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Synthesis and antitrypanosomal profile of new functionalized 1,3,4-thiadiazole-2-arylhydrazone derivatives, designed as non-mutagenic megazol analogues

Samir A. Carvalho,[b,c] Edson F. da Silva,[c] Ricardo M. Santa-Rita,[c,d] Solange L. de Castro[d] and Carlos A.M. Fraga[a,b,*]

[a]*Laboratório de Avaliação e Síntese de Substâncias Bioativas, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, PO Box 68023, 21944971 Rio de Janeiro, RJ, Brazil*
[b]*Instituto de Química, Universidade Federal do Rio de Janeiro, 21949900 Rio de Janeiro, RJ, Brazil*
[c]*FioCruz-Fundação Oswaldo Cruz, Instituto de Tecnologia em Fármacos-Far Manguinhos, 21041250 Rio de Janeiro, RJ, Brazil*
[d]*Laboratório de Biologia Celular, DUBC, Instituto Oswaldo Cruz, FIOCRUZ, 21041250 Rio de Janeiro, RJ, Brazil*

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Abstract—In this work we reported the synthesis and the trypanocidal profile of new 1,3,4-thiadiazole-2-arylhydrazone derivatives of nitroimidazole series (**4**) or phenyl series (**5**), designed by exploring the molecular hybridization approach between megazol (**2**) and guanyl hydrazone derivative (**3**). The evaluation of the activity against bloodstream trypomastigote forms of *Trypanosoma cruzi* forms lead us to identify a new potent trypamomicide prototype, that is, brazilizone A (**4k**), which present an IC_{50}/24 h = 5.3 μM.

Chagas' disease is one of the most important parasitic infections of Latin America, with over 17 million people infected, mainly in endemic areas, and at least 120 million people at risk.[1] It is caused by the hemoflagellate protozoan *Trypanosoma cruzi*, which infected humans through the bite of a triatomine insect vector or blood transfusion.[2] The infective trypomastigote form of the parasite penetrates into mammalian cells and undergoes differentiation into proliferative amastigotes. Rupture of these cells leads to liberation of the parasites and perpetuation of the infection, which after several years can lead to the chronic forms of the disease, cardiac and/or digestive.[3] Nowadays, the available chemotherapy for Chagas' disease relies only in the nitro-heterocyclic drug benznidazole (**1**), being unsatisfactory due to limited efficacy in the chronic phase of the disease and to severe side effects.[4] Megazol (**2**), a 5-nitroimidazole derivative synthesized in 1968 by Berkelhammer and Asato[5] as an antimicrobial agent, was later characterized by Brener's group[6,7] as a powerful trypanocide agent. In spite of its impressive antiprotozoal profile, which was associated with its interference with oxygen metabolism as well as its role as thiol scavenger for trypanothione, cofactor for trypanothione reductase,[8,9] megazol (**2**) development was discontinued due to the toxicity and mutagenicity induced by its use in animals.[10,11] Trying to circumvent this undesired profile several megazol analogues were synthesized. However, none of these derivatives have showed to be more potent than the prototype.[12] Considering this panorama, we decided to construct a new class of 1,3,4-thiadiazole-2-arylhydrazone derivatives (**4**) as attractive candidates to antichagasic drugs, designed by molecular hybridization between megazol (**2**) and guanylhydrazone derivative (**3**), which showed to be able to lyse trypomastigote forms of *T. cruzi*, with an IC_{50}/24 h = 17.1 μM.[13] The design concept of these compounds explored the introduction of the arylhydrazone moiety (**A**, Fig. 2) in the heterocyclic framework of (**2**), in order to act, in the structural pattern of the lead-compound, as a radical scavenger group,[14,15] which could compete and avoid the oxidative stress induced by formation of toxic nitro radical species. Additionally, it is well known that some functionalized hydrazone-related derivatives[16] presented trypanocidal activity, which could be correlated with an action on essential

