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Synthesis, QSAR and hypoglycemic activity of substituted 2, 4-thiazolidinediones

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ABSTRACT

This paper report synthesis and structural characteristic of several analogues of the substituted 2,4-thiazolidinediones. These compounds were evaluated for hypoglycemic activity after oral administration at dose 30mg/kg in alloxan induced diabetic mice. Blood glucose levels were measured and compared with pioglitazone (30mg/kg) as the reference drug. Oral glucose tolerance test (OGTT) was also performed. Quantitative structure activity relationship (QSAR) studies of these substituted 2,4-thiazolidinediones was performed using multiple linear regression analysis.A series of substituted 2,4-thiazolidinediones were synthesized and the structures of synthesized compounds were confirmed by chromatographic and spectral analysis. Compounds 2d, 2e, 2a and 2i have shown significant reduction in Blood glucose level. Improvement in glycemic control was also observed with compounds 2a, 2b, 2c, 2d, 2e and 2i with OGTT. QSAR studies of these substituted 2,4-thiazolidinediones revealed the importance of lipophilic and electronic parameters.

Keywords: Diabetes, TZDs, QSAR, PPAR γ, MLR.

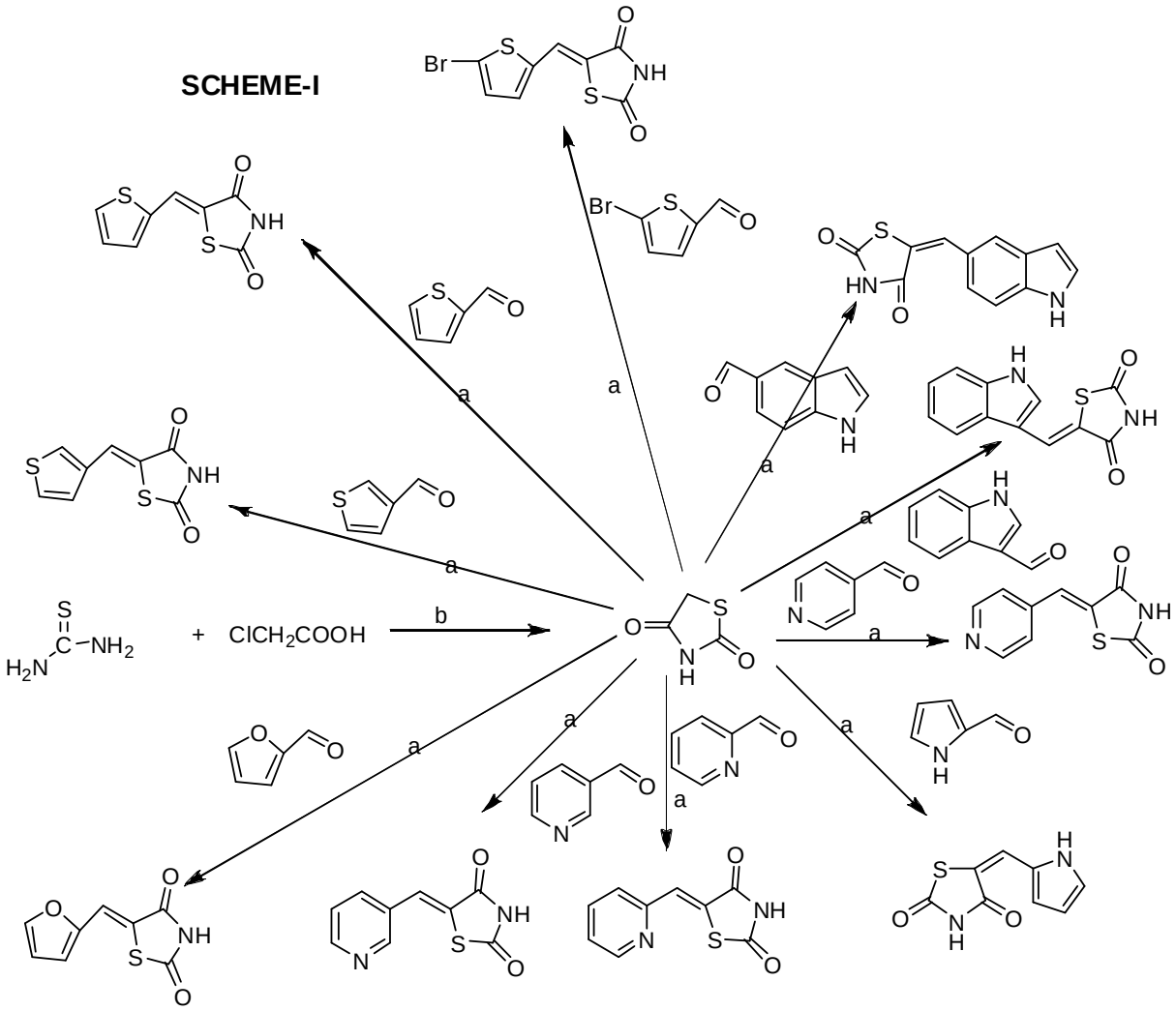
INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, glycosuria, negative nitrogen balance and sometimes ketonaemia. It causes a number of complications like retinopathy, neuropathy and peripheral vascular insufficiencies[1]. Diabetes mellitus, long considered disease of minor significance to world health, is now taking its places as one of the main threads to human wealth in 21st century. The incidence of the disease currently estimated is to reach 210 million by the year 2010 and 300 million by the year 2025[2]. The rising prevalence of type 2 diabetes and obesity in the worldwide population has fueled an intensified search for new therapeutic treatment options. Current pharmacological approaches are unsatisfactory in

