A Facile Synthesis and Antimicrobial Activity Evaluation of Sydnonyl-Substituted Thiazolidine Derivatives

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Abstract: Some new sydnonyl-substituted thiazolidine derivatives were synthesized in high yields by the modified Knoevenagel condensation of 3-aryl-4-formylsydnones with thiazolidine-2,4-dione and 2-thioxo-thiazolidine-4-one, respectively. All the synthesized thiazolidine derivatives were screened by paper-disc method to identify their antimicrobial activities against three bacteria viz. Staphylococcus aureus, Proteus vulgaris and Escherichia coli, and two fungal cultures viz. Aspergillus niger and Penicillium citrinum. The reference drugs were Norfloxacin and Griseofulvin, respectively. The screening data indicated that the tested sydnonyl-substituted thiazolidine derivatives exhibited no obvious antibacterial activity compared with the standard drug Norfloxacin. However, thiazolidine derivatives displayed significant antifungal activities against Penicillium citrinum and Aspergillus niger. Notably, all of the tested compounds showed growth inhibitory activity 1.5-4.4 times higher than that of the standard drug Griseofulvin against the two fungi.

Keywords: Knoevenagel reaction; sydnones; thiazolidines; antimicrobial activity
1. Introduction

Thiazolidines, thiazolidinones and their derivatives have attracted continuing interest over the years because of their varied biological activities, such as antitumor [1–3], anticancer [4–6], anti-inflammatory [7], antimicrobial [8–10] and anti-Toxoplasma gondii activities [11]. Consequently, chemists still enthusiastically pursue the syntheses and activity evaluation of thiazolidine or thiazolidinone derivatives [12–16]. 3-Aryl-4-formylsydnones 1 have extensively been studied since their discovery [17] and their applications have been investigated [18–24]. Several sydnone derivatives are also associated with a wide range of pharmacological activities, exhibiting antimicrobial, anti-inflammatory, antioxidant, antitumor and anticancer properties [25–28]. Thus, syntheses of sydnone derivatives substituted with thiazolidine or thiazolidinone group at a suitable position by a convenient method is an important part of developing new and potentially biologically active compounds. In view of the continued interest in the development of simpler and more convenient synthetic routes for preparing sydnonyl-substituted heterocyclic systems, an efficient and useful method is reported herein to synthesize some new sydnonyl-substituted thiazolidinone derivatives by the modified Knoevenagel condensation of 3-aryl-4-formylsydnones 1a–d with thiazolidine-2,4-dione (2) and 2-thioxo-thiazolidin-4-one (3), respectively. All the synthesized thiazolidinone derivatives 4a–d, 5a–d were screened by paper-disc method to identify their antimicrobial activities against three bacteria viz. Staphylococcus aureus, Proteus vulgaris and Escherichia coli, and two fungal cultures viz. Aspergillus niger and Penicillium citrinum.

2. Results and Discussion

2.1. Synthetic Chemistry

Knoevenagel condensation is now a very established method for the synthesis of α,β-unsaturated carbonyl compounds by condensation of aldehydes or ketones with C-H acidic methylene group-containing compounds. In general, this condensation is usually carried out homogeneously using bases such as ammonia and ammonium salts, aliphatic amines and their salts as catalysts [29–32]. It can also be performed in the heterogeneous phase using an inorganic catalyst such as titanium tetrachloride, tellurium tetrachloride or aluminum oxide [33–35].

