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Original article

Synthesis, antimicrobial and cytotoxic activities of some 5-arylidene-4-thioxo-thiazolidine-2-ones

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ABSTRACT

Several 5-arylidene-4-thioxo-thiazolidine-2-ones (**3a**–**n**) were synthesized and evaluated as antimicrobial agents against representative strains, including multidrug-resistant strains of clinical isolates. Also, the antiproliferative activity was evaluated against two human carcinoma cell lines (NCI-H292 and HEp-2). The compounds containing the 5-arylidene subunit presented greater antimicrobial activities against Gram positive bacteria, including the multidrug-resistant clinical isolates, than the 4-thioxo-thiazolidine-2-one. Important SAR information was also gathered, such as the contribution of thiocarbonyl attached at 4-position on the thiazolidine heterocyclic for antimicrobial properties. None of the derivatives exhibited significant antiproliferative activity against the human carcinoma cell lines.

1. Introduction

Infectious diseases are responsible for great number of deaths in the world population. The reduction of sensibility to antimicrobial agents in current use has been increasing for a great variety of pathogens and the resistance to multiple drugs is common for several microorganisms, especially for Gram positive bacteria. Infection by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) presents a difficult problem for medicine [1–4]. In addition, the treatment of infectious diseases is more complicated in immuno-suppressed patients, such as those infected with the HIV, undergoing anticancer therapy or transplants. Given the evidence for the rapid global spread of resistant clinical isolates and the appearance of drug-resistant strains among community acquired infections, the need for discovery or optimization of antimicrobial agents active against these resistant strains is of paramount importance. In this context, thiazolidines show remarkable antimicrobial activity [5,6], in addition to various biological properties, such as antiproliferative [7,8], antituberculosis [9], antihyperglycemic [10], antiinflammatory [11], among others [12].

Concurring with previous reports in the literature, illustrating various thiazolidinone derivatives, 5-arylidene-4-thioxo-thiazolidine-2-one has a number of structural contributors which enable antimicrobial activity. For example, the arylidene group at 5-position [13,14], the presence of halides in arylidene group [13,15,16] and non-substitution in nitrogen N-3. In one recent work, the alkylation at N-3 has not contributed to improve the antimicrobial properties [17]. Although extensive studies exist regarding the SAR between thiazolidinone analogues and biological activity, very little is known about the contribution of thiocarbonyl and the presence of arylidene subunit attached in 5-position to its antimicrobial activity.

In view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents, thirteen 5-arylidene-4-thioxo-thiazolidine-2-ones (**3a**–**n**) were synthesized and evaluated as antimicrobial agents, including against multidrug-resistant clinical isolates. In addition, the cytotoxicity of these compounds was tested against two human carcinoma cell lines.

2. Results and discussion

2.1. Chemistry

The title compounds were synthesized as outline in Scheme 1. Thiazolidine-2,4-dione (**1**) was prepared using a prior method [18]. Then, 4-thioxo-thiazolidine-2-one (**2**) was obtained using Lawesson's Reagent as an effective thionation reagent [19]. The compounds **3a**–**n** were prepared by a simple and direct method, which involves the Knoevenagel condensation with different

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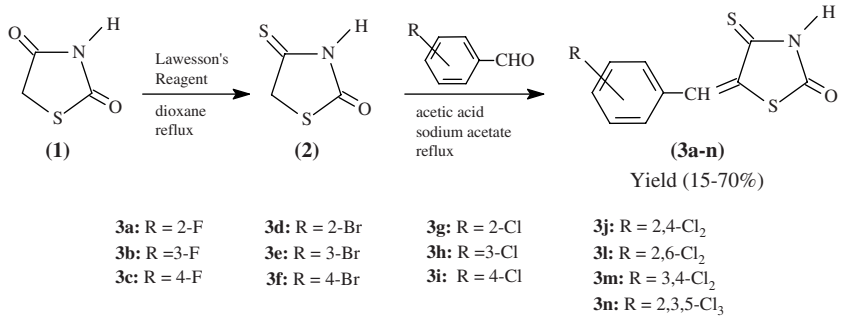

Scheme 1. Synthesis of 5-arylidene-4-thioxo-thiazolidine-2-ones (**3a**–**n**).

aromatic aldehydes [19]. The structures of the desired compounds were determined by ^{1}H NMR, ^{13}C NMR, IR and elemental analyses.

