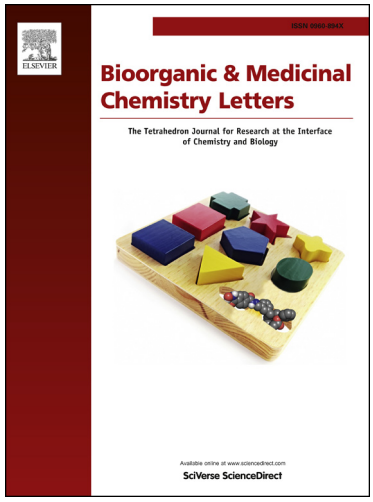


Antidiabetic effect of novel benzenesulfonylureas as PPAR-γ agonists and their anticancer effect

Chetna Kharbanda, Mohammad Sarwar Alam, Hinna Hamid, Kalim Javed, Abhijeet Dhulap, Sameena Bano, Yakub Ali

PII: S0960-894X(15)00884-7
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.08.062
Reference: BMCL 23056

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 5 March 2015
Revised Date: 14 August 2015
Accepted Date: 19 August 2015

Please cite this article as: Kharbanda, C., Alam, M.S., Hamid, H., Javed, K., Dhulap, A., Bano, S., Ali, Y., Antidiabetic effect of novel benzenesulfonylureas as PPAR-γ agonists and their anticancer effect, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.08.062

Antidiabetic effect of novel benzenesulfonylureas as PPAR-γ agonists and their anticancer effect.

Chetna Kharbanda[a], Mohammad Sarwar Alam[a], Hinna Hamid[a], Kalim Javed[a], Abhijeet Dhulap[b], Sameena Bano[a], Yakub Ali[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.* [c]*CSIR Unit for Research and Development of Information Products, Pune -411038.*

Correspondence to Prof. Mohammad Sarwar Alam; Email ID msalam@jamiahamdard.ac.in., msalam5555@gmail.com Phone:+91 11 26059688 (5555), Fax:+91 11 26059663

Abstract

Twenty one pyrazoline containing benzenesulfonylureas were synthesized and docked against PPAR-γ target. All the compounds were first screened for their antidiabetic potential by oral glucose tolerance test and then six active compounds were assessed on STZ diabetic model. It was found that five compounds showed significantly high antidiabetic activity in comparison to glibenclamide as well as rosiglitazone (standard drugs). The active compounds were evaluated for their effect on body weight since weight management is one of the main concerns associated with sulfonylureas. Finally, the most active compound **6f** was shown to elevate PPAR-γ gene expression. The synthesized compounds were also screened for anticancer activity by National Cancer Institute. Five compounds (**5i, 6e, 6g, 6i** and **6j**) were selected at one dose level and showed potency against cancers.

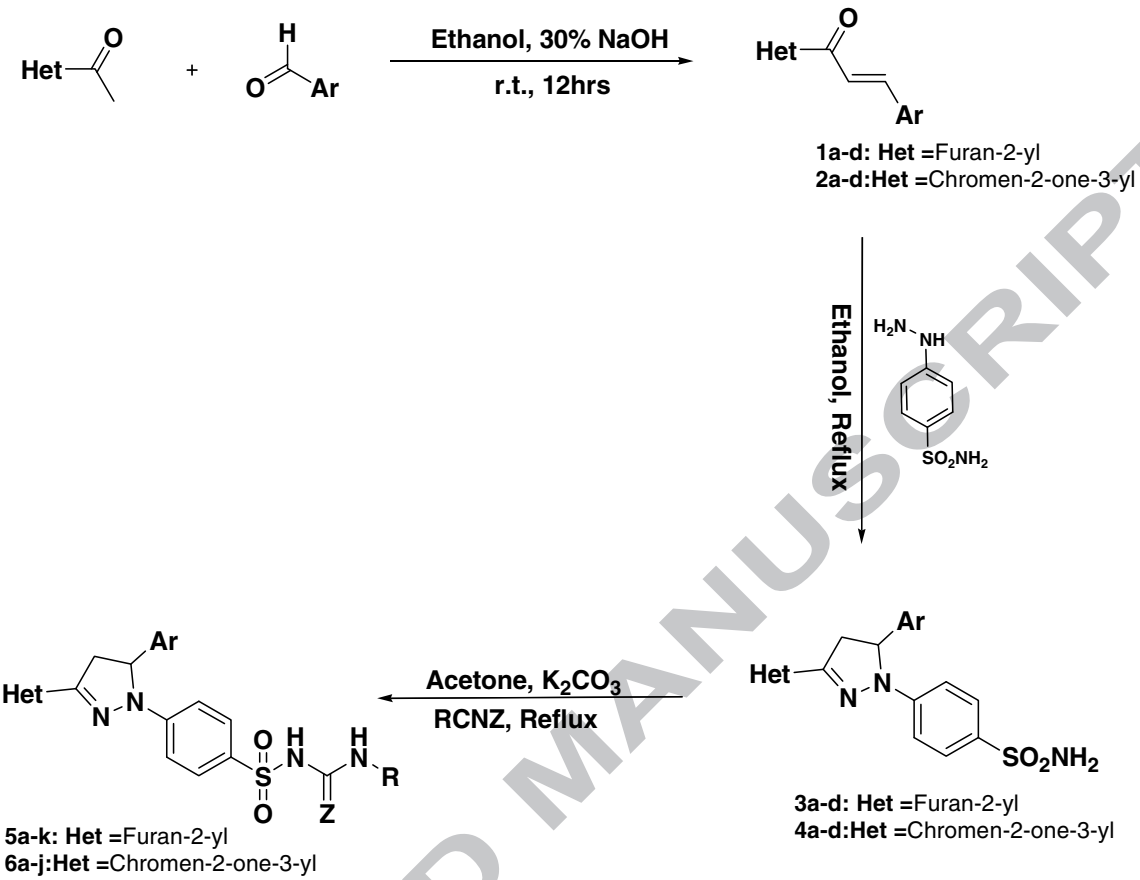
Keywords: Sulfonylureas, PPAR-γ, antidiabetic, anticancer, gene expression.

