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Research Note

Design, synthesis and anti-diabetic activity of piperazine sulphonamide derivatives as dipeptidyl peptidase-4 inhibitors

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Abstract

Type II diabetes (T2DM) is considered one of the most prevalent metabolic disorders in the world. It is known as insulin resistance and persistent hyperglycemia. Over the past decade, inhibition of the enzymatic dipeptidyl peptidase-4 (DPP-4) has indeed been demonstrated to be an efficient and safe intervention for type 2 diabetes. In order to develop innovative DPP-4 inhibitors, several in silico techniques including 3D-QSAR, molecular docking, *in-silico* toxicity has been performed to confirm a total of 18 novel piperazine and pyridine derivatives to be synthesized from many designed molecules. These molecules have indeed been docked onto the protein surface of the DPP-4 enzyme, and ADMET characteristics have also been generated *in silico*. The compounds were then developed and analysed using FT-IR. Then, these compounds were investigated for DPP-4 inhibition *in vitro*. The most promising compound 8h showed 27.32% inhibition at 10μmol L^{-1} concentration over DPP-4 so selected for further *in-vivo* anti-diabetic evaluation. Compound 8h decreased blood glucose excursion in a dose-dependent manner during the OGTT and STZ-induced glucose models in normal Albino Wistar rats. Low-dose streptozotocin-induced type 2 diabetes in Albino Wistar rats treated chronically for 21 days with compound 8h resulted in a reduction in serum glucose levels. This highlighted that 8h is a moderately strong and specific blockbuster molecule that can be structurally modified to boost its effectiveness and overall pharmacological profile as a DPP-4 inhibitor.

Keywords

dipeptidyl peptidase (DPP-4), type II diabetes mellitus, piperazine and pyridine derivatives, molecular docking, biological study

1. Introduction

Numerous variables contribute to the aetiology of diabetes, which represents among the most prevalent chronic illnesses in the globe. In 2013, The World Health Organization (WHO) reported that 347 million people have been identified with Type-II diabetes mellitus, with an unprecedented rise expected in the coming decade. (Schwehm et al. 2015). Type 2 diabetes mellitus (T2DM) is a metabolic condition that is projected to impact 366 million individuals globally by 2030 (Association 2014). To present, Dipeptidyl peptidase-4 (DPP-4) has been featured to be type 2 diabetes's most potential treatment target. The half-life of Glucagon-like peptide-1 (GLP-1) is only 1 to 2 minutes, and this is due to the serine peptidase dipeptidyl peptidase-4 (Deacon et al. 1995; Lambeir et al. 2003). Insulin synthesis and secretion from pancreatic cells can be stimulated by GLP-1, which is secreted in the presence of food availability by gastrointestinal endocrine cells (Drucker and Nauck 2006; Baggio and Drucker 2007).

Reducing DPP-4 levels has been linked to an increase in insulin secretion (Nauck et al. 1986). An increase in the production of incretins (GLP-1 and GIP) in response to a meal prevents the release of the hormone glucagon, which then in turn leads to a rise in insulin release and a fall in blood sugar levels (Glucagon-like Peptide-1, a New Hormone of the Entero-Insular Axis - PubMed 2022). Consequently, DPP-4 inhibitors have the potential to be anti-diabetic medications since they would increase the half-life of activated GLP-1 and extend the favorable impacts of such a incretin hormone on maintaining a normal blood sugar level. Several chemically synthesized DPP-4 inhibitors, including sitagliptin, vildagliptin, saxagliptin, alogliptin, and linagliptin, have shown promise as a new family of medications for the treatment of type 2 diabetes. Hypoglycemia is less likely to occur with DPP-4 inhibitors since their glucose-lowering impact is glucose-dependent (Ji et al. 2009). The trifluorophenyl subunit was among the sitagliptin-derived DPP-4 inhibitors found to occupy the S1 hydrophobic pocket, and the -amino group, that develops hydrogen bonding interactions with both the side chains of a tyrosine (Tyr662) and 2 glutamate residues (Glu205 and Glu206), was found to be crucial for their inhibitory potential against DPP-4 in a past analysis reporting their structure-activity relationship. Most of the contemporary work has been done to enhance the P2-binding component (the fused heterocyclic ring), but relatively limited work has been done to improve the trifluorophenyl moiety. We explain our hypothesis and verifiable evidence as stereochemical change of piperazine moiety in sitagliptin to pyridine and designed novel structures to increase the potency if methoxy and Fluro groups involved in piperazine and pyrdine moieties could inhibit DPP-4 enzyme more efficiently.

