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Research Article

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL ACTIVITY OF 3, 6-DISUBSTITUTED 2-PYRIDINECARBOXAMIDE DERIVATIVES AS GLUCOKINASE ACTIVATOR

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ABSTRACT

Glucokinase (GK) is the key enzyme expressed in β-cells of pancreas and liver hepatocytes and helps in the maintenance of blood glucose levels in normal range. Activators of GK are the novel category of drug candidates which activate GK enzyme allosterically and show their antidiabetic activity. A new series of 3, 6-disubstituted 2-pyridinecarboxamide derivatives have been synthesized and evaluated for antidiabetic activity. These compounds evaluated by IR, ^{1}H-NMR and Mass spectroscopy. Amongst the synthesized derivatives, compounds 5d, 5e showed maximum GK activation in the *in vitro* GK assay. Furthermore, the results of antihyperglycemic activity indicated that substitution with hydrophobic group like trifluoromethyl at position-3 of pyridine ring (5d and 5e) attached to carboxamide led to increased antidiabetic activity in OGTT assay results.

Keywords: Diabetes mellitus, Glucokinase activator, 3, 6-disubstituted 2-pyridinecarboxamide, Structure activity relationship, Spectroscopy, Oral glucose tolerance test.

1. INTRODUCTION

Pyridinecarboxamide derivatives shows varieties of therapeutic activities like antioxidant [1], antitumor [2], anti-allergic [3], antihypertensive [4], antibacterial [5], antipsychotic[6], histamine H_3 receptor antagonist [7] and anti-diabetic activity [8-12].Some other moieties also exhibit anti-diabetic activity [13-18]. Pyridinecarboxamide is a heterocyclic compound containing pyridine ring. This compound contains one oxygen atom and two nitrogen atom having formula $C_6H_6N_2O$ and molecular weight of 122.127. Pyridinecarboxamide is a small organic molecule having wide range of action.

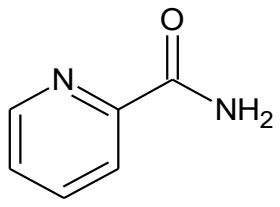


Fig. 1: Structure of pyridinecarboxamide

Diabetes mellitus is a long lasting disease of food metabolism characterized by hyperglycemia due to defect in insulin secretion. No single drug is helpful in achieving long term management of blood glucose level. Glucokinase catalyzes the phosphorylation of glucose toglucose-6-phosphate and is predominantly expressed in liver and pancreatic β-cells. GK acts as a glucose sensor regulating hepatic glucose metabolism and glucose dependent insulin secretion. Based on the dual hepatic and pancreatic effects, GK activators represent novel and promising approach for the treatment of type 2 diabetes. Various groups have reported that the glucokinase activators demonstrated antidiabetic efficacy in rodent models. Many pharmaceutical companies have actively pursued a program aiming at the development of GK activators. From these, some companies including Roche and OSI/Prosidion have entered their compounds into clinical study [8, 9].

2. EXPERIMENTAL

Chemical used for the synthesis of molecules were of analytical grade. Thin layer chromatography was performed on ready-made Aluminum backed TLC GF254 plates as well as on microscopic slides (2 x 7.5 cm) coated with silica gel G and spots were visualized by exposure to iodine vapors and UV radiation. Melting points were reported by open capillary tube method.IR spectra were done on FT-IR on 8400S Shimadzu by using KBr pellets in the range of 4000-400 cm^{-1}. ^{1}H-

NMR spectra were done on Bruker400 MHz instrument in solvent (DMSO-d_6, CHC_{l3}).Mass spectra were done on LC-MS Spectrometer using Model Q-ToF Micro Waters. The anti-diabetic activity of some synthesized compounds was carried out by OGTT method.

