Synthesis and Antimicrobial Studies of New Trifluoromethylpyrimidine Analogues

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Abstract: This paper describes the synthesis of a new series of trifluoromethylpyrimidine and their potential antimicrobial evaluation. We have prepared 10 novel pyrimidine derivatives in high yields and short reaction time using microwave irradiation. Compounds 4a,b and 6a,b showed good antimicrobial activity against Gram-positive bacteria; *Clostridium perfringens*, *Bacillus pumilus* and *Enterococcus faecalis*. These compounds were found to be the most potent antimicrobials compared to ampicillin, tetracycline, and streptomycin that showed no activity against *Enterococcus faecalis*. Compounds 4a,b and 6a,b exhibited great antimicrobial potency against all tested bacteria strains at a MIC of 3.125-100 μg/mL whereas only 4a showed the antimicrobial activity against Gram-negative bacteria *Klebsiella pneumonia* with a MIC value 12.5 μg/mL.

Key words: Synthesis, microwave, fluoropyrimidine, antimicrobial.

1. Introduction

In spite of the remarkable growth in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still representing major threats to public health. Their impact is particularly large in developing countries due to the relative unavailability of medicines, while the excessive use of antimicrobial drugs has led to the emergence of widespread bacterial resistance [1]. In 2003, a number of substituted pyrimidines were synthesized and intensively studied as potent and selective inhibitors of Gram positive bacterial DNA polymerase IIIC [2]. Over the last decades, development of drug resistance as well as the appearance of undesirable side effects of some antibiotics [3] has initiated the search for new antimicrobial agents to overcome some of the disadvantages of the existing drugs [4]. Fluorinated pyrimidine derivatives have attracted more attention especially in biological and medicinal chemistry fields because of the unique features of fluorine compounds and their physiological activity [5,6]. The introduction

of fluorine atoms into organic compounds often permits dramatic changes in their chemical and pharmaceutical properties [7]. The presence of pyrimidine nucleus in compounds containing fluorine atoms was found enhancing the biological activities, such as anti-viral [8], anti-malarial [9], adenosine receptor [10], anti-cancer agents [11], as well as compounds targeting delayed-type hypersensivity agents [12].

Hence, there is a never lasting demand for synthesis of novel antimicrobial agents with high potency, efficacy and minimum side effects; this work aims to synthesize novel fluoropyrimidine derivatives with high potency and efficiency against different bacterial strains. Our synthetic approach is based on microwave protocols to enhance the yields in shorter times for the targeted compounds 3-5a,b and 6a-f. The antimicrobial activities of the newly synthesized fluoropyrimidine derivatives 4a,b and 6a,b will be tested against different bacteria strains such as Gram-positive bacteria, *Clostridium perfringens*, *Bacillus pumilus* and *Enterococcus faecalis* and Gram-negative bacteria, *Klebsiella pneumonia*. Results out of this work will establish a new structure-activity relationships based

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on substitutions at C-2 and C-4 of the pyrimidine ring.

2. Materials and Methods

General: Microwave synthetic protocol was done using CEM Microwave system. Melting points were determined on (Pyrex capillary) Gallenkamp apparatus. Infrared spectra was recorded with a Thermo Nicolet Nexus 470 FT-IR spectrometer in the range 4,000-400cm^{-1} on samples in potassium bromide disks. ^{1}H-NMR spectra, ^{13}C-NMR spectra were obtained on Varian Gemini 400 MHz FT NMR spectrometer in CDCl$_3$ and DMSO-d_6; chemical shifts were recorded in δ (ppm) units, relative to Me$_4$Si as an internal standard. All exchangeable protons were confirmed by addition of D$_2$O. Thin-layer chromatography (TLC) was carried out on precoated Merck silica gel F$_{254}$ plates and UV light was used for visualization. Column chromatography was performed on a Merck silica gel. The reagents were purchased from Aldrich and used without further purification. Elemental analysis performed on Leco Model CHN-600 elemental analyzer.

2.1 *Microwave Synthesis of 2-Hydroxypyrimidine Analogous (3a,b)*

A mixture of 1,3-diketone 1a,b (2.0 mmol), urea (120 mg, 2.0 mmol) and 2 drops of HCl (6.0 M) in ethanol (8 mL) was mixed in 10 mL CEM-microwave vial. The vial was sealed and irradiated in CEM-microwave reactor at 135°C for 5-10 min. The reaction was verified for completion by TLC and recrystallized from a proper solvent to give 3a,b in yields 89% and 84% respectively.

4-(Thien-2′-yl)-6-(trifluoromethyl)pyrimidin-2-ol (3a): yellow crystals; yield 89%; mp 230 °C; IR (KBr, cm^{-1}): 3,459 (br, OH), 3,088 (C-H aromatic), 1,680 (CONH); ^{1}H-NMR [DMSO-d_6, 400 MHz]: (δ, ppm) 7.29 (s, 1H, H-5 pyrimidine), 7.30 (t, 1H, thien-2′-yl H-4′, J = 4.0 Hz), 7.93 (d, 1H, thien-2′-yl H-5′, J = 5.0 Hz), 8.29 (d, 1H, thien-2′-yl H-3′, J = 4.0 Hz), 12.88 (1H, s, OH exchangeable with D$_2$O); ^{13}C-NMR

[DMSO-d_6, 100 MHz]: (δ, ppm) 103.6 (C-5), 120.6 (CF$_3$, q, J = 274 Hz), 129.1, 131.1, 132.8 (C-3′, C-4′, C-5′ thien-2′-yl), 140.5 (C-2′ thien-2′-yl), 160.7 (C-6 pyrimidine), 163.5 (C-4 pyrimidine), 165.1 (C-2 pyrimidine). Anal. Calcd for C$_9$H$_5$F$_3$N$_2$OS: C, 43.90; H, 2.05; N, 11.38; S, 13.02. Found: C, 44.35; H, 2.12; N, 11.66; S, 13.30.

