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Design, Synthesis, Biological Screening and Molecular Docking Studies of Piperazine-Derived Constrained Inhibitors of DPP-IV for the Treatment of Type 2 Diabetes

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Running Title:

Piperazine-derived constrained inhibitors of DPP-IV

Abstract

Novel piperazine-derived conformationally constrained compounds were designed, synthesized, and evaluated for *in vitro* Dipeptidyl peptidase-IV *(*DPP-IV) inhibitory activities. From a library of compounds synthesized, 1-(2-(4-(7-Chloro-4-quinolyl)piperazin-1-yl)acetyl)pyrrolidine (**2g**) was identified as a potential DPP-IV inhibitor exhibiting better inhibitory activity than P32/98, reference inhibitor. The *in vivo* studies carried out in STZ and *db/db* mice models indicated that the compound **2g** showed moderate antihyperglycemic activity as compared to the marketed drug Sitagliptin. A two-week repeated dose study in *db/db* mice revealed that compound **2g** significantly declined blood glucose levels with no evidence of hypoglycemia risk. Furthermore, it showed improvement in insulin resistance reversal and antidyslipidemic properties. Molecular docking studies established good binding affinity of compound **2g** at the DPP-IV active site and are in favor of the observed biological data. These data collectively suggest that compound **2g** is a good lead molecule for further optimization studies.

Keywords:

Antihyperglycemic; Constrained DPP-IV inhibitor; Dipeptidyl Peptidyl-IV (DPP-IV) inhibitor; Molecular docking; Oral glucose tolerance test; Piperazine.

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1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic, severe and progressive disease, comprises more than 95% of diabetes cases and is characterized by hyperglycemia resulting from insufficient insulin secretion, insulin resistance and other abnormalities. In case of improper treatment, type 2 diabetes can cause some serious complications such as coronary artery disease, hypertension, stroke, peripheral vascular disease, neuropathy, blindness and renal failure.[1,2] Among the potential approaches for T2DM reported so far, glucagon-like peptide (GLP-1) and glucose-dependent insulinotropic peptide (GIP) have recently come to the center stage and are being considered as a game changer towards treatment of type 2 diabetes.[3] GLP-1 and GIP collectively known as incretin peptide hormones, are secreted from the gastrointestinal tract in response to meal ingestion and stimulate insulin secretion from β-cells in a glucose-dependent manner.[4] These hormones play a significant role in blood glucose homeostasis.[5] The elevated levels of GLP-1 improve the glycemic control by reducing the blood glucose and glycosylated haemoglobin (HbA_{1c}) levels.[6] Dipeptidyl peptidase-IV (DPP-IV), a serine protease, controls the activities of GLP-1 and GIP by cleaving N-terminal dipeptide to their inactive forms. Inhibition of DPP-IV enzyme prevents the inactivation of GLP-1 and GIP, and enhances approximately two to three folds endogenous levels of GLP-1 and GIP.[7] Therefore, inhibition of DPP-IV has emerged as a novel and promising new target for treatment of type 2 diabetes. Thus, extensive efforts have been done in order to explore DPP-IV inhibitors as oral antidiabetic agents.[8,9] As a result, several inhibitors of DPP-IV have currently been approved for clinical usage in certain countries and some are under the late-stage clinical trials.[10,11]

In the past few years, many of the constrained DPP-IV inhibitors were synthesized[12] and thus, a concerted effect in this direction led to the discovery of piperazine containing gliptins,

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namely; teneligliptin and gosogliptin.[13,14] These observations and the diverse pharmacological and medicinal potentialities of piperazine[15,16] prompted us to investigate the conformationally constrained and low molecular weight inhibitors of DPP-IV that have common basic piperazine structure. Herein, we describe the rationale designing, synthesis, biological evaluation and molecular docking simulations of piperazine-derived conformationally constrained small molecules as potential DPP-IV inhibitors. In order to explore these novel DPP-IV inhibitors, the cyanopyrrolidine and its mimetic such as pyrrolidine, thiazolidine, piperidine and morpholine were incorporated at P1 site and the electron-deficient aryl and fused bicyclic heteroarylpiperazine moieties were introduced at P2 site in which the piperazine structure provides natural constaint (Fig. 1).

MATERIALS AND METHODS

1.1. *In vitro* DPP-IV inhibitory assay

All the synthesized compounds presented in Table 1 were assayed with human recombinant DPP-IV enzyme and the DPP-IV inhibitory activity was determined by measuring the p-nitroaniline (pNA) released from the chromogenic substrate hydrolysis (H-Gly-Pro-pNA). The DPP-IV Drug Discovery Kit- BML-AK 499 used for assay was taken from Enzo Life Sciences International, Inc., USA. The DPP-IV inhibitor P32/98 was selected as standard inhibitor. The principle of the assay was given in the kit and described in the literature.[17,18] The recombinant soluble human DPP-IV enzyme, chromogenic substrate, assay buffer, DPP-IV inhibitor and Calibration Standard were present in the kit. The assay was carried out in 96-well flat bottomed microtiter plates. The stock solutions of the test compounds were prepared in DMSO and diluted in buffers (50 mM Tris-HCl) to final concentrations ranging from 10-1000

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µM in the assay. 10 µl of each compounds were added in each reaction well. The final concentration of DMSO in the assay well is 1% which is found to have no demonstrable effect on DPP-IV enzyme activity.[17] The control well of the assay contains assay buffer, DPP-IV enzyme and substrate; and assay well contains assay buffer, DPP-IV enzyme, inhibitor and substrate. After addition of assay components in the assay well, the plate was incubated at 37ºC for 10 minutes and read continuously at 405 nm in a double beam spectrophotometer. The percentage inhibition of test compounds against DPP-IV enzyme was calculated on the basis of activity in the control tube as 100% from three independent sets of experiments.

