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Antimicrobial and Cytotoxicity Evaluation of New 3-Allyl-2-iminothiazolidin-4-ones

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Abstract

A series of novel 3-allyl-2-iminothiazolidin-4-one derivatives (**4-17**) was synthesized, through the reaction of 3-allyl-2-((3,4-dichlorophenyl)imino)thiazolidin-4-one (**3**) with different aromatic aldehydes. The chemical stability of four representative thiazolidinone derivatives (**3**, **11**, **13** and **14**) was evaluated against γ-irradiation, at a radiation dose of 25 kGy. The compounds were found to be stable with no observed degradation in liquid chromatography–mass spectrometry (LC-MS) experiments. The synthesized thiazolidinone derivatives (**3-17**) were evaluated for their antimicrobial and anticancer activities. Among the tested compounds, compounds **5**, **7**, **14** and **16** exhibited slight antibacterial activity against a multi-drug resistant *Staphylococcus aureus* (ATCC 43300 strain), at a concentration of 32 µg/mL. Compound **3** fully inhibited the growth of the fungal pathogen *Cryptococcus neoformans*, at a tested concentration of 32 µg/mL. Besides, compound **12** inhibited the biofilm formation of *S. aureus* (HG001 strain), with a percentage of 54% at a concentration of 64 µg/mL. Compared to the other tested compounds, compound **11** showed higher *in vitro* anticancer activity against melanoma and breast cancer cells, with growth inhibition values ranging from 42% to 73% at a concentration of 10 µM. Interestingly, only compounds **3**, **8** and **16** showed weak cytotoxicity to murine fibroblast L929 cells, at a tested concentration of 50 µM. The other tested compounds were not cytotoxic to fibroblasts, which suggests the relative safety of the synthesized compounds to normal mammalian cells.

Keywords: 1,3-Thiazolidin-4-one; Chemical stability; Antibacterial; Antifungal; Antibiofilm; Anticancer

1. Introduction

Infectious diseases and cancer could cause each other, as some infectious diseases interact with human cells and alter humans' essential cell signaling that could be the reason for chronic inflammation and eventually tumors formation [1]. Therefore, preventing and tackling of infectious agents have substantially affected malignant growth anticipation. Additionally, cancer is an indirect cause of infections and the occurrence of their complications due to the deficient immunity in cancer patients, especially who are under therapeutic regimens acting on the myeloproliferative cells of the bone marrow [2].

On the other hand, infections worsen the prognosis of cancer diseases and promote metastasis [3]. Bacterial infections followed by fungal infections are the most common types of cancer-associated infections. Bacterial sepsis keeps on being the main

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cause of morbidity in pediatrics under intensive cancer treatment [4]. Furthermore, antimicrobial chemotherapy played a vital role in bacteremia prophylaxis in children with acute leukemia [5]. Therefore, there is a persistent need for more new anticancer drugs and to decrease the morbidity and mortality of microbial infections in cancer patients.

4-Thiazolidinones have been reported to be a pharmacophoric core of numerous synthetic compounds endowed with different biological actions and drug-like features [6-9]. In particular, 2-substituted-1,3-thiazolidin-4-one derivatives were reported as effective antimicrobial agents [7,10,11]. 4-Thiazolidinones were also reported to have a very good ability to inhibit biofilm formation in staphylococcal bacterial strains [9,12,13]. Moreover, 2-phenylimino-4-thiazolidinones are promising compounds in the development of anticancer agents. They were reported as growth inhibitors for human lung cancer cell lines (H460 and H460/TaxR) and leukemia cell lines with lower toxicity to normal fibroblasts [14,15].

Halogenation is a pervasive approach in drug discovery and a significant number of drugs used in the clinics are halogenated [16]. Halogen atoms have a role in the interaction between proteins and their ligands through non-covalent halogen bonds [17,18]. In general, inserting halogens in molecules, such as chlorine, results in increasing the lipophilicity and consequently the biological membrane permeability without an evidence of developed toxicity [16,19]. Reported studies on the antimicrobial activity of 1,3-thiazolidinones showed also a remarkable effect of the presence of a halogenated aryl substitution at C2 [20,23].

Among the reported biologically active 1,3-thiazolidin-4-ones, the 2,4-dichlorophenyl thiazolidinone derivative (**I**) was reported to have significant antibacterial activity against *Escherichia coli*, while its brominated derivative (**III**) showed anticancer activity against the breast cancer cell line MDA-MB-231 [20]. The alkylation of 2-phenylimino-4-thiazolidinones at N3 with small alkyl group, in particular the allyl moiety, was reported to enhance the antitumor and antimicrobial activities [24-26]. Compounds (**II**) and (**IV**), with an allyl residue at N3, were reported to exhibit significant antibacterial and antiproliferative activities, respectively [27-29]. 5-Ene-4-thiazolidinones demonstrated potent activities against various biotargets. They are potential phosphate mimics and pyrophosphate bioisosteres [8,30]. In this study, a series of 3-allyl-5-arylidene-2-((3,4-dichlorophenyl)imino)-thiazolidin-4-one derivatives (**4-17**) was synthesized by introducing different arylidene moieties at C5 of the 1,3-thiazolidin-4-one ring (Figure 1).

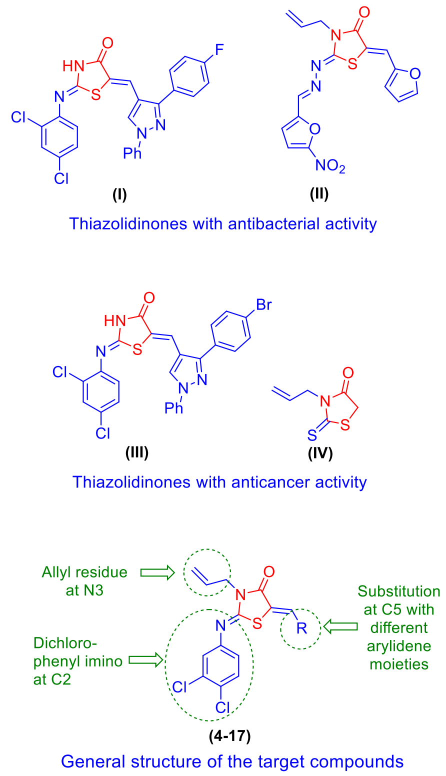


Figure 1. Reported biologically active 1,3-thaizolidin-4-ones (**I-IV**) and the design of the target compounds (**4-17**)

All the synthesized thiazolidinones **3-17** were tested for their antimicrobial (antibacterial, antibiofilm and antifungal) activity, against a panel of pathogenic bacterial and fungal strains. Five selected compounds were evaluated for their antitumor activity against 59 human-cancer cell lines. In addition, the cytotoxicity of the synthesized compounds against murine fibroblast L929 cells were evaluated. The chemical stability of four representative compounds against γ-irradiation was also studied.

