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Design and Discovery of Novel 1,3,5-Triazines as Dipeptidyl Peptidase-4 Inhibitor against Diabetes

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Keywords
1,3,5-triazine · Sulfonamide · Dipeptidyl peptidase-4

Abstract
This study aims at synthesizing novel di-morpholine 1,3,5-triazine derivatives as antidiabetic agent via inhibition of dipeptidyl peptidase-4 (DPP-4). The molecules were developed via sequential nucleophilic reaction to afford target derivatives 5(a–f) and subsequently tested for inhibitory potency against DPP iso-enzymes, such as DPP-4, DPP-8, and DPP-9. The in vitro inhibition assay suggested that these derivatives prominently and selectively inhibit DPP-4 over DPP-8 and DPP-9. These molecules also showed no presence of cardiotoxicity, as confirmed by no activity against human Ether-à-go-go related gene channel. The study disclosed compound 5c as the most potent inhibitor of DPP-4 with IC_{50} of 1.10 nmol/L as compared to the standard. Compound 5c was further evaluated for oral glucose tolerance test (OGTT) and antidiabetic activity in ICR mice and Wistar rats, respectively. In OGTT, compound 5c showed dose-dependent improvement of glucose tolerance with a maximum at 30 mg/kg. It also showed reduction in area under the curve from 0 to 120 min, similar to alogliptin (standard). In Wistar rats, compound 5c causes reduction in the blood glucose level, total cholesterol, triglyceride, low density lipoprotein (LDL) and very LDL level as compared to the diabetic control group, whereas the level of high-density lipoprotein was found to be increased. Compound 5c causes improvement in antioxidant defense mechanism, as confirmed via improving superoxide dismutase, catalase, glutathione peroxidase and reducing the malondialdehyde level as compared to normal control group rats.

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Introduction

Among the different metabolic disorders that affect humans, type 2 diabetes mellitus (T2DM) is a serious concern for mankind and is responsible for elevating the blood glucose levels above normal for an extended time [1]. This results from the improper management of insulin, either due to impaired secretion of insulin from pancreas or inadequate utilization of insulin by the body, or both [2]. The raised blood sugar level causes many serious complications, which include ketoacidosis, hypoosmolar coma, cardiac diseases, stroke, non-functioning of kidney, ulcers of foot, and retinopathy [3]. Thus, if not treated at early stages, it may be fatal. According to International Diabetes Federation, it was estimated that diabetes alone results in 4.9 million deaths worldwide, making it the leading cause of morbidity and mortality among diseases of metabolic disorders [4].

Currently, the available therapeutics to manage type 2 diabetes are based on the increasing availability of insulin (via enhancing insulin secretion, or by direct administration), increasing insulin sensitivity, delaying absorption of carbohydrate from gastrointestinal tract, and increasing the secretion of glucose in urine [5–7]. As a matter of fact, agents acting by these mechanisms are associated with serious limitations or side-effects which compromise their clinical utility [8]. In recent times, agents belonging to newer therapeutic class are widely researched. Among such agents, glucagon-like peptide-1-based therapies (e.g., dipeptidyl peptidase-4 [DPP-4] inhibitors, glucagon-like peptide-1 receptor agonists) are widely effective in controlling the glucose level in the body [9, 10]. It exerts its effect via several mechanisms, such as induction of glucose-dependent insulin release, delayed gastric emptying time, decrease of postprandial glucagon [11, 12].

The mechanism of action of these agents is based on the inhibition of DPP-4 (also known as CD26) enzyme [13]. This enzyme is well-known for the catalytic degradation of incretins and can have a direct effect on the availability of insulin via enhancing its blood level [14]. Owing to excellent pharmacological activity, lack of cardiotoxicity and ease of administration, DPP4 inhibitors have a profound role in the antidiabetic therapeutic regime [15].

Derivatives originating from 1,3,5-triazine showed diverse pharmacological activities which include antibacterial [16, 17], antifungal [18], antimalarial [19], anticancer [20], and are also involved in cystic fibrosis [21]. Recent studies showed 1,3,5-triazine exerts excellent DPP-4 inhibition and profound antidiabetic activity [22, 23]. Gao et al. [22] showed that 1,3,5-triazine substituted with benzenesulfonamide-containing morpholine exerts excellent pharmacological benefit against diabetes and strong inhibition of DPP-4 with no cardiotoxicity. Encouraged by these results, we intended to synthesize more potent class of novel 1,3,5-triazines as potent DPP-4 inhibitors, with additional morpholine ring and after removal of phenyl bridge to connect sulfonamide with 1,3,5-triazine core.