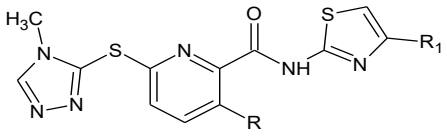


Fig. 2: Moiety

Table 1: Physical properties of the synthesized compounds 5a-5f

Compound Name	R	R_1	Molecular formula	Molecular weight	M.P.	% Yield
5a	-H	-H	$C_{12}H_{10}N_6OS_2$	318.3 g/mol	220-224°C	80%
5b	-H	$-CH_3$	$C_{13}H_{12}N_6OS_2$	332.4 g/mol	210-215°C	85%
5c	-H	$-CH_2CH_3$	$C_{14}H^{14}N_6OS_2$	346.4 g/mol	239-243°C	90%
5d	$-CF_3$	-H	$C_{13}H_9F_3N_6OS_2$	386.3 g/mol	217-221°C	90%
5e	$-CF_3$	$-CH_3$	$C_{14}H_{11}F_3N_6OS_2$	400.4 g/mol	240-244°C	82%
5f	$-CF_3$	$-CH_2CH_3$	$C_{15}H_{13}F_3N_6OS_2$	414.4 g/mol	225-230°C	82%

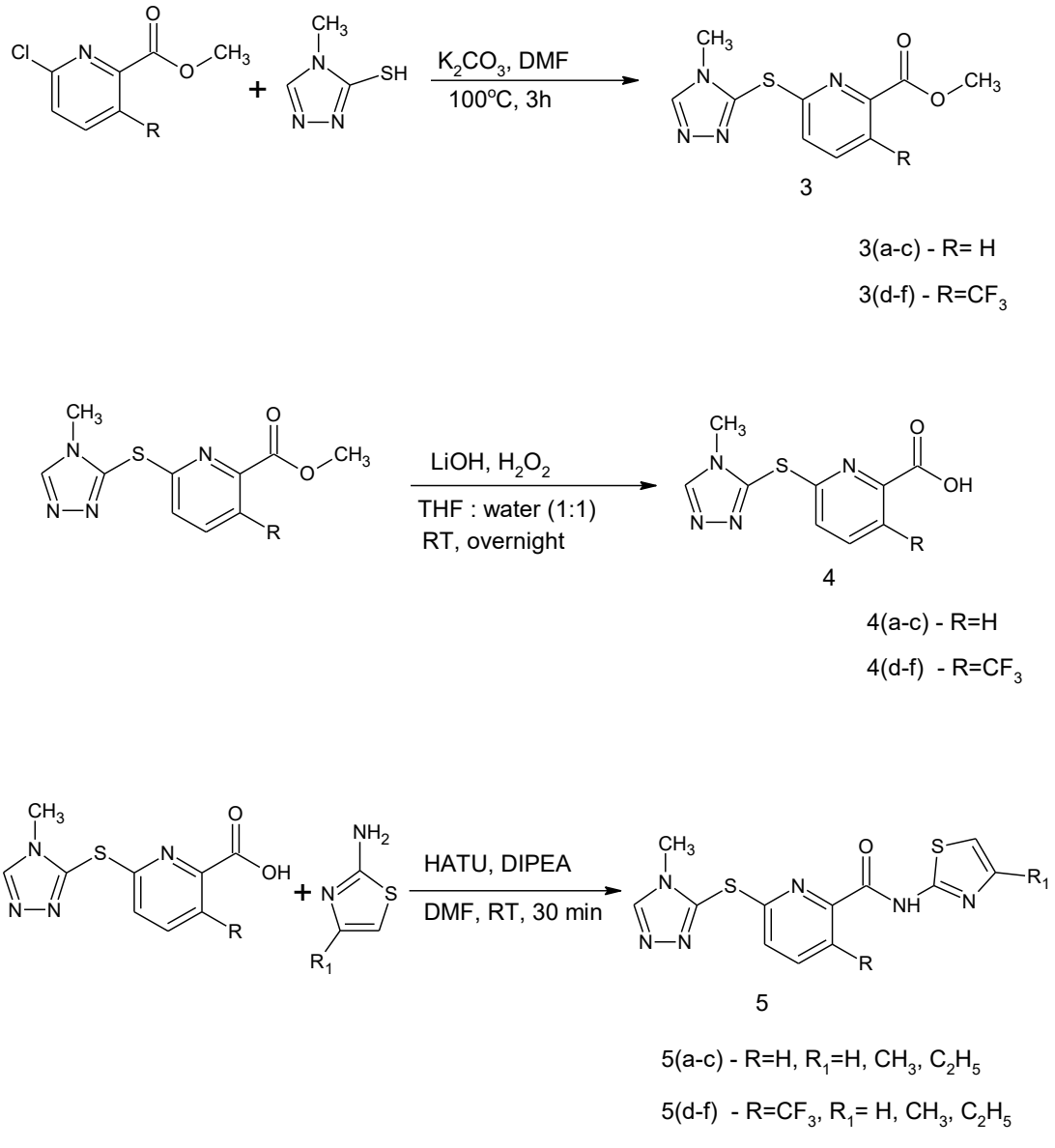


Fig.3: Reaction scheme for the synthesis of compounds 5a -5f

2.1. Synthetic procedure

2.1.1. General procedure for the synthesis of methyl 6-((4-methyl-4H-1,2,4-triazol-3-yl) thio)picolinate (3a-3f)

A mixture of methyl 6-chloropicolinate (1a; 16g, 0.08 mol), 4-methyl-4H-1,2,4-triazole-3-thio (9.6 g, 0.09 mol), potassium carbonate (12.4g, 0.09mol), dimethyl-formamide (72 ml), and water (8ml) was stirred at room temperature (30-35°C) for 4-5 h. Dilution of the mixture with water (300ml) then led to precipitation of a solid which was isolated by suction, dried and purified by column chromatography on silica gel using chloroform and chloroform/methanol (70/30) as eluents. Product 3 was obtained by elution with chloroform/ methanol and recrystallized from methanol.

2.1.2. Procedure for the synthesis of 6-((4-methyl-4H-1,2,4-triazol-3-yl)thio) picolinic acid (4a-4f)

