Bioorg. Med. Chem. Lett. 34 (2021) 127760

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Microwave-assisted efficient synthesis of pyrazole-fibrate derivatives as stimulators of glucose uptake in skeletal muscle cells

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ARTICLE INFO

*Keywords:*
Pyrazolo[3,4-*d*]pyrimidinones
GLUT4*myc* translocation
Diabetes mellitus

ABSTRACT

The design and synthesis of a series of pyrazolo[3,4-*d*]pyrimidinones containing fibrate side chains have been accomplished by utilizing the concept of molecular hybridization. All the synthesized compounds were evaluated for the glucose uptake stimulatory effect in L6 rat skeletal muscle cells. Four compounds (**3f**, **3g**, **3j** and **3q**) were found to show significant stimulation of glucose uptake. Further these four compounds have been examined for their Glut4 translocation stimulatory effect in L6-Glut4*myc* myotubes. Compound **3q** was found to exert maximum increase in GLUT4*myc* translocation.

Diabetes mellitus (DM) is a multifactorial metabolic syndrome marked by high blood glucose level due to defective insulin secretion, impaired insulin action or both.[1] The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications as well as an increased threat for cardiovascular diseases (CVD).[2] Diabetes has been considered as the fast growing epidemic worldwide. Type 2 diabetes mellitus is the most common type of diabetes that accounts for 90–95% of all cases of diabetes.[3] Insulin resistance is the main pathophysiological feature of type 2 diabetes mellitus, characterized by the reduced ability of insulin sensitive tissues to respond effectively to normal levels of insulin.[4]

Insulin resistance in major insulin sensitive tissues, including skeletal muscles, liver and fat tissue leads to imbalance of glucose homoeostasis,[5] resulting in establishment of hyperglycemia. Skeletal muscle has major contribution in postprandial glucose disposal, which accounts for more than 80% of insulin-dependent glucose disposal in human.[6] Glucose uptake is the rate-limiting step in skeletal muscle.[7] In skeletal muscle cells, glucose uptake results from the enhanced translocation and redistribution of insulin sensitive glucose transporter 4 (GLUT4) to the cell membrane, which acts as a shuttle to move sugar across the cell surface.[8] Under insulin resistance, the translocation of GLUT4 gets reduced, resulting in a consequent defect in the insulin-stimulated glucose uptake.[9] Thus, interventions with ability to stimulate GLUT4 translocation are useful for the treatment of diabetes mellitus.[3]

Current therapeutics for diabetes are often associated with undesirable side effects such as, weight gain, which consequently increases the risk of insulin resistance leading to enhance in drug dose.[10] Therefore, there is a need to introduce new and improved novel antidiabetic agents. Large numbers of structurally diverse molecules were designed and biologically evaluated against type 2 diabetes mellitus. In the design and synthesis of novel antidiabetic compounds, we found several reports in which compounds containing pyrazole and pyrimidone rings were endowed with potent antidiabetic activity due to their unique heterocyclic structure.[11] For example, Balaglitazone, which contains pyrimidone, has been used in clinical trials to manage blood glucose levels in type 2 diabetes mellitus.[12] In addition linagliptin,[13] alogliptin,[14] teneligliptin, drugs from liptin family are used to treat type 2 diabetes mellitus.[15] These drugs contain either pyrazole or pyrimidone in their structure. Narihiro et al. have reported pyrazole containing compound showing potent glucose lowering effects.[16] Eduardo et al. have designed pyrazole containing rimonabant derivatives and evaluated them for their antidiabetic properties.[17] Vasu et al. have synthesized a novel

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https://doi.org/10.1016/j.bmcl.2020.127760
Received 16 June 2020; Received in revised form 9 December 2020; Accepted 20 December 2020
Available online 25 December 2020

