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MOLECULAR DOCKING STUDIES &EVALUATING ANTI-DIABETIC ACTIVITY OF THIAZOLIDINEDIONES DERIVATES

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ABSTRACT

Currently available therapies for type 2 diabetes include insulin and various oral agents such as sulfonylureas, metformin, acarbose, and more recently pioglitazone. Each of these agents suffers from generally inadequate efficacy (as monotherapy) and a number of serious adverse effects such as hypoglycemia, GI disturbances and cardiovascular disorders. As a consequence, there continues to be a high demand for new oral antidiabetic drugs devoid of these shortcomings. Novel Thiazolidinediones derivatives was synthesized purified, characterized and subjected to appropriate screening for acute oral toxicity and hypoglycemic activity in Albino mice and Wistar rats respectively. All computational experiments were performed on computer having genuine Intel Pentium Dual Core Processor and Windows XP operating system using the VLifeMDS software package of VLife Technologies Pvt. Ltd. Pune (www.vlifesciences.com). (version 4.3).All the chemicals were of laboratory reagent grade and were obtained from Thomas Baker, Loba Chemie and Sigma-Aldrich. Melting points were taken in one end sealed glass capillary using Omega melting point apparatus. Analytical thin-layer chromatography was performed on 60F254 Precoated silica gel plates (Merck) to establish identity of reactants and products monitored in between reactions as well at the end for completion of reaction. The spots were visualized in UV chamber or by iodine vapors in an enclosed chamber. The solvent system used for Thin-Layer Chromatography was Chloroform: Methanol (5:5) Infra-Red spectra of compounds were recorded using KBr disc method on a shimadzu 1000 FTIR spectrometer in the range of 4000-200 cm^{-1}Proton (^1H) Nuclear Magnetic Resonance spectra of compounds were recorded on Bruker Avance II 400 NMR Spectrophotometer using DMSO solvent, at SAIF, Punjab University, Chandigarh. Mass spectra of compounds were recorded on DMSO, Q-TOF MICROMASS (LC-MS) at SAIF, Punjab University, Chandigarh. Based on the favorable Antidiabetic activity of thiazolidinedione derivatives, it could be concluded that this hydrophobic interaction played an important effect in the activity. The end amino cation of PHE287A was also formed a π-cation interaction with the triazole ring of the compound MCR-013-14/15 (distance: 4.56 A^0), which enhanced the binding action between receptor PPAR-γ and the ligand compound MCR-013-14/15. The compounds MCR-011 and MCR-014 showed highest percentage decrease of blood glucose level at the dose of $1/10^{th}$ that of LD_{50} among the evaluated compounds compared to control**.** Acute toxicity study was done for determining LD_{50}. The LD_{50} was found to be 2000, 1098, 1098,305 mg/Kg for synthesized compounds. Two doses were selected for the hypoglycemic evaluation of the compounds. The compounds MCR-011 and MCR-014 showed highest percentage decrease of blood glucose level at the dose of $1/10^{th}$ that of LD_{50} among the evaluated compounds compared to control. The analysis of structural features revealed that substitution of thiadiazole group enhanced the hypoglycemic potential of the synthesized compounds.

Key Words: Thiadiazole, MCR-011, Triazole.

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INTRODUCTION

Diabetes Mellitus is a metabolic disorder due to sedentary lifestyle, smoking, obesity, high blood pressure and others. The forecast is grim as diabetes related deaths around the world, would increase many fold.

Thiazolidinediones have come in existence since 1982. Though they are effective therapeutic agents for diabetes, they suffer from one or more deficiencies; some of them have been even withdrawn from market. So there is a challenge to develop novel Thiazolidinediones derivatives. Novel thiazolidinedione synthesized having less side effects. Our extensive literature review reveals that thiazolidinediones (TZDs) have been the focus of recent studies to assess their potential as antidiabetic, antibacterial, anticancer, anti-inflammatory agents (Labouta IM *et al*., 1987; Goes AJS *et al*., 1991; DeLima MCA *et al*., 1992; Cantello BCC 1994; Sushil DP, 2014). Present work we synthesize novel Thiazolidinediones incorporating the required elements of a suitable pharmacophore.

