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Design, Synthesis and Evaluation of Substituted 5-(2-methoxybenzylidene)-rhodanine Ester Analogs as Aldose Reductase Inhibitors

***Jyoti Pandey*[1], *Ritu Gilhotra*[1] *and Arun K. Gupta*[2]**

[1]*School of Pharmacy, Suresh Gyan Vihar University, Jaipur (Rajasthan), India*
[2]*Chameli Devi Institute of Pharmacy, Indore, (Madhya Pradesh), India*

(Corresponding author: Jyoti Pandey)
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ABSTRACT: Aldose reductase (ALR) enzyme plays a significant role in conversion of excess amount of glucose into sorbitol in diabetic condition, inhibitors of which decrease the secondary complication of diabetes mellitus. The AR active site can adapts itself to bind firmly to special inhibitors; this happens together upon binding to the inhibitor's hydrophobic, hydrophilic heads, and at the specificity pockets of AR, and capable to alter their nature through special conformational changes of the identical residues.

Newer (E)-2-(5-(4-(benzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid and (E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl)phenyl benzoate derivatives were applied for molecular docking studies, synthesized, and these compound were evaluated for their ARI and antidiabetic activity.

A docking study was also applied to envisage the interactions between the aldose reductase and designed series of compounds. The results of this present study might be useful in the designing of more potent rhodanine derivatives as Aldose Reductase Inhibitor. ARI activity of synthesized compounds was found in the range of IC_{50} (µM) 5.47 to 20 at 5µg/mL. Similarly, synthesized compounds decrease blood glucose level in the range of 60.4-75.4 mg/dl at 15 mg/kg body weight.

(E)-2-(5-(4-(benzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid and (E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl)phenyl benzoate analogs shows comparable ARI as well as antidiabetic activity. These new class of rhodanine compounds might be address the diabetic complications with safety.

Keywords: Aldose Reductase Inhibitors, Docking, Diabetes, Substituted 5-(2-methoxybenzylidene)-rhodanine Ester Analogs.

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INTRODUCTION

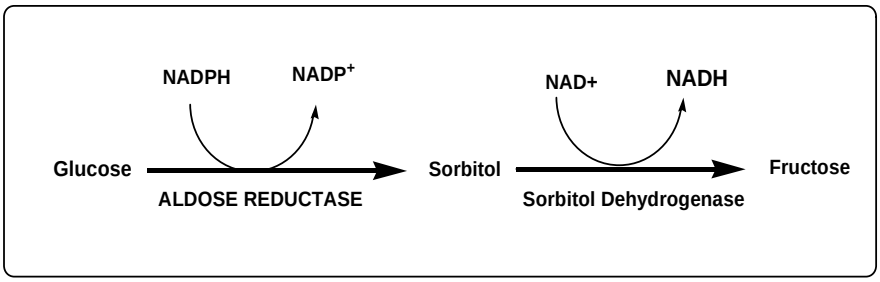
Diabetes mellitus is affecting quality of patient's life because it includes micro vascular and macro vascular complications. Current report on survey of diabetes patient shows that it is the 21st century most challenging heath problem for human being with occurrence of 387 million and it may be 592 million by 2035. India was home to 61.3 million diabetes patients, as it was known as 'Diabetes Capital of The World'. Now India comes in second after china which is home for 92.3 million diabetics. The estimation of international diabetes federation was the numbers of diabetic patients was doubled between 1995 and 2015 and 70 millions diabetics may be by 2025 (IDF, Diabetes Atlas, 2013 & Wild, 2004). DM is a multisystem disorder comprising metabolic and vascular abnormalities resulting from insulin deficiency, with or without insulin resistance.

Diabetes is a prevalent, costly condition that causes significant illness, disability, and premature death.

Insulin deficiency, in turn, leads to chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism. (Zubin *et al.*, 2018). The diabetic complications and the United Kingdom prospective diabetes study demonstrated that strict and sustained control of glucose excursions through interventions including intensive insulin therapy reduces the risk of developing these complications in types 1 and 2 diabetes (Bastaki, 2005 & Fowler, 2008). Under normal glucose condition glucose first gets converted into glucose- 6 phosphate through phosphorylation by hexokinase enzyme. Some amount of glucose which is non- phosphorylated enters into another pathway, called polyol pathway.

In this reaction enzyme aldose reductase cause the reduction of glucose and converted into sorbitol. Aldose reductase (ALR) contributes to the development of secondary diabetic complications. It is; therefore, believe to be a promising drug target. At present, there is no specific therapy available for diabetic complications. A metabolic approach is to control excess glucose flux in diabetic tissue through the first step of polyol pathway by aldose reductase inhibitors (ARIs) (Agrawal YP *et al*., 2013 Yabe-Nishimura C, 1998, Costantino *et al.,* 1999).

Aldose Reductase: Aldose reductase is the enzyme which catalyses the formation of sorbitol from glucose through polyol pathway it is first and rate limiting enzyme of polyol pathway the second enzyme sorbitol dehydrogenase which catalyze the formation of fructose from sorbitol.



Aldose redutase (AR, AKR1B1, and EC1.1.21) is the member of aldo/keto super family. This enzyme involved in metabolism of glucose through polyol pathway. (Seung Hwan Hwang *et al.,* 2018) AR foremost catalyse the construction of sorbitol by the NADPH dependent reduction of aldehyde type of glucose later than that sorbitol dehydrogenase enzyme uses NAD and oxidize the sorbitol into fructose (Rosanna and Rosaria, 2015). As the action of polyol path increases it will source elevation of sorbitol stage in cells and obtain accumulated which leads to osmotic stress on cells this mostly influences the retina, kidney and nervous system as a result polyol pathway is generally good enough and important method for accepting pathogenesis of micro vascular diabetic complication (El-Kabbani O *et al*., 1998 & Urzhumtsev A. *et al*., 1997).

