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Antimicrobial activity of some novel 2-(2-iodophenylimino)-5-arylidenethiazolidin-4-one derivatives

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Abstract

Background: Infectious diseases, especially those caused by multidrug-resistant bacteria, are becoming a serious problem worldwide because of the lack of effective therapeutic agents. Moreover, most antifungal drugs exhibit low efficacy and high toxicity because of the similarity between fungal and human cells. These issues warrant the search for potential new agents.

Objectives: To synthesize potent 2-(2-iodophenylimino)-5-arylidenethiazolidin-4-one derivatives, improve the synthetic process, elucidate their structures, and determine their antimicrobial activity.

Methods: 2-Iodoaniline was used as an initial reactant in a 3-step process for the synthesis of 2-(2-iodophenylimino)-5-arylidenethiazolidin-4-one derivatives, including an acylation reaction, a cyclization reaction, and aldol condensation reactions. The structures of the obtained derivatives were investigated and elucidated using spectral methods. Antimicrobial activity toward 5 bacterial strains and 2 fungal strains was determined using Kirby–Bauer and agar dilution methods.

Results: We successfully synthesized 12 novel compounds and elucidated their structures. The derivatives had no antifungal activities. By contrast, they showed remarkable antibacterial activities. Some of them with minimum inhibitory concentrations (MICs) ≤8 μg/mL in both *Staphylococcus aureus* and methicillin-resistant *S. aureus*.

Conclusions: A simple and flexible way to synthesize new compounds with a thiazolidin-4-one ring was determined. Some of these new compounds have outstanding effects with low MICs for bacteria. Their further investigation as therapeutic agents is warranted.

Keywords: antimicrobial, derivatives, MRSA, synthesis, thiazolidin-4-one

Infectious diseases are among the most dangerous and deadly diseases worldwide. This is highlighted by the emergence of the *Staphylococcus aureus* superbug, a multidrug-resistant strain that can resist vancomycin, an antibiotic of last resort [1]. The ineffectiveness and toxicity of antifungal drugs, because of the similarity between fungal and mammalian cells, warrant the search for potential new agents [2, 3].

Thiazolidin-4-one is a 5-membered heterocyclic ring structure including sulfur and nitrogen located at positions 1 and 3, respectively, and a carbonyl group on the fourth carbon. This

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structure has been acknowledged as a powerful pharmacophore because of its therapeutic effects on almost all kinds of diseases [4]. Some notable actions of thiazolidin-4-one are antiproliferative [5], anti-HIV [6], antioxidant [7], anticonvulsant [8], and antimicrobial [9–13] effects. An aryl substitution at position 5 of thiazolidin-4-one can dramatically increase the potency of pharmacological effects [4]. Related derivatives including 5-arylidene-2-imino-4-thiazolidinones have respectable activities on both bacteria (*S. aureus*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*, *Aspergillus niger*) [9]. To our knowledge, none of the studies conducted so far have investigated the antimicrobial effect of thiazolidin-4-one derivatives with a halogenic substitution. Halogen atoms, when incorporated into heterocyclic rings (i.e., azole), may bind to the cell wall or membranes of microbes via hydrogen or covalent bonds and can consequently destroy the microbes [14, 15].

Chavan and Pai suggested a simple method for synthesizing a 5-arylidenethiazolidin-4-one ring from initial aromatic amine derivatives [9]. However, the yield of their products was <70%. By adopting their procedure, we aimed to modify the synthetic process to increase the yield.

The objective of this study was to synthesize the 2-(2-iodophenylimino)-5-arylidenethiazolidin-4-one derivatives from 2-iodoaniline, improve the synthetic procedures, and evaluate the antimicrobial activities of the products.

Materials and methods

Materials

Reagents: 2-iodoaniline, chloroacetic acid, K_2CO_3, potassium thiocyanate (KSCN), ammonium thiocyanate (NH_4SCN), dimethylamine, triethylamine, and aromatic aldehydes (4-methoxybenzaldehyde; 2,4-dichlorobenzaldehyde; 4-chlorobenzaldehyde; 4-fluorobenzaldehyde; 4-methylbenzaldehyde; 4-nitrobenzaldehyde; 2-nitrobenzaldehyde; 2-hydroxybenzaldehyde; 4-hydroxy-3-methoxybenzaldehyde; benzaldehyde; 5-bromo-2-hydroxybenzaldehyde; and 4-dimethylaminobenzaldehyde) were reagent grade or better and imported from Sigma-Aldrich (Singapore).

Solvents: ethanol, dimethylformamide (DMF), glacial acetic acid, toluene, ethyl acetate, methanol, chloroform, dioxane, and dimethylsulfoxide (DMSO) were purchased from Merck (USA) and were laboratory grade or better.