MATERIALS AND METHODS

All computational experiments were performed on computer having genuine Intel Pentium Dual Core Processor and Windows XP operating system using the VLifeMDS software package of VLife Technologies Pvt. Ltd. Pune (www.vlifesciences.com). (version 4.3).

All the chemicals were of laboratory reagent grade and were obtained from Thomas Baker, Loba Chemie and Sigma-Aldrich. Melting points were taken in one end sealed glass capillary using Omega melting point apparatus. Analytical thin-layer chromatography was performed on 60F254 Precoated silica gel plates (Merck) to establish identity of reactants and products monitored in between reactions as well at the end for completion of reaction. The spots were visualized in UV chamber or by iodine vapors in an enclosed chamber. The solvent system used for Thin-Layer Chromatography was Chloroform: Methanol (5:5) Infra-Red spectra of compounds were recorded using KBr disc method on a shimadzu 1000 FTIR spectrometer in the range of 4000-200 cm^{-1}Proton (^1H) Nuclear Magnetic Resonance spectra of compounds were recorded on Bruker Avance II 400 NMR Spectrophotometer using DMSO solvent, at SAIF, Punjab University, Chandigarh. Mass spectra of compounds were recorded on DMSO, Q-TOF MICROMASS (LC-MS) at SAIF, Punjab University, Chandigarh Hypoglycemic study has been done using the animal model already reported in the literature. The animal model being used is Sucrose Loaded Model (Godkar PB and Godkar DP, 2003), though other models are available (Chaturvedi D *et al*., 2008; Sharma PK *et al*., 2010; Ram VJ *et al*., 2003), blood glucose estimation has been done using a glucometer (Pattan SR *et al*., 2009).

The synthetic protocol of thiazolidinedione derivatives presented here is shown in Scheme (Fig. 1).

Experimental

Step I: Synthesis of 2, 4 thiazolidinedione (Prashantha KBR *et al*., 2006; Sushil DP *et al.,* 2015)

A mixture was prepared containing Chloracetic Acid **(**0.6mol**)** in 60ml of water and Thiourea **(**0.6mol**)** in 60ml of water. Above mixture was stirred for 15min to form white precipitate, accompanied by considerable cooling. To this mixture then added slowly 60ml of concentrated HCL drop wise, the flask was then connected with reflux condenser and gentle heat applied to effect complete solution, after which the reaction mixture was stirred and refluxed for 10-15hrs at 100-110^{0}C. Upon cooling the content of the flask were solidified to a cluster of white needles. The product was filtered and washed with cold water to remove traces of hydrochloric acid and dried.It was purified by recrystallization from ethyl alcohol. The yield was 80%.

Step II: Synthesis of 5-Substituted 2,4-thiazolidinediones

In 250ml three necked round bottom flask provided with a Dean-Stark apparatus, a substituted benzaldehyde (0.188mol) and 2,4-thiazolidinedione (0.188mol) were together suspended in toluene. To this a catalytic amount of pyridine (1ml) was added. The mixture was stirred and refluxed. After the complete removal of water and when the temperature reached above 110^{0}C the reaction mixture was stirred for further 6-7 hrs. On cooling, the product precipitated out from toluene. The product was filtered and washed with cold dry ethanol. The yield was 83-90%.

Step III: Synthesis of Ethyl 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)acetate

The product of step II (1 mol) was suspended in dry acetone and to this anhydrous K_2CO_3 (1 mol)was added. To this mixture, ethyl-2-chloroacetate (1 mol) was then added dropwise. This was then stirred for 35-40 hrs at room temperature and reaction was monitored by TLC. The product was then filtered and washed with water. It was purified by recrystallization from ethanol. The yield was 95 % and 81 % respectively.