The prevalence of diabetes worldwide is the matter of serious concern with the exponential increase in diabetic subjects every year.[1] Besides many oral therapies present in the market, there is still urgent requirement to guide appropriate therapy in order to overcome side effects of presently available treatments. Sulfonylureas are the oldest known but are still being used as potential oral antidiabetic agents. Sulfonylureas like glibenclamide, glimepiride and glicazide etc were reported to induce transcriptional activity of PPAR-γ in a competitive manner than known PPAR-γ agonists, rosiglitazone and pioglitazone.[2-6] The activation of PPAR-γ also affiliates to

antineoplastic prodifferentiation which in turn, slows down the progression of cancer. PPAR-γ agonists therefore induce inhibition of angiogenesis and metastasis leading to suppression of tumor development and growth which is usually augmented by prolonged persistence of diabetic condition.[7] Therefore, in continuation of our previously reported study,[8] twenty one novel pyrazoline derived sulfonylureas were synthesized and were evaluated for their action as PPAR-γ agonists. The effect on body weight was studied during the duration of antidiabetic activity. The synthesized compounds were also evaluated for their effect on different tumor cell lines.

The chalcones (**1a-d and 2a-d**) obtained by reacting compound **1** and **2** with appropriate aldehydes resulted in the synthesis of corresponding pyrazoline intermediates **(3a-d** and **4a-d).** These pyrazolines were then reacted with appropriate aryl isocyanate and aryl isothiocyanate by conventional method in presence of anhydrous potassium carbonate and acetone.[9] The reaction mixture was refluxed until the reactants were consumed. After the completion of reaction, the mixture was concentrated and poured on ice. The resulting solid was filtered, and dried. The product was crystallized from acetone (**Scheme 1**) and further purified by recrystallization from ethanol. The synthesis of desired product was confirmed by ^{1}H NMR, ^{13}C NMR, IR, Mass spectra and CHNS data. ^{1}H NMR spectra was recorded at 300-400 MHz and expressed as parts per million (ppm). The formation of pyrazoline ring was confirmed by the appearance of three double doublets in the range of δ 3.00-6.00 ppm which appeared due to protons present on C-4 of pyrazoline ring. ESI-MS spectra exhibited the presence of peaks at $[M+H]^{+}$.

Scheme 1:

