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TRIFLUOROMETHYL PYRIMIDINE: SYNTHESIS AND ANTIDIABETIC EVALUATION OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS

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In this study, two series of new trisubstituted pyrimidine derivatives were designed and synthesized using an efficient route, and these derivatives were evaluated for GLP-1 receptor agonist activity. In the first series, new pyrimidines substituted at positions 2 with a p-fluorobenzoyl moiety was synthesized in a good yield. In the second series, the designed pyrimidine templates were substituted at positions 2 with different cycloaliphatic amines, and these compounds were successfully produced with yields of 77-89%. In vitro experiments in cultured cells showed that compounds 4a and 6a (10-15 to 10-9 M) significantly increased insulin secretion compared to the control cells in the absence and presence of 2.8 mM glucose. Compounds 4b and 6d demonstrated significance in the absence of glucose. These results suggest a valuable starting point for the design and discovery of orally active GLP-1 receptor agonists.

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1. INTRODUCTION

Type 2 diabetes mellitus (DM) is a metabolic disease in which the body loses its ability to control glucose levels within normal range because of alterations in insulin secretion from the pancreas or the development of insulin resistance by different organs, especially skeletal muscle [1]. This disease is a chronic disease with high morbidity and mortality, and it poses an economic burden in developing countries [2]. A recent study from the world health organization (WHO) revealed that approximately 200 million people globally, ages 20-80 years, suffered from diabetes, and this figure is expected to increase to 366 million by the year 2030 [3]. Prolonged exposure to uncontrolled hyperglycemia in diabetic patients leads to several diabetic complications [4], such as retinopathy [5], neuropathy [6], cataracts [7], nephropathy [8] and cardiovascular complication [9]. Several drugs, such as sulfonylureas and biguanides, are available to reduce hyperglycemia in diabetes mellitus. However, these drugs produce substantial side effects, and the search for a new class of compounds is essential to overcome these problems [10]. Therefore, the urgent need to identify novel drug scaffolds with minimal side effects remains a challenge to medicinal chemists [11]. A new, advancing agent for the management of DM is incretin-based therapies. Incretin is a gastrointestinal hormone [12] that decreases blood glucose levels [13]. A typical incretin is glucagon-like peptide 1 (GLP-1) [14]. GLP-1 is a peptide hormone that is secreted from intestine L-

cells in response to food intake. GLP-1 binds to GLP-1 receptors, and its activation results in multiple metabolic benefits that are attractive for antidiabetic agents. Rapid inactivation of GLP-1 in the blood stream leads to the development of degradation-resistant peptide drugs. Nonetheless, orally active small molecule agonists of GLP-1 receptor are needed. The present study reports the synthesis and structural characterization of new trifluoromethyl pyrimidine derivatives, which were evaluated for GLP-1 receptor agonist activity. We used short synthetic steps that are tolerant of the presence of various functional groups and appropriate for parallel operations to allow for the rapid generation of libraries of various, structurally complex, small molecules. Elemental analysis, IR, and NMR spectra were used to characterize all compounds.

2. MATERIAL AND METHODS

2.1. GENERAL

All reagents and chemicals were purchased from Sigma-Aldrich and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel glass plates (Silica gel, 60 F_{254}, Fluka) and spots were visualized under UV lamp. Column chromatography was performed on Kieselgel S (Silica gel S, 0.063-0.1mm). Melting points recorded on a Gallenkamp apparatus and are uncorrected. Infrared spectra were measured using KBr pellets on a Thermo Nicolet model 470 FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on Varian, 400 MHz instruments by using DMSO-d_6 and $CDCl_3$ solutions and tetramethylsilane (TMS) as an internal reference. Elemental Analysis performed on Euro Vector EA 3000. Microwave-assisted reactions performed using a microwave reactor (Discover–CEM Corporation). The reactor uses a continuous, focused microwave power-delivery system with an operator-selectable power output of up to 300W. The temperature of the reaction mixture controlled using a calibrated infrared temperature controller mounted under the reaction vessel.

2.2 2-Hydroxypyrimidines 3a,b

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

2.2.1 4-(Thien-2′-yl)-6-(trifluoromethyl) pyrimidin-2-ol **3a**. Yellow crystals; yield 89%; mp 230°C; IR (KBr, cm^{-1}): 3459 (br, OH), 3088 (C-H aromatic), 1680 (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_2O); ^{13}C-NMR [DMSO-d_6, 100 MHz]: (*δ, ppm*) 103.6 (C5-pyrimidine), 120.6 (CF_3, q, *J = 274.0 Hz*), 129.1, 131.1, 132.8 (C3′, 4′,5′-thien-2′-yl), 140.5 (C2′-thien-2′-yl), 160.7 (C6-pyrimidine), 163.5 (C4-pyrimidine), 165.1 (C2-pyrimidine); Anal. Calcd for $C_9H_5F_3N_2OS$: 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.

