Bioorganic & Medicinal Chemistry Letters 23 (2013) 5740–5743

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of thiazolyl-phthalazinone acetamides as potent glucose uptake activators via high-throughput screening

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ARTICLE INFO

Article history:
Received 14 March 2013
Revised 24 July 2013
Accepted 30 July 2013
Available online 7 August 2013

Keywords:
Thiazolyl-phthalazinone acetamides
Glucose uptake activators
Type 2 diabetes
Obesity
PPAR-γ

ABSTRACT

With the aim to discover orally active small molecules that stimulate glucose uptake, high throughput screening of a library of 5000 drug-like compounds was conducted in differentiated skeletal muscle cells in presence of insulin. *N*-Substituted phthalazinone acetamide was identified as a potential glucose uptake modulator. Several novel derivatives were synthesized to establish structure activity relationships. Identified lead thiazolyl-phthalazinone acetamide (7114863) increased glucose uptake (EC_{50} of 0.07 ± 0.02 μM) in differentiated skeletal muscle cells in presence of insulin. Furthermore, 7114863 was superior to rosiglitazone under similar experimental conditions without inducing PPAR-γ agonist activity thus making it a very interesting scaffold.

Type 2 diabetes is a chronic metabolic disorder that refers to a condition created by non-responsiveness to or deficiency of insulin. Causes for this disease are many and quite complex. It has been observed that about 80% of the type 2 diabetes patients are obese. Obesity indicates an increase in the number or size of adipocytes leading to an overproduction of certain hormones like leptin and cytokines, some of which appear to cause cellular resistance to insulin.[1] While adipocytes contribute significantly towards the development of insulin resistance, skeletal muscles are also known to play an important role in glucose homestatsis.[2–4] It has been observed that people with type 2 diabetes have reduced insulin dependent glucose transport in skeletal muscles.[5] At present, various treatments for type 2 diabetes mellitus aim at reducing these hyperglycemic conditions. The therapeutic regimens include among other classes, sulfonylureas, metformin, PPAR-γ agonists (thiazolidinediones), α-glucosidase inhibitors and insulin itself. PPAR-γ full agonists enhance insulin action and are the mainstay drugs for the treatment of type 2 diabetes. However in recent times thiazolidinedione treatment has been linked to increased incidences of weight gain and congestive heart failure.[6] It is therefore essential to develop a therapeutic that follows alternate pathways to reverse the effect of insulin resistance and yet not produce any undesirable side effect.

In an attempt to discover orally active small molecules that stimulate glucose uptake, we devised a high throughput screening (HTS) assay to identify compounds which could improve the ability of skeletal muscle to take up glucose under in vitro conditions.[4,5] A HTS assay measuring glucose uptake in differentiated skeletal muscle cells pulsed with radioactive glucose in presence of insulin was established. A library comprising of 5000 drug-like compounds was screened and the compound 7114863 was found to be most potent compound in inducing glucose uptake in differentiated skeletal muscle cells (EC_{50} = 0.070 ± 0.002 μM). This compound increased glucose uptake without activating PPAR-γ. We evaluated its potential by measuring the effect on glucose uptake in human skeletal muscle cells (Fig. 1, Table 1). The activity of 7114863 (**1a**) was superior to that of rosiglitazone under similar experimental conditions without inducing PPAR-γ agonist activity thus making it a very interesting scaffold. Based on these interesting results, we explored structure–activity relationship around 7114863 (**1a**) molecule. Chemistry and biological results are discussed in this Letter.

The active molecule obtained from high-throughput screening 7114863 (EC_{50} of 0.070 ± 0.002 μM) was synthesized to validate its biological activity. Retrosynthetic analysis of 7114863 revealed that a key precursor acid **6a** was required. The resynthesized

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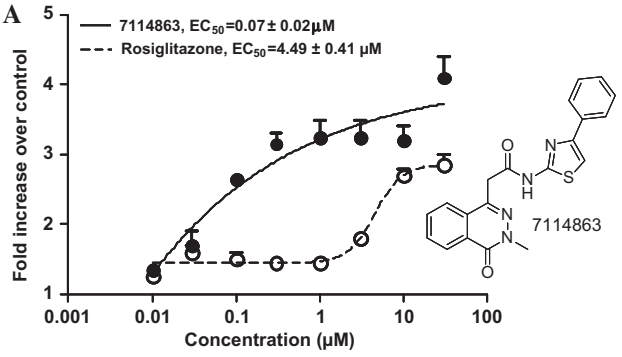
http://dx.doi.org/10.1016/j.bmcl.2013.07.067