A variety of ARIs have been reported; however, in clinical studies, many of them have exhibited low efficacy or a narrow spectrum of tissue activity,generally because of unfavorable pharmacokinetics, or have proved to produce toxic side-effects. At present, epalrestat is the only ARI available on the market (Malik *et al.,* 2011). Literature reveals that in the last few years, numerous 5-arylidene-2,4-thiazolidinediones derivatives produced appreciable ALR inhibition (Bruno G. *et al*., 2002 & Agrawal YP *et al*., 2015), but their effectiveness generally decreases *in vivo*, probably due to their poor penetrability to key target tissues, in particular, peripheral nerves (Lee YS *et al*., 2003 & El-Kabbani *et al.,* 2004). Thus, the aim of this work to develop new ARIs, using docking simulation to get improved physicochemical properties and better bioavailability. In this study, new active bioisoster analogs of 5-arylidene-2, 4-dioxothiazolidines were identified and synthesized as potent *in vitro* ARI and *in vivo* antidiabetic activity.

MATERIAL AND METHOD

Docking: The X-ray crystal structure of the protein aldose reductase (4LAU) was retrieved from protein data bank (http://www.pdb.org/pdb/explore/explore.do?structureId=4LAU). The opportunity of binding, accurate and specific location of binding site and the approach of binding of the ligand was conceded by an automated docking software molegro virtual docker 2013, version 6.0.0. The probable orientations and conformations were analyzed by clustering method implanted in MVD. Docking studies were performed using the construct aldose reductase molecular model complexes with inhibitor of *E)-2-(5-(4-(benzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid* and *(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl benzoate* derivatives. The molecular structure of aldose reductase active domain was arranged from the synchronized sets of 4LAU. All water molecules were remaining expelled from the active site set to their crystal place during the whole docking process. The active binding site of the enzyme was computed within a cavity volume 129.024 Å and surface 396.8Å such that the binding site of aldose reductase was well sampled with grid resolution of 0.3 Å The value of population size and maximum interactions 50 and 1500 respectively were used for each run.

Synthesis of Selected Molecules: All the chemicals used in the synthesis of designed compounds were of synthetic grade, and they were procured from Sigma, Loba, Highmedia, and E. Merck.

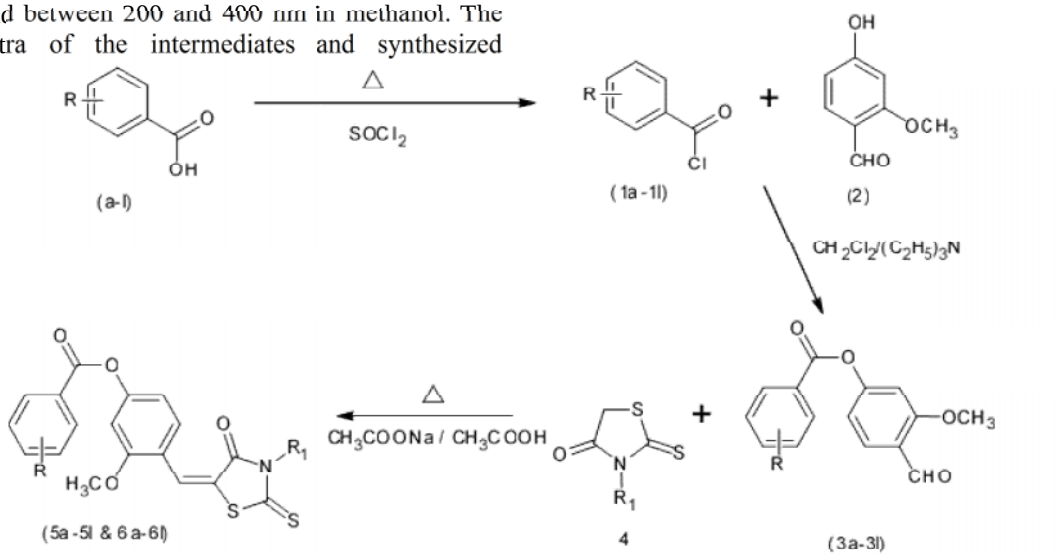
The progress of the reactions was monitored through Thin layer chromatographic method, thin layer chromatography (TLC) was performed using silica gel-G on glass plate in different solvent systems. Iodine vapor and UV detector (long wavelength) were used as detecting agents. The purification of intermediates and final compounds was carried out through re-crystallization and 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. The melting points of synthesized compounds and intermediates were determined by open capillary method, which were uncorrected.

The absorption maxima (λ_{max}) of the intermediate and synthesized compounds were determined on Shimadzu 1700 UV-visual spectrophotometer by scanning the compound between 200 and 400 nm in methanol. The IR spectra of the intermediates and synthesized compounds were recorded on ABB spectrophotometer. The samples were sent to IISER, Bhopal for mass spectrometry (MS), and Punjab University, Chandigarh for nuclear magnetic resonance (NMR). Chemical shifts are given in δ units (ppm) relative to internal standard tetramethylsilane and refer to dimethyl sulfoxide (DMSO)-D6 and $CDCl_3$ as solvent.

General Method of Synthesis

General synthesis of substituted benzoyl chloride (1a-1l)

Substituted benzoic acid (a-l) (0.01 mol) was refluxed with thionyl chloride for 3-4 hrs, and the progress of reaction monitored through TLC (Scheme 1). After completion of the reaction, excess thionyl chloride was distilled off under reduced pressure to afford the corresponding crude benzoyl chloride (1a-1l). The syrupy mass of benzoyl chloride was used as such in next step.



Scheme 1: General Synthesis of Rhodanine Analogs.

