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Synthesis and Biological Evaluation of Some New Rhodanine Analogues as Aldose Reductase Inhibitors (ARIs)

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting long-term secondary complication. Aldose reductase, the rate-limiting enzyme of the polyol pathway, plays a key role in the treatment of diabetic complications. Appropriately, inhibition of this enzyme is emerging as a major therapeutic strategy for the pathogenesis of secondary complication. In this study, we describe a series of 5 aryl benzylidene -thiazolidine, 4-dione derivatives, F3 synthesized as aldose reductase inhibitors. Besides inhibiting efficiently the target enzyme, F4 and F5 showed additional AR inhibitory as well as hypoglycaemic activity (146.15 and 175.20 mg/dl) thus emerging as novel dual acting compounds. The bezylidene derivative F3, the most promising of the whole series, showed a well-balanced, consisting of ALR2 inhibitory efficacy (83.00% at 10μg/mL), similarly, F3 have lower blood glucose level in the range of 131.11 mg/dl at 15 mg/kg body weight. This compound show robust in vitro and in vivo efficacy, and could be considered as promising dual target antidiabetic drug candidates.

Keywords: Diabetes mellitus, hyperglycemia, Aldose reductase inhibitors

Introduction

Diabetes is a devastating disease increasing rapidly all over the world, epidemiological studies have estimated at over 171 million people worldwide were suffering with this disease in 2000 and the prevalence is expected to grow more than twice and reach to 366 million by 2030.[1] Although, diabetic patients in China have reached 92 million in 2007, and even escalate to 114 million in 2010.[2,3]

The increase in prevalence rate are as a result of authorization, westernization and their associated lifestyle changes, increase in life expectancy at birth, obesity, physical inactivity, and possibly a genetic predisposition.

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting long-term complications, such as neuropathy, nephropathy, cataracts, retinopathy due to defects in insulin action, insulin secretion or both.

Aldose reductase (ALR2; EC: 1.1.1.21) plays a pivotal role in development of chronic diabetic complications, belongs to aldoketo reductases super family. It is the first rate limiting enzyme in polyol pathway and reduces glucose to sorbitol by utilizing NADPH as a cofactor. Sorbitol dehydrogenase is the enzyme responsible for the conversion of sorbitol into fructose.

Generally, ALR2 has a low activity and only a small amount of the glucose is metabolized by polyol pathway. In hyperglycemia, however, ALR2 is activated and one third of the total metabolic glucose turns to the alternative polyol pathway, which leads to the accumulation of sorbitol in tissues possessing insulin-independent uptake of glucose, such as kidney, lens, retina, peripheral nerves.[4]

Sorbitol is hardly excreted across cell membranes due to strong polarity, and therefore its intracellular accumulation would lead to osmotic imbalance, cell swelling, and changes of membrane permeability, mainly in lens. Also, the abnormal level of NADPH and NAD+ caused by the polyol pathway would induce modification in cellular redox potentials, and unconventionally activated enzymes such as nitric oxide synthase (NOS) and glutathione reductase would give rise to the cellular oxidative stress. As a consequence, the imbalance between increased production of radical oxygen species (ROS) and reduced intracellular antioxidant defense occurs.[5]

In the recent few years, the aromatic heterocyclic structures including thiazolidenedione, benzamide and other derivatives have been found to be excellent scaffold for the ARI drug design, which led to a number of novel and potent inhibitors as promising drug candidates for the treatment of diabetic complications.

Heterocyclic compounds are an important part of the synthetic medicinal chemistry. They offer a high degree of structural variety and have proven to be widely useful as therapeutic agents. Heterocyclic compounds play an important role in the biological processes. They are widespread as natural products. Heterocycles have huge potential as the most promising molecules as lead structures for the design of new drugs.[6]

Rhodanine is a five-membered heterocyclic molecule containing a thiazole nucleus with thioxo group on second carbon and carbonyl group on fourth carbon. Structural modifications of rhodanine derivatives consequently, compounds with a broad spectrum of pharmacological activities. [7, 8]

