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Original article

Synthesis and evaluation of anti-*Toxoplasma gondii* and antimicrobial activities of thiosemicarbazides, 4-thiazolidinones and 1,3,4-thiadiazoles

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ABSTRACT

In this work we reported the synthesis and evaluation of anti-*Toxoplasma gondii* and antimicrobial activities *in vitro* of three new compound series obtained from ethyl(5-methyl-1-*H*-imidazole-4-carboxylate): acylthiosemicarbazide analogues **3a**–**d**, 4-thiazolidinone analogues **4a**–**d** and 1,3,4-thiadiazole analogues **5a**–**d**. All synthesized compounds were characterized by IR, ^{1}H, ^{13}C NMR and HRMS. The majority of the tested compounds show excellent anti-*T. gondii* activity when compared to hydroxyurea and sulfadiazine. In addition it was also shown that most of the compounds in this study have a better performance against intracellular tachyzoites. The results for antimicrobial activity evaluation showed weak antibacterial and antifungal activities for all the tested molecules, when compared with the standard drugs (chloramphenicol and rifampicin for antibacterial activity; nistatin and ketoconazole for antifungal activity).

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1. Introduction

Toxoplasma gondii infections are subclinical, unless located in the uterus or developed in patients with serious immunosuppressive condition. However, early studies also showed that they are associated with lymphadenopathy, fever, weakness and debilitation and ophthalmitis in immunocompetent individuals [1]. In addition toxoplasmic encephalitis has been increasing worldwide since the 1980s [2]. The treatments of toxoplasmic infections use effective combination of pyrimethamine and sulphadiazine (or clindamycin) with folinic acid [3]. Unfortunately, up to 40–50% of patients treated develop severe adverse effects that force the change or interruption of the therapy [4].

In immunocompetent patients, the infection with *T. gondii* can cause symptoms as fever, headache or myalgia. However, serious cases can result in toxoplasmic encephalitis (characterized by intracerebral mass lesions) with mortality rates exceeding 30% [5–9].

Therefore becomes necessary the discovery of less-toxic and more-efficacious parasite-specific drugs against the infection by *T. gondii* and treatment of toxoplasmosis [10–16]. In recent work, our group reported the synthesis and evaluation of anti-*T. gondii* activity of 4-thiazolidinones substituted at arylhydrazone moiety with electron-withdrawing or electron-donating groups, and at N-3 position with H, methyl, ethyl and phenyl substituents (Fig. 1). According to biological results, various compounds showed best values of LD_{50} for both infected cells and intracellular parasites, when compared with hydroxyurea and sulfadiazine (reference substances) [17,18]. Thiosemicarbazones and 4-thiazolidinones are known to inhibit the cell cycle in the G1/S phase, mainly inactivating ribonucleotide reductase, an enzyme that mediates the conversion of ribonucleotides to deoxyribonucleotides [19].

Compounds containing imidazole ring have been described as antiprotozoal agents [20–22]. Most of these works report an antitrypanosomal activity for these molecules. Megazol (2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole) (Fig. 2), synthesized in 1968 by Berkelhammer and Asato [23] as an antimicrobial agent and characterized later as a powerful trypanocide agent, was discarded because of its mutagenic risk [24]. Carvalho et al. [25] reported new megazol analogues, containing a 2-arylhydrazone moiety, where one of these compounds showed to be a new potent trypanocide prototype. Imidazole derivatives are

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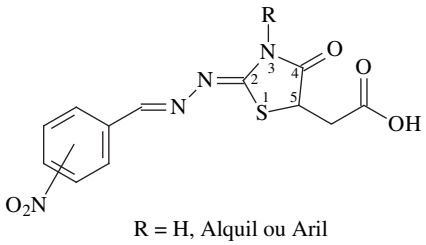


Fig. 1. 2-hydrazolyl-4-thiazolidinones.

known to possess a large range of biological activities, amongst which are antiprotozoal (metronidazole, benznidazole, and megazol) and antifungal (miconazole and ketoconazole) (Fig. 2).

Considering this panorama and perspectives, our research group decided to synthesize new 4-thiazolidinones **4** and 1,3,4-thiadiazoles **5** from substituted acylthiosemicarbazides **3**, containing the imidazole ring, utilizing ethyl(5-methyl-1-*H*-imidazole-4-carboxylate) **1** as a start compound. All compounds were screened for *in vitro* anti-*T. gondii* activity, and evaluated for antimicrobial activity. The aim of these biological assays is to discover new drugs with antimicrobial and antiprotozoal potential, once the available drugs are inefficient due to parasites elimination and show an increasing number of resistant properties developed by the pathogens [26].

2. Results and discussion

2.1. Chemistry

The acylthiosemicarbazide analogues **3a**–**d** were synthesized by an addition reaction between the hydrazide **2** (previously obtained by condensation reaction between ethyl(5-methyl-1-*H*-imidazole-4-carboxylate) **1**, and hydrazidehydrate 80%) and the substituted isothiocyanates. From these analogues were obtained two new series of compounds: 4-thiazolidinones **4a**–**d**, by thia-Michael reaction, involving maleic anhydride as Michael acceptor [18] and 1,3,4-thiadiazoles **5a**–**d** by cyclodehydration with sulfuric acid [27]. The synthetic route for the preparation of these molecules is outlined in Scheme 1. The acylthiosemicarbazide analogue **3a**–**d** and 1,3,4-thiadiazole analogue **5a**–**d** were prepared with satisfactory yield, but for the synthesis of 4-thiazolidinones **4a**–**d**, the yields ranged from 10 to 16%, due to formation of considerable quantities of polar subproducts, which remain as residues in the column chromatography on silica gel.