4-Phenyl-6-(trifluoromethyl)pyrimidin-2-ol (3b): white powder; yield 84%; mp 234 °C, from hexane; IR (KBr, cm^{-1}): 3,489 (br, OH), 3,076 (CH-aromatic), 1,676 (CONH); ^{1}H-NMR [DMSO-d_6, 400 MHz]: (δ, ppm) 7.22 (s, 1H, H-5 pyrimidine), 7.52-7.61 (m, 3H, aromatic), 8.16-8.17 (m, 2H, aromatic), 12.88 (s, 1H, OH; exchangeable with D$_2$O); ^{13}C-NMR [DMSO-d_6, 100 MHz]: (δ, ppm) 109.4 (C-5), 122.8 (CF$_3$, q, J = 274 Hz), 129.0, 130.4, 133.4, 139.8 (phenyl carbons), 161.7 (C-6 pyrimidine), 165.3 (C-4 pyrimidine), 167.8 (C-2 pyrimidine). Anal. Calcd. for C$_{11}$H$_7$F$_3$N$_2$O: C, 55.01; H, 2.94; N, 11.66; Found: C, 55.46; H, 3.01; N, 11.94.

2.2 *Microwave Synthesis of 2-Pyrimidine Benzoyl Esters (5a,b)*

To a solution of 3a,b (2 mmol) in 15 mL pyridine, p-fluorobenzoyl chloride (5 mmol, 0.59 mL) was added gradually with stirring in ice bath. After the addition is completed, the reaction mixture was heated under microwave irradiation at 100°C for about 10 min. The progress of the reaction was monitored by TLC, The solid obtained was washed with water and crystallized from ethanol to give the desired compounds 4a,b.

4-(Thien-2′-yl)-6-(trifluoromethyl)-2-pyrimidinyl-4″-fluorobenzoate (4a): white crystals, yield 85%; mp 105-7 ℃; IR (KBr, cm^{-1}): 3,116 (C-H, aromatic), 1,757 (C=O), 1,603 (C=C), 1,429 (C=N); ^{1}H-NMR [CDCl$_3$, 400 MHz]: (δ, ppm) 7.17-7.22 (m, 3H, phenyl & thien-2′-yl H-4′), 7.64 (dd, 1H, thien-2′-yl H-5′, J = 4.9 Hz), 7.79 (s, 1H, H-5 pyrimidine), 7.92 (dd, 1H, thien-2′-yl H-3′, J = 3.7 Hz), 8.24 (m, 2H, aromatic); ^{13}C-NMR [CDCl$_3$, 100 MHz]: (δ, ppm) 111.9 (C-5 pyrimidine), 115.41 (phenyl carbon), 122.8 (CF$_3$, q, J =

274 Hz), 127.2, 127.3, 127.4, 128.9, 129.0 (Ar-carbons), 148.3 (C-2′ thien-2′-yl), 161.5 (C-6 pyrimidine), 163.2 (C-4 pyrimidine), 165.4 (C=O), 165.5 (C-F, aromatic), 165.7 (C-2 pyrimidine). Anal. Calcd for $C_{16}H_8F_4N_2O_2S$: C, 52.18; H, 2.19; N, 7.61; S, 8.71; Found: C, 52.63; H, 2.26; N, 7.89; S, 8.99.

4-Phenyl-6-(trifluoromethyl)pyrimidin-2-yl 4′-fluorobenzoate (4b): white powder; yield 83%; mp 114 ℃; IR (KBr, cm^{-1}): 3,118 (C-H, aromatic), 1,758 (C=O), 1,602 (C=C), 1,428 (C=N); ^{1}H-NMR [DMSO-d_6, 400 MHz]: (δ, ppm) 7.26 (1H, s, H-5 pyrimidine), 7.53-7.61 (m, 5H, aromatic), 7.93-7.95 (m, 2H, aromatic), 8.25-8.27 (m, 2H, aromatic); ^{13}C-NMR [DMSO-d_6, 100 MHz]: (δ, ppm) 109.2 (C-5 pyrimidine), 115.1 (aromatic carbons), 122.8 (CF$_3$, q, J = 274 Hz), 127.2, 127.3, 128.9, 129.0, 129.8, 133.4 (aromatic carbons), 148.8 (C-6 pyrimidine), 161.5 (C-4 pyrimidine), 163.2 (C=O), 165.3 (C-F, aromatic), 165.8 (C-2 pyrimidine). Anal. Calcd for $C_{18}H_{10}F_4N_2O_2$: C, 59.68; H, 2.78; N, 7.73; Found: C, 60.13; H, 2.85; N, 8.01.

2.3 *Microwave Synthesis of 2-Chloropyrimidine Derivatives (5a,b)*

In 10 mL CEM-microwave vessel, two drops of pyridine were added to a mixture of 3a,b (2.0 mmol) and POCl$_3$ (4.0 mmol, 0.37 ml). The vial was sealed and the mixture was heated under microwave irradiation at 100°C for 25 min. The reaction mixture was cooled to room temperature then poured into an ice-cold water (10 mL) under vigorous stirring. The pH was adjusted to pH-8 and the resulting mixture was stirred for 15 minutes. The obtained light brown solid was filtered, washed with water (2 ×10 mL) and dried under reduced pressure for 2 hours.

2-Chloro-4-(thien-2′-yl)-6-(trifluoromethyl)pyrimidine (5a): brown crystals; yield 96%; mp 109 °C; ^{1}H-NMR [DMSO-d_6, 400 MHz]: (δ, ppm) 7.30-7.33 (t, 1H, thien-2'-yl H-4′, J = 4.0 Hz), 7.84 (1H, s, H-5 pyrimidine), 7.92 (d, 1H, thien-2'-yl H-5′, J = 5.0 Hz), 8.21 (d, 1H, thien-2'-yl H-3′, J = 4.0 Hz); ^{13}C-NMR

[DMSO-d_6, 100 MHz]: (δ, ppm) 105.2 (C-5 pyrimidine), 122.0 (CF$_3$, q, J = 274 Hz), 129.7, 131.5, 133.3 (C-3′, C-4′, C-5′ thien-2′-yl), 140.1 (C-2′ thien-2′-yl), 163.6 (C-6 pyrimidine), 164.7 (C-4 pyrimidine), 165.1 (C-2 pyrimidine).