Synthesis of 5-(4-((5-amino-1,3,4-thiazole-2-yl)methoxy) benzaldehyde)thiazolidine-2,4-dione(MCR-011-14/15)

Mixture of 2.34g (0.01mol)of Ethyl 2-(4-((2,4-dioxothiazolidin-5ylidene)methyl)phenoxy)acetate product of step III and 0.91g (0.01 mol) thiosemicarbazide in concentrated sulphuric acid (10 ml) were taken and heated in water bath for 1h, cooled, poured in 25 g of crushed ice. Product which separated was collected under pressure by suction pump and dried. It was then recrystallized from ethanol to yield crystals.

Synthesis of 5-(4-((5-amino-4H-1,2,4-triazol-3-yl)methoxy)benzylidene)thiazolidine-2,4-dione(MCR-012-14/15)

Mixture of 2.34g (0.01mol) of Ethyl 2-(4-((2,4-dioxothiazolidin-ylidene)methyl)phenoxy)acetate product

of step III and 0.91g (0.01 mol) thiosemicarbazide in concentrated sodium hydroxide 10% (10 ml) ,ammonia were taken and heated in water bath for 1h, cooled, poured in 25 g of crushed ice. Product which separated was collected under pressure by suction pump and dried. It was then recrystallized from ethanol to yield crystals.

Synthesis of N-(5-((4-((2,4-dioxothiazolidin-5-ylidene) methyl)phenoxy)methyl)-4H-1,2,4-triazol-3-yl)benzamide(MCR-013-14/15)

1g (0.003mol) of MCR-012-14/15 was dissolved in 20 ml of 10% NaOH solution. 3 g (0.021mol) of benzoyl chloride was added drop wise with vigorous shaking and alkaline pH was maintained. While shaking product was separated rapidly, which was collected, dried, recrystallized by ethanol to yield crystals.

Synthesis of N-(5-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)-1,3,4-thiadiazol-2-yl)benzamide(MCR-014-14/15)

1g (0.003mol) of MCR-011-14/15 was dissolved in 20 ml of 10% NaOH solution. 3 g (0.021mol) of benzoyl chloride was added drop wise with vigorous shaking and alkaline pH was maintained. While shaking product was separated rapidly, which was collected, dried, recrystallized by ethanol to yield crystals.

5-(4-((5-amino-1,3,4-thiazole-2-yl)methoxy)benzaldehyde)thiazolidine-2,4-dione
Percentage Yield (%)73.20, Rf value0.6, ketone (C=O) 1703 cm^{-1}, alkene (-C=C-) 1529, disubstituted benzene ring at 754 cm^{-1}, imine C=N 1633 cm^{-1}, primary amines (-NH_2) 3439cm^{-1},secondary amine (N-H) 3410, ether 1114 and S=C=O 1732 cm^{-1}.

5-(4-((5-amino-4H-1,2,4-triazol-3-yl)methoxy)benzylidene)thiazolidine-2,4-dione,
Percentage Yield (%)83, Rf value0.7 , ketone (C=O) 1732 cm^{-1}, alkene (-C=C-) 1512, disubstituted benzene ring 758 cm^{-1}, imine C= 1660 cm^{-1}, primary amines (-NH_2) 3452cm^{-1},two signals for secondary amine (N-H) at 3334 and 3352 cm^{-1}, ether 1120,C-N 1319 $cm^{-1,}$ C=N 1660 cm^{-1}.

N-(5-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)-4H-1,2,4-triazol-3-yl)benzamide Percentage Yield (%)83.33, Rf value0.8, (C=O) 1732 cm^{-1}, alkene (-C=C-) 1647, disubstituted benzene ring at 804 cm^{-1}, imine C=N 1685 cm^{-1},three signals for secondary amine (N-H) at 3336,3361 and 3325 respectively, ether 1128,monosubstituted benzene 707 cm^{-1} .

N-(5-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)-1,3,4-thiadiazol-2-yl)benzamide Percentage Yield (%)89.62, Rf value0.6, ketone (C=O) 1722 cm^{-1}, disubstituted benzene ring 734 cm^{-1}, imine C=N shows at 1683 cm^{-1}, secondary amine (N-H) 3317 and 3360 repectively,ether 1074 and -S-C 1722 cm^{-1}.