improving the consequences of insulin resistance. There is no single approach to treat this disease and usually a combination therapy is adopted to treat the disease[3].

The insulin-sensitizing thiazolidindiones (TZDs), which are selective ligands of the nuclear transcription factor peroxisome proliferator activated receptor γ-(PPARγ), are the first drugs to address the basic problem of insulin resistance in patients with type 2 diabetes[4].The first in the class was Ciglitazone which was discontinued during early clinical trials[5]. In 1997 troglitazone was launched into the market, but had to be withdrawn in 2000 because of idiocynceatic liver failure[6]. Since 1999 rosiglitazone and pioglitzone are available as second line drugs restricted to combination therapy[7]. It was demonstrated that the molecular target of the TZDs is part of the nuclear receptor called PPARγ which controls the differentiation of adipocytes[8], the metabolism of fatty acids and alters the expression of genes that are also regulated by insulin[9]. The TZDs or glitazones are oral hypoglycemic agents which act mainly by increasing tissue sensitivity especially adoipose tissue to insulin[10]. Structure activity relationship showed structural requirements for these types of compounds[11].

SCHEME-I



b=conc. HCl, H_2O
a=C_2H_5OH, Piperidine

This paper report the synthesis and structural characteristic of several analogues of the substituted 2,4-thiazolidinediones. These compounds were evaluated for their hypoglycemic activity after oral administration at dose 30mg/kg in alloxan induced diabetic mice. Blood glucose levels were measured and compared with pioglitazone (30mg/kg) as the reference drug. Oral glucose tolerance test (OGTT) was also performed for some selected compounds of these series. Quantitative structure activity relationship (QSAR) studies of these substituted 2,4-thiazolidinediones was performed using multiple linear regression (MLR) analysis.

The substituted 2,4-thiazolidinediones,2a-j, were prepared by condensation of the 2,4-thiazolidinediones 1 at 5-position by using different heterocyclic aldehydes, 2a-j, Initially 2,4-thiazolidinediones was synthesized by reacting chloroacetic acid and thiourea in the presence of conc. HCl and water[12]. 2,4-thiazolidinediones condensed with heterocyclic aldehydes in the presence of ethanol and piperidine[13].(Scheme-1) The structures of synthesized compounds were confirmed by chromatographic and spectral analysis.

Pharmacology

Swiss Albino mice of either sex weighing between 25-30 gm ± 5 gm were used in antidiabetic screening. Animals were housed under standard condition of temperature of the experimental room was maintained constant at 25°C and lightening was kept artificial. The sequence being 12 hrs light and 12 hrs dark. Conventional laboratory diets and water were provided *ad-libitum*. Studies were carried out at Poona College of Pharmacy, Pune. College animal House used for housing of animals. Approval was taken from committee for the purpose of control and supervision of experiments on animals (CPCSEA) and Institutional animal ethical committee (IAEC).

