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Research Article

Synthesis and Evaluation of Thiouracil Derivatives as Dipeptidyl Peptidase IV Inhibitors

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A series of thiouracil derivatives were designed, synthesized and screened for *in vitro* inhibition of dipeptidyl peptidase IV. The SAR study indicated the influence of substituted chemical modifications on thiouracil scaffold. Compounds 8 (IC_{50} = 0.32 μM), 9 (IC_{50} = 0.29 μM), and 12 (IC_{50} = 0.25 μM) showed excellent dipeptidyl peptidase IV inhibition having heterocyclic substituted piperazine with acetamide linker resulted as most potent dipeptidyl peptidase IV inhibitors among all the compounds screened. Single dose (10 mg/kg) of the compounds 8, 9, and 12 significantly reduced glucose excursion during oral glucose tolerance test in streptozotocin-induced diabetic rat model. The present study on substituted thiouracil derivatives shows good-to-moderate inhibitory potential of dipeptidyl peptidase IV enzyme.

Key words: diabetes, DPP IV, microwave synthesis, thiouracil

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Incretin hormones represent a promising approach for treatment of type 2 diabetes. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones responsible for 50–70% of postprandial insulin release from pancreatic beta cells (1). Chronic infusion of GLP-1 has significantly improved both blood glucose and hemoglobin A1c (HbA1c) level in patients with T2D (2). Additionally, GLP-1 also plays beneficial role in treatment of diabetes by regulating various metabolic changes as improving insulin biosynthesis, inhibiting glucagon secretion, reducing food intake, slow gastric emptying time, and feeling of satiety (3). However, its therapeutic efficacy is limited due to short half-life and rapid degradation in plasma by serine protease dipeptidyl peptidase IV (DPP IV) (4,5). Inhibition of DPP IV will result in regulating level of endogenous intact incretin hormones by extending its half-life, consequently potentiating insulin secretion (6,7). Therefore, DPP IV inhibitors have emerged as novel class of antidiabetic drugs, which prevent proteolytic degradation of incretin hormones and stimulate glucose-dependent insulin secretion (8).

Pyrimidine and related fused heterocyclic derivatives, such as purines (9), aminomethylpyrimidines (10,11), pyridyl acetamide (12), pyridopyrimidines (13), phenylethylamines (14), quinoxaline diones (15), quinazolinone (16), pyrimidinone, and pyrimidinedione (17), are well-known pharmacophores in drug discovery and are also responsible for providing series of potent DPP IV inhibitors (Figure 1). The earlier studies show that conserving heterocyclic integrity of the pharmacophore may establish a magnetic strategy for development of DPP IV inhibitors with desirable bioactivity. The extended investigation on heterobicyclic systems led us to examine thiouracil derivatives against DPP IV inhibitory activity. Thus, in an attempt to establish new therapeutic agents, we designed a series of substituted thiouracil derivatives as structurally modified and targeted DPP IV inhibitors. Our interest in the synthesis of heterocyclic core as thiouracil made us to study the stabilized interactions that may happen to occur with conserved features of the core. We envisioned that targeted substitution on the pharmacophore can prove to be an attractive strategy for synthesis of DPP IV inhibitors with significant bioactivity. In an effort to find more targeted compounds, we focused on affinity of substituted thiouracil derivatives, which may be responsible for DPP IV inhibition. However, the designed heterocycle core revealed the close resemblance with various available DPP IV inhibitors (Figure 1) (18,19).

Herein, we report proposed methodology with the result in respect to the synthesis, SAR of synthesized thiouracil derivatives across the pharmacophore. The synthetic compounds have been investigated by *in vitro* DPP IV inhibition and *in vivo* antihyperglycemic activity.

Methods and Materials

Experimental

All commercial chemicals and solvents were purchased from Sigma-Aldrich Chemical Company, St. Louis, USA and Spectrochem Pvt. Ltd (USA), India. Chemicals and solvents were used as such without further purification. ^{1}H spectra were recorded on a Bruker NMR spectrometer operating at 300 MHz in $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an

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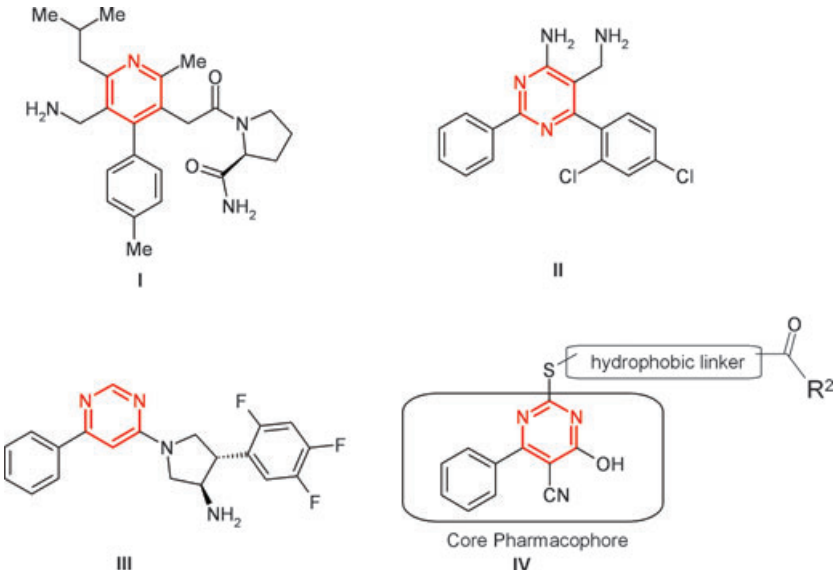


