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ARTICLE TYPE

# Sulfonamide-1,3,5-triazine-thiazoles: Discovery of novel class of antidiabetic agent *via* inhibition of DPP-4

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**Dipeptidyl peptidase-4 (DPP-4) inhibitors are a novel class of antidiabetic drugs used for treating type 2 diabetes mellitus. In the present study, a novel hybrid sulphonamide 1,3,5-triazine thiazole derivatives were synthesized and characterized by $^{1}$H-NMR, $^{13}$C-NMR, FT-IR, mass and elemental analysis. The result showed that, among the tested compound 8c was found as more potent inhibitor of DPP-4 (2.32nM) than alogliptin as a standard. Moreover, molecular docking results showed that, ligand 8c was efficiently docked into the active site of the catalytic triad of Ser630, Asp708 and His740 encompassing both S1 and S2 pocket with CDOCKER interaction energy of 57.80. The in-vitro results were further substantiated by *in-vivo* blood glucose lowering effect in experimental subject. The results of the investigation showed that, compound 8c exhibit concentration-dependent improvement of glucose tolerance in ICR after oral administration. It has been also found that, compound 8c (30mg/kg) showed reduction in the area under the curve from 0 to 120 min (AUC) 0–120 min to 37.46%, which found approximately similar to the hypoglycemic profile of alogliptin (Standard). The activity of compound 8c was also investigated in STZ-induced diabetic rats, where it showed dose-dependent decrease in blood glucose level together with improvement in insulin level probably via inhibition of DPP-4. The effect of compound 8c was also investigated on lipid profile and antioxidant enzyme system.**

## Introduction

Diabetes mellitus (DM) is considered as a chronic metabolic disorder which either results from the impaired secretion of insulin from pancreas or inadequate utilization of the insulin by the body, or both [1]. It is considered as a major public health concern across the world, affecting nearly 285 million people and expected to rise to 439 million people by 2030 [2]. Most of the DM patients are categorized as Non-insulin-dependent diabetes mellitus (NIDDM) due to resistance to insulin action, improper secretion of insulin and excessive production of hepatic glucose [3,4]. As a result, currently several drugs that are used to maintain the DM condition is often associated with hyperglycemia [5]. The efficacy of these drugs has been seriously compromised owing to various side effects, such as, hepato-toxicity, abdominal pain, flatulence, diarrhea, and hyperglycemia [6]. Therefore, the discovery of new antidiabetic agent with improved efficacy has been much sought to provide better relief. In this regard, the therapy of Glucagon-like peptide-1 (GLP-1) has emerged as a promising treatment for type 2 diabetes [7]. The incretin hormone, GLP-1 causes stimulation of the biosynthesis and secretion of insulin in a glucose-dependent manner together with suppression of glucagon [8]. However, the active GLP-1 has been rapidly cleaved to its inactive form by the action of dipeptidyl peptidase 4 (DPP-4) resulting in alteration of insulin levels. Thus, inhibition of DPP-4 offers selective blood glucose lowering effect by increasing the level of active GLP-1 with improved patient compliance, such as, reduction in appetite [9,10].

Numerous DPP-4 inhibitors are used in clinical practice, including Sitagliptin [11], Vildagliptin [12] and Saxagliptin [13]. The omarigliptin (MK-3102), a sulfonamide based DPP-4 inhibitor developed by Merc proved to exert potent antidiabetic activity. The pharmacokinetic studies showed that, omarigliptin is suitable for once weekly dosing, which makes it unique among the other DPP-4 inhibitors [14]. Imeglimin is the first novel class of the - Glimins – belonging to the class of 1,3,5-triazine for the treatment of type 2 diabetes [15]. The drug has been acting via a unique mechanism of action targeting mitochondria bioenergetics, which in-turn improve mitochondrial function, resulting in the restoration of both glucose-dependent insulin secretion and insulin action [16]. Moreover, it can also be able to entirely protect the beta cells or endothelial cells from the death triggered by oxidative stress. 1,3,5-triazines also showed significant inhibition of DPP-4 activity in-vitro [17]. In a recent study, it has been found that thiazole derivatives showed DPP-4 inhibitory activity. Moreover, it also exhibits potent anti-diabetic activity in oral glucose tolerance test [18].

Based on the distinct and specific pharmacological advantages inherited with sulfonamide, thiazole and 1,3,5-triazine scaffolds, we intended to develop a hybrid skeleton of sulfonamide-1,3,5-triazine-thiazole in a search novel and potent DPP-4 inhibitor as antidiabetic agent, Figure 1.

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<Figure 1>

## Result and discussion

### *Chemistry*

The synthesis of the target hybrid compounds were achieved in excellent yield via facile synthetic route as shown in Scheme 1. Briefly, the commercially available starting materials cyanuric chloride (**1**) was allowed to react with ammonia gas under 0-5 °C to afford 4,6-dichloro-1,3,5-triazin-2-amine (**2**). Further, the mono-substituted 1,3,5-triazine derivative was further allowed to react with morpholine at 40-45 °C to afford 4-chloro-6-morpholino-1,3,5-triazin-2-amine (**3**). The resulting compound was then reacted with 4-amino-N-(thiazol-2-yl)benzenesulfonamide to afford 4-((4-amino-6-morpholino-1,3,5-triazin-2-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (**4**). The second part of the synthesis deals with the development of structural fragment needed for the induction of diversity to the core scaffold. This step corresponds to the preparation of N-substituted chloracetamides **7**(**a-j**) in excellent yield by nucleophilic substitution reaction of various distinguished amines **6** (**a-j**) with chloroacetyl chloride (**5**). The various substituted acetamides derivative (**7**) was further allowed to react with the previously synthesized 4-((4-amino-6-morpholino-1,3,5-triazin-2-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide (**4**) to afford corresponding final target derivatives **8** (**a-j**) in efficient manner. The structure of the synthesized target derivatives was ascertained by the FT-IR, $^{1}$H-NMR, $^{12}$C-NMR, mass and elemental analysis. FT-IR spectra of all derivatives **8**(**a-j**) were characterized by the appearance of a strong band at 3383-3398 $cm^{-1}$ attributed to N-H group. Furthermore, another band at 3078-3092 $cm^{-1}$ attributed to the stretching vibrations of the aromatic C-H group. Many strong absorption bands appear at 1714-1726 $cm^{-1}$ which confirm the existence of C=O group. The band corresponds to C=N triazine ring appears at 1608-1617 $cm^{-1}$. Moreover, the appearance of a strong band at 1621-1629 $cm^{-1}$ confirmed the presence of C=C group of aromatic ring. The $NO_2$ group of aromatic ring appears at 1524 $cm^{-1}$. Compound **8b** exhibited bands at 821 $cm^{-1}$ attributed to the stretching vibrations of C-Cl group. The $^{1}$H NMR spectrums of title compounds **8(a-j)** reveal a doublet corresponding to the phenyl ring at 7.47–7.27 ppm. The 1,3,5-triazine NH proton appear at 3.96 ppm. The resonance due to morpholine proton found in the range of 3.67-3.54 ppm. Further, the resonance at 3.16 ppm confirmed the presence of side chain methylene proton with a single peak of secondary amide appeared at 7.23 ppm. The thiazole ring proton appeared as two singlet peaks at 7.26-6.97 ppm. Finally, all the structure of synthesized derivatives **8** (**a-j**) was confirmed by elemental analysis and mass spectra.

### *DPP inhibitory activity*

The various synthesized derivatives were assessed for their potential inhibitory activity against numerous DPP enzymes, for instance, DPP-4, DPP-8 and DPP-9 and their inhibitory profile has been tabulated in Table 1. The compounds were designed in a manner to assess the effect of minor structural variation on the biological activity. The results of the inhibitory data depict that, entire set of compounds showed considerable inhibitory activity against DPP-4 in enzyme based in-vitro assay. Among the tested derivatives, compound **8c** was found more potent than alogliptin as a standard. Moreover, as seen in Table 1, none of the compound showed inhibitory activity against DPP-8 and DPP-9 suggesting that, designed molecules showed selective inhibition of DPP-4 in comparison of DPP-8 and DPP-9.

