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Synthesis, anti-*Toxoplasma gondii* and antimicrobial activities of benzaldehyde 4-phenyl-3-thiosemicarbazones and 2-[(phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids

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Abstract—In the present communication, a new series of 2-[(phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids (**2a**–**p**) have been synthesized. Benzaldehyde 4-phenyl-3-thiosemicarbazones substituted (**1a**–**p**) were also obtained and used as intermediate to give the title compounds. All synthesized compounds were characterized by IR, ^{1}H and ^{13}C NMR. The in vitro anti-*Toxoplasma gondii* activity of **1a**–**p** and **2a**–**p** was evaluated. The 4-thiazolidinones (**2a**–**p**) were screened for their *in vitro* antimicrobial activity. For anti-*Toxoplasma gondii* activity, in general, all compounds promoted decreases in the percentage of infected cells leading to parasite elimination. These effects on intracellular parasites also caused a decrease in the mean number of tachyzoites. In addition, most of the 4-thiazolidinones showed more effective toxicity against intracellular parasites, with IC_{50} values ranging from 0.05 to 1 mM. According to results of antimicrobial activity, compounds **2f**, **2l**, and **2p** showed best activity against *Mycobacterium luteus*, **2c** was more active against *Mycobacterium tuberculosis*, and **2g**, **2l**, and **2n** showed same activity as nistatin (standard drug) against *Candida* sp. (4249).

1. Introduction

Parasitic diseases, as toxoplasmosis, affect millions of people, and they are responsible for some of the most important and prevalent diseases of humans and domestic animals. These diseases result in considerable morbidity and mortality worldwide, especially in developing countries.[1–3] The toxoplasmosis is caused by an intracellular parasite, *Toxoplasma gondii*, and is associated with severe pathologies, including pneumonia, myocarditis, pulmonary necrosis, and severe neurological disorders as psychomotor or mental retardation. In humans, infection with *T. gondii* commonly occurs either following ingestion of food or water infected with cysts, transplacental transmission of the parasite to the fetus, or immunosuppression in adults.[4–7]

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%.[6,8,9] The current therapy for clinical treatment of toxoplasmosis is isolation or combination of chemotherapy using pyrimethamine and sulfadiazine. This utilization is limited, primarily because of high toxicity and serious side effects for the host individual and its ineffectiveness in eliminating intracellular parasites. So, the discovery of less-toxic and more efficacious parasite-specific drugs becomes necessary against the infection by *T. gondii* and treatment of toxoplasmosis.[10–17]

Keywords: Thiosemicarbazone; 4-Thiazolidinone; Antimicrobial activity; Anti-*Toxoplasma gondii* activity.
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In recent works, Tenório et al.[18] have reported the synthesis and evaluation of anti-*T. gondii* activity of thiosemicarbazones substituted at arylhydrazone moiety with nitro substituents at *ortho*, *meta*, and *para* positions, and 4-thiazolidinones substituted at *N*-3 position with phenyl, methyl and hydrogen substituents, and with the same groups at arylhydrazone moiety (Chart 1). This paper was pioneer, because of the inexistence of studies in the literature involving the action of these two classes of compounds on intracellular *T. gondii*. It is important to show a structural similarity between 4-thiazolidinones synthesized from thiosemicarbazones, as both derivatives possess the Ph–C=N–N–C–(S)–N moiety (Chart 1; A). According to biological results, the 4-thiazolidinone derivatives with a phenyl substituent at *N*-3 position showed best values of IC_{50} for both infected cells and intracellular parasites. In order to investigate the anti-*T. gondii* activity of new structural analogues, we describe the synthesis, elucidate the structure and in vitro biological activities of thirty-two new compounds, by fixing the phenyl group at *N*-4 and *N*-3 positions in the thiosemicarbazones (**1a**–**p**) and 4-thiazolidinones (**2a**–**p**), respectively (Chart 1; B), and introducing electron-withdrawing or electron-donating substituents at arylhydrazone moiety in both derivatives (Chart 1; C).

According to the literature, thiosemicarbazones, as a class, and compounds which have the 4-thiazolidinone ring are reported to possess various biological activities, as antimicrobial, anti-inflammatory, antiviral, antiparasitic, and antituberculosis.[19–25] As part of our studies to discover new active compounds, thiosemicarbazones (**1a**–**p**) and 4-thiazolidinones (**2a**–**p**) were screened for in vitro anti-*T. gondii* activity. As thiosemicarbazones synthesized by Tenório et al.[18] do not show activity against various bacteria and fungal species, only the 4-thiazolidinones (**2a**–**p**) were evaluated for antimicrobial activity. The aim of these biological assays is to discover new drugs with antimicrobial and antiparasitic potential, due to inefficient parasite elimination and an increasing number of resistant properties developed by the pathogens.[17]

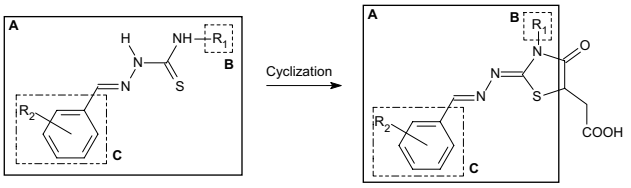


Chart 1.

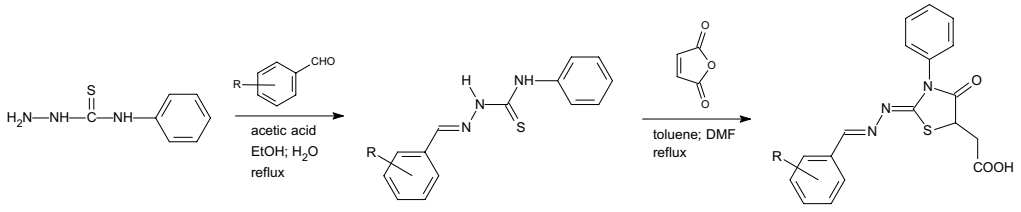
2. Results and discussion

2.1. Chemistry

The syntheses of the thiosemicarbazones (**1a**–**p**) and 4-thiazolidinones (**2a**–**p**) have been carried out utilizing the same methodology previously reported by Tenório et al.[18] (Scheme 1). Thiosemicarbazones (**1a**–**p**) were characterized by ^{1}H NMR, where signals at 9.89–11.95 and 9.13–10.25 ppm were attributed to NH and NH–Ar groups.[18] The IR spectra of the 4-thiazolidinones (**2a**–**p**) showed absorption bands at about 1731–1707 cm^{-1} characteristic for C=O stretching vibration (acid group), and 1621–1606 cm^{-1} associated with C=O amide I band. In addition, absorption bands around 1360–1337 cm^{-1} characteristic for NCS bending vibration provided confirmatory evidence for ring closure.[19,26–28] Further support was obtained from the ^{1}H NMR spectra, where it did not display signs of the thiosemicarbazone protons (NH), and peaks resonated at 171.5–171.8, 161.6–167.0, and 42.3–42.5 ppm in the ^{13}C NMR spectra assigned for C=O, C=N, and SCH moieties. On the other hand, ^{1}H NMR spectra exhibited resonance assigned to the SCH group of the thiazolidine ring appearing as triplet or double doublet (ABX pattern) at 4.42–4.59 ppm due to the interaction with methylene protons of the acetyl group.[29] The CH=N protons in these structures were observed in the 8.13–8.43 ppm region.[30] In summary, all the synthesized compounds exhibited satisfactory spectral data consistent with the structures.

2.2. Biological activities

The synthesized and purified thiosemicarbazones (**1a**–**p**) and 4-thiazolidinones (**2a**–**p**) were submitted to incubation for 24 h in cultures of Vero cells infected with *T. gondii*. After drug treatment, the cultures showed decreasing percentage of infection. As a consequence the mean number of normal tachyzoites decreased (Tables 1–3). The compounds **1h**, **2b**, **2e**, **2k**, and **2l** had an effective action on intracellular parasite multiplication. The action of these compounds for a 50% inhibition of parasite growth was ⩽0.1 mM, which represents a range of about 12.5–30 μg/mL. However, sulfadiazine concentration for 50% inhibition is the same



Scheme 1. **a**; **R** = hydrogen, **b**; **R** = 4-chloro, **c**; **R** = 2,4-dichloro, **d**; **R** = 3,4-dichloro, **e**; **R** = 3-methyl, **f**; **R** = 2-fluoro, **g**; **R** = 4-fluoro, **h**; **R** = 3-methoxy-4-hydroxy, **i**; **R** = 4-dimethylamino, **j**; **R** = 3-chloro, **k**; **R** = 4-methyl, **l**; **R** = 3-methoxy, **m**; **R** = 4-methoxy, **n**; **R** = 2,4-dimethoxy, **o**; **R** = 3,4,5-trimethoxy, **p**; **R** = 3,5-di-*tert*-butyl-4-hydroxy.

