Original Article

Mahidol Univ J Pharm Sci 2016; 43 (1), 45-53

Synthesis and antimicrobial activity screening of 2-(2-fluorophenylimino)-5-arylidenethiazolidin-4-one derivatives

D.T. Pham[*1,4], *T.M.H. Vo*[2], *P. Truong*[3], *P.T. Ho*[2], *N. Khorana*[4]

[1] *Department of Pharmaceutical Technology, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam.*

[2] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho, Vietnam.*

[3] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy, Ho Chi Minh city, Vietnam.*

[4] *Department of Pharmaceutical Technology and Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, Thailand.*

Abstract

Twelve derivatives of 2-(2-fluorophenylimino)-5-arylidenethiazolidin-4-one (F1-F12) were synthesized using 2-fluoroaniline as a reactant through 3-step process including acylation reaction, cyclisation reaction and aldol condensation reaction. All synthesized compounds were elucidated the structure by infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR) and mass spectra (MS). None of the synthesized compounds inhibited *Candida albicans* or *Aspergillus niger*. Compounds F4 and F8 showed antibacterial activity on *Staphylococcus aureus* with MIC ≤ 64 µg/ml and methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC ≤ 64 µg/ml. These compounds have potential for further investigation and modification to become potential therapeutic drugs in the future.

Keyword: thiazolidin-4-one, synthesis, antibacterial, antifungal, MRSA.

1. INTRODUCTION

According to WHO (2014), infectious diseases were reported as one of the major public health issue due to the high rate of mortality and difficulty in treatment. Bacteria and fungi resistant strains are significantly increased and caused many serious health problems[1]. Therefore, new antifungal and antibacterial agents with outstanding activity are needed. Thiazolidin-4-one is a five-member ring with sulfur and nitrogen located at position 1 and 3, respectively, and a ketone group at position 4 (Figure 1)[2]. The previous studies showed the importance of substitution at position 5 of thiazolidin-4-one with aryl group which dramatically increased the pharmacological effect[3]. The 5-arylthiazolidin-4-one derivatives demonstrated various therapeutic effects included anticancer[4], antioxidants[5], anticonvulsants[6] and antimicrobial[7-12]. Vinay V. and his group reviewed the effect antimicrobial activities of thiazolidin-4-one derivatives, but none of the studies investigated the effect of thiazolidin-4-one derivatives with halogen substitution[12]. Halogen, especially fluorine (F), which contains free pairs of electron is expected to interact with microbes' cell wall or membrane via van der Waals linkage or covalent bonding and might consequently destroy the microbes[2]. The objective of this research was to synthesize the 2-(2-fluorophenylimino)-5-arylidenethiazolidin-4-one derivatives and to evaluate the antimicrobial activities of the products. Various strains of bacteria and fungi were screened for the activity.

***Corresponding author:** E-mail: pdtoan@ctump.edu.vn

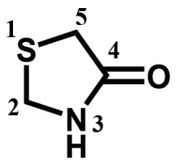


Figure 1. Thiazolidin-4-one structure

2. MATERIALS AND METHODS

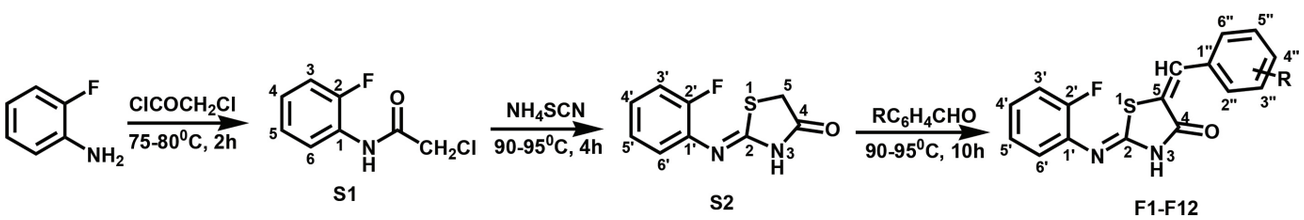
2.1. Materials

The chemical used in this study such as; solvents: ethanol, methanol, glacial acetic acid, dimethylformamide (DMF), ethyl acetate, toluene, dioxane, chloroform; reagents: chloroacetic acid, ammonium thiocyanate, aromatic aldehydes; reactants: 2-fluoroaniline; catalyst: dimethylamine; and thin layer chromatography (TLC) plates, were purchased from Merck Inc. and were qualified as laboratory grade.