In the case of DPP-4 inhibition, compound **8a**, which has no substituent on the scaffold, showed least inhibitory activity than other tested derivatives. Whereas, on introducing the *p*-chloro, the inhibitory potency of the compound has been significantly improved (24.03nM). More surprisingly, the addition of fluoro (**8c**) in the position of chloro, displayed the most potent inhibition of DPP-4 (2.32nM) than rest of the analogues. In response of that, the next analogue **8d**, was synthesized having tri-fluoro methyl to assess the effect of additional fluoro group. The results showed that, introduction of additional fluoro causes mild drop in activity. The presence of non-halogen electron withdrawing *p*-nitro (**8e**) also revealed mild inhibitory activity. In next instance, the study was aimed to define the effect of electron donating group on the inhibitory potency (**8f**, **8g** and **8h**). Disappointingly, these groups do not influence the activity. As shown in Table 1, compound **8f**, **8g** and **8h** displayed a progressive decline in the activity exhibiting only moderate inhibition of DPP-4. The rest of compounds were synthesized to analyze the effect of additional aromaticity on the activity (**8i** and **8j**). The comparative inhibitory results revealed that, compound **8i** and **8j** showed mild to moderate inhibitory activity, i.e., 277.33nM and 392.45nM, respectively.

The structure-activity relationship studies suggest that, changes performed in the core skeleton have profound influence on the inhibitory activity. It has been clearly depicted that, compounds containing electron withdrawing group showed more pronounced inhibitory activity in comparison to the electron donating substituent. Moreover, it has been also found that, the presence of additional aromaticity does not influence the activity. These results suggest that, the receptor pocket is little enough to accommodate only small particle, not the bigger fragments, because of potential steric clashes.

In accordance with new FDA guidelines, the novel therapies for Type 2 DM should be devoid of any cardiovascular risk. Consequently, it is worthwhile to determine the effect of these novel inhibitors on the human Ether-à-go-go Related Gene (hERG) study. Thus, these constitutive compounds were analyzed for their ability to inhibit hERG ion channel. As shown in Table 1, none of the synthesized derivative exhibit inhibition of hERG ion channel at the tested dose. Results suggest that, these compounds have potentially devoid of cardiac side-effect.

<Table 1>

### *Docking analysis of compound 8c*

In order to explicate the key structural parameter vital for DPP-4 inhibition, a molecular docking of compound **8c** was carried out in the active site region of the DPP-4 (2FJP.pdb) enzyme by the CDOCKER module of Discovery Studio 2.5. Numerous structural analysis of the DPP-4 protein model suggested that, the enzyme has been broadly classified two various domains, in which one domain represented by eight-stranded β-propeller

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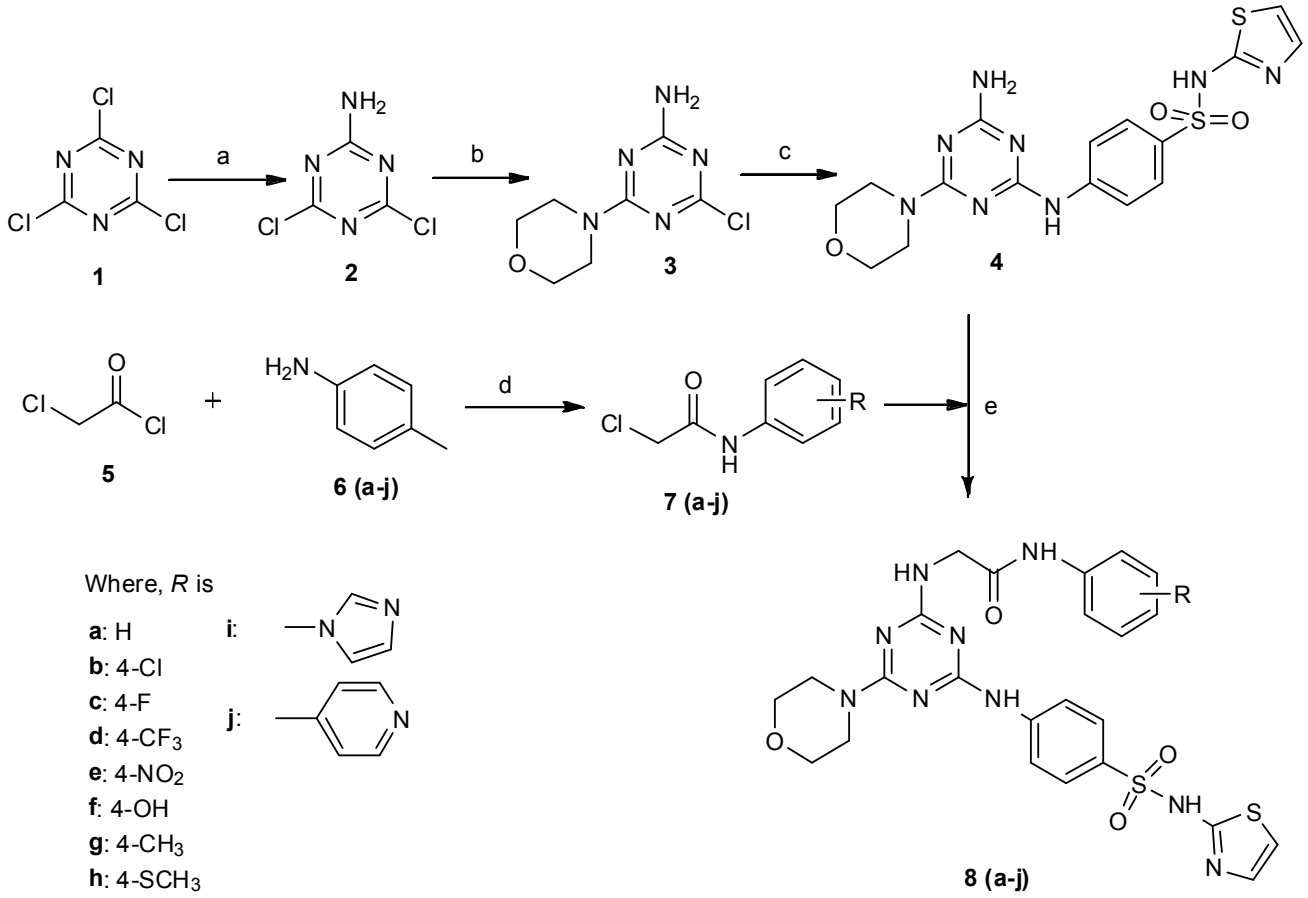
domain at the N-terminus. Whereas, the other domain consists of α/β-hydrolase at the C-terminus.

<Figure 2>

Particularly, the binding site of the DPP-4 enzyme has been divided into three specific regions, such as, the S1 pocket, the N-terminal recognition region, and last S2 pocket. Docking results showed that, ligand **8c** was efficiently docked in the active site of the catalytic triad of Ser630, Asp708 and His740 encompassing both S1 and S2 pocket showed in Figure 2. The morpholine fragment of the compound **8c** was deeply engulfed in the S1 pocket lined by with residues like Tyr631, Val656, Trp659, Tyr662, Tyr666 and Val711 which found adjacent to the catalytic triad of active site via formation of H-bond with Ser630. The thiazole sulphonamide fragment of ligand was found positioned deep in the N-terminal recognition region formed by Glu205, Glu206, and Tyr662 with the formation of two H-bond with Arg358. It has been also found that, terminal phenyl bearing fluoro interact with Tyr547 via formation non-covalent pi-pi interaction with S2 pocket. Moreover, the morpholine and 1,3,5-triazine revealed to create pi-sigma and pi-cation with Arg125 and Tyr662, respectively. In terms of scoring, compound **8c** showed CDOCKER interaction energy of 57.80. The results of the docking investigation, suggested that, compound 8c was able to bind efficiently with all three specific regions (the S1 pocket, the N-terminal recognition region, and last S2 pocket) of the DPP-4 enzyme active site and this might be the possible reason for its excellent inhibitory activity.