Table 1. Effect of **1a**–**p** and **2a**–**p** on cultures of Vero cells infected with *T. gondii*

Compound	% Infected Vero cells[a]					
	Untreated (control)	Treated (mM)				
		0.1	2	5	8	20
1a	83.07 ± 6.3	82.5 ± 4.2	77.82 ± 10.6	31.83 ± 8.4	13.52 ± 1.4	0
1b	77.64 ± 0.17	70.01 ± 5.6	34.8 ± 9.19	38.7 ± 5.6	21.05 ± 0.8	0
1c	61.09 ± 2.12	49.33 ± 14	33.28 ± 13.4	31.32 ± 4.9	12.08 ± 2.82	0
1d	59 ± 1.4	49.18 ± 7.7	38.02 ± 22.6	21.96 ± 6.36	4.54 ± 1.4	0
1e	56.98 ± 6.36	66.23 ± 9.89	39.82 ± 4.9	26.86 ± 9.89	22.72 ± 1.41	0
1f	93.16 ± 3.53	91.5 ± 3.5	28.13 ± 0.7	14.32 ± 0.7	29.29 ± 0.71	0
1g	55.76 ± 4	46.59 ± 13.5	35.2 ± 2	28 ± 10	19.83 ± 6	0
1h	80.4 ± 0.7	80.05 ± 11.3	19.7 ± 0.17	19.3 ± 0.17	2.6 ± 0.53	0
1i	74.06 ± 22	76.14 ± 31	35.81 ± 0.7	18.14 ± 5.6	13.46 ± 2.12	0
1j	69.21 ± 10.4	44.52 ± 2.12	60.69 ± 19	52.06 ± 12	24.49 ± 0.7	0
1k	61.33 ± 13.4	51.08 ± 18.3	36.78 ± 6.3	27.84 ± 18.4	2.96 ± 0.7	0
1l	64.09 ± 15	53.6 ± 16.9	34.82 ± 17	9.7 ± 2.8	5.9 ± 1.41	0
1m	70.32 ± 5.6	50.43 ± 3.53	29.39 ± 0.7	19 ± 7.01	12.36 ± 0.7	0
1n	83.3 ± 36	73 ± 19	41.44 ± 7.7	9.2 ± 4.2	3.6 ± 2.8	1.49
1o	80.73 ± 19.09	76.37 ± 9.89	72.04 ± 8.4	24.32 ± 16	23.17 ± 2.8	0
1p	62.66 ± 0.53	63.44 ± 3.53	64.07 ± 12	30.51 ± 4.9	6.42 ± 7.12	0
2a	78.8 ± 2.12	69.7 ± 4.9	48.5 ± 4.2	14.6 ± 6.3	0	0
2b	82.46 ± 4.9	68.15 ± 19	35.3 ± 2.47	0	0	0
2c	68.08 ± 10.6	63.23 ± 0.17	18.08 ± 4.2	19.05 ± 0.7	0	0
2d	108 ± 6	101 ± 6	0	0	0	0
2e	63.93 ± 5.6	66 ± 4.2	15.54 ± 2.8	16.19 ± 2.82	10.13 ± 3.53	0
2f	59.88 ± 2.82	70.79 ± 2.12	16.14 ± 4.2	0	0	0
2g	62.9 ± 17	21.7 ± 8.8	10.2 ± 4.8	0	0	0
2h	51.45 ± 10.9	46.02 ± 1.4	41.96 ± 5.65	3.5 ± 0.01	0	0
2i	71.66 ± 10.6	50.58 ± 2.12	0	0	0	0
2j	53.73 ± 1.4	53.74 ± 9.19	34.8 ± 3.53	33.5 ± 7	0	0
2k	83.63 ± 0.7	39.2 ± 4.2	1.23 ± 0.17	0	0	0
2l	41.47 ± 15	34.16 ± 12.7	28.71 ± 1.41	7.3 ± 7.7	4.16 ± 0.71	0
2m	32.5 ± 0.7	27.32 ± 7	4.51 ± 0.35	5.9 ± 0.7	0	0
2n	72.51 ± 0.7	66.36 ± 2.12	39.5 ± 4.9	0	0	0
2o	64.85 ± 8.4	55.57 ± 14	14.33 ± 6.36	15.63 ± 0.7	7.2 ± 0.7	0
2p	57.47 ± 3.67	53.55 ± 8.83	0	0	0	0
Hydroxyurea	61.18 ± 0.17	58.23 ± 5.65	22.78 ± 9.19	10.4 ± 5.65	7.37 ± 0.88	0
Sulfadiazine	80.3 ± 7.7	53.84 ± 0.7	48.71 ± 0.7	29.55 ± 0.7	6.84 ± 1	0

[a] Values are mean ± SD ($n = 3$).

for host cell and parasites, about 3 mM (Table 3), justifying its toxic effects. Moreover, some thiosemicarbazones and 4-thiazolidinones have been found to be more effective than hydroxyurea (reference drug).

In the concentrations of 2, 5, and 8 mM, some compounds were highly toxic because very few or no infected cells and intracellular parasites could be observed, especially for **2d**, **2i**, **2k**, and **2p** (in the concentration of 2 mM), and for **2b**, **2f**, **2g**, **2h**, and **2n** (in the concentration of 5 mM). For higher concentrations, the parasitophorous vacuole was enlarged and contained distorted parasites. These cytostatic effects were dependent on the drug concentrations. In the concentration of 20 mM for all drugs, no infected cells or intracellular parasites were observed. In general, 4-thiazolidinones were more effective that thiosemicarbazones for interrupting the *T. gondii* growth in lower concentrations. According to Table 3, the toxicity of all compounds was more effective against intracellular parasites, except for **2g**, **2i**, **2j**, **2m**, and **2p**. Similar values were obtained with hydroxyurea but not with sulfadiazine.

It was the difficulty to access intracellular taquizoites that are harnessed by the chemical structure of the intracellular environment, which led us to the use of these compounds in the mM (millimolar) range. However, these results are interesting because they demonstrate the effective anti-*T. gondii* action of these new compounds. Future researches can be done from these molecules' structures to enable them to access the *T. gondii* intravacuolar and make them effective in micro- to nanomolar range.

4-Thiazolidinone derivatives (**2a**–**p**) were preliminarily tested for antimicrobial activity by the disc diffusion method, and the results are reported in Tables 4 and 5. These results indicated that **2p** showed best activities against *Staphylococcus aureus*, *Streptococcus faecalis*, *Mycobacterium phlei*, *M. smegmatis*, and *M. tuberculosis*. **2p** and **2o** were more effective against *Bacillus subtilis*, **2m** against *Candida albicans*, and various compounds showed significant inhibition against *Micrococcus luteus* and *Candida* sp. (IMUR 4249). None of the compounds showed inhibitory effects against *Klebsiella pneumoniae*, *C.* sp. (IMUR 1224), and *C.* sp. (IMRU 720). Only **2n** showed a little activity against *Saccharomyces cerevisiae*.

