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ARTICLE TYPE

Synthesis of substituted 2*H*-benzo[e]indazole-9-carboxylate as potent antihyperglycemic agent that may act through IRS-1, Akt and GSK-3β pathways†

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Based on high throughput screening of our chemical library, we identified two 4,5-dihydro-2*H*-benzo[*e*]indazole derivatives (**5d** and **5g**), which displayed significant effect on glucose uptake in L6 skeletal muscle cells. Based on these lead molecules, a series of benzo[*e*]indazole derivatives were prepared. Among all the synthesized dihydro-2*H*-benzo[*e*]indazoles, 8-(methylthio)-2-phenyl-6-p-tolyl-4,5-dihydro-2*H*-benzo[e]indazole-9-carboxylate **(5e)** showed significant glucose uptake stimulation in L6 skeletal muscle cells even better than lead compounds. Additionally, **5e** decreased glucagon-induced glucose release in HepG2 hepatoma cells. The 2*H*-benzo[*e*]indazole **5e** exerted antihyperglycemic effect in normal, sucrose challenged streptozotocin-induced diabetic rats and type 2 diabetic *db/db* mice. Treatment with **5e** at the dose of 30 mg/kg in *db/db* mice caused a significant decrease in triglyceride and total cholesterol levels, and increased the HDL-C level in a significant manner. The mechanistic studies revealed that 2*H*-benzo[*e*]indazole **5e** significantly stimulated insulin-induced signaling at the level of IRS-1, Akt and GSK-3β in L6 skeletal muscle cells and possibly by inhibiting protein tyrosine phosphatase-1B. This new 2*H*-benzo[*e*]indazole derivative has potential for the treatment of diabetes with improved lipid profile.

Introduction

Diabetes mellitus is a debilitating metabolic disorder which represents a huge social and economic burden owing to its long term complications and morbidity. International Diabetes Federation's (IDF) most recent estimates have indicated that 415 million people have diabetes, and the number is set to rise beyond 642 million by 2040.[1] Type 2 diabetes is characterized by impaired insulin secretion from β-cells with associated increasing insulin resistance in hepatic and peripheral tissues[2-4] and by chronic hyperglycemia.[5] It possess a large risk of secondary complications such as diabetic retinopathy,[6] diabetic neuropathy,[7,8] diabetic nephropathy,[9,10] cerebrovascular[11,12] and cardiovascular complications.[13] Initially glycaemic control is achieved by lifestyle changes such as increased physical activity and dietary modifications but, when this is no longer sufficient, pharmacological intervention is required.[14]

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Current regimen of drugs for the treatment of type 2 diabetes include use of sulfonylureas,[15] biguanides,[16,17] alpha-glucosidase inhibitors,[18] glitazones,[19] glucagon-like peptide 1 (GLP-1) analogues,[20] gliptins[21] and sodium-glucose co-transporter 2 (SGLT2) inhibitors.[22] However, a major challenge in the treatment of type 2 diabetes is that, glycaemic control may still deteriorate, despite of the aggressive therapy followed.[2] Furthermore, weight gain and hypoglycaemia are common associated events.[23] In addition, other adverse events such as gastrointestinal discomfort with the use of biguanides, and possible oedema, cardiac failure or fractures with the use of thiazolidinediones, occur frequently during treatment.[24-27] Therefore, new therapies that can correct glycaemia on a long term basis without causing adverse events are highly desirable.

We initiated a program to identify new anti-diabetic molecules for the management of T2DM treatment. To discern new small-molecule insulin sensitizers, a high-throughput screening of our chemical library of diverse scaffolds was conducted and it was observed that two partially hydrogenated indazole derivatives showed significant stimulation of glucose uptake in the L6 skeletal muscle cells (Supplementary Figure S1†). On the basis of literature survey, we found that benzene derivatives fused with

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nitrogen containing five membered heterocyclic moieties in a