Methyl 6-((4-methyl-4H-1,2,4-triazol-3-yl)thio) picolinate (2.5 g, 7.6 mmol) was suspended in 140 mL of THF in a 500-mL round-bottomed flask. In a separate flask, 2.5 g of lithium hydroxide was dissolved in 140 mL of deionized water. Both mixtures were chilled to 4°C and combined to form a turbid white mixture. After 1 h of stirring, the mixture became homogeneous. After 24 h, 50 mL of 3 M HCl was added, and the mixture was allowed to warm to room temperature. Following the addition of a 100-mL portion of saturated aqueous NaCl solution, the mixture was extracted four times with 100-mL portions of EtOAc, and the combined organic layers were evaporated. Yield 2.28 g (7.25 mmol, 95%).

2.1.3. Procedure for the synthesis of 6-((4-methyl-4H-1,2,4-triazol-3-yl)thio)-N-(thiazol-2-yl)picolinamide (5a-5f)

Thiazol-2-amine and 6-((4-methyl-4H-1,2,4-triazol-3-yl)thio)picolinic acid (0.5 g, 0.00218 mol 1.0 eq) and substituted thiazol-2-amine was dissolved in dry DMF (10 cm^3). The solution was stirred for 10 min at ambient temperature. Acid (1.0 eq) was added, followed by HATU (1.2 eq) and Diisopropyl ethylamine (2 eq).The reaction mass was heated to 45-50°C for 1-2 hr, the completion of reaction was monitored by TLC. After completion of the reaction, the reaction mass was diluted with ethyl acetate. The organic layer was washed with water followed by 10% sodium bicarbonate solution, brine solution dried over anhydrous sodium sul-phate. The organic layer was concentrated under vacuum and the crude was purified by column chromatography.

