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Evaluation of thiazolidinedione derivatives for acute toxicity and potential antidiabetic activity

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ABSTRACT

The new TZD derivatives prepared (i.e. ***A1, A2, A5, B2, C1 and D1****) were screened in vivo for acute oral toxicity study for the doses of 30 and 100 mg/kg in Wistar rats and were found to be safe as they did not show any treatment related adverse effects. So these six derivatives were evaluated for antidiabetic activity using the experimental model of diabetes induced by alloxan in rats for acute as well as subacute study.The results of the study revealed that most of the compounds tested showed moderate to good antidiabetic activity. The compound* ***C1*** *was found to be the most active in all synthesized compounds which have nearly similar glucose lowering activity as that of standard drugs i.e. metformin and pioglitazone at dose of 100 mg/kg of body weight.*

Keywords: Thiazolidinediones, alloxan, antidibetic activity.

INTRODUCTION

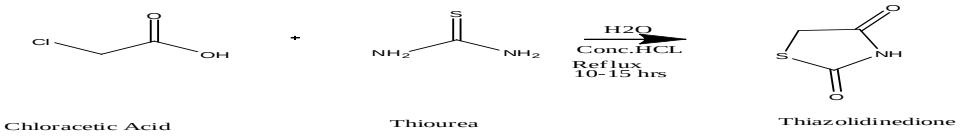
Present work is approved by the IAEC under the title “Synthesis & Bioogical Evaluation of Thizolidinedione derivatives for Antidiabetic Activity” at S.M.B.T. College of Pharmacy, Nashik. Thiazolidinediones molecule has basic pharmacophore which shows wide range of Pharmacological Activity like Antidiabetic, Anticancer, Antimicrobial, and Anti-inflammatory, this review paper shows different derivatives & biological activity of thiazolidinedione[1]. Thiazolidinediones acts by binding to PPARs (Peroxisome Proliferator Activated Receptors) [2], a group of receptor molecule inside the cell nucleus. The normal ligands for PPAR receptor are free fatty acids & eicosanoids. PPARs have been identified in three forms: alpha, gamma and delta[3]. Thiazolidinediones derivatives synthesized as per ref.[4] an performed acute toxicity studies & antidibetic activity.

MATERIALS AND METHODS

1.1. Experimental

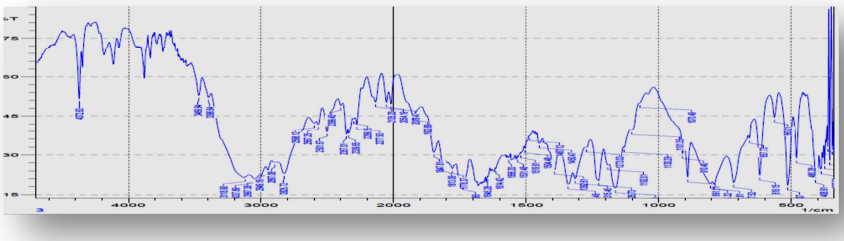
The melting points were determined by open cup capillary method and are uncorrected. TLC analyses were performed on glass plates using silica gel G60 and spots were visualized either by ultraviolet light or by iodine

vapours. IR spectra were recorded as KBr pellets, using JASCO 4100 FTIR spectrophotometer. 1H-NMR were obtained with BRUKER AVANCE II 400 NMR spectrometer and are reported as parts per million (ppm) downfield to TMS. A mass spectrum was recorded on PRA-O-336 wiff Turbo Spray mass spectrometer.



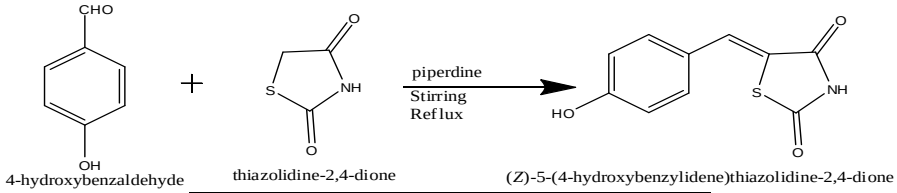
Chloracetic Acid Thiourea Thiazolidinedione

