The Pharma Innovation Journal 2017; 6(12): 390-398

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(12): 390-398

www.thepharmajournal.com
Received: 29-10-2017
Accepted: 30-11-2017

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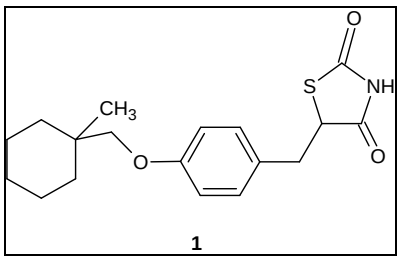
Design synthesis and evaluation of novel thiazolidinedione derivatives as antidiabetic agents

Kawade Dadasaheb, Nitin Jain, Jadhav Vijay and Girish Kashid

Abstract
A Series of thiazolidinedione derivatives were synthesized by reacting 5-(4-hydroxybenzylidene)-1,3-thiazolidine-2,4-one and 5-(4-hydroxy-3-methoxybenzylidene)-1,3-thiazolidine-2,4-one with aromatic N-substituted acetamide at room temperature. Synthesized compounds were characterised by IR, ^{1}H-NMR and mass spectroscopy and evaluated for antidiabetic activity in dexamethasone induced Wister albino mice animal model. Compound C and Compound D have exhibited promising hypoglycemic activity by comparing against Pioglitazone and metformin.

Keywords: Thiazolidinedione, acetamide, anti-hyperglycaemic, Dexamethasone induced diabetes

Introduction
Generally two type of diabetes has been seen in patient; one is insulin dependent diabetic mellitus, known as type-1 diabetes while another is Non-insulin dependent diabetes mellitus, known as type-2 diabetes. Type-2 diabetes is observed by insulin resistance and hyperglycemia [1]. Insulin resistance is considered to be the underlying mechanism in the pathogenesis of type 2 diabetes, which also leads to dyslipidemia, hypertension and obesity, termed together as metabolic syndrome [2, 3]. Treatment of type-2 diabetic mellitus including oral hypoglycemic agents; such as insulin and insulin analogues [4-5], sulfonylureas [6], glinides [7], biguanides [8, 9], glitazone [10] (Thiazolidinedione), α-glucosidase inhibitors [11]. Following the initial report of a novel antidiabetic agent ciglitazone [12] (1) the PPAR-γ agonists have emerged a new class of antidiabetic agents to treat type 2 diabetes. A new class of drugs Pioglitazone (2) & Rosiglitazone (3) called glitazones [12, 13] was approved by FDA for the treatment of type 2 diabetes [14, 15] Although their exact mechanism of action has not been completely elucidated, it has been demonstrated that TZDs elicit their pharmacological actions by binding and activating nuclear receptor PPAR-γ [16, 17] Recently, several compounds was reported to have both PPAR-α/γ dual agonistic activation. Among these compounds, KRP-297 (Kyorin/Merck) (4) [18, 19], Netoglitazone (Mitsubishi/J & J) (5) [20] and AZ-242 (Astra Zeneca) (6) [21] were comprised.



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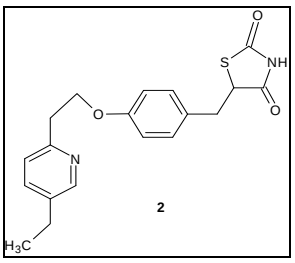

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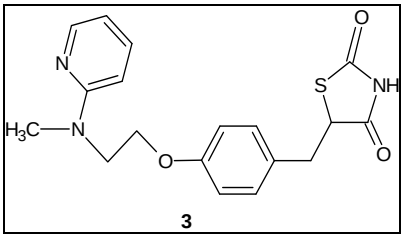

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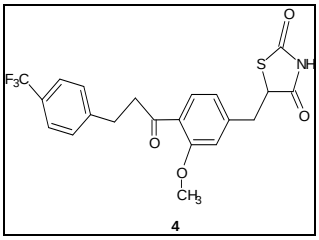

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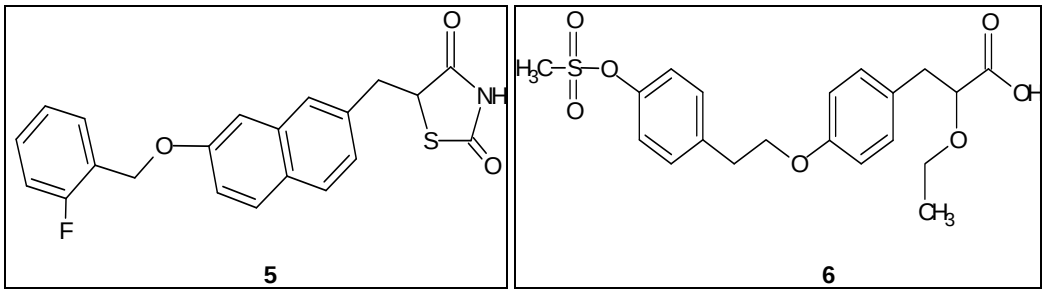


Thiazolidinediones (TZDs) are a group of pharmacological agents that enhance insulin action (insulin sensitizes) and promote glucose utilization in peripheral tissues hence act as antidiabetic agents. There were several thiazolidinedione derivatives act as antidiabetic agents [22].