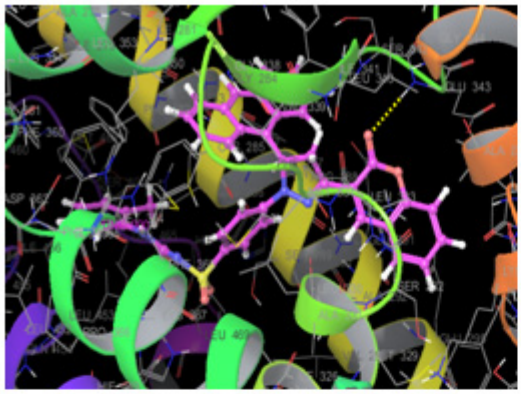


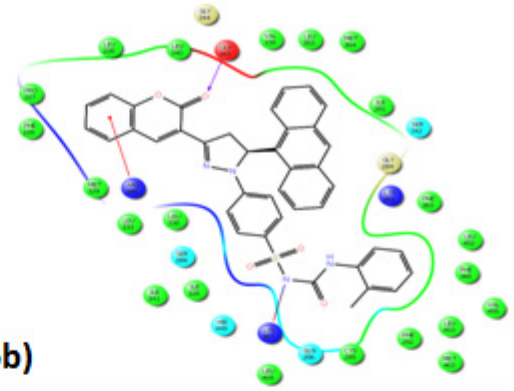
Compound	Ar	R	Z
5a, 6a	anthracen-9-yl	Phenyl	O
5b, 6b	anthracen-9-yl	*o*-tolyl	O
5c, 6c	anthracen-9-yl	Phenyl	S
5d, 6d	anthracen-9-yl	*p*-methoxyphenyl	O
5e, 6e	*N,N*-dimethylphenyl	phenyl	O
5f, 6f	*N,N*-dimethylphenyl	*o*-tolyl	O
5g, 6g	*N,N*-dimethylphenyl	*p*-methoxyphenyl	O
5h	*N,N*-dimethylphenyl	Phenyl	S
5i	*p*-methoxyphenyl	Phenyl	O
6h	*p*-methoxyphenyl	Phenyl	S
5j, 6i	*p*-methoxyphenyl	*o*-tolyl	O
5k	*p*-methoxyphenyl	*p*-methoxyphenyl	O
6j	thiophen-2-yl	Phenyl	O

Recent reports on sulfonylureas acting as PPAR-γ agonists prompted us to carry out *in silico* molecular docking of synthesized compounds with ligand binding domain of PPAR-γ before performing *in vivo* studies. It was found that out of twenty one compounds, ten compounds (**5a, 5c-f, 5i, 6b, 6f, 6h and 6i**) were found to exhibit higher dock score than glibenclamide and thirteen compounds showed dock score higher than rosiglitazone. Some of the compounds could not be docked inside PPAR-γ receptor site due to their bulky structure and rigid confirmation. It should be noted that eight synthesized compounds (**5a, 5c-f, 6f, 6b,** and **6i**) showed dock scores more than -8 where the standards, glibenclamide and rosiglitazone showed -7.43 and -5.72, respectively. Therefore, indicating the effective interaction of these synthesized compounds with receptor site of PPAR-γ. On examination of docking images of the ligands, it was clear that these compounds formed H-bonding, π-π stacking or both with amino acid residues of receptor site. Some compounds showed multiple H-bonds with the amino acid residues of receptor while some of the compounds did not show any visible binding with amino acid residues but were deeply buried inside the site (**Fig 1**). Also, the drug likeness parameters of the molecules were evaluated by computing the physicochemical properties of all the compounds. The Lipinski's rule of five defined the standard properties for compounds to behave as a good drug. These includes the molecular weight less than 500 gms/mol, H-bond donors upto 5, H-bond acceptors upto 10 and octanol-water partition coefficient (log P) not greater than 5. All the synthesized molecules have nearly acceptable ranges of the Lipinski's rule of 5.

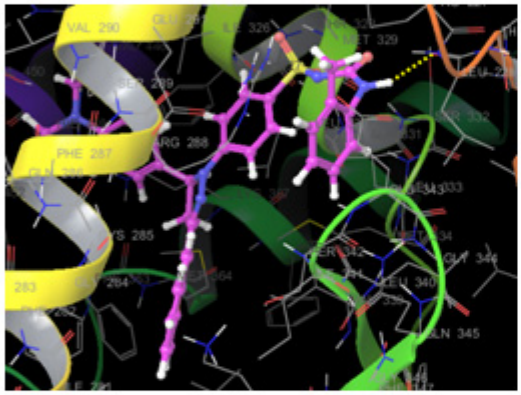
The molecular weight of the compounds ranges in 530-694, while the predicted octanol/water partition coefficient (Log P o/w) are in the range of 3.4–7.5. Both the acceptable molecular weight and log P values attributes to better absorption of the synthesized compounds as oral drugs. Furthermore, the number of hydrogen bond donors and acceptors are within the standard range and are 1-2 & 5-8 respectively contributing to overall better fat solubility and cell wall permeability. Additionally, the lower solubility property (log S) values contributes to better absorption and distribution characteristics and are ranging from -11.73 to -4.5. Comprehensively, the synthesized compounds showed better ADME properties as summarized in Table S1.

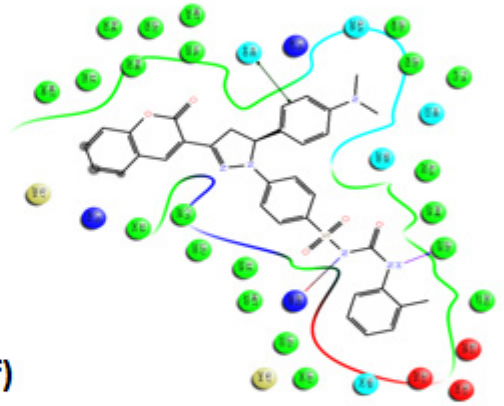
Fig 1: Images of the compounds (**3w, k, l**) in PPAR-γ active site.