In theory, two geometrical isomers (*E* and *Z*) around the exocyclic double bond (CH═C) are possible for the above 5-arylidene-4-thioxo-thiazolidine-2-ones (**3a**–**n**). However, all the synthesized compounds exhibited only the configuration *Z*, as expected from the literature and verified by the ^{1}H NMR spectra. The literature records that the condensation of imidazolidine-2,4-dione with aromatic aldehydes in acid medium, led only to the *Z* isomer [20]. Also, the data of coupled ^{13}C NMR spectra indicated that 5-benzylidene thiazolidines and imidazolidines exhibit the *Z* configuration [19,21,22]. This assignment was also confirmed by X-ray crystallographic data [23].

The ^{1}H NMR spectra of the compounds (**3a**–**n**) showed one singlet at δ 7.91–8.29 ppm, correspondent to the methine proton, which is in agreement with the *Z* configuration, due to the deshielding effect of the C═S adjacent. The earlier literature reports that the methine proton in *E* isomers appears at lower chemical shift values, due to the lesser deshielding effect of the sulphur atom at 1-position [17,24].

2.2. Pharmacology

2.2.1. Antimicrobial activity

The in vitro antimicrobial activity was performed using the disk diffusion method and the Minimum Inhibitory Concentration (MIC) with different strains, including multidrug-resistant clinical isolates. Ampicillin and kanamycin were used as positive controls for bacteria and ketoconazole for yeast. In a preliminary assay (Table 1) 5-arylidene-4-thioxo-thiazolidine-2-ones (**3a**–**n**) and the intermediate (**2**) showed activity against Gram positive and *Candida albicans* strains, but did not exhibit activity against Gram negative strains. Thiazolidine-2,4-dione (**1**) was inactive for all microorganisms tested, however, demonstrating that the replacing of the carbonyl by thiocarbonyl increased the antimicrobial activity.

The values of the MIC against the microorganisms susceptible in the preliminary test are reported in Table 2. The results showed significant inhibitory effects, and most compounds exhibit MIC values in the 2–16 μg/mL range. This class of compounds presented high activity against *S. aureus*, especially the 5-substituted compounds, when the derivatives **3h** and **3j** were as active as the standard drug, ampicillin, but less active than cefalexin, the second standard used. Besides, the activity against *Bacillus subtilis* was found for compounds **3f**, **3j** and **3n**; while last one also was active against *Enterococcus faecalis*, in potency comparable with ampicillin. The derivatives **3f**, **3j** and **3l** exhibited significant values of MIC for *Mycobacterium smegmatis*. It is noteworthy that the intermediate **2** was able to inhibit the proliferation of yeast when it was compared with compounds **3a**–**n** however, with less potency than ketoconazole (the reference drug). In contrast, the biological data indicated that 5-arylidene subunit is essential for antibacterial activity.

The compounds **2** and **3a**–**n** were also evaluated for antibacterial activity against a large number of clinical isolates of multidrug-resistant Gram positive bacteria. The values of the MIC are given in Table 3 compared with ampicillin and cefalexin, used as standards. All compounds showed significant inhibitory effects, especially 5-arylidene derivatives, confirming the importance of this substitution in increasing activity to inhibit growth of Gram positive bacteria. The compounds **3a**–**n** were more potent than cefalexin against most of the microorganisms tested. The most relevant results were verified for strains *S. aureus* **III** (H.C. 21036) and **VI** (H.C. 20905), whose inhibitory effect was much greater than that of ampicillin and cefalexin. It is interesting to note that the derivative **3n** showed a greater inhibitory capacity. This suggests that the introduction of three atoms of the halogens to the arylidene group may have exercised an important role to increase antibacterial properties.

Table 1
Inhibitory zone (diameter, mm) of compounds against bacterial and yeast strains using the disk diffusion method.