Synthesis of substituted 4-formyl-2-methoxyphenyl benzoate (3a-3l)

Dichloromethane (20 mL) was added to the syrupy mass (substituted benzoyl chloride analogs 1a-1l) in pre-cooled RBF at 0°C, subsequently triethylamine (0.03 mol) was added slowly to this with constant stirring. 2-methoxy p-hydroxy benzaldehyde (2) (0.01 mol), was added to the reaction mixture with constant stirring. The reaction mixture was further stirred at 0°C for another 2hrs and stirring was continued at RT for overnight. Progress of the reaction mixture was monitored through TLC, on completion of reaction; mixture was washed with saturated solution of sodium bicarbonate, brine solution and water respectively. The organic phase was separated and passes through anhydrous Na_2SO_4. Solvent was removed under vacuum to afford the corresponding crude benzoate(3a-3l) and the crude product was re-crystallizing through ethanol.

Synthesis of substituted (E)-2-(5-(4-(benzoyloxy)-2-methoxybenzylidene)-4-oxo-2thioxothiazolidin-3-yl) acetic acid (5a-5l) and substituted(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl benzoate (6a-6l)

Substituted benzoate (3a-3l) (0.0025 mol) and rhodanine analogs (4) (0.0025 mol) were taken in glacial acetic acid. A catalytic amount of sodium acetate (0.080 gm) was added to the reaction mixture. The reaction mixture was heated at 100-105°C with continuous stirring for 10-12 hrs. The progress of the reaction mixture was monitored through TLC. After completion of reaction, mixture was kept aside for overnight at RT. The crude was filtered, washed with cold acetic acid and purified by column chromatography to afford the corresponding product (5a-5l and 6a-6l).

Biological Evaluation of Compounds:

In vitro biological evaluation

Enzyme Preparation: A purified goat lens extract was prepared in accordance with the method of Hayman and Kinoshita with slight modifications (18). Lenses were quickly removed from goat eye following euthanasia and homogenized (Glass-Potter) in 5 volume of cold deionized water. The homogenate was centrifuged at 10,400 rpm at 0-4°C for 30 minutes. Saturated ammonium sulfate solution was added to the supernatant fraction to form a 40% solution, which was stirred for 30 minutes at 0-4°C and then centrifuged at 10,400 rpm for 20 minutes. The recovered supernatant was subsequently fractionated with saturated ammonium sulfate solution using first a 50%, and then a 75% salt saturation. The precipitate recovered from the 75% saturated fraction, possessing ALR2 activity, was re-dissolved in 0.05 M NaCl and dialyzed overnight in 0.05 M NaCl. The dialyzed material was used for the enzymatic assay.

Enzymatic Assay: ALR2 activity was assayed at 30°C 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°C for 10 minutes; the substrate was then added to start the reaction, which was monitored for 5 minutes. Enzyme activity was calibrated by diluting the enzymatic solution to obtain an average reaction rate of 0.011±0.0010 absorbance units/minute for the sample. AR inhibitory activity of the synthesized compounds was determined using same procedure.

In vivo biological evaluation

Induction of non-insulin dependent DM: The acclimatized animals were kept fasting for 24 hrs with water *ad-libitm.*1% W/V of alloxan monohydrate (120 mg/kg i.p) in saline was administered,after 1 hr of administrations; the animals were given *ad-libitm.* A 5% dextrose solution was given in feeding bottle for a day to overcome the early hypoglycemic phase. The blood glucose regulator was monitored after alloxination by withdrawing a drop of blood from the tail vein by Tail tipping method. The blood was dropped on the dextrostix reagent pad. The strip was inserted into microprocessor digital blood glucometer and readings were noted.

Experimental Design: After a period of three days the rats with a blood glucose levels greater than 200mg/dl were considered for the study. The alloxan induced diabetic rats were randomly assigned into twenty six groups (1-26) of six rats (n=6) each as follows, namely Group 1- Received normal saline; Group2- Received reference standard (pioglitazone 4 mg/kg body weight), and Group 3-26 for synthesized compounds (15 mg/kg i.p body weight for acute study). The blood glucose level was monitored at different times 0, 1, 3, and 6 hrs, respectively.

RESULT AND DISCUSSION

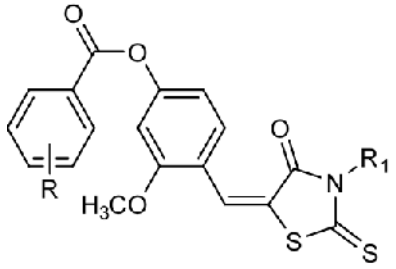
Docking of designed compounds showed hydrogen bond interaction with His110 and Lys 77. the phenyl ring fall in the hydrophobic pocket constituted from TRP111, PHE115, PHE122, VAL130, TRP219, CYS298, LEU300, ALA301, CYS303, TYR309 and forms a π-π stacking with aromatic ring of TRP219, whereas another phenyl ring participating in π-π stacking with aromatic ring of TRP20 which is imperative for potency. The docking score of designed compound are shown in Table 1 while representation of rhodanine analogs interaction with aldose reductase enzyme is shown in Fig. 1.

In this study, a series of 24 new compounds were synthesized. Scheme 1 illustrates the synthetic route for the preparation of target compounds. The purity of the compounds was checked via TLC using various mobile bases and the compounds of this study were identified by spectral data. Both analytical and spectral data (^{1}H NMR, IR spectroscopy, Mass Spectroscopy) of all the synthesized compounds were in full agreement with the proposed structures. The analytical data of compound are as follows:

(E)-2-(5-(4-(benzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl) acetic acid (5a): Yield 78%; M.P. 239-241°C;R_f0.68; IR (υ in cm^{-1}) 2998 (C-H), 1522 and 1505 (aromatic C=C), 1184 (C-O), 1740 (C=O ester), 3397 (O-H); ^{1}H NMR (δ value) 11.12 (s, 1H, -COOH), 7.55-7.8 (m, 5H, Ar-H), 7.28-7.44 (m, 3H, Ar-H &1H, ethylene CH), 3.88 (s, 2H, CH_2), 3.69 (s, 3H, OCH_3); MS (ESI):430$[M+H]^+$.