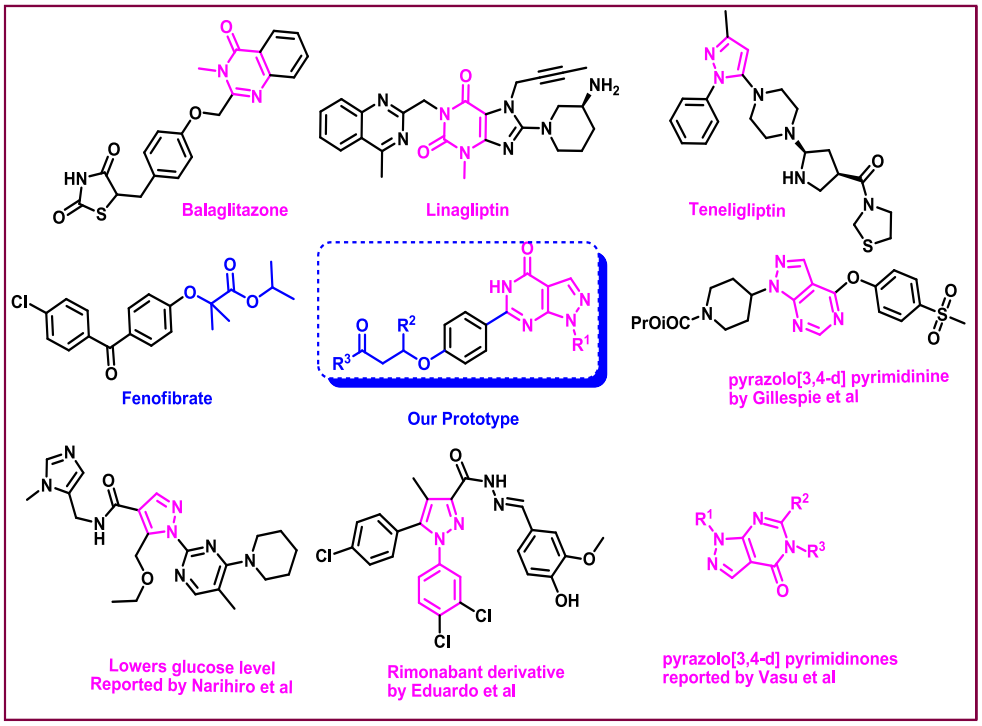
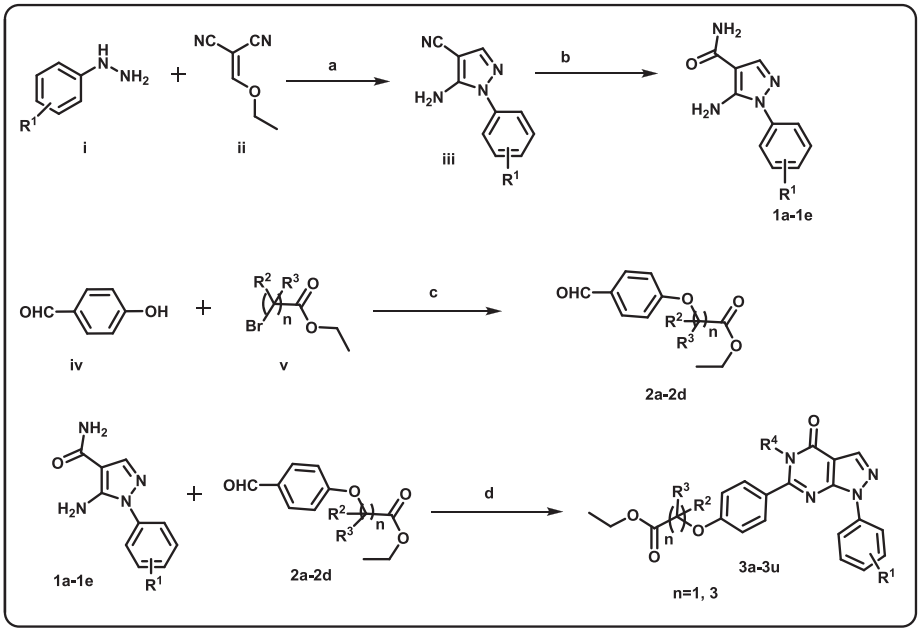