Table-1 Effect of Oral administration of 2a-2j on SG levels in diabetic mice

Compd	Dose (mg/kg)	Percentage Change in Serum Glucose at			
		2hr.	4hr.	6hr.	24hr.
2a	**30mg/kg**	-40.31±6.12	-38.14±7.12	-36.78±8.02	-29.61±7.19
2b	**30mg/kg**	-21.67±4.52	-18.54±6.48	-17.65±5.22	-14.01±5.38
2c	**30mg/kg**	-20.13±3.68	-17.81±5.21	-15.08±6.31	-12.34±4.81
2d	**30mg/kg**	-46.82±5.33	-43.28±5.03	-40.61±4.81	-30.62±6.24
2e	**30mg/kg**	-43.37±8.13	-41.71±9.91	-42.17±5.27	-28.21±8.01
2f	**30mg/kg**	+3.72±1.84	+5.05±1.98	+7.21±2.33	+2.68 ±1.49
2g	**30mg/kg**	+4.27±1.94	+6.93±2.41	+9.16±2.58	+3.98 ±1.83
2h	**30mg/kg**	-15.67±4.61	-13.14±5.13	-12.54±6.41	-10.98±6.12
2i	**30mg/kg**	-38.65±4.52	-35.76±4.31	-32.76±2.71	-26.51±3.91
2j	**30mg/kg**	-9.56±3.27	-7.02±2.43	-5.87±3.21	-4.98±1.96
Alloxan	**70mg/kg**	1.71±0.19	2.16±1.79	2.81±1.59	1.46±1.25
Vehicle	**-**	0.98±0.08	1.16±0.21	1.92±0.43	1.18±0.65
Pioglitazone	**30mg/kg**	-26.16±5.28	-28.70±4.49	-20.84±3.98	-17.24±4.37

All values are expressed as mean = SEM,
+ indicates increase in blood glucose, - indicates decrease in blood glucose

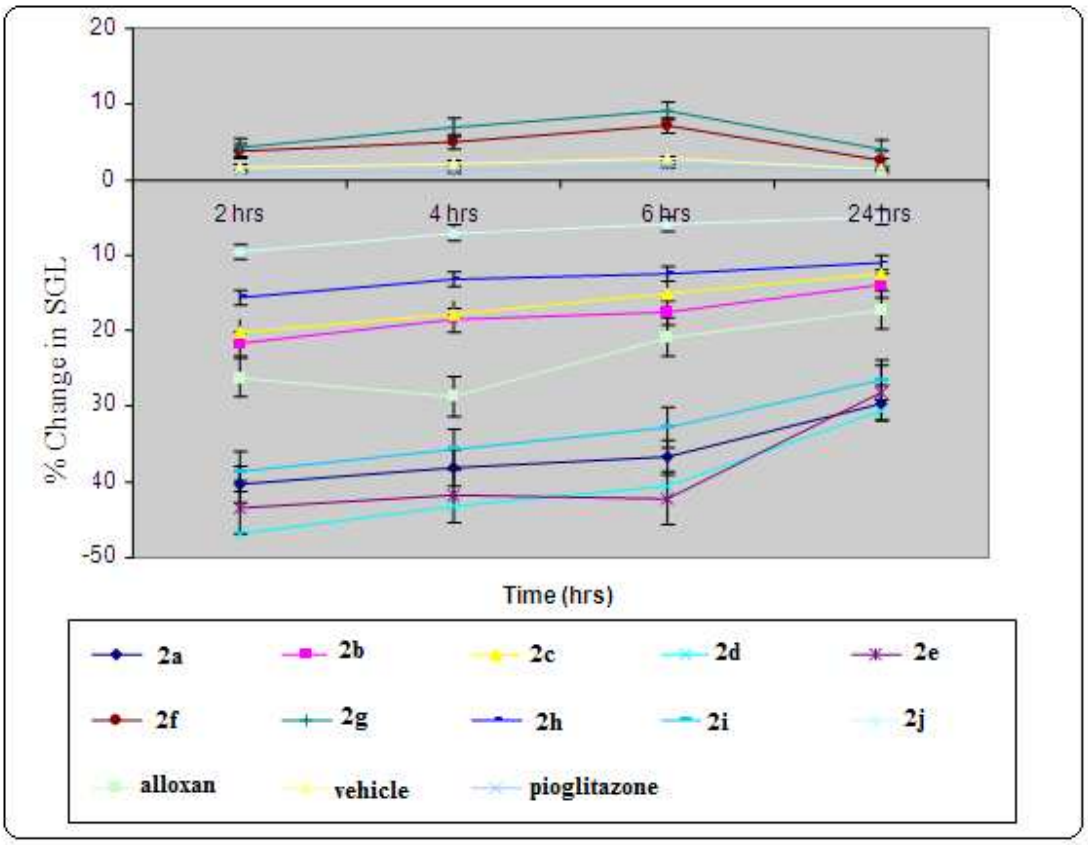
The initial fasting SG was estimated by glucose oxidase peroxidase (GOD/POD) method using kit obtained from Accurex Biomedical, Mumbai. The animals showing SG 80-120 mg/dl were selected for study. Injected with alloxan (70mg/dl) i.v. alloxan monohydrate was purchased from spectrochem (Batch No. 3170145). India. After 48 hr of alloxan injection the blood was removed

