Research Article

Synthesis and biological evaluation 0f novel 5-benzylidene-[3-(diethyl amino) methyl]-thiazolidine-2, 4-dione derivatives having anti-diabetic activity

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ABSTRACT

Seven derivatives of novel5-Benzylidene- [3-(diethyl amino) methyl] Thiazolidine-2, 4- Dione derivatives (5a-5g) were synthesized by 3 steps. At first Preparation of 1, 3-Thiazolidine 2,4-Dione (1) then condensation between 1,3-Thiazolidine 2,4-Dione (1) with various substituted Benzaldehyde derivatives (2a-2g) in the presence of Piperidine to obtain Benzylidine intermediates (3a-3g). Finally N-Mannich base (beta-amino-ketone) derivatives (5a-5g) were synthesized by the reaction of Benzylidine intermediates (3a-3g) with diethyl amine (4) in the presence of formaldehyde and DMF. Chemical structures of these compounds were confirmed by means of FTIR spectra. The compounds were assayed for anti diabetic activity by using animal models (male wistar rats). Animals when treated with Glibenclamide (0.45 mg/kg, s.c) and final derivatives **5a, 5b, 5e and 5f** (0.50 mg / kg, s.c) **(G-IV, V, VIII and IX)** showed significant decrease in blood glucose levels when compared to control group (G-II) on day 2 and 4 respectively ($p<0.001$)

Keywords: Antibacterial activity, 1, 3-thiazolidine 2, 4- Dione, Beta-amino-ketone, FTIR spectra, Glibenclamide.

INTRODUCTION

Thiazolidinediones (TZD's) or glitazones forms a significant class of drugs which exhibits various biological activities. 1, 3-thiazolidine 2, 4- Dione contains basic skeleton of thiazole or thiazolidine.[1] Presence of one carbonyl group in thiazole at 4th position makes it thiazolidine-4-one which is known for various activities such as anti-inflammatory and presence of another carbonyl group at 2nd position makes it thiazolidine-2, 4 Dione which is basically known for its anti-diabetic activity. Its derivatives constitute an interesting class of compounds due to their synthetic versatility and effective biological activity. In the last few years, considerable attention has been focused on Thiazolidinediones due to their interesting biological activities ranging from anti-diabetic [2], anti-inflammatory [3], anti-microbial[4], antipyretic[5], Anti-Hyperglycemic[6], Anticonvulsant[7], aldose reductase inhibitor[8] and anti-cancer. A Mannich base is a beta-amino-ketone, which is formed in the reaction of an amine, formaldehyde (or an aldehyde) and a carbon acid.[9] The Mannich base is an end product in the Mannich reaction, which is nucleophilic addition reaction of a non-enolizable aldehyde and any primary or secondary amine to produce resonance stabilized imine (iminium ion or imine salt). The addition of a carbanion from a CH acidic compound (any enolizable carbonyl compound, amide, carbamate, hydantoin or urea) to the imine gives the Mannich base.[10] Seven derivatives of novel 5-benzylidene-[3-(diethyl amino) methyl]-thiazolidine-2, 4-diones were synthesized by 3 steps. At first Preparation of 1, 3-Thiazolidine 2, 4-Dione (1) then condensation between 1, 3-Thiazolidine 2, 4-Dione (1) with various substituted Benzaldehyde derivatives (2a-2g) in the presence of Piperidine to obtain Benzylidine intermediates (3a-3g). Finally N-Mannich base (beta-amino-ketone) derivatives (5a-5g) were synthesized by the reaction of Benzylidine intermediates (3a-3g) with diethyl amine (4) in the presence of formaldehyde and DMF.

MATERIALS AND METHODS

All the required chemicals used were obtained from Fischer chemicals, Finar Chemicals, Nice chemicals, Sd-fine chemicals and Avra Labs. All the solvents used were of laboratory grade. Each reaction was monitored by TLC by using appropriate solvent system, which was selected by trial and error method. Pre-coated TLC plates (0.25 mm silica gel) were obtained from E. Merck. All the synthesized compounds were purified by re-crystallization. Melting points were determined on Bio-Techniques India melting point apparatus and they were uncorrected.

