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Anti-hyperglycemic evaluation of 2,4-thiazolidinedione and rhodanine derivatives

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ABSTRACT

A novel series comprising of 10 derivatives of two chemical entities thiazolidinedione and rhodanine were synthesized and characterized physicochemically as well as by spectral means. The synthesized derivatives (THP, RHP, RHB, MB01-MB09) were then screened for anti-hyperglycemic activity in fructose-induced diabetic animal model. It can be concluded that presence of piperazine and N-methyl piperazine at N-3 of thiazolidinedione and rhodanine derivatives have shown significant activity in comparison to aromatic amine substitution. The presence of ether linkage is the primary requisite for anti-hyperglycemic activity. Condensation of benzylidene with lipophilic moiety i.e. benzothiazole enhanced the activity of the synthesized derivatives. Piperazine substituted compound, ***MB01****was most potent anti-hyperglycemic activity that might be due to the availability of free nitrogen at receptor site.*

Keywords: Pioglitazone, antidiabetic, thiazolidinediones, blood glucose level, anti-hyperglycemic, rhodanine

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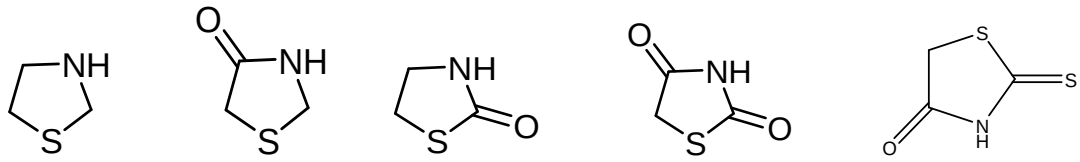
INTRODUCTION

Type 2 diabetes mellitus (non-insulin dependent diabetes mellitus or NIDDM) is a metabolic disorder involving dysregulation of glucose metabolism and insulin resistance, and long term complications involving the eyes, kidneys, nerves and blood vessels**.** It is described as the body's inability to make either sufficient insulin (abnormal insulin secretion) or its inability to effectively use insulin (resistance to insulin action in target organs and tissues) [1]. Recent and emerging anti-diabetes targets are Glucagon-like Peptide & its mimetic (GLP-1), Glucose Insulinotropic Peptide (GIP) & its mimetic, Di-peptidyl Protease Inhibitors (DPPIV), PTP-IB inhibitors, GSK3 inhibitors, Fructose-1,6-bisphosphatase inhibitors, Glycogen phosphorylase a inhibitors [1].

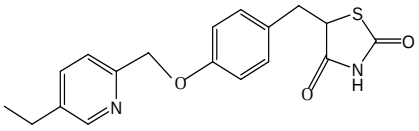
Five-member heterocyclic molecules rhodanine and 2, 4-thiazolidinedione derivatives have broad spectrum of pharmacological activities. 1, 3-Thiazolidine-2, 4-dione contains basic skeleton of thiazole or thiazolidine (A). Presence of one carbonyl group in thiazole at 4th position makes it thiazolidine-4-one (B) which is known for various activities and presence of another carbonyl group at 2nd position (C) makes it thiazolidine-2, 4-dione (D) and replacing O at 2nd position makes it rhodanine (E) which is basically known for its anti-hyperglycemic activity.



(A) (B) (C) (D) (E)

Various researchers have synthesized derivatives of thiazolidinedione and rhodamine and evaluated them as potential anti-hyperglycemic agents [2, 3]. In our work, Pioglitazone was taken as the standard prototype drug and retained the pharmacophore, i.e., TZD ring and made the changes in lipophilic group to develop new congeners with better efficacy and less toxicity. Tanis *et al.* [4] have synthesized the putative ketone metabolite and have described their potential as a pioglitazone anti-hyperglycemic congener with somewhat greater potency and a better metabolic profile. Munj *et al.* [5] synthesized new moieties changing the lipophilic part and introducing carbonyl carbon. They reported significant increase in blood glucose reduction. Sohda *et al.*[6] reported that benzothiazole and benzoxazole would also fit well into the binding site and thiazolidinedione with benzothiazole moiety has been proved to be effective anti-hyperglycemic agent (European Patent Specification, 1999). We further extended the work by synthesizing new moieties, changing lipophilic part, keeping the carbonyl carbon and unsaturation intact, and introducing fourth modification i.e. formation of mannich bases in view of developing some potential anti-hyperglycemic agents. Main four modifications in the structure of pioglitazone are (Fig 1):

1. Novel lipophilic moiety
2. Introduction of carbonyl carbon
3. Unsaturation
4. Formation of Mannich bases



Pioglitazone

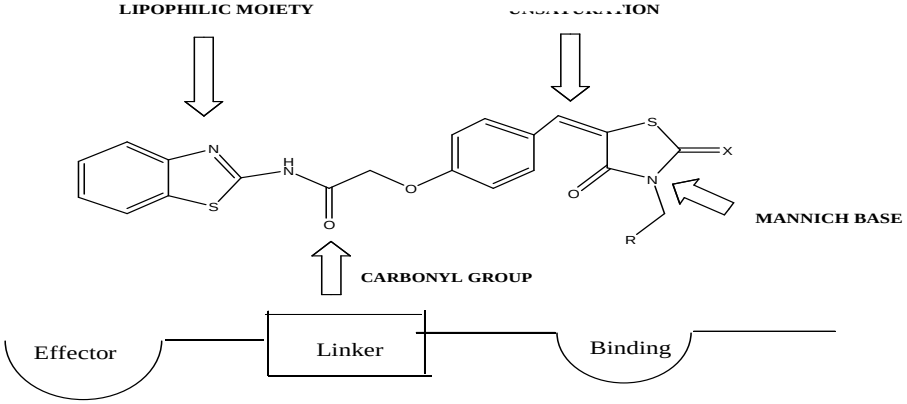


Fig. 1. Showing four main modifications in pharmacophore

MATERIALS AND METHODS

The starting materials and the reagents used were of analytical grade and used further without purification. Melting points were determined on ELICO melting point apparatus and are uncorrected. Infrared spectra were recorded using KBr pellets on a Perkin-Elmer FTIR spectrophotometer. ^{1}H NMR spectra were recorded on Brucker Advance II 400 MHz spectrometer using TMS (tetramethylsilane) as an internal standard. The chemical shifts are expressed

in parts per million (δ, ppm). All compounds exhibited ^{1}H NMR and IR spectral data consistent with the proposed structures. The progress of the reaction was monitored by TLC using silica gel G as adsorbent.