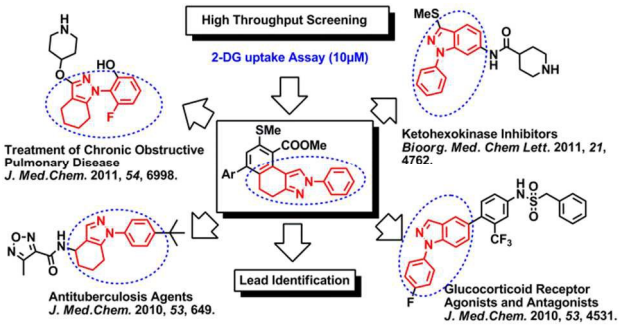


Figure 1: Biologically active benzo[*e*]indazole derivatives

rigid or flexible conformation constitute an interesting class of compounds such as benzimidazole,[28] pyrazole,[29] pyrazolopyrimidine,[30] that possess potential anti-hyperglycemic activity. Indazoles belong to privileged class of molecules with diverse biological activity (Figure 1).[31-34] Motivated by these results, we thus, synthesized functionalized dihydro-2*H*-benzo[*e*]indazole derivatives with donor (D) and acceptor (A) functionalities in anticipation to evaluate their anti-hyperglycemic activity and gain mechanistic insight. Herein, we report discovery[35] of functionalized dihydro-2*H*-benzo[*e*]indazole derivative **5e**, which exhibited good antihyperglycemic activity in *in vivo* animal models.

Results and discussion

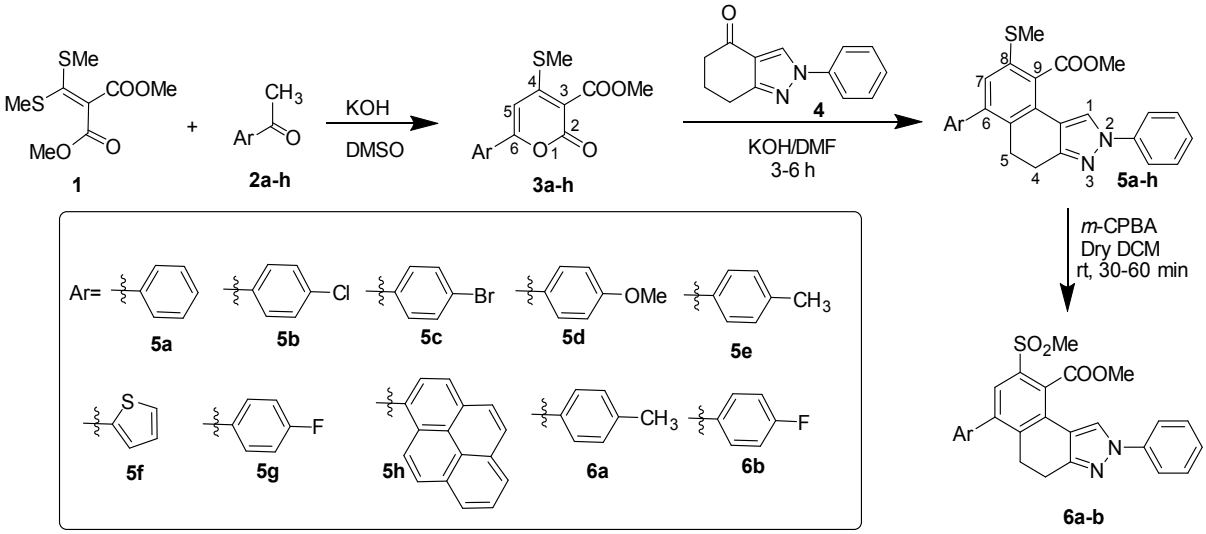
Chemistry

2*H*-Benzoindazoles have attracted significant interest in the pharmaceutical industry owing to their potential biological activity. These molecules have been used for anticryptosporidial,[36] selective cyclooxygenase-2 inhibitor,[37] human dopamine D4 receptor,[38] antiproliferative,[39] and antifungal activity.[40] The reported strategies in the literature for the synthesis of 2*H*-benzo[*e*]indazoles, generally require several steps which suffer with one or more drawbacks including reactive starting materials, long reaction time, low yields and harsh reaction conditions. These systems have been prepared earlier by the reaction of 1-acetyl-2-tetralone or related materials with hydrazine,[41-43] dilithiated 2-tetralone phenylhydrazone and aromatic esters[44] or by condensation of dilithiated 2-tetralone carboalkoxyhydrazones with aromatic esters followed by acid cyclization of C-acylated intermediates.[45] Sivaprasad et al.[46] synthesized 2*H*-benzo[*e*]indazoles by Vilsmeier-Haack reaction of 2-tetralone phenylhydrazones. The quest for new, simple, efficient and cost effective synthesis is essentially required due to limitations of the earlier procedures. Therefore, the development of mild and novel approaches to 2*H*-benzo[*e*]indazole derivatives is rewarding because of their extreme significance. We describe a novel and direct synthesis of dihydro-2*H*-benzo[*e*]indazoles **5a-h** in one-step through ring transformation of 6-aryl-4-(methylthio)-2-oxo-2*H*-pyran-3-carboxylates[48] **3a-h** from 2-phenyl-6,7-dihydro-2*H*-indazol-4(5*H*)-one[47] **(4)** at room temperature in excellent yields (Scheme 1). The ring transformation proceeded via Michael addition of the conjugate base of **4** to the lactones **3a-h** at position 6 followed by intramolecular cyclization and subsequent loss of CO_2. Oxidation of methylthio group to methylsulfonyl group was achieved in 58-75 % yield by stirring an equimolar mixture of **5e,g** with meta-chloro perbenzoic acid (*m*-CPBA)[49] in dry dichloromethane for 30-60 minute at ambient temperature to afford the methyl 8-(methylsulfonyl)-2-phenyl-6-aryl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylates **6a,b** (Scheme 1). All the new synthesized derivatives of 2*H*-benzo[*e*]indazole-9-carboxylates (**5a-c**,**e,f**,**h** and **6a,b**) were characterized by spectroscopic analyses. Furthermore, both the lead compounds **5d** and **5g** were also prepared and evaluated for biological activity together with newly synthesized 2*H*-benzo[*e*]indazole derivatives.

