INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 3 | Jul - Sep- 2019

Journal Home page: www.ijpar.com

Research article *Open Access*

Synthesis, Physical characterisation and Biological Evaluation of novel 1, 3- thiazolidine-2, 4-dione derivatives as ALR-2 inhibitors

VenkataSowjanya.T *, Dr. I.V.Ramarao[1], Dr. Y.A. Chowdary[1],N.Nagamalleswari[2], G. Usha kiran[2]

*****Associate Professor, department of Pharmaceutical Chemistry, NRI College of Pharmacy, Pothavarappadu, Vijayawada*

[1]*Department of Pharmaceutical chemistry, NRI College of Pharmacy, Pothavarappadu, Vijayawada*

[2]*Department of pharmacology, NRI College of Pharmacy, Pothavarappadu, Vijayawada*

***Corresponding Author: VenkataSowjanya.T**

ABSTRACT

In our research we particularly designed some new 5- (substituted benzylidene)-3-(2-(morpholin-4-yl) ethyl)-1,3-thiazolidine-2,4-diones. The 5-arylidene moiety contained a phenyl ring bearing a different potential *electron donating* as well as *electron withdrawing*groups, this was found to be a favorable feature for ALR-2 inhibition. The structures of newly synthesized compounds were confirmed by physical parameters like Melting point, solubility, chromatographic methods (TLC) and at last by Spectroscopic methods (IR, ^{1}HNMR and MS). The Antidiabeticactivity of synthesized compounds were carried out on Wistar rats using ***Pioglitazone*** as standard drug. Apart from this activity thiazolidine-2,4- diones derivatives found to have diverse pharmacological actions like anti-inflammatory, anti-microbial, anti-proliferative, antiviral, anticonvulsant, anti-cancer etc.

Keywords: 1, 3-thiazolidine-2, 4-diones, Pioglitazone, Antidiabetic activity.

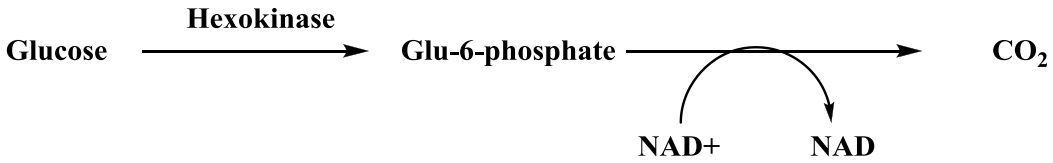
INTRODUCTION

Diabetes mellitus is a major and growing public health problem throughout the world characterized by high levels of blood glucose resulting from defects in insulin production, action or both. There are four main categories for the etiology of diabetes [1].

1. Type-1 diabetes occurs due to the failure of the pancreatic beta cells to produce insulin. The onset of Type-1 diabetes is childhood or adulthood.
2. Second category of diabetes is gestational diabetes; it is a form of glucose intolerance observed during pregnancy.
3. Third category of diabetes caused by genetic defects in insulin action or beta cell function.
4. The fourth category, Type-2 diabetes characterized by insulin resistance initially, but overtime inadequate pancreatic production of

insulin occurs. Type-2 is associated with obesity, history of gestational diabetes, impaired glucose tolerance.

Under normoglycemic conditions most of the cellular glucose is phosphorylated to glucose-6-phosphate by hexokinase [2]. Non-phosphorylated glucose enters the *polyol pathway*, alternative route for glucose metabolism.



Mechanism under normoglycemic conditions

POLYOL PATHWAY

Aldose reductase a member of aldo-ketoreductase (AKR) super family of enzymes, together with sorbitol dehydrogenase forms a polyol pathway. In this pathway, aldose reductase which is first enzyme of polyolcatalyses NADPH-dependent reduction of glucose to sorbitol.Sorbitol dehydrogenase which in turn utilizing NAD^+oxidises this sorbitol to fructose [3].

Under hyperglycemic conditions, hexokinase is rapidly saturated and polyol pathway becomes activated. Sorbitol is formed more rapidly than it is converted to Glu-6-Phosphate (normoglycemia). This results in increased intracellular accumulation of sorbitol [4]. The accumulation of sorbitol increases cellular osmolarity; in tissues possessing insulin dependent glucose transport (kidney, retina, lenses), which in turn leads to development of complications like nephropathy, cataracts, neuropathy etc [5].