Compound	Mean zone inhibition[a]								
	Sa	Bs	Ml	Ef	Pa	Ec	Sm	Ms	Ca
1	–	–	–	–	–	–	–	–	–
2	19	21	26	22	–	–	–	18	19
3a	24	23	25	20	–	–	–	20	16
3b	28	20	27	22	–	–	–	20	16
3c	29	26	29	24	–	–	–	25	17
3d	25	19	18	19	–	–	–	21	13
3e	28	26	29	25	–	–	–	25	14
3f	23	18	21	17	–	–	–	17	15
3g	25	17	18	16	–	–	–	20	12
3h	31	25	29	22	–	–	–	24	13
3i	27	22	25	17	–	–	–	20	12
3j	23	24	24	18	–	–	–	19	15
3l	20	20	18	–	–	–	–	17	15
3m	25	27	27	20	–	–	–	20	14
3n[b]	16	–	21	19	–	–	–	–	–
Ampicillin	38	28	45	24	NT	20	NT	NT	NT
Kanamycin	NT	NT	NT	NT	20	15	45	40	NT
Ketoconazole	NT	NT	NT	NT	NT	NT	NT	NT	28

Ampicillin (10 μg/disc), Kanamycin (30 μg/disc) and Ketoconazole (25 μg/disc) were used as positive references; Compounds **1**, **2** and **3a–n** (300 μg/disc); –, indicates no sensitivity or mean zone inhibition lower than 7 mm; NT, not tested; Sa, *S. aureus*; Bs, *B. subtilis*; Ml, *M. luteus*; Ef, *E. faecalis*; Pa, *P. aeruginosa*; Ec, *E. coli*; Sm, *S. marcescens*; Ms, *M. smegmatis*; Ca, *C. albicans*.

[a] Values are mean ($n = 3$).

[b] Solubility problems were observed at higher doses.

Table 2
Inhibitory activity of compounds **2** and **3a–n** expressed as MIC (μg/mL).

Compound	Microorganism					
	S. aureus	*B. subtilis*	*M. luteus*	*E. faecalis*	*M. smegmatis*	*C. albicans*
2	8	4	4	64	32	8
3a	8	32	32	32	32	32
3b	4	16	16	32	32	64
3c	4	16	16	32	16	32
3d	4	8	16	32	32	32
3e	4	8	8	16	16	64
3f	4	2	8	16	4	32
3g	4	16	16	16	16	32
3h	2	8	8	8	16	32
3i	4	16	32	32	32	64
3j	2	2	8	16	4	32
3l	4	8	16	16	4	32
3m	4	4	4	8	32	32
3n	4	2	4	2	32	64
Ampicillin	2	2	2	2	64	NT
Cefalexin	≤1	≤1	≤1	64	>128	NT
Ketoconazole	NT	NT	NT	NT	NT	≤ 1

NT, not tested.

2.2.2. Cytotoxic activity

The in vitro cytotoxic activity was performed by MTT assay [25,26] against two human carcinoma cell lines: NCI-H292 (obtained from mucoepidermoid carcinoma of lung) and HEp-2 (obtained from epidermoid carcinoma of the larynx). The inhibitory effects of compounds **3a–n** on the growth of the two cell lines are shown in Table 4. All compounds showed lower cytotoxicity when they were compared with vincristine, the standard drug used ($IC_{50} = 0.04$ and 0.003 μg/mL for NCI-H292 and HEp-2, respectively). Moreover, they were not able to inhibit 50% of cell proliferation even at the highest dose used (10 μg/mL). The antiproliferative effect was only achieved at 45.03% for NCI-H292 cells and at 30.10% for HEp-2 cells. Also, we observed that the NCI-H292 line was more sensitive to synthesized compounds than the HEp-2 line.

Compounds containing thiazolidinedione ring have already been reported as apoptosis inducers in cancer cell strains and more recently have been shown to be active against drug-resistant lung cancer cells. Thus, although this inhibition is still not sufficient, we feel that further research would be able to improve the inhibitory property. This could be done by putting one or more bulky group(s) in the arylidene ring [27,28].

3. Conclusion

This study demonstrated that the bioisosteric replacement of thiocarbonyl instead of carbonyl in thiazolidine ring, results in an enhancement of antimicrobial activity. Due to its antibacterial properties, especially against multidrug-resistant strains of clinical isolates, the 5-arylidene-4-thioxo-thiazolidine-2-ones identified here may represent useful starting points for further lead optimization. Studies involving the mechanism of action are necessary for a complete understanding of their antimicrobial activity, as well as structural modifications on the title compounds to improve antiproliferative activity.