Materials and Methods

General Procedure for the Synthesis of 4,4'-(6-Chloro-1,3, 5-Triazine-2,4-Diyl) Dimorpholine 3

2,4,6-Tri-chloro 1,3,5-triazine (1; 0.1 mol) was dissolved in 30 mL acetone, and morpholine (2; 0.2 mol) was added constantly in the above solution at 0–5°C for 1 h followed by drop-wise addition of $NaHCO_3$ solution (0.1 mol), taking care that reaction mixture does not become acidic. The temperature was raised to 40–45°C and stirred for 3 h. The reaction was monitored by TLC using benzene:ethyl acetate (9:1) as mobile phase. The product was filtered, washed with water, and recrystallized with ethanol to afford pure compound 3.

General Procedure for the Synthesis of 4,6-Dimorpholino-1,3, 5-Triazin-2-Amine 4

4,4'-(6-Chloro-1,3,5-triazine-2,4-diyl)dimorpholine (3; 0.01 mol), strong ammonia solution (0.01 mol) and K_2CO_3 (0.01 mol) were dissolved in 1,4-dioxane. The resulting mixture was refluxed for 8–9 h, and the reaction was monitored by TLC using benzene:ethyl acetate (9:1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by ethanol to afford the desired product 4.

Synthesis of 1,3,5-Triazine Benzenesulfonamide Hybrids 5a – 5f

4,6-Dimorpholino-1,3,5-triazin-2-amine (4; 0.1 mol) and various substituted phenyl sulfonyl chloride (a–f) and K_2CO_3 (0.1 mol) were added to 50 mL of 1,4-dioxane and the mixture refluxed at 110–115°C for 12–13 h. The product was filtered and washed with cold water and recrystallized with ethanol to afford the title compound 5 (a–f).

2,5-Dichloro-N-(4,6-Dimorpholino-1,3,5-Triazin-2-yl) Benzenesulfonamide (5a)

Yield: 81%; MP: 218–219 °C; MW: 475.35; R_f: 0.72; FT-IR (ν_{max}; cm^{-1} KBr): 3,394 (N-H), 3,059 (Ar C-H str), 1,631 (C=C str), 1,612 (C=N str), 1,532 (N-H bend), 1,349 and 1,172 (asymmetric and symmetric SO_2 str), 1,129 (aromatic C-C str), 984 (C-N str), 828 (C-Cl str) 643 (C-S str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 8.02 (d, 1H, J = 7.9 Hz, ArH), 7.74 (d, 1H, J = 7.2 Hz, ArH), 7.41 (d, 1H, J = 1.9 Hz, Ar-H), 3.97 (s, 1H, N-H), 3.74–3.67 (m, 16H, 4 × CH_2, Morpholine); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm:

183.6, 177.4, 141.2, 133.5, 132.6, 130.8, 129.4, 128.1, 66.8, 48.9; Mass: 476.38 (M + 1); Elemental analysis for $C_{17}H_{20}Cl_2N_6O_4S$: Calculated: C, 42.95; H, 4.24; N, 17.68; Found: C, 42.98; H, 4.26; N, 17.68.

2,4,6-Trichloro-N-(4,6-Dimorpholino-1,3,5-Triazin-2-yl) Benzenesulfonamide (5b)

Yield: 73%; MP: 228–230°C; MW: 509.79; R_f: 0.65; FT-IR (ν_{max}; cm^{-1} KBr): 3,397 (N-H), 3,068 (Ar C-H str), 1,629 (C=C str), 1,618 (C=N str), 1,539 (N-H bend), 1,342 and 1,176 (asymmetric and symmetric SO_2 str), 1,123 (aromatic C-C str), 985 (C-N str), 827 (C-Cl str) 638 (C-S str) cm^{-1}; ^{1}H-NMR (400MHz, DMSO, TMS) δ ppm: 7.74 (d, 2H, J = 2.4 Hz, ArH), 3.98 (s, 1H, N-H), 3.74–3.67 (m, 16H, 4 × CH_2, Morpholine); ^{13}C-NMR (100 MHz, CDCl3) δ ppm:183.5, 177.4, 140.4, 138.2, 134.3, 128.8, 66.8, 48.9; Mass: 510.78 (M + 1); Elemental analysis for $C_{17}H_{19}Cl_3N_6O_4S$: Calculated: C, 40.05; H, 3.76; N, 16.49; Found: C, 40.02; H, 3.78; N, 16.48.

