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Novel benzenesulfonylureas containing thiophenylpyrazoline moiety as potential antidiabetic and anticancer agents

Chetna Kharbanda [a], Mohammad Sarwar Alam [a,*], Hinna Hamid [a], Kalim Javed [a], Syed Shafi [a], Yakub Ali [a], Perwez Alam [b], M. A. Q. Pasha [b], Abhijeet Dhulap [c], Sameena Bano [a], Syed Nazreen [a], Saqlain Haider [a]

[a] Department of Chemistry, Faculty of Science, Hamdard University, New Delhi 110 062, India
[b] Functional Genomics Unit, CSIR-Institute of Genomics & Integrative Biology, Delhi, India
[c] CSIR Unit for Research and Development of Information Products, Pune 411038, India

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ABSTRACT

In the present study a library of twenty six benzenesulfonylureas containing thiophenylpyrazoline moiety has been synthesized. All the compounds were docked against PPAR-γ target. Most of the compounds displayed higher dock score than standard drugs, glibenclamide and rosiglitazone. All the synthesized compounds were primarily evaluated for their antidiabetic effect by oral glucose tolerance test. Further assessment of antidiabetic potential of sixteen active compounds was then done on STZ induced diabetic model. The results of in vivo activity by both the methods were found to be consistent with each other as well as with docking studies. Change in body weight of STZ induced animals post treatment was also assessed at the end of study. In vitro PPAR-γ transactivation assay was performed on active compounds in order to validate docking results and the most active compound **3k** was also shown to elevate gene expression of PPAR-γ. Furthermore, the compounds were screened by National Cancer Institute, Bethesda for anticancer effect and two compounds **3h** and **3i** were selected at one dose level since they exhibited sensitivity towards tumor cell lines (mainly melanoma).

The peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors which are involved in the regulation of lipid and glucose metabolism.[1] PPARs are classified into three subclasses-α, β and γ which are coded by different genes and associated with activation of different target genes by binding to their regulatory region. One of these, PPAR-γ regulates gene expression in response to lipophilic ligands since its ligand binding pocket is quite spacious.[2] PPAR-γ increases fatty acid flux in adipocytes which on contrary alleviates insulin resistance.[3] In addition, many reports contemplated that during adipocyte differentiation, PPAR-γ exerts antimitotic action, thereby reduces the risk of cell proliferation. The activation of PPAR-γ in fibroblasts leads to retraction of cell cycle in tumor cells which have exponential growth rate. It has also been reported that the mutations in PPAR-γ resulting in loss of function were found to be associated with risk of cancer.[4] Therefore, it is evident that there is close relation between PPAR-γ and cancer.

The utility of sulfonylureas have been proved as effective oral antidiabetic agents till date.[5] Consequently, the research is still being pursued by scientists to synthesize new sulfonylurea derivatives with lesser side effects. The mode of action of sulfonylureas is mainly to stimulate insulin secretion by binding to sulfonylurea receptors (SURs) of ATP assisted potassium ion (K_{ATP}) channels present in pancreatic β-cells.[6] Their mechanistic action on molecular level has not yet been too intensively studied. The activation of PPAR-γ is the general known mechanism of thiazolidinediones for improving a glycemic condition.[7,8] But in some recent studies it has been reported that several marketed sulfonylureas also induce PPAR-γ transcriptional activity. Sulfonylureas like glibenclamide, glimepiride, gliquidone and glipizide has been reported to stimulate pancreatic insulin secretion by acting as PPAR-γ agonist in addition to binding with sulfonylurea receptor (SUR) on the plasma membrane of pancreatic β-cells.[9] It has also been studied that glibenclamide binds to PPAR-γ receptor site in a competitive manner with respect to marketed PPAR-γ agonists like pioglitazone and rosiglitazone. Therefore, in the present study we are presenting a library of twenty six sulfonylurea derivatives which have been showing significant antidiabetic activity by acting as PPAR-γ agonists. Increase in body weight which is one of the major side effects

* Corresponding author. Tel.: +91 11 26059688 (5555); fax: +91 11 26059663.
E-mail addresses: msalam@jamiahamdard.ac.in, msalam5555@gmail.com (M.S. Alam).