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* Corresponding author. Tel.: +55 2122609192; fax: +55 2125626644; e-mail: cmfraga@pharma.ufrj.br

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enzymes of *T. cruzi* such as cruzain and trypanothione reductase. Our second goal was investigate the effects of the isosteric substitution of toxicophoric nitroimidazole ring (**B**, Fig. 1), present in the more active hydrazone derivatives of series (**4**), to a simple phenyl group, producing the corresponding 1,3,4-thiadiazole derivatives of series (**5**). The synthetic route used for the preparation of the title compounds (**4**) and (**5**) is outlined in the Scheme 1. Megazol[17] (**2**) and 2-amino-5-phenyl-1,3,4-thiadiazole (**7**) were employed as starting material. Derivative **7** was obtained in 94% yield from condensation of benzoic acid (**6**) and thiosemicarbazide in phosphorus oxychloride.[18]

Amino-heterocyclic derivatives (**2**) and (**7**) were converted in the corresponding chlorides (**8**) and (**9**) in 90% and 85% yield, respectively, exploiting the diazotation and Sandmeyer reaction with CuCl generated in situ.[19] Heteroaromatic nucleophilic substitution of the chlorine atom of the derivatives (**8**) and (**9**) using 85% aq hydrazine hydrate,[20] furnished the heterocyclic hydrazines (**10**) and (**11**) in 97% and 96% yield, respectively. Finally, the target hydrazone derivatives (**4**) and (**5**) were obtained, in good yields, by acid catalyzed condensation of the hydrazines (**10**) and (**11**) with the corresponding aromatic aldehydes (ArCHO) in ethanol,[21] as described in the Scheme 1 and Table 1. The analysis of the ^{1}H NMR spectra and HPLC chromatograms of the synthesized compounds of series (**4**) and (**5**) were consistent with the presence of geometric (*Z*)-isomers at C=N bond level in almost all derivatives prepared, which presented the NMR signal of imine

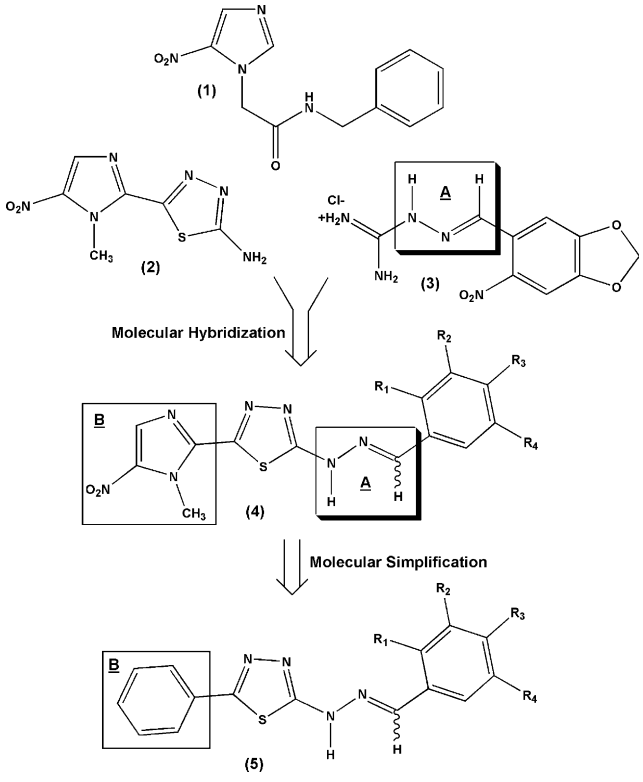
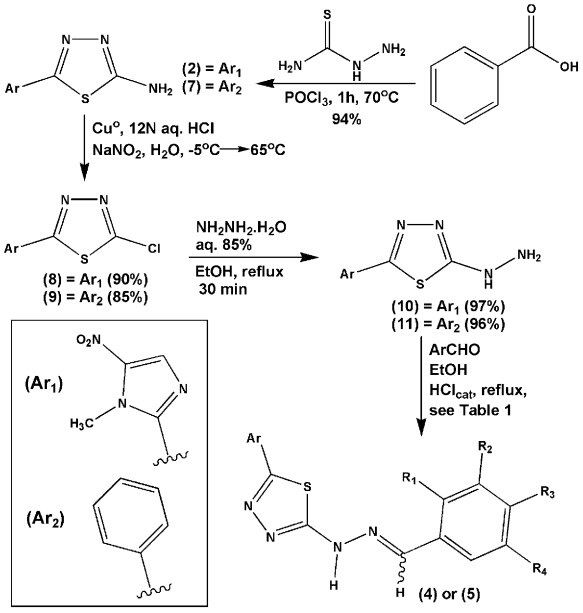