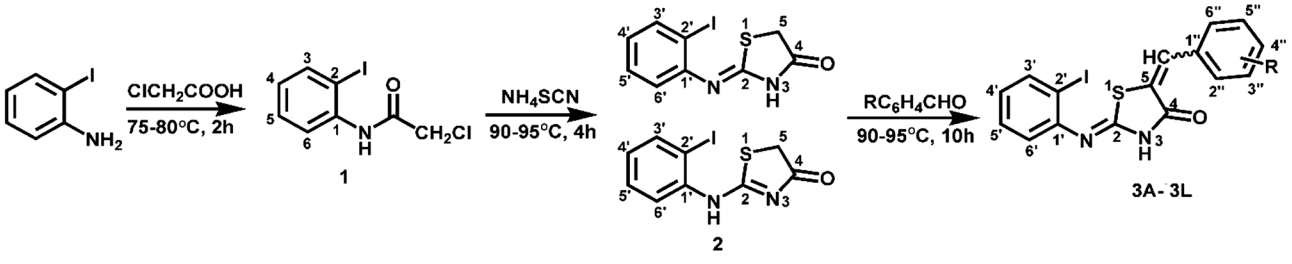
Test medium and positive reference: Mueller–Hinton agar (MHA) was purchased from Sigma-Aldrich, and cephalexin with a purity of >99% was purchased from DHG (Vietnam).

Bacterial strains: *Escherichia coli* American Type Culture Collection (ATCC) 25922, *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and MRSA ATCC 43300 were imported from ATCC (USA).

Fungal strains: *C. albicans* ATCC 10231 and *A. niger* ATCC 16404 were imported from ATCC.

Chemical synthesis

The synthetic process was modified from a previous study and is shown in **Scheme 1** [9]. Briefly, the first step was the acylation of 2-iodoaniline (0.1 mol) in 50 mL DMF and chloroacetic acid (0.3 mol), using thionyl chloride as a coreagent, with or without K_2CO_3, at 75–80°C for 2 h to make **1**. Then, a refluxed cyclization reaction of **1** (0.1 mol) and KSCN or NH_4SCN (0.1 mol) was conducted in ethanol at 90–95°C for 4 h to make **2**. The final step was the aldol condensation reaction of **2** (0.01 mol) and aromatic aldehydes (0.03 mol) in glacial acetic acid using dimethylamine or triethylamine as a catalyst at 90–95°C for 10 h to make **3A**–**3L**. The products from each step were purified by solvent recrystallization with a mixture of ethanol and water (ratio was dependent on the compound). Thin-layer chromatography (TLC; silica gel 60 GF_{254} from Sigma-Aldrich) was used to follow the reactions with mobile phase consisted of toluene–ethyl acetate–methanol (5:4:1 v:v:v).



Scheme 1. Synthesis process for 2-iodophenylimino-5-arylidenethiazolidin-4-one. The initial reactant, 2-iodoaniline, underwent the first acylation with chloroacetic acid, a second cyclization with ammonium thiocyanate, and a final aldol condensation with various aromatic aldehydes.

Purity testing

All compounds synthesized (**1**, **2**, **3A**–**3L**) were analyzed by TLC (silica gel 60 GF_{254} from Sigma-Aldrich) in 4 different solvent mixtures with various polarities, including (1) toluene–ethyl acetate–methanol (5:4:1 v:v:v); (2) ethyl acetate–ethanol (8:2 v:v); (3) toluene–dioxane (7:3 v:v); and (4) chloroform–ethanol (7:3 v/v) to test their purities before conducting other experiments. The mobile phase conditions were developed by our group based on the retardation factor (R_f) values of investigated compounds. The 4 systems were chosen because of their diverse polarity, and hence, gave R_fs ranging from 0.2 to 0.8, which were suitable for early screening of compounds' purities.

Structure elucidation

The melting points (mps) were measured using an open capillary method with Stuart SMP3 apparatus (Barloworld Scientific, UK). Infrared (IR) spectra were obtained on an FTIR Bruker Alpha T spectrophotometer (Bruker, USA), using a KBr pellet technique. Ultraviolet (UV) spectra were obtained on a UV U2800 spectrophotometer (Hitachi, Japan). Nuclear magnetic resonance (NMR) spectra were obtained using an AV 500 MHz NMR spectrometer (Bruker, USA), with tetramethylsilane as an internal standard and deuterated DMSO (DMSO-d_6) as a solvent. Mass spectra (MS) and molecular weight were obtained using Fourier transform ion cyclotron resonance (FT-ICR) apparatus (Perkin-Elmer, USA) at room temperature with an electrospray ionization (ESI) method. Elemental analysis was conducted on a Perkin-Elmer 2400 CHNS/O elemental analyzer (USA). Structures of the compounds are given in **Table 1**.

Compound **1**: Yield 75%, white crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 105–107°C; UV λ_{max} 227.5 nm; IR (KBr) ν_{max} (cm^{-1}): 3245.4 (NH amide), 1669.1 (C=O amide), 1576.1, 1535.4, 1434.1 (C=C benzene), 568.3 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 9.76 (s, 1H: N*H*), 7.90 (d, *J* = 7.5 Hz, 1H: H-6), 7.48 (d, *J* = 8 Hz, 1H: H-3), 7.41 (t, *J* = 7.5 Hz, 1H: H-5), 7.02 (t, *J* = 7.5 Hz, 1H: H-4), 4.33 (s, 2H: CH_2); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 164.9 (C=O), 139.0 (C-3), 138.7 (C-1), 128.8 (C-5), 128.0 (C-4), 126.8 (C-6), 96.0 (C-2), 43.0 (*C*H_2Cl); ESI-MS *m/z* [M – H]$^-$ 293.89 C_8H_7-ONICl (calcd 294.93); Anal. Calcd (%): C, 32.52; H, 2.39; N, 4.74. Found: C, 32.50; H, 2.40; N, 4.73.

