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Anti-inflammatory, Hypoglycemic, and Anti-diabetic activity of Novel Sesamol substituted Thiazolidine-4-one Hybrids

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ABSTRACT: Background: From our lab, twenty one compounds of sesamol substituted with thiazolidine-4-one derivatives were synthesized, characterized and evaluated for their antioxidant and anticancer potentials, six derivatives (compounds 5, 6, 7, 9, 12, and 13) exerted modest anticancer activity. **Aim:** The present study is an extension of the earlier work, and is designed to establish their *in vitro* anti-inflammatory and anti-diabetic therapeutic potential. **Methods:** I*n vitro* test such as inhibition of bovine serum albumin was conducted to assess the possible anti-inflammatory activity of the reported compounds. The compounds were also tested for *in vitro* and *in vivo* anti-diabetic activities. **Results:** Here in, compounds 3, 5, 8, 12, 13, and 17 showed best acute oral glucose tolerance test (OGTT) and hypolipidemic activity in lean mice when compared to pioglitazone. Compounds 3, 5, 12, 13, and 17 showed best PPARγ over expression, enhanced glucose uptake in skeletal muscle cell lines, and merited anti-diabetic activity. Compounds 3, 12, and 13 up regulated modest GLUT4 gene expression and were significant when compared to standard pioglitazone. **Conclusion:** Overall, the study supports the anti-diabetic activity of novel sesamol substituted thiazolidine-4-one hybrids.

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INTRODUCTION:

Sesamol (3, 4-methylenedioxyphenol) is a constituent of roasted sesame seeds has been found to be a good antioxidant. It has a benzodioxole group, which is known to scavenge hydroxyl radicals to produce 1, 2-dihydroxybenzene. Sesamol is a potent phenolic antioxidant which is a component of sesame oil. It is white crystalline powder, sparingly soluble in water, and miscible with most oils. Several papers have been

published in the past on the synthesis of 3, 4-methylenedioxyphenol derivatives and reported to posses' radioprotective, anti-cancer, gastroprotective, antiplatelet and anti-diabetic activity [1]. Since, sesamol and thiazolidin-4-one structure with various pharmacological potentials has proved to be an important scaffold in the process of developing a wide variety of biologically active molecules with therapeutic benefits. Thus, this renewed interest prompted us to evaluate novel sesamol substituted thiazolidine-4-one hybrids for their possible *in vitro* anti-inflammatory, hypoglycemic, hypolipidemic and anti-diabetic activities.

MATERIAL AND METHODS:

All the chemicals and solvents used were purchased from standard Sigma – Aldrich, INDIA and were 98 % pure. Different cell lines used in this study were procured from Amla cancer research centre, thrissur, Kerala and were maintained in good condition in our research lab. *In vitro* anti-cancer and anti-diabetic studies were carried out at Radiant Research Labs Pvt. Ltd, Peenya industrial area, Bangalore, Karnataka.

IN VITRO ANTI-INFLAMMATORY ACTIVITY:

Inhibition of bovine serum albumin denaturation:

Test or Standard solutions (1 ml) containing different concentrations of drug were mixed with 1 ml of 1 mM albumin solution in a phosphate buffer and incubated at 27 ± 1 ºC for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 ºC in a water bath for 10 min. After cooling the turbidity was measured at 660 nm (UV-1660-Shimadzu Spectrophotometer). Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken [2].

ANTI-DIABETIC ACTIVITIES:

Oral glucose tolerance test (OGTT) in lean mice:

Animals were fasted overnight and divided into six groups (n = 6) based on basal blood glucose levels. Distilled water or glucose load of 2 g/kg/p.o. were administered immediately after treatment with 0.5 % w/v carboxymethylcellulose (CMC) or compounds (100 mg/kg) by p.o. and i.p. route. Blood samples were collected by retro-orbital plexus at 0, 15, 30, 60, and 120 min after the glucose challenge. Plasma glucose was measured spectrophotometrically using colorimetric kits (Aspen Limited, India). The reduction in blood glucose produced by test compounds was calculated using area under the curve ($AUC_{0\text{-}120min}$) [3]. The results were expressed in plasma glucose in mg/dl and AUC $_{0\text{-}120min.}$ The percentage reduction in plasma glucose was also calculated from the AUC graph.