2. Experimental

2.1. Chemistry

2.1.1. Materials and methods

Solvents, reagents and chemicals were bought from Alfa-Aesar and Sigma-Aldrich and used without any further purification or refining. Thin layer chromatography (TLC) plates were used to check the reactions' progress. The TLC plates were silica gel 60 F254 plated on aluminum sheets and purchased from Merck. UV light at a wave length of 254 nm was used for the visualization of the TLC plates. The used eluting solvent was a mixture of acetone/cyclohexane (3:7). Stuart apparatus was used to measure the melting points of the synthesized compounds in capillary tubes. Infrared (IR) spectroscopy for the synthesized compounds were performed by VERTEX 70 FT-IR spectrophotometer. Nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were performed in δ scale given in ppm on Varian spectrophotometer (400 MHz for ^{1}H-NMR and 100.6 for ^{13}C-NMR) and alluded to Trimethylsilane (TMS) as internal indicator. Mass spectrometry was performed by Waters ACQUITY Xevo TQD framework (Waters Corp., Milford, MA, USA), which composed of XevoTM TQD triple-quadrupole tandem mass spectrometer and ACQUITY UPLC H-Class system, with electrospray ionization (ESI) interface. The eluting solvents consisted of water containing 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Purity of the compounds was detected by HPLC (flow rate 200 μL/min).

2.1.2. *Synthesis of 1-allyl-3-(3,4-dichlorophenyl)-thiourea* (**2**)

The thiourea derivative **2** was synthesized by the reaction of 3,4-dichlorophenylisothiocynate (**1**) with allylamine, according to the reported procedure [31].

2.1.3. Synthesis of 3-allyl-2-((3,4-dichlorophenyl)-imino)thiazolidin-4-one (**3**)

Ethyl bromoacetate (2.5 g, 15 mmol) was added to a solution of compound **2** (10 mmol) and anhydrous sodium acetate (0.82 g, 10 mmol) in glacial acetic acid (15 mL) or ethanol (20 mL). The reaction mixture was refluxed for 12–20 h. The solvent was evaporated and 20 mL of water was added. The formed precipitate was filtrated and crystallized from EtOH/H_2O (15:2) [32].

2.1.4. General procedure for the synthesis of compounds **4-17**

A mixture of compound **3** (0.5 mmol) and the proper aromatic aldehyde (0.6 mmol) was refluxed in 20 mL of ethanol/piperidine (30:1), for 2-12 h. The reaction was then left for cooling. The formed precipitate was filtered and crystalized from ethanol to obtain compounds **4**-**17**.