Compound **2**: Yield 70%, white crystals, odorless, insoluble in water, soluble in ethanol, chloroform, methanol, acetone, DMF, DMSO; mp 163–165°C; UV λ_{max} 228 nm; IR (KBr) ν_{max} (cm^{-1}): 2788.5 (NH lactam), 1725.8 (C=O lactam), 1625.7 (C=N imine), 1460.3, 1400.9, 1316.4 (C=C benzene), 506.8 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 11.99 (s, 1H: N*H*), 7.85 (d, *J* = 8 Hz, 1H: H-6′), 7.36 (t, *J* = 8 Hz, 1H: H-5′), 6.96 (d, *J* = 7.5 Hz, 1H: H-3′), 6.88 (t, *J* = 7.5 Hz, 1H: H-4′), 3.99 (s, 2H: CH_2); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 173.9 (C=O), 158.1 (C-2), 149.8 (C-1′), 138.9 (C-3′), 129.3 (C-4′), 126.0 (C-5′), 121.0 (C-6′), 92.9 (C-2′), 34.3 (C-5); ESI-MS *m/z* [M – H]$^-$ 316.92 $C_9H_7ON_2SI$ (calcd 317.93); Anal. Calcd (%): C, 33.98; H, 2.22; N, 8.81; S, 10.08. Found: C, 33.99; H, 2.21; N, 8.81; S, 10.06.

Compound **3A**: Yield 88%, yellow crystals, odorless, insoluble in water, soluble in ethanol, methanol, acetone, DMF, DMSO; mp 195–196°C; UV λ_{max} 351 nm; IR (KBr) ν_{max} (cm^{-1}): 2992.3 (NH lactam), 1701.5 (C=O lactam), 1645.8 (C=N imine), 1595.8 (C=C benzene), 528.4 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.56 (s, 1H: N*H*), 7.90 (d, *J* = 7 Hz, 1H: H-6′), 7.62 (s, 1H: C*H*), 7.42 (m, 3H: H-2″, H-6″, H-4′), 7.06 (d, *J* = 7.5 Hz, 1H: H-3′), 7.03 (d, *J* = 8.5 Hz, 2H: H-3″, H-5″), 6.95 (t, *J* = 7.5 Hz, 1H: H-5′); 3.77 (s, 3H: CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 160.6 (C-4″), 139.0 (C-3′), 131.7 (C-2″, C-6″), 129.8 (*C*H), 129.6 (C-5′), 126.5 (C-4′), 125.7 (C-1″), 119.6 (C-5), 114.8 (C-3″, C-5″), 55.4 (O*C*H_3); ESI-MS *m/z* [M – H]$^-$ 434.96 $C_{17}H_{13}O_2N_2SI$ (calcd 435.97); Anal. Calcd (%): C, 46.80; H, 3.00; N, 6.42; S, 7.35. Found: C, 46.84; H, 2.98; N, 6.43; S, 7.37.

Compound **3B**: Yield 91%, light yellow crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 237–238°C; UV λ_{max} 334.5 nm; IR (KBr) ν_{max} (cm^{-1}): 3136.2 (NH lactam), 1702.6 (C=O lactam), 1641.1 (C=N imine), 1601 (C=C benzene), 821.2 (C-Cl), 572.2 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.70 (s, 1H: N*H*), 7.89 (d, *J* = 8 Hz, 1H: H-6′), 7.63 (s; 1H: C*H*), 7.50 (m, 4H: H-2″, H-3″, H-5″, H-6″), 7.41 (t, *J* = 7.5 Hz, 1H: H-4′), 7.05 (d, *J* = 7.5 Hz, 1H: H-3′), 6.96 (t, *J* = 7.5 Hz, 1H: H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 139.1 (C-3′), 134.6 (C-1′), 132.2 (C-4″), 131.4 (C-2″, C-6″), 129.6 (*C*H), 129.4 (C-3″, C-5″), 128.6 (C-5′), 126.7 (C-4′), 123.6 (C-1″), 121.2 (C-6′), 92.6 (C-2′); ESI-MS *m/z* [M – H]$^-$ 438.90 $C_{16}H_{10}ON_2SICl$ (calcd 439.92); Anal. Calcd (%): C, 43.61; H, 2.29; N, 6.36; S, 7.28. Found: C, 43.64; H, 2.25; N, 6.38; S, 7.30.