B

Compound	% hPPAR-γ activation (n=3)
7114863	0.3
Rosiglitazone, 1 μM	100

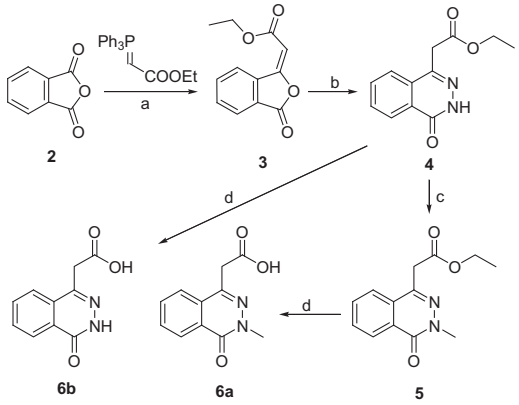
Figure 1. Effect of 7114863 and rosiglitazone on (A). Glucose uptake by L6 myotubes and (B). PPAR-γ activity in human PPAR-γ receptor transfected CV1 cells.

compound (**1a**) showed a reproducible effect on glucose uptake with an EC_{50} of 0.090 ± 0.006 μM. The key precursor **6a** along with its non-methylated analog **6b** were synthesized from commercially available phthalic anhydride in 3–4 steps as depicted in Scheme 1. Phthalic anhydride on treatment with Witting reagent PPh_3=CH–COOEt in chloroform as solvent under reflux conditions produced the desired ester **3** in 51% yield. Ester **3** was then treated with hydrazine hydrate to yield the phthalazine product **4** (71% yield). The phthalazine ester on further treatment with methyl iodide, followed by ester hydrolysis using LiOH produced the desired phthalazine acetic acid **6a**. Similarly its non-methylated analog **6b** was synthesized.

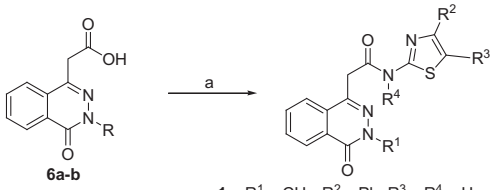
After synthesis of key precursors, acid **6a** was treated with corresponding thiazol-2-yl amine to get desired 7114863 (**1a**) in 46% yield. Similarly by varying acids and different thiazole-amines, a series of analogs **1b**–**1s** were synthesized as depicted in Scheme 2.

Phthalizine ester **4** on treatment with phenacyl bromide in dry DMF in presence of potassium *tert*-butoxide produced *N*-alkylated product **7** in 70% yield, which further on LiOH hydrolysis produced corresponding acid **8** (Scheme 3).

Phthalazine acetic acid **6b** on treatment with hydrazine hydrate under reflux conditions for 30 min produced the corresponding



Scheme 1. Reagents and conditions: (a) $CHCl_3$, reflux, 5 h, 51%; (b) hydrazine hydrate, EtOH, addition at rt and then reflux, 90 °C, 2 h, 71%; (c) MeI, KO*t*Bu, DMF, 30 min, 80%; (d) LiOH, THF:MeOH:water (1:1:1), rt, 8–10 h, 81%.