2.2.2 4-Phenyl-6-(trifluoromethyl) pyrimidin-2-ol **3b**. White powder; yield 84%; mp 234°C, from hexane; IR (KBr, cm^{-1}): 3489 (br, OH), 3076 (CH-aromatic), 1676 (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_2O); ^{13}C-NMR [DMSO-d_6, 100 MHz]: (*δ, ppm*) 109.4 (C5-pyrimidine), 122.8 (CF_3, q, *J = 274.0 Hz*), 129.0, 130.4, 133.4, 139.8 (aromatic carbons), 161.7 (C6-pyrimidine), 165.3 (C4-pyrimidine), 167.8 (C2 pyrimidine); Anal. Calcd for $C_{11}H_7F_3N_2O$: C, 55.01; H, 2.94; N, 11.66; Found: C, 55.46; H, 3.01; N, 11.94.

2.3 Synthesis of 2-Pyrimidine Benzoyl Esters 4a,b

To a solution of 4-phenyl-6-(trifluoromethyl) pyrimidin-2-ol **3a,b** (2.0 mmol) in 20 ml acetonitrile and 0.3 ml of pyridine, *p*-fluorobenzoyl chloride 142 (5.0 mmol, 0.59 ml) was added gradually with stirring in an ice bath. After the addition was completed, the reaction

mixture was heated under microwave irradiation at 100°C for 10 min. The progress of the reaction was monitored using TLC. The solid obtained was washed with water and crystallized from ethanol to give the desired products **4a,b**.

2.3.1 4-(Thien-2′-yl)-6-(trifluoromethyl)-2-pyrimidinyl-4″-fluorobenzoate **4a**. White crystals, yield 85%; mp 105-7°C; IR (KBr, cm^{-1}): 3116 (C-H, aromatic), 1757 (C=O), 1603 (C=C), 1429 (C=N); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 7.18-7.22 (m, 3H, phenyl & thien-2′-yl $H_{4'}$), 7.65 (dd, 1H, thien-2′-yl $H_{5'}$, *J = 4.9 Hz*), 7.80 (s, 1H, H_5 pyrimidine), 7.94 (dd, 1H, thien-2′-yl $H_{3'}$, *J = 3.7 Hz*), 8.25 (m, 2H, aromatic); ^{13}C-NMR [$CDCl_3$, 100 MHz]: (*δ, ppm*) 111.9 (C5-pyrimidine), 115.4 (aromatic carbon), 122.8 (CF_3, q, J = 274 Hz), 127.2, 127.3, 127.4, 128.9, 129.0 (aromatic carbon), 148.3 (C2′-thien-2′-yl), 161.5 (C6-pyrimidine), 163.2 (C4-pyrimidine), 165.4 (C=O), 165.5 (C-F, aromatic), 165.7 (C2-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.

2.3.2 **4**-Phenyl-6-(trifluoromethyl)pyrimidin-2-yl 4′-fluorobenzoate **4b**. White powder; yield 83%; mp 114°C; IR (KBr, cm^{-1}): 3118 (C-H, aromatic), 1758 (C=O), 1602 (C=C), 1428 (C=N); ^{1}H-NMR [DMSO, 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, 100 MHz]: (*δ, ppm*) 109.2 (C5 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 (C6-pyrimidine), 161.5 (C4-pyrimidine), 163.2 (C=O), 165.3 (C-F, aromatic), 165.8 (C2-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.

2.4 Synthesis of 2-Chloropyrimidines **5a,b**

In a 10-ml CEM-microwave vessel, two drops of pyridine were added to a mixture of 4-aryl-6-(trifluoromethyl)pyrimidin-2-ol **3a,b** (2.0 mmol) and $POCl_3$ (4.0 mmol, 0.37 ml). The vial was sealed, and the mixture was irradiated at 100 °C for 25 min. The reaction mixture was cooled to room temperature then poured into ice-cold water (100 ml) with vigorous stirring. The pH was adjusted to pH 8, and the resulting mixture was stirred for 15 min. The obtained light brown solid was filtered, washed with water (2×10 ml) and dried.

2.4.1 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 (C5-pyrimidine), 122.0 (CF_3, q, *J = 274 Hz*), 129.7, 131.5, 133.3 (C3′, 4′, 5′ -thien-2′-yl), 140.1 (C2′-thien-2′-yl), 163.6 (C6-pyrimidine), 164.7 (C4-pyrimidine), 165.1 (C2-pyrimidine).

2.4.2 2-Chloro-4-phenyl-6-(trifluoromethyl) pyrimidine **5b**. Brown crystals; yield 95%; mp 105°C, from ethanol; ^{1}H-NMR [DMSO-*d*$_6$, 400 MHz]: (*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, 100 MHz]: (*δ, ppm*) 104.9 (C5-pyrimidine), 122.5 (CF_3, q, J = 274.0 Hz), 129.7, 131.4, 132.9, 136.4 (aromatic), 157.5 (C6-pyrimidine), 162.7 (C2-pyrimidine), 165.1 (C4-pyrimidine).