**Fig. 1.** Designing of pyrazole-fibrate derivatives as T2DM agents.

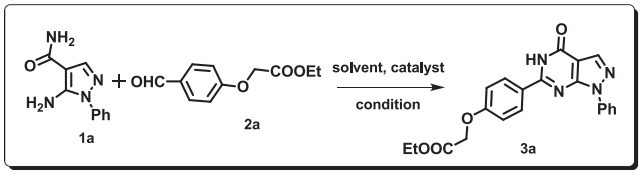
series of fused pyrazolo[3,4-*d*] pyrimidinones and found them to exhibit promising *in vivo* blood glucose-lowering activity.[15] Gillespie et al. have discovered potent pyrazolo[3,4-*d*]pyrimidine derivatives with promising pharmacokinetic profile, which reduced post-prandial glucose levels in mice.[18] Likewise, fibrates are also reported in the literature as an interesting class of compound. Fibrates are used for the treatment of dyslipidemia are agonists of PPARα. It lowers plasma triglycerides via activation of lipid catabolism. Fibrates also increase plasma HDL and are therefore hypolipidemic agents.[19] These are structurally and pharmacologically related to the thiazolidinediones,[20] a class of anti-diabetic drugs. Herein, we describe a novel and simple method to synthesize pyrimidinone ring starting from 5-aminopyrazole-4-carboxamide **1** and aryl aldehyde **2**. With our long standing interest in the concept of molecular hybridization,[21] we have hybridised pyrazole-pyrimidone moiety with fibrate side chain to obtain twenty-one compounds and evaluated them for glucose uptake stimulatory effect in skeletal muscle cells (Fig. 1).

Previously several methods were reported in the literature for the synthesis of pyrazolo[3,4-*d*]pyrimidinones by the cyclisation of 5-aminopyrazole-4-carboxamide derivatives with carboxylic acid,[22] acid




**Scheme 1.** Synthesis of novel pyrazole-fibrate derivatives. Reagent and Conditions: a) Ethanol, reflux, 6 h, b) $H_2SO_4$, toluene 60 °C, 3–4 h, c) $K_2CO_3$, Acetonitrile, reflux 3 h, d) AcOH, DDQ, 140 °C, MW irradiation, 30 min.