2.2. Methods

Chemical synthesis

2-(2-Fluorophenylimino)-5-arylidenethiazolidin-4-one derivatives were synthesized through three steps as shown in Scheme 1. The first step was the acylation process of 2-fluoroaniline (0.1 mol) in 50 ml DMF and chloroacetic acid (0.3 mol) at 75-80^{0}C for 2 hours to make 2-chloro-N-(2-fluorophenyl)acetamide (S1)[2]. The solid product was washed with purified water to eradicate excess acid and recrystallized in ethanol:water (2:1 v/v). The second step was the cyclisation reaction of intermediate S1 (0.1 mol) and ammonium thiocyanate (0.1 mol) in ethanol and refluxed at 90-95^{0}C for 4 hours to make 2-(2-fluorophenylimino)thiazolidin-4-one (S2)[3]. The obtained product was recrystallized in ethanol:water (3:1 v/v). The final step was the aldol condensation reaction of intermediate S2 (0.01 mol) and corresponding aromatic aldehyde (0.03 mol) in glacial acetic acid by using dimethylamine as catalyst[11] and refluxed at 90-95^{0}C for 10 hours to make F1-F12 (Table 1). The recrystallization process of products was made with a mixture of ethanol:water (ratio depends on the compound). TLC method was used to follow the reactions with a mobile phase of toluene: ethyl acetate: methanol (5:4:1 v/v/v).



Scheme 1. Synthetic procedure of 2-fluorophenylimino-5-arylidenethiazolidin-4-one

Structure elucidation

Melting points and spectral methods (UV, IR, MS and NMR) were used to identify all compounds. The melting points were recorded by open capillary method using Stuart SMP3 apparatus – Barloworld Scientific (UK). IR spectra were recorded on FTIR Bruker Alpha T spectrophotometer – Bruker (USA), using KBr pellet technique. UV spectra were done using UV U2800 spectrophotometer – Hitachi (Japan). NMR spectra were recorded on AV 500MHz NMR spectrophotometer – Bruker (USA), using tetramethylsilane as an internal standard and deuterated dimethylsulfoxide (DMSO-*d6*) as solvent. Mass spectra and molecular weight were recorded on FT-ICR apparatus – Perkin-Elmer (USA) at room temperature with electrospray ionization method (ESI).

Antimicrobial assay

Screening of antibacterial and antifungal activities of twelve compounds (F1-F12) was done by Kirby-Bauer method in Mueller-Hinton Agar (MHA)[13]. There were five bacteria strains

which used for testing including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and MRSA ATCC 43300. The fungi strains was tested including *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. All testing compounds were dissolved in DMSO 2% at a concentration of 2,048 µg/ml. The amount of 10 µl of the prepared solution was put into small 3 mm-diameter-holes located on the agar plates by using DMSO 2% as the reference. The plates were then incubated at 35-37^{0}C for 24 h.

The tested discs that showed a clear zone of inhibition, with diameter larger than the negative reference, indicated the activity on the microorganisms. The active compounds were selected to determine the minimum inhibitory concentration (MIC) with agar dilution method[14]. MIC experiment was performed by dissolved the tested compounds in DMSO 2% and diluted with MHA medium to get accurate concentrations ranging from 2 to 1024 µg/ml. Each testing concentration was poured into the tested plates. Put 1-2 µl of suspension containing experimental strains (10^4 CFU/ml) into the plates and incubated at 35-37^{0}C for 20-24 hours. The concentration in which bacteria or fungi cannot grow was MIC value. All assays were performed in duplicate.

3. RESULTS

3.1. Chemistry

Compound S1

Formula C_8H_7NOFCl, molecular weight (MW) 178.00, yield 75%, white crystal, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, melting point (m.p.) 88-90^{0}C, UV: λ_{max}=227.5 nm, IR (cm^{-1}): 3448.2 (NH amide); 1669.1 (C=O amide); 1590.1; 1488.2 (C=C benzene); 1258.7 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 9.76 (s, 1H: NH); 7.9 (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 (125MHz, DMSO-*d6*, δ ppm): 164.95 (C=O); 139.02 (C_3); 138.67 (C_1); 128.80 (C_5); 128.02 (C_4); 126.81 (C_6); 95.98 (C_2); 43.03 (CH_2Cl). ESI-MS: $[M+Na]^+$ (m/z) 201.00.

Compound S2

Formula $C_9H_7N_2OSF$, MW 210.00, yield 70%, white crystal, odourless, insoluble in water, soluble in ethanol, chloroform, methanol, acetone, DMF, DMSO, m.p. 150-152^{0}C, UV: λ_{max}=228 nm, IR (cm^{-1}): 3448.5 (NH lactam); 1695.2 (C=N imine); 1600.3; 1503.9 (C=C benzene); 1268.8 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 11.99 (s, 1H: NH); 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 (125MHz, DMSO-*d6*, 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 (C2'); 34.3 (C5). ESI-MS: $[M+Na]^+$ (m/z) 233.00.