^{1}H-NMR spectrum of the compound MCR-011-14/15, Ha, Hb, Hc, Hd of benzene ring shows doublet at δ 7.1, δ 7.1, δ 7.62, δ 7.62, respectively, Primary amine shows singlet at δ 7.00, Hf singlet δ 8.06, Hg amino group singlet δ 3.14.

^{1}H-NMR spectrum of the compound MCR-012-14/15, Ha, Hb, Hc, Hd of benzene ring doublet δ 6.83, δ 6.83, δ 7.55, δ 7.55, respectively, Primary amine shows singlet δ 6.83, Hf singlet at δ 7.96, Hg amino singlet δ 3.66.

The ^{1}H-NMR spectrum of the compound MCR-013-14/15, Ha, Hb, Hc, Hd of disubstituted benzene ring doublet δ 6.83, δ 6.83, δ 7.55, δ 7.55, respectively, He, Hf, Hg, Hh of monosubstituted benzene ring doublet δ 8.03, δ 8.03, δ 7.61, δ 7.61, respectively, Hi singlet at δ 8.02,Hj,Hk of secondary amines singlet δ 8.14 and δ 12.07.

^{1}H-NMR spectrum of the compound MCR-013-14/15, Ha, Hb, Hc, Hd of disubstituted benzene ring doublet at δ 6.60, δ 6.60, δ 7.57, δ 7.57, respectively, He, Hf, Hg, Hh of monosubstituted benzene ring doublet δ 8.00, δ 8.00, δ 7.55, δ 7.55, respectively, Hi singlet δ 7.93,Hj of secondary amine singlet δ 8.83.

PHARMACOLOGICAL EVALUATION

Acute oral toxicity studies of synthesized compounds

ACUTE ORAL TOXICITY TEST

OECD guidelines (no.A25) were followed for toxicity studies. Acute oral toxicity study for mice were carried out for determining median lethal dose (LD_{50}). Animals were dose two at a time at a minimum of 48 hours interval doses were selected from sequence 2000,550, 175,55,17.5,5.5,1.75 mg/kg with 5 animals per group. Each animal were observed carefully for the signs of toxicity as well as mortality for first 30 minutes after dosing and then occasionally further for 4 hours and daily thereafter for the period of 14 days. The number of mice dying during 48 hours periods was recorded. The test compounds were administered orally during acute oral toxicity testing.

In vivo estimation of Blood glucose level

Oral glucose tolerance test (OGTT)

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee (IAEC), and all experiments were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy wistar rats of both sexes weighing 200 to 250 gm included in this study. Animals were randomly

divided into four groups, six animals in each group. This test was performed as per reference.

RESULT AND DISCUSSION

Docking studies on synthesized Thiazolidinedione derivatives

Hardware and Software

All Docking studies and conformational analysis were performed using the Molecular Design Suite (VLife MDS software package, version 4.3;from VLife Sciences, Pune, India)

Structure Confirmation Generation

Structure of compounds was sketched using the 2D structure draw application VLife2Ddraw and converted to 3D structures. All the structures were minimized and optimized with the AMBER method taking the root mean square gradient (RMS) of 0.01 kcal/mol A^0 and the retention limit to 10,000. Confirmers for each structure were generated using Monte Carlo be applying AMBER force field method and least energy confirmer was selected for further study.

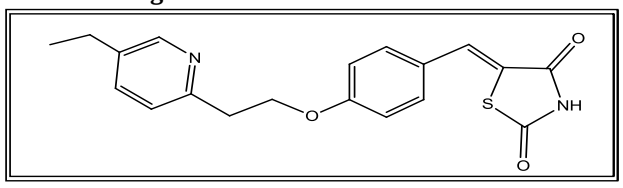
Docking methodology

Docking study was performed on VlifeMDS version 4.2 on Lenovo computer, i3 processor with XP operating system. The Grip ligand docking with approximated a systematic search of positions, orientations, and conformations of the ligand in the enzyme binding pocket via a series of hierarchical filters..