Compound **3C**: Yield 84.4%, greenish yellow powder, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 234–235°C; UV λ_{max} 340.5 nm; IR (KBr) ν_{max} (cm^{-1}): 3125.2 (NH lactam), 1716.0 (C=O lactam), 1602.5 (C=N imine), 1575.9 (C=C benzene), 763.6 (C-Cl); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.82 (s, 1H: N*H*), 7.90 (d, *J* = 7.5 Hz, 1H: H-6′), 7.78 (s, 1H: C*H*), 7.75 (s, 1H: H-3″), 7.52 (d, *J* = 8.5 Hz, 1H: H-6″), 7.42 (d, *J* = 8 Hz, 1H: H-5″), 7.39 (t, *J* = 7.5 Hz, 1H: H-4′), 7.05 (d, *J* = 7.5 Hz, 1H: H-3′), 6.96 (t, *J* = 7.5 Hz, 1H: H-5′);

^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 172.9 (C-4), 139.1 (C-3′), 135.0 (C-2″), 135.0 (C-1″), 130.3 (C-4″), 129.9 (C-6″), 129.8 (C-3″), 129.6 (C-5′), 128.4 (C-4′), 126.8 (C-5′), 123.8 (C-6′); ESI-MS *m/z* [M – H]$^-$ 472.87 $C_{16}H_9ON_2SICl_2$ (calcd 473.89); Anal. Calcd (%): C, 40.45; H, 1.91; N, 5.90; S, 6.75. Found: C, 40.43; H, 1.94; N, 5.92; S, 6.75.

Compound **3D**: Yield 87.7%, orange crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 215–216°C; UV λ_{max} 365 nm; IR (KBr) ν_{max} (cm^{-1}): 3406.9 (OH phenol), 2796.6 (NH lactam), 1709.3 (C=O lactam), 1645.7 (C=N imine), 1586.2 (C=C benzene), 616.1 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.59 (s, 1H: N*H*), 9.82 (s, 1H: O*H*), 7.90 (d, *J* = 8 Hz, 1H: H-6′), 7.59 (s, 1H: C*H*), 7.41 (t, *J* = 8 Hz, 1H: H-4′), 7.14 (s, 1H: H-2″), 7.06 (d, *J* = 7.5 Hz, 1H: H-3′), 6.95 (t, *J* = 7.5 Hz, 1H: H-5′), 6.88 (m, 2H: H-5″, H-6″), 3.75 (s, 3H: CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 149.0 (C-3″), 147.9 (C-4″), 139.0 (C-3′), 130.7 (*C*H), 129.6 (C-5′), 124.7 (C-1″), 122.6 (C-6″), 116.3 (C-5″), 115.3 (C-2″), 55.7 (O*C*H$_3$); ESI-MS *m/z* [M – H]$^-$ 450.96 $C_{17}H_{13}O_3N_2SI$ (calcd 451.97); Anal. Calcd (%): C, 45.15; H, 2.90; N, 6.19; S, 7.09. Found: C, 45.18; H, 2.87; N, 6.18; S, 7.10.

Compound **3E**: Yield 89%, light yellow crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 212–213°C; UV λ_{max} 330 nm; IR (KBr) ν_{max} (cm^{-1}): 3313.7 (NH lactam), 1704.2 (C=O lactam), 1640.2 (C=N imine), 1593.5 (C=C benzene), 1230 (C-F), 528 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.67 (s, 1H: N*H*), 7.91 (d, *J* = 7.5 Hz; 1H: H-6′), 7.69 (s, 1H: C*H*), 7.56 (t, *J* = 8 Hz, 2H: H-2″, H-6″), 7.43 (t, *J* = 7.5 Hz, 1H: H-4′), 7.31 (t, *J* = 7.5 Hz, 2H: H-3″, H-5″), 7.07 (d, *J* = 7.5 Hz, 1H: H-3′), 6.96 (t, *J* = 7.5 Hz, 1H: H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 163.5 (C-4″), 161.5 (C-2), 139.0 (C-3′), 132.2 (C-2″), 132.1 (C-6″), 129.9 (C-1″), 129.6 (*C*H), 128.9 (C-5′), 126.6 (C-4′), 122.4 (C-5), 121.1 (C-6′), 116.5 (C-3″), 116.3 (C-5″); ESI-MS *m/z* [M + H]$^+$ 424.95 $C_{16}H_{10}ON_2SIF$ (calcd 423.95); Anal. Calcd (%): C, 45.30; H, 2.38; N, 6.60; S, 7.56. Found: C, 45.28; H, 2.42; N, 6.62; S, 7.55.

Compound **3F**: Yield 71%, greenish yellow crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 230–231°C; UV λ_{max} 330.5 nm; IR (KBr) ν_{max} (cm^{-1}): 3012.6 (NH lactam), 1711.9 (C=O lactam), 1660.3 (C=N imine), 1573.3 (C=C benzene), 532.2 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.66 (s, 1H: N*H*), 7.91 (d, *J* = 8 Hz; 1H: H-6′), 7.90 (s, 1H: C*H*), 7.68 (m, 6H: H-4′, H-2″, H-3″, H-5″, H-4″, H-6″), 7.07 (d, *J* = 7.5 Hz, 1H: H-3′); 6.96 (t, *J* = 7.5 Hz, 1H: H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 139.0 (C-3′), 133.2 (C-1″), 129.9 (*C*H), 129.8 (C-5′), 129.7 (C-3″, C-5″), 129.5 (C-4′), 129.2 (C-2″, C-6″), 126.7 (C-4″), 122.7 (C-5), 121.1 (C-6′); ESI-MS *m/z* [M – H]$^-$ 404.93 $C_{16}H_{11}ON_2SI$ (calcd 405.96); Anal. Calcd (%): C, 47.30; H, 2.73; N, 6.90; S, 7.89. Found: C, 47.33; H, 2.70; N, 6.92; S, 7.87.