IR Spectra



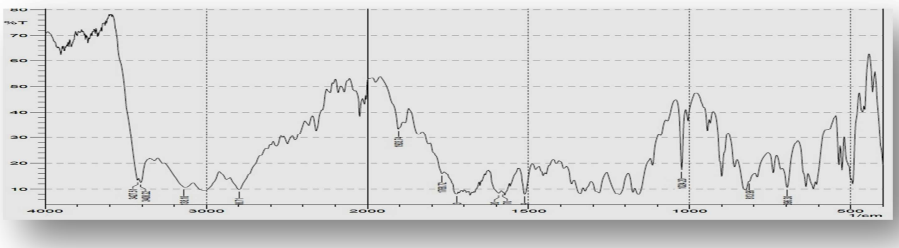
Sr.No.	Groups	Observed (cm^{-1})	Reported (cm^{-1})
1	-C=O	1737	1745
2	-S-C=O	1778	1730
3	-NH	3400	3200-3400
4	$-CH_2$	2949	2900-3000

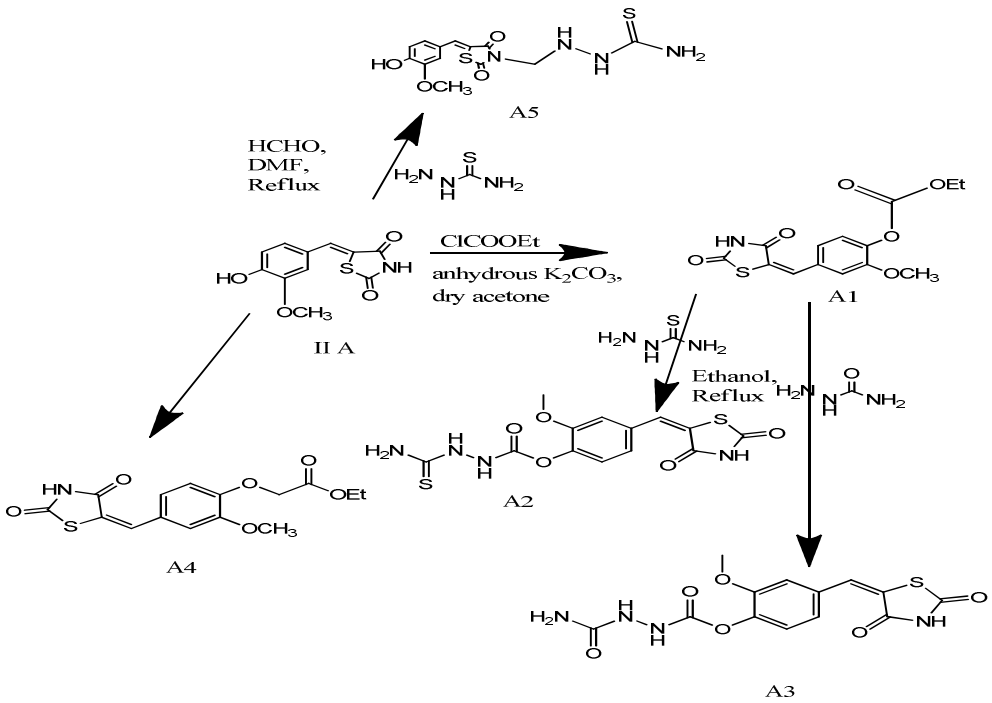
Step- II :- 5-substituted-2,4-thiazolidinedione



4-hydroxybenzaldehyde thiazolidine-2,4-dione (Z)-5-(4-hydroxybenzylidene)thiazolidine-2,4-dione

Sr no.	Groups	Observed (cm^{-1})	Reported (cm^{-1})
1	**-C=O**	1722	1745
2	**-C=O**	1768	1730
3	**-NH**	3408	3200-3400 Sharp
4	**-OH**	3138	3200-3400 Broad
5	**-C=C**	1593	1600
6	**-C-O**	1024	1100