2.5 Amination Procedure

To a solution of 4-aryl-2-chloro-6-(trifluoromethyl) pyrimidine **5a,b** (10 mmol) in toluene (15 ml), an excess of amine was added at room temperature in a 35-ml CEM microwave vial. The reaction mixture was heated under microwave irradiation at 80-100°C for 5-10 min. The progress of the reaction was monitored using TLC. After completion, the reaction mixture was quenched with water (0.5 ml), and a solution of sodium carbonate (2 mmol, 0.10 g) was added with stirring at room temperature. The product was extracted with ether, and the organic layer was dried over anhydrous $MgSO_4$. The product was purified using silica gel column chromatography with ethyl acetate:hexane (6:4) to give pure products **6a-f**.

2.5.1 2-(*N*-Cyclopentyamino)-4-(thien-2′-yl)-6-(trifluoromethyl) pyrimidine **6a**. Pale yellow crystal, yield 86%; mp 99°C; IR (KBr, cm^{-1}): 3544 (NH), 3089 (C-H, aromatic), 2931 (aliphatic C-H), 1478 (C=N); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 1.47-1.73 (m, 8H, cyclopentyl), 4.30-4.36 (m, 1H, cyclopentyl), 5.35 (d, 1H, NH, exchanges with D_2O, *J = 8.0 Hz*), 7.08 (1H, s, H_5-pyrimidine), 7.12-7.15 (m, 1H, $H_{4'}$-thien-2′-yl), 7.49-7.51 (m, 1H, $H_{5'}$-thien-2′-yl), 7.70 (m, 1H, thien-2′-yl $H_{3'}$); ^{13}C-NMR [$CDCl_3$, 100 MHz]: (*δ, ppm*) 23.7 (C3,4-cyclopentyl), 33.1 (C2,5-cyclopentyl), 53.2 (C1-cyclopentyl), 99.6 (C5-pyrimidine), 120.7 (CF_3, q, *J = 274.0 Hz*), 128.3, 130.4, 132.4 (C3′,4′,5′-thien-2′-yl), 142.6 (C2′-thien-2′-yl), 156.5 (C6-pyrimidine), 161.8 (C4-pyrimidine), 162.8 (C2-pyrimidine); Anal. Calcd for $C_{14}H_{14}F_3N_3S$: 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.

2.5.2 2-(*N*-Cycloheptylamino)-4-(thien-2′-yl)-6-(trifluoromethyl)pyrimidine **6b**. White powder, R_f = 0.66 (ethylacetate:hexane 1:1), yield 77%; mp 111-13°C; IR (KBr, cm^{-1}): 3531 (NH), 3054 (C-H, aromatic), 2884 (aliphatic C-H), 1600 (C=C), 1463 (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_2O, *J = 4.0 Hz*), 7.07 (1H, s, H_5-pyrimidine), 7.13 (t, 1H, $H_{4'}$-thien-2′-yl, *J = 4.0 Hz*), 7.50-7.51 (d, 1H, $H_{5'}$-thien-2′-yl, *J = 4.0 Hz*), 7.73 (m, 1H, thien-2′-yl $H_{3'}$); ^{13}C-NMR [$CDCl_3$, 100 MHz]: (*δ, ppm*) 24.2 (C3,6-cycloheptyl), 30.9 (C4,5-cycloheptyl), 34.6 (C2,7-cyclopentyl), 53.2 (C1-cycloheptyl), 101.0 (C5-pyrimidine), 120.8 (CF_3, q, *J = 274.0 Hz*), 127.1, 128.8, 131.2 (C3′, 4′,5′-thien-2′-yl), 141.9 (C2′-thien-2′-yl), 156.9 (C6- pyrimidine), 161.7 (C4-pyrimidine), 167.2 (C2-pyrimidine); Anal. Calcd for $C_{16}H_{18}F_3N_3S$: 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.

2.5.3 *2*-(*N*-Methylpiperazin-1′-yl)-4-(thien-2′-yl)-6-(trifluoromethyl)pyrimidine **6c**. White powder, R_f = 0.16 (ethyl acetate:hexane 1:1), yield 89%; mp 109-11°C; IR (KBr, cm^{-1}): 3095 (C-H, aromatic), 2911 (aliphatic C-H), 1593 (C=C), 1452 (C=N), 1254 (C-N); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 2.35 (s, 3H, methyl group), 2.49-2.50 (m, 4H, piperazine), 3.93-3.95 (m, 4H, piperazine), 7.05 (1H, s, H_5-pyrimidine), 7.14 (t, 1H, $H_{4'}$-thien-2′-yl, *J = 4.0 Hz*), 7.50 (dd, 1H, $H_{5'}$-thien-2′-yl, *J = 4.9 Hz*), 7.72 (dd, 1H, $H_{3'}$-thien-2′-yl, *J = 3.7 Hz*); ^{13}C-NMR [$CDCl_3$, 100 MHz]: (*δ, ppm*) 43.6 (CH_3), 46.2 (piperazine), 54.9 (piperazine), 99.1 (C5-pyrimidine), 120.8 (CF_3, q, *J = 274.0 Hz*), 127.8, 128.3, 130.2 (C3′, 4′,5′-thien-2′-yl), 142.7 (C2′-thien-2′-yl), 156.6 (C6-pyrimidine), 161.4 (C4-pyrimidine), 161.5 (C2-pyrimidine); Anal. Calcd for $C_{14}H_{15}F_3N_4S$: 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.