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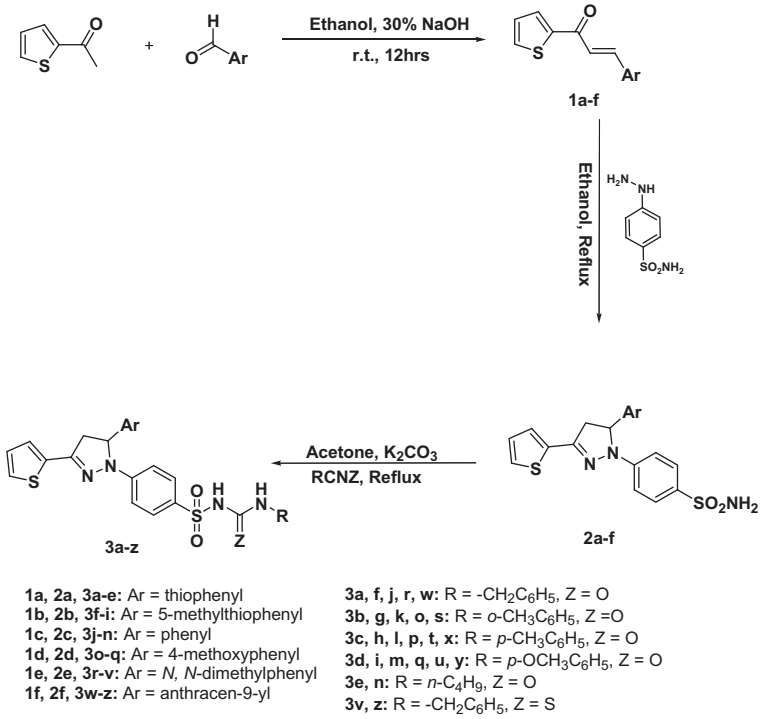

Scheme 1.

of sulfonylurea agent has also been evaluated for their effect on body weight.

The chalcones (**1a–f**) obtained by reacting 2-acetyl thiophene with appropriate aldehyde resulted in the synthesis of key intermediates, that is, pyrazolines (**2a–f**). The pyrazolines so obtained were made to react with appropriate aryl isocyanate and aryl isothiocyanate in the presence of anhydrous potassium carbonate and acetone by conventional reactions.[10,11] The resulting solid was filtered, washed with water, dried, and crystallized from acetone (Scheme 1). All reagents were procured from commercial source and used without further purification unless indicated. The progress of reaction was monitored by using silica gel GF245 plates (Merck Pvt. Ltd, Germany). The purification of compounds was carried out by recrystallization from ethanol. ^{1}H NMR, IR, Mass spectra and CHNS data was also presented to confirm the synthesis of desired product. ^{1}H NMR spectra of all the synthesized compounds was recorded in parts per million (ppm) using TMS as the standard and at 300–400 MHz frequency. The appearance of three double doublets in the range of δ 3.00–6.00 ppm confirmed the formation of pyrazoline ring. The coupling with isocyanate/isothiocyanate was verified by increase in aromatic protons depending on the substituent. ^{1}H NMR of the compound **3k** at 400 MHz showed singlet at chemical shift 2.09 ppm corresponding to three methyl protons present on tolyl group attached to sulfonylurea linkage. The formation of pyrazoline ring was confirmed by the presence of three double doublets at 3.13 (J = 5.6 Hz, 17.2 Hz), 3.94 (J = 12.0 Hz, 17.2 Hz) and 5.54 ppm (J = 5.6 Hz, 12.0 Hz). Remaining aromatic protons appeared as multiplet in the region 6.69–7.84 ppm. Mass spectra showing peaks at $[M+H]^+$ or $[M-H]^+$ further confirmed the syntheses of desired products.

