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GROWTH INHIBITORY EFFECT OF NAPHTHOFURAN AND NAPHTHOFURANQUINONE DERIVATIVES ON *Trypanosoma cruzi* EPIMASTIGOTES.

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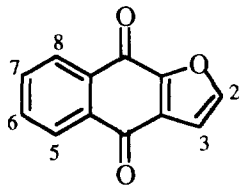
Abstract: Synthetic and natural naphthofuranquinones were tested *in vitro* against three *Trypanosoma cruzi* strains with different drug susceptibility to nifurtimox and benznidazole *in vivo*. Four compounds exhibited high trypanocidal activity. No difference in drug susceptibility was found among the strains, therefore showing no correlation between the *in vitro* activity of those compounds and the *in vivo* results with the classic drugs.

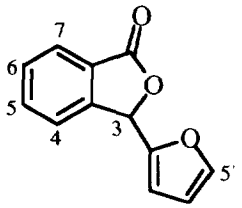
Chagas' disease, or American trypanosomiasis, is a zoonosis caused by the flagellate protozoan *Trypanosoma cruzi*. It has been estimated that at least 24 million people are currently infected, with an additional 65 million people either living in endemic areas or at risk of infection.[1] The disease is restricted to tropical and subtropical countries of Latin America with few cases reported in North America.[2] The chemotherapy of Chagas' disease is limited to the drugs nifurtimox, a 5-nitrofuran derivative, and benznidazole, a nitroimidazole derivative, both presenting low efficacy and severe side effects.[3] Furthermore, they are effective only in the acute phase of the disease, with uncertain results in the chronic phase.[4] The necessity of drugs effective in both phases of the disease, with less or no side effects and a short schedule of administration, has stimulated the search for new compounds. Moreover, drugs that can be used to sterilize blood to prevent transmission by blood transfusion in endemic and non-endemic areas are also important targets of research in Chagas' disease.

The anti-protozoan activity of naphthoquinones has been known for many years.[5-9] Their activity against *T. cruzi* blood trypomastigote has only been described recently.[10] In the present work we screened natural and synthetic quinones, as well as some synthetic intermediates, against culture forms of the SC-28, Y and CL strains of *T. cruzi*. These strains are known to display low, medium and high sensitivity to nifurtimox and benznidazole *in vivo*, respectively.[11] The natural quinones 2-(1-hydroxyethyl)-6-methoxy-naphtho[2,3-*b*]furan-4,9-quinone (**1**), 2-(1-hydroxyethyl)-7,8-dimethoxy-naphtho[2,3-*b*]furan-4,9-quinone (**2**) and 2-acetyl-7,8-dimethoxy-naphtho [2,3-*b*] furan-4,9-quinone (**3**) were isolated from the trunkwood of *Tabebuia ochracea*.[12] The synthetic 2-acetyl-7-methoxy-naphtho[2,3-*b*]furan-4,9-quinone (**4**), 5,6-dimethoxy-naphtho[2,3-*b*]furan-4,9-quinone (**5**), 7,8-dimethoxy-naphtho[2,3-*b*]furan-4,9-quinone (**6**), naphtho[1,2-*b*]furan-4,5-quinone (**7**) and their synthetic precursors (**8-12**) were obtained *via* the *ortho*-directed metalation strategy.[13,14]

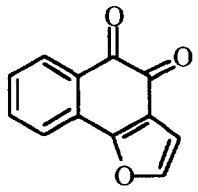
Figure 1: Structures and percent growth inhibition (GI) at 4μg/ml (13-17 μM) of the compounds tested against *T. cruzi* epimastigotes, Y strain.



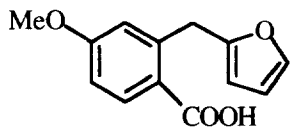
Compound	Substituents						Growth
	C-2	C-3	C-5	C-6	C-7	C-8	inhib. (%)
1	MeCH(OH)-	H	H	OMe	H	H	100
2	MeCH(OH)-	H	H	H	OMe	OMe	22
3	MeC(O)-	H	H	H	OMe	OMe	45
4	MeC(O)-	H	H	H	OMe	H	100
5	H	H	OMe	OMe	H	H	45
6	H	H	H	H	OMe	OMe	25



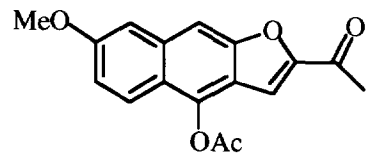
Compound	Substituents					Growth
	C-4	C-5	C-6	C-7	C-5'	inhib. (%)
8	OMe	H	H	H	H	19
9	H	OMe	H	H	H	18
10	H	OMe	H	H	-C(O)Me	17



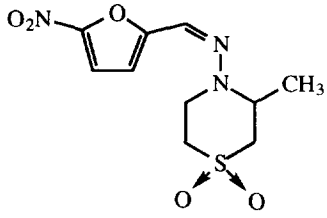
7
(GI=100%)



