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Original Article

Design, Synthesis and Oral Glucose Tolerance Screening of Some 5-substituted benzylidene-3-[2-(2-methyl-*1H*-benzimidazol-1-yl)-2-oxoethyl]-1,3-thiazolidine-2,4-dione Derivatives on Rats

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ABSTRACT

Objective: As a part of research project, the knowledge gained by various researches has suggested that substituted benzimidazole which is the structural isosteres of nucleotides allow them to interact easily with the biopolymers, possess good pharmacological activity with lower toxicities. In recent years 2-methyl-1*H*-benzimidazole shows promising antihyperglycaemic activity. So, substituting or adding a new moiety to the parent lead compound thus by making gradual changes in the structure of compound resulting gradual change in physicochemical properties and biological activities of drug. All the synthesized compounds were characterized by UV, IR, Mass and some of by ^{1}H-NMR spectroscopy & report of them supports the structures of compounds. Compounds **40a, 40b, 40e and 40h** were found to be having significant glucose lowering effect as compared to control and standard. Other compounds like 40c, 40d, 40f and 40g were found to be lower active as compared to standard and other derivatives. Evaluation of antidiabetic activity revealed that compounds with acetylated benzimidazole having the thiazolidinedione moiety gives best biological activity having electron donating group at the 4th position of the phenyl ring i.e., **40a, 40b, 40e and 40h** are most active with electron releasing functional groups are having good antidiabetic property. All the synthesized final compounds were screened for antidiabetic activity by Oral Glucose Tolerance Test (OGTT) method against standard reference drug pioglitazone.

Activity Scale: 40a>40b>40h>40e>40c>40d>40f>40g

Keywords: 2-Methyl benzimidazole derivatives, OGTT, Knovenagel condensation, Physicochemical properties, Antidiabetic activity.

INTRODUCTION

- Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both.
- Diabetes is generally state of increased blood glucose level, Diabetes Mellitus mainly categorised in two categories: Type 1 (IDDM) & Type 2 (NIDDM).
- On the basis of etiology type 1 is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases in Europe) which is thought to be due to immunological destruction of pancreatic ß-cells resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with ß-cell destruction.
- Autoimmune diseases such as Grave's disease, Hashimoto's thyroiditis and Addison's disease may be associated with type 1 diabetes mellitus.
- Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus diabetes covers a wide range of heterogeneous diseases.
- According to the ADA recommendation changes in 1997, the fasting glucose concentration should be used in routine screening for diabetes as well as epidemiological studies; the threshold for fasting glucose was changed from 7.8 mmol/L (140 mg/dL) to 7.0 mmol/L (126 mg/dL); however the 2-h glucose criterion remains as = 11.1 mmol/L (200 mg/dL.[1-3]

MATERIALS AND METHODS

- The entire chemicals were supplied by S. D. Fine Chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and Loba Chemie. Pvt. Ltd. (Mumbai).
- Melting points were determined by open tube capillary method and were uncorrected.
- Purity of compounds was checked by thin layer chromatography (TLC) on silica gel-G in solvent system hexane-ethyl acetate (1:1) and the spots were located under iodine vapours and UV light.
- IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr.
- Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.

General Procedure for synthesis of 2-methyl-(1*H*)-benzimidazole

o-Phenylenediamine (5g, 0.046 mole) and sufficient quantity of (2.76g, 0.046 mole) glacial acetic acid were mixed under harsh dehydrating reaction condition and refluxed it at 70-80ºC temperature for 8 hrs. Then the reaction mixture was cooled and 10% NaOH solution was added slowly with stirring till mixture became alkaline. The precipitates

were filtered and washed 3-4 times with water, dried and recrystallized finally from Methanol.[4]

General Procedure for synthesis of 2-chloro-1-(2-methyl-1*H*-benzimidazole-1-yl) ethanone