Biological evaluation

***In vitro* effect of compound on insulin response in target tissues**

To evaluate the anti-hyperglycemic potential of above synthesized compounds of the 4,5-dihydro-2*H*-benzo[*e*]indazole series, effect on glucose uptake was monitored in L6 skeletal muscle cells



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Scheme 1. Synthesis of 4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylates **5a-h** and **6a,b**.

(Supplementary Figure S2†). The activity profile of the screened compounds revealed that 4-methylphenyl group (**5e**) at position C6 showed significant glucose uptake stimulation, while other derivatives in which this group was replaced by other aryl groups showed less (**5d, g**) or no (**5a-c, 5f, h**) significant effect on glucose uptake in L6 skeletal muscle cells. Oxidation of methylthio group to methyl sulfonyl group **(6a,b)** also showed no significant effect on glucose uptake. Hence, methyl 8-methylthio-2-phenyl-6-p-tolyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate **5e** exhibited higher glucose uptake stimulation than lead compounds **5d, g** and was selected for further study. In the L6 myotubes, **5e** increased the rate of glucose uptake in a concentration-dependent fashion with significant effect at the minimal concentration of 2.5 μM ($P < 0.05$) and caused around 2.0-fold stimulation at 10 μM concentration ($P < 0.001$). Since insulin is the physiological stimulator of glucose uptake in skeletal muscle cell,[50] we used it as positive control, which caused around 2.3-fold stimulation ($P < 0.001$) upon acute exposure at 100 nM for 20 min (Figure 2).

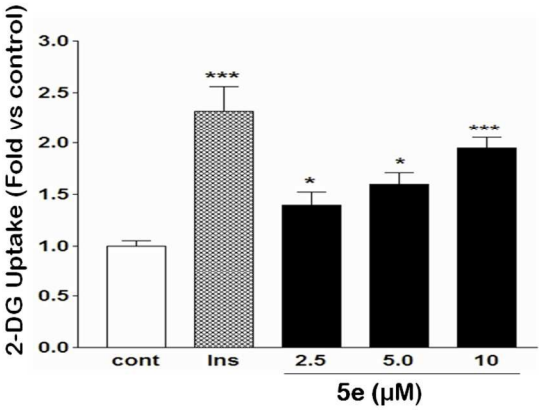

Figure 2: Dose-dependent effect of **5e** on ^{3}H-2-Deoxyglucose uptake by L6 myotubes. Significance: *p < 0.05, ***p < 0.001 relative to control condition.

The glucose uptake stimulatory effect of **5e** was further validated in hepatic cells, which is another major target tissue of insulin action. In human hepatic cell lines (HepG2), **5e** significantly increased the uptake of glucose, the effect comparable to standard antidiabetic drug metformin (Figure 3). Metformin has been established to exert its antidiabetic potential through regulating hepatic glucose metabolism.[51] In hepatic tissues, activation of insulin signaling inhibits the release of glucose via gluconeogenesis.[52] Here, treatment with **5e** significantly decreased the glucagon-mediated release of glucose in HepG2 cells ($P < 0.001$) (Figure 4). Moreover, **5e** exerted these cellular effects without any significant change in cell viability, as established by MTT assay (Supplementary Figure S3†). Results validate the potential of **5e** to enhance insulin response in sensitive tissues.