Experimental section

Material and Methods

Chemicals: Chemicals used in the synthesis of the titled compounds were purchased from Thomas bekar and Research lab. They were Thiourea, Chloroacetic acid, conc.HCl, substituted aromatic aldehydes, Piperidine, chloroacetyl chloride, and substituted amine.

Instruments and Software: The melting points of the organic compounds were determined by open capillary in a heavy liquid paraffin bath. FT-IR spectra were recorded on Bruker spectrophotometer by using KBr pellets. ^{1}HNMR spectra were recorded on sophisticated multinuclear NMR Spectrometer Brucker Ascend 500 with TMS as an internal standard DMSO-d_6 used as Solvent and Mass spectra were recorded on Mass spectrometer of Bruker impact HD in Center for Instrumentation Faculty (CIF) Savitribai Phule Pune University, Pune, with acquisition Parameter such as source type ESI, ion polarity positive etc. Docking was performed on the Vlife MDS version: 4.6 of Vlife science, Pune.

Molecular Docking: [23]

Steps for Docking

Ligand preparation

Step 1: Ligands were Drawn in 2D and converted from 2D to 3D: From modules selecting the module VLife Engine, Further using the option Tools >> Converting 2D to 3D, convert the ligands. Specifying the folder where sdf file is saved along with folder to save converted ligands

Step 2: Ligand Optimization: From option Compute>> Batch Minimize, optimize the folder of ligands using Numerical type and RMS gradient 1, and lated using analytical type and RMS gradient 0.01/0.1 (as required).
The software was created folder to save the output.

Protein Preparation

Step 1: The PDB ID: 2PRG file was downloaded from rcsb.org and open the protein in Vlife MDS, Switch on the module>VLife engine and go to menu Tools and add hydrogen's.

Step 2: Output window was checked for any errors in the protein structure. Switch on module>biopredicta for correcting errors.

Step 3: Unwanted chains of the protein were removed by selecting the chain and delete form option edit>delete.

Step 4: The co-crystal ligand was identified and extracts the co-crystal from the option edit>extract and save the co-crystal ligand and protein in same coordinates.

Step 5: Other ligands and ions present in the structure were deleted from menu edit>delete.

Step 6: protein was Check for criss cross residues, & local geometry check form option Biopredicta tools.
For local geometry check: settings > bond length 20, bond angle 20 and bond length 10 %.

Step 7: protein was optimized using Compute>minimize>force field option.
Following is the protein structure after preparation (figure 1)

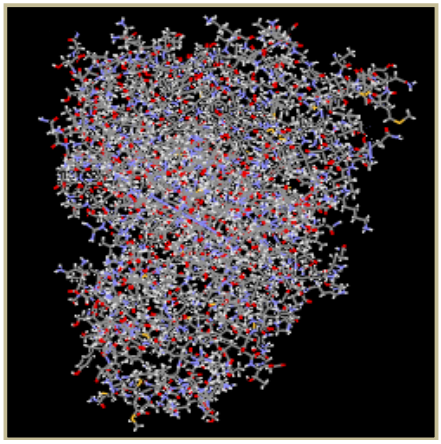


Fig 1: protein structure

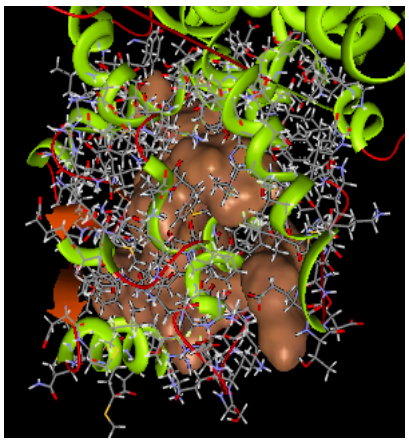


Fig 2: Cavity for docking

Step 1: Selecting option Biopredicta Tools>>Docking>>Grip

Step 2: Protein file was selected to be used for docking

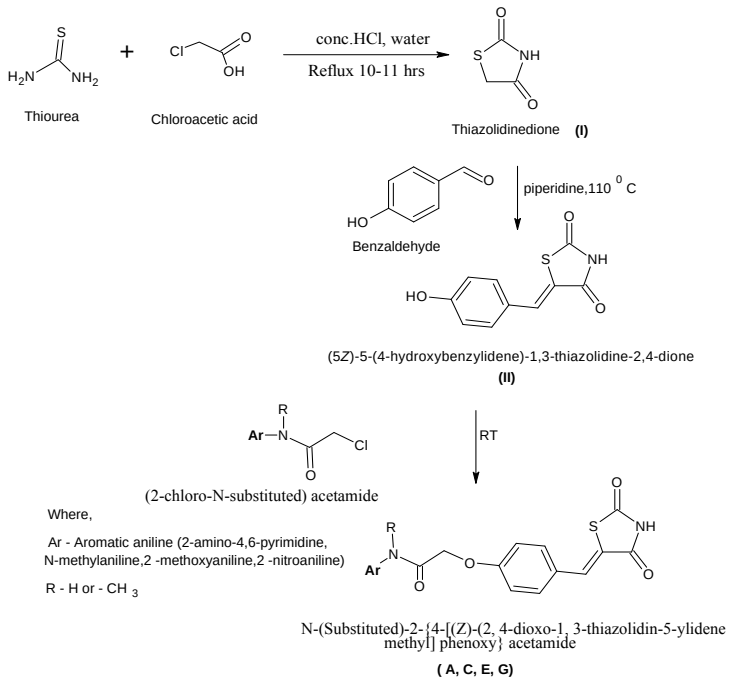
Step 3: Cavity was specified for docking either by selecting the pre saved co-crystal ligand or selecting the cavity number based on the residues of active site.
(Figure 2: cavity for docking)