To a solution of 2-methylbenzimidazole (5.5g, 0.05mole) and anhydrous Potassium carbonate (6.4g, 0.05mole) in Acetone (10ml), chloroacetyl chloride (0.6g, 0.1mole) was added drop wise at room temperature. The mixture was refluxed for about 7-8hrs and solid was separated out.[5]

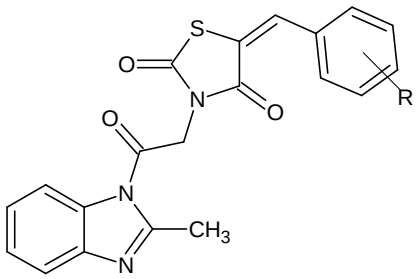
General Procedure for synthesis of 5-(4-methylbenzyl)-1, 3-thiazolidine-2,4-Dione

A mixture of 2, 4-Thiazolidinedione (2.07g, 0.047mole), *p*-methylbenzaldehyde (2g, 0.047mole) and piperidine 0.2ml in 150ml ethanol was refluxed for 6hrs. Then after it was poured in cold water and acidified with acetic acid to obtain product which was recrystallized with DMF.[6]

General Procedure for synthesis of 3-[2-(2-methyl-1*H*-benzimidazol-1-yl)-2-oxoethyl]-5-(4-methylbenzylidene)-1, 3- thiazolidine-2,4-dione

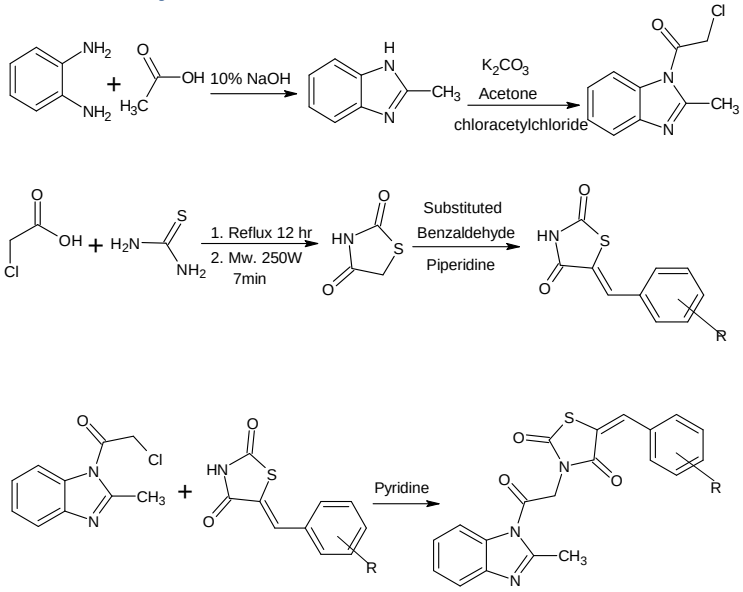
2-chloro-1-(2-methyl-1*H*-benzimidazole-1-yl-) ethanone (3.5g, 0.01mole) and 5-(4-methylbenzylidene)-1,3-thiazolidine-2, 4-dione (2.05g, 0.01mole) was mixed in pyridine and refluxed for 16hrs. After cooling the content, few drops of conc. HCl was added to precipitate out the product and finally recrystallized with DMF. The reaction mixture was monitored by TLC.[7,8]

Physical characteristics of 5-substituted benzylidene-3-[2-(2-methyl-*1H*-benzimidazol-1-yl)-2-oxoethyl]-1,3-thiazolidine-2,4-dione



For details see Table no 1

Scheme of Synthesis



(40a-h)

Spectral characteristics of 5-substituted benzylidene-3-[2-(2-methyl-*1H*-benzimidazol-1-yl)-2-oxoethyl]-1,3-thiazolidine-2,4-dione

For details see Table no 2

Biological evaluation

Oral glucose tolerance test

Experimental design and procedure

- A total number of 26 Albino Wistar rats weighing about 250-300 gm.
- Special diets are fed for 30 to 90days prior to the OGTT. We carry out the OGTT by fasting animals for 18hrs,

taking a blood sample from the tail under local anaesthesia, and then gavaging with 12mL/kg of a 50% glucose solution, which delivers 6g of glucose per kg of body weight. Blood samples are taken 30, 60, 90 and 120minutes after the glucose meal and analyzed for blood glucose with a clinical glucometer. The reference drug and the synthesized compounds were administered orally with oral feeding tube to the rats.