General procedure for thiazolidine-2, 4-dione (1) [7]
The equimolar quantity (1:1) of chloroacetic acid (56.4 g, 0.6mol) in 60 ml of water was added to the solution of thiourea (45.6 g, 0.6mol) in 60 ml of water. The mixture was stirred for 15 min. and precipitates were obtained after cooling. Then added slowly 60 ml of concentrated hydrochloric acid from a dropping funnel. Once the mixture got converted to solution form, it was refluxed for 8-10 hour at 100-110°C. On cooling, the contents of the flask solidified to a cluster of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was purified by recrystallization from ethyl alcohol. Yield: 85%; m.p.:123-125°C.

General procedure for compound (2) [7]
4-Hydroxy benzaldehyde (0.188mol) and 2, 4-thiazolidinedione/ rhodanine (0.188mol) were together suspended in ethanol. To this, a catalytic amount of piperidine (1 ml) was added. The mixture was stirred and refluxed. After the complete removal of water and when the temperature reached above 110°C the reaction mixture was stirred for a further1 hour. On cooling, the product precipitated out from ethanol. The compound was filtered and washed with cold dry toluene and dry ethanol. Yield: 93%; m.p.:240-242°C.

General procedure for compound (3) [8]
2-Amino benzothiazole (15 gm, 0.1mol) in chloroform (10 ml) was stirred in a conical flask and to this, chloroacetyl chloride (12.01ml, 0.15mol) was added drop wise under cold condition. Reaction mixture was stirred till completion of reaction, which was monitored by TLC.

General procedure for compound (4) [8]
5-Benzylidene-2,4-thiazolidinedione/ 5-Benzylidene-2-thioxothiazolidin-4-one (22.12gm, 0.1mol) and anhydrous potassium carbonate (20.72gm, 0.15mol) in dimethyl formamide (DMF) was stirred in a flask and to this reaction mixture, above synthesized compound **(3)** (34.0gm, 0.15mol) in DMF was added. Reaction mixture was stirred at room temperature till the completion of reaction, which was monitored by TLC. After completion of reaction, water was added to get the solid final product.

General procedure for compound (5) (MB01-MB09) [9]
To a solution of above synthesized compound **(4)** (0.01 mol) in DMF, formaldehyde (0.6ml, 0.02 mol) was added under stirring. The reaction-mixture was stirred at RT for 0.5 hr and yielded methyl derivative of compound. To this, the solution of secondary amine in DMF was added drop wise and refluxed 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 recrystallization from chloroform to get the desired compound. The completion of reaction was confirmed by single spot TLC.

The synthesized derivatives were characterized by their physical parameters such as R_f, melting point and % yield.The results are summarized in Table I.

Table I: Physicochemical Characterization of the Synthesized Compounds

S.No.	Product code	Molecular formula	Molecular weight	Melting Point	R_f	% yield
1.	TZP	$C_{19}H_{13}O_4N_3S_2$	411.454	168-180	0.61	58.4
2.	RHP	$C_{19}H_{13}O_3N_3S_3$	427.52	170-185	0.86	60.3
3.	MB01	$C_{24}H_{23}O_4N_5S_2$	509.601	62-64	0.73	67.89
4.	MB02	$C_{32}H_{24}O_4N_4S_2$	592.687	56-60	0.72	48.56
5.	MB03	$C_{22}H_{20}O_4N_4S_2$	468.549	54-58	0.65	56.7
6.	MB04	$C_{25}H_{25}O_4N_5S_2$	523.627	60-64	0.70	44.9
7.	MB05	$C_{34}H_{28}O_4N_4S_2$	620.741	63-65	0.82	78.25
8.	MB06	$C_{24}H_{24}O_4N_4S_2$	496.602	55-57	0.76	62.8
9.	MB07	$C_{24}H_{23}O_3N_5S_3$	525.666	58-62	0.60	53.65
10.	MB08	$C_{25}H_{25}O_3N_5S_3$	539.693	60-65	0.79	70.48
11.	MB09	$C_{22}H_{20}O_3N_4S_3$	484.614	55-59	0.57	64.45
12.	RHB	$C_{10}H_7O_2NS_2$	237.298	150-155	0.67	78.42

TLC Mobile Phase: Benzene:Methanol ; 8.5:1.5 (v/v)

Spectral data

(E)-5-(4-hydroxybenzylidene) thiazolidine-2, 4-dione: IR (KBr, cm^{-1}): 3405 (NH str.), 3125 (C-H str., aromatic), 1720 and 1678 (C=O str., cyclic imide), 1510 (C=C bend, aromatic), 1279 (C-O str.), 1156 (C-N str.), 614 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 6.92 (d, 2H, aromatic), 7.46 (d, 2H, aromatic), 7.695 (s, 1H, benzylidene proton)

N-(Benzothiazol-2-yl)-2-chloroacetamide: IR (KBr, cm^{-1}): 3368 (NH str.), 1695 (C=O str., amide), 1450 (C=C bend, aromatic), 1268 (C-O str.), 1177 (C-N str.), 677 (C-Cl str.)

