ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and Evaluation of Some Novel 5-[4-(substituted) benzylidene] 2.4 thiazolidinediones as Oral Antihyperglycemic Agents

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ABSTRACT

Thiazolidinediones(TZDs) are a new class of antidiabetic drugs, having an insulin sensitizing effect in patients with type 2 diabetes. A few 5-[4-(substituted) benzylidene]-2,4-thiazolidinediones have been designed, synthesized and evaluated as oral antihyperglycemic agents by fructose loaded model. 1,3-thiazolidine-2,4dione was prepared with the simple reaction of chloroacetic acid (mono) and thiourea. Knoevenagel condensation was also used for preparation of Thiazolidinediones. After dehydrohalogenation with respective moiety with free amino group, title compounds were prepared. Primary antihyperglycemic screening of synthesized compounds was confirmed the activity of compound AB_2 and AB_8. Compound AB_2 and AB_8 were further screened for getting reproducible results by using model of fructose induced diabetic male Albino Wistar rats. Compound AB_2 and AB_8 was significantly decreased blood glucose levels in fructose induced diabetic male Albino Wistar rats.

Keywords: Thiazolidinediones, Antihyperglycemic activity, Knoevenagel condensation

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ISSN: 0975-8585

INTRODUCTION

Non-insulin dependent diabetes mellitus (NIDDM) is characterized by insulin resistance in the liver and peripheral tissues together with a pancreatic-cell defect. The insulin resistant state at the peripheral level causes impaired glucose utilization leading to hyperglycemia [1]. This insulin resistance together with the hyper-insulinemia that is often present may also play a role in the etiology of a wider spectrum of metabolic disorders such as obesity, hypertension and atherosclerosis. A new series of 5(4'-alkoxy benzyl)-2, 4-thiazolidinediones were reported by Sohda as antihyperglycemic agents [2].

A large number of antihyperglycemic 5-(substituted) benzyl-2,4-thiazolidinediones have been reported during the last twenty years or so after the initial report of the activity of the ciglitazone, the prototype of this class of the drugs. Ciglitazone was never clinically used because of the toxicity associated with it but led to the development of more potent and clinically useful "glitazones" such as troglitazone , pioglitazone, englitazone and rosiglitazone. Troglitazone was first marketed in the US in 1997 under the trade name Rezulin but had been banned and withdrawn from the US market due to hepatotoxicity in February 2000 [3].

In the present work, it has been tried to introduce some of the structural features of 2-(P-aminobenzenesulphonamido)-5-isopropyl -1,3,5-thiadiazole (IPTD) and those of clinically useful 2,4-thiazolidinediones in designing and synthesizing a series of 5-[4-(substituted) benzylidene)-2,4-thiazolidinediones. In this series, 2,4-thiazolidinedione moiety serves as the head end and the heteroaromatic or the aromatic moiety serves as the tail end of the molecules, and the two ends are linked through the 5-position of 2,4-thiazolidinediones via p-aminobenzylidene and p-sulfonamidobenzylidene moiety. The p-aminobenzylidene and p-sulfonamidobenzylidene nitrogen is attached to various lipophilic heterocyclic or carbocyclic moieties so that the acidity of the sulfonamido hydrogen changes marginally and may play a role in binding with the receptor. Initially, only a limited number of heterocyclic and aromatic systems were introduced so as to verify whether the final thiazolidinediones possess significant activity with the aim to develop better and safer drugs than the available ones, and also to verify the usefulness of such moieties in maintaining the antihyperglycemic activity with low tolerable toxicity.

Studies in rats have demonstrated that high intake of fructose produced a decline of insulin sensitivity in the liver and peripheral tissues. Thus, fructose has been implicated as the useful tool to induce insulin resistance in animals. In the present study, we employed rats with insulin resistance induced by administration of 40%w/v aq. fructose solution, 4g/kg through oral gavage to investigate the effects of newly synthesized TZDs.