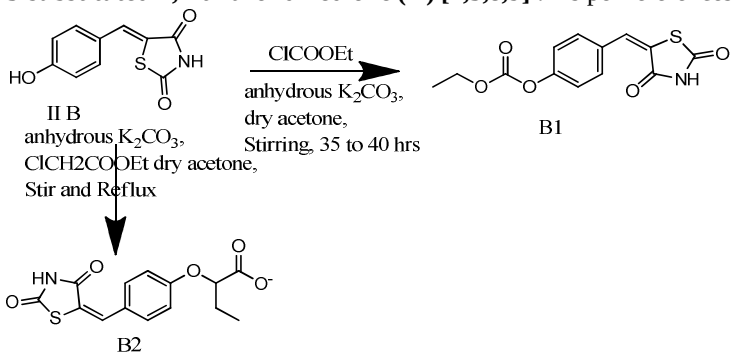




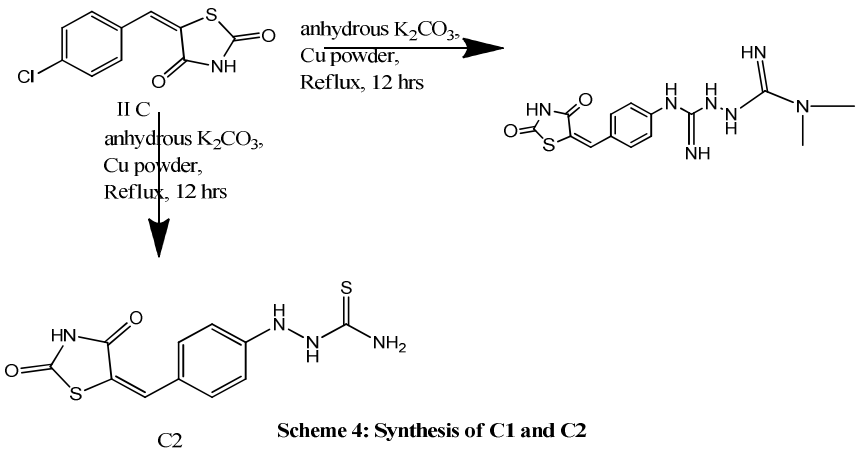
Scheme 2: Synthesis of A1-A5

1.1.1. Synthesis of 2,4-thiazolidinedione (I)[4,5,6,7] : As per references.

1.1.2. Synthesis of 5-substituted-2,4-thiazolidinedione (II) [4,5,8,9] : As per references.

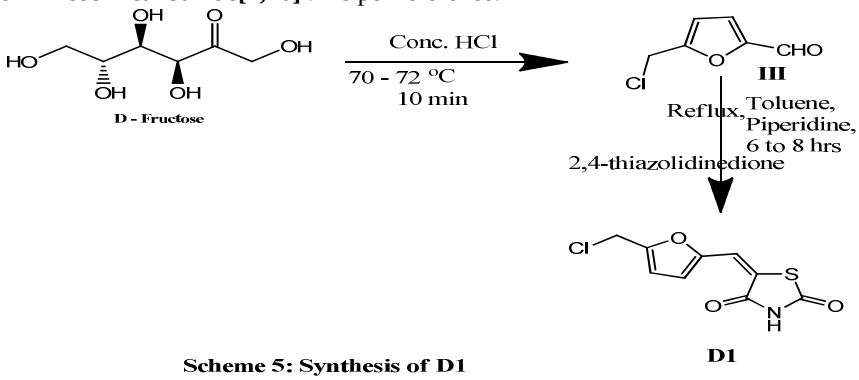


Scheme 3: Synthesis of B1 and B2



Scheme 4: Synthesis of C1 and C2

1.1.3. Synthesis of Thiosemicarbazide[4,10] *:* As per reference.



Scheme 5: Synthesis of D1

1.1.4. Synthesis of 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl]-2-methoxyphenyl ethyl carbonate (A1) and 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl ethyl carbonate (B1)[4,11]

As per ref.[2] $C_{14}H_{13}NO_6S$;Yield: 95 %; m.p.: 200-202 ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.72. IR cm-1: Imide C=O str.(1705,1756); ester C=O str.(1786); C-O-C (ether) str. (1262,1216); =C-H str.(2995); N-H str.(3223); C-N str.(1302); C-S str.(679); Aro. C=C str.(1547). 1H NMR (δ ppm, DMSO): 1.27(t,3H,CH3); 2.58(s,DMSO); 3.91(s,3H,OCH_3); 4.52(q,2H,CH2); 7.11-7.75 (m,3H,Aro. CH); 7.93(s,H,CH-benzylidene); 11.76(s,H,N-H of TZD).