**Scheme 1:** Synthesis of target compounds 8 (a-j). Reagent and conditions: a) $NH_3$, 0-5 °C, 3h, $NaHCO_3$ b) Morpholine, 40-45 °C, 4 h, $NaHCO_3$ c) 4-amino-N-(thiazol-2-yl)benzenesulfonamide, pyridine, $K_2CO_3$, , reflux, 10h; d) NHR (a–j), triethyl amine, 1,4-dioxan, reflux, 6-8 h; e) pyridine, 1,4-dioxan, reflux 70–80 °C,9 h.

***In Vivo Pharmacological activity of Compound 8c***

Owing to excellent *in-vitro* potency and selectivity, compound **8c** (3, 10, and 30 mg/kg), Alogliptin (3 mg/kg) or vehicle (0.5% methylcellulose aqueous solution) was orally administered to 12 h-fasted ICR mice (n = 8 in each group) 30 min prior to an oral glucose challenge (2.5 g/kg). The blood glucose level of the mice was examined at definite interval of time, i.e., -30, 0, 30, 60 and 120 min. The results shown in Figure 3, indicated that, compound **8c** found to exhibit concentration-dependent improvement of glucose tolerance in ICR after oral administration. It has been also found that, compound **8c** (30mg/kg) showed reduction in area under the curve from 0 to 120 min $(AUC)_{0-120}$ min to 37.46%, which found approximately similar to the hypoglycemic profile of Alogliptin (Standard).

<Figure 3>

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**Antidiabetic Activity in STZ-induced diabetes in rats**

***Effect of Compound 8c on the blood glucose level and body weight***

The antidiabetic effect of compound **8c** has been presented in the Table 2. The results confirmed that, the STZ induced diabetic rats showed increase in the blood glucose level at the end of the experimental study, which was significantly reduced by the effect of compound **8c** in a dose-dependent manner. The maximum blood glucose reduction was observed in the **8c** group treated with 30 mg/kg. On the other hand, the glibenclamide also significantly causes decrease in the blood glucose at the end of the study. As shown in fig. 4, diabetic rat showed significant reduction in body weight of the rats on the 28$^{th}$ day, whereas, compound 8c treated rats showed significant improvement in body weight as compared to diabetic control.

<Table 2>

<Figure 4

***Effect of compound 8c on the plasma insulin***

The STZ treated group rats showed significant decline in the serum insulin as compared to the normal control group rats. Moreover, the STZ induced diabetic rat treated with different doses of **8c** significantly improved the plasma insulin level as compared to the diabetic control group rat (Table 2).

**Experimental**

The chemical used in the present study was procured from Sigma Aldrich (USA). The spectra of $^{1}$H NMR and $^{13}$C NMR were recorded on Bruker Avance 400 and Bruker Avance 500 Spectrophotometer, respectively. The chemical shifts are expressed in parts per million (ppm), and coupling constants are expressed in Hertz (Hz). The Low-resolution mass spectrum (MS) was recorded on a Waters ZQ LC/MS single quadrupole system equipped with an electrospray ionization (ESI) source. The elemental analysis of the final derivatives was performed via Vario Elemental analyser. The Thin-layer chromatography was performed on 0.25 mm Merck silica gel plates (60F-254) and visualized under UV light.

***General procedure for the synthesis of 4,6-dichloro-1,3,5-triazin-2-amine.* 2**

The synthesis of mono-substituted 1,3,5-triazine was performed in accordance with earlier reported procedure. The solution of 2,4,6-tri chloro-1,3,5-triazine (1) (0.1 mol) in 25 mL acetone maintain the temperature 0-5°C. Ammonia solution was added constantly to above solution and stirred for 3 h followed by addition of $NaHCO_3$. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products [19].

***General procedure for the synthesis of 4-chloro-6-morpholino-1,3,5-triazin-2-amine.* 3**

4,6-dichloro-1,3,5-triazin-2-amine (2) (0.1 mol) was added into 50 mL of acetone maintaining temperature 40–45°C. Morpholine was added constantly to above solution and stirred for 4 h followed by addition of $NaHCO_3$. The product was filtered and washed with cold water and recrystallized with ethanol to afford the corresponding pure product **3** [20].

***Effect of compound 8c on the lipid profile***

Effect of different doses of compound **8c** on lipid profile level was estimated at the end of the study and results have been shown in Table 2. The level of TC, TG, LDL and VLDL were found to be increased together with an increase in HDL level in STZ induced diabetic rat as compared to normal control group rats. Moreover, the oral administration of different doses of **8c** considerably causes reduction in TC, TG, LDL and VLDL level compared to the diabetic control rat group.Whereas, the level of HDL was found to be considerably increased in comparison to diabetic control rat.

***Effect of 8c on the antioxidant enzymes***

As shown in Table 3, the antioxidant activity of SOD, CAT, GPx and MDA in the normal and diabetic rat was investigated. The level of SOD, CAT, GPx was found to be decreased significantly together with an increase in MDA level in the STZ induced diabetic group rats. The oral administration of different doses of compound **8c** significantly improved the SOD, CAT, GPx and declined the MDA as compared to normal control group rats. The results of the investigation have confirmed the protective effect of compound **8c** in the diabetic rats via improvement in the level of various antioxidant enzymes.

<Table 3>

***General procedure for synthesis of 4-((4-amino-6-morpholino-1,3,5-triazin-2-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide.* 4**

The above synthesized compound **3** (0.1 mol), 4-amino-N-(thiazol-2-yl)benzenesulfonamide (0.1 mol), pyridine (0.1 mol) and $K_2CO_3$ (0.1mol) were taken in a round-bottomed flask, containing 100 mL1,4-dioxane, fitted with a reflux condenser. The content was refluxed for 10 h at 100-120°C. When the reaction was completed, the resulting solution was poured on crushed ice. The product was filtered off and washed several times with cold distilled water to free it from chloride. The product was recrystallized with alcohol to furnish **4**.

***General procedure for synthesis of N-substituted chloracetamides 7(a-j)***

Different aromatic amines **6**(**a-j**) (0.01 mol) were added in dry 1,4-dioxane (50 mL), maintaining temperature 0–5°C. The chloroacetyl chloride (**5**) (0.02 mol) and triethyl amine (0.01 mol) were added with stirring. These different reaction mixtures were, further, refluxed for 6 to 8 h and excess of solvent then distilled off. The resultant mixtures were poured onto crushed ice to afford compounds **7**(**a-j**).

***General procedure for synthesis of final title derivatives 8 (a-j)***

A solution of the N-substituted chloracetamides **7**(**a-j**) (0.01mol) in 1,4-dioxane (5mL) was added drop wise to a mixture of 4-((4-amino-6-morpholino-1,3,5-triazin-2-yl)amino)-N-(thiazol-2-yl)benzenesulfonamide. (**4**) (0.01 mol), pyridine (0.015 mol) and dioxane (10mL) with constant stirring at 0-5 °C for 60 min. The temperature was raised to 70 -80 °C and further stirred for 9 h. The mixture was then poured in to water (50mL) and the formed precipitate was filtered, washed with water and recrystallized with alcohol to furnish **8 (a-j)**.

*2-((4-morpholino-6-((4-(N-(thiazol-2-*

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*yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)-N-phenylacetamide* (8a)

Yield: 74%; MP: 221-223°C; MW: 567.64; $R_f$: 0.63; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3387 (N-H), 3078 (Ar C-H str), 2987 (ali C-H str), 1714 (C=O str), 1628 (C=C str), 1608 (C=N str), ), 1341 and 1165 (asymmetric and symetric $SO_2$ str), 1138 (aromatic C-Cstr), 989 (C-N str ), 781 $cm^{-1}$; $^1$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.12 (d, 2H, *J* = 7.9 Hz, ArH), 7.95 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.47 (d, 2H, *J* = 4.7 Hz, Ar-H), 7.27 (d, 2H, *J* = 4.6 Hz, ArH), 7.26 (s, 1H, Thiazole), 7.23 (s, 1H, Sec. Amide,), 7.06 (s, 1H, *J* = 1.2 Hz, Ar-H), 6.97 (s, 1H, Thiazole), 3.96 (s, 3H, Aromatic C-NH), 3.76 (s, 2H, Methylene), 3.67-3.54 (m, 8H, Morpholine) ; $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.5, 165.7, 142.2, 138.5, 137.1, 130.2, 129.8, 128.8, 128.1, 121.7, 113.9, 112.2, 66.4, 55.3, 48.7; Mass :568.62 (M+1); Elemental analysis for $C_{24}H_{25}N_9O_4S_2$: Calculated: C, 50.78; H, 4.44; N, 22.21; Found: C, 50.79; H, 4.44; N, 22.23.