In this work, 3-aryl-4-formylsydnones 1 were at first treated with active methylene compounds, thiazolidine-2,4-dione (2) or 2-thioxo-thiazolidin-4-one (3) in piperidine/glacial acetic acid buffer system. However, the sydnone ring itself is sensitive to acids, bases and heat. Sydnone compounds are sometimes decomposed during reaction and/or work-up. Hence, the Knoevenagel condensations were controlled at low temperature by successive addition of glacial acetic acid, methylene compounds with piperidine to the sydnone ethanol solution in order. However, the starting materials 1 were still unavoidably decomposed. Several tests established glacial acetic acid was first added to the ice-cooled ethanol solution of sydnone 1 since sydnone compound was absolutely not decomposed in acetic acid. Then, the ice-cooled ethanol solution of the active methylene compound with sodium acetate was slowly added to the above acidic solution and the mixture was stirred to precipitate out the solid. We also detected two or more products in the reaction mixture. Finally, directly using glacial acetic acid as solvent rather than ethanol improved the reaction result, we found only one product in the reaction by
TLC detection. Consequently, under optimal experimental condition, starting materials 1a–d reacted with activated methylene compound thiazolidine-2,4-dione (2) at 80 °C in glacial acetic acid/sodium acetate buffer system to give condensation products 4a–d successfully. Compounds 5a–d were obtained by the reaction of 2-thioxo-thiazolidin-4-one (3) with starting materials 1a–d through the same reaction condition mentioned above (Scheme 1). The pH value about 4.7 of glacial acetic acid/sodium acetate buffer system was very suitable for sydnone derivative syntheses and the sydnone ring was absolutely not decomposed.

Scheme 1. Synthesis of thiazolidinone derivatives 4a–d, 5a–d from sydnone compounds 1a–d.

a: Ar = C₆H₅; b: Ar = p-CH₃C₆H₄; c: Ar = p-CH₃OC₆H₄; d: Ar = p-C₂H₅OC₆H₄.

All these synthesized products were spectrally characterized by IR, ¹H-NMR, ¹³C-NMR, MS and Elemental Analyses. Among these new compounds 4a–4d, 5a–5d, the crystals 4b, and 5a were also analyzed by X-ray diffraction. Figures 1 and 2 show the molecular structures of compounds 4b and 5a. Details of the crystal data of compounds 4b and 5a are given in Table 1. Moreover, view along the b axis of packing diagram of compound 5a is displayed in Figure 3.

Figure 1. ORTEP drawing of 5-[3-(4-methylphenyl)sydnon-4-ylmethylene]thiazolidine-2,4-dione (4b).
**Table 1. Crystal Data of compounds 4a and 5b.**

| Compounds | 4b                          | 5a                          |
|-----------|-----------------------------|-----------------------------|
| Diffractometer | Nonius Kappa CCD         | Nonius Kappa CCD           |
| Formula   | C_{13}H_{9}N_{3}O_{4}S     | C_{12}H_{7}N_{3}O_{3}S_{2}  |
| Formula weight | 303.29                  | 305.33                     |
| Crystal system | Monoclinic              | Monoclinic                  |
| Space group | P2(1)/c                  | P2(1)/c                    |
| a/Å       | 7.46360(10)               | 16.749(3)                  |
| b/Å       | 22.0840(5)                | 4.9332(10)                 |
| c/Å       | 8.2955(2)                 | 24.777(5)                  |
| α/°       | 90.00                     | 90.00                      |
| β/°       | 98.2900(14)               | 140.16(3)                  |
| γ/°       | 90.00                     | 90.00                      |
| V/Å³      | 1353.03(5)                | 1311.7(5)                  |
| Z         | 4                          | 4                           |
| D_{calc} (g·cm⁻³) | 1.489                    | 1.546                      |
| F₀₀₀      | 624.00                    | 624                        |
| Absorption coefficient (mm⁻¹) | 0.259                  | 0.416                      |
| Crystal size/mm | 0.30 × 0.25 × 0.20 | 0.30 × 0.25 × 0.20         |
| Temperature (K) | 295(2)                 | 295(2)                     |
| θ range, deg | 1.84–27.47              | 1.65–27.49                 |
| Reflections collected | 8949                   | 13460                      |
| Independent reflections | 3075 [R(int) = 0.0318] | 2940 [R(int) = 0.1322]    |
| Refinement method | Full-matrix least-squares on F² | Full-matrix least-squares on F² |
| Final R indices [I > 2.00σ(I)] | R₁ = 0.0427, wR₂ = 0.1123 | R₁ = 0.0585, wR₂ = 0.1584 |
| R indices (all data) | R₁ = 0.0653, wR₂ = 0.1339 | R₁ = 0.0814, wR₂ = 0.1904 |
| GoF       | 1.096                     | 1.052                      |