Compound **3G**: Yield 86%, yellow powder, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, mp 202–203°C, UV λ_{max} 340.5 nm, IR (KBr) ν_{max} (cm^{-1}): 2994.7 (NH lactam), 1644.6 (C=O lactam), 1573.6 (C=N imine), 1509.0 (C=C benzene), 594.5 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.60 (s, 1H: N*H*), 7.89 (d, *J* = 8 Hz, 1H: H-6′), 7.62 (s, 1H: C*H*), 7.41 (t, *J* = 7.5 Hz, 1H: H-4′), 7.35 (d, *J* = 8 Hz, 2H: H-2″, H-6″), 7.25 (d, *J* = 7.5 Hz, 2H: H-3″, H-5″), 7.05 (d, *J* = 7.5 Hz, 1H: H-3′), 6.94 (t, *J* = 8 Hz, 1H: H-5′), 2.29 (s, 3H: CH_3); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 167.4 (C-2), 149.6 (C-1′), 140.1 (C-4″), 139.0 (C-3′), 130.4 (C-1″), 129.9 (*C*H), 129.8 (C-3″, C-5″), 129.7 (C-2″, C-6″), 129.5 (C-5′), 126.5 (C-4′), 121.5 (C-5), 121.2 (C-6′), 92.6 (C-2′), 21.0 (*C*H$_3$); ESI-MS *m/z* [M + H]$^+$ 420.97 $C_{17}H_{13}ON_2SI$ (calcd 419.98); Anal. Calcd (%): C, 48.58; H, 3.12; N, 6.67; S, 7.63. Found: C, 48.57; H, 3.15; N, 6.65; S, 7.65.

Compound **3H**: Yield 89%, light orange powder, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 238–239°C; UV λ_{max} 355.5 nm; IR (KBr) ν_{max} (cm^{-1}): 2787.0 (NH lactam), 1649.8 (C=O lactam), 1609.4 (C=N imine), 1511.2 (C=C benzene), 767.2 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.90 (s, 1H: N*H*), 8.28 (d, *J* = 8.5 Hz, 2H: H-3″, H-5″), 7.92 (d, *J* = 8 Hz, 1H: H-6′), 7.76 (t, *J* = 8 Hz, 3H: H-2″, H-6″, C*H*), 7.43 (t, *J* = 7.5 Hz, 1H: H-4′), 7.08 (d, *J* = 7.5 Hz, 1H: H-3′), 6.98 (t, J=8 Hz, 1H: H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 147.1 (C-4″), 139.6 (C-1″), 139.0 (C-3′), 130.7 (C-2″, C-6″), 129.6 (*C*H), 127.3 (C-4′), 126.7 (C-5′), 124.3 (C-3″, C-5″); ESI-MS *m/z* [M – H]$^-$ 449.94 $C_{16}H_{10}O_3N_3SI$ (calcd 450.95); Anal. Calcd (%): C, 42.59; H, 2.23; N, 9.31; S, 7.11. Found: C, 42.58; H, 2.26; N, 9.33; S, 7.11.

Compound **3I**: Yield 80%, red crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 197–199°C; UV λ_{max} 355.5 nm; IR (KBr) ν_{max} (cm^{-1}): 3002.9 (NH lactam), 1731.1 (C=O lactam), 1650.6 (C=N imine), 1572.9, 1524.3, 1460.2 (C=C benzene), 1175.6 (C-NO$_2$), 532.9 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.81 (s, 1H: N*H*), 8.15 (d, *J* = 8 Hz, 1H: H-3″), 7.88 (s, 1H: C*H*), 7.86 (d, *J* = 7.5 Hz, 1H: H-6′), 7.79 (t, *J* = 7.5 Hz, 1H: H-4″), 7.65 (t, *J* = 7.5 Hz, 1H: H-5″), 7.59 (d, *J* = 7.5 Hz, 1H: H-6″), 7.38 (m, 1H: H-4′), 7.03 (d, *J* = 7.5 Hz, 1H: H-3′), 6.93 (t, *J* = 7.5 Hz, 1H: H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 147.8 (C-2″), 139.0 (*C*H), 134.8 (C-3′), 130.6 (C-5″), 129.5 (C-6″), 129.10 (C-5, C-4′), 128.9 (C-1″), 126.6 (C-6′), 126.0 (C-3″), 125.3 (C-4″), 121.1 (C-5′), 79.2 (C-2′); ESI-MS *m/z* [M – H]$^-$ 449.95 $C_{16}H_{10}O_3N_3SI$ (calcd 450.95); Anal. Calcd (%): C, 42.59; H, 2.23; N, 9.31; S, 7.11. Found: C, 42.59; H, 2.25; N, 9.29; S, 7.10.