***N-(Benzothiazol-2-yl)-2-(4-((2, 4-dioxothiazolidin-5 ylidene)methyl)phenoxyacetamide* (TZP):** IR (KBr, cm^{-1}): 3197 (NH str.), 3065 (H-C=C str.), 1733 and 1677 (C=O str., cyclic imide), 1267 (C-O str.), 1176 (C-N str.), 611 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 8.5 (2H, s, Ar-H), 8.3 (1H, s, NH), 7.56 (2H, s, Ar-H), 7.14 (2H, m, Ar-H), 4.68 (2H, s, CH_2)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*RHP*): IR (KBr, cm^{-1}): 3190 (NH str.), 3070 (H-C=C str.), 1643 (C=O, aliphatic) and 1664 (C=O str., imide), 1271 (C-O str.), 1170 (C-N str.), 621 and 632 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 7.96 (1H, s, NH), 7.75 (2H, s, Ar-H), 7.32 (3H, m, Ar-H), 6.68 (2H, s, Ar-H), 3.7 (2H, s, CH_2)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((2, 4-dioxo-3-(piperazin-1-ylmethyl) thiazolidin-5-ylidene) methyl)phenoxy) acetamide (*MB01*): IR (KBr, cm^{-1}): 3402 (NH str.), 2935 (H-C=C str.), 1664 (C=O str., imide), 1459 (CH_2 str.), 1278 (C-O str.), 1169 (C-N str.), 620 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 8.25 (2H, s, Ar-H), 7.95 (1H, s, NH), 7.10 (3H, m, Ar-H), 6.8 (3H, m, Ar-H), 3.34 (2H, s, CH_2), 2.5 (4H, m, CH_2)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((diphenylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*MB02*): IR (KBr, cm^{-1}): 3385 (NH str.), 3045 (H-C=C str.), 1680 and 1656 (C=O str., cyclic imide), 1494 (C=C bend, aromatic), 1477 (CH_2, bend), 1234 (C-O str.), 1172 (C-N str.), 615 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 8.14 (1H, s, NH), 7.2 (4H, m, Ar-H), 7.07 (6H, d, Ar-H), 6.8 (1H, s, H-C=C)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((dimethylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*MBO3*): IR (KBr, cm^{-1}): 3381 (NH str.), 3061 (H-C=C str.), 1664 (C=O str., imide), 1593 (C=C bend, aromatic), 1471 (CH_2, bend), 1273 (C-O str.), 1176 (C-N str.), 619 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 7.9 (1H, s, NH), 7.72 (4H, m, Ar-H), 7.33 (4H, m, Ar-H), 6.84 (1H, s, H-C=C), 3.4 (4H, s, CH_2), 2.5 (6H, s, CH_3)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((4-methylpiperazin-1-yl)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl) phenoxy)acetamide (*MB04*): IR (KBr, cm^{-1}): 3288 (NH str.), 3062 (H-C=C str.), 1681 and 1598 (C=O str., cyclic imide), 1494 (C=C bend, aromatic), 1377 (CH_3, bend), 1273 (C-O str.), 1188 (C-N str.), 617 (C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 8.1 (2H, s, Ar-H), 7.96 (1H, s, NH), 7.17 (4H, m, Ar-H), 6.98 (1H, s, H-C=C), 3.42 (4H, s, CH_2), 2.5 (8H, s, CH_2), 2.2 (3H, s, CH_3)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((diethylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*MBO5*): IR (KBr, cm^{-1}): 3413 (NH str.), 2921 (CH_3 str., aliphatic), 1666 (C=O str., imide), 1494 (C=C bend, aromatic), 1377 (CH_3, bend), 1273 (C-O str.), 1188 (C-N str.), 617 (C-S str.)

***(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((dibenzylamino)methyl)-2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (MB06)*:** IR (KBr, cm^{-1}): 3406 (NH str.), 1664 (C=O str., imide), 1506 (C=C bend, aromatic), 1467(-CH_2-), 1250 (C-O str.), 1176 (C-N str.), 622(C-S str.); ^{1}H NMR ($DMSOd_6$) δ (ppm): 7.95 (1H, s, NH), 7.68 (4H, m, Ar-H), 7.09 (4H, m, Ar-H), 3.34 (4H, s, CH_2), 2.5 (4H, s, CH_2), 1.2 (6H, s, CH_3)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((4-oxo-3-(piperazin-1-ylmethyl)-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*MB07*): IR (KBr, cm^{-1}): 3240 and 3304 (NH str.), 3078 (H-C=C str.), 1666 (C=O str., amide), 1460 (CH_2, bend), 1278 (C-O str.), 1159 (C-N str.), 646 (C-S str.)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((4-methylpiperazin-1-yl)methyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*MB08*): IR (KBr, cm^{-1}): 3213 (NH str.), 3061 (H-C=C str.), 1664 (C=O str., amide), 1481 (C=C bend, aromatic), 1465 (CH_2 bend), 1365 (CH_3, bend), 1278 (C-O str.), 1151 (C-N str.), 624 (C-S

str.); ^{1}H NMR (DMSOd_6) δ (ppm): 8.23 (2H, s, Ar-H), 7.89 (1H, s, NH), 7.23 (4H, m, Ar-H), 6.91 (1H, s, H-C=C), 3.38 (4H, s, CH_2), 2.43 (8H, s, CH_2), 2.32 (3H, s, CH_3)

(E)-N-(benzo[d]thiazol-2-yl)-2-(4-((3-((dimethylamino)methyl)-4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)acetamide (*MB09*): IR (KBr, cm^{-1}): 3381 (NH str.), 3061 (H-C=C str.), 1664 (C=O str., amide), 1593 (C=C bend, aromatic), 1471 (CH_2, bend), 1273 (C-O str.), 1176 (C-N str.); ^{1}H NMR (DMSOd_6) δ (ppm): 7.85 (1H, s, NH), 7.69 (4H, m, Ar-H), 7.39 (4H, m, Ar-H), 6.78 (1H, s, H-C=C), 3.5 (4H, s, CH_2), 2.8 (6H, s, CH_3)

(E)-5-(4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (*RHB*): IR (KBr, cm^{-1}): 3383 (NH str.), 3101 (C-H str., aromatic), 1685 (C=O str., amide), 1506 (C=C bend, aromatic), 1282 (C-O str.), 1174 (C-N str.), 624 (C-S str.); ^{1}H NMR (DMSOd_6) δ (ppm): 10.4 (1H, s, NH), 7.13 (3H, m, Ar-H), 6.68 (2H, m, Ar-H)

Anti-Hyperglycemic Activity

The anti-hyperglycemic activity of the synthesized compounds was determined using Fructose Induced Diabetic Rat Model. Adult albino wistar rats (100-120 g) were purchased from the Disease Free Small Animal House, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana). Animals were housed separately in groups of 6 per cage in the animal facility of the Department of Pharmaceutical Sciences, G.J.U S & T, Hisar, under standard conditions of temperature (25±2ºC) and 12 hr light/dark cycles. The Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of committee for the purpose of Control and supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (IAEC/ 136-144).