Hypoglycemic and hypolipidemic activity in hypercholesterolemia mice:

Four week-old male Swiss albino mice were placed on the feeding of high fat content providing different percentages of energy and 20 % sucrose in drinking water. The detailed composition of diet where 30% energy source (kilocalories) was from fat given below in table 9. After 4 weeks of HFD feeding, mice displayed hyperglycemia and hypercholesterolemia. Animals with similar degrees of hyperglycemia and hypercholesterolemia were randomly divided into six groups (n=6). The normal pellet diet fed mice were used as non-diabetic controls. The diabetic control and the normal control groups received the vehicle (0.25 % CMC, 10 ml/kg), while the treatment groups were given Pioglitazone (30 mg/kg/p.o.) and compounds – 3, 5, 8, 12, 13, and 17 (100 mg/kg/p.o.), suspensions in 0.3 % CMC (10 ml/kg). All the treatments were given for 28 days. The drug treatments were administered once a day in morning at 8:30 to 9.00 am. Plasma glucose and total cholesterol were monitored at the end of the study [4].

In vitro glucose uptake activity of test compounds in a skeletal muscle cell line (L6):

***Preparation of test solution*:**

For cytotoxicity studies, 10 mg of all the test substances were separately dissolved and the volume was made up with DMEM-HG supplemented with 2 % inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by 0.22µ syringe filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies [5].

***Cell line and culture medium*:**

L6 (Rat skeletal muscle) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in their respective media viz., DMEM-HG supplemented with 10 % inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/ml) in a humidified atmosphere of 5 % CO_2 at 37 °C until confluent. The cells were dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in25 cm^2 culture

flasks and all experiments were carried out in 96 well microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

***Cytotoxicity studies*:**

The monolayer cell culture was trypsinized and the cell count was adjusted to 1, 00, 000cells/ml using respective media viz., DMEM-HG containing 10 % FBS. To each well of the 96 well microtiter plate, 0.1 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100 ml of different test concentrations of test substances were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37 ºC for 72 h in a 5 % CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval.

Determination of cell viability by MTT assay:

After 72 h incubation, the drug solutions in the wells were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37 ºC in 5 % CO_2 atmosphere. The supernatant was removed and 100 ml of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the standard formula and the concentration of test substances needed to inhibit cell growth by 50 % (CTC_{50}) values was generated from the dose-response curves for each cell line.

***In vitro* glucose uptake assay:**

The glucose uptake activity of the test substance was determined in differentiated L-6 cells. In brief, the 24 h cell cultures with 70 to 80 % confluence in 40mm Petri plates were allowed to differentiate by maintaining in DMEM with 2 % FBS for 5 to 7 days. The extent of differentiation was established by observing the multinucleate of cells. The differentiated cells were serum starved overnight and at the time of experiment cells were washed with HEPES buffered Krebs Ringer Phosphate solution (KRP buffer) once and incubated with KRP buffer with 0.1 % BSA for 30min at 37 ºC. Cells were treated with different non-toxic concentrations of test and standard drugs for 30 min along with negative controls at 37 ºC. About 20µl of D-glucose solution was added simultaneously to each well and incubated at 37 ºC for 30 min. After incubation, the uptake of the glucose was terminated by aspiration of solutions from wells and washing thrice with ice-cold KRP buffer solution. Cells were lysed with 0.1M NaOH solution and an aliquot of cell lysates were used to measure the cell-associated glucose. The glucose levels in cell lysates were measured using a glucose assay kit (ERBA). Two independent experimental values in duplicates were taken to determine the percentage enhancement of glucose uptake over controls.