Docking studies

The compounds evaluated in silico (docking) to distinguish their hypothetical binding mode using the X-ray crystal structure of thiazolidinedione [PDB ID: 2PRG]. To pre-asses the anti-diabetic behaviour of designed ligands on structural basis, docking studies were carried out and scoring functions, their binding affinities and orientation of designed compounds having blocking property of active site of the receptor.

Standard Pioglitazone



General structure of Pioglitazone

The dock score of Pioglitazone is -61. It shows the following interaction with residues of receptor at active site cavity 1.

Docking studies of Synthesised Thiazolidinedione Derivatives.

The compounds were evaluated in *silico* (docking) to distinguish there hypothetical binding mode using the X-ray crystal structure of Peroxisome proliferative activated gamma receptor 2PRG protein. To pre-asses the anti-diabetic behaviour of designed ligands on structural basis, docking studies were carried out and scoring functions, their binding affinities and orientation of designed compounds having blocking property of active site of the receptor. The protein-ligand complex was constructed based on the X-ray crystallized structure of receptor. The designed compounds built using Vlife2Ddraw converted into 3D structure and energy minimized by using Merck Molecular Force Field (MMFF). Conformers were generated by using Monte Carlo conformational search ring flip method.

Molecules which show minimum dock score shows more affinity for synthesized compounds inhibition and Dock score shown in Table 1.

Docking Interactions.(Interaction of ligands with receptor)

Above: 3Dmodel shows interaction of Pioglitazone, MCR-013-14/15 with their respective RXR binding site. The H-bond (green lines) is displayed as dotted lines, and the π-cation interaction is shown as yellow lines and the hydrophobic interaction is shown as dotted blue lines.

2D model shows interaction between compound Pioglitazone, MCR-013-14/15 with their RXR binding site. The H-bond (dark blue arrows) is displayed as dotted arrows. And the π-cation interaction is shown as pink lines and hydrophobic bond is shown as faint blue lines.

In the binding model, compound MCR-013-14/15 was nicely bound to the PPAR-γ with its ethyl –group of A287 (PHE 287), forming a more optimal hydrophobic interaction (distance: = 3.99A^0). Based on the favourable Antidiabetic activity of thiazolidinedione derivatives, it could be concluded that this Hydrophobic interaction played an important effect in the activity. The end amino cation of PHE287A was also formed a π-cation interaction with the triazole ring of the compound MCR-013-14/15 (distance: 4.56 A^0), which enhanced the binding action between receptor PPAR-γ and the ligand compound MCR-013-14/15.

NMR Spectra of compound MCR-014-14/15- N-(5-((4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)methyl)-1,3,4-thiadiazol-2- yl)benzamide

Pharmacological Evaluation

Acute oral toxicity testing (OECD guidelines, 2000)

The main test consists of a single ordered dose progression in which animals are dosed, one at a time, at a minimum of 48 hr intervals. The acute toxicity test was performed for four compounds, to ascertain the LD_{50} values as per OECD guidelines. The experimental dose was selected between

the minimum effective dose and maximal non-lethal dose. A total of 28 Swiss albino mice were required for this study. Thus the LD_{50} was found to be2000, 1098, 1098 and 305 mg/kg for MCR-011 to MCR-014 respectively.

Oral glucose tolerance test (OGTT)

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee (IAEC), and all experiments were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Table 1. Chemical structure and dock scores TZD