Step 4: Selecting the folder of Ligands and the folder to save docking results.

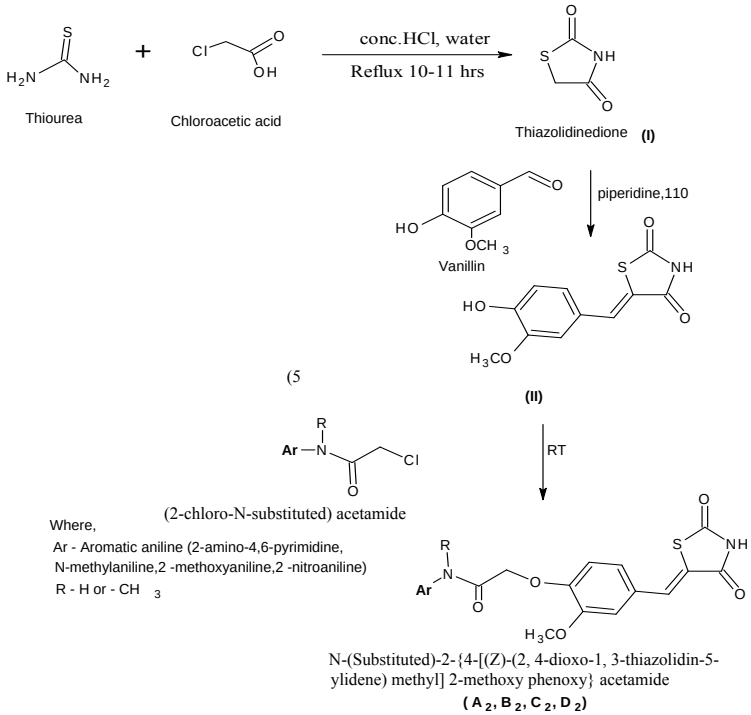
Step 5: Selecting docking parameters and click ok, check the docking job in Task manager.
After docking best dock score molecule was selected for Synthesis of new thiazolidin-2, 4-dione derivatives following by two schemes as below.

Scheme of Synthesis: [24, 25]

Scheme I



Scheme II



Synthesis of thiazolidin-2, 4-Dione (I)
Solution of Chloro acetic acid (5.64g, 0.06mol) in 6 ml of water was mixed with solution of thiourea (4.56g, 0.06mol) in 6 ml of water in a 100 ml RBF. The mixture was stirred for 15 minutes, the white solid separated out. To this solid, 6ml of concentrated HCl was added slowly from dropping funnel. The flask was connected to reflux condenser, and heated gently to effect complete solution after which the reaction mixture is stirred and refluxed for 8-10 hours at 100-110^{0}C. On cooling the contents of the flask, the cluster of colourless crystalline product was separated out. The product was filtered, washed with water to remove traces of HCl and dried. It was purified and recrystallized by hot water. Yield 85%, M.P. 123-125^{0}C. Rf: 0.43 (Benzene: Methanol 2:0.5), IR-3132.84 cm^{-1}, 2946.88 cm^{-1}, 1741.81 cm^{-1}, 1228.00 cm^{-1}, 612.93 cm^{-1}.

Synthesis of 5-(4-hydroxybenzylidene)-1, 3-thiazolidine-2,4-Dione (IIa)
In a 250ml round bottom flask, 4-Hydroxybenzaldehyde (5.12gm, 0.042 M) and 2, 4-thiazolidinedione (4.91g, 0.042 M) was together suspended in dry toluene (10 ml). 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 and dry ethanol. Yield 80%, M.P.296-298^{0}C. Rf: 0.38 (Benzene: Methanol 2:0.5), IR-3407.67 cm^{-1}, 3130.19 cm^{-1}, 3007.80 cm^{-1}, 2793.49 cm^{-1}.