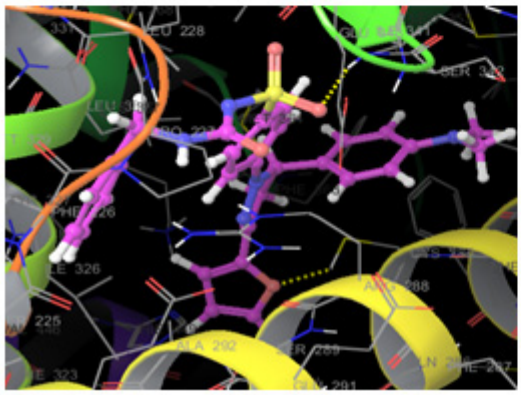


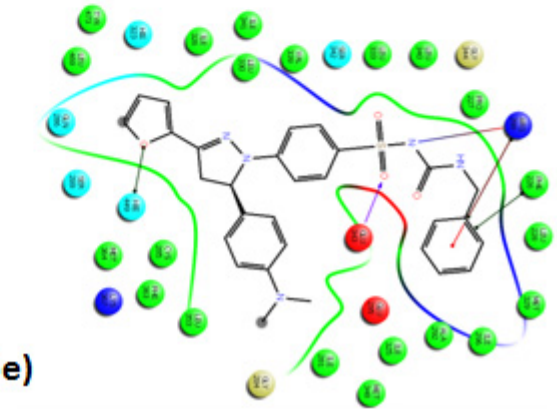
(6b)





(6f)



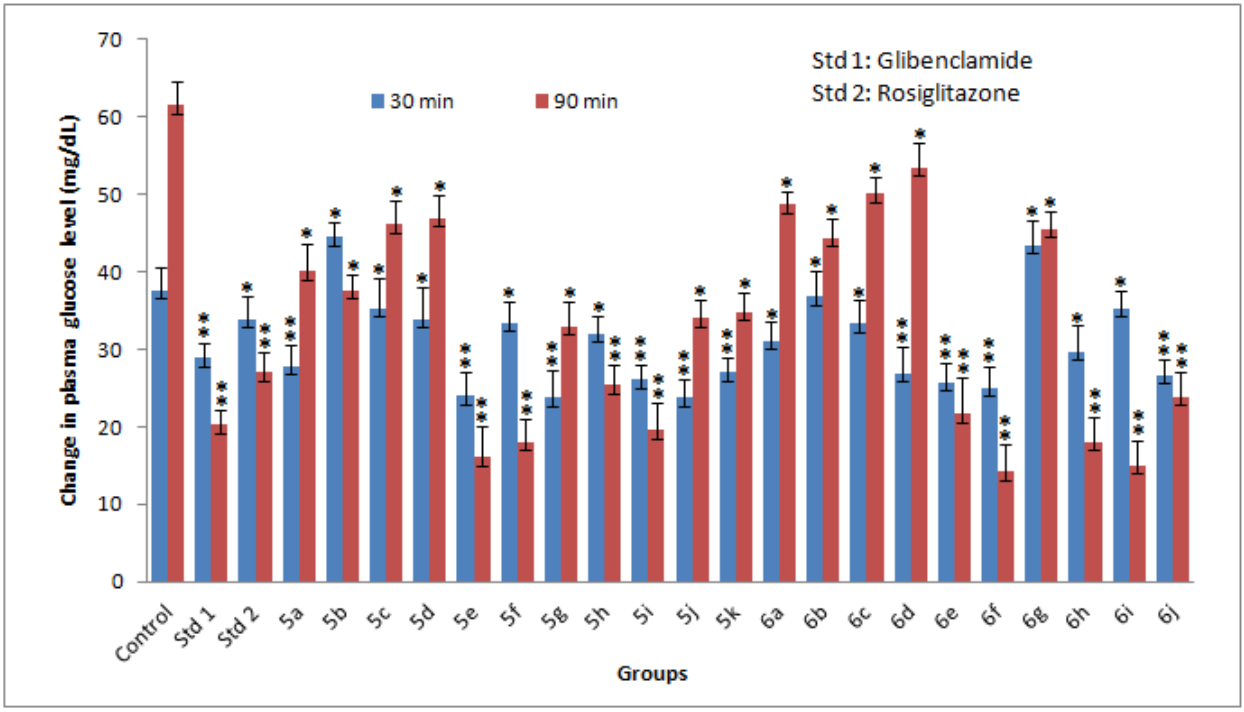


(5e)