(E)-2-(5-(4-(2-chlorobenzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5b): Yield 68%; M.P. 235-238°C;R_f 0.46; IR (υ in cm^{-1}) 2984 (C-H), 1184 (C-O), 1743 (C=O ester) 3426 (O-H); ^{1}H NMR (δ value)11.14 (s, 1H, -COOH), 6.5-7.2 (m, 3H, Ar-H &1H, ethylene CH), 7.28-7.44 (m, 4H, Ar-H), 3.86 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3); MS (ESI):464 $[M+H]^+$.

Table 1: Structure of Designed Compounds and their Dock Score



S. No.	Comp. Name	Substitution		Mol Dock Score
		R_1	R	
1	5a	CH_2COOH	H	-157.879
2	5b	CH_2COOH	2-Cl	-157.728
3	5c	CH_2COOH	4-Cl	-163.182
4	5d	CH_2COOH	2,4-Cl	-167.273
5	5e	CH_2COOH	4-OCH_3	-165.124
6	5f	CH_2COOH	2-OCH_3	-164.198
7	5g	CH_2COOH	2-CH_3	-161.788
8	5h	CH_2COOH	3,4-OCH_3	-189.226
9	5i	CH_2COOH	4-CH_3	-162.386
10	5j	CH_2COOH	4-Br	-162.055
11	5k	CH_2COOH	4-F	-164.553
12	5l	CH_2COOH	2-Br	-165.048
13	6a	H	H	-166.144
14	6b	H	2-Cl	-174.881
15	6c	H	4-Cl	-173.974
16	6d	H	2,4-Cl	-179.051
17	6e	H	4-OCH_3	-174.111
18	6f	H	2-OCH_3	-181.457
19	6g	H	2-CH_3	-170.05
20	6h	H	3,4-OCH_3	-181.269
21	6i	H	4-CH_3	-168.92
22	6j	H	4-Br	-170.807
23	6k	H	4-F	-174.435
24	6l	H	2-Br	-163.72

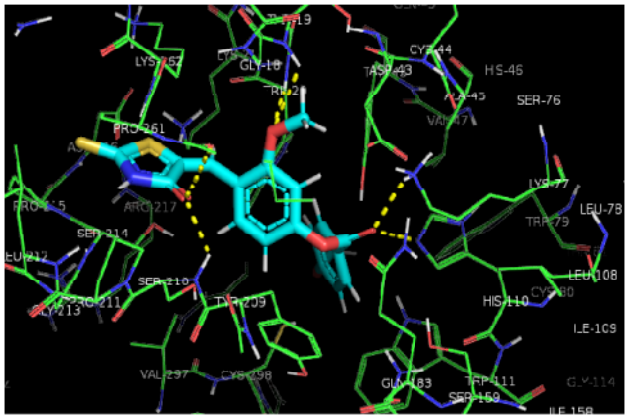


Fig. 1. Illustrative representation of rhodanine analogs interaction with aldose reductase enzyme.

(E)-2-(5-(4-(4-chlorobenzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5c): Yield 78% and M.P. 235-237°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.48; IR (υ in cm^{-1}) 2926 (C-H), 1786 (C=O ester), 1635 (amide C=O), 1595 (aromatic C=C), 1179 (C-O ether); ^1H NMR (δ value)10.97 (s, 1H, COOH), 6.5-7.2 (m, 3H, Ar-H & 1H, ethylene CH), 7.28-7.44 (m, 4H, Ar-H), 3.86 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3); MS (ESI): 464 $[M+H]^+$.

(E)-2-(5-(4-(2,4-dichlorobenzoyloxy)-2-methoxy benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5d): Yield 77% and M.P. 243-245°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.45; IR (υ in cm^{-1}) 3200 (COOH carboxyl acid), 2884 (C-H), 1757 (C=O ester), 1695 (amide C=O), 1516 (aromatic C=C), 1026 (C-O ether);

^{1}H NMR (δ value)11.12 (s, 1H, O-H), 6.7-7.9 (m, 6H, arom. CH; 1H, ethylene CH), 3.88 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3); MS (ESI): 498 $[M+H]^+$.

(E)-2-(5-(2-methoxy-4-(4-methoxybenzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5e): Yield 68% and M.P. 239-242°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.73,IR (υ in cm^{-1})3232 (OH carboxyl acid),3066 (aromatic CH), 1757 (C=O ester), 1693 (amide C=O), 1514 & 1548 (aromatic C=C), 1022 (C-O ether); ^{1}H NMR (δ value)10.98 (s, 1H, O-H), 6.7-8.0 (m, 7H, arom. CH; 1H, ethylene CH), 3.92 (s, 2H, methylene), 3.73 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3); MS (ESI): 460 $[M+H]^+$.

(E)-2-(5-(2-methoxy-4-(2-methoxy benzoyloxy) benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5f): Yield 62% and M.P. 240-243°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.64; IR (υ in cm^{-1}) 3277 (OH carboxyl acid), 3002 (aromatic CH), 1757 (C=O ester), 1696 (amide C=O) 1551 & 1515 (aromatic C=C); ^{1}H NMR (δ value)11.03 (s, 1H, O-H), 6.5-7.6 (m, 7H, arom. CH; 1H, ethylene CH), 3.94 (s, 2H, methylene)3.75 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3);MS (ESI): 460 $[M+H]^+$.

