

Research Article

Synthesis, Biological Screening and *in Silico* Studies of Chalcone Based Novel Phenyl Urea Derivatives as Potential Antihyperglycemics

Satarupa Acharjee Sengupta¹, Tapan. K. Maity^{1*}, Subir Samanta²

¹Department of Pharmaceutical Technology, Synthetic and Natural Product Research Laboratory, Jadavpur University, Jadavpur, Kolkata-700032, West Bengal, India.

² Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India.



DOI:10.18579/jprkc/2017/16/3/118765

Received on: 10/07/2017

Revised on: 08/08/2017

Accepted on: 22/08/2017

Corresponding author:

TK Maity

Department of Pharmaceutical Technology,
Synthetic and Natural Product Research Laboratory, Jadavpur University,
Jadavpur, Kolkata, 700032,
West Bengal, India.
jutkmaity@gmail.com

ABSTRACT

Aim: Current work was aimed to explore the effect of synthesized novel chalcone based 1-(4"-methoxyphenyl)-3-{4-[(2E)-3-phenylprop-2-enoyl] phenyl} urea derivatives, on adult male Wistar rats in an experimental model of type 2 diabetes.

Methods: Type 2 Diabetes was induced in overnight fasted rats by a high fat diet followed by a single intraperitoneal injection of low dose streptozotocin (35mg/kg body weight) leading to hyperglycemia and considerable augmentation in blood urea nitrogen and creatinine levels. Treatment with compounds 2(a-d) and 3(a-c) (dose; 30mg/kg) continued for 28 days followed by oral glucose tolerance test. Docking studies of synthesized compounds with ligand binding domain of the human peroxisome proliferator activated receptor gamma-2 (PPARγ) were performed to correlate the antihyperglycemic activity for possible mode of action.

Result: Oral administration of compounds 2(a-d) and 3(a-c) in 35mg/kg for 4 weeks reduced blood glucose level significantly by 68-71% and 71-75% respectively comparable to that of glipizide (80%) in a dose of 1mg/kg/day coupled with significant reduction ($p \leq 0.05$) in the increased blood urea nitrogen and serum triglyceride level. Compound 2c have been found most active on the PPAR gamma receptor displaying docking score (-21) comparable to reference ligands Rosiglitazone and pioglitazone.

Conclusion: This observation indicates that chalcone based novel phenylureas have the potential to be an effective antihyperglycemic agent with probable PPAR gamma agonistic action.

Keywords: chalcone, urea, glucose tolerance test, PPAR gamma.

INTRODUCTION

India has a big challenge to face in management of type 2 diabetes mellitus (T2DM) with an alarming figure of more than 50 million people currently suffering from the disease¹.

T2DM is characterized by insulin resistance and it occurs when the β -cells do not secrete enough insulin to counter balance insulin resistance followed by decreased sensitivity of muscle and adipose cells to insulin². Peroxisome proliferator-activated receptor- γ (PPAR- γ) activators, thiazolidinediones, are the most popular ligands till date in the management of T2DM but adverse effects of hepatotoxicity, weight gain, and edema still persist³. Hence the search for newer molecular targets and better agonists/inhibitors is still the foremost need to control the accelerating diabetic population which can also cater to the management of long term complications of T2DM. During research into new antidiabetic agents, new 2, 4-thiazolidinediones with aryl sulfonylurea moieties were reported to be potent antidiabetic agents⁴. Several studies have

demonstrated significant antihyperglycemic as well as hypoglycemic effect of natural and synthetic chalcones in *in-vitro* and *in-vivo* models^{5,6}. Interestingly, both chalcones and benzenesulfonylureas having pyrazoline and thiophenyl pyrazoline rings have been reported to have shown antidiabetic effect via PPAR activation^{7,8}. We used the chalcone moiety as the parent compound backbone and used the phenyl urea group to design compounds with modifiable groups prepared in this work. The aim of this study was to investigate the antihyperglycemic activity of synthesized chalcone based novel urea derivatives (Fig.1) on an experimental model of T2DM and elucidate its possible mode of action.

MATERIAL AND METHODS

Chemistry

Chemicals and solvents required for the work were obtained from Merck, Spectra chem and S.D fine. ¹H NMR spectra were recorded at 300 MHz on Bruker DRX-300 respectively. The melting points were taken in open capillary and were uncorrected. The synthetic route used to synthesize title compounds 2(a-d) and 3(a-c) was outlined in Fig. 1. 1-(4-acetylphenyl)-3-(4-methoxyphenyl) urea (1a) and 1-(4-acetylphenyl)-3-(3,4-dichlorophenyl) urea (1b) were prepared according to method reported in literature⁹ using p-amino acetophenone (FS grade, Sigma Aldrich, India)

and p-methoxy phenylisocyanate and 3, 4 dichloro phenylisocyanate (Sigma Aldrich, India).

*General procedure for the synthesis of 1-{4-[(2E)-3-(substituted phenyl) prop-2-enoyl] phenyl}-3-(substituted phenyl) urea (2a-d), 3(a-c)*¹⁰

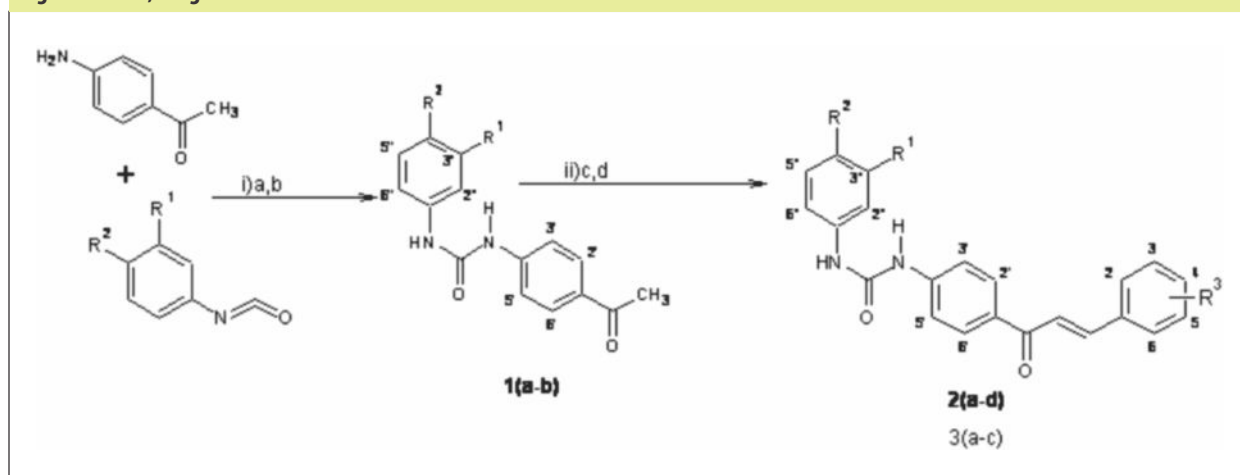
1a (0.01M) /1b(0.01M) and substituted benzaldehydes (0.01M) were dissolved in 95% ethanol, 40% NaOH was added drop by drop till the suspension turns slightly colored and stirred for 24-48 hrs. 6N HCl was added after cooled to acidify using pH indicator paper till neutral. The mixture was extracted with ether and the ether extract was washed with sat. NaHCO₃. The extract was dried till the solvent evaporates off and the solid product was recrystallised with acetone /methanol to get yellow to orange colored crystals (Table 1).