1.1.5. General Procedure for synthesis of 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl]-2-methoxyphenyl 2-carbamothioylhydrazinecarboxylate(A2) and 4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenyl 2-carbamoylhydrazinecarboxylate (A3)[12]:

As per ref.[2] $C_{13}H_{12}N_4O_5S_2$; Yield: 65%; m.p.: 218-220ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.57. IR cm-1: Imide C=O str.(1608,1705); Ar-O-C=O str.(1746); C-O-C (ether) str.(1266, 1300); =C-H str.(2998); NH_2 str.(3459); N-H str.(3219, 3423); NH_2-CS-NH str.(1169); C=S str.(1369); C-S str. (680); C=C str. (1518). 1H NMR (δ ppm, DMSO): 3.90(s,3H,OCH_3); 2.59 (s,DMSO); 7.10-7.66(m,3H,Aro. CH); 7.73(s,H,CH-benzylidene);8.64(s,2H,NH_2); 10.05-10.38 (m,2H,NH of TSC group); 12.31(s,H,NH of TZD).

4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]-2-methoxyphenyl-2-carbamoyl-hydrazinecarboxylate(A3):

$C_{13}H_{12}N_4O_6S_2$; Yield: 60%; m.p.: 174-176ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.34. IR cm-1: Imide C=O str.(1608, 1704); Ar-O-C=O str.(1745); NH_2-C=O (1517); C-O-C(ether) str.(1262, 1302); =C-H str.(3072); NH_2 str.(3463); N-H str.(3226, 3419); NH_2-CS-NH str.(1172); C=S str.(1369); C-S str. (680), Aro. C-H str. (2997).

1.1.6. General Procedure for synthesis of ethyl {4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl]-2-methoxyphenoxy}acetate (A4) and ethyl {4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}acetate (B2)[13] *:*

As per ref.[2] $C_{15}H_{15}NO_6S$; Yield:82%; m.p.: 114-116ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.89. IR cm-1: Imide C=O str.(1730, 1689);ester C=O str.(1766), C-O-C(ether) str.(1226, 1261); =C-H str.(2990); N-H str.(3445); C-S str. (670).

1.1.6.1. Ethyl-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}acetate(B2): $C_{14}H_{13}NO_5S$; Yield:79%; m.p.: 148-150ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.8. IR cm-1: Imide C=O str.(1670, 1721); ester C=O (1751); Aro. C=C str.(1509); C-S str. (686); alkyl C-H str.(2948); Aro. C-H str.(2995); N-H str. (3454).

1.1.7. Synthesis of 2-{[(5E)-5-(4-hydroxy-3-methoxybenzylidene)-2,4-dioxo-1,3-thiazolidin-3-yl]methyl} hydrazine carbothioamide(A5)[14] :

As per ref.[2] $C_{13}H_{14}N_4O_4S_2$; Yield: 76%; m.p.: 202-204ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.64. IR cm-1: Imide C=O str.(1690,1734); C-O-C (ether) str.(1281); =C-H str.(3056); Aro. C=C str.(1514); NH_2 str.(3451); NH_2 ben.(1591); NH ben.(1514); NH-CS-NH_2 (1149); C=S str.(1213); Ali.C-N str.(1033), C-S str. (686);alkyl C-H str.(2776); Aro. C-H str.(2929). 1H NMR (δ ppm, DMSO): 3.87(s,3H,OCH_3); 2.55(s,DMSO); 4.21(d,2H,CH_2); 6.91-7.05(m,3H, Aro. CH); 7.67(s,H,CH- benzylidene); 8.03-8.29(m,4H,NH and NH_2 of TSC gr.);9.80 (s,H,OH).

1.1.8. General Procedure for synthesis of N'-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenyl}-N,Ndimethylimidodicarbonimidic diamide(C1) and 2-{4-[(E)-(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl] phenyl}hydrazinecarbothioamide (C2)[15] :

As per ref.[2] $C_{14}H_{16}N_6O_2S$; Yield: 67%; m.p.: 250-252ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.19. IR cm-Imide C=O str.(1670,1617); =C-H str.(2954); Aro.C=C str.(1547); =NH str.(3352); NH str. (3174); NH ben.(1489);Ali.C-N str.(1227); Aro.C-N(1310); C-S str. (676); alkyl C-H str.(2847); Aro.C-H str.(2923). 1H NMR (δ ppm, DMSO): 2.54 (s,6H,2CH3); 3.38-4.09(m,4H,NH and =NH); 7.39-7.51(m,4H, Aro.CH); 8.15(s,H,CH-benzylidene); 11.46 (s,H,N-H of TZD); MS: m/z : (M-2)+ = 330.3.