Compound F1

Formula $C_{16}H_9N_2OSFCl_2$, MW 365.98, yield 84.4%, yellow crystal, odourless, insoluble in water, soluble in ethanol, methanol, acetone, DMF, DMSO, m.p. 224-226^{0}C, UV λ_{max}=339 nm, IR (cm^{-1}): 3448.6 (NH lactam); 1731.1 (C=O lactam); 1654.4 (C=N imine); 1583.9; 1461.8 (C=C benzene); 1247.8 (C-F); 754.9 (C-Cl). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.56 (s, 1H: NH); 7.78 (s, 1H: =CH); 7.72 (d, J=9 Hz, 1H: $H_{6'}$); 7.49 (d, J=8.5 Hz, 1H: $H_{6''}$); 7.42 (d, J=8.5 Hz, 1H: $H_{5''}$); 7.29 (m, 1H: $H_{4'}$); 7.20 (d, J=7 Hz, 2H: $H_{3'''}$, $H_{3'}$); 7.12 (t, J=9 Hz, 1H: $H_{5'}$). ^{13}C-NMR (125MHz, DMSO -*d6*, δ ppm): 167.02 (C_4); 154.49 (C_2); 152.53 ($C_{2'}$); 135.08 ($C_{2''}$); 134.95 ($C_{1'}$); 130.19 ($C_{1''}$); 129.84 (=CH-); 129.73 ($C_{6''}$); 128.26 ($C_{3''}$); 127.02 ($C_{4''}$); 126.48 ($C_{4'}$); 125.10 ($C_{5''}$); 125.07 ($C_{5'}$); 123.76 ($C_{6'}$); 116.45 ($C_{3'}$); 116.29 (C_5). ESI-MS: $[M+Na]^+$ (m/z) 388.98.

Compound F2

Formula $C_{16}H_{10}N_2OSFCl$, MW 332.52, yield 91%, light yellow crystal, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 234-236^{0}C, UV λ_{max}=339.5

nm, IR (cm^{-1}): 3410.2 (NH lactam); 1675.5 (C=O lactam); 1627.6 (C=N imine); 1601.2; 1478.0 (C=C benzene); 1248.8 (C-F); 750.8 (C-Cl). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.7 (s, 1H: NH); 7.67 (s; 1H: -CH=); 7.53 (t, J=9 Hz, 4H: $H_{2"}$, $H_{3"}$, $H_{5"}$, $H_{6"}$); 7.31 (t, J=4.5 Hz, 1H: $H_{4'}$); 7.23 (d, J=4.5 Hz, 2H: $H_{3'}$, $H_{6'}$); 7.15 (t, J=4.5 Hz, 1H: $H_{5'}$). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.53 (C_4); 154.19 (C_2); 152.48 ($C_{2'}$); 136.59 ($C_{1'}$); 134.49 (=CH); 133.69 ($C_{4"}$); 132.08 ($C_{1"}$); 131.35 ($C_{2"}$, $C_{6"}$); 129.30 ($C_{5"}$, $C_{6"}$); 128.60 ($C_{4'}$); 126.11 ($C_{5'}$); 125.13 (C_6'); 116.45 ($C_{3'}$); 116.29 (C_5). ESI-MS: $[M+Na]^+$ (m/z) 355.52.

Compound F3

Formula $C_{16}H_{11}N_2OSF$, MW 298.06, yield 81%, yellow powder, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 211-213^{0}C, UV λ_{max}=332.5 nm, IR (cm^{-1}): 3406.6 (NH lactam); 1675.5 (C=O lactam); 1627.6 (C=N imine); 1611.5; 1478.0 (C=C benzene); 1248.8 (C-F). ^{1}H-NMR (500 MHz, DMSO-*d6*, δ ppm): 12.65 (s, 1H: NH); 7.67 (s, 1H: -CH=); 7.48 (m, 4H: $H_{6'}$, $H_{2"}$, $H_{4"}$, $H_{6"}$); 7.41 (t, J=7 Hz, 1H: $H_{4'}$); 7.31 (m, 1H: $H_{5'}$); 7.22 (m, 2H: $H_{3"}$, $H_{5"}$); 7.16 (d, J=8 Hz, 1H: $H_{3'}$). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.51 (C_4); 154.53 (C_2); 152.59 ($C_{2'}$); 135.33 ($C_{1'}$); 133.17 ($C_{1"}$); 129.92 (=CH-, $C_{4'}$); 129.67 ($C_{3"}$, $C_{5"}$); 129.18 ($C_{2"}$, $C_{6"}$); 126.26 ($C_{4"}$); 125.08 ($C_{6'}$); 123.23 ($C_{5'}$); 116.43 ($C_{3'}$); 116.26 (C_5). ESI-MS: $[M+Na]^+$ (m/z) 321.06.