2.2. Evaluation of anti-diabetic activity

2.2.1. In vitro enzyme assay

The GK activity of the synthesized compounds was evaluated using a coupled reaction with glucose-6-phosphatedehydrogenase (G-6-PDH) spectrometrically. All the compounds were prepared in dimethyl sulfoxide (DMSO) and the assay was performed in a final volume of 2000 µL containing 2-(4-(2-hydroxyethyl)piperazin-1-yl) ethanesulfonic acid (25 mM, pH 7.4), glucose (10 mM), potassium chloride (25 mM), magnesiumchloride (1 mM), dithiothreitol (1 mM), ATP (1 mM), NAD (1 mM), G-6-PDH (2.5 U/mL), GK (0.5 µg), and compounds under investigation (10 µM). Absorbance was measured at 340 nm after 3 min incubation period and GK activation fold by the synthesized com-poundsand GK fold activation was calculated compared to control (GK activation by the control (*i.e.*, DMSO only) was considered as 100%) [19-22].

2.2.2. Oral glucose tolerance test (OGTT)

This assay is designed to identify genetically modified mice that exhibit alterations of metabolism associated with diabetes, obesity and cardiovascular disease. An oral glucose tolerance test (OGTT), in which the mice are challenged with a bolus of glucose and blood glucose and insulin levels are measured across a two hour time course is performed one week prior to the initiation of the high fat diet (HFD) challenge. The mice are subjected to a seven-week HFD challenge using a Western diet. (Circulating levels of insulin, adiponectin, and cholesterol from samples taken before and after the HFD can also be measured, as well as the distribution of cholesterol in the VLDL, LDL, and HDL sub-fractions both pre and post the HFD challenge are analyzed using a polyacrylamide based system (Lipoprint).) The post HFD OGTT results are compared to those obtained before the start of the diet. The ability of genetically modified or pharmacologically treated mice to handle an oral glucose load, in combination with changes in insulin and adiponectin levels in response to the HFD, are assessed to identify genes or pharmacological agents affecting development of a diabetic state. Differences in cholesterol levels and cholesterol distribution are examined to establish if the genetic modification or the compound alters the response to the HFD.

Healthy Sprague-Dawley rats (150-200 g) were procured and kept at controlled room temperature and fed with the normal pellet diet and water *ad libitum*, prior to the dietary manipulation. Consent was taken from Institutional Animal Ethics Committee to conduct this study (Approval No. CPCSEA/IAEC/0254/09/20/216). Based on the results of *in vitro* GK assay, selected synthesized derivatives were evaluated in rat OGTT model. Rats were divided into different groups containing six animals in each group and all the rats were fasted overnight for at least 8 h before experiment. Control group was administered vehicle only (5% DMSO, p.o.), standard group was administered with metformin (30 mg/kg, p.o.), and test groups were administered compounds 5a to 5f (50 mg/kg, p.o.). All the animals were loaded with glucose (3 g/kg, p.o.) 30 min after drug administration. Blood samples were collected just prior to drug administration, and 0, 30, 60, and 120 min after oral glucose administration. Blood glucose level was measured immediately and glucose area under curve (AUC) was calculated from the data (from 0 to 2 h). The OGTT assay results were statistically analyzed by two-way ANOVA [8-9, 23].

3. RESULTS AND DISCUSSION

3.1. Structural analysis of synthesized compounds

3.1.1. 6-[(4-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]-N-(1,3-thiazol-2-yl)pyridine-2-carboxamide (5a)

R_f = 0.8, FTIR ($CHCl_3$, υ/(cm^{-1}): 3261 (N-H), 3207 (NH), 3044 (Ar C-H), 2962 (C-H of CH_3alky), 1715 (Ar C=O), 740 (C-S-C). ^{1}H NMR (500 MHz, $CHCl_3$): δ (ppm), 3.61 (3H, s), 7.61 (1H, d, J = 6.6 Hz), 7.62 (1H, d, J = 6.6 Hz), 7.86 (1H, dd, J = 8.2, 1.7 Hz), 7.93 (1H, dd, J = 8.1, 1.7 Hz), 8.02 (1H, dd, J = 8.2, 8.1 Hz), 8.53 (1H, s), 10.36 (1H, s). ESI Mass (*m/z*, %): 319 (M^+, 1), 164 (100).