In the present study, Piperazine oxadiazole sulphonamide and pyridine oxadiazole compounds were designed, synthesised, and evaluated in this work as DPP-4 inhibitors. The ability of these compounds to inhibit DPP-4 was examined in vitro. Animal models of diabetes treatment included evaluations of *in-vivo* anti-diabetic activity.

2. Experimental procedure

2.1. Molecular docking studies

Docking study was performed on the DPP-4 protein and its co-crystallized ligand (PDB ID: 5Y7K) because their X-ray structures are extremely comparable. The protein had its water and cognate ligand removed next. ChemDraw 15.0 (Mills 2006) was used to create the structures of the desired compounds, and chimera 1.16 was used to decrease their energy (Ramachandran et al. 2011) . The correct protein and ligand pdbqt format were prepared using MGL Tools version 1.5.7 (http://mgltools.scripps.edu). Autodock 1.5.6 (Morris et al. 2009) was used for the docking analysis. The ligands were considered flexible, while the receptors were considered rigid. Kollman balanced out the partial charges of the atoms, polar hydrogens were incorporated into protein molecules. Utilizing the BIOVIA Discovery Studio 2021, protein-ligand relationships were investigated (Sharma et al. 2019).

2.2. Chemistry

Solvents and chemicals were obtained from institutional suppliers including Sigma Aldrich and Siscon (Southern-India scientific corporation). They were put to use before any additional cleaning was done. After drying on three or four molecular sieves, solvents were distilled. Analytical thin-layer chromatography (TLC) plates pre-coated with silica gel from Merck were used to evaluate reaction development (they lacked a fluorescent indicator, though). Lab Intelligence Appliances' SMP 203 electronic melting point device was used to get the results. The compounds' infrared spectra have been obtained using the KBr dispersion method on a Schimadzu spectrometer.

2.2.1. General methodology for synthesis of 4-ethyl-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8a–8i)

In a solution of 20 ml ethanol, aldehyde (1) of 0.03 mole and semi-carbazide (2) of 0.05 mole are added and stirred for 5–10 min with a solution of 10ml sodium acetate, under reflux for 3–4 hours. To this resulting intermediate compound (3), a mixture of 0.01mole sodium carbonate dissolved in water with potassium iodide 0.01 mole, was put to reflux for 2–3 hours. To this intermediate mixture (4) chlorosulfonic acid ($ClSO_3$) (5) of 0.01 mole with ethanol has been added and refluxed for 3–4 hours at a temperature of 60–70 °C. For the final resulting intermediate compound (6), 10% potassium hydroxide (KOH) 20 ml with ethanol of 20ml and 0.01mole of N- ethyl Piperazine (7) has been added and refluxed for 3–4 hours and the final product (8) has been washed, filtered and dried for further recrystallization. The TLC for the intermediate compounds and final products were checked for the progress of reaction where single spot is obtained with ethyl acetate and dichloromethane (DCM) solvent system of 4:6 ratio.

4-ethyl-N-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8a)
Yield: 45%; C14H19N5O4S; Yellow solid; m.p: 107–114 °C; Rf: 0.603446; FT-IR (KBr, cm-1): 3446 (-O-H Stretch),1597 (-C=O Stretch).

4-ethyl-N-(5-(4-hydroxy-3-methoxyphenyl)-1,3,4-oxadiazol-2-yl)piperazine-1-sulfonamide (8b)
Yield: 60.4%; C15H21N5O5S; Yellow solid; m.p: 100–110 °C; Rf: 0.51; FT-IR (KBr, cm-1):2081 (-C≡C Stretch), 1595 (-C=O Stretch).

4-ethyl-N-(5-phenyl-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8c)
Yield:50%; $C_{14}H_{19}N_5O_3S$; White solid; m.p: 103–115 °C; R_f: 0.40; FT-IR (KBr, cm^{-1}): 3454 (-O-H Stretch), 1589 (-C=O Stretch), 821 (C-Cl).