**Figure 2.** ORTEP drawing of 5-(3-phenylsydnon-4-ylmethylene)-2-thioxothiazolidin-4-one (5a).
2.2. Evaluation of Antimicrobial Activity

All these compounds were screened to determine their antimicrobial activity in vitro against three bacteria viz. Staphylococcus aureus, Proteus vulgaris and Escherichia coli, and two fungal cultures viz. Aspergillus niger and Penicillium citrinum. The reference drugs were Norfloxacin (N) for antibacterial and Griseofulvin (G) for antifungal tests, respectively. The tests were performed with the title compounds and the reference drugs, under identical conditions by the paper-disc method using the adequate quantity (30 μg) of the substance in 50 μL of DMF. The total inhibition area was calculated by the inhibition zone, in comparison with the reference drug, as follows: Relative inhibition % = 100(X − Y)/(Z − Y); X = total inhibition area in the test compound; Y = total inhibition area in DMF; Z = total inhibition area in the reference drug [36,37].

The screening data indicated that the tested compounds 4a–d and 5a–d did not show very obvious degree of antibacterial activities against Staphylococcus aureus, Proteus vulgaris and Escherichia coli, compared with the standard drug Norfloxacin. The growth inhibitory activities of all test compounds are lower than that of the standard drug Norfloxacin against three bacteria, and the relative Inhibition (%) are about 11%–77%. However, compounds 4a–d and 5a–d displayed significant antifungal activities against both fungi Aspergillus niger and Penicillium citrinum, as listed in Table 2. Notably, all of the
tested compounds showed growth inhibitory activity 1.5–4.4 times higher than that of the standard drug Griseofulvin against the two fungal cultures. Among these thiazolidine derivatives, compounds 5a–d carrying 2-thioxothiazolidin-4-one group displayed better antimicrobial activity against both fungi than thiazolidine-2,4-diones 4a–d did. Especially, compounds 5c and 5d with 3-(4-methoxyphenyl)sydnon-4-yl and 3-(4-ethoxyphenyl)sydnon-4-yl moiety, respectively, exhibited the best antimicrobial activity against both fungi, Aspergillus niger and Penicillium citrinum, as shown in Table 2 and Figure 4. The study of the syntheses of new sydnonyl-substituted thiazolidines with antimicrobial activity might support the development of new drugs and improve the treatment of infectious diseases.

Table 2. Antifungal activity of sydnonyl-substituted thiazolidine derivatives 4a–d, 5a–d.

| Compounds | Aspergillus N. * | Penicillum C. * |
|-----------|-----------------|----------------|
|           | Inhibition Zone (mm) | Relative Inhibition (%) | Inhibition Zone (mm) | Relative Inhibition (%) |
| 4a (Ar = C6H5) | 19 | 282.88 ± 5.91 | 20 | 175.01 ± 3.81 |
| 4b (Ar = p-CH3C6H4) | 19 | 282.88 ± 6.61 | 19 | 154.70 ± 1.62 |
| 4c (Ar = p-CH3OC6H4) | 20 | 320.04 ± 9.32 | 20 | 175.07 ± 8.51 |
| 4d (Ar = p-C2H5OC6H4) | 20 | 320.06 ± 11.21 | 21 | 196.38 ± 5.05 |
| 5a (Ar = C6H5) | 19 | 282.91 ± 9.34 | 23 | 242.24 ± 6.51 |
| 5b (Ar = p-CH3C6H4) | 21 | 351.11 ± 7.93 | 23 | 242.23 ± 7.59 |
| 5c (Ar = p-CH3OC6H4) | 23 | 442.92 ± 9.39 | 24 | 266.68 ± 4.56 |
| 5d (Ar = p-C2H5OC6H4) | 23 | 442.87 ± 12.37 | 24 | 266.67 ± 2.89 |
| G (Griseofulvin) | 13 | 100 | 16 | 100 |
| B (DMF) | 8 | - | 8 | - |

* Concentration of tested compounds in the antifungal activity: 30 μg/50 μL.