ISSN: 0975-8585

MATERIALS AND METHODS

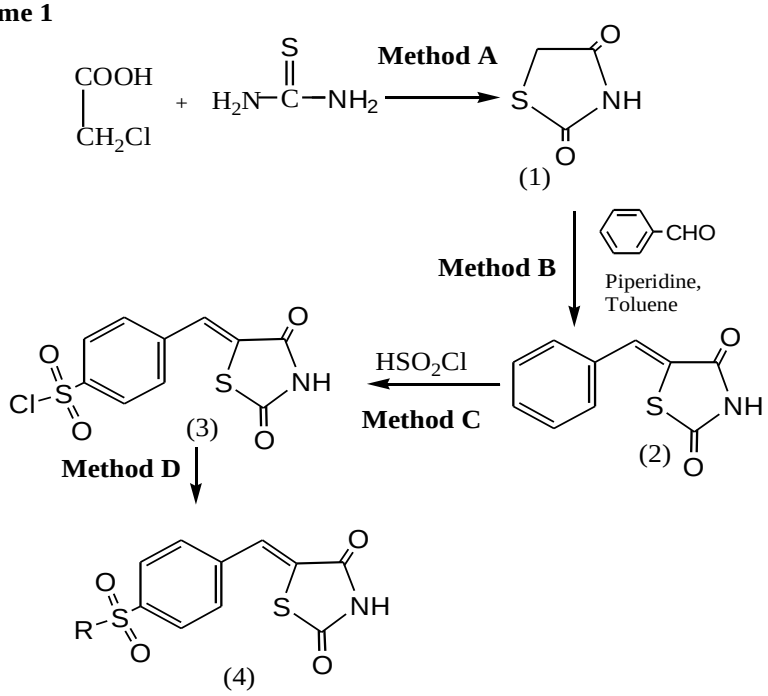
EXPERIMENTAL

The melting point of the compounds was determined on a Elico melting point apparatus and the values were uncorrected. I.R spectras of the compounds were recorded in KBr on Perkin-Elmer AC-1 Spectrophotometer, Glenmark Pharmaceuticals Ltd. Nashik. 1H NMR spectra were recorded on BROOT spectrophotometer (800 MHz) using DMSO-d_6 as solvent, at Analytical Centre, university of Pune. Mass spectra were recorded on Shimadzu QP2010, MGV college of Pharmacy, Nashik.

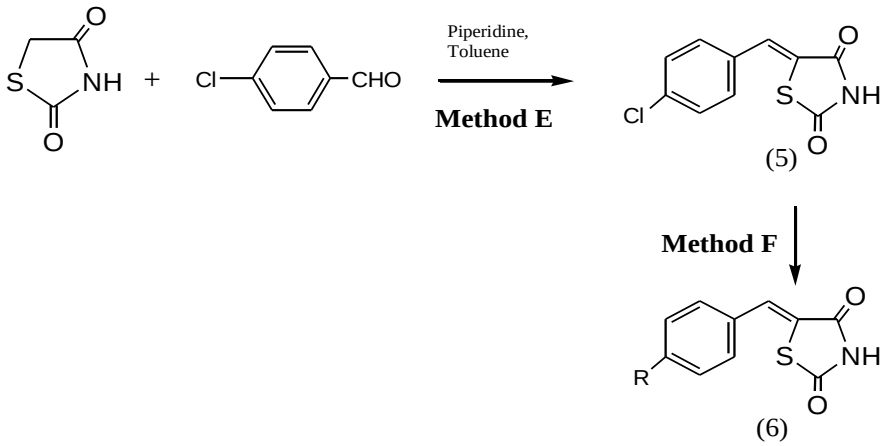
All the solvents used were of analytical grade. Reactions were monitored with the help of TLC.

Reaction Schemes followed for the synthesis of compounds [4]

Scheme 1



Scheme 2



Method A: Chloroacetic acid, Thiourea, Water, Conc. HCl. Method B: 2,4 Thiazolidine-dione, Benzaldehyde, Dry toluene, Piperidine. Method C: Benzylidine-2,4 Thiazolidine- dione, Chlorosulphonic acid. Method D: R (aryl/heterocyclyl moiety with free amino group), Dry Pyridine, Acetic anhydride. Method E: 2,4 Thiazolidinedione, p-chlorobenzyldehyde, Piperidine, Dry toluene. Method F: R (aryl/heterocyclyl moiety with free amino group, Dry Pyridine, Acetic anhydride.