PPAR γ protein expression in Human skeletal cell lines:

RNA isolation and Complementary DNA synthesis:

The Human skeletal muscle cells treated with drug were subjected to cell lysis by treating with Tri-extract reagent. Chloroform was added, to isolate the total RNA from the sample and subjected for centrifugation. Out of the three distinct layers observed, the upper layer was collected in a fresh tube and an equal volume of isopropanol was added and incubated at -20 ºC for 10 mins. After the incubation followed by centrifugation, appropriate volume of ethanol was added to resuspend the pellet. After incubation and centrifugation, the pellet was air dried and an appropriate volume of TAE buffer was added. The isolated total RNA was further used for cDNA synthesis. cDNA was synthesized by priming with oligo dT primers followed by reverse transcriptase enzyme treatment according to manufacturer's protocol (Thermo scientific). The cDNA thus synthesized was taken up for PCR for the amplification of PPAR-γ and GAPDH (internal control) [6].

Semi quantitative PCR:

The mRNA expression level of PPAR-γ was carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, the L-6 cells were cultured in a 60 mm petridish and maintained in DMEM medium for 48 hrs. The DMEM medium was supplemented with FBS and amphotericin. To the dish was added the required concentration of test sample i.e. Compounds-3, 5, 12, 13, 17 and standard pioglitazone incubated for 24 hr. Total cellular RNA was isolated from the untreated (control) and treated cells using Tri Reagent according to the manufacturer's protocol. cDNA was synthesized from total isolated RNA by reverse transcriptase kit according to manufacturer's instructions (Thermo scientific). Then 50 µl of the reaction mixture was subjected to PCR for amplification of PPAR-γ cDNAs using specifically designed primers procured

from Eurofins India and as an internal control the housekeeping gene GAPDH was co-amplified with each reaction.

***In vitro* Expression of Glucose transporter (GLUT4) in human skeletal cell lines:**

***RNA isolation and Complementary DNA synthesis*:**

The Human skeletal muscle cells treated with drug were subjected to cell lysis by treating with Tri-extract reagent. Chloroform was added, to isolate the total RNA from the sample and subjected for centrifugation. Out of the three distinct layers observed, the upper layer was collected in a fresh tube and an equal volume of isopropanol was added and incubated at - 20 ºC for 10 mins. After the incubation followed by centrifugation, appropriate volume of ethanol was added to resuspend the pellet. After incubation and centrifugation, the pellet was air dried and an appropriate volume of TAE buffer was added. The isolated total RNA was further used for cDNA synthesis. cDNA was synthesized by priming with oligo dT primers followed by reverse transcriptase enzyme treatment according to the manufacturer's protocol (Thermo scientific). The cDNA thus synthesized was taken up for PCR for the amplification of Glut 4 gene and GAPDH (internal control) [7].

***Semi quantitative PCR*:**

The mRNA expression level of GLUT-4 was carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Briefly, the L-6 cells were cultured in a 60 mm petri dish and maintained in DMEM medium for 48 hrs. The DMEM medium was supplemented with FBS and amphotericin. To the dish was added the required concentration of test sample i.e. Compounds-3, 5, 12, 13, 17 and standard pioglitazone incubated for 24 h. Total cellular RNA was isolated from the untreated (control) and treated cells using Tri Reagent according to the manufacturer's protocol. cDNA was synthesized from total isolated RNA by reverse transcriptase kit according to the manufacturer's instructions (Thermo scientific). Then 50µl of the reaction mixture was subjected to PCR for amplification of GLUT4 cDNAs using specifically designed primers procured from Eurofins India and as an internal control the housekeeping gene GAPDH was co-amplified with each reaction.

Statistical analysis:

Data represent the mean ± standard error mean (SEM) of the indicated number of experiments. Graphs were prepared by GraphPad Prism 5.0 version software. Statistical analysis of the data was carried out by one-way ANOVA (GraphPad Prism 5.0 version) followed by post hoc Dunnett's test. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION:

Fig 1 summarizes the list of sesamol substituted thiazolidien-4-ones derivatives which were reported in the previous work from our lab and reported to possess anti-cancer activity [8]. In the present study the same compounds were evaluated for their possible anti-inflammatory, hypoglycemic, hypolipidemic and anti-diabetic activities.

Among the compounds tested for *in vitro* anti-inflammatory activity using Inhibition of Bovine Serum Albumin denaturation method, Compounds (3 to 21) showed the best *in vitro* anti-inflammatory activity of percentage inhibition at 250 µg/ml concentrations. However, standard Diclofenac showed a maximum inhibition percentage of 77.38 at 250 µg/ml concentration. Values more than 25 % inhibition are significant. The activity of these compounds might be due to electrostatic forces and hydrophobic bonding involved in the stabilization of albumin against denaturation. The results are tabulated in Table 1.