2-Chloro-4-phenyl-6-(trifluoromethyl)pyrimidine (5b): brown crystals; yield 95%; mp 105°C, from ethanol; ^{1}H-NMR [DMSO-d_6, 400MHz]: (δ, ppm) 7.58-7.67 (m, 3H, aromatic), 7.75 (s, 1H, H-5 pyrimidine), 8.16-8.17 (m, 2H, aromatic); ^{13}C-NMR [DMSO-d_6, 100MHz]: (δ, ppm) 104.9 (C-5 pyrimidine), 122.5 (CF$_3$, q, J = 274 Hz), 129.7, 131.4, 132.9, 136.4 (aromatic carbons), 157.5 (C-6 pyrimidine), 162.7 (C-2 pyrimidine), 165.1 (C-4 pyrimidine).

2.4 *Microwave Amination Procedure (6a-f)*

To a solution of 4-aryl-2-chloro-6-(trifluoro methyl)pyrimidine 5a,b in toluene (15 mL), an excess of amine was added at room temperature in 35 mL CEM microwave vial. The vial was sealed and the reaction mixture was heated under microwave irradiation at 80-100°C for 5-10 min. The progress of the reaction was monitored by TLC and after completion; the reaction mixture was quenched with water (0.5 mL) and a solution of sodium carbonate (0.1 g, 2 mmol) was added with stirring at room temperature. The reaction mixture was extracted in ether and the organic layer was dried over anhydrous MgSO$_4$. The product purified by silica gel column chromatography with ethylacetate: hexane (6:4) to give pure products 6a-f.

2-(*N*-Cyclopentyamino)-4-(thien-2′-yl)-6-(trifluoro methyl)pyrimidine (6a): pale yellow crystal, yield 86 %; mp 99 ℃; IR (KBr, cm^{-1}): 3,544 (-NH), 3,089 (C-H, aromatic), 2,931 (aliphatic C-H), 1,478 (C=N); ^{1}H-NMR [CDCl$_3$, 400 MHz]: (δ, ppm) 1.47-1.73 (m, 8 H, cyclopentyl), 4.30-4.36 (m, 1H, cyclopentyl), 5.35 (d, 1H, -NH, exchanges with D$_2$O, J = 6 Hz), 7.08 (1H, s, H-5 pyrimidine), 7.12-7.15 (m, 1H, thien-2′-yl H-4′), 7.49-7.51 (m, 1H, thien-2′-yl H-5′), 7.70 (m, 1H,

thien-2′-yl H-3′); ^{13}C-NMR [CDCl$_3$, 100 MHz]: (δ, ppm) 23.7 (C-3, C-4 cyclopentyl), 33.1 (C-2, C-5 cyclopentyl), 53.2 (C-1 cyclopentyl), 99.6 (C-5 pyrimidine), 120.7 (CF$_3$, q, J = 274 Hz), 128.3, 130.4, 132.4 (C-3′, C-4′, C-5′ thien-2′-yl), 142.6 (C-2′ thien-2′-yl), 156.5 (C-6 pyrimidine), 161.8 (C-4 pyrimidine), 162.8 (C-2 pyrimidine). Anal. Calcd. for C$_{14}$H$_{14}$F$_3$N$_3$S: C, 53.66; H, 4.50; N, 13.41; S, 10.23; Found: C, 54.11; H, 4.57; N, 13.69; S, 10.51.

2-(*N*-Cycloheptylamino)-4-(thien-2′-yl)-6-(trifluoro methyl)pyrimidine (6b): white powder, R$_f$ = 0.66 (ethylacetate:hexane 1:1), yield 77 %; mp 111-13 ℃; IR (KBr, cm^{-1}): 3,531 (-NH), 3,054 (C-H, aromatic), 2,884 (aliphatic C-H), 1,600 (C=C), 1,463 (C=N); ^{1}H-NMR [CDCl$_3$, 400 MHz]: (δ, ppm) 1.55-1.64 (m, 10H, cycloheptyl), 2.05-2.06 (m, 2H, cycloheptyl), 4.09 (m, 1H, cycloheptyl), 5.36 (d, 1H, -NH, exchanges with D$_2$O, J = 4.0 Hz), 7.07 (1H, s, H-5 pyrimidine), 7.13 (t, 1H, thien-2′-yl H-4′, J = 4.0 Hz), 7.50-7.51 (d, 1H, thien-2′-yl H-5′, J = 4.0 Hz), 7.73 (m, 1H, thien-2′-yl H-3′); ^{13}C-NMR [CDCl$_3$, 100 MHz]: (δ, ppm) 24.2 (C-3, C-6 cycloheptyl), 30.9 (C-4, C-5 cycloheptyl), 34.6 (C-2, C-7 cyclopentyl), 53.2 (C-1 cycloheptyl), 101.0 (C-5 pyrimidine), 120.8 (CF$_3$, q, J = 274 Hz), 127.1, 128.8, 131.2 (C-3′, C-4′, C-5′ thien-2′-yl), 141.9 (C-2′ thien-2′-yl), 156.9 (C-6 pyrimidine), 161.7 (C-4 pyrimidine), 167.2 (C-2 pyrimidine). Anal. Calcd for C$_{16}$H$_{18}$F$_3$N$_3$S: C, 56.29; H, 5.31; N, 12.31; S, 9.39; Found: C, 56.74; H, 5.38; N, 12.59; S, 9.67.