N-(4,6-Dimorpholino-1,3,5-Triazin-2-yl)-4-Methoxybenzenesulfonamide (5c)

Yield: 76%; MP 194–196°C; MW: 436.49; R_f: 0.65; FT-IR (ν_{max}; cm^{-1} KBr): 3,392 (N-H), 3,062 (Ar C-H str), 2,987 (ali C-H str), 1,635 (C=C str), 1,611 (C=N str), 1,538 (N-H bend), 1,352 and 1,168 (asymmetric and symmetric SO_2 str), 1,134 (aromatic C- Cstr), 987 (C-N str), 648 (C-S str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 7.74 (d, 2H, J = 7.2 Hz, ArH), 7.12 (d, 2H, J = 4.6 Hz, ArH), 3.98 (s, 1H, N-H), 3.82 (s, 3H, OCH_3), 3.74–3.64 (m, 16H, 4 × CH_2, Morpholine); ^{13}C-NMR (100MHz, $CDCl_3$) δ ppm: 182.5, 177.9, 163.4, 132.1, 126.2, 114.8, 66.8, 55.9, 48.9; Mass: 437.51 (M + 1); Elemental analysis for $C_{18}H_{24}N_6O_5S$: Calculated: C, 49.53; H, 5.54; N, 19.25; Found: C, 49.56; H, 5.56; N, 19.24.

4-Bromo-N-(4,6-Dimorpholino-1,3,5-Triazin-2-yl) Benzenesulfonamide (5d)

Yield: 85%; MP 211–212°C; MW: 485.36; R_f: 0.69; FT-IR (ν_{max}; cm^{-1} KBr): 3,398 (N-H), 3,064 (Ar C-H str), 1,638 (C=C str), 1,619 (C=N str), 1,532 (N-H bend), 1,359 and 1,161 (asymmetric and symmetric SO_2 str), 1,137 (aromatic C-C str), 1,095 (C-Br str), 985 (C-N str), 652 (C-S str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 7.79 (d, 2H, J = 7.8 Hz, ArH), 7.57 (d, 2H, J = 4.9 Hz, ArH), 3.97 (s, 1H, N-H), 3.73–3.65 (m, 16H, 4 × CH_2, Morpholine); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 182.7, 177.2, 138.9, 131.8, 129.4, 126.5, 66.8, 48.9; Mass: 486.38 (M + 1); Elemental analysis for $C_{17}H_{21}BrN_6O_4S$: Calculated: C, 42.07; H, 4.36; N, 17.32; Found: C, 42.04; H, 4.38; N, 17.32.

N-(4,6-Dimorpholino-1,3,5-Triazin-2-yl)-4-Methylbenzenesulfonamide (5e)

Yield: 76%; MP 189–191°C; MW: 420.49; R_f: 0.78; FT-IR (ν_{max}; cm^{-1} KBr): 3,391 (N-H), 3,069 (Ar C-H str), 2,987 (ali C-H str), 2,939 (CH_3 str), 1,642 (C=C str), 1,614 (C=N str), 1,536 (N-H bend), 1,352 and 1,164 (asymmetric and symmetric SO_2 str), 1,132 (aromatic C-Cstr), 987 (C-N str), 659 (C-S str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 7.73 (d, 2H, J = 7.9 Hz, ArH), 7.38 (d, 2H, J = 4.6 Hz, ArH), 3.96 (s, 1H, N-H), 3.74–3.66 (m, 16H, 4 × CH_2, Morpholine), 2.32 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ, ppm: 182.6, 177.9, 137.8, 136.9, 129.4, 128.6, 66.5, 48.9, 21.6; Mass: 421.49 (M + 1); Elemental analysis for $C_{18}H_{24}N_6O_4S$: Calculated: C, 51.41; H, 5.75; N, 19.99; Found: C, 51.40; H, 5.78; N, 19.97.