Compound **3J**: Yield 90%, dark yellow crystals, odorless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO; mp 250–251°C; UV λ_{max} 330 nm; IR (KBr) ν_{max} (cm^{-1}): 3564.6 (OH phenol), 3125.7 (NH lactam), 1702.5 (C=O lactam), 1639.9 (C=N imine), 1595.3, 1455.7, 1348.7 (C=C benzene), 758.3 (C-O), 694.6 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.54 (s, 1H: N*H*), 10.41 (s, 1H: O*H*), 7.90 (s, 1H: C*H*), 7.89 (d, *J* = 10.5 Hz, 1H: H-6′), 7.40 (t, *J* = 7.5 Hz, 1H: H-4′), 7.23 (m, 1H: H-5″), 7.18 (d, *J* = 7.5 Hz, 1H: H-3″), 7.05 (d, *J* = 7.5 Hz, 1H: H-3′), 6.93 (m, 2H: H-6″, H-4″), 6.85 (t, *J* = 7.5 Hz, 1H: H-5′); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 156.8 (C-1′), 138.9 (*C*H), 131.7 (C-5, C-3′), 128.2 (C-4′, C-5′), 129.5 (C-4″), 126.4 (C-2″), 125.1 (C-6′), 121.1 (C-3″), 120.2 (C-1″), 119.6 (C-5″), 116.0 (C-3″); ESI-MS *m/z* [M – H]$^-$ 420.95 $C_{16}H_{11}O_2N_2SI$ (calcd 421.96); Anal. Calcd (%): C, 45.51; H, 2.63; N, 6.63; S, 7.59. Found: C, 45.54; H, 2.63; N, 6.60; S, 7.61.

Compound **3K**: Yield 87%, yellow powder, odorless, insoluble in water, methanol, soluble in ethanol, acetone, DMF, DMSO; mp 254–255°C; UV λ_{max} 340.5 nm; IR (KBr) ν_{max} (cm^{-1}): 3363.6 (OH phenol), 3055.8 (NH lactam), 1713.7 (C=O lactam), 1635.5 (C=N imine), 1603.5, 1575.5 (C=C benzene), 767.6 (C-Br), 545.8 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.64 (s, 1H: N*H*), 10.80 (s, 1H: O*H*), 7.90 (d, *J* = 8 Hz, 1H: H-6′), 7.76 (s, 1H: C*H*), 7.41 (m, 2H: H-3″, H-4′), 7.25 (d, *J* = 2 Hz, 1H: H-6″), 7.07 (d, *J* = 7.5 Hz, 1H: H-3′), 6.96 (t, *J* = 7.5 Hz, 1H: H-5′), 6.88 (d, *J* = 8 Hz, 1H: H-4″); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 155.9 (C-2″), 149.3 (C-1′), 139.1 (C-3′, *C*H), 133.9 (C-4″, C-6″), 130.3 (C-4′, C-5′), 129.6 (C-5), 123.9 (C-5″), 123.1 (C-6′), 122.6 (C-1″), 118.2 (C-3″), 110.3 (C-2′); ESI-MS *m/z* [M – H]$^-$ 500.86 (95%), 499 (100%) $C_{16}H_{10}O_2N_2SIBr$ (calcd 499.87); Anal. Calcd (%): C, 38.35; H, 2.01; N, 5.59; S, 6.40. Found: C, 38.33; H, 2.05; N, 5.59; S, 6.40.

Compound **3L**: Yield 60%, red powder, odorless, insoluble in water, ethanol, soluble in methanol, acetone, DMF, DMSO; mp 225–226°C; UV λ_{max} 351.0 nm; IR (KBr) ν_{max} (cm^{-1}): 2977.1 (NH lactam), 1644.2 (C=O lactam), 1574.8 (C=N imine), 1572.6 (C=C benzene), 1015.6 (C-NR_2), 595.4 (C-I); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.35 (s, 1H: N*H*), 7.90 (d, *J* = 7 Hz, 1H: H-6′), 7.54 (s, 1H: C*H*), 7.42 (t, *J* = 7.5 Hz, 1H: H-4′), 7.31 (d, *J* = 8.5 Hz, 2H: H-2″, H-6″), 7.06 (d, *J* = 7.5 Hz, 1H: H-3′), 6.95 (t, *J* = 7.5 Hz, 1H: H-5′), 6.76 (d, *J* = 8 Hz, 2H: H-3″, H-5″), 2.97 (s, 6H: (CH_3)$_2$-N); ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 175.7 (C-4), 174.4 (C-2), 158.1 (C-1′), 138.9 (*C*H), 135.3 (C-3′), 131.6 (C-5′, C-4′), 130.8 (C-6″), 129.5 (C-5), 126.2 (C-2″), 121.2 (C-1″), 120.1 (C-6′), 112.0 (C-3″, C-5″), 68.0 ((CH_3)$_2$-N); ESI-MS *m/z* [M – H]$^-$ 448.00 $C_{18}H_{16}ON_3SI$ (calcd 449.01); Anal. Calcd (%): C, 48.12; H, 3.59; N, 9.35; S, 7.14. Found: C, 48.10; H, 3.62; N, 9.32; S, 7.17.