2.5.4 2-(*N*-Cyclopentylamino)-4-phenyl-6-(trifluoromethyl) pyrimidine **6d**. Pale yellow crystal, R_f = 0.66 (ethyl acetate: hexane 1:1), yield 78%; mp 103°C; IR (KBr, cm^{-1}): 3542 (NH), 3056 (C-H, aromatic), 2918 (aliphatic C-H), 1597 (C=C), 1456 (C=N); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 1.51-1.53 (m, 2H, 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_2O, *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 (C2,5-cyclopentyl), 53.2 (C1-cyclopentyl), 99.6 (C5-pyrimidine), 120.4 (CF_3, q, *J = 274 Hz*), 128.3, 130.4, 132.4, 142.6 (aromatic carbons), 156.5 (C6- pyrimidine), 161.8 (C4-pyrimidine), 162.0 (C2-pyrimidine); Anal. Calcd. for $C_{16}H_{16}F_3N_3$: C, 62.53; H, 5.25; N, 13.67; Found: C, 62.98; H, 5.32; N, 13.95.

2.5.5 2-(*N*-Cycloheptylamino)-4-phenyl-6-(trifluoromethyl) pyrimidine **6e**. White powder, R_f = 0.68 (ethyl acetate:hexane 1:1), yield 83%; mp 115-18°C; IR (KBr, cm^{-1}): 3548 (-NH), 3088 (C-H, aromatic), 2923 (aliphatic C-H), 1585 (C=C), 1461 (C=N); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 1.56-1.73 (m, 10H, cycloheptyl), 2.06 (m, 2H, cycloheptyl), 4.17 (br,1H, cycloheptyl), 5.42 (d, 1H, NH, exchanges with D_2O, *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 (C3,6-cyclopentyl), 28.3 (C4,5-cycloheptyl), 34.7 (C2,7-cycloheptyl), 52.1 (C1-cycloheptyl), 101.0 (C5-pyrimidine), 120.8 (CF_3, q, *J = 274 Hz*), 127.1, 128.8, 131.2, 136.6 (aromatic carbons), 156.2 (C6-pyrimidine), 161.8 (C4-pyrimidine), 167.2 (C2-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.

2.5.6 2-(*N*-Methylpiperazin-1′-yl)-4-phenyl-6-(trifluoromethyl)pyrimidine **6f**. White powder, R_f = 0.15 (ethyl acetate:hexane 1:1), yield 82%; mp 114-16°C; IR (KBr, cm^{-1}): 3083 (C-H, aromatic), 2924 (aliphatic C-H), 1583 (C=C), 1457 (C=N); ^{1}H-NMR [$CDCl_3$, 400 MHz]: (*δ, ppm*) 2.35 (s, 3H, methyl), 2.49-2.50 (m, 4H, piperazine), 3.98 (m, 4H, piperazine), 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 (C2,6- methylpiperazine), 54.9 (C3,5-methylpiprazine), 100.6 (C5-pyrimidine), 120.9 (CF_3, q, *J = 274 Hz*), 127.2, 128.8, 131.2, 136.7 (aromatic), 156.4 (C6-pyrimidine), 161.7 (C4-pyrimidine), 166.8 (C2-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.

2.6 ANTIDIABETIC ACTIVITY

2.6.1 In vitro testing

2.6.1.1 Cell culture

*β*TC6 cells, a mouse immortalized insulin-secreting pancreatic beta cell line (T-SV40), were grown in DMEM culture medium containing 25.0 mM glucose, 1.0 mM sodium pyruvate, 4.0 mM L-glutamine, 44.0 mM sodium bicarbonate, 15.0% (v/v) FBS, and 50.0 µg/ml gentamicin in a 5.0% CO_2 incubator at 37°C. The medium was replaced every 48 hrs with fresh culture medium and cells sub-cultured as needed to prevent over-confluence. Cells were passaged by treatment with 0.25% trypsin and 0.91 mM EDTA at passages 6-8.