IR spectra were recorded on Shimadzu FTIR thermonicolet instrument by KBr disc method.

The final compounds were synthesized as given below

I. Preparation of 1, 3-Thiazolidine 2, 4-Dione (1)

II. Preparation of Benzylidine derivatives (3a-3f)

III. Preparation of Novel *N*- Mannich bases (5a-5f)

I) Preparation of 1, 3-Thiazolidine 2, 4- Dione (1)

In a 250 ml round bottom flask, a solution containing 50 g (0.52 M) of Chloroacetic acid in 50 ml of water and 40.27 g (0.52 M) of Thiourea was added and the reaction mixture was stirred for 15 min to obtain a white precipitate, accompanied by considerable cooling. The flask was then connected with a reflux condenser and gentle heating was applied to effect complete dissolution, after which the reaction mixture was stirred and refluxed for 40 hr at 100-110°C. On cooling the contents of flask solidified into a cluster of white needles. The product was

filtered and washed with water to remove the traces of un-reacted species. It was then dried and purified by re-crystallization using ethanol.

i) Preparation of 5-(4-flourobenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3a)

In a 250ml round bottom flask, 4-flourobenzaldehyde (6.34 g, 0.042 moles) and 2, 4-thiazolidinedione (5.0g, 0.042 M) was together suspended in dry toluene (10 ml). To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110°C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol.

ii) Preparation of 5-(4-chlorobenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3b)

In a 250ml round bottom flask, 4-chlorobenzaldehyde (5.90 g, 0.042 M) and 2, 4-thiazolidinedione (5.0 g, 0.042 M) was together suspended in dry toluene (10 ml). To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110 ^{0}C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol

iii) Preparation of 5-(4-nitrobenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3c)

In a 250ml round bottom flask, 4-nitrobenzaldehyde (5.15 g, 0.034 M) and 2, 4-thiazolidinedione (4.0 g, 0.034 M) was together suspended in dry toluene (10 ml). To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110^{0}C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol.

iv) Preparation of 5-(4-methoxybenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3d)

In a 250ml round bottom flask, 4-methoxybenzaldehyde (6.34 g, 0.042 M) and 2,4-thiazolidinedione (5.0g, 0.042 M) were together suspended in dry toluene. To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110 ^{0}C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol.

v) Preparation of 5-(3-nitrobenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3e)

In a 250ml round bottom flask, 3-nitrobenzaldehyde (6.44g, 0.04 M) and 2, 4-thiazolidinedione (5.0 g, 0.04 M) was together suspended in dry toluene. To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110°C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol.

vi) Preparation of 5-(2-hydroxybenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3f)

In a 250ml round bottom flask, 2-hydroxybenzaldehyde (4.16g / 3.63ml, 0.034 M) and 2, 4-thiazolidinedione (4.0 g, 0.034 M) was together suspended in dry toluene. To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110°C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol.

vii) Preparation of 5-(2-methylbenzylidene)-1, 3-Thiazolidine-2, 4-Dione (3g)

In a 250ml round bottom flask, 2-methylbenzaldehyde (6.34 g, 0.042 M) and 2,4-thiazolidinedione (5.0g, 0.042 M) were together suspended in dry toluene. To this catalytic amount of Piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110°C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol.

III) Preparation of novel n-mannich bases (5a-5f)

i) Synthesis of 5-(4-flourobenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5a)

5a was prepared in a Mannich-type reaction from Benzylidine derivative of 2, 4-Thiazolidinedione, formaldehyde and the diethyl amine. To a solution of compound 3a in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3a. To this, the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give final compound.

ii) Synthesis of 5-(4-chlorobenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5b)

5b was prepared in a Mannich-type reaction from Benzylidine derivative of 2,4-Thiazolidinedione, formaldehyde and the diethyl amine. To a solution of compound 3b in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3. To this the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give final compound.

iii) Synthesis of 5-(4-nitrobenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5c)

5c was prepared in a Mannich-type reaction from Benzylidine derivative of 2,4-Thiazolidinedione, formaldehyde and the diethyl amine. To a solution of compounds 3c in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3. To this, the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give the final compound.