4-Chloro-N-(4,6-Dimorpholino-1,3,5-Triazin-2-yl) Benzenesulfonamide (5f)

Yield: 85%; MP: 203–204°C; MW: 440.90; R_f: 0.77; FT-IR (ν_{max}; cm^{-1} KBr): 3,397 (N-H), 3,054 (Ar C-H str), 1,635 (C=C str), 1,618 (C=N str), 1,539 (N-H bend), 1,352 and 1,174 (asymmetric and symmetric SO_2 str), 1128 (aromatic C-Cstr), 989 (C-N str), 824 (C- Cl str) 648 (C-S str) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO, TMS) δ ppm: 7.82 (d, 2H, J = 7.8 Hz, ArH), 7.62 (d, 2H, J = 6.3 Hz, ArH), 3.98 (s, 1H, N-H), 3.72–3.65 (m, 16H, 4 × CH_2, Morpholine); ^{13}C- NMR (100 MHz, $CDCl_3$) δ ppm: 182.8, 177.2, 137.9, 137.4, 129.2, 128.9, 66.4, 48.9; Mass: 441.92 (M + 1); Elemental analysis for $C_{17}H_{21}ClN_6O_4S$: Calculated: C, 46.31; H, 4.80; N, 19.06; Found: C, 46.33; H, 4.82; N, 19.08.

Pharmacological Activity

In vitro Assays for DPP-4, DPP-8 and DPP-9

Briefly, the corresponding enzymes and associated chemicals were allowed to dilute in an assay buffer composed of 50 mmol/L Tris, pH 7.5 and 0.1% BSA at pH 7.4. The premixed sample was then placed in 96-well plates and then incubated for 10 min at room temperature in dark. In an assay buffer, the enzymatic reaction was initiated after adding 100 mmol/L of Gly-Pro-aminomethyl coumarin (AMC; in assay buffer) and allowed to incubate again for 20 min at room temperature. The microplate reader (ELx800 microplate reader, BioTek Instruments, Inc., Winooski, VT, USA) was used to estimate the amount of AMC released upon breakdown 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 the mean of IC_{50} values were calculated.

Assay of Nonradioactive Rb^+ Efflux

The present study was conducted to confirm the ability of designed analogs to inhibit the potassium channel. Approximately, 50,000 HEK cells were seeded into 96 well-plates and incubated for 24 h at 37 °C. The medium was then discarded after incubation. The open channel buffer (198 mL) along with the test compound solution (stock solution: 30 nmol/L to 300 mmol/L) loaded into well plates. The control wells received no test compound. The media of the plate was further replaced by a fresh mixture of Rb^+ load buffer and test compound for incubation for 3 h at 37 °C, and the resulting cell layers were washed to remove extracellular Rb^+. The plates were refilled with a mixture of channel opening buffer and test compound except for the control wells for activating the human Ether-à-go-go related gene (hERG) channel. After incubation for 5 min, the supernatant was carefully removed, collected, and the cells were lysed by the addition of lysis-buffer. The samples were analyzed on the Ion Channel Reader 8,000.

Oral Glucose Tolerance Test

The male ICR mice (10 weeks old, 18–22 g) were procured from Central animal house facility and housed 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 categorized 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. Subsequently, glucose (20% aqueous glucose solution, 2.5 g/kg) was 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 using 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 experiment, 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 intraperitoneally (i.p.) in a single dose (40 mg/kg b.w.).

Experimental Design. After the successful induction of diabetes, the animals were randomly divided into 7 groups and each group consisted of 6 rats.

Group I: normal control rats, vehicle only

Group II: normal control rats administered 5c (30 mg/kg body weight)

Group III: diabetic control rats administered drinking water alone

Group IV: 5c (3 mg/kg body weight)

Group V: 5c (10 mg/kg body weight)

Group VI: 5c (30 mg/kg body weight)

Group VII: glibenclamide (10 mg/kg body weight)

The 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 4,000 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 the group rats was performed by glucose oxidase-peroxidase method using Glucose estimation kit. Other serum estimation was done by spectrophotometric method using standard kit following the manufacturer's instruction. The serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol were analyzed using the standard kit. While the level of low-density lipoprotein (LDL) and very LDL (VLDL) were estimated with the help of the following formulae:

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

VLDL = TGs (mg/dL)/5

Estimation of Antioxidant Enzymes

For the estimation of antioxidant enzymes, the group rats' liver was 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 previously reported method [24–26].

Statistical Analysis and Ethical Approval

Results are expressed as mean ± SEM. The significance of differences between the data was established by Student t test. p values less than 0.05 were considered significant. All experimental designs and protocols were conducted according to the recommendations of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Jinan University Institutional Animal Care and Use Committee (Permit No. 2330056).