*N-(4-chlorophenyl)-2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)acetamide* (8b)

Yield: 67%; MP: 243-245°C; MW: 602.09; $R_f$: 0.68; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3389 (N-H), 3076 (Ar C-H str), 2984 (ali C-H str), 1716 (C=O str), 1629 (C=C str), 1612 (C=N str), ), 1345 and 1162 (asymmetric and symetric $SO_2$ str), 1135 (aromatic C-Cstr), 992 (C-N str ), 821 (C-Cl str) $cm^{-1}$; $^1$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.14 (d, 2H, *J* = 7.8 Hz, ArH), 7.93 (d, 2H, *J* = 7.7 Hz, Ar-H), 7.74 (d, 2H, *J* = 5.4 Hz, Ar-H), 7.42 (d, 2H, *J* = 5.3 Hz, ArH), 7.22 (s, 1H, Thiazole), 7.24 (s, 1H, Sec. Amide,), 6.98 (s, 1H, Thiazole), 3.98 (s, 3H, Aromatic C-NH), 3.74 (s, 2H, Methylene), 3.69-3.52 (m, 8H, Morpholine); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.4, 171.8, 168.6, 165.7, 142.2, 137.1, 136.8, 133.4, 130.2, 129.7, 129.1, 120.5, 113.9, 112.2, 66.4, 55.2, 48.7; Mass :603.08 (M+1); Elemental analysis for $C_{24}H_{24}ClN_9O_4S_2$: Calculated: C, 47.88; H, 4.02; N, 20.94; Found: C, 47.89; H, 4.03; N, 20.94.

*N-(4-fluorophenyl)-2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)acetamide* (8c)

Yield: 78%; MP: 234-235°C; MW: 585.63; $R_f$: 0.72; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3383 (N-H), 3078 (Ar C-H str), 2986 (ali C-H str), 1714 (C=O str), 1627 (C=C str), 1615 (C=N str), ), 1347 and 1163 (asymmetric and symetric $SO_2$ str), 1157 (C-F str), 1138 (aromatic C-Cstr), 989 (C-N str ), cm $^{-1}$; $^1$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.12 (d, 2H, *J* = 7.9 Hz, ArH), 7.92 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.73 (d, 2H, *J* = 5.8 Hz, Ar-H), 7.27 (s, 1H, Sec. Amide), 7.24 (s, 1H, Thiazole), 7.08 (d, 2H, *J* = 5.2 Hz, ArH), 6.96 (s, 1H, Thiazole), 3.95 (s, 3H, Aromatic C-NH), 3.72 (s, 2H, Methylene), 3.67-3.51 (m, 8H, Morpholine); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.4, 171.8, 168.6, 165.9, 162.8, 142.2, 137.3, 134.2, 130.4, 129.8, 120.8, 115.8, 113.9, 112.2, 66.4, 55.3, 48.7; Mass :586.64 (M+1); Elemental analysis for $C_{24}H_{24}FN_9O_4S_2$: Calculated: C, 49.22; H, 4.13; N, 21.53; Found: C, 49.23; H, 4.12; N, 21.53.

*2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)-N-(4-(trifluoromethyl)phenyl)acetamide* (8d)

Yield: 72%; MP: 254-256°C; MW: 635.64; $R_f$: 0.62; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3387 (N-H), 3083 (Ar C-H str), 2989 (ali C-H str), 1719 (C=O str), 1624 (C=C str), 1612 (C=N str), ), 1349 and 1162 (asymmetric and symetric $SO_2$ str), 1254 ($CF_3$ str), 1141 (aromatic C-Cstr), 984 (C-N str ), cm $^{-1}$; $^1$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.09 (d, 2H, *J* = 7.8 Hz, ArH), 7.94 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.56 (d, 2H, *J* = 5.3 Hz, Ar-H), 7.21 (d, 2H, *J* = 5.1 Hz, ArH), 7.25 (s, 1H, Sec. Amide), 7.23 (s, 1H, Thiazole), 6.97 (s, 1H, Thiazole), 3.97 (s, 3H, Aromatic C-NH), 3.74 (s, 2H, Methylene), 3.69-3.49 (m, 8H, Morpholine); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.3, 171.8, 168.5, 165.8, 142.2, 141.9, 137.2, 132.4, 130.2, 129.8, 125.4, 124.2, 118.9, 113.9, 112.1, 66.4, 55.2, 48.7; Mass :636.65 (M+1); Elemental analysis for $C_{25}H_{24}F_3N_9O_4S_2$: Calculated: C, 47.24; H, 3.81; N, 19.83; Found: C, 47.25; H, 3.82; N, 19.81.

*2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)-N-(4-nitrophenyl)acetamide* (8e)

Yield: 77%; MP: 263-264°C; MW: 612.64; $R_f$: 0.72; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3392 (N-H), 3089 (Ar C-H str), 2984 (ali C-H str), 1723 (C=O str), 1627 (C=C str), 1614 (C=N str), 1524 ($NO_2$ str), 1345 and 1165 (asymmetric and symetric $SO_2$ str), 1146 (aromatic C-Cstr), 987 (C-N str ), cm $^{-1}$; $^1$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.22 (d, 2H, *J* = 7.9 Hz, ArH), 8.09 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.95 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.35 (d, 2H, *J* = 8.4 Hz, ArH), 7.27 (s, 1H, Sec. Amide), 7.21 (s, 1H, Thiazole), 6.95 (s, 1H, Thiazole), 3.94 (s, 3H, Aromatic C-NH), 3.76 (s, 2H, Methylene), 3.71-3.49 (m, 8H, Morpholine); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.6, 165.7, 144.6, 143.7, 142.2, 137.1, 130.2, 129.8, 124.2, 119.8, 113.7, 112.1, 66.3, 55.2, 48.7; Mass :613.67 (M+1); Elemental analysis for $C_{24}H_{24}N_{10}O_6S_2$: Calculated: C, 47.05; H, 3.95; N, 22.86; Found: C, 47.06; H, 3.94; N, 22.86.

*N-(4-hydroxyphenyl)-2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)acetamide* (8f)

Yield: 64%; MP: 251-252°C; MW: 583.64; $R_f$: 0.68; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3596 (OH str) 3398 (N-H), 3082 (Ar C-H str), 2989 (ali C-H str), 1714 (C=O str), 1621 (C=C str), 1617 (C=N str), 1348 and 1161 (asymmetric and symetric $SO_2$ str), 1149 (aromatic C-Cstr), 992 (C-N str ), cm $^{-1}$; $^1$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.14 (d, 2H, *J* = 7.8 Hz, ArH), 7.97 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.31 (d, 2H, *J* = 5.4 Hz, Ar-H), 7.28 (s, 1H, Sec. Amide), 7.24 (s, 1H, Thiazole), 6.98 (s, 1H, Thiazole), 6.67 (d, 2H, *J* = 5.2 Hz, ArH), 5.34 (s, 1H, Ar-OH), 3.97 (s, 3H, Aromatic C-NH), 3.78 (s, 2H, Methylene), 3.74-3.52 (m, 8H, Morpholine); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.5, 165.7, 154.2, 142.2, 137.1, 130.2, 129.8, 131.2, 123.1, 116.2, 113.9, 112.2, 66.4, 55.2, 48.7; Mass :584.65 (M+1); Elemental analysis for $C_{24}H_{25}N_9O_5S_2$: Calculated: C, 49.39; H, 4.32; N, 21.60; Found: C, 49.40; H, 4.31; N, 21.60.

