Synthesis and Evaluation of 4-Acyl-2-thiazolylhydrazone Derivatives for Anti-Toxoplasma Efficacy in Vitro

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · AUGUST 2009

Impact Factor: 5.45 · DOI: 10.1021/jm9005862 · Source: PubMed

11 AUTHORS, INCLUDING:

Daniela Secci
Sapienza University of Rome
112 PUBLICATIONS **2,227** CITATIONS
SEE PROFILE

Simone Carradori
Università degli Studi G. d'Annunzio Chieti e …
95 PUBLICATIONS **994** CITATIONS
SEE PROFILE

Daniela Rivanera
Sapienza University of Rome
40 PUBLICATIONS **488** CITATIONS
SEE PROFILE

Lori V Brando
Johns Hopkins Medicine
39 PUBLICATIONS **710** CITATIONS
SEE PROFILE

4574 J. Med. Chem. 2009, 52, 4574–4577
DOI: 10.1021/jm9005862

Journal of Medicinal Chemistry Letter

Synthesis and Evaluation of 4-Acyl-2-thiazolylhydrazone Derivatives for Anti-*Toxoplasma* Efficacy in Vitro

Franco Chimenti,[†] Bruna Bizzarri,*[,†] Adriana Bolasco,[†] Daniela Secci,[†] Paola Chimenti,[†] Simone Carradori,[†] Arianna Granese,[†] Daniela Rivanera,[‡] Nathan Frishberg,[§] Claudia Bordón,[§] and Lorraine Jones-Brando[§]

[†]*Dipartimento di Chimica e Tecnologie del Farmaco and* [‡]*Dipartimento di Scienze di Sanità Pubblica, Università "La Sapienza", P.le A. Moro 5, 00185 Rome, Italy, and* [§]*Stanley Medical Research Institute, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Blalock 1105, Baltimore, Maryland 21287*

Received May 6, 2009

Abstract: A new series of 4-acyl-2-thiazolylhydrazone derivatives was synthesized and screened for its in vitro activity against *Toxoplasma gondii*. We evaluated parasite growth inhibition and cytotoxicity, inhibition of replication, and inhibition of parasite invasion of host cells. The biological results indicated that some substances had an antiproliferative effect against intracellular *T. gondii* tachyzoites cultivated in vitro.

The intracellular protozoan *Toxoplasma gondii* is an opportunistic pathogen responsible for asymptomatic parasitic infection. It is generally acquired by ingesting food or water contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts. After host cell invasion, the rapidly replicating form of *T. gondii*, the tachyzoite, localizes in the cytoplasm within a membrane-bounded vacuole. It multiplies at intervals of 6−8 h and thus initiates the acute stage of infection.[1] Following the acute stage, the parasite forms cysts (latent stage) in various organs, especially the brain, heart, and skeletal muscle. Primary infection in immunocompetent individuals results in chronic infection. In chronically infected people, immunosuppression may result in reactivation of a latent infection that can cause life-threatening encephalitis.[2−4] In pregnant women, *T. gondii* infection may cause severe congenital defects in the fetus.[5]

The standard chemotherapy against human toxoplasmosis is a combination of pyrimethamine and sulfa drugs like sulfadiazine.[6,7] This combination therapy targets folate metabolism enzymes such as dihydrofolate reductase. However, current therapy has been limited by high toxicity, resistance, and poor absorption of efficient drugs. In addition, this traditional therapeutic regime is not always suitable for prolonged treatment because of the appearance of undesirable side effects like allergic reactions, which are common among patients and which further limit the treatment in some cases.[8−10] Identifying novel antitoxoplasmic compounds effective against chronic stages of the parasite would help in overcoming such disadvantages.