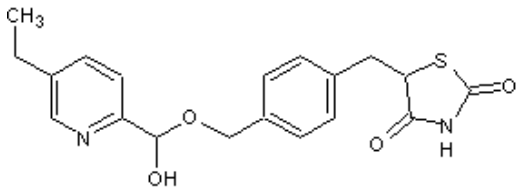
- Group I stands for normal control group. Group II is treated with Pioglitazone (30mg/kg body weight). The synthesized compounds were dissolved 0.5 % CMC in according to 20mg/ kg of body weight. Then the solution was administered orally to the glucose fed rats and blood was collected from the rat by cutting the tail. Blood sample was taken in a strip and then measured the glucose concentration level by glucometer and Plasma glucose level in mg/dL was being monitored at 0, 30, 60 90, 120minutes. Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's Multiple Comparison Test and the values were considered statistically significant when P <0.05.[9-11]

IN-VIVO ANTIDIABETIC ACTIVITY BY OGTT

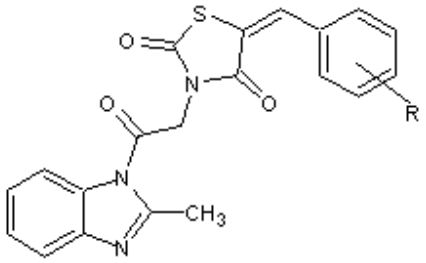
One-way ANOVA (*, p<0.05 consider for significance) followed by post test, Dunnett's multiple comparison tests.

Reading of concentration of blood glucose level in mg/dL at different time interval 0, 30, 60, 90, 120min

For details see Table no 3.



Pioglitazone



Synthesized Moiety

DISCUSSION

- All the synthesized compounds are characterized by using IR, Mass and some by NMR spectroscopy.
- All the synthesized compounds activity is compared with the standard drug as a Pioglitazone.
- From the above activity table it is evident that the entire synthesized compounds give a good antidiabetic activity.
- Compounds **40a, 40b, 40e** and **40h** were found to be having significant glucose lowering effect as compared to control and standard.
- Other compounds like 40c, 40d, 40f and 40g were found to be lower active as compared to standard and other derivatives.
- Synthesized compounds were screened for antidiabetic activity by OGTT method.
- Upon data showed in table, we can say that the synthesized compounds 40a, 40b, 40e and 40h decreases blood glucose level significantly with time and results are approximately near with std.

pioglitazone, so it having similar antidiabetic activity like standard.

CONCLUSION

- All the synthesized compounds were characterized by using UV, IR, Mass and some of by ^{1}H-NMR spectroscopy & report of them support the structures of compounds.
- As per synthetic point of view all the synthesized final compounds have good yield in range of 70.43-78.33%w/w except compound 40f, 40g and 40h have yield 59.92, 52.24 and 56.11%w/w respectively because of the steric hindrance by the *ortho* substituent of benzaldehyde.
- All the synthesized final compounds were screened for antidiabetic activity by Oral Glucose Tolerance Test Method against standard reference drug pioglitazone.
- Synthesized compounds 40a, 40b having mainly electron donating group gives best activity furthermore other *para* substituted compounds like 40e, 40h also gives good antidiabetic activity.
- Evaluation of antidiabetic activity revealed that compounds with acetylated benzimidazole having the thiazolidinedione moiety gives best biological activity having electron donating group at the 4th position of the phenyl ring i.e., 40a, 40b, 40e and 40h are most active with electron releasing functional groups are having good antidiabetic property.