iv) Synthesis of 5-(4-methoxybenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5d)

5d was prepared in a Mannich-type reaction from Benzylidine derivative of 2,4-Thiazolidinedione, formaldehyde and the diethyl amine To a solution of compounds 3d in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3. To this, the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give final compound.

v) Synthesis of 5-(3-nitrobenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5e)

5e was prepared in a Mannich-type reaction from Benzylidine derivative of 2, 4 Thiazolidine Dione, formaldehyde and the diethyl amine. To a solution of compounds 3e in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3. To this, the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give the final compound.

vi) Synthesis of 5-(2-hydroxybenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5f)

5f was prepared in a Mannich-type reaction from Benzylidine derivative of 2,4-Thiazolidinedione, formaldehyde and diethyl amine. To a solution of compounds 3f in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3. To this, the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give the final compound.

vii) Synthesis of 5-(2-methylbenzylidene-[3-(diethyl amino) methyl]-1, 3-Thiazolidine-2, 4-Dione (5g)

5g was prepared in a Mannich-type reaction from Benzylidine derivative of 2, 4-Thiazolidinedione, formaldehyde and diethyl amine. To a solution of compounds 3g in DMF, formaldehyde (0.2 M) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and yield methylol derivative of compound 3. To this, the solution of diethyl amine in DMF was added drop wise and reflux for 2 hr. The reaction was poured into ice cold water and filtered off and wash with hot water. Finally it was dried and purified by re-crystallization with chloroform to give the final compound.

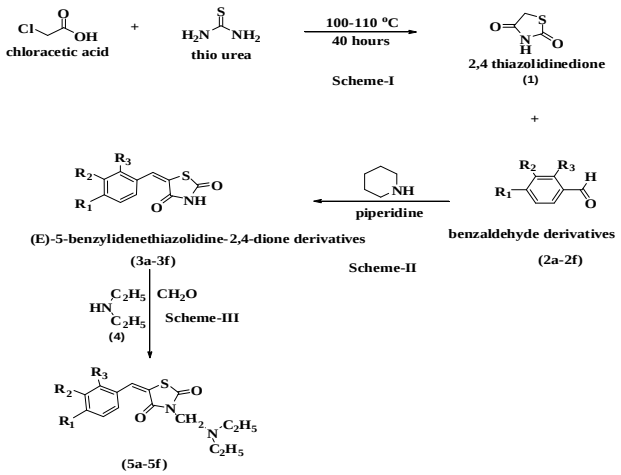
RESULTS AND DISCUSSION

Seven derivatives of novel5-Benzylidene- [3-(diethyl amino) methyl] Thiazolidine-2, 4- Dione derivatives (5a-5g) were synthesized by 3 steps. At first Preparation of 1,3-Thiazolidine 2,4-Dione (1) then condensation between 1,3-Thiazolidine 2,4-Dione (1) with various substituted Benzaldehyde derivatives (2a-2g) in the presence of Piperidine to obtain Benzylidine intermediates (3a-3g). Finally n-Mannich base derivatives (5a-5g) were synthesized by the reaction of Benzylidine intermediates (3a-3g) with diethyl amine in the presence of formaldehyde and DMF.