1.2. *In vivo* antihyperglycemic evaluation in STZ Model

Male albino rats of Sprague-Dawley strain (8 to 10 weeks of age body weight 160 ± 20 g) were selected for this study. Streptozotocin (Sigma, USA) was dissolved in 100 mM citrate buffer pH 4.5 and the calculated amount of the fresh solution was injected into overnight fasted rats (60 mg/kg) intraperitoneally. Fasting blood glucose was checked 48 h later by glucometer, using glucostrips and animals showing blood glucose values over 270 mg/dl were selected and randomly divided into groups of five animals each. Rats of the experimental groups were administered suspension of standard drug and the desired test samples orally (made in 1.0% gum acacia) at a dose of 100 mg/kg body weight. Animals of the control group were given an equal amount of 1.0 % gum acacia.The blood glucose levels of each animal were determined just before the administration of standard drug and test samples (0 min) and thereafter at 30, 60, 90, 120, 180, 240, 300 and 1440 min. Food but not water was withdrawn from the cages during 0 to 300 min. The average lowering in blood glucose level between 0 to 300 min and 0 to 1440 min was calculated by plotting the blood glucose levels on the y-axis and time on x-axis and determining the area under the curve (AUC).[19] Comparing the AUC of experimental group with

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that of control group determined the percent lowering of blood glucose levels during the period. A statistical analysis was made by Dunnett's test (Prism Software).

1.3. *In vivo* antihyperglycemic, antidyslipidemic and insulin resistance reversal evaluation in db/db mice model

Male C57BL/KsJ strain of mouse (db/db mouse) 10-12 weeks of age and around 40 ±3 g of body weight were procured from the animal colony of the Institute. The work with these animals was cleared by the institutional ethics committee for animal study and was conducted in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. The institute has taken permission from the animal ethical committee for the work (No. 129/07/Biochem/IAEC). The animals were housed four or five in a polypropylene cage in the animal house. The following norms were always followed for the animal room environment: temperature 23 ± 2°C; humidity 50-60%; light 300 lux at floor level with regular 12 h light cycle; noise level 50 decibel; ventilation 10-15 air changes per hour. After randomization into groups, the mice were acclimatized for 2-3 days in the new environment before initiation of the experiment. Standard pellets were used as a basal diet during the experimental period.

The animals were allocated into groups of 5 animals each. Prior to start of the sample feeding, a vehicle training period was followed from day -3 to day 0 during which all the animals were given vehicle (1% gum acacia) at a dose volume of 10 ml/kg body weight. At day 0 animals having blood group levels between 350 to 500 mg/dl were selected and divided into four groups containing 5 animals in each group. One group was considered as a control group while the other groups were considered as the experimental groups. The experimental groups were given a

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suspension of compounds **2g** and **4c** at 30.0 mg/kg and reference drug Sitagliptin at 10 mg/kg body weight dose. The control group was given an equal amount of the vehicle. All the animals had normal diet and were free to access fresh water. Random blood glucose of each mouse was checked daily at 10.00 pm. On day 10^{th} and day 15^{th} oral glucose tolerance (OGTT) test was performed on overnight fasted animals. On the following day their blood glucose profiles were measured at -30 min and 0 min (baseline) and then at 30, 60, 90 and 120 min post an oral glucose load of 3.0 g/kg body weight to study the effect of the compound on glucose tolerance. Blood has been withdrawn from the retro-orbital plexus of mice eye for the estimation of lipid profile on DIALAB DTN-410-K and insulin levels by Mercodia Uppsala Insulin ELISA Kit.

1.4. Statistical analysis

The homeostatic model assessment (HOMA) was used to calculate relative insulin resistance as follows: [Fasting blood glucose (mg/dl) × Fasting serum insulin (μIU/ml)/405]. All statistical calculations were performed using Graph-Pad Prism version 3.02 for Windows (GraphPad Software). Data were expressed as mean +SE. The criterion for statistical analysis was significant (*$p<0.05$), more significant (**$p<0.01$), highly significant (***$p<0.001$) and not significant (ns).

1.5. Molecular docking method

The molecular docking study of compound **2g** was performed using the Surflex-Dock module with standard protocols in SYBYL-X 1.3 (Tripos Inc, St. Louis, MO, USA) modeling package[20] which was installed on an HP Z400 desktop workstation equipped with a dual 3.20 GHz Intel® Xeon® processor running Windows 7 Professional (Service Pack 1) operating system. Teneligliptin is a prototype of piperazine containing constrained inhibitor of DPP-IV resembling more closely to the compound **2g**. Therefore, it is selected as a reference for docking

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studies. The X-ray crystal structure of DPP-IV complexed with Teneligliptin was retrieved from the RCSB Protein Data Bank (PDB code: 3VJK).[21] During the protein preparation in the Surflex-Dock protocol, the ligand was extracted; and substructures including N-acetyl-D-glucosamine and crystallographic water molecules were also removed from the crystal structure. Hydrogen atoms were added to the protein structure and ligand to define the correct configuration and tautomeric states. Charges were also added using Gasteiger-Huckel charge for both protein and the ligand. Energy-minimizations were performed with standard protocol using the Tripos force field with a Gasteiger-Huckel charge, distance-dependent dielectric and the Powell conjugate gradient algorithms with a convergence criterion of 0.01 kcal/mol. The extracted ligand was used for a protomol generation and was also used as a template reference molecule. Molecular docking simulation was performed by binding to the binding site of DPP-IV by an empirical scoring function to score the ligand and protomol guided docking. Thus, this protomol-based method and empirically derived scoring function (e.g. Total score, crash score, polar etc.) were used to calculate the binding affinities.