2.1.4.1. 3-Allyl-5-benzylidene-2-((3,4-dichlorophenyl)imino)thiazolidin-4-one (**4**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with benzaldehyde. Physical form: white crystals; Yield = 90.7%; m.p. = 105-107 ºC; IR, cm^{-1}: 3056 (CH aromatic), 2972, 2873 (CH aliphatic), 1707 (C=O), 1629 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.48 (d, J = 5.2 Hz, 2H, N-CH_2-), 5.16-5.26 (m, 2H, -CH=C$\underline{H_2}$), 5.88-6.00 (m, 1H, -C$\underline{H}$=CH_2), 7.02 (dd, J = 2.4 and 8.4 Hz, 1H, aromatic), 7.29 (d, J = 2.4 Hz, 1H, aromatic), 7.39-7.60 (m, 5H, aromatic), 7.64 (d, J = 8.4 Hz, 1H, aromatic), 7.79 (s, 1H, S-C=C$\underline{H}$); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.18 (N-CH_2-), 117.77 (-CH=$\underline{C}H_2$), 121.01 (S-$\underline{C}$=CH), 122.04, 123.51, 127.40, 129.77 (2C), 130.37 (2C), 130.70, 131.59, 131.63, 131.89, 132.20, 133.51, 148.31, 151.77 (C=N), 165.82 (C=O); LC-MS (*m/z*): calculated for $C_{19}H_{14}Cl_2N_2OS$ = 388.02, found = 389.05 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.2. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(4-methylbenzylidene)-thiazolidin-4-one (**5**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 4-methylbenzaldehyde. Physical form: yellow needle crystals; Yield = 95.3%; m.p. = 130-132 ºC; IR, cm^{-1}: 3019 (CH aromatic), 2967, 2906 (CH aliphatic), 1712 (C=O), 1623 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 2.31 (s, 3H, -CH_3), 4.47 (d, J = 5.2 Hz, 2H, N-CH_2), 5.16-5.24 (m, 2H, -CH=C$\underline{H_2}$), 5.87-5.98 (m, 1H, -C$\underline{H}$=CH_2), 7.02 (dd, J = 8.4 and 2.4 Hz, 1H, aromatic), 7.26-7.32 (m, 3H, aromatic), 7.44 (d, J = 8.4 Hz, 2H, aromatic), 7.65 (d, J = 8.4 Hz, 1H, aromatic), 7.76 (s, 1H, S-C=C$\underline{H}$); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 21.54 (CH_3), 45.14 (N-CH_2), 117.73 (-CH=$\underline{C}H_2$), 119.77 (S-$\underline{C}$=CH), 122.06, 123.52, 127.35, 130.39 (2C), 130.44 (2C), 130.77, 131.67, 131.68, 131.88, 132.19, 140.95, 148.36, 151.83 (C=N), 165.89 (C=O); LC-MS (*m/z*): calculated for $C_{20}H_{16}Cl_2N_2OS$ = 402.04, found = 403.07 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.3. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(2-methoxybenzylidene)-thiazolidin-4-one (**6**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 2-methoxybenzaldehyde. Physical form: canary yellow needle crystals; Yield = 67.8%; m.p. = 98-100 ºC; IR, cm^{-1}: 3055 (CH aromatic), 2934, 2832 (CH aliphatic), 1711 (C=O), 1627 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 3.86 (s, 3H, O-CH_3), 4.47 (d, J = 5.2 Hz, 2H, N-CH_2-), 5.17-5.25 (m, 2H, -CH=C$\underline{H_2}$), 5.88-5.99 (m, 1H, -C$\underline{H}$=CH_2), 7.01 (dd, J = 8.4 and 2.4 Hz, 1H, aromatic), 7.05 (d, J = 7.2 Hz, 1H, aromatic), 7.12 (d, J = 8.4 Hz, 1H, aromatic), 7.29 (d, J = 2.4 Hz, 1H, aromatic), 7.34 (dd, J = 7.8 and 1.4 Hz, 1H, aromatic), 7.40-7.46 (m, 1H, aromatic), 7.63 (d, J = 8.4 Hz, 1H, aromatic), 7.98 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.12 (N-CH_2-), 56.27 (O-CH_3), 112.29 (aromatic), 117.75 (-CH=<u>C</u>H_2), 120.98, 121.49, 121.99, 122.07, 123.53, 126.13, 127.33, 128.88, 131.66, 131.85, 132.16, 132.71, 148.35, 152.00 (C=N), 158.31 (2-OCH_3-<u>C</u>), 165.89 (C=O); LC-MS (*m/z*): calculated for $C_{20}H_{16}Cl_2N_2O_2S$ = 418.03, found = 419.10 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.4. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(3-methoxybenzylidene)-thiazolidin-4-one (**7**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 3-methoxybenzaldehyde. Physical form: pale yellow needle crystals; Yield = 50.3%; m.p. = 84-85 ºC; IR, cm^{-1}: 3056 (CH aromatic), 2944, 2833 (CH aliph.), 1713 (C=O), 1618 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 3.74 (s, 3H, O-CH_3), 4.47 (d, J = 5.2 Hz, 2H, N-CH_2-), 5.17-5.24 (m, 2H, -CH=C<u>H_2</u>), 5.88-5.98 (m, 1H, -C<u>H</u>=CH_2), 6.99-7.05 (m, 2H, aromatic), 7.08 (d, J = 7.6 Hz, 1H, aromatic), 7.12 (t, J = 2.0 Hz, 1H, aromatic), 7.29 (d, J= 2.4 Hz, 1H, aromatic), 7.40 (t, J = 8.0 Hz, 1H, aromatic), 7.64 (d, J = 8.8 Hz, 1H, aromatic), 7.76 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.17 (N-CH_2-), 55.71 (O-CH_3), 116.22 and 116.42 (aromatic), 117.74 (-CH=<u>C</u>H_2), 121.44, 121.76, 122.04, 123.51, 127.42, 130.95, 131.55, 131.59, 131.88, 132.18, 134.89, 148.22, 151.70 (C=N), 160.01 (3-OCH_3-<u>C</u>), 165.75 (C=O); LC-MS (*m/z*): calculated for $C_{20}H_{16}Cl_2N_2O_2S$ = 418.03, found = 419.08 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.5. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(4-methoxybenzylidene)-thiazolidin-4-one (**8**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 4-methoxybenzaldehyde. Physical form: yellow needle crystals; Yield = 89.8%; m.p. = 117-118 ºC; IR, cm^{-1}: 3017 (CH aromatic), 2937, 2830 (CH aliphatic), 1707 (C=O), 1626 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 3.78 (s, 3H, O-CH_3), 4.47 (d, J = 5.2 Hz, 2H, N-CH_2-), 5.15-5.25 (m, 2H, -CH=C<u>H_2</u>), 5.88-5.99 (m, 1H, -C<u>H</u>=CH_2), 6.99-7.09 (m, 3H, aromatic), 7.29 (d, J = 2.4 Hz, 1H, aromatic), 7.51 (d, J = 8.8 Hz, 2H, aromatic), 7.64 (d, J = 8.8 Hz, 1H, aromatic), 7.75 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.08 (N-CH_2-), 55.87 (O-CH_3), 115.37 (2C), 117.67, 117.79, 122.11, 123.53, 126.03, 127.29, 131.60, 131.71, 131.86, 132.17, 132.44 (2C), 148.49, 152.01 (C=N), 161.28 (4-OCH_3-<u>C</u>), 165.98 (C=O); LC-MS (*m/z*): calculated for $C_{20}H_{16}Cl_2N_2O_2S$ = 418.03, found = 419.11 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.6. 3-Allyl-5-(3-chlorobenzylidene)-2-((3,4-dichlorophenyl)imino)-thiazolidin-4-one (**9**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 3-chlorobenzaldehyde. Physical form: white flakes; Yield = 50.8%; m.p. = 113-114 ºC; IR, cm^{-1}: 3074 (CH aromatic), 2972, 2930 (CH aliphatic), 1715 (C=O), 1638 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.48 (d, J = 5.2 Hz, 2H, N-CH_2-), 5.17-5.26 (m, 2H, -CH=<u>CH_2</u>), 5.88-5.99 (m, 1H, -<u>C</u>H=CH_2), 7.02 (dd, J = 8.4 and 2.4 Hz, 1H, aromatic), 7.30 (d, J = 2.4 Hz, 1H, aromatic), 7.45-7.68 (m, 5H, aromatic), 7.79 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.28 (N-CH_2-), 117.84 (-CH=<u>C</u>H_2), 122.00 (S-<u>C</u>=CH), 122.85, 123.50, 127.50, 127.83, 130.00, 130.30, 130.54, 131.56, 131.64, 131.91, 132.22, 134.31, 135.71, 148.14, 151.33 (C=N), 165.60 (C=O). LC-MS (*m/z*): calculated for $C_{19}H_{13}Cl_3N_2OS$ = 421.98, found = 423.03 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.7. 3-Allyl-5-(4-chlorobenzylidene)-2-((3,4-dichlorophenyl)imino)-thiazolidin-4-one (**10**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 4-chlorobenzaldehyde. Physical form: yellow flakes; Yield = 70.7%; m.p. = 152-153 ºC; IR, cm^{-1}: 3055 (CH aromatic), 2962, 2889 (CH aliphatic), 1717 (C=O), 1640 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.48 (d, J = 4.8 Hz, 2H, N-CH_2-), 5.17-5.26 (m, 2H, -CH=C<u>H_2</u>), 5.88-5.99 (m, 1H, -C<u>H</u>=CH_2), 7.02 (dd, J = 8.4 and 2.4 Hz, 1H, aromatic), 7.30 (d, J = 2.4 Hz, 1H, aromatic), 7.52-7.60 (m, 4H, aromatic), 7.65 (d, J = 8.4 Hz, 1H, aromatic), 7.80 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.24 (N-CH_2-), 117.78 (-CH=<u>C</u>H_2), 121.81 (S-<u>C</u>=CH), 122.05, 123.47, 127.45, 129.85 (2C), 130.24, 131.58, 131.91, 132.04 (2C), 132.21, 132.43, 135.23, 148.28, 151.52 (C=N), 165.73 (C=O). LC-MS (*m/z*): calculated for $C_{19}H_{13}Cl_3N_2OS$ = 421.98, found = 423.06 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.8. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(4-(dimethylamino)-benzylidene)thiazolidin-4-one (**11**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 4-(dimethylamino)benzaldehyde. Physical form: yellowish orange needle crystals; Yield = 88.5%; m.p. = 150-152 ºC; IR, cm^{-1}: 3057 (CH aromatic), 2963, 2852 (CH aliphatic), 1707 (C=O), 1622 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 2.96 (s, 6H, $N(CH_3)_2$), 4.45 (d, J = 5.2 Hz, 2H, N-CH_2-), 5.15-5.23 (m, 2H, -CH=C<u>H_2</u>), 5.87-5.98 (m, 1H, -C<u>H</u>=CH_2), 6.77 (d, J =