Compounds (1 to 21) were screened for acute oral glucose tolerance test (OGTT) in lean mice to evaluate for their possible anti-diabetic activity. Herein, Compounds (3, 5, 8, 12, 13, and 17) exhibited maximum reduction in plasma glucose. Compounds (5 and 17) showed 69.17 6.78 and 68.67 3,83 maximum percent blood glucose lowering activity additionally at 120 minutes following glucose administration when compared to standard pioglitazone and was found to be significant. The hypoglycemic activity of these compounds (5 and 17) might be due the presence of thiophene moiety and dimethoxy substitution with its affinity interaction with receptors in the tissue has been implicitly accepted. The results are tabulated in Table 2 and 3.

Based on the results obtained from oral glucose tolerance test (OGTT) compounds (3, 5, 8, 12, 13 and 17) were tested for hypoglycemic and hypolipidemic activity in hypercholesterolemic mice. The mice fed with a high fat diet for 4 weeks showed a statistically significant ($p<0.05$) rise in plasma glucose and serum cholesterol levels compared with animals fed with the normal pellet diet, thus confirming the presence of

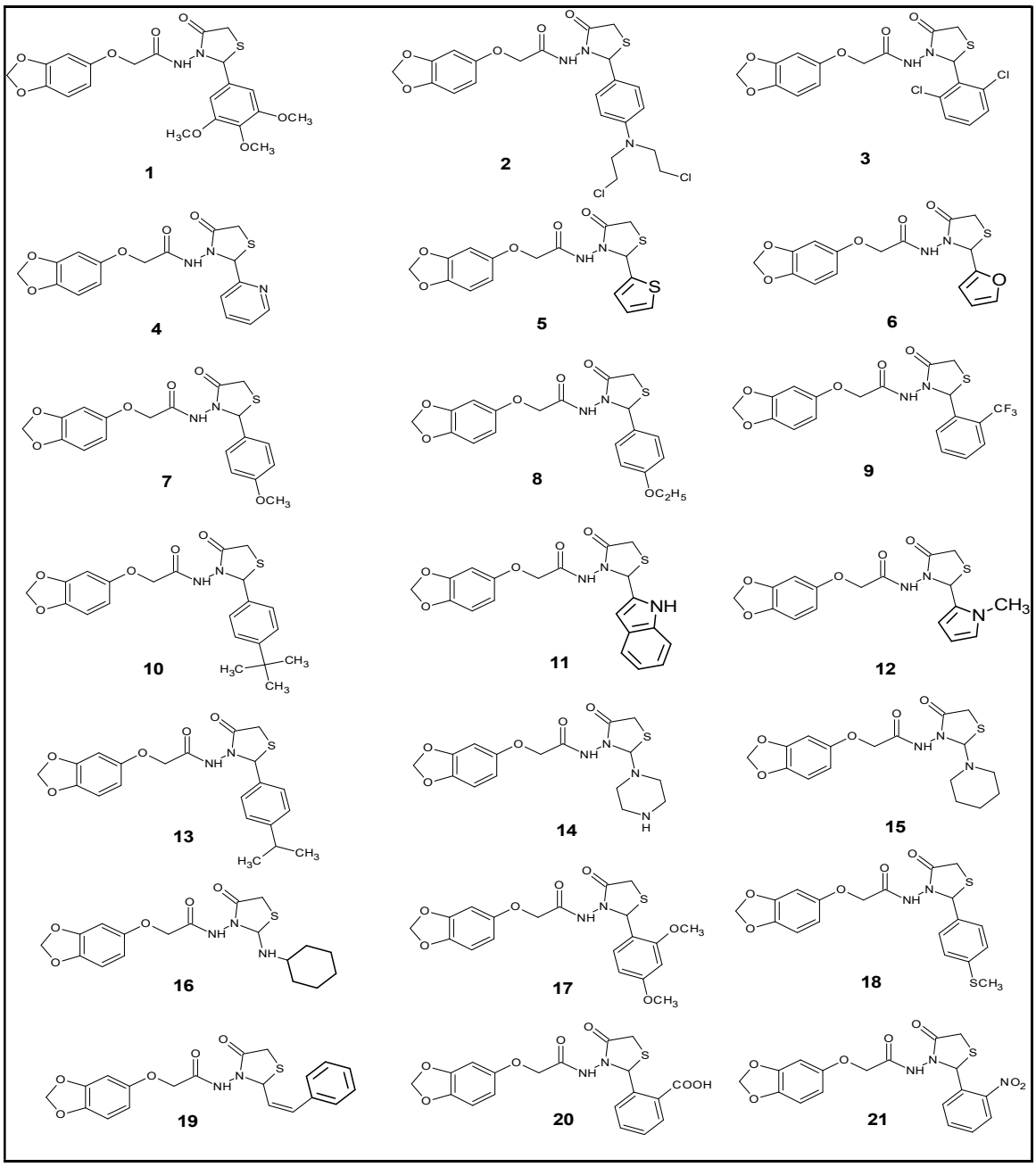