Spectral data of compounds

1-(4-acetylphenyl)-3-(4"-methoxyphenyl) urea (1a)

Yield 80%; mp 198–200 C (from ethanol); IR (KBr) $\nu_{\max}$ 3440(NH) 1630 (CO) cm⁻¹, 9.05 δ ppm (br, s, -NH), 8.59 δ ppm (br, s, -NH), 2.49 δ ppm (s, -COCH₃, 3H), 7.36 δ ppm (d, H_{2'}-6', 2H, J=8.9), 6.87 δ ppm (d, H_{3'}-5', 2H, J=8.9), 7.88 δ ppm (d, H_{2''}-6'', 2H, J=8.6), 7.56 δ ppm (d, H_{3''}-5'', 2H, J=8.6), FAB-MS: (M⁺): 284. Anal. Calcd. for C₁₆H₁₆N₂O₃: C, 67.59; H, 5.67; N, 9.85, O, 16.8; Found: C, 67.0; H, 5.47; N, 9.90, O, 17

Fig.1: Scheme, Reagents and conditions:



(i) a) Δ , 1-2 hrs b) acetone (ii) c) substituted aromatic aldehyde, 40% NaOH d) stirring, rt, 12-24 hrs

Table 1: Synthesized compounds (2a-2d)(3a-3c)

Compound Code	R1	R2	R3
2a	-H	4-methoxy	-H
2b	-H	4-methoxy	-4 methoxy
2c	-H	4-methoxy	-2,5 methoxy
2d	-H	4-methoxy	-3,4,5 trimethoxy
3a	3 Cl	4Cl	-H
3b	3 Cl	4Cl	-4-methoxy
3c	3 Cl	4Cl	-4-Cl

1-(4"-methoxyphenyl)-3-{4-[(2E)-3-phenylprop-2-enoyl]phenyl} urea (2a)

Yield 70%; mp 178–179° C (from methanol); IR(KBr) v max 3296 (NH), 1638 (CO) cm⁻¹.

9.04δppm (br,s,-NH),8.61 δppm (br, s,-NH),8.1 δ ppm (d, H 2'-6', 2 H, J=8.4), 7.6 δ ppm (d, H 3'-5'; 2H, J=8.4Hz), 7.83δ ppm (d, H 2"-6", 2H, J=8Hz), 7.01 δ ppm(d, H3"-5"; 2H, J=8Hz), d, 7.37δppm(αH, 1H, J=9Hz), d, 6.88 δppm(βH, 1H, J=9Hz), s, 3.80 δ ppm(3H, 4 O-CH₃), 7.76δppm(d, H2-6, 2H, J=7.8Hz), 7.68δppm(d, H3-5, 2H, J=7.8Hz), FABMS: 372. Anal. Calcd. for C₂₃H₂₀N₂O₃, C, 74.18, H, 5.41, N, 7.52, O, 12.89, Found: C, 73.89; H, 5.00; N, 7.50, O, 13.0

1-(4"-methoxy phenyl)-3-{4-[(2E)-3-phenylprop-2-enoyl] 4-methoxyphenyl} urea (2b)

Yield 70%; mp 210-212° C (from methanol); IR (KBr) v max 3330 (NH), 1652 (CO) cm⁻¹, 9.04 δppm (br, s,-NH), 8.61 δppm (br, s,-NH), 8.1 δ ppm (d, H2'-6', 2H, J=8.4Hz), d, 7.6 δ ppm (d, H3'-5'; 2H, J=8.4Hz), 7.83δppm(d, H2"-6", 2H, J=8Hz), 7.01δppm(d, H3"-5", 2H, J=8Hz), 7.37δppm(d, αH, 1H, J=9.2Hz), 6.88 δppm(d, βH, 1H, J=9.2Hz), 3.80 δ ppm(s, 3H, 4 O-CH₃), 3.7 δ ppm(s, 3H, 4"O-CH₃), 7.76 δppm (d, H2-6, 2H, J=8.4Hz), 7.68 δ ppm (d, H 3-5, 2 H, J=8.4 H z), F A B - M S : 403, Anal. Calcd. for C₂₄H₂₂N₂O₄, C, 65.63, H, 5.51, N, 6.96, O, 15.9, Found: C, 65.30; H, 5.00; N, 7.00, O, 15.75.

1-(4"-methoxy phenyl)-3-{4-[(2E)-3-phenylprop-2-enoyl] 2,5-methoxyphenyl} urea (2c)

Yield 70%; mp 180–182° C (from methanol); IR (KBr) v max 3445(NH) 1672 (CO) cm⁻¹; 1H NMR (300 MHz, DMSO-d₆) 9.31δppm (br, s,-NH), 9.17 δppm (br, s,-

NH), 7.91 δ ppm (d, H2'-6', 2H, J=8.9Hz), d, 7.64 δ ppm(d, H3'-5'; 2H, J=8.9Hz), 3.80 δ ppm (s, 3H, 4"OCH₃), 7.83δ ppm (H 2"-6", 2H, J=8Hz), d, 7.01 δ ppm(H3"-5"; 2H, J=8Hz), 7.37δppm(d, αH, 1H, J=9Hz), d, 6.88 δppm (d, βH, 1H, J=9Hz), 3.82 δ ppm(s, 3H, 2 O-CH₃), 3.78 δ ppm(s, 3H, 5 O-CH₃), 7.76 δppm (s, H3, 4, 2H), 7.68δppm(s, H 6, 2H), FAB-MS: (M⁺): 432, Anal. Calcd. for C₂₅H₂₄N₂O₅: C, 69.43, H, 5.59, N, 6.48, O, 18.5 Found: C, 70.0; H, 5.54; N, 6.44, O, 19.0.