Synthesis of 5-(4-hydroxy-3-methoxybenzylidene)-1, 3-thiazolidine-2, 4-dione (II b)
In a 250ml round bottom flask, 4-Hydroxy-3-Methoxybenzaldehyde (Vanillin) (10.55g, 0.042 M) and 2, 4-thiazolidinedione (4.91g, 0.042 M) was together suspended in dry toluene (20 ml). 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 and dry ethanol. Afford 86% of the product as a yellow powder. M.P. 210-212^{0}C, Rf = 0.35 (n-hexane/ethyl acetate = 2:1), IR- 3462.68 cm^{-1}, 3186.24 cm^{-1}, 3036.26 cm^{-1}, 1679.45 cm^{-1}

Synthesis of 2-chloro-*N*-(4, 6-dimethylpyrimidin-2-yl) acetamide (III a)
2-amino-4, 6-dimethylpyrimidine (4.99g, 0.025mol) in chloroform (20ml) was stirred in a conical flask and to this reaction mixture chloroacetyl chloride (4.23gm, 0.0375mol) was added drop wise under cold condition. Reaction mixture was stirred till completion of reaction, which was monitored by TLC. Yield 72.00%, M.P.40-42^{0}C, Rf: 0.36. (Benzene: methanol 2:0.5) IR- 3352.87 cm^{-1}, 1747.74 cm^{-1}, 1262.28 cm^{-1}, 787.68 cm^{-1}.

Synthesis of 2-chloro-*N*-methyl-*N*-phenylacetamide (III c)
Following the same procedure mention in step-III by using N-methyl aniline (5.35g, 0.025mol), Yield 45.58%, M.P. 66-68^{0}C. Rf: 0.38, IR- 3326.54 cm^{-1}, 3047.42 cm^{-1}, 2960.16 cm^{-1}, 1687.37 cm^{-1}, 784.46 cm^{-1}.

Synthesis of 2-chloro-*N*-(2-methoxyphenyl) acetamide (III e)
Following the same procedure mention in step-III by using 2-methoxyaniline (3.07g, 0.025mol), Yield 59.62%, M.P.40-42^{0}C, Rf: 0.42 IR-3404.19 cm^{-1}, 3181.69 cm^{-1}, 2922.46 cm^{-1}, 1725.44 cm^{-1}, 1242.96 cm^{-1}, 795.85 cm^{-1}.

Synthesis of 2-chloro-*N*-(2-nitrophenyl)acetamide (III g)
Following the same procedure mention in step-III by using 2-nitroaniline (3.45g, 0.025mol), Yield 64.80%, M.P.180-183^{0}C, Rf: 0.40 IR-3296.68 cm^{-1}, 3041.36 cm^{-1}, 2949.16 cm^{-1}, 1692.73 cm^{-1}, 1248.99 cm^{-1}, 786.18 cm^{-1}.

Synthesis of N-(4, 6-dimethylpyrimidin-2-yl)-2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] phenoxy} acetamide (A_1): [24]
5-(4-Hydroxybenzylidene)-2,4-thiazolidenedione (II a) (4.385g, 0.02mol) and potassium carbonate (1.68g, 0.03mol) in dimethyl sulfoxide (DMSO) was stirred in a flask and to this reaction mixture, 2-chloro-*N*-(3,5-dimethylphenyl) acetamide, (5.98g, 0.03mol) in DMSO was added. Reaction mixture was stirred at room temperature (4Hrs.) till the completion of reaction, which was monitored by TLC. After completion of reaction, water was added to get the solid, final product which was recrystallized using solvent ethanol. Yield 68%, M.P.188-190^{0}C, Rf: 0.40 (Benzene: methanol 2:0.5), IR (cm^{-1}): 3369.97 (N-H stretching of imide), 3296.11(N-H stretching of amide), 3171.84 (C-H stretching of aromatic ring), 1728.96 (C=O stretching of acetamide carbonyl), 1566.86-1585.60 (C=C stretching), 1332.80-1307.78 (C-O-C stretching). ^{1}HNMR (DMSO-d6): 2.512 (6H-singlet of methyl), 4.547(2H-singlet of methylene), 6.710-7.171 (4H-multiplet of Benzene), 6.968(1H-singlet of pyrimidine), 7.563(1H-singlet of methylene), 7.981(1H-singlet of sec.amide), 9.786 (1H-singlet of imide).

Synthesis of 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] phenoxy}-*N*-methyl-*N*-phenylacetamide (B_1): Following the same procedure as mention in step-IV by using 2-chloro-*N*-methyl-*N*-phenylacetamide (5.0gm, 0.03mol), Yield 64%, M.P.162-164^{0}C, Rf: 0.38 IR (cm^{-1}): 3380.78 (N-H stretching of imide), 3321.11 (N-H stretching of amide), 3063.20 (C-H stretching of aromatic ring), 1736.21 (C=O stretching of acetamide carbonyl), 1599.89-1510.75 (C=C stretching), 1379.34 (C-O-C stretching).

Synthesis of 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] phenoxy}-*N*-(2-metho xyphenyl) acetamide (C_1): Following the same procedure as mention in step-IV by using 2-chloro-*N*-(2-methoxyphenyl)acetamide (5.98gm, 0.03mol), Yield 74%,M.P.138-140^{0}C, Rf: 0.36, IR (cm^{-1}): 3370.11(N-H stretching of imide), 3175.83 (N-H stretching of amide), 3059.77 (C-H stretching of aromatic ring), 1742.91 (C=O stretching of acetamide carbonyl), 1593.29-1510.85 (C=C-stretching), 1379.34 (C-O-C stretching).