Fig 1. The chemical structures of sesamol substituted thiazolidine-4-one hybrids.

Table 1. Inhibition of bovine serum albumin denaturation by Test compounds and Diclofenac.

Test Compounds	Inhibition of denaturation (%), Mean Concentrations (µg/ml)				
	250	200	150	100	50
Compound 1	18.27	17.25	15.10	13.11	13.00
Compound 2	11.23	4.46	3.49	-	-
Compound 3	35.25	25.69	20.85	15.64	14.83
Compound 4	50.51	30.47	29.34	26.33	24.40
Compound 5	69.43	44.81	28.80	23.91	21.71
Compound 6	41.64	26.01	24.40	20.26	18.75
Compound 7	65.56	27.19	14.24	16.12	11.12
Compound 8	44.76	41.43	35.30	31.43	28.86
Compound 9	62.71	37.51	26.06	18.86	18.59
Compound 10	26.11	16.87	16.01	14.72	5.00
Compound 11	43.69	29.82	27.35	13.33	9.99
Compound 12	35.89	32.40	30.31	22.30	17.57

Compound 13	33.37	29.29	23.75	16.82	15.69
Compound 14	9.67	8.92	-	-	-
Compound 15	22.25	19.72	17.84	16.98	14.72
Compound 16	24.13	22.41	22.25	20.74	15.64
Compound 17	33.32	29.12	21.33	19.94	4.62
Compound 18	40.52	29.29	27.24	21.49	16.01
Compound 19	38.15	35.52	26.17	21.39	16.01
Compound 20	44.87	33.85	30.95	18.70	18.32
Compound 21	49.97	38.15	25.90	24.13	16.23
Standard (Diclofenac)	77.38	50.99	45.14	34.28	29.50

Table 2. Effect of Test compounds on oral glucose tolerance test (OGTT) in lean mice at 100 mg/kg/p.o.