2.6.1.2 Insulin secretion assay

*β*TC6 cells (0.1 x 10^6 cells/ml) was cultured in a 24-well plate for 48 hr in 5% CO_2 incubator at 37°C. The cells were then preincubated for 30 minutes in modified Krebs/Ringer buffer (KRB) (118.5 mM NaCl, 25 mM $NaHCO_3$, 4.74 mM KCl, 1.19 mM $MgSO_4$, 2.54 mM $CaCl_2$, 10 mM HEPES, 1.19 mM KH_2PO_4, 0.1% BSA, pH 7.4) in the CO_2 incubator. Subsequent cells were washed and incubated for another 30 min with fresh buffer. Solutions of pyrimidine compounds (10^{-6}-10^{-12} M) were prepared by diluting the stock standard solutions by KRB. Solutions having 0.000004% DMSO were obtained. 250 µl of the different concentrations were added to the cells and incubated in a 5% CO_2 incubator at 37°C for 120 min in the absence and presence of 2.80 mM glucose solution. Total reaction volume was 1 ml for each experiment. To maintain total volume of 1 ml, either 750 µl or 500 µl KRB was added first followed by 250 µl of 4 time's concentrated dose of compound and glucose (Basal experiment: 750 µl KRB+4X 250 µl test drug. Glucose stimulated experiment: 500 µl KRB+4X 250 µl glucose 2.8 mM+4X 250 µl test drug). After incubation, the supernatant layers were collected and subjected to sandwich ELISA using high range insulin assay kit according to the manufacturer's instruction. As per instructions from the manufacturer of the kit, 10 µl samples were incubated with enzyme conjugate solutions on shaker plates for 2.0 hrs at room temperature. The plates were washed, TMB was added for 15 min and the reaction was stopped. The color intensity of solutions was read at 450 nm with a Tecan microplate reader. The sensitivity of insulin ELISA was 216 *p*mol/L. The average intra and inter assay coefficients of variation were 3.37 and 2.29%, respectively and the levels of insulin were expressed as *p*mol/L.

2.6.3 Statistical analysis

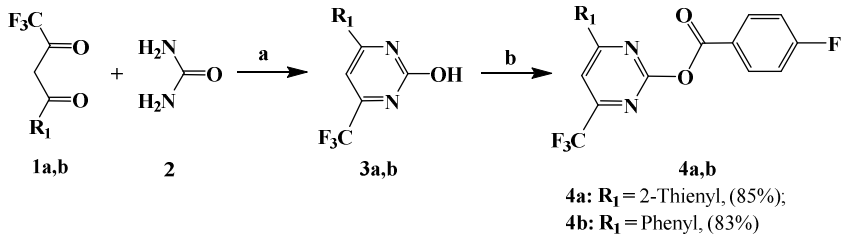
Experimental results were expressed as mean±SEM and statistically assessed by SPSS-20.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic strategy for the two series of trifluoromethyl pyrimidine derivatives is illustrated in Schemes 1-2. All of the synthetic steps were carried out using microwave irradiation. The first series **4a,b** were prepared by the reaction between trifluorobutane-1,3-dione **1a,b** and urea **2** via nucleophilic addition

reaction, followed by cyclization to form 6-trifluoromethyl pyrimidin-2-ol **3a,b** in 89 % and 84% respectively (Scheme 1).

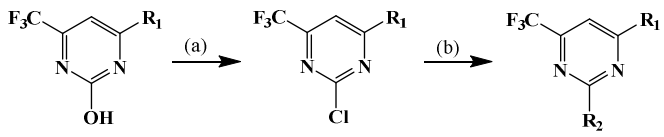


Scheme 1: *Reagents and conditions*: (a) i- MW, 135°C, ethanol-HCl; (b) *p*-fluorobenzoylchloride, pyridine, MW; 100°C.

The structure of **3a** was confirmed using IR-spectroscopy which revealed by the appearance of broad band at υ = 3459 cm^{-1} corresponding to a tautomeric hydroxyl group (N=C-OH). While, a sharp band appeared at υ = 1680 cm^{-1} assigned for the amide carbonyl (CONH) in **3a**. The ^{1}H-NMR 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-5′ appeared as triplet at δ = 7.30 ppm with coupling constant *J* = 4.0 Hz, the H-4′ resonated as a triplet at δ = 7.94 ppm with coupling constant $J_{H5',H4'}$ = 8.0 Hz while the H-3′ appeared as doublet at δ = 8.30 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* exchangeable with D_2O. The ^{13}C-NMR spectrum 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 quartet at 120.6 *ppm*. Thiophene carbons resonate at δ = 129.1, 131.1, 132.8 and 140.5 *ppm*.

p-Fluorobenzoyl chloride allowed to react with 2-hydroxypyrimidine **3a,b** in present of pyridine to give **4a,b** in 85% and 83% yields, respectively (Scheme 1). The structure of **4a** was confirmed using IR spectroscopic analysis. The IR spectrum of compound **4a** showed a new absorption bands at 1757 cm^{-1} due to the carbonyl of the newly formed ester group. The band at 1603 cm^{-1} accounted for (C=C) stretch in the aromatic system. While the ether linkage (C-O-C) appeared as two sharp signals at 1056 cm^{-1} and 1246 cm^{-1}. In addition, the ^{1}H-NMR spectrum revealed the appearance of new two signals attributed to *p*-fluorophenyl ring at δ = 7.17 and 8.24 ppm. The ^{13}C-NMR spectrum confirm the proposed structure due to the appearance of a signal at δ = 165.4 ppm corresponding to the carbonyl carbon of the ester group at pyrimidine C-2, as well as the change in the chemical shift of pyrimidine C-5 to resonate at δ = 111.9 ppm. 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.