2-(*N*-Methylpiperazin-1′-yl)-4-(thien-2′-yl)-6-(triflu oromethyl)pyrimidine (6c): white powder, R$_f$ = 0.16 (ethylacetate:hexane 1:1), yield 89 %; mp 109-11 ℃; IR (KBr, cm^{-1}): 3,095 (C-H, aromatic), 2,911 (aliphatic C-H), 1,593 (C=C),1,452 (C=N), 1,254 (C-N); ^{1}H-NMR [CDCl$_3$, 400 MHz]: (δ, *ppm*) 2.35 (s, 3H, methyl group), 2.49-2.50 (m, 4H, piprazine ring), 3.93-3.95 (m, 4H, piprazine ring), 7.05 (1H, s, H-5 pyrimidine), 7.14 (t, 1H, thien-2′-yl H-4′, J = 4.0 Hz), 7.50 (dd, 1H, thien-2′-yl H-5′, J = 4.9 Hz), 7.72 (dd, 1H, thien-2′-yl H-3′, J = 3.7 Hz); ^{13}C-NMR [CDCl$_3$,

100 MHz]: (δ, ppm) 43.6 (CH$_3$), 46.2 (piprazine), 54.9 (piprazine), 99.1 (C-5), 120.8 (CF$_3$, q, J = 274 Hz), 127.8, 128.3, 130.2 (C-3′, C-4′, C-5′ thien-2′-yl), 142.7 (C-2′ thien-2′-yl), 156.6 (C-6 pyrimidine), 161.4 (C-4 pyrimidine), 161.5 (C-2 pyrimidine). Anal. Calcd. for C$_{14}$H$_{15}$F$_3$N$_4$S: C, 51.21; H, 4.60; N, 17.06; S, 9.77; Found: C, 51.66; H, 4.67; N, 17.34; S, 10.02.

2-(*N*-Cyclopentylamino)-4-phenyl-6-(trifluorometh yl)pyrimidine (6d): pale yellow crystal, R$_f$ = 0.66 (ethylacetate: hexane 1:1), yield 78 %; mp 103 ℃; IR (KBr, cm^{-1}): 3,542 (-NH), 3,056 (C-H, aromatic), 2,918 (aliphatic C-H), 1,597 (C=C), 1,456 (C=N); ^{1}H-NMR [CDCl$_3$, 400 MHz]: (δ, ppm) 1.51-1.53 (m, 2 H, cyclopentyl), 1.64-1.75 (m, 4H, cyclopentyl), 2.08-2.11 (m, 2H, cyclopentyl), 4.40 (br, 1H, cyclopentyl), 5.41 (d, -NH, exchanges with D$_2$O, J = 6.8 Hz), 7.22 (1H, s, H-5 pyrimidine), 7.48-7.49 (m, 3H, aromatic), 8.05 (m, 2H, aromatic); ^{13}C-NMR [CDCl$_3$, 100 MHz]: (δ, ppm) 23.7 (C-3, C-4 cyclopentyl), 33.1 (C-2, C-5 cyclopentyl), 53.2 (C-1 cyclopentyl), 99.6 (C-5 pyrimidine), 120.4 (CF$_3$, q, J = 274 Hz), 128.3, 130.4, 132.4, 142.6 (aromatic carbons), 156.5 (C-6 pyrimidine), 161.8 (C-4 pyrimidine), 162.0 (C-2 pyrimidine). Anal. Calcd. for C$_{16}$H$_{16}$F$_3$N$_3$: C, 62.53; H, 5.25; N, 13.67; Found: C, 62.98; H, 5.32; N, 13.95.

2-(*N*-Cycloheptylamino)-4-phenyl-6-(trifluorometh yl)pyrimidine (6e): white powder, R$_f$ = 0.68 (ethylacetate:hexane 1:1), yield 83%; mp 115-18 ℃; IR (KBr, cm^{-1}): 3,548 (-NH), 3,088 (C-H, aromatic), 2,923 (aliphatic C-H), 1,585 (C=C), 1,461 (C=N); ^{1}H-NMR [CDCl$_3$, 400 MHz]: (δ, ppm) 1.56-1.73 (m, 10 H, cycloheptyl), 2.06 (m, 2H, cycloheptyl), 4.17 (br,1H, cycloheptyl), 5.42 (d, 1H, NH, exchanges with D$_2$O, J = 8.0 Hz), 7.21 (1H, s, H-5 pyrimidine), 7.48-7.50 (m, 3H, aromatic), 8.05 (m, 2H, aromatic); ^{13}C-NMR [CDCl$_3$, 100 MHz]: (δ, ppm) 24.1 (C-3, C-6 cyclopentyl), 28.3 (C-4, C-5 cycloheptyl), 34.7 (C-2, C-7 cycloheptyl), 52.1 (C-1 cycloheptyl), 101.0 (C-5 pyrimidine), 120.8 (CF$_3$, q, J = 274 Hz), 127.1, 128.8, 131.2, 136.6 (aromatic carbons), 156.2 (C-6

pyrimidine), 161.8 (C-4 pyrimidine), 167.2 (C-2 pyrimidine). Anal. Calcd. for $C_{18}H_{20}F_3N_3$: C, 64.46; H, 6.01; N, 12.53; Found: C, 64.91; H, 6.08; N, 12.81.