Figure 1: Dipeptidyl Peptidase IV inhibitors I[11], II[10], III[12b], IV.

internal standard. The Chemical shifts are reported as parts per million (ppm, δ), and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). All the Fourier transform infrared (FT-IR) spectra were recorded on Jasco-410 spectrophotometer. Elemental analysis was carried out using Vario EL III C, H, N elemental analyzer, and the values were found within acceptable limits. Melting points of compounds were determined in open capillary tubes using a Hicon melting point instrument and are uncorrected. Microwave-assisted synthetic reactions were performed using a CEM Discover Benchmate microwave reactor (CEM Microwave technology ltd., Buckingham). All reactions were carried out under dried condition and performed using oven-dried glassware.

Synthetic procedures

Step I: synthesis of 4-hydroxy-6-phenyl-2-sulfanylpyrimidine-5-carbonitrile (1)

The compound **1** is prepared by the previously reported procedure (20) obtained as white solid after neutralization with 1 M hydrochloric acid. Resultant compound identity was confirmed by further characterization. White solid; yield: 93%; mp: 145–149 °C; R_f: 0.65; ^{1}H NMR (300MHz, $CDCl_3$) δ ppm: 7.43 (t, J = 15, 1H), 7.55 (d, J = 9, 2H), 7.94 (d, J = 9, 2H), 8.18 (s, 1H); FT-IR (ν_{max}; per cm, KBr): 2954 (C-$H_{stretch}$), 1628 (C=N), 1549 (C=C ring$_{stretch}$), 1453 (C-N), 1247 (C-$S_{stretch}$), 1153 (C-O), 765 (Phenyl); Anal. Calcd. For $C_{11}H_7N_3OS$: C, 57.63; H, 3.08; N, 18.33; found: C, 57.65; H, 3.05; N, 18.39.

Step II: General procedure for synthesis of *N*-substituted amides (2–7)

To a solution of secondary amine (2.9 mol) in 1, 4-dioxan (10 mL) was added chloroacetyl chloride (3.33 mol) followed immediately by stirring under reflux for 2 h then at room temperature for 30 min. The progress of reaction was continuously monitored by thin-layer chromatography (TLC) (Developing solvent: chloroform/methanol = 9.5:0.5). The reaction solution was concentrated under reduced pressure, and the resulting residue was neutralized with saturated bicarbonate solution. The resulting residue was diluted with water and extracted with chloroform. The chloroform layer was washed with water and brine and dried over anhydrous sodium sulfate. The organic layers were evaporated to dryness under reduced pressure using rotary evaporator. The resultant intermediates (**2–7**) were obtained in excellent yields.

2-chloro-1-[4-(pyrimidin-2-yl)piperazin-1-yl]ethanone (2)

Yellow oil; yield: 87%; R_f: 0.71; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.70–3.64 (m, 4H), 3.91–3.73 (m, 4H), 4.28 (s, 2H), 6.66 (t, J = 9, 1H), 8.38 (d, J = 9, 2H); FT-IR (ν_{max}; per cm, neat): 2931 (C-$H_{stretch}$), 1629 (C=O), 1493 (C=N), 640 (C-$Cl_{stretch}$); Anal. Calcd. For $C_{10}H_{13}ClN_4O$: C, 49.90; H, 5.44; N, 23.28; found: C, 49.94; H, 5.39; N, 23.24.

2-chloro-1-[4-(pyridin-2-yl)piperazin-1-yl]ethanone (3)

Brown oil; yield: 87%; R_f: 0.70; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.71–3.66 (m, 4H), 3.91–3.73 (m, 4H), 4.43 (s, 2H), 6.73 (t, J = 12 Hz, 1H), 6.99 (d, J = 9, 1H), 7.71 (t, J = 15.3, 1H), 8.17 (s, 1H); FT-IR (ν_{max}; per cm, neat): 3004 (C-$H_{stretch}$), 2920 (C-$H_{stretch}$), 1647 (C=O), 1541 (C=N), 775 (C-$Cl_{stretch}$); Anal. Calcd. For $C_{11}H_{14}ClN_3O$: C, 55.12; H, 5.89; N, 17.53; found: C, 55.17; H, 5.85; N, 17.58.

2-chloro-1-[4-(4-chlorophenyl)piperazin-1-yl]ethanone (4)

Yellow solid; yield: 84%; mp: 129–133 °C; R_f: 0.69; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.23–3.15 (m, 4H), 3.72–3.68 (m, 4H), 4.12 (s, 2H), 6.88 (d, J = 6 Hz, 2H), 7.28 (d, J = 6 Hz, 2H); FT-IR (ν_{max}; per cm, KBr): 2917 (C-$H_{stretch}$), 1594 (C=O), 1497 (C=C $ring_{stretch}$), 667 (Ar-$Cl_{stretch}$), 624 (C-$Cl_{stretch}$); Anal. Calcd. For $C_{12}H_{14}Cl_2N_2O$: C, 52.76; H, 5.17; N, 10.26; found: C, 52.79; H, 5.19; N, 10.29.