4-ethyl-N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8d)
Yield:70%; $C_{15}H_{21}N_5O_4S$; White solid; m.p: 110–118 °C; R_f: 0.30; FT-IR (KBr, cm^{-1}): 2924 (-C-H Stretch), 1745 (-C=O Stretch).

N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-4-ethylpiperazine-1-sulfonamide (8e)
Yield:65%; $C_{14}H_{18}ClN_5O_3S$; White solid; m.p: 105–112 °C; R_f : 0.52;FT-IR (KBr, cm^{-1}): 3514 3454 (-O-H Stretch), 1678 (-C=C Stretch), 1510 (-C=O Stretch).

4-ethyl-N-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8f)
Yield:50%; $C_{14}H_{18}N_6O_5S$; Yellow solid; m.p: 103–111 °C; R_f: 0.45; FT-IR (KBr, cm^{-1}):3479 (-O-H Stretch), 3064 (Ar-H Stretch), 1575 (-C=O Stretch).

N-(5-(2,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-4-4ethylpiperazine-1-sulfonamide (8g)
Yield:50%; $C_{16}H_{23}N_5O_5S$; Yellow solid; m.p: 108–113 °C; R_f: 0.50; FT-IR (KBr, cm^{-1}):3446 (-O-H Stretch),2441 (-C=C Stretch), 1573 (-C=C Stretch).

4-ethyl-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8h)
Yield:67%; $C_{15}H_{21}N_5O_3S$; White solid; m.p: 109–115 °C; R_f: 0.63; FT-IR (KBr, cm^{-1}):3456 (-O-H Stretch), 2920 (-C-H Stretch), 1595 (-C=O Stretch).

4-ethyl-N-(5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8i)
Yield:40%; $C_{14}H_{18}FN_5O_3S$; White solid; m.p: 109–114 °C; R_f: 0.55; FT-IR (KBr, cm^{-1}):3454 3456 (-O-H Stretch), 1899 (-C=O Stretch), 1589 (-C=O Stretch).

2.2.2. General procedure for synthesis of 1-(pyridin-4-yl)-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) methanimine (22a-22i)

A mixture of aldehyde 0.03 (18) mole and semi-carbazide (2) 0.05 mole with 20ml of ethanol along with 10ml of sodium acetate solution and refluxed for 3–4 hours. To the intermediate (19) obtained a mixture of sodium carbonate of 0.01 mole and potassium iodide of 0.01 mole dissolved in water has been refluxed for 3–4 hours. To the final intermediate (20) pyridine-4- carbaldehyde (21) 0.03 mol and ethanol of 20 ml were added and refluxed for 3–4 hours at 70°–80 °Celsius temperature. Then the final product (22) has been washed, filtered and dried for further recrystallization. The TLC for the intermediate compounds and final products were checked for the progress of reaction where single spot is obtained with ethyl acetate and dichloromethane (DCM) solvent system of 4:6 ratio.

(Z)-2-(5-((pyridin-4-ylmethylene) amino)-1,3,4-oxadiazol-2-yl) phenol (22a)
Yield:60.4%; $C_{14}H_{10}N_4O_2$; Yellow solid; m.p: 113–119 °C; R_f: 0.40; FT-IR (KBr, cm^{-1}):3514 (-O-H Stretch), 1899 (-C=O Stretch), 1589 (-C=O Stretch).

(Z)-2-methoxy-4-(5-((pyridin-4-ylmethylene) amino)-1,3,4-oxadiazol-2-yl) phenol (22b)
Yield:70.1%; $C_{15}H_{12}N_4O_3$; Yellow solid; m.p: 109–115 °C; R_f: 0.35; FT-IR (KBr, cm^{-1}): 3452 3514 (-O-H Stretch), 1647 (-C=C Stretch).

(Z)-N-(5-phenyl-1,3,4-oxadiazol-2-yl)-1-(pyridin-4-yl) methanimine (22c)
Yield:49.2%; $C_{14}H_{10}N_4O$; White solid; m.p: 110–115 °C; R_f: 0.50; FT-IR (KBr, cm^{-1}): 3446 (-O-H Stretch), 1678 (-C=C Stretch), 1597 (-C=O Stretch).