Figure 1. Design concept of new 1,3,4-thiadiazole-2-arylhydrazone derivatives (**4**) and (**5**).



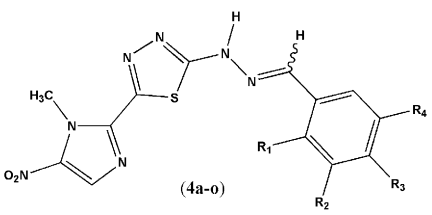
Scheme 1. Synthetic route for the preparation of the new 1,3,4-thiadiazole-2-arylhydrazone derivatives (**4**) and (**5**).

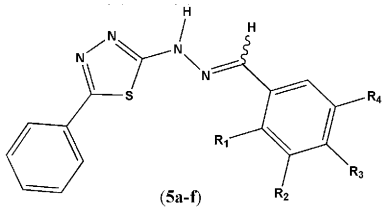
hydrogen ranging from 8.22 to 8.28 ppm (Fig. 2). However, *ortho*-hydroxylated derivatives (**4i**), (**4j**) and (**5d**) were obtained as a mixture of (*E*)- and (*Z*)-diastereomers due to the relative stabilization of (*E*)-diastereomer induced by a possible intramolecular hydrogen bond between the electron pair of imine nitrogen and the hydroxyl hydrogen. The NMR signal of the imine hydrogen of (*E*)-diastereomers of these derivatives appear in a range from 8.43 to 8.48 ppm. These results, which were supported by several NOE experiments (Fig. 2), are in total agreement with those previously described by Karabatsos et al.,[22] showing that the NMR signal of the imine hydrogen of the (*E*)-diastereomer of phenylhydrazone derivatives is downfielded by 0.2–0.3 ppm from that of the corresponding (*Z*)-diastereomer.

The antitrypanosomal profile of the new 1,3,4-thiadiazole-2-aryllhydrazones of nitroimidazole series (**4**) was carried out using the trypomastigote form of *T. cruzi* obtained from mice intraperitoneally inoculated with 10^5 parasites of the Y strain.[13] The stock solutions of the compounds were prepared in DMSO, and the assays were performed in Dulbecco's modified Eagle medium. The final concentration of the solvent never exceeded 0.5%, which has no deleterious effect on the parasite. All tests were performed by mixing 100 μL of cell suspension with an equal volume of the desired test-compound solution to make a final drug concentration ranging from 1.5 to 200 μM, and incubating at 4 °C for 24 h. Untreated and megazol-treated parasites were used as controls. The results were analyzed by plotting % lysis of *T. cruzi* against the concentration of the test compound. The values of IC_{50} corresponded to the concentration that led to 50% lysis of the parasite and are summarized in Table 1.