(E)-2-(5-(2-methoxy-4-(2-methylbenzoyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5g): Yield 65% and M.P. 246-248°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.66.IR (υ in cm^{-1}) 3281 (COOH carboxyl acid), 3000 (aromatic (C-H), 1757 (C=O ester),1694 (amide C=O) 1550 & 1514 (aromatic C=C), 1021 (C-O ether); ^{1}H NMR (δ value)11.1 (s, 1H, O-H), 6.2-7.8 (m, 7H, arom. CH; 1H, ethylene CH), 3.85 (s, 2H, methylene), 3.73 (s, 3H, OCH_3), 2.78 (s, 3H, CH_3); MS (ESI): 444 $[M+H]^+$.

(E)-2-(5-(4-(3,4-dimethoxybenzoyloxy)-2-methoxy benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid(5h): Yield 55% and M.P. 249-253°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.65; IR (υ in cm^{-1}) 3276 (COOH carboxyl acid), 2979 (aromatic CH), 1757 (C=O ester) 1670 (amide C=O) 1566 & 1524 (aromatic C=C), 1044 (C-O ether); ^{1}H NMR (δ value)11.21 (s, 1H, O-H), 6.5-7.9 (m, 6H, arom. CH; 1H, ethylene CH), 3.90 (s, 2H, methylene); 3.78-3.73 (s, 9H, OCH_3);MS (ESI): 490 $[M+H]^+$.

(E)-2-(5-(2-methoxy-4-(4-methylbenzoyloxy)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5i): Yield 65% and M.P. 232-234°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.57;IR (υ in cm^{-1}) 3276 (COOH carboxyl acid),3001 (aromatic CH), 1757 (C=O ester),1679 (amide C=O) 1551 & 1516 (aromatic C=C); ^{1}H NMR (δ value)10.97 (s, 1H, O-H), 6.5-7.8 (m, 7H, arom. CH; 1H, ethylene CH), 3.90 (s, 2H, methylene),3.72 (s, 3H, OCH_3), 2.81 (s, 3H, CH_3); MS (ESI): 444 $[M+H]^+$.

(E)-2-(5-(4-(4-bromobenzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5j): Yield 72% and M.P. 262-265°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.51; IR (υ in cm^{-1}) 3248 (COOH carboxyl acid),3012 (aromatic), 1757 (C=O ester) 1682 (amide C=O), 1549 & 1535 (aromatic C=C), 1021 (C-O ether); ^{1}H NMR (δ value)10.59 (s, 1H, O-H), 6.3-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.79 (s, 2H, methylene), 3.74 (s, 3H, OCH_3); MS (ESI): 508 $[M+H]^+$.

(E)-2-(5-(4-(4-fluorobenzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5k) Yield 62% and M.P. 246-248°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.53;IR (υ in cm^{-1}) 3198 (COOH carboxyl acid),3021 (aromaticCH), 1541 (aromatic C=C), 1757 (C=O ester) 1654 (amide C=O), 1016 (C-O ether); ^{1}H NMR (δ value)10.92 (s, 1H, O-H), 6.2-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.87 (s, 2H, methylene), 3.74 (s, 3H, OCH_3); MS (ESI): 448 $[M+H]^+$.

(E)-2-(5-(4-(2-bromobenzoyloxy)-2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid (5l): Yield 68% and M.P. 248-252°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.58;IR (υ in cm^{-1}) 3221 (COOH carboxyl acid), 3003 (aromatic CH), 1757 (C=O ester),1659 (amide C=O), 1539 (aromatic C=C), 1026 (C-O ether); ^{1}H NMR (δ value)10.79 (s, 1H, O-H), 6.6-8.1 (m, 7H, arom. CH; 1H, ethylene CH), 3.89 (s, 2H, methylene),3.72 (s, 3H, OCH_3); MS (ESI):508 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl benzoate (6a): Yield 60% and M.P. 222-225°C, R_f (Pet. Ether: ethyl acetate (3:2)) 0.71; IR (υ in cm^{-1}) 3427 (N-H sec. amine), 3062 (aromaticC-H), 1757 (C=O ester) 1697 (amide C=O), 1550 & 1515 (aromatic C=C), 1068 (C-O ether); ^{1}H NMR (δ value) 6.8-7.9 (m, 8H, arom. CH; 1H, ethylene CH), 3.73 (s, 3H, OCH_3); MS (ESI):372 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 2-chlorobenzoate (6b): Yield 35% and M.P. 229-231°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.49. IR (υ in cm^{-1}) 3394 (N-H sec. amine), 3064 (aromatic C-H), 1757 (C=O ester),1694 (amide C=O), 1548 & 1515 (aromatic C=C), 1052 (C-O ether); ^{1}H NMR (δ value)6.3-7.6 (m, 7H, arom. CH; 1H, ethylene CH), 3.71 (s, 3H, OCH_3); MS (ESI): 406 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 4-chlorobenzoate (6c); Yield 69% and M.P. 230-234°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.71.IR (υ in cm^{-1}) 3394 (N-H sec. amine), 3001 (aromatic C-H), 1757 (C=O ester),1694 (amide C=O), 1513 (aromatic C=C), 1033 (C-O ether); ^{1}H NMR (δ value) 6.6-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.70 (s, 3H, OCH_3); MS (ESI): 406 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 2,4-dichlorobenzoate (6d); Yield 80% and M.P. 232-235°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.47. IR (υ in cm^{-1}) 3427 (N-H sec. amine), 3003 (aromatic C-H), 1756 (C=O ester),1694 (amide C=O), 1515 (aromatic C=C), 1031 (C-O ether); ^{1}H NMR (δ value)6.5-7.9 (m, 6H, arom. CH; 1H, ethylene CH), 3.71 (s, 3H, OCH_3); MS (ESI): 440 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 4-methoxybenzoate (6e); Yield 80% and M.P. 238-242°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.76. IR (υ in cm^{-1}) 3428 (N-H sec. amine), 3003 (aromatic C-H), 1756 (C=O ester), 1695 (amide C=O), 1548 & 1513 (aromatic C=C), 1030 (C-O ether); ^{1}H NMR (δ value)6.5-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.76 (s, 6H, OCH_3); MS (ESI): 402 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 2-methoxybenzoate (6f); Yield 70% and M.P. 240-242°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.74,IR (υ in cm^{-1}) 3394 (N-H sec. amine), 3001 (aromaticC-H), 1757 (C=O ester),1693 (amide C=O), 1514 (aromatic C=C), 1024 (C-O ether); ^{1}H NMR (δ value)6.4-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.74 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3); MS (ESI): 402 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 2-methylbenzoate (6g); Yield 81% and M.P. 240-242°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.66. IR (υ in cm^{-1}) 3393 (N-H sec. amine), 3001 (aromaticC-H), 1756 (C=O ester),1694 (amide C=O), 1513 (aromatic C=C), 1027 (C-O ether); ^{1}H NMR (δ value)6.5-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.71 (s, 3H, OCH_3) 2.78 (s, 3H, CH_3); MS (ESI):386 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 3,4-dimethoxybenzoate (6h); Yield 85% and M.P. 243-245°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.63. IR (υ in cm^{-1}) 3427 (N-H sec. amine), 3002 (aromatic C-H), 1755 (C=O ester), 1694 (amide C=O), 1550 & 1514 (aromatic C=C), 1025 (C-O ether); ^{1}H NMR (δ value)6.5-7.9 (m, 6H, arom. CH; 1H, ethylene CH), 3.76 (s, 3H, OCH_3)3.72-3.71 (s, 6H, OCH_3); MS (ESI): 432 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 4-methylbenzoate (6i); Yield 84% and M.P. 238-240°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.69.IR (υ in cm^{-1}) 3427 (N-H sec. amine), 3000 (aromatic C-H), 1757 (C=O ester),1694 (amide C=O), 1548 & 1513 (aromatic C=C), 1022 (C-O ether),;^{1}H NMR (δ value) 6.6-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.72 (s, 3H, OCH_3), 2.82 (s, 3H, CH_3); MS (ESI):386 $[M+H]^+$.