Rhodanine is a five-membered heterocycle containing thio ether and amino groups at positions 1 and 3, respectively. It is structurally related to thiazolidine-2,4-dione and 2-iminothiazolidine-4-one that include an oxo or imino group, respectively, instead of the thioxo group at position 2. It is also related to 4-thioxothiazolidin-2-one, which bears oxo and thioxo groups at positions opposite to those in rhodanine. However these heterocycles reveal to be very similar at first gaze, compounds derivatives based on these framework usually differ in their biological activities. The rhodanine ring allow the formation of several types of ligand protein interaction with the amino acid residues like in hydrogen bonds, a hydrogen bond acceptor or donor, hydrophobic and $p-p$ and cation–p interactions with amino acids with aromatic or charged side chains in the case of aromatic 5-benzylidene rhodanines. All these possibilities give the rhodanine framework special properties to compounds possessing high biological activity.[9-15]

There are currently two main classes of AR inhibitors, cyclic imides such as sorbinil and carboxcylic acid derivatives such as tolrestat and epalrestat, is only drug marketed to treat the diabetes complications.

Although a large no of synthetic ARIs have shown to inhibit the enzyme and have been tested in clinical trials, the clinical efficacy of these compounds is not satisfactory and some have also shown deleterious side effect.[16] Furthermore recent study was explored to improving the activity via heterocyclic analogues like Thiazolidinediones and Benzimidazole rhodanine derivatives which improve glycaemic control type 2 diabetes now as effective antihyperglycaemic agents and AR inhibitors

Consequently it is need to develop new analogues of aldose reductase inhibitors which could be devoid from the toxicity.

In this study, we have determined the probable inhibitory effects of some rhodanine derivatives, already known to have antidiabetic activities but their effectiveness generally decreases in vivo, probably due to their poor penetrability to key target tissues, in particular, peripheral nerves. Hence, the aim of this work to develop new ARIs, active on relevant targets involved in the control of glucose level to hyperglycaemic conditions, are presented as promising antidiabetic compounds.

At present study, some new analogs of 5-arylidene-2, 4-dioxothiazolidines were synthesized and biological

evaluated as potent ARI such as imine derivatives of rhodanine possess anti diabetes properties.

Material and Methods

Chemistry

All the chemicals and reagents used in the synthesis of designed compounds were of synthetic grade, and commercially procured from Loba, Highmedia, and E. Merck. The melting points were determined using open capillary tubes and are uncorrected. Purity of the all synthesized compounds was checked by thin layer chromatography technique (0.2 mm thickness of silica gel G plates) and iodine was used as visualizing agent and recrystallize with column chromatography technique. For the purpose of chromatography glass column (high 18″ with internal diameter 20 mm), column grade silica gel mesh #240-400 as the stationary phase and appropriate solvent system as mobile phase were used. Absorption maxima (λmax) of the intermediate and synthesized compounds were determined on Shimadzu 1800 UV-visual © 2018 spectrophotometer by scanning the compound IR spectra were recorded on Shimadzu8400s at BR Nahata college of Pharmacy, Mandsaur (M.P.). The samples for NMR and Mass were tested at IIT Indore (M.P.).

In-vitro biological evaluation

Enzyme preparation[14].

Eye ball was removed from goat immediately after sacrifice and stored in ice-cold container. Lenses were removed by lateral incision of the eye, washed with ice-cold distilled water and kept cold. The lenses were homogenized in 10 volumes of 100 mM ice- cold potassium phosphate buffer, pH6.2 and centrifuged at 15,000 xg for 30 minutes at 4℃. The resulting supernatant was used as the source of aldose reductase. Saturated ammonium sulphate (100%) was added to the supernatant from the homogenate to reach 40% saturation and then allowed to stand for 15 min with occasional stirring to ensure the completeness of precipitation. It was then centrifuged and the precipitate was discarded. The same procedure was repeated for the resulting supernatant using 50% and 75% ammonium sulphate saturations. The final supernatant was used as the partially purified aldose reductase. The precipitate recovered from the 75% saturated fraction, possessing ALR2 activity, was Redissolved in 0.05 M NaCl and dialyzed overnight in 0.05 M NaCl. The dialyzed material was used for the enzymatic assay.