**Table 1**
Optimization of reaction conditions.[a]

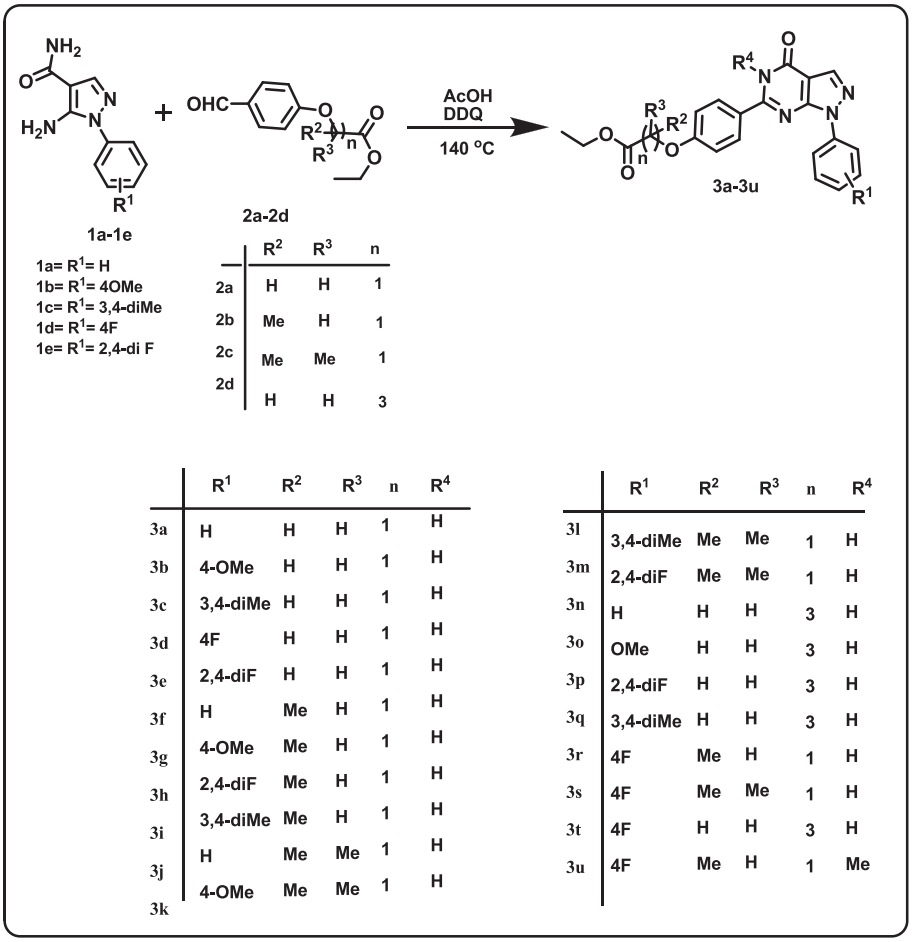



| Entry | Solvent | Catalyst | Condition | Yield |
|---|---|---|---|---|
| 1 | Toluene | *p*TSA(20 mol%) | reflux/6h | NR |
| 2 | Water | – | 120 °C/8h | NR |
| 3 | DMF | – | 100 °C/4h | 20% |
| 4 | DMF | $I_2$(0.5 equiv.) | 100 °C/6h | 44% |
| 5 | EtOH | $CuCl_2{\cdot}H_2O$(20 mol%) | reflux/8h | 25% |
| 6 | DMSO | – | 120 °C/8h | 30% |
| 7 | AcOH | – | 120 °C/6h | 30% |
| 8 | AcOH | $I_2$(0.5 equiv.) | 120 °C/8h | 40% |
| 9 | AcOH | $KMnO_4$(1 equiv.) | 120 °C/8h | 45% |
| 10 | AcOH | DDQ(1 equiv.) | 140 °C/ 8 h | 60% |
| **11** | **AcOH** | **DDQ(2 equiv.)** | **140 °C/8 h** | **88%** |
| **12**[b] | **AcOH** | **DDQ(2 equiv.)** | **140 °C/30 min** | **90%** |

Bold represents optimized reaction conditions.
[a] All reactions were carried out using **1a** (0.5 mmol), **1b** (0.5 mmol), solvent (4 mL) at indicated time.
[b] Microwave irradiation, NR no reaction.

chloride,[23] ester,[24] ketone,[25] aldehyde[26] in different reaction conditions. Herein we are reporting the synthesis of pyrazolo[3,4-*d*]pyrimidinones from 5-aminopyrazole-4-carboxamide derivatives and substituted aryl aldehyde derivatives. The reaction proceeds via condensation of aryl aldehyde (**2a–2d**) and pyrazole-4-carboxamide (**1a–1e)** to afford Schiff base, followed by the cyclisation leading the formation of pyrazolo[3,4-*d*]pyrimidinones derivatives. In the literature this cyclocondensation reaction is reported in various catalytic systems and solvents such as AcOH,[26] $I_2$/DMF,[27] piperidine/EtOH,[28] *p*TSA/benzene,[29] and $I_2$/CAN.[30] Most of the reported method possessed drawbacks like prolonged reaction time, low yield. Herein we report a convenient method which gives cyclocondensation product with high yield in relatively short duration. We found in AcOH and DDQ medium the reaction occurs within 30 min at 140 °C under microwave irradiation. The pyrazole-4-carboxamides (**1a–1e**) were prepared from substituted 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitriles via hydrolysis in toluene medium using $H_2SO_4$ (2:1) at 60 °C. The substituted benzaldehydes (**2a**–**2d**) were obtained via substitution reaction of 4-hydroxybenzaldehyde with appropriate bromo esters in the presence of $K_2CO_3$ in acetonitrile under reflux conditions. An efficient cyclocondensation reaction took place between suitable amide derivatives (**1a–1e**) and substituted benzaldehyde derivatives (**2a–d**) using DDQ in AcOH at 140 °C which provided final pyrazole pyrimidone-fibrate hybrids (**3a–3u**) in good to excellent yields (Scheme 1).