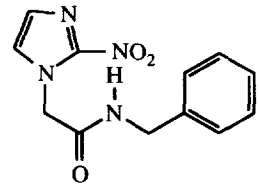
11
(GI=0%)



12
(GI=100%)



nifurtimox
(GI=100%)



benznidazole
(GI=100%)

All compounds were initially screened at 4μg/ml against the medium sensitivity Y strain epimastigotes of *T. cruzi*.[15] As shown in Figure 1, compounds **1**, **4**, **7**,**12** and the controls nifurtmox and benznidazole completely inhibited the growth of the parasites. The IC_{50}'s of these compounds were then determined in order to establish their relative efficacy (Table 1). It can be noted that the three *T. cruzi* strains were equally susceptible to each compound *in vitro*, independent of their drug sensitivity *in vivo*. Compounds **1**, **4**, **7** and **12** were more active than nifurtimox and benznidazole, compound **4** being the most active.

Table 1: IC_{50} of active compounds against the epimastigote form of different *T. cruzi* strains.

Compounds	IC50 (μM)*		
	Trypanosoma cruzi strain		
	CL	**Y**	**SC-28**
Nifurtimox	3.81±0.43	3.29±0.24	3.55±0.35
Benznidazole	8.95±0.38	8.05±0.64	8.97±0.31
1	2.65±0.91	2.82±0.24	3.05±0.29
4	0.53±0.04	0.49±0.06	0.57±0.07
7	2.55±0.11	3.02±0.36	2.80±0.38
12	1.86±0.25	1.92±0.21	2.17±0.33

* Mean average with standard deviation of three different experiments in triplicates for each strain. The statistical analysis was made using Tukey's test [23] with a confidence level of 95%.

The mechanism of action of naphthoquinone derivatives involves their absorption by parasites and reduction to semiquinones and quinols.[7] These compounds are capable of reducing molecular oxygen into hydrogen peroxide and superoxide anions, forming hydroxyl radicals which are known to cause damage to the parasite plasma membrane and to inhibit biosynthetic pathways.[17-21] Although inconclusive due to the limited number of compounds tested, some structural features which could account for the differences in inhibitory activity among the naphthofuranquinones might be suggested. Besides steric factors, the number and position of the methoxy groups are expected to interfere in the redox potential of the quinonoid ring, altering its ability to be reduced to toxic semiquinone and quinols. Indeed, all quinones with two methoxy groups, including one in the *peri* position (**2**, **3**, **5** and **6**), were much less active than those with only one methoxy substituent (**1** and **4**). The same difference in activity was observed when **1** and **2** were evaluated against *Plasmodium falciparum*.[9] The presence of a substituent at C-2 in the studied quinones seems to have negligible effect on their activity. Thus, the low activity of the quinone **6** was not significantly altered by the introduction of 1-hydroxyethyl or acetyl substituent at C-2 position, as in quinone **2** and **3**, respectively. The high activity of the synthetic precursor **12** can be rationalized in terms of its conversion to the quinone (**4**) through the hydrolysis of the acetyl group and oxidation of the resulting furanonaphthol by the action of the medium or the parasite. The activity of the compounds, measured as IC_{50}, showed no differences among the strains with distinct drug susceptibility *in vivo*. Thus, no correlation between the susceptibility to the classic drugs *in vivo* and the *in vitro* susceptibility to the compounds tested could be observed. This lack of correlation has already been described for other compounds.[22] On the other hand, some naphthoquinone derivatives have shown high activity against protozoan parasites both *in vitro* and *in vivo*.[6,10]

Although the drugs were screened only *in vitro* against *T. cruzi* epimastigotes, the vertebrate non-infective form of the parasite, they exhibited higher inhibitory activity than the classical drugs benznidazole and nifurtimox. These results are encouraging, and further *in vitro* and *in vivo* experiments must be performed to investigate their potential for use in chemotherapy and/or prophylaxis, or as tools for understanding the mechanism of drug toxicity in *T. cruzi*.

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14 All new compounds show analytical and spectral (IR, NMR, MS) data in full accord with the indicated structures. Details of the synthetic work will be published separately.

15 All parasites were maintained and tested in liver infusion tryptose (LIT) culture medium enriched with calf serum.[16] Stock solutions of compounds were prepared in dimethylsulfoxide (DMSO) and maintained at -20°C until use. Parasites at a final concentration of 10^6 epimastigotes/ml and the compounds at the stated concentrations were incubated in 24 well tissue culture plates (LINBRO, Flow Laboratories) at a final volume of 1.25 ml/well, in triplicate. Controls without drug, with DMSO (0.4%), with nifurtimox and with benznidazole, were run in parallel. The plates were incubated at 28°C for 96h. After this period the parasite number was determined by an electronic cell counter (Coulter Electronics Inc. Hialeah, FLA, USA, Model D2) and the growth rate determined and compared with the non-treated controls.

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