Induction of Experimental Fructose induced Diabetes.

The acclimatized animals were kept fasting for 24 hrs with water ad libitum and 25 % fructrose solution was given in feeding bottle for 20 days. After 20 days, animals having blood glucose level beyond 200 mg/dl of blood were selected for study and divided into 12 groups. The quantity of 2, 4-thiazolidinedione derivatives equivalent to average human intake 200 mg/ kg at a time was calculated for single dose 36 mg/kg (for acute study). The test compounds were administered orally by mixing with CMC (0.25 %) solution and those of control animals were given CMC (0.25% w/v). Pioglitazone was used as standard. The blood glucose level was monitored by withdrawing a drop of blood from the tail vein by Tail tipping method. The blood was dropped on the dextrostrix reagent pad. The strip was inserted into microprocessor digital blood glucometer and readings were noted. The blood glucose level was monitored at different time 0 hr, 1hr, 3 hr, and 6 hr respectively [10].

RESULTS AND DISCUSSION

Chemistry

The mannich bases of thiazolidinedione and rhodanine derivatives were synthesized using scheme 1 and scheme 2 respectively (**Fig 2**). Thiazolidinedione **(1)** and rhodanine was condensed with benzaldehyde by Knoevenagel condensation to form 5-benzylidene thiazolidinedione and 5-benzylidene rhodanine which was condensed with acetamide derivative of 2-aminobenzothiazole to form compound **(3)**. This compound was further reacted with formaldehyde by stirring and then refluxed with desired secondary amines to get the mannich bases.

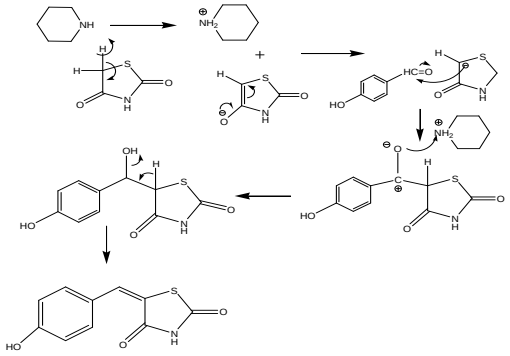


Fig 1. Mechanism of Knoevenagel condensation

A Knoevenagel condensation is a nucleophilic addition of an active hydrogen compound to a carbonyl group followed by a dehydration reaction in which a molecule of water is eliminated (hence condensation) (Fig 1). The product is often an alpha, beta conjugated enone. Knoevenagel reaction of aldehyde with the cyclic dione in the presence of a catalytic amount of base such as piperidine and an acid such as benzoic acid can provide the phenoxy cyclic dione. The Knoevenagel reaction is typically performed in an aprotic solvent such as toluene at a temperature preferably between 100-200°C [8].

The structure of the synthesized compounds was confirmed by IR, ^{1}HNMR. The IR spectrum of benzylidene 2, 4-thiazolidinedione illustrates the presence of H-C=C bond at 3065cm^{-1} which confirmed the unsaturation at C-5 in all the synthesized derivatives. The absence of O-H peak at 3600 cm^{-1} in TZP confirmed the formation of ether linkage in the pharmacophore.

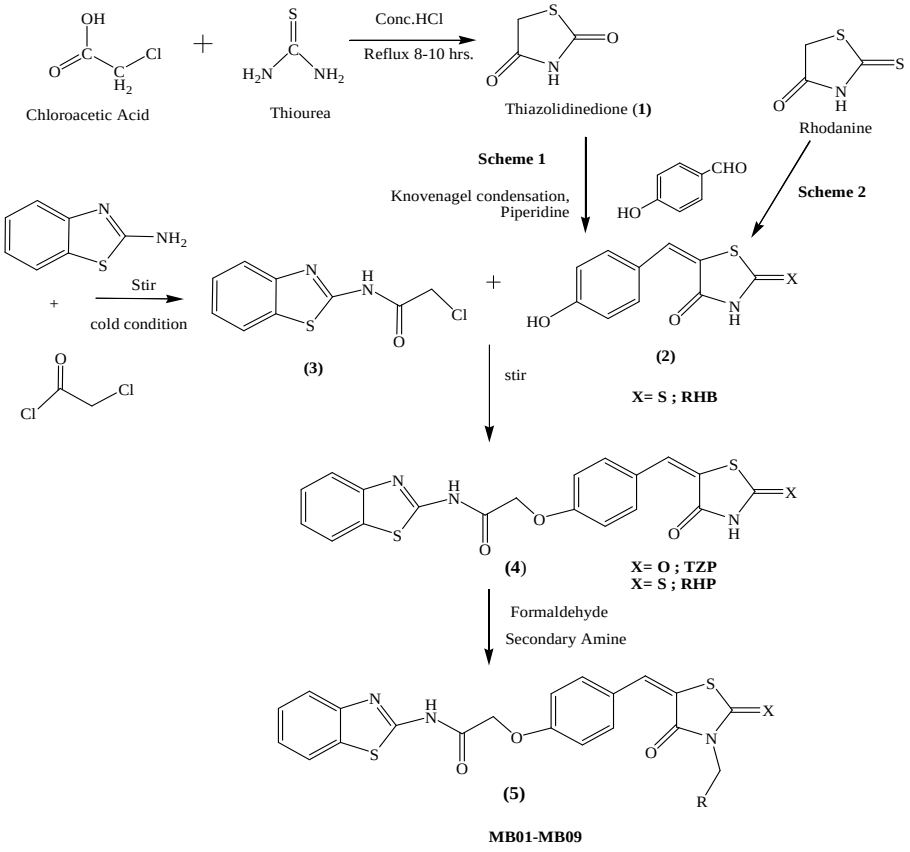
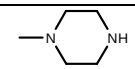
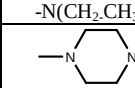
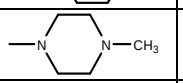
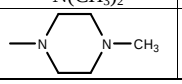