4. Experimental

4.1. Chemistry

All melting points were determined using QUIMIS (model 320.23) and are uncorrected. FTIR spectra were recorded on a BRUKER IFS-66 IR spectrophotometer in KBr pellet. ^{1}H NMR and ^{13}C NMR spectra were measured on VARIAN UNITY PLUS (300 MHz for ^{1}H and 75.4 MHz for ^{13}C). The chemical shifts are reported in δ units and the coupling constants (J) are reported in hertz. C, H, N and S analyses were performed with a Carlo Erba model EA1108 elemental analyzer. Thin layer chromatography was performed on pre-coated silica plates (Merck Kiesegel 60 F_{254}) and column chromatography using silica gel (mesh 70–230). The spots could be visualized easily under ultraviolet light.

4.2. General procedure for the preparation of 4-thioxo-thiazolidine-2-one (*2*)

A mixture of thiazolidine-2,4-dione (**1**) (0.1 mol) and Lawesson's reagent (0.03 mol) in anhydrous dioxane, was heated under reflux for 24 h. Part of the solvent was evaporated and, after cooling, the precipitate was filtered, washed with *n*-hexane and recrystallized in ethanol. Yield 68%, mp: 140–141 °C. IR (KBr, cm^{-1}): 3115 (NH); 1710 (C═O). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 13.54 (s, 1H, NH); 4.60 (s, 2H, $-CH_2-$). Anal. Calcd. for $C_3H_3NOS_2$ (133.18): C,

Table 3
MIC values (in μg/mL) against clinical isolate of multidrug-resistant Gram positive bacterial strains.

Compound	*S. aureus*						*E. faecalis*				Coagulase-negative *Staphylococcus*	
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
2	32	32	32	64	32	32	32	32	32	32	64	64
3a	4	8	2	4	8	8	32	32	32	32	32	16
3b	2	16	2	2	2	2	16	8	8	8	16	16
3c	4	32	2	2	2	2	32	32	16	16	32	32
3d	4	16	4	4	4	4	8	16	16	16	2	16
3e	16	32	16	8	8	16	8	16	8	16	64	64
3f	4	4	4	4	2	4	8	8	8	8	4	4
3g	4	8	2	2	2	2	16	32	16	32	32	8
3h	2	8	2	2	2	2	4	4	4	4	16	2
3i	2	8	2	2	2	2	16	16	16	16	4	4
3j	2	4	8	2	2	2	4	4	8	8	4	2
3l	8	8	8	4	4	4	8	8	16	16	4	4
3m	2	2	4	2	2	4	8	4	16	16	4	2
3n	2	2	2	2	2	2	4	4	4	4	2	2
Ampicillin	2	≤1	32	≤1	2	64	2	≤1	2	2	≤1	≤1
Cefalexin	8	2	>128	2	4	>128	64	64	64	64	128	128

S. aureus: H.C. 20981 (**I**), H.C. 21141 (**II**), H.C. 21036 (**III**), H.C. 20489 (**IV**); H.C. 20794 (**V**) and H.C. 20905 (**VI**); *E. faecalis*: H.C. 21752 (**VII**), H.C. 21944 (**VIII**), H.C. 24708 (**IX**) and H.C. 24962 (**X**); Coagulase-negative *Staphylococcus*: H.A.M. 278 (**XI**) and H.A.M. 338 (**XII**).

Table 4
Growth inhibitory effects (%) of **3a–n** on human carcinoma cell lines.

