Research Article

Design, Synthesis, Biological Evaluation of Tnf-α Inhibi- Tors As Antidiabetic Agents

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Abstract:

The weight loss in obese patients is associated with reduced Tumor necrosis factor-α (TNF-α) production and ameliorated insulin resistance. TNF-α has been shown to be an important mediator of insulin resistance linked to obesity. TNF-α interferes with insulin-signaling by inhibiting tyrosine kinase activity of the insulin receptor and the serine phosphorylation of insulin receptor substrate-1 (IRS-1). Thiazolidinediones serve as a boom in the antidiabetic therapy by increasing the sensitivity of insulin receptors towards insulin. The present study aims at designing novel thiazolidinediones with TNF-α inhibitory action thereby increasing the insulin sensitivity. The goal of our research work is to synthesize newer thiazolidinedione derivatives via convenient and efficient synthetic pathways which will offer an advantage of having both TNF-α inhibitory and antidiabetic activities particularly in obese people. The hybrid molecules with two basic pharmacophores *viz* Chalcone and Thiazolidinediones having potential to show both TNF-α inhibitory activity as well as antidiabetic activity are synthesized. A series of **5-(4-(4-(3-(4-substituedphenyl) prop-2-en-1-one) phenoxy)benzylidene)thiazolidine-2,4-dione** are synthesized by designing the appropriate schemes and characterized further by TLC, FTIR and ^{1}H NMR. The synthesized compounds were further evaluated for antidiabetic activity and TNF-α inhibitory activity. The results show that the compounds inhibit TNF-α along with comparable antidiabetic activity. The study can be extended further to establish the correlation of TNF-α inhibition with increased insulin sensitivity.

Key Words: Thiazolidinedione, TNF-alpha, antidiabetic

Introduction:

Diabetes mellitus is a permanent bodily state in which there is a derangement in carbohydrate metabolism, and sometimes also in fat and protein metabolism[(1)]. The main problem in diabetes is that sometimes cells become resistant to insulin. Thiazolidinediones serve as a boom in the antidiabetic therapy by increasing the sensitivity of insulin receptors towards insulin. Hence they are also called ''insulin sensitizers''.

Stimulation of PPARγ is regarded as the principle mechanism through which thiazolidinediones enhance insulin sensitivity[(2)]. PPARγ is expressed at highest levels in adipose tissue, and less so in muscle and liver. PPARγ operates in association with the retinoid X receptor. The resulting heterodimer binds to nuclear response elements, thereby modulating transcription of a range of insulin-sensitive genes, in the presence of necessary cofactors.

Many of the genes activated or suppressed by thiazolidinediones are involved in lipid and carbohydrate metabolism. Stimulation of PPARγ by a thiazolidinedione promotes differentiation of pre-adipocytes with accompanying lipogenesis, effects that promote or enhance the local effects of insulin[(3)]. Thiazolidinediones increase glucose uptake via glucose transporter-4 in skeletal muscle, and some reports indicate that rates of gluconeogenesis in the liver are reduced. Stimulation of lipogenesis via PPARγ reduces circulating non-esterified fatty acid (NEFA) concentrations through cellular uptake and triglyceride synthesis. The reduction in plasma NEFA concentrations is associated with increased glucose utilization and reducing gluconeogenesis by reducing operation of the glucose-fatty acid cycle; reductions in ectopic lipid deposition in muscle and liver may contribute to the improvements on glucose metabolism. Thiazolidinediones block TNF-α mediated inhibition of insulin signaling. TNF-α has been shown to be an important mediator of insulin resistance linked to obesity[(4)]. This cytokine induces insulin resistance, at least in part, through inhibition of the tyrosine kinase activity of the insulin receptor. Small-molecule anti-TNF-α drugs are being investigated as potential therapeutics[(5)]. TNF-α antagonists, including antibodies and soluble receptors, are being used to treat Crohn's disease, rheumatoid arthritis, and asthma, and recently it is investigated for use in treatment of type II diabetes[6]. Dysregulation of TNF-α production has been implicated in a variety of human diseases, including Alzheimer's disease, cancer, major depression and inflammatory bowel disease (IBD).