The chemical structures were established using NMR, IR and HRMS spectroscopy. The ^{1}H NMR spectra for acylthiosemicarbazides **3a**–**d** showed signals at 12.41–9.48 ppm, attributed to NH, NH (imidazole) and NH-aromatic groups. On the other hand, ^{13}C NMR spectra exhibited resonated at 181.13–181.01 and 163.13–162.57 ppm assigned for C=S and C=O moieties, respectively. For 4-thiazolidinones **4a**–**d** the IR spectra showed bands around 1397–1378 cm^{-1} characteristic for NCS bending vibration, providing evidence for ring closure [28–31]. Further confirmation was obtained from the ^{1}H NMR spectra, which exhibited resonance assigned to the SCH group appearing as triplet or double doublet (ABX pattern) at 4.56–4.62 ppm, due to the interaction with methylene protons of the acetyl group [32]. The formation of the 1,3,4-thiadiazoles **5a**–**d** was confirmed through the presence of bands at 1104–1082 cm^{-1} attributed to N=C–S–C=N vibrations. Moreover, the absence of the signals at 181.13–181.01 and 163.13–162.57 ppm in ^{13}C NMR spectra, attributed to C=S and C=O moieties, was also a parameter considered for the confirmation of the cyclodehydration reaction.

2.2. Biological activities

Cultures of Vero cells were previously infected with *T. gondii* and incubated for 24 h at 37 °C. The synthesized and purified acylthiosemicarbazides **3a**–**d**, 4-thiazolidinones **4a**–**d** and 1,3,4-thiadiazoles **5a**–**d** were added to the cultures and incubated for another 24 h at 37 °C. Untreated cultures showed intracellular proliferation of *T. gondii* leading to the lysis of some cells after 48 h. In the same time the cultures treated with the compounds showed decreased percentage of infection with intracellular tachyzoites elimination. During this drugs incubation the

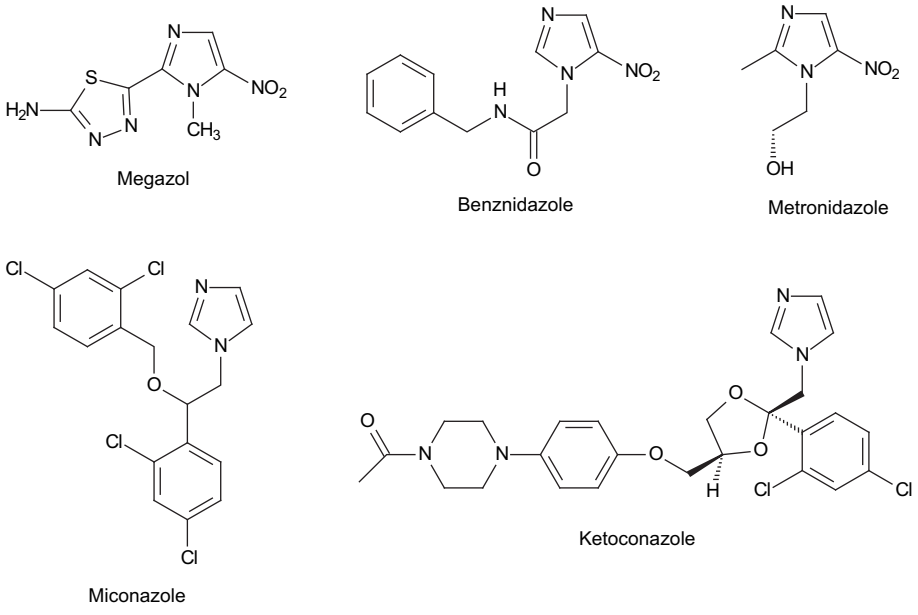
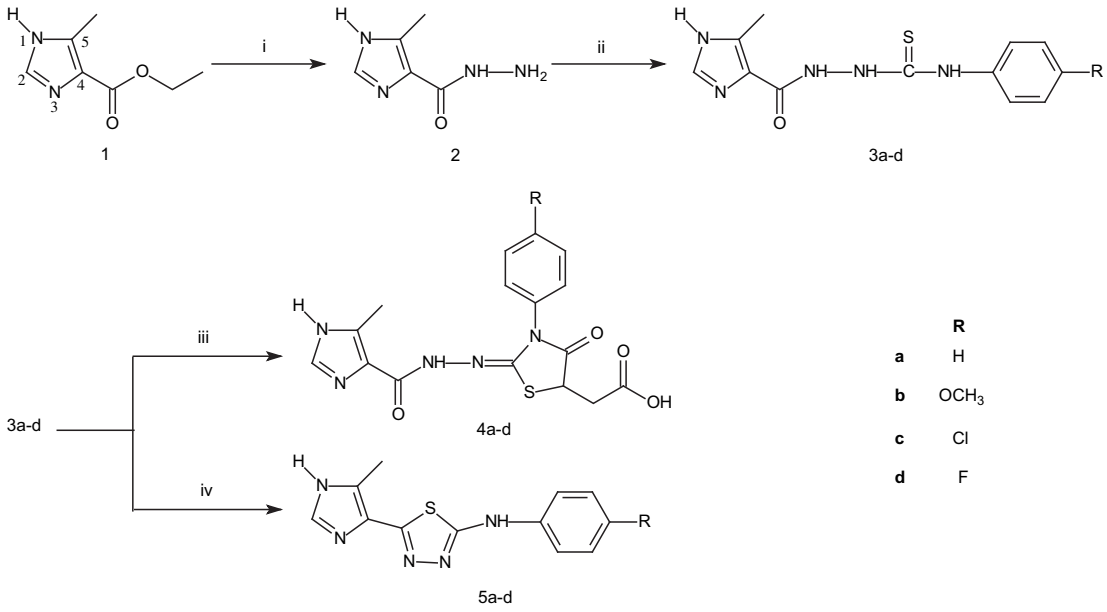


Fig. 2. Drugs containing Imidazole ring.