1-(1,4'-bipiperidin-1'-yl)-2-chloroethanone (5)

Yellow oil; yield: 64%; R_f: 0.65; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm : 1.15–1.20 (t, J = 15, 2H), 1.5–1.7 (m, 4H), 2.15–2.26 (m, 4H), 2.78–2.86 (m, 4H), 3.06–3.11 (m, 4H), 5.02 (s, 2H), 5.19–5.30 (m, 1H);); FT-IR (ν_{max}; per cm, neat): 2940 (C-$H_{stretch}$), 1645 (C=O), 1150 (C-$N_{stretch}$), 659 (C-$Cl_{stretch}$); Anal. Calcd. For $C_{12}H_{21}ClN_2O$: C, 58.89; H, 8.65; N, 11.45; found: C, 58.92; H, 8.63; N, 11.47.

1-(4-aminopiperidin-1-yl)-2-chloroethanone (6)

Yellow oil; yield: 75%; R_f: 0.69; ^{1}H NMR (300MHz, $CDCl_3$) δ ppm: 2.00 (q, J = 12, 4H), 3.2 (t, J = 9, 4H), 4.46 (d, J = 12, 2H), 4.8 (s, 2H); FT-IR (ν_{max}; per cm, neat): 2936 (C-$H_{stretch}$), 1649 (C=O), 1169 (C-$N_{stretch}$), 654 (C-$Cl_{stretch}$); Anal. Calcd. For $C_7H_{13}ClN_2O$: C, 47.60; H, 7.42; N, 15.86; found: C, 47.62; H, 7.45; N, 15.89.

1-(azepan-1-yl)-2-chloroethanone (7)

Light brown oil; yield: 78%; R_f: 0.69; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.79–1.73 (m, 4H), 1.87–1.82 (m, 4H), 3.58–3.42 (m, 4H), 4.11 (s, 2H); FT-IR (ν_{max}; per cm, neat): 1647 (C=O), 1099 (C-$N_{stretch}$), 651 (C-$Cl_{stretch}$); Anal. Calcd. For $C_8H_{14}ClNO$: C, 54.70; H, 8.03; N, 7.97; found: C, 54.74; H, 8.05; N, 7.99.

General procedures for synthesis of 8–14

A solution of 4-hydroxy-6-phenyl-2-sulfanylpyrimidine-5-carbonitrile **1** (0.43 mol), diisopropylethylamine (0.86 mol) in dry tetrahydrofuran was placed in a 10-mL microwave vial. A solution of *N*-substituted acetamide/alkyl chloride (0.43 mol) in dry tetrahydrofuran (3 mL) was then added to the reaction mixture and subjected to microwave irradiation for 10 min at 120 °C. The completion of reaction was optimized and confirmed through TLC (Developing solvent: ethyl acetate: hexane = 2:8) visualized under iodine vapors. The reaction solution was concentrated under reduced pressure. The resulting mixture was diluted with cold water and extracted with chloroform. The organic layer was washed with water and brine. The extraction was repeated twice, and combined organic layers were dried over anhydrous sodium sulfate. The organic layers were further concentrated to the dryness using rotary evaporator. Compounds obtained as solid were recrystallized using appropriate solvent, and the oily compounds were purified by column chromatography (5% MeOH in DCM). The final desired synthetic compounds were obtained in good yields.

2-{2-[4-(4-Chloro-phenyl)-piperazin-1-yl]-2-oxo-ethylsulfanyl}-4-hydroxy-6-phenyl-pyrimidine-5-carbonitrile (8)

Yellow solid; yield: 89%; mp: 155–158 °C; R_f: 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.1–3.15 (m, 4H), 3.62–3.69 (m, 4H), 4.13 (s, 2H), 6.79–6.82 (d, J = 9, 2H), 7.17–7.20 (d, J = 9, 2H), 7.39–7.42 (t, J = 9, 2H), 8.28–8.30 (d, J = 6, 1H), 8.69–8.71 (d, J = 6, 2H); FT-IR (ν_{max}; per cm, KBr): 2922 (C-$H_{stretch}$), 1727 (C=O), 1632 (C=N), 1496 (C=C $ring_{stretch}$), 1454 (C-N), 1232 (C-$S_{stretch}$), 640 (Ar-$Cl_{stretch}$), 764 (Phenyl); Anal. Calcd. For $C_{21}H_{18}ClN_7O_2S$: C, 53.90; H, 3.88; N, 20.95; found: C, 53.87; H, 3.91; N, 20.93

4-hydroxy-2-({2-oxo-2-[4-(pyridin-2-yl)piperazin-1-yl]ethyl}sulfanyl)-6-phenylpyrimidine-5-carbonitrile (9)

Light brown solid; yield: 75%; mp: 151–153 °C; R_f: 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.42–3.57 (m, 8H), 5.47 (s, 2H), 6.65–6.69 (t, J = 12, 2H), 6.83–6.86 (d, J = 9, 2H), 7.32–7.35 (d, J = 9, 2H), 7.54–7.58 (t, J = 12, 2H), 8.12–8.13 (d, J = 3, 1H); FT-IR (ν_{max}; per cm, KBr): 2919 (C-$H_{stretch}$), 1725 (C=N), 1638 (C=O), 1561 (C=C $ring_{stretch}$), 1478 (C-N), 1232 (C-$S_{stretch}$), 772 (Phenyl); Anal. Calcd. For $C_{22}H_{20}N_6O_2S$: C, 61.10; H, 4.66; N, 19.43; found: C, 61.07; H, 4.69; N, 19.40.