Fig 2. : The general synthetic scheme for synthesis of mannich bases of thiazolidinedione and rhodanine derivatives

S. No.	Product code	R	X	S.No.	Product code	R	X
1.	TZP	-	O	**7.**	MB05	$-N(CH_2.C_6H_5)_2$	O
2.	RHP	-	S	**8.**	MB06	$-N(CH_2.CH_3)_2$	O
3.	MB01		O	**9.**	MB07		S
4.	MB02	$-N(C_6H_5)_2$	O	**10.**	MB08		S
5.	MB03	$-N(CH_3)_2$	O	**11.**	MB09	$-N(CH_3)_2$	S
6.	MB04		O	**12.**	RHB	-	S

The IR spectra of synthesized derivatives (MB01-MB09) exhibited the absorption bands for aromatic ring vibrations in the region of 3150-3050 cm^{-1}. The carbonyl stretching band was observed at 1680 and 1664 cm^{-1}.The $–CH_2$- bend was observed at 1465cm^{-1} which confirmed the presence of methylene group in mannich bases. The synthesized compounds also showed C-O str. in the range of 1270-1278cm^{-1}, C-N str. in the range of 1100-1200cm^{-1} and C-S str. in the range of 620-630cm^{-1} which indicated the synthesis of respective compounds.

The ^{1}HNMR spectrum of benzylidene derivatives of thiazolidinedione and rhodanine displayed the characteristic peak at δ 7.1 for unsaturation at C-5 of the ring and NH peak at δ 10.0. The pharmacophore showed the peak at δ 4.7 for $–CH_2$ which confirmed the condensation of 2-amino benzothiazole ring with benzylidene thiazolidinedione and rhodanine, with the peaks for aromatic hydrogen at δ 7.2 and δ 7.7. The NH peak at 8.0 for amide linkage at benzothiazole and NH peak at δ 10.0 for unsubstituted N-3 can be easily distinguished in the spectrum of TZP and RHP. The mannich bases of thiazolidinedione gave characteristic peak for $–CH_2$ at δ 2.2-3.5 which confirmed the methylene group between secondary amines and N-3. The absence of NH peak at δ 10.0 further confirms the substitution at N-3 of thiazolidinedione and rhodanine instead of at amide linkage of benzothiazole which was also one of the probable sites for substitution of secondary amine. The peaks of respective amines containing hydrogens were also noted. The spectral data thus confirmed the synthesis of mannich bases of thiazolidinedione and rhodanine derivatives with respective amines at N-3 of TZP and RHP.

Evaluation of Anti-hyperglycemic Activity

The synthesized compounds were evaluated for anti-hyperglycemic activity in fructose induced diabetic rat model using pioglitazone as standard drug in this study. The results calculated are shown in Table II. Most of the synthesized derivatives of benzylidene-2, 4-thiazolidinedione and rhodanine showed moderate to significant anti-hyperglycemic activity. MB01, MB03, MB06, MB08 and RHP have shown good blood glucose reduction. The maximum activity was shown by MB01 (46.46% Blood glucose reduction). MB06 and MB08 showed significant anti-hyperglycemic activity with 42.46% and 41.44% blood glucose reduction respectively. All the derivatives have shown significant rise in activity at the 6th hour of the study. Some of the derivatives (MB01, MB03, MB04, MB05 and MB06) showed a great increase in blood glucose reduction during the interval of 1 hour to 3 hour. Thus the peak concentration of synthesized compounds in serum can be estimated to be near about 3 hours.

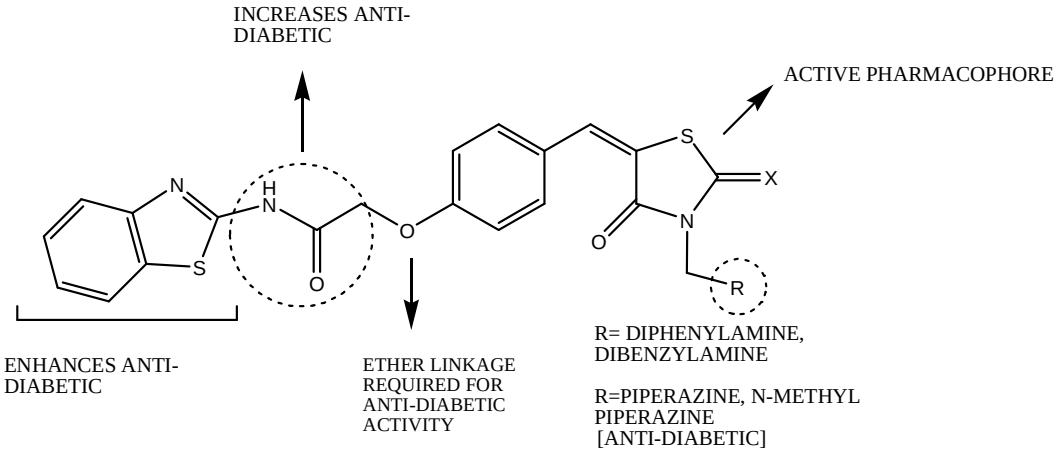


Fig 3. Various active sites of the synthesized derivatives contributing to the anti-hyperglycemic activity