Compound F4

Formula $C_{16}H_{10}N_3O_3SF$, MW 343.04, yield 65%, dark yellow crystal, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 201-203^{0}C, UV λ_{max}=341.5 nm, IR (cm^{-1}): 3418.6 (NH lactam); 1716.5 (C=O lactam); 1648.5 (C=N imine); 1600.6; 1490.5 (C=C benzene); 1227.9 (C-F); 1311.0 (NO_2). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.8 (s, 1H: NH); 8.15 (d, J=8.5 Hz, 1H: $H_{3"}$); 7.87 (s, 1H: -CH=); 7.79 (t, J=7.5 Hz, 1H: $H_{4'}$); 7.64 (m, 2H: $H_{4"}$, $H_{5"}$); 7.27 (m, 1H: $H_{5'}$); 7.17 (m, 2H: $H_{6'}$, $H_{6"}$); 7.11 (d, J=7 Hz, 1H: $H_{3'}$). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.05 (C_4); 154.16 (C_2); 152.42 ($C_{2'}$); 147.76 ($C_{2"}$); 136.62 ($C_{1'}$); 134.42 (=CH); 130.66 ($C_{5"}$); 129.16 ($C_{4'}$); 128.95 ($C_{1"}$); 127.65 ($C_{4"}$); 126.33 ($C_{6"}$); 125.28 ($C_{5'}$); 125.06 ($C_{6'}$); 123.19 ($C_{3"}$); 116.41 ($C_{3'}$); 116.26 (C_5). ESI-MS: $[M+H]^+$ (m/z) 344.04.

Compound F5

Formula $C_{16}H_{10}N_2OSF_2$, MW 316.05, yield 87%, light yellow crystal, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 226-228^{0}C, UV λ_{max}=332 nm; 230.5 nm, IR (cm^{-1}): 3453.5 (NH lactam); 1686.0 (C=O lactam); 1635.2 (C=N imine); 1602.3; 1484.0 (C=C benzene); 1230.8 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.63 (s, 1H: NH); 7.68 (s, 1H: -CH=); 7.58 (m, 2H: $H_{3'}$, $H_{4'}$); 7.31 (m, 4H: $H_{2"}$, $H_{3"}$, $H_{5"}$, $H_{6"}$); 7.22 (m, 2H: $H_{5'}$, $H_{6'}$). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.41 (C_4); 161.56 ($C_{4"}$); 154.54 (C_2); 152.59 ($C_{2'}$); 135.39 ($C_{1'}$); 132.14 ($C_{2"}$,$C_{6"}$); 129.83 ($C_{1"}$); 128.86 (=CH-); 126.27 ($C_{4'}$); 125.1 ($C_{5'}$); 123.24 ($C_{6'}$); 122.41 ($C_{3'}$); 116.43 ($C_{3"}$, $C_{5"}$); 115.8 (C_5). ESI-MS: $[M+Na]^+$ (m/z) 339.05.

Compound F6

Formula $C_{17}H_{13}N_2O_2SF$, MW 328.07, yield 90%, yellow crystal, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 226-227^{0}C, UV λ_{max}=354 nm, IR (cm^{-1}): 3455.7 (NH lactam); 1676.9 (C=O lactam); 1630.6 (C=N imine); 1597.0; 1478.8 (C=C benzene); 1257.8 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.67 (s, 1H: NH); 7.63 (m, 1H: -CH=); 7.46 (d, J=8.5 Hz, 2H: $H_{2"}$, $H_{6"}$); 7.30 (m, 1H: $H_{4'}$); 7.22 (t, J=3 Hz, 2H; $H_{3'}$, $H_{5'}$); 7.14 (s, 1H: $H_{6'}$); 7.05 (d, J=8.5 Hz, 2H: $H_{3"}$, $H_{5"}$); 3.78 (s, 3H: -OCH_3). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.33 (C_4); 160.62 ($C_{4"}$); 154.57 (C_2); 152.63 ($C_{2'}$); 135.52 ($C_{1'}$); 131.69 ($C_{2"}$, $C_{6"}$); 129.95 (=CH-); 126.21 ($C_{4'}$); 125.68 ($C_{1"}$); 125.09 ($C_{5'}$); 123.29 ($C_{6'}$); 116.43 ($C_{3'}$); 116.28 (C_5); 114.82 ($C_{3"}$, $C_{5"}$) ; 55.37 (-OCH_3). ESI-MS: $[M+Na]^+$ (m/z) 351.07.

Compound F7

Formula $C_{17}H_{13}N_2OSF$, MW 312.07, yield 87%, yellow powder, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 216-217^{0}C, UV λ_{max}=340.5 nm, 3455.7 (NH lactam); 2946.1 (C-H alkyl); 1713.4 (C=O lactam); 1649.2 (C=N imine); 1597.8; 1487.8 (C=C benzene); 1257.8 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.77 (s, 1H: NH); 7.63 (s, 1H: -CH=); 7.40 (d, J=8 Hz, 2H: $H_{2"}$,$H_{6"}$); 7.30 (s, 1H: $H_{4'}$); 7.29 (d, J=8 Hz, 2H: $H_{3'}$, $H_{6'}$); 7.22 (m, 2H: $H_{3"}$, $H_{5"}$); 7.15 (s, 1H: $H_{5'}$); 2.32 (s, 3H: CH_3). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 130.0 (=CH-); 129.83 ($C_{3"}$, $C_{5"}$); 129.74 ($C_{2"}$, $C_{4"}$); 126.22 ($C_{4'}$); 125.07 ($C_{5'}$); 123.24 ($C_{6'}$); 116.42 ($C_{3'}$); 21.0 (–CH_3). ESI-MS: $[M+Na]^+$ (m/z) 335.07.