As discussed earlier, the mode of action of action of sulfonylureas can also be through the activation of PPAR-γ. Such reports on sulfonylureas prompted us to carry out molecular docking studies of synthesized compounds. In order to get better insight of ligand–protein interaction, all the synthesized compounds were docked and their dock scores were compared to that of standard drugs glibenclamide and rosiglitazone. It has been found that glibenclamide (−7.42) exhibited higher docking score than rosiglitazone (−5.72). Captivatingly, docking score of nineteen compounds (**3a–n**, **3p**, **3r**, **3t**, **3v**, **3w**) was found to be higher than rosiglitazone and sixteen compounds (**3a**, **3c–f**, **3h**, **3i–n**, **3p**, **3r**, **3t**, **3w**) showed higher docking score than glibenclamide. On careful examination of different substitutions, it could be inferred that most of the compounds containing unsubstituted benzyl ring exhibited higher docking score than both the standards. The alignment of compounds in active PPAR-γ site was such that the most of the compounds formed H-bond with different amino acid residues. In some cases, more than one H-bond formation or π–π stacking was also found. The images of the compounds displaying high docking score have been shown in Figure 1 and dock scores were given in Table S1 (Supplementary material).

The efficacy of administration of all the synthesized compounds as oral antidiabetic agents has been assessed primarily by loading glucose on normal rats. It has been observed that in comparison to standard drug glibenclamide, seven compounds (**3d**, **3i–n**) significantly controlled increase in plasma glucose level. However, sixteen compounds (**3a**, **3c–f**, **3h**, **3i–n**, **3p**, **3r**, **3t**, **3v**) exhibited significant lowering in plasma glucose level than PPAR-γ agonist, rosiglitazone. The change in plasma glucose level was illustrated graphically in Figure 2. The result of Glucose Tolerance Test on in vivo model was consistent with docking studies. Few exceptions from docking results were related to the compounds with bulky aryl substitution. For instance for compound **3w** docking score was as high as −11.54 but in vivo antidiabetic activity was less significant to standard drug glibenclamide which showed docking score of −7.42.

The compounds which showed significant glucose tolerance were further evaluated for their effect in STZ induced diabetic model.[12] It was found that the results of both the studies are in concurrence. The change in plasma glucose level on 7th and 15th day of study was assessed and given in Figure 3. The supplementation of diabetic rats with glibenclamide as well as rosiglitazone