1-(4"-methoxy phenyl)-3-{4-[(2E)-3-phenylprop-2-enoyl] 3,4,5-methoxyphenyl} urea (2d)

Yield 30%; m.p 200-206° C (from methanol); IR (KBr) v max 3230(NH) 1645 (CO) cm⁻¹; 1H NMR (300 MHz, DMSO-d₆) 9.07δppm (br,s,-NH), 8.62 δppm (br,s,-NH), 8.62δ ppm (d, H2'-6', 2H, J=7.8Hz), 7.90 δ ppm(d, H3'-5'; 2H, J=7.8 Hz), 7.63δ ppm (d, H 2"-6", 2H, J=8.5Hz), 7.54 δ ppm (d, H3"-5"; 2H, J=8.5 Hz), 8.14 δppm(d, αH, 1H, J=9.2 Hz), 6.88 δppm (d, βH, 1H, J=9.2 Hz), s, 3.70 δ ppm(6H, 3, 5 O-CH₃), s, 3.69 δ ppm(3H, 4O-CH₃), s, 3.85 δ ppm(3H, 4"O-CH₃), 7.76 δppm (s, H2-6, 2 H), F A B - M S (M +) : 463. Anal. Calcd. for C₂₆H₂₆N₂O₆: C, 67.52; H, 5.67; N, 6.06, O, 20.7, Found: C, 67.48; H, 5.6; N, 5.99, O, 20.7.

1-(4-acetylphenyl)-3-(3",4"-dichlorophenyl) urea (1b)

Yield 80%; mp 238-240° C (from ethanol); IR (KBr) v max 3453(NH) 1646 (CO) cm⁻¹, 1H NMR (300 MHz, DMSO-d₆): 9.22δppm (br,s,-NH), 9.10 δppm (br, s,-NH), 2.50 δ ppm (s, -COCH₃, 3H), 7.91 δ ppm (d, H2'-6', 2H, J=8.7Hz), 7.58 δ ppm(d, H3'-5'; 2H, J=8.7Hz), 7.35 δ ppm (d, H6", 2H, J=8.8Hz), 7.56δppm(d, H5", 1H, J=8.8Hz), FAB-MS: (M⁺): 322. Anal. Calcd. for C₁₅H₁₂Cl₂N₂O₂: C, 55.75; H, 3.74; N, 8.67, O, 9.9, Cl, 21.94; Found: C, 55.0; 4.02, N, 8.47; O, 9.7, Cl, 20.

1-(3", 4"-dichlorophenyl)-3-{4-[(2E)-3-(4-methoxy phenyl) prop-2-enoyl] phenyl} urea (3a)

Yield: 65%, mp: 238-240° C (from methanol); IR (KBr) v max 3557(NH) 1687 (CO) cm⁻¹, 1H NMR (300 MHz, DMSO-d₆) 9.26 δppm (br s, 1H, NH), 9.14 (br s, 1H, NH), 7.7-7.29δppm (m, 3H, H2"-5"-6"), 7.85 δppm (d, 2H, H2'-6', J = 8.4 Hz), 7.65 δppm (d, 2H, H3'-5', J = 8.4Hz), 7.52 δppm (d, 1H, Ha, J = 9Hz), 6.66 δppm (d, 1H, Hb, J = 9 Hz), 8.13 δppm (d, 2H, H2-6, J=8.6), 7.03

δ ppm (d, 2H, H 3- 5, J=8.68Hz), FAB-MS: (M⁺):441, Anal. Calcd. for C₂₃H₁₈Cl₂N₂O₃: C, 62.66; H, 4.11; N, 6.35, O, 10.88, Cl.16.07 Found: C, 62.84; H, 4.10; N, 6.00; O, 11.20; Cl.15.99

1-(3'',4''-dichlorophenyl)-3-{4-[(2E)-3-(4-chlorophenyl)prop-2-enoyl]phenyl}urea(3b)

Yield: 40%, mp:210-215°C (from methanol) IR (KBr) ν max 3547(NH) 1645 (CO) cm⁻¹, ¹H NMR (300 MHz, DMSO-d₆), 9.27 δ ppm (br, s, -NH), 9.14 δ ppm (br, s, -NH), 7.52-7.48 δ ppm (m, 3H, H2''-5''-6''), 8.63 δ ppm (d, 1H, Ha, J = 9Hz), 7.35 δ ppm (d, 1H, Hb, J = 9 Hz), 7.55 δ ppm (d, 2H, H2'-6', J = 8.1 Hz), 7.49 δ ppm (d, 2H, H3'-5', J = 8.1 Hz), 7.54 δ ppm (d, 2H, H 3- 5, J = 8.1 Hz), 7.53 (d, 2H, H2-6, J = 8.1 Hz), FAB-MS: (M⁺):446. Anal. Calcd. for C₂₂H₁₅Cl₃N₂O₂: C, 59.28; H, 3.39; N, 6.28, O, 7.18, Cl, 23.86. Found: C, 60.05; H, 4.10; N, 6.05; O, 7.00; Cl, 24.02

1-(3'', 4''-dichlorophenyl)-3-{4-[(2E)-3-phenylprop-2-enoyl]phenyl}urea (3c)

Yield: 40%, mp:258-262°C (from methanol); IR (KBr) ν max 3433(NH) 1629 (CO) cm⁻¹, ¹H NMR (300 MHz, DMSO-d₆), 9.27 δ ppm (br, s, , NH), 9.04 δ ppm (br, s, -NH), 7.7-7.38 δ ppm (m, 3H, H2''-5''-6''), 8.8 δ ppm (d, 1H, Ha, J = 9Hz), 7.05 δ ppm (d, 1H, Hb, J = 9 Hz), 7.45 δ ppm (d, 2H, H2'-6', J = 7.8 Hz), 7.30 δ ppm (d, 2H, H3'-5', J = 7.8 Hz), 7.54 δ ppm (d, 2H, H 3 - 5, J = 8.1 Hz), 7.73 (d, 2H, H2-6, J = 8.1 Hz), FAB-MS: (M⁺):411. Anal. Calcd. for C₂₂H₁₆Cl₂N₂O₂: C, 64.25; H, 3.92 N, 6.81, O, 7.78, Cl, 17.24. Found: C, 64.20; H, 4.10; N, 7.01, O, 7.99; Cl; 17.20

Pharmacology

The animal studies were performed after receiving approval of the Institutional animal ethics committee (IAEC Regd. No. 1458/PO/E/11/CPCSEA) in NSHM college of pharmaceutical technology, Kolkata, India (IAEC approval number: NCPT/IAEC-14/2015) Acute Oral Toxicity Study (LD50).