Synthesis of 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}-N-(2-nitrophe nyl) acetamide (D_1): Following the same procedure as mention in step-IV by using 2-chloro-*N*-(2-nitrophenyl) acetamide (7.21gm, 0.03mol) Yield 72%, M.P.142-144^{0}C, Rf:0.40,

IR (cm^{-1}): 3479.10 (N-H stretching of imide), 3351.47 (N-H stretching of amide), 3172.63 (C-H stretching of aromatic

ring), 1736.20 (C=O stretching of acetamide carbonyl), 1566.86-1585.60 (C=C stretching), 1332.80-1307.78 (C-O-C stretching).

^{1}HNMR (DMSO-d6): 4.547(2H-Singlet of methylene), 6.938-7.899(4H-multiplet of Benzene), 7.540(1H-singlet of ethylene), 7.986 (1H-singlet of sec.amide), 10.421(1H-singlet of imide).

Synthesis of *N*-(4, 6-dimethylpyrimidin-2-yl)-2-{4-[(2,4-dioxo-1,3thiazolidin-5-ylidene)methyl]-2-methoxyphenoxy}acetamide (A_2): [25]
5-(4-hydroxy-3-methoxybenzylidene)-1, 3-thiazolidine-2,4-dione (4.385g, 0.02mol) and potassium carbonate (1.68g, 0.03mol) in dimethyl sulfoxide (DMSO) was stirred in a flask and to this reaction mixture, 2-chloro-*N*-(3,5-dimethylphenyl) acetamide, (5.98g, 0.03mol) in DMSO was added. Reaction mixture was stirred at room temperature (4Hrs.) till the completion of reaction, which was monitored by TLC. After completion of reaction, water was added to get the solid, final product which was recrystallized using solvent ethanol. yield 72%%, M.P. 294-296^{0}C, Rf: 0.52 (Benzene: methanol 2:0.5), IR (cm^{-1}): 3369.89 (N-H stretching of imide), 3292.96 (N-H stretching of amide), 3014.75 (C-H stretching of aromatic ring), 1674.78 (C=O stretching of acetamide carbonyl), 1512.11 (C=C-stretching), 1368.67-1305.18 (C-O-C stretching).

Synthesis of 2-{4-[(2,4-dioxo-1,3-thiazolidin-5-ylidene) methyl] - 2- methoxyphen oxy}- *N* – methyl – *N* - phenyl acetamide (B_2): Following the same procedure as mention in step-IV by using 2-chloro-*N*-methyl-*N*-phenylacetamide (5.0gm, 0.03mol)**,** Yield 60%, M.P. 276-278^{0}C, Rf: 0.42, IR (cm^{-1}): 3462.68 (N-H stretching of imide), 3185.24 (N-H stretching of amide), 3036.26 (C-H stretching of aromatic ring), 1679.45 (C=O stretching of acetamide carbonyl), 1515.86 (C=C- stretching), 1339.25 (C-O-C stretching).

Synthesis of 2-{4-(2,4-dioxo-1, 3-thiazolidin-5-ylidene)methyl]-2-methoxypheoxy}-*N*-(2-methoxyphenyl) acetamide(C_2): Following the same procedure as mention in step-IV by using 2-chloro-*N*-(2-methoxyphenyl)acetamide (5.98gm, 0.03mol), Yield 75%, M.P. 294-296^{0}C, Rf: 0.68, IR (cm^{-1}): 3372.50 (N-H stretching of imide), 3317.40 (N-H stretching of amide) 3062.15 (C-H stretching of aromatic ring), 1682.00 (C=O stretching of acetamide carbonyl), 1595.93-1518.34 (C=C stretching), 1375.13 (C-O-C stretching).

Synthesis of 2 - {4-[(2, 4-dioxo-1, 3-thiazolidin-5-ylidene)methyl] – 2 - methoxyphenoxy}-*N*-(2-nitrophenyl)acetamide(D_2): Following the same procedure as mention in step-IV by using 2-chloro-*N*-(2-nitrophenyl)acetamide (7.21gm,Yield 70%, M.P. 320-322^{0}C, Rf: 0.68

IR (cm $^{-}$1): 3487.03 (N-H stretching of imide), 3372.68 (N-H stretching of amide), 3173.73 (C-H stretching of aromatic ring), 1683.45 (C=O stretching of acetamide carbonyl), 1582.21-1506.36 (C=C- stretching), 1377.28 (C-O-C stretching).

MS (ESI$^+$): 430.07[M+H]$^+$
Antidiabetic Activity: [26]
Model: Dexamethasone induced insulin resistance in mice [26]:

Chemicals: Dexamethasone sodium phosphate Injection (Agdex, Aglomed Ltd., Mumbai, India)

Apparatus: Johnson & Johnson one touch select glucose monitor with 10 strips glucometer.