Activity Scale:

40a>40b>40h>40e>40c>40d>40f>40g

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Table 1. Physicochemical parameters

Comp. code	R	Molecular Formula	Molecular Weight (g/mol)	MP (ºC)	Yield (%w/w)	R_f
(40a)	*p*-CH_3	$C_{21}H_{17}N_3O_3S$	391.44	228-230	72.13	0.61
(40b)	*p*-OCH_3	$C_{21}H_{17}N_3O_4S$	407.44	238-240	76.70	0.40
(40c)	*p*-Cl	$C_{20}H_{14}ClN_3O_3S$	411.86	232-234	70.43	0.62
(40d)	*p*-Br	$C_{20}H_{14}BrN_3O_3S$	456.31	218-220	78.33	0.67
(40e)	*p*-OH	$C_{20}H_{15}N_3O_4S$	393.41	253-255	71.12	0.42
(40f)	*o*-OH	$C_{20}H_{15}N_3O_4S$	393.41	250-252	59.92	0.40
(40g)	*m*-NO_2	$C_{20}H_{14}N_4O_5S$	422.41	250-252	52.24	0.53
(40h)	*p*-NO_2	$C_{20}H_{14}N_4O_5S$	422.41	258-260	56.11	0.59

Table 2. Spectral parameters

Comp. code	UV λ_{max} (nm)	IR (υ, cm^{-1})	Mass (m/z)	^{1}H-NMR (δ, ppm)
(40a)	329	1737.14 (C=O), 1687 (N-C=O), 1342.36 (-C=N), 639(C-S)	393 [M+1]	6.77-7.54 (m, 10H, ArH), 4.59 (s, 2H, CH_2), 2.50-2.51 (s,
(40b)	350	1737.14 (C=O), 1687 (N-C=O), 1342.36 (-C=N), 1255.57 (C-O-C), 639 (C-S)	406 [M-1]	-
(40c)	328	1687(N-C=O), 1737.14(C=O), 1342.36 (-C=N), 639(C-S), 626 (C-Cl)	412.56 [M+], 414 [M+2]	7-7.53 (m, 9H, ArH), 4.58 (s, 2H, CH_2), 2.50-2.51 (s,
(40d)	331	1726.17 (C=O), 1610.45 (N-C=O), 1325.01 (-C=N), 1166.85 (C-Br), 696.25 (C-S)	457.11 [M+1], 458 [M+2]	-
(40e)	346	3440.77 (-OH), 1722.31 (C=O), 1676.03 (N-C=O), 1323.08 (-C=N), 696.25 (C-S)	392 [M-1]	6.77-7.54 (m, 9H, ArH), 5.53 (s, 1H, OH), 4.59 (s, 2H,
(40f)	340	1724.24 (C=O), 1330 (-C=N), 690.47(C-S), 1677.95 (N-C=O), 3421.48 (-OH)	394.13 [M+1]	-
(40g)	320	2899.02 (Ar C-H), 1733.11(C=O), 1677.95(N-C=O), 1479.30 (-N=O), 1334.65 (-C=N), 669.25 (C-S)	421 [M-1]	-
(40h)	328	2925.81 (Ar C-H), 1710.74 (C=O), 1608.52 (N-C=O), 1510.16 (-N=O), 1344.29 (-C=N), 690.47 (C-S)	423.71 [M+1]	-