2. RESULTS AND DISCUSSION

2.1. Chemistry

In our present research, five series of piperazine-derived constrained derivatives bearing different levels of substituents were prepared. Schemes 1-2 outline the syntheses of compounds presented in Table 1. Compounds **1(a-g)** were synthesized using intermediate (*S*)-1-(2-chloroacetyl)pyrrolidine-2-carbonitrile (**10**). Thus, in order to synthesize compounds **1(a-g),** commercially available proline was protected with Boc anhydride, followed by conversion of Boc-proline (**9**) to Boc-prolinamide (**8**) using the mixed anhydride protocol. After Boc-deprotection with 4N HCl-dioxane,[22] obtained L-prolinamide hydrochloride (**9**) was treated with

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chloroacetyl chloride in DMF in the presence of triethyl amine at -15°C and formed amide bond was dehydrated to nitrile using cyanuric chloride in one-pot protocol,[23] which resulted to the intermediate compound **10**. The final compounds **1(a-g)** were synthesized by nucleophilic substitution reaction with mono substituted aryl piperazine compounds **11(a-g)** at room temperature in excellent yields (Scheme 1). Compounds **2(a-i)**, **3(a-d)**, **4(a-g)**, and **5(a-g)** were also synthesized as in scheme-1 using chloroacetamide intermediates (**16-19**). These intermediates were prepared either by treating of chloroacetyl chloride with respective cyclic amines (**12-15**) in DCM under cold condition (-15°C) or by coupling reaction with chloroacetic acid using DCC/HOBt protocol (Scheme 2).[24]

2.2. Biological Activities

2.2.1. *In vitro* DPP-IV Inhibitory Activities

In order to reveal the structure-activity relationship (SAR) and to get potent DPP-IV inhibitors, various degrees of substituent were introduced on the aryl piperazine moieties. It is well known that the introduction of an electron-deficient arylpiperazine scaffold at the S2 site is favorable for improved potency and long lasting DPP-IV inhibition.[12b] Therefore, we introduced various electron-withdrawing groups on the phenyl ring to improve the DPP-IV inhibitory activity and to explore the effect of electron-withdrawing groups on DPP-IV enzyme inhibition. The hydrophobic moieties such as Cl and CF_3 are of great interest and have important contributions towards DPP-IV inhibitory activity. Thus, introduction of electron withdrawing groups such as nitrile, nitro and chloro groups on the phenyl or pyridyl moieties, and the core structures at the P1 site influenced the DPP-IV activity. The SAR analysis revealed that

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compounds **1a** and **1b** had little more DPP-IV inhibitory activities than others (**1c-1f**) in the cyanopyrrolidine series (**1**(**a-g**)). Therefore, in order to get the potential and chemically stable inhibitor, cyanopyrrolidine mimetics such as pyrrolidine, thiazolidine, piperidine and morpholine moieties, were incorporated in place of cyanopyrrolidine moiety to probe the hypothesis that the proline moiety could be replaced with other heterocycles as well as a large ring to enhance the DPP-IV inhibitory activity. In the pyrrolidine series, the introduction of pyridyl moiety with pyrrolidine (**2c**) core structure exhibited 44.0% of DPP-IV inhibition. In order to improve inhibitory activity, electron withdrawing groups (Cl, NO_2, and CF_3) were introduced on the phenyl, but they diminished the activities (**2b, 2e, 2f**). Introduction of chlorine on 3-position of phenyl ring showed very weak inhibition in all series (**2b, 5b**), while it exhibited 72.9% inhibition in piperidine series (**6c**). In the thiazolidine series, introduction pyridyl moiety with thiazolidine (**3b**) exhibited 78.9% inhibition. On the other hand, the introduction of the fused bicyclic moiety, i.e. 7-Chloro-4-quinolyl, with pyrrolidine (**2g**) and morpholine (**5e**) showed good inhibitory activities with 80.4% and 75.4% inhibition, respectively, and with cyanopyrrolidine, thiazolidine and piperidine had little activities (**1f, 3d, 4e**); whereas the introduction of a trifluoromethyl group in 7-position of 4-quinolyl diminished the activity (**1g, 2h**). In particular, despite the lack of an electrophilic nitrile group at P1 site, compounds **3b** and **5e** showed moderate activity while **2g** and **4c** showed exceptionally good DPP-IV inhibitory activity *in vitro* compared to the reference inhibitor, P32/98 and less active than sitagliptin. Among all constrained piperazine derivatives, compound **2g** is identified as the most potent inhibitor. The IC_{50} values were determined only for the most active compounds having more than 50% of DPP-IV inhibition.

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2.2.2. *In vivo* activities

Based on the *in vitro* data presented in table-1, only four compounds namely **2g**, **3b**, **4c** and **5e** exhibiting inhibitory activity with the reference compound P32/98 were considered appropriate to evaluate in the in vivo models in order to assess the potential of this new scaffold and to enable fine-tuning of the biological activity. Towards this objective, these compounds were evaluated for *in vivo* antihyperglycemic activity in streptozotocin-induced diabetic rat model, and compounds exhibiting significant activity were subjected to evaluate their antidiabetic activity in a genetically diabetic and obese C57BL/KsJ-db/db mouse model.