1a: R^1 = CH_3, R^2 = Ph, R^3 = R^4 = H
1b: R^1 = CH_3, R^2 = Ph(3-F), R^3 = R^4 = H
1c: R^1 = R^3 = CH_3, R^2 = Ph(4-Br), R^4 = H
1d: R^1 = CH_3, R^2 = Ph, R^3 = COOEt, R^4 = H
1e: R^1 = R^3 = CH_3, R^2 = Ph, R^4 = H
1f: R^1 = CH_2CH_2OH, R^2 = Ph, R^3 = R^4 = H
1g: R^1 = CH_3, R^2 = Ph(2-OH), R^3 = R^4 = H
1h: R^1 = CH_3, R^2 = Ph, R^3 = COOH, R^4 = H
1i: R^1 = CH_3, R^2 = R^4 = H, R^3 = Ph
1j: R^1 = CH_3, R^2 = Ph(4-F), R^3 =R^4 = H
1k R^1 = CH_3, R^2 = Ph(4-Br), R^3 = R^4 = H
1l: R^1 = CH_3, R^2 = CH_3, R^3 = COOEt, R^4 = H
1m:R^1 = CH_3, R^2 = Ph(4-CF_3), R^3 = R^4 = H
1n: R^1 = CH_3, R^2 =P h(4-OCH_3), R^3 = CH_3, R^4 = H
1o: R^1 = CH_3, R^2 = R^3 = R^4 = H
1p: R^1 = CH_3, R^2 = Ph(4-Cl), R^3 = R^4 = H
1q R^1 = R^3=R^4 = H, R^2 = Ph
1r: R^1 = CH_3, R^2 = Ph, R^3 = H, R^4 = CH_2COOEt
1s R^1 = R^4 = CH_3, R^2 = Ph, R^3 = H

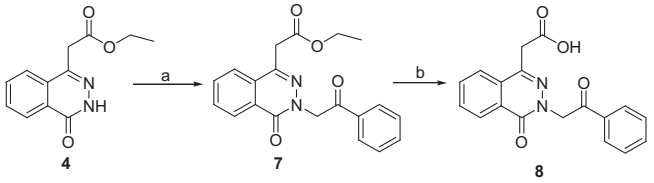
Scheme 2. Reagents and conditions: (a) TBTU, DIPEA, heterocyclic amine, CH_2Cl_2, rt, 48 h, 46%.

hydrazide **9** in 70% yield. Hydrazide **9** on treatment with 4-phenyl substituted acyl chloride (prepared by treating corresponding acid with oxalyl chloride) produced product **10** in 25% yield (Scheme 4).

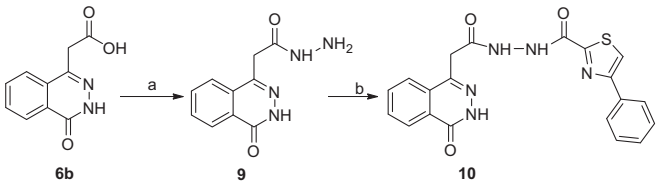
Table 1
Results of preliminary screening at 10 μM[a]

Entry	Fold increase over control	Remarks	Entry	Fold increase over control	Remarks
7114863	1.8	Active	**1s**	1.8	Active
1a	1.8	Active			
1c	3.4	Active	**7**	1.1	Not active
1d	1.3	Not active	**8**	0.9	Not active
1e	3.4	Active	**10**	0.9	Not active
1f	0.8	Not active	**11a**	1.2	Not active
1g	1.3	Not active	**11b**	1.9	Active
1h	1.3	Not active	**11c**	1.3	Not active
1i	1.6	Not active	**11d**	2.0	Active
1j	2.7	Active	**11e**	1.0	Not active
1k	3.6	Active	**11f**	0.7	Not active
1l	0.9	Not active	**11g**	4.9	Active
1m	3.2	Active	**11h**	1.3	Not active
1n	2.9	Active	**11i**	0.8	Not active
1o	1.7	Active	**14a**	0.9	Not active
1p	3.4	Active	**14b**	1.2	Not active
1q	1.8	Active	Rosiglitazone	1.8	Active
1r	1.9	Active			