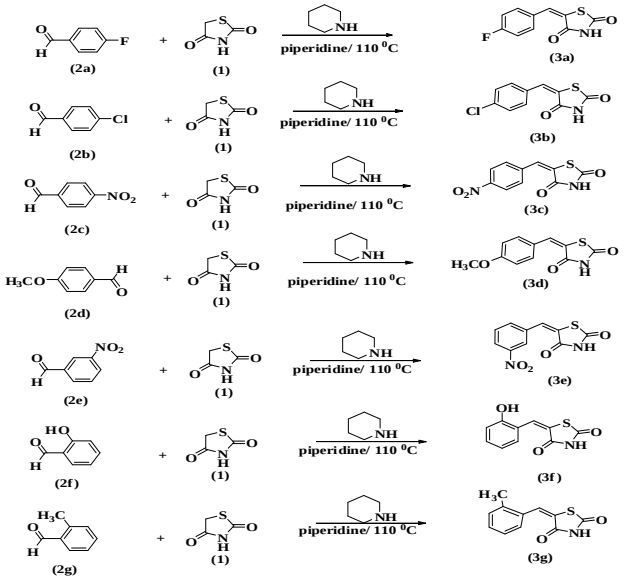
Table 1: Benzaldehyde Derivatives

Derivative	R_1	R_2	R_3
2a	-F	-H	-H
2b	-Cl	-H	-H
2c	$-NO_2$	-H	-H
2d	$-OCH_3$	-H	-H
2e	-H	$-NO_2$	-H
2f	-H	-H	-OH
2g	-H	-H	$-CH_3$

The total reactions are depicted in following scheme.



Reactions of (E)-5-benzylidenethiazolidine-2,4-dione derivatives(3a-3g)



Reactions of N-Mannich bases (5a-5g)

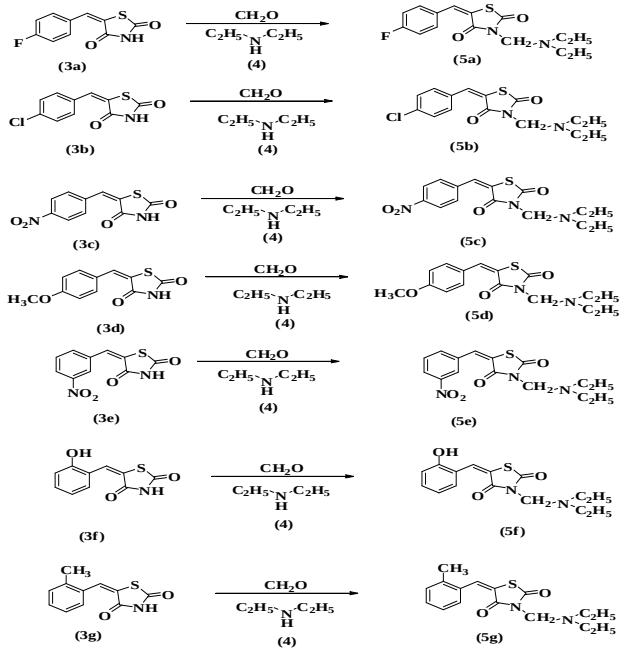


Table 2: Physical and Chemical data of Benzylidine derivatives (3a-3f)

Benzylidine Derivative	Chemical Formula	Molecular Weight	Melting Point	Yield
3a	$C_{10}H_6FNO_2S$	223.22 g	130-135°C	78%
3b	$C_{10}H_6ClNO_2S$	239.68 g	238-242°C	72%
3c	$C_{10}H_6N_2O_4S$	250.23 g	266-268°C	81%
3d	$C_{11}H_9NO_3S$	235.26 g	240-242°C	76%
3e	$C_{10}H_6N_2O_4S$	250.23 g	189-192°C	79%
3f	$C_{10}H_7NO_3S$	221.23 g	232-235°C	77%
3g	$C_{11}H_9NO_2S$	219.26 g	240-242°C	86%

Table 3: Physical and Chemical data of N-Mannich base derivatives (5a-5f)

N-Mannich bases	Chemical Formula	Molecular Weight	Melting Point	Yield
5a	$C_{15}H_{17}FN_2O_2S$	308.37 g	385-390°C	62%
5b	$C_{15}H_{17}ClN_2O_2S$	324.83 g	412-416°C	44%
5c	$C_{15}H_{17}N_3O_4S$	335.38 g	422-425°C	67%
5d	$C_{16}H_20N_2O_3S$	320.41 g	367-372°C	56%
5e	$C_{15}H_{18}N_2O_3S$	306.38 g	425-430°C	63%
5f	$C_{15}H_{17}N_3O_4S$	335.38 g	484-487°C	72%
5g	$C_{16}H_{20}N_2O_2S$	304.41 g	396-398°C	76%

Screening for Anti Diabetic Activity of Novel N-Mannich Bases

Wistar albino rats of either sex were used for the study. The animals were housed in groups of six and maintained under standard conditions (27±2°C, relative humidity 44 - 56% and light and dark cycles of 10 and 14 hours respectively) and fed with standard rat diet and purified drinking water ad libitum for 1 week before and during the experiments. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of MLR Institute of Pharmacy (Regd.no:1567/CO/a/11/CPCSEA). Alloxan was procured from Avra Labs Pvt Ltd. IND. Glibenclamide, was received as a gift drug from Aventis Pharma Ltd. All other chemicals used were of analytical grade obtained from Sd-Fine, India.