Figure 4. (a) The relative inhibition activity of compounds 4a–d and 5a–d against Aspergillus niger; (b) The relative inhibition activity of compounds 4a–d and 5a–d against Penicillium citrinum. G: Griseofulvin.
3. Experimental Section

3.1. General

All melting points were determined on an England Electrothermal Digital Melting Point apparatus and were uncorrected. IR spectra were recorded on a MATTSON/SATELLITE 5000 FT-IR spectrophotometer (Madison, WI, USA). Mass spectra were measured on a high-resolution mass spectrometer JEOL JMS-700 (Tokyo, Japan) and Bruker FT-MS APEX II (Rheinstetten, Germany). 1H-NMR spectra were run on a Bruker AV 400 NMR spectrometer (Rheinstetten, Germany), using TMS as an internal standard. 13C-NMR spectra were carried out with complete 1H decoupling and assignments were made through additional DEPT experiments. Elemental analyses were taken with an Elementar Vario EL-III Analyzer (Hanau, Germany). X-ray spectra were performed on Nonious Kappa CCD Single-crystal XRD (Bruker, Germany). 3-Aryl-4-formylsydnones (1a–d) were prepared from the corresponding 3-arylsydrones according to the literature [17].

3.2. Syntheses of 5-(3-Arylsydnon-4-ylmethylene)thiazolidine-2,4-diones 4a–d

To a solution of thiazolidine-2,4-dione (2, 176.0 mg, 1.5 mmol) and sodium acetate (246.1 mg, 3.0 mmol) in 3 mL of glacial acetic acid, 3-phenyl-4-formylsydnone (1a, 190.2 mg, 1.0 mmol) was slowly added at room temperature. The mixed solution was heated at 80 °C for 1–3 days until the reaction was completed and then cooled. The precipitating yellow powder (216.2 mg) was collected by filtration and washed with ice-cold water, cold ethanol, and after recrystallization from acetone-ethanol afford 199.8 mg of 5-(3-phenylsydnon-4-ylmethylene)thiazolidine-2,4-dione (4a) as yellow needles, yield 69%. The chemical and physical spectral characteristics of these products 4a–d are given below.

5-(3-Phenylsydnon-4-ylmethylene)thiazolidine-2,4-dione (4a): Yellow needles from CH3COCH3/EtOH; yield 69%; mp 260–261 °C; IR (KBr) 3144, 3039, 2762, 1744, 1678, 1598, 1323, 1290, 1012, 769, 552 cm−1; 1H-NMR (DMSO-d6) δ 6.89 (s, 1H, HC=C), 7.72–7.87 (m, 5H, ArH), 12.53 (s, 1H, NH); 13C-NMR (DMSO-d6) δ 107.01, 113.86, 122.25, 125.95, 130.63, 132.71, 133.28, 164.71, 167.36, 168.61; FABMS m/z (%): 290 ([M+H]+, 100), 289 (M+, 38); Anal. Calcd for C12H7N3O4S: C, 49.83; H, 2.44; N, 14.53. Found: C, 49.94; H, 2.56; N, 14.55.

5-(3-(4-Methylphenyl)sydnon-4-ylmethylene)thiazolidine-2,4-dione (4b): Yellow crystals from CH3COCH3/EtOH; yield 77%; mp 239–240 °C; IR (KBr) 3126, 3037, 2791, 1749, 1692, 1600, 1332, 1277, 1174, 826, 612 cm−1; 1H-NMR (DMSO-d6) δ 2.52 (s, 3H, CH3), 6.92 (s, 1H, HC=C), 7.69 (d, J = 7.6 Hz, 2H, ArH), 7.73 (d, J = 7.6 Hz, 2H, ArH), 12.53 (s, 1H, NH); 13C-NMR (DMSO-d6) δ 21.12, 106.95, 113.96, 122.11, 125.64, 130.23, 130.95, 143.67, 164.75, 167.36, 168.63; FABMS m/z (%): 304 ([M+H]+, 100), 303 (M+, 80); Anal. Calcd for C13H11N3O4S: C, 51.48; H, 2.99; N, 13.85. Found: C, 51.45; H, 3.01; N, 13.82. X-ray analytical data is listed in Table 1. Further details have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 1048337.