To optimize the reaction conditions for the synthesis of pyrazole pyrimidone-fibrate hybrid, the reactions were carried out using carboxamide intermediates (**1a**) and substituted benzaldehydes derivative




| | R2 | R3 | n |
|---|---|---|---|
| 2a | H | H | 1 |
| 2b | Me | H | 1 |
| 2c | Me | Me | 1 |
| 2d | H | H | 3 |

| | R1 | R2 | R3 | n | R4 |
|---|---|---|---|---|---|
| 3a | H | H | H | 1 | H |
| 3b | 4-OMe | H | H | 1 | H |
| 3c | 3,4-diMe | H | H | 1 | H |
| 3d | 4F | H | H | 1 | H |
| 3e | 2,4-diF | H | H | 1 | H |
| 3f | H | Me | H | 1 | H |
| 3g | 4-OMe | Me | H | 1 | H |
| 3h | 2,4-diF | Me | H | 1 | H |
| 3i | 3,4-diMe | Me | H | 1 | H |
| 3j | H | Me | Me | 1 | H |
| 3k | 4-OMe | Me | Me | 1 | H |

| | R1 | R2 | R3 | n | R4 |
|---|---|---|---|---|---|
| 3l | 3,4-diMe | Me | Me | 1 | H |
| 3m | 2,4-diF | Me | Me | 1 | H |
| 3n | H | H | H | 3 | H |
| 3o | OMe | H | H | 3 | H |
| 3p | 2,4-diF | H | H | 3 | H |
| 3q | 3,4-diMe | H | H | 3 | H |
| 3r | 4F | Me | H | 1 | H |
| 3s | 4F | Me | Me | 1 | H |
| 3t | 4F | H | H | 3 | H |
| 3u | 4F | Me | H | 1 | Me |

**Scheme 2.** Synthetic strategy for the synthesis of mini library of compounds.

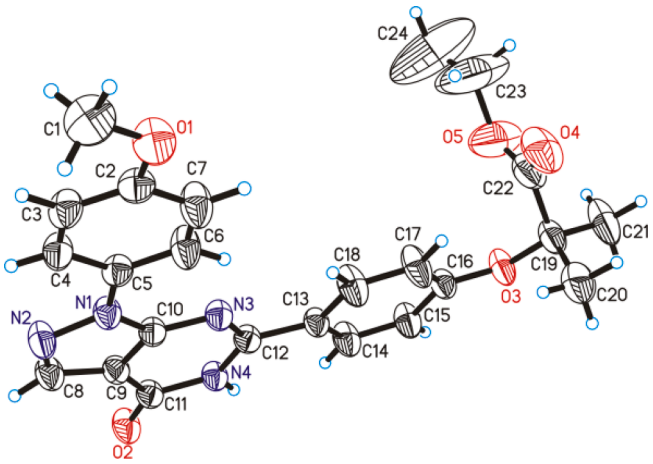



**Fig. 2.** ORTEP diagram drawn with 30% ellipsoid probability for non-H atoms of the crystal structure of compound **3k** determined at 293 K.

**Table 2**
Effect on *in vitro* glucose uptake in L6-GLUT4myc skeletal muscle cells of compound **3a–3u**.

| Compound number | % Glucose uptake stimulation[a] |
|---|---|
| 3a | −8.60 |
| 3b | 13.10 |
| 3c | −16.80 |
| 3d | 23.70 |
| 3e | −1.60 |
| **3f** | **34.00** |
| **3g** | **34.40** |
| 3h | −14.70 |
| 3i | −17.20 |
| **3j** | **34.40** |
| 3k | 5.00 |
| 3l | 12.90 |
| 3m | −12.80 |
| 3n | −8.30 |
| 3o | 10.40 |
| 3p | 2.90 |
| **3q** | **54.00** |
| 3r | 0.60 |
| 3s | 0.60 |
| 3t | 6.40 |
| 3u | 2.90 |
| Metformin[b] | 48.0 |

Bold represents compounds considered as lead for further analysis.
[a] Glucose uptake activity of compounds was done at 10 μM concentration.
[b] Metformin used was at 500 μM concentration.