As an obligate intracellular parasite, *T. gondii* depends on an efficient host cell invasion strategy to support its survival and transmission. In this process, an important role is played by a sequential release of secretory proteins such as cysteine protease. Studies performed on *Trypanosoma* species report that cysteine protease inhibitors can be potent antiprotozoal agents.[11,12] In particular, thiosemicarbazones (**a**) and 4-thiazolidinone derivatives (**b**) possess a better in vitro specific activity against *T. gondii* than hydroxyurea (Chart 1).[13,14]

In the search for new anti-infective agents, we took into account the importance of a thiazole ring, which is one of the possible molecular complications of thiosemicarbazone moiety. It is present in the structure of many antimicrobial and antiparasitic substances like the antihelmintic thiabendazole (**c**) and the antischistosomial myridazole (**d**) (Chart 1).[15] The contribution of the presence of a hydrazine moiety is also important, as reported before.

As a result of these observations, we decided to synthesize a series of new thiazolylhydrazone derivatives bearing different substitutions at 2 and 4 positions of the thiazole ring. To increase the variability of the imine position and to improve the drug penetration within the parasite-infected cell, we designed a series of 2-hydrazothiazoles substituted at the *N*-hydrazone moiety with cycloaliphatic rings or heterocyclic rings (all structures provided with a suitable lipophilic character) such as thiophene, furan, pyridine, naphthalene, indole, and coumarin. The introduction of an ester or an acid group in the 4 position of the thiazole ring gave us insight into the influence of this substitution on the anti-*Toxoplasma* activity. Thus, our objective was to deepen the study by evaluating these new compounds for activity against *T. gondii*. We found that some analogues could moderately block the parasite's invasion and replication in vitro.

The synthesis of new derivatives was performed knowing that different carbonyl compounds react directly with thiosemicarbazide in the presence of catalytic amounts of acetic acid in ethanol. The obtained thiosemicarbazones were subsequently condensated with ethyl ester of bromopyruvic acid to give the 2,4-disubstituted 1,3-thiazoles (Hantzsch reaction) as shown in Scheme 1 (**1**−**22**). Our choice of ethanol as solvent allowed the final products to precipitate without further purification. Subsequently some of the derivatives were hydrolyzed to the corresponding carboxylic acid by mild alkaline hydrolysis ($LiOH \cdot H_2O$ in H_2O/MeOH, 1/4) to obtain the derivatives **23**−**30**.

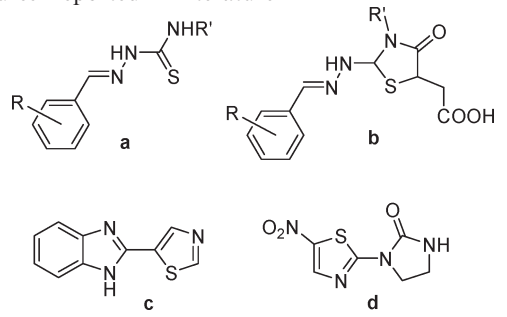
All synthesized compounds, which show calculated log $P <$ 5, were fully characterized by analytical and spectral data as listed in Tables 1, 2, and 3 (see also Supporting Information). The synthesis of **10** and **13** has been described and was performed with slight changes; their analytical and spectral data were in full agreement with those reported in the literature.[16,17]

For all the synthesized compounds, the relative ability to inhibit the growth of the tachyzoites of *T. gondii* was determined using a published method.[18] The tests were performed on genetically modified parasites constitutively expressing β-galactosidase (β-gal) (strain 2F derived from strain RH; ATCC, VA) and human foreskin fibroblast (HFF; ATCC) host cells plated in 96-well (12 columns × 8 rows) plates. Prior to adding the parasite to cells and beginning with column 2 of the plate, drugs were serially diluted across the plate by dilutions of 0.5 $\log_{10}$, leaving column 11 drug-free (parasite control). Following this, *T. gondii* tachyzoites were added to

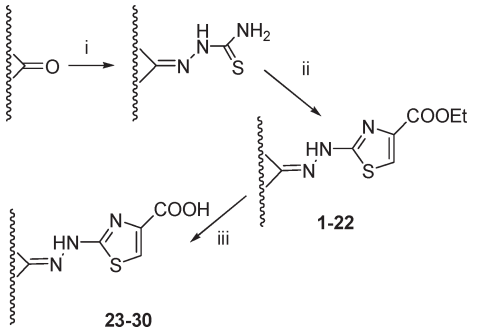
*To whom correspondence should be addressed. Phone: +39 064 9693242. Fax: +39 064 991 3772. E-mail: bruna.bizzarri@uniroma1.it.