In addition to osmotic imbalance, increase in activity of aldose reductase-2 (ALR-2) during hyperglycemia causes a substantial imbalance in the free cytosolic co-enzyme ratios NADPH / $NADP^+$ and NAD^+ /NADPH.

ALR-2 shares its detoxification role with aldose reductase (ALR-1) closely related enzyme. Both ALR-1 and ALR-2 belong to AKR superfamily of enzymes, possessing structural homology with identity in their amino acid sequences. Therefore the drug should be able to inhibit ALR-2 without affecting the detoxification activity of ALR-1.

Inhibition of ALR-2 is therefore a useful therapeutic strategy to prevent the onset, severity, and progression of diabetic complications.

Structural requisites for the ALR-2 inhibition [6]

- An acidic hydrogen and H-bond acceptor groups which can bind the positively charged polar recognition region of ALR-2 active site formed by Try48, His110, Trp111 residues and nicotinamide ring of cofactor $NADP^+$.
- An aromatic moiety, which can establish hydrophobic interactions with lipophilic contact zone of the catalytic cleft lined by Leu 300 and Trp 111.

With this brief literature background, we thought of synthesizing thiazolidinedione biheterocyclic system,

Containing morpholine moiety as a heterocyclic partner and evaluate these derivatives towards their hypoglycemic activity [7].

METHODOLOGY

Procedure for the preparation of thiazolidene-2,4-dione (1)

Into a 100 ml clean and dry round bottom flask introduced a mixture of chloro acetic acid (0.106 mol) and thiourea (0.106 mol) in 15 ml water. The mixture was refluxed for 40 h [8]. The separated solid product thiazolidene-2,4-dione **(1)** was collected by filtration and recrystallized from water, the yield was 75 % and melting point was 120 ^{o}C.

Procedure for the preparation of 5-(benzylidene)-1, 3-thiazolidene-2,4-dione (2a-2g)

Into a 100 ml clean and dry round bottom flask containing 10 ml of glacial acetic acid, introduced thiazolidene-2,4-dione (0.02 mol, 2.34 gm) **(1)**, appropriate benzaldehyde (0.02 mol) and sodium acetate (0.015 mol, 2.46 gm) was added, the reaction mixture was refluxed for 12 h [9]. After completion of reaction, the mixture was allowed to cool to room temperature and the separated solid was filtered, washed with water and dried. The obtained benzylidene derivatives **(2a-2g)** were recrystallized from ethanol , the yield was 65-90 %.

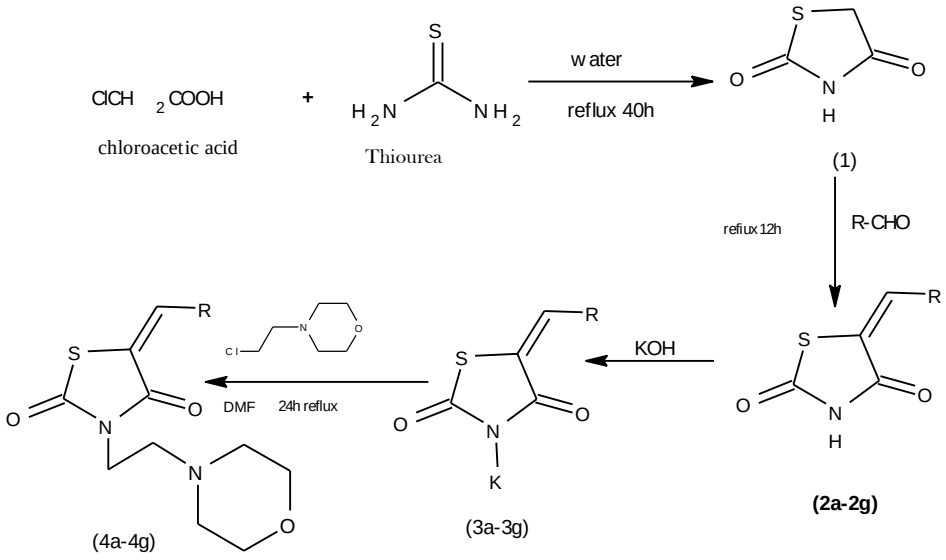
Procedure for the preparation of potassium salts of 5-(benzylidene)-1,3-thiazolidene-2,4-dione (3a-3g).