4-hydroxy-2-({2-oxo-2-[4-(pyrimidin-2-yl)piperazin-1-yl]ethyl}sulfanyl)-6-phenylpyrimidine-5-carbonitrile (10)

Yellow solid; yield: 79%; mp: 152–154 °C; R_f: 0.67; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.4–3.6 (m, 8H), 5.47 (s, 2H), 6.67–6.65 (t, J = 6, 1H), 6.80–6.86 (t, J = 18, 2H), 7.32–7.35 (d, J = 9, 2H), 7.44 (t, J = 6, 1H), 8.12–8.13 (d, J = 3, 2H); FT-IR (ν_{max}; per cm, KBr): 2923 (C-$H_{stretch}$), 1631 (C=N), 1585 (C=O), 1549 (C=C $ring_{stretch}$), 1442 (C-N), 1230 (C-$S_{stretch}$), 765 (Phenyl); Anal. Calcd. For $C_{21}H_{19}N_7O_2S$: C, 58.19; H, 4.42; N, 22.62; found: C, 58.21; H, 4.45; N, 22.59.

2-({2-[1-(4'-bipiperidinyl)-1'-yl]-2-oxoethyl}sulfanyl)-4-hydroxy-6-phenylpyrimidine-5-carbonitrile (11)

Yellow solid; yield: 65%; mp: 147–149 °C; R_f: 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.15–1.20 (t, J = 15, 2H), 1.5–1.7 (m, 4H), 2.15–2.26 (m, 4H), 2.78–2.86 (m, 4H), 3.06–3.11 (m, 4H), 5.02 (s, 2H), 5.19–5.30 (m, 1H) 7.21–7.33 (m, 3H), 7.69–7.71 (d, J = 6, 2H); FT-IR (ν_{max}; per

cm, KBr): 2939 (C-$H_{stretch}$), 1627 (C=N), 1542 (C=C ring-$_{stretch}$), 1449 (C-N), 1241 (C-$S_{stretch}$), 764 (Phenyl); Anal. Calcd. For $C_{23}H_{27}N_5O_2S$: C, 63.13; H, 6.22; N, 16.01; found: C, 63.11; H, 6.25; N, 16.04.

2-{[2-(4-aminopiperidin-1-yl)-2-oxoethyl]sulfanyl}-4-hydroxy-6-phenylpyrimidine-5-carbonitrile (12)

Yellow solid; yield: 79%; mp: 145–148 °C; R_f: 0.59; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.62 (m, 2H), 2.02 (m, 2H), 2.35 (m, 1H), 3.16 (d, J = 3, 2H), 3.30–3.31 (t, J = 3, 2H), 3.78 (s, 2H), 6.83–6.85 (t, J = 6, 3H), 7.49 (m, 2H); FT-IR (ν_{max}; per cm, KBr): 3430 (N-$H_{stretch}$), 2932 (C-$H_{stretch}$), 1631 (C=O), 1543 (C=C $ring_{stretch}$), 1459 (C-N), 1248 (C-$S_{stretch}$), 712 (Phenyl); Anal. Calcd. For $C_{18}H_{19}N_5O_2S$: C, 58.52; H, 5.18; N, 18.96; found: C, 58.49; H, 5.20; N, 18.91.

2-{[2-(azepan-1-yl)-2-oxoethyl]sulfanyl}-4-hydroxy-6-phenylpyrimidine-5-carbonitrile (13)

Off white solid; yield: 70%; mp: 152–155 °C; R_f: 0.62; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 1.25 (m, 4H), 2.36 (m, 4H), 3.36–3.41 (t, J = 15, 4H), 3.76 (s, 2H), 6.85 (m, 3H), 7.49 (m, 2H); FT-IR (ν_{max}; per cm, KBr): 2930 (C-$H_{stretch}$), 1717 (C=O), 1623 (C=N), 1566 (C=C $ring_{stretch}$), 1447 (C-N), 1242 (C-$S_{stretch}$), 765 (Phenyl); Anal. Calcd. For $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21; found: C, 61.96; H, 5.45; N, 15.19.

4-hydroxy-2-[(oxiran-2-ylmethyl)sulfanyl]-6-phenylpyrimidine-5-carbonitrile (14)

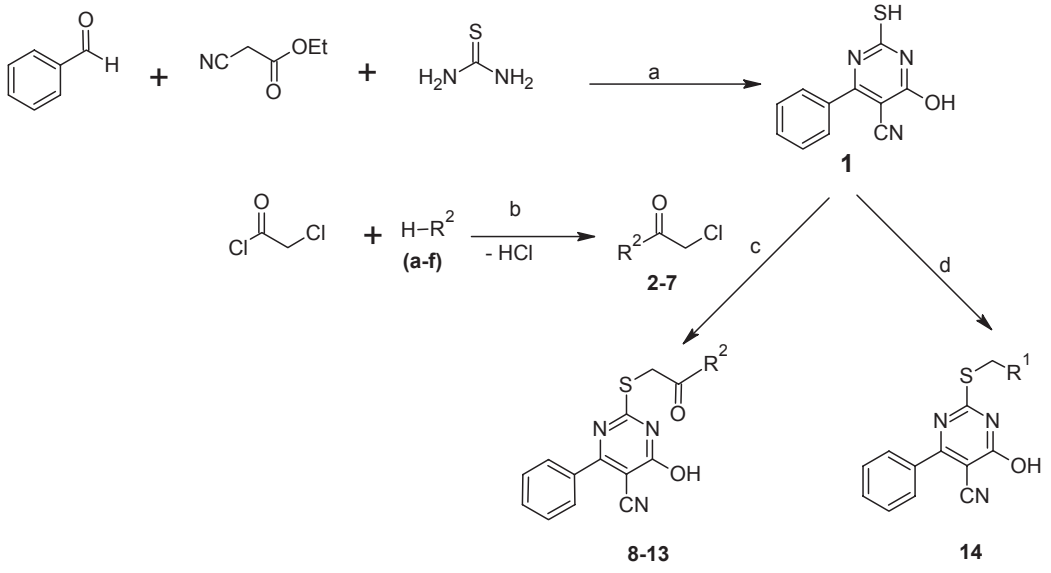
Yellow solid; yield: 68%; mp: 149–151 °C; R_f: 0.65; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.11–2.18 (q, J = 21, 2H), 3.68–3.79 (m, 1H), 4.99–5.02 (d, J = 9, 2H), 5.23–5.30 (m, 2H), 7.26–7.33 (m, 3H), 7.86–7.88 (t, J = 6, 2H); FT-IR (ν_{max}; per cm, KBr): 2930 (C-$H_{stretch}$), 1725 (C=O), 1547 (C=C $ring_{stretch}$), 1453 (C-N), 1240 (C-$S_{stretch}$), 764 (Phenyl); Anal. Calcd. For $C_{14}H_{11}N_3O_2S$: C, 58.93; H, 3.89; N, 14.73; found: C, 58.90; H, 3.91; N, 14.75.