Acute toxicity study was performed following the guidelines of the Organization for Economic Co-operation and Development (OECD) for testing of chemicals, TG 420 (adopted—December 2001) using various doses (2000, 200, 100, and 30 mg/kg) body

weight of test compounds via gastric intubation to eight groups of 24 female BALB/c mice (nulliparous and nonpregnant; 22±5 g body weight) viz., control and test groups. The control group received 0.5 % carboxy methyl cellulose (prepared in double distilled water) as vehicle at a dose volume of 10 ml/kg body weight. The animals were observed up to 3 days post drug treatment¹¹.

In vivo antihyperglycemic activity

The test compounds were evaluated for their anti hyperglycemic activity in streptozotocin induced type 2 diabetic model of male Wister albino rats. Diabetes was induced in overnight fasted rats by a high fat diet followed by a single intraperitoneal injection of low dose streptozotocin (STZ) (35mg/kg body weight¹². STZ was dissolved in citrate buffer (pH 4.5). Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours by using Glucostrips (Johnson & Johnson®). The animals with blood glucose concentration more than 200mg/dl were used for study. Treatment with compounds (2a-d), 3(a-c) (35mg/kg) started after 3 days of STZ injection and continued for 28 days. On the 28th day of treatment rats were sacrificed and pancreatic β -cell function was assessed by measuring blood urea nitrogen (BUN), serum triglycerides, HDL cholesterol, LDL cholesterol, serum total proteins. Glipizide (1mg/kg/day) was used as a reference.

Oral glucose tolerance test (OGTT):

Compounds were evaluated in vivo for their antidiabetic activity using an OGTT on diabetic rats in a rat model of T2DM. OGTTs were performed after a single PO administration of 35 mg/kg of each synthesized compound. The oral glucose tolerance test was performed on the second week of treatment and same rats were further treated for four weeks. In short, the base line blood glucose level was measured. The respective groups of rats were administered the respective compounds (i.e. control, standard and test compounds) in specified dose used in previous procedure of induced NIDDM. All the animals were given glucose (2 g/kg p.o.) 15 min after dosing. Blood samples were collected prior to and after 0, 30, 60, 90, 120 minutes of the glucose loading, then the

glucose levels were measured by semi-auto biochemical analyzer¹³.

In vitro antioxidant activity of complex by diphenylpicrylhydrazyl (DPPH) method¹⁴

The antioxidant activity of compounds 2(a-d), 3(a-c) was evaluated using DPPH radical scavenging method. Different concentrations (25 µg, 50 µg, 75 µg, 100 µg /ml) of compounds were taken. Then 1 ml of the sample and 3 ml of DPPH solution and the reaction mixture was shaken vigorously and kept in completely dark for a better reaction. The reduction of DPPH absorbance was followed by monitoring at 517 nm every 5 min for about 30–35 min (As). As a control the absorbance of blank solution of DPPH was also determined at 517 nm (Ac). The percentage of radical scavenging activity (RSA %) was calculated according to the following equation: $RSA\% = 100 / (Ac - As) / Ac$.

Docking and Absorption, distribution, metabolism, excretion (ADME) studies:

The structures of title compounds (2a–d) and 3(a–c) were drawn using ChemDraw ultra v 10.0 (Chemical Structure Drawing Standard; Cambridge Soft corporation, USA), copied to Chem3D ultra v 10.0 to create a 3D model and, finally subjected to energy minimization using molecular mechanics (MM2). Energy minimized structures are considered for molecular docking studies. The crystal structures of ligand binding domain of human peroxisome proliferator activated receptor gamma (PDBID: 2PRG) was accessed from Protein Data Bank (PDB) (www.rcsb.org). The selection of protein for molecular docking studies is based upon several factors, that is, structure should be determined by X-ray diffraction, and resolution should be between 2.5–3.0 Å, it should contain a cocrystallized ligand; the selected protein should not have any protein breaks in their 3D structure. Molecular docking experiments were performed against PPARγ utilizing FlexX software LEADIT 2.13, to understand the interaction of ligands with the receptor. Evaluations of ADME properties were done by using QikProp 3.0 to check for the possibility of oral delivery.

Statistical Analysis

Data were analyzed through one-way analysis of

variance (ANOVA) using the *GraphPad Prism 5* (Version 5.01 GraphPad Software Inc. USA). Post Dunnett test was applied with ANOVA. $P < 0.05$ was considered statistically significant difference.

RESULT AND DISCUSSION

The IR spectrum of all the compounds (2a–d) and 3(a–c) exhibited the characteristic absorptions at various frequencies correspondingly at 3400–3100 cm⁻¹ and 1620–1715 cm⁻¹ suggesting the presence of a secondary amine group and α, β-unsaturated carbonyl group, respectively. The ¹H NMR spectra of compounds (2a–d) and 3(a–c), exhibited characteristic peaks of H-α and H-β protons of α, β unsaturated carbonyl group as two doublets, one at around 8.01 ppm (H-a, J = 9 Hz) and the other one around at 6.88 ppm (H-b J = 9 Hz). Two singlets integrating for two protons of -NH of substituted phenyl urea was observed in between δ 8.8–9.2 ppm as two broad signals. Further, the FAB-Mass data shows the presence of (M⁺) and (M+1) ion indicating the compounds being structurally pure.

