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Research Article

Synthesis and Biological Evaluation of A New Substituted 4-Carboxy Benzamide Derivative to Provide New Therapeutic Strategy for Diabetes Mellitus.

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Abstract

Diabetes mellitus is a major affective disorder, common in the general population. Worldwide there is a renewed interest in the development of an effective medicine against the most dreaded diseases. Earlier known the class of Phenyl substituted carboxylic acids and Benzamide Derivatives are found to be good candidate for inhibitors of various PTPs, especially as PTP-1B. In the present study we propose to develop a new substituted 4-Carboxy Benzamide derivative, these can be prepared and can be checked for antidiabetic activity. The experimental work has been divided into two main parts: 1. Synthesis Work 2. Biological Studies, therefore which prove the Inhibition of PTPase and Activation of insulin receptor and downstream functions could provide new therapeutic strategy for diabetes mellitus.

Keywords: *PTPs, Carboxy Benzamide Derivatives, Ant Diabetic Activity.*

Introduction

Diabetes is often referred by **diabetes mellitus**, which describes as a group of metabolic disorder in which the person has high blood glucose (blood sugar) either because insulin production is inadequate or because the body's cells do not respond properly to insulin or both. Patients with high blood sugar experience polyuria (frequent urination), increasingly thirsty (polydipsia) and hungry (polyphagia) [1]. Hyperglycemia may be defined as a condition in which fasting blood glucose is more than 7.0 m mole/l (126mg%) and post prandial plasma glucose is more than 11.1 mmole/l (200mg%) [1]. Diabetes currently affects 246 million people worldwide and is expected to affect 592 million by 2035. Diabetes is the fourth leading cause of global death causing by disease [2]. Figure 1.

Amide(-CONH2) is an organic compound consisting of a benzene ring with a amide substituent [3]. Piperidine is an organic compound with the molecular formula (CH2)5NH. This heterocyclic amine consists of a six-membered ring containing five methylene bridge(-CH2-) and one amine bridge (-NH-) [3]. Benzaldehyde (C6H5CHO) is an organic compound consisting of a benzene ring with a formyl substituent [3].

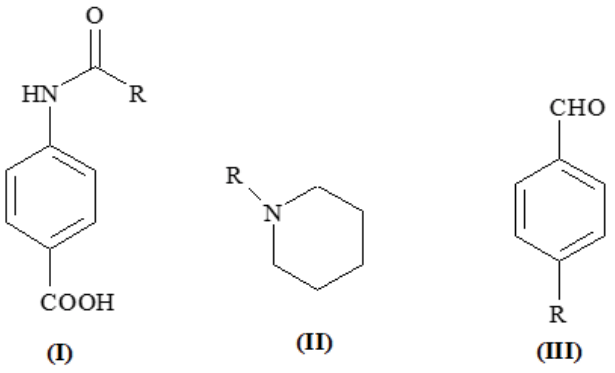

Figure 1

As per patent review class of Phenyl substituted carboxylic acids and benzamide derivatives are found to be good candidate for inhibitors of various PTPs, especially as PTP-1B.as per review the new substituted 4-Carboxy Benzamide derivatives can be prepared and can be checked for antidiabetic activity [4-9]. Therefore, Inhibition of PTPase and Activation of insulin receptor and downstream functions could provide new therapeutic strategy for diabetes mellitus.

Material and Methods

Synthesis of the Benzamide derivatives started with a reaction of p-amino benzoic acid with chloro acetylchloride in the presence of dimethyl formamide as solvents under reflux condition for 24 hrs to give the (3) PHK-100 [10-13]. Different substituent's were synthesised via oxidation reaction in the presence of Acetone and potassium carbonate for 6 hrs at 60.$_c$ to give the PHK-200 reacted with piprazene and PHK-300 when reacted with pipridine.In the scheme different substituent's were prepared from PHK-200(400-a) PHK-300(500$_{a-c}$) followed by triturated the benzaldehyde and sodium salt of benzene [14-19]. The chemical structure was identified by 1HNMR and IR, and MASS, confirming the procedure of scheme.

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Synthesis Scheme

Figure 2.