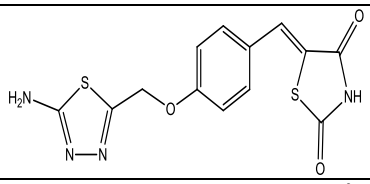
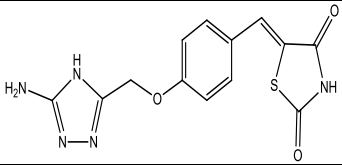
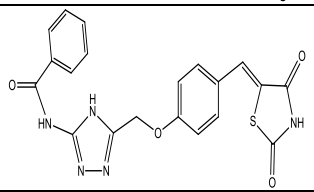
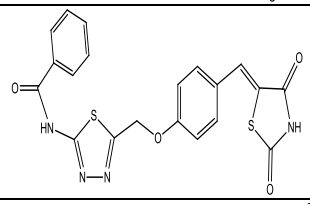
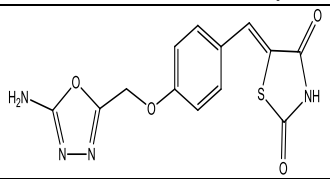
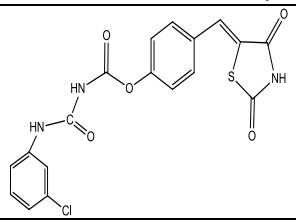
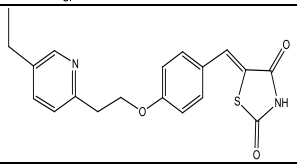
Compound code	Chemical structures of molecules	Dock Score
MCR-011-14/15		-62.586
MCR-012-14/15		-64.445
MCR-013-14/15		-71.561
MCR-014-14/15		-66.33
TZD-E		-67.60
TZD-F		-64.33
PIOGLITAZONE		-61.97

Table 2. Novel Synthesized Compounds Screened for Acute Oral Toxicity

Sr.no	Compound Code	LD_{50}
1	MCR-011-14/15	2000
2	MCR-012-14/15	1098

3	MCR-013-14/15	1098
4	MCR-014-14/15	305

Table 3. Hypoglycemic effects of synthesized test compounds

Treatment	Blood Glucose Level (mg/dl)		
	30min	60min	120min
Pioglitazone	142±3.44	96.25±6.98	90.25±1.31
MCR-011-14/15	136±1.95	113.5±2.21	102±1.58
MCR-012-14/15	131±1.68	117.5±1.70	107.75±3.01
MCR-013-14/15	133±1.58	111.25±1.10	98.5±1.04
MCR-014-14/15	138.75±1.49	120.25±2.13	110.5±2.59
Glucose control	150.5±14.26	149±0.70	131±2.17
Saline control	119±2.27	116.5±2.39	114±2.16

Values are expressed as mean ± S.E.M

Table 4. Effect of test compounds on % decrease in blood glucose level

Treatment	% Decrease in Blood Glucose Level (mg/dl)		
	30min	60min	120min
Pioglitazone	136.1	122.07	111.94
MCR-011-14/15	124.2	103.19	93.15
MCR-012-14/15	114.97	77.26	73.07
MCR-013-14/15	122.29	102.29	90.57
MCR-014-14/15	125	108.33	99.54
Glucose control	115.85	111.38	108.53
Saline control	100.21	98.1	96