In the oral acute toxicity test, all the tested compounds were found to be safe upto a dose (Lethal Dose 50) of 100 mg kg⁻¹ body weight. No changes in any vital functions were observed throughout the study period. In the STZ induced diabetic rats, oral administration of compounds 2(a–d) and 3(a–c) in 35 mg/kg for 4 weeks reduced elevated blood glucose level significantly by 68–71% and 71–75% respectively in a triphasic response comparable to glipizide (1 mg/kg/day) treatment which decreased BGL by 80% (Fig. 2). Table 2 presents the average blood glucose profile of normal control and the experimental groups at various time intervals in oral glucose tolerance. Reference standard glipizide caused nearly 19% inhibition at a dose of 1 mg/kg/day 60 minutes after glucose load whereas compounds 2a, 2c, 2d showed significant ($p \leq 0.05$) reduction of elevated BGL by 56, 46, 55%, in 60 min, a better profile compared to pattern exhibited by glipizide. The above finding suggests that the compounds may have therapeutic potential in post prandial hyperglycemia. Significant reduction ($p \leq 0.05$) in plasma insulin levels (Table 3) in diabetic rats compared to normal control can be

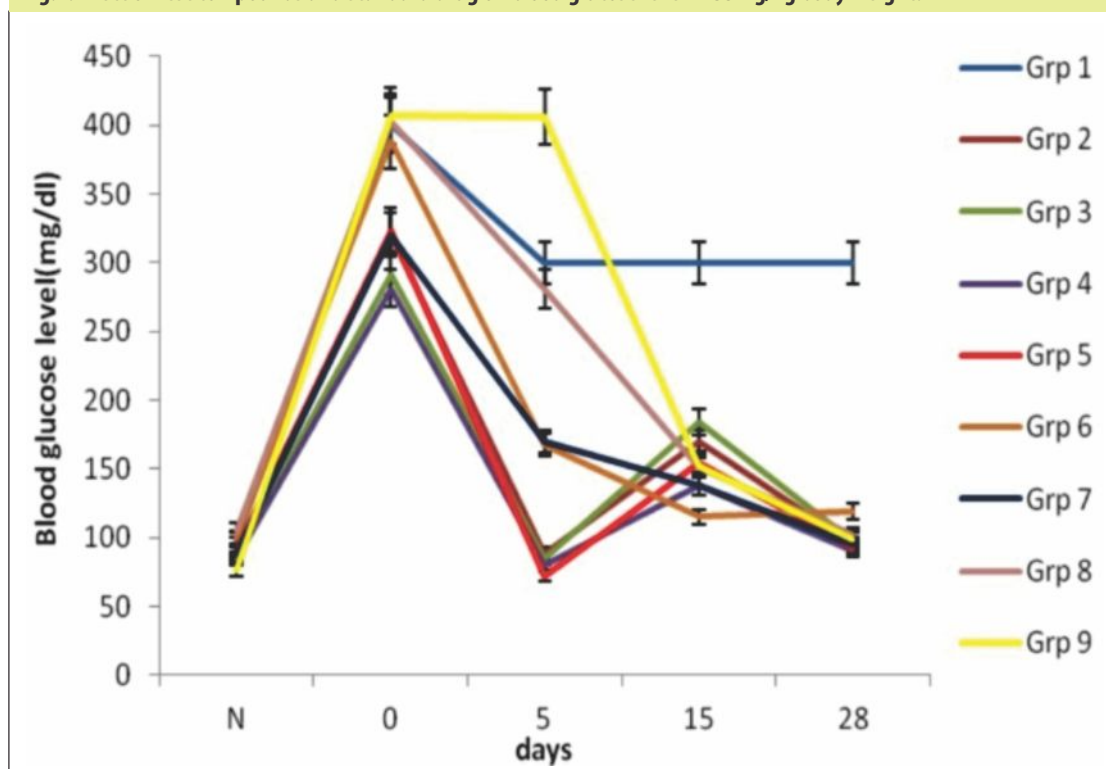
Table 2: Effect of oral glucose tolerance test in different animal groups.

Treatment Groups	Just prior to glucose load (2g/kg p.o)	0 hr (After glucose load)	1hr	2hr	Percent reduction in BGL post 2hrs
Normal	82.9±0.4	95.6 ±0.3	97.2±0.4 1	10± 0.5	NA
Group I	95.5±0.39	436.5±0.31	438.1±0.3	430±0.4	
Group II	83.5±0.95	213.3±7.6	98.3±4.23***	93.5±5.56***	53
Group III	90.5±0.56	338.5±2.78	265.8±3.96***	85.67±1.2***	21
Group IV	90.5±0.5	303.7±1.1	162.2±1.9***	91.17±1.7***	46
Group V	93.7±1.5	337.5±3.3	149.8±1.7***	119.8±5.4***	55
Group VI	90.5±0.5	298.7±1.1	268.4±4.4	85.1±1.7***	10
Group VII	85.5±0.6	400.2±2.7	342±4.8	150.3±1.8***	14
Group VIII	87.2±0.5	305.6±1.5	265.7±0.2	133.2±1.5***	13
Group IX	95.7±0.6	210.3±0.6	170.9±0.33	82.8±0.5***	19

Group I: Diabetic control, **Group II:** Diabetic+ compound 2a, **Group III:** diabetic +compound 2b, **Group IV:** Diabetic+ compound 2c, **Group V:** Diabetic+ compound 2d, **Group VI:** Diabetic+ compound 3a **Group VII:** Diabetic+ 3b, **Group VIII:** Diabetic +3c, **Group IX:** Diabetic +glipizide

Data is expressed as mean± (SEM), n=6, Significant at***p ≤ 0.05 in relation to hyperglycemic control.

Significant at##p ≤ 0.05 in relation to normal control, Ns= non significant. BGL :Blood glucose level

Fig.2:Effect of Test compounds and standard drug on blood glucose level in 35mg/kg body weight.


Group I: Diabetic control, **Group II:** Diabetic+ compound 2a, **Group III:** diabetic +compound 2b, **Group IV:** Diabetic+ compound 2c, **Group V:** Diabetic+ compound 2d, **Group VI:** Diabetic+ compound 3a, **Group VII:** Diabetic+ 3b, **Group VIII:** Diabetic +3c, **Group IX:** Diabetic +glipizide

0 day corresponds to day of recording elevated BGL post STZ administration

5, 15, 20, 28 days corresponds to days post administration of test compounds and standard drug.