The second series of pyrimidine derivatives **6a-f** were synthesized by chlorination of 2-hydroxypyrimidine **3a,b** followed by amination. The chlorination of 2-hydroxypyrimidine **3a,b** carried out using two equivalents of phosphorus oxychloride ($POCl_3$) under microwave irradiation which afford the target products **5a,b** in ~95% yields after 25 min. The formation of 2-chloro-4-(thien-2′-yl)-6-trifluoromethyl pyrimidine **5a** was confirmed by ^{1}H-NMR analysis. The shifting in the pyrimidine H-5 signal from δ = 7.29 ppm to the higher chemical shift at δ = 7.84 ppm. Also, the ^{13}C-NMR showed the same shift of the pyrimidine C-5 from δ =103.6 ppm to a low field at δ = 105.2 ppm. Amination of 2-chloro-6-trifluoromethyl pyrimidine **5a,b** were carried out using commercially available amines. The reaction produced the desired compounds **6a-f** in 77-89 % isolated yields (Scheme 2).



3a,b 5a,b 6a-f

6a: R_1= 2-Thienyl, R_2= Cylopentylamine (86%); **6b:** R_1 = 2-Thienyl, R_2 = Cycloheptylamine (77%);
6c: R_1= 2-Thienyl, R_2 = *N*-Methylpiperazine (89%); **6d**: R_1 = Phenyl, R_2= Cylopentylamine (78%);
6e: R_1= Phenyl, R_2 = Cycloheptylamine (83%); **6f:** R_1 = Phenyl, R_2= *N*-Methylpiperazine (82%).

Scheme 1: *Reagents and Conditions*: a) i- MW; 100°C, $POCl_3$, Pyridine; b) 2 equiv. of cyclopentylamine, cycloheptylamine or *N*-Methylpiperazine in toluene, MW; 112°C

The formation of compound **6b** was confirmed by elemental analysis, IR, ^{1}H NMR, and ^{13}C-NMR. The IR spectrum of compound **6b** showed absorption bands at 3531, 3054, 2884 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 δ = 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 δ = 5.36 ppm with coupling constant J = 4.0 Hz was attributed to the -NH proton exchanged with D_2O. While, a singlet corresponding to pyrimidine H-5 appeared at δ = 7.07 *ppm*. The 2′-thienyl protons (H-4′, H-5′ and H-3′) resonated at δ = 7.13, 7.50 and 7.73 ppm respectively. ^{13}C-NMR spectrum showed that the cycloheptyl carbons resonated as four signals at δ = 24.2, 30.9, 34.6, 53.2 ppm. The pyrimidine C-5 appeared at δ = 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 δ = 127.1, 128.8 and 131.2 ppm respectively while, the pyrimidine C-2 resonated at δ = 141.9 ppm.

3.2 ANTIDIABETIC ACTIVITY

3.2.1 Effects of trisubstituted pyrimidines on insulin secretion of *β*TC6 cell line

The glucose dependence of the insulin secretory activity of new trifluoromethyl pyrimidines as GLP-1R agonists has been measured using the high-range insulin Sandwich ELISA kit. Fig. 1 shows the glucose response of the βTC6 cells in the absence of drugs. Glucose at 2.8 mM induced a mild insulin secretion of nearly 3000 pmol/L, which was used in subsequent testing of the new pyrmidine compounds and the positive control, exenatide. Exenatide drug showed a significant increase in insulin secretion compared to basal secretion from the βTC6 cells. Moreover, in the presence of 2.8 mM glucose, 10^{-12} and 10^{-5} M exenatide significantly increased insulin secretion compared to that of control (i.e., 2.88 mM glucose alone) (Fig. 2a). The *in vitro* effects of compounds **4a** and **6a** at 10^{-15}, 10^{-12}, and 10^{-9} M on insulin secretion in the absence and presence of 2.8 mM glucose are shown in Fig. 2b and 2c. In the absence of glucose, 10^{-15} and 10^{-12}, of compounds **4a** and **6a** significantly increased insulin secretion compared to that of the basal control. In the presence of 2.8 mM glucose, 10^{-15} M of **4a** and **6a** significantly increased insulin secretion compared to that of the control (i.e., with 2.8 mM glucose alone, Fig. 2b and 2c). Regarding compounds **4b** and **6d,** Fig. 2d and 2e shows that, in the absence of glucose, 10^{-15} and 10^{-12} M of compounds **4b** and **6d** significantly increased insulin secretion compared to that of the basal control, while compounds **4b** and **6d** had no significant effect on insulin secretion in the presence of 2.8 mM glucose (Fig. 2d and 2e). Compounds **4a** and **6a** showed similar effects as did exenatide in stimulating insulin secretion from βTC6 cells in absence and presence of 2.8 mM glucose. We have briefly investigated different SAR for functionalized pyrimidine derivatives. These modifications result in changes the antidiabetic activity of the synthesized compounds. In this contrast, first changing the substituent on the substituted pyrimidine ring by