Scheme 1. Reagents and conditions: (i) EtOH, $NH_2NH_2 \cdot H_2O$ 80%, reflux, 6 h; (ii) EtOH, RPhNCS, reflux, 4–8 h; (iii) dried toluene, maleic anhydride, reflux, 8–41 h; (iv) H_2SO_4, 50 °C, 2 h.

tachyzoites showed complete morphological disorganization before were completely eliminated. As a consequence the mean number of tachyzoites decreased (Table 1). The acylthiosemicarbazides **3a**–**d** and 1,3,4-thiadiazoles **5a**–**d** were the most active compounds on the infection at 0.1 mM concentration, while the 4-thiazolidinones **4a**–**d** compounds had an efficient effect only at 1 mM concentration. In spite of the compounds **3c** and **5b**–**d** had been less-toxic at 10 mM, all the other compounds showed high toxicity at 10 mM concentration and cells were eliminated from the culture (LD_{50}).

The majority of the tested compounds were more effective than hydroxyurea and sulfadiazine (standard drugs). In general, among the tested derivatives, the acylthiosemicarbazides **3a**–**d** and 1,3,4-thiadiazoles **5a**–**d** were the most active against *T. gondii*. The values of LD_{50} (Table 2), which corresponded to the concentration that leads to 50% lethal death, showed a low cytotoxicity in the uninfected cells for all compounds (≥10 mM), except for **3a**, **3b** (1 mM), and **4a**–**c** (5 mM). All compounds had a range of IC_{50} values between 0.05–1 mM for infected cells and 0.05–1.5 mM for parasites, indicating a more effective action than those standard drugs (sulfadiazine and hydroxyurea). In accordance with the obtained results, the derivative **5a** was the most selective compound against *T. gondii* intracellular parasite, among the molecules with low cytotoxicity. According to SAR exploration, in both the compound series, the presence of electron-withdrawing or electron-donating substituents on the phenyl moiety did not dramatically alter the anti-*T. gondii* activity.

The synthesized derivatives were also tested for antimicrobial activity by the disc diffusion method. In general, these results indicated weak antimicrobial activities for all compounds. However, some compounds showed significant mean zone inhibition (MZI), for bacterial strains: **3a** (18 mm and 27 mm), **3b** (19 mm and 22 mm), **3c** (25 mm and 29 mm), **3d** (20 mm and 28 mm) and **4a** (19 mm and 27 mm) against *Staphylococcus aureus* and *Bacillus subtilis*, respectively; **3d** (21 mm) against *Escherichia coli*; **3c** (21 mm) and **3d** (20 mm) against *Mycobacterium smegmatis*; for

Table 1
Effect of **3a**–**d**, **4a**–**d**, and **5a**–**d** on cultures of Vero cells infected and intracellular multiplication of *T. gondii*.

Compound	% Infected Vero cells[a]				Mean number of intracellular parasites[a]			
	Untreated (control)	Treated (mM)			Untreated (control)	Treated (mM)		
		0.1	1	10		0.1	1	10
3a	59 ± 7	18 ± 8	6 ± 2	0	788 ± 107	77 ± 31	14 ± 4	0
3b	73 ± 4	22 ± 6	12 ± 2	0	730 ± 98	76 ± 28	25 ± 4	0
3c	80 ± 1	11 ± 3	8 ± 3	1 ± 1.8	739 ± 148	26 ± 4	12 ± 5	3.06 ± 3.09
3d	78 ± 4	14 ± 3	8 ± 2	0	706 ± 26	51 ± 15	7.02 ± 2	0
4a	74 ± 6	69 ± 5	42 ± 5	0	748 ± 87	718 ± 64	413 ± 37	0
4b	76 ± 5	73 ± 4	17 ± 6	0	806 ± 53	750 ± 104	123 ± 33	0
4c	65 ± 5	63 ± 8	51 ± 6	0.19 ± 0.2	513 ± 56	440 ± 57	308 ± 49	0.06 ± 0.07
4d	58 ± 14	59 ± 26	22 ± 6	0	861 ± 142	439 ± 43	193 ± 36	0
5a	72 ± 7	13 ± 4	10 ± 1	0.36 ± 0.2	780 ± 76	103 ± 32	6 ± 1.6	2 ± 1.9
5b	56 ± 8	28 ± 9	14 ± 4	0	444 ± 75	194 ± 40	79 ± 16	0
5c	70 ± 7	12 ± 2	8 ± 1	1 ± 0.3	595 ± 45	147 ± 46	42 ± 12	11 ± 2.5
5d	56 ± 4	22 ± 7	6 ± 2	3 ± 0.5	507 ± 56	139 ± 52	34 ± 15	13 ± 1.2
Hydroxyurea	59 ± 9	53 ± 7	4 ± 0.3	1 ± 0.3	487 ± 69	452 ± 58	6 ± 1.1	3 ± 2.8
Sulfadiazine	79 ± 11	64 ± 11	48 ± 15	36 ± 18	716 ± 191	570 ± 101	259 ± 114	115 ± 68

[a] Values are mean ± SD ($n = 3$).