Fig 1. Scheme design for synthesis of thiazolidinedione derivatives

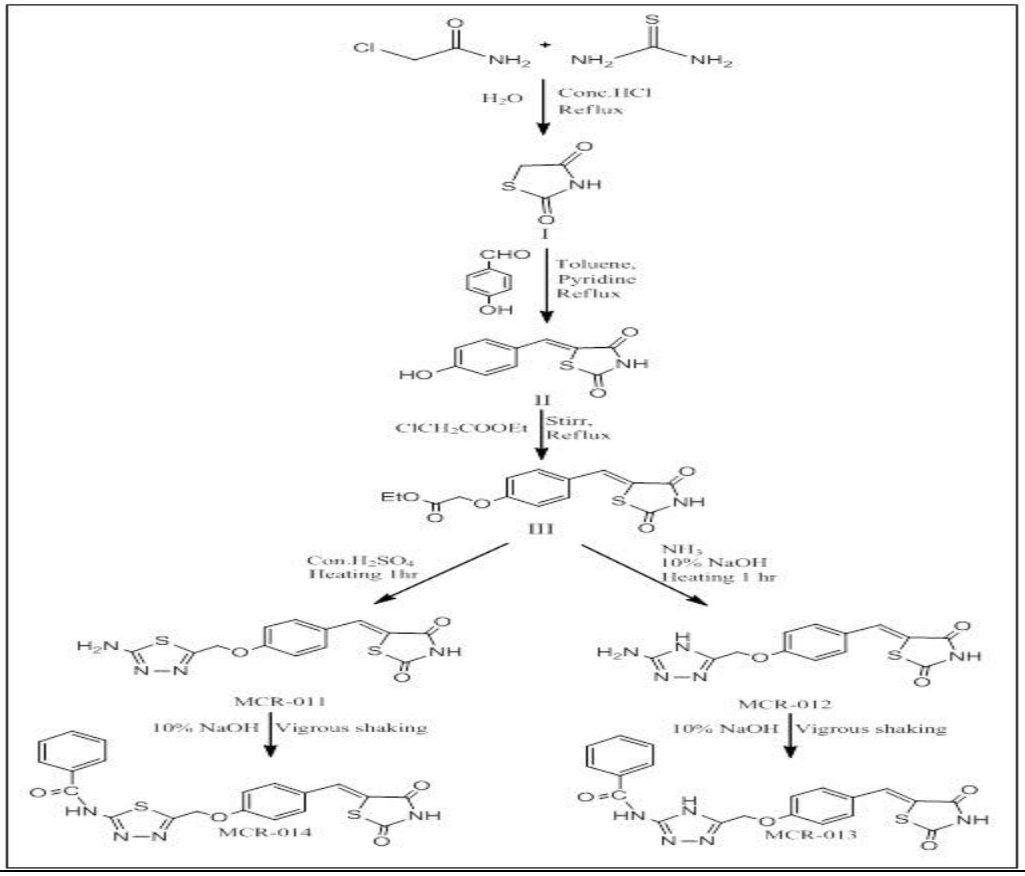


Fig 2. Hydrogen bonding and Pi-stackingof Pioglitazone with active receptor

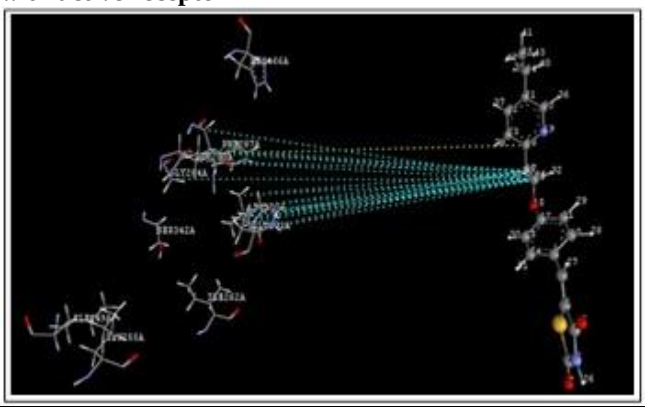


Fig 3. Active site of 2PRG receptor cavity

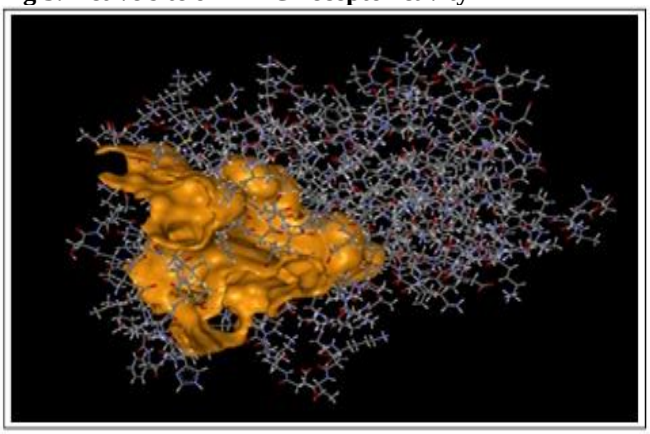


Fig 4. 3D view –Molecular docking modelling of Parent compound Pioglitazone and newly designed compound MCR-013-14/15 with PPAR-γ: for clarity, only interacting residues are displayed.