It can be further concluded that

- Presence of piperazine and N-methyl piperazine at N-3 of thiazolidinedione and rhodanine derivatives have shown significant activity in comparison to aromatic amine substitution.
- The presence of ether linkage is the primary requisite for anti-hyperglycemic activity.
- Condensing benzylidene with lipophilic moiety i.e. benzothiazole enhanced the activity of the synthesized derivatives.
- Piperazine substituted compound, MB01was most potent anti-hyperglycemic activity that might be due to the availability of free nitrogen at receptor site which further supports the results by Gaul *et al.* [12]

Table II: Anti-hyperglycemic evaluation of synthesized compounds

S.NO.	PRODUCT CODE	PERCENT BLOOD GLUCOSE REDUCTION		
		1 Hr	3 Hr	6 Hr
1	PIOGLITAZONE	19.35±0.6131**	44.21±0.1378**	67.97±0.7189**
2	TZP	14.97±0.5709**	23.54± 0.2541**	35.52± 0.1970**
3	RHP	9.71±0.2260**	29.31±0.1746*	40.44±0.2390**
4	MB01	16.97±0.2529**	35.34±0.2648**	46.46±0.2045**
5	MB02	15.02±0.2975**	23.09± 0.1624**	37.32±0.1723**
6	MB03	14.02±0.2529**	31.59±0.1586*	40.16±0.1428**
7	MB04	8.97±0.2986**	28.18±0.4264**	35.38±0.2347**
8	MB05	11.0±0.3013**	28.49±0.2646**	40.24±0.3199**
9	MB06	9.23 ±0.1453**	28.41±0.2092**	42.47±0.2512**
10	MB07	13.9 ±0.238**	21.54±0.2183**	35.46±0.2294**
11	MB08	10.87±0.1872**	23.27±0.1767**	41.44±0.2072**
12	MB09	14.59±0.2992**	22.43±0.2249**	35.50±0.2153**
13	RHB	16.35±0.1685**	19.32±0.136**	36.46±0.2147**

*The basal blood glucose level considered as 100% respectively for calculation of % decrease n=3.The results expressed as Mean ±SEM and the data analyzed using One-way ANOVA followed by Dunnett test; ***p<0.001, **p<0.01, *p<0.05*

Graphical representation of lowering of blood glucose level by synthesized derivatives of thiazolidinedione and rhodamine can be seen in Fig 4 to 10 respectively.