Results and Discussion

The synthesis of title derivatives 5 (a–f) were accomplished in 3 steps. In the first step, synthesis of *4,4'-(6-chloro-1,3,5-triazine-2,4-diyl)dimorpholine* (3) was achieved by the reaction of 2,4,6-trichloro 1,3,5-triazine with (1) morpholine (2) in the presences of acetone. In the second step, synthesis of *4,6-dimorpholino-1,3,5-triazin-2-amine* (4) was accomplished by nucleophilic substitution of the Cl atom of 1 with ammonia, in the presence of $NAHCO_3$ as a base. Whereas, synthesis of title compounds 5(a–f) were accomplished by reaction of *4,6-dimorpholino-1,3,5-triazin-2-amine* (4) with various substituted phenyl sulfonyl chloride derivatives (a–f) in the presence of 1,4 dioxane, as shown in Figure 1. The title compounds were synthesized in good yields. Furthermore, the structure of title compounds was confirmed by different spectroscopic analysis such as FT-IR, NMR and Mass spectrometry. The FT-IR spectra of title hybrid compounds 5(a–f) were found in the range 3,397–3,391 cm^{-1} which can be attributed to aromatic $N\text{-}H_{stretch}$. Whereas, aromatic $C\text{-}H_{stretch}$ was observed in the range of 3,069–3,054 cm^{-1}. Furthermore, $C{=}N_{stretch}$ was observed in the range of 1,619–1,611 cm^{-1}. Whereas, SO_2 group appeared in the range 1,359–1,342 cm^{-1}. But the other peak such as 828–824 was attributed to the Cl group. The methyl group appeared at 2,939 cm^{-1}. ^1H NMR spectra of the title compounds 5(a–f) showed a proton of phenyl moiety as doublets at the chemical shift range of 8.02–7.41 ppm. Furthermore, the aliphatic proton of the morpholine moiety appeared as multiplet in the range at 3.74–3.67 ppm. The methoxy group of the compound 5c appeared as singlet at 3.82 ppm. The aliphatic protons of methyl groups of compound 5e appeared as singlet at 2.32 ppm. The N-H group proton