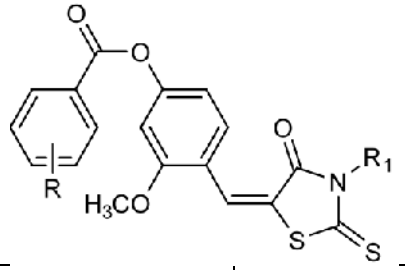
(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 4-bromobenzoate (6j);Yield 80% and M.P. 246-248°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.59; IR (υ in cm^{-1}) 3429 (N-H sec. amine), 3000 (aromatic C-H), 1757 (C=O ester),1694 (amide C=O), 1514 (aromatic C=C), 1024 (C-O ether); ^{1}H NMR (δ value)6.5-7.8 (m, 7H, arom. CH; 1H, ethylene CH), 3.73 (s, 3H, OCH_3); MS (ESI): 450 $[M+H]^+$.

(E)-4-fluorophenyl 3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)benzoate (6k); Yield 80% and M.P. 230-232°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.43. IR (υ in cm^{-1}) 3428 (N-H sec. amine), 3065 (aromatic C-H), 1754 (C=O ester),1692 (amide C=O), 1550 & 1515 (aromatic C=C), 1026 (C-O ether); ^{1}H NMR (δ value)6.3-7.9 (m, 7H, arom. CH; 1H, ethylene CH), 3.72 (s, 3H, OCH_3); MS (ESI):390 $[M+H]^+$.

(E)-3-methoxy-4-((4-oxo-2-thioxothiazolidin-5-ylidene)methyl)phenyl 2-bromobenzoate (6l);Yield 82% and M.P. 244-247°C, Rf (Pet. Ether: ethyl acetate (3:2)) 0.55; IR (υ in cm−1) 3427 (N-H sec. amine), 3002 (aromaticC-H), 1757 (C=O ester),1694 (amide C=O), 1548 & 1514 (aromatic C=C), 1028 (C-O ether); ^{1}H NMR (δ value) 6.5-7.8 (m, 7H, arom. CH; 1H, ethylene CH), 3.75 (s, 3H, OCH_3); MS (ESI): 450 $[M+H]^+$.

In vitro Activity: The synthesized compounds were screened for their aldose reductase inhibitory activity IC_{50} of the synthesized compounds along with the standard drugs for comparison are reported in Table 2 and Fig. 2. The preliminary structure activity relationship reveled that *N*-acetic acid rhodanine analogs are more potent than rhodanine analogs

Table 2: Aldose reductase inhibitory activity data of synthesized compounds.