Compound F8

Formula $C_{17}H_{13}N_2O_3SF$, MW 344.06, yield 89%, white powder, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 214-216^{0}C, UV λ_{max}=341.0 nm, IR (cm^{-1}): 3371.2 (OH phenol); 3230.8 (NH lactam); 2982.6 (C-H alkyl); 1711.8 (C=O lactam); 1651.8 (C=N imine); 1609.4; 1511.2 (C=C benzene); 1259.2 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.5 (s, 1H: NH); 9.8 (s, 1H: OH); 7.6 (s, 1H: -CH=); 7.31 (s, 1H: $H_{3'}$); 7.3 (s, 1H: $H_{5'}$); 7.28 (s, 1H: $H_{6'}$); 7.2 (s, 1H: $H_{2"}$); 7.15 (s, 1H: $H_{4'}$); 6.9 (d, J=8 Hz, 1H: $H_{6"}$); 6.87 (d, J=8 Hz, 1H: $H_{5"}$); 3.7 (s, 3H: CH_3). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.33 (C_4); 154.57 (C_2); 152.62 ($C_{2'}$); 149.01 ($C_{3"}$); 147.86 ($C_{4"}$); 136.52 ($C_{1'}$); 130.73 (=CH-); 129.95 ($C_{4'}$); 126.22 ($C_{1"}$); 125.05 ($C_{5'}$); 124.59 ($C_{6'}$); 122.73 ($C_{6"}$); 116.40 ($C_{3'}$); 116.20 ($C_{5"}$); 115.50 (C_5); 115.17 ($C_{2"}$); 55.68 (-OCH_3). ESI-MS: $[M+H]^+$ (m/z) 345.06.

Compound F9

Formula $C_{16}H_{10}N_3O_3SF$, MW 343.04, yield 50%, light yellow powder, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 293-294^{0}C, UV: λ_{max}= 355.5 nm, IR (cm^{-1}): 3432.9 (NH lactam); 1677.7 (C=O lactam); 1627.7 (C=N imine); 1600.9; 1482.2 (C=C benzene); 1456.1 (C-NO_2); 1246.3 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.8 (s, 1H: NH); 8.3 (d, J=9 Hz, 2H: $H_{3"}$, $H_{5"}$); 7.7 (d, J=7.5 Hz, 3H: $H_{2"}$, $H_{6"}$, -CH=); 7.3 (t, J=7 Hz, 1H: $H_{4'}$); 7.2 (m, 2H: $H_{3'}$, $H_{6'}$); 7.15 (t, J=8 Hz, 1H: $H_{5'}$). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.63 (C_4); 154.53 (C_2); 152.50 ($C_{2'}$); 147.13 ($C_{4"}$) ; 139.52 ($C_{1"}$); 135.67 ($C_{1'}$); 130.63 ($C_{2"}$,$C_{6"}$); 127.35 (-CH=); 126.47 ($C_{4'}$); 125.15 ($C_{5'}$); 125.12 ($C_{6'}$); 124.19 ($C_{3"}$, $C_{5"}$); 116.48 ($C_{3'}$); 116.32 (C_5). ESI-MS: $[M+Na]^+$ (m/z) 366.04.

Compound F10

Formula $C_{18}H_{15}N_2O_3SF$, MW 358.08, yield 90%, dark yellow crystal, odourless, insoluble in water, soluble in ethanol, acetone, DMF, DMSO, m.p. 240-241^{0}C, UV: λ_{max}=356 nm, IR (cm^{-1}): 3449.3 (NH lactam); 2970.8 (C-H alkyl); 1728.9 (C=O lactam); 1692.3 (C=N imine); 1595.3; 1455.7 (C=C benzene); 1139.4 (C-O); 1278.0 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.7 (s, 1H: NH); 7.6 (s, 1H: -CH=); 7.3 (t, J=10 Hz, 1H: $H_{4'}$); 7.2 (s, 2H: $H_{5'}$, $H_{2"}$); 7.18 (s, 1H: $H_{6'}$); 7.15 (s, 1H: $H_{3'}$); 7.1 (d, J=8.5 Hz, 1H: $H_{6"}$); 7.0 (d, J=8.5 Hz, 1H: $H_{5"}$); 3.8 (s, 3H: CH_3); 3.7 (s, 3H: CH_3). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.53 (C_4); 154.28 (C_2); 152.63 ($C_{2'}$); 150.43 ($C_{3"}$); 148.84 ($C_{4"}$); 135.60 ($C_{1'}$); 130.32 (=CH-); 126.17 ($C_{4'}$); 125.96 ($C_{1"}$); 125.02 ($C_{5'}$); 123.26 ($C_{6'}$); 122.33 ($C_{6"}$); 119.81 (C_5); 116.39 ($C_{3'}$); 114.24 ($C_{5"}$); 112.14 ($C_{2"}$); 55.59 (-OCH_3); 55.56 (-OCH_3). ESI-MS: $[M+H]^+$ (m/z) 359.08