Table 2. Effect of **1a**–**p** and **2a**–**p** on the intracellular multiplication of *T. gondii*

Compound	Mean number of intracellular parasites[a]					
	Untreated (control)	Treated (mM)				
		0.1	2	5	8	20
1a	413 ± 1.4	429 ± 45	367 ± 3.5	32 ± 11	2 ± 2	0
1b	446 ± 33	434 ± 101	109 ± 19	63 ± 16	15 ± 12	0
1c	431 ± 46	359 ± 47	103 ± 11	60 ± 2.12	0	0
1d	717 ± 57	577 ± 43	371 ± 90	140 ± 4.2	1 ± 0.7	0
1e	575 ± 83	510 ± 12	232 ± 14	25 ± 2.82	0	0
1f	461 ± 9.19	406 ± 8.4	67 ± 13.4	10 ± 14.14	0	0
1g	541 ± 61	332 ± 132	195 ± 4.5	11 ± 7.5	1 ± 1	0
1h	574 ± 66	368 ± 42	7 ± 10	5 ± 0.7	1 ± 0.7	0
1i	462 ± 25	290 ± 35	143 ± 25	20 ± 14	26 ± 4.2	0
1j	663 ± 103	526 ± 45	367 ± 51	218 ± 52	31 ± 25	0
1k	445 ± 92	330 ± 31	326 ± 40	42 ± 10	0	0
1l	565 ± 55	409 ± 22	68 ± 24	7 ± 1.4	2 ± 0.17	0
1m	545 ± 65	439 ± 72	112 ± 7	16 ± 2	7 ± 2.8	0
1n	771 ± 62	684 ± 29	352 ± 7.07	15 ± 18	0	0
1o	596 ± 48	461 ± 14	277 ± 5.6	96 ± 26	61 ± 3.53	0
1p	504 ± 2.82	517 ± 57	401 ± 101	138 ± 41	2 ± 2.8	0
2a	467 ± 24	336 ± 71	176 ± 25	8 ± 2.19	0	0
2b	341 ± 58	235 ± 92	32 ± 0.35	0	0	0
2c	614 ± 49	530 ± 33	100 ± 24	5 ± 7.7	0	0
2d	890 ± 15	822 ± 85	0	0	0	0
2e	442 ± 20	266 ± 47	44 ± 2.82	34 ± 7.77	16 ± 10	0
2f	512 ± 7.7	504 ± 7.7	29 ± 14	0	0	0
2g	163 ± 42	12 ± 9	2 ± 4	0	0	0
2h	458 ± 80	385 ± 29	71 ± 24	0	0	0
2i	558 ± 144	146 ± 2.82	0	0	0	0
2j	369 ± 79	273	181 ± 40	145 ± 9.1	0	0
2k	417 ± 33	91 ± 17	0	0	0	0
2l	447 ± 153	144 ± 24	28 ± 0.7	1 ± 2.12	0	0
2m	278 ± 0.7	202 ± 26	35 ± 113	28 ± 2.12	0	0
2n	560 ± 58	401 ± 18	50 ± 10	0	0	0
2o	391 ± 65	249 ± 70	59 ± 24	13 ± 13	1 ± 0.53	0
2p	224 ± 22	190 ± 9.12	0	0	0	0
Hydroxyurea	804 ± 36	463 ± 125	12 ± 3.5	7 ± 0,7	6 ± 1.4	0
Sulfadiazine	487 ± 22	448 ± 9.19	393 ± 30	28 ± 24	0	0

[a] Values are mean ± SD ($n = 3$).

The values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of the compounds with mean zone inhibition (MZI) above or equal to 18 mm are reported in Tables 6 and 7. The results showed that the compounds **2f**, **2l**, and **2p** have less MIC and MBC values against *M. luteus* when compared with the standard drug (chloramphenicol). Similarly, **2c** is more active that rifampicin against *M. tuberculosis*. In addition, the compounds **2f**, **2g**, **2l**, and **2n** showed equal values for MIC and MFC against *Candida* sp. (IMUR 4249) when compared with nistatin.

3. Conclusion

The thiosemicarbazone (**1a**–**p**) and 4-thiazolidinone (**2a**–**p**) derivatives were synthesized and characterized based on their physical, analytical, and spectral data. All the prepared compounds were evaluated in vitro against *T. gondii* intracellular. All of them significantly reduced the percentage of infected cells and mean number of tachyzoites per cell in 2 mM concentration. Various compounds showed best results when compared with hydroxyurea and sulfadiazine. The toxicity of majority compounds was more effective against intracellular parasites, with IC_{50} values ranging from 0.05 to 1 mM.

The 4-thiazolidinones (**2a**–**p**) were evaluated in vitro against bacteria and fungal species. The compounds **2f** (MIC = 40 μg/mL; MBC = 30 μg/mL), **2l** (MIC = 40 μg/mL; MBC = 30 μg/mL), and **2p** (MIC = 35 μg/mL; MBC = 30 μg/mL) were more active than chloramphenicol against *M. luteus*, **2c** (MIC = 70 μg/mL; MBC = 60 μg/mL) was more active than rifampicin against *M. tuberculosis*, and the compounds **2g**, **2l**, and **2n** (MIC = 80 μg/mL; MBC = 40 μg/mL) showed same activity as nistatin against *Candida* sp. (IMUR 4249).

It can be concluded that thiosemicarbazones and 4-thiazolidinones provide interesting leads for anti-*T. gondii* drug discovery. Modifications to improve the potency of these derivatives by diversification of the ring at hydrazone moiety are currently under progress in our laboratory. Further studies involving mechanistic action are necessary to fully understand their anti-*T. gondii* activity. In addition, the antimicrobial results confirm

Table 3. IC_{50} values of **1a–p** and **2a–p** for uninfected cells, infected cells and intracellular parasites in mM

Compound	IC_{50} (mM)[a]		
	Uninfected cells	Infected cells	Intracellular parasites
1a	5 ± 0.05	5 ± 0.05	4 ± 0.05
1b	10 ± 1	7 ± 1	1.5 ± 0.05
1c	6 ± 1	2 ± 0.05	1 ± 0.05
1d	6 ± 1	5 ± 0.05	2 ± 0.05
1e	5 ± 0.05	5 ± 0.05	2 ± 0.05
1f	4 ± 0.05	1.5 ± 0.05	1 ± 0.05
1g	10 ± 1.5	2 ± 0.05	1 ± 0.05
1h	6 ± 0.05	1 ± 0.05	0.1 ± 0.05
1i	6 ± 0.05	2 ± 0.05	1 ± 0.05
1j	10 ± 1	7 ± 0.05	3 ± 0.05
1k	8 ± 1	4 ± 1	3 ± 0.05
1l	5 ± 0.05	2 ± 0.05	1 ± 0.05
1m	6 ± 0.06	2 ± 0.05	1 ± 0.05
1n	5 ± 0.05	2 ± 0.06	2 ± 0.05
1o	10 ± 1	3 ± 1	2 ± 0.05
1p	10 ± 1.5	5 ± 1	4 ± 1
2a	5 ± 0.05	4 ± 0.05	1 ± 0.05
2b	2 ± 0.06	1.5 ± 0.05	0,5 ± 0.05
2c	2 ± 0.05	1.5 ± 0.05	1 ± 0.05
2d	4 ± 0,05	1.5 ± 0,05	1 ± 0,05
2e	9 ± 1	1 ± 0.05	0.5 ± 0.05
2f	2 ± 0.05	2 ± 0.05	1 ± 0.05
2g	2 ± 0.05	0.05	0.05 ± 0.02
2h	0.1 ± 0.005	3 ± 0.05	1 ± 0.05
2i	1 ± 0.05	0.05	0.05 ± 0.03
2j	10 ± 0.05	3 ± 0.05	2 ± 0.05
2k	1 ± 0.05	0.1 ± 0.05	0.05 ± 0.02
2l	5 ± 1	3 ± 0.05	0.1 ± 0.05
2m	6 ± 0.05	0.5 ± 0.05	0.5 ± 0.05
2n	5 ± 0.06	2 ± 0.05	0.5 ± 0.05
2o	2 ± 0.05	1 ± 0.05	0.5 ± 0.05
2p	1 ± 0.006	1 ± 0.05	1 ± 0.05
Hydroxyurea	1 ± 0.05	0.5 ± 0.05	0.1 ± 0.05
Sulfadiazine	8 ± 0.05	3 ± 0.05	3 ± 0.05

[a] Values are mean ± SD (n = 3).

the fact that the 4-thiazolidinone ring has great biological potential.

4. Experimental

The melting points were determined on BÜCHI-535 apparatus and are uncorrected. IR spectra were measured on BRUKER IFS-66 IR spectrophotometer. ^{1}H NMR were recorded on UNITY PLUS-300 MHz-VARIAN spectrometer by using tetramethylsilane as internal standard. The chemical shifts are reported in δ units, and coupling constants (J) are reported in hertz. TLC development was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck).

4.1. Representative procedure for (1a–q). Benzaldehyde 4-phenyl-3-thiosemicarbazone (1a)

To a solution of 0.0119 mol of 4-phenylthiosemicarbazide in 11 mL of EtOH and 22 mL of water were added 0.0125 mol of benzaldehyde and 0.55 mL of acetic acid. The mixture was stirred under reflux for another 1 h and cooled to ambient temp. After, the precipitate was collected with filter under vacuum and washed with water. White crystals; yield 91%; mp 193–195 °C; IR (KBr): 3297 and 3158 (NH), 1540 (C=N), 1442 (N–CS–N), 1265 and 1198 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.47 (s, 1H, NH), 9.22 (s, 1H, NH–Ar), 8.00 (s, 1H, CH=N), 7.65–7.68 (m, 4H, Ar–H), 7.40–7.45 (m, 5H, Ar–H), 7.29–7.30 (m, 1H, Ar–H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.8 (C=S), 142.8 (CH=N), 137.7 (Cq Ar), 132.9 (Cq Ar), 130.7 (CH Ar), 128.9 (CH Ar), 128.8 (CH Ar), 127.4 (CH Ar), 126.2 (CH Ar), 124.5 (CH Ar).