by retro orbital plexus technique (ROP) and SG was estimated. The animals showing SG levels above 200 mg/dl were selected for study.

Anti diabetic Activity

All the synthesized compound were tested for hypoglycemic activity *in vivo* by alloxan induced diabetic mice model[14,15] using pioglitazone as standard. For acute study animals were kept for fasting overnight and SG, 0 hr, levels were calculated. Now the test compounds and standard (pioglitazone) were administered at a fixed dose of 30 mg/kg body weight orally (homogenized suspension in Carboxy methyl cellulose (1%) and Tween80 (0.1%)). Blood samples were removed from all animals at 2, 4, 6, and 24 hr and percentage change in SG was calculated. The data obtained were analyzed by one-way ANOVA followed by Dunnet test[16]. The results were expressed as mean ± standard error of mean (SEM) for each group, $P<0.01$ was considered as statistically significant (Table-1). Oral administration of compounds 2a-j (30 mg/kg) reduced SG level in alloxan (70 mg/kg) induced diabetic mice significantly ($P<0.01$) as compared to control group, 2d, 2e, 2a and 2i have shown good activity. While other compounds exhibited moderate hypoglycemic activity.

Figure No. 1 Hypoglycemic activity of substituted 2, 4- thiazolidinedione in diabetic mice



SGL = Serum glucose level

Oral glucose tolerance test

Owing to better activity compounds 2d, 2e, 2a and 2i were selected for OGTT which was performed on diabetic animals after 21 days of treatment with test compound as dose of 30 mg /kg daily. In this test mice were kept on fasting overnight and challenged with glucose (2.5g/kg). Blood samples were collected at 0, 30, 60 and 120 min for measuring SG level. It was found that pretreatment of the animals with synthesized compounds and pioglitazone improved the glucose tolerance 2d, 2e, 2a and 2i and pioglitazone has shown significant ($P<0.01$) decrease in blood glucose at 0, 60 and 120 min but at 30 min it was less when compared to control group.

Quantitative Structure Activity Relationship

The structure of all the compounds were drawn using Chem Draw Ultra ver. 8.0 and copied to 3D Ultra ver.8.0 in order to create its 3-D model. The energy minimization of all compounds was done using Allinger MM2 force field until RMS value of 0.01 kcal/mol A° is achieved followed by semiempirical AM1 (Austin model) Hamiltonian method available in MOPAC module by fixing root mean square gradient as 0.001 Kcal/mol A˚.

Thus, minimized structure of each compound was generated and used for calculating various physicochemical descriptors like thermodynamic, steric, electronic and lipophilic.

A QSAR study was carried out for synthesized 2,4-thiazolidinedione derivatives. All the QSAR parameters were calculated using CS Chem. Office, 2004 molecular modeling software ver.8.0[17]. All the structures were added to TSAR. Charges were assigned using TSAR 3.3. Minimization using corina was performed and used for generation of descriptors.

Stepwise multiple linear regression (MLR) between physicochemical descriptors and biological activity was carried out. Multiple linear regression (MLR) analysis was performed to obtain correlation between physicochemical descriptors and the biological activity. The validity of the regression was confirmed by correlation coefficient (r), standard deviation (s), F-test value. The best models were selected on the basis of higher r, lower s and higher F-test values. The percent change in SG level determined at 24 hr was considered as BA for the QSAR study (Table-2) From the QSAR analysis many models were generated using different set of descriptors. The most significant model generated was derived as best model –

Table 2: Best QSAR Model

No	Equations	n	r	r^2	F	s	$r^2_{(CV)}$
1	Log 1/ BA= - 0.035 Moment Of Inertia1(Whole Molecule) + 0.0670 Lipole_X + 3.3592	10	0.96	0.93	50.868	0.11	0.59

The equation reveals that there is a significant influence of Inertia Moment (whole Molecule) and Lipole_X on the biological activity.