5-(3-(4-Methoxyphenyl)sydnon-4-ylmethylene)thiazolidine-2,4-dione (4c): Yellow powder from CH3COCH3/EtOH; yield 67%; mp 225–226 °C; IR (KBr) 3157, 3067, 2769, 1754, 1717, 1607, 1510, 1319, 1262, 1171, 836, 610 cm−1; 1H-NMR (DMSO-d6) δ 3.89 (s, 3H, CH3O), 6.91 (s, 1H, HC=C),
7.29 (d, J = 9.2 Hz, 2H, ArH), 7.76 (d, J = 9.2 Hz, 2H, ArH), 12.50 (s, 1H, NH); \(^{13}\)C-NMR (DMSO-\(d_6\)) \(\delta\) 56.11, 107.04, 114.15, 115.63, 121.90, 125.17, 127.47, 162.49, 164.78, 167.35, 168.64; FABMS m/z (%): 320 ([M+H]+, 100), 319 (M+, 38); Anal. Calcd for C\(_{13}\)H\(_9\)N\(_3\)O\(_5\)S: C, 48.90; H, 2.84; N, 13.16. Found: C, 48.74; H, 2.92; N, 13.05.

5-[3-(4-Ethoxyphenyl)sydnon-4-ylmethylene]thiazolidine-2,4-dione (4d): Yellow needles from CH\(_3\)COCH\(_3\)/EtOH; yield 67%; mp 219–220 °C; IR (KBr) 3176, 3074, 2761, 1759, 1722, 1605, 1509, 1318, 1260, 1173, 844, 610 cm\(^{-1}\); \(^{1}\)H-NMR (DMSO-\(d_6\)) \(\delta\) 1.37 (t, J = 6.8 Hz, 3H, CH\(_3\)), 4.17 (q, J = 6.8 Hz, 2H, CH\(_2\)), 6.91 (s, 1H, HC=C), 7.26 (d, J = 8.8 Hz, 2H, ArH), 7.74 (d, J = 8.8 Hz, 2H, ArH), 12.50 (s, 1H, NH); \(^{13}\)C-NMR (DMSO-\(d_6\)) \(\delta\) 14.60, 64.20, 107.02, 114.16, 115.94, 121.85, 124.99, 127.45, 161.78, 164.77, 167.32, 168.63; FABMS m/z (%): 334 ([M+H]+, 100), 333 (M+, 35); Anal. Calcd for C\(_{14}\)H\(_{11}\)N\(_3\)O\(_5\)S: C, 50.45; H, 3.33; N, 12.61. Found: C, 50.32; H, 3.32; N, 12.45.

3.3. Syntheses of 5-(3-Arylsydnon-4-ylmethylene)-2-thioxothiazolidin-4-ones 5a–d

To a solution of 2-thioxo-4-thiazolidinone (3, 199.8 mg, 1.5 mmol) and sodium acetate (246.1 mg, 3.0 mmol) in 3 mL of glacial acetic acid, 3-phenyl-4-formylsydnone (1a, 190.2 mg, 1.0 mmol) was slowly added at room temperature. The mixed solution was heated at 80 °C for 1–2 d until the reaction was completed and then cooled. The precipitating orange red powder (395.5 mg) was collected by filtration and washed with ice-cold water, cold ethanol, and after recrystallization from acetone-ethanol afford 268.8 mg of 5-(3-phenylsydnone-4-ylmethylene)-2-thioxothiazolidin-4-one (5a) as orange red needles, yield 88%. The chemical and physical spectral characteristics of these products 5a–d are given below.