9.2 Hz, 2H, aromatic), 7.02 (d, *J* = 8.4 and 2.4 Hz, 1H, aromatic), 7.29 (d, J= 2.4 Hz, 1H, aromatic), 7.37 (d, *J* = 8.8 Hz, 2H, aromatic), 7.64 (d, *J* = 8.4 Hz, 1H, aromatic), 7.66 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 40.66 (2C, N(CH_3)$_2$), 44.91 (N-CH_2-), 112.51 (2C) and 113.23 (aromatic), 117.53 (-CH=<u>C</u>H_2), 120.42 (S-<u>C</u>=CH), 122.22, 123.61, 127.08, 131.82, 131.88, 132.12, 132.44 (2C), 132.63, 148.78, 151.77, 152.45, 166.18 (C=O); LC-MS (*m/z*): calculated for $C_{21}H_{19}Cl_2N_3OS$ = 431.06, found = 432.15 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.9. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(4-nitrobenzylidene)-thiazolidin-4-one (**12**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 4-nitrobenzaldehyde. Physical form: dark yellow flakes; Yield = 85.4%; m.p. = 150-152 ºC; IR, cm^{-1}: 3083 (CH aromatic), 2968, 2928 (CH aliphatic), 1712 (C=O), 1646 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.50 (d, *J* = 5.2 Hz, 2H, N-CH_2-), 5.18-5.229 (m, 2H, -CH=C<u>H$_2$</u>) , 5.89-6.00 (m, 1H, -C<u>H</u>=CH_2), 7.03 (dd, *J* = 8.4 and 2.4 Hz, 1H, aromatic), 7.31 (d, *J* = 2.4 Hz, 1H, aromatic), 7.66 (d, *J* = 8.4 Hz, 1H, aromatic), 7.81 (d, *J* = 8.8 Hz, 2H, aromatic), 7.90 (s, 1H, S-C=C<u>H</u>), 8.29 (d, *J* = 8.8 Hz, 2H, aromatic); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.39 (N-CH_2-), 117.88 (-CH=<u>C</u>H_2), 122.04 (S-<u>C</u>=CH), 123.43, 124.76 (2C), 125.54, 127.60, 128.97, 131.33 (2C), 131.48, 131.94, 132.25, 139.83, 147.76, 148.11, 151.18 (C=N), 165.51 (C=O); LC-MS (*m/z*): calculated for $C_{19}H_{13}Cl_2N_3O_3S$ = 433.01, found = 434.10 $[M+H]^+$; HPLC purity %: 98.47%.

2.1.4.10. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(3-(trifluoromethyl)-benzylidene)thiazolidin-4-one (**13**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 3-(trifluoromethyl)benzaldehyde. Physical form: pale yellow needle crystals; Yield = 52.6%; m.p. = 150-152 ºC; IR, cm^{-1}: 3061 (CH aromatic), 2980, 2935 (CH aliph.), 1711 (C=O), 1639 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.49 (d, *J* = 5.2 Hz, 2H, N-CH_2-), 5.18-5.27 (m, 2H, -CH=C<u>H$_2$</u>), 5.89-6.00 (m, 1H, -C<u>H</u>=CH_2), 7.03 (dd, *J* = 8.8 and 2.4 Hz, 1H, aromatic), 7.31 (d, *J* = 2.4 Hz, 1H, aromatic), 7.66 (d, *J* = 8.4 Hz, 1H, aromatic), 7.69-7.81 (m, 3H, aromatic), 7.93 (s, 1H, S-C=CH), 8.00 (s, 1H, aromatic); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.32 (N-CH_2-), 117.84 (-CH=<u>C</u>H_2), 122.03 (S-<u>C</u>=CH), 123.28, 123.48, 126.81 (q, J= 3.9 Hz, aromatic), 126.92 (q, J= 269.8 Hz, -CF_3), 127.52, 127.93 (q, J= 3.9 Hz), 129.95, 130.37 (q, J= 32.2 Hz, <u>C</u>-CF_3), 131.00, 131.55, 131.91, 132.22, 132.60, 134.69, 148.12, 151.26 (C=N), 165.58 (C=O); LC-MS (*m/z*): calculated for $C_{20}H_{13}Cl_2F_3N_2OS$ = 456.01, found = 457.12 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.11. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(2,4-dimethoxy-benzylidene)thiazolidin-4-one (**14**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 2,4-dimethoxybenzaldehyde. Physical form: canary yellow flakes; Yield = 69.8%; m.p. = 123-124 ºC; IR, cm^{-1}: 3017 (CH aromatic), 2933, 2857 (CH aliphatic), 1707 (C=O), 1628 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 3.79 (s, 3H, 4-OCH_3), 3.86 (s, 3H, 2-OCH_3), 4.45 (d, *J* = 5.2 Hz, 2H, N-CH_2-), 5.16-5.23 (m, 2H, -CH=C<u>H$_2$</u>), 5.5.87-5.98 (m, 1H, -C<u>H</u>=CH_2), 6.62-6.68 (m, 2H, aromatic), 7.01 (dd, *J* = 8.6, 2.6 Hz, 1H, aromatic), 7.25-7.30 (m, 2H, aromatic), 7.63 (d, *J* = 8.4 Hz, 1H, aromatic), 7.92 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.01 (N-CH_2-), 56.04 (4-OCH_3), 56.42 (2-OCH_3), 99.05, 107.01 and 114.83 (aromatic), 117.47, 117.65, 122.14, 123.55, 126.08, 127.21, 130.33, 131.74, 131.83, 132.13, 148.53, 152.24 (S-C=N), 160.10 (2-OCH_3-<u>C</u>), 163.33 (4-OCH_3-<u>C</u>), 166.09 (C=O); LC-MS (*m/z*): calculated for $C_{21}H_{18}Cl_2N_2O_3S$ = 448.04, found = 449.16 $[M+H]^+$; HPLC purity %: 100%.