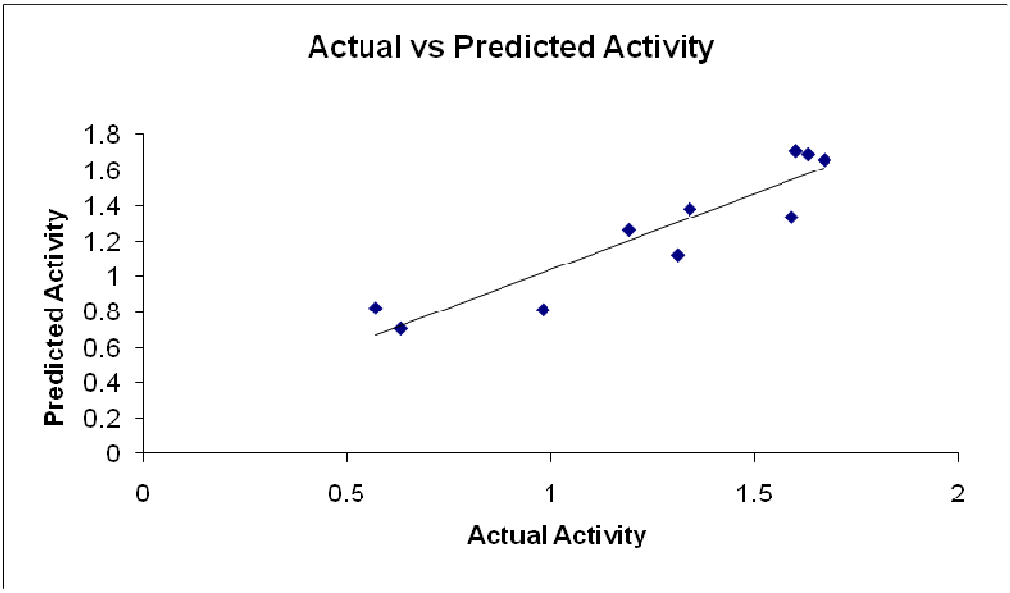
Lipole_X Component is a Lipophilic descriptor, which represents Lipophilicity of a group along X-axis. It is positively correlated with activity. The lipole of a molecule is a measure of the lipophilic distribution. It is calculated from the summed atomic log P values, as dipole is

calculated from the summed partial charges of a molecule. It indicates that a more lipophilic group should be present along X-axis. Thus, higher the Lipole_X, more will be the activity.

Moment of inertia is structural property parameter for QSAR. Moment of inertia assumes mean atomic masses for constituent atoms (parameterized for elements up to atomic number 100). The moments of inertia and principal axis of inertia for a molecule are calculated using the inertia tensor. The length of each axis is inversely proportional to the moment of inertia around that axis. Thus increase in the surface area of the molecule decrease in the moment of inertia.

QSAR study revealed that importance of lipophilic and electronic parameters for hypoglycemic activity.

Figure No. 2 Plot between Actual Vs Predicted Activity



MATERIALS AND METHODS

Experimental

Melting points were taken on electrothermal apparatus as well as in open capillary tubes and are uncorrected. In order to find purity and homogeneity of synthesized compounds, thin layer chromatography was carried out by using plates composed of silica gel with a fluorescent indicator and the R_f values were calculated. IR spectra were recorded in KBr pellets on a JASCO 5300V" and SHIMADZU –FTIR 3100 spectrometer. ^{1}H-NMR spectra were recorded in DMSO and $CDCl_3$ solution on "FT-NMR Varian-NMR 300 MERCURY" spectrometer using tetramethylsilane (TMS) as internal standard.