Table 2
LD50 values of **3a**–**d**, **4a**–**d**, and **5a**–**d** for uninfected cells, infected cells, and intracellular parasites, respectively, in millimolar concentration.

Compound	LD_{50} (mM)[a]		
	Uninfected cells	Infected cells	Intracellular parasites
3a	1	0.05	0.05
3b	1	0.07	0.06
3c	>10	0.06	0.06
3d	10	0.06	0.05
4a	5	5	1.5
4b	5	1	0.5
4c	>10	1	1.5
4d	5	1	0.1
5a	>10	0.6	0.05
5b	>10	0.1	0.05
5c	>10	0.08	0.05
5d	>10	0.07	0.05
Hydroxyurea	>10	>10	0.5
Sulfadiazine	>10	>10	6

[a] Values are mean ± SD ($n = 3$).

Table 3
Minimum inhibitory concentration (MIC) in $\mu g\,mL^{-1}$ of selected compounds.

Organism	Compound	MIC[a] ($\mu g\,mL^{-1}$)
S. aureus	**3a**	190
	3b	210
	3c	190
	3d	190
	4a	250
	Chloramp.	35
B. subtilis	**3a**	140
	3b	160
	3c	130
	3d	130
	4a	270
	Chloramp.	30
E. coli	**3d**	250
	Chloramp.	14
M. smegmatis	**3c**	150
	3d	160
	Rifampicin	55
C. albicans	**4c**	280
	Nistatin	50
	Ketoconazole	25
Candida sp. (UFPEDA 2224)	**4c**	300
	Nistatin	50
	Ketoconazole	40
A. niger	**4d**	350
	Nistatin	50

[a] MIC, minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth).

fungal strains: **4c** (20 and 20 mm) against *Candida albicans* and *Candida* sp. (UFPEDA 2224); **4d** (18 mm) against *Aspergillus niger*.

The values of minimum inhibitory concentration (MIC) of the compounds with MZI above or equal to 18 mm are reported in Table 3. The results showed that the tested molecules possess weak antibacterial and antifungal activities, when compared with the standard drugs (chloramphenicol and rifampicin for antibacterial activity; and nistatin and ketoconazole for antifungal activity). The best results for antibacterial activity were **3c** (MIC = 130 $\mu g\,mL^{-1}$) and **3d** (MIC = 130 $\mu g\,mL^{-1}$) against *B. subtilis*. For antifungal properties, the low activities of the tested molecules ranged from 280 μg/mL to 350 μg/mL.

3. Conclusion

The acylthiosemicarbazides **3a**–**d**, 4-thiazolidinones **4a**–**d** and 1,3,4-thiadiazoles **5a**–**d**, obtained from ethyl(5-methyl-1-*H*-imidazole-4-carboxylate), were synthesized and characterized based on their physical, analytical and spectral data. The compounds of these three series were evaluated *in vitro* against *T. gondii* intracellular. Results showed a significant decrease in the percentage of infected cells and in the mean number of tachyzoites per cell from the concentrations of 0.1, 1 and 10 mM, when compared with hydroxyurea and sulfadiazine (standard drugs). Acylthiosemicarbazides **3a**–**d** and 1,3,4-thiadiazoles **5a**–**d** showed the best results. The majority presented high toxicity for both infected cells and intracellular parasites, where the determined DL_{50} values ranged from 0.05 to 1 mM. The derivative **5a** was the most selective against intracellular parasites and showed low toxicity. On the other hand, the synthesized compounds were evaluated *in vitro* against bacteria and fungal species, showing weak antimicrobial activities. Finally, it can be concluded that thiosemicarbazides, 4-thiazolidinones and 1,3,4-thiadiazoles, obtained from ethyl(5-methyl-1-*H*-imidazole-4-carboxylate), provide interesting lead for anti-*T. gondii* drugs discovery. Modifications to improve the potency for these derivatives by structural diversification are currently under progress in our laboratory.

4. Experimental

4.1. Chemistry protocols

The melting points were determined on BÜCHI-535 apparatus and are uncorrected. IR spectra were measured on BRUKER IFS-66 IR spectrophotometer. NMR spectra were recorded on UNITY PLUS-300 MHz-VARIAN spectrometer by using tetramethylsilane as an internal standard. HRMS were recorded on Varian MAT 711 spectrometer 70 eV electron impact, Kratos profile spectrometer 70 eV electron impact. The chemical shifts are reported in δ units, and coupling constants (J) are reported in hertz (Hz). TLC development was conducted on 0.25-mm silica gel plates ($60F_{254}$, Merck). The purification of 4-thiazolidinones **4a**–**d** was achieved by passage through column chromatography on silica gel 60 (mesh 230–400, E. Merck) with the indicated solvent system.