S.No.	CODE	R_1	R	IC_{50} (µM)
1	5a	CH_2COOH	H	0.40
2	5b	CH_2COOH	2-Cl	6.36
3	5c	CH_2COOH	4-Cl	6.94
4	5d	CH_2COOH	2,4-dichloro	5.42
5	5e	CH_2COOH	4-OCH_3	10.48
6	5f	CH_2COOH	2-OCH_3	8.87
7	5g	CH_2COOH	2-CH_3	19.73
8	5h	CH_2COOH	3,4-dimethoxy	0.712
9	5i	CH_2COOH	4-CH_3	5.98
10	5j	CH_2COOH	4-Br	8.32
11	5k	CH_2COOH	4-F	6.14
12	5l	CH_2COOH	2-Br	10.65
13	6a	H	H	7.08
14	6b	H	2-Cl	4.72
15	6c	H	4-Cl	5.73
16	6d	H	2,4-dichloro	13.51
17	6e	H	4-OCH_3	4.79
18	6f	H	2-OCH_3	6.99
19	6g	H	2-CH_3	7.77
20	6h	H	3,4-dimethoxy	8.13
21	6i	H	4-CH_3	12.63
22	6j	H	4-Br	20.51
23	6k	H	4-F	3.23
24	6l	H	2-Br	16.23
25	Sorbinil	-	-	1.90

**IC50 (µM) (95% C.L.) or % inhibition at given conc[n]*

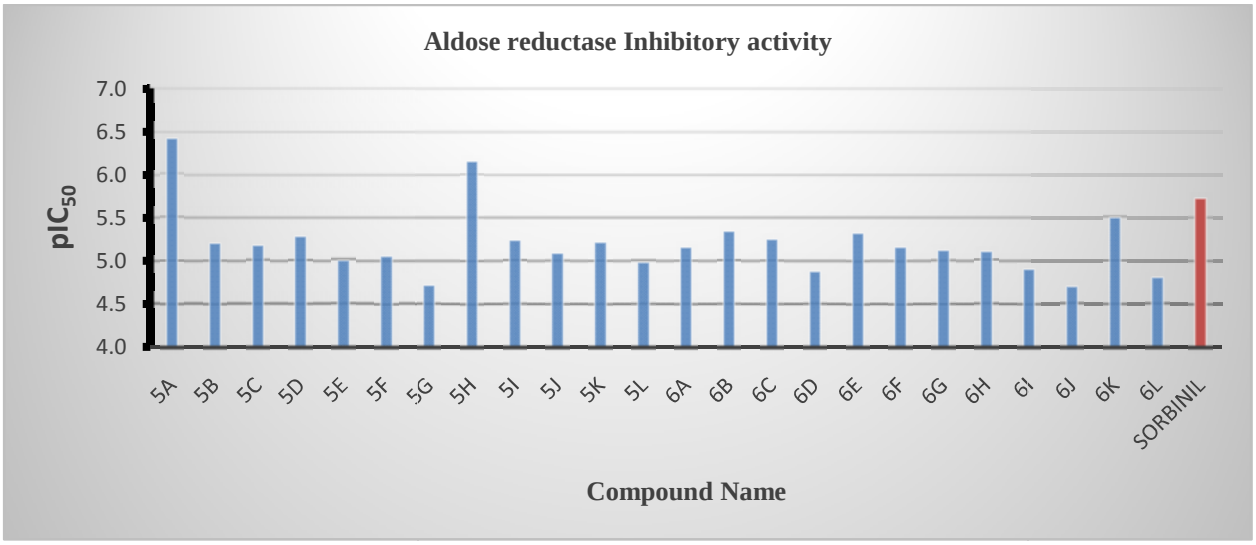


Fig. 2. Graphical representation of negative logarithm of aldose reductase inhibitory concentration in mole (pIC_{50} value) of synthesized compound and reference drug.

In vitro Activity: The blood glucose level was monitored at different interval of time 0, 1, 3, and 6h respectively. Pioglitazone(4 mg/kg body weight) in 2% acacia was used as standard drug. The decrease in blood glucose level against each compounds are shown in Table 3 and Fig. 3.

Table 3: Antidiabetic activities of the synthesized N-acetic acid-2,4-thiazolidinedione analogs.

Compound code	Decrease in blood glucose level mg/dl			
	0 hr	1 hr	3 hr	6 hr
Control	257.50±6.11	245.33±2.50	238.33±5.54	235.13±3.14
Standard	239.33±6.86	224.50±3.61**	195.67±11.49***	134.26±5.57***
5a	252.00±2.91	246.65±7.00	191.17±6.89***	154.83±3.63***
5b	231.33±7.64	224.83±2.49	193.91±7.12***	171.67±6.24***
5c	219.00±4.38	206.39±2.03*	184.15±7.67***	156.38±3.09***
5d	226.00±5.14	215.36±3.86	167.00±8.56***	148.83±4.09***
5e	230.17±3.66	218.67±4.41	164.67±7.23***	151.50±3.66***
5f	266.67±6.91	254.67±3.39	221.33±8.32***	171.50±3.58***
5g	230.17±8.83	221.00±7.15	175.67±5.67***	146.17±3.79***
5h	221.00±6.02	214.33±4.83	167.33±3.95***	139.95±3.09***
5i	241.00±5.86	228.83±3.39*	173.00±7.26***	146.33±2.62***
5j	223.17±5.67	216.00±3.61	182.17±3.61***	156.14±4.98***
5k	233.50±5.33	226.33±4.56	186.17±5.11***	168.83±2.26***
5l	251.83±4.73	238.00±4.13*	194.67±5.67***	164.67±2.67***
6a	220.00±3.64	206.26±4.14*	156.62 ±9.03***	138.11±3.08***
6b	237.20±5.53	228.50±4.68	178.67±7.86***	153.50±3.50***
6c	240.00±3.99	233.00±4.45	204.00±7.03***	191.00±3.03***
6d	235.67±3.26	228.87±6.20	186.26±4.87***	163.45±1.58***
6e	243.33±3.03	235.50±4.69	178.83±6.09***	156.83±3.57***
6f	239.00±6.05	238.33±7.43	194.50±6.10***	173.50±2.62***
6g	208.33±8.68	206.50±7.13	186.17±6.47**	158.50±1.96***
6h	227.17±5.94	219.33±6.78	198.83±3.42**	185.67±2.22***
6i	216.00±5.34	211.00±5.65	183.17±7.22***	164.17±2.56***
6j	254.50±6.04	249.67±7.24	214.17±6.32***	192.50±4.05***
6k	242.17±5.10	236.67±6.46	183.33±3.20***	164.67±2.03***
6l	200.50±5.67	196.67±5.54	178.50±9.94**	151.67±2.16***