Into a 100 ml clean and dry round bottom flask 5-(benzylidene)-1,3-thiazolidene-2,4-dione(0.008 mol, 2 gm) **(2a)** was dissolved in sufficient quantity of ethanol and a solution of KOH (0.01 mol, 1 gm) in ethanol was added drop wise and reaction mixture was stirred and warmed on a water bath for 2 h. After cooling the separated potassium salt was collected and dried. The yield was 90 %. The other potassium salts of this series i.e. **(3b-3g)** were prepared by using same procedure**..**

Procedure for the preparation of 5-(benzylidene)-3-(2-(morpholin-4-yl) ethyl)-1,3-thiazolidine-2,4-dione (4a-4g)

Into a 100 ml clean and dry round bottom flask potassium salt of appropriate 5-(benzylidene)-1,3-thiazolidene-2,4-dione(0.002 mol, 0.5 gm) **(3a)** was dissolved in sufficient quantity of DMF and 4-(*2-chloroethyl*)*morpholine hydrochloride* (0.0019 mol, 0.36 gm) was added. The reaction mixture was refluxed for 24 h. After completion of the reaction, it was poured into ice and water mixture and the solid separated was collected by filtration. The obtained products **(4a- 4g)** were recrystallized from ethanol.

SCHEME



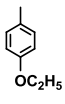
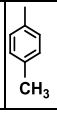
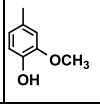
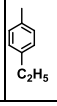
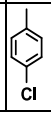
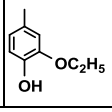
	a	b	c	D	E	f	g
R							

Table 1: Physical characterization data of synthesized compounds

Compound code	Molecular formula	Molecular wt.	M.P ^{o}C	% yield
1	$C_3H_3NSO_2$	117	120	75 %
2a	$C_{12}H_{11}NSO_3$	249	174-176	50
2b	$C_{11}H_9NSO_2$	219	220-224	82.5
2c	$C_{11}H_9NSO_4$	251	220-222	60.3
2d	$C_{11}H_9NSO_3$	235	210-212	43.4
2e	$C_{12}H_{11}NSO_2$	233	182-184	82.6
2f	$C_{10}H_6NSO_2Cl$	239	214-218	53
2g	$C_{12}H_{11}NSO_4$	265	198-202	35
3a	$C_{12}H_{10}NSO_3K$	287	>250	90
3b	$C_{11}H_8NSO_2K$	257	>250	95
3c	$C_{11}H_8NSO_4K$	289	>290	89
3d	$C_{11}H_8NSO_3K$	273	260-66	95
3e	$C_{12}H_{10}NSO_2$ K	271	>250	82
3f	$C_{10}H_5NSO_2Cl$ K	277	>250	87
3g	$C_{12}H_{10}NSO_4$ K	303	>250	85
4a	$C_{18}H_{22}N_2SO_4$	362	112-114	50
4b	$C_{17}H_{20}N_2SO_3$	332	130-136	72
4c	$C_{17}H_{20}N_2SO_5$	364	146-150	60
4d	$C_{17}H_{20}N_2SO_4$	348	112-118	72
4e	$C_{18}H_{22}N_2SO_3$	346	110-114	50
4f	$C_{16}H_{17}N_2SO_3Cl$	352	86-90	68
4g	$C_{18}H_{22}N_2SO_4$	362	146-150	35

Table 2. Spectral data of synthesized compounds

Compound code	IR (KBr) cm^{-1}	1HNMR (δ ppm)	MS (m/z)
1	3129 (N-H stretching); 2949, 2826 (CH of -CH_2 stretching); 1738, 1681 (-C=O stretching).	12.0 (1H, s, 1H of -NH); 4.1 (2H, s, 2H of –CH2)	117 (M^+) 116 (M-1)
2a	3152 (N-H stretching); 3040, 2981 (Ar-CH=CH stretching); 1759, 1692 (-C=O stretching)	12.4 (1H, s, 1H of -NH); 7.7 (1H, s, 1H of =CH); 6.9-7.6 (4H, m, 4H of Ar-H); 3.9-4.2 (2H, q, 2H of -CH_2 of -OCH_2CH_3); 1.2-1.4 (3H, t, 3H of CH_3 of -OCH_2CH_3).	249 (M^+) 248 (M-1)
2c	3152 (N-H stretching); 3040, 2981 (Ar-CH=CH stretching); 1759, 1692 (-C=O stretching)	12.4 (1H, s, 1H of -NH); 9.9 (1H, s, 1H of -OH); 7.7 (1H, s, 1H of =CH); 6.9-7.1 (3H, m, 3H of Ar-H); 3.7-3.9 (3H, s, 3H of -OCH_3).	251(M^+) 250(M-1)
2g	3152 (N-H stretching); 3040, 2981 (Ar-CH=CH stretching);	12.5 (1H, s, 1H of -NH); 7.8 (1H, s, 1H of =CH-); 7.3-7.7 (4H, m, 4H of Ar-H); 2.6-2.7 (2H, q, 2H of -CH_2 of -CH_2CH_3); 1.1-1.2 (3H, t, 3H of -CH_3 of -CH_2CH_3).	265(M^+) 264(M-