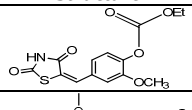
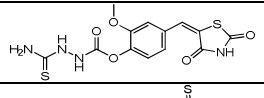
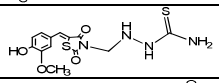
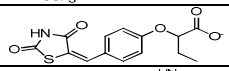
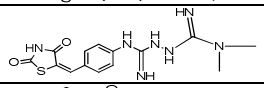
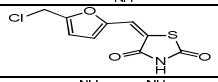
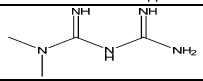
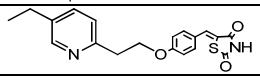
1.1.9. Synthesis of (5E)-5-{[5-(chloromethyl)furan-2-yl]methylidene}-1,3-thiazolidine-2,4-dione (D1) :

As per ref.[2] $C_9H_6ClNO_3S$; Yield: 85%; m.p.:>250ºC; TLC- Benzene:Ethyl acetate (8:2), Rf value: 0.48. IR cm-1: Imide C=O str.(1741,1705); =C-H str.(2923); C-S str. (665); alkyl C-H str. (2853); Furan ring skeleton (1595); C-Cl str. (777); C-H of Furan (1011), N-H str.(3447). 1H NMR (δ ppm, DMSO): 3.37(s,2H,CH2); 2.66(s,DMSO); 7.23 (d,H,CH); 7.66(d,H,CH); 8.10(s,H,CH Benzylidene); 10.28(s,H,NH of TZD).

RESULTS AND DISCUSSION

A1 A2 A5 B2 C1 D1 compounds were subjected for the screening for acute oral toxicity and antidiabetic activity.

Table no1: Novel Compounds Screened for Acute Oral Toxicity and Antidiabetic activity

Sr. No.	Comp. Code	Structure	PASS Prediction[16]
1.	**A1**		**0.830**
2.	**A2**		**0.779**
3.	**A5**		**0.602**
4.	**B2**		**0.589**
5.	**C1**		**0.905**
6.	**D1**		**0.814**
7.	**Metformin**		**0.656**
8.	**Pioglitazone**		**0.975**

I) Acute Oral Toxicity:

The experiments were carried out on normal healthy rats for acute toxicity studies. The novel compounds were tested for dose of 30 and 100 mg/kg of body weight. The animals were observed for 48 hrs for any signs of acute toxicity such as increased or decreased motor activity, convulsions, skin changes, salivation and lacrimation. No mortality of the animals was observed even after 48 hrs. The behaviour of the treated rats appeared normal and no toxic effects were seen even with the dose of 100 mg/kg of body weight. The body weight was normal. Hence the dose of 100 mg/kg was considered as safe dose and used for evaluation of antidiabetic activity.

I) Antidiabetic activity: The effects of the new TZD derivatives prepared were screened *in vivo* using the experimental model of diabetes induced by alloxan in rats. Hyperglycemia was induced by alloxan monohydrate at dose of 120 mg/kg, i.p. The biological activity was evaluated for acute response and subacute response with daily doses of 100 mg/kg/day, administered orally for 14 days of treatment, and specific reduction in levels of Blood Glucose was measured at various time intervals and compared with‘0’ hr BGL of respective group. Metformin and

pioglitazone were used as reference drugs at doses of 100 mg/kg/day. Statistical comparisons between the groups were performed on all the groups using two-way ANOVA and Dunnet's multiple comparison test on Graph Pad Prism 4.0 software for Windows [17]. All the compounds showed remarkable hypoglycemic activity, however with a degree of variation and time. Blood glucose changes in treatment of diabetic rats with synthesized TZD derivatives are presented in **Table no.2 &3.** Oral administration of compounds **A1, A2, A5, B2, C1** and **D1** (100 mg/kg) reduced blood glucose level in diabetic rats at various time intervals.