The most active hydrazone compound of series (**4**) was 3,4-dihydroxyphenyl derivative (**4k**), which present an IC_{50} = 5.3 μM, twofold more potent than the prototype

Table 1. Physical and spectral properties of the 1,3,4-thiadiazole-2-arylhydrazone derivatives (**4**) and (**5**)





Compd	R_1	R_2	R_3	R_4	Molecular formula[a]	Molecular weight	Yield (%)	Mp (°C)	Diastereoselection[b,c]	IC_{50}[d]
4a	H	H	H	H	$C_{13}H_{11}N_7O_2S$	329.07	63	292–294	*Z*	>200
4b	H	H	F	H	$C_{13}H_{10}FN_7O_2S$	347.06	63	300–302	*Z*	>200
4c	H	H	Cl	H	$C_{13}H_{10}ClN_7O_2S$	363.03	61	280–281	*Z*	38.4 ± 3.0
4d	H	H	Br	H	$C_{13}H_{10}BrN_7O_2S$	406.98	60	293–294	*Z*	17.0 ± 0.8
4e	H	H	NO_2	H	$C_{13}H_{10}N_8O_4S$	374.05	84	305–307	*Z*	22.0 ± 4.0
4f	H	H	OCH_3	H	$C_{14}H_{13}N_7O_3S$	359.08	66	282–283	*Z*	>200
4g	H	H	OCF_3	H	$C_{14}H_{10}F_3N_7O_3S$	413.05	60	300–301	*Z*	>200
4h	H	H	OH	H	$C_{13}H_{11}N_7O_3S$	345.06	95	299–300	*Z*	11.6 ± 0.6
4i	OH	H	H	H	$C_{13}H_{11}N_7O_3S$	345.06	70	308–309	*E*/*Z*	54.2 ± 4.4
4j	OH	OCH_3	H	H	$C_{14}H_{13}N_7O_4S$	375.07	67	307–308	*E*/*Z*	>200
4k	H	OH	OH	H	$C_{13}H_{11}N_7O_4S$	361.06	66	299–300	*Z*	5.3 ± 0.6
4l	H	OH	OCH_3	H	$C_{14}H_{13}N_7O_4S$	375.07	77	303–304	*Z*	50.5 ± 0.6
4m	H	OCH_3	OH	H	$C_{14}H_{13}N_7O_4S$	375.07	78	298–300	*Z*	>200
4n	H	OCH_2O		H	$C_{14}H_{11}N_7O_4S$	373.06	71	300–302	*Z*	>200
4o	H	*t*-Bu	OH	*t*-Bu	$C_{21}H_{27}N_7O_3S$	457.19	92	289–291	*Z*	33.2 ± 6.4
5a	H	H	H	H	$C_{15}H_{12}N_4S$	280.08	80	256–257	*Z*	>200
5b	H	H	NO_2	H	$C_{15}H_{11}N_5O_2S$	325.06	63	270–272	*Z*	>200
5c	H	H	OH	H	$C_{15}H_{12}N_4OS$	296.07	70	255–257	*Z*	>200
5d	OH	H	H	H	$C_{15}H_{12}N_4OS$	296.07	65	229–232	*E*/*Z*	>200
5e	H	OH	OH	H	$C_{15}H_{12}N_4O_2S$	312.07	65	205–207	*Z*	63.4 ± 9.1
5f	H	*t*-Bu	OH	*t*-Bu	$C_{23}H_{28}N_4OS$	408.20	88	260–261	*Z*	135.6 ± 11.0
Megazol	—	—	—	—	—	—	—	—	—	9.9 ± 0.8

[a] The analytical results for C, H, N, S were within ±0.4% of calculated values.
[b] Determined by HPLC by using a Rexchrom 5 μm RP-18 column (125 × 4.6 mm) and a mixture of methanol–water (7:3 v/v) as eluent at flow rate of 1 mL/min.
[c] Data obtained at 200 MHz, using DMSO-d_6 as solvent.
[d] Mean ± standard deviation of at least four separated experiments.