4.1.1. Representative procedure for (**3a**–**d**)

*4.1.1.1. 1-[(5-Methyl-1H-imidazole-4-yl)carbonyl]-4-phenyl-thiosemicarbazide (**3a**).* To a solution of hydrazide **2** (1 g, 0.0071 mol) in 30 mL of EtOH, was added, dropwise a 0.71 mL (0.007 mol) of phenylisothiocyanate. The mixture was stirred under reflux for 6 h and cooled to room temperature. The solvent was removed under reduced pressure to get a crude product that was purified by recrystallization from EtOH. White fine powder; yield 80%; mp 160–162 °C; IR (KBr): ν_{max} 3178 cm^{-1} (N–H), 1657 cm^{-1} (C=O), 1583 and 1536 cm^{-1} (NC=O), 1200 and 656 cm^{-1} (C=S); ^1H NMR (300 MHz, DMSO-d_6): δ 12.41 (br s, 1H, NH imidazole), 9.84 (s, 1H, NNHCS), 9.59 (br s, 2H, CONHN and CSNHPh), 7.62 (s, 1H, CH imidazole), 7.49 (d, 2H, $J = 9$ Hz, Ar-H), 7.30 (t, 2H, $J = 9$ Hz, Ar-H), 7.12 (t, 2H, $J = 9$ Hz, Ar-H), 2.45 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 180.93 (C=S), 162.84 (C=O), 139.32 (Cq Ar), 133.70 (C_2 imidazole), 132.10 (C_4 imidazole), 128.56 (C_5 imidazole), 128.01 (CH Ar), 124.00 (CH Ar), 124.62 (CH Ar), 10.54 (CH_3); HRMS (EI+): calcd. ($C_{12}H_{13}N_5OS$) 275.0840; found: 275.0924.

*4.1.1.2. 1-[(5-Methyl-1H-imidazole-4-yl)carbonyl]-4-(4-methoxyphenyl])-thiosemicarbazide (**3b**).* White fine powder; yield 97%; mp 161–162 °C; IR (KBr): ν_{max} 3339 cm^{-1} (N–H), 1668 cm^{-1} (C=O), 1594 and 1555 cm^{-1} (NC=O), 1244 and 643 cm^{-1} (C=S); ^1H

NMR (300 MHz, DMSO-d_6): δ 12.39 (br s, 1H, NH imidazole), 9.77 (s, 1H, NNHCS), 9.48 (br s, 2H, CONHN and CSNHPh), 7.61 (s, 1H, CH imidazole), 7.31 (d, 2H, $J = 9$ Hz, Ar-H), 6.86 (d, 2H, $J = 9$ Hz, Ar-H), 3.73 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 181.09 (C═S), 162.57 (C═O), 156.51 (Cq Ar), 136.90 (Cq Ar), 133.66 (C_2 imidazole), 132.14 (C_4 imidazole), 128.41 (C_5 imidazole), 126.79 (CH Ar), 123.20 (CH Ar), 55.20 (OCH_3), 10.61 (CH_3); HRMS (EI+): calcd. ($C_{13}H_{15}N_5O_2S$) 305.0946; found: 305.0937.

*4.1.1.3. 1-[(5-Methyl-1H-imidazole-4-yl)carbonyl]-4-(4-chlorophenyl)-thiosemicarbazide (**3c**).* White fine powder; yield 73%; mp 217–218 °C; IR (KBr): ν_{max} 3355 cm^{-1} (N–H), 1670 cm^{-1} (C═O), 1593 and 1547 cm^{-1} (NC═O), 1263 and 640 cm^{-1} (C═S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.42 (br s, 1H, NH imidazole), 9.84 (s, 1H, NNHCS), 9.69 (br s, 2H, CONHN and CSNHPh), 7.63 (s, 1H, CH imidazole), 7.54 (d, 2H, $J = 9$ Hz, Ar-H), 7.36 (d, 2H, $J = 9$ Hz, Ar-H), 2.46 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 181.01 (C═S), 163.13 (C═O), 138.38 (Cq Ar), 133.71 (C_2 imidazole), 132.21 (C_4 imidazole), 128.56 (C_5 imidazole), 127.82 (CH Ar), 127.01 (CH Ar), 124.44 (CH Ar), 10.57 (CH_3); HRMS (EI+): calcd. ($C_{13}H_{15}N_5O_2S$) 309.0451; found: 309.0398.

*4.1.1.4. 1-[(5-Methyl-1H-imidazole-4-yl)carbonyl]-4-(4-fluorophenyl)-thiosemicarbazide (**3d**).* White fine powder; yield 54%; mp 187–188 °C; IR (KBr): ν_{max} 3184 cm^{-1} (N–H), 1663 cm^{-1} (C═O), 1590 and 1541 cm^{-1} (NC═O), 1200 and 656 cm^{-1} (C═S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.44 (br s, 1H, NH imidazole), 9.78 (s, 1H, NNHCS), 9.62 (br s, 2H, CONHN and CSNHPh), 7.62 (s, 1H, CH imidazole), 7.46 (t, 2H, $J = 6$ Hz, Ar-H), 7.14 (t, 2H, $J = 6$ Hz, Ar-H), 2.45 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 181.13 (C═S), 162.75 (C═O), 159.21 (d, $J = 240.5$ Hz, Cq Ar), 135.67 (Cq Ar), 133.69 (C_2 imidazole), 132.32 (C_4 imidazole), 128.37 (C_5 imidazole), 127.41 (CH Ar), 114.41 (d, $J = 23.3$ Hz, CH Ar), 10.60 (CH_3); HRMS (EI+): calcd. ($C_{13}H_{15}N_5O_2S$) 293.0746; found: 293.0686.