In-vitro Enzyme inhibition assay of aldose reductase

ALR2 activity has been assayed at 30℃ in a reaction mixture containing 0.75 mL of 10 mM D,L-glyceraldehyde, 0.5 ml of 0.104 mM NADPH, 0.75 mL of 0.1 M sodium phosphate buffer (pH=6.2), 0.3 mL of enzyme extract and 0.7 mL of deionized water in a total volume of 3 ml. All the above reagents, except D, L-glyceraldehyde, were incubated at 30℃ for 10 minutes; then substrate was added to start the reaction, which was monitored for 5 minutes. Enzyme activity calibrated by dilutes the enzymatic solution to obtain an average reaction rate of 0.011±0.0010 absorbance units/minute for the sample. AR percentage inhibitory activity of the synthesized compounds (15 μL, 5μg/mL) has been determined using same procedure.
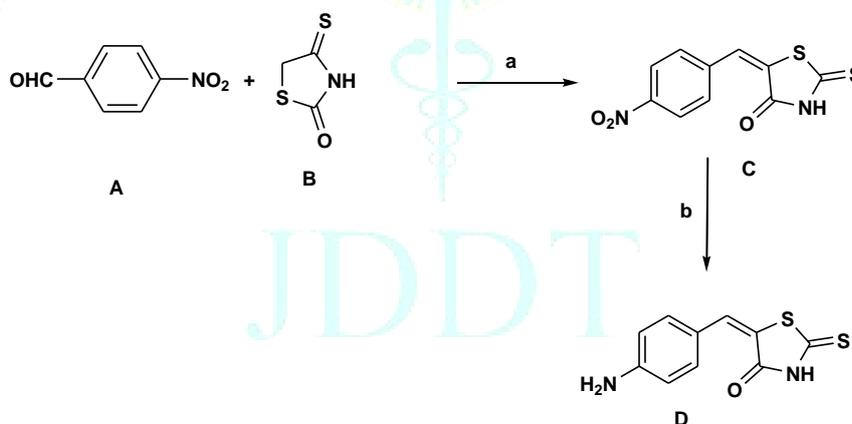
In-vivo Biological Evaluation

Alloxan Induced Diabetic Model

The diabetes in Wister rats (150 ± 20 g) was experimentally induced by intra peritoneal administration of alloxan monohydrate (dose 120 mg/kg body weight) in saline (1% w/v NaCl). Prior to this rat were fasted for 18 h but were allowed free access to drinking water. The rats were kept for next 24 h on 5% glucose solution in bottles in their cages to prevent hypoglycemia. The blood glucose level was checked after 72 h. Animals with serum glucose levels 200–350 mg/dl were considered diabetic and were used for the study. A 5% dextrose solution was given in feeding bottle for a day to overcome the early hypoglycemic phase. The blood glucose regulator has been monitored after alloxination by withdrawing a drop of blood from the tail vein by Tail tipping method. The blood dropped on the dextrostix reagent pad. The strip was inserted into microprocessor digital blood glucometer and readings were noted.
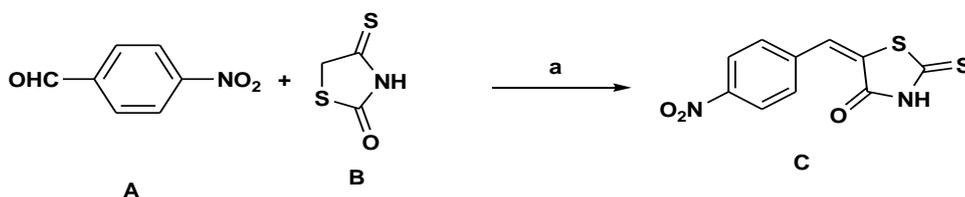
Experimental Anti diabetic Evaluation

Experimental Animals Adult Wister rats weighing (120–150 g) of either sex were used as experimental animals. All the animals were housed in cage at a temperature of 22 ±1C and a relative humidity of 55± 5%and 12 h dark and 12 h light cycle was followed during the experiments. Animals were allowed free access to food and water *ad libitum.* During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were followed and experimental study has been approved by Institutional Animal Ethical Committee (IAEC), Protocol no. is PBRI/IAEC/PN-17049a.