(Z)-N-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)-1-(pyridin-4-yl) methanimine (22d)
Yield:63.4%; $C_{15}H_{12}N_4O_2$; White solid; m.p: 115–118 °C; R_f: 0.50 ; FT-IR (KBr, cm^{-1}):3454 3446 (-O-H Stretch), 2987 (-C-H Stretch), 1587 (-C=O Stretch).

(Z)-N-(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)-1-(pyridin-4-yl) methanimine (22e)
Yield:75%; $C_{14}H_9ClN_4O$; White solid; m.p: 113–119 °C; R_f: 0.44; FT-IR (KBr, cm^{-1}): 3441 (-O-H Stretch), 2362 (-C≡C Stretch), 1593 (-C=O Stretch).

(Z)-N-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)-1-(pyridin-4-yl) methanimine (22f)
Yield:60.2%; $C_{14}H_9N_5O_3$; Yellow solid; m.p: 115–120 °C; R_f: 0.50; FT-IR (KBr, cm^{-1}): 3228 (-O-H Stretch),1575 (-C=O Stretch).

(Z)-N-(5-(2,5-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)-1-(pyridin-4-yl)methanimine (22g)
Yield:63.3%; $C_{16}H_{14}N_4O_3$; Yellow solid; m.p: 111–119 °C; R_f: 0.55; FT-IR (KBr, cm^{-1}): 3485 (-O-H Stretch), 3053 (Ar-H Stretch), 1563 (-C=O Stretch).

(Z)-N-(5-(4-fluorophenyl)-1,3,4- oxadiazol-2-yl)-1-(pyridin-4-yl)methanimine (22h)
Yield:73.5%; $C_{14}H_9FN_4O$; White solid; m.p: 109–116 °C; R_f: 0.42; FT-IR (KBr, cm^{-1}): 3061 (Ar-H Stretch),2113 (-C=C Stretch), 1595 (-C=O Stretch).

(Z)-1-(pyridin-4-yl)-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) methanimine (22i)
Yield:69.7%; $C_{15}H_{12}N_4O$; White solid; m.p: 115–120 °C; R_f: 0.48; FT-IR (KBr, cm^{-1}):3456 (-O-H Stretch),2362 (-C=C Stretch), 1591 (-C=O Stretch).

3. *In vitro* assay for the inhibition of dipeptidyl peptidase-4 (DPP-4)

The necessary reagents as well as compounds have been obtained from a major supplier (Sigma Aldrich). In Dimethyl Sulfoxide, solution of 4-ethyl-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8a–8i) and 1-(pyridin-4-yl)-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) methanimine (22a–22i) with 10 nM concentration was produced in (DMSO), dilution of prepared concentrations

in assay buffer were done. 20 millimolar Tris of (pH 7.4), 20 millimolar (KCL) potassium chloride, and 0.1 mg/mL Albumin from Bovine Serum were combined to make the test buffer (BSA). At a final concentration of 10 mM, A-P-7-amido-4-trifluoromethylcoumarin (AP-7-AT-FMC) served as the substrate. The test chemical and buffer solution were then incubated at room temperature for 15 minutes before the addition of the substrate, during which time human dipeptidyl peptidase-4 (DPP-4) was introduced. Each well in the 96-well microtiter plate contained 100 mL of the reaction mixture during the test. The kinetics of the reaction were monitored for 10–15 minutes at room temperature using excitation at 400 nm and emission at 505 nm.

4. *In-vivo* anti-diabetic studies

4.1. Acute toxicity study

According to the OECD-423 limit test protocol, the product's acute oral toxicity was tested on healthy female albino wistar rats. Three female albino Wistar rats were employed, ranging in age from 7 to 10 weeks. Before and after receiving the crude product, all rats fasted (without drinking water) for 3 hours. For the first test subject, a maximum dose of 300 mg/kg was administered. A total of four further animals were dosed in sequence based on the findings. The animals were maintained individually and observed for the initial 30 minutes and then every 4 hours for 24 hours for any signs of gross physical and behavioral toxicity, including variations in epidermis, urinary incontinence, excessive drooling, reduced in feeding activity, arousal, paw licking, elevated breathing rate, impaired motor activity, diarrhoea, loss of weight, and quadriplegia (OECD 2002).