4.1.1. 4-Chlorobenzaldehyde 4-phenyl-3-thiosemicarbazone (1b). White crystals; yield 92%; mp 199–201 °C; IR (KBr): 3305 and 3134 (NH), 1537 (C=N), 1445 (N–CS–N), 1265 and 1194 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.21 (s, 1H, NH), 9.16 (s, 1H, NH–Ar), 7.92 (s, 1H, CH=N), 7.60–7.66 (m, 4H, Ar–H), 7.38–7.45 (m, 4H, Ar–H), 7.26–7.30 (m, 1H, Ar–H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.7 (C=S), 141.6 (CH=N), 137.5 (Cq Ar), 136.6 (Cq Ar), 131.4 (Cq Ar), 129.2 (CH Ar), 128.8 (CH Ar), 128.5 (CH Ar), 126.4 (CH Ar), 124.7 (CH Ar).

4.1.2. 2,4-Dichlorobenzaldehyde 4-phenyl-3-thiosemicarbazone (1c). White crystals; yield 91%; mp 186–187 °C; IR (KBr): 3251 and 3142 (NH), 1537 (C=N), 1439 (N–CS–N), 1262 and 1199 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.33 (s, 1H, NH), 9.15 (s, 1H, NH–Ar), 8.32 (s, 1H, CH=N), 7.87 (d, 1H, J = 8.4 Hz, Ar–H), 7.65 (dd, 1H, J = 8.4, 1.5 Hz, Ar–H), 7.66 (s, 1H, Ar–H), 7.38–7.43 (m, 3H, Ar–H), 7.23–7.30 (m, 2H, Ar–H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.8 (C=S), 138.5 (CH=N), 137.5 (Cq Ar), 136.7 (Cq Ar), 135.1 (Cq Ar), 129.9 (CH Ar), 129.2 (Cq Ar), 128.8 (CH Ar), 127.8 (CH Ar), 127.6 (CH Ar), 126.3 (CH Ar), 124.4 (CH Ar).

4.1.3. 3,4-Dichlorobenzaldehyde 4-phenyl-3-thiosemicarbazone (1d). White crystals; yield 86%; mp 206–208 °C; IR (KBr): 3310 and 3134 (NH), 1545 (C=N), 1466 (N–CS–N), 1268 and 1191 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.95 (s, 1H, NH), 10.25 (s, 1H, NH–Ar), 8.34 (d, 1H, J = 1.8 Hz, Ar–H), 8.11 (s, 1H, CH=N), 7.81 (dd, 1H, J = 8.4, 1.8 Hz, Ar–H), 7.67 (d, 1H, J = 8.4 Hz, Ar–H), 7.20-7.53 (m, 5H, Ar–H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.6 (C=S), 142.0 (CH=N), 133.3 (Cq Ar), 132.7 (Cq Ar), 131.9 (Cq Ar), 131.4 (Cq Ar), 130.8 (CH Ar), 129.2 (CH Ar), 129.1 (CH Ar), 127.9 (CH Ar), 125.5 (CH Ar), 125.3 (CH Ar).

4.1.4. 3-Methylbenzaldehyde 4-phenyl-3-thiosemicarbazone (1e). White crystals; yield 89.5%; mp 165–166 °C; IR (KBr): 3297 and 3147 (NH), 1540 (C=N), 1444 (N–CS–N), 1265 and 1202 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.05 (s, 1H, NH), 9.21 (s, 1H, NH–Ar), 7.91 (s, 1H, CH=N), 7.67 (d, 2H, J = 8.1 Hz, Ar–H), 7.40-7.49 (m, 4H, Ar–H), 7.23-7.34 (m, 3H, Ar–H), 2.39 (s, 3H, CH_3), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.6 (C=S), 143.4 (CH=N), 138.6 (Cq Ar), 137.7 (Cq Ar), 132.9 (Cq Ar), 131.5 (CH Ar),

Table 4. Inhibitory zone diameters (mm) of **2a**–**p** against tested bacterial strains by disc diffusion method

Compound	Mean zone inhibition (MZI) in mm[a]								
	Sa	Bs	Ml	Ec	Kp	Sf	Mp	Ms	Mt
2a	—	15	20	—	—	12	07	09	14
2b	—	13	21	—	—	15	12	15	17
2c	10	17	20	—	—	15	12	16	22
2d	11	16	19	—	—	11	12	13	18
2e	—	—	22	—	—	11	—	—	—
2f	—	15	25	—	—	16	07	13	14
2g	—	14	15	—	—	16	07	12	14
2h	—	—	—	—	—	—	—	—	—
2i	—	—	10	—	—	—	—	—	—
2j	—	—	16	—	—	12	—	—	—
2k	—	—	16	—	—	12	—	—	—
2l	14	09	22	—	—	11	—	—	—
2m	—	11	16	—	—	—	—	—	—
2n	—	—	15	—	—	—	—	—	—
2o	—	19	25	—	—	—	—	—	—
2p	26	19	22	—	—	18	16	26	23
Rifampicin	31	20	49	14	NT	20	25	30	28
Chloramphenicol	25	27	50	27	25	NT	NT	NT	NT

Rifampicin (100 μg/disc) and chloramphenicol (100 μg/disc) were used as positive reference; compounds **2a**–**p** (300 μg/disc).
—, indicates no sensitivity or MZI lower that 7 mm.
NT, not tested.
Sa, *S. aureus*; Bs, *B. subtilis*; Ml, *M. luteus*; Ec, *E. coli*; Kp, *K. pneumoniae*; Sf, *S. faecalis*; Mp, *M. phlei*; Ms, *M. smegmatis*; Mt, *M. tuberculosis*.
[a] Values are mean ($n = 3$).

128.7 (CH Ar), 128.7 (CH Ar), 127.9 (CH Ar), 126.2 (CH Ar), 124.7 (CH Ar), 21.2 (CH_3).

4.1.5. 2-Fluorobenzaldehyde 4-phenyl-3-thiosemicarbazone (1f). White crystals; yield 93%; mp 181–182 °C; IR (KBr): 3297 and 3165 (NH), 1535 (C=N), 1442 (N–CS–N), 1263 and 1198 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.40 (s, 1H, NH), 9.22 (s, 1H, NH–Ar), 8.19 (s, 1H, CH=N), 7.85 (t, 1H, $J = 7.2$ Hz, Ar–H), 7.66 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.38 (t, 3H, $J = 7.8$ Hz, Ar–H), 7.26 (t, 3H, $J = 7.2$ Hz, Ar–H), 7.19 (t, 3H, $J = 7.2$ Hz, Ar–H), 7.14 (t, 3H, $J = 9.3$ Hz, Ar–H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.6 (C=S), 160.5 (Cq Ar), 136.1 (Cq Ar), 132.3 (CH Ar), 132.1 (CH Ar), 128.8 (CH Ar), 126.8 (Cq Ar), 126.2 (CH Ar), 124.5 (CH Ar), 124.4 (CH Ar), 124.3 (Cq Ar), 116.2 (CH Ar).

4.1.6. 4-Fluorobenzaldehyde 4-phenyl-3-thiosemicarbazone (1g). White crystals; yield 95%; mp 178 °C; IR (KBr): 3313 and 3134 (NH), 1548 (C=N), 1447 (N–CS–N), 1230 and 1198 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.38 (s, 1H, NH), 9.16 (s, 1H, NH–Ar), 7.96 (s, 1H, CH=N), 7.63–7.70 (m, 4H, Ar–H), 7.39–7.45 (m, 2H, Ar–H), 7.25–7.30 (m, 1H, Ar–H), 7.08–7.14 (t, 2H, $J = 7.8$ Hz, Ar–H), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.6 (C=S), 165.8 (Cq Ar), 162.4 (Cq Ar), 142.0 (CH=N), 137.6 (Cq Ar), 129.3 (CH Ar), 129.2 (CH Ar), 128.8 (CH Ar), 126.3 (Cq Ar), 124.7 (CH Ar), 116.1 (CH Ar).