2-thienyl at position 4 instead of phenyl produced a relatively significant increase antidiabetic activity. Secondly, the presence of the linkage (–OC=O) show relatively significant antidiabetic activity when compared with (-NH) linker in position 2 of pyrimidine ring. The above SAR correlation studies reveal that; the nature of the substitution and type of linkage influences the antidiabetic activity.

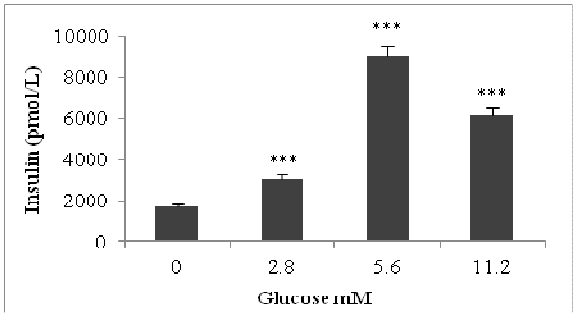
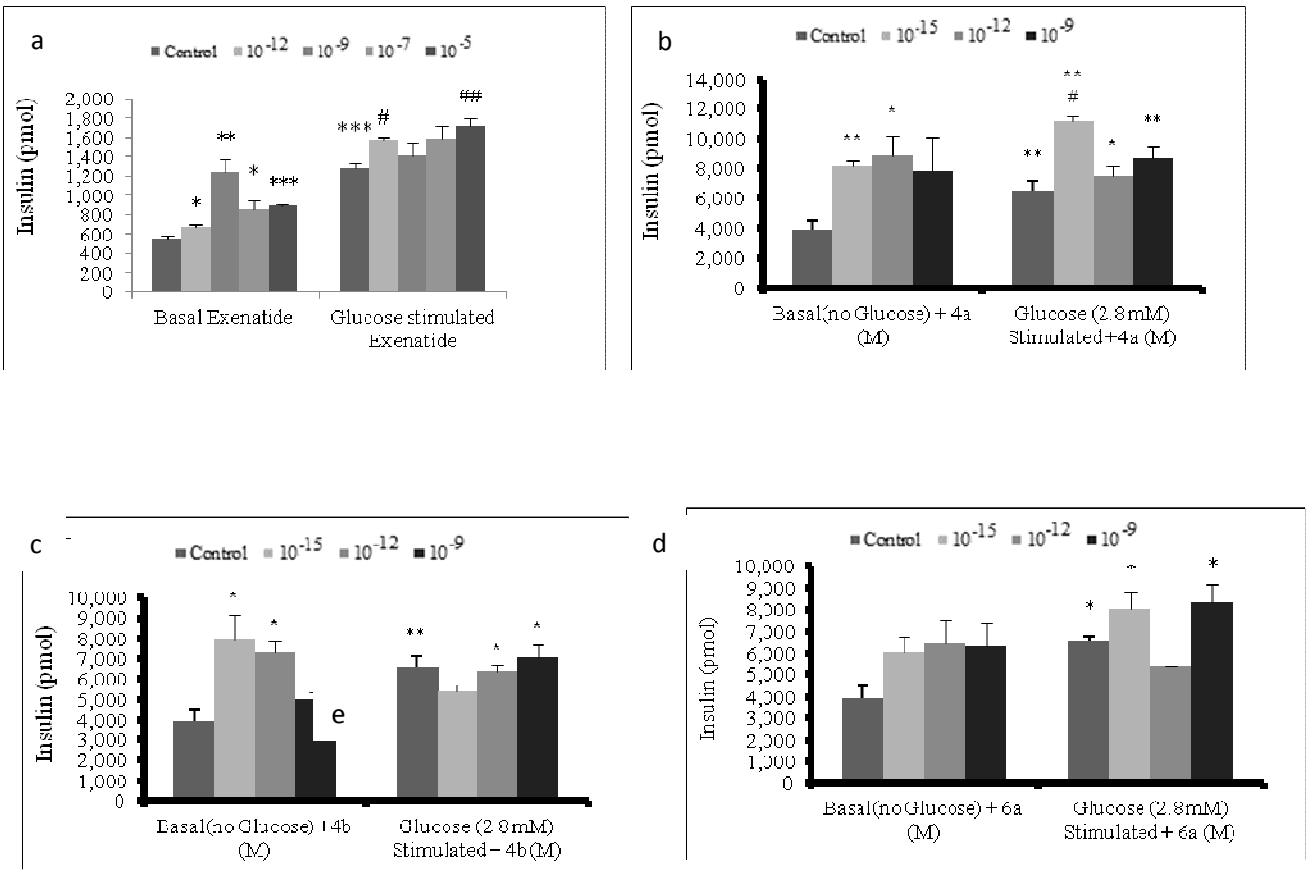