All the synthesized compounds were first evaluated by performing oral glucose tolerance test to discern their effect on plasma glucose level **(Fig 2)**. The efficacies of compounds **5a-k** and **6a-j** were compared with glibenclamide and rosiglitazone. The results showed that six compound **(5e, 5f, 5i, 6f, 6h and 6i)** lowered plasma glucose level more significantly than the standard drugs, glibenclamide as well as rosiglitazone. Five compounds out of remaining compounds (**5g, 5h, 5j, 6e** and **6j**) showed comparably significant antidiabetic activity with respect to standard

drugs. Comparing the results of *in vivo* antidiabetic activity with docking results showed that some of the compounds that showed very high dock score were not dynamically contributing in plasma glucose level maintenance while performing in *in vivo* system. All these compounds that displayed high degree of deviation in results contained bulky aryl group like anthracen-9-yl. Although the presence of bulky aryl group facilitated the formation of H-bonding and other interactions inside the receptor ligand domain of PPAR-γ (predicted by *in silico* study) but their presence might affect other factors that were needed for the bioavailability of compound in *in vivo* rat model.

Fig 2: Antidiabetic effect of treating wistar rats with synthesized compounds by Oral Glucose Tolerance Test.

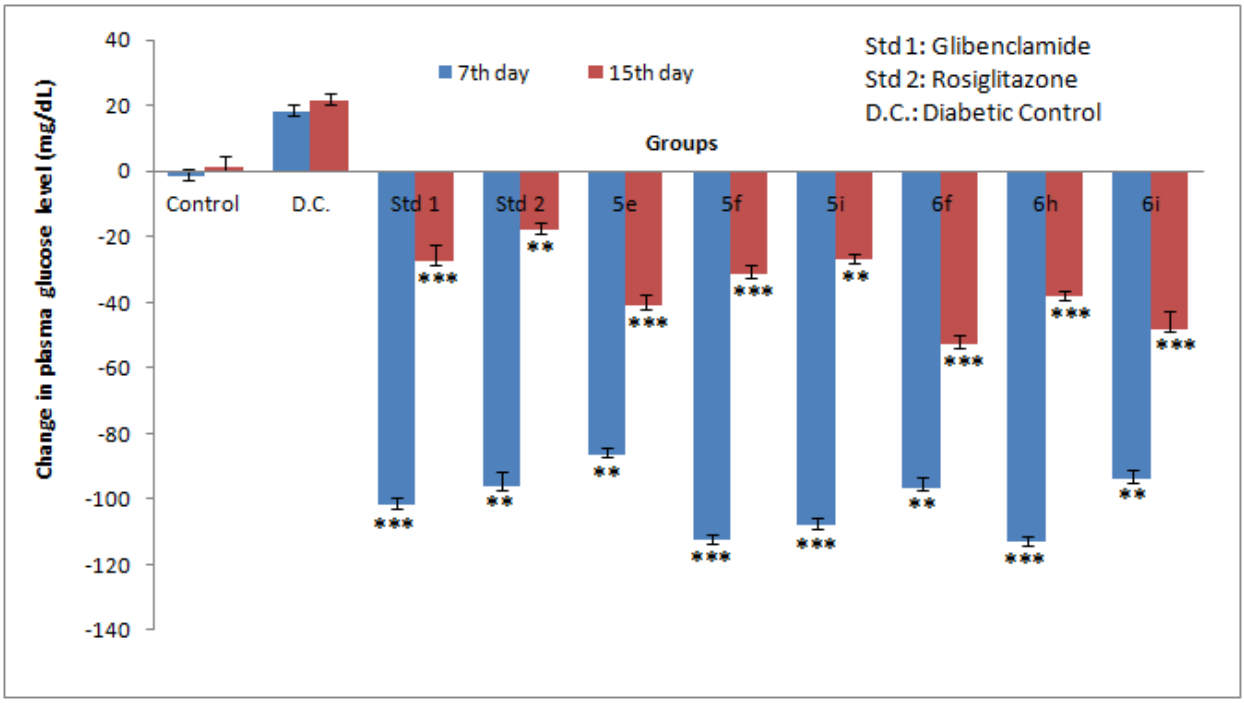


Data is analyzed by one way ANOVA followed by Dunnett test and expressed as mean ± SEM from six observations; * represents change as compared to control; ** indicates $p < 0.01$ & * indicates $p < 0.05$.