Table No.2 : Hypoglycemic effects of synthesized test compounds (Acute study)

Treatment	Blood Glucose Level (mg/dl)			
	0 hr	3 hr	6 hr	24hr
Metformin	441.8±18.71	399.4±17.72***	289.4±18.46***	219.6±18.40***
Pioglitazone	402.2±28.7	363.4±26.08	302.4±26.87***	232.2±20.53***
A1	362±22.04	330.4±22.19	303.6±23.19*	269.8±23.62**
A2	376.4±21.00	342.8±21.58	315.2±21.66*	276±21.79***
A5	357.8±24.43	328±24.56	303±24.91	268.2±25.46**
B2	326.2±25.32	300±25.03	278.2±25.76	245.2±25.91**
C1	355±24.59	322.8±24.10	253.8±23.45***	231.4±23.48*
D1	369.2±20.54	343.6±20.33	342.6±22.32	308.2±22.33*
Vehicle Control	304.2±36.81	308.2±36.85	309±37.92	310.4±39.57
Diabetic control	322.2±22.96	337±23.59	347±24.01	363.4±24.0
Normal control	120.33±7.76	125.66±2.08	126.66±3.05	129.33±1.52

In acute study, no one test compound was found to be significant at 3 hrs of treatment. After 6 hrs, the compound **C1** decreased BGL which is highly significant ($p<0.0001$) and **A1, A2** exhibited significant hypoglycaemic activity ($p<0.05$). At 24hrs, the compounds **C1** and **A2** decreased BGL which is highly significant ($p<0.0001$) and others showed more significant ($p<0.01$) decrease in BGL. So in acute study, the compound **C1** was found to be more significant amongst test compounds.The continuous treatment with these compounds for 7 and 14 days at dose of 100 mg/kg/day showed highly significant ($p<0.0001$) decrease in blood glucose for all the test compounds.

Table No.3: Hypoglycemic effects of synthesized test compounds (Subacute study)

Treatment	Blood Glucose Level (mg/dl)		
	0 hr	7 days	14 days
Metformin	441.8 ± 18.71	153.8 ± 19.63***	112.8 ± 16.84***
Pioglitazone	402.2 ± 28.77	167 ± 20.51***	123.8 ± 16.94***
A1	362 ± 22.04	221.4 ± 24.81***	158.2 ± 19.26***
A2	376.4 ± 21.00	218.8 ± 22.34***	146.4 ± 20.50***
A5	357.8 ± 24.43	215.8 ± 26.07***	147.6 ± 23.46***
B2	326.2 ± 25.32	195.6 ± 25.74***	134.8 ± 22.80***
C1	355 ± 24.59	164.6 ± 22.46***	123 ± 18.68***
D1	369.2 ± 20.	54 257.4 ± 23.14***	217.8 ± 25.08***
Vehicle Control	304.2 ± 36.81	343.2 ± 41.07	353.2 ± 36.70
Diabetic control	322.2 ± 22.96	393.6 ± 24.36	411 ± 23.69
Normal control	120.33 ± 7.76	114 ± 10.14	116 ± 12.28

*Values are expressed as mean ±S.E.M; n = 5; (*** p < 0.0001), (**p < 0.01) , (* p < 0.05)*

Table No.4: Effect of test compounds on % decrease in blood glucose

Treatment	**% decrease in Blood Glucose Level***				
	3 hr	6 hr	24hr	7 days	14 days
Metformin	9.73	34.49	50.22	65.19	73.83
Pioglitazone	9.64	24.81	42.27	58.48	69.22
A1	8.73	16.14	25.47	38.84	56.29
A2	8.93	16.26	26.68	41.87	61.10
A5	8.33	15.32	25.04	39.69	58.75
B2	8.03	14.72	24.83	40.04	58.67
C1	9.07	28.50	34.81	53.63	65.35
D1	6.93	7.21	16.52	30.28	41.01

** 0 hr the basal blood glucose level in mg/dl considered as 100% respectively for calculation of % decrease.*

Among all the derivatives, compound **C1** (65.35%) was found to be better hypoglycemic agent like the standard drug pioglitazone (69.22%) at dose of 100mg/kg b.w. in reducing the blood glucose level after 14 days.