3.1.2. N-(4-methyl-1,3-thiazol-2-yl)-6-[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] pyridine-2-carboxamide (5b)

R_f = 0.6, FTIR ($CHCl_3$, υ/(cm^{-1}): 3299 (N-H), 3201 (NH), 3054 (Ar C-H), 2931 (C-H of CH_3alky), 1713 (Ar C=O), 764 (C-S-C). ^{1}H NMR (500 MHz, $CHCl_3$): δ (ppm), 2.13 (3H, s), 3.60 (3H, s), 6.30 (1H, s), 7.62 (1H, dd, J = 8.2, 1.7 Hz), 7.86 (1H, dd, J = 8.1, 1.7 Hz), 7.93 (1H, dd, J = 8.2, 8.1 Hz), 8.10 (1H, s), 10.35 (1H, s). ESI Mass (*m/z*, %): 333 (M^+, 1), 164 (100).

3.1.3. N-(4-ethyl-1,3-thiazol-2-yl)-6-[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] pyridine-2-carboxamide (5c)

R_f = 0.6, FTIR ($CHCl_3$, υ/(cm^{-1}): 3290 (N-H), 3207 (NH), 3026 (Ar C-H), 2931 (C-H of CH_3alky), 1726 (Ar C=O), 848 (C-S-C). ^{1}H NMR (500 MHz, $CHCl_3$): δ (ppm), 1.25 (3H, t, J = 7.3 Hz), 2.52 (2H, q, J = 7.3 Hz), 4.56 (3H, s), 6.43 (1H, s), 7.32 (1H, dd, J = 8.2, 1.7 Hz), 7.51 (1H, dd, J = 8.1, 1.7 Hz), 7.92 (1H, dd, J = 8.2, 8.1 Hz), 8.23 (1H, s), 10.25 (1H, s). ESI Mass (*m/z*, %): 347 (M^+, 1), 164 (100).

3.1.4. 6-[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(1,3-thiazol-2-yl)-3-(trifle oromethyl)pyridine-2-carboxamide (5d)

R_f = 0.6, FTIR ($CHCl_3$, υ/(cm^{-1}): 3323 (N-H), 3170 (NH), 3014 (Ar C-H), 3002 (C-H of CH_3alky), 1649 (Ar C=O), 844 (C-S-C). ^{1}H NMR (500 MHz, $CHCl_3$): δ (ppm), 7.61 (1H, d, J = 6.6 Hz), 7.63 (1H, d, J = 8.8 Hz), 7.92 (1H, d, J = 8.8 Hz), 8.14 (1H, s), 10.43 (1H, s). ESI Mass (*m/z*, %): 387 (M^+, 1), 232 (100).

3.1.5. N-(4-methyl-1,3-thiazol-2-yl)-6-[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] -3-(trifle-oromethyl)pyridine-2-carboxamide (5e)

R_f = 0.6, FTIR ($CHCl_3$, υ/(cm^{-1}): 3279 (N-H), 3231 (NH), 3034 (Ar C-H), 2962 (C-H of CH_3alky), 1711 (Ar C=O), 848 (C-S-C). ^{1}H NMR (500 MHz, $CHCl_3$): δ (ppm), 2.10 (3H, s), 3.64 (3H, s), 6.30 (1H, s), 7.72 (1H, d, J = 8.8 Hz), 7.89 (1H, d, J = 8.8 Hz), 8.15 (1H, s), 10.33 (1H, s). ESI Mass (*m/z*, %): 402 (M^+, 1), 232 (100).