TNF- α has been demonstrated to disrupt insulin signaling at a number of peripheral sites which are dependent on IRS phosphorylation. As indicated, some of these actions can be mimicked by protein kinase C isoforms, cell permeable ceramides, neutral sphingomylinase and the protein phosphatase inhibitor, okadaic acid. In addition, chronic exposure to TNF- α can suppress the expression of many adipocyte-specific genes including the insulin-sensitive glucose transporter Glut 4. Pre-treatment with the insulin sensitizingthiazolidinediones (TZD) has been shown to reverse at least two actions of TNF- α. TNF- α can also stimulate the production of additional mediators such as free fatty acids and leptin which may act to potentiate its actions on insulin resistance in adipocytes. A series of potent and selective antidiabetic agents mostly from substituted thiazolidinediones has been developed and their blood glucose level lowering activities were mainly examined in genetically obese and insulin resistant ob/ob mouse.

Chalcones are the compounds which are also known as benzalacetophenone or benzylidene acetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcones are a, β-unsaturated ketones containing the reactive ketoethylenic group–CO-CH=CH-. These are colored compounds because of the presence of the chromophore -CO-CH=CH-, which depends in the presence of other auxochromes. Chalcone is an important chemotype that has attracted great research interest for decades due to the high natural abundance of chalcone compounds, their easy synthesis, and most importantly, their diverse biological activities. Indeed, many natural chalcones demonstrated a wide variety of bioactivities, including anti-cancer, anti-inflammatory, anti-diabetic, cancer chemopreventive, anti-oxidant, and anti-microbial activities[7-9]. Different classes like phthalidomide, pyrimidines, quinazolines, thiazolidinediones and also some natural flavanoids and biaryl based chalcones are used as TNF-α inhibitors[10]. Present TNF-α inhibitors cause the side effects like increased risk of cardiovascular events, increased risk of bladder cancer, hepatitis, polyneuropathy, fatigue etc. Thiazolidinedione derivatives and chalcones possess good TNF-α inhibitory activity and are used in treatment of type 2 diabetes. Since TNF-α is found to be important mediator of insulin resistance linked in obesity, TNF-α inhibition by antidiabetic drug may enhance the efficacy of drug. Thus we proposed to synthesize the hybrid molecule having both TNF-α inhibitory activity as well as antidiabetic activity leading us to synthesize hybrid analogues of two pharmacophores, *viz.* chalcones and thiazolidinediones to achieve compounds having potentially combined biological activities as TNF-α inhibitory activity and antidiabetic activity.

Materials And Methods:

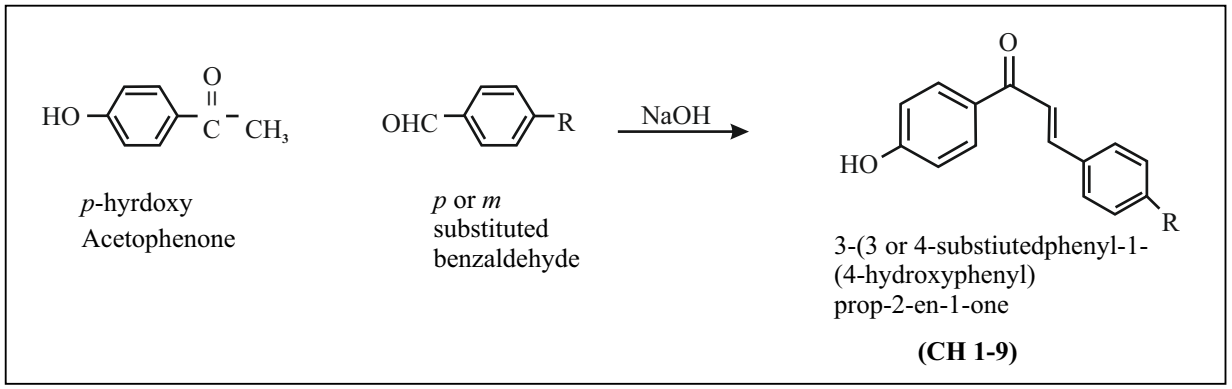
Experimental :

All the chemicals used in the synthesis were of laboratory grade. The Melting points were determined in open capillary on Veego (model: -VMP-D) electronic apparatus and are uncorrected. The IR spectra of synthesized compounds were recorded on Shimadzu 8400 - S FTIR Spectrophotometer using potassium bromide. The ^{1}H NMR spectra of synthesized compound were recorded on NMR Varian-Mercury 300 MHz spectrometer. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on precoated aluminium silica gel-G, using chloroform-methanol, chloroform- ethyl acetate, as the solvent systems and the spots were visualized by exposure to UV cabinet.