Figure 1. The glucose response of the βTC6 cells in the absence of drugs. Plotted values are means of triplicates ± SEM*; P < 0.05, ** P < 0.01, *** P < 0.001 versus 0 mM glucose



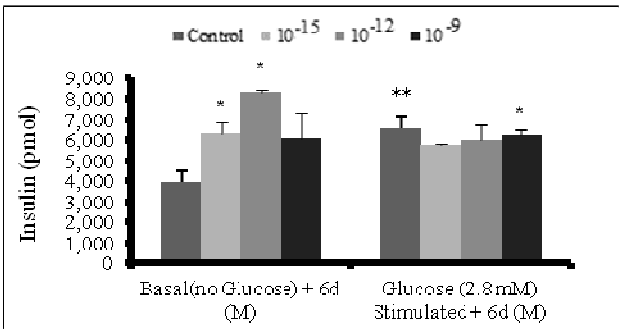


Figure 2. The effects of exenatide (10^{-12} –10^{-5} M, panel a) and test compounds 4a (panel b), 4b (panel c), 6a (panel d) and 6d (panel e) (10^{-15} –10^{-9} M) on insulin secretion in βTC6 cells in the absence (Basal, left bars) and in the presence of 2.8 mM glucose concentration (right bars). Results are means of triplicates ± SEM; *P < 0.05, ** P < 0.01, *** P < 0.001 versus basal control, and #P < 0.05, ## P < 0.01 versus 2.8 mM glucose alone.

4. CONCLUSION

This study reports the synthesis and antidiabetic activity of trifluoromethyl pyrimidines as GLP-1R agonists. The *in-vitro* anti-diabetes activities results showed that compounds **4a**, and **6a** could significantly increase insulin secretion compared to that of the control cells in the absence and presence of 2.8 mM glucose; compounds **4b** and **6d** established significance in the absence of glucose. These results suggesting that trifluoromethyl pyrimidines could be served as moderately potential small-molecule GLP-1R agonists which can be further optimized structurally to increase the efficacy and the pharmacological profile of these compounds which may result in more potent GLP-1R agonists.

ACKNOWLEDGMENT

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5. REFERENCES

1. DeFronzo, R.A., Diabetologia, 1992. **35**: p. 389-397.
2. M.M. Engelgau, L.S.G., J.B. Saaddine, J.P. Boyle, S.M. Benjamin,E.W. Gregg, E.F. Tierney, N. Rios-Burrows, A.H. Mokdad, E.S. Ford, G. Imperatore, K.M. Venkat Narayan, Ann. Intern. Med., 2004. **140**: p. 945-950.
3. S. Wild, G.R., A. Green, R. Sicree, H. King and 1053., Diabetes Care, 2004. **27**: p. 1047-1053.
4. P.F. Kador, J.H.K., N.E. Sharpless, J. Med. Chem. , 1985. **28**: p. 841-849.
5. G.B. Reddy, A.S., N. Balakrishna, R. Ayyagari, M. Padma, K. Viswanath, J.M. Petrash, Mol. Vis., 2008. **14**: p. 593-601.
6. R.J. Young, D.J.E., B.F. Clarke, Diabetes, 1983. **32**: p. 938-942.
7. W.G. Robinson Jr., P.F.K., J.H. Kinoshita, *Science* 1983. **221**: p. 1177-1179.
8. M. Dunlop, Kidney Int., 2000. **58**: p. S3-S12.
9. M. Pyörälä, H.M., P. Halonen, M. Laakso, K. Pyörälä, Arterioscler., Thromb. Vasc. Biol., 2000. **20**: p. 538-544.
10. Kamaeswara R B, G.R., Kesavulu M M and Apparao C. , *Effect of oral administration of bark extracts of Pterocarpus santalinus L. on blood glucose level in experimental animals.* J. Ethnopharmacol 2001. **74**: p. 69-74.
11. Mariappan G, S.B.P., Datta S, Kumar D and Haldar PK., *Design, synthesis and antidiabetic evaluation of oxazolone derivatives.* ChemInform, 2011. **123**: p. 335-341.
12. Drucker, D.J., *The biology of incretin hormones.* Cell Metabolism, 2006. **3**(3): p. 153-165.

13. Shaffer, C., *Incretin mimetics vie for slice of type 2 diabetes market.* Nature Biotechnology, 2007. **25**(3): p. 263.
14. B. Kreymann, G.W., M. A. Ghatei, and S. R. Bloom, *Glucagon-like peptide-1 7-36: a physiological incretin in man.* The Lancet, 1987. **2**: p. 1300-1304.