 Published on Web 07/20/2009

Chart 1. Antimicrobial and Antiparasitic Thiazole-Based Structures Reported in Literature

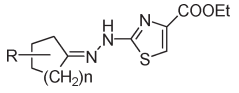


Scheme 1[a]



[a] Reagents and conditions: (i) thiosemicarbazide, acetic acid (cat.), EtOH; (ii) ethyl ester of bromopyruvic acid, EtOH; (iii) (1) $LiOH \cdot H_2O$, H_2O/MeOH, (2) HCl, 3 N.

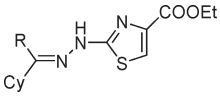
Table 1. Chemical−Physical Data of Derivatives **1**−**8**



compd	R	*n*	mp (°C)	yield (%)	ClogP
1	H	1	153−158	99	1.87
2	2-CH_3	1	143−148	91	2.39
3	3-CH_3	1	134−139	79	2.39
4	H	2	146−147	99	2.43
5	2-CH_3	2	146−148	79	2.95
6	3-CH_3	2	125−132	74	2.95
7	4-CH_3	2	109−112	99	2.95
8	H	3	149−140	85	2.99

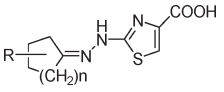
6 out of 8 wells in each column, leaving 2 wells in each column parasite-free for cytotoxicity testing. The substrate for β-gal, chlorophenol red-β-D-galactopyranoside (CPRG), was added to the *Toxoplasma* wells after 4 days of incubation at 37 °C/5% CO_2. Further incubation for 20 h was followed by addition of the cell viability reagent, CellTiter 96Aqueous One Solution Reagent (Promega Corp., WI) to the uninfected cytotoxicity wells. Color reactions in all wells were read in a Vmax microplate reader (molecular Devices, CA) after 3 h of incubation. The amount of absorbance (570−650 nm) in wells containing drug, *Toxoplasma*, and CPRG was compared to that in parasite control wells. The amount of absorbance in these wells is directly proportional to the amount of β-gal activity and thus to the amount of viable tachyzoites. Thus, a decrease in amount of absorbance indicates an inhibition of enzyme activity and, by extension, parasite growth. In the cytotoxicity wells, the bioreduction of the cell viability reagent

Table 2. Chemical−Physical Data of Derivatives **9**−**22**



compd	Cy	R	mp (°C)	yield (%)	ClogP
9	thiophen-2′-yl	CH_3	205−207	74	2.42
10	thiophen-2′-yl	H	198−199	89	2.69
11	fur-2′-yl	H	183−185	99	2.12
12	fur-2′-yl	CH_3	208−210	77	1.95
13	pyridin-2′-yl	CH_3	215−216	80	1.28
14	pyridin-3′-yl	H	211−212	98	1.66
15	pyridin-3′-yl	CH_3	210−215	77	1.28
16	pyridin-4′-yl	H	251−254	98	1.66
17	pyridin-4′-yl	CH_3	215−218	72	1.28
18	naphthalen-1′-yl	H	189−190	75	4.12
19	naphthalen-2′-yl	CH_3	228−230	99	3.95
20	benzodioxol-5′-yl	H	217−218	99	3.19
21	indol-3′-yl	H	232−235	93	3.42
22	coumarin-3′-yl	CH_3	193−195	99	1.11