2.2.2.1. *In vivo* antihyperglycemic activity in STZ-induced diabetic rat model

In order to assess the potential antihyperglycemic activity of compounds **2g, 3b, 4c, 5e** and Sitagliptin were screened on streptozotocin-induced diabetic rats at 100 mg/kg dose level. It was found that compounds **2g, 4c,** and Sitagliptin potentially declined the blood-glucose profile of streptozotocin-induced diabetic rats during 0-300 min and 0-1440 min of the experiments. The overall improvement in glucose AUC during 0-300 min was calculated to be around 20.2, 16.2 and 20.9 % ($p<0.01$) by **2g, 4c** and standard drug Sitagliptin, respectively, whereas an improvement of 25.5, 24.6 and 31.9 % ($p<0.01$) was observed during 0-1440 min of the experiment, respectively, while **3b** and **5e** showed moderate improvement on the blood-glucose profile as compared to **2g** and **4c**. It was found that **3b** and **5e** improved the glucose AUC of streptozotocin-induced diabetic rats to the tune of 15.5% ($p<0.01$) and 13.7 % ($p<0.05$) during 0-300 min and by 18.4 and 17.4 % ($p<0.01$) during 0-1440 min of the experiment, respectively (Fig. S1, supp. Info.).

The results obtained from streptozotocin-induced diabetic rats clearly suggest that the improvement of diabetic state and the blood-glucose profile during 0-300 min and 0-1440 min

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post administration of the test samples, is probably because of their continuous inhibition of circulating DPP-IV, which is required for the optimal efficacy. Therefore, compounds **2g** and **4c** were further subjected to *in vivo* antihyperglycemic and antidyslipidemic activities, and improvement in insulin resistance studies in *db/db* mice.

2.2.2.2. *In vivo* antihyperglycemic, antidyslipidemic and insulin resistance reversal activities in *db/db* mice

Compounds **2g** and **4c** were orally gavaged for 15 consecutive days at 30 mg/kg and reference drug Sitagliptin at 10 mg/kg. The effect of **2g, 4c** and Sitagliptin on postprandial blood-glucose profile of *db/db* mice during 14 days of consecutive dosing is shown in Fig. 2. We found that compound **2g** effectively declined the postprandial blood glucose from day 11, and its effect persisted until the end of the experiment, whereas **4c** showed a significant decline in postprandial blood glucose from day 12, and its effect prolonged up to the end of the experiment, while the effect of reference drug Sitagliptin was observed from day 9 and continued up to the end of the experiment (Fig. 2).

It is well known that the DPP-IV inhibitors improve the glycemic control by potentiating the action of endogenously secreted GLP-1 and GIP.[25] In the present study repeated oral gavages of compounds **2g** and **4c** significantly improved postprandial blood glucose and oral glucose tolerance and insulin resistance of *db/db* mice. Thus, to observe the effect of compound **2g** and **4c** on the glucose tolerance, an OGTT was performed on the day 10^{th} and 15^{th} during course of study. Compounds **2g** and **4c** significantly improved the glucose tolerance by 14.6 and 11.8 % ($p<0.01$) on day 10^{th}, and 26.4 and 19.4 % ($p<0.01$) on day15^{th}, respectively at dose of 30 mg/kg body weight whereas the standard drug Sitagliptin exhibited a significant improvement of 21.9

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and 29.5 % ($p<0.01$) at a dose of 10 mg/kg on the day 10^{th} and 15^{th}, respectively as compared to control group (Fig. 3, Table 2). The improvement on oral glucose tolerance revealed that circulating DPP-IV activity was chronically inhibited by compounds **2g** and **4c** during consecutive days of dosing and also during OGTT which is probably associated with elevation in plasma active GLP-1.

Serum lipid profile of *db/db* mice after 15 days of consecutive dosing was investigated to observe the additional beneficial effect of the tested compounds. Compounds **2g, 4c** and standard antidiabetic drug, Sitagliptin declined the plasma triglycerides (TG) by 14.1, 11.1 and 8.8%; cholesterol by 39.1 ($p<0.05$), 13.7 and 14.2%; plasma low-density lipoproteins (LDL-c) by 36.8, 27.5, and 35.1% ($p<0.05$); and enhanced the level of plasma high-density lipoproteins (HDL-c) by 37.1, 28.2 and 38.3% ($p<0.05$), respectively (Fig. 4). Apart from antihyperglycemic properties, an applausable effect of compound **2g** and **4c** on serum lipid profile of *db/db* post 15 days of consecutive treatment was obtained.

An interesting feature of compounds **2g** and **4c** is that in addition to significant *in vivo* efficacy as potential antihyperglycemic agents, these compounds also improve diabetic dyslipidemia in *db/db* mice by elevating HDL-C level. In the diabetic condition, the level of plasma lipids is usually elevated and the results of the present study have revealed that compounds **2g** and **4c** significantly reduce the levels of serum triglycerides (TG), total cholesterol and LDL-cholesterol as well as significantly increase the level of HDL-cholesterol, cardio protective factor, which is a desirable effect.