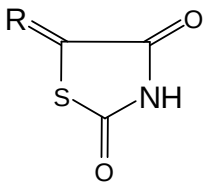
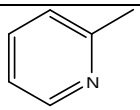
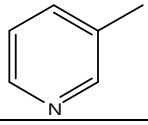
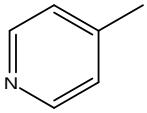
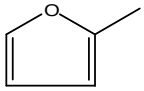
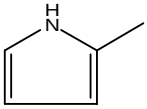
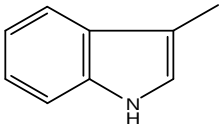
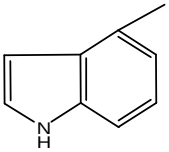
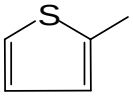
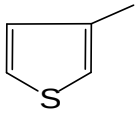
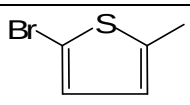


Table- 3, Structural and biological activity data of 2a-2j in logarithm form

Comd	R	Log 1/BA
2a		1.6
2b		1.34
2c		1.31
2d		1.67
2e		1.63
2f		0.57
2g		0.63
2h		1.19
2i		1.59
2j		0.98

Preparation of 2, 4-thiazolidinedione (1)

In a 250 ml three necked flask a solution containing (56.4 gm, 0.6 mol) of chloroacetic acid and (45.6 gm, 0.6 mol) of thiourea dissolved in 60 ml of water was taken. The mixture was stirred for 15 minutes. To the contents of the flask was now added slowly 60 ml concentrated hydrochloric acid from dropping funnel, after which the reaction mixture was stirred and refluxed for 10-12 hr at 100-110^{0}C. On cooling the contents of the flask solidified to a mass of clusters of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was re-crystallized from ethanol.

Preparation of 2, 4-thiazolidinedione derivatives (2a-2j)

Dissolved 2,4-thiazolidinedione (2.34 gm, 0.02 mol) kept in a 250 ml RBF in ethanol. To this solution, was added 2-pyridinecarboxaldehyde (picolinaldehyde) (1.92 ml, 0.02 mol) and (0.2 ml) piperidine. The resulting solution was refluxed for 4 hrs. The reaction mixture was cooled and poured on to crushed ice with stirring. The solid product was separated and dried, washed with toluene and recrystallized it from ethanol.

2a) Yield: 54%; m.p: 225-228˚C; R_f: 0.68 [Chloroform: Methanol :: 4 : 1]
IR (KBr): 3447 (NH- stretching), 3043 (Aromatic C-H stretching), 2826 (Aliphatic C-H stretching), 1741, 1680 (C=O stretching), 1581, 1471 (Aromatic C=C, C=N stretching), 704 (C-S stretching), cm^{-1}.

^{1}H NMR (δ, ppm, $CDCl_3$): 6.976-7.469 (m, 4H, Proton of pyridine ring), 7.808 (s, 1H, CH), 8.590 (s, 1H, Proton of imido group).

Compound 2b - 2j were prepared by above described procedure.
2b) Yield: 51% ; m.p: 230-232˚C; R_f: 0.67 [Chloroform: Methanol :: 4 : 1]
IR (KBr): 3240 (NH-stretching), 3072 (Aromatic C-H stretching), 2841 (Aliphatic C-H stretching), 1741, 1682 (C=O stretching), 1581, 1508 (Aromatic C=C,C=N stretching), 684 (C-S stretching), cm^{-1}.

2c) Yield: 56%; m.p: 235-2337˚C; R_f: 0.65 [Chloroform: Methanol :: 4 : 1]
IR (KBr): 3242 (NH- stretching), 3043(Aromatic C-H stretching), 2822 (Aliphatic C-H stretching), 1724, 1693 (C=O stretching), 1577, 1518 (Aromatic C=C, C=N stretching), 702 (C-S stretching), cm^{-1}.

2d) Yield: 80%; m.p: 240-242˚C; R_f: 0.58 [benzene: ethyl acetate :: 4 : 1]
IR (KBr): 3447 (NH-stretching), 3034 (Aromatic C-H stretching), 2824 (Aliphatic C-H stretching), 1734, 1685 (C=O stretching), 1219 (C-O-C stretching), 688 (C-S stretching), cm^{-1}.
^{1}H NMR (δ, ppm, DMSO): 6.662-7.541 (m, 3H, Proton of Furan ring), 7.935 (s, 1H, CH), 9.269

(s, 1H, Proton of imido group).
2e) Yield: 52%; m.p: 259-262˚C; R_f: 0.62 [benzene: ethyl acetate :: 4 : 1]
IR (KBr): 3392, 3204 (NH- stretching), 3011 (Aromatic C-H stretching), 2813 (Aliphatic C-H stretching), 1709, 1672 (C=O stretching), 1427 (Aromatic C-N stretching), 682 (C-S stretching), cm^{-1}.