3.1.6. N-(4-ethyl-1,3-thiazol-2-yl)-6-[(4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]-3 (trifluoromethyl)pyridine-2-carboxamide (5f)

R_f = 0.6, FTIR ($CHCl_3$, υ/(cm^{-1}): 3269 (N-H), 3203 (NH), 3033 (Ar C-H), 2942 (C-H of CH_3alky), 1713 (Ar C=O), 875 (C-S-C). ^{1}H NMR (500 MHz, $CHCl_3$): δ (ppm), 1.06 (3H, t, J = 7.3 Hz), 2.38 (2H, q, J = 7.3 Hz), 3.60 (3H, s), 6.43 (1H, s), 7.86 (1H, d, J = 8.8 Hz), 7.91 (1H, d, J = 8.8 Hz), 8.14 (1H, s), 10.34 (1H, s). ESI Mass (*m/z*, %):

3.2. Evaluation of anti-diabetic activity

3.2.1. In vitro GK activity

The results of *in vitro* GK assay (fold activation) are presented in Table 2. Amongst the synthesized derivatives, compounds 5d, 5e showed maximum GK

activation in the *in vitro* GK assay (fold activation around 2 compared to control). Compounds 5c, 5b, and 5a showed moderate fold activation (around 1.5 compared to control) of GK enzyme. Further the results of antihyperglycemic activity assay indicated that substitution of substituted pyridine ring attached to carboxamide with alkyl groups like methyl, ethyl led to decreased antidiabetic activity. The results of *in vitro* GK assay depicted that substitution of the pyridine-2-yl ring attached to CONH with 4 -methyl group resulted in decreased GK activity compared to unsubstituted pyridine-2-yl ring. Amongst the compounds bearing 3-substituted-pyridin-2-yl ring only compound 5d having 3 trifluoromethyl showed good GK activation (fold activation of 2.42) compared to compounds bearing unsubstituted pyridine-2-yl ring attached to 1,3-thiazol-2-yl amide nucleus 5a.

The 3-trifluoromethyl-pyridin-2-yl ring substituted benzamide derivatives bearing 4-ethyl-1,3-thiazol-2-yl and 4-methyl-1,3-thiazol-2-yl (compounds 5f and 5e) displayed potent GK fold activation of 1.85 and 2.05, respectively.

The results of in vitro GK assay demonstrated that substitution with hydrophobic group like trifluoromethyl at position-3 of pyridine ring showed increased in activity whereas substitution with hydrophilic groups decreased the GK activity.

3.2.2. *In vivo* antihyperglycemic activity

In vivo antihyperglycemic activity based on screening by *in vitro* GK assay studies selected compounds (5a-5f) were further evaluated for their glucose lowering effect in animal models by means of OGTT assay (Table 2). The results of antihyperglycemic activity (*i.e.,* OGTT assay) were measured as blood glucose levels (μg/dL) at different time intervals (0, 30, 60 and 120 min after oral glucose administration) and glucose AUC represented in Fig. 4, respectively. The results of antihyperglycemic activity assay depicted that amongst compounds evaluated in OGTT assay, compound 5d were found to be highly active in OGTT assay. Compound 5d was almost equipotent to standard antidiabetic drug metformin at 30 and 60 min and decreased the blood glucose levels equivalent to that of standard antidiabetic drug metformin at 120 min interval. Compound 5d was found to reduce significantly glucose AUC compared to control and analogous to that of standard antidiabetic drug. Compound **5e** displayed appreciable reduction in blood glucose levels compared to that of standard drug (metformin) in OGTT assay and these compounds significantly reduced glucose AUC compared to control group. The results of in vivo antihyperglycemic activity assay indicated that the compounds **5d** and **5e** followed the similar pattern in blood glucose lowering as that of the standard antidiabetic drug metformin. Compound 5b was found to be fairly effective in the in vivo antihyperglycemic activity assay compared to standard antidiabetic drug metforminat 120 min interval. All the compounds tested for antihyperglycemic activity reduced blood glucose in safe range at time interval of 120 min during OGTT assay (*i.e.,* no hypoglycaemic effect was observed during assay period). The control group (vehicle *i.e.,* DMSO only) didn't produce any significant effect on blood glucose levels as well as blood glucose AUC in the OGTT assay. The results of antihyperglycemic activity assay indicated that substitution with hydrophobic group like trifluoromethyl at position-3 of pyridine ring attached to carboxamide led to increased antidiabetic activity which can be seen from the OGTT assay results of compounds 5d and 5e.