(**2a**) as model substrate. The reaction conditions were optimized by changing catalyst and the solvent. The results are summarized in Table 1. Initially, 20 mol% of *p*TSA as catalyst in toluene under reflux condition failed to give any product. Likewise, no product formation was observed when water was used as solvent (entries 1 and 2). However, using DMF as solvent afforded slight formation of product (20%) after 4 h at 90–100 °C but most of the reactant was recovered (entry 3). Repeating the same reaction using $I_2$ (0.5 equiv.) as catalyst gave 44% of product (entry 4). Further, 20 mol% of $CuCl_2$ as catalyst in refluxing ethanol as medium offered 25% of **3a** (entry 5). Replacing, DMSO as solvent at 120 °C for 8 h does not favor the reaction by giving only 30% yields (entry 6). Subsequently, AcOH was choosen as solvent without presence of any catalyst resulted 30% of **3a** (entry 7). Therefore, in the same solvent the reaction was carried out using $I_2$ (entry 8) and $KMnO_4$ (entry 9) as catalysts yielded 40% and 45% of product, respectively. Replacing the catalysts with DDQ (1 equiv.) at 140 °C for 8 h displayed a better result than $KMnO_4$ and $I_2$ by giving 60% of yields. Surprisingly, increasing the amount of DDQ to 2 equiv., improved the yield of **3a** to

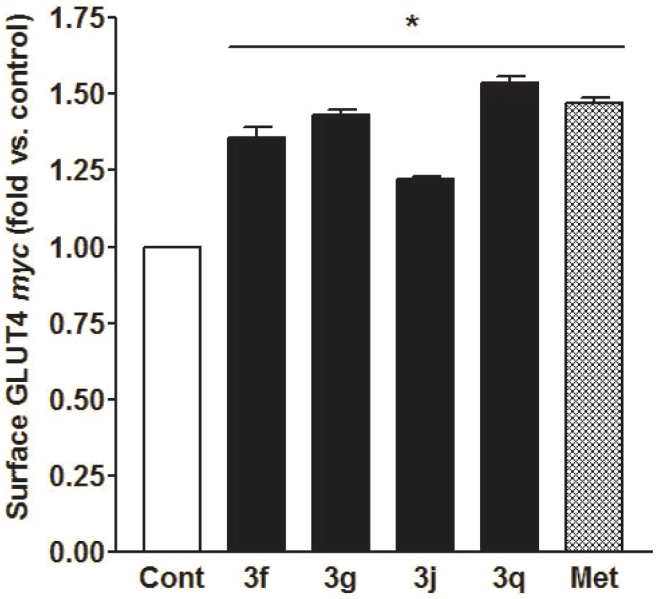



**Fig. 3.** Effect of compounds on Glut4 translocation in L6-Glut4myc myotubes. All the experiments were done in triplicate and expressed as mean ± SE. *p < 0.05 relative to the un-stimulated control.

88% (entry 11). The 2 equiv. of DDQ is required in the reaction probably due to inhibition the activity of DDQ molecule by water molecule formed in the cyclisation between **1a** and **2a**. To optimize the reaction time, the reaction was then carried out under microwave (MW) irradiation. Interestingly, the product was formed within 30 min with 90% yields (entry 12). This was considered as optimal reaction codition. With the optimum conditions in hand, a variety of pyrazolo[3,4-*d*]pyrimidinones derivatives were then synthesized and the results are summarized in Scheme 2.