[a]*mean ±S.E.M.(n=6); *** P<0.001; **P<0.01; *P<0.05*

CONCLUSION

Rhodanine *and N*-acetic acid rhodanine derivatives with various substitutions on benzylidene moiety were studied. A molecular docking study was performed using MVD to envisage the interactions between the aldose reductase active site and designed rhodanine series of compounds. Synthesized compound shows ARI activity in the range of 0.712 to 20.51 µM. The *N*-acetic acid analogs are more potent as compare to *N*-unsubstituted rhodanine analogs. In terminal phenyl ring at para position no substitution or fluoro substitution is favour for the activity while compound with bromo substitution on terminal phenyl ring shows minimum inhibition as compared to other analogs. The 3,4-dimethoxy substituted derivative proved to be the most active among these substituted compounds. *In vivo* study of *N*-acetic acid rhodanine derivatives were carried out by alloxan induced tail tipping method. Synthesized compound shows decrease in blood glucose level in the range of 28.80-10.64% after 3hrs while 39.28-18.26% after 6 hrs. The activity data of all compounds have p<0.001 after 6 hrs. Synthesized Compound (5i) that is (E)-2-(5-(2-methoxy-4-(4-methylbenzoyloxy)benzylidene)-4-oxo-2-thioxothiazolidin -3-yl) acetic acid showed comparable antidiabetic activity with standard drug (pioglitazone). Current study offers substituted ester of (E)-2-(5-(2-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)acetic acid will be used as promising scaffolds for a future drug discovery program.

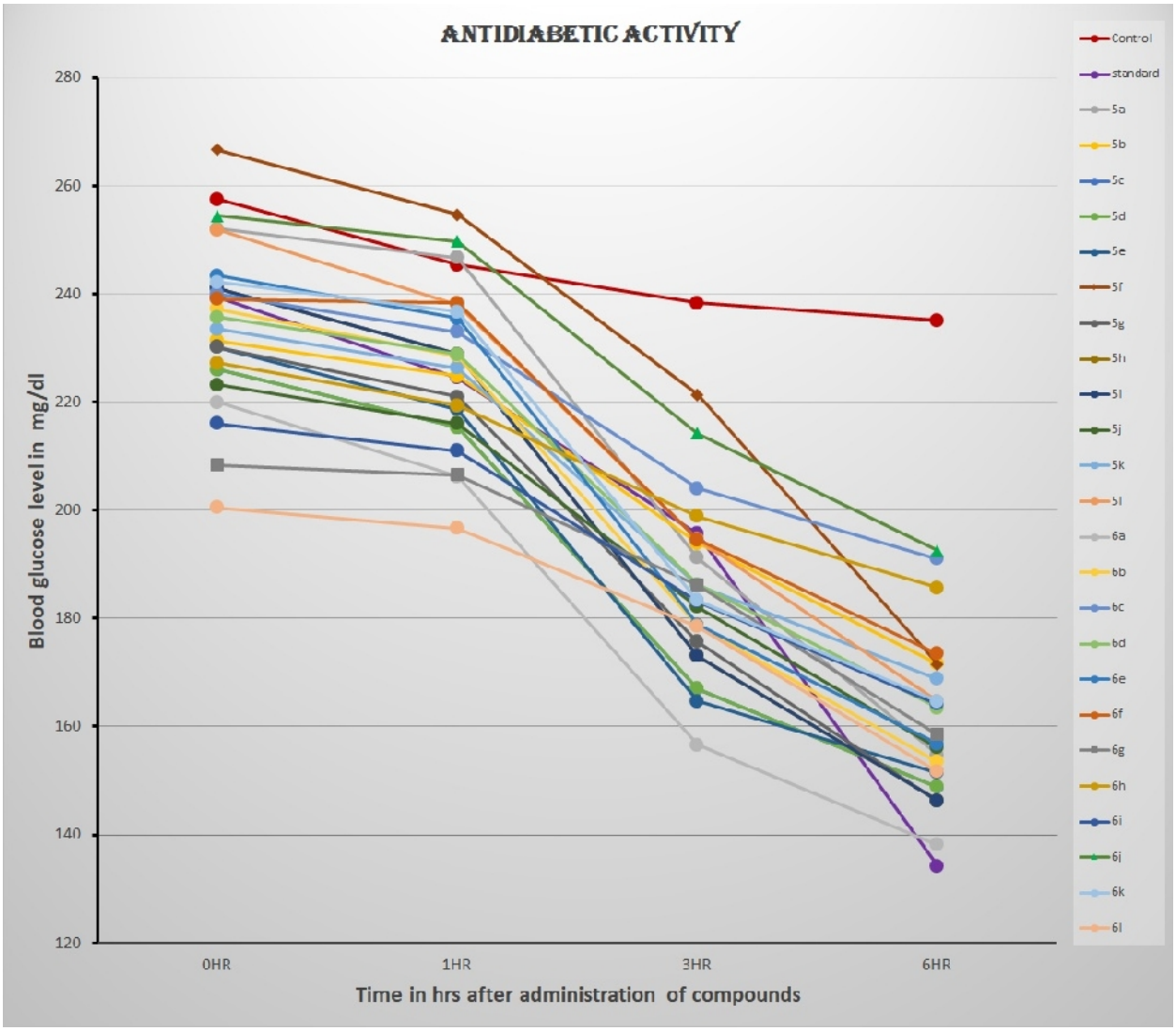


Fig. 3. Graphical representation of antidiabetic activity of synthesized compound and reference drug.

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