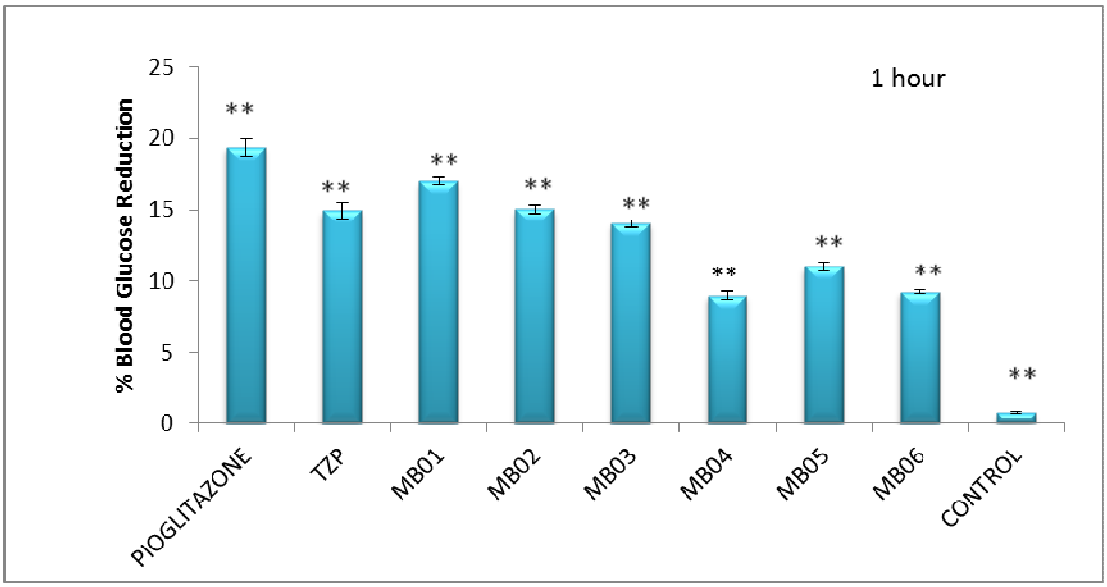


Fig 4. Blood glucose reduction by thiazolidinedione derivatives at one hour

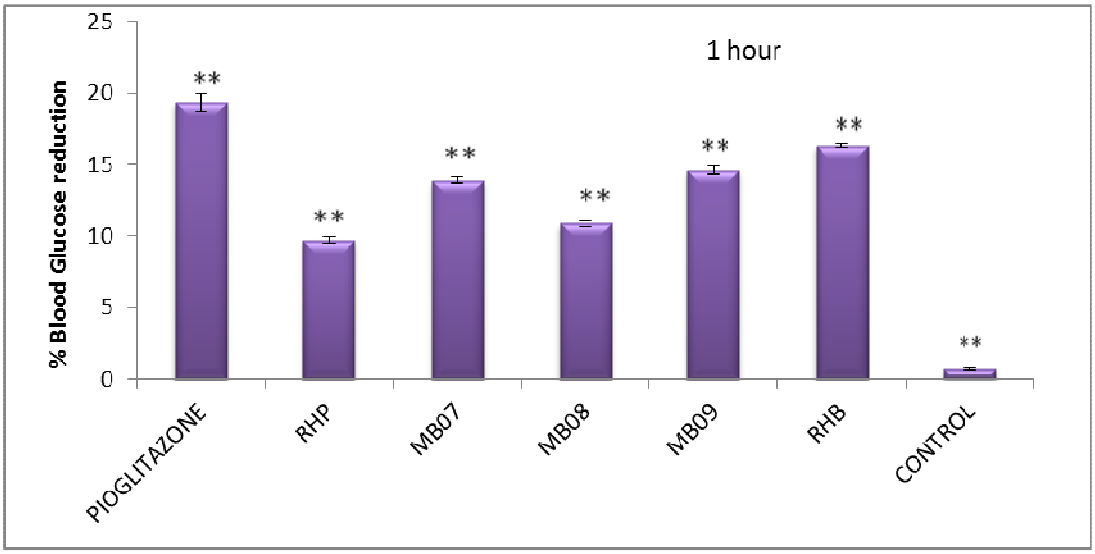


Fig 5. Blood glucose reduction by rhodanine derivatives at one hour

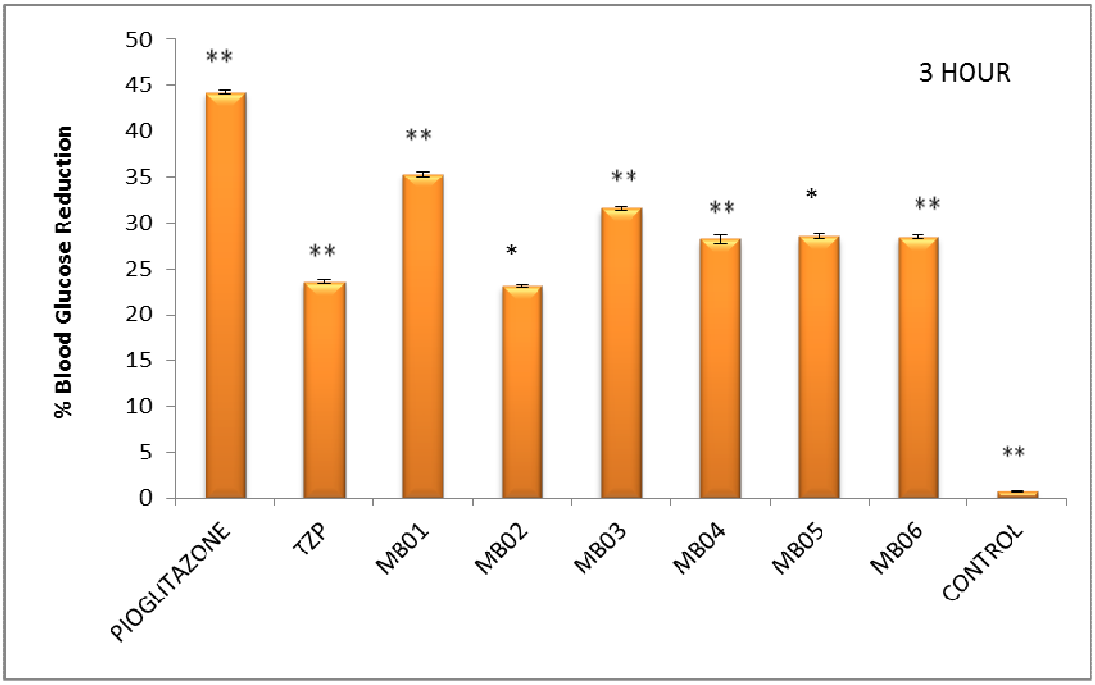


Fig 6. Blood glucose reduction by thiazolidinedione derivatives at three hour

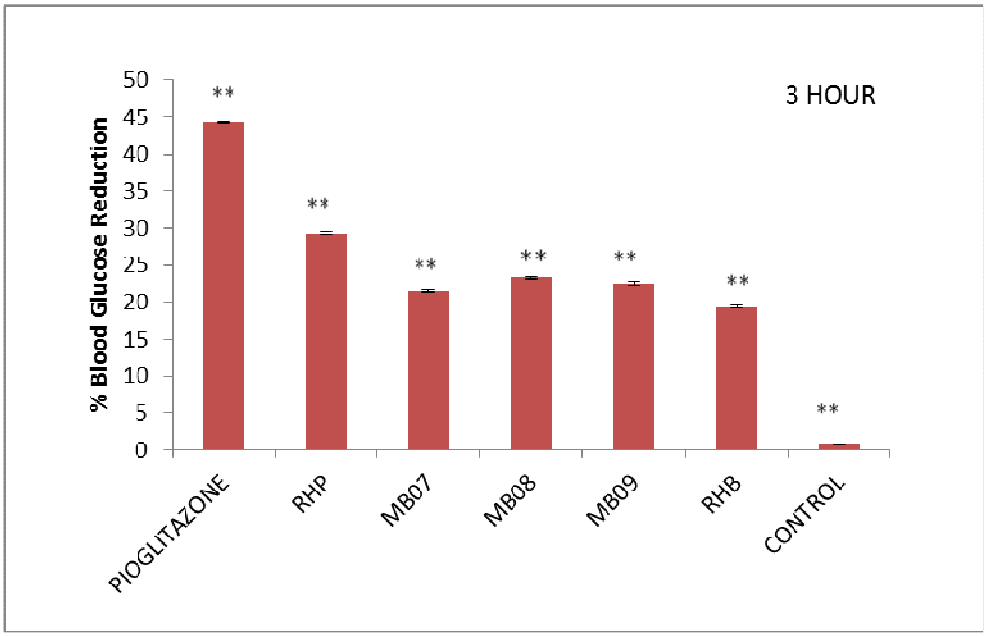


Fig 7. Blood glucose reduction by rhodanine derivatives at three hour

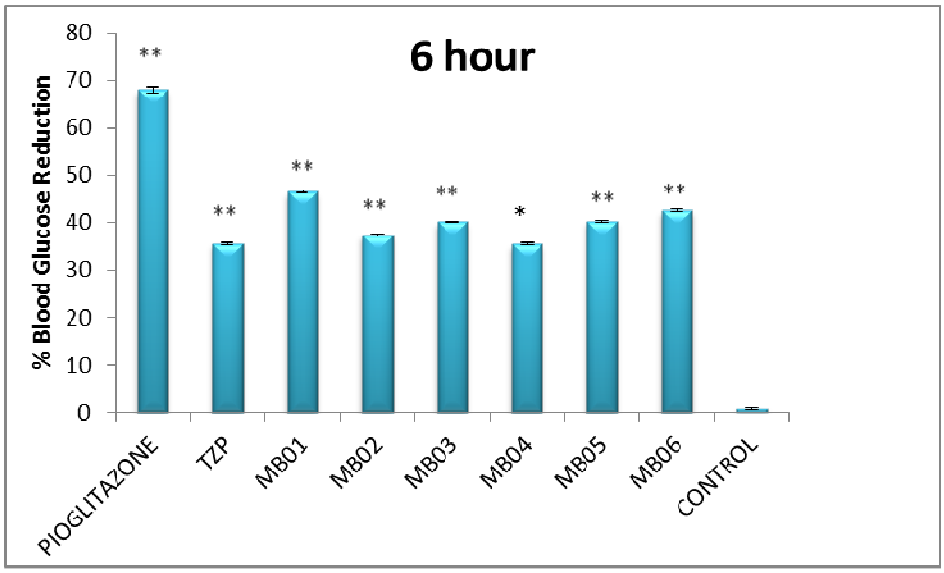


Fig 8. Blood glucose reduction by thiazolidinedione derivatives at six hour

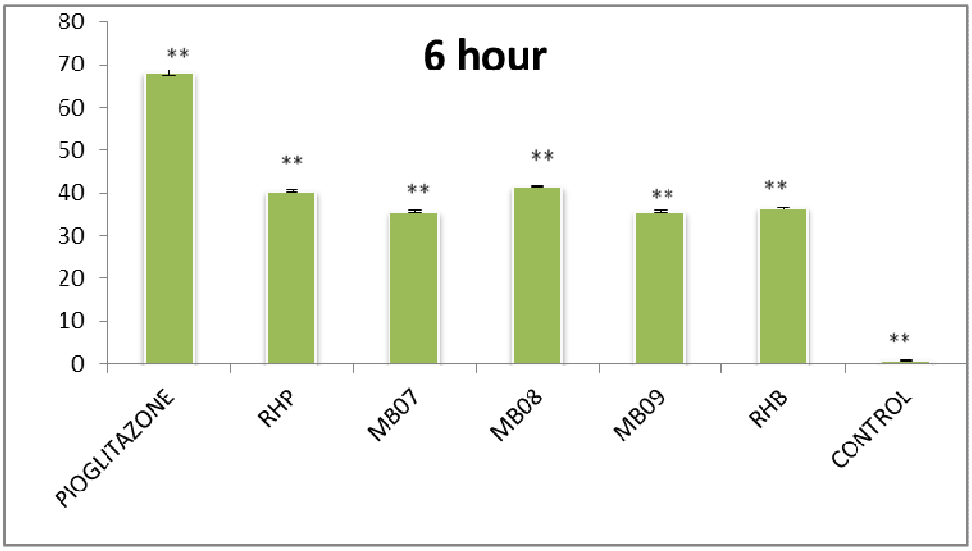