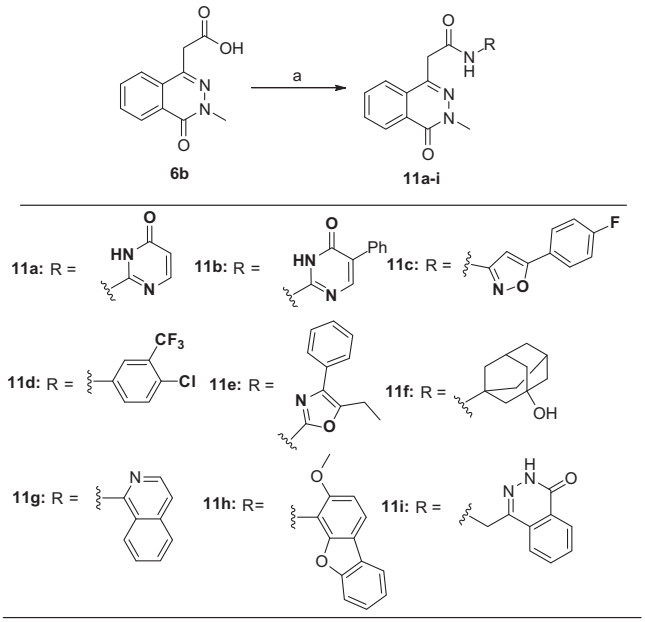
[a] Fold increase of insulin (200 nM) over control was found to be 1.3. Values comparable to the effect of rosiglitazone at the same concentration were considered as active.



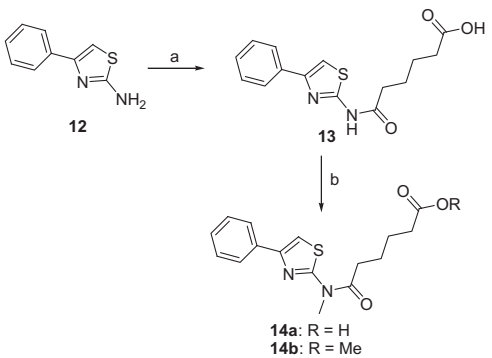
Scheme 3. Reagents and conditions: (a) phenacyl bromide, pot. *tert*-butoxide, DMF, rt, 30 min, 70%; (b) LiOH, THF:MeOH:water (1:1:1), rt, 8–10 h, 80%.



Scheme 4. Reagents and conditions: (a) hydrazine hydrate, reflux, 30 min, 70%; (b) Et_3N, rt, heterocyclic amine, 48 h, 25%.



Scheme 5. Reagents and conditions: (a) TBTU, DIPEA, heterocyclic amine, CH_2Cl_2, rt, 48 h, 40–70%.



Scheme 6. Reagents and conditions: (a) polymeric adipic anhydride, pyridine, rt, overnight, 46%; (b) MeI, DMF, pot-*tert*-butoxide, rt, 30 min, two products **14a** and **14b**.

Table 2
Dose response studies of the actives

Entry	EC_{50} ± SE (μM)	Entry	EC_{50} ± SE (μM)
7114863 [a]	0.070 ± 0.002	**1q**	0.040 ± 0.009
1a[a]	0.090 ± 0.006	**1r**	0.28 ± 0.17
1c	0.003 ± 0.0004	**1s**	5.96 ± 2.7
1e	0.010 ± 0.002		
1k	0.1 ± 0.02	**11b**	7.47 ± 1.7
1l	0.04 ± 0.03	**11d**	2.56 ± 0.85
1n	0.009 ± 0.002	**11g**	0.17 ± 0.02
1o	0.12 ± 0.01	Rosiglitazone	4.49 ± 0.41

[a] 7114863 and **1a** are same compounds, 7114863 was purchased from ChemBridge library and **1a** was synthesized by us.

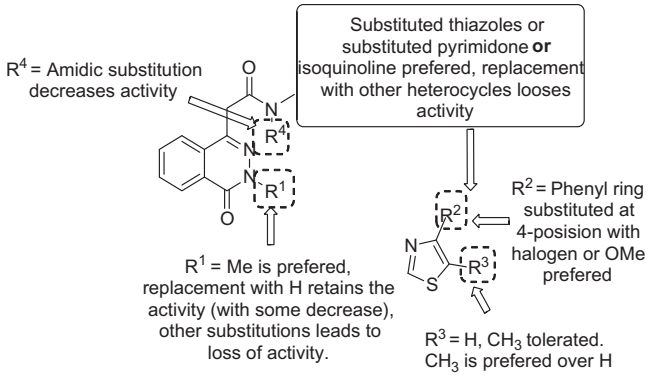
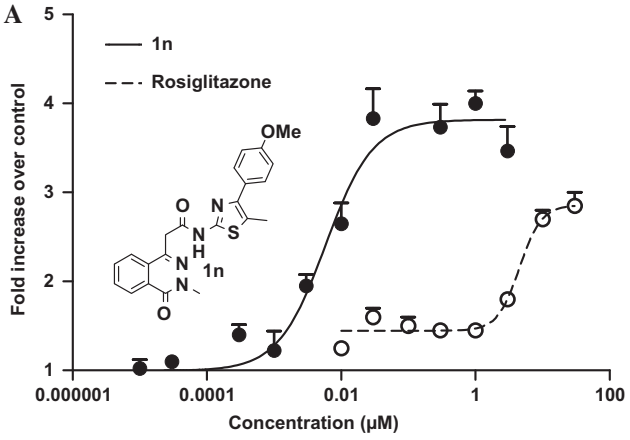