Table 3. OGTT Screening report

Control	Baseline	Time (min) ± SEM				
		0	30	60	90	120
	85±2.08	173.66±1.76	165±1.15	152±1.52	141.33±185	126±2.30
Pioglitazone	87.66±1.85	124.66±2.96	116.66±3.17	111.33±3.52	87.66±0.88	67.66±3.17
40a	83.33±2.02	137.66±2.02	123.66±2.90	115.66±2.02	94.66±2.33	73.33±1.45
40b	75.33±2.33	153.66±1.76	127.33±2.02	124±2.08	102.66±3.71	82±3.78
40c	83±2.30	140.33±1.45	133.66±1.76	125.33±1.76	114.66±2.60	97±3.05
40d	92.66±2.02	145.33±1.76	140.66±1.45	128.66±2.51	126±1.15	107.33±4.37
40e	91±2.08	156.33±2.72	133.33±2.02	127.33±1.76	99±1.15	92.66±2.33
40f	90.33±1.76	146±1.73	139.66±1.45	128.66±1.45	123±1.15	114±2.64
40g	82±1.52	153±1.45	152±2.08	144.33±2.40	138.33±1.45	131±1.15
40h	82.33±2.40	161±1.52	126.66±2.96	120.66±3.28	106.33±4.70	83±4.16

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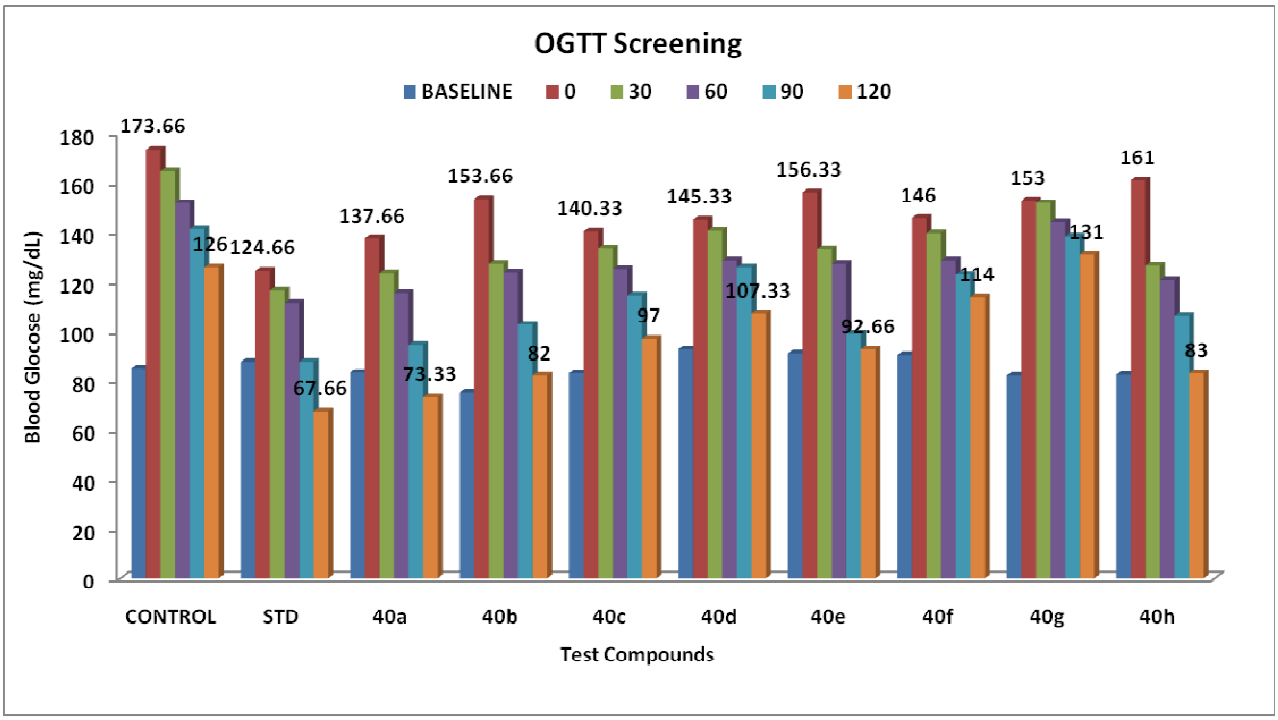

Figure.1. Histogram of OGTT