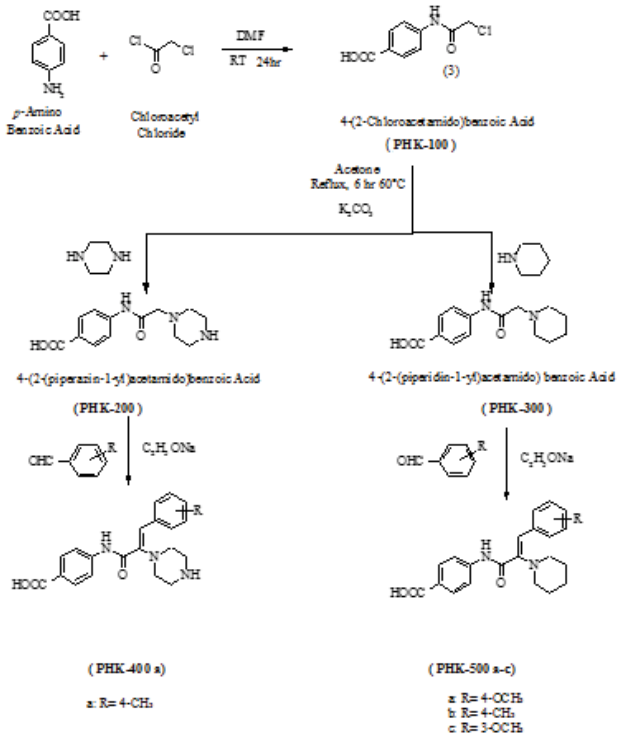

Figure 2

Results and Discussion: Derivatives was prepared in one step with a modified oxidation reaction. It is essential to keep the reaction continuing under the reflux condition to ensure high yield.

(PHK-400a)

Figure 3. Table A.

(PHK-500a)

Figure 4. Table B.

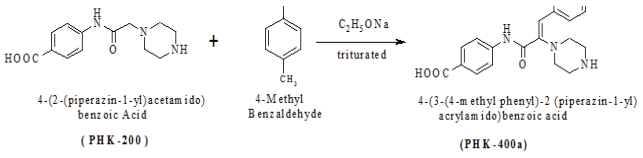

Figure 3

Table A

IR (KBr, cm^{-1})	C=O stretch of acid (1922), -C=O stretch of ketone (1684), aromatic C-H stretching (2948), -C=C- alkene (1602), para substitution (844), Carboxylic acid-OH broad (2550)
Mass	365(M+)
NMR (DMSO)	11.2[1H, s, COOH], 6.9-7.8[8H, m, Ar-H], 6.15[1H, s, =CH], 4.4[1H, s, -NH], 3.4[3H, s, -CH3], 2.1-2.5[4H, t, -CH2-piperazine], 2[4H, s, -CH2-piperazine], 1.5 [1H, s, -NH]

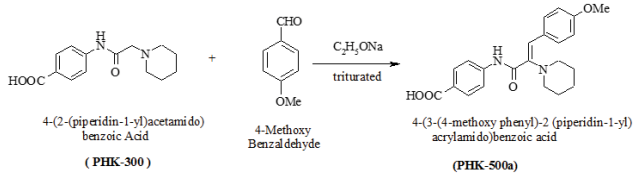

Figure 4

Table B

IR (KBr, cm^{-1})	C=O stretch of acid (1921), -C=O stretch of ketone (1685), aromatic C-H stretching (2948), -C=C- alkene (1603), para substitution (841), Carboxylic acid-OH broad (2554)
Mass	381(M+1), 295, 179, 121
NMR (DMSO)	11.4[1H, s, -COOH], 6.9-8.1[8H, m, Ar-H], 6.35[1H, s, =CH], 4.1[1H, s, -NH], 2.7[3H, s, -OCH3], 2.3-2.6[4H, t, -CH2piperidine], 1.3-1.7[6H, m, -CH2piperidine]

(PHK-500b)

Figure 5. Table C.

(PHK-500c)

Figure 6. Table D.

Biological Evaluation

Induction of diabetes:

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline solution. T2DM was induced in overnight fasted rats by a single intraperitoneal injection of 65 mg/kg streptozotocin, 15 min after the I.P. administration of 110 mg/kg of nicotinamide. Hyperglycemia was confirmed by the elevated glucose concentration in plasma, determined at 72 h by glucometer. The animals with blood glucose concentration higher 250 mg/dl were used for the antidiabetic screening.

In vivo antidiabetic assay (T2DM model):

The diabetic animals were divided into groups of six animals each (n = 6). Rats were orally administered a suspension of the compounds (prepared in 10% Tween 80) (50 mg/kg body weight) and a similar suspension of dry acetone extract (100 mg/kg body weight). Control group animals were also fed with 10% Tween 80. Rapaglitamide (5 mg/kg) was used as hypoglycemic reference drug. Blood samples

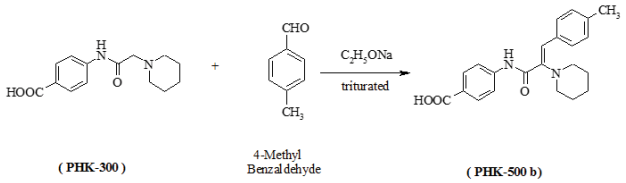

Figure 5

Table C

IR (KBr, cm^{-1})	C=O stretch of acid (1921), -C=O stretch of ketone (1684), aromatic C-H stretching (2929), -C=C- alkene (1603), para substitution (841), Carboxylic acid-OH broad (2559)
Mass	365(M+1), 364(M+), 277, 181
NMR(DMSO)	12.6[1H, s, -COOH], 6.8-7.9[8H, m, Ar-H], 6[1H, s, =CH], 4.4[1H, s, -NH], 3.7-3.8[4H, t, -CH2piperidine], 2.5 [3H, s, -CH3], 1.2-1.4[6H, m, -CH2piperidine]