***In vivo* anti-diabetic effect of compound in animal models**

Before screening in *in vivo* models, compound **5e** was checked for its purity by HPLC method and was found >98% pure (Supplementary Figure S4, S5†).

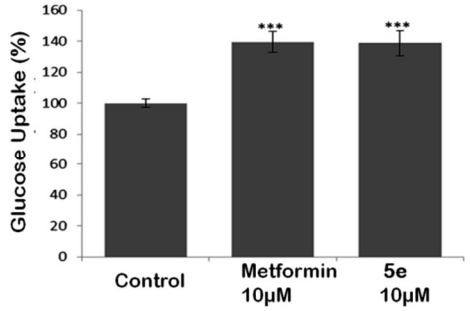

Figure 3: Effect of **5e** on glucose uptake in HepG2 liver cells**.** Significance: *p < 0.05, ***p < 0.001 relative to control condition.

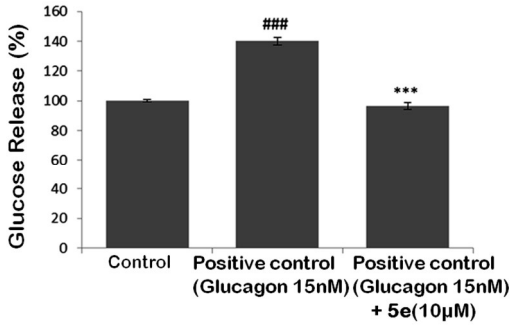

Figure 4: Effect of **5e** on glucose release from HepG2 liver cells. Significance: $^{###}p < 0.001$, ***p < 0.001 relative to control condition.

First, the effect of compound **5e** on the improvement of oral glucose tolerance post sucrose load (SLM) on normal rats was evaluated. Figure 5 showed the acute effect of **5e** on postprandial hyperglycemia post sucrose load in normal albino rats. Treatment with **5e** significantly prevented the postprandial rise in blood glucose. The percent inhibition on the rise of postprandial hyperglycemia by **5e** was calculated to be ~ 35.8% at the dose of 100 mg/kg. Further the compound was evaluated for oral glucose tolerance in sucrose challenged streptozotocin-induced diabetic rats. Figure 6 shows the blood glucose profile of treated and control animals at various time interval post sucrose load. It is evident from the results that compound **5e** inhibited the rise in postprandial hyperglycemia. The percent improvement in glucose tolerance by **5e** was calculated to be ~ 23.1% after 5h post sucrose load in STZ-induced diabetic rat.

As an interesting chemical entity, the compound **5e** was further evaluated for antihyperglycemic effect in the *db/db* mice, a genetic model of type 2 diabetes. The animals were dosed orally

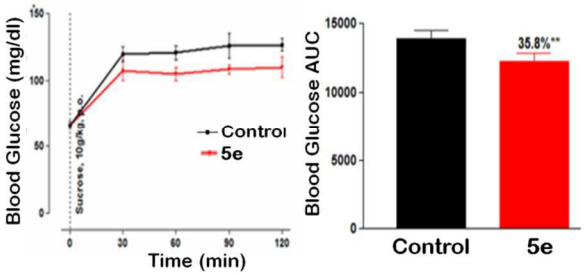

Figure 5: Effect of **5e** at oral dose of 100 mg/kg b.wt. on (A) blood glucose profile, (B) glucose AUC (0–2h) of sucrose loaded (oral) normal

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rats. Values are mean % change in 5 animals of a group; significance: ** $p < 0.01$ compared with control.

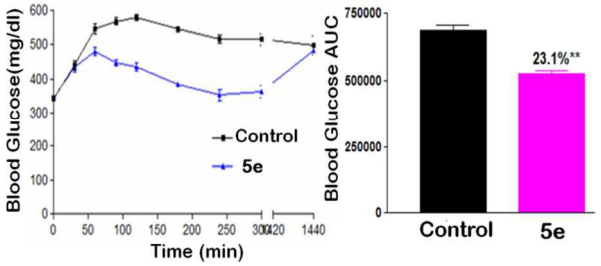

Figure 6: Effect of **5e** at oral dose of 100 mg/kg b.wt. on (A) blood glucose profile, (B) glucose AUC (0–24h) of streptozotocin-induced diabetic rats with sucrose challenge. Values are mean % change in 5 animals of a group; significance: **$p < 0.01$ when compared with control.