attributed to partial destruction of pancreatic beta cells owing to low dose streptozotocin (30mg/kg)¹⁵ which further leads to impaired glucose stimulated insulin release and insulin resistance, both typical markers of T2DM. The marked elevated plasma triglyceride, LDL, BUN and creatinine levels in diabetic rats, may be the consequence of the uninhibited actions of lipolytic hormones on fat depots¹⁶ which occurs when there is a dwindling insulin level in blood mimicking the pattern observed in diabetic patients¹⁷. The efficacy of the test compounds in attenuating PPG level may be due to increased glucose stimulated insulin secretion. (GSIS). This is indicated by significantly ($p \leq 0.05$) increased insulin level in treated rats compared to diabetic rats which is correlated to possible improvement of tissue glucose uptake. It is interesting to note that all the test compounds 2(a-d) and 3(ac) which displayed significant antihyperglycemic activity also exhibited moderate DPPH radical scavenging activity, though nonsignificantly (data not shown). Amongst them, compound 2a and 2c and compound 3a showed enhancement of anti-oxidant activity in a dose dependent manner with IC₅₀ value of 104.8 µg/ml, 101.8 µg/ml and 108.4 µg/ml respectively, although maximum percentage inhibition shown by 2c, 3a is 54% and 2a being 65%. at a maximum dose of 125 µg/ml which is quite lesser as compared to 90% as shown by ascorbic acid (IC₅₀ of ascorbic acid: 71.5 µg/ml). The modest anti oxidant protection rendered by test compounds 2(a-d) and 3a-c) could also be a promoting factor for restoring insulin secretion leading to enhanced GSIS. All the seven novel

chalcone based novel phenylureas exhibited notable amelioration of augmented lipid (trig, LDL) and renal (BUN, creatinine) parameters (Table 3) which suggests these could be beneficial in preventing diabetic complications as well. Increased GSIS increases fatty acid oxidation and reduces serum triglyceride and LDL levels². The reduction in urea and creatinine levels in treated rats indicates reduced proteolysis¹⁸ and this might be the reason for the increase in the body weight of the animals in four weeks of treatment after an initial loss¹⁹ (Table 4).

Though the docking results were not so promising, compound 2c was the most potential candidate exhibiting docking score of -21 (Table 5) compared to reference ligands. (Rosiglitazone: -24 and pioglitazone: -25). The polar group in full agonists, Rosiglitazone and Pioglitazone forms intramolecular H bonds with His323, Ser289, His449 and Tyr473 via carbonyl group present in thiazine ring. Similarly His 323 and Tyr473 has been detected to form two hydrogen bonds with the electron donating methoxy group in phenyl urea rings in the analysis of ligand-receptor interaction of most active agonist 2c. (Fig. 3) These HBs are responsible for the conformational change of helix 12 (H12) of the receptor and subsequently, the activation of PPAR γ ²¹.

A comprehensible study published by Mahapatra et.al and Jung et.al report that chalcones having methoxy group at position 4/5 of ring B play key role in PPAR γ selective agonism showed potent PPAR γ agonistic activity [3,22]. Based on the findings of our investigation it can be concluded that, the plausible

Table 3: Effect on various biochemical parameters in different animal groups

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX	Normal
Insulin (mIU/ml)	13.9±0.3	29.2±0.7	35.8±0.3	26.8±0.4	24.3±0.3	22.2±1.3	25.5±0.2	20±1.5	22.5±0.2	20±0.1
Creatinine (mg/dl)	1.58±0.04	0.63±0.03	0.6±0.02	0.71±0.0	0.61±0.01	1±0.02	0.85±0.05	0.8±0.02	0.72±0.00	0.35±0.0
Ld I (mg/dl)	20.3±0.08	17.3±0.08	17.6±0.13	14.38±0.09	16.2±0.13	17.2±0.05	15.5±0.03	15.7±0.15	15.28±0.07	16.38±0.09
TRIG (mg/dl)	105.3±2.9	80±1.6	78±1.7	75.6±0.84	62.5±1.5	95.2±1.2	77.5±0.7	90±1.1	66.3±1.2	60.6±0.3
BUN (mg/dl)	32.08±0.8	16.47±0.3	16.27±0.13	19.7±0.1	20.38±0.6	18.5±1.3	20±0.5	22.3±0.15	21.47±0.4	20.7±0.3

Group I: Diabetic control, **Group II:** Diabetic+ compound 2a, **Group III:** diabetic + compound 2b, **Group IV:** Diabetic+ compound 2c, **Group V:** Diabetic+ compound 2d,

Group VI: Diabetic+ compound 3a **Group VII:** Diabetic+ 3b, **Group VIII:** Diabetic + 3c, **Group IX:** Diabetic + glipizide

Data is expressed as mean±(SEM), n=6, Significant at *** $p \leq 0.05$ in relation to hyperglycaemic control, TRIG= Serum triglycerides, LDL =LDL cholesterol, BUN= Blood urea nitrogen

Fig.3: Binding mode pose of compound...2c with 2-PRG

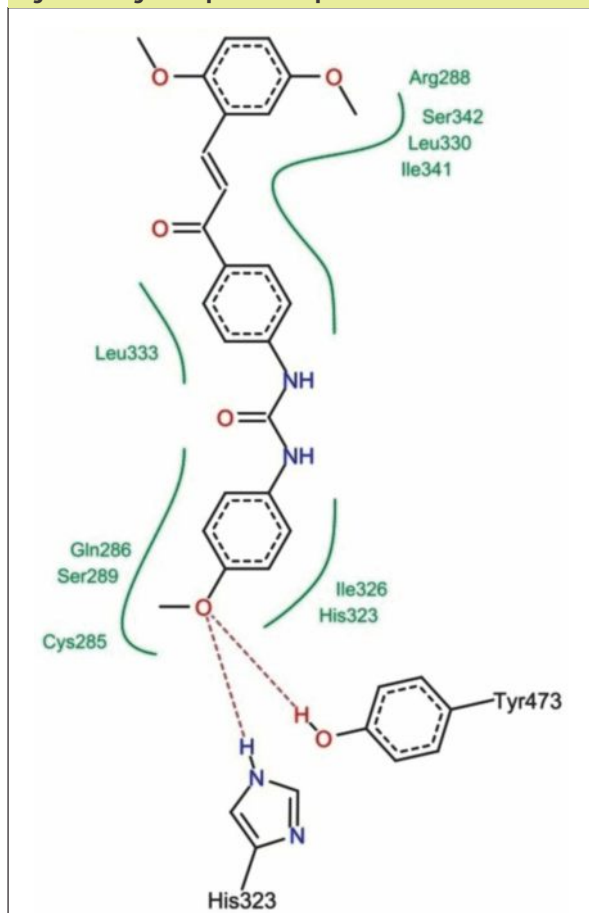


Table 5: Docking scores of test compound and reference ligands

Compound Code	Docking Score
2a	-18.53
2b	-18
2c	-21.71
2d	-
3a	-14.56
3b	18.26
3c	-
Glipizide	-18
Rosiglitazone	-25.28
Pioglitazone	-23

mechanism of action of novel chalcones being PPAR γ activation, which is anticipated to play an important role in regulating glucose sensing insulin secretion resulting in reduced BGL levels shown by the treated type 2 diabetic rats [17], a theory supported by the fact that chalcones have also been reported to increase expression of PPAR γ 4. The test compounds exhibit predicted 95-100% human oral absorption rate, great blood brain barrier permeability (QPP MDCK $\geq$ 500) as well as gut blood barrier permeability (QPP CACO $\geq$ 500). The advantage of the predicted physicochemical data compatible with desirable pharmacokinetic