Table 3. Chemical−Physical Data of Derivatives **23**−**30**



compd	R	*n*	mp (°C)	yield (%)	ClogP
23	H	1	242−250	78	2.22
24	2-CH_3	1	215−217	72	2.73
25	3-CH_3	1	203−205	77	2.73
26	H	2	242−247	72	2.73
27	2-CH_3	2	221−224	78	3.29
28	3-CH_3	2	231−235	75	3.29
29	4-CH_3	2	236−238	78	3.29
30	H	3	251−253	73	3.29

by viable cells into a soluble, colored formazan product was captured by reading the plates at 490−650 nm. The median inhibitory concentration (IC_{50}) and median cytotoxic dose (TD_{50}) were calculated using CalcuSyn software (Biosoft, Cambridge, U.K.). For each compound, a therapeutic index (TI) was calculated with the formula $TI = TD_{50}/IC_{50}$. This number reflects the specific activity of a compound against *Toxoplasma*. The TIs of the test compounds were compared to the TI of trimethoprim, a folate antagonist commonly employed for toxoplasmosis therapy in humans.

Of the 30 thiazolylhydrazone derivatives tested, the ester analogues **9** and **15** displayed a moderate efficacy relative to control trimethoprim. They were similar in potency, inhibiting *T. gondii* growth with IC_{50} ranging from 34 to 52 μM. Analogue **22** proved to be relatively more effective in inhibiting *T. gondii* growth with $IC_{50} = 21\ \mu M$. In addition, as shown in Table 4, the four derivatives proved to be noncytotoxic ($TD_{50} \geq 320\ \mu M$).

Compound **13** appeared to be quite effective against *Toxoplasma* ($IC_{50} = 3\ \mu M$); however, this was due to its strong cytotoxic action ($TD_{50} = 6\ \mu M$) (Table 4 and Figure 1).

As shown in Figure 1, when compounds were tested for the ability to inhibit the intracellular replication of *T. gondii* tachyzoites, derivative **22** was found to be moderately inhibitory while the cytotoxic derivative **13** showed the largest reduction of the number of tachyzoites per vacuole.

Table 4. Biological Data of **1**−**30** Derivatives and Drug Reference

compd	IC_{50} (μM)	IC_{90} (μM)	TD_{50} (μM)	TI[a]
1	92	239	≥320[c]	6
2	130	248	≥320	4
3	59	190	≥320	10
4	160	638	≥320	4
5	185	508	≥320	3
6	15	46	84	6
7	60	151	≥320	9
8	77	226	≥320	7
9	37	136	≥320	15
10	≥320	ND[b]	≥320	ND
11	≥320	ND	≥320	ND
12	49	161	241	5
13	3	7	6	2
14	162	398	≥320	4
15	34	86	≥320	17
16	≥320	ND	≥320	ND
17	64	188	≥320	9
18	52	182	≥320	11
19	43	88	127	3
20	479	2590	≥320	1
21	280	2235	≥320	2
22	21	55	≥320	27
23	≥320	ND	≥320	ND
24	≥320	ND	≥320	ND
25	38	70	82	2
26	≥320	ND	≥320	ND
27	128	310	≥320	4
28	≥320	ND	≥320	ND
29	≥320	ND	≥320	ND
30	179	3872	≥320	3
T[d]	14	ND	≥320	41

[a] TI, therapeutic index determined by TD_{50}/IC_{50}. [b] ND, not determined. [c] Toxicity endpoint not reached; a value of $^1/_4 \log_{10}$ greater than the concentration tested was used to compute the TI. [d] **T**, trimethoprim.