2.1.4.12. 3-Allyl-5-(3,4-dichlorobenzylidene)-2-((3,4-dichlorophenyl)imino)-thiazolidin-4-one (**15**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 3,4-dichlorobenzaldehyde. Physical form: pale buff flakes; Yield = 55.7%; m.p. = 143-144 ºC; IR, cm^{-1}: 3072 (CH aromatic), 2980, 2935 (CH aliphatic), 1714 (C=O), 1635 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.48 (d, *J* = 5.2 Hz, 2H, N-CH_2-), 5.17-5.27 (m, 2H, -CH=C<u>H$_2$</u>), 5.88-5.99 (m, 1H, -C<u>H</u>=CH_2), 7.02 (dd, *J* = 8.8 and 2.4 Hz, 1H, aromatic), 7.30 (d, *J* = 2.4 Hz, 1H, aromatic), 7.48 (dd, *J* = 8.4 and 2.0 Hz, 1H, aromatic), 7.66 (d, *J* = 8.4 Hz, 1H, aromatic), 7.73 (d, *J* = 8.4 Hz, 1H, aromatic), 7.79 (s, 1H, S-C=C<u>H</u>), 7.90 (d, *J* = 2.0 Hz, 1H, aromatic); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.33 (N-CH_2-), 117.85 (-CH=<u>C</u>H_2), 122.01 (S-C=<u>C</u>H), 123.39, 123.48, 127.54, 128.97, 128.99, 131.53, 131.93 (2C), 132.24, 132.40, 132.84, 132.94, 134.29, 148.13, 151.17 (C=N), 165.54 (C=O); LC-MS (*m/z*): calculated for $C_{19}H_{12}Cl_4N_2OS$ = 455.94, found = 457.05 $[M+H]^+$; HPLC purity %: 98.41%.

2.1.4.13. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(3,4,5-trimethoxy-benzylidene)thiazolidin-4-one (**16**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 3,4,5-trimethoxybenzaldehyde. Physical form: canary yellow needle crystal; Yield = 43.7%; m.p. = 102-104 ºC; IR, cm^{-1}: 3063 (CH aromatic), 2930, 2833 (CH aliphatic), 1707 (C=O), 1629 (C=N); ^{1}H-NMR

(DMSO-d_6, δ [ppm]): 3.69 (s, 3H, 4-OCH_3), 3.75 (s, 6H, 3,5-dimethoxy), 4.48 (d, J= 5.2 Hz, 2H, N-CH_2-), 5.19-5.25 (m, 2H, -CH=C<u>H</u>$_2$), 5.88-5.99 (m, 1H, -C<u>H</u>=CH_2), 6.86 (s, 2H, aromatic), 7.05 (dd, *J* = 8.4 and 2.4 Hz, 1H, aromatic), 7.33 (d, *J* = 2.4 Hz, 1H, aromatic), 7.63 (d, *J* = 8.4 Hz, 1H, aromatic), 7.76 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.17 (N-CH_2-), 56.55 (2C, 3,5-dimethoxy), 60.66 (4-OCH_3), 108.05 (2C, aromatic), 117.70 (-CH=<u>C</u>H_2), 120.13 (S-<u>C</u>=CH), 122.12, 123.63, 127.34, 129.19, 131.66, 131.79, 131.95, 132.13, 139.89, 147.96, 151.50, 153.62 (2C, (3,5-dimethoxy)<u>C</u>$_2$), 165.69 (C=O); LC-MS (*m/z*): calculated for $C_{22}H_{20}Cl_2N_2O_4S$ = 478.05, found = 479.13 $[M+H]^+$; HPLC purity %: 99.02%.

2.1.4.14. 3-Allyl-2-((3,4-dichlorophenyl)imino)-5-(thiophen-2-ylmethylene)-thiazolidin-4-one (**17**)