	1759, 1692 (-C=O stretching)		1)
4a	2974, 2926 (Ar-CH=CH- stretching); 1733, 1517 (-C=O stretching).	7.8 (1H, s, 1H of =CH); 6.9-7.7 (4H, m, 4H of Ar-H); 4.1-4.2 (2H, q, 2H of -CH_2 of –OC_2H_5); 3.8-3.9 (2H, t, 2H of –N-CH_2-**CH_2**-N-); 3.6-3.7 (4H, t, 4H of –$O(CH_2)_2$ of morpholine); 2.6-2.7 (2H, t, 2H of –N-**CH_2**-CH_2-N-); 2.4-2.6 (4H, t, 4H of -$N(CH_2)_2$ of morpholine); 1.4-1.5 (3H, m, 3H of -CH_3 of –OC_2H_5).	362(M^+) 363 (M+1)
4c	3400 (-OH stretching); 2957, 2826 (Ar-CH=CH stretching); 1731, 1585 (-C=O stretching).	9.9 **(**1H, s**,** 1H of -OH**);** 7.8 (1H, s, 1H of =CH); 6.8-7.2 (3H, m, 3H of Ar-H); 3.9 (3H, s, 3H of –OCH_3); 3.7-3.8 (2H, t, 2H of –N-**CH_2**-CH_2-N-); 3.3-3.5 (6H, m, 4H of -$O(CH_2)_2$ of morpholine and 2H of N-CH_2-**CH_2**-N); 2.3 -2.5 (4H, dd, 4H of –N-$(CH_2)_2$- of morpholine).	364(M^+) 365 (M+1)
4g	2951, 2816 (Ar-CH=CH stretching); 1732, 1600 (-C=O stretching)	7.8 (1H, s, 1H of =CH); 7.2-7.7 (4H, m, 4H of Ar-H); 3.7-4.0 (2H, t, 2H of -N-CH_2-**CH_2**-N-); 3.6-3.7 (4H, dd, 4H of –$O(CH_2)_2$ of morpholine); 2.4-2.7 (8H, m, [4H of –N-$(CH_2)_2$ of morpholine], [2H of -N-**CH_2**-CH_2-N-] and [2H of –CH_2 of –CH_2CH_3)]); 1.2-1.3 (3H, t, 3H of -CH_3 of -CH_2CH_3).	346 347 (M+1)

BIOLOGICAL EVALUATION

Method for determination of Antidiabetic activity

EXPERIMENTAL ANIMALS

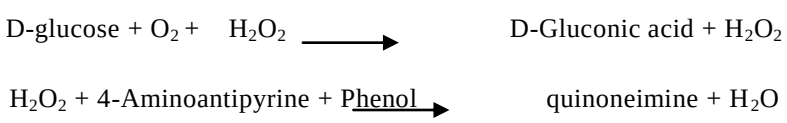
Adult Wistar rats weighing (160-220g) of either sex were used as experimental animals. All the animals were housedin the cage at a temperature 25±1^{0}C and a relative humidity of 45-55%. A 12h dark and 12h light cycle was followed during experiments [9]. Animals were followed to free access of food and water ad libitum. During the study period, the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animal Ethics Committee (IAEC) were followed for maintenance of animals

Induction of diabetes by Alloxan

Rats of either sex weighing between 160-220 g were selected and fasted for 18 h prior to experiment and water supplied *ad-libitum*. The rats were injected intraperitoneally with a single dose of 100mg/kg Alloxan [10] dissolving it in distilled water. After the injection, they had free access to feed and 5% glucose solution was given overnight to counter the hypoglycemic shock. The development of diabetes was confirmed after 48 h,the blood (0.3-0.5 ml) was collected into centrifuge tubes, from retro-orbital puncture and was centrifuged at 3000 rpm at room temperature. The Wistar having fasting blood glucose level more than 200 mg/dl were selected for experiment. Serum glucose levels of collected blood samples were estimated by GOD/POD method.