*4.1.2. Representative procedure for (**4a**–**d**)*

*4.1.2.1. 3-Phenyl-2-[(5-methyl-1H-imidazole-4-yl)carbonyl]hydrazono-4-oxo-1,3-thiazolidin-5-yl-acetic acid (**4a**).* A mixture of 1 g (0.0036 mol) of 1-[(5-methyl-1*H*-imidazole-4-yl)carbonyl]-4-phenyl-thiosemicarbazide **3a** and 1.058 g (0.0108 mol) of maleic anhydride in 50 mL of dried toluene was stirred under reflux. The mixture was stirred in the same conditions till the completion of the reaction (8 h). The solvent was evaporated at reduced pressure, and the crude product was extracted with ethyl acetate. The organic layer was treated with anhydrous sodium sulfate and evaporated again. Finally the pure product was obtained by column chromatography on silica gel using hexane–ethyl acetate and methanol–ethyl acetate as an eluent. White fine powder; yield 12%; mp 205–210 °C (decomposed); IR (KBr): ν_{max} 3504 cm^{-1} (O–H), 3347 cm^{-1} (N–H), 1709 (C═O), 1643 (NC═O), 1585 (C═N), 1378 (NCS), 1278 (N–N═C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.48 (br s, 1H, NH imidazole), 10.43 (br s, 1H, CO_2H), 10.27 (br s, 1H, CONHN), 7.65 (s, 1H, *H*-imidazole), 7.35 (t, 2H, $J = 9$ Hz, Ar-H), 7.11 (t, 1H, $J = 9$ Hz, Ar-H), 6.83 (t, 2H, $J = 9$ Hz, Ar-H), 4.59 (dd, 1H, $J = 3$ and 12 Hz, CH), 3.16 (m, 1H, CH_{2a}), 2.73 (m, 1H, CH_{2b}), 2.45 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 171.38 (CO_2H), 170.38 (CO 4-thiazolidinone), 151.72 (CONH), 147.65 (C═N), 135.1 (Cq Ar), 133.90 (C_4 imidazole), 133.40 (C_2 imidazole), 129.22 (C_5 imidazole), 124.21 (CH Ar), 120.63 (CH Ar), 120.36 (CH Ar), 42.50 (CH_2), 36.70 (CH), 10.36 (CH_3); HRMS (EI+): calcd. ($C_{16}H_{15}N_5O_4S$) 373.0844; found: 373.0712.

*4.1.2.2. 3-(4-Methoxyphenyl)-2-{[(5-methyl-1H-imidazole-4-yl)carbonyl]hydrazono}-4-oxo-1,3-thiazolidin-5-yl-acetic acid (**4b**).* White fine powder; yield 15%; mp 205–210 °C (decomposed); IR (KBr): ν_{max} 1731 (C═O), 1643 (NC═O), 1505 (C═N), 1397 (NCS), 1295 (N–N═C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.54 (br s, 1H, NH imidazole), 10.49 (br s, 1H, CO_2H), 10.28 (br s, 1H, CONHN), 7.68 (s, 1H, *H*-imidazole), 6.92 (d, 2H, $J = 6$ Hz, Ar-H), 6.82 (d, 2H, $J = 6$ Hz, Ar-H), 4.60 (dd, 1H, $J = 3$ and 12 Hz, CH), 3.73 (s, 3H, OCH_3), 3.18 (m, 1H, CH_{2a}), 2.73 (m, 1H, CH_{2b}), 2.44 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 171.47 (CO_2H), 170.55 (CO 4-thiazolidinone), 159.38 (CH Ar), 151.69 (CONH), 149.24 (C═N), 138.71 (Cq Ar), 134.14 (C_4 imidazole), 133.74 (C_2 imidazole), 128.66 (CH Ar), 128.32 (C_5 imidazole), 119.97 (CH Ar), 55.25 (OCH_3), 42.24 (CH_2), 34.03 (CH), 10.42 (CH_3); HRMS (EI+): calcd. ($C_{17}H_{17}N_5O_5S$) 403.0950; found: 403.0875.

*4.1.2.3. 3-(4-Chlorophenyl)-2-{[(5-methyl-1H-imidazole-4-yl)carbonyl]hydrazono}-4-oxo-1,3-thiazolidin-5-yl-acetic acid (**4c**).* Yellow oil; yield 10%; ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.51 (br s, 1H, NH imidazole), 10.52 (br s, 1H, CO_2H), 10.32 (br s, 1H, CONHN), 7.66 (s, 1H, *H*-imidazole), 7.39 (d, 2H, $J = 9$ Hz, Ar-H), 6.88 (d, 2H, $J = 9$ Hz, Ar-H), 4.64 (dd, 1H, $J = 3$ and 9 Hz, CH), 3.17 (m, 1H, CH_{2a}), 2.73 (m, 1H, CH_{2b}), 2.44 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 171.53 (CO_2H), 170.69 (CO 4-thiazolidinone), 152.94 (CONH), 146.62 (C═N), 161.00 (Cq Ar), 142.27 (Cq Ar), 134.09 (C_4 imidazole), 133.25 (C_2 imidazole), 129.33 (C_5 imidazole), 128.41 (CH Ar), 127.63 (CH Ar), 40.96 (CH_2), 35.60 (CH), 10.48 (CH_3); HRMS (EI+): calcd. ($C_{16}H_{14}ClN_5O_4S$) 407.0455; found: 407.0590.