The title compound was synthesized according to the general procedure by the reaction of compound **3** with 2-thiophenecarboxaldehyde. Physical form: canary yellow flakes; Yield = 78.4%; m.p. = 117-118 ºC; IR, cm^{-1}: 3076 (CH aromatic), 2937, 2889 (CH aliphatic), 1710 (C=O), 1631 (C=N); ^{1}H-NMR (DMSO-d_6, δ [ppm]): 4.47 (d, J= 5.2, 2H, N-CH_2-), 5.16-5.25 (m, 2H, -CH=C<u>H</u>$_2$), 5.87-5.99 (m, 1H, -C<u>H</u>=CH_2), 7.04 (dd, *J* = 8.8 and 2.4 Hz, 1H, aromatic), 7.24 (dd, *J* = 5.0 and 3.8 Hz, 1H, aromatic), 7.32 (d, *J* = 2.4 Hz, 1H, aromatic), 7.64 (d, *J* = 3.6 Hz, 1H, aromatic), 7.67 (d, *J* = 8.8 Hz, 1H, aromatic), 7.91 (d, *J* = 4.8 Hz, 1H, aromatic), 8.06 (s, 1H, S-C=C<u>H</u>); ^{13}C-NMR (DMSO-d_6, δ [ppm]): 45.29 (N-CH_2-), 117.72 (-CH=<u>C</u>H_2), 118.44 (S-<u>C</u>=CH), 122.10, 123.54, 124.93, 127.44, 129.35, 131.65, 131.90, 132.19, 133.20, 134.71, 137.64, 148.38, 151.37 (C=N), 165.59 (C=O); LC-MS (*m/z*): calculated for $C_{17}H_{12}Cl_2N_2OS_2$ = 393.98, found = 395.03 $[M+H]^+$; HPLC purity %: 97.82%.

2.2. Irradiation of the synthesized compounds

The tested compounds, in solid form, were collected in polypropylene vials and wrapped with aluminum sheet. The vials were subjected to γ-radiation dose of 25 kGy. γ-Irradiation was performed by using a ^{60}Co source, with a dose rate of 1.119 kGy/h, utilizing Indian-Gamma Cell (Ge 4000 A).

2.3. Biological evaluation

2.3.1. Screening against bacterial and fungal strains

The antimicrobial activity was tested against 5 bacterial strains (*Staphylococcus aureus* (MRSA) ATCC 43300, *E. coli* ATTCC 25922, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606) and 2 fungal strains (*Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 208821). The antimicrobial screening was performed by the Community for Antimicrobial Drug Discovery (CO-ADD), funded by the Wellcome Trust (United Kingdom) and University of Queensland (Australia).

2.3.2. Inhibition of biofilm formation

The synthesized compounds were evaluated for their biofilm inhibition activity in a microtiter format utilizing a reported procedure [33]. Overnight culture of *S. aureus* (HG001) was diluted 1:100 in 0.5X Tryptic soy broth appended with 1% glucose (culture medium). Compounds' solutions in DMSO and bacterial suspensions were added in the wells of the 96-well plates. The final concentration of DMSO in each well would be ≤ 3%. The covered plates were incubated at 37 °C for 24 hours in the incubator. The optical density of the overall bacterial growth was measured spectrophotometrically at 630 nm for each well. The plates wells were washed by using a washer device (Biotek ELx50) after the removal of the bacterial suspension. 50 µL of watery 0.06% (w/v) crystal violet solution to each well to stain the attached biofilm. The solution was removed, and the wells were washed three times with distilled water and dried, followed by the addition of 200 µL of acetic acid (30%) in each well to elute crystal violet. Direct quantification of the biofilm by using microplate reader (Biotek ELx800) at 630 nm. The data are presented as per cent inhibition of *S. aureus* (HG001) biofilm for the synthesized compounds prorated to the negative control (DMSO).

2.3.3. Anticancer evaluation

Five compounds (**4**, **11**, **13**, **14** and **17**) were selected by the National Cancer Institute (NCI), Bethesda, MD, USA, for evaluating their anticancer activity. The selected compounds were subjected to a primary *in vitro* one-dose (10 µM) assay against 59 human tumor cell lines. The cell lines used in the screening included leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer cells.

2.3.4. Cytotoxicity evaluation against murine fibroblast (L929) cells

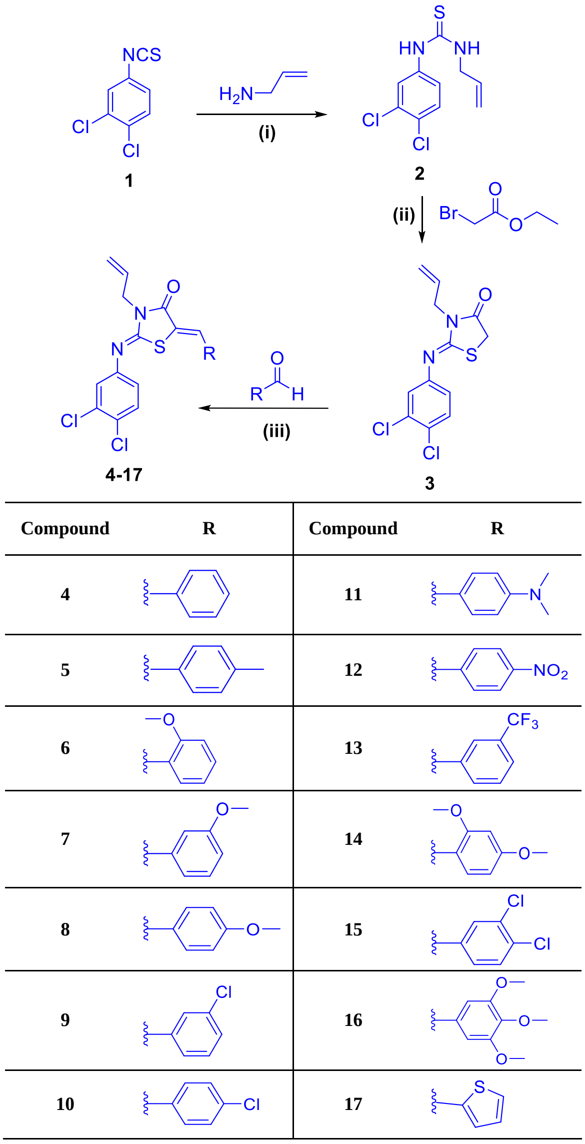
The compounds were screened for their cytotoxicity using the alamarBlue reagent [ThermoFischer Scientific], a resazurin based solution to measure cell viability. L929 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1.56*104 cells/mL was seeded in 96-well cell culture plates with a total volume of 60 µL. The plates with cells were incubated for 24 h at 37 °C and 10 % CO_2. Subsequently, 0.6 µL of compound solutions were added per well (for final a concentration of 100 µM). The plate was placed on a plate shaker for 15 seconds at 1000 rpm to ensure the optimal mixing. After 72 h of incubation at 37 °C and 10 % CO_2, 5 µL of alamarBlue reagent was added to each well. The fluorescence intensity of each well was determined after 2.5 h of incubation at 37 °C and 10 % CO_2, using an extinction wavelength of (λmax, abs= 540 nm) and an emission wavelength of 600 nm (λmax, em= 600 nm). For data analysis, the mean value and standard deviation were determined from three replicates. DMSO and water were used as vehicle controls; cells without compounds and pure medium without cells and compounds as negative controls. The final concentration of DMSO in each well did not exceed 1%. Compounds **3**, **5**, **8**, **11**, **14**, **16** and **17** were further tested at concentrations of 50, 30, 12.5, 5, 1.3 and 0.4 µM.