Elevated fasting blood glucose, hyperinsulinemia, and insulin resistance are characteristic features of type 2 diabetes mellitus as well as of *db/db* mice. Serum samples of **2g** and **4c** treated

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db/db mice were analyzed to evaluate the effect on fasting blood glucose and serum insulin, and it was found that **2g, 4c** and Sitagliptin significantly improved the fasting blood glucose by 29.9, 16.0 % ($p<0.05$) and 27.7 ($p<0.01$), respectively (Fig. 5a), while a significant decline of 10.1 and 14.2 ($p<0.05$) by **2g** and Sitagliptin in serum insulin level was observed, respectively (Fig. 5b). The improvement in fasting blood glucose and fasting serum insulin profile eventually leads to the improvement in insulin resistance, which is reflected in the homeostatic model assessment (HOMA) index. It was found that compound **2g,** and Sitagliptin significantly decline the HOMA-index to the tune of 29.8 and 38.0 % ($p<0.05$) (Fig. 5c). Thus, the improvement in fasting blood glucose by compound **2g** and **4c** refers the improvement of insulin sensitivity in *db/db* mice. In contrast, to enhance the insulin secretion by DPP-IV inhibitors in the present study it was found that repeated oral gavages of compound **2g** and **4c** declined the serum insulin level of treated *db/db* mice, which indicates the improvement in the insulin sensitivity and signaling, and reversal on insulin resistance, a hallmark of type 2 diabetes mellitus. The improvement in insulin sensitivity by compound **2g** and **4c** was also proved with the downswing in the HOMA-index, a pointer of insulin resistance inversely correlated with insulin sensitivity. Thus, the improvement on glucose homeostasis and hyperinsulinemia were evocative of improved insulin sensitivity. Based on results obtained, it cannot be ruled out that 15 days of consecutive treatment with compounds **2g** and **4c** have improved both glucotoxicity and lipotoxicity which also contribute to the improvement of β-cell functions.

2.3. Molecular docking of compound 2g

In order to support the observed *in vitro* and *in vivo* biological activities in hand and to get more insight to predict the binding mode of compound **2g** at the active site of DPP-IV enzyme, molecular docking was performed. All the conformers/poses of compound **2g** into the

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active site of DPP-IV were generated based on the scores. The conformer/pose of compound **2g** targeting the similar residues as teneligliptin was chosen for analyzing the binding features**.** Thus, the docking results revealed that the binding mode of **2g** is similar to teneliglipin in the DPP-IV active site. The pyrrolidine moiety fully occupies the S1 hydrophobic pocket and the remaining part of the compound **2g** binds to the S2 and S2 extensive pockets. The carbonyl group of the amide bond forms strong hydrogen bonds with the side chains of ASN-710 and ARG-125. The 7-chloroquinolin-4-yl moiety occupies the S2 extensive pocket and is stacked with the side chain of Phe357 via a π-π interaction. This moiety is also stabilized with hydrophobic residues such as SER-209, ARG-358, PHE-357 (Fig. 6). These decisive interactions with ARG-125, ASN-710 and PHE-357 are desirable for the promising inhibitory activity of **2g** against DPP-IV. The Total_score and polar scores of **2g** are less than Teneligliptin (Table S1, supp. Info.) which might be the reason for the observed moderate *in vitro* inhibitory activity.

3. CONCLUSIONS

In this paper, we have synthesized the piperazine-derived conformationally constrained derivatives and evaluated for their DPP-IV inhibitory activity *in vitro* and antihyperglycemic, antidyslipidemic and insulin reversal activities *in vivo* in *db/db* mice. The results showed that compound **2g** exhibited better *in vitro* inhibitory activity than reference inhibitor P32/98 and moderate *in vivo* antihyperglycemic, antidyslipidemic and insulin resistance reversal activities as compared to standard antidiabetic drug namely, Sitagliptin. Molecular docking studies have also exhibited good binding affinity of compound **2g** at the active site of DPP-IV complementing the biological activity. Therefore, the piperazine-derived constrained compound **2g** has remarkable promise for further exploration as potential small-molecule DPP-IV inhibitor. Thus,

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modifications of this novel series are ongoing in our laboratories and the results will be reported in due course.

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CONFLICTS OF INTEREST

The authors declared that there is no conflict of interest

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Supporting information

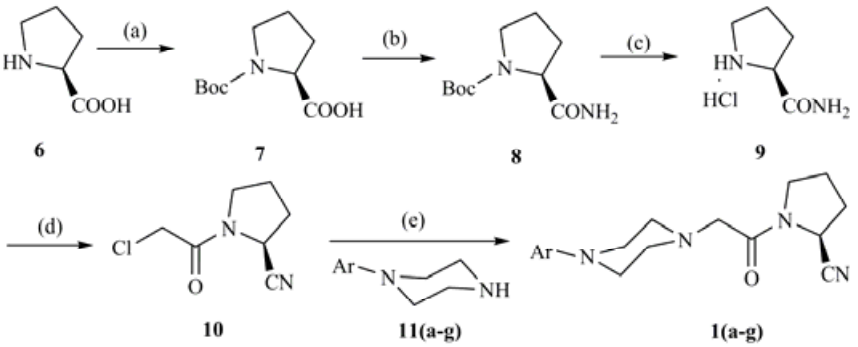
Supporting Information may be found in the online version of this article:

Oral Glucose Tolerance Test (OGTT) in streptozotocin-induced (STZ) diabetic rats (Fig. S1).

Docking Scores of compounds (Table S1)

Experimental details and characterization for the synthesized compounds.

Scheme 1 Synthetic route of targeted compounds **1(a-g).**



Reagents and conditions: (**a**) $(Boc)_2O$, NaOH, Dioxane: water (1:1), 0°C-RT, 4 hrs; (**b**) NMM, Isobutyl chloroformate, aq. NH_3, THF, -15°C; 30 Min.; (**c**) 4N HCl/dioxane, RT, 30 min; (**d**) (**i**) Chloroacetyl chloride, TEA, DMF, -15°C, 1 hr; (**ii**) Cyanuric chloride, 0°C-18°C, 50 min.; (**e**) TEA, THF, RT, 6-8 hrs; (**f**) TEA, THF or neat, RT or heat.