Pharmacological Studies

Oral glucose tolerance test

Rats were fasted overnight and divided into ten groups with 2 animals in each group. Group-I serves as normal.

Group-III animals were treated with Glibenclamide (0.45 mg / kg, s.c) to serve as standard. Group-IV to group-X animals were treated with Glibenclamide and n-Mannich base derivatives (5a – 5g) (0.45 and 0.50 mg /kg, s.c) in different doses. The group's normal, standard and test were treated with drugs 30 minutes prior to the glucose load (3 g/kg, s.c). Blood samples were collected at 0, 15, 30, 60, 90, 120 and 180 min after glucose loading. Serum

was separated and glucose levels were measured immediately using standard procedures.

Anti diabetic study-Interaction between Glibenclamide and n-Mannich base derivatives (5a – 5g)

In the present study, diabetes was induced by subcutaneous injection of alloxan (100 mg / kg) [11]. The alloxan was freshly prepared by dissolving 100 mg in 1ml of normal saline solution. The animals were allowed to drink glucose solution overnight to overcome the drug induced hypoglycemia. 48 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of >200 mg/dl were selected.

The rats were divided randomly into ten groups consisting of two rats each and the animals were treated for 8 days as shown in table 6.

Table 4: Spectral data of Benzylidine derivatives (3a-3g)

Compound	FTIR Absorption values (cm^{-1})
3a	3041.36 – Aliphatic C-H stretching; 2768.41 – Aromatic C-H stretching; 1697.35-Aromatic C=O Stretching; 1238.47-Aromatic C-F stretching; 1150.03 - C-N stretching; 523.60 - C-S stretching.
3b	3221.21 – Aliphatic C-H stretching; 2950.61 – Aliphatic C-H stretching; 1655.91- Aliphatic C=O Stretching; 1135.42 - C-N stretching; 706.19- Aromatic C-Cl stretching; 555.52 - C-S stretching.
3c	3199.56 – Aromatic C-H stretching; 2950.78 – Aliphatic C-H stretching; 1674.66 - Aromatic C=O Stretching; 1521.81-Aromatic N=O stretching; 1131.55 - C-N stretching; 540.01- C-S stretching.
3d	3220.85 – Aromatic C-H stretching; 2950.86 – Aliphatic C-H stretching; 1695.54-Aromatic C=O Stretching; 1181.89-Aromatic C-N stretching; 530.36 - C-S stretching.
3e	2954.01 – Aliphatic C-H stretching; 1662.11-Aromatic C=O stretching; 1516.08 N=O Stretching; 1201.43 - C-N stretching; 517.98 - C-S stretching.
3f	2949.98- Aliphatic C-N stretching; 1652.53-Aromatic C=O stretching; 1207.38-Aromatic O-H (C-O) stretching; 1129.09 - C-N stretching; 543.22 - C-S stretching.
3g	3162- Aromatic C-H stretching; 3049.52-Aliphatic C-H stretching; 2771.24 - C-H stretching in CH_3 group; 1687.78 - C-N stretching; 1151.86 - C-N stretching.