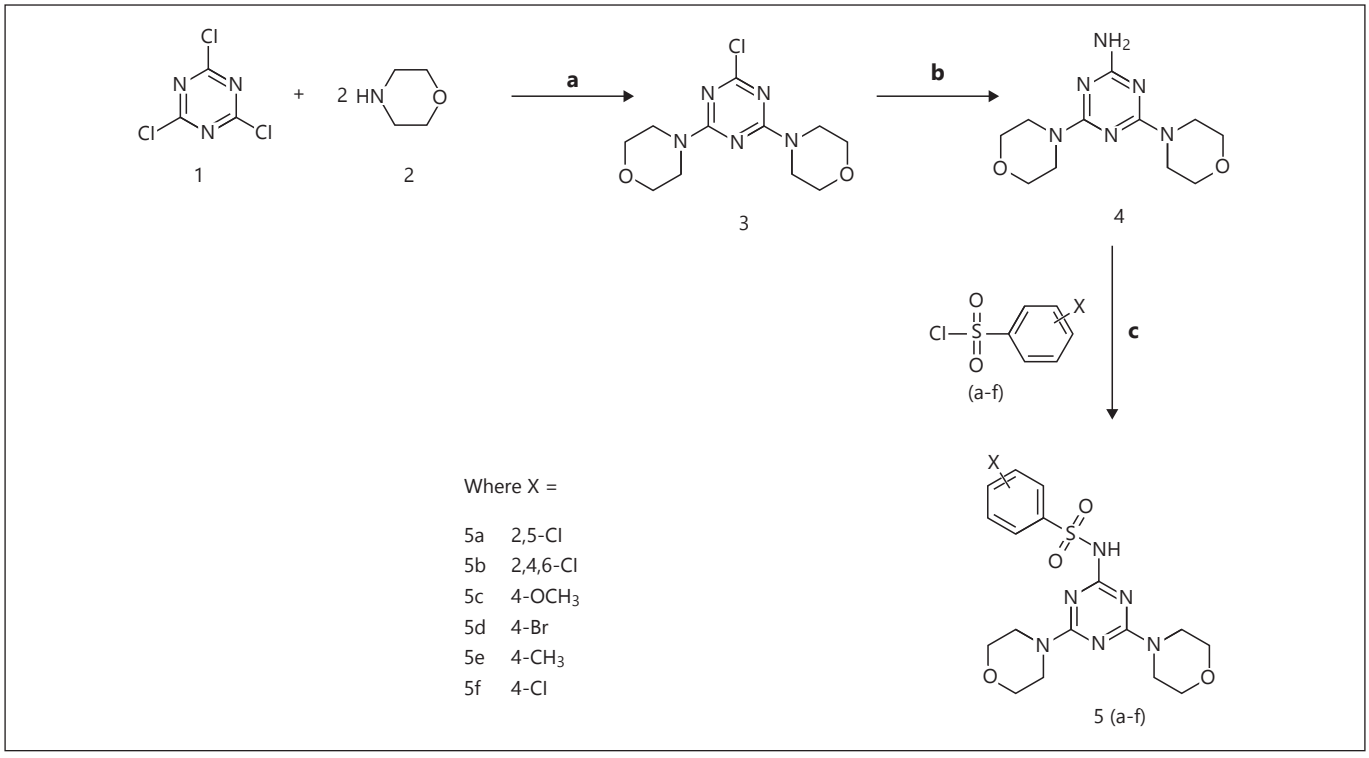

Fig. 1. Reagents and condition: (a–f) various substituted phenyl sulfonyl chloride (**a**) Acetone, stir 1 h at 0–5° C then stir 3 h at 40–45° C, $NaHCO_3$, (**b**) NH_3,K_2CO_3, 1,4 dioxane, reflux for 8–9 h, (**c**) 1,4 dioxane, reflux for 12–13 h at 110–115° C, K_2CO_3.

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

Compound	DPP-4 IC_{50}, nmol/L	DPP-8 IC_{50}, nmol/L	DPP-9 IC_{50}, nmol/L	hERG, μmol/L
5a	152.22	>25,000	>25,000	NA
5b	235.31	>25,000	>25,000	NA
5c	1.10	>25,000	>25,000	561.32
5d	27.11	>25,000	>25,000	NA
5e	10.32	>25,000	>25,000	350.25
5f	72.05	>25,000	>25,000	431.00
Aloglptin	3.22	–	–	>39

NA, not available; DPP, dipeptidyl peptidase; hERG, human ether-à-go-go related gene.

appeared at 3.96–3.98 ppm as singlet. Finally, the structure of the title compounds was confirmed by Mass spectrometry and elemental analysis.

DPP Inhibitory Activity

The synthesized derivatives were assessed for the estimation of inhibitory potential of various isoforms of DPP enzymes in an enzymatic assay. The inhibitory potential of compounds against DPP-4, DPP-8, and DPP-9 was provided in Table 1. The results illustrate that compounds showed considerable activity against DPP-4, not against DPP-8 and DPP-9 isoform. The compound 5c showed most potent inhibition of DPP-4 as compared to alogliptin as standard with IC_{50} of 1.10 nmol/L. In the next instance, compound 5e was found as second most potent molecule among the tested series. The activity was further lost on introduction of electron withdrawing group in the place of methyl, 5d. The activity was further decreased