Sl. No.	Test compounds	0 min Mean ± S.E.M.	15 min Mean ± S.E.M.	30 min Mean ± S.E.M.	60 min Mean ± S.E.M.	120 min Mean ± S.E.M.
1	Glucose Control	90.17 ± 3.04	277.5 ± 3.01	227.3 ± 7.38	192.3 ± 5.85	119 ± 4.35
2	Compound 1	92 ± 4.86	276 ± 19.28	253.5 ± 17.46	182.7 ± 17.76	108 ± 5.48
3	Compound 2	82.5 ± 5.12	257.3 ± 7.91	221.3 ± 11.19	170.7 ± 15.32	99.83 ± 8.20
4	Compound 3	94.67 ± 3.22	214 ± 7.75	151.5 ± 15.26	109.8 ± 6.38	95 ± 3.55
5	Compound 4	65.83 ± 5.41	252.3 ± 5.32	190.7 ± 13.31	152 ± 16.15	100.3 ± 7.25
6	Compound 5	61 ± 6.20	197 ± 12.42	180.3 ± 3.87	122.8 ± 18.69	69.17 ± 6.78
7	Compound 6	92.83 ± 4.30	382.3 ± 12. 5	329.8 ± 11.59	216.8 ± 24.38	112 ± 12.31
8	Compound 7	74.33 ± 6.48	273 ± 18.31	253.3 ± 15.61	207.8 ± 18.91	110.7 ± 3.11
9	Compound 8	72.17 ± 6.84	280 ± 9.62	252.5 ± 21.28	178.8 ±19.22	98.33 ± 6.41
10	Compound 9	69.5 ± 7.50	263.7 ± 6.59	279.7 ± 6.51	212.8 ± 8.96	112 ± 5.41
11	Compound 10	71.17 ± 5.86	361.5 ± 13.44	313.3 ± 22.46	213.3 ± 27.33	107 ± 7.55
12	Compound 11	71.67 ± 8.14	204.3 ± 8.97	186.5 ±14.0	116.8 ± 13.78	82.5 ± 5.66
13	Compound 12	73.33 ± 5.64	189.5 ± 4.17	128.7 ± 8.57	102.8 ± 7.23	90.5 ± 7.18
14	Compound 13	99.5 ± 4.99	301.5 ± 10.91	287 ± 17.27	201.7 ± 10.39	109.8 ± 6.98
15	Compound 14	70.83 ± 4.36	269.7 ± 7.18	233.3 ± 8.46	187.7 ± 9.34	135 ± 6.12
16	Compound 15	86.83 ± 2.75	278.8 ± 7.41	243.3 ± 9.00	189 ± 7.20	131.7 ± 7.35
17	Compound 16	57.83 ± 5.79	253.7 ± 7.47	230.3 ± 13.29	204 ± 18.27	139.5 ± 5.73
18	Compound 17	58.33 ± 4.66	210.2 ± 12.62	175 ± 20.27	126.5 ± 14.36	68.67 ± 3.83
19	Compound 18	91.17 ± 6.76	272.3 ± 16.19	270.5 ± 6.86	198.8 ± 10.43	107.7 ± 5.25
20	Compound 19	81.67 ± 4.03	283.3 ± 6.70	217.3 ± 13.99	149.5 ± 6.14	99.17 ± 5.21
21	Compound 20	72.33 ± 4.76	198.7 ± 17.18	193.5 ± 12.94	163.7 ± 16.68	90 ± 4.84
22	Compound 21	94 ± 1.86	270 ± 7.76	232.8 ± 12.64	174.2 ± 10.38	109.7 ± 5.81

Table 3. Effect of Test compounds on plasma glucose (AUC) in lean mice at 100 mg/kg/p.o.

Test compounds	Plasma glucose (AUC), Mean ± S.E.M.	% reduction in glucose (mg/dL)
Glucose Control	833 ± 10	--
Compound 1	812.2 ± 53.0	2.57
Compound 2	741 ± 30	11.14
Compound 3	570.2 ± 28.8	31.60
Compound 4	675.4 ± 29.5	18.98
Compound 5	565 ± 34	32.17
Compound 6	1031 ± 44	-23.75
Compound 7	827 ± 42	0.80
Compound 8	637.0 ± 43.9	23.59
Compound 9	847 ± 31	- 1.61
Compound 10	977.3 ± 58.9	- 17.22
Compound 11	827 ± 21	0.82
Compound 12	503 ± 17	39.65

Compound 13	585 ± 41	29.83
Compound 14	793.6 ± 24.2	4.80
Compound 15	820.4 ± 25.7	1.58
Compound 16	786.7 ± 41.7	5.63
Compound 17	575.2 ± 41.7	31.00
Compound 18	841.1 ± 27.8	- 0.88
Compound 19	740 .6 ±30.4	11.16
Compound 20	797 ± 42	4.41
Compound 21	778.8 ± 27.7	6.55