Table 5: Spectral data of n- Mannich bases (5a-5f)

Compound	FTIR Absorption values(cm^{-1})
5a	3404.96- N-H stretching; 2919.46-Aliphatic C-H stretching; 1678.7- Aromatic C=O stretching; 1240.79-Aromatic C-F stretching; 1159.51- C-N stretching; 528.45 - C-S stretching.
5b	3234.97 - N-H stretching; 1679.41-Aromatic C=O stretching; 1081.70 - C-N stretching; 753.02-Aromatic C-Cl stretching; 520.76 - C-S stretching.
5c	3269.31 – Aromatic C-H stretching; 2850.68 – Aliphatic C-H stretching; 1644.66 - Aromatic C=O Stretching; 1921.81-Aromatic N=O stretching; 1151.45 - C-N stretching; 540.01- C-S stretching.
5d	3320.15 – Aromatic C-H stretching; 2856.86 – Aliphatic C-H stretching; 1795.44-Aromatic C=O Stretching; 1281.09-Aromatic C-N stretching; 530.36 - C-S stretching.
5e	3207.40 - N-H stretching; 1681.24-Aromatic C=O stretching; 1530.31- N=O stretching; 1254.98 C-N stretching; 546.01 - C-S stretching.
5f	3252.29 - N-H stretching; 2933.74-Aliphatic C-H stretching; 1679.10-Aromatic C=O stretching; 1258.62,1387.87-Aromatic O-H (C-O stretching) 1082.50 - C-N stretching; 519.90 - C-S stretching
5g	3360- Aromatic C-H stretching; 3069.42-Aliphatic C-H stretching; 2671.34 - C-H stretching in CH_3 group; 1787.75- C-N stretching; 1351.88 - C-N stretching.

Table 6: Treatment schedule for assessing pharmacodynamic interactions between n-Mannich base derivatives (5a-5g) and Glibenclamide in diabetic rats

Group	Treatment	Purpose
Normal	Normal	To serve as normal
Control	Alloxan (100mg/kg, s.c)	To serve as disease control
Standard	Alloxan (100mg/kg, s.c) + Glibenclamide (0.45 mg / kg, s.c)	To serve as standard
5a	Alloxan (100mg/kg, s.c) + 5a (0.5 g/kg, s.c)	To study the effect of 5a
5b	Alloxan(100mg/kg, s.c) + 5b (0.50g/kg, s.c)	To study the effect of 5b
5c	Alloxan(100mg/kg, s.c) + 5c (0.5 g/kg, s.c)	To study the effect of 5c
5d	Alloxan(100mg/kg, s.c) + 5d (0.5 g/kg, s.c)	To study the effect of 5d
5e	Alloxan(100mg/kg, s.c) + 5e (0.5 g/kg, s.c)	To study the effect of 5e
5f	Alloxan(100mg/kg, s.c) + 5f (0.5 g/kg, s.c)	To study the effect of 5f
5g	Alloxan(100mg/kg, s.c) + 5g (0.5 g/kg, s.c)	To study the effect of 5g

Table 7: Effect of n-mannich base derivatives (5a-5g) and Glibenclamide on serum glucose levels

Group	Treatment	Blood Glucose Levels (Mg/Dl)		
		0th day	2nd day	4th day
Normal	Normal	78 ± 2.121	80 ± 1.826	74 ± 1.826
Control	Alloxan (100mg/kg, s.c)	310 ± 6.377[a]	280 ± 7.292[a]	297 ± 2.944[a]
Standard	Alloxan (100mg/kg, s.c) + Glibenclamide (0.45 mg / kg, s.c)	302 ± 1.581	113 ± 3.555[b]	90 ± 0.816[b]
5a	Alloxan (100mg/kg, s.c) + 5a (0.5 g/kg, s.c)	293 ± 1.581	116 ± 3.555[b]	87 ± 0.816[b]
5b	Alloxan(100mg/kg, s.c) + 5b (0.50g/kg, s.c)	299.5 ± 1.041	88.5 ± 1.771[b]	82.2 ± 2.698[b]
5c	Alloxan(100mg/kg, s.c) + 5c (0.5 g/kg, s.c)	278 ± 2.287	121 ± 2.345[b]	93.5 ± 3.116[b]
5d	Alloxan(100mg/kg, s.c) + 5d (0.5 g/kg, s.c)	2876.5 ± 2.872	131 ± 1.909[b]	113.5 ± 1.531[b]
5e	Alloxan(100mg/kg, s.c) + 5e (0.5 g/kg, s.c)	296.8 ± 1.181	84.5 ± 1.771[b]	83.2 ± 2.698[b]
5f	Alloxan(100mg/kg, s.c) + 5f (0.5 g/kg, s.c)	299 ± 2.287	98 ± 2.345[b]	83.5 ± 3.116[b]
5g	Alloxan(100mg/kg, s.c) + 5g (0.5 g/kg, s.c)	297 ± 2.287	127 ± 2.345[b]	98.5 ± 3.116[b]

a<0.001 when compared to normal (I); b<0.001 compared to control (II)