2-(N-Methylpiperazin-1′-yl)-4-phenyl-6-(trifluorom ethyl)pyrimidine (6f): white powder, R_f = 0.15 (ethylacetate:hexane 1:1), yield 82%; mp 114-16 ℃; IR (KBr, cm^{-1}): 3,083 (C-H, aromatic), 2,924 (aliphatic C-H), 1,583 (C=C), 1,457 (C=N); ^{1}H-NMR [CDCl$_3$, 400MHz]: (δ, ppm) 2.35 (s, 3H, methyl), 2.49-2.50 (m, 4H, piprazine ring), 3.98 (m, 4H, piprazine ring), 7.19 (1H, s, H-5 pyrimidine), 7.46-7.48 (m, 3H, aromatic), 8.03-8.05 (m, 2H, aromatic); ^{13}C-NMR [CDCl$_3$, 100 MHz]: (δ, *ppm*) 43.7 (CH$_3$), 46.2 (C-2, C-6 methylpiprazine), 54.9 (C-3, C-5 methylpiprazine), 100.6 (C-5 pyrimidine), 120.9 (CF$_3$, q, J = 274 Hz), 127.2, 128.8, 131.2, 136.7 (aromatic carbons), 156.4 (C-6 pyrimidine), 161.7 (C-4 pyrimidine), 166.8 (C-2 pyrimidine). Anal. Calcd. for $C_{16}H_{17}F_3N_4$: C, 59.62; H, 5.32; N, 17.38; Found: C, 60.07; H, 5.39; N, 17.66.

2.5 Determination of MIC (Minimum Inhibitory Concentration)

2.5.1 Microdilution Method

The 96-well microtitre assay using resazurin as the indicator of cell growth [13] was employed for the determination of the minimum inhibitory concentration. Resazurin is an oxidation-reduction indicator used for the evaluation of microbial growth. The blue non-fluorescent dye turned into pink color with fluorescent when reduced to resorufin by oxidoreductase within cells. A 50 μL sterile deionized water was added to each well. A 50 μL purified test compound was added in the first well of horizontal row and double diluted horizontally in each well. Last well was added with 100 μL of sterile deionized water without test compound, used as control. A 100 μL double strength nutrient broth was added in each well then 10 μL test organisms (OD at 600 nm ~1) added to each well. This was followed by the addition of 1 μL resazurin (1% stock prepared). The microtitre plate was incubated at 37 ℃ for 18-24 h. The well with blue color (no viable bacteria) just before the pink well (viable bacteria) was taken as MIC value. The inoculated plates incubated. MIC was defined as the lowest concentration of the tested plant extracts that prevented resazurin color change from blue to pink.

2.5.2 Determination of Zones of the Inhibition

All the synthesized compounds were tested for their *in vitro* growth inhibitory activity against a panel of standard strains of pathogenic microorganism including Gram-positive and Gram-negative bacteria. Gram-positive bacteria are *Clostridium perfringens*, *Bacillus pumilus and Enterococcus faecalis* and Gram-negative bacteria's are *Klebsiella pneumonia* and *Pseudomonas aeruginosa.* The efficacy was determined by zone of inhibition values using disk diffusion technique [14]. To each petri-plate, 20 mL of sterilized medium was added. After the agar had set, 10% of inoculum of each microorganism culture was added to each petri-plate and spread thoroughly. Sterilized Whatmann no. 1 filter papers discs (diameter 6 mm) were thoroughly moistened with the synthesized compounds of specific concentrations 100 μg/mL in DMSO and placed on seeded agar plates. Paper discs moistened with DMSO were considered as negative control. Discs saturated with Ampicillin, Tetracycline and Streptomycin at the same concentrations were taken as standard (positive control). The plates were incubated at 37 ℃ for 24 h. The clear zone of inhibition around disc-paper demonstrated the relative susceptibility towards the synthesized derivatives.

3. Results and Discussion

Fluorine has played a pivotal role in novel drug discovery for modulating physical and biological properties of molecules [15-18]. Incorporation of one or several fluorine atoms into an organic molecule may enhance its biological activity, bioavailability, metabolic stability and lipophilicity due to intrinsic properties of fluorine atoms such as high electronegativity and small atomic radius [19].

3.1 Chemical Synthesis

All of the synthetic steps described in this paper were carried out under controlled microwave irradiation. The conversion of diketone to the targeted substituted pyrimidine 3-6 involving cyclization, chlorination and amination carried out under microwave irradiation yielded 3a,b (84-89%), 5a,b (95-96%) and 6a-f (77-89%) respectively (Table 1). Synthetic methodologies began by the reaction between trifluorobutane-1,3-dione 1a,b and urea via nucleophilic substitution at the vinyl carbon atom, followed by cyclization to form 6-trifluoromethyl pyrimidin-2-ol 3a,b. The structure and properties of the final products obtained have been established by their melting point, elemental analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The structure of 2-hydroxy-4-(thien-2′-yl)-6-trifluoromethyl pyrimidine 3a was confirmed using IR-spectroscopy which revealed by the appearance of broad band at υ = 3,459 cm^{-1} corresponding to a tautomeric hydroxyl group (N=C-OH). While, a sharp band appeared at υ = 1,680 cm^{-1} assigned for the keto-group (CONH) in 3a. The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound 3a showed a sharp signal at δ = 7.29 ppm assigned to the H-5 of pyrimidine. The thiophene protons showed the following splitting pattern: the H-4′ appeared as triplet at δ = 7.30 ppm with coupling constant J = 4.0 Hz, the H-5′ resonated as a doublet of doublet at δ = 7.93 ppm with coupling constant $J_{H5′,H4′}$= 5.0 Hz while the H-3′ appeared as doublet of doublet at δ = 8.29 ppm with coupling constant $J_{H3′,H4′}$= 4.0 Hz.

The hydrogen proton of the hydroxyl group resonates as singlet at δ = 12.88 ppm. ^{13}C-NMR (100 MHz, DMSO-d_6) showed a signal at δ = 103.6 ppm assigned for the C-5 of pyrimidine ring. The C-4 of pyrimidine resonates at δ =163.5 ppm and C-6 appeared at δ = 160.7 ppm, while the signal appeared at δ = 165.1 assigned to C-2 of pyrimidine ring. The CF$_3$ group split as quadratic at 120.6 ppm. Thiophene carbons resonate at δ = 129.1, 131.1, 132.8 and 140.5 ppm.