*4.1.2.4. 3-(4-Fluorophenyl)-2-{[(5-methyl-1H-imidazole-4-yl)carbonyl]hydrazono}-4-oxo-1,3-thiazolidin-5-yl-acetic acid (**4d**).* White fine powder; yield 16%; mp 200–205 °C (decomposed); IR (KBr): ν_{max} 1742 (C═O), 1646 (NC═O), 1502 (C═N), 1388 (NCS), 1283 (N–N═C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.54 (br s, 1H, NH imidazole), 10.55 (br s, 1H, CO_2H), 10.34 (br s, 1H, CONHN), 7.68 (s, 1H, *H*-imidazole), 7.18 (t, 2H, $J = 9$ Hz, Ar-H), 6.89 (dd, 2H, $J = 3$ and 9 Hz, Ar-H), 4.65 (dd, 1H, $J = 3$ and 12 Hz, CH), 3.18 (m, 1H, CH_{2a}), 2.75 (m, 1H, CH_{2b}), 2.45 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 171.80 (CO_2H), 170.71 (CO 4-thiazolidinone), 161.38 (CONH), 159.10 (d, $J = 240$ Hz, Cq Ar), 152.96 (C═N), 144.16 (Cq Ar), 133.90 (C_2 imidazole), 133.31 (C_4 imidazole), 127.66 (C_5 imidazole), 122.46 (d, $J = 8.2$ Hz, CH Ar), 116.06 (d, $J = 22.3$ Hz, CH Ar), 45.36 (CH_2), 34.20 (CH), 10.53 (CH_3); HRMS (EI+): calcd. ($C_{16}H_{14}FN_5O_4S$) 391.0750; found: 391.0464.

*4.1.3. Representative procedure for (**5a**–**d**)*

*4.1.3.1. N-Phenyl-5-(5-methyl-1H-imidazole-4-yl)-1,3,4-thiadiazole-2-amine (**5a**).* White fine powder; yield 93%; mp 249–250 °C; a mixture of 0.28 g (0.001 mol) of 2-[(5-methyl-1*H*-imidazole-4-yl) carbonyl]-4-phenyl-thiosemicarbazide **3a** and 0.5 mL of conc. H_2SO_4 was heated at 50 °C for 2 h, according to procedure stated in literature [26]. The resulting solution was cooled, poured into crushed ice, and treated with sodium carbonate to pH 6. The precipitate was collected by filtration under vacuum and washed with water. IR (KBr): ν_{max} 1657 cm^{-1} (C═N), 1566 (C═C), 1100 (N═C–S–C═N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.65 (br s, 2H, NHPh and NH-imidazole), 7.66 (d, 2H, $J = 6$ Hz, H-Ar), 7.65 (s, 1H, *H*-imidazole), 7.33 (t, 2H, $J = 6$ Hz, H-Ar), 6.97 (t, 1H, $J = 6$ Hz, H-Ar), 2.53 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 162.27 (Cq thiadiazole), 155.80 (Cq thiadiazole), 140.95 (Cq Ar), 134.98 (C_2 imidazole), 129.05 (CH Ar), 127.68 (C_4 imidazole), 126.24 (C_5 imidazole), 121.49 (CH Ar), 117.19 (CH Ar), 10.70 (CH_3); HRMS (EI+): calcd. ($C_{12}H_{11}N_5S$) 257.0735; found: 257.0924.

*4.1.3.2. N-(4-Methoxyphenyl)-5-(5-methyl-1H-imidazole-4-yl)-1,3,4-thiadiazole-2-amine (**5b**).* White fine powder; yield 84%; mp 279–280 °C; IR (KBr): ν_{max} 1623 cm^{-1} (C═N), 1546 cm^{-1} (C═C), 1082 cm^{-1} (N═C–S–C═N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.47

(br s, 2H, NHPh and NH-imidazole), 7.92 (s, 1H, *H*-imidazole), 7.55 (d, 2H, $J = 9$ Hz, H-Ar), 6.95 (d, 2H, $J = 9$ Hz, H-Ar), 3.73 (s, 3H, OCH_3), 2.50 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 162.11 (Cq thiadiazole), 158.22 (Cq Ar), 151.95 (Cq thiadiazole), 137.40 (Cq Ar), 134.92 (C_2 imidazole), 127.66 (C_4 imidazole), 124.47 (CH Ar), 126.35 (C_5 imidazole), 123.45 (CH Ar), 49.03 (OCH_3), 10.66 (CH_3); HRMS (EI+): calcd. ($C_{13}H_{13}N_5OS$) 287.0840; found: 287.0925.

*4.1.3.3. N-(4-Chlorophenyl)-5-(5-methyl-1H-imidazole-4-yl)-1,3,4-thiadiazole-2-amine (**5c**).* White fine powder; yield 71%; mp 256–257 °C; IR (KBr): ν_{max} 1618 cm^{-1} (C=N), 1560 cm^{-1} (C=C), 1095 cm^{-1} (N=C–S–C=N); ^1H NMR (300 MHz, DMSO-d_6): δ 10.42 (br s, 2H, NHPh and NH-imidazole), 7.69 (s, 1H, *H*-imidazole), 7.64 (d, 2H, $J = 9$ Hz, H-Ar), 7.35 (d, 2H, $J = 9$ Hz, H-Ar), 2.50 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 161.90 (Cq thiadiazole), 156.32 (Cq thiadiazole), 139.79 (Cq Ar), 134.97 (C_2 imidazole), 128.87 (CH Ar), 127.78 (C_4 imidazole), 126.09 (C_5 imidazole), 124.87 (Cq Ar), 118.71 (CH Ar), 10.61 (CH_3); HRMS (EI+): calcd. ($C_{12}H_{10}ClN_5S$) 291.0345; found: 291.0668.