Antimicrobial assay

Antibacterial and antifungal activities of the 12 compounds (**3A**–**3L**) were screened using a Kirby–Bauer method in MHA [16]. Compounds to be tested were dissolved in DMSO 2% at 2048 μg/mL. The prepared solution (10 μL) was put into small 3-mm-diameter holes located on agar plates using DMSO 2% as a negative reference and cephalexin as a positive reference. The plates were then incubated at 35–37°C for 24 h.

The minimum inhibitory concentration (MIC) was determined using an agar dilution method [17]. Tested compounds in DMSO 2% were diluted with MHA medium to obtain accurate concentrations ranging from 2 to 1024 μg/mL. Each concentration was poured into plates contained 1–2 μL of suspension containing experimental strains (10^4 CFU/mL) and incubated at 35–37°C for 20–24 h. All assays were conducted in duplicate using cephalexin as the positive reference.

Results and discussion

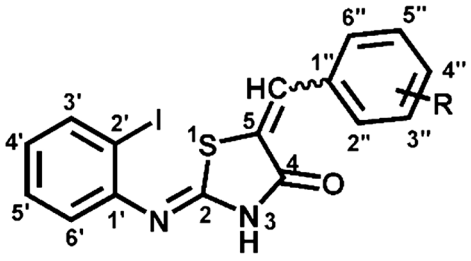
Synthesis

The yield of the intermediate **1** from the first acylation reaction with and without K_2CO_3 was not substantially different. For the second cyclization reaction, NH_4SCN produced a better outcome with the percent yield of **2** at 70% compared with KSCN with only 62% yield. The catalytic effects of dimethylamine and triethylamine in the aldol condensation reactions were similar (i.e., no substantial differences were observed). All reactions were conducted 3 times, and the percentage yields shown are averages of these.

In the original method, the main purpose of adding K_2CO_3, a weak base, into the acylation reaction was to create a basic condition and consequently reduce the acidic products and shift the reaction in the desired direction [9]. However, adding K_2CO_3 had no apparent effect on our reaction. This may be a consequence of adding DMF. We used DMF to dissolve 2-iodoaniline; fortunately, DMF can protect the highly reactive chloroacetic acid structure [18] and simultaneously catalyze the aromatic acylation reaction [19]. Therefore, the inclusion of K_2CO_3 as a catalyst, in our case, was unnecessary.

For the cyclization reaction, the use of NH_4SCN resulted in a better yield than with KSCN. This may be explained by the decomposition reaction of the products. NH_4Cl can decompose at high temperature to make ammonia, whereas KCl is stable at the same temperature. Therefore, the reaction equilibrium with NH_4SCN was shifted to the right, affording more products.

Table 1. Antimicrobial activity of the synthesized compounds, **3A**–**3L**, on various strains of bacteria and fungi



Structure	Name	R	Bacteria					Fungi	
			E. coli	*P. aeruginosa*	*E. faecalis*	*S. aureus*	MRSA	*C. albicans*	*A. niger*
3A	2-(2-Iodophenylimino)-5-(4-methoxybenzylidene)thiazolidin-4-one	4″-OCH_3	–	–	–	>1024	>1024	–	–
3B	2-(2-Iodophenylimino)-5-(4-chlorobenzylidene)thiazolidin-4-one	4″-Cl	–	–	–	8	4	–	–
3C	2-(2-Iodophenylimino)-5-(2,4-dichlorobenzylidene)thiazolidin-4-one	2″-Cl 4″-Cl	–	–	–	>1024	>1024	–	–
3D	2-(2-Iodophenylimino)-5-(4-hydroxy-3-methoxybenzylidene) thiazolidin-4-one	4″-OH 3″-OCH_3	–	–	–	16	16	–	–
3E	2-(2-Iodophenylimino)-5-(4-fluorobenzylidene)thiazolidin-4-one	4″-F	–	–	–	8	8	–	–
3F	2-(2-Iodophenylimino)-5-benzylidenethiazolidin-4-one		–	–	–	>1024	>1024	–	–
3G	2-(2-Iodophenylimino)-5-(4-methylbenzylidene)thiazolidin-4-one	4″-CH_3	–	–	–	>1024	128	–	–
3H	2-(2-Iodophenylimino)-5-(4-nitrobenzylidene)thiazolidin-4-one	4″-NO_2	–	–	–	8	8	–	–
3I	2-(2-Iodophenylimino)-5-(2-nitrobenzylidene)thiazolidin-4-one	2″-NO_2	–	–	>1024	8	32	–	–
3J	2-(2-Iodophenylimino)-5-(2-hydroxybenzylidene)thiazolidin-4-one	2″-OH	–	–	–	>1024	>1024	–	–
3K	2-(2-Iodophenylimino)-5-(5-bromo-2-hydroxybenzylidene) thiazolidin-4-one	2″-OH 5″-Br	–	–	–	64	64	–	–
3L	2-(2-Iodophenylimino)-5-(4-dimethylaminobenzylidene) thiazolidin-4-one	4″-$N(CH_3)_2$	–	–	–	–	–	–	–
PC	Cephalexin				32	4	32		

The tests were performed in duplicate; (–) indicates negative result (clear zone diameter was less than that for the negative control, DMSO); the number indicates the mean of the MIC (μg/mL) of compounds active against specific microbes.