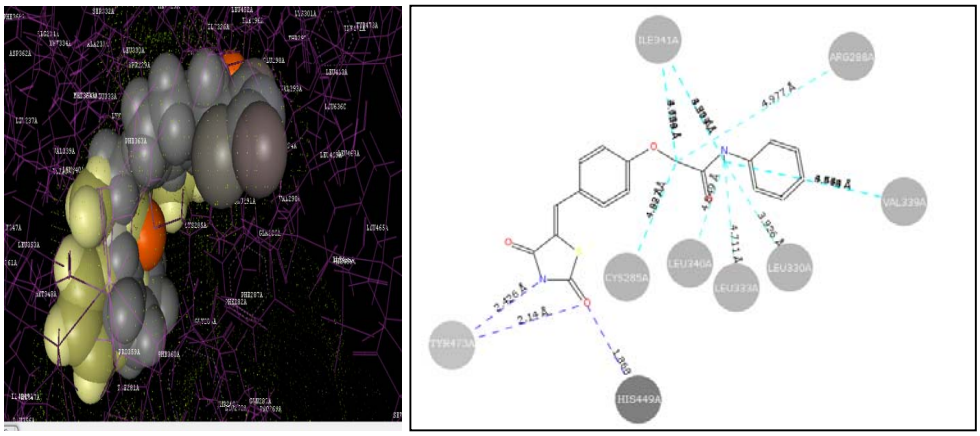
Animals and Treatment
Wister Albino mice of either sex weighing between 25-30 gm were selected for the study. Animals were housed in polypropylene cages and under standard condition of temperature (25 ± 5 ^{0}C), 12h/12h, light/dark cycles and were fed with standard mice pelleted diet and water was given *ad libitum*. All the study protocols related to hypoglycemic activity testing were approved from the Institutional Animal Ethics Committee. Ref. No. KBHSST/IAEC/2015-16/03 and CPCSEA-1566/PO/a/11/CPCSEA.

Experimental design
Sixty six mice were rendered hyperglycaemic by daily administration of a prestandardized dose of dexamethasone (1 mg=kg body wt., IM.) [27] for 7 consecutive days and then divided into Eleven groups of six each.
Group I- Dexamethasone control- Received 0.25% CMC p.o and Dexamethasone 1 mg/Kg IM.
Group II- Pioglitazone Treated- Received Pioglitazone 30 mg/Kg in 0.25% CMC p.o.
Group III-Metformin Treated- Received Metformin 30 mg/Kg in 0.25% CMC p.o.
Group IV- Received Sample A, 30 mg/Kg in 0.25% CMC p.o.
Group V- Received Sample B, 30 mg/Kg in 0.25% CMC p.o.
Group VI- Received Sample C, 30 mg/Kg in 0.25% CMC p.o.
Group VII- Received Sample D, 30 mg/Kg in 0.25% CMC p.o.
Group VIII- Received Sample E, 30 mg/Kg in 0.25% CMC p.o.
Group IX- Received Sample F, 30 mg/Kg in 0.25% CMC p.o.
Group X- Received Sample G, 30 mg/Kg in 0.25% CMC p.o.
Group XI- Received Sample H, 30 mg/Kg in 0.25% CMC p.o.
On 7th Day, after overnight fasting, blood samples were collected from all the animals by puncturing the retro orbital plexus under mild ether anaesthesia for 0hrs, 2hrs, 4hrs, 6hrs, & 12hrs time interval. After that plasma glucose level was estimated by using digital blood glucometer (Johnson & Johnson one touch select glucose monitor with 10 strips glucometer). Statistical analysis was analyzed using analysis of variance, and group means were compared with Dunnett's comparison one way ANOVA test by using prism graph pad software. The antidiabetic activities of the synthesized compounds are shown in Table 3.

Result and Discussion
1. Molecular Docking: A series of 8 compounds were designed and proposed for docking study to evaluate the selectivity and activity of the designed compounds as PPAR-γ agonist using grip docking on Vlife MDS version: 4.6 of Vlife science, Pune. Most of the prepared TZD derivatives revealed agonistic activity for PPAR-γ and not PPAR-α. All ligands after preparation were docked with 2PRG receptor. The structure of PPAR-γ Protein was downloaded from protein data bank (PDB ID-2PRG) http://www.rcsb.org/pdb, where residue were bonded more closely to rosiglitazone agonist co-crystallized to PPAR-γ. After completion of docking it was found that compound C and Compound D were highly consisted with chain length, and most active Compound was