Experimental Design

Experimental design Animals were divided into 6 rats in each group. The animals found diabetic after induction of alloxan monohydydrate have been selected for the further study. Group 1 for diabetic control (Alloxan induced), Group 2 for reference standard (rosiglitazone 4 mg/kg) and Group 3-8 for synthesized compounds (15 mg/kg for acute study). Antidiabetic activities of the synthesized compounds were tested by using alloxan induced diabetic model in Wistar rats. The dose of the synthesized compounds (15 mg/kg body weight) and rosiglitazone (4 mg/kg body weight) were administered orally in 2% acacia. The blood glucose level was monitored at different times 0, 1, 3, and 9 hours respectively.

GENERAL SYNTHESIS AND SCHEME

Synthesis of following compounds is shown

1. Method for the Synthesis of 2-thiaoxozolidin-4- one nitro benzylidene derivatives (C)

2. Method for the Synthesis of 2-thiaoxozolidin-4- oneamino benzyl derivatives (D)

3. Method for the Synthesis of substituted benzylidene 2,4-dioxothiazolidin derivatives (F_1-F_5)

4. Methods for the Synthesis (Z)-N-(4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl) phenyl)benzamide F_6

Reagents conditions: (a) CH_3COONa, CH_3COOH, Reflux; (b) Tin granules, HCl, Heat, NaoH;
Scheme1: General synthesis scheme of 2-thiaoxozolidin-4- one benzylidene derivatives.

Method for the Synthesis of 2-thiaoxozolidin-4- one nitro benzylidene derivatives (C)

Equimolar concentration of derivative of 4 nitro benzaldehyde (0.025 mol) and rhodanine (0.025 mol) was taken in round bottom flask containing glacial acetic acid. To this, catalytic amount (0.080gm) was added the reaction mixture was stirred and heated at 100-105 ºC for 10 -12 hrs. Progress of the reaction mixture was checked through TLC. After completion of reaction, mixture was kept aside for overnight at RT crystalline product was filtered, washed with cold acetic acid and used in next step.

Reagent condition: a=CH_3COOH, heat and stir

Method for the Synthesis of 5 aryl (amino benzylidene)-2-thioxothiazolidin-4-one (D): The crude amount C (0.025 mol) and granulated tin (0.038 mol) were taken in RBF equipped with reflux condenser. 10 ml HCl was added in step to control vigorous reaction. After complete addition of HCl,

the reaction mixture was heated on water bath and progress of the reaction mixture was checked through TLC. After completion of reaction gradually sodium hydroxide solution (7.5gm in 12 ml) was added and amine was separated out.
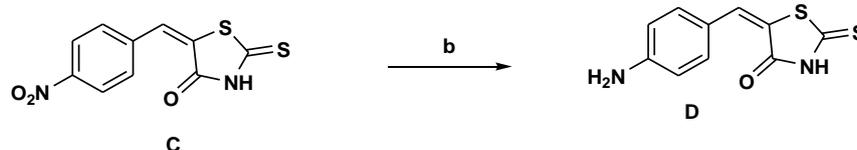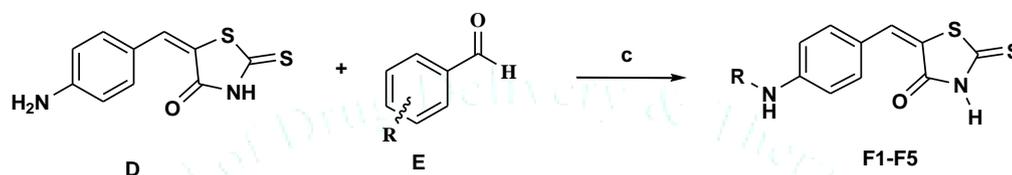
Reagent condition: b= granulated tin, HCl and heat

Synthesis of 5 aryl (amino benzylidene)-2-thioxothiazolidin-4-one (D)

Method for the of substituted 5 aryl (amino benzylidene)-2-thioxothiazolidin-4-one: Equimolar concentration of compound (D) and substituted benzaldehyde reflux with ethanol (3.5ml), and acetic acid (2-

3drop).The reaction mixture was stirred and heated at 100-105 ºC for 4 hrs. Progress of the reaction mixture was checked through TLC. After completion of reaction, product was filtered and washed.