Compound	10 μg/mL		5 μg/mL		2.5 μg/mL		1.25 μg/mL	
	NCI-H292	HEp-2	NCI-H292	HEp-2	NCI-H292	HEp-2	NCI-H292	HEp-2
3a	40.41	11.21	25.18	8.86	7.78	1.66	4.29	−8.34
3b	26.78	20.93	13.14	19.83	3.43	7.03	2.10	0.84
3c	38.00	30.10	31.55	20.56	17.49	−4.51	7.32	−10.82
3d	36.87	21.23	22.94	16.23	16.19	10.33	13.27	3.14
3e	42.36	23.63	22.03	−1.04	11.14	−1.82	4.67	−5.3
3f	36.45	18.23	16.61	15.43	1.19	−0.36	1.10	−3.16
3g	45.03	21.15	34.19	5.97	19.88	−11.53	10.60	−11.34
3h	30.87	18.83	21.61	17.63	11.19	5.44	3.35	−0.36
3i	41.39	22.25	35.83	16.07	28.55	3.91	10.01	0.92
3j	38.37	24.83	29.28	20.03	14.69	15.33	12.11	6.83
3l	32.80	8.73	10.34	−2.39	7.70	−4.24	4.36	−9.16
3m	43.28	16.41	35.83	11.02	19.36	5.03	9.18	−5.45
3n	40.50	23.54	39.68	22.84	33.01	21.75	14.21	12.58

27.06; H, 2.27; N, 10.52; S, 48.14. Found: C, 27.18; H, 2.47; N, 10.22; S, 48.74.

4.3. General procedure for the preparation of 5-arylidene-4-thioxo-thiazolidine-2-ones (**3a–n**)

To a solution of compound **2** (0.01 mol) and anhydrous sodium acetate (0.01 mol) in glacial acetic acid, was added the respective aromatic aldehyde. The mixture was stirred under reflux for 1–10 h and then poured into ice-cold water. Then, the precipitate was filtered, washed with water and *n*-hexane, dried and purified by column chromatography.

4.3.1. 5-(2-Fluorobenzylidene)-4-thioxo-thiazolidine-2-one (**3a**)

Yield 30%, mp: 159–160 °C. IR (KBr, cm^{-1}): 3163–3080 (NH); 1690 (C=O); 1592 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 13.97 (s, 1H, NH); 8.14 (s, 1H, –CH=); 7.62–7.54 (m, 2H, Ar); 7.41–7.35 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm, DEPT): δ 116.35 (d, $J_{CF} = 21.3$), 125.53 (d, $J_{CF} = 3.0$), 126.39 (d, $J_{CF} = 6.97$), 128.61, 133.23 (d, $J_{CF} = 9.0$) (CH); 121.50 (d, $J_{CF} = 11.0$), 131.88, 161.09 (d, $J_{CF} = 251.4$) (Cq); 170.43 (C=O); 195.41 (C=S). Anal. Calcd. for $C_{10}H_6FNOS_2$ (239.28): C, 50.20; H, 2.53; N, 5.85; S, 26.80. Found: C, 50.42; H, 2.54; N, 5.79; S, 21.09.

4.3.2. 5-(3-Fluorobenzylidene)-4-thioxo-thiazolidine-2-one (**3b**)

Yield 20%, mp: 103–104 °C. IR (KBr, cm^{-1}): 3080 (NH); 1700 (C=O); 1586 (C=C). ^{1}H NMR (Acetone-d_6, 300 MHz, ppm): δ 10.53 (s, 1H, NH); 8.12 (s, 1H, –CH=); 7.66–7.16 (m, 4H, Ar). Anal. Calcd. for $C_{10}H_6FNOS_2$ (239.28): C, 50.20; H, 2.53; N, 5.85; S, 26.80. Found: C, 50.60; H, 2.54; N, 5.70; S, 21.19.

4.3.3. 5-(4-Fluorobenzylidene)-4-thioxo-thiazolidine-2-one (**3c**)

Yield 18%, mp: 126–127 °C. IR (KBr, cm^{-1}): 3068 (NH); 1727 (C=O); 1573 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 13.89 (s, 1H, NH); 8.06 (s, 1H, –CH=); 7.76–7.71 (m, 2H, Ar); 7.41–7.35 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm, DEPT): δ 116.70 (d, $J_{CF} = 22.05$), 133.06 (d, $J_{CF} = 9.0$), 134.73 (CH); 129.58 (d, $J_{CF} = 2.55$), 130.12 (d, $J_{CF} = 3.0$), 163.10 (d, $J_{CF} = 250.87$) (Cq); 170.54 (C=O); 195.43 (C=S). Anal. Calcd. for $C_{10}H_6FNOS_2$ (239.28): C, 50.20; H, 2.53; N, 5.85; S, 26.80. Found: C, 50.40; H, 2.74; N, 5.29; S, 21.11.