3. Results and discussion

3.1. Chemistry

The synthetic route of compounds **2-17** is illustrated in Scheme 1. The reported thiourea derivative **2** was obtained by nucleophilic addition reaction of 3,4-dichlorophenyl-isothiocyanate (**1**) with allylamine, according to the reported method [24, 31, 32]. The next step was S_N2 nucleophilic substitution rection of the thiourea derivative (**2**) with ethyl bromoacetate, catalyzed by the presence of sodium acetate anhydrous, followed by intramolecular cyclization reaction to afford the previously reported thiazolidinone derivative (**3**) [32, 34]. The preparation of thiazolidinone derivative **3** was performed in glacial acetic acid, or in absolute ethanol where the reaction yield was higher. The target 5-ene-4-thiazolidinones (**4-17**) were obtained as pure crystals through a Knoevenagel condensation reaction of the active methylene (C5) of compound **3** with the carbonyl group of the corresponding aldehyde in a mixture of ethanol and piperidine (30:1) [35].

IR spectroscopy, ^{1}H-NMR, ^{13}C-NMR and LC-MS were used to confirm the structures of the target compounds. FT-IR spectra of compounds (**4-17**) revealed a shift in the value of the carbonyl bands from 1760 cm^{-1} to a range of 1707-1717 cm^{-1}. This bathochromic shift is due to the conjugation of the carbonyl group with the arylidene moiety [36]. ^{1}H-NMR spectra of compounds **4-17** showed signals for the one olefinic proton (S-C=CH) at δ ranging from 7.6 to 8 ppm, which confirms the occurrence of the reaction between compound **3** and the corresponding aldehydes. Therefore, the exocyclic C=C bond of the synthesized thiazolidinone derivatives (**4-17**) was confirmed to be in *Z*-configuration by those singlet signals of olefinic protons of all derivatives, which resonate at higher chemical shifts due to the deshielding effect of the adjacent carbonyl group. Otherwise, the olefinic proton would resonate towards the right at δ value less than 6.64 ppm if it were in *E*-configuration [37,38].



Scheme 1. Synthesis of compounds **2-17**. Reagents and conditions: **(i)** Toluene, room temperature, 1 h or ethanol, reflux, 6 h **(ii)** Sodium acetate anhydrous, ethyl bromoacetate, ethanol or glacial acetic acid, reflux, 20 h **(iii)** Ethanol/piperidine (30:1), reflux 2-12 h.

The purity and the molecular masses of the synthesized compounds were tested by LC-MS analyses. With the purpose of evaluating the chemical stability of the thiazolidinone derivatives (**3-17**), four compounds (**3**, **11**, **13** and **14**) were selected and exposed to γ-irradiation at single dose of 25 kGy [39-42]. The physical and the chemical properties of these tested compounds, in their solid state, were studied before and after the irradiation process. The results revealed no changes in the physical and the chemical properties, including the color, form and solubility. Thin layer chromatography (TLC) experiments showed no change in the R_f values of the tested compounds and no additional spots were observed. In addition, LC-MS experiments were performed for the four tested compounds (**3**, **11**, **13** and **14**), before and after irradiation. The results of the LC-MS experiments revealed no change in the mass nor in the purity of any of the tested compounds, which indicates the chemical stability of the synthesized compounds.

3.2. Biological evaluation

All the target compounds (**3-17**) were tested for their *in vitro* antibacterial activity against five pathogenic bacterial strains (Table 1). The used bacterial strains included the Gram-positive multi-drug resistant *S. aureus* (MRSA) ATCC 43300, and the Gram-negative bacteria *E. coli* ATTCC 25922, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853 and *A. baumannii* ATCC 19606. Compounds **5**, **7**, **14** and **16** exhibited a slight antibacterial activity against MRSA, with a growth inhibition percentage of 21%, 24%, 26% and 23% at a concentration of 32 μg/mL, respectively. It was clearly observed that the presence of a methyl group (compound **5**) and methoxy groups (in compounds **7**, **14** and **16**), in the meta and para positions, enhanced the antibacterial activity of the tested compounds against MRSA. The other compounds showed lower activity against the used Gram-positive and Gram-negative bacteria (Table 1).

The synthesized 1,3-thiazolidine-4-one derivatives (**3-17**) were also evaluated for their antifungal activity against *C. albicans* ATCC 90028 and *C. neoformans* var. *grubii* H99; ATCC 208821 (Table 1). Compound **3** exhibited a potent *in vitro* antifungal activity against *C. neoformans*, with a full growth inhibition (100% inhibition), at a concentration of 32 μg/mL. The other tested compounds displayed weak or no antifungal activity against the tested fungal strains. The results of the antifungal screening confirmed that the unsubstituted C5 of the thiazolidinone ring is essential for the antifungal activity against *C. neoformans*. Insertion of substitutions on C5 diminished the antifungal activity.