*2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)-N-(p-tolyl)acetamide* (8g)

Yield: 81%; MP: 248-249°C; MW: 581.67; $R_f$: 0.72; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3394 (N-H), 3082 (Ar C-H str), 2982 (ali C-H str), 2924 ($CH_3$ str), 1721 (C=O str), 1623 (C=C str), 1612 (C=N str), 1343 and 1164 (asymmetric and symetric $SO_2$ str), 1151

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(aromatic C-Cstr), 994 (C-N str ), cm $^{-1}$; $^{1}$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.17 (d, 2H, *J* = 7.8 Hz, ArH), 7.94 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.18 (d, 2H, *J* = 5.1 Hz, Ar-H), 7.26 (s, 1H, Sec. Amide), 7.26 (s, 1H, Thiazole), 7.08 (d, 2H, *J* = 4.2 Hz, ArH), 6.93 (s, 1H, Thiazole), 3.95 (s, 3H, Aromatic C-NH), 3.76-3.49 (m, 8H, Morpholine), 3.73 (s, 2H, Methylene), 2.28 (s, 3H, Ar-$CH_3$); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.5, 165.7, 142.2, 137.1, 136.9, 135.5, 130.2, 129.6, 129.1, 121.5, 113.8, 112.1, 66.4, 55.2, 48.8, 21.4; Mass :582.68 (M+1); Elemental analysis for $C_{25}H_{27}N_9O_4S_2$: Calculated: C, 51.62; H, 4.68; N, 21.67; Found: C, 51.63; H, 4.68; N, 21.65.

*N-(4-(methylthio)phenyl)-2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)acetamide* (8h)

Yield: 78%; MP: 268-269°C; MW: 613.73; $R_f$: 0.61; FT-IR ($\nu_{max}$; cm$^{-1}$ KBr): 3397 (N-H), 3085 (Ar C-H str), 2984 (ali C-H str), 2934 ($CH_3$ str), 1726 (C=O str), 1629 (C=C str), 1614 (C=N str), 1348 and 1161 (asymmetric and symetric $SO_2$ str), 1154 (aromatic C-Cstr), 997 (C-N str ), cm $^{-1}$; $^{1}$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.14 (d, 2H, *J* = 7.9 Hz, ArH), 7.97 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.34 (d, 2H, *J* = 5.4 Hz, Ar-H), 7.28 (s, 1H, Sec. Amide), 7.24 (s, 1H, Thiazole), 7.14 (d, 2H, *J* = 5.2 Hz, ArH), 6.95 (s, 1H, Thiazole), 3.97 (s, 3H, Aromatic C-NH), 3.74-3.52 (m, 8H, Morpholine), 3.72 (s, 2H, Methylene), 2.48 (s, 3H, Ar-$SCH_3$); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.6, 165.8, 142.1, 137.2, 135.2, 134.8, 131.7, 130.2, 129.7, 120.5, 113.9, 112.1, 66.3, 55.2, 48.8, 14.8; Mass :614.73 (M+1); Elemental analysis for $C_{25}H_{27}N_9O_4S_3$: Calculated: C, 48.92; H, 4.43; N, 20.54; Found: C, 48.93; H, 4.43; N, 20.53.

*N-(4-(1H-imidazol-1-yl)phenyl)-2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)acetamide* (8i)

Yield: 58%; MP: 273-274°C; MW: 633.70; $R_f$: 0.68; FT-IR ($\nu_{max}$; cm$^{-1}$ KBr): 3394 (N-H), 3087 (Ar C-H str), 2981 (ali C-H str), 1723 (C=O str), 1627 (C=C str), 1612 (C=N str), 1345 and 1164 (asymmetric and symetric $SO_2$ str), 1157 (aromatic C-Cstr), 994 (C-N str ), cm $^{-1}$; $^{1}$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.22 (d, 2H, *J* = 7.9 Hz, ArH), 8.14 (s, 1H, Imidazole), 7.94 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.76 (d, 2H, *J* = 5.3 Hz, Ar-H), 7.67 (d, 1H, *J* = 1.9 Hz, Imidazole), 7.64 (d, 2H, *J* = 5.1 Hz, ArH), 7.27 (s, 1H, Thiazole), 7.25 (s, 1H, Sec. Amide), 7.02 (d, 1H, *J* = 1.4 Hz, Imidazole), 6.98 (s, 1H, Thiazole), 3.94 (s, 3H, Aromatic C-NH), 3.76-3.53 (m, 8H, Morpholine), 3.74 (s, 2H, Methylene); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.5, 165.4, 142.1, 138.2, 137.1, 135.5, 132.5, 130.3, 130.1, 129.7, 122.5, 118.3, 117.2, 113.9, 112.1, 66.4, 55.1, 48.9; Mass :634.70 (M+1); Elemental analysis for $C_{27}H_{27}N_{11}O_4S_2$: Calculated: C, 51.17; H, 4.29; N, 24.31; Found: C, 51.18; H, 4.30; N, 24.31.

*2-((4-morpholino-6-((4-(N-(thiazol-2-yl)sulfamoyl)phenyl)amino)-1,3,5-triazin-2-yl)amino)-N-(4-(pyridin-4-yl)phenyl)acetamide* (8j)

Yield: 64%; MP: 296-298°C; MW: 644.73; $R_f$: 0.57; FT-IR ($\nu_{max}$; cm$^{-1}$ KBr): 3397 (N-H), 3092 (Ar C-H str), 2985 (ali C-H str), 1719 (C=O str), 1623 (C=C str), 1615 (C=N str), 1343 and 1158 (asymmetric and symetric $SO_2$ str), 1159 (aromatic C-Cstr), 991 (C-N str ), cm $^{-1}$; $^{1}$H-NMR (400MHz, DMSO, TMS) δ ppm: 8.62 (d, 2H, *J* = 4.8 Hz,Pyridine), 8.18 (d, 2H, *J* = 7.9 Hz, ArH), 7.97 (d, 2H, *J* = 7.5 Hz, Ar-H), 7.71 (d, 2H, *J* = 4.8 Hz, Ar-H), 7.52 (d, 2H, *J* = 4.5 Hz,Pyridine), 7.48 (d, 2H, *J* = 4.3 Hz, ArH), 7.28 (s, 1H, Thiazole), 7.27 (s, 1H, Sec. Amide), 6.94 (s, 1H, Thiazole), 3.97 (s, 3H, Aromatic C-NH), 3.74-3.51 (m, 8H, Morpholine), 3.75 (s, 2H, Methylene); $^{13}$C-NMR (100MHz, $CDCl_3$) δ,ppm:176.2, 171.8, 168.5, 165.7, 149.6, 147.2, 142.1, 139.1, 137.8, 137.2, 130.1, 129.7, 127.4, 120.7, 119.6, 113.9, 112.2, 66.4, 55.3, 48.7; Mass :645.74 (M+1); Elemental analysis for $C_{29}H_{28}N_{10}O_4S_2$: Calculated: C, 54.02; H, 4.38; N, 21.73; Found: C, 54.03; H, 4.38; N, 21.72.

***In vitro assays for DPP-4, DPP-8 and DPP-9***

Briefly, the DPP-4 enzymes and chemicals were diluted in an assay buffer (50 mM Tris, pH 7.5 and 0.1% bovine serum albumin, pH 7.4). The above mixture was premixed and poured into the 96-well plates. The filled plates were incubated for 10min at room temperature in dark to provide the viable condition for enzyme/inhibitor reaction. The assay was started after the addition of 100 mmol/L of Gly-Pro-AMC (in assay buffer), which act as initiator to induce the enzymatic reaction. The mixture was further incubated for 20 min at room temperature. The liberation of aminomethylcoumarin (AMC) was quantified by microplate reader after the cleavage of Gly-Pro-AMC at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The experiments were performed in triplicates and mean of $IC_{50}$ values were calculated by a curve-fitting.

***DPP-8 and DPP-9 Inhibitory assay***

Recombinant Human DPP-8 (abcam) and DPP-9 (R&D systems) enzyme activity were performed according to manufacturer protocols. Briefly, each enzyme was diluted 1250× to a final volume of 50 µL in assay buffer (100 mM Tris-HCl, 100 mM NaCl, pH 7.8) and added to 96-well flat-bottom microtiter plates, followed by the addition of 10 µL inhibitor and 40 µL substrate (H-Gly-Pro-AMC, final concentration in the assay, 303 µM). The plates were incubated at 37 °C for 10 min. After incubation, fluorescence was measured using a microplate reader using the excitation 380 nm and emission 460 nm).