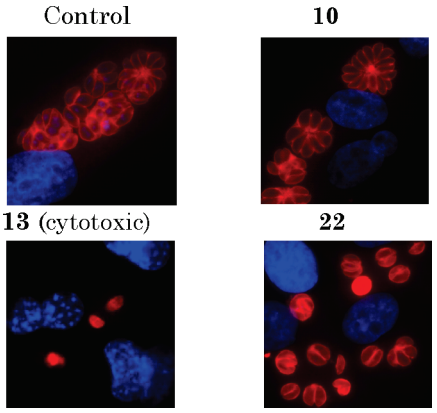


Figure 1. Replication assay. Compounds were tested using a conventional method.[19] HFF monolayers were inoculated with tachyzoites and then incubated for 2 h. Compounds (final 25 μM) or DMSO (control) was then added to the medium. Parasite replication proceeded for 26 h, after which time the monolayers were fixed, permeabilized, and immunolabeled with Rb anti-p30 (SAG1) (AbD Serotec, Oxfordshire, U.K.) followed by goat antirabbit Alexa Fluoro 594 (red) (Invitrogen, CA). DAPI (Invitrogen) was used for visualizing host cell nuclei. Slides were examined by reflected fluorescence. The numbers of vacuoles containing 1, 2, 4, or 8+ parasites/vacuole were enumerated. Shown are representative fluorescence images of the most common sized vacuole for the active compounds **13** and **22**, an example of an inactive compound **10**, and control. Tachyzoites within vacuoles are immunostained red and host nuclei stained blue.

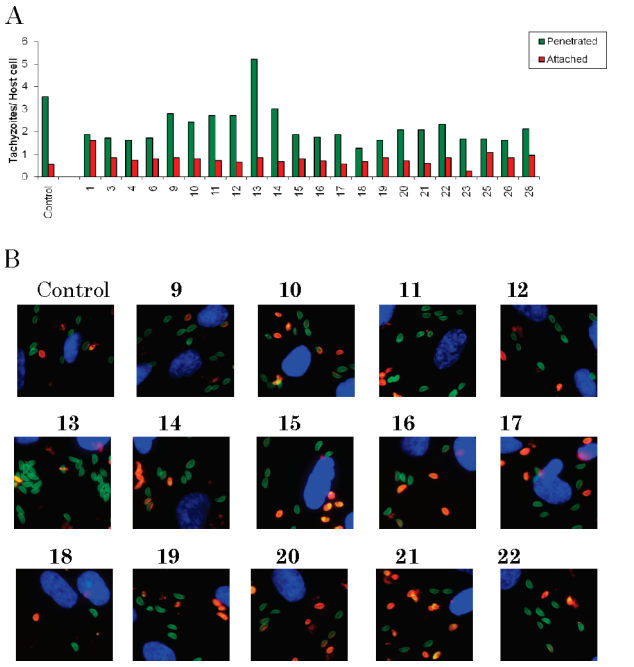


Figure 2. Invasion assay. (A) The red/green invasion assay was performed with published methods.[20] Tachyzoites were mixed with DMSO or compound (final 25 μM), left at room temperature for 20 min, and then added to HFF monolayers. After 1 h at 37 °C, 5% CO_2, the cells were rinsed and fixed. Attached/extracellular parasites were detected using Rb anti-p30 followed by Alexa Fluoro 594 (red). After permeabilization, penetrated/intracellular parasites were stained with mAb 9e11 anti-SAG1 (Argene Inc., NY) followed by Gt antimouse Alexa Fluoro 488 (green). DAPI was added to secondary antibody. Slides were visualized as in Figure 1. Numbers of green and red tachyzoites per host cell nucleus were enumerated. (B) Representative fluorescence images of fibroblast monolayers infected with strain 2F tachyzoites that have been treated with experimental compounds or DMSO. Attached/extracellular parasites are stained red, penetrated/intracellular parasites are stained green, and host nuclei are stained blue.

The sequential staining of the red/green invasion assay[20] permitted us to evaluate the compounds for inhibitory effects on tachyzoite host cell invasion and to distinguish *T. gondii* tachyzoites that had actively penetrated the cells from those parasites that were simply attached to host cells. A moderate decrease in the number of penetrated parasites relative to the control penetrated (indicating inhibition of invasion) was observed with **3** (48%), **4** (45%), **6** (48%), **16** (49%), **18** (35%), **19** (45%), **25** (47%), and **26** (45%), while derivative **13** appeared to enhance invasion (147%) (Figure 2A). As demonstrated by previous studies,[21] an overall decrease in both attached and invaded parasites strongly indicates an inhibitory effect on parasite attachment, an essential prerequisite for penetration. A moderate decrease in the number of total parasites (penetrated + attached relative to same of control) was observed with **18** (47%) and **23** (47%) (Figure 2A and Figure 2B). An increased number of attached vs penetrated parasites (within one compound) indicates inhibition of penetration. No compounds showed such activity.