Table 4: Relative body weights of treated animals in 28 days study

Treatment Groups	0	5	15	20	28
Normal	210.5 $\pm$ 2.5	198.1 $\pm$ 4.1 ^{ns}	194.2 $\pm$ 3 ^{ns}	201.5 $\pm$ 2.4 ^{ns}	203.0 $\pm$ 1.6 ^{ns}
Group 1	213.2 $\pm$ 4.8	181.7 $\pm$ 7 ^{ns}	179.3 $\pm$ 3.3 ^{***}	179.3 $\pm$ 3.3 ^{***}	179.3 $\pm$ 3.3 ^{***}
Group II	208.0 $\pm$ 4.3	173.3 $\pm$ 4.94 ^{***}	150.2 $\pm$ 3.7 ^{***}	165.0 $\pm$ 2.9 ^{***}	189.0 $\pm$ 5.3 [*]
Group III	198.2 $\pm$ 6.7	170.2 $\pm$ 18.4 ^{ns}	172.2 $\pm$ 5.3 ^{ns}	180.7 $\pm$ 5.6 ^{ns}	188.3 $\pm$ 5.8 ^{ns}
Group IV	208.0 $\pm$ 5.9	195.3 $\pm$ 3.9 ^{ns}	179.5 $\pm$ 4.6 ^{***}	185.2 $\pm$ 3.7 [*]	199.3 $\pm$ 4 ^{ns}
Group V	231.3 $\pm$ 6.3	225.0 $\pm$ 3.6 [*]	190.8 $\pm$ 3.7 ^{***}	200.2 $\pm$ 3.9 ^{***}	210.3 $\pm$ 2.9 ^{***}
Group VI	197.3 $\pm$ 4.8	175.3 $\pm$ 4.2 ^{ns}	165.5 $\pm$ 3.7 [*]	174.0 $\pm$ 3.2 ^{ns}	184.0 $\pm$ 2.7 ^{ns}
Group VII	228.2 $\pm$ 2.3	200.0 $\pm$ 5.4 [*]	191.4 $\pm$ 5.6 ^{***}	200.2 $\pm$ 5.4 ^{**}	210.5 $\pm$ 2 ^{ns}
Group VIII	220.5 $\pm$ 2.5	198.3 $\pm$ 3.5 [*]	191.5 $\pm$ 2.8 ^{ns}	197.3 $\pm$ 4.2 ^{ns}	199.4 $\pm$ 3.5 ^{ns}
Group IX	235.5 $\pm$ 5.4	202.2 $\pm$ 4.3 ^{***}	185.6 $\pm$ 3.4 [*]	190.5 $\pm$ 2.5 ^{ns}	195.5 $\pm$ 2.3 ^{ns}

Group I: Diabetic control, **Group II:** Diabetic+ compound 2a, **Group III:** diabetic +compound 2b, **Group IV:** Diabetic+ compound 2c, **Group V:** Diabetic+ compound 2d, **Group VI:** Diabetic+compound 3a, **Group VII:** Diabetic+ 3b, **Group VIII:** Diabetic +3c, **Group IX:** Diabetic +glipizide. Data is expressed as mean $\pm$ (SEM), n=6, Significant at *** $p \leq 0.05$ in relation to hyperglycemic control, significant at ** $p \leq 0.01$ in relation to hyperglycemic control, STZ =Streptozotocin

Table 6: ADME properties of test compounds

COMPOUND CODE	MOL WT	CNS	QPL OGP o/w	QPL OGS	QP log HERG	QPP Caco	QPPM DCK	Qplog KP	QplogK hsa	% HUMAN ORAL ABS
2a	372.42	-2	4.048	-5.92	-6.385	642.6	649.2	-1.04	0.37	100
2b	402.4	-2	4.251	-6.6	-6.244	642.6	449.1	-1.14	0.395	100
2c	432.4	-2	4.430	-6.66	-6.297	642.8	449.2	-1.22	0.434	100
2d	462.5	-2	4.447	-6.4	-5.959	642.7	449.2	-1.24	0.406	100
3a	411.28	-1	4.863	-7.3	-6.31	642.82	305.2	-1.24	0.5	100
3b	441.3	-1	5.070	-7.27	-6.166	642.82	305.3	-1.34	0.06	94
3c	445.7	0	5.458	-7.78	-6.194	644.6	569.1	-1.41	0.699	96
Recommended range	130-725	-2+2	-2-6.5	-6-0.5	Concern below-5	≤ 2500 ≥ 500 great	≤ 2500 ≥ 500 great	-8 - -1	-1.5-1.5	≥ 80%high ≤ 25%poor

CNS: predicted central nervous system activity, **QplogPo/w:** predicted octanol water partition coefficient, **QP log S:** predicted aqueous solubility, **QplogHERG:** predicted IC50 values for blockade of K⁺ HERG channels, **QPPCaco:** predicted apparent caco-2 cell permeability, a model for gut-blood barrier, **QPPMDCK:** predicted apparent MDCK cell permeability, a good mimic for blood-brain barrier, **QplogKP:** predicted skin permeability, **QplogKhsa:** prediction of binding to human serum albumin, **% HUMAN ORAL ABS:** predicted qualitative human oral absorption

properties makes the novel compounds suitable candidate for oral formulations, which definitely need to be validated by suitable preformulation and further in vivo studies (Table 6).