hyperglycemia and hypercholesterolemia. No significant differences were observed between the groups on day 0 with respect to plasma glucose levels. On the 28th day the plasma glucose levels (Mean ± S.E.M) for HFD + vehicle, HFD + test compounds (3, 5, 8, 12, 13 and 17) were found to be 115.3 ± 2.29, 225.5 ± 3.21, 157.8 ± 2.52, 164.0 ± 2.66, 193.5 ± 1.57, 167.2 ± 1.90, 165.0 ± 2.08, 192.3 ± 3.11, respectively. However, standard pioglitazone exhibited 148.3 ± 2.43 reductions in plasma glucose levels Data indicates that Compound (3) showed statistically significant fall in plasma glucose level on day 28th compared with that of HFD + vehicle treatment. No significant changes in plasma glucose levels were observed in Normal Pellet Diet (NPD) fed mice between days 0 and 28. Moreover, no significant differences were observed between the groups on day 0 with respect to serum cholesterol levels. Perusal of the data shows that neither pioglitazone nor test compounds could reverse the effect of high fat-diet on serum cholesterol. The results are tabulated in Table 4 and 5.

Skeletal muscle is a major tissue involved in insulin-induced stimulation of glucose uptake. The L6 cell line is the established cellular model for glucose uptake because they have been used extensively to elucidate the mechanisms of glucose uptake in muscle. Test compounds (3, 5, 8, 12, 13 and 17) were also evaluated for *in vitro* glucose uptake activity by using human skeletal muscle cell line (L6). The perusal of the data showed that L6 cell line enhance the glucose uptake of 47.20 ± 0.52 at 500 µg/ml for compound (12), 41.64 ± 0.52 at 250 µg/ml for compound (5) and 40.39 ± 0.62 at 500 µg/ml for compound (3), respectively. Among the tested compounds, compound (12) showed the best utilization of glucose uptake by L6 cell lines, which was significant when compared to standard pioglitazone and merited its anti-diabetic potential. The anti-diabetic activity might be due to the presence of a sesamol scaffold substituted with more lipophilic N-methylpyrrole moiety when compared with other substitutions like thiophene or dichlorophenyl moiety. The results are tabulated in Table 6.

Table 4. Effect of Test compounds and Pioglitazone on plasma glucose in hyperglycemic Mice

Test Compounds	Day 0 Mean ± S.E.M	Day 28 Mean ± S.E.M
NPD	113.7 ± 5.47	115.3 ± 2.29
HFD + vehicle	201.8 ± 3.11	225.5 ± 3.21
HFD + Compound-3	200.5 ± 4.12	157.8 ± 2.52
HFD + Compound-5	201.8 ± 4.74	164.0 ± 2.66
HFD + Compound-8	200.7 ± 2.16	193.5 ± 1.57
HFD + Compound-12	201.3 ± 1.94	167.2 ± 1.90
HFD + Compound-13	201.0 ± 1.88	165.0 ± 2.08
HFD + Compound-17	201.2 ± 3.65	192.3 ± 3.11
HFD + Pioglitazone	199.7 ± 3.81	148.3 ± 2.43

Table 5. Effect of Test compounds and Pioglitazone on plasma cholesterol in Hypercholesterolemia mice.

Test Compounds	Day 0 Mean ± S.E.M	Day 28 Mean ± S.E.M
NPD	114.7 ± 3.45	122.2 ± 4.23
HFD + vehicle	181.5 ± 4.25	194.7 ± 2.15
HFD + Compound-3	181.8 ± 5.26	170.2 ± 4.47
HFD + Compound-5	181.0 ± 3.15	170.2 ± 0.47
HFD + Compound-8	182.0 ± 4.71	186.0 ± 1.71
HFD + Compound-12	180.0 ± 5.11	174.7 ± 3.19
HFD + Compound-13	180.5 ± 4.63	170.3 ± 1.83
HFD + Compound-17	181.2 ± 4.43	175.3 ± 4.23
HFD + Pioglitazone	180.2 ± 4.46	168.8 ± 3.03

Based on the data obtained from *in vitro* glucose uptake assay, test Compounds (3, 5, 12, 13 and 17) were also screened for modulator effect on PPARγ protein expression by using Human skeletal cell lines. Among

the tested compounds, compound (3) exhibited an increase in the upregulation of PPARγ gene expression by 1.45 in terms of the fold at a concentration 500 µg/ml and was found to be significant when compared to that of standard pioglitazone. The results are tabulated in Table 7. Perusal of the data shows that expression of PPAR*γ* increases insulin sensitivity of skeletal muscle cells and merited the anti-diabetic potential of the tested compounds.