Pharmacological activity

The Institutional Animal Ethical Committee (IAEC) approved the experiments for oral glucose tolerance test (OGTT). Healthy Wistar rats used for the study were obtained from Animal House of Delhi Institute of Pharmaceutical Sciences and Research. Rats were housed in colony cages (three female and one male rats per cage for breeding), at an ambient temperature of 25 °C with 12-h light/12-h dark cycle. Animals were kept in polypropylene cages and were fed with rat food as pellets and water *ad libitum*. All experimental procedures were performed according to IAEC (Protocol No.1/DIPSAR/IAEC/2010) and CPCSEA guidelines.

Induction of experimental diabetes

The experimental diabetes was induced using freshly prepared solution of streptozotocin (STZ; Sigma-Aldrich) at the



8, R^2 = (4-chlorophenyl)piperazin - 1 -yl
9, R^2 = (pyridin-2-yl)piperazin -1-yl
10, R^2 = (pyrimidyl-2-yl)piperazin-1-yl
11, R^2 = 1,4'-bipiperidynyl
12, R^2 = 4 - aminopiperidinyl
13, R^2 = azepanyl
14, R^1 = (oxirane-2-yl)methyl

Scheme 1: Reagents and Conditions: (A) potassium carbonate, ethanol, reflux, 6 h, (B) H-R^2 (A–F) secondary amines, diisopropylethylamine, 1,4-dioxan, reflux, 2 h, (C) 2–7, dry tetrahydrofuran, MW, 10 min, 120 °C, (d) RCl, dry tetrahydrofuran, MW, 10 min, 120 °C.

dose level of 90 mg/kg (I.P.) in 0.1 M freshly prepared citrate buffer pH 4.5 to 2-day-old neonatal pups (21,22). A total of 30 diabetic and six non-diabetic male rats were used in the study. After 6 weeks of injection, animals were screened for fasting blood glucose level. Diabetic rats were further divided into four groups with six rats per group. Animals showing fasting glucose levels >150 mg/dL were considered diabetic and selected for screening of compounds.

Antihyperglycemic activity

Male Wistar rats weighing 150–200 g having blood glucose level >150 mg/dL were selected for OGTT. Animals of diabetic control group received oral administration of vehicle (0.5% w/v methylcellulose), and standard group received vildagliptin (10 mg/kg). Animals of experimental group were administered suspension of desired synthetic compound orally (made in 0.5% w/v methylcellulose) at a single dose of 10 mg/kg body weight (9,23). A glucose load (2 g/kg) was given orally to overnight fasting rats after 30 min prior to administration of the test compound/vehicle. Blood samples were taken from the tail vein at regular intervals of 0, 30, 60, 90, 120 min after glucose load. The blood glucose level was monitored using glucometer (ACCU-CHEK, Active; Roche Diagonistics, Mannheim, Germany).

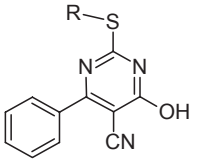
Statistical evaluation

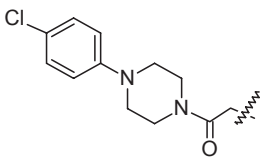
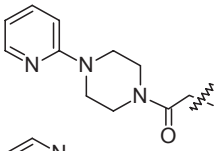
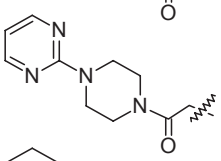
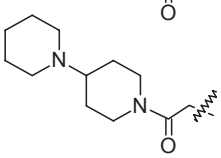
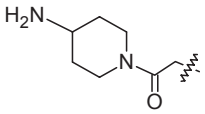
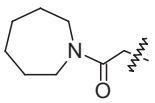
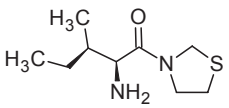
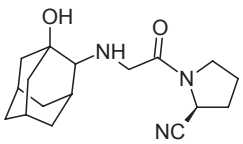
Statistical evaluation was performed by one-way ANOVA followed by Dunnett's post-test and expressed as mean ± SEM. IC_{50} values were determined using nonlinear regression analysis and expressed as results obtained from three independent experiments. Statistical studies and data analyses were performed using GRAPHPAD PRISM Version 5.0 (San Diego, CA, USA).