In acute study, the compound **C1** showed almost same activity (9.07%) as that of pioglitazone (9.64%) after 3 hrs and greater activity(28.50%) after 6 hrs. But after 24 hrs, the activity was slightly less (34.81%). The compounds **A1** , **A2, A5** and **B2** also showed good activity. The compound **A2** containing hydrazide group as lipophilic moiety showed more activity than **A1** and **B2** which contains ester group. The compound **A5** containing 3-N substitution of thiosemicarbazide separated by methylene group also showed good activity. The compound **D1,** showed poor activity amongst all the tested compounds.In subacute study, after 7 days treatment, the compound **C1** showed highest (53.63%) activity among all the compounds and which is nearer to standard drugs used in this study. The compound **A2** , containing hydrazide moiety, showed more activity than **A1** and **B2** which contains ester group. Interestingly, the compound **B2,** containing ethyl acetate moiety as lipophilic chain and lacks methoxy group at *meta* to thiazolidinedione ring, showed more activity than **A1** which contains ethyl formate moiety and methoxy substitution. After 14 days treatment, the compound **C1** remains the compound having highest (65.35%) activity among all the tested compounds followed by **A2.** The compound **A5**, containing 3-N substitution of thiosemicarbazide separated by methylene group, exhibited considerable activity as compared to acute study. The compound **D1** showed poor activity than other tested compounds even after 14 days treatment.For confirmation of our results, the % decrease in blood glucose level is futher calculated by the formula[18] given below and presented in **Table 4** :% decrease = 1− [(TT/OT)/(TC/OC)] ×100 where,TT : readings for test compound at time T;OT : readings for test compound at time 0;TC : readings for vehicle control at time T;OC: readings for vehicle control at time 0

Table No. 4: % decrease in BGL calculated with formula

Treatment	**% decrease in Blood Glucose Level***				
	3 hr	6 hr	24hr	7 days	14 days
Metformin	88.09	63.48	47.77	29.85	21.53
Pioglitazone	88.18	73.01	55.58	35.80	25.51
A1	89.09	81.56	72.04	53.21	36.64
A2	88.89	81.44	70.86	50.52	32.50
A5	89.48	82.36	72.46	52.46	34.53
B2	89.78	82.96	72.66	52.15	34.59
C1	88.75	69.38	62.88	40.09	30.73
D1	90.86	90.35	80.81	60.79	49.80

** for calculation of % decrease, the formula: 1−[(TT/OT)/(TC/OC)]×100 , is used.*

The same results were observed in calculation using formula and that of % decrease calculated by considering the 0 hr glucose level of respective group. The compound **C1** have shown nearly similar activity as that of standard compounds.The BGL was measured in mg/dl and significance was calculated by comparing with vehicle control. The % reduction in BGL was calculated for easier comparison with standard compounds used.

In this study, we have done modifications at 3-N, phenoxyalkyl linker and lipophilic chain. All the compounds prepared have shown good activity in subacute study and moderate activity in acute study. In case of compounds **A1** , **A2, B2** and **C1** ,we have modified lipophic portion and phenoxyalkyl linker also, except in **B2** , we have kept phenoxyalkyl portion as such. Out of these compounds, **C1** containing guanidine group (metformin moiety) attached directly to phenyl ring showed better activity. This activity may be due to amine group present in guanidine moiety which may forms hydrogen bonding with receptor. The compounds **A1** and **A2** possess ester and hydrazide groups respectively attached to oxygan of phenoxy ring. In addition, they also possess methoxy group *meta* to thiazolidinedione ring. The compound having hydrazide moiety (**A2**) showed almost same activity in acute study and more activity than compound containing only ester group (**A1**). The compound **B2,**containing ester group attached to methylene of phenoxyalkyl linker, showed less activity than **A1** and **A2** . The modification done on 3-NH of thiazolidinedione ring (**A5**) i.e. replacement of 'H' by methylene thiosemicarbazide showed good activity and is almost similar to that of **A2** which also contains thiosemicarbazide group but attached at different position. This shows that, the thiosemicarbazide moiety is responsible for reducing blood glucose level together with other pharmacophores in the compounds.The incorporation of 5-membered heterocyclic ring (compound **D1**) in place of phenoxyalkyl group showed poor activity amongst tested compounds but is significant as compared to vehicle control in subacute study.

CONCLUSION

We find that all our synthesized TZD derivatives showed potency to decrease blood glucose level. A brief summary of the observed SAR is presented below.**A.** Keeping other pharmacophore constant, we have replaced aromatic lipophilic portion with aliphatic chain. The activity profile or potency can be given as: C1 > **A2** > **A1** > **B2**

B. The modification at 3-N of thiazolidinedione ring and methoxy substitution at *m*-phenyl ring, without aryl lipophilic group (**A5**) also showed activity.

C. The replacement of phenoxyalkyl linker with substituted furan ring (**D1**) exhibited poor activity as compare to other tested compounds.

From the observed results, it is concluded that almost all the tested compounds reduced glucose level in diabetic rats. However, the effect of **C1** is more pronounced in alloxan induced diabetic rats.Hence we have concluded that suitably modified TZDs offer rich potential for the development of new drug candidates for diabetes mellitus.

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