^{1}H NMR (δ, ppm, DMSO): 6.306 -7.174 (m, 3H, Proton of pyrrole ring), 7.613 (s, 1H, CH), 8.989 (proton of pyrrole nitrogen), 9.564 (s, 1H, Proton of imido group).
2f) Yield: 82% ; m.p: 278-280˚C; R_f: 0.56 [benzene: ethyl acetate :: 4 : 1]

IR (KBr): 3490, 3227 (NH- stretching), 3012 (Aromatic -C-H stretching), 2928 (Aliphatic C-H stretching), 1722, 1695 (C=O stretching), 1593, 1437(Aromatic C=C, C=N stretching), 684 (C-S stretching), cm^{-1}. ^{1}H NMR (δ, ppm, DMSO): 7.833-7.154 (m, 5H, Proton of indole ring), 8.012 (s, 1H, CH), 9.223, (s, 1H, Proton of imido group), 9.393 (s, 1H, Proton of indole nitrogen).
2g) Yield: 80%; m.p: 282-285˚C; R_f: 0.58 [benzene: ethyl acetate :: 4 : 1]

IR (KBr): 3248, 3225 (NH- stretching), 3010 (Aromatic C-H stretching), 2928 (Aliphatic C-H stretching), 1722, 1693 (C=O stretching), 1577, 1439 (Aromatic C=C, C=N stretching), 684 (C-S stretching), cm^{-1}.

2h) Yield: 56%; m.p: 250-252˚C; R_f: 0.48 [benzene: ethyl acetate :: 4 : 1]
IR (KBr): 3439 (NH- stretching), 3061 (Aromatic -C-H stretching), 2926 (Aliphatic C-H stretching), 1736, 1685 (C=O stretching), 1597 (Aromatic C=C stretching), 711,700 (C-S stretching) cm^{-1}. ^{1}H NMR (δ, ppm, $CDCl_3$): 7.186-7.672 (m, 3H, Proton of thiophene ring), 8.623 (s, 1H, CH), 8.903 (s, 1H, Proton of imido group).

2i) Yield**:** 53%; m.p: 257-258˚C; R_f: 0.47 [benzene: ethyl acetate :: 4 : 1]
IR (KBr): 3445 (NH- stretching), 3040 (Aromatic -C-H stretching), 2822 (Aliphatic C-H stretching), 1736, 1685(Aromatic C=O stretching), 1597(C=C stretching), 711, 698 (C-S stretching), cm^{-1}.

2j) Yield: 62%; m.p: 245-248˚C; R_f: 0.52 [benzene: ethyl acetate :: 4 : 1]
IR (KBr): 3447 (NH- stretching), 3077 (Aromatic C-H stretching), 2978 (Aliphatic C-H stretching), 1738, 1680 (C=O stretching), 1593(Aromatic C=C stretching), 696, 650 (C-S stretching), 547 (C-Br stretching) cm^{-1}.

RESULTS AND DISCUSION

The antidiabetic activity of test compound along with commercially available Pioglitazone, was evaluated by comparing the reduced serum glucose level (SGL) in alloxan (70mg/kg) induced diabetic mice significantly ($P< 0.01$). The results indicate that the compounds 2d, 2e, 2a and 2i shown good hypoglycemic activity comparable to pioglitazone at the dose of 30mg/kg. Compounds 2a, 2b, 2c, 2d, 2e and 2i were also tested for oral glucose tolerance test (OGTT). Quantitative structure activity relationship (QSAR) study of these substituted 2,4-thiazolidinediones revealed the importance of lipophilic and electronic parameters.

CONCLUSION

A series of substituted 2,4-thiazolidinediones were synthesized successfully and the structures of synthesized compounds were confirmed by chromatographic and spectral analysis. In conclusion, series of 2, 4-thiazolidinedione derivatives may be potent alternative to some of

other antidiabetic agents. The work can be further extended to find safe and effective antidiabetic compounds.

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