4.2. An oral glucose tolerance test (OGTT)

After 14 hours of abstinence, male albino wistar rats (150–170 g) were split into three groups (n = 6 in each group) and given either Piperazine compound (20 mg/kg), sitagliptin (3 mg/kg), or saline (control group). 30 minutes following therapy, the rats were given a oral glucose loading dosage (2 g/kg). After glucose was injected into the subjects' veins, blood was drawn at 0, 30, 60, and 120 minutes. A diagnostic kit was used to detect glucose levels in blood taken from the retro-orbital plexus (Klein et al. 2021).

4.3. *In-vivo* study of antihyperglycemic activity in a STZ-induced T2DM model

Albino male Wistar rats (bought from TANUVAS in Chennai, Tamil Nadu, India) were approved by Institute Animal Ethics Committee (IAEC) with approval number of IAEC/252/2021 were used for the experiment. The minimal dose streptozotocin (STZ) model for type 2 diabetes mellitus in rats was used to generate diabetes according to the outlined protocol. The occurrence of diabetes is proven by a fasting blood glucose level greater than 140 mg/dl after three days of inducing diabetes. The remaining thirty rats were randomised into 5 groups (n = 6 in each) with nearly identical fasting blood glucose levels. Confirmation of diabetes led to the initiation of treatment in the following groups: normal control group, diabetic control group, low dose piperazine compound (20 mg/kg), high dose piperazine (40 mg/kg), sitagliptin (3 mg/kg), and normal control group and diabetic control group (saline) for 21 days. The dosage given was 1 ml/kg, whether it was the active ingredient or a placebo. After 14 hours of fasting on 0, 7, 14 and 21 days blood glucose levels were tested as mentioned above.

4.4. Statistical analysis

Mean standard error of the mean is presented. We used GraphPad PRISM for the statistical analysis (version 6.01). When comparing groups over many time points, the two-way ANOVA was followed by the Bonferroni test. Considered statistically significant at $p < 0.0001$.

5. Results and discussion

5.1. Molecular docking

To test the aforementioned hypothesis and learn more about the affinity of the newly created molecules for DPP-4, we used Autodock 1.5.6. The docking outcomes showed that the proposed molecules occupy the DPP-4 catalytic site with the appropriate spacing GLU361, ILE407, PHE461, LYS463, and GLU8. The substituents placed on benzyl ring of the piperazine and pyridine moieties which have designed are changed and the 8d,8h, 22d,22h were obtained with better docking score compared to the others. They have either phenyl methoxy group or Fluro phenyl groups which has shown the enhancing activity through the good binding affinity towards DPP-4 enzyme. Therefore, the synthesis of these proposed compounds with a higher docking score has been investigated.

5.2. Chemistry

The designed 4-ethyl-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8a–8i) derivatives and 1-(pyridin-4-yl)-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) methanimine (22a–22i) The fundamental methods mentioned in schemes 1 and 2 were used to synthesis derivatives with yields ranging from 50 to 75%. Target molecules were quickly produced with a scheme-1 of 3-step synthesis and scheme-2 of 2- step synthesis respectively. Briefly, starting with commercially available semi-carbazide using several substituted aldehydes, and

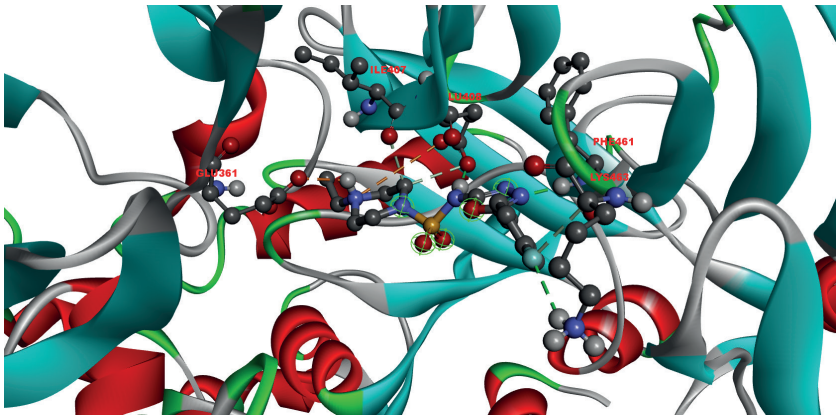

Figure 1. Molecular docking of compound 8h with GLU361, ILE407, PHE461, LYS463, and GLU8 amino acids

2-(4-methylbenzylidene) hydrazine-1-carboxamide in presence of sodium carbonate and water along chlorosulfonic acid and finally by adding N-ethyl piperazine the final product of scheme-1 is obtained. In scheme-2 in only 2-step synthesis the final product is obtained using Pyridine-4-carbaldehyde. Several types of mobile phases were utilised to test the purity of all substances by thin-layer chromatography. Each component was pure and stable. Utilizing 1HNMR, 13C-NMR, and mass spectrometry, the structures of the newly synthesised compounds were verified and confirmed.