megazol (IC_{50} = 9.9 μM). In order to evaluate the different contribution of its hydroxyl groups at C-3 and C-4, we performed the investigation of the antiprotozoal profile of the hydroxylated analogues (**4h–j**) and (**4l–n**), which are not so potent as (**4k**), indicating that both unsubstituted hydroxyl groups in catechol subunit have a pharmacophoric character to the action over *T. cruzi*. From this comparative study we were able to identify the interesting bioprofile of the 4-OH derivative (**4h**) and 3,5-di*t*-Bu,4-OH derivative (**4o**), which showed, respectively, IC_{50} values of 11.6 and 33.2 μM. On the other hand, the introduction of *para*-substituents presenting different electronic and lipophilic properties, for example, compounds (**4a–g**), indicated to us that the best ones are those with strong lipophilic characteristics, such as the *para*-bromo derivative (**4d**) (IC_{50} = 17.0 μM). Nevertheless, the nitro derivative (**4e**) also presented an important activity against *T. cruzi*, that is, IC_{50} = 22.0 μM, due to the special ability of nitro group to form radical species toxic to the parasite.

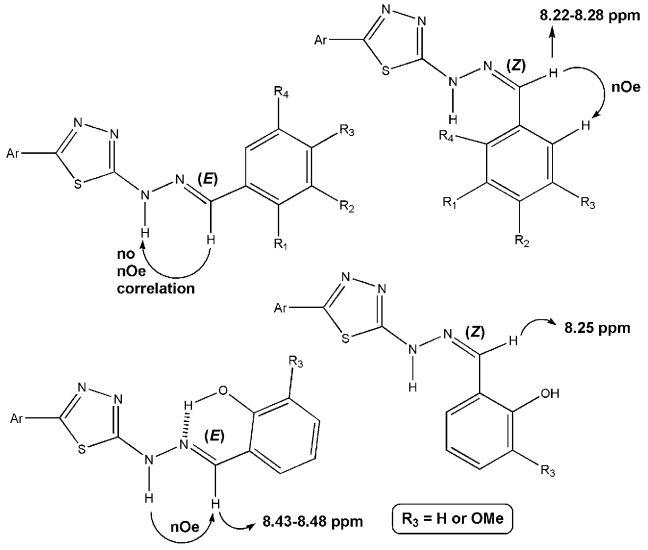


Figure 2. NMR shifts and NOE correlations of benzylidene hydrogen of (*E*)- and (*Z*)-diastereomers of hydrazone derivatives (**4**) and (**5**).

With the results of the trypanocidal profile of series (**4**) in hand, we elected some of the most active substituents (4-NO_2, 4-OH, 2-OH, 3,4-diOH and 3,5-di*t*-Bu-4-OH) to construct the corresponding 5-phenyl-1,3,4-thiadiazole analogues of series (**5a–f**). All of these compounds were less active than the corresponding ones of the series (**4**), reinforcing the pharmacophoric contribution of nitroimidazole group to the mechanism of action against

T. cruzi. On the other hand, we are able to identify the interesting profile of 3,4-dihydroxy derivative (**5e**), which in spite of the absence of the nitro-heterocyclic framework was able to kill the trypomastigote forms of *T. cruzi* with an $IC_{50} = 63.4\,\mu M$, indicating clearly the compensatory effect promoted by introduction of the arylhydrazone subunit.

To acquire preliminary information about the radical scavenger activity of the 3,4-dihydroxy derivative (**4k**) we evaluated by UV spectrometry its effect over stable DPPH radical.[23] In fact, hydrazone (**4k**) was able to inhibit in 91% the UV absorption of DPPH radical while megazol (**2**) used as standard inhibited 32%, at the same molar concentration (0.1 mM). This result indicated that, as anticipated in the design of series (**4**) and (**5**), the introduction of arylhydrazone framework in megazol structure (**2**) changed its redox behaviour and could eventually improve its therapeutic profile and safety.

As concluding remarks, we discovered the new potent hydrazone-containing trypanocide prototype (**4k**), named *brazilizone A*, which possibly acts through the interference in some step of the oxidative metabolism of trypomastigote *T. cruzi* forms.

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