Compound F11

Formula $C_{19}H_{17}N_2O_4SF$, MW 388.08, yield 92%, light yellow crystal, odourless, insoluble in water, methanol, soluble in ethanol, acetone, DMF, DMSO, m.p. 201-203^{0}C, UV: λ_{max}=352.5 nm, IR (cm^{-1}): 3448.7 (NH lactam); 2980.1 (C-H alkyl); 1704.7 (C=O lactam); 1660.7 (C=N imine); 1606.4; 1504.9 (C=C benzene); 1320.6 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.7 (s, 1H: NH); 7.6 (s, 1H: -CH=); 7.3 (t, J=10 Hz, 2H: $H_{4'}$, $H_{5'}$); 7.21

(d, J=5 Hz, 1H: $H_{3'}$); 7.17 (d, J=5 Hz, 1H: $H_{6'}$); 6.8 (s, 2H: $H_{2''}$, $H_{6''}$); 3.7 (s, 6H: OCH_3, OCH_3), 3.69 (s, 3H: OCH_3). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.57 (C_4); 154.28 (C_2); 152.63 ($C_{2'}$); 151.44 ($C_{3''}$, $C_{5''}$); 140.84 ($C_{4''}$); 135.56 ($C_{1'}$); 132.52 (=CH-); 128.18 ($C_{4'}$); 127.96 ($C_{1''}$); 126.02 ($C_{5'}$); 124.26 ($C_{6'}$); 119.81 (C_5); 117.39 ($C_{3'}$); 110.24 ($C_{2''}$, $C_{6''}$); 60.80 (-OCH_3); 56.56 (-OCH_3). ESI-MS: $[M+H]^+$ (m/z) 389.08.

Compound F12

Formula $C_{16}H_{11}N_2O_2SF$, MW 314.05, yield 74%, red powder, odourless, insoluble in water, ethanol, soluble in methanol, acetone, DMF, DMSO, m.p. 246-248^{0}C, UV: λ_{max}=362.0 nm, IR (cm^{-1}): 3448.2 (OH phenol); 3267.8 (NH lactam); 1723.2 (C=O lactam); 1655.1 (C=N imine); 1596.9; 1455.9 (C=C benzene); 1253.8 (C-F). ^{1}H-NMR (500MHz, DMSO-*d6*, δ ppm): 12.5 (s, 1H: NH); 10.4 (s, 1H: OH); 7.9 (s, 1H: -CH=); 7.3 (m, 2H: $H_{4'}$, $H_{6''}$); 7.2 (t, J=7 Hz, 3H: $H_{3''}$, $H_{5'}$, $H_{6'}$); 7.14 (d, J=8 Hz, 1H: $H_{3''}$); 6.9 (t, J=8 Hz, 1H: $H_{4''}$); 6.86 (t, J=8 Hz, 1H: $H_{5''}$). ^{13}C-NMR (125MHz, DMSO-*d6*, δ ppm): 167.63 (C_4); 156.87 ($C_{2''}$); 154.53 (C_2); 152.50 ($C_{2'}$); 135.99 ($C_{1'}$); 131.72 (=CH); 128.21 ($C_{4''}$); 125.25 ($C_{6''}$); 125.05 ($C_{4'}$); 123.24 ($C_{1''}$); 120.15 ($C_{5''}$); 119.6 ($C_{6'}$); 117.1 (C_5); 116.4 ($C_{3'}$); 115.98 ($C_{3''}$); 115.89 ($C_{5'}$). ESI-MS: $[M+H]^+$ (m/z) 315.05.

Antimicrobial activity

The antimicrobial activity is showed in Table 1 and Table 2.