Synthesis of *para* or *meta* substituted Chalcones [11]:

Figure 1 : Solution of 2 gm of sodium hydroxide in 18.18 ml of water and 11.5 ml of rectified spirit was taken in a 500 ml bolt head flask provided with a mechanical stirrer. The flask was immersed in a bath of crushed ice, 4.7 (0.43 mol) of *p*-hydroxyacetophenone was poured in flask, the stirrer was started and then 4.10gm of *para* or *meta* substituted

Figure 1: Scheme of Synthesis

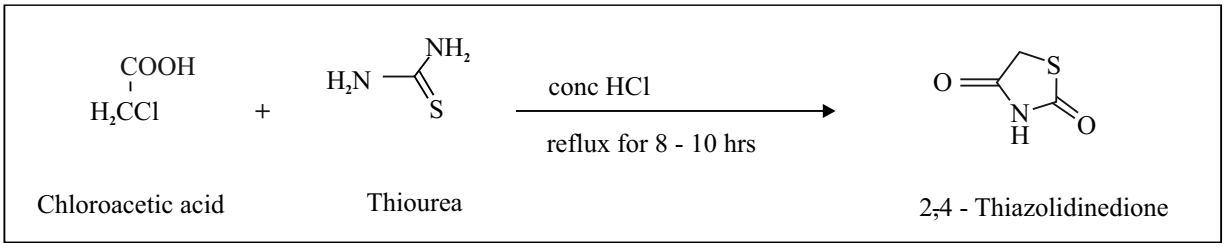


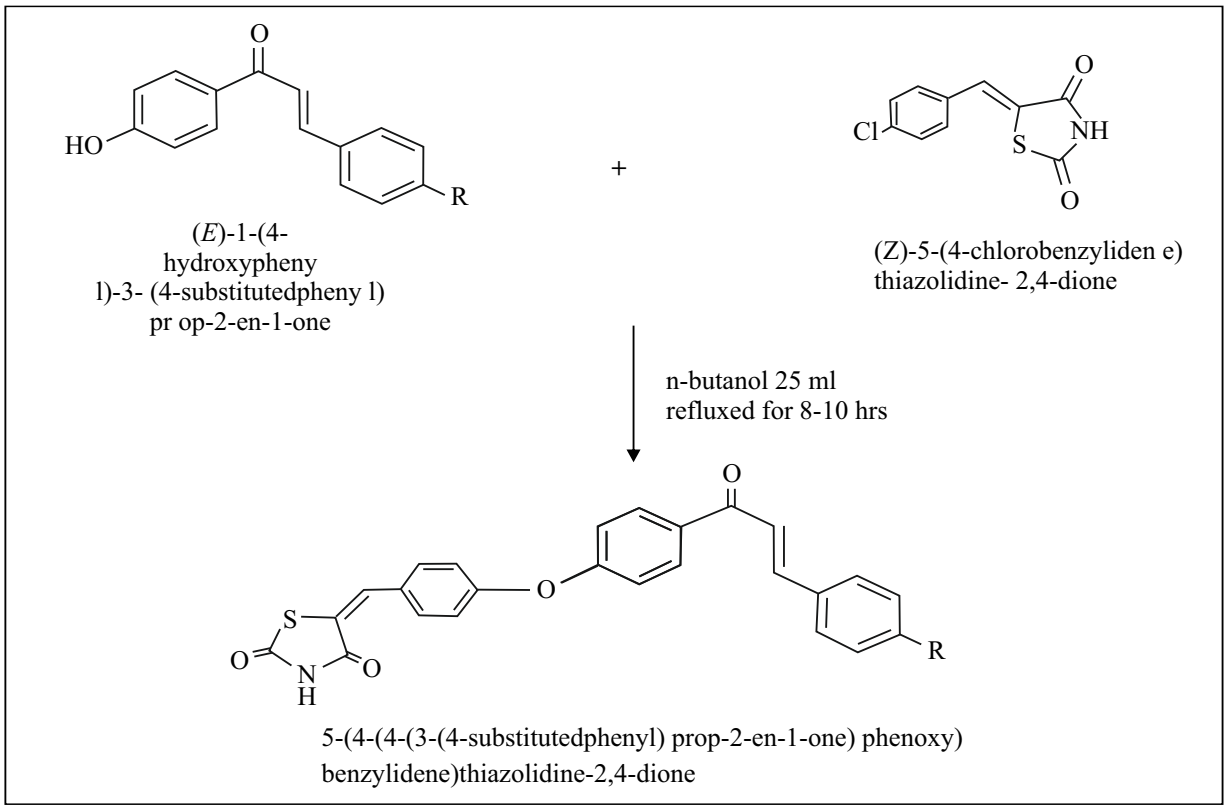
p-hyrdoxy Acetophenone

p or *m* substituted benzaldehyde

3-(3 or 4-substiutedphenyl-1-(4-hydroxyphenyl) prop-2-en-1-one

(CH 1-9)





benzaldehyde was added. The temperature of mixture was kept at about 25°C and stirred for 7-8 hrs. The reaction mixture was poured in cold water containing 10% HCl and the product was allowed to precipitate, the product was washed with water until neutral to litmus. The product was recrystallized from ethanol.

Synthesis of Thiazolidine-2,4-dione:[12,13]

In 250 ml three necked round bottom flask solution containing 2 gm, (0.6 mol) of chloroacetic acid in 2.12 ml of water and 1.61gm, (0.6 mol) of thiourea dissolved in 2.12 ml of water was placed. The mixture was stirred for 15 minutes to form a white precipitate, accompanied by considerable cooling. To the contents of the flask was then added slowly 2.12 ml of conc. HCl from a dropping funnel, the flask was then connected with a reflux condenser and gentle heat supplied to the effect complete