Reagent condition: **c=** ethanol, acetic acid, heat and stirring

Scheme **2**: Synthesis scheme of substituted 5 aryl (amino benzylidene)-2-thioxothiazolidin-4-one

Synthesis of benzoyl chloride

Benzoic acid (0.01 mol) was refluxed with thionyl chloride for 3-4 hrs, and the reaction was monitored through TLC.

After completion of reaction, evaporate excess of thionyl chloride under reduced pressure, and the crude solid was used as such in next step.
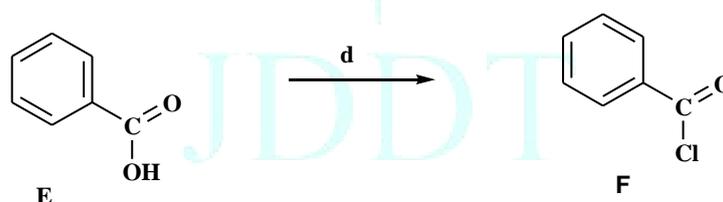
Reagents and conditions: (d) SOCl2, Reflux

Method for the synthesis of *N*(Z)-*N*-(4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl) **benzamide F6:** crude product was taken in RBF containing anhydrous dichloromethane. To this, a catalytic amount of triethylamine (0.001) was added. To the reaction mixture benzoyl chloride

was added slowly with constant stirring. Progress of the reaction mixture was checked though TLC. After evaporation of the solvent under reduced pressure, the crude solid was purified by column chromatography.
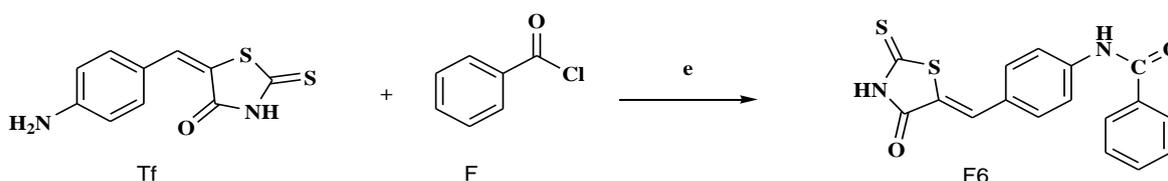
Reagents and conditions: (d) CH₂Cl₂, N(CH₂CH₃)₃,C₆H₅Cl and reflux

RESULT AND DISCUSSION

A series of rhodanine derivatives were synthesized. The structures of these compounds were established by means of IR, 1H NMR, and elemental analysis study shown in table.

Table 1: IR, NMR and mass studied of synthesized compound

1		IR (KBr, cm-1)3591(N-H), 3016(C-H), 1867 (C=O), 1651 (C=C), 1560(C=N);^{1}H NMR (400 MHz, DMSO) δ 10.95 (s, 1H), 8.81 (s, 1H), 7.75 – 7.61 (m, 2H), 7.37 (d, J = 5.9 Hz, 2H), 7.33 – 7.13 (m, 6H). m/e: 324.04; C, 62.94; H, 3.73; N, 8.63; O, 4.93; S, 19.77
2		IR (KBr, cm-1)3280(N-H), 3155(C-H), 1722(C=O), 1721 (C=C), 1207(C=N)^{1}H NMR (500 MHz, DMSO) δ 8.66 (s, 1H), 7.97 (s, 1H), 7.61 – 7.52 (m, 3H), 7.50 (s, 1H), 7.41 (s, 1H), 7.32 – 7.18 (m, 2H), 7.16 (s, 1H).m/e: 369.02 ;C, 55.27; H, 3.00; N, 11.37; O, 12.99; S, 17.36
3		IR (KBr, cm-1)3340(N-H), 3100(C-H), 1842(C=O), 1629 (C=C), 1157(C=N);^{1}H NMR (400 MHz, DMSO) δ 10.96 (s, 1H), 8.82 (s, 1H), 8.62 (s, 1H), 8.27 (s, 1H), 8.04 (s, 1H), 7.55 (s, 1H), 7.34 – 7.23 (m, 5H). m/e: 369.02 ;C, 55.27; H, 3.00; N, 11.37; O, 12.99; S, 17.36
4		IR (KBr, cm-1)3201(N-H), 3051(C-H), 1867 (C=O), 1452 (C=C), 1639(C=N);^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 8.70 (s, 1H), 8.11 – 7.93 (m, 2H), 7.87 – 7.70 (m, 2H), 7.39 – 7.19 (m, 5H). m/e: 369.02 ;C, 55.27; H, 3.00; N, 11.37; O, 12.99; S, 17.36
5		IR (KBr, cm-1)3340(N-H), 3100(C-H), 1842(C=O), 1629 (C=C), 1157(C=N);^{1}H NMR (400 MHz, DMSO) δ 9.97 (s, 3H), 7.89 – 7.75 (m, 6H), 7.47 – 7.31 (m, 19H), 7.31 (s, 2H), 7.13 (s, 3H).m/e: 369.02 ;C, 55.27; H, 3.00; N, 11.37; O, 12.99; S, 17.36
6		IR (KBr, cm-1)3379(N-H), 2808(C-H), 1869(C=O), 1541(C=C), 1689(C=N); 1H NMR (400 MHz, DMSO) δ 10.98 (s, 1H), 8.81 (s, 1H), 7.34 – 7.19 (m, 5H), 7.08 (d, J = 11.7 Hz, 2H), 7.01 (s, 1H), 6.67 (s, 1H), 2.91 – 2.86 (m, 6H). m/e: C, 62.10; H, 4.66; N, 11.43; O, 4.35; S, 17.45