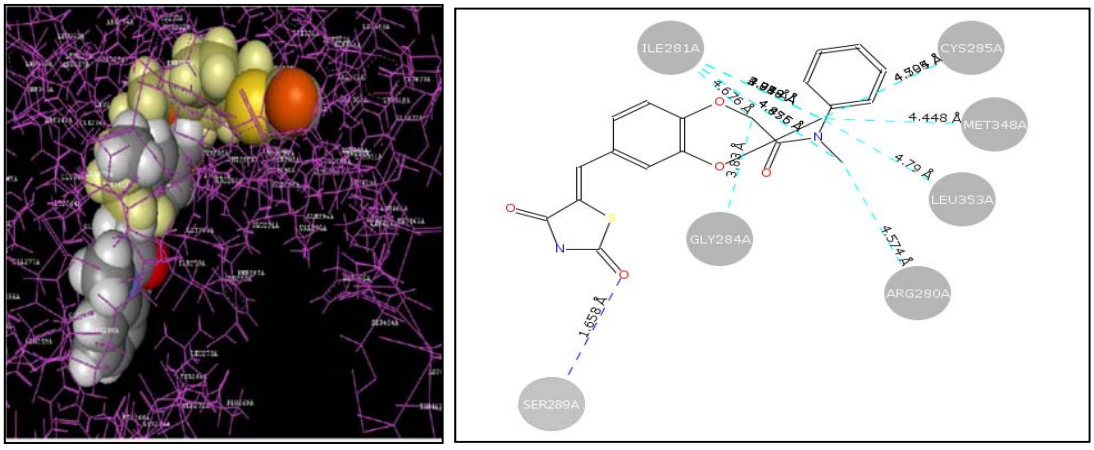
Compound C, suggesting that introduction of extra hydrophobic group may increase the contact with the receptor in the hydrophobic sub pocket of the active site of PPAR-γ (2PRG-Protien). Effect of substitution with methyl group at the acetamide nitrogen compound C is that having strong hydrophobic interaction with Leu340A, Leu333A, Leu330A, Val339A, & Ile341 which may increase the contact with the receptor in the hydrophobic sub pocket shown in figure-3(B), & subsequently increase activity. Importance of linker size was also examined. The chain length between the terminal nitrogen of amide and the oxygen atom adjacent to the benzene ring had also significant influence on activity. When the chain was lengthened to that of ethylene (n=2), with introducing carbonyl group at neighbouring carbon atom of terminal nitrogen was selected as best linker. Other happenings were introducing the methoxy group at 3rd position of aromatic center of benzene ring had significant influence on activity Compound D. These results suggest that optimal chain length is critical for controlling the conformation of the molecule and hence the way it binds to and activate PPAR-γ. We examined the Compound C & D having good score -60.272500 & -54.851932 with pioglitazone -55.739572.

We also examined the mode of binding of Compounds C to PPAR-γ shown in figure 3(A). Compound C was docked to the ligand binding domain (LBD) of PPAR-γ as identified in the crystal structure of PPAR-γ – rosiglitazone complex. Reported crystal structures have shown that two carbonyl groups of thiazolidinedione are co-ordinated by two histidines (His323, His449) and a tyrosine (Tyr 473) in the AF2 helix with roughly U-shaped conformation. Compound C occupies the hydrophobic pocket formed by residue Val339A, Leu330A, Leu333A, Cys285A, Ile341A etc. from which Val339A is matching, while acidic head having hydrogen bonding with His449 A & Tyr 473 with slightly U-shaped conformation. Figure 4 (A) indicate the mode of binding of compound D to PPAR-γ which was compare with co-crystal ligand of rosiglitazone. Rosiglitazone bind to PPAR- γ had strong interaction of acidic head group with histamine (His323, His449) and a tyrosine (Tyr 473) in roughly U-shaped conformation. Compound D had weak interaction of acidic head group and binding with serine (SER289A). Figure 4 (B) indicate interaction of compound D with various amino acids. Compound D had methoxy group at 3rd position of phenoxy ring increase the binding activity in to the hydrophobic sub pocket by interacting with ILE281A, CYS285A, MET348A & LEU353 while methyl group at acetamide nitrogen interact with ARG280A & ILE281A it indicate that in compound D methoxy group take participation in interaction with hydrophobic sub pocket was more than that of methyl group.



(A) **(B)**

Fig 3: (A) - mode of binding of Compounds C to PPAR-γ. **(B)** Compound C interaction with amino acid



(A) **(B)**

Fig 4: (A) - mode of binding of Compounds D to PPAR-γ. **(B)** Compound D interaction with amino acid

2. Prediction for Lipinski's Rule

Table 1: Property prediction by Lipinski's rule of five using Molinspiration software.

Compound code	Mol. wt	H-bond donors	H-bond acceptors	- log p	No. of violations	Dock score
A_1	384.40	2	8	1.82	0	-41.439904
A_2	414.43	2	9	1.41	0	-47.398258
B_1	368.40	1	6	2.59	0	-60.272500
B_2	398.43	1	7	2.18	0	-54.851932
C_1	384.40	2	7	2.33	0	-41.153022
C_2	414.43	2	8	3.04	0	-46.464608
D_1	399.37	2	10	1.84	0	-41.547182
D_2	429.40	2	9	2.25	0	-46.906676
Pioglitazone	356.42	1	5	3.07	0	-55.739572
Metformin	129.16	5	5	-1.13	0	-
Normal Range	<500	<5	<10	-0.4 to +5.6	0	-

Our approach was based initially around the preparation of compounds with calculated -Log P values close to that of Pioglitazone and led to the discovery of a series of new thiazolidinedione derivatives all compounds was showed optimum values, there were no violations in Lipinski's rule. Further all dock molecules which were obeyed the Lipinski's rule (table-1) which was acceptable for oral bioavailability and chosen for synthesis. In the present work Synthesis of four compounds of 2-{4-(2, 4-thiazolidinedione-5-ylidine)N-substituted phenoxy}phenyl acetamide derivatives and four compounds of novel 2-(4-((2,4-dioxothiazolidin-5-ylidene) methyl)-2-methoxyphenoxy)-N-substituted acetamide derivatives were synthesized and characterized as follows;

3. Characterization of synthesized compound

Final compounds were characterized using TLC. Determine the R*f* value, % yield and Melting Point by open capillary method of each comp. as shown in Table 2.