5.3. *In vitro* DPP-4 assay and structure-activity relationship studies

A summary of data on the DPP-4 inhibitory action of the synthesized derivatives and the positive control, sitagliptin, is shown in Table 1. All of the final products displayed inhibition, whereas substances 8a–8i and 22a–22i exhibited exceptional inhibitory activity, ranging from 8.07 to 28.73% inhibition at 10 mol L^{-1}. The methoxy group and fluro group attached to phenyl ring where Piperazine and pyridine ring are the main nucleus they showed exceptionally good inhibition compared to other groups attached at 4th position of phenyl showed better inhibitory activity. The biological activity was firstly influenced by the electrostatic volume of the N-aryl moiety, and the DPP-4 inhibitory activity was considerably influenced by the electronegativity difference between molecules.

Table 1. Results are expressed as an average of three readings (mean ± sem).

S.no	Compounds code	Concentration	% Inhibition
1	8a	10μmol L^{-1}	20.34
2	8b	10μmol L^{-1}	26.52
3	8c	10μmol L^{-1}	8.07
4	8d	10μmol L^{-1}	28.73
5	8e	10μmol L^{-1}	6.58
6	8f	10μmol L^{-1}	12.34
7	8g	10μmol L^{-1}	25.48
8	8h	10μmol L^{-1}	27.32
9	8i	10μmol L^{-1}	15.8
10	22a	10μmol L^{-1}	18.24
11	22b	10μmol L^{-1}	22.34
12	22c	10μmol L^{-1}	10.15
13	22d	10μmol L^{-1}	26.39
14	22e	10μmol L^{-1}	11.39
15	22f	10μmol L^{-1}	13.20
16	22g	10μmol L^{-1}	23.27
17	22h	10μmol L^{-1}	24.32
18	22i	10μmol L^{-1}	12.58
19	Sitagliptin	1μmol L^{-1}	100

5.4. *In-vivo* study of antihyperglycemic activity in a STZ-induced T2DM model

Compound 8h demonstrated adequate DPP4 inhibitory action was chosen for additional *in-vivo* research over the other compounds because it possessed superior physicochemical features, including a greater level of DMSO solubility. The *in-vivo* anti - hyperglycaemic effectiveness of 8h was investigated in a persistent Streptozotocin type II diabetes animal study, according to Fig. 3. A A 2-way

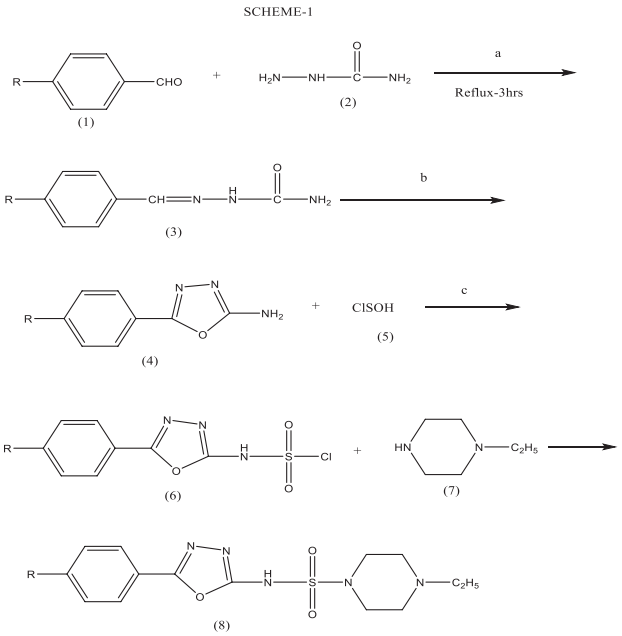

Figure 2. Scheme-1, Reagents and conditions: (**a**) CH_3COONa, reflux for 3hours (**b**) Na_2CO_3, H_2O, reflux for 3-4 hours (**c**) - H_2O.