In vitro *DPP IV enzyme inhibition assay*

All the synthesized compounds were evaluated for *in vitro* DPP IV enzyme inhibition. The enzyme assay was performed using a DPP IV drug discovery kit (BML-AK 499; Enzo Life Sciences, Pennsylvania, USA). The activity of test compounds was assayed using human recombinant DPP IV enzyme, chromogenic substrate (H-Gly-Pro-AMC, Km 114 μM), DPP IV inhibitor (P32/98), assay buffer, and calibration standard as provided in the kit. The assay was performed using 96-well flat-bottomed microtiter plate followed by addition of assay buffer, DPP IV enzyme, and chromogenic substrate (HGly-Pro-pNA). The assay principle and procedure were followed as per a manufacturer's guidelines. Solutions of the test compounds were made in dimethyl sulfoxide (DMSO) at different concentrations of 25, 50, 100, 200 μg/mL, and 20 μL were added to each well after further dilutions. The plate was incubated at 37 °C for 10 min to allow enzyme-inhibitor reaction and read continuously at A-405 nm using Bio-Rad Elisa Plate Reader Philadelphia, PA, USA.

Table 1: Dipeptidyl peptidase IV inhibitory effect of thiouracil derivatives **1**, **8**–**14**



Compound	R	IC_{50} (μM)[a]
1	H-⧘-	29.19
8		0.32
9		0.29
10		1.03
11		10.38
12		0.25
13		4.33
14		33.79
P 32/98		2.5
Vildagliptin		<0.05

[a]IC_{50} represents inhibitory concentration determined by nonlinear regression analysis using GRAPHPAD PRISM software. Values are expressed as mean of three independent experiments.

Results and Discussion

Chemistry

A convergent synthesis was designed for synthesis of targeted compounds as outlined in Scheme 1. 4-hydroxy-6-phenyl-2-sulfanylpyrimidine-5-carbonitrile **1** was synthesized by cyclo-condensation of benzaldehyde with thiourea and ethylcyanoacetate according to the reported procedure (20). The second part of the scheme included synthesis of *N*-substituted acetamide derivatives **2–7** as versatile intermediate. The synthesis of *N*-substituted acetamide derivatives was accomplished by reaction of various secondary amines with chloroacetyl chloride by nucleophilic substitution of chlorine by amines. Condensation of both parts was carried out via S-alkylation on heterocyclic core under microwave irradiation to obtain the targeted compounds **8–14** (Scheme 1). The structures of the titled analogs were confirmed by spectral analysis.

Biological activity

In vitro DPP IV inhibitory activity

All the synthesized compounds **8–14** were investigated for DPP IV inhibition, and the results of initial SAR development for the series are reported as their micromolar inhibitory concentration, IC_{50} (μM). With respect to *in vitro* DPP IV inhibition as shown in Table 1, almost all the compounds showed good-to-moderate response against DPP IV inhibition. Among the synthesized analogs, parent compound **1** (IC_{50} = 29.2 μM) showed weak inhibitory activity. The *in vitro* study indicated that different substitutions over SH group of thiouracil ring by *N*-substituted acetamide derivatives exerted variable DPP IV inhibitory activity. The *N*-substituted acetamide derivatives (**2–7**) were synthesized using secondary amines and chloroacetyl chloride and have been explored due to their substrate specificity for DPP IV inhibition (24). Significant inhibition was recorded with 4-chlorophenyl piperazine derivative **8** (IC_{50} = 0.32 μM). It was noted that the presence of electron withdrawing chloro group at 4-position of phenyl ring may be responsible for enhancement of activity. This indication prompted us to prepare pyridyl piperazine derivative **9** (IC_{50} = 0.29 μM) having an electron-deficient aromatic pyridyl moiety, leading the most potent compound of series. The replacement by the pyridyl piperazine moiety resulted in a more potent inhibitor than the replacement by the pyrimidyl piperazine moiety **10** (IC_{50} = 1.03 μM). No considerable difference in activity was seen with 4-piperidino piperidine derivative **11** (IC_{50} = 10.38 μM) with the