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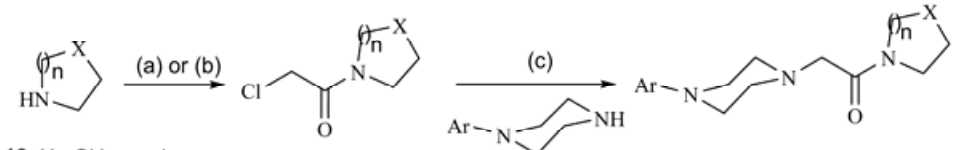
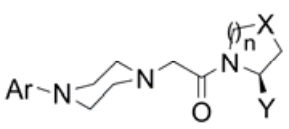
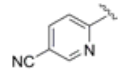
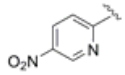
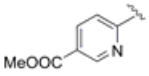
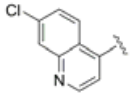
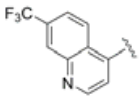
Scheme 2 Synthetic route of targeted compounds **2(a-i), 3(a-d), 4(a-g)** and **5(a-g).**



Reagents and conditions: (a) Chloroacetyl chloride, TEA, DCM, -15°C to 0°C, 2 hrs; (b) Chloroacetic acid, DCC, DCM, 0°C, 4 hrs; (c) TEA, THF, RT, 6-8 hrs.

Table 1. DPP-IV inhibitory Profile of Piperazine-derived Constrained DPP-IV inhibitors



Compounds	Ar	X	n	Y	% Inhibition* (IC_{50} in µM)
1a		CH_2	1	CN	34.60
1b		CH_2	1	CN	46.40
1c		CH_2	1	CN	NI
1d		CH_2	1	CN	NI
1e		CH_2	1	CN	3.94
1f		CH_2	1	CN	18.01
1g		CH_2	1	CN	NI
2a		CH_2	1	H	10.00
2b		CH_2	1	H	6.28
2c		CH_2	1	H	44.00

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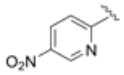
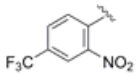
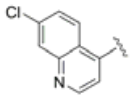
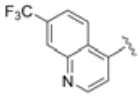
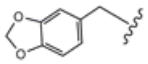
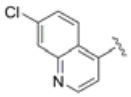
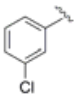
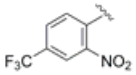
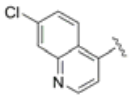
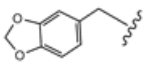
2d		CH_2	1	H	NI
2e		CH_2	1	H	4.26
2f		CH_2	1	H	3.19
2g		CH_2	1	H	80.40 (3.73)
2h		CH_2	1	H	11.94
2i		CH_2	1	H	4.64

Table-1 continued…

Compounds	Ar	X	n	Y	% Inhibition* (IC_{50} in µM)
3a		S	1	H	7.45
3b		S	1	H	78.90 (6.08)
3c		S	1	H	47.3
3d		S	1	H	NI
4a		CH_2	2	H	8.90
4b		CH_2	2	H	8.00
4c		CH_2	2	H	72.90 (6.06)
4d		CH_2	2	H	23.98
4e		CH_2	2	H	15.88
4f		CH_2	2	H	5.5
5a		O	2	H	36.00

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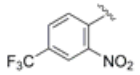
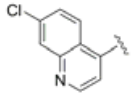
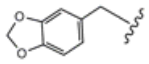
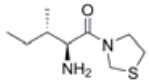
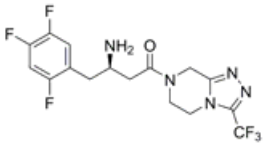
5b		O	2	H	19.50
5c		O	2	H	16.9
5d		O	2	H	0.10
5e		O	2	H	75.40 (5.96)
5f		O	2	H	23.9
P32/98**		-	-	-	76.43
Sitagliptin**		-	-	-	(18 nM)

NI denotesNo inhibition; *DPP-IV assay was done at 10μM; **Reference compound.

Table 2 Antihyperglycemic and antidyslipidemic activity in *db/db* mice (% efficacy, 10 and 15 days)

Compounds	**Antihyperglycemic**		**Antidyslipidemic**				**Insulin resistant reversal**		
	10 days	15 days	TG	CHOL	LDL	HDL	Fasting blood glucose	Serum insulin level	HOMO-index
2g	14.6	26.4	14.1	39.1	36.8	37.1	29.9	10.1	29.8
4c	11.8	19.4	11.1	13.7	27.5	28.2	16.0	6.08	21.1
Sitagliptin	21.5	29.5	8.83	14.2	35.1	38.3	27.7	14.2	38.0