4.3.4. 5-(2-Bromobenzylidene)-4-thioxo-thiazolidine-2-one (**3d**)

Yield 39%, mp: 214–215 °C. IR (KBr, cm^{-1}): 3161–3080 (NH); 1710 (C=O); 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.06 (s, 1H, NH); 8.25 (s, 1H, –CH=); 7.80 (d, 1H, Ar); 7.62–7.52 (m, 2H, Ar); 7.45–7.39 (m, 1H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm): δ 126.00, 128.69, 129.10, 132.38, 132.80, 133.73, 133.64, 133.73; 170.54 (C=O); 195.25 (C=S). Anal. Calcd. for $C_{10}H_6BrNOS_2$ (300.19): C, 40.01; H, 2.01; N, 4.67; S, 21.36. Found: C, 40.07; H, 2.01; N, 4.79; S, 21.19.

4.3.5. 5-(3-Bromobenzylidene)-4-thioxo-thiazolidine-2-one (**3e**)

Yield 40%, mp: 214–215 °C. IR (KBr, cm^{-1}): 3149–3064 (NH); 1685 (C=O); 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 13.96 (s, 1H, NH); 8.02 (s, 1H, –CH=); 7.87 (s, 1H, Ar); 7.71–7.63 (m, 2H, Ar); 7.49 (t, 1H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm, DEPT): δ 122.59, 128.53, 131.45, 133.09, 133.29 (CH); 131.32, 133.86, 135.82 (Cq); 170.37 (C=O); 195.35 (C=S). Anal. Calcd. for $C_{10}H_6BrNOS_2$ (300.19): C, 40.01; H, 2.01; N, 4.67; S, 21.36. Found: C, 40.21; H, 2.01; N, 4.41; S, 21.56.

4.3.6. 5-(4-Bromobenzylidene)-4-thioxo-thiazolidine-2-one (**3f**)

Yield 22%, mp: 188–189 °C. IR (KBr, cm^{-1}): 3161–3075 (NH); 1688 (C=O); 1582 (C=C). ^{1}H NMR (Acetone-d_6, 300 MHz, ppm): δ 8.09 (s, 1H, –CH=); 7.78–7.73 (m, 2H, Ar); 7.68–7.63 (m, 2H, Ar). ^{13}C NMR (Acetone-d_6, 75.4 MHz, ppm): δ 126.15, 130.28, 132.09, 133.09, 133.62, 134.08, 134.59, 135.95; 170.83 (C=O); 196.75 (C=S). Anal. Calcd. for $C_{10}H_6BrNOS_2$ (300.19): C, 40.01; H, 2.01; N, 4.67; S, 21.36. Found: C, 40.03; H, 2.31; N, 4.79; S, 21.49.

4.3.7. 5-(2-Chlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3g**)

Yield 40%, mp: 166–167 °C. IR (KBr, cm^{-1}): 3166–3080 (NH); 1715 (C=O); 1581 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 14.05 (s, 1H, NH); 8.29 (s, 1H, –CH=); 7.65–7.61 (m, 2H, Ar); 7.54–7.49 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm): δ 128.19, 128.93, 130.47, 130.82, 131.59, 132.21, 132.74, 135.13; 170.48 (C=O); 195.31 (C=S). Anal. Calcd. for $C_{10}H_6ClNOS_2$ (255.74): C, 46.97; H, 2.36; N, 5.48; S, 25.07. Found: C, 47.07; H, 2.41; N, 5.48; S, 25.27.

4.3.8. 5-(3-Chlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3h**)

Yield 17%, mp: 112–113 °C. IR (KBr, cm^{-1}): 3081 (NH); 1686 (C=O); 1589 (C=C). ^{1}H NMR (Acetone-d_6, 300 MHz, ppm): δ 12.26 (s, 1H, NH); 8.08 (s, 1H, –CH=); 7.70 (s, 1H, Ar); 7.66–7.52 (m, 3H, Ar). ^{13}C NMR (Acetone-d_6, 75.4 MHz, ppm): δ 129.77, 131.67, 131.88, 132.53, 132.79, 135.49, 136.29, 137.45; 189.88 (C=O); 196.56 (C=S). Anal. Calcd. for $C_{10}H_6ClNOS_2$ (255.74): C, 46.97; H, 2.36; N, 5.48; S, 25.07. Found: C, 47.17; H, 2.21; N, 5.48; S, 25.27.