In conclusion we report herein the synthesis and the characterization of 4-acyl-2-thiazolylhydrazone derivatives based on their physical, analytical, and spectral data. The prepared compounds were evaluated for their in vitro antiprotozoal properties against *T. gondii*. The results confirm that the 2,4-disubstituted 1,3-thiazole moiety could be an important pharmacophoric requirement in displaying

anti-*Toxoplasma* efficacy in vitro. By comparison of the biological data of derivatives **1**−**8** with those of **9**−**22**, it emerges that the substitution of a cycloaliphatic ring with heteroaromatic ones could be useful for the activity. The **9**, **15**, **18**, and **22** derivatives, which bear a heterocyclic moiety, were moderately effective in inhibiting *T. gondii* growth with IC_{50} ranging from 21 to 52 μM, and they were also noncytotoxic ($TD_{50} \geq 320$ μM). Furthermore, derivatives **9**, **12**, **13**, **15**, **17**, **19**, and **22**, bearing a methyl substituent at the R position of the hydrazone moiety, display a better activity than an unsubstituted one. Derivatives **23**−**30**, bearing a carboxylic acid function at the C4 position of thiazole ring, show a decrease of *Toxoplasma* inhibition or an increase in their cytotoxicity. Finally compound **22**, which had an effective action on intracellular parasite replication causing a 50% decrease in the number of tachyzoites per vacuole, could be used as a tool for determining the mode of action of this new thiazole-based scaffold. In conclusion, it is possible to state that some derivatives of this series could act in two different ways (inhibition of replication and decrease of host cell invasion).

In the present work, we have focused our attention on the synthesis and the biological evaluation of new 4-acyl-2-thiazolylhydrazone derivatives. We plan to extend our studies on the substitution of the nucleus linked to the hydrazone, which seems to have a major impact on the anti-*Toxoplasma* activity or cytotoxicity. Furthermore, our efforts will be oriented toward the comprehension of how these compounds could inhibit not only the replication of parasite but also host cell attachment and invasion.

Acknowledgment. This work was supported by MURST (Italy) and the Stanley Medical Research Institute (U.S.).