CONCLUSION

In summary, a series of novel chalcone based phenyl ureas were synthesized through a short synthetic route and tested, identifying a series of compounds with an interesting profile as potential antidiabetic agents. The high bioactivity of compound 2c coupled with good docking score comparable to that of reference ligand make it a suitable lead to develop new chemical entities as promising PPAR γ activators in the treatment of T2DM. Additional studies needs to be performed on the pharmacology of the described compounds for an evaluation of their mechanistic and pharmacokinetic properties and for a deeper investigation of their activity profile as antidiabetic agents for management of T2DM.

ACKNOWLEDGEMENT

The authors are thankful to the Director, Department of pharmaceutical Chemistry, NSHM Knowledge campus and Department of pharmaceutical Technology, Jadavpur University for providing laboratory facilities and Department of pharmaceutical sciences and technology, BIT Mesra

for providing drug design laboratory for carrying out docking studies.

CONFLICT OF INTEREST: Nil

REFERENCES

1. <http://timesofindia.indiatimes.com/life-style/health-fitness/health-news/India-is-the-diabetes-capital-of-the-world/articleshow/50753461.cms>, Malik R, 2016, [Cited 2016, Jan 28]
2. Gisela W, Insulin and insulin resistance, Clin. Biochem. Rev.2010; 26:119-139.
3. MahapatraDK, AsatiV, BhartiSK. Chalcones and their therapeutic targets for the management of diabetes: structural and pharmacological perspectives. Eur. J Med. Chem.2015; 92: 839-865.
4. JawaleDV, PratapUR, RahujaN, SrivastavaAK, ManeRA. Synthesis and antihyperglycemic evaluation of new 2,4-thiazolidinediones having biodynamic arylsulfonylurea moieties, Bioorg. & Med. Chem. Lett.2012; 22:436-439.
5. ShuklaP, SinghAB, SrivastavaAK, PratapR. Chalcone based aryloxypranolamines as potential antihyperglycemic agent, Bioorg.med chem.2007;17: 799-802.
6. SeoWD, JinHK, KangJE, RyuHW, LongMJC, LeeHS Yang MS, ParkKH, Sulfonamide chalcone as a new class of alpha-glucosidase inhibitors, Bioorg. Med. Chem. Lett.2005;15: 5514-5516.
7. KharbandaC, AlamMS, HamidH, JavedK, DhulapA, BanoS, AliY, Antidiabetic effect of novel benzenesulfonylureas as PPAR- γ agonists and their anticancer effect, Bioorg.Med.Chem.Lett.2015;25: 4601-4605.
8. KharbandaC, AlamMS, HamidH, JavedK, DhulapA, BanoS, Ali Y, Novel benzenesulfonylureas containing thiophenylpyrazoline moiety as potential antidiabetic and anticancer agents, Bioorg.Med.chem.Lett.2015;24: 5298-5303.

9. FrankRL,SmithPV.Org. Synth.1948;28: 89, DOI: 10.15227/orgsyn.028.0089, [Accessed on 20 May, 2014].
10. Vasudeva RA, Yejella RP, Annapurna A, Guntuku GS, Doddi BR, Vutla VR, Anagani SR et.al, Synthesis, characterization and biological evaluation of some novel 2,4- thiazolidinediones as potential cytotoxic, antimicrobial and antihyperglycemic agents, Bioorg. Med. Chem. Lett.2012; 22:6442–6450.
11. Bandgar BP, Gawande SS, Bodade RG, Totre JV, Khobragade CN, Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, antiinflammatory and antioxidant agents, Bioorg. Med. Chem.2010; 18:1364–1370.
12. SkovsoS. Modeling type 2 diabetes in rats using high fat diet and streptozotocin.J Diabetes Investig.2014; 5: 349-358,DOI:10.1111/jdi.12235.
13. L Crane, Anastassiadou M, Salomé Ehage, StiglianiJL, Baziard-Mouysset G, Payard, JM..Leger M, Bizot-Espiard JG, Ktorza A., Imidazoline derivatives with potent antihyperglycemic activity in a rat model of type 2 diabetes, Bioorg Med chem.2006;14:7419-33.
14. Roy S, Majumdar S, Singh AK, Ghosh B, Ghosh N, Manna S, Chakraborty S, Mallick S, Synthesis, Characterization, Antioxidant Status, and Toxicity Study of Vanadium–Rutin Complex in Balb/c Mice, Biol. Trace. Elem. Res.2015; 166 (2):183-200.
15. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res, 2005; 52: 313–320.
16. Saha HR, Sarkar BC, Khan SA, Sana NK, Choudhury S, A comparative study of thyroid hormone and lipid status in diabetic and non diabetic adults.Open. Access.Sci.Reports.2012; 1(9):1–5.
17. HenryNG, Yuan-LiZ, AntonioH, Regulation of plasma triglycerides in insulin resistance and diabetes. Arch Med Res.2005; 36: 232–240.
18. Oliveira HC, Dos S, Grigulo MP, Lima LL, Martins DTO, Lima JCS, Stoppiglia LF, Lopes, Kawashita CF, Hypoglycemic and antihyperglycemic effect of begonia malabarica in STZrats, N.F. J Ethnopharmacol.2008;115: 515–519.
19. Jangra M, Sharma S, Kumar M, Evaluation of antihyperglycemic activity of dodonaea viscosa leaves in normal and stzdiabetic rats.Int. J Pharm. Pharm. Sci.2011;3: 69-74.
20. Tsakovskal, Al SharifM, Alov P, Diukendjieva A, Fioravanzo E, CroninMTD, Pajeva I, Molecular Modelling Study of the PPAR γ Receptor in Relation to the Mode of Action/Adverse Outcome Pathway Framework for Liver Steatosis,Int. J. Mol. Sci.2014;15: 7651-7666.
21. GuaschL, Sala E, Castell-Auví A, CedóL, Liedl KR, Wolber G, Muehlbacher M, MuleroM, PinetM, ArdévolA, Identification of PPAR γ partial agonists of natural origin (I): Development of a virtual screening procedure and in vitro validation. PLoS One.2012; 7: e50816.
22. Jung SH, Park SY, Kim-pakY, Lee HK, Park KS, Shin KH, OhuchiK, Keum SR, Lim SS, Synthesis and PPAR- γ Ligand-Binding Activity of the New Series of 2-Hydroxychalcone and Thiazolidinedione Derivatives, Chem. Pharm. Bull.2006;54: 368—371.
23. HI Kim, YH Ahn, Role of Peroxisome Proliferator–Activated Receptor in the glucose sensing apparatus of liver and cells, Diabetes.2004; 1:560-65.