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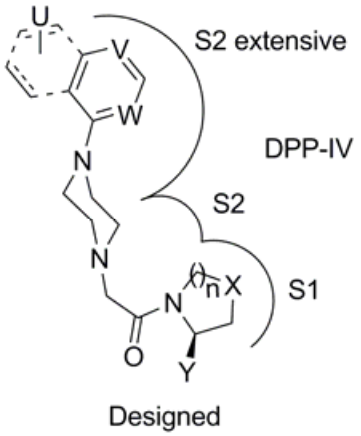
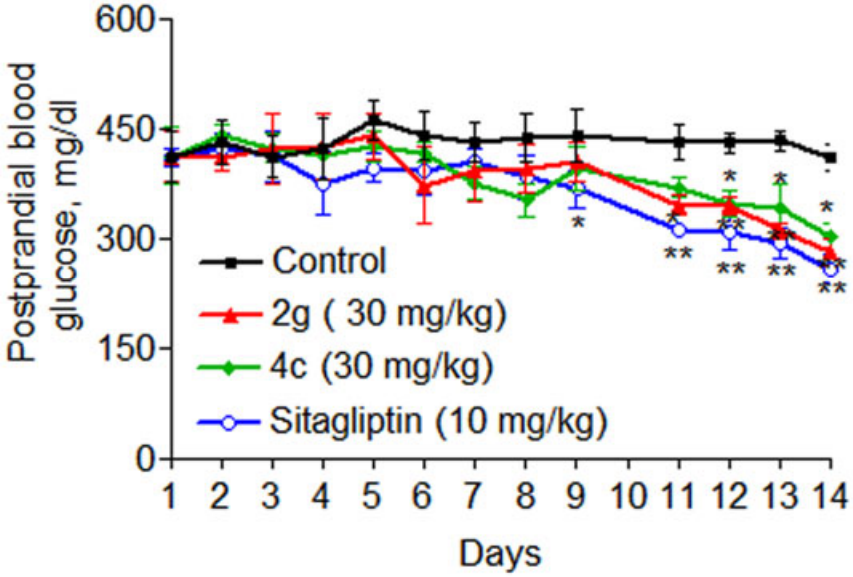


1, X= CH_2, Y= CN, n= 1;
2, X= CH_2, Y= H, n= 1;
3, X= S, Y= H, n= 1;
4, X= CH_2, Y= CN, n=2;
5, X= O, Y= H, n= 2;



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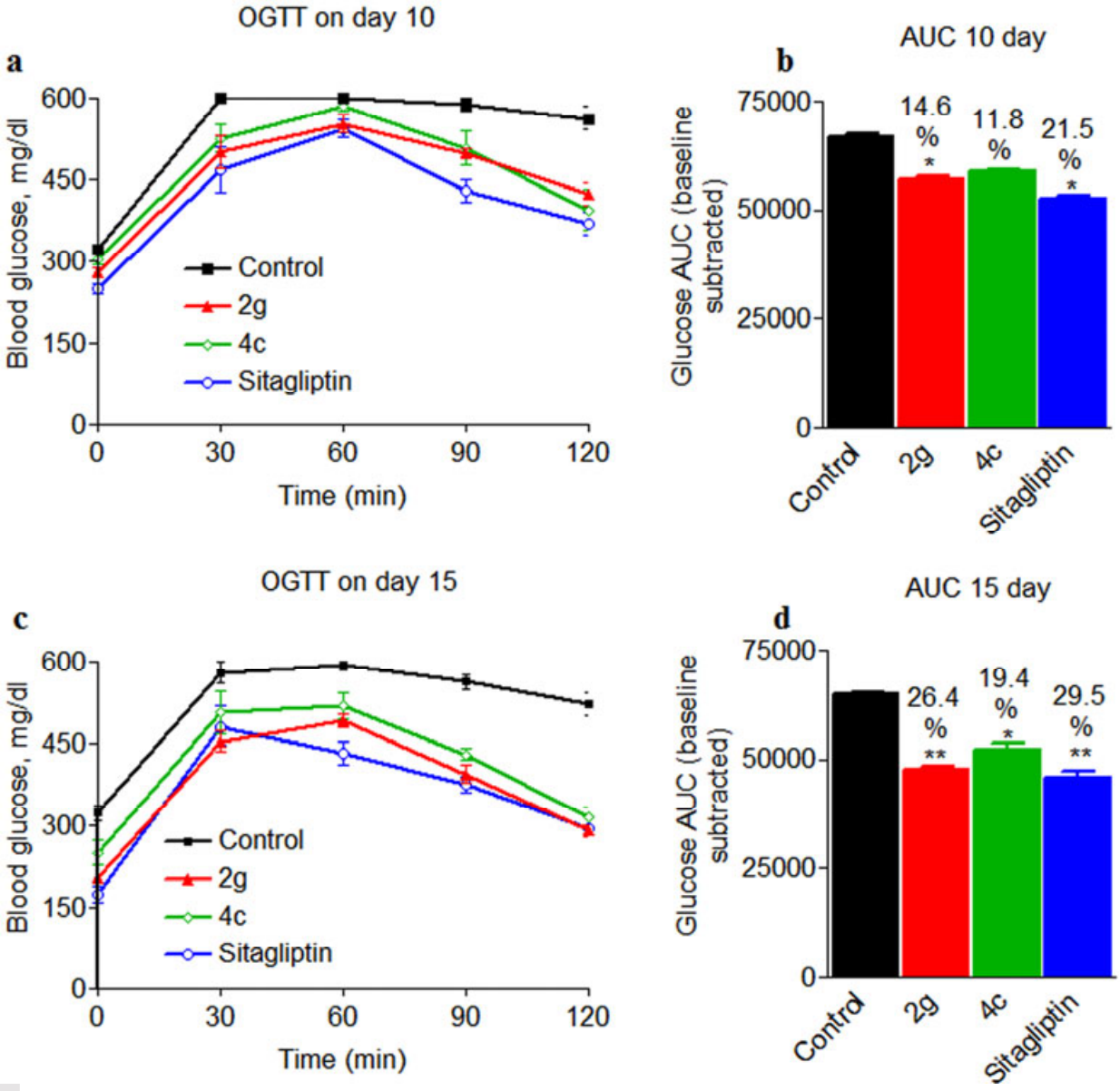