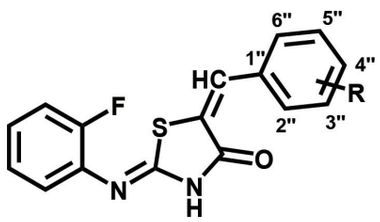
3. DISCUSSION

All synthesized compounds, S1, S2 and F1-F12, were characterized by physical appearances, melting points, spectral method to demonstrate the expected products. Almost all compounds showed a medium peak of C=O stretching at a frequency in the range of 1680-1730 cm^{-1} and a broad peak with one spike in the range of 3200-3500 cm^{-1} for N-H and O-H stretching which might overlap with each other in the IR spectrum. This phenomenon might be the keto-enol tautomerization of the target compounds. With some exception, F9 showed a stronger peak of C=O than others. The electron withdrawing group of NO_2 at para-position might be the reason to stabilize the molecule as a ketone form. In addition, compound S2 clearly showed the effect of resonance property and the IR spectra demonstrated the strong C=O peak. This issue might be explained by the effect of substitution at 5-aryl on thiazolidin-4-one ring through the electron delocalization in the molecule.

The fact that compounds F4 and F9 had lower yield (65% and 50%, respectively) than other derivatives. It might be due to the nitro group substituted on the phenyl ring. Either nitro on ortho- or para-position might retarded the aldol reaction from the withdrawing or steric properties. However, this effect did not detect in the aldol reaction of other structures such as chalcone15. It might need more research to confirm the chemical reaction property.

Based on the antimicrobial screening by clear zone assay, only six compounds, F1, F3, F4, F8, F11 and F12, demonstrated antimicrobial activity either on *S. aureus*, MRSA or *C. albican* at a concentration of 2,048 μg/ml. These six compounds were selected for further investigation on MIC assay. None of the tested compounds showed the activity on fungi strains. Only two compounds were active in bacterial testing. Compound F4 showed potent antibacterial action on *S. aureus* and MRSA at minimum concentration of 16 μg/ml and 32 μg/ml, respectively, and compound F8 showed activity at 64 μg/ml for both bacteria strains. This result might be explained by the influence of type, position and the structure of substituted groups at 5-aryl group on thiazolidin-4-one ring. The derivative containing nitro group at ortho-position (F4) was likely to interact with the target site of *S. aureus* and MRSA better that other substitution group. This indicated that the position of nitro group was important for the activity. Compound F9 with nitro group at para-position was not presented the similar action. The substitution

Table 1. Antimicrobial activity of the synthesized compounds F1-F13 on the various strains of bacteria and fungi.



Ab.	Name	R	Bacteria					Fungi	
			E. coli	P. aeruginosa	S. faecalis	S. aureus	MRSA	C. albican	A. niger
F1	2-(2-fluorophenylimino)-5-(2,4-dichlorobenzylidene) thiazolidin-4-one	2”-Cl 4”-Cl	-	-	-	+	+	-	-
F2	2-(2-fluorophenylimino)-5-(4-chlorobenzylidene)thiazolidin-4-one	4”-Cl	-	-	-	-	-	-	-
F3	2-(2-fluorophenylimino)-5-benzylidenethiazolidin-4-one	-H	-	-	-	-	-	+	-
F4	2-(2-fluorophenylimino)-5-(2-nitrobenzylidene)thiazolidin-4-one	2”-NO_2	-	-	-	+	+	+	-
F5	2-(2-fluorophenylimino)-5-(4-fluorobenzylidene)thiazolidin-4-one	4”-F	-	-	-	-	-	-	-
F6	2-(2-fluorophenylimino)-5-(4-methoxybenzylidene)thiazolidin-4-one	4”-OCH_3	-	-	-	-	-	-	-
F7	2-(2-fluorophenylimino)-5-(4-methylbenzylidene)thiazolidin-4-one	4”-CH_3	-	-	-	-	-	-	-
F8	2-(2-fluorophenylimino)-5-(-4-hydroxy-3-methoxybenzylidene)thiazolidin-4-one	3”-OCH_3 4”-OH	-	-	-	+	+	+	-
F9	2-(2-fluorophenylimino)-5-(4-nitrobenzylidene)thiazolidin-4-one	4”-NO_2	-	-	-	-	-	-	-
F10	2-(2-fluorophenylimino)-5-(3,4-dimethoxybenzylidene) thiazolidin-4-one	3”-OCH_3 4”-OCH_3	-	-	-	-	-	-	-
F11	2-(2-fluorophenylimino)-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one	3”-OCH_3 4”-OCH_3 5”-OCH_3	-	-	-	-	-	+	-
F12	2-(2-fluorophenylimino)-5-(-2-hydroxybenzylidene)thiazolidin-4-one	2”-OH	-	-	-	-	-	+	-

Note: Testing solution at concentration 2,048 μg/ml, the study was performed in duplicate.
(-) : negative, clear zone diameter is lower than reference one (+) : positive, clear zone diameter is higher than reference one

Table 2. MIC of antimicrobial compounds

	MIC (µg/ml)		
	Staphylococcus aureus	*MRSA*	*Candida albicans*
F1	>1024	>1024	-
F3	-	-	>1024
F4	16 ± 1	32 ± 2	>1024
F8	64 ± 3	64 ± 3	>1024
F11	-	-	>1024
F12	-	-	>1024