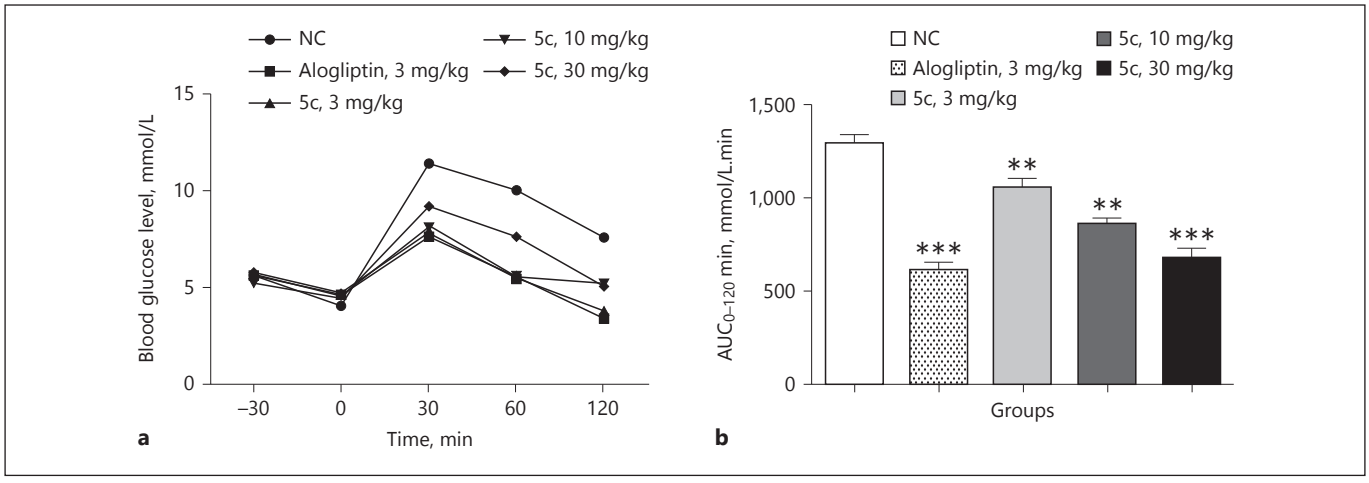

Fig. 2. Effect of compound 5c throughout an OGTT in male ICR mice. **a** Time-dependent effect on blood glucose after the oral administration of compounds, followed by 2.5 g/kg oral glucose challenge. Date in (**b**) represent AUC_{0-120} min of blood glucose levels. Values are mean ± SEM ($n = 8$). ** $p \leq 0.01$ compared to vehicle-treated ICR mice by Student t test; *** $p \leq 0.001$ compared to vehicle-treated ICR mice by Student t test. AUC_{0-120}, area under the curve from 0 to 120 min.

manifolds after replacing bromo with chloro, 5f against DPP-4. The introduction of additional chloro group on the aromatic moiety, di-chloro (5a) and tri-chloro (5b) showed least activity among the tested compounds, that is, 152.22 and 235.31 nmol/L, respectively. On closely inspecting the results, the structure-activity relationship studies suggest that the electron donating group have profound effect on the pharmacological activity, whereas the compounds containing electron withdrawing group have detrimental effect. These results suggest that the structural variation among the compounds have pronounced effect of DPP-4 inhibitory activity.

In the next instance, the entire set of compounds were tested for the effect on hERG, due to significant cardiovascular risk of newer T2DM therapies. This study was conducted based on the recommendation of FDA, which suggests that newer therapies of T2DM do not have any cardiovascular effects. The results, as presented in Table 1, confirmed that none of the compound showed inhibition of hERG ion channel at the tested dose.

In accordance with new FDA guidelines, the novel therapies for T2DM should be devoid of any cardiovascular risk. Consequently, it is worthwhile to determine the effect of these novel inhibitors on the 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. Thus, it could be suggested that these molecules might be devoid of any cardiovascular effects.

In vivo Pharmacology

The excellent in vitro DPP-4 inhibitory profile of compound 5c has prompted us to determine its antidiabetic effect in experimental animal (ICR mice). Thus, the hypoglycemic activity compound 5c was tested at numerous doses (3, 10, and 30 mg/kg) as compared to standard (alogliptin, 3 mg/kg) or vehicle (0.5% methylcellulose aqueous solution). These were administered orally to 12 h-fasted ICR mice ($n = 8$ in each group) 30 min prior to an oral glucose challenge (2.5 g/kg). At definite period of time interval, that is, –30, 0, 30, 60, and 120 min, the blood glucose of mice will be evaluated. Compound 5c showed dose-dependent improvement of glucose tolerance with a maximum at 30 mg/kg. Compound 5c at 30 mg/kg also showed reduction in the area under the curve from 0 to 120 min in a similar fashion to alogliptin (standard; Fig. 2).

Antidiabetic Activity of 5c in STZ-Induced Diabetes in Rats

Effect on the Blood Glucose Level and Body Weight

The results, as presented in Table 2, confirmed that compound 5c causes reduction in the blood glucose level, which was found to be elevated at the end of the study in a concentration-dependent manner with maximum pharmacological effect at 30 mg/kg. The glibenclamide significantly causes decrease in the blood glucose at the end of the study. The next part of the study suggested that diabetic rats showed significant reduction in the body weight

Table 2. Effect of compound 5c on biochemical parameters in STZ-induced diabetic rats