5-(3-Phenylsydnon-4-ylmethylene)-2-thioxothiazolidin-4-one (5a): Orange red needles from CH\(_3\)COCH\(_3\)/EtOH; yield 88%; mp 243–244 °C; IR (KBr) 3134, 3036, 2843, 1761, 1683, 1578, 1314, 1215, 1077, 768, 589 cm\(^{-1}\); \(^{1}\)H-NMR (DMSO-\(d_6\)) \(\delta\) 6.71 (s, 1H, HC=C), 7.64–7.92 (m, 5H, ArH), 13.72 (s, 1H, NH); \(^{13}\)C-NMR (DMSO-\(d_6\)) \(\delta\) 107.71, 113.18, 123.97, 125.95, 130.66, 132.59, 133.36, 164.87, 169.40, 196.43; FABMS m/z (%): 306 ([M+H]+, 100), 305 (M+, 26); Anal. Calcd for C\(_{12}\)H\(_7\)N\(_3\)O\(_3\)S\(_2\): C, 47.20; H, 2.31; N, 13.76. Found: C, 47.35; H, 2.45; N, 13.71. X-ray analytical data is listed in Table 1. Further details have been deposited at the Cambridge Crystallographic Data Center (www.ccdc.cam.ac.uk/conts/retrieving.html) and allocated the deposition number CCDC 1048338.

5-[3-(4-Methylphenyl)sydnon-4-ylmethylene]-2-thioxothiazolidin-4-one (5b): Orange red crystals from CH\(_3\)COCH\(_3\)/EtOH; yield 79%; mp 245–246 °C; IR (KBr) 3149, 3048, 2867, 1743, 1690, 1587, 1318, 1225, 1178, 819, 679 cm\(^{-1}\); \(^{1}\)H-NMR (DMSO-\(d_6\)) \(\delta\) 2.51 (s, 3H, CH\(_3\)), 6.74 (s, 1H, HC=C), 7.64–7.92 (m, 5H, ArH), 13.74 (s, 1H, NH); \(^{13}\)C-NMR (DMSO-\(d_6\)) \(\delta\) 70.71, 113.18, 123.97, 125.95, 130.66, 132.59, 133.36, 164.87, 169.40, 196.43; FABMS m/z (%): 320 ([M+H]+, 100), 319 (M+, 33); Anal. Calcd for C\(_{13}\)H\(_{8}\)N\(_3\)O\(_3\)S\(_2\): C, 48.89; H, 2.84; N, 13.16. Found: C, 49.13; H, 3.00; N, 12.76.

5-[3-(4-Methoxyphenyl)sydnon-4-ylmethylene]-2-thioxothiazolidin-4-one (5c): Orange red needles from CH\(_3\)COCH\(_3\)/EtOH; yield 85%; mp 230–231 °C; IR (KBr) 3154, 3004, 2962, 1770, 1683, 1579,
5-[3-(4-Ethoxyphenyl)sydnon-4-ylmethylene]-2-thioxothiazolidin-4-one (5d): Orange red needles from CH$_3$COCH$_3$/EtOH; yield 87%; mp 244–245 °C; IR (KBr) 3134, 3041, 2848, 1759, 1682, 1568, 1508, 1312, 1256, 1225, 839, 682 cm$^{-1}$; $^1$H-NMR (DMSO-$d_6$) $\delta$ 1.37 (t, $J = 7.2$ Hz, 3H, CH$_3$), 4.17(q, $J = 7.2$ Hz, 2H, CH$_2$O), 6.73 (s, 1H, HC=C), 7.27 (d, $J = 9.2$ Hz, 2H, ArH), 7.75(d, $J = 9.2$ Hz, 2H, ArH), 13.71 (s, 1H, NH); $^{13}$C-NMR (DMSO-$d_6$) $\delta$ 14.60, 54.23, 107.68, 113.53, 116.00, 123.58, 124.87, 127.48, 161.87, 164.90, 196.44; FABMS m/z (%): 350 ([M+H]$^+$, 100), 349 (M$^+$, 35); Anal. Calcd for C$_{14}$H$_{11}$N$_3$O$_4$S$_2$: C, 48.13; H, 3.17; N, 12.03. Found: C, 48.24; H, 3.23; N, 11.91.