General methods of preparation.

Method A: Preparation of 2,4-thiazolidinedione (1) (Scheme 1) :

In a 250ml three-necked flask was placed, a solution containing 56.6 g (0.6 M) of chloroacetic acid in 60 ml of water and 45.6 g (0.6M) of thiourea dissolved in 60 ml of water. The mixture was stirred for 15 min to obtain a white precipitate, accompanied by considerable cooling. To the contents of the flask was then added slowly 60 ml conc. Hydrochloric acid from a dropping funnel. The flask then connected with the reflux condenser and gentle heating was applied to effect complete dissolution, after which the reaction mixture was stirred and refluxed for 8-10 hr at 100-10^{0}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 hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol. Yield 85%, m.p. 123-25^{0}C.

Method B: Preparation of 5-benzylidene-2,4-thiazolidinedione (2) (Scheme 1) :

In a 250ml three-necked flask provided with a Dean-Stark apparatus, benzaldehyde (20 g, 0.188 mole) and 2,4-thiazolidinedione (22 g, 0.188 mole) were together suspended in dry toluene. To this catalytic amount of piperidine (1ml) 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. Yield 89-93%, m.p. 240-42^{0}C.

Method C: Preparation of 4'chlorosulphonylbenzylidene-2,4-thiazolidinedione (3) (Scheme 1) :

Benzylidene-2,4-thiazolidinedione (8g, 0.0388 mole) was placed in a 100ml RBF equipped with a condenser and dropping funnel. Chlorosulphonic acid (18.08 g, 0.155 mole) was added at room temperature using dropping funnel. The reaction was found to be exothermic. After addition of chlorosulphonic acid was over, the mixture was refluxes for 1 hr on a water bath. The reaction mass was cooled and poured in a thin stream with stirring into crushed ice. It was filtered and dried. Yield 68%, m.p. 180-81^{0}C.

Method D: Preparation of 5-[4-(substituted) sulfonyl benzylidene] 2,4-thiazolidinedione (4) (Scheme 1) :

Substituted amine (or heterocycle) (0.1 mole) and 4'chlorosulphonylbenzylidene-2,4-thiazolidinedione (0.1 mole) were added to a mixture of 4ml of dry pyridine and 20 ml of acetic anhydride. The mixture was refluxed for 2 hr, the reaction mixture was then poured into 20 ml of ice-water and solid obtained was filtered and purified by recrystallization from ethanol.

Method E: Preparation of 5-(4-Chlorobenzylidene)-2,4-thiazolidinedione (5) (Scheme 2) :

In a 250ml three-necked flask provided with a Dean-Stark apparatus, P-Chlorobenzaldehyde (26.41 g, 0.188 mole) and 2,4-thiazolidinedione (22 g, 0.188 mole) were together suspended in dry toluene. To this catalytic amount of piperidine (1ml) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110^0C the reaction mixture was stirred for further 30 min. On cooling, the product precipitated out from toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol. Yield 89-93%, m.p. 240-42^0C.

Method F: Preparation of 5-[4-(substituted) benzylidene] 2,4-thiazolidinedione (6) (Scheme 2):

Substituted amine (or heterocycle) (0.1 mole) and 5-(4-chlorobenzylidene)-2,4-thiazolidinedione (0.1 mole) were added to a mixture of 4ml of dry pyridine and 20 ml of acetic anhydride. The mixture was refluxed for 2 hr, the reaction mixture was then poured into 20 ml of ice-water and solid obtained was filtered and purified by recrystallization from ethanol.