S. no	Biochemical parameter	Normal control	Normal control + 5c (30 mg/kg)	STZ control	STZ + 5c (3 mg/kg)	STZ + 5c (10 mg/kg)	STZ + 5c (30 mg/kg)	STZ + glibenclamide (10 mg/kg)
1	Fasting plasma glucose, mg/dL	78.5±0.223	67.2±0.125	430.2±21.431[a]	322.4±12.10[b]	280.5±5.34[b]	195.5±10.49[b]	100.5±8.835[b]
2	Fasting plasma insulin, μU/mL	10.2±1.034	9.2±1.102	2.3±0.865[a]	3.2±0.341[b]	6.3±0.225[b]	9.1±1.003[b]	11.2±1.562[b]
3	Hexokinase (μg/mg of tissue)	140±6.20	135.2±11.762	70.3±2.551[a]	102.1±5.964[b]	120±1.606[b]	135.5±1.340[b]	142.2±3.340[b]
4	Glucose-6-phosphatase (unit/mg of tissue)	9.2±0.552	8.0±0.501	18.2±0.405[a]	7.3±0.361[b]	9.8±0.117[b]	10.2±01.271[b]	10.3±0.220[b]
5	Fructose-1-6-biphosphatase (unit/mg of tissue)	29.4±2.003	28.2±1.651	65.5±4.561[a]	55±2.634[b]	48.5±2.405[b]	33±0.325[b]	30.3±0.221[b]
6	TC, mg/dL	60.2±5.334	58.6±4.604	162.6±5.631[a]	140.5±2.450[b]	120.1±4.550[b]	102.6±8.980[b]	72.1±3.822[b]
7	TGs, mg/dL	70.3±2.346	72.3±1.563	152.2±5.326[a]	141.3±2.472[b]	130.2±4.206[b]	101.5±4.673[b]	95.5±4.451[b]
8	Total HDL cholesterol, mg/dL	60.3±2.343	45.4±2.326	20.2±3.412[a]	31.5±3.452[b]	38.2±2.814[b]	41.3±2.453[b]	50.6±2.403[b]
9	Total LDL cholesterol, mg/dL	6.5±0.221	6.3±0.457	62.4±0.442[a]	55.21±0.364[b]	51.4±0.524[b]	10.2±2.346[b]	8.4±0.112[b]
10	Total VLDL cholesterol, mg/dL	12.5±0.352	13.2±0.331	30.4±0.440[a]	28.2±0.226[b]	20.5±0.281[b]	16.3±0.472[b]	12.5±0.334[b]

Values are given as mean ± SEM of 6 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).
HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very LDL; ns, non significant; TC, total cholesterol; TG, triglyceride.

Fig. 3. Effect of varying dose of compound 5c on the body weight of 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. ** $p < 0.05$ as compared with normal rats, # $p < 0.05$ as compared to diabetic control.

at the end of the study, whereas compound 5c-treated rats showed significant improvement in body weight as compared to diabetic control due to antihyperglycemic and prevention of muscle wasting effect (Fig. 3).

Moreover, as shown in Table 2, compound 5c causes significant improvement in the plasma insulin level as compared to diabetic control. The level of TC, TG, LDL, and VLDL was found to increase together with a decrease in HDL level in STZ-induced diabetic rat as compared to normal control group rats. Moreover, the oral administration of different doses of 5c considerably causes reduction in elevated levels of TC, TG, LDL, and VLDL as compared to the diabetic control group, whereas the level of HDL was found to be considerably increased. Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular; thus, it is imperative to study the effect of compound 5c on the activity of SOD, CAT, GPx, and MDA in the normal and diabetic rat. The levels 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 5c significantly improved the SOD, CAT, GPx and declined the MDA as compared to normal control group rats (Table 3). Thus, according to the above results compound 5c might have a protective role in diabetic rats via improving the oxidative defense mechanism.

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)	8.2±0.905	8.2±0.361	2.6±0.645[a]	3.8±0.204[b]	4.9±0.492[b]	6.9±0.721[b]	7.2±0.333[b]
2	CAT (U/mg of protein)	75.2±3.340	76.3±3.251	29.1±2.336[a]	36.008±1.068[b]	43.3±2.346[b]	54.8±1.003[b]	59.4±2.324[b]
3	GPx (nmol/mg of protein)	30.5±1.402	29.03±1.342	10.2±0.224[a]	14.4±0.382[b]	18.7±0.434[b]	23.4±0.718[b]	28.5±0.642[b]
4	MDA (nmol/mg of protein)	0.20±0.014	0.27±0.008	0.67±0.014[a]	0.50±0.015[b]	0.39±0.064[b]	0.30±0.562[b]	0.29±0.034[b]

Values are given as mean ± SEM of 6 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).
CAT, catalase; GPx, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; ns, non significant.

Conclusion

This study provides a fresh insight into the development of novel DPP-4 inhibitor, which is devoid of any cardiac toxicity. The most active compound 5c showed in vivo DPP-4 inhibition accompanied with blood glucose lowering effect in the experimental subject. Compound 5c also showed improvement in the oxidative system of experimental rats for the beneficial effect against DM. Further studies are needed to get more insight into the development of novel and potent DPP-4 inhibitors against DM.

Disclosure Statement

Authors declare no conflict of interest.

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