Table 1. Antibacterial, antifungal and antibiofilm activities of the synthesized compounds

Compound	**Antibacterial activity** Growth inhibition percent (GI%) of bacterial strains, at a concentration of 32 μg/mL					**Antifungal activity** Growth inhibition percent (GI%) of fungal strains, at a concentration of 32 μg/mL		**Anti-biofilm activity** Inhibition percent of biofilm formation of *S. aureus* HG001	
	MRSA ATCC 43300	*E. coli* ATTCC 25922	*K. pneumniae* ATCC 700603	*P. aeruginosa* ATCC 27853	*A. baumannii* ATCC 19606	*C. albicans* ATCC 90028	*C. neoformans* Var. *grubii* H99; ATCC 208821	32 μg/mL	64 μg/mL
3	<10	<10	<10	<10	<10	10	100	<10	<10
4	15	14	11	11	<10	12	19	<10	<10
5	21	17	11	13	<10	<10	18	<10	<10
6	16	12	<10	11	<10	<10	18	<10	<10
7	24	18	<10	13	<10	<10	17	<10	22
8	19	15	16	<10	<10	<10	13	<10	<10
9	<10	<10	<10	<10	<10	<10	<10	<10	19
11	<10	12	<10	10	<10	<10	<10	<10	13
12	18	16	11	<10	13	<10	<10	35	54
13	10	<10	<10	<10	<10	10	<10	<10	26
14	26	16	13	15	22	<10	<10	<10	<10
15	17	16	20	<10	<10	<10	<10	<10	<10
16	23	20	19	12	16	<10	<10	<10	<10

S. aureus belongs to "ESKAPE" pathogens; pathogenic bacteria with the highest impact in bacterial resistance [43]. In many cases, persistent infections caused by *S. aureus* are due to its high capability to resist antibiotics via biofilm formation [44]. Therefore, new molecules capable of defeating bacterial biofilm are needed to overcome resistant infections. The ability of the synthesized compounds to inhibit the biofilm formation of *S. aureus* HG001 was also evaluated. Among the tested compounds, the 3-allyl-2-iminothiazolidin-4-one derivative **12** inhibited biofilm formation with percentages of 35% and 54% at tested concentrations of 32 and 64 µg/mL, respectively (Table 1).

Five 5-(arylidene)thiazolidin-4-one derivatives (compounds **4**, **11**, **13**, **14** and **17**) were selected by the NCI (National Cancer Institute - Developmental Therapeutic Program), to be evaluated for their *in vitro* anticancer activity. The five selected compounds were tested against a panel of 59 human-cancer cell lines, including leukemia, non-small cell lung cancer, ovarian cancer, CNS cancer, renal cancer, melanoma, prostate cancer, colon cancer and breast cancer cells (Table S1 in the supplementary file). Out of the used 59 cell lines, the five tested compounds exhibited anticancer activity against 18 cell lines, as presented in Table 2. The 5-(4-dimethyl-aminobenzylidene)-thiazolidin-4-one derivative **11** was the most active compound. Compound **11**, bearing a dimethylamino moiety, inhibited the growth of MDA-MB-435, UACC-62 and MCF7 cells with growth inhibition values of 73%, 45% and 42%, respectively, at concentration of 10 µM. The other tested compounds showed weaker anticancer activity, with growth inhibition values ranging from < 20% up to 47%.

Cytotoxicity of compounds **3**-**17** was tested against murine fibroblast L929 cells. The test was performed at a concentration of 100 µM with an incubation period of 3 days. Cell viability was measured using alamarBlue reagent method. Only compounds **3**, **5**, **8**, **11**, **14**, **16** and **17** showed slight cytotoxicity at the tested concentration (100 µM), while the other tested compounds did not show cytotoxicity to murine fibroblast L929 cells. Compounds **3**, **5**, **8**, **11**, **14**, **16** and **17** were further tested to calculate their IC_{50} values. No significant cytotoxicity was observed for the tested compounds (IC_{50} values ranging from 80.32 to >250 µM), which suggests their relative safety on fibroblasts (Table 3).

Table 2. Growth inhibition percent (GI%) of human-cancer cell lines, at a concentration of 10 µM

Cell line		Compound				
		4	**11**	**13**	**14**	**17**
Leukaemia	SR	<20	29	<20	<20	<20
Non-Small Cell Lung Cancer	HOP-92	<20	25	20	<20	<20
	NCI-H522	<20	32	23	<20	24
Colon Cancer	HCT-116	<20	<20	32	<20	<20
	HCT-15	<20	26	28	<20	<20
	HT29	<20	<20	33	<20	20
	KM12	<20	20	<20	<20	<20
	SW-620	<20	22	<20	<20	<20
CNS Cancer	SNB-75	<20	<20	<20	25	<20
Melanoma	MALME-3M	<20	24	<20	<20	<20
	MDA-MB-435	<20	73	<20	<20	<20
	UACC-62	21	45	31	20	25
Renal Cancer	A498	<20	35	<20	<20	30
	UO-31	24	31	<20	22	<20
Prostate Cancer	PC-3	<20	23	<20	<20	<20
Breast Cancer	MCF7	<20	42	47	<20	20
	BT-549	<20	22	<20	<20	<20
	T-47D	24	<20	<20	<20	<20

Table 3. Cytotoxicity against murine fibroblast (L929) cells

Compound	Growth inhibition % at 50 µM	Calculated IC_{50} in µM
3	34.25	94.3
5	10.62	214.14
8	25.41	80.32
11	8.89	>250
14	8.29	146.6
16	26.08	95.61
17	14.4	225.9

4. Conclusion

We report the synthesis of new halogenated 3-allyl-2-iminothiazolidin-4-ones (**4**-**17**). The synthesized compounds were subjected to antibacterial, antifungal, anti-biofilm formation and anticancer screening. Four compounds (**5**, **7**, **14** and **16**) exhibited slight antibacterial activity. The common feature of the four compounds **5**, **7**, **14** and **16** was the presence of methyl or methoxy groups in the meta or the para positions of the benzylidene group. Compound **3**, with no substitution at C5 of the 1,3-thiazolidine-4-one, caused a complete inhibition of the growth of the fungal pathogen *C. neoformans*. The antifungal activity of compound **3** proves that unsubstituted C5 is important for the antifungal activity. It was also clearly observed that compound **11**, with a dimethylamino group at the para position, has a remarkable *in vitro* anticancer activity. Melanoma cells were the most sensitive cancer cells to compound **11**, followed by breast cancer cells. The other tested compounds showed lower anticancer activity. Moreover, all the synthesized thiazolidinone derivatives did not show significant cytotoxicity to murine fibroblast cells. Taken together, the results of this study showed a clear relationship between the structures and the biological activity of the tested compounds. Consequently, these results could be a guide to synthesize more 1,3-thaizolidin-4-one derivatives, in order to develop new more active compounds.

5. Acknowledgement

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6. Conflict of interest

The authors declare no conflict of interest.

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