Six active compounds **(5e, 5f, 5i, 6f, 6h and 6i)** that exhibited significantly higher antidiabetic activity in comparison to both glibenclamide and rosiglitazone were subsequently assessed for their effect on STZ-induced diabetic rat model (**Fig 3**). It was observed that all the screened compounds showed excellent antidiabetic potential. Compounds **(5e, 5f, 6f, 6h and 6i)**

alleviated plasma glucose level more significantly in comparison to both the standard drugs on 15th day of study. The ameliorative effect of compound **5i** was found to be more significant than rosiglitazone but slightly less appreciable than glibenclamide. The order of activity followed by *in vivo* antidiabetic activity of these compounds on STZ-induced diabetic rats was similar to that observed in OGT test.

Fig 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 $p < 0.001$.

On the basis of the above studies, the activity of synthesized compounds can be related to the structures as follows (**Fig S1**):

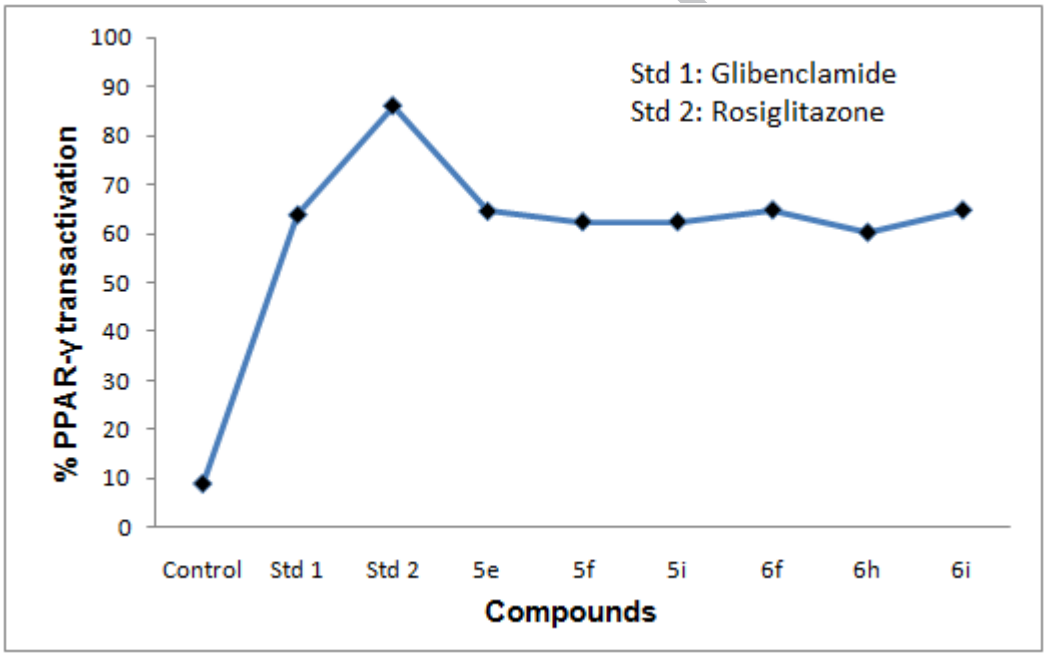
- The compounds substituted with bulky aryl groups showed very high dock score but very less *in vivo* antidiabetic activity.
- Substitution of *N,N*-dimethyl on the para position of aryl group resulted in significant increase in *in vivo* antidiabetic activity.
- Methoxy substitution at para position of aryl group also increased the biological activity but less significantly than *N,N*-dimethyl substitution

- Different substitutions at R position affected the activity in the order: o-tolyl>p-tolyl>benzyl>p-methoxyphenyl.

However, no particular differentiation on the activity was observed on the basis of different heterocycles used.

