In Vitro Evaluation of the Activity of Aromatic Nitrocompounds against *Trypanosoma cruzi*

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Fourteen compounds were evaluated for their activity against Trypanosoma cruzi *blood stream forms at the concentration of 500 µg/ml. Six compounds were active and re-tested at lower concentrations.*

Key words: nitrocompounds - *Trypanosoma cruzi* - bloodstream forms

Nifurtimox 1, Benznidazole 2 and Megazol 3 (Fig. 1) are nitroheterocycles which exhibit, as do other nitrocompounds, activity against *Trypanosoma cruzi*, the causative agent of Chagas disease (Albuquerque & Perie 1999). Their activity is due at least in part to the well-documented sensitivity of trypanosomes and particularly *T. cruzi* towards oxidative stress. Upon one electron reduction these compounds generate radical anions ($R\text{-}NO_2$/ $R\text{-}NO_2^{\bullet}$), which interfere with oxygen metabolism (La-Scalea 1998, Viodé et al. 1999). For Benznidazole, the generation of reduced reactive species that react with parasite macromolecules is also proposed (Urbina 1999).

Differently from mammalian host cells, *T. cruzi* is deficient in antioxidant enzymes, which are essential to prevent oxidative damage. Trypanothione reductase (TR) is the key enzyme involved in the protection of *T. cruzi* against the oxidative stress (Zhang et al. 1996). The radical anions generated by the nitrocompounds upon reduction lead to TR depletion and, as consequence, toxicity to *T. cruzi*.

Taking into account the low efficacy and the side effects of Benznidazole (Neves et al. 1995, Urbina 1999), the current clinically available drug, development of new drugs for the treatment of Chagas disease is mandatory.

In this work, we report the evaluation of in vitro activity of nitroaromatic derivatives 4-13 against trypomastigotes of *T. cruzi*. Compounds 14 and 15 were included for comparison with the corresponding nitrocompounds 5 and 8. Some secondary products (16 and 17) were also evaluated.

The rationale behind the choice of the compounds was that the *o*-nitrobenzyl halides and acetates can generate the alkylating agents quinonimine methide upon reduction to the corresponding hydroxylamino or amino derivatives (Teicher & Sartorelli 1980, Wakselman et al. 1990). These reactive species could alkylate important biomolecules of the parasite, as nucleic acids and proteins (Fig. 2c). As the reduction of nitroaromatics generate superoxide anion (Fig. 2a), these compounds may potentially have a dual mode of action, through oxidative stress and/or alkylation. The *o*-nitrobenzyl halides can also lead to the reactive benzyl radicals formed by one-electron reduction and subsequent fragmentation (Fig 2b), which is sufficient to expel a good leaving group such as Br^- but not a acetate anion (Kirkpatrick et al. 1986). The carboxyl group in the aromatic nucleus allows the preparation of derivatives (esters and amides) for structure-activity relationship purposes. The presence these electron-withdrawing group (e.g., COOH, CONHR and COOMe) is expected to facilitate the reduction of the nitro group (Palmer et al. 1992). The carbohydrate moiety (compounds 11 and 12) was incorporated to improve solubility properties. The piperazino derivative 13 constitutes a potential bis-alkylating agent (Delgado & Remer 1991).

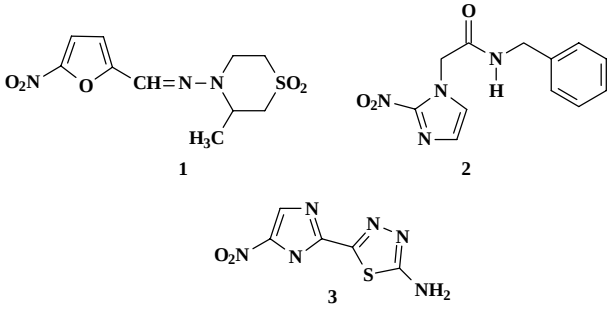

Fig. 1: chemical structures of known nitroheterocycles active against *Trypanosoma cruzi*. 1: Nifurtimox; 2: Benznidazole; 3: Megazol

MATERIALS AND METHODS

Chemistry - The compounds 4-10 and 13-17 were synthesised from the 4-methylbenzoic acid. The compounds 4-7 and 15 were prepared according to literature procedures (Rich & Gurwara 1975, Barany & Albericio 1985, Lloyd-Williams et al. 1991). The carbohydrate derivatives 11 and 12 were prepared in six steps from commercially available methyl-α-D-glucopyranoside. All compounds were fully characterised by IR, ^1H and ^{13}C NMR (Oliveira 1998).