solution, after which the reaction mixture was stirred and refluxed for 8-10 hrs at 100-110°C. On cooling the contents of the flask solidified to a cluster of white needles. The product was filtered and washed with water to remove traces of HCl and dried. It was recrystallized from ethanol.

Synthesis of intermediate compound:

In a three necked round bottom flask 1 gm (0.188 mol) of *p*-substituted benzaldehyde and 0.83 gm, (0.188 mol) of 2, 4-thiazolidinedione were together suspended in 25 ml of dry toluene. To this catalytic amount of piperidine (2-3 drops) was added. The mixture was refluxed with stirring. After the complete removal of water and when the temperature crossed 110°C the reaction mixture was stirred for further 1 hr. On cooling the product precipitated out from toluene. The compound was filtered and washed with cold dry toluene. Product was recrystallized from acetic acid.

Synthesis of target compound:

In a round bottom flask 1 gm, (1.5 mol) of 3-(4-chlorophenyl-1-(4-hydroxyphenyl) prop-2-en- 1-one and 0.83 gm, (1 mol) of 5-(4- chlorobenzylidene) thiazolidine-2,4-dione were together suspended in 25 ml of n-butanol. Reaction was refluxed for about 8-10 hours. After completion of reaction the reaction mixture was cooled to room temperature. On cooling the product precipitated out from n-butanol. The compound was filtered and washed with n-butanol. The product was recrystallized from ethanol.

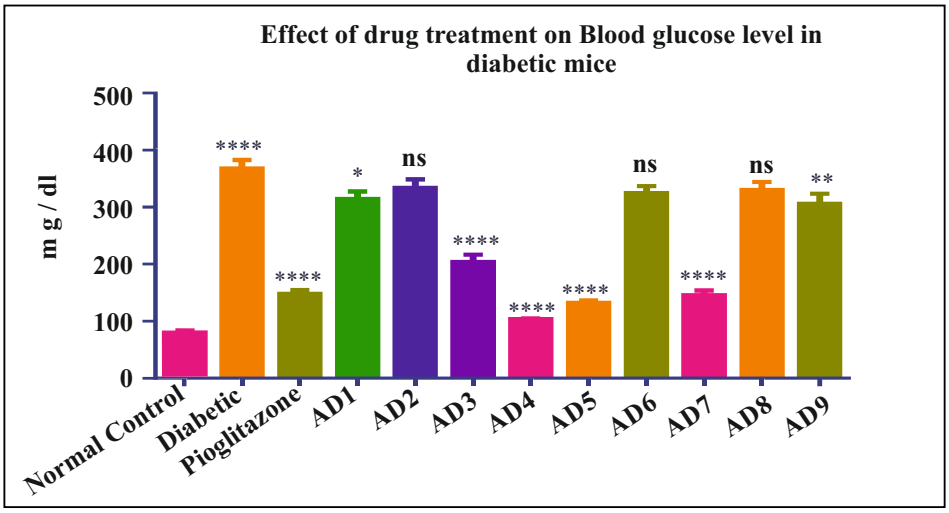
Pharmacological Evaluation: Antidiabetic Activity:

Figure 2 : All the target compounds AD (1-9) were screened for their antidiabetic activity by using Alloxan induced diabetes method.[14]

Animals and Treatment:

Wister Albino mice of either sex weighing between 25-30 gm were selected for the study. Animals were housed in polypropylene cages and under standard condition of

Figure 2: Graph of Effect of Drug Treatment on BSL in Diabetic Mice



temperature (25 ± 50°C), 12h/12h, light/dark cycles and were fed with standard mice pelleted diet and water was given. Blood sample was collected through the tail vein and the basal fasting blood glucose (BG) was estimated by digital Blood Glucometer. The mice were injected with alloxan (120 mg/kg) and made i. p. The blood glucose level was again monitored after 48 hrs. The animals showing elevated blood glucose (BG) levels above 250 mg/dl were selected for study.

Acute Antidiabetic activity:

Study animals were fed with standard pelleted diet and water was given and on next day Blood Sugar Level was checked. The synthesized compounds AD1-9 were homogenized and suspended in 0.5% CMC, and was administered at a fixed dose of 30 mg/ kg i.p. Blood glucose levels of the treated animals was measured after 2,4,6 and 24 hrs. Blood drop was removed from the tail vein for testing BSL with the help of Glucometer (Glucopoint meter). The results were expressed as mean ± standard error of mean (SEM) and percent decrease in blood glucose was calculated. The data obtained was analyzed by one-way ANOVA followed by Bonferroine's test, $p<0.01$ is considered as statistically significant for evaluation of hypoglycemic activity.

TNF-α inhibitory activity:

Assay Procedure: All reagents and samples were brought to room temperature before use. All reagents, working standards, and samples were prepared as directed in the previous sections. Excess microplate strips were removed from the plate frame, and returned to the foil pouch containing the desiccant pack, and were resealed. 100 µL of Assay Diluents RD1-27 was added to each well. 100 µL of Standard, sample, or control was added in each well and covered with the adhesive strip provided. All solutions were incubated for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout was provided to record standards and samples assayed. Each well was aspirated and washed, repeating the process three times for a total of four washes. It was washed by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Further 200 µL of Human TNF-α Conjugate was added to each well and covered with a new adhesive strip. All solutions were incubated for 2 hours at room temperature on the shaker. 100 µL of Working Glo Reagent was added to each well and incubated for 5 - 20 minutes at room temperature on the benchtop protecting from light. The RLU of each well was determined using a luminometer set with the following parameters; 1.0 min. lag time; 0.5 sec/well read time; summation mode; auto gain on.

Results And Discussion:

Step 1: Synthesis of substituted Chalcones CH (1-9)

Table 1, 2, 3 : Substituted chalcones **CH (1-9)** were synthesized using *p*-hydroxyacetophenone and substituted benzaldehydes in presence of strong base NaOH. It is **Claisen-schmidt condensation** reaction. The compounds **CH (1-9)** obtained are solids melting at around the range of 178-210°C. All the substituted chalcones synthesized were

