

Synthesis of thiosemicarbazone and 4-thiazolidinone derivatives and their in vitro anti-*Toxoplasma gondii* activity

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Received 2 December 2004; revised 8 March 2005; accepted 14 March 2005

Available online 9 April 2005

Abstract—Thiosemicarbazone and 4-thiazolidinone derivatives were synthesized in one and two step, respectively, from thiosemicarbazide, in satisfactory yields. Then, the synthesized compounds were submitted to evaluation against host cells infected with *Toxoplasma gondii*. The present studies showed that thiosemicarbazones **2** and 4-thiazolidinone derivatives **3** were effective against intracellular *T. gondii*.

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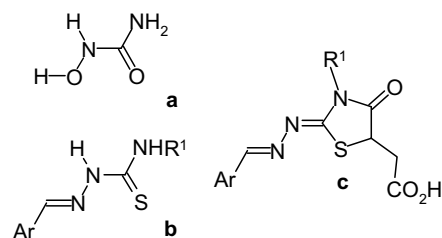
1. Introduction

Toxoplasma gondii is an obligate intracellular protozoa able to infect a large number of vertebrate cells.¹ After host cell invasion, the infected form of *T. gondii*, called tachyzoite, localized in the cytoplasm within a membrane-bounded vacuole, known as parasitophorous vacuole (PV), multiplying at intervals of 6 h.² In humans, it is responsible for toxoplasmosis, a disease known for its great importance to immunodepressed patients due to its opportunistic character.³ With the advent of AIDS, the worldwide incidence of infections caused by *T. gondii* has been rising.⁴ In the USA, between 18% and 25% of patients with AIDS will suffer from symptomatic toxoplasmosis during the course of their illness.³ Besides this, the current chemotherapy against human toxoplasmosis involve undesirable side effects and it is not effective in eliminating the parasite located in the central nervous system.^{5,6}

Recent studies using hydroxyurea (HU) demonstrate its efficacy, in vitro, in eliminating the intracellular parasites of *T. gondii*, *Trypanosoma cruzi* and *Leishmania amazonensis* (Fig. 1a).⁷ HU acts by blocking the activity of the enzyme ribonucleotide reductase, arresting the

cell cycle in the G1/S phase and, then, inactivating the DNA synthesis.² However, HU has a low affinity for the ribonucleotide reductase and a short half-life in humans.⁸ On the other hand, thiosemicarbazones are known inhibitors of this enzyme, being approximately 1000 times more potent than hydroxyurea (Fig. 1b).⁹

Biological activities of these thiosemicarbazones are related to their abilities to form complex with metal cations, by bonding through the sulfur and azomethine nitrogen atoms.¹⁰ Several works demonstrate their activity against extracellular protozoan as *Plasmodium falciparum*, *Trichomonas vaginalis*, *T. cruzi* and other parasites.^{10–12} However, no study about their biological properties against intracellular *T. gondii* was even related.



Ar = aryl group; R¹ = H, alkyl, aryl

Figure 1. Hydroxyurea (a), thiosemicarbazone (b) and 4-thiazolidinone (c).

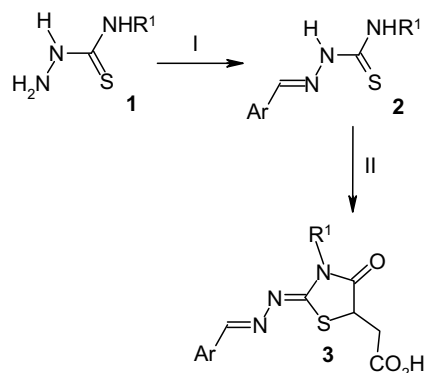
Keywords: Thiosemicarbazones; 4-Thiazolidinones; *Toxoplasma gondii*.

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In addition, thiosemicarbazones may also be seen as structure analogues of 4-thiazolidinones carrying a hydrazone moiety in the two position (Fig. 1c). Compounds bearing a 4-thiazolidinone heterocycle are usually of biological interest, due to their large spectrum of activity.^{13–17} In this communication, we described a cost-effective synthesis, in one and two steps, of thiosemicarbazone and 4-thiazolidinone derivatives, their structure elucidation and in vitro biological activities against *T. gondii*.