Principle

Glucose is oxidized by the enzyme glucose oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of enzyme peroxidase (POD) oxidizes phenol, which combines with 4-Amino–antipyrine to produce red colored quinoneimine dye which is measured at 505 nm and the intensity of the colour produced is proportional to glucose concentration in the sample.



Experimental design

Animals were dived into 9 groups of six animals in each group . Group 1 (control) normal animals received distilled water. Group 2 diabetic animals received standard drug Pioglitazone 30 mg/kg bwt. Group 3-9 diabetic wistars received test drugs of respective concentrations as specified in table [3].

Assay Procedure (End Point method) [14]

Pipette into clean dry micro-centrifuge tubes labeled as blank, standard and test

Pipette into test tube labeled as	Blank	Standard	Test
Sample	-	-	10µl
Standard	-	10µl	-
Enzyme reagent	1.0 ml	1.0 ml	1.0ml

As mentioned in the above table blank, standard and sample was prepared by considering 1ml of working reagent and 1ml each of distilled water, standard and sample respectively, later all the samples were incubated at 37^0c for 1o min, aspirated individually and absorbance was recorded at 505 nm [15]. The details of the treatment protocol was given below in ***table-3.***.

The blood samples were collected at prefixed time intervals 2.0, 6.0, 12.0 and 24.0 h and were analyzed for glucose levels. The percentage reduction in serum glucose level at time "t" was calculated by using the following equation.

Percentage reduction in serum glucose at time "t"

$$t = A\text{-}B/A \times 100$$

Where **A** is serum glucose concentration at time "0" and

B is serum glucose concentration at time "t".

RESULTS AND DISCUSSION

Statistical analysis

The results are expressed as the mean ± SEM and were analyzed by one-way ANOVA followed by Dunnett's multiple comparison "t" test. Data was computed for statistical analysis by using Graph Pad PRISM Software.

Table 3: Blood glucose levels in normal and alloxan induced diabetic rats.

Group	Compound Mg/Kg bwt	Time in hours 0h	2h	6h	12h	24h
1	**Control**	102.94 ±3.26	110.33 ±4.38	102.52 ±3.44	105.28 ±3.09	102.82 ±4.84
2	**Standard Pioglitazone(30 mg)**	274.78 ±9.73	228.98 ±14.03	172.38 ±1.13	130.35 ±10.29	107.2 ±10.72
3	**4a (200 mg/kg)**	225.5 ±19.57	149.25 ±12.55	135.02 ±12.90	111.63 ±13.24	104.47 ±6.8
4	**4b(200 mg/kg)**	236.38 ±1.59*	191.98 ±3.2**	179.63 ±3.35	173.68 ±3.43	113.84 ±2.9
5	**4c(200 mg/kg)**	256.87 ±6.57	167.53 ±12.55	157.18 ±12.9	150.38 ±13.2	113.92 ±6.8
6	**4d(200 mg/kg)**	247.22 ±12.7	222.4 ±6.13	139.7 ±4.4	148.05 ±3.16	115.87 ±3.3
7	**4e(180 mg/kg)**	251.2 ±4.71	176.3 ±4.12	146.32 ±3.31	122.58 ±4.22	100.87 ±4.84
8	**4f(200 mg/kg)**	257.83 ±7.7	195.8 ±5.79	182.38 ±2.45	157.35 ±2.59	103.51 ±4.72
9	**4g(150 mg/kg)**	285.9167 ±7.20	200.93 ±2.86	142.25 ±2.69	118.9667 ±3.05	112.45 ±1.08

From the observations and data obtained illustrates that the compounds under antidiabetic evaluation have shown significant reduction in the blood sugar when compared with Pioglitazone standard drug employed for the study. To conclude the total results data after 2 h and 24 h have been taken.

Data at 2 h

The data at 2 h (**Table 3**) shows that some of the synthesised compounds are **4a**,(149.25±12.55) ; **4c**, (167.53±12.55); **4e**, (176.3 ±4.12); **4f**, (195.8 ±5.79) exhibited better activity than standard (228.98±14.03).