Table 1: Spectral Characterization of the Synthesized Compounds

Sr. No.	Comp code	R	FT-IR spectral data	$^{(1)}$H NMR spectral data
1	AD1	4-H	Ar C-H str (3055), N-H str (3147), C=O str (1751), C=C str (1612), C-N str (1243), C-O str (1165), C-S(663)	δ 7.0 – 7.8 (m, 12H, Ar), 7.7 (d,1H, CH=CH), 7.8 (d, 1H, CH=CH), 7.35 (s,1H, CH), 12.72 (s, 1H, NH)
2	AD2	4- OCH_3	Ar C-H str (3063), N-H str (3117), C=O str (1751), C=C str (1604), C-N str (1247), C-O str (1165), C-S (662)	δ 3.89 (s, 3H, OCH_3), 6.99 – 7.8 (m, 12H, Ar), 7.7 (d, 1H, CH=CH), 7.76 (d, 1H, CH=CH),7.35 (s,1H, CH), 10.38 (s, 1H, NH)
3	AD3	4-$N(CH_3)^2$	Ar C-H str (3020), N-H str (3324), C=O str (1751), C=C str (1604), C-N str (1249), C-O str (1165),C-S str (661)	δ 4.35 (s, 6H, $N(CH_3)_2$), 6.99 – 7.8 (m, 12H, Ar), 7.7 (d, 1H, CH=CH), 7.76 (d, 1H, CH=CH), 7.35 (s,1H, CH), 11.88 (s, 1H, NH)
4	AD4	4-NO_2	Ar C-H str (3047), N-H str (3147), C=O str (1751), C=C str (1612), C-N str (1239), C-O str (1165), C-S str (660)	δ 6.99 – 7.8 (m, 12H, Ar), 7.7 (d, 1H, CH=CH), 7.76 (d, 1H, CH=CH), 7.35 (s,1H, CH), 11.88 (s, 1H, NH)
5	AD5	3,4 - OCH_3	Ar C-H str (3047), N-H str (3147), C=O str (1751), C=C str (1612), C-N str (1248), C-O str (1165), C-S str (658)	δ 4.0 (s, 1H, CH), 4.4 (s, 6H, OCH_3), 7.2 – 9 (m, 12H, Ar), 7.7 (d, 1H, CH=CH), 7.75 (d, 1H,CH=CH), 12 (s, 1H, NH)
6	AD6	4-Cl	Ar C-H str (3063), N-H str (3147), C=O str (1751), C=C str (1612), C-N str (1245), C-O str (1165), C-Cl str (694),C-S str (659)	δ 7.1 – 8.2 (m, 13H, Ar), 8.0 (d, 1H, CH=CH), 7.55 (d, 1H, CH=CH), 9.0 (s,1H, CH), 11.0 (s, 1H, NH)
7	AD7	3-Cl	Ar C-H str (3039), N-H str (3109), C=O str (1751), C=C str (1612), C-N str (1245), C-O str (1165), C-Cl str (771), C-S str (660)	δ 6.9 –9.1 (m, 12H, Ar), 6.9 (d, 1H, CH=CH), 7.89 (d, 1H, CH=CH), 8.7 (s,1H, CH), 11.7 (s,1H, NH)
8	AD8	3-NO_2	Ar C-H str (3039), N-H str (3326), C=O str (1751), C=C str (1612), C-N str (1276), C-O str (1165), C-Cl str (693), C-S str (661)	δ 7.0 – 8.7 (m, 12H, Ar), 7.3 (d, 1H, CH=CH), 7.45 (d, 1H, CH=CH), 9.0 (s,1H, CH), 11.2 (s,1H,NH)
9	AD9	3-Br	Ar C-H str (3047), N-H str (3124), C=O str (1751), C=C str (1612), C-N str (1239), C-O str (1165), C-Br str (694), C-S str (663)	δ 6.8 – 8.55 (m, 12H, Ar), 7.8 (d, 1H, CH=CH), 8.0 (d, 1H, CH=CH), 8.9 (s,1H, CH), 12.0 (s,

Table 2 - Blood glucose level in mice plasma after treatment with synthesized compound

Sr. No.	Animal group	Blood Glucose Level (mg/ dl) Mean ± SEM
1.	Normal Control	78.5±3.96
2.	Diabetic Control	368±15.27 ****
3.	Pioglitazone	148±6.82
4.	AD1	314.8±12.69 ****
5.	AD2	334±14.59 ****
6.	AD3	204±12.73*
7.	AD4	106±2.315 ns
8.	AD5	132.2±4.277 ns
9.	AD6	324.8±12.2 ****
10.	AD7	145±9.09 ns
11.	AD8	330±14.47****
12.	AD9	306±17.62****

All the values are expressed as mean ± S.E.M compared with diabetic control and normal control compared with diabetic control Data analyzed by one way ANOVA followed by Bonferroni's multiple comparison tests.**** indicates p <0.0001, *** indicates p <0.001, ** indicates p <0.01,* indicates p<0.1 when compared with diabetic group. ns- indicate non-significant with diabetic stressed group.