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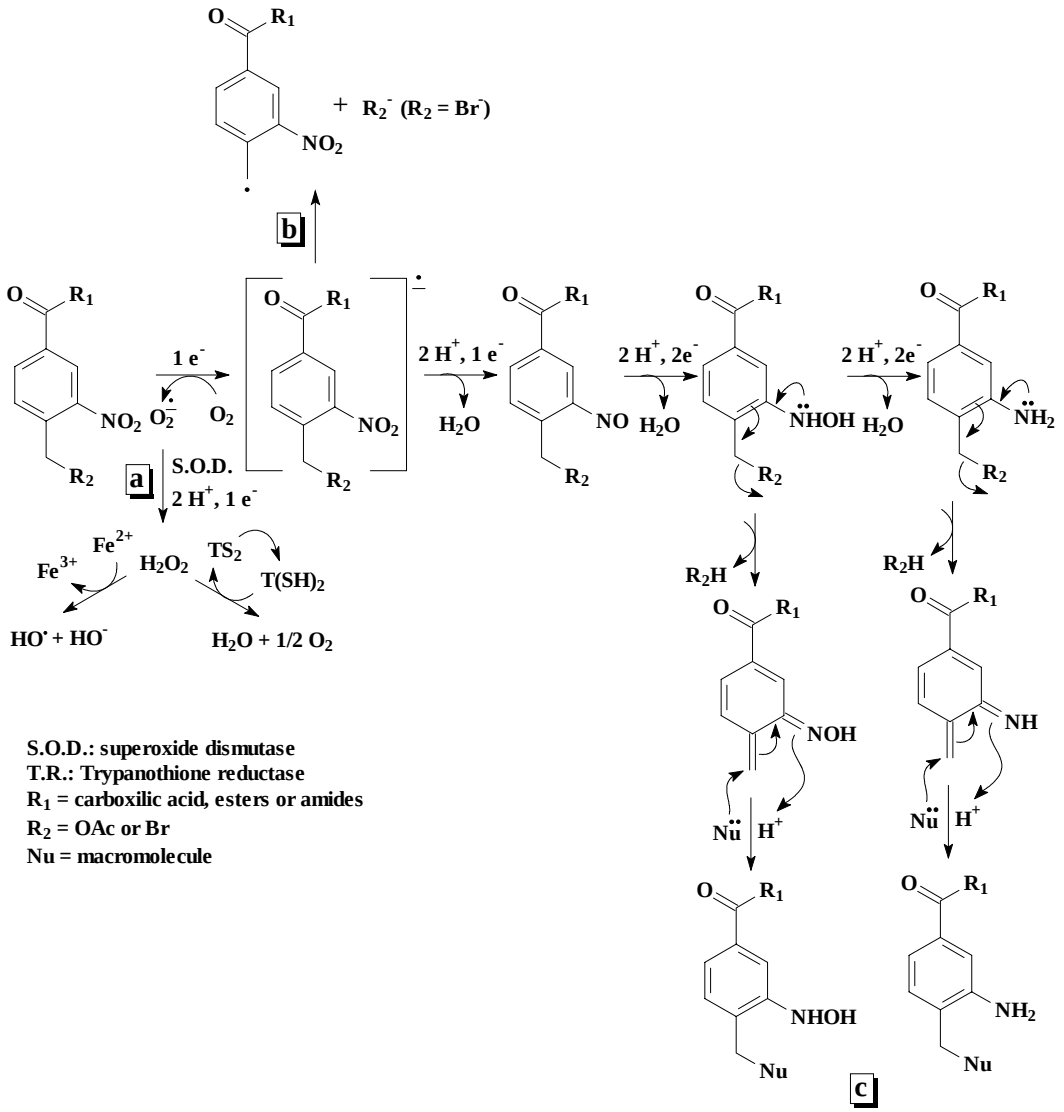


Fig. 2: bioreductive pathway of *o*-nitrobenzyl halides and acetates. a: oxidative stress; b: generation of nitrobenzyl radical; c: generation of quinonimine methide

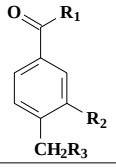
Biological assays - Blood of mice infected with trypomastigotes of the Y strain of *T. cruzi* was used in the experiments. Drug tests were made in 96 well plates. To each well containing 195 µl of infected blood (2 x 10^6 parasites/ml), 5 µl of the drug solution in DMSO was added, resulting in a final concentration of 500 µg/ml. The plates were then maintained at 4ºC for 24 h. Afterwards, the parasite concentration was evaluated using an optical microscope with a 400 X magnification. The parasite concentration reduction (parasite lysis) was determined in comparison with untreated parasites. Positive (7.5 µg/ml crystal violet) and solvent controls (2.5% v/v DMSO) were performed in all experiments. In the specified concentration crystal violet causes 50% reduction of parasite concentration (IC_{50}). DMSO and almost all compounds tested cause no morphological alterations or lysis either of the parasites, erythrocytes or leukocytes. The only exception was compound 8 that presented a moderated erythrocyte lysis at higher concentrations. The IC_{100} of crystal violet is 30 µg/ml. The experiments were made in duplicate and repeated. The active compounds, those that presented reduction in parasite concentration of approximately 100% (5, 8 and 14-17), were re-tested at lower concentrations.