Data at 24 h

The data at 24 h (**Table 3**) indicates that some of the synthesised compounds such as **4a**, (104.47 ± 6.8); **4e**, (100.87 ± 4.84) ; **4f** (103.51 ±4.72), exhibited good hypoglycemic activity than the standard (107.2±10.72

CONCLUSION

In conclusion, we have described a simple protocol for synthesis of new 1, 3 -thazolidine-2,4-diones with remarkable yields. All the compound structures were characterized by physical and Spectroscopical methods. After physical characterization all compounds were screened for anti- diabetic activity and most of them found to be significantly active as AldoseRreductase Inhibitors(ARI s). The results clearly suggest that still there is a scope for the synthesis of structurally diverse 2, 4-TZDs and explore them for better hypoglycemic agents than present derevatives of TZDs available in the market.Further more , an extensive toxicological studies are recommended to asses safety of these novel drugs.

REFERENCES

[1]. Rosanna M, Rosaria O, Rosella C, Maria G, Vigorita. Evaluation of *in-vitro* aldose reductase inhibitory activity of 5-arylidene-2,4-thiazolidinediones. Bioorg MedChemLett 17, 2007, 3886-93.

[2]. Rosanna M, Rosella C, Marco G, Mario C, Roberta M, Antonella DC et al. Identification of new non-carboxylic acid containing inhibitors of aldose reductase. Bioorg Med Chem 18, 2010, 4049-55.

[3]. Michael CVZ, Evelyn OS, Erin EM, Kerry JC, Brenda F, Diane RS et al. Design and synthesis of highly potent and selective (2-arylcarbamoyl phenoxy)-acetic acid inhibitors of aldose reductase for treatment of chronic diabetic complications. Bioorg MedChem 12, 2004, 5661-75.

[4]. Pattan SR, Kekare P, Dighe 1NS, Bhawar SB, Nikalje A, Pati A et al. Synthesis and evaluation of some new thiazolidinedione derivatives for their antidiabetic activities. Asian J ResChem 2, 2009, 123-6.

[5]. Rosanna M, Rosaria O, Rosella C, Maria GV, Dietmar R, Theodora S et al. Evaluation of *in-vitro* aldose redutase inhibitory activity of 5-arylidene-2,4-thiazolidinediones. Bioorg Med ChemLett 17, 2007, 3886-93.

[6]. Makoto M, Buichi F, Hiroyuki M. Synthesis and aldose reductase inhibitory activity of a new series of 5-[[2-(ω-carboxyalkoxy) aryl]methylene]-4-oxo-2-thioxo thiazolidine derivatives. Eur J Med Chem 34, 1999, 1061-70.

[7]. David SH Bell.Type 2 diabetes mellitus: what is the optimal treatment regimen? The Am J Med 2004, 116.

[8]. Cleiton D, Angelic A, Tiago B, Karime BRI, Anekecia L, Teresinha GS et al. Synthesis and anti-inflammatory activity of new arylidene-thiazolidine-2,4-diones as PPARγ ligands. Bioorg Med Chem 18, 2010, 3805-11.

[9]. F. alam, Biplab Kr. Dey et al. Synthesis Characterisation of thiazolidinone Derivatives as Oral Hypoglycemic agents. Indian Journal Of Pharmaceutical Sciences and research; 5, 2015, 67-71.

[10]. Rosanna M, Rosaria O, Rosella C, Maria G, Vigorita. Evaluation of *in-vitro* aldose reductase inhibitory activity of 5-arylidene-2,4-thiazolidinediones. Bioorg MedChemLett 17, 2007, 3886-93.

[11]. Ying W, Hsin-Hsiung T, Hoon C. Synthesis and SAR of thiazolidinedione derivatives as 15-PGDH inhibitors. Bioorg Med Chem 18, 2010, 1428-33.

[12]. Dietmar R, Rosanna M, Rosaria O, Maria GV. *In-vitro* aldose reductase inhibitory activity of 5-benzyl-2,4-thiazolidinediones. Bioorg Med Chem 14, 2006, 567-74.
[13]. Minoru O, Kunio W, Hidehito H, Asako T, Tomoko K, Sachiko S et al. Molecular design, synthesis, and hypoglycemic activity of a series of thiazolidine-2,4-diones. J Med Chem 43, 2000, 3052-66.
[14]. Pattan SR, Kekare P, Dighe N, Bhawar SB, Nikalje A, Pati A et al. Synthesis and evaluation of some new thiazolidinedione derivatives for their antidiabetic activities. Asian J Res Chem 2, 2009, 123-6.
[15]. Hiroshi K, Mitsuharu N, Hiroki T, Nobuharu G. Hybridization of non-sulfonylurea insulin secretagogue and thiazolidinedione derived insulin sensitizer. Bioorg Med ChemLett 10, 2000, 2453-6.