4.1.7. 4-Hydroxy-3-methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (1h). White crystals; yield 88.5%; mp 176–177 °C; IR (KBr): 3325 and 3166 (NH), 1551 (C=N), 1447 (N–CS–N), 1268 and 1199 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.69 (s, 1H, NH), 9.98 (s, 1H, NH–Ar), 9.54 (s, 1H, OH), 8.05 (s, 1H, CH=N), 7.55 (t, 3H, $J = 8.4$ Hz, Ar–H), 7.37 (t, 2H, $J = 7.5$ Hz, Ar–H), 7.20 (t, 2H, $J = 8.4$ Hz, Ar–H), 6.81 (d, 1H, $J = 7.5$ Hz, Ar–H), 3.84 (s, 3H, OCH_3), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 175.5 (C=S), 148.3 (Cq Ar), 146.9 (Cq Ar), 143.4 (CH=N), 137.8 (Cq Ar), 128.8 (CH Ar), 126.3 (Cq Ar), 125.3 (CH Ar), 124.8 (CH Ar), 122.8 (CH Ar), 114.6 (CH Ar), 108.2 (CH Ar), 56.1 (OCH_3).

4.1.8. 4-Dimethylaminobenzaldehyde 4-phenyl-3-thiosemicarbazone (1i). Light yellow crystals; yield 81.5%; mp 208–210 °C; IR (KBr): 3325 and 3298 (NH), 1594

Table 5. Inhibitory zone diameters (mm) of **2a**–**p** against tested fungal strains by disc diffusion method

Compound	Mean zone inhibition (MZI) in mm[a]				
	S. cerevisiae	*Candida* sp. (IMUR 1224)	*Candida* sp. (IMUR 720)	*C. albicans*	*Candida* sp. (IMUR 4249)
2a	—	—	—	08	—
2b	—	—	—	08	20
2c	—	—	—	07	14
2d	—	—	—	07	21
2e	—	—	—	09	—
2f	—	—	—	07	20
2g	—	—	—	08	22
2i	—	—	—	—	16
2j	—	—	—	—	11
2l	—	—	—	—	18
2m	—	—	—	15	16
2n	12	—	—	13	20
2o	—	—	—	10	15
2p	—	—	—	09	—
Nistatin	20	30	20	26	32

Nistatin (100 μg/disc) was used as positive reference; compounds **2a**–**p** (300 μg/disc).
—, indicates no sensitivity or MZI lower that 7 mm.
[a] Values are mean ($n = 3$).

Table 6. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in μg/mL of selected compounds

Organism	Compound	MIC[a] (μg/mL)	MBC[b] (μg/mL)
M. luteus	**2a**	100	90
	2b	90	80
	2c	60	50
	2d	60	50
	2e	60	55
	2f	40	30
	2l	40	30
	2o	110	100
	2p	35	30
	Chloramphenicol	50	40
S. faecalis	**2p**	200	190
	Chloramphenicol	70	60
S. aureus	**2p**	50	45
	Chloramphenicol	25	20
M. smegmatis	**2p**	150	140
	Rifampicin	140	130
M. tuberculosis	**2c**	70	60
	2d	100	90
	2p	110	100
	Rifampicin	110	100

[a] MIC, minimum inhibitory concentration (the lowest concentration that inhibited the bacterial growth).
[b] MBC, minimum bactericidal concentration (the lowest concentration at which no bacterial growth was observed).

Table 7. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) in μg/mL of selected compounds

Compound	MIC[a](μg/mL)	MFC[b] (μg/mL)
2b	150	140
2d	120	100
2f	80	70
2g	80	70
2l	80	70
2n	80	70
Nistatin	80	70

Microorganism: *Candida* sp. (IMUR 4249).
[a] MIC, minimum inhibitory concentration (the lowest concentration that inhibited the fungal growth).
[b] MFC, minimum fungicidal concentration (the lowest concentration at which no fungal growth was observed).

(C═N), 1442 (N–CS–N), 1257 and 1182 (C═S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.59 (s, 1H, NH), 9.92 (s, 1H, NH–Ar), 8.03 (s, 1H, CH═N), 7.70 (d, 2H, J = 8.7 Hz, Ar–H), 7.59 (d, 2H, J = 7.5 Hz, Ar–H), 7.35 (t, 2H, J = 7.8 Hz, Ar–H), 7.18 (t, 1H, J = 7.5 Hz, Ar–H), 7.72 (d, 2H, J = 8.7 Hz, Ar–H), 2.97 (s, 6H, $N(CH_3)_2$), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 174.8 (C═S), 151.5 (Cq Ar), 143.9 (CH═N), 139.2 (Cq Ar), 129.0 (CH Ar), 127.9 (CH Ar), 125.5 (CH Ar), 125.0 (CH Ar), 121.1 (Cq Ar), 111.6 (CH Ar).

4.1.9. 3-Chlorobenzaldehyde 4-phenyl-3-thiosemicarbazone (1j). White crystals; yield 96%; mp 194–195 °C; IR (KBr): 3297 and 3139 (NH), 1551 (C═N), 1442 (N–CS–N), 1265 and 1194 (C═S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.93 (s, 1H, NH), 10.25 (s, 1H, NH–Ar), 8.18 (br s, 1H, Ar–H), 8.13 (s, 1H, CH═N), 7.74 (m, 1H, Ar–H), 7.52 (m, 2H, Ar–H), 7.40 (m, 4H, Ar–H), 7.22 (m, 1H, Ar–H), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 176.3 (C═S), 141.7 (CH═N), 139.0 (Cq Ar), 136.3 (Cq Ar), 133.7 (Cq Ar), 130.4 (CH Ar), 129.6 (CH Ar), 128.0 (CH Ar), 127.0 (CH Ar), 126.4 (CH Ar), 126.1 (CH Ar), 125.5 (CH Ar).

4.1.10. 4-Methylbenzaldehyde 4-phenyl-3-thiosemicarbazone (1k). White crystals; yield 77.5%; mp 189–190 °C; IR (KBr): 3297 and 3142 (NH), 1548 (C═N), 1439 (N–CS–N), 1260 and 1198 (C═S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.19 (s, 1H, NH), 9.21 (s, 1H, NH–Ar), 7.93 (s, 1H, CH═N), 7.66 (dt, 2H, J = 1.2, 8.1 Hz, Ar–H), 7.57 (d, 2H, J = 8.1 Hz, Ar–H), 7.42 (dt, 2H, J = 1.5, 6.6 Hz, Ar–H), 7.27 (dt, 2H, J = 1.2, 8.1 Hz, Ar–H), 7.22 (d, 1H, J = 8.1 Hz, Ar–H), 2.39 (s, 3H, CH_3), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.6 (C═S), 143.2 (CH═N), 141.2 (Cq Ar), 137.7 (Cq Ar), 130.2 (Cq Ar), 129.6 (CH Ar), 128.7 (CH Ar), 127.4 (CH Ar), 126.2 (CH Ar), 124.6 (CH Ar).

4.1.11. 3-Methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (1l). White crystals; yield 94%; mp 154 °C; IR (KBr): 3325 and 3155 (NH), 1545 (C═N), 1442 (N–CS–N), 1276 and 1194 (C═S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.75 (s, 1H, NH), 9.21 (s, 1H, NH–Ar), 7.98 (s, 1H, CH═N), 7.65 (d, 2H, J = 8.4 Hz, Ar–H), 7.42 (t, 2H, J = 7.8 Hz, Ar–H), 7.20-7.35 (m, 4H, Ar–H), 6.97 (dt, 1H, J = 1.2, 8.1 Hz, Ar–H), 3.84 (s, 3H, OCH_3), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.7 (C═S), 159.8 (Cq Ar), 143.0 (CH═N), 137.6 (Cq Ar), 134.3 (Cq Ar), 129.9 (CH Ar), 128.8 (CH Ar), 126.3 (CH Ar), 124.7 (CH Ar), 120.4 (CH Ar), 116.6 (CH Ar), 111.9 (CH Ar), 55.3 (OCH_3).

4.1.12. 4-Methoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (1m). White crystals; yield 94%; mp 178–179 °C; IR (KBr): 3325 and 3145 (NH), 1543 (C═N), 1447 (N–CS–N), 1249 and 1198 (C═S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.89 (s, 1H, NH), 9.18 (s, 1H, NH–Ar), 7.87 (s, 1H, CH═N), 7.64 (dt, 4H, J = 2.1, 9 Hz, Ar–H), 7.41 (dt, 2H, J = 2.1, 7.2 Hz, Ar–H), 7.25 (dt, 1H, J = 1.5, 7.2 Hz, Ar–H), 6.93 (dt, 2H, J = 1.8, 7.8 Hz, Ar–H), 3.85 (s, 3H, OCH_3), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.4 (C═S), 161.7 (Cq Ar), 142.9 (CH═N), 137.8 (Cq Ar), 129.1 (CH Ar), 128.7 (CH Ar), 126.1 (CH Ar), 125.5 (Cq Ar), 124.5 (CH Ar), 114.3 (CH Ar), 55.4 (OCH_3).