The synthetic pathway of the new p-fluorobenzoyl pyrimidine analogues 4a,b is shown in Scheme 1. p-Fluorobenzoyl chloride allowed to react with 2-hydroxypyrimidine analogues 3a,b in present of a catalytic amount of pyridine under microwave protocol to give the final products 4a,b in 85% and 83% yields, respectively. The structure of 4-(thien-2′-yl)-6-trifluoromethyl pyrimidin-2-yl 4″-fluorobenzoate 4a was confirmed using IR spectroscopic analysis. The IR spectrum of compound 4a showed a new absorption bands at 1,757 cm^{-1} due to the carbonyl of the newly formed ester group. The band at 1,603 cm^{-1} accounted for C=C stretch in the aromatic system. While the ether linkage (C-O-C) appeared as two sharp signals at 1,056 cm^{-1} and 1,246 cm^{-1}. In addition, the ^{1}H NMR spectrum revealed the appearance of new two doublets of doublets at δ = 7.64 and 7.92 ppm with coupling constant J = 4.9 and 3.7 Hz respectively assigned to the thiophene protons (H-3′ & H-5′). The ^{13}C-NMR spectrum proved the proposed structure due to the appearance of a signal at δ = 165.4 ppm corresponding to the carbonyl carbon of the newly formed ester group at pyrimidine C-2, as well as the

Compound	R$_1$	Yield %
4a	2-thienyl	85
4b	phenyl	83
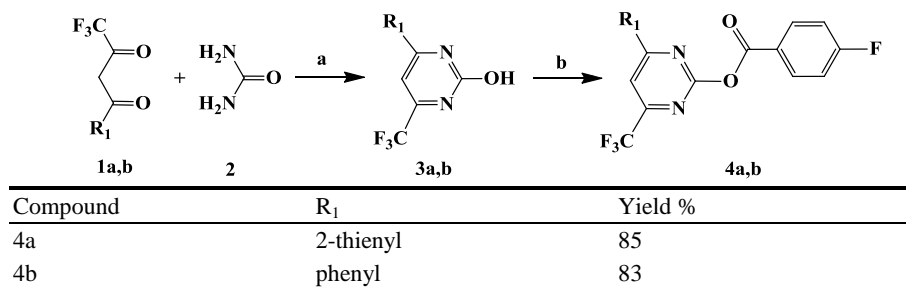
Scheme 1. Microwave synthetic pathways of compounds 4a,b.

Reagents and conditions: (a) i- MW; 135 °C, ethanol-HCl, ii- MW; 135 °C, acetic acid; (b) p-fluorobenzoylchloride, pyridine, MW; 100 °C.

change in the chemical shift of pyrimidine C-5 to resonate at $\delta = 111.9$ ppm. Moreover, the elemental analysis of compound 4a with chemical formula $C_{16}H_8F_4N_2O_2S$ showed the Anal. Calcd. C, 52.18; H, 2.19; N, 7.61; S, 8.71. found: C, 52.63; H, 2.26; N, 7.89; S, 8.99.

A 35 mL CEM Microwave reactor containing 6-trifluoromethyl pyrimidin-2-ol 3a,b and 2 equivalents of phosphorus oxychloride (POCl$_3$) in ethanol with a catalytic amount of pyridine during 25 min furnished the 2-chloro-6-trifluoromethyl pyrimidine 5a,b in ~95% yields (Scheme 2). Under conventional conditions, this substitution reaction is typically carried out using POCl$_3$ as a solvent (100 °C, 3-5 h) yielded 5a,b in 65-71% respectively. The structure of the obtained products 5a,b was confirmed using ^{1}H, and ^{13}C-NMR. The formation of 2-chloro-4-(thien-2′-yl)-6-trifluoromethyl pyrimidine 5a was confirmed by shifting the signal corresponding to the pyrimidine H-5 from $\delta = 7.29$ ppm to $\delta = 7.84$ ppm. Also, the ^{13}C-NMR showed the same shift of the pyrimidine C-5 shifted from $\delta = 103.6$ ppm to a low field at $\delta = 105.2$ ppm.

Amination of 2-chloro-6-trifluoromethyl pyrimidine 5a,b was performed in CEM Microwave using commercially available amines (2 equiv.) in toluene (15 mL) at 100 °C and 150 W for 10 min. The reaction produced the desired 2-(*N*-cycloalkylamino)-6-trifluoromethyl pyrimidine 6a-f in 77-89 % isolated yields (Scheme 2).

The formation of 2-(*N*-cycloheptylamino)-4-(thien-2′-yl)-6-trifluoromethyl pyrimidine 6b was confirmed by elemental analysis, IR, ^{1}H NMR, and ^{13}C-NMR. The IR spectrum of compound 6b showed absorption

bands at 3,531, 3,054, 2,884 cm^{-1} corresponding to the stretching vibration of NH, C-H aromatic and C-H aliphatic respectively. The ^{1}H-NMR spectrum of compound 6b showed three multiplets resonated at $\delta = 1.55$-1.64 ppm, 2.05-2.06 ppm and 4.09 ppm corresponding to 13 protons of cycloheptyl ring. A doublet observed at $\delta = 5.36$ ppm with coupling constant J = 4.0 Hz was attributed to the -NH proton. While, a singlet corresponding to pyrimidine H-5 shifted to $\delta = 7.07$ *ppm*. The 2′-thienyl protons (H-4′, H-5′ and H-3′) resonated at $\delta = 7.13, 7.50$ and 7.73 ppm respectively. ^{13}C-NMR (100 MHz, DMSO-d_6) showed that the cycloheptyl carbons resonated as four signals at $\delta = 24.2, 30.9, 34.6, 53.2$ ppm. The pyrimidine C-5 shifted to $\delta = 101.0$ ppm, and signals assigned for C-2, C-4 and C-6 of the pyrimidine ring resonated at 167.2, 161.7 and 156.9 respectively. Thiophene C-3′, C-4′ and C-5′signals appeared at $\delta = 127.1, 128.8$ and 131.2 ppm respectively while, the thiophene C-2′ resonated at $\delta = 141.9$ ppm.