*4.1.3.4. N-(4-Fluorophenyl)-5-(5-methyl-1H-imidazole-4-yl)-1,3,4-thiadiazole-2-amine (**5d**).* White fine powder; yield 94%; mp 259–261 °C; IR (KBr): ν_{max} 1632 cm^{-1} (C=N), 1572 cm^{-1} (C=C), 1104 cm^{-1} (N=C–S–C=N); ^1H NMR (300 MHz, DMSO-d_6): δ 10.54 (br s, 2H, NHPh and NH-imidazole), 8.49 (s, 1H, *H*-imidazole), 7.68 (dd, 2H, $J = 6$ and 9 Hz, H-Ar), 7.20 (t, 2H, $J = 9$ Hz, H-Ar), 2.51 (s, 3H, CH_3); ^{13}C NMR (75.4 MHz, DMSO-d_6): δC 163.68 (Cq thiadiazole), 157.32 (d, $J = 238.1$ Hz, Cq Ar), 150.03 (Cq thiadiazole), 137.06 (Cq Ar), 134.90 (C_2 imidazole), 127.62 (C_4 imidazole), 123.48 (C_5 imidazole), 119.17 (d, $J = 7.5$ Hz, CH Ar), 115.69 (d, $J = 22.3$ Hz, CH Ar), 10.52 (CH_3); HRMS (EI+): calcd. ($C_{12}H_{10}FN_5S$) 275.0640; found: 275.0643.

4.2. Biological assays

4.2.1. Assay for anti-T. gondii activity

Tachyzoites from the virulent RH strain of *T. gondii* were obtained by intraperitoneal passages in Swiss mice and collected in Ringer's solution at pH 7.2, 48 h after infection. Vero cells (kidney fibroblasts from African green monkeys) were incubated (37°) with *T. gondii* tachyzoites (parasite/host cell 10:1 relationship) for 1 h, washed twice with phosphate-buffered saline solution (PBS) to remove extracellular parasites, and incubated for 24 h at 37 °C in the presence of medium 199 supplemented with 5% fetal calf serum (FCS). Cells infected with *T. gondii* were incubated with test compounds in the concentrations of 0.1, 1, and 10 mM for 24 h (37°). Hydroxyurea and sulfadiazine were utilized as reference substances. All compounds were added to the infected cells during intense parasite proliferation. The infected cultures were washed thrice with PBS, fixed with Bouin's fixative, stained with Giemsa, and observed under a light microscope (63× objective Axioplan, Zeiss, Jena, Germany). The percentage of infected cells and the mean number of intracellular parasites were determined by the examination of at least 400 cells [33,34]. Statistical analysis was carried out using the Student's *t*-test. *P* values <0.05 were considered as significant. Data shown are representative of 13 assays in triplicate. Finally, the LD_{50} values for infected cells and intracellular parasites for all compounds were obtained after 24-h exposure in the concentrations ranged of 0.01–10 mM, in triplicate per assay, by a non-linear regression using exclusion test with trypan blue [20].

4.2.2. Assay in vitro for antimicrobial activity

Bacterial and fungal strains used in the antimicrobial evaluation were obtained from the Departamento de Antibióticos and from the Instituto de Micologia culture collections, Universidade Federal de Pernambuco, Brazil. Namely, *S. aureus* (UFPEDA 02), *B. subtilis* (UFPEDA 16), *Micrococcus luteus* (UFPEDA 100), *E. coli* (UFPEDA 224), *Klebisiella pneumoniae* (UFPEDA 396), *Pseudomonas aeruginosa* (UFPEDA 416), *M. smegmatis* (UFPEDA 71), *Mycobacterium tuberculosis* (UFPEDA 82), *Streptococcus faecalis* (UFPEDA 138), *Saccharomyces cerevisiae* (UFPEDA 1012), *Candida* sp. (URM 720), *Candida* sp. (UFPEDA 2224), *Candida krusei* (UFPEDA 1002), *C. albicans* (UFPEDA 1007), *Malassezia furfur* (URM 4849), *A. niger* (UFPEDA 2003), *Fusarium moniliforme* (UFPEDA 2409), and *Fusarium oxysporum* (UFPEDA 2414). The antibacterial and antifungal activities were reported preliminary utilizing disc diffusion method [35]. In this method, discs containing known amounts of an antimicrobial agent were placed on the surface of an agar plate that has been inoculated with a standardized suspension of microorganisms to be tested. Paper discs with only DMSO or CH_2Cl_2 (tests involving ketoconazole) were used as negative controls. The MZI for chloramphenicol and rifampicin (antibacterial) and nistatin and ketoconazole (antifungal) was used as reference values (millimeter). All experiments were conducted in triplicate and repeated if the results differed. All compounds used having MZI larger or equal to 18 mm were selected to MIC.

For MIC assays [36,37], a stock solution (1 mg mL^{-1}) of each test compound was prepared in dimethylsulfoxide solvent. Further, the serial dilution of test compounds was carried out and the concentrations used ranged from 50 to 400 μg mL^{-1}. Test compounds at various concentrations were added to culture medium in a test tube and different strains were inoculated at 108 bacteria mL^{-1} concentration. Tryptic soy agar and nutrient agar (for antibacterial) and Sabouraud liquid medium (for antifungal) were utilized as culture medium. The tubes were incubated at 37 °C (antibacterial) or 30 °C (antifungal) for 24–48 h and then examined for the presence or absence of growth organisms tested. Chloramphenicol, rifampicin, nistatin, and ketoconazole were used as antibacterial and antifungal substances. The MIC values were obtained from the lowest concentration of the test compounds where the tubes remained clear, indicating that the bacterial or fungal growth was completely inhibited at this concentration. MIC values were expressed in μg mL^{-1}.

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