already mentioned. The number of trypanosomes originally in the subculture decreased progressively even though a certain amount with apparent motility was maintained (Table IV). The fact that the culture forms continued to show motility in the presence of the drug during the experiment indicates that the products do not by themselves have a trypanolytic action. Results in Tables I, II and III show that, at higher drug concentrations active epimastigote forms still appear after 108 hours, therefore, the drugs seem to act only the reproduction of the protozoon, apparently blocking DNA and RNA synthesis. This type of phenomenon has been described by GARCÍA-GANCEDO et al.[7] in viruses and by BRAÑA et al.[3] in cancer cells, where the products prevented DNA replication and transcription.

In the Giemsa stained preparations, nuclear alterations, irregular chromatic condensations, and clear zones within the nucleus have been observed, which can be partially explained by the affinity that these products show for DNA.

Figs. 2, 3 and 4 — **Percentages of growth inhibition produced in the cultures of** T. cruzi **epimastigotes by M-12210, M-4212 and FA-142**



O = 0.05 µg/ml ◑ = 0.10 µg/ml ● = 0.20 µg/ml ◍ = 0.30 µg/ml

□ = 0.40 µg/ml ◪ = 0.50 µg/ml ■ = 0.60 µg/ml ▣ = 0.70 µg/ml

Δ = 0.80 µg/ml ◭ = 0.90 µg/ml ▲ = 1.00 µg/ml

Fig. 2

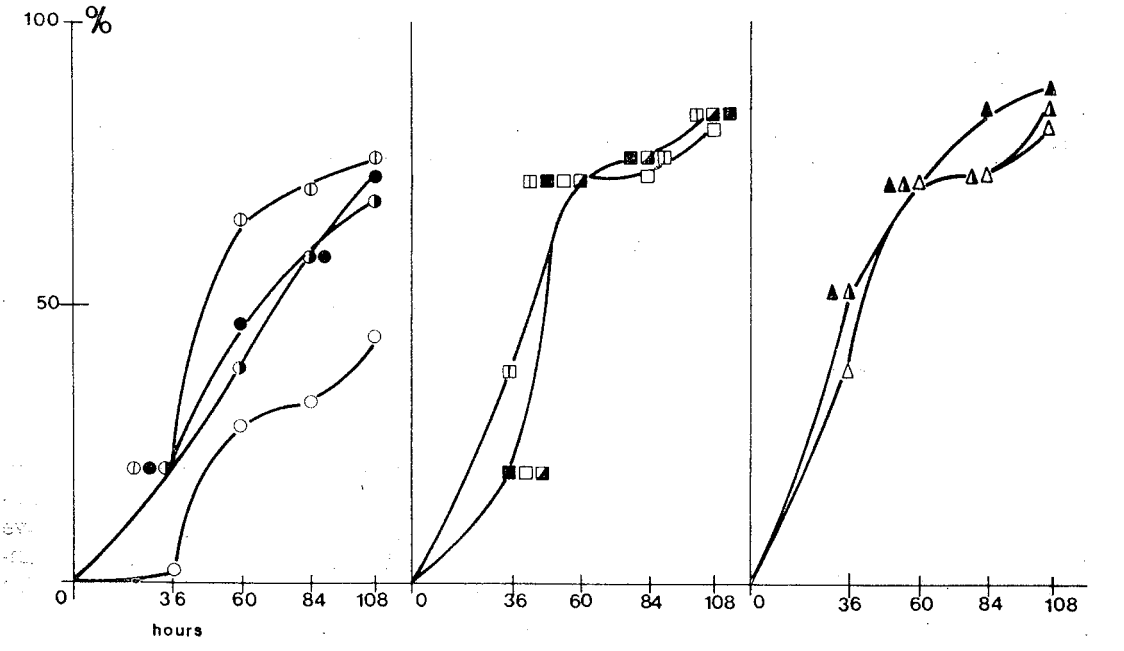


Fig. 3

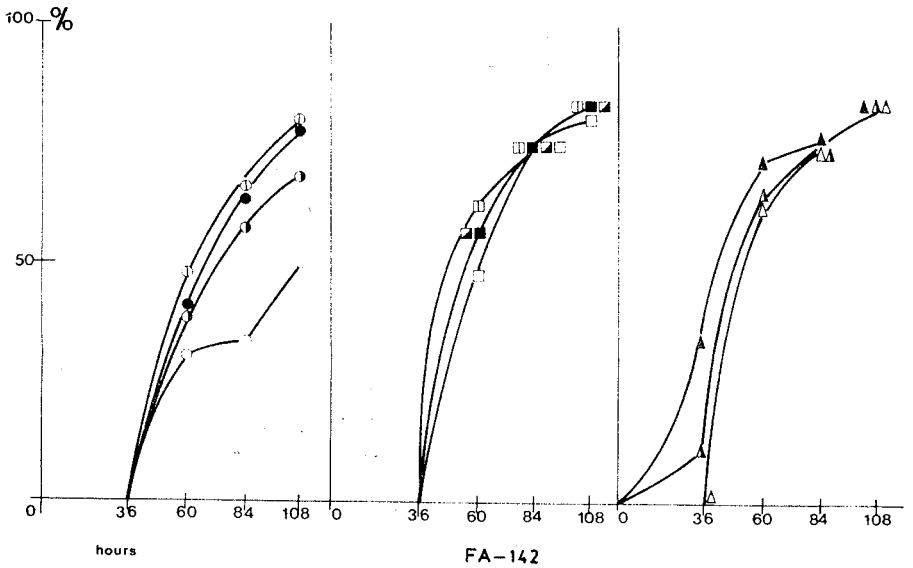


Fig. 4

T A B L E I

Effect of M-12210 on the growth of **T. cruzi** epimastigotes in LIT medium. Initial inoculum was 2.00x10⁵ parasites/ml **Average values of five experiments**

Concentration of drug (μg/ml)	Organism number (x10⁵ org./ml) 36 hrs	60 hrs	84 hrs	108 hrs
—	3.40	4.60	5.30	6.80
0.05	3.20	2.50	2.10	1.80
0.10	2.56	1.70	1.90	1.60
0.20	2.32	1.63	1.54	1.60
0.30	2.08	1.60	1.32	1.33
0.40	1.76	1.20	1.16	1.20
0.50	1.70	1.20	1.16	1.10
0.60	1.70	1.20	1.16	1.10
0.70	1.70	1.20	1.16	1.10
0.80	1.70	1.20	1.16	1.10
0.90	1.70	1.20	1.16	1.10
1.00	1.70	1.20	1.07	1.10

T A B L E II

Effect of M-4212 on the growth of **T. cruzi** epimastigotes in LIT medium. Initial inoculum was 2.00x10⁵ parasites/ml **Average values of five experiments**