4.1.13. 2,4-Dimethoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (1n). Light yellow crystals; yield 93.5%; mp 201–202 °C; IR (KBr): 3309 and 3194 (NH), 1543 (C═N), 1453 (N–CS–N), 1284 and 1206 (C═S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.71 (s, 1H, NH), 10.00 (s, 1H, NH–Ar), 8.43 (s, 1H, CH═N), 8.19 (d, 1H, J = 8.7 Hz, Ar–H), 7.57 (d, 2H, J = 7.2 Hz, Ar–H), 7.36 (t, 2H, J = 8.1 Hz, Ar–H), 7.19 (t, 1H, J = 7.2 Hz, Ar–H), 6.62 (d, 1H, J = 1.8 Hz, Ar–H), 6.58 (d, 1H, J = 8.7 Hz, Ar–H), 3.82 (s, 6H, OCH_3), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 175.3 (C═S), 162.5 (Cq Ar), 159.3 (Cq Ar), 139.1 (CH═N), 138.6 (Cq Ar), 127.9 (CH Ar), 127.7 (CH Ar), 125.7 (CH

Ar), 125.1 (CH Ar), 114.7 (Cq Ar), 106.3 (CH Ar), 97.8 (CH Ar), 55.7 (OCH_3), 55.4 (OCH_3).

4.1.14. 3,4,5-Trimethoxybenzaldehyde 4-phenyl-3-thiosemicarbazone (1o). Light yellow crystals; yield 91%; mp 161–162 °C; IR (KBr): 3299 and 3177 (NH), 1556 (C=N), 1417 (N–CS–N), 1262 and 1191 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.31 (s, 1H, NH), 9.13 (s, 1H, NH–Ar), 7.88 (s, 1H, CH=N), 7.63 (d, 2H, J = 8.1 Hz, Ar–H), 7.43 (t, 2H, J = 8.1 Hz, Ar–H), 7.28 (t, 1H, J = 7.5 Hz, Ar–H), 6.88 (s, 2H, Ar–H), 3.92 (s, 9H, OCH_3), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.4 (C=S), 153.4 (Cq Ar), 143.6 (CH=N), 140.2 (Cq Ar), 137.6 (Cq Ar), 128.7 (CH Ar), 128.3 (Cq Ar), 126.4 (CH Ar), 125.1 (CH Ar), 104.5 (CH Ar), 60.8 (OCH_3), 56.1 (OCH_3).

4.1.15. 3,5-Bis(1,1-dimethylethyl)4-hydroxybenzaldehyde-4-phenyl-3-thiosemicarbazone (1p). Yellow crystals; yield 100%; mp 204–205 °C; IR (KBr): 3317 and 3137 (NH), 1535 (C=N), 1439 (N–CS–N), 1268 and 1201 (C=S); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.68 (s, 1H, NH), 9.17 (s, 1H, NH–Ar), 7.84 (s, 1H, CH=N), 7.67 (dd, 2H, J = 1.5, 8.4 Hz, Ar–H), 7.48 (s, 2H, Ar–H), 7.42 (dt, 2H, J = 1.5, 7.5 Hz, Ar–H), 7.26 (dt, 1H, J = 1.2, 7.8 Hz, Ar–H), 5.56 (s, 1H, OH), 1.45 (s, 18H, CH_3), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 175.2 (C=S), 156.5 (Cq Ar), 144.5 (CH=N), 137.9 (Cq Ar), 136.5 (Cq Ar), 128.8 (CH Ar), 126.1 (CH Ar), 124.7 (CH Ar), 124.5 (CH Ar), 124.2 (Cq Ar), 34.3 (Cq $C(CH_3)_3$), 30.1 (CH_3).

4.2. Representative procedure for (2a–q). 2-[(Phenylmethylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2a)

A solution of 0.0078 mol of benzaldehyde 4-phenyl-3-thiosemicarbazone and 0.0352 mol of maleic anhydride in 50 mL of dried toluene was stirred until reflux, and 2 mL of DMF was added until complete solubilization. The mixture was stirred under the same conditions till the completion of the reaction (5–9 h). After, the solvent was evaporated at reduced pressure and the crude product was extracted with ethyl acetate twice. The organic layer was treated with anhydrous sodium sulfate and evaporated again. Finally the product was purified by recrystallization from MeOH/water. White crystals; yield 76%; mp 212–214 °C; IR (KBr): 1707 (C=O), 1621 (NC=O), 1582 and 1555 (C=N), 1344 (NCS), 1252 (N–N=C), 1030 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.83 (br s, 1H, CO_2H), 8.33 (s, 1H, CH=N), 7,74 (dd, 2H, J = 2.1, 6.6 Hz, Ar–H), 7.38–7.56 (m, 8H, Ar–H), 4.57 (t, 1H, J = 6.0 Hz, CH), 3.13 (d, 2H, J = 6.0 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.8 (C=O), 164.8 (C=N), 157.7 (CH=N), 135.1 (Cq Ar), 134.0 (Cq Ar), 130.8 (CH Ar), 129.0 (CH Ar), 128.8 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 127.7 (CH Ar), 42.5 (CH), 36.7 (CH_2).

4.2.1. 2-[[(4-Chlorophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2b). White crystals; yield 56.5%; mp 245–247 °C; IR (KBr): 1721 (C=O), 1623 (NC=O), 1575 and 1548 (C=N), 1341 (NCS), 1248 (N–N=C), 1034 (CS); ^{1}H NMR (75.4 MHz, DMSO-d_6): δ 12.84 (br s, 1H, CO_2H), 8.34 (s, 1H, CH=N), 7.74–7.77 (m, 2H, Ar–H), 7.38-7.56 (m, 7H, Ar–H), 4.57 (t, 1H, J = 5.7 Hz, CH), 3.13 (d, 2H, J = 5.7 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.8 (C=O), 165.4 (C=N), 154.8 (CH=N), 135.3 (Cq Ar), 135.1 (Cq Ar), 132.9 (Cq Ar), 129.3 (CH Ar), 129.1 (CH Ar), 128.9 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 42.5 (CH), 36.7 (CH_2).

4.2.2. 2-[[(2,4-Dichlorophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2c). White crystals; yield 63%; mp 236–237 °C; IR (KBr): 1724 (C=O), 1614 (NC=O), 1565 and 1538 (C=N), 1337 (NCS), 1244 (N–N=C), 1037 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.85 (br s, 1H, CO_2H), 8.43 (s, 1H, CH=N), 7.98 (d, 1H, J = 8.7 Hz, Ar–H), 7.72 (d, 1H, J = 2.1 Hz, Ar–H), 7.38–7.57 (m, 6H, Ar–H), 4,59 (t, 1H, J = 5.8 Hz, CH), 3.13 (d, 2H, J = 5.8 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.8 (CO_2H), 171.8 (C=O), 167.0 (C=N), 152.3 (CH=N), 135.9 (Cq Ar), 135.9 (Cq Ar), 134.4 (Cq Ar), 130.1 (Cq Ar), 129.5 (CH Ar), 129.1 (CH Ar), 128.8 (CH Ar), 128.1 CH Ar), 42.6 (CH), 36.6 (CH_2).

4.2.3. 2-[[(3,4-Dichlorophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2d). White crystals; yield 45.5%; mp 254–256 °C; IR (KBr): 1730 (C=O), 1611 (NC=O), 1569 and 1536 (C=N), 1340 (NCS), 1241 (N–N=C), 1033 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.82 (br s, 1H, CO_2H), 8.34 (s, 1H, CH=N), 7,92 (s, 1H, Ar–H), 7.72 (s, 2H, Ar–H), 7.38-7.56 (m, 5H, Ar–H), 4.59 (t, 1H, J = 6.0 Hz, CH), 3.14 (d, 2H, J = 6.0 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.8 (CO_2H), 171.8 (C=O), 166.2 (C=N), 155.4 (CH=N), 135.1 (Cq Ar), 134.8 (Cq Ar), 133.0 (Cq Ar), 131.7 (Cq Ar), 131.2 (CH Ar), 129.2 (CH Ar), 129.1 (CH Ar), 128.8 (CH Ar), 128.1 (CH Ar), 127.2 (CH Ar), 42.6 (CH), 36.6 (CH_2).