Scheme 1 shows the synthetic route employed for the preparation of the compounds. As can be seen, a simple synthesis was carried out, initiating from the commercially available benzaldehyde derivatives and the appropriate thiosemicarbazide substitutes **1**, in water/ethanolic media containing a few drops of acetic acid as the catalyst. At the end of the reaction, the isolated benzaldehyde thiosemicarbazone derivatives **2** were obtained as solids of yellow colour. This procedure was adapted from an article previously reported by Bharti et al.¹² and gave us satisfactory yields.

Furthermore, the obtained benzaldehyde thiosemicarbazone derivatives **2** were utilized for the preparation of 4-thiazolidinones substituted in the five position with an acetic acid group. Since the presence of an acid function



Scheme 1. Reagents and conditions: (I) ArCHO, H₂O, EtOH, acid acetic, reflux (56–97%); (II) dry toluene, anhydride maleic, DMF, Dean–Stark, reflux (26–95%).

introduces an additional diversity point to further structural tuning, we utilized a thia-Michael addition reaction with the employment of anhydride maleic as the acceptor of Michael.¹⁸ The reaction was carried out in dry toluene and DMF and, as expected, gave us the 4-thiazolidinones substituted **3**.

The synthesized compounds were obtained in satisfactory yields (Table 1). The structures of all compounds

Table 1. Synthesized compounds, their yields, melting points and in vitro activities against *T. gondii*

Compounds	R ¹	R ²	Yield (%)	Mp (°C)	% Infected Vero cells		Mean number of intracellular parasites	
					Untreated	Treated	Untreated	Treated
2a	–H	<i>m</i> -NO ₂	93	226–228	76 ± 9	4 ± 2	768 ± 142	3 ± 3
2b	–H	<i>p</i> -NO ₂	56	229–230	65 ± 12	5 ± 2	698 ± 94	4 ± 6
2c	–CH ₃	<i>o</i> -NO ₂	75	234–235	81 ± 4	9 ± 5	783 ± 84	2 ± 3
2d	–CH ₃	<i>m</i> -NO ₂	70	233–234	84 ± 3	9 ± 3	642 ± 92	1 ± 1
2e	–CH ₃	<i>p</i> -NO ₂	87	230–232	89 ± 6	12 ± 4	683 ± 60	72 ± 28
2f	–CH ₂ CH ₃	<i>o</i> -NO ₂	60	232–233	81 ± 4	8 ± 2	783 ± 84	1 ± 2
2g	–CH ₂ CH ₃	<i>m</i> -NO ₂	60	207–208	76 ± 9	7 ± 3	768 ± 142	5 ± 6
2h	–CH ₂ CH ₃	<i>p</i> -NO ₂	96	228–229	86 ± 4	17 ± 3	852 ± 140	9 ± 2
2i	–C ₆ H ₅	<i>o</i> -NO ₂	96	197–198	84 ± 3	7 ± 3	642 ± 92	3 ± 2
2j	–C ₆ H ₅	<i>m</i> -NO ₂	97	189–191	65 ± 15	7 ± 4	442 ± 69	11 ± 12
3a	–H	<i>m</i> -NO ₂	95	249–250	80 ± 6	15 ± 8	840 ± 116	4 ± 3
3b	–H	<i>p</i> -NO ₂	52	267–268	85 ± 3	16 ± 5	732 ± 69	4 ± 3
3c	–CH ₃	<i>o</i> -NO ₂	26	247–248	84 ± 3	15 ± 5	888 ± 143	3 ± 3
3d	–CH ₃	<i>m</i> -NO ₂	36	194–196	85 ± 2	11 ± 4	628 ± 74	2 ± 3
3e	–CH ₃	<i>p</i> -NO ₂	26	202–204	87 ± 4	13 ± 6	726 ± 115	2 ± 2
3f	–CH ₂ CH ₃	<i>o</i> -NO ₂	33	213–215	86 ± 4	14 ± 5	663 ± 16	3 ± 2
3g	–CH ₂ CH ₃	<i>m</i> -NO ₂	60	187–188	85 ± 3	19 ± 5	732 ± 69	3 ± 2
3h	–CH ₂ CH ₃	<i>p</i> -NO ₂	56	220–221	80 ± 2	7 ± 4	627 ± 81	2 ± 2
3i	–C ₆ H ₅	<i>o</i> -NO ₂	65	238–240	83 ± 4	8 ± 3	687 ± 103	2 ± 2
3j	–C ₆ H ₅	<i>m</i> -NO ₂	50	215–217	72 ± 22	13 ± 4	786 ± 79	2 ± 2
HU ^a	—	—	—	—	80 ± 5	26 ± 6	913 ± 123	186 ± 52