Table 3: TNF-*α* inhibitory activity of compounds

Test Name Ref. Range TUMOUR NECROSIS FACTOR (TNF)- ALPHA, SERUM (CMI)	Results	Units
AD1 <8.10	5.00	pg/mL
Ad4 <8.10	3.75	pg/mL
Ad7	4.13	pg/mL

freely soluble in DMSO and in ethanol on heating. The solid state IR (KBr, $cm^{(-1)}$) spectra of these compounds revealed the characteristic chalcone a,β- unsaturated ketone C=C at 1650 $cm^{(-1)}$ and C=O at 1700-1751cm^{-1} in addition to the aromatic C=C at 1500- 1600 $cm^{(-1)}$ and OH stretch at 3100-3300 $cm^{(-1)}$ were also seen. $H^{(1)}$ NMR spectrum exhibited aromatic ring peaks between 7-8 δ ppm as multiplet.

Step 2 - Synthesis of (Z)- 5 -(4 - chlorobenzylidene) thiazolidine-2, 4-dione

(Z)-5-(4-chlorobenzylidene) thiazolidine - 2, 4-dione was synthesized using 2, 4- thiazolidinedione with *p*-chlorobenzaldehyde using piperidine as catalyst. It is **Knoevenagel condensation** reaction in which equimolar quantities of thiazolidine 2, 4- dione was reacted with *p*-chlorobenzaldehyde in presence of piperidine as catalyst & toluene as solvent to yield 5-(4-chlorobenzylidene) thiazolidene 2,4- dione by elimination of water molecule. The compound obtained is solid and melts in the range of 240-241^{0}C. The synthesized compound is freely soluble in DMSO and in acetic acid on heating.

Step 3-Synthesis of target compounds (AD1- 9)

5-(4-(4-(3-(4-substitutedphenyl) prop-2-en-1- one) phenoxy) benzylidine) thiazolidine-2,4- dione was synthesized using substituted chal- cones and (Z)-5-(4-chlorobenzylidene) thiazolidine-2,4-dione in presence of n-butanol to yield the target compound. The compounds **AD (1-9)** obtained are solids melting at around the range of 176-229^{0}C. All the substituted tar- get compounds were freely soluble in DMSO and in ethanol on heating.

Antidiabetic Activity:

The investigation of acute antidiabetic screen- ing data revealed that all the tested compounds showed moderate to good blood sugar lowering activity in diabetes condition. The *in vivo* acute antidiabetic activity was evaluated by alloxan induced diabetes method. In this method about 120 mg/kg dose of alloxan was injected in mice as per the weight of the individual mouse. After injecting the dose of alloxan in mice by i. p. route diabetes was induced after 48 hrs and the mouse in which the blood sugar level elevated above 250 mg/dl were selected for the activity. The synthesized compounds were injected in mice in dose of 200mg/kg of human dose of pioglitazone which is used as the standard drug and about 30 mg/kg of the dose of the synthe- sized compound was homogenized in 0.5% of CMC solution and 0.5 ml of this suspension was injected by i.p. route. Compounds AD4, AD5, AD7 showed good blood sugar lowering activity and compound AD3 showed moderate blood sugar lowering activity whereas com- pounds AD1, AD2, AD6, AD8 and AD9 showed very poor blood sugar lowering activity as compared to the standard drug. Compound AD4 exhibited the significant BSL activity which is equivalent to that of the standard drug.

TNF-*α* Inhibitory Activity:

TNF-α inhibitory activity was performed by Chemilluminescence Immunoassay (CMI) this assay employs the quantitative sandwich en- zyme immunoassay technique. In this assay a monoclonal antibody specific for TNF-α was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal anti- body specific for TNF-α is added to the wells. Following a wash to remove any unbound anti- body-enzyme reagent, an enhanced luminol/ peroxide substrate solution is added to the wells and light is produced in proportion to the amount of TNF-α bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted. Three synthesized compounds AD1, AD4, AD7 of nine were in-vestigated for TNF-α inhibitory activity by CMI technique

and it can be concluded that these compounds showed TNF-α inhibitory activity since the results of the activity are obtained within the reference range.