RESULTS AND DISCUSSION

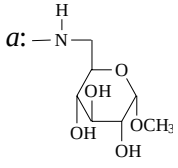
As shown in the Table, the most active compounds are benzyl bromide derivatives, with one exception (compound 17). As compounds 14 and 15 bearing no nitro substituent were highly active, their activity might be associated to their intrinsic alkylating properties. The introduction of a nitro group in the aromatic ring resulted in an increase of the activity, as seen for compound 5 and 8. This is supposed to be due to the presence of both nitro and benzylic bromine substituents, acting probably, as initially rationalized (Fig. 2). Nitrocompound 4 bearing no

TABLE

Activity of aromatic nitrocompounds against *Trypanosoma cruzi* bloodstream forms



Compound	R_1	R_2	R_3	Concentration (µg/ml)	Parasite concentration reduction (%)
4	OH	NO_2	H	500	43
5	OH	NO_2	Br	500	100
5	OH	NO_2	Br	250	100
5	OH	NO_2	Br	125	53
5	OH	NO_2	Br	62.5	13
6	OH	NO_2	OAc	500	3
7	OH	NO_2	OH	500	2
8	OCH_3	NO_2	Br	500	98
8	OCH_3	NO_2	Br	250	86
8	OCH_3	NO_2	Br	125	55
8	OCH_3	NO_2	Br	62.5	37
9	$NHCH(CH_3)_2$	NO_2	OAc	500	30
10	$N(C_6H_{11})CONH(C_6H_{11})$	NO_2	OAc	500	37
11	Carbohydrate [a]	NO_2	Br	500	1
12	Carbohydrate [a]	NO_2	OAc	500	25
13	piperazine	NO_2	OAc	500	2
14	OH	H	Br	500	98
14	OH	H	Br	250	54
14	OH	H	Br	125	8
15	OCH_3	H	Br	500	99
15	OCH_3	H	Br	250	57
15	OCH_3	H	Br	125	9
16	$NHCH_2Ph$	H	Br	500	92
16	$NHCH_2Ph$	H	Br	250	50
16	$NHCH_2Ph$	H	Br	125	22
17	$NHCH_2Ph$	H	$NHCH_2Ph$	500	100
17	$NHCH_2Ph$	H	$NHCH_2Ph$	250	99
17	$NHCH_2Ph$	H	$NHCH_2Ph$	125	38



benzylic bromine has less than 50% of the activity of the above compounds, in the highest concentration tested. The very low activity of compound 11 was unexpected and can be related to inadequate physicochemical properties of the hydrophilic carbohydrate moiety.

Since benzyl acetates cannot generate the nitrobenzyl radical as do benzyl bromide, the benzyl acetates 6, 9, 10, 12 and 13 need to be reduced to the corresponding hydroxylamino or amino derivatives to act as alkylating agents via the formation of the highly reactive quinonimine methide species. The inefficient reduction of the nitro group by *T. cruzi* bloodstream forms may explain the low activities of these compounds. Further experiments are underway testing these same compounds against tissue culture forms (amastigotes) of the parasite. The hydrolysis of the esters before reduction to give the poor active benzyl alcohol 7 cannot be ruled out.

Surprisingly, compound 17, bearing no alkylating or nitro functionalities, was one of the most active. In consequence, the mode of action of 17 might differ from that of the other compounds of this series. This compound was obtained as a by-product in the synthesis of 16.

In conclusion, 2-nitrobenzyl bromides and acetates bearing either carboxyl, ester or amide derivatives were prepared and assayed in vitro against the bloodstream forms of *T. cruzi*. Some compounds displayed significant activity. Compound 5, bearing an *orto*-nitrobenzyl bromide moiety was the most active. Compound 17, bearing none of those functional groups, was also very active, suggesting the involvement of other(s) mechanism(s) of action against *T. cruzi* bloodstream forms. Further studies are necessary to assess the real potential of these compounds or derivatives thereof as drug leads. Work in this direction is in progress.

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