Characterization of synthesized rhodanine compounds

Table 2: λmax, , Rf, MW, % yield of synthesized compound

Comp no.	R	MW	%Yield	Rf value	λmax(nm) in Methanol
F1		250–255ºC	60.0	0.59	352
F2		240–243ºC	35.5	0.52	359
F3		230–235ºC	55.0	0.79	368
F4		225–228ºC	60.2	0.75	370
F5		200-210ºC	79.8	0.48	338
F6		220-223ºC	33.4	0.59	358

Aldose reductase Inhibitory activity

All the synthesized 5 aryl 2-thioxothiazolidin-4-one derivatives were evaluated for their ability to inhibit the *in vitro* reduction of D, L glyceraldehydes by partially purified ALR from goat lenses; sorbinil was used as a reference drug (Table 3 and Fig 1)

Table 3: Aldose reductase percentage inhibitory activity of synthesized 5 aryl benzylidene 2-thioxothiazolidin-4-one

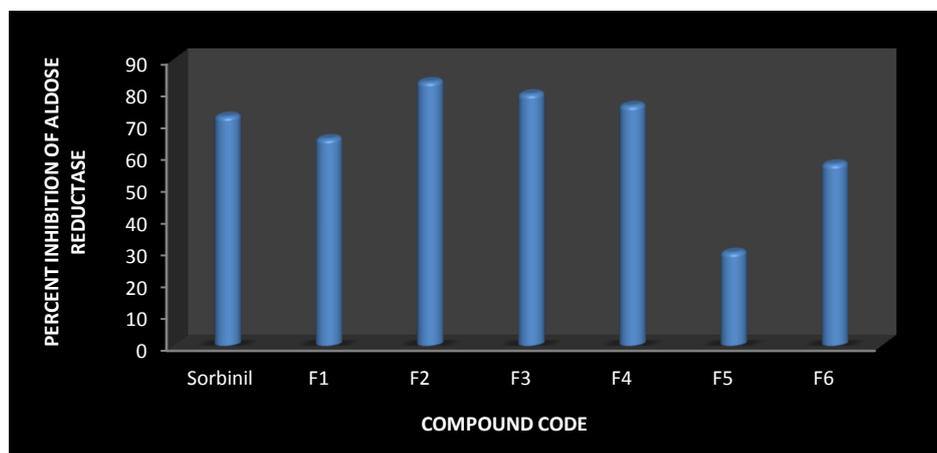| S.N.	Comp Code	% Inhibition
1	Standard (Sorbinil)	70.2
2	F1	65.05
3	F2	29.00
4	F3	83.14
5	F4	76.51
6	F5	72.32
7	F6	57.13