4.3.9. 5-(4-Chlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3i**)

Yield 15%, mp: 183–184 °C. IR (KBr, cm^{-1}): 3161–3080 (NH); 1691 (C=O); 1577 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz, ppm): δ 13.93 (s, 1H, NH); 8.04 (s, 1H, –CH=); 7.69–7.66 (m, 2H, Ar); 7.61–7.58 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm, DEPT):

δ 129.54, 132.07, 134.33 (CH); 130.43, 132.28, 135.50 (Cq); 170.43 (C=O); 195.39 (C=S). Anal. Calcd. for $C_{10}H_6ClNOS_2$ (255.74): C, 46.97; H, 2.36; N, 5.48; S, 25.07. Found: C, 46.09; H, 2.51; N, 5.48; S, 25.37.

4.3.10. 5-(2,4-Dichlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3j**)

Yield 54%, mp: 162–163 °C. IR (KBr, cm^{-1}): 3041 (NH); 1733 (C=O); 1573 (C=C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 8.22 (s, 1H, –CH=); 7.85 (d, 1H, Ar); 7.66 (m, 2H, Ar). ^{13}C NMR (Acetone-d_6, 75.4 MHz, ppm): δ 129.73, 131.40, 131.67, 132.68, 133.46, 134.74, 137.84, 138.08; 170.87 (C=O); 196.61 (C=S). Anal. Calcd. for $C_{10}H_5Cl_2NOS_2$ (290.18): C, 41.39; H, 1.74; N, 4.83; S, 22.10. Found: C, 41.56; H, 1.91; N, 4.78; S, 22.27.

4.3.11. 5-(2,6-Dichlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3l**)

Yield 41%, mp: 191–192 °C. IR (KBr, cm^{-1}): 3190–3111 (NH); 1715 (C=O); 1604 (C=C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 7.91 (s, 1H, –CH=); 7.60–7.57 (m, 2H, Ar); 7.51–7.45 (m, 1H, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm): δ 128.58, 130.15, 131.93, 132.19, 133.04, 138.02, 168.50; 170.43 (C=O); 195.39 (C=S). Anal. Calcd. for $C_{10}H_5Cl_2NOS_2$ (290.18): C, 41.39; H, 1.74; N, 4.83; S, 22.10. Found: C, 41.57; H, 1.96; N, 4.78; S, 22.37.

4.3.12. 5-(3,4-Dichlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3m**)

Yield 55%, mp: 183–184 °C. IR (KBr, cm^{-1}): 3161–3080 (NH); 1685 (C=O); 1583 (C=C). ^1H NMR (DMSO-d_6, 300 MHz, ppm): δ 13.99 (s, 1H, NH); 8.00 (s, 1H, –CH=); 7.94 (d, 1H, $J_m = 2.1$, Ar); 7.78 (d, 1H, $J_o = 8.4$, Ar); 7.60 (dd, 1H, $J_o = 8.3$, $J_m = 2.1$, Ar). ^{13}C NMR (DMSO-d_6, 75.4 MHz, ppm, DEPT): δ 129.27, 131.51, 132.50, 132.77 (CH); 131.73, 132.20, 133.14, 134.11 (Cq); 168.49 (C=O); 170.22 (C=S). Anal. Calcd. for $C_{10}H_5Cl_2NOS_2$ (290.18): C, 41.39; H, 1.74; N, 4.83; S, 22.10. Found: C, 41.46; H, 1.92; N, 4.79; S, 22.27.

4.3.13. 5-(2,3,5-Trichlorobenzylidene)-4-thioxo-thiazolidine-2-one (**3n**)

Yield 70%, mp: 186–187 °C. IR (KBr, cm^{-1}): 3103–3057 (NH); 1753 (C=O); 1600 (C=C). ^1H NMR (Acetone-d_6, 300 MHz, ppm): δ 8.24 (s, 1H, –CH=); 7.82 (d, 1H, $J_m = 2.4$, Ar); 7.62 (d, 1H, $J_m = 2.4$, Ar). Anal. Calcd. for $C_{10}H_4Cl_3NOS_2$ (324.63): C, 37.00; H, 1.24; N, 4.31; S, 19.75. Found: C, 37.02; H, 1.25; N, 4.32; S, 19.85.