***Assay of Nonradioactive $Rb^+$ efflux***

This analysis was used to determine the ability to inhibit hERG potassium channel. Briefly, approximately fifty thousand HEK 293 cells were seeded into 96-well culture plates. The plates were incubated for 24 h at 37 °C and after incubation the medium was then discarded. The well of the plates were filled with open channel buffer (198mL) and 2 mL of test compound solution (stock solution: 30 nM to 300 mM) except for the control wells. The media of the plate was further replaced by a fresh mixture of 198 mL $Rb^+$ load buffer and 2 mL of test compound for incubation for 3 h at 37 °C. The resulting cell layers were then washed quickly in order to remove extracellular $Rb^+$. In next instance, to activate the hERG channel, the plates were refilled with a mixture of 198 mL channel opening buffer and 2 mL of test compound except for the control wells. After incubation for 5 min, the supernatant was carefully removed, collected and cells were lysed by the addition of 200 mL of lysis-buffer. The samples were analyzed on the Ion Channel Reader 8000.

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***Oral glucose tolerance test***

For this study, briefly male ICR mice aged 10 weeks (18–22 g) were procured from Institutional animal house facility. The mice were kept under optimal temperature and humidity conditions with a continuous 12 h/12 h light-dark cycle and had free access to laboratory food and water for 1 week before the experimental period. The male ICR mice were fasted overnight (12 h), weighted, and randomly categorised into groups (n = 8). Mice were dosed orally with vehicle (0.5% methylcellulose aqueous solution), alogtliptin (suspended in vehicle; 3 mg/kg) or compound 8c (suspended in vehicle; 3, 10 and 30 mg/kg) at -30 min. The blood samples were collected at -30 and 0 min by tail bleeding for the determination of glucose concentration. After that, glucose (20% aqueous glucose solution, 2.5 g/kg) was subsequently administered orally (at 0 min). Moreover, the additional blood samples were collected at 30, 60 and 120 min after glucose load for glucose determinations. The blood glucose was measured by automatic gluocometer.

***Antidiabetic Activity in STZ-induced diabetes***

*Animals*

Adult male Wistar rats weighing 150–250 g were used in this study. The animal experiments were processed following the internationally accepted ethical guidelines for the care of laboratory animals. They were fed with a standard pellet and water ad libitum and maintained at 22 °C temperature, and 12 h fasting cycle. Before the experiments, animals were fasted overnight but allowed free access to water.

*Induction of experimental diabetes*

The STZ was dissolved in saline immediately before use and injected intraperitonially (i.p.) in a single dose (40 mg/kg b.w.).

*Experimental Design*

After successful induction of diabetes, the animals were then randomly divided into seven groups and each group contain six rats.

Group I: normal control rats administered vehicle only
Group II: normal control rats administered 8c (30 mg/kg body weight)
Group III: diabetic control rats administered drinking water alone
Group IV: tested rats administered 8c (3 mg/kg body weight)
Group V: tested rats administered 8c (10 mg/kg body weight)
Group VI: tested rats administered 8c (30 mg/kg body weight)
Group VII: tested rats administered glibenclamide (10 mg/kg body weight)

Entire group rats received different doses of highly active compound 8c and glibenclamide using intragastric tube once daily for 28 days, continuously. The blood samples of each animal were collected from puncturing the retro-orbital plexus and were later preserved by using anticoagulating agents. The blood sample was then centrifuged at 4000 RPM for 15 min and used for analysis of various biochemical parameters. The plasma insulin level was assayed by the radio-immunoassay method. The serum glucose analysis of entire group rats was performed by glucose oxidase-peroxidase (GOD-POD) method using Glucose estimation kit. Other serum estimation was done by spectrophotometric method using standard kit following the manufacturer's instruction. The serum triglyceride, total cholesterol, HDL (High Density Lipoprotein) cholesterol were analyzed using standard kit. While the level of LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein) were estimated with the help of the following formulas.

LDL = TC/1.19 + TG/1.9 – HDL/1.1 – 38 (mg/dL)
VLDL = triglycerides (mg/dL) / 5

***Estimation of antioxidant enzymes***

For the estimation of the antioxidant enzymes, all groups rats' liver successfully removed and made homogenate. The liver homogenate was prepared with ice chilled 10% potassium chloride solution and it was used to measure the levels and activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and Malondialdehyde (MDA) by the method of Kumar et al., [21, 22].

*Statistical analysis and ethical approval*

Results are expressed as mean ± SEM. The significance of differences between the data was established by Student's t-test. P-values less than 0.05 were considered significant. The entire animal experiments have been duly approved by the Institutional Animal Ethical Committiee of Logistics University of Chinese People's Armed Police Force, China.

**Conclusion**

As a concluding remark, it has been corroborated from the present investigation that, entire set of the compound showed selective inhibition of DPP-4 than DPP-8 and DPP-9. These compounds also found devoid of any cardiac overactivity confirmed by the hERG ion channel activity, which can pose risk to its drug likeness ability. The most active compound **8c** showed in vivo DPP-4 inhibition accompanied with blood glucose lowering effect in experimental subject. It also found to posses favorable pharmacokinetic profile. It also showed improvement of blood glucose level in dose-dependent manner in STZ-induced diabetic rats via significant improvement of insulin level and antioxidant enzyme systems. The present study provides a novel series of potent DPP-4 inhibitor.

**Conflict of interest:** Authors declare no conflict of interest

**Notes and references**

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**List of Figures:**

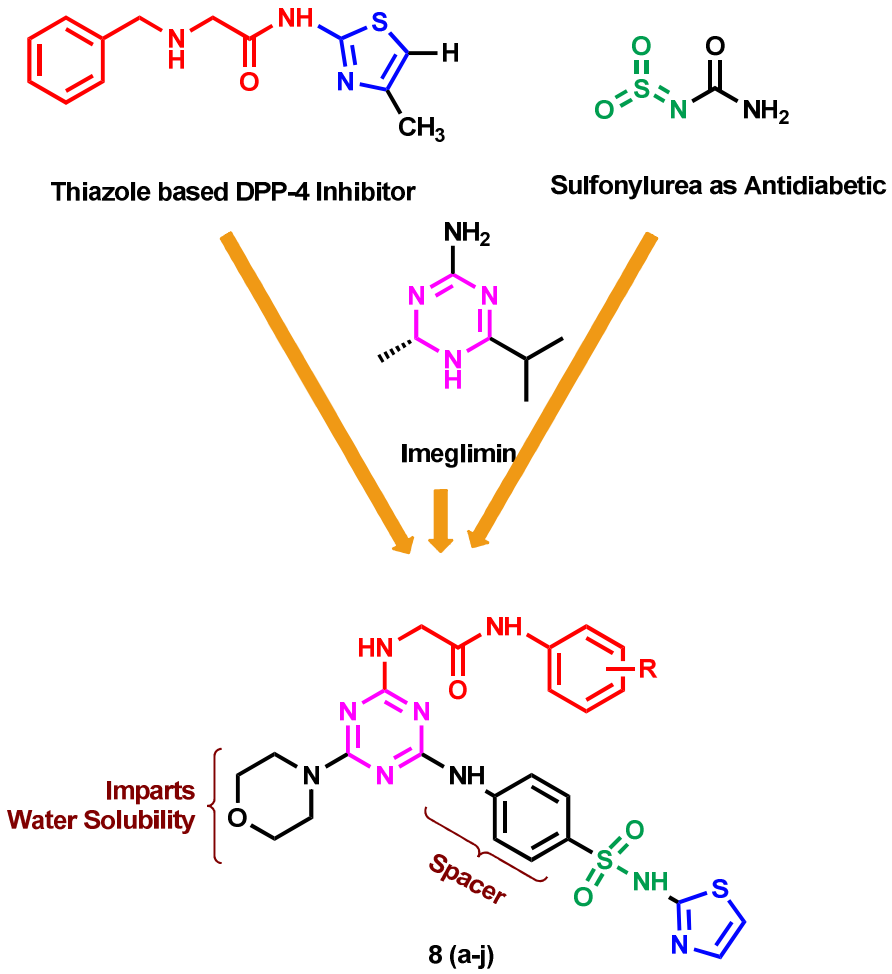


Figure 1: Design strategy of target compounds 8 (a-j)