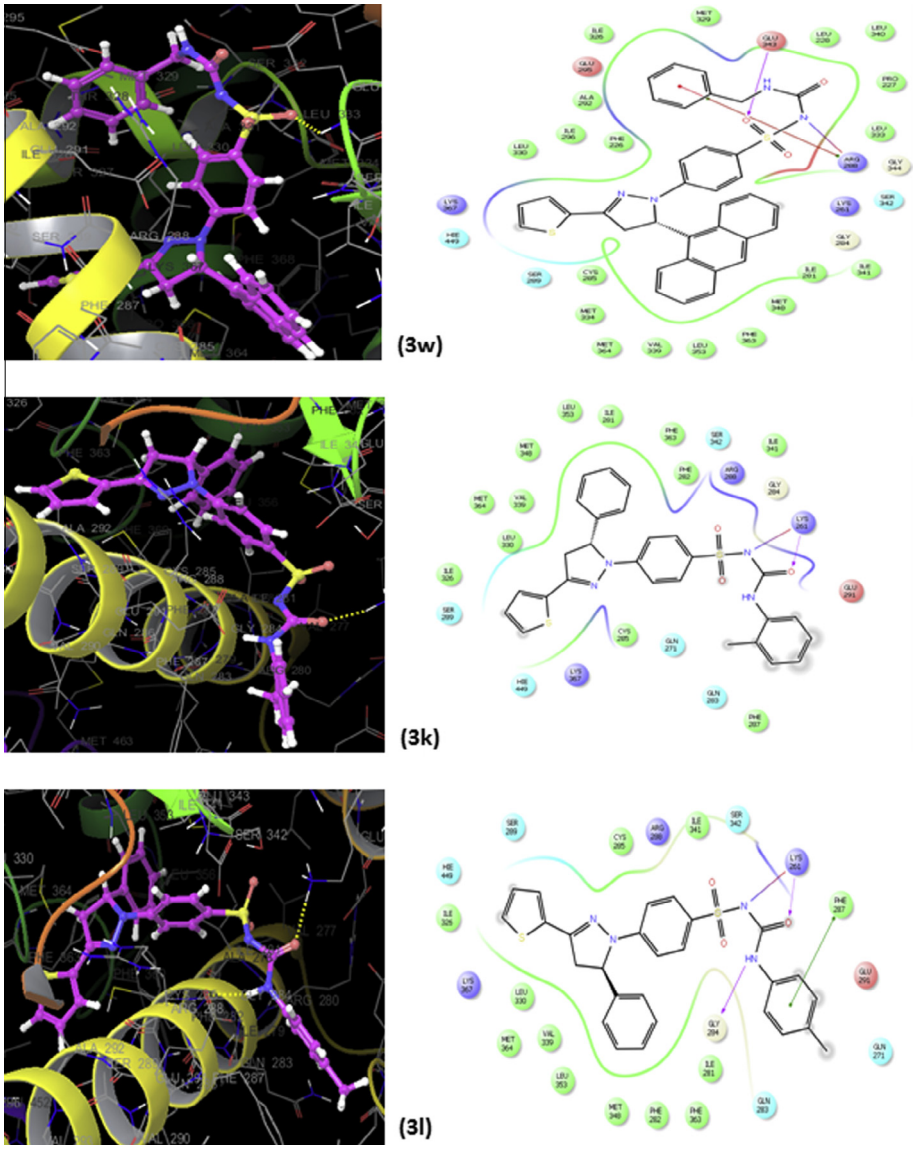

Figure 1. Images of the compounds (**3w**, **k**, **l**) in PPAR-γ active site.

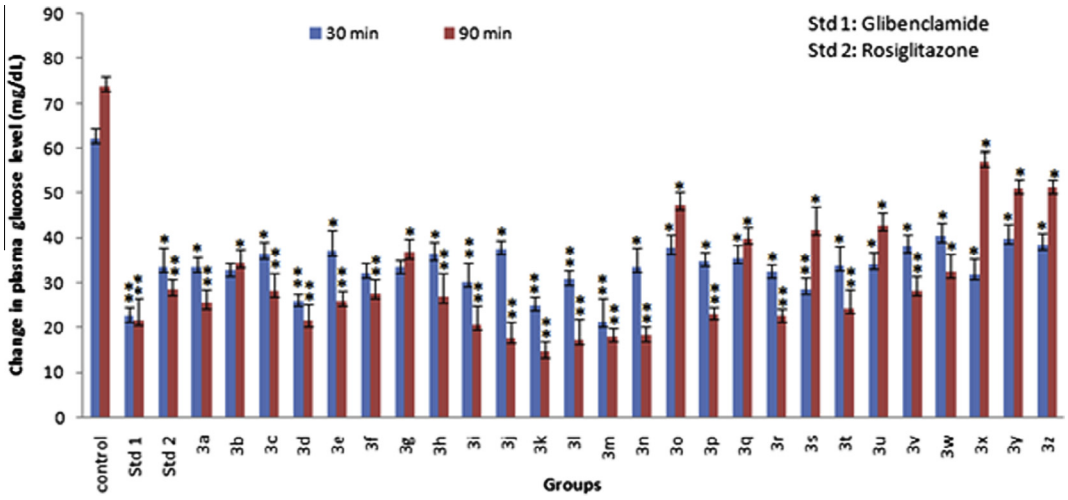

Figure 2. Antidiabetic effect of treating wistar rats with synthesized compounds by Oral Glucose Tolerance Test. Data is analyzed by one way ANOVA followed by Dunnett test and expressed as mean ± SEM from six observations; * represents change as compared to control; ** indicates $p < 0.01$ & * indicates $p < 0.05$.