Spectral Analysis [5, 6, 7, 8, 9, 10]

AB_1: 5-[4-(4-methoxy anilinyl) benzylidene] 2, 4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3370.00 (N-H TZD str.), 3350.00 (N-H sec.amide str.), 3055.00 (C-H Ar. str.), 1610.49,1580.00,1511.32,1488.67 (C=C Ar.str.), 1705.00,1740.00 (C=O str.) 1169.57,1402.57 (C-N str.), 771.57 (C-S-C str.)
^{1}H NMR (DMSO-d_6,δ ppm): 12.660 (s, br.1H, N-H TZD), 9.756 (s, sh.1H, N-H sec.amide), 7.732 (s, sh. 1H Arylidene), 6.987 (d, m, 2H, Ar.), 7.479 (d, m, 2H, Ar.), 7.172 (d, m, 2H, Ar.), 7.451 (d, m, 2H, Ar.), 3.76 (s, sh, 3H, OCH_3).
Mass: m/z: 326(M^+)

AB_2: 5-{4-[(4-methoxy anilinyl) sulfonyl] benzylidene} 2, 4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3380.00 (N-H TZD str.), 3234.70 (N-H sulfonamide str.), 3022.00 (C-H Ar. str.), 1611.00,1570.00,1509.61 and 1460.00 (C=C Ar.str.), 1705.00,1747.68 (C=O str.) 1162.22, 1410.00 (C-N str.), 751.42 (C-S-C str.), 1190.00,1320.00 (S=O str.)
^{1}H NMR (DMSO-d_6, δ ppm): 10.063 (s, br.1H, N-H TZD), 7.797 (s, sh.1H, N-H sulfonamide), 7.797 (s, sh. 1H Arylidene), 7.278 (d, m, 2H, Ar.), 7.551 (d, m, 2H, Ar.), 7.307 (d, m, 2H, Ar.), 7.577 (d, m, 2H, Ar.), 3.759 (s, sh, 3H, OCH_3).
Mass: m/z: 390(M^+)

AB_3: 5-[4-(4-methoxy, 2- nitro anilinyl) benzylidene] 2, 4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}); 3360.00 (N-H TZD str.), 3340.00 (N-H sec.amide str.), 3022.00 (C-H Ar. str.), 1609.76,1586.89,1537.11 and 1450.00 (C=C Ar.str.), 1705.00,1747.00 (C=O str.) 1187.98, 1402.81 (C-N str.), 755.00 (C-S-C str.)
^{1}H NMR (DMSO-d_6,δ ppm); 12.664 (s, br.1H, N-H TZD), 10.08 (s, sh.1H, N-H sec.amide), 7.827 (s, sh. 1H Arylidene), 7.457 (s, m, 1H, Ar.), 7.671 (d, m, 2H, Ar.), 7.569 (d, m, 2H, Ar.), 7.754 (d, m, 2H, Ar.), 3.895 (s, sh, 3H, OCH_3).
^{13}C NMR (DMSO-d_6,δ ppm); 124.236 (C-5), 167.195, 167.589 (C=O), 130.360 (C-H), 129.294, 131.555, 131.855 and 134.966 (C-Aromatic), 39.98 (OCH_3).

AB_4: 5-{4[(4-methoxy,2-nitro anilinyl) sulfonyl] benzylidine} 2,4- Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3418.14 (N-H TZD str.), 3250.00 (N-H sulfonamide str.), 3050.00 (C-H Ar. str.), 1648.00,1609.00,1554.00 and 1500.00 (C=C Ar.str.), 1710.00, 1746.85 (C=O str.) 1044.00, 1402.49 (C-N str.), 755.35 (C-S-C str.), 1188.68, 1342.07 (S=O str.)
^{1}H NMR (DMSO-d_6, δ ppm): 8.071 (s, sh.1H, N-H sulfonamide), 7.504 (s, sh. 1H Arylidene), 6.839 (d, m, 2H, Ar.), 7.479 (s, m, 1H, Ar.), 6.550 (d, m, 2H, Ar.), 7.132 (d, m, 2H, Ar.), 4.093 (s, sh, 3H, OCH_3).

AB_5: 5-[4-(4-amino antipyrinyl) benzylidine] 2,4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3360.00 (N-H TZD str.), 3220.00 (N-H sec.amide str.), 1609.95, 1580.00, 1500.00 and 1488.79 (C=C Ar.str.), 1700.00,1741.00 (C=O str.) 1011.51, 1402.85 (C-N str.), 770.06 (C-S-C str.), 1334.13 (C-N Ar.str.)
^{13}C NMR (DMSO-d_6,δ ppm); 129.616 (C-5), 174.556, 179.997 (C=O), 130.216 (C-H), 129.616, 105.71, 111.499, 114.196, 137.307, 147.54, 157.292 and 157.997 (C-Aromatic), 55.8 (OCH_3).