Figure 1: Graphical representation of aldose reductase inhibitory activity of 5 aryl benzylidene 2-thioxothiazolidin-4-one
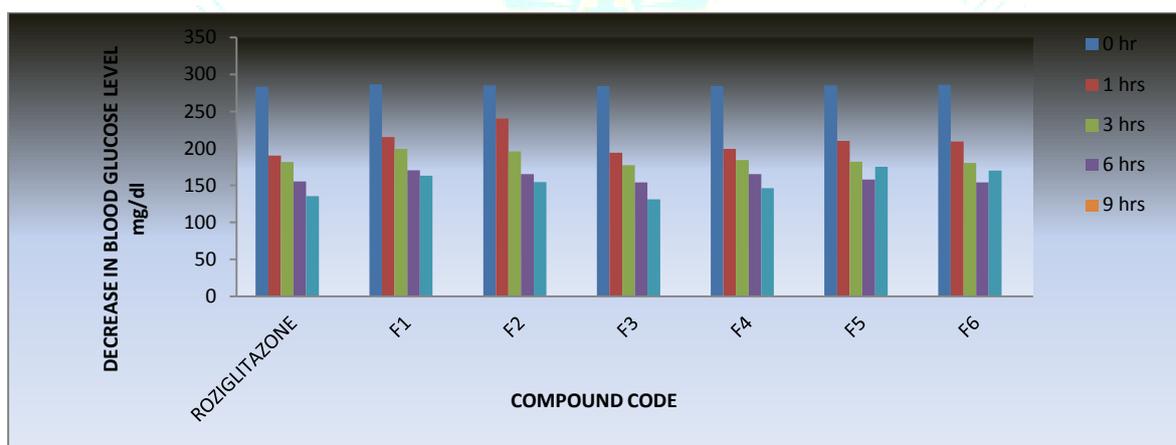

Figure 2: Graphical representation of antidiabetic activity of 5 aryl benzylidene 2 thioxothiazolidin-4-one

Antidiabetic activity

In vivo biological evaluation of all the synthesized rhodanine also evaluated for their antidiabetic activity using rosiglitazone as reference drug. The decrease in blood glucose level against each compound is shown in Table 3 and Fig. 3.

Table 4: Antidiabetic activities of the synthesized 5arylbenzylidene 2, thioxothiazolidin-4-one

Comp code	Decrease in blood glucose level mg/dl				
	0 hrs	1 hrs	3 hrs	6 hrs	9 hrs
Cntrol	75.20±1.306	77.25±1.120	78.50±1.478	80.32±1.789	85.20±1.200
Std(rosiglitazo)	283.35±1.250***	190.25±2.220**	181.45±2.120**	155.20±1.230***	135.35±1.185***
F1	286.51±2.520**	215.31±2.250**	199.35±2.325**	170.38±3.322*	163.30±1.340***
F2	283.16±2.355**	240.50±1.320***	196.00±4.300*	165.20±1.255***	154.30±2.270**
F3	282.15±1.410***	194.15±1.230***	177.12±1.220***	154.17±1.300***	131.11±1.150***
F4	284.15±1.400**	199.15±1.235***	184.22±1.225***	165.26±2.325**	146.15±1.280***
F5	285.24±2.420**	210.15±1.250***	182.22±2.330**	158.00±3.320*	175.20±1.300***
F6	285.24±2.420**	209.15±1.250***	180.22±2.330**	154.00±3.320*	170.20±1.300***

CONCLUSION

The present work was studied on 5 aryl benzylidene - thiazolidine, 4-dione derivatives, this family constitutes a scaffold from which more potent dual acting (aldose reductase inhibitory action and antihyperglycemic action) compounds could be designed by acting on ALR2. In the present case compound F3 may show good aldose reductase inhibitory action but optimum antihyperglycemic action, that most of the electron-withdrawing substituents in the aromatic aldehydes can stabilize the creation of stable iminium ion. Moreover, F4 and F5 both the actions were good and have been optimized. All significant scaffold of compound increased the interaction of the inhibitor with the hydrophobic region of the ALR2 active pocket with these targets. It is explore that rhodanine derivatives gives potential dual action but significant in aldose inhibitory activity. It is evident from the present study that rhodanine may be considered as a new class of potent and selective inhibitors of ALR2. The compounds can be further modified to get promising ALR2 inhibitors for the prevention and treatment of diabetic complications.

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