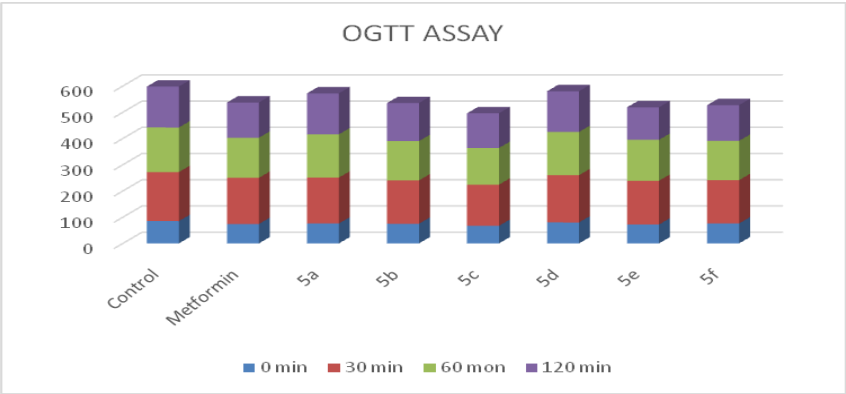
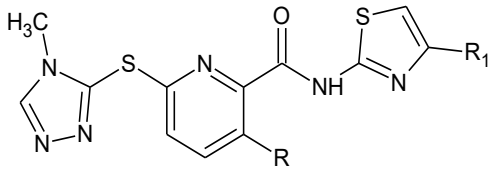


Fig.4: Blood glucose levels of selected molecules at different time intervals. All the values are mean of six measurements ± SE

Table 2: *In vitro* GK potency and metabolic stability for 5a-5f



S.No.	Compound No.	R	R_1	R_f vaue	Percen t yield	GK potency EC50 (μM) (*invitro*)	GK activity	Metabolic stability FH[a] (*invitro*)
1	5a	-H	-H	0.80	80	0.076	1.42±0.06	72
2	5b	-H	$-CH_3$	0.60	85	0.040	1.45±0.08	68
3	5c	-H	$-CH2CH_3$	0.60	90	0.038	1.57±0.09	64
4	5d	$-CF_3$	-H	0.60	90	0.001	2.42±0.02	100
5	5e	$-CF_3$	$-CH_3$	0.60	82	0.012	2.05±0.09	98
6	5f	$-CF_3$	$-CH2CH_3$	0.60	82	0.032	1.85±0.06	95

4. CONCLUSION

From the present experimental study, it was observed that 3, 6-disubstituted 2-pyridinecarboxamide moiety gives better anti-diabetic activity. Amongst the synthesized derivatives, compounds 5d, 5e showed maximum GK activation in the *in vitro* GK assay (fold activation around 2 compared to control). Furthermore, the results of antihyperglycemic activity assay indicated that substitution with hydrophobic group like trifluoromethyl at position-3 of pyridine ring attached to carboxamide led to increased antidiabetic activity which can be seen from the OGTT assay results of compounds 5d and 5e.These compounds further can be taken for evaluation of other studies.

5. ACKNOWLEDGEMENT

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