4.2.4. 2-[[(3-Methylphenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2e). White crystals; yield 67%; mp 221–222 °C; IR (KBr): 1724 (C=O), 1615 (NC=O), 1582 and 1546 (C=N), 1351 (NCS), 1232(N–N=C), 1034 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.83 (br s, 1H, CO_2H), 8.28 (s, 1H, CH=N), 7.25–7.54 (m, 9H, Ar–H), 4.57 (t, 1H, J = 6 Hz, CH), 3.13 (d, 2H, J = 6 Hz, CH_2), 2.33 (s, 3H, CH_3), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.8 (C=O), 164.7 (C=N), 157.8 (CH=N), 138.0 (Cq Ar), 135.2 (Cq Ar), 134.0 (Cq Ar), 131.5 (CH Ar), 129.1 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 127.9 (CH Ar), 125.2 (CH Ar), 42.5 (CH), 36.7 (CH_2), 20.9 (CH_3).

4.2.5. 2-[[(2-Fluorophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2f). Light yellow crystals; yield 60%; mp 217 °C; IR (KBr): 1721 (C=O), 1621 (NC=O), 1582 and 1548 (C=N), 1344 (NCS), 1232 (N–N=C), 1034 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.85 (br s, 1H, CO_2H), 8.37 (s, 1H, CH=N), 7.90 (dt, 1H, J = 1.5, 8.4 Hz, Ar–H), 7.43-

7.56 (m, 4H, Ar–H), 7.38–7.41 (m, 2H, Ar–H), 7.24-7.31 (m, 2H, Ar–H), 4.58 (t, 1H, J = 6.0 Hz, CH), 3.13 (d, 2H, J = 6.0 Hz, CH_2). ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.8 (CO_2H), 171.8 (C=O), 166.1 (C=N), 162.6 (Cq Ar), 159.2 (CH=N), 150.6 (CH Ar), 135.1 (Cq Ar), 132.9 (CH Ar), 129.1 (CH Ar), 128.8 (CH Ar), 128.1 (CH Ar), 127.4 (CH Ar), 124.9 (CH Ar), 121.4 (Cq Ar), 42.6 (CH), 36.7 (CH_2).

4.2.6. 2-[[(4-Fluorophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2g). White crystals; yield 56%; mp 227–229 °C; IR (KBr): 1731 (C=O), 1621 (NC=O), 1592 and 1550 (C=N), 1347 (NCS), 1226 (N–N=C), 1040 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.83 (br s, 1H, CO_2H), 8.34 (s, 1H, CH=N), 7.80 (dd, 2H, J = 8.7, 5.7 Hz, Ar–H), 7.38-7.56 (m, 5H, Ar–H), 7.29 (t, 2H, J = 8.7 Hz, Ar–H), 4.57 (t, 1H, J = 6.0 Hz, CH), 3.13 (d, 2H, J = 6.0 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.8 (C=O), 165.2 (Cq Ar), 164.9 (C=N), 161.9 (Cq Ar), 156.6 (CH=N), 135.2 (Cq Ar), 130.7 (Cq Ar), 130.0 (CH Ar), 129.1 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 42.5 (CH), 36.7 (CH_2).

4.2.7. 2-[[(4-Hydroxy-3-methoxyphenyl)methylene]-hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2h). Beige crystals; yield 44.5%; mp 219–221 °C; IR (KBr): 1724 (C=O), 1621 (NC=O), 1592 and 1562 (C=N), 1347 (NCS), 1249 (N–N=C), 1030 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.82 (br s, 1H, CO_2H), 9.67 (s, 1H, Ar–OH), 8.18 (s, 1H, CH=N), 7.36–7.55 (m, 5H, Ar–H), 7.31 (d, 1H, J = 1.8 Hz, Ar–H), 7.16 (dd, 1H, J = 1.8, 8.1 Hz, Ar–H), 6.83 (d, 1H, J = 8.1 Hz, Ar–H), 4.55 (t, 1H, J = 5.7, CH), 3.79 (s, 3H, OCH_3), 3.13 (d, 2H, J = 5.7, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.8 (C=O), 162.9 (C=N), 157.9 (CH=N), 149.6 (Cq Ar), 147.8 (Cq Ar), 135.3 (Cq Ar), 129.1 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 125.5 (Cq Ar), 122.6 (CH Ar), 115.5 (CH Ar), 110.2 (CH Ar), 55.5 (OCH_3), 42.4 (CH), 36.8 (CH_2).

4.2.8. 2-[[(4-Dimethylaminophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2i). Dark red crystals; yield 36%; mp 230–231 °C; IR (KBr): 1724 (C=O), 1606 (NC=O), 1529 (C=N), 1360 (NCS), 1239 (N–N=C), 1030 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.82 (br s, 1H, CO_2H), 8.13 (s, 1H, CH=N), 7.36–7.56 (m, 7H, Ar–H), 6.71 (d, 2H, J = 8.7 Hz, Ar–H), 4.53 (t, 1H, J = 5.7 Hz, CH), 3.11 (d, 2H, J = 5.7 Hz, CH_2), 2.95 (s, 6H, $N(CH_3)_2$), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.6 (CO_2H), 171.8 (C=O), 161.6 (C=N), 157.9 (CH=N), 151.9 (Cq Ar), 135.3 (Cq Ar), 129.2 (CH Ar), 129.0 (CH Ar), 128.6 (CH Ar), 128.2 (CH Ar), 121.2 (Cq Ar), 111.6 (CH Ar), 42.4 (CH), 39.7 ($N(CH_3)_2$), 36.9 (CH_2).

4.2.9. 2-[[(3-Chlorophenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2j). Beige crystals; yield 58%; mp 237–239 °C; IR (KBr): 1711 (C=O), 1621 (NC=O), 1573 (C=N), 1341 (NCS), 1258 (N–N=C), 1040 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.84 (br s, 1H, CO_2H), 8.33 (s, 1H, CH=N), 7.69–7.75 (m, 2H, Ar–H), 7.37–7.55 (m, 7H, Ar–H), 4.58 (t, 1H, J = 5.4 Hz, CH), 3.13 (d, 2H, J = 5.4 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.8 (CO_2H), 171.8 (C=O), 168.4 (CH=N), 165.9 (C=N), 136.2 (Cq Ar), 135.1 (Cq Ar), 133.6 (Cq Ar), 130.8 (CH Ar), 130.4 (CH Ar), 129.1 (CH Ar), 128.8 (CH Ar), 128.2 (CH Ar), 127.0 (CH Ar), 126.2 (CH Ar), 42.5 (CH), 36.7 (CH_2).

4.2.10. 2-[[(4-Methylphenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2k). White crystals; yield 54%; mp 224–226 °C; IR (KBr): 1727 (C=O), 1615 (NC=O), 1573 and 1556 (C=N), 1347 (NCS), 1236 (N–N=C), 1034 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.84 (br s, 1H, CO_2H), 8.27 (s, 1H, CH=N), 7.62 (d, 2H, J = 8.4 Hz, Ar–H), 7.36-7.55 (m, 5H, Ar–H), 7.25 (d, 2H, J = 8.4 Hz, Ar–H), 4.55 (t, 1H, J = 6 Hz, CH), 3.11 (d, 2H, J = 6 Hz, CH_2), 2.33 (s, 3H, CH_3), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.8 (C=O), 164.3 (C=N), 157.7 (CH=N), 140.8 (Cq Ar), 135.2 (Cq Ar), 131.3 (Cq Ar), 129.4 (CH Ar), 129.0 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 127.7 (CH Ar), 42.5 (CH), 36.8 (CH_2), 21.1 (CH_3).

4.2.11. 2-[[(3-Methoxyphenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2l). White crystals; yield 57%; mp 235–237 °C; IR (KBr): 1727 (C=O), 1615 (NC=O), 1582 and 1559 (C=N), 1341 (NCS), 1241 (N–N=C), 1030 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.76 (br s, 1H, CO_2H), 8.28 (s, 1H, CH=N), 7.28–7.55 (m, 8H, Ar–H), 7.01-7.04 (m, 1H, Ar–H), 4.56 (t, 1H, J = 5.7 Hz, CH), 3.78 (s, 3H, OCH_3), 3.13 (d, 2H, J = 5.7 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.6 (CO_2H), 171.5 (C=O), 164.6 (C=N), 159.4 (Cq Ar),157.5 (CH=N), 135.3 (Cq Ar), 135.1 (Cq Ar), 129.8 (CH Ar), 128.9 (CH Ar), 128.6 (CH Ar), 128.0 (CH Ar), 120.2 (CH Ar), 116.4 (CH Ar), 112.6 (CH Ar), 55.0 (OCH_3), 42.4 (CH), 36.7 (CH_2).