A. niger, *Aspergillus niger*; *C. albicans*, *Candida albicans*; DMSO, dimethylsulfoxide; *E. coli*, *Escherichia coli*; *E. faecalis*, *Enterococcus faecalis*; MIC, minimum inhibitory concentration; *P. aeruginosa*, *Pseudomonas aeruginosa*; PC, positive control; *S. aureus*, *Streptococcus aureus*.

Nevertheless, further investigations are needed to substantiate this hypothesis.

Chemistry

All compounds synthesized demonstrated acceptable physicochemical properties as predicted. Notably, the mass spectrum of compound **3K** shows 2 sharp peaks at *m*/*z* 500.86 and 499.00 with a height ratio of 9.5:10. This occurs because of the natural isotopes of bromine. Elemental bromine has 2 stable isotopes, namely ^{79}Br and ^{81}Br with abundance of 50.5 and 49.5, respectively. Therefore, they contributed their mass to **3K** molecular weight in an appropriate manner, resulted in 2 separate peaks.

Compound **3L** had lower yield at 60% than other derivatives, which might be the result of a steric effect of the bulky substituent tertiary amino group on the phenyl ring that may hinder the efficiency of the aldol condensation reaction. However, this hypothesis is not well supported and there may be other reasons for the low yield.

Antimicrobial activity

As shown in **Table 1**, none of the 12 compounds tested had antifungal activity. However, 11 of 12 investigated compounds, from **3A** to **3K**, exhibited antibacterial activity either on *S. aureus*, MRSA, or *E. faecalis* qualitatively. By contrast, MIC tests indicated that only 6 compounds, namely **3B**, **3D**, **3E**, **3H**, **3I**, and **3K**, have potential therapeutic effects.

Compound **3B** showed the most potent antibacterial action on *S. aureus* and MRSA at MICs of 8 μg/mL and 4 μg/mL, respectively, and compound **3E** showed the activity at 8 μg/mL for both bacterial strains. This result might be explained by the better interaction of a halogenic group (i.e., chloride and fluoride) with the target site on *S. aureus* and MRSA compared with other substituents. This hypothesis can be confirmed with compound **3K**. With *o*-hydroxy and *o*-bromo groups, **3K** destroys the *S. aureus* and MRSA at very low MICs (64 μg/mL) compared with the ineffective compound **3J** with only an *o*-hydroxy group. It was possible that the properties of halogenic groups might play a major role in binding to and killing bacteria. However, that compound **3C** did not possess the same effect may be the result of an interaction between ortho- and para-substitution and steric effects on the molecule and receptor.

Nevertheless, the effect of a nitro group on killing bacteria was clearly demonstrated on compounds **3H** and **3I**. Overall, we can conclude that the 5-aryl substituent on the thiazolidin-4-one ring required a halogenic group and/or a nitro group at either the ortho- or para- position for the inhibitory activity on *S. aureus* and MRSA. The number of compounds tested is limited, and further research with more compounds to fully explore these hypotheses is warranted.

The MICs of 6 compounds illustrated good activity, especially compounds **3B**, **3E**, and **3H** with MICs of ≤8 μg/mL for *S. aureus* and MRSA, compared with the positive control, and novel compounds in previous studies [9–11, 20]. Therefore, these compounds can be further investigated in the quest for new antimicrobial agents. Although some compounds showed promising activities on *S. aureus* and MRSA, only one strain of each type of bacteria was tested in our study. To confirm their effectiveness, other clinical isolates of these 2 bacteria should be investigated in future studies. The safety of the newly discovered compounds is considered as one of the most important aspects in the drug development process. Their relative lack of water solubility may limit their formulation. Our study is limited because no cytotoxicity test was conducted. Other methods such as 96-well plate microdilution, recommended by the Clinical Laboratory Standards Institute (CLSI), could be used to confirm our findings in further studies.

Conclusion

In the present study, we achieved 3 objectives. The first was synthesis of 12 novel derivatives of 2-(2-iodophenylimino)-5-arylidenethiazolidin-4-one, which are not yet to be found in chemical databases such as ChemSpider and PubChem. The second was to improve the synthetic process with simple laboratory equipment. The third was to investigate the antimicrobial activity of the synthesized compounds. Among them, 3 compounds, **3B**, **3E**, and **3H**, show good antibacterial activities with low MICs (≤8 μg/mL) on *S. aureus* and MRSA. The result could be a starting point for the development of future antimicrobial agents.

Author contributions. DTP, TMHV, and PT contributed substantially to the conception and design of the study. DTP, PTH, and MQN acquired the data and TMHV and PT contributed substantially to its interpretation. All the authors were involved in drafting and critical revision of the article, approved the final version, and take responsibility for the statements in the published article.

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Conflict of interest statement. The authors have each completed and submitted an International Committee of Medical Journal Editors Form for Disclosure of Potential Conflicts of Interest. None of the authors has any conflict of interest to disclose.

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