Table 2: Physicochemical & TLC data of the synthesized compounds (scheme I & II).

Comp.	Mol. Formula	Mol. Wt.	Melting point (^{0}C)	R_f Value	Yield %
A_1	$C_{18}H_{16}N_4O_4S$	384.409	188-190	0.40	68%
A_2	$C_{19}H_{18}N_4O_5S$	414.435	294-296	0.52	72%
B_1	$C_{19}H_{16}N_2O_4S$	368.406	162-164	0.38	64%
B_2	$C_{20}H_{18}N_2O_5S$	398.432	276-278	0.42	60%
C_1	$C_{19}H_{16}N_2O_5S$	384.405	138-140	0.36	74%
C_2	$C_{20}H_{18}N_2O_6S$	414.431	294-296	0.68	75%
D_1	$C_{18}H_{13}N_3O_6S$	399.377	142-144	0.40	72%
D_2	$C_{19}H_{15}N_3O_7S$	429.403	320-322	0.68	70%

(TLC Mobile Phase Benzene: Methanol 2:0.5)

5. Evaluation for Antidiabetic Activity

For antidiabetic activity there are various types of model to evaluate antidiabetic activity. *In-vivo* dexamethasone induced insulin resistance is good choice for evaluation of antidiabetic activity. The statistical significance, Dunett test using one ANOVA, & 95% confidence limits (95% CL) were calculated using Graph Pad Prism software. Group I for dexamethasone control, Group IV to Group XI was administered orally with synthesized compounds in 0.25 % CMC (30 mg/kg body weight) while Group-II with Pioglitazone (30 mg/kg body weight) and metformin (30 mg/kg body weight) in 0.25 % CMC respectively. The blood glucose level was monitored at different interval of time 0, 2, 4, 6, and 12 hrs respectively. The decrease in blood glucose level against each compounds are shown in following table-3:

Table 3: Antidiabetic activity of synthetic Compound.

Compound	Blood glucose level mg/dl (Mean ± SEM)				
	0 hour	2hour	4hour	6hour	12hour
Control	339.4±2.055	337.2±2.585	337.2±2.585	334.1±4.232	330.1±3.395
Pioglitazone	320.2±9.056*	164±0.9397****	150.2±1.874****	132.8±1.466****	120.2±1.249****
Metformin	311.7±3.398*	171.1±2.764****	157±1.342****	137.6±1.655****	122.7±1.329****
Sample A	329.5±5.78	293.1±3.383**	285.8±1.857***	241.2±2.183***	197.9±2.936***
Sample B	321.9±3.362	285.3±2.465***	275.9±2.061***	262.3±1.241***	183.8±2.261***
Sample C	312.6±1.713***	218.2±1.857****	156.3±4.148****	136.4±2.303****	113.7±1.067****
Sample D	318.4±0.7973**	240.6±2.113****	196.8±2.221****	150.7±2.43****	129.9±1.744****
Sample E	325.5±2.27	282.3±2.875***	275.3±3.013****	177.7±2.658****	154.1±3.311****
Sample F	320.2±2.447	307.9±1.846***	281.6±1.231***	261.6±1.893***	193.9±2.245***
Sample G	322.3±3.394	311.3±4.136***	296.7±2.164***	274.7±1.702***	201.6±2.23***
Sample H	326±2.342	303.6±3.881**	291.5±2.55***	281.1±1.043***	205.3±2.461***

Where (n=6); **** P<0.0001*** P<0.001; **P<0.01; *P<0.05

Blood glucose level after 0hrs, 2hrs, 4hrs, 6hrs, and 12hrs Administration of samples was described that the sample C more significant than pioglitazone and metformin, while sample D was moderately decrease in blood glucose level. All

samples after 12 hrs exhibits P value 0.0001 means all Samples were significantly changes in blood glucose level when comparable with diabetic control.

Conclusion

We conclude from the result & discussion, that

- From the molecular docking studies it was concluded that the compound C had better PPAR-γ agonist activity.
- Synthesis of four compounds of 2-{4-(2,4-thiazolidinedione-5-ylidine)N-substituted phenoxy}phenyl acetamide derivatives and four compounds of novel 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)-2-methoxyphenoxy)-N-substituted acetamide derivatives were synthesized and confirm the structures of the synthesized compounds by IR, ^{1}H-NMR and Mass.
- Dexamethasone induced hyperglycaemic model shows that compound C having significant activity than pioglitazone at 30mg/kg body weight. All compounds were screened by one way ANNOVA following Dunnett's multiple comparison; it was shown all compounds were statistically significant have P value < 0.0001 after 12 hr.

Acknowledgements: We are grateful to the Principal of KBHSST'S College of pharmacy, Malegaon for their guidance and providing facilities of animal activity for my research work. We also thankful of Analyst of CIF of Savitribai Phule Pune University, Pune to provide me facilities of NMR and Mass.

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