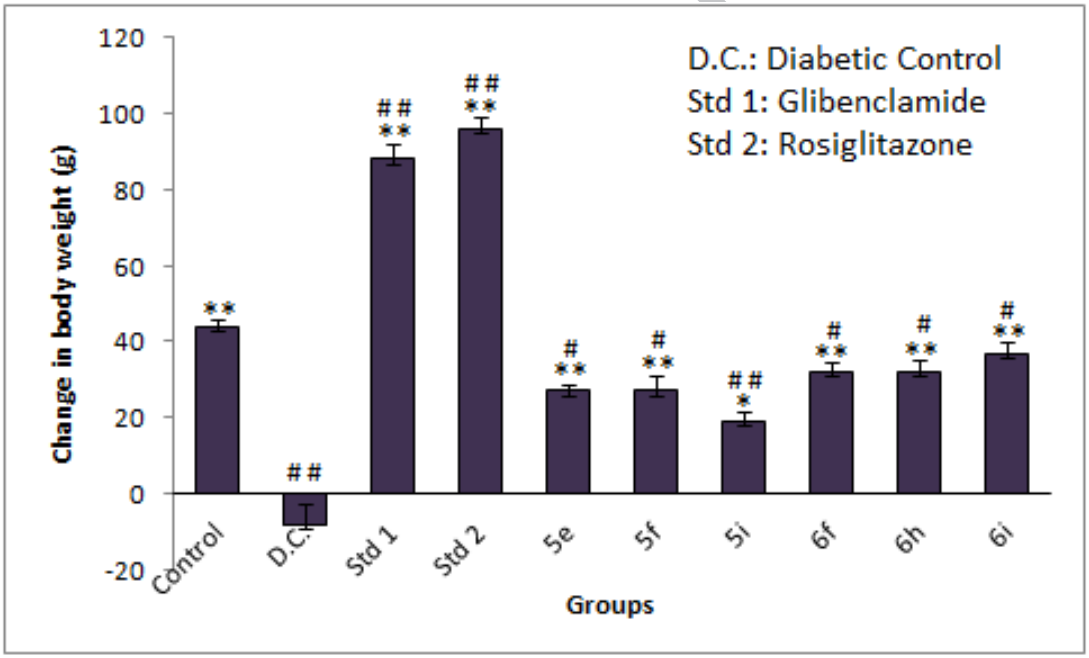
Since *in vivo* biological activity of active compounds followed similar pattern as ensued from docking study, *in vitro* PPAR transactivation assay was performed to further corroborate that these compounds are acting as PPAR agonists. Six selected compounds **(5e, 5f, 5i, 6f, 6h and 6i)** transactivated PPAR more or less as significantly as glibenclamide but contrary to *in vivo* results, all these compounds as well as glibenclamide showed less significant results in comparison to rosiglitazone. This variation showed that the synthesized compounds opted PPAR transactivation as an additional pathway apart from binding to SURs in pancreatic β-cells (**Fig. 4**).

Fig. 4: *In vitro* PPAR-γ transactivation assay on 3T3-L1 cell line.



It has earlier been reported that the diabetic subjects treated with sulfonylureas are more susceptible to weight gain. Moreover, the activation of PPAR-γ being closely related to increased adipose differentiation leads to increase in body weight. Therefore, it was necessary to study the effect on body weight of diabetic rats after supplementation with synthesized compounds. In the present study, it was observed that the animals of diabetic control underwent weight loss during the study period of 15 days while the groups treated with both the standards, glibenclamide and rosiglitazone observed obtrusive gain in weight (**Fig. 5**). However, the body weights of diabetic animals were substantially maintained even after administration of active synthesized compounds (**5e, 5f, 5i, 6f, 6h and 6i**). Therefore, the incorporation of pyrazoline moiety in sulfonylurea derivatives might direct researchers towards antidiabetic drugs with lesser side effects.

Fig 5: Change in body weight after 15 day study on STZ-induced diabetic rats.

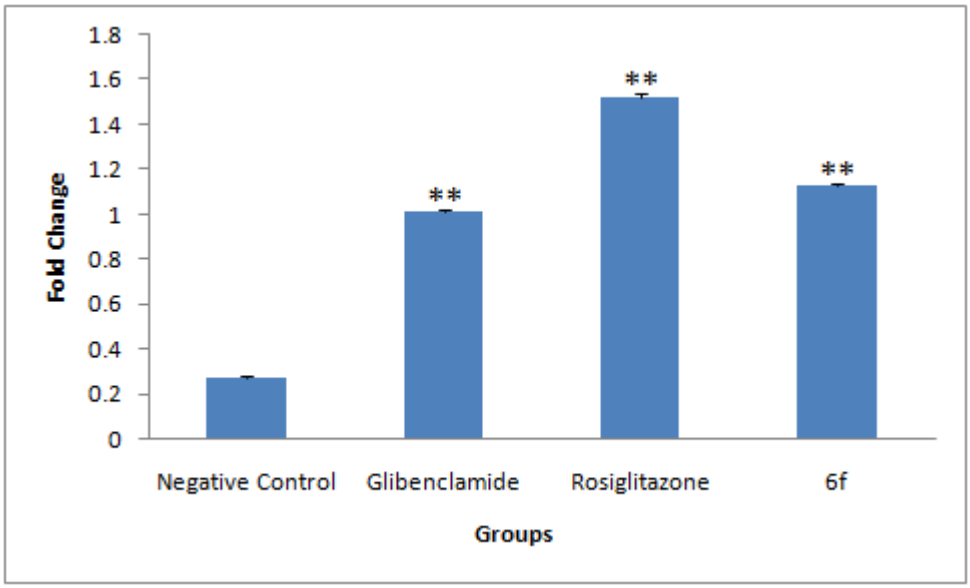


Data is analyzed by one way ANOVA followed by Bonferroni ´t´ test and expressed as mean ± SEM from six observations; * represents significant change as compared to diabetic control; # represents significant change as compared to normal control.** indicates $p < 0.01$ & * indicates $p < 0.05$; ## indicates $p < 0.05$ & # indicates $p < 0.01$.