The structures of synthesized compounds were characterized by $^1H$ NMR, $^{13}C$ NMR and mass spectral techniques, which were found to be in good agreement with the proposed structures. The structure of **3k** was further confirmed by X-ray crystallography (Fig. 2).

All the synthesized pyrazole-pyrimidone-fibrate hybrids (**3a–3u**) were screened for their effect on glucose uptake in L6 rat skeletal muscle cells. For the compounds that were found active in primary screening, their dose-dependent effect on glucose uptake were also determined.

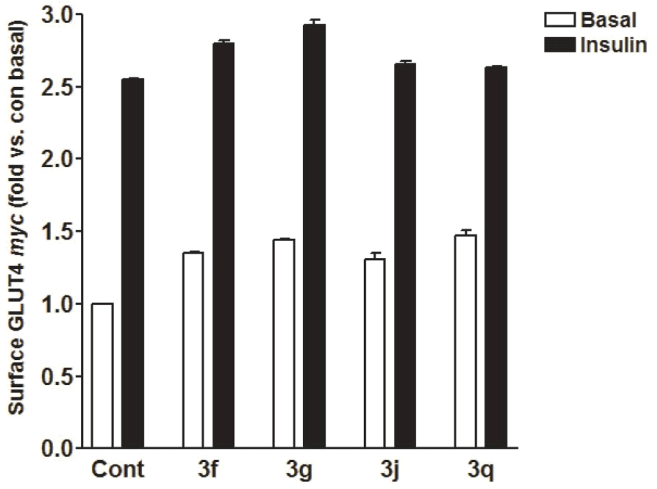



**Fig. 4.** Effect of compounds on insulin-stimulated Glut4 translocation in L6-Glut4myc myotubes. All the experiments were done in triplicate and expressed as mean ± SE.

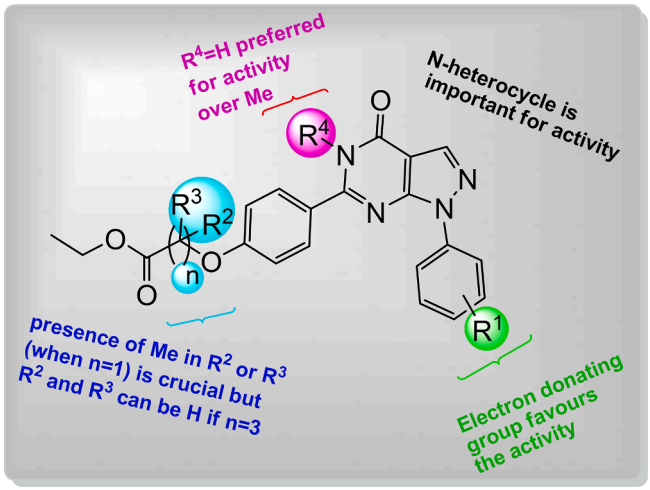



**Fig. 5.** Pictorial representation of structural activity relationship (SAR).

Skeletal muscles are the major insulin-target tissues responsible for the maintenance of whole-body glucose homeostasis. In skeletal muscle, the rate of glucose uptake determines its utilization rate and gets impaired under diabetic stage. Thus, to assess the *in vitro* antidiabetic potential, all the synthesized compounds (**3a–3u**) were evaluated for their effect on glucose uptake in L6-GLUT4*myc* skeletal muscle cells, at a single dose of 10 μM concentration. The results of the glucose uptake stimulatory effect of compounds are summarized in Table 2. Among all the tested compounds, **3f**, **3g**, **3j** and **3q** showed significant stimulation of glucose uptake with 1.34 ($p < 0.01$), 1.34 ($p < 0.01$), 1.34 ($p < 0.01$) and 1.54 ($p < 0.05$) fold increase, respectively, at 10 μM concentration. Interestingly, compound **3q** was found to be the most potent in the series. Metformin was used as a positive control, which showed 1.48-fold increase in glucose uptake at 500 μM concentration.