with different doses (3, 10, 30 mg/kg) of compound or standard pioglitazone (10 mg/kg) once a day for 15 consecutive days with daily measurement of the blood glucose levels, and oral glucose tolerance test (OGTT) at day 10 and day 15. The pioglitazone has been established to stimulate insulin signaling,[53] thereby used as positive control to compare the antidiabetic efficacy of **5e**. The effect on blood glucose during the course of treatment was illustrated in Figure 7. Treatment with compound **5e** led to a continuous fall in blood glucose level over time, in a dose-dependent manner. Significant decrease in blood glucose level was observed from day 7 upon treatment with compound (30 mg/kg), whereas upon pioglitazone (10 mg/kg) significant lowering in blood glucose level was observed from day 4 (Figure 7). There was no effect of compound on body weight and food intake during the course of treatment (data not shown).

Glucose tolerance pattern of each animal was tested on day 10 and day 15. The postprandial rise in blood glucose levels of compound treated animals were found to be significantly lowered compared to that of the control group, in a dose-dependent manner. It showed significant improvement in their glucose tolerance pattern in comparison to control animals. The overall improvement in the glucose tolerance was calculated to be around 7.8 %, 13.9 %, and 25.7 % at the doses of 3, 10, and 30 mg/kg, at day 10 (Figure 8A). The improvement was further enhanced to 13.2 %, 20.1 % and 31.6 % at the doses of 3, 10, and 30 mg/kg, respectively at day 15 (Figure 8B). These effects of **5e** in *db/db* mice are associated with decrease in fasting blood glucose and serum insulin levels, in a dose-dependent manner and improvement in lipid profile of the animals. As shown in Figure 9, treatment with **5e** at the dose of 30 mg/kg caused a significant decrease in triglyceride (18.2 %, $p < 0.01$) and total cholesterol (22.7 %, $p < 0.05$) levels, and increased the HDL-C level (20.5%, $p < 0.05$) in a significant manner.

Mechanism Studies in L6 Skeletal Muscle

To investigate the signaling pathways involved in the glucose uptake by **5e** in skeletal muscle, western blot analysis was carried out. From literature, it is known that insulin stimulation in target tissues results in phosphorylation and activation of the signaling cascade IR/IRS1/PI3K/Akt/GSK3β leading to enhanced glucose utilization in peripheral tissues and decreased hepatic glucose production.[54] Given the sensitizary potential of the **5e**, we assessed its cellular activity in the insulin signal

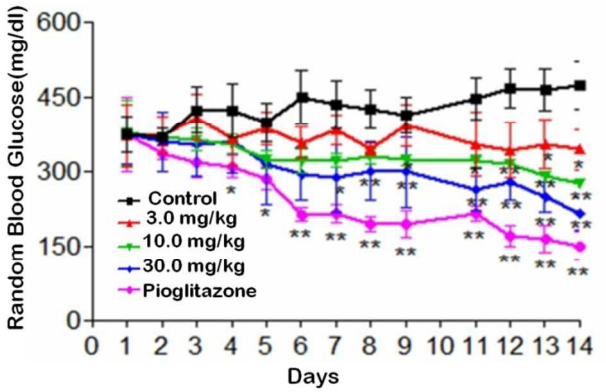

Figure 7: Antihyperglycemic effect of **5e** in db/db mice. Blood glucose profile of *db/db* mice treated with vehicle, compound **5e** at oral dose of 3 mg/kg, 10mg/kg, 30 mg/kg b.wt. and standard drug Pioglitazone at oral dose of 10 mg/kg b.wt. Values are mean % change in 5 animals of a group; significance: *$p < 0.05$, **$p < 0.01$ when compared with control.

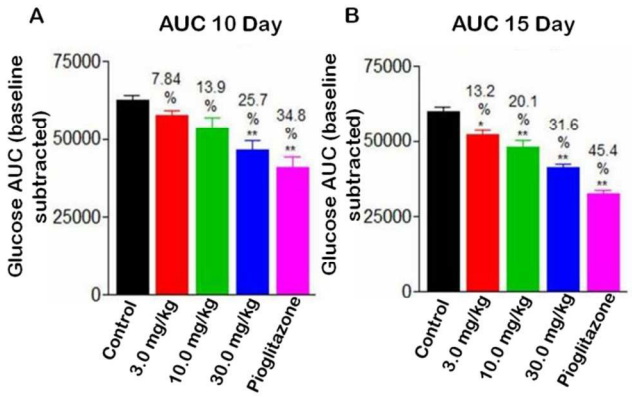

Figure 8: Dose dependent effect of compound **5e** on oral glucose tolerance (OGTT) of *db/db* mice. Values are mean % change in 5 animals of a group; significance: *$p < 0.05$, **$p < 0.01$ when compared with control.