^a Hydroxyurea; data is reported as mean ± SD of four independent experiments ($p < 0.05$) compared to untreated cells (Student *t*-test).

were established on the basis of their NMR spectroscopic data, IR and, only for the 4-thiazolidinones, mass spectroscopy.¹⁹

2. Biological studies

2.1. In vitro activity against *T. gondii*

The cultures infected with tachyzoites were treated with the drugs as described by Melo et al.² and Melo and Beiral.⁷ Concisely Vero cells (kidney fibroblast from African green monkeys) were infected with tachyzoites of *T. gondii* (1:10) and incubated at 37 °C for 24 h. After that the cultures were allowed in presence of thiosemicarbazone compounds **2** (1 mM), 4-thiazolidinone derivatives **3** (1 mM) and hydroxyurea (4 mM) for 24 h. These concentrations were screened in early studies to shown anti-parasite effects. After that the cultures were processed in light microscopy and counted using AXIOPLAN microscope equipped with x63 objective. Statistical analysis was carried out using the Student *t*-test, with the level of significance set at $p < 0.05$. The data shown are representative of four experiments in triplicate.

In presence of thiosemicarbazones (**2a–j**) and 4-thiazolidinone derivatives (**3a–j**), a drastic decrease in infected host cells with *T. gondii* was observed (Table 1). This reduction of infected host cells was due to elimination of intracellular parasites (Table 1). The treated host cells not present morphologic alterations after 24 h of drugs treatment and they continued their development after drugs were withdrawn from the medium (data not shown). This potentiality of the compounds as inhibitors of reductase activity suggests further that arrest parasite cycle induced to decrease of the infection and elimination of intracellular parasites. These new compounds also were most effective than hydroxyurea (HU) to eliminate intracellular *T. gondii* (Table 1, Fig. 2). The two series of compounds showed little differences in the anti-*T. gondii* effect at 1 mM (Fig. 2). The IC₅₀ of some representative compounds was determined (Table 2).

Table 2. IC₅₀ values for representative compounds of the two series

Compounds	Infected cells IC ₅₀ (mM)	Intracellular parasites IC ₅₀ (mM)
2a	2 ± 0.05	0.1 ± 0.06
2b	1.5 ± 0.05	0.5 ± 0.06
2c	2 ± 0.06	0.5 ± 0.05
2d	1.5 ± 0.04	0.5 ± 0.05
2e	10 ± 1	3 ± 0.4
2f	2 ± 0.05	0.5 ± 0.05
2g	1 ± 0.05	0.08 ± 0.002
2h	2.5 ± 0.05	0.5 ± 0.05
2i	1 ± 0.05	0.5 ± 0.05
2j	1 ± 0.05	0.08 ± 0.004
3a	2 ± 0.05	0.5 ± 0.06
3b	2.5 ± 0.04	0.5 ± 0.04
3c	2 ± 0.07	0.5 ± 0.06
3h	2 ± 0.06	0.5 ± 0.06
HU ^a	0.1 ± 0.004	0.1 ± 0.003
SULF ^b	0.09 ± 0.04	0.1 ± 0.07

^a Hydroxyurea.

^b Sulfadiazine; each single compound was tested in a series dilution (0.1, 1, 10, 20, 30 mM) in triplicate per assay. The IC₅₀ values (mean ± SD) were determinate by a non-linear regression using exclusion test with trypan blue.

After 24 h exposure in the concentrations range of 0.01–30 mM, the IC₅₀ values of most compounds were observed (Table 2). In the infected cells, the IC₅₀ values of **2a–f,h** were ≥1.5 mM whereas **2g,i,j** were 1 mM. However, all compounds showed lower cytotoxicity than hydroxyurea and sulfadiazine. The toxicity of all compounds were more effective against intracellular parasites (Table 2) where IC₅₀ were ≤0.5 mM with exception to compounds **2g,j** (0.08 mM) and **2a** (0.1 mM). Some studies showed that thiosemicarbazones have been effective against extracellular parasites.^{20,21} However, very little is known about the effect of thiosemicarbazones on intracellular parasites. The present studies showed that the presence of thiosemicarbazones **2** and 4-thiazolidinone derivatives **3** were also effective against intracellular *T. gondii*. Studies using these compounds