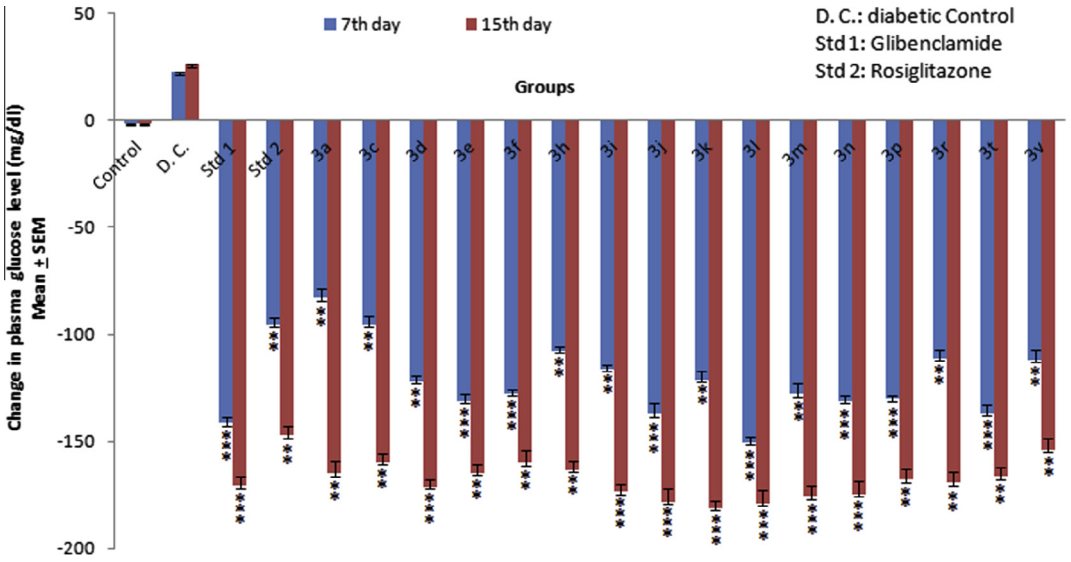

Figure 3. Antidiabetic effect of treating STZ-induced wistar rats with synthesized compounds. Data is analyzed by one way ANOVA followed by Dunnett test and expressed as mean ± SEM from six observations; * represents change as compared to diabetic control; ** indicates $p < 0.01$ & *** indicates $p < 0.001$.

restored plasma glucose level near to normal but the alleviation effect of seven synthesized compounds (**3d**, **3i–n**) was comparatively more pronounced.

Structure activity relationship has been generated to understand the effect of different substitutions placed around pyrazoline ring and correlations that have been drawn from it are as follows (Fig. 4):

- Most of the compounds containing unsubstituted phenyl group showed maximum docking score as well as significant in vivo antidiabetic activity.
- However substitution on aryl ring linked to urea/thio urea linkage did not show any particular trend in regards to activity or docking score.
- Substituting bulky aryl groups like anthracene significantly lowered the in vivo antidiabetic activity even if docking score was high.
- Replacing aryl ring with thiophenyl ring exhibited in vivo activity almost as significant as benzyl ring substitution. However, thiophenyl ring substituted with methyl group did not bring significant variation in comparison to unsubstituted thiophenyl ring.
- Replacing urea linkage with thiourea linkage significantly lowered the in vivo antidiabetic activity as well as dock score.

The active compounds (**3a**, **3c–f**, **3h**, **3i–n**, **3p**, **3r**, **3t**, **3v**) were scrutinized by in vitro PPAR transactivation assay to validate their mechanism of action. The result of transactivation activity of these

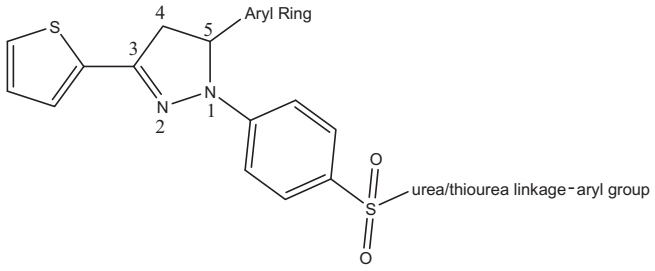

Figure 4. General structure of synthesized sulfonylurea.

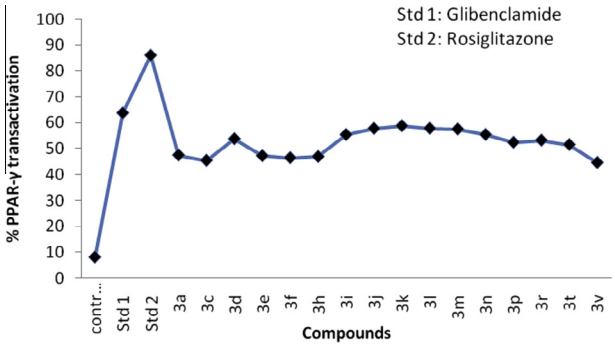

Figure 5. In vitro PPAR-γ transactivation assay on 3T3-L1 cell line.

compounds was presented in Figure 5. In contrast to in vivo results, the transactivation activity of glibenclamide as well as active compounds was found to be quite less than the reference drug, rosiglitazone. The elevated in vivo activity might be due to the fact that sulfonylureas not only activate PPAR-γ but also bind to SU receptors present in pancreatic β cells when acts in vivo. Additionally, the transactivational assay involves different extent of co-activator recruitment by different ligands which are also responsible for changes in conformation of target protein.