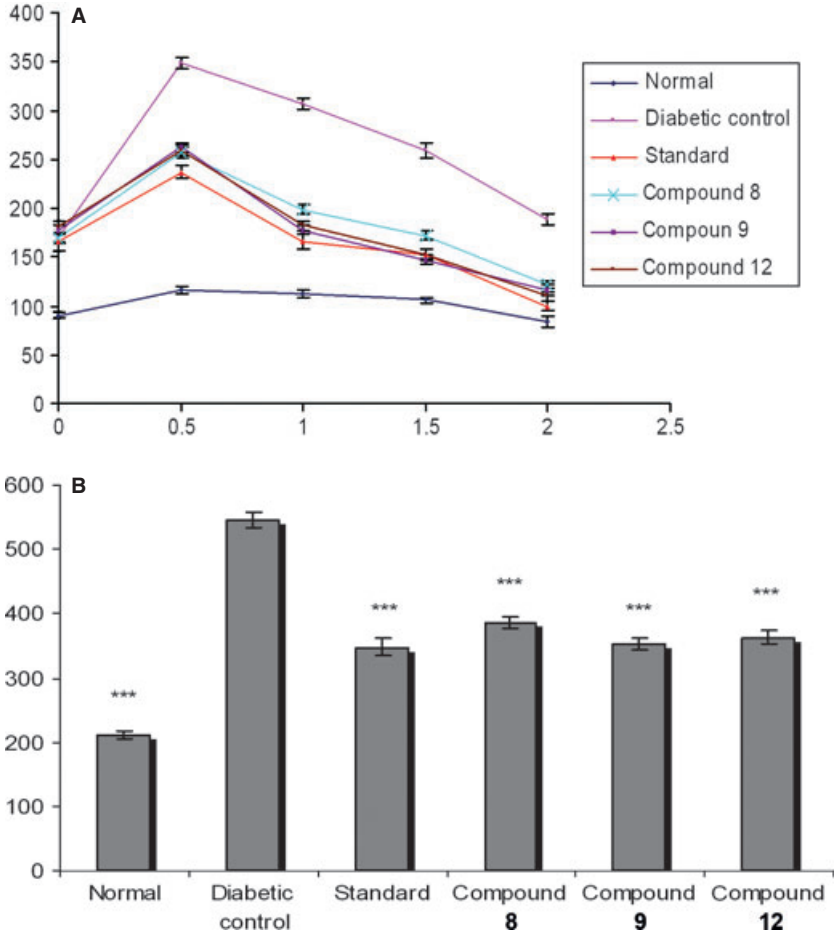


Figure 2. Effect of compounds **8**, **9** and **12** on blood glucose level in an oral glucose tolerance test (OGTT) using streptozotocin-induced diabetic rats. The test compounds were given orally (10 mg/kg), vildagliptin (standard, 10 mg/kg) and 0.5% CMC (diabetic control) followed by oral glucose challenge (2 g/kg) after 30 min: (A) time–course of changes in blood glucose level and (B) area under the blood glucose concentration–time curve (AUC) for the period of 0–2 h during OGTT. Data are expressed as mean ± SEM for six animals in each group. ***p < 0.0001 versus diabetic group.

exception of incorporation of substituted 4-aminopiperidine ring **12** (IC_{50} = 0.25 μM). The slight retention in activity was encountered with seven membered ring analog, azepenyl derivative **13** (IC_{50} = 4.33 μM) may be tolerated as substitution. The epichlorohydrin derivative **14** (IC_{50} = 33.79 μM) displays marked decrease in activity, which suggested that removal of the carbonyl group was unfavorable for DPP IV inhibition. Analysis of structural activity relationship of synthesized derivatives revealed that replacement of SH by *N*-substituted acetamide derivatives effectively influences the DPP IV inhibitory activity. These results indicated that effect of different cyclic analogs did not produce much impact with regards to DPP IV inhibitory effect. However, presence of the carbonyl oxygen of an acetamide linker is necessary for DPP IV inhibition as evidenced by synthetic compounds **8**, **9**, and **12**.

Antihyperglycemic activity

Encouraged by strong *in vitro* DPP IV enzyme inhibitory potential, compounds **8**, **9**, and **12** were chosen for *in vivo* study. These compounds were orally administered at a dose of 10 mg/kg. In a single-dose study, the compounds lead to lower blood glucose levels beginning 30 min after glucose loading, and a 10 mg/kg dose significantly suppressed hyperglycemia as compared with the control group. The results indicate that the area under blood glucose concentration–time curve (AUC) and OGTT significantly reduced (<0.0001) glucose excursion during 0–2 h as shown in Figure 2A–B. Vildagliptin showed 41.0% reduction in blood glucose at time of OGTT (at 1.5 h) at 10 mg/kg. Compounds **8**, **9**, and **12** also showed glucose lowering up to 33.6%, 43.3%, and 40.9%, respectively, at same dose and at the same time. These results showed that the *in vivo* efficacy of compounds **8**, **9**, and **12** was comparable to vildagliptin at 10 mg/kg as shown in Figure 2A–B.

Thus, improved glycemic control was seen with oral dosing of compounds **8**, **9**, and **12**, which significantly decreased elevated glucose challenge and possesses therapeutic efficacy.

Conclusion

In conclusion, we synthesized a series of thiouracil derivatives that were evaluated for inhibitory activity against the DPP IV enzyme. Among these, compounds **8** (IC_{50} = 0.32 μM), **9** (IC_{50} = 0.29 μM), and **12** (IC_{50} = 0.25 μM) showed excellent DPP IV inhibition and exhibited pronounced *in vivo* antihyperglycemic activity in streptozotocin-induced diabetic rat model. Furthermore, heterocyclic substituted piperazines with an acetamide linker were indicated to offer the most potent DPP IV inhibitors. Thus, the thiouracil nucleus offers a potential bioactive core for design of DPP IV inhibitors with desirable bioactivity for type 2 diabetes in the future.

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Conflict of Interest

None.