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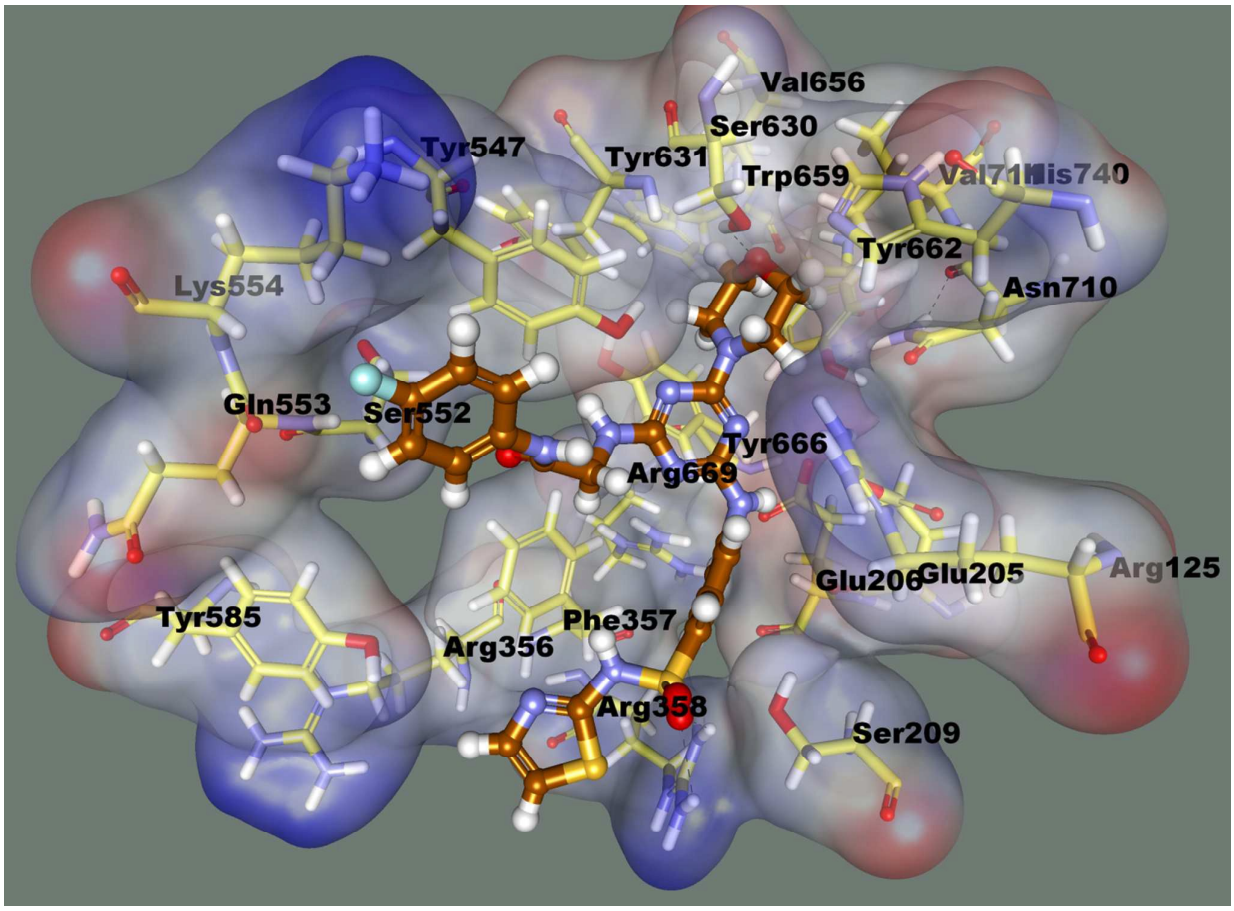


Figure 2: Docked orientation of compound 8c in the active site of DPP-4 enzyme.

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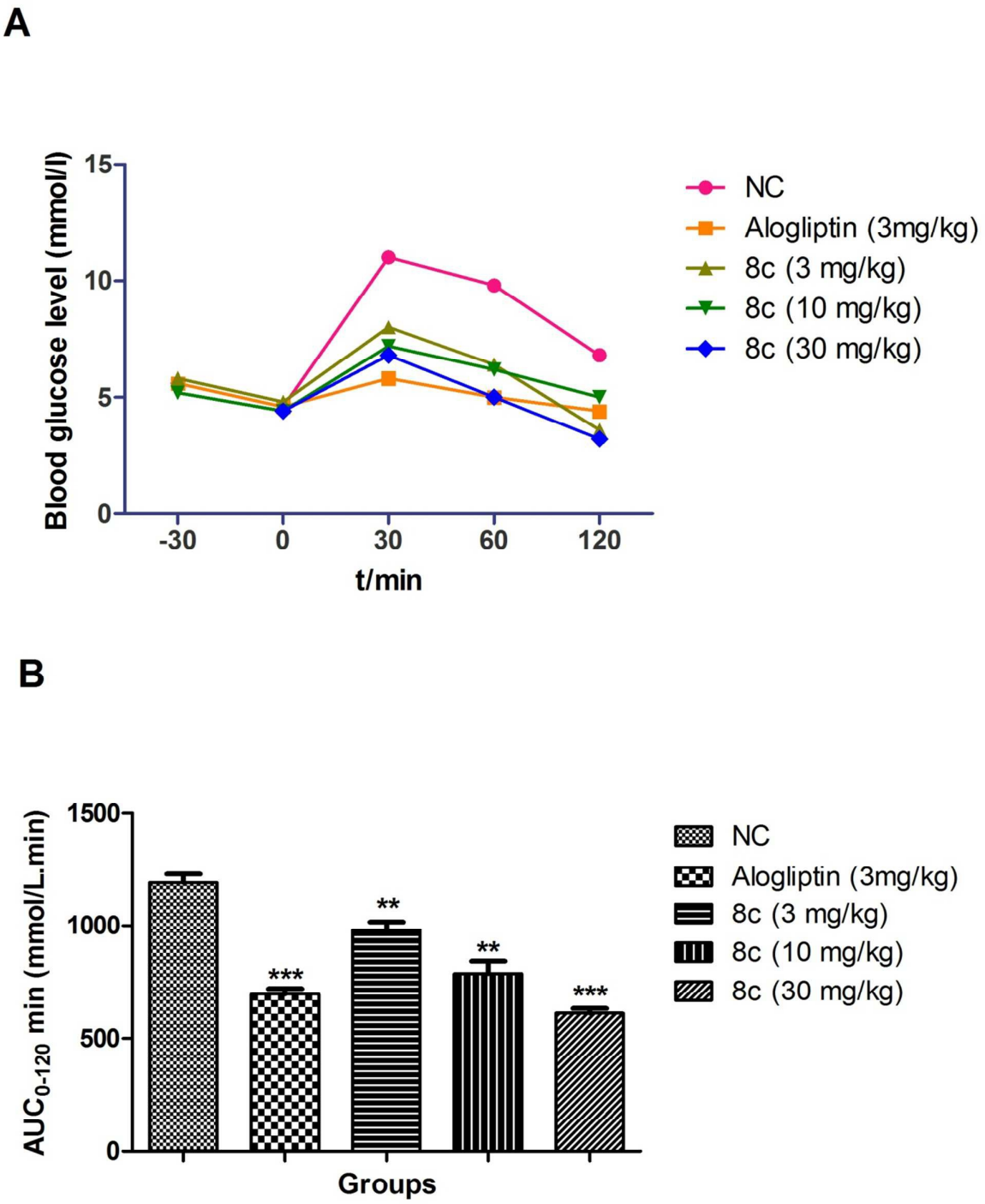


Figure 3: Effect of compound **8c** throughout an OGTT in male ICR mice. (A) time-dependent effect on blood glucose after oral administration of compounds, followed by 2.5 g/kg oral glucose challenge. Date in (B) represent $AUC_{0-120}$ min of blood glucose levels. Values are mean ± SEM (n = 8). **P≤0.01 compared to vehicle-treated ICR mice by Student's t test; ***P ≤0.001 compared to vehicle-treated ICR mice by Student's t test.