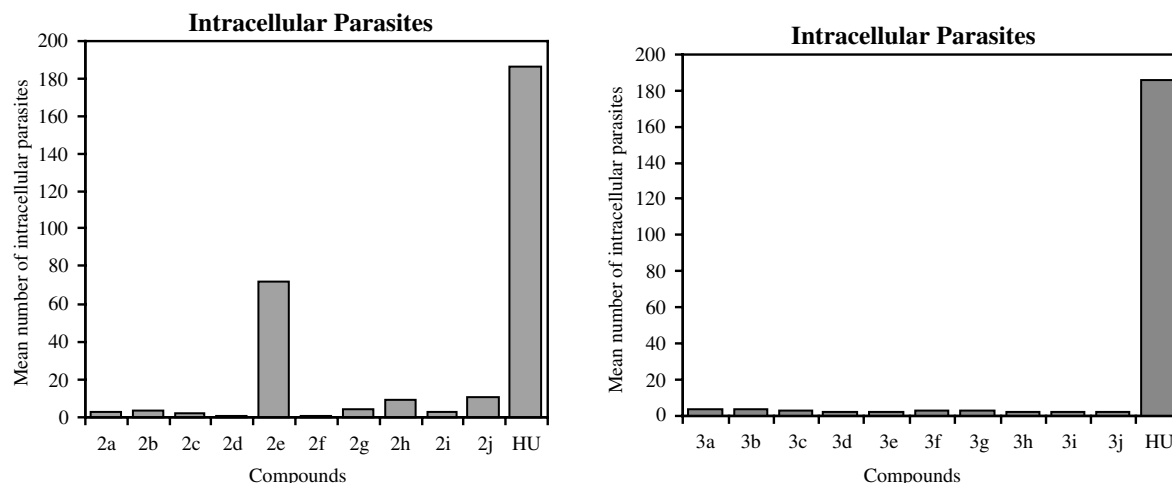


Figure 2. Relative number of intracellular parasites in infected host cells after treatment with the compounds.

against other intracellular parasites are also currently being conducted in our laboratory.

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We also thank CAPES and the Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ) for the student fellowships.

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- All the compounds were fully characterized. Structural data for **2c** and **3c** is present here. Compound **2c**: FTIR (ν cm⁻¹ KBr): 3370 (NHCH₃), 1513 (C=N), 1098 (C=S); ¹H NMR (DMSO-*d*₆, 300 MHz/ppm): 11.8 (1H, s, NH), 8.66 (1H, br q, *J* = 4.5 Hz, NHCH₃), 8.47 (1H, s, CH=N), 8.42 (1H, dd, *J* = 8.1 Hz and *J* = 1.2 Hz, CH arom), 8.03 (1H, dd, *J* = 8.1 Hz and *J* = 1.2 Hz, CH arom), 7.74–7.79 (1H, m, CH arom), 7.60–7.65 (1H, m, CH arom), 3.02 (3H, br d, *J* = 4.5 Hz, NHCH₃). Compound **3c**: FTIR (ν cm⁻¹ KBr): 1714 (C=O; CO₂H), 1617 (C=O; lactam), 1356 (NCS); ¹H NMR (DMSO-*d*₆, 300 MHz/ppm): 12.73 (1H, br s, CO₂H), 8.74 (1H, s, CH=N), 8.07 (1H, dd, *J* = 8 Hz and *J* = 1.2 Hz, CH arom), 8.02 (1H, dd, *J* = 8 Hz and *J* = 1.2 Hz, CH arom), 7.79–7.85 (1H, m, CH arom), 7.68–7.74 (1H, m, CH arom), 4.43 (1H, dd, *J* = 9 Hz and *J* = 4 Hz, CH), 3.19 (3H, s, CH₃), 3.08 (1H, dd, *J* = 18 Hz and *J* = 4 Hz, CH₂), 2.91 (1H, dd, *J* = 18 Hz and *J* = 9 Hz, CH₂); ¹³C NMR (DMSO-*d*₆, 75.4 MHz/ppm): 29.50 (CH₃), 36.64 (CH₂), 42.79 (CH), 124.62 (CH arom), 128.22 (C_q, arom), 129.05 (CH arom), 131.31 (CH arom), 133.65 (CH arom), 148.36 (C_q, arom), 153.58 (CH, CH=N), 166.44 (C_q, C=N), 171.69 (C_q, C=O lactam), 173.81 (C_q, CO₂H); Mass *m/e*, *M*⁺ = 335.
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