Note: The study was performed in duplicate, results are shown in average value.
(-) : not determined.

at para-position might require the appropriate size to enter into the target site. With this hypothesis, the result of compound F8 with m-OCH¬3 and p-OH substitution was also shown antibacterial activity for both *S. aureus* and MRSA but the corresponding F10 with adding a methyl group on the p-hydroxyl group of F8 clearly reduced the antibacterial activity. It was possible to conclude that the steric property at para-position of 5-aryl group might play a major role in binding. From the result, it was more likely that the 5-aryl substituted on thiazolidin-4-one ring required electron withdrawing group at ortho-position and a specific size or the H-bond donor group (OH) at para-position for the inhibitory activity on *S. aureus*. However, the tested compounds were in limit number, further studies are needed to confirm the hypothesis and to improve the antibacterial activity.

The MIC of both F4 and F8 showed moderate activity compared to the previous studies[3,7,8,9-12] but it showed the potential activity for MRSA. Accordingly, these compounds were very interesting for further investigated.

4. CONCLUSION

In this research, twelve derivatives of 2-(2-fluorophenylimino)-5-arylidenethiazolidin-4-one were synthesized by a three-step process with easy-to-find, inexpensive ingredients and simple laboratory equipments. All the synthesized compounds were new compounds which were not found in chemical sources like Chemspider and PubChem. Some compounds, especially F4 and F8, can be a good starting point for further exploration to develop a new antimicrobial agent in the future.

5. ACKNOWLEDGEMENTS

Authors are thankful to the department of Pharmaceutical Chemistry, Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Viet Nam for providing us chemical reagents and facilities in the synthesis process. We also acknowledge the department of Biology, University of Medicine and Pharmacy, Ho Chi Minh city for antimicrobial testing equipments and the Ha Noi Institute of Chemistry for NMR and MS analysis.

REFERENCES

1. World Health Organization. Antimicrobial resistance. 2014. Available from: http://www.who.int/mediacentre/factsheets/fs194/en/
2. Brown FC. 4-Thiazolidinones. Chem Rev 1961;71(3):81-104.
3. Abhinit M, Ghodke M, Pratima NA. Exploring potential of 4-thiazolidinone: a brief review. Int J Pharm Pharm Sci 2009; 1(1):47-64.

4. Havrylyuk D, Zimenkovsky B, Lesyk R. Synthesis and anticancer activity of novel nonfused bicyclic thiazolidinone derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements. 2009;184(3):638-50.
5. Buvana C, Mahalaksmi A, Bijo M, Sukumar M. Synthesis of some thiazolidinone derivatives from indole 2-Carboxylic acid and evaluation of its xanthine oxidase inhibitory and antioxidant activity. Int J ChemTech Res 2011;3(2):671-5.
6. Gursoy A, Terzioglu N. Synthesis and isolation of new regioisomeric 4-thiazolidinones and their anticonvulsant activity. Turkish J Chem 2005;29:247-54.
7. Chavan A, Pai NR. Synthesis and antimicrobial screening of 5-aryliden-4-thiazolidinones. Arkivoc 2007;16:148-55.
8. Desai NC, Bhatt N, Mukesh K, Autul M. Synthesis, characterization and antimicrobial activity activity of some 3-(2-(1H-benzo [d]imidazol-2-yl)phenyl)-2-arylthiazolidin-4-ones. Indian J Chem 2011;50B:941-5.
9. Parekh HH, Parikh KA, Parikh AR. Synthesis of Some 4-Thiazolidinone Derivatives as Antitubercular Agents. Journal of Sciences – Islamic Republic of Iran. 2004;15(2): 143-8.
10. Shelar V, Ravi TR, Venkataramana CHS. Synthesis and antimicrobial activity of some novel thiazolidinone derivatives. Der Pharmacia Lettre. 2010;2(5):146-54.
11. Vicini P, Geronikaki A, Incerti M, Zani F, Dearden J, Hewitt M. 2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones with antimicrobial activity: synthesis and structure–activity relationship. Bioorg Med Chem 2008;16:3714-24.
12. Vinay V, Khurana L. A review on anti-microbial activity of 4-thiazolidinone derivatives. IJRPS 2011;1(1):17-27.
13. Andrews JM, Howe RA. BSAC standardized disc susceptibility testing method. J Antimicrob Chemother 2001; 66(12):2726-57.
14. MacGowan AP, Wise R. Establishing MIC breakpoints and interpretation of in vitro susceptibility tests. J Antimicrob Chemother 2001;48(1):17-28.
15. Farouq EH, Lana HC, Diler DG. Solvent-free synthesis and spectroscopic identification of some chalcones and imines derived from p-amino acetophenone. Tikrit Journal of Pure Science. 2010; 15(2):78-86.