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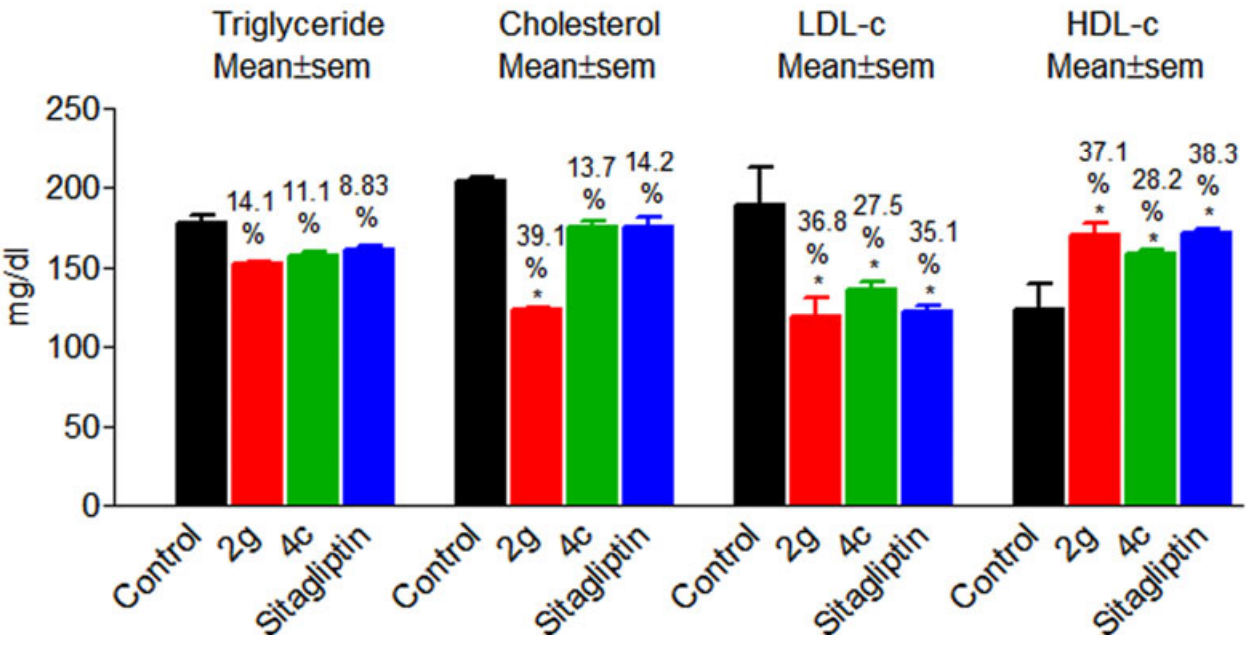

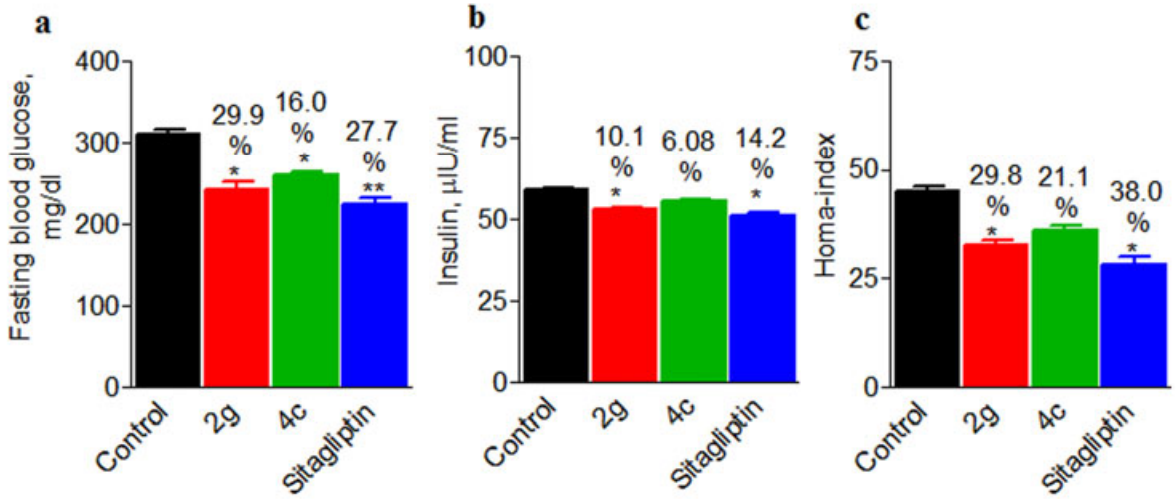

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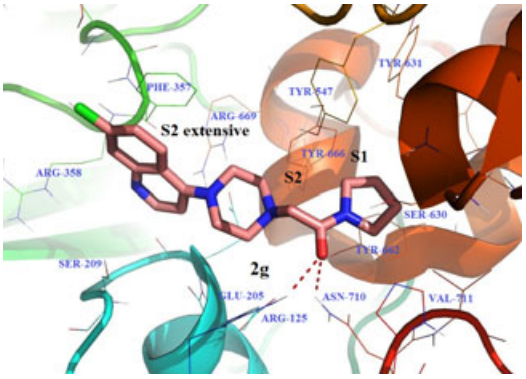

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