References

1. Matuszek B., Lenart Lipińska M., Nowakowski A. (2007) Incretin hormones in the treatment of type 2 diabetes Part I: influence of insulinotropic gut-derived hormones (incretins) on glucose metabolism. Endokrynol Pol;58:522–85.
2. Ahren B, Schmitz O. (2004) GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. Horm Metab Res;36:867–76.
3. Verspohl E.J. (2009) Novel therapeutics for type 2 diabetes: incretin hormone mimetics (glucagons-like peptide-1 receptor agonists) and dipeptidyl peptidase-4 inhibitors. Pharmacol Therapeut;124:3–138.
4. Mest H. J., Mentlein R. (2005) Dipeptidyl peptidase inhibitors as new drugs for the treatment of type 2 diabetes. Diabetologia;48:616–620.
5. Green D.E. (2007) New therapies for diabetes. Clin Cornerstone;8:58–65.
6. Bo Ahre'n (2004) Enhancement or prolongation of GLP-1 activity as a strategy for treatment of type 2 diabetes. Drug Discov Today Ther Strateg;1:207–212.
7. Holst J.J, Gromada J. (2004) Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am J Physiol Endocrinol Metab;287:E199–206.
8. Chyan Y.J., Chuang L.M. (2007) Dipeptidyl peptidase-IV Inhibitors: an evolving treatment for type 2 diabetes from the incretin concept. Recent Pat Endocr Metab Immune Drug Discov;1:15–24.
9. Yamazaki K., Yasuda N., Inoue T, Nagakura T., Kira K., Shinoda M., Saeki T., Tanaka I. (2006) 7-But-2-ynyl-9-(6-methoxy-pyridin-3-yl)-6-piperazin-1-yl-7,9- dihydropurin-8-one is a novel competitive and selective inhibitor of dipeptidyl peptidase IV with an antihyperglycemic activity. J Pharmacol Exp Ther;319:1253–1257.
10. Peters J. U., Weber S., Kritter S., Weiss P., Wallier A., Boehringer M., Hennig M., Kuhn B., Loeffler B. M. (2004) Aminomethylpyridines as novel DPP-IV inhibitors: a 10^5-fold activity increase by optimization of aromatic substituents. Bioorg Med Chem Lett;14:1491–1493.
11. Peters J. U., Weber S., Kritter S., Weiss P., Wallier A., Zimmerli D., Boehringer M., Steger M., Loeffler B. M (2004) Aminomethylpyridines as DPP-IV inhibitors. Bioorg Med Chem Lett;14:3579–3580.

12. Miyamoto Y., Banno Y., Yamashita T., Fujimoto T., Oi S., Moritoh Y., Asakawa T. *et al.* (2011) Design and synthesis of 3-pyridylacetamide derivatives as dipeptidyl peptidase IV (DPP-4) inhibitors targeting a bidentate interaction with Arg125. Bioorg Med Chem Lett;19:172–185.
13. Feng J., Gwaltney S., Lam B., Zhang Z. (2007) Dipeptidyl peptidase inhibitors. WO Pat Apl;2006019965:2005.
14. Backes B.J., Longenecker K., Hamilton G.L., Stewart K., Lai C., Kopecka H. *et al.* (2007) Pyrrolidine-constrained phenethylamines: the design of potent, selective, and pharmacologically efficacious dipeptidyl peptidase IV (DPP4) inhibitors from a lead-like screening hit. Bioorg Med Chem Lett;17:2005–2012.
15. Cheon H.G., Lee C.M., Kim B.T., Hwang K.J. (2004) Lead discovery of quinoxalinediones as an inhibitor of dipeptidyl peptidase-IV (DPP-IV) by high-throughput screening. Bioorg Med Chem Lett;14:2661–2664.
16. Feng J., Zhang Z., Wallace M. B., Stafford J. A., Kaldor S. W., Kassel D. B., Navre M., Shi L., Skene R. J., Asakawa T., Takeuchi K., Xu R., Webb D. R., Gwaltney S. L. (2008) Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. J Med Chem;51:4357.
17. Zhang Z., Wallace M.B., Feng J., Stafford J.A., Skene R.J., Shi L., Lee B., Aertgeerts K., Jennings A., Xu R., Kassel D.B., Kaldor S.W., Navre M., Webb D.R., Gwaltney S.L. (2011) Design and synthesis of pyrimidinone and pyrimidinedione inhibitors of dipeptidyl peptidase IV. J Med Chem;54:510–524.
18. Havale S.H., Pal M. (2009) Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. Bioorg Med Chem Lett;17:1783–1802.
19. Szczepankiewicz B.G, Kurukulasuriya R. (2007) Aromatic heterocycle-based DPP-IV inhibitors: xanthines and related structural types. Curr Top Med Chem;27:569–578.
20. Ding Y., Girardet J.L., Smith K.L., Larson G., Prigaro B., Wu J.Z., Yao N. (2006) Parallel synthesis of 5-cyano-6-aryl-2-thiouracil derivatives as inhibitors for hepatitis C viral NS5B RNA-dependent RNA polymerase. Bioorg Med Chem;34:26–38.
21. Weir G.C., Clore E.E., Zma-Chinsky C.J., Bonnier-weir S. (1981) Islet secretion in new experimental model of non-insulin dependent diabetes. Diabetes Metab Rev;30:590–594.
22. Abeeleh M.A., Ismail Z.B., Alzaben K.R., Halaweh S., Al-Essa M.K., Abuabeeleh J., Alsmady M.M. (2009) Induction of diabetes mellitus in rats using intraperitoneal streptozotocin: a comparison between 2 strains of rats. Eur J Sci Res;32:398–402.
23. Singh S., Sethi S., Khanna V., Benjamin B., Kant R., Sattigeri J., Bansal V.S., Bhatnagar P.K., Davis J.A. (2011) RBx-0597, a potent, selective and slow-binding inhibitor of dipeptidyl peptidase IV for the treatment of type 2 diabetes. Eur J Pharmacol;652:157–163.
24. Gupta R.C., Chhipa L., Mandhare A.B., Zambad S.P., Chauthaiwale V., Nadkarni S.S., Dutt C. (2009) Novel N-substituted 4-hydrazino piperidine derivative as a dipeptidyl peptidase IV inhibitor. Bioorg Med Chem Lett;19:5021–5025.