AB_6: 5-{4-[(4-amino antipyrinyl) sulfonyl] benzylidine} 2,4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3360.00 (N-H TZD str.), 3220.00 (N-H sulfonamide str.), 3060.09 (C-H Ar. str.), 1607.67, 1580.00, 1500.00, 1410.00 (C=C Ar.str.), 1705.00, 1747.68 (C=O str.) 1060.00, 1410.00 (C-N str.), 751.26 (C-S-C str.), 1164.53, 1318.49 (S=O str.)

AB_7: 5-[4-(8-amino quinolinyl) benzylidine] 2,4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3350.00 (N-H TZD str.), 3310.00 (N-H sec.amide str.), 3050.00 (C-H Ar. str.) 1610.13, 1586.92, 1500.00 and 1488.79 (C=C Ar.str.), 1700.00,1740.00 (C=O str.) 1220.24, 1402.80 (C-N str.), 767.88 (C-S-C str.), 1334.37 (C-N Ar.str.)

AB_8: 5-{4-[(8-amino quinolinyl) sulfonyl] benzylidine} 2,4-Thiazolidinedione.

I.R. (KBr, Cm^{-1}): 3421.81 (N-H TZD str.), 3220.00 (N-H sulfonamide str.), 3030.00 (C-H Ar. str.), 1609.71,1580.00, 1500.00 and 1402.85 (C=C Ar.str.), 1705.00, 1746.02 (C=O str.) 1240.70, 1402.85 (C-N str.), 780.00 (C-S-C str.), 1189.15, 1341.63 (S=O str.), 1310.00 (C-N Ar.str.)

PHARMACOLOGICAL SCREENING

The studies were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India) and Institutional Animal Ethical Committee (IAEC/ 2009).

Animals

Male Wistar rats weighing 130–170g, age 6–8 weeks were used for the study [11]. Animals were housed in colony cages at ambient temperature of 25 ± 2 ºC, 12 hour light: 12 hour dark cycle and 50 ± 5 % relative= humidity with free access to food and water ad libitum.

Experimental design

Experiments are performed in healthy male albino Wistar rats (National Institute of Biosciences, Pune), weighing in the range of 130-170g. Rats are fed with the normal diet (Pranav Agro Industries Ltd., Sangli) containing following contents.

Energy	: 3625 Kcal/Kg.
Crude Protein	: 22.10%
Crude Oil	: 4.02 %
Crude Fibre	: 4.09%
Ash	: 10.02%
Sand Silica	: 0.70 %

Animals were divided into 11 groups each containing 5 animals. Food but not water was deprived before 8 hour and during the experiment. All the experiments were carried out during the light period (09:00-17:00 h).

A) **Untreated Group**

Group I : Normal Control Group (Fed with normal diet without fructose).

B) **Treated Groups**

(All treated groups are fed on normal diet with 40%w/v aq.fructose solution 4g/kg/day, by oral gavage, for 21 days).

Group II : Fructose Treatment Control Group
Group III : Pioglitazone as a standard [12] (20mg/Kg.p.o.)
Group IV : AB_1 (100mg/Kg.p.o.)
Group V : AB_2 (100mg/Kg.p.o.)
Group VI : AB_3 (100mg/Kg.p.o.)
Group VII : AB_4 (100mg/Kg.p.o.)
Group VIII : AB_5 (100mg/Kg.p.o.)
Group IX : AB_6 (100mg/Kg.p.o.)
Group X : AB_7 (100mg/Kg.p.o.)
Group XI : AB_8 (100mg/Kg.p.o.)