4.2.12. 2-[[(4-Methoxyphenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2m). White crystals; yield 55.5%; mp 211–213 °C; IR (KBr): 1711 (C=O), 1615 (NC=O), 1579 and 1552 (C=N), 1344 (NCS), 1245 (N–N=C), 1024 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.74 (br s, 1H, CO_2H), 8.24 (s, 1H, CH=N), 7.68 (d, 2H, J = 8.4 Hz, Ar–H), 7.37-7.55 (m, 5H, Ar–H), 7.00 (d, 2H, J = 8.4 Hz, Ar–H), 4.55 (t, 1H, J = 5.4 Hz, CH), 3.80 (s, 3H, OCH_3), 3.10 (d, 2H, J = 5.4 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.5 (CO_2H), 171.5 (C=O), 163.2 (C=N), 161.3 (Cq Ar),157.2 (CH=N), 135.1 (Cq Ar), 129.3 (CH Ar), 128.9 (CH Ar), 128.5 (CH Ar), 128.0 (CH Ar), 126.6 (Cq Ar), 114.2 (CH Ar), 55.2 (OCH_3), 42.3 (CH), 36.7 (CH_2).

4.2.13. 2-[[(2,4-Dimethoxyphenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2n). White crystals; yield 62.5%; mp 202–203 °C; IR (KBr): 1717 (C=O), 1607 (NC=O), 1555 (C=N), 1344 (NCS), 1244 (N–N=C), 1023 (CS); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.73 (br s, 1H, CO_2H), 8.37 (s, 1H, CH=N), 7.79 (d, 1H, J = 8.1 Hz, Ar–H), 7.36-7.55 (m, 5H, Ar–H), 6.61 (m, 2H, Ar–H), 4.54 (t, 1H, J = 6 Hz, CH), 3.81 (s,

3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.10 (d, 2H, J = 6 Hz, CH_2), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.4 (CO_2H), 171.5 (C=O), 163.1 (C=N), 162.7 (Cq Ar), 159.6 (Cq Ar), 152.5 (CH=N), 135.1 (Cq Ar), 128.8 (CH Ar), 128.4 (CH Ar), 128.0 (CH Ar), 127.3 (CH Ar), 114.6 (Cq Ar), 106.6 (CH Ar), 98.1 (CH Ar), 55.6 (OCH_3), 55.4 (OCH_3), 42.3 (CH), 36.8 (CH_2).

4.2.14. 2-[[(3,4,5-Trimethoxyphenyl)methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2o). Light brown crystals; yield 45%; mp 119–120 °C; IR (KBr): 1724 (C=O), 1615(NC=O), 1585 and 1559 (C=N), 1354 (NCS), 1239 (N–N=C); ^1H NMR (300 MHz, $CDCl_3$): δ 12.80 (br s, 1H, CO_2H), 8.18 (s, 1H, CH=N), 7.26–7.54 (m, 5H, Ar–H), 6.94 (s, 2H, Ar–H), 4.42 (dd, 1H, J = 3.6, 8.1 Hz, CH), 3.87 (s, 9H, OCH_3), 3.31 (dd, 1H, J = 3.6, 17.7 Hz, CH_2a), 3.18 (dd, 1H, J = 8.1, 17.7 Hz, CH_2b), ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 173.7 (CO_2H), 171.7 (C=O), 164.1 (C=N), 157.7 (CH=N), 153.1 (Cq Ar), 135.2 (Cq Ar), 129.5 (Cq Ar), 129.0 (CH Ar), 128.7 (CH Ar), 128.2 (CH Ar), 104.9 (CH Ar), 60.1 (OCH_3), 55.8 (OCH_3), 42.5 (CH), 36.6 (CH_2).

4.2.15. 2-[[(3,5-Bis(1,1-dimethylethyl)4-hydroxyphenyl)-methylene]hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acid (2p). White crystals; yield 77%; mp 246–247 °C; IR (KBr): 1730 (C=O), 1615 (NC=O), 1585 (C=N), 1358 (NCS), 1245 (N–N=C), 1030 (CS); ^1H NMR (300 MHz, DMSO-d_6): δ 12.81 (br s, 1H, CO_2H), 8.20 (s, 1H, CH=N), 7.35–7.54 (m, 7H, Ar–H), 4.52 (t, 1H, J = 5.7 Hz, CH), 3.11 (d, 2H, J = 5.7 Hz, CH_2), 1.39 (s, 18H, $C(CH_3)_3$), ^{13}C NMR (75.4 MHz, DMSO-d_6): δ 173.7 (CO_2H), 171.7 (C=O), 162.7 (C=N), 158.5 (CH=N), 156.7 (Cq Ar), 138.9 (Cq Ar), 135.3 (Cq Ar), 129.0 (CH Ar), 128.6 (CH Ar), 128.2 (CH Ar), 125.4 (Cq Ar), 124.7 (CH Ar), 42.4 (CH), 36.7 (CH_2), 34.4 (Cq $C(CH_3)_3$), 30.1 (CH_3).

4.3. Assay for anti-*Toxoplasma gondii* activity

Tachyzoites from the virulent RH strain of *T. gondii* were maintained by intraperitoneal passages in Swiss mice and were collected in Ringer's solution at pH 7.2, 48 h after infection. Animals were used following Experimental Research Ethical International Committees. Vero cells (Kidney fibroblasts from African green monkeys) were incubated with *T. gondii* Tachyzoites (parasite/host cell 5:1 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). After, cells infected with *T. gondii* were incubated with test compounds in the concentrations of 0.1, 2, 5, 8, and 20 mM for 24 h. 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 examination of at least 400 cells.[31,32] Statistical analysis was carried out using the Student's t test. P values <0.05 were considered as significant. Data shown are representative of thirteen in triplicate. Finally, the IC_{50} values for infected cells and intracellular parasites for all compounds were obtained after 24 h exposure in the concentrations ranging of 0.01–30 mM, in triplicate per assay, by a non-linear regression using exclusion test with trypan blue.[18]

4.4. Assay in vitro for antimicrobial activity

Bacteria and fungal species used in the antimicrobial evaluation were obtained from Departamento de Antibióticos and Instituto de Micologia cultures collections, Universidade Federal de Pernambuco, Brazil. Namely, *Staphylococcus aureus* (ATTC 6538), *Bacillus subtilis* (UFPEDA 16), *M. luteus* (ATTC 2225), *Escherichia coli* (ATTC 25922), *K. pneumoniae* (ATTC 29665), *Streptococcus faecalis* (ATTC 6057), *Mycobacterium phlei* (UFPEDA 70), *Mycobacterium smegmatis* (UFPEDA 71), *Mycobacterium tuberculosis* (DAUFPE 82), *Saccharomyces cerevisiae* (UFPEDA 07), *Candida* sp. (IMUR 720), *Candida* sp. (IMUR 1224), *Candida* sp. (IMUR 4249), and *Candida albicans* (UFPEDA 1007) species. The antibacterial and antifungal activities are reported preliminarily utilizing disc diffusion method.[33] In this method, disks 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 tested. Paper discs with only DMSO were used as negative controls. The MZI (Mean zone inhibition) for chloramphenicol and rifampicin (antibacterial), and nistatin (antifungal) was referred to as a reference value (mm). All experiments were carried out three times and repeated if the results differed. All compounds having MZI of more or equal to 18 mm were selected for MIC and MBC or MFC.

For MIC and MBC or MFC assays,[34,35] a stock solution (1 mg/mL) of test compounds was prepared in dimethylsulfoxide solvent. Further, the serial dilution of test compounds was carried out and the concentrations used ranged from 10 to 220 μg/mL. Test compounds at various concentrations were added to culture medium in a test tube and different strains were inoculated at 10^8 bacteria/mL concentration. Tryptic Soy Agar and Nutrient Agar (for antibacterial), and Sabouraud Liquid Medium (for antifungal) were utilized for 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, and nistatin 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. The MBC or MFC values were measured by inoculating the broths used for MIC determinations onto drug-free medium. The MBC or MFC were the first dilution where no growth is observed. The MIC, MBC, and MFC values were expressed in μg/mL.

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