3.2 Antibacterial Screening

Among the antimicrobial agents, derivatives containing thiophene like cephalothin, cephalorodine and cefoxitin exhibit high antimicrobial potency [19]. It is also noticed that the antibacterial activities enhance by the present of 2-thienyl ring at position-4 and this might be based on the fact that 2-thiophene has shown an array of biological activities ranging from antibacterial [20-24], antifungal [25,26], antioxidant [27], and anti-inflammatory activity [28].

The antibacterial assay is based on the comparison of growth inhibition of micro-organisms by measured known concentrations of test compounds with that
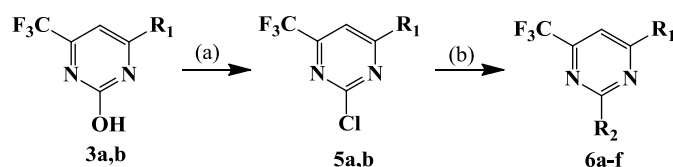
Scheme 2. Synthetic pathway of compounds 5a,b and 6a-f.

Reagents and Conditions: a) i- MW; 100 °C, POCl$_3$, Pyridine, or ii- reflux, excess of POCl$_3$; b) 2 equiv. of cyclopentylamine, cycloheptylamine or *N*-Methylpiprazine in toluene, MW; 112 °C.

produced by known concentrations of standard antibiotics [29]. All of the newly synthesized compounds evaluated against Gram-positive bacteria (*Clostridium perfringen*s, *Bacillus pumilus and Enterococcus faecalis*), and Gram-negative bacteria (*Klebsiella pneumonia)*. A total of eight compounds were screened for *in vitro* antibacterial activity. The screening results (Table 2) showed that compounds 4,6a,b were found to possess appreciable antibacterial activity with a zone of inhibition greater than 10 mm against the Gram-positive bacteria compared to the results obtained from three standard drugs, whereas compound 4b showed no activity against *Bacillus pumilus.* Surprisingly, our synthesized analogues with 4-fluorobenzoyloxy 4a,b or *N*-cycloalkylamine 6a,b substituted at pyrimidine C-2 displayed great antibacterial enhancement against *Enterococcus faecalis* with an inhibition zones ranging between 11-12 mm while, the standard drugs used showed zero activity (Fig. 1). When compounds 4a,b tested against Gram-positive bacteria (*Bacillus pumilus*), 4a showed better activity with inhibition zone of 16 mm (MIC 12.5 μg/mL) while, 4b gave zero activity when tested against the same bacteria stream. Interestingly, compound 4b was found to have two folds more active

than 4a when both derivatives 4a and 4b tested against Gram-positive bacteria (*Clostridium perfringens)* with inhibition zones of 11 mm observed from both compounds 4a,b. The MIC result (6.25 μg/mL) for compound 4a indicates that an enhancement in the activity observed (MIC = 3.125 μg/mL) when 4b was used. Results obtained from the screening against Gram-negative bacteria (*Klebsiella pneumonia)* indicate that only compound 4a showed activity with a zone of inhibition of 11 mm (MIC 12.5 μg/mL). The inhibition zones of the newly synthesized compounds are shown in Figs. 2 and 3.

Individual minimum inhibitory concentration (MIC, μg/mL) values of active compounds 4a,b and 6a,b against the test microbes listed in Table 3. The data derived from the MIC test can be correlated with the data obtained from *in vitro* to estimate the efficacy of the new synthesized derivatives 4a,b and 6a-f.

4. Conclusion

In conclusion, novel fluoropyrimidine analogues 3-6a-f have been synthesized using microwave protocols. The antimicrobial screening of the newly synthesized compounds bearing 2-thienyl group substituted at the pyrimidine ring 4a,6a,b showed better

Table 1   Yields obtained for compounds 5,6a-f under microwave and conventional methods.

Compound	R_1	Yield % (t)		Compound	R_1	R_2	Yield %
		Microwave	Conventional				Microwave
5a	2-thienyl	96 (25 min)	65 (3.5 h)	6a	2-thienyl	cylopentylamine	86
5b	phenyl	95 (25 min)	71 (4 h)	6b	2-thienyl	cycloheptylamine	77
				6c	2-thienyl	*N*-methylpiprazine	89
				6d	phenyl	cylopentylamine	78
				6e	phenyl	cycloheptylamine	83
				6f	phenyl	*N*-methylpiprazine	82

Table 2   Inhibition zones (mm) as a criterion of antibacterial activity of the active compounds 4,6a,b.

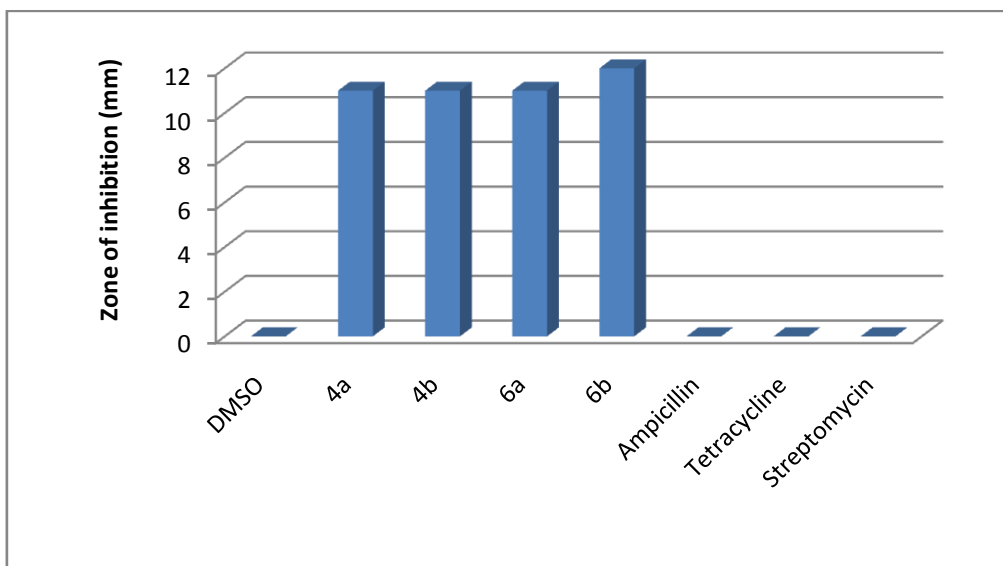| Bacteria	Inhibition zones (mm)							
	Compounds				DMSO	Ampicillin	Tetracycline	Streptomycin
	4a	4b	6a	6b				
Clostridium perfringens	11	11	10	13	0	20	31	32
Bacillus pumilus	16	NA	11	18	0	33	30	32
Enterococcus faecalis	11	11	11	12	0	NA	NA	NA
Klebsiella pneumonia	11	NA	NA	NA	0	27	32	30