Fig 9. Blood glucose reduction by rhodanine derivatives at six hour

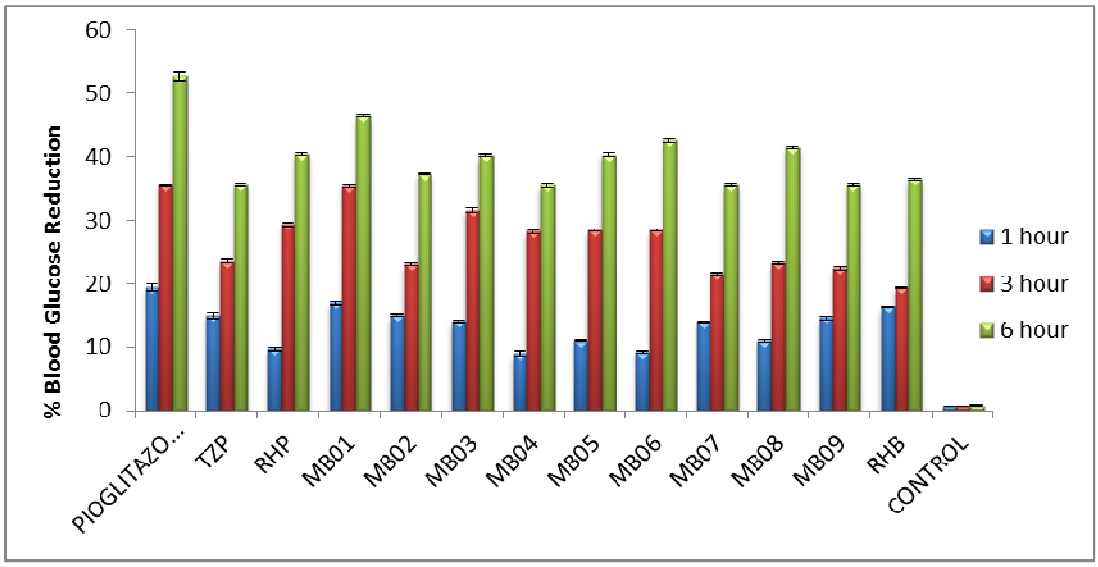


Fig 10. Blood glucose reduction of all synthesized derivatives

CONCLUSION

The mannich bases of 2, 4-thiazolidinedione and rhodanine derivatives were synthesized and were found to be in their agreement with the assigned molecular structure by means of IR, NMR spectral analysis. The derivatives were evaluated for their *in-vivo* anti-hyperglycemic activity. The results can be summarized as follows: The highest anti-hyperglycemic activity was shown by **MB01** with maximum blood glucose reduction of 46.46%.

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