3.4. Biological Evaluation (Antimicrobial Activity)

The antimicrobial activities of synthesized compounds 4a–d and 5a–d were investigated in vitro using five microorganisms. These organisms included three bacteria (1, *Staphylococcus aureus* ATCC-12600; 2, *Proteus vulgaris* ATCC-13315; 3, *Escherichia coli* CCRC-10316), and two fungi (4, *Aspergillus niger* ATCC-42418 and 5, *Penicillium citrinum*. ATCC-8506). The microorganisms were provided by the Culture Collection and Research Center of FIRDI in Taiwan and the American Type Culture Collection, Manassas, VA, USA. The tests were carried out with the synthesized compounds 4a–d and 5a–d and the reference drugs, under identical conditions using the paper-disc method with the adequate quantity (30 $\mu$g) of the substance in 50 $\mu$L of DMF. The reference drugs used were Norfloxacin for antibacterial activities and Griseofulvin for antifungal activity, respectively. First, the testing plates with double layer agar were prepared. The base layer contains nutrient agar for bacteria and potato dextrose agar for fungi, respectively. Meanwhile, the upper layer comprises water agar containing bacteria or fungi. The discs of the filter paper (8 mm diameter) were placed in a petri dish and sterilized at 125 °C for 2 h. Following cooling, 50 $\mu$L of the compound solution was added to each paper-disc. After drying in the laminarflow, the paper-disc containing test compound was placed on a petri dish with a double layer of nutrient agar and water agar. The plates were incubated at a suitable temperature (37 °C for bacteria, 26 °C for fungi) for 1–4 days. The inhibition zones were observed and determined. All the tests were undertaken on four replicates and the results were averaged. The total inhibition area was calculated using the inhibition zone, in comparison with the reference drug, as follows: Relative inhibition % = 100($X - Y$)($Z - Y$); $X$ = total area of inhibition in the test compound; $Y$ = total area of inhibition in DMF; $Z$ = total area of inhibition in the reference drug [36,37].

4. Conclusions

For detailed study of biological activity, some new sydnonyl-substituted thiazolidine derivatives were successfully synthesized by the modified Knoevenagel condensation of 3-aryl-4-formylsydnones with thiazolidine-2,4-dione and 2-thioxo-thiazolidin-4-one respectively. The evaluation of antimicrobial activity indicated that the synthesized compounds 4a–d and 5a–d showed less antibacterial activities
against *Staphylococcus aureus*, *Proteus vulgaris* and *Escherichia coli*, comparable to the standard drug Norfloxacin. However, compounds 4a–d and 5a–d showed growth inhibitory activity 1.5–4.4 times higher than that of the standard drug Griseofulvin against *Penicillium citrinum* and *Aspergillus niger*. Among these thiazolidinone derivatives, compounds 5a–d carrying 2-thioxo-thiazolidin-4-one group displayed better antimicrobial activity against both fungi than thiazolidine-2,4-diones 4a–d did. Especially, substituents 4-methoxy and 4-ethoxy groups on the 3-arylsydnone ring of compounds 5c and 5d respectively, increased the antifungal activity compared with compounds 5a and 5b against both of fungi, *Aspergillus niger* and *Penicillium citrinum*. 

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Author Contributions

Mei-Hsiu Shih planned and supervised the project and wrote the manuscript. Yu-Yuan Xu carried out the synthesis of new compounds and participated in the characterization of all synthesized compounds. Yu-Sheng Yang carried out the syntheses of starting materials and performed their physical and chemical characterization. Guan-Ling Lin evaluated the antimicrobial activities of tested compounds. All the authors have read and approved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds 4b, 4d, 5a, 5c and 5d are available from the authors.

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