NA: no activity observed.

Fig. 1 Comparison of inhibition zones' values of the synthesized compounds 4,6a,b against *Enterococcus faecalis vs* standards antimicrobial drugs: ampicillin, tetracycline, and streptomycin.
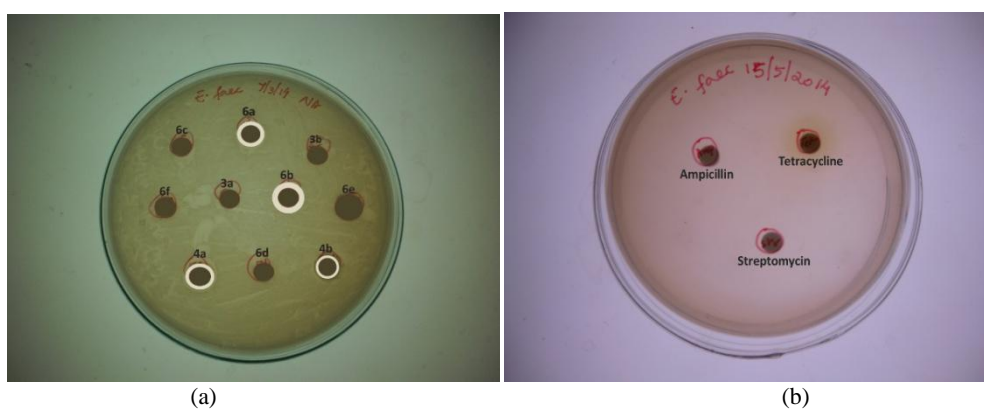

(a) (b)

Fig. 2 (a) zone of inhibition by 4a,b, and 6a,b (b) zone of inhibition by standard antimicrobials.
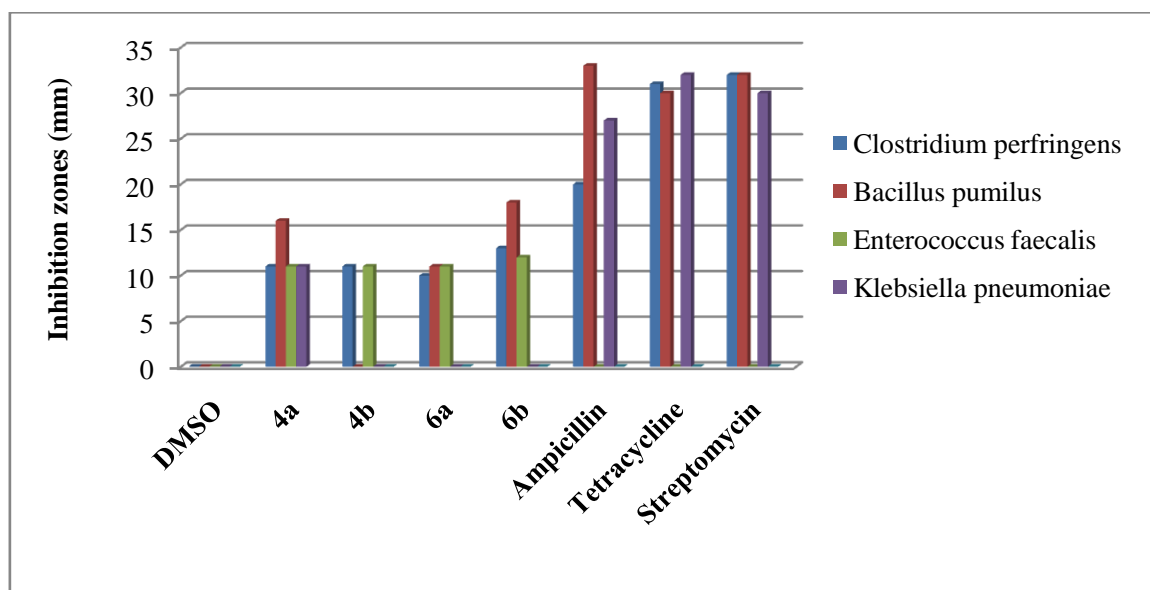

Fig. 3 Inhibition zones (mm) of the newly synthesized compounds 4,6a,b.

Table 3　Minimal inhibitory concentration (MIC) for compounds 4,6a,b.

Compound	MIC (μg/mL)			
	Enterococcus faecalis	*Bacillus pumilus*	*Clostridium perfringens*	*Klebsiella pneumoniae*
4a	50	12.5	6.25	12.5
4b	50	-	3.125	-
6a	25	25	100	-
6b	12.5	100	50	-

antimicrobial activities than other derivatives. For instance, compounds 4,6a,b were found to be more effective than the reference drugs, ampicillin, tetracycline and streptomycin, when tested against Gram-positive bacteria (*Enterococcus faecalis*). Compound 4a was found to be the only active compound when tested against Gram-negative bacteria (*Klebsiella pneumonia*). Thus, in the future, this class of compounds can be used as template to design new derivatives that might help to enhance the activity when used to defeat the bacterial infection.

Acknowledgments

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