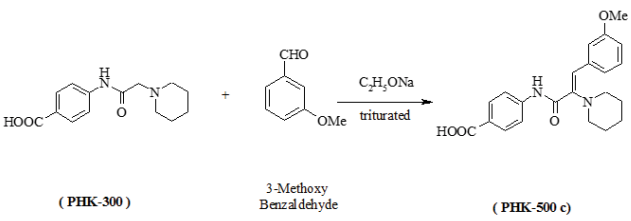

Figure 6

Table D

IR (KBr, cm^{-1})	C=O stretch of acid (1920), -C=O stretch of ketone (1687), Aromatic C-H stretching (2926), -C=C- alkene (1602), para substitution (835), O-H stretch (3443)
Mass	381(M+1), 295, 179, 121

were collected from the caudal vein at 0, 1, 3, 5 and 7 hrs after vehicle, sample and drug administration. Blood glucose concentration was estimated by enzymatic glucose oxidase method using a commercial glucometer. The percentage variation of glycemia for each group was calculated in relation to initial (0 h) level, according to: %Variation of glycemia = $[(Gx - G_0)/G_0]$ 100, where G_0 were initial glycemia values and Gx were the glycemia values at +1, +3, +5 and +7 h respectively. Table 1.

The synthesized compounds were evaluated for anti-diabetic activity using Calbiochem® PTP1B colorimetric assay kit and the PTP-1B inhibitor assay suramin is taken as a control. The activity profile of the compounds is given in following Table. The compound PHK 500b and PHK 400a shows highest anti diabetic activity among all synthesized compounds. Table E.

Phosphate Standard Curve Data. Table F.

% Inhibition of test samples as compared to Control (Suramine)

Conclusion

Result of biological evaluation on anti-diabetic shows that substituted 4-carboxy benzamide derivatives possess moderate to high activity. The 4-methyl derivatives were found to be more active. **Compounds PHK 500-b and PHK 400-a**, shows maximum activity in synthesized compounds. The 4-carboxy benzamide derivatives **(PHK 500b & PHK 400a)** with 4-methyl phenyl derivatives more active than others derivative due to presence methyl group at para positon. The methoxy phenyl substituted 4-carboxy benzamide (PHK400a and PHK 400c) gives less activity as compared to other derivatives.

Table1: Result of *in vivo* antidiabetic activity.

Test Samples	Dose (mg/kg)	% Variation of glycemic ± S.E.M. (mg/dl)				
		Zero hour	First hour	Third hour	Fifth hour	Seventh hour
Vehicle	-	0 ± 0.0	1.16 ± 1.55	0.144 ± 0.72	-0.35 ± 1.14	-2.99 ± 1.18
Rapaglitamide	3	0 ± 0.0	-4.65 ± 0.94*	-12.34 ± 0.66*	-21.32 ± 0.62*	-33.51 ± 0.88*
PHK-500a	50	0 ± 0.0	-2.74 ± 0.99*	-1.2 ± 0.72*	-7.44 ± 0.82*	-2.88 ± 1.7*
PHK-500b	50	0 ± 0.0	-3.94 ± 0.76*	-8.59 ± 0.95*	-15.75 ± 0.96*	-23.15 ± 1.96*
PHK-500 c	50	0 ± 0.0	-1.56 ± 0.88*	-3.37 ± 1.45*	-1.25 ± 1.34*	-5.08 ± 1.8*
PHK-400a	50	0 ± 0.0	-3.5 ± 0.84*	-10.32 ± 0.87*	-14.78 ± 1.22*	-21.71 ± 1.7*

Value represent the mean ± S.E.M (n=6). $p < 0.05$ compared with control group. The negative value (-) indicates decrease in glycemia.

Table E

Concentration of Phosphate (nmole)	Absorbance (620 nm)
0.00	0.092
0.25	0.123
0.50	0.138
1.0	0.212
2.0	0.312
3.0	0.381

Table F

Compounds	Absorbance At 620 nm	nmol of phosphate	% inhibition as compare with Suramine	Conc. of Compounds (µM)
Suramine	0.109	0.118	100	20
PHK-400a	0.107	0.098	83.05	20
PHK-500a	0.104	0.068	57.63	20
PHK-500b	0.108	0.108	91.52	20
PHK-500c	0.101	0.038	32.20	20

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