Figure 2. Structure–activity relationship for thiazolyl-phthalazinone acetamide series as potent glucose uptake activators.



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Compound	% hPPAR- γ activation (n=3)
1n	1
Rosiglitazone, 1 μM	100

Figure 3. Effect of **1n** and rosiglitazone on (**A**). Glucose uptake by L6 myotubes and (B). PPAR-γ activity in human PPAR-γ receptor transfected CV1 cells.

Phthalazine acetic acid **6b** on treatment with different heterocyclic amines in presence of TBTU and DIPEA produced corresponding amides **11a–i** as depicted in Scheme 5.

We also synthesized adipic acid linked 4-phenyl thiazole-2-yl compounds **14a** and **14b** as depicted in Scheme 6. All synthesized compounds were characterized by NMR and MS data.[7]

In order to establish the structure–activity relationship around identified novel lead compound, all synthesized analogs were

screened for glucose uptake in L6 rat skeletal muscle cells[8,9] and human skeletal muscle myotubes[10,11] and results are summarized in Table 1. From the biological results, we found that compounds from Schemes 3, 4 and 6 were inactive. The most promising compounds were found from Schemes 2 and 5.

Based on preliminary screening results, active compounds were selected for dose–response studies and EC_{50} values were determined as depicted in Table 2.

Based on the obtained biological results, key structural features essential for glucose uptake activity have been identified. The *N*-substituted phthalazimyl acetamides are tolerated. As depicted in Figure 2, the presence of substituted thiazoles **1**, substituted pyrimidone **11b** and isoquinoline **11d** yielded most active compounds. Among all active analogs, most of them consist of the thiazole moiety linked to phthalazine-acetamide skeleton. Further there was a significant effect of substituent variation on the thiazole-linked phthalazine acetamide skeleton. Methyl substitution on *N1* of phthalazine is preferred over H or other alkyl chains. Substitution on amidic NH resulted in decreased activity. A halogen or OMe substituted phenyl ring at 4th position of thiazole ring is preferred. Methyl substitution at 5th position of thiazole is preferred over unsubstituted position.

In conclusion, we have discovered new scaffolds **1** and **11** for increased glucose uptake in skeletal muscle cells with potential for anti-diabetic activity. Of the above mentioned scaffolds our lead molecule **1n** showed eightfold improvement in glucose uptake activity as compared to 7114863, without inducing PPAR-γ agonist activity (Fig. 3). Additionally, a 23-fold increase in glucose uptake activity for compound **1c** over 7114863 was observed. No further studies were performed on the latter due to the presence of undesirable bromine atom on the molecule.

Acknowledgements

The authors wish to thank Dr. Somesh Sharma, Dr. Rajiv Sharma, Dr. Veena Agarwal and Dr. Arun Balakrishnan of Piramal Enterprises Limited, India for supporting this study and providing the infrastructure needed for carrying out the research work reported in this Letter. We also thank Mr. T.T. Matan and Mr. S. Senthil Kumar for their excellent technical support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.07.067.