The treatment with sulfonylurea agents is potentially associated with increase in body weight. Therefore, in the present study the change in body weight was carefully observed over the period of 15 days of study. The induction of STZ resulted in significant weight loss after 15th day of study (Fig. 6). The standard groups and groups treated with compounds **3e**, **3h**, **3n**, **3r** showed significant increase in body weight. However, this increase in body weight was appreciably controlled in groups treated with compounds **3a**, **3c–k**, **3m**, **3p**, **3t**, **3v** in comparison to glibenclamide and rosiglitazone treated groups.

Finally, the effect of the most potent compound **3k** on the expression of PPAR-γ target genes were measured in 3T3-L1 fibroblasts in comparison to standard drugs, rosiglitazone and glibenclamide (Fig. 7). The compound **3k** significantly enhanced the expression of PPAR-γ genes but the result was not as significant as rosiglitazone. It was found that rosiglitazone augmented gene expression relatively by 1.5 folds whereas the compound **3k**

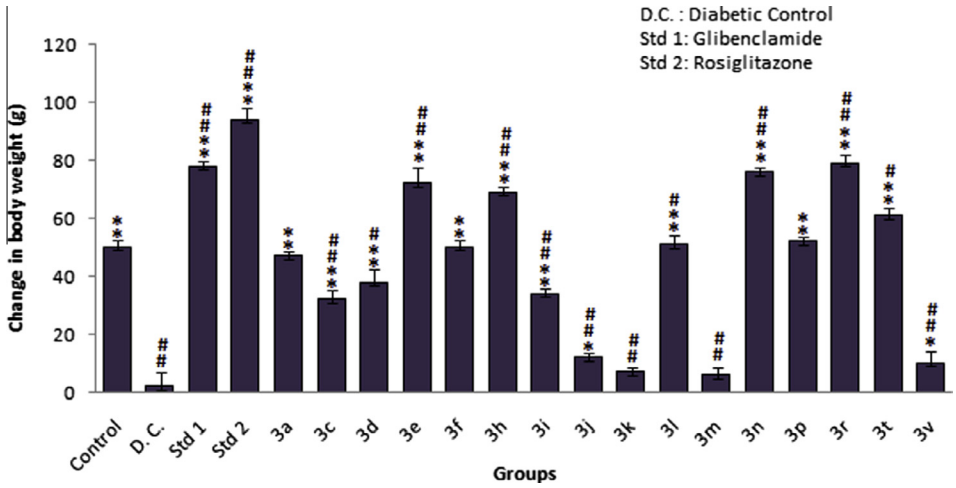

Figure 6. Change in body weight after 15 day study on STZ-induced diabetic rats. Data is analyzed by one way ANOVA followed by Bonferroni '*t*' test and expressed as mean ± SEM from six observations; * represents significant change as compared to diabetic control; # represents significant change as compared to normal control. ** indicates $p < 0.01$ & * indicates $p < 0.05$; ## indicates $p < 0.05$ & # indicates $p < 0.01$.

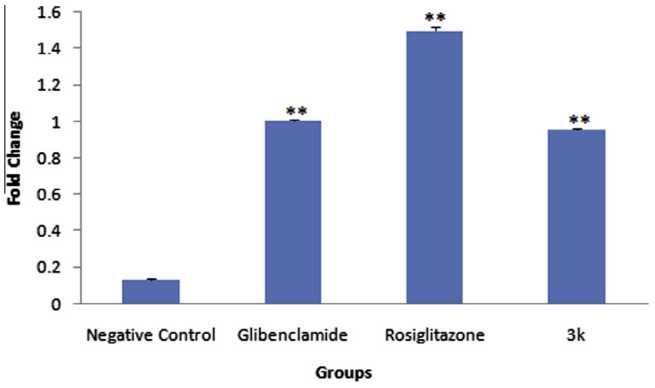

Figure 7. PPAR-γ gene expression evaluation of compound **3k** in comparison to standards, glibenclamide and rosiglitazone. Data is analyzed by one way ANOVA followed by Dunnett test and expressed as mean ± SEM from three observations; * represents change as compared to control; ** indicates $p < 0.01$.

caused 0.95 fold elevation. On the other hand, the gene expression was ameliorated by one fold on treatment with glibenclamide.