Conclusion:

These target compounds AD (1-9) were synthe- sized and were biologically screened for antidiabetic activity and TNF alpha inhibitory activity. All the synthesized compounds were evaluated for its antidiabetic activity by alloxan induced diabetes method in mice. The *in vivo* Blood sugar lowering activity of the synthe- sized compounds indicated that the compounds AD1, AD2, AD6, AD8 and AD9 showed lower blood sugar lowering activity in diabetes. Compound AD3 showed moderate blood sugar lowering activity in diabetes. The compounds AD4, AD5 and AD7 showed good blood sugar lowering activity. Compound AD4 showed the best blood sugar lowering activity. Compounds AD1, AD4 and AD7 were screened for TNF- alpha inhibitory activity by CMI assay method and showed TNF- alpha inhibitory activity. The synthesized compounds have a potential for further development as novel antidiabetic agents with TNF-alpha inhibitory activity.

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Conflict of Interest: Nil

References:

1. Skyler JS. Diabetes mellitus: Pathogenesis and treatment strategies. J Med Chem. 2004; 47: 4113-4117.
2. Schoonjans K, Auwerx J. Thiazolidin- ediones: an update. Lancet. 2000; 355: 1008-1010.
3. Smith SA. Peroxisome proliferator- activated receptor and the regulation of mammalan lipid metabolism. Biochem Soc Trans. 2002; 30(6): 1086-1090.Fukuzawa
4. M, Satoh J, Qiang X, Miyaguchi S ,Sakata Y, et al. Inhibition of tumor necrosis factor-α with anti-diabetic agents. Diabetes Research and Clinical Practice. 1999 March; 43(3) : 147-154.Tweedie D,
5. Sambamurti K, Greig NH. TNF-α Inhibition as a Treatment Strategy for Neurodegenerative Disorders: New Drug Candidates and Targets. Current Alzheimer Research. 2007 September; 4 (4) : 378-385.
6. Lin J, Ziring D, Desai S, Kim S, Wong M, Korin Y, et al. TNF blockade in human diseases: an overview of efficacy and safety. Clin Immunol 2008;126:13–30.
7. Batovska DI, Todorova IT. Trends in utilization of the pharmacological potential of chalcones. Curr Clin Pharmacol. 2010; 5: 1-29.
8. Sahu NK, Balbhadra SS, Choudhary J, Kohli DV. Exploring pharmacological significance of chalcone scaffold: a review. Curr Med Chem. 2012; 19: 209-225.
9. Singh P, Anand A, Kumar V. Recent developments in biological activities of chalcones: a mini review. Eur J Med Chem,2014; 85: 758-777.
10. Bandgar BP, Hote BS, Dhole NA, Gacche RN. Synthesis and biological evaluation of novel series of chalcone derivatives as inhibitors of cyclooxygenase and LPS-induced TNF-a with potent antioxidant properties. Med Chem Res. 2012; 21: 2292– 2299.
11. Borse SL, Patel MR, Borse LB. Microwave Assisted Synthesis and Biological Evaluation of Substituted Chalcones. International Journal of Pharmacy &Technology. 2011 June; 3(2) : 2465-2479.
12. Swathi N, Ramu Y, Subrahmanyama CVS, Satyanarayana K. Synthesis, Quantum Mechanical Calculation And Biological Evaluation Of 5-(4-Substituted Aryl/ Hetero Aryl Methylidene)-1,3-Thiazolidine-2, 4-Diones. Int J Pharm Pharm Sci. 2012; 4 (2): 561-566.
13. Pattan SR, Kekare P, Patil A, NikaljeA , Kittur BS. Studies on the Synthesis of Novel 2,4-Thiazolidinedione Derivatives with Antidiabetic Activity . Iranian Journal of Pharmaceutical Sciences. 2009; 5(4): 225-230.
14. Nikalje APG, Deshpande D Une H. Facile Synthesis and in vivo Hypoglycemic Activity of Novel 2,4-Thiazolidinedione Derivatives. European Journal of Experimental Biology 2012; 2(2); 343-353.