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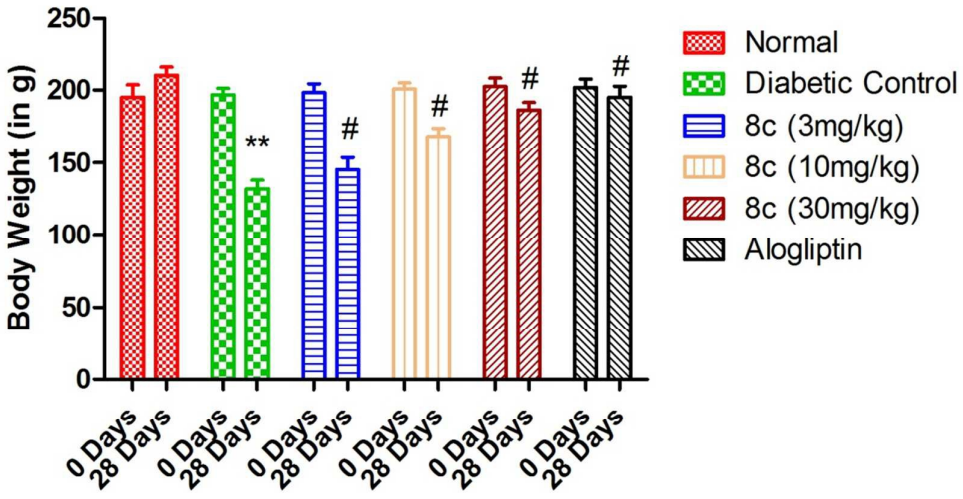


Figure 4 Effect of varying dose of compound 8c on the body weight of the normal and STZ induced diabetic rats. Values are expressed as Mean ± SD (n = 8 rats in each group). Statistical significance was evaluated by one-way analysis of variance (ANOVA). **p<0.05 as compared with normal rats, #p<0.05 as compared to diabetic control

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## List of Tables

**Table 1:** Inhibitory activities of target compounds against DPP-4, DPP-8, DPP-9, and hERG.

| Compound | DPP-4 $IC_{50}$ (nM) | DPP-8 $IC_{50}$ (nM) | DPP-9 $IC_{50}$ (nM) | hERG (μM) |
|---|---|---|---|---|
| 8a | 544.35 | >25,000 | >25,000 | NT |
| 8b | 24.03 | >25,000 | >25,000 | NT |
| 8c | 2.32 | >25,000 | >25,000 | 505.21 |
| 8d | 18.54 | >25,000 | >25,000 | 486.05 |
| 8e | 43.62 | >25,000 | >25,000 | 450.62 |
| 8f | 63.32 | >25,000 | >25,000 | 410.00 |
| 8g | 145.63 | >25,000 | >25,000 | 320.55 |
| 8h | 205.18 | >25,000 | >25,000 | 310.14 |
| 8i | 277.33 | >25,000 | >25,000 | 250.45 |
| 8j | 392.45 | >25,000 | >25,000 | NT |
| Aloglptin | 3.56 | - | - | >34 |

Where NA: Not available

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**Table 2:** Effect of compound **8c** on biochemical parameters in STZ-induced diabetic rats.

| S. No. | Biochemical parameter | Normal Control | Normal Control+8c (30 mg/kg) | STZ Control | STZ +8c (3 mg/kg) | STZ+8c (10 mg/kg) | STZ+8c (30 mg/kg) | STZ +Glibenclamide (10 mg/kg) |
|---|---|---|---|---|---|---|---|---|
| 1 | Fasting plasma glucose (mg/dL) | 82.1± 0.302 | 77.2± 0.365 | 365.3± 7.242[a] | 243.2± 4.34[b] | 187.3± 0.434[b] | 98.3±4 .439[b] | 111±4.5 84[b] |
| 2 | Fasting Plasma Insulin (µU/mL) | 12.1± 2.139 | 12.3± 2.344 | 2.8±0.9 33 [a] | 4.9±0.8 39 [b] | 7.2±0.3 82 [b] | 11.2±1 .328 [b] | 10.2±1.0 39 [b] |
| 3 | Hexokinase (µg/mg of tissue) | 154± 4.64 | 155.3 ±5.32 4 | 80.4±3. 103 [a] | 109.3± 3.432 [b] | 128±1. 839 [b] | 150.2± 1.327 [b] | 144.2±2. 123 [b] |
| 4 | Glucose-6-Phosphatase (unit/mg of tissue) | 8.1±0 .831 | 8.2±0. 802 | 14.9±0. 734 [a] | 12.9±0. 839 [b] | 11.8±0. 436 [b] | 8.6±0. 439 [b] | 9.6±0.43 5 [b] |
| 5 | Fructose-1-6-biphosphatase (unit/mg of tissue) | 27.2± 1.323 | 27.5± 1.928 | 59.3±3. 435[a] | 47±1.9 32 [b] | 40.2±1. 738 [b] | 33±0.3 25 [b] | 35.2±0.3 24 [b] |
| 6 | Total Cholesterol (mg/dL) | 66.4± 3.564 | 67±3. 454 | 141.5± 2.837[a] | 130.1± 1.932 [b] | 102.1± 2.321 [b] | 77.2±3 .901 [b] | 84;3±4.3 43 [b] |
| 7 | Triglycerides (mg/dL) | 78.1± 2.434 | 78.6± 2.839 | 145.1± 4.53[a] | 131.9± 2.843 [b] | 111±2. 344 [b] | 91.6±3 .545 [b] | 98.4±2.3 23 [b] |
| 8 | Total HDL Cholesterol (mg/dL) | 57.3± 2.343 | 57.1± 3.874 | 26.1±2. 432 [a] | 39.1±2. 432 [b] | 45.1±1. 343 [b] | 52.1±1 .023 [b] | 53.9±1.8 23 [b] |
| 9 | Total LDL Cholesterol (mg/dL) | 6.8±0 .323 | 6.9±0. 834 | 87.2±0. 383 [a] | 65.2±0. 432 [b] | 36.2±0. 938 [b] | 8.6±1. 928 [b] | 10.8±0.3 23 [b] |
| 10 | Total VLDL Cholesterol (mg/dL) | 15.8± 0.92 | 15.9± 0.324 | 29.1±0. 326 [a] | 26.4±0. 454 [b] | 22.1±0. 543 [b] | 18.5±0 .982 [b] | 10.8±0.3 23[b] |

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Values are given as mean±S.E.M. of six rats in each group. [a]($P$ <0.01) compared with the corresponding value for normal control animals (group I); [b]($P$ <0.01) compared with the corresponding value for diabetic control animals (group III); ns-non significant

**Table 3:** Effect of 8c on antioxidant marker in STZ-induced diabetic rats.

| S. No. | Antioxidant Parameters | Normal Control | Normal Control +8c (30 mg/kg) | STZ Control | STZ +8c (3 mg/kg) | STZ+8c (10 mg/kg) | STZ+8c (30 mg/kg) | STZ +Glibenclamide (10 mg/kg) |
|---|---|---|---|---|---|---|---|---|
| 1 | SOD (U/mg of protein) | 7.9±0.432 | 7.8±0.435 | 2.4±0.432[a] | 4.4±0.783[b] | 5.4±0.432[b] | 7.1±0.343[b] | 6.8±0.432[b] |
| 2 | CAT (U/mg of protein) | 72.7±2.43 | 70.8±1.43 | 38.6±1.930[a] | 45.8±1.068[b] | 53.8±1.158[b] | 68±1[b] | 66.1±1.434[b] |
| 3 | GPx (nmole/mg of protein) | 33.2±1.43 | 32.9±1.432 | 13.8±0.849[a] | 19.8±0.948[b] | 24.9±0.434[b] | 31.7±0.435[b] | 30.4±0.435[b] |
| 4 | MDA (nmole/mg of protein) | 0.22±0.014 | 0.24±0.008 | 0.59±0.014[a] | 0.43±0.011[b] | 0.34±0.012[b] | 0.28±0.003[b] | 0.29±0.006[b] |

Values are given as mean±S.E.M. of six rats in each group. [a]($P$ <0.01) compared with the corresponding value for normal control animals (group I); [b]($P$ <0.01) compared with the corresponding value for diabetic control animals (group III); ns-non significant.