Several reports have been extended to prove the anticancer potential of PPAR-γ and sulfonylureas. Therefore, the structures of twenty six compounds have been submitted to NCI for evaluating their anticancer activity. Among all the synthesized compounds, two compounds (**3h**, **3i**) have been selected by NCI, Bethesda at one dose level study. Both these compounds **3h** and **3i** showed mean growth percent of 89.29% and 87.76%, respectively (Table 1). Both these compounds engaged in mild sensitivity towards melanoma (MALME-3M, M14, MDA-MB-435, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62) with growth percent less than 62%. The compound **3i** also exhibited pronounced sensitivity (5.45%) towards A549/ATCC cell line cultured from non-small cell lung cancer.

It can be concluded that ten compounds out of twenty six compounds have been found to possess significant antidiabetic potential without inducing increase in body weight. Mechanistically, these compounds are engaged in the activation of PPAR-γ as observed from in vitro transactivation assay. One of the compound **3k**, which exhibited remarkably high antidiabetic activity has also shown significant increase in PPAR-γ gene expression. Two compounds **3h** and **3i** also exhibited potency against cancer. Therefore, these sulfonylurea derivatives can be explored further

Table 1
Anticancer data of compounds **3h** and **3i** provided by NCI

Panel	Cell line	Growth percent	
		NSC: 777382/1 (**3i**)	NSC: 777381/1 (**3h**)
Leukemia	CCRF-CEM	88.12	87.89
	HL-60(TB)	102.86	126.37
	K-562	111.39	113.81
	MOLT-4	94.70	94.41
	RPMI-8226	95.29	83.37
	SR	75.83	84.69
Non-small cell lung cancer	A549/ATCC	5.45	101.55
	HOP-62	81.02	76.92
	HOP-92	87.06	84.91
	NCI-H226	110.15	110.48
	NCI-H23	97.55	98.53
	NCI-H322M	102.37	103.57
	NCI-H460	108.95	103.16
	NCI-H522	87.33	91.51
Colon cancer	COLO 205	−63.47	−54.62
	HCC-2998	111.31	108.61
	HCT-116	98.77	85.12
	HCT-15	102.46	99.31
	HT29	119.52	127.85
	KM12	98.51	97.36
	SW-620	107.16	106.34
CNS cancer	SF-268	97.85	95.62
	SF-295	104.70	100.07
	SF-539	104.31	98.31
	SNB-19	103.16	108.18
	SNB-75	99.87	93.74
	U251	98.07	94.12
Melanoma	LOX IMVI	96.81	99.74
	MALME-3M	31.15	40.64
	M14	52.12	58.39
	MDA-MB-435	52.30	61.95
	SK-MEL-28	57.56	60.91
	SK-MEL-5	48.27	47.78
	UACC-257	49.98	61.42
	UACC-62	30.03	40.91
		31.00	38.15
Ovarian cancer	IGROV1	108.05	107.76
	OVCAR-3	106.38	98.17
	OVCAR-4	102.84	106.40
	OVCAR-5	113.48	114.78
	OVCAR-8	106.90	99.82
	NCI/ADR-RES	103.44	102.87

Table 1 (*continued*)

Panel	Cell line	Growth percent	
		NSC: 777382/1 (**3i**)	NSC: 777381/1 (**3h**)
	SK-OV-3	82.55	78.27
Renal cancer	786-0	100.87	103.79
	A498	101.86	109.00
	ACHN	93.16	96.60
	CAKI-1	94.74	95.78
	RXF 393	—	—
	SN12C	111.79	111.21
	TK-10	96.03	96.78
	UO-31	87.18	85.69
Prostate cancer	PC-3	96.33	86.34
	DU-145	105.62	102.33
Breast cancer	MCF7	99.04	97.09
	MDA-MB-231/ ATCC	113.39	116.79
	HS 578T	97.52	96.05
	BT-549	116.23	74.25
	T-47D	85.68	78.79
	MDA-MB-468	—	—
Mean	87.76	89.29	

to design more potent antidiabetic and anticancer agents with lesser side effects.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.044.

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