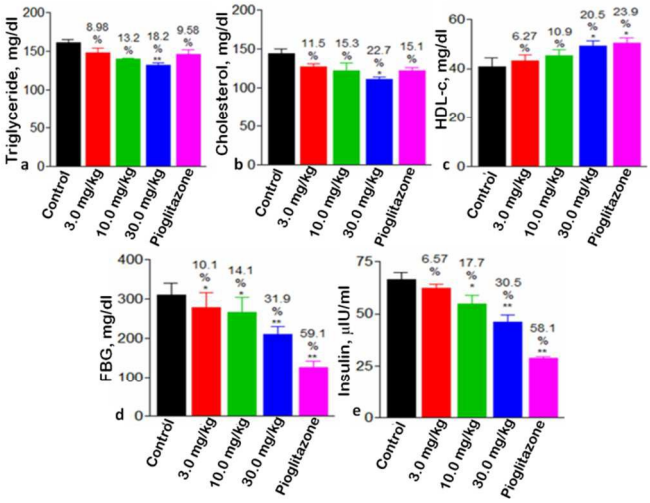

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Figure 9: Dose dependent effect of compound **5e** on serum lipid profile, insulin and fasting blood glucose level in db/db mice. Values are mean % change in 5 animals of a group; significance: *p < 0.05, **p < 0.01 when compared with control.

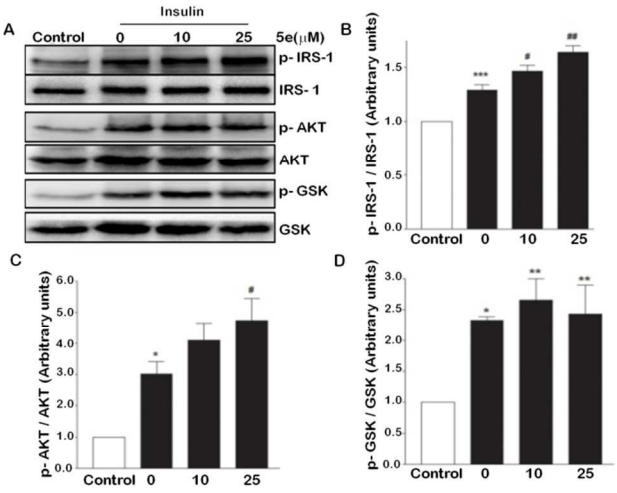

Figure 10: Effect of **5e** on activation of the components of insulin signaling cascade in L6 myotubes. Cells were treated with indicated concentration of **5e** for 16h and stimulated with insulin (100 nM) for 10 min, followed by cell lysis and western analysis. Shown are representative immunoblots (A) and densitometric quantification of p-IRS-1 (B), p-AKT (C) and p-GSK (D) relative to their respective total forms. Significance: *P < 0.05, **P < 0.01 realtive to control; #P < 0.05, ##P < 0.01 relative to control insulin treated condition.

transduction pathway in skeletal muscle, the major depot for postprandial glucose utilization. Treatment of L6 myotubes with **5e** increased the insulin-stimulated tyrosine phosphorylation of IRS-1 in a concentration-dependent manner, which is further validated by the enhanced insulin-stimulated phosphorylation of the downstream targets Akt (Ser-473) and GKS-3β (Ser-9) (Figure 10). Results indicated the ability of **5e** to activate insulin signaling pathway in skeletal muscle cells.

Again, from the literature it is known that PTP-1B inhibition plays a key role in insulin signaling through dephosphorylation of the insulin receptor.[55-57] Hence, the above observations of the activation of insulin signalling cascade in L6 myotubes prompted us to check the protein tyrosine phosphatase-1B inhibitory (PTP-1B) activity of compound **5e**.[58] The compound **5e** was found to inhibit the activity of PTP-1B enzyme in a concentration-dependent manner and the IC_{50} value was calculated to be 7.12 μM (Supplementary Table 1†). Thus, PTP-1B may be involved for the action of compound **5e**.

Conclusion

In conclusion, we discovered two dihydro-2*H*-benzo[*e*]indazoles through screening of our chemical library as anti-hyperglycemic agents. A series of 2*H*-benzo[*e*]indazoles were prepared by reacting substituted 2*H