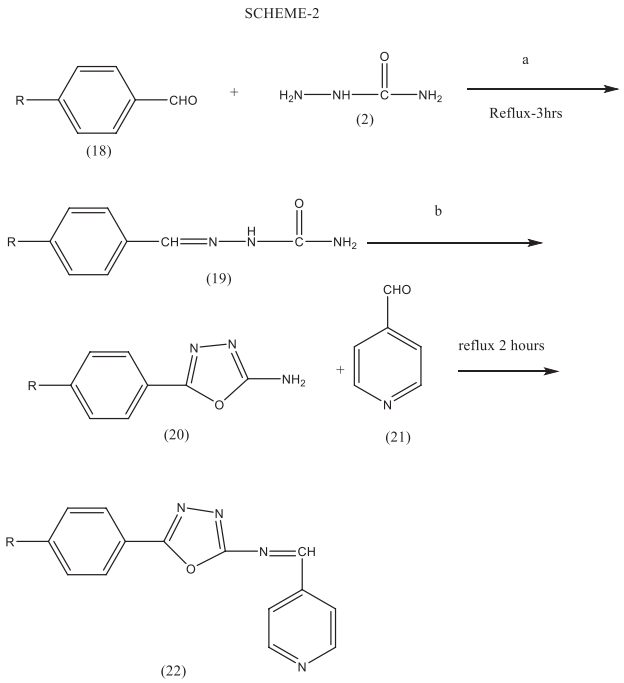

Figure 3. Scheme 2, Reagents and conditions: (**a**) CH_3COONa, reflux for 3 hours (**b**) Na_2CO_3, H_2O, reflux for 3-4 hours.

ANOVA and consequent post hoc test showed a statistically significant difference between hyperglycaemic and healthy rats' serum blood sugar ($p < 0.001$). In addition, a course of treatment lasting 21 days with 2 mg/kg of sitagliptin after the first week, there was a considerable decrease in the fasting blood glucose. ($p < 0.001$) and following the second week ($p < 0.001$) relative to the diabetic animals which were treated with saline. In addition, 21 number of days of treatment with compound 8h (20 mg/kg) and (40 mg/kg) after the first week, there was a considerable decrease in the animal's fasting blood glucose ($p < 0.0001$) as well as upon the second week ($p < 0.0001$) comparing to the diabetic animals which were given saline as treatment.

5.5. Oral glucose tolerance test in rats and the response of test substances on blood sugar levels

Compound 8h (20 mg/kg), sitagliptin (3 mg/kg), or saline (in the control group) were injected orally 30 minutes prior to a solitary oral glucose load (2.5 g/kg) to 6h-Fasting Wistar albino rats (n = 6 for each group). Blood glucose levels were assessed at baseline (time 0), 30 minutes, 60 minutes, and 120 minutes following glucose loading. As depicted in Fig. 5, sugar levels amount peaked 30 minutes after glucose injection. At 60 and 120 minutes after glucose load, blood sugar levels in the 8h-therapy and sitagliptin-treated groups remained considerably reduce than in the group treated with saline ($p < 0.001$).

6. Conclusion

In conclusion, a set of new of 4-ethyl-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) piperazine-1-sulfonamide (8a–8i) derivatives and 1-ethyl-N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) piperazine-1-s (pyridin-4-yl) With good yields, -N-(5-(p-tolyl)-1,3,4-oxadiazol-2-yl) methanimine (22a–22i) derivatives have been synthesised and characterised by IR spectroscopy. In vitro DPP-4 inhibitory effectiveness studies were conducted on all newly developed analogues.