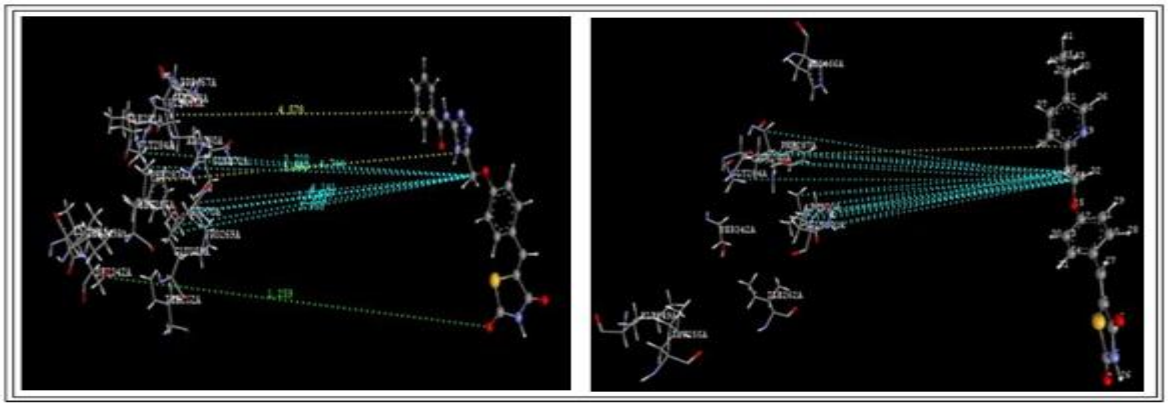
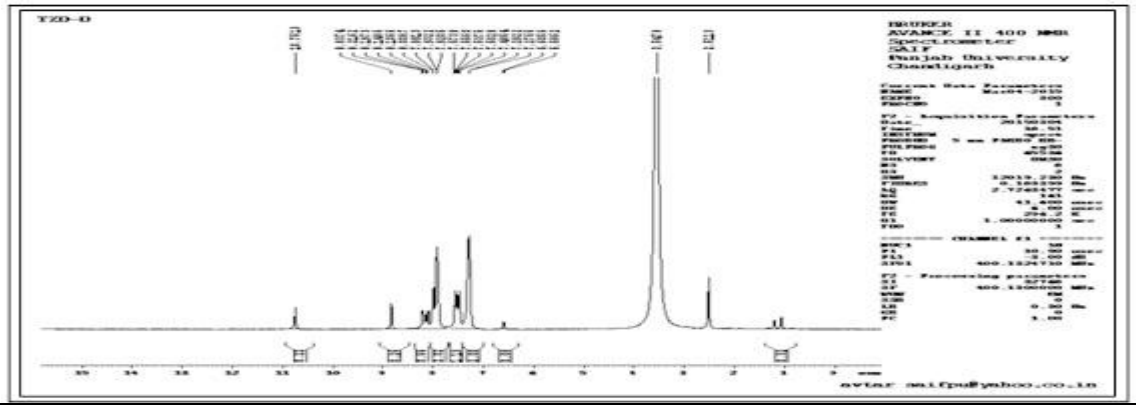


Fig 5. ^{1}H NMR Spectra of Compound MCR-014-14/15



CONCLUSION

Acute toxicity study was done for determining LD_{50}. The LD_{50} was found to be 2000, 1098, 1098,305 mg/Kg for synthesized compounds. Two doses were selected for the hypoglycemic evaluation of the compounds. The compounds MCR-011 and MCR-014 showed highest percentage decrease of blood glucose level at the dose of $1/10^{th}$ that of LD_{50} among the evaluated compounds compared to control. The analysis of structural features revealed that substitution of thiadiazole group enhanced the hypoglycemic potential of the synthesized compounds.

ACKNOWLEDGEMENT

None

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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