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7. Spectral data for selected compounds. 2-(3-Methyl-4-oxo-3,4-dihydrophthalazin-1-yl)-*N*-(4-phenylthiazol-2-yl)acetamide (**1a**): $R_f = 0.4$ (petroleum ether:EtOAc, 1:1); yield: 1.4 g (46%); purity (HPLC): 96.64%; mp 306–308 °C; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.68 (s, 1H), 8.32 (d, $J = 7.8$ Hz, 1H), 7.85–7.97 (m, 5H), 7.64 (s, 1H), 7.44 (t, $J = 7.5$ Hz, 2H), 7.31–7.36 (m, 1H), 4.26 (s, 2H), 3.73 (s, 3H); ESI-MS: m/z 377.1 $[M+H]^+$, 399 $[M+Na]^+$; HRMS: m/z observed 377.1074 $[M+H]^+$ for $C_{20}H_{16}N_4O_2S + H^+$ (377.1067); analysis ($C_{20}H_{16}N_4O_2S$). *N*-(4-(4-Bromophenyl)-5-methylthiazol-2-yl)-2-(3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (**1c**): yield: 51%; purity (HPLC): 98.16%; ^{1}H NMR (DMSO, 300 MHz): δ 12.54 (br s, 1H), 8.32 (d, $J = 7.5$ Hz, 1H), 7.94–7.84 (m, 3H), 7.66 (d, $J = 8.7$ Hz, 2H) 7.61 (d, $J = 8.7$ Hz, 2H), 4.22 (s, 2H), 3.72 (s, 3H), 2.45 (s, 3H); ESI-MS: m/z 471.1 $[M+H]^+$. *N*-(4-(4-Methoxyphenyl)-5-methylthiazol-2-yl)-2-(3-methyl-4-oxo-3,4-dihydrophthalazin-1-yl)acetamide (**1o**): yield: 14 mg; purity (HPLC): 96.64%; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.50 (s, 1H), 8.31 (d, $J = 7.8$ Hz, 1H), 7.85–7.95 (m, 3H), 7.50 (d, $J = 3.6$ Hz, 1H), 7.22 (d, $J = 3.6$ Hz, 1H), 4.23 (s, 2H), 3.72 (s, 3H); ESI-MS: m/z 301.1 $[M+H]^+$, 323.1 $[M+Na]^+$. *N*-(4-(4-Chlorophenyl)thiazol-2-yl)-2-(3-methyl-4-oxo-3,4-dihydroph-thalazin-1-yl)acetamide (**1q**): yield: 15 mg; purity (HPLC): 96.84%; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.66 (s, 1H), 8.29 (d, $J = 7.8$ Hz, 1H), 7.82–8.0 (m, 5H), 7.64 (s, 1H), 7.42–7.47 (m, 2H), 7.31–7.36 (m, 1H), 4.22 (s, 2H); ESI-MS: m/z 363 $[M+H]^+$, 385 $[M+Na]^+$.
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9. Glucose uptake assay in L6 myotubes. L6 rat skeletal muscle cells (ATCC, USA) were cultured in 96 well plates (Nunc) in MEM alpha medium (AMIMED) containing 10% serum and 1% penicillin–streptomycin solution. Differentiation was induced in confluent myotube cultures by culturing the cells in 2% serum. The differentiated fused myotubes were further starved in serum free media, treated with the samples and incubated overnight. After 24 h the cells were pulsed with C14 tagged deoxy glucose (GE Healthcare, UK) for 10 min in presence of insulin (Sigma, St. Louis, MO, USA). Glucose uptake measured by lysing the cells with Microscint PS (Perkin Elmer, USA). The plates were read using a Top Count Reader (Perkin Elmer, USA).
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11. *PPAR-γ luciferase assay:* CV-1 cells were seeded in a 96 well plate (25,000 cells/well) and incubated overnight. Cells were then transiently co-transfected with a luciferase gene under the control of Gal4 DNA binding elements and a plasmid containing the ligand binding domain for PPAR-γ fused to the GAL4 DNA binding domain. All transfections were performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Six hours after transfection, cells were treated with test compounds and incubated for an additional 24 h and the luciferase assay (Promega) was performed according to the manufacture's protocol. Luminescence was measured using POLARStar Optima (BMG-Labtech). Transactivation of human PPAR-γ by 1 μM rosiglitazone was considered as 100% activation and hence data for test compounds were calculated in comparison to rosiglitazone which was used as a positive control in the assay. The entire assay was performed in triplicates and repeated twice.