Collection of blood samples

The blood samples were withdrawn on 2nd, 4th, 6th and 8th day from the retro orbital plexus of rats under anesthesia using a glass capillary tube after a fast of 6 hr and the blood was centrifuged (2,500 rpm for 10 min) to get serum. The serum was used for biochemical estimation of blood glucose.

Effect on glucose tolerance

The blood glucose levels in the control group (G-II) increased up to 60 min after glucose load. Group III animals treated with Glibenclamide (0.45 mg/kg, s.c) didn't show significant increase in the blood glucose levels upon glucose load. Group IV to X are treated with combination of Alloxan(100mg / kg, s.c) and n-Mannich base derivatives (5a – 5g) (0.50 mg / kg, s.c) also prevented raise of blood glucose levels upon glucose load, when compared with control animals (G-II). This suggests that hypoglycemic effect of Glibenclamide is unaffected when given in combination with Mannich base derivatives (5a – 5g). Moreover, the combination was found to show additive effect.

Effect on blood glucose levels

Animals treated with Alloxan (100 mg / kg, s.c) (G-II) alone showed a significant increase in blood glucose levels on 0, 2nd and 4th day when compared to normal animals (G-I) (p<0.001) (Table-7).

Discussion on synthesized compounds

Treatment with Glibenclamide (0.45 mg/kg, s.c) (G-III) caused a significant decrease in blood glucose levels on 2nd and 4th day when compared to the control group (G-II) (p<0.001).

N-Mannich base derivatives (5a – 5g) were administered (0.45 and 0.50 mg /kg, s.c) (G-III to X), among that 5a, 5b, 5e and 5f showed a significant decrease in blood glucose levels on 2nd and 4th day when compared to the control group (G-II) respectively (p<0.001). This decrease in blood glucose level was found to be dose dependent.

Animals when treated with Glibenclamide (0.45 mg/kg, s.c) and n-Mannich base derivatives (5a, 5b, 5e and 5f) (0.50 mg / kg, s.c) (G-IV, V, VIII and IX) also showed significant decrease in blood glucose levels when compared to control group (G-II) on day 2 and 4 respectively (p<0.001). The decrease in blood glucose levels were within the normal range.

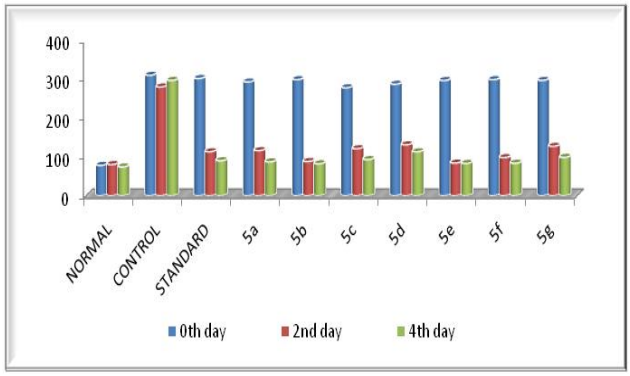


Figure 1: BAR Diagram of Effect of n-Mannich base derivatives (5a- 5g) and Glibenclamide on Blood glucose levels

CONCLUSION

The results obtained in this investigation indicate that among the compounds assayed for anti diabetic activity by using animal models (male wistar rats). Animals when treated with Glibenclamide (0.45 mg/kg, s.c) and n-Mannich base derivatives **5a, 5b, 5e and 5f** (0.50 mg / kg, s.c) **(G-IV, V, VIII and IX)** also showed significant decrease in blood glucose levels when compared to control group (G-II) on day 2 and 4 respectively (p<0.001).

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