4.4. In vitro assay for antimicrobial activity

The microorganisms used in this study are *S. aureus* (UFPEDA 01), *B. subtilis* (UFPEDA 16), *M. luteus* (UFPEDA 06), *E. faecalis* (UFPEDA 138), *Pseudomonas aeruginosa* (UFPEDA 39), *Escherichia coli* (UFPEDA 224), *Serratia marcescens* (UFPEDA 398), *M. smegmatis* (UFPEDA 71) and *C. albicans* (UFPEDA 1007) obtained from the cultures collection of the Antibiotics Department of the Federal University of Pernambuco (UFPE), Brazil. Strains of multidrug-resistant clinical isolates were also used and consisted of *S. aureus* (20981, 21141, 21036, 20489, 20794 and 20905), *E. faecalis* (21752, 21944, 24708 and 24962) obtained from Bacteriology Department of University Hospital of the Federal University of Pernambuco and Coagulase-negative *Staphylococcus* (278 and 338), obtained from Bacteriology Department of the Agamenon Magalhães Hospital, Pernambuco, Brazil. The clinical isolates were collected from various patients hospitalized in several clinics.

The antimicrobial activity was reported preliminarily using the disk diffusion method [29]. In this method, paper disks (6 mm) containing specific amounts of an antimicrobial agent (300 μg for the synthesized compounds) were placed on the surface of an agar plate inoculated with a standardized suspension of the microorganisms tested. The plates were incubated at 35 °C for 24 and 48 h, for bacteria and yeast respectively. Ketoconazole (25 μg) for yeast, ampicillin (10 μg) for Gram positive bacteria and kanamycin (30 μg) for Gram negative bacteria, were used as standard drugs. Paper disks with only DMSO were utilized as negative controls.

A twofold serial dilution technique [30,31] was followed to determine the minimum inhibitory concentration (MIC) of the compounds against the susceptible microorganisms in the preliminary test (yeast and Gram positive bacteria) and against strains of clinical isolates of multidrug-resistant Gram positive bacteria. Test compounds, dissolved in DMSO, were added to culture media (Mü ller–Hinton agar for bacteria and Sabouraud Liquid Medium for yeast) to obtain final concentrations ranging from 128–1 μg/mL. A plate (tube for *C. albicans*) containing only the culture medium and DMSO was used as negative control. The final amount applied was of 10^5 CFU/plate for bacteria and 10^3 CFU/tube for yeast. The MIC values were read after incubation at 35 °C for a period of 20 h (bacteria) and 48 h (yeast). The lowest concentration of the test substance that completely inhibited the growth of the microorganism was recorded as the MIC, expressed in μg/mL. Ampicillin, cefalexin (bacteria) and ketoconazole (yeast) were used as standard drugs. All experiments were carried out three times.

4.5. In vitro assay for cytotoxic activity

The human lung carcinoma cell line (NCI-H292) and the human larynx carcinoma cell line (HEp-2) were purchased from the Adolfo Lutz Institute, São Paulo, Brazil. A DMEM (Dulbecco's Modified Eagle's Medium), enriched with 10% of fetal bovine serum, 1% of L-glutamine and 1% of antibiotics (penicillin and streptomycin), was used for cell cultivation and to perform the tests.

The cytotoxic activity was investigated using the MTT assay (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide). Cell suspensions were diluted to 10^5 cells/mL, suitably prepared and distributed in plates of culture with 96 wells (225 μL in each well), then incubated at 37 °C in a humid atmosphere with 5% of CO_2. After 24 h, 25 μL of either the synthesized compounds or the reference drug (vincristine) was added to each well. The plates were incubated again at 37 °C for 72 h. Then, 25 μL of MTT solution (5 mg/mL) was added to each well, and the mixture was incubated at 37 °C for 2 h. At the end of this period, the culture medium with the MTT excess was aspirated and after that, 100 μL of DMSO was added to each well to dissolve the formazan crystals. The optical density (OD) of the wells was measured at 540 nm and compared to the control (cells with medium only). The assay was conducted in triplicate.

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