(Test compounds and pioglitazone were formulated in suspension by using 2% w/v sodium carboxy methyl cellulose and distilled water before dosing) All animals were fed with the normal diet. Experimental animals were given 40%w/v aq. solution of fructose (4g/kg/day), by oral gavage for three weeks, while untreated control animals were given 2% w/v sodium carboxy methyl cellulose in distilled water through oral gavage. This dose was well-tolerated by the animals with no evidence of diarrhea as reported in the literature[13]. Induction of hyperglycemia in male rats was confirmed by measuring the blood glucose level on 0, 7^{th} , 14^{th} and 21^{st} day of the experiment and all hyperglycemic rats were used for the experimental study. Important parameters like body weight, food intake, water intake, righting reflex, pupil size, mood, reactivity were daily monitored during the study. Fructose treatment was discontinued from 21^{st} day of the experiment. First dose treatment was given on 21^{st} day and second dose treatment was given on 24^{th} day of the experiment to the 8 hour fasted rats. Blood glucose level of the treated animals was measured after 2, 5 and 24 hour. Blood drop is removed from tail vein for testing plasma glucose level with the help of glucometer (Accusure, TaiDoc Technology Corporation, Taiwan.).

To economize the study, Antihyperglycemic activity of the test compounds (TZDs) was confirmed by first trial on the 8 hour fasted, fructose induced hyperglycemic male Albino Wistar rats (n = 2) and selected active test compounds (TZDs) were further screened for getting reproducible results.

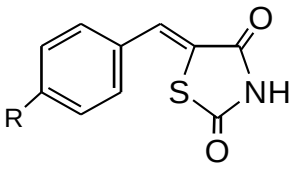
Statistical Analysis

All the data were expressed as mean ± S.E.M. Statistical differences were calculated for each of the above mentioned parameters using the Student's t-test and one way ANOVA followed by dunnett's test for multiple comparisons. A probability level of $p < 0.05$ was taken to indicate a significant difference between means.

ISSN: 0975-8585

RESULT AND DISCUSSION

Table – 1: Structures of the synthesized TZD derivatives



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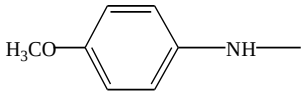
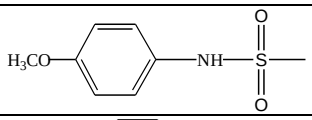
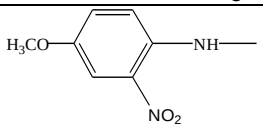
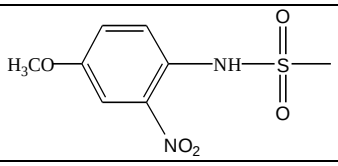
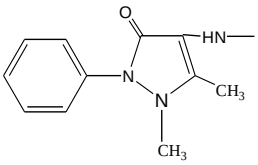
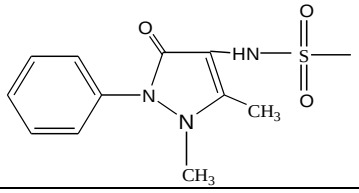
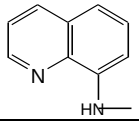
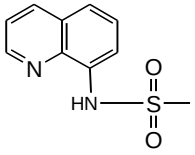
1	AB_1	
2	AB_2	
3	AB_3	
4	AB_4	
5	AB5	
6	AB6	
7	AB7	
8	AB8	

ISSN: 0975-8585

Table – 2: Physical data of 5-[4-(substituted) benzylidene] 2.4 thiazolidinediones derivatives

Compound	R	Molecular Formula	Melting Point ^0C	% Yield	Rf Value
AB_1	p-methoxy anilinyl	$C_{17}H_{14}N_2O_3S_1$	218-219	89.09	0.28
AB_2	p-anisidine N-sulfonyl	$C_{17}H_{14}N_2O_5S_2$	247-248	49.23	0.41
AB_3	2-Nitro, p-methoxy anilinyl	$C_{17}H_{13}N_3O_5S_1$	230-231	86.33	0.31
AB_4	2-Nitro, p-anisidine, N-sulfonyl	$C_{17}H_{13}N_3O_7S_2$	290-291	91.99	0.53
AB_5	4-Antipyrinyl amino	$C_{21}H_{18}N_4O_3S_1$	217-218	82.72	0.64
AB_6	4- Antipyrinyl amino, N- sulfonyl	$C_{21}H_{18}N_4O_5S_2$	161-162	61.27	0.76
AB_7	8-Quinolinyl amino	$C_{19}H_{13}N_3O_2S_1$	215-216	73.06	0.52
AB_8	8-Quinolinyl amino, N-sulfonyl	$C_{19}H_{13}N_3O_4S_2$	272-273	61.34	0.57

Table – 3: Effect of Fructose feeding on body weight, food intake and water intake of rats before the treatment of drugs.