During screening against DPP-4 in vitro, molecules 8d,8h,22d,22h were discovered to have stronger inhibitory activity than other groups involved in the structure with percentage inhibition of 28.73, 27.32, 26.39, 24.32 respectively. According to SAR analyses of the produced compounds, methoxy and Fluro groups attached to the phenyl group at fourth position of piperazine and pyridine derivatives exhibited better activity by inhibiting DPP-4 enzyme. Compound 8h significantly decreased serum glucose levels during *in-vivo* OGTT. In addition, at the completion of a 21-day research in Wistar albino rats with STZ-induced T2DM,

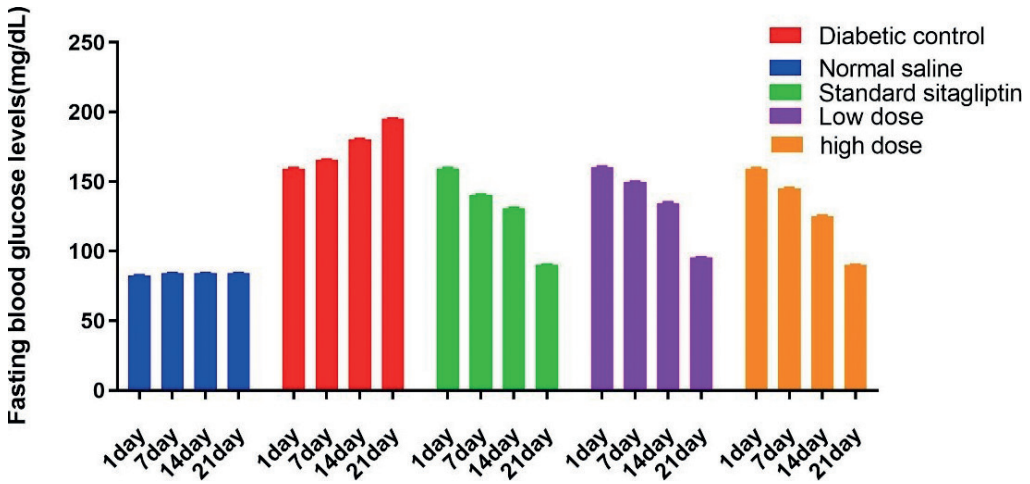

Figure 4. The effect of the chemical being treated with 8h (20 mg/kg, p.o., once daily) and (40 mg/kg, p.o., one time in a day) sitagliptin (2 mg/kg, p.o., one time in a day) based on overnight glucose levels at the commencement of treatment, After the week is up, two weeks, and after three weeks of treatment. Data were presented as mean ± SEM (n = 6 per group) $p < 0.001$ marked contrast to diabetic group given saline at the same period $p < 0.001$ in comparison to the healthy batch.

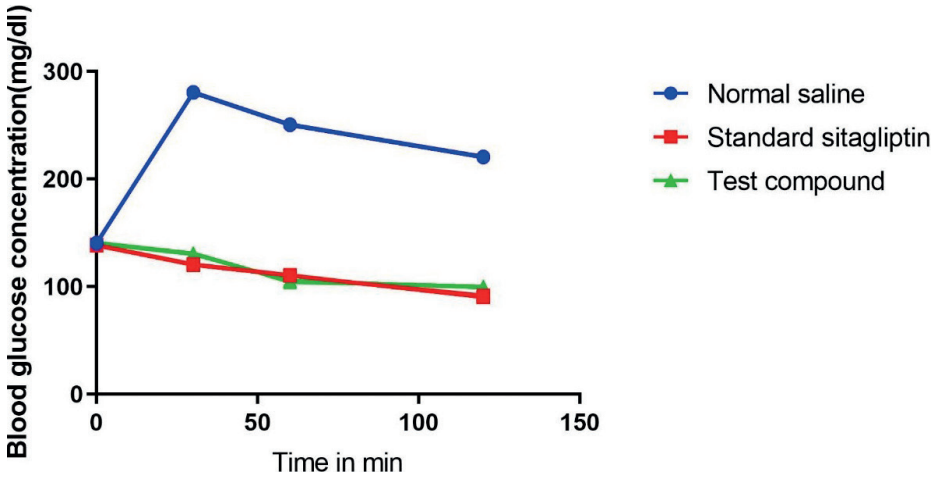

Figure 5. Glucose concentrations throughout a test of oral glucose tolerance (OGTT) in Wistar albino rats treated with 8h or sitagliptin. The data were presented as the mean standard error of the mean (n = 6 for each group). $p < 0.001$ significant difference in comparison to the group treated at almost the same time point with saline.

compound 8h had a substantial antihyperglycemic effect at 20 mg/kg once-daily oral dose, whereas no Observed glucose effect in the control animals. These data imply that 8h could be They are potential candidates for inhibiting the DPP-4 enzyme, and their efficacy and selectivity can be enhanced.

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