Supporting Information Available: Synthesis procedures, analytical and spectral data for new compounds, and pharmacological studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pfefferkorn, E. R. Cell Biology of *Toxoplasma gondii*. In *Modern Parasite Biology*; Wyler, D. J., Ed.; W.H. Freeman and Co.: New York, 1990; pp 26−50.
(2) Mamidi, A.; De Simone, J. A.; Pomerantz, R. J. Central nervous system infections in individuals with HIV-1 infection. *J. Neurovirol.* **2002**, *8*, 158–167.
(3) Lane, H. C.; Laughon, B. E.; Falloon, J.; Kovacs, J. A.; Davey, R. T.Jr.; Polis, M. A.; Masur, H. NIH conference. Recent advances in the management of AIDS-related opportunistic infections. *Ann. Intern. Med.* **1994**, *120* (11), 945–955.
(4) Klepser, M. E.; Lepser, T. B. Drug treatment of HIV-related opportunistic infections. *Drugs* **1997**, *53*, 40–73.
(5) Montoya, J. G.; Liesenfeld, O. Toxoplasmosis. *Lancet* **2004**, *363*, 1965–1976.
(6) Schurmann, D.; Bergmann, F.; Albrecht, H.; Padberg, J.; Wunsche, T.; Grunewald, T.; Schurmann, M.; Grobusch, M.; Vallee, M.; Ruf, B.; Suttorp, N. Effectiveness of twice-weekly pyrimethamine-sulfadoxine as primary prophylaxis of *Pneumocystis carinii* pneumonia and toxoplasmic encephalitis in patients with advanced HIV infection. *Eur. J. Clin. Microbiol. Infect. Dis.* **2002**, *21* (5), 353–361.
(7) Petersen, E.; Schmidt, D. R. Sulfadiazine and pyrimethamine in the postnatal treatment of congenital toxoplasmosis: What are the options?. *Expert Rev. Anti-Infect. Ther.* **2003**, *1*, 175–182.
(8) Joss, A. W. L. In *Human Toxoplasmosis*; Ho-Yen, D. O., Joss, A. W. L., Eds.; Oxford University Press: New York, 1992; Vol. 120, p 5.
(9) Subauste, C. S.; Remington, J. S. Immunity to *Toxoplasma gondii*. *Curr. Opin. Immunol.* **1993**, *5*, 532–537.
(10) Weller, I. V. D.; Williams, I. G. ABC of AIDS. Treatment of infections. *Br. Med. J.* **2001**, *322*, 1350–1354.
(11) Du, X.; Guo, C.; Hansell, E.; Doyle, P. S.; Caffrey, C. R.; Holler, T. P.; McKerrow, J. H.; Cohen, F. E. Synthesis and structure−activity relationship study of potent trypanocidal thio semicarbazone inhibitors of the trypanosomal cysteine protease cruzain. *J. Med. Chem.* **2002**, *45*, 2695–2707.
(12) McKerrow, J. H.; Engel, J. C.; Caffrey, C. R. Cysteine protease inhibitors as chemotherapy for parasitic infections. *Bioorg. Med. Chem.* **1999**, *7*, 639–644.
(13) Tenorio, R. P.; Carvalho, C. S.; Pessanha, C. S.; de Lima, J. G.; de Faria, A. R.; Alves, A. J.; de Melo, E. J. T.; Goes, A. J. S. Synthesis of thiosemicarbazone and 4-thiazolidinone derivatives and their in vitro anti-*Toxoplasma gondii* activity. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2575–2578.
(14) de Aquino, T. M.; Liesen, A. P.; da Silva, R. E. A.; Lima, V. T.; Carvalho, C. S.; de Faria, A. R.; de Araujo, J. M.; de Lima, J. G.; Alves, A. J.; de Melo, E. J. T.; Goes, A. J. S. Synthesis, anti-*Toxoplasma gondii* and antimicrobial activities of benzaldehyde 4-phenyl-3-thiosemicarbazones and 2-[(phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids. *Bioorg. Med. Chem.* **2008**, *16*, 446–456.
(15) de Souza, M. V. N. Synthesis and biological activity of natural thiazoles: an important class of heterocyclic compounds. *J. Sulfur Chem.* **2005**, *26*, 429–449.
(16) Usol'tseva, S. V.; Andronnikova, G. P.; Nikolaeva, S. L. Synthesis of 2-hydrazono-4-ethoxycarbonylthiazoles. *Zh. Org. Khim.* **1991**, *27* (11), 2445–2450.
(17) Foye, W. O.; Ghosh, K. Superoxide dismutase mimicking activity of copper(II) complexes of bis(methylthio) and methylthio amino derivatives of 1-methylpyridinium-(and quinolinium)-2-dithioacetic acids and other heterocyclic ligands. *Trends Heterocycl. Chem.* **1990**, *1* (1), 9–17.
(18) Jones-Brando, L.; Torrey, E. F.; Yolken, R. Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii*. *Schizophr. Res.* **2003**, *62*, 237–244.
(19) Huynh, M. H.; Rabenau, K. E.; Harper, J. M.; Beatty, W. L.; Sibley, L. D.; Carruthers, V. B. Rapid invasion of host cells by *Toxoplasma* requires secretion of the MIC2-M2AP adhesive protein complex. *EMBO J.* **2003**, *22*, 2082–2090.
(20) Silverman, J. A.; Hayes, M. L.; Luft, B. J.; Joiner, K. A. Characterization of anti-*Toxoplasma* activity of SDZ 215-918 a cyclosporin derivative lacking immunosuppressive and peptidyl-prolyl-isomerase-inhibiting activity: possible role of a P glycoprotein in *Toxoplasma* physiology. *Antimicrob. Agents Chemother.* **1997**, *41*, 1859–1866.
(21) Huynh, M. H.; Carruthers, V. *Toxoplasma* MIC2 is a major determinant of invasion and virulence. *PLoS Pathog.* **2006**, *2*, 753–762.