Group ⟶ Parameter ↓	NCG	FCG	PFG	AB_2FG	AB_8FG
Body Weight (g)	164.2 ± 3.36	181.2 ± 4.59	179.4 ± 4.20	180 ± 6.85	174.8 ± 7.11
Food Intake per group (g)	33.27 ± 0.66	24.31 ± 0.64***	24.18 ± 0.59***	24.18 ± 0.40***	25.04 ± 0.37***
Water Intake per group (ml)	55.68 ± 0.754	79.59 ± 0.64***	81.18 ± 0.80***	80.36 ± 0.65***	79.31 ± 0.57***

NCG: Normal control group, FCG: Fructose control group, PFG: Pioglitazone with Fructose treatment group. AB_2FG: AB_2 with fructose treatment group. AB_8FG: AB_8 with fructose treatment group.
Values are expressed as Mean ± S.E.M. for five animals in each group (n = 5).
All groups are compared with control treatment group
* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs Control croup
One-way ANOVA followed by Dunnett's test.

Table – 4: Effect of Fructose feeding on body weight and glucose of rats before the treatment of drugs.

Group	Blood Glucose (mg/dL)			
	#Before	After First Dose Drug Treatment		
		2h	5h	24h
NCG	97.8 ± 1.68	96.2 ± 2.08	96± 1.22	89.4 ± 1.63
FCG	156 ± 3.04	146± 2.32**	143.2± 2.05**	139.4± 2.48**
PFG	152.6 ± 3.98	112.2± 1.31*	92.2± 1.35**	120.4± 1.63*
AB_2FG	147.2 ± 2.97	121± 1.22*	100.2± 3.49**	121.2 ± 0.8*
AB_8FG	151 ± 2.91	126.4 ± 3.73*	94.4± 1.43**	128 ± 1

ISSN: 0975-8585

NCG: Normal control group; FCG: Fructose control group; PFG: Pioglitazone with fructose treatment group; AB_2FG:AB_2 with fructose treatment group, AB_8FG: AB_8 with fructose treatment group. # Before the drug treatment; †After the second dose treatment
Values are expressed as Mean ± S.E.M. for five animals in each group (n = 5).
Fructose control group was compared with Normal control group (Unpaired Student's t-test). Other groups were compared with fructose control group (One-way ANOVA followed by Dunnett's test.). $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

Table – 5: Effect of AB_2 and AB_8 on blood glucose on fructose induced diabetic rats.

Group	Blood Glucose (mg/dL)			
	#Before	After First Dose Drug Treatment		
		2h	5h	24h
NCG	97.8 ± 1.68	96.2 ± 2.08	96± 1.22	89.4 ± 1.63
FCG	156 ± 3.04	146± 2.32**	143.2± 2.05**	139.4± 2.48**
PFG	152.6 ± 3.98	112.2± 1.31*	92.2± 1.35**	120.4± 1.63*
AB_2FG	147.2 ± 2.97	121± 1.22*	100.2± 3.49**	121.2 ± 0.8*
AB_8FG	151 ± 2.91	126.4 ± 3.73*	94.4± 1.43**	128 ± 1

NCG: Normal control group; FCG: Fructose control group; PFG: Pioglitazone with fructose treatment group; AB_2FG: AB_2 with fructose treatment group, AB_8FG: AB_8 with fructose treatment group. # Before the drug treatment; † After the second dose treatment
Values are expressed as Mean ± S.E.M. for five animals in each group (n = 5).
Fructose control group was compared with Normal control group (Unpaired Student's t-test). Other groups were compared with fructose control group (One-way ANOVA followed by Dunnett's test.).
$p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$

TZDs sensitizing effect on insulin increases the efficiency of available insulin in the body. This is turn helps the pancreas allowing it to produce, store less insulin and to release less insulin in the blood circulation without hampering normoglycemic condition. Because of these special advantages of TZDs, the physicians around the world are preferring use of TZDs over other available drugs. These prompted pharmaceutical industries and researchers to deeply investigate the ideal structural requirements of TZDs with a view to obtain better and safer TZDs than the existing ones.