Table 6. *In vitro* glucose uptake studies for test compounds in L-6 cell line.

Sl. No.	Test Compounds	Conc. (µg/ml)	Glucose uptake over control (%)
1	Compound - 3	500	40.39 ± 0.62
		250	20.68 ± 1.55
2	Compound - 5	250	41.64 ± 0.52
		125	24.93 ± 0.31
3	Compound - 8	1000	12.03 ± 0.73
		500	3.09 ± 0.52
4	Compound - 12	1000	47.20 ± 0.52
		500	26.54 ± 0.52
5	Compound - 13	500	44.49 ± 0.62
		250	28.23 ± 0.41
6	Compound - 17	500	30.94 ± 0.52
		250	12.18 ± 0.31
7	Standard (Pioglitazone)	100	54.68 ± 0.73

Moreover, GLUT4 is the major glucose transporter isoform in tissues that exhibits insulin-stimulated glucose uptake such as adipose tissue and skeletal muscle tissues. In this view, the test compounds (3, 5, 12, 13 and 17) were also screened for Glut4 gene expression in skeletal muscle cells to elucidate the molecular basis of anti-diabetic potential. Among the tested compounds, Compounds (3, 12 and 13) up-regulated modest GLUT4 gene expression and was significant when compared to standard pioglitazone. The results are tabulated in Table 8. Our data revealed a significant increase in the expression of GlUT4 in compound (13) treated cells and this can be accounted for by blood glucose reduction through glucose utilization by muscle tissue. Compound (13) has a potential to be further developed as a potential molecule to decrease insulin resistance in the future.

CONCLUSION:

Compounds (5, 7, 9, 20, and 21) showed modest inhibition of thermal denaturation of albumin and were significant when compared with standard diclofenac. Compounds (3, 5, 8, 12, 13, and 17) showed maximum reductions in plasma glucose levels when compared to standard pioglitazone indicating its anti-diabetic activity *in vitro*. Compounds (3, 5, 12, 13, and 17) also showed significant hypolipidemic and hypoglycemic activity when screened *in vivo* in mice when compared to standard pioglitazone meriting its potent anti-diabetic activity. Furthermore, same compounds were screened for possible *PPARγ* agonist and inhibitory effect of GLUT 4 expressions in human skeletal muscle cell line by MTT assay as an approach for mechanistic action and how these compounds elicit anti-diabetic activity. The data suggest that the said Compounds (3, 5, 12, 13, and 17) exhibited potent anti-diabetic activity, and the effect may be due to the presence of thiazolidin-4-one scaffold. A thorough structure–activity relationship (SAR) has not been carried out. With more detailed SAR studies, it will be possible to develop molecules that have greater and more selective activity. It will also be possible to develop molecules that have multiple beneficial effects such as anti-inflammatory, anti-hyperlipedimic and anti-diabetic activities in the same molecule. Future work could possibly address the lacunae in the current study.

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Table 7. Effect of Test compounds on PPAR γ protein expression in Human skeletal cell lines.

Test compounds	Concentrations (µg/ml)	Regulation in terms of Folds	Concentrations (µg/ml)	Regulation in terms of Folds
Cell Control	--	1.51	--	--
Compound -3	500	1.45	250	1.31
Compound -5	250	1.33	125	1.22
Compound -12	1000	1.39	500	1.33
Compound -13	500	1.29	250	1.14
Compound -17	500	1.39	250	1.29
Standard Control (Pioglitazone)	100	1.21	--	--

Table 8. Effect of Test compounds on Glucose transporter 4 (GLUT 4) expression in Human skeletal cell lines

Test compounds	Concentrations (µg/ml)	Regulation in terms of Folds	Concentrations (µg/ml)	Regulation in terms of Folds
Cell Control	--	1.21	--	--
Compound -3	500	1.65	250	1.58
Compound -5	250	1.30	125	1.21
Compound -12	1000	1.66	500	1.53
Compound -13	500	1.68	250	1.42
Compound -17	500	1.34	250	1.22
Standard Control (Pioglitazone)	100	1.11	--	--

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