Concentration of drug (μg/ml)	Organism number (x10⁵ org./ml) 36 hrs	60 hrs	84 hrs	108 hrs
—	3.40	4.60	5.30	6.80
0.05	3.36	3.30	3.60	3.70
0.10	2.72	2.80	2.10	2.20
0.20	2.72	2.50	2.10	1.90
0.30	2.72	1.60	1.60	1.70
0.40	2.72	1.30	1.50	1.30
0.50	2.72	1.30	1.40	1.20
0.60	2.72	1.30	1.40	1.20
0.70	2.08	1.30	1.40	1.20
0.80	2.08	1.30	1.40	1.20
0.90	1.60	1.30	1.40	1.10
1.00	1.60	1.30	0.80	0.90

T A B L E III

Effect of FA-142 on the growth of **T. cruzi** epimastigotes in LIT medium. Initial inoculum was 2.00x10⁵ parasites/ml **Average values of five experiments**

Concentration of drug (μg/ml)	Organism number (x10⁵ org./ml) 36 hrs	60 hrs	84 hrs	108 hrs
—	3.40	4.60	5.30	6.80
0.05	3.40	3.03	3.43	3.40
0.10	3.40	2.80	2.20	2.10
0.20	3.40	2.70	1.90	1.50
0.30	3.40	2.40	1.80	1.40
0.40	3.40	2.40	1.30	1.30
0.50	3.40	2.00	1.30	1.10
0.60	3.40	1.70	1.30	1.10
0.70	3.40	1.70	1.30	1.10
0.80	3.40	1.70	1.30	1.10
0.90	3.00	1.60	1.30	1.10
1.00	2.20	1.30	1.20	1.10

T A B L E IV

Average number per ml of total flagellate forms in the subculture, after removal of M-12210. The experiment was repeated five times

Concentration of drug (μg/ml)	Organism number (x10⁵ org./ml) 0 hrs	72 hrs	96 hrs
—	6.80	36.80	58.62
0.05	1.80	8.80	12.60
0.10	1.60	8.00	10.02
0.20	1.60	6.60	8.20
0.30	1.33	2.00	1.10
0.40	1.20	1.20	0.40
0.50	1.10	0.40	0.40
0.60	1.10	0.40	0.40
0.70	1.10	0.35	0.20
0.80	1.10	0.30	0.10
0.90	1.10	0.20	0.02
1.00	1.10	0.20	0.02

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Comparative studies seem to suggest that these new drugs have more profound effects upon **T. cruzi** cultivated **"in vitro"**, than the intercalant agents derivates of ellipticine, also tested by BÉNARD et al. [1,2].

RESUMO

Ação "in vitro" de três derivados benzo (de) isoquinolina-1,3-dionas contra Trypanosoma cruzi

Investigou-se a ação "in vitro" contra **Trypanosoma cruzi,** forma epimastigota, de três derivados benzo (de) isoquinolina 1,3 dionas, (5-nitro-2-(2-dimetilaminoetil); 5-nitro 2-(2-(1-pirrolidina)-etil) e 5-amino-2-(2-dimetilaminoetil)).

Os resultados obtidos neste trabalho, mostram que os três compostos inibem o crescimento normal da forma de cultura de **Trypanosoma cruzi.**

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