The prevalence of insulin resistance and associated diseases has risen seriously around the world. The general view of insulin action places this hormone at the point of multiple organ adaptations to the ingested nutrients, in particular, dietary carbohydrates. It has been established that insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertension and hyperlipidemia are associated with fructose intake in animal models [14]. Increasing consumption of dietary fructose might be one of the factors responsible for the development of obesity and the accompanying insulin resistance syndrome. Thus, rats received 21 days 40%w/v

aq. fructose solution (4g/kg) administered through oral gavage could be served as a reliable model than the other models for the investigation of insulin resistance.

On critical scrutiny of the structure of rosiglitazone and pioglitazone, one can draw conclusions that Thiazolidinedione hypoglycemic agents can be viewed as being composed of an acidic head group connected to a lipophilic tail by a alkoxy benzyl linker. From the available SAR literature, it is clear that in 5-(substituted benzyl)-2,4-thiazolidinediones, 5-benzyl-2,4-thiazolidinedione moiety is essential for activity and possibly has important role in binding with the receptor [15]. Some reported 5-(substituted benzylidene)-2,4-thiazolidinediones are also having better activity [16]. The weakly acidic imido hydrogen of the 2,4-thiazolidinedione moiety probably forms hydrogen bond with a receptor moiety and therefore, seems essential for activity as a number of N-substituted 2,4-thiazolidinediones are reported to possess either very less activity or no activity.

Also distance between lipophilic tail group and acidic head group appears to be important for binding with the receptor and activity. As per SAR study, increase or decrease of this distance significantly affects activity.

IR spectra supports well with the structures of the tile compounds. H^1NMR and C^{13}NMR spectral data of representative compounds were taken, and the same fully supported the structure of those compounds. Two of the compounds were analyzed for mass spectroscopy for final confirmation of structure.

Feeding of 40%w/v aq. fructose solution (4g/kg) to rats by oral gavage for 21 days shown significant increase in water intake, blood glucose, while significant decrease in food intake and non significant increase in body weight as compared to normal control group (Table No.4).

Primary antihyperglycemic screening of all synthesized compounds shown that AB_2, AB_8, Pioglitazone were decreasing the blood glucose level in fructose induced diabetic rats. Sulfonyl linkage appears to be optimum for activity while direct linking of substituted amines to the benzylidene 2,4 thiazolidene leads to the loss of activity.

Compound AB_2 and Pioglitazone was significantly decreased the blood glucose level after 2h, 5h and 24h while compound AB_8 was significantly decreased the blood glucose level after 2h and 5h as compared to fructose control group. (Table No.5)

CONCLUSON

With the introduction of sulfonyl linker moiety the R_f value of the target molecules were increased and also characteristic IR peaks of N-H (TZD) stretching band of TZD molecules shifted to higher value. This shows an increase in lipophilicity and decrease in the acidity of imido hydrogen. It is concluded that compound AB_2 and AB_8 decreases the blood glucose level in fructose induced diabetic rats though about five times less potent than the standard drug

pioglitazone. This showed that sulfonyl moiety as a spacer between acidic head group and lipophilic tail end of the two target molecules (AB_2 and AB_8) can be useful for the activity but possibly requires suitable moiety in other parts of the molecules. To optimize activity and toxicity of such compounds more work on synthesis and screening are necessary in future.

Interestingly, the compound AB_2 showed longest duration of action among all synthesized compounds. This gives impression that suitable modification of AB_2 can lead to potent long-acting TZDs, and this work can be undertaken in future to design and synthesize better and safer TZDs

ACKNOWLEDGEMENTS

The authors are thankful to Analytical Department, University of Pune. Also thankful to N.D.M.V.P. Samaj's College of Pharmacy, Nashik for providing facilities to carry out research work.

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