Finally the most active compound **6f** was assessed for its effect on the expression of PPAR-γ gene (**Fig. 6**). Compound **6f** augmented PPAR-γ gene expression significantly by 1.13 fold whereas glibenclamide exhibited only 1.01 fold increase. Rosiglitazone on the other hand,

enhanced the expression of PPAR-γ gene by 1.52 fold. Therefore, it is clear that the compound **6f** acted as PPAR-γ agonist.

Fig. 6: PPAR-γ gene expression evaluation of compound **6f** in comparison to 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 $p < 0.01$.

PPAR-γ activators have been reported to induce growth arrest in order to inhibit cellular transformation and angiogenesis of tumor cell. This phenomenon consequently suppresses tumor growth and development.[10] Therefore, the structures of all the synthesized compounds were screened for their anticancer potential at National Cancer Institute (NCI), Bethesda. Five compounds named **5i, 6e, 6g, 6i and 6j** were evaluated at one dose level and exhibited growth percent of 101.49, 65.75, 95.53, 93.37 and 102.80%, respectively (**Table S2**). The compound **6e** exhibited sensitivity towards all the tumor cell lines but the effect was more pronounced for SR (leukemia), HCT-15 (colon cancer), SNB-75 (CNS cancer) and UACC-62 (melanoma) with only 19.69, 32.79, 27.14 and 37.65% growth, respectively.

Finally, it can be concluded that among all the synthesized compounds six compounds **5e, 5f, 5i, 6f, 6h and 6i** exhibited significant antidiabetic potential while five compounds **5i, 6e, 6g, 6i and 6j** showed moderate potency against cancer. The compound **6f** was found to be the most active antidiabetic agent and was observed to have activity greater than rosiglitazone and

glibenclamide in *in vivo* system. The synthesized compounds were engaged in the activation of PPAR-γ responsible for increased insulin sensitivity without any noteworthy weight gain. Therefore, the present library of compounds can be modified further to enhance their efficacy against both diabetes as well as cancer in order to get new drugs with less associated side effects.

Acknowledgements

The authors wish to express their thanks to Dr G N Qazi, Vice Chancellor, Jamia Hamdard for providing necessary research facilities and to the staff members of National Cancer Institute (NCI), USA, for performing *in vitro* anticancer screening. The authors are thankful to Dr. Javed Iqbal, former Director, Institute of Life Sciences (ILS), Hyderabad, for providing necessary *in vitro* facilities and Dr. Parimal Misra for his help in performing *in vitro* studies. One of the authors, CK is also thankful to University Grant Commission for providing financial assistance.

References

1. Wild, S., Roglic, G., Green, A., Sicree, R., King, H. *Diabetes Care.* **2004**, *27,* 1047.
2. Fukuen, S.; Iwaki, M.; Yasui, A.; Makishima, M.; Matsuda, M.; Shimomura, I. *J. Biol. Chem.* **2005**, *280*, 23653.
3. Scarsi, M.; Podvinec, M.; Roth, A.; Hug, H.; Kersten, S.; Albrecht, H.; Schwede, T.; Meyer, U.A.; Rucker, C. *Mol. Pharmacol.* **2007**, *71*, 398.
4. Arrault, A.; Rocchi, S.; Picard, F.; Maurois, P.; Pirotte, B.; Vamecq, J. *Biomed. Pharmacother.* **2009**,*63*,56.
5. Lee, K.W.; Ku, Y.H.; Kim, M.; Ahn, B.Y.; Chung, S.S.; Park, K.S. *Diabetes Metab. J.* **2011**, *35*, 340.
6. Inukai, K.; Watanabe, M.; Nakashima, Y.; Takata, N.; Isoyama, A.; Sawa, T.; Kurihara, S.; Awata, T.; Katayama, S. *Biochem. Biophys. Res. Commun.* **2005**, *328*, 484.
7. Rosen, E.D.; Spiegelman, B.M. *J. Biol. Chem.* **2001,** *276,* 37731.
8. Kharbanda, C., Alam, M.S., Hamid, H., Javed, K., Shafi, S., Ali, Y., Alam, P., Pasha, M.A.Q., Dhulap, A., Bano, S., Nazreen, S., Haider, S. *Bioorg. Med. Chem. Lett*. **2014,** *24*, 5298.
9. Arrault, A.; Rocchi, S.; Picard, F.; Maurois, P.; Pirotte, B.; Vamecq, J. *Biomed. Pharmacother*. **2009**, *63*, 56.
10. Xin, X., Yang, S., Kowalski, J., Gerritsen, M.E. *J. Biol. Chem.* 1999, *274*, 9116.

Graphical Abstract