In skeletal muscle, an increase in glucose uptake is associated with enhanced translocation of GLUT4 to cell membrane, where they facilitate the entry of glucose inside the cell. In a quest to assess the effect of compounds to stimulate GLUT4 translocation to the cell surface, L6-Glut4*myc* cells were treated with the compounds **3f**, **3g**, **3j** and **3q** at 10 μM concentration for 16 h, followed by measurement for cell surface level of GLUT4*myc*. As shown in Fig. 3, treatment with the compounds caused a significant increase in the surface level of GLUT4*myc* ($p < 0.05$ *vs*. control) under basal conditions. Metformin, a known anti-diabetic drug was used as the positive control, which brought about 1.45-fold increase under identical experimental conditions.

Subsequently, we tested the effect of compounds on insulin response to increase GLUT4 translocation in muscle cells. Cells were treated with compounds for overnight period with the final three hours in serum-free medium. Then cells were stimulated with 100 nM insulin for 20 min, and the surface level of GLUT4*myc* was monitored. As shown in Fig. 4, insulin alone caused 2.5-fold increase in the surface level of GLUT4*myc*; prior treatment with compounds did not cause any significant difference in insulin-induced GLUT4*myc* translocation. Among the four compounds, **3g** was found to exert maximum increase in GLUT4*myc* translocation.

The structure-activity relationship (SAR) correlates the series of compounds with similar structural properties and its biological activity. The analysis of SAR facilitates the discovery of the chemical groups responsible for biological activity. Based on the *in vitro* glucose uptake stimulatory effect, we can give a brief structure-activity relation of synthesized compounds. In this screening process, we have found compound **3f**, compound **3g**, compound **3j** and compound **3q** were active. While correlating this activity with structures of the compounds it has been found that electron-donating groups like 4-OMe, 3, 4 dimethyl groups are preferred for activity on $R^1$ rather than any electron withdrawing group. The free –NH of pyrimidone ring i.e. $R^4 = H$ is crucial for activity over –Me protected nitrogen. In the case of $R^2$ and $R^3$, presence of –Me group is found beneficial for activity when $n = 1$. However, when $R^2 = R^3 = H$, the n is optimized to 3 for the most active compound. This SAR allows modification of a potent compound's activity by changing its chemical structure and test the modifications for their biological effects. This study will be helpful for further developing these molecules with better activity. The pictorial representation of this SAR is shown in Fig. 5.

In conclusion, we have designed and synthesized novel pyrazole-fibrate derivatives. The synthetic strategy used is concise, efficient, high yielding and flexible enough to create diversity. In our study, we have tested the glucose uptake stimulatory effect of the synthesized compounds. Four compounds (**3f**, **3g**, **3j** and **3q**) were found to show significant stimulation of glucose uptake with respective 1.34 ($p < 0.01$), 1.34 ($p < 0.01$), 1.34 ($p < 0.01$) and 1.54 ($p < 0.05$) fold increase at 10 μM concentration. Taking these compounds, we evaluated the effect of active compounds on Glut4 translocation in L6-Glut4myc myotubes. Compound **3q** exhibited maximum GLUT4*myc* translocation. Further studies with detailed mechanistic and pharmacokinetic studies are ongoing in our lab to establish structure-activity relationship and optimize GLUT4 translocation stimulatory activity.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

*Acknowledgments*

The authors acknowledge CSIR-CDRI, Govt. of India, for financial and infrastructural support. Instrumentation facilities from SAIF, CDRI, are gratefully acknowledged. We acknowledge Dr. S.P. Singh (Technical Officer), CSIR-CDRI, for technical assistance. We thank Dr. Tejender S. Thakur of Molecular and Structural Biology Division, CSIR-Central Drug Research Institute for supervising the X-ray Data collection and structure determination of our compound reported in this paper. The author SG, LRS acknowledge CSIR, New Delhi and SP is thankful to UGC, AKR is thankful to ICMR, New Delhi for the financial assistance in the form of fellowships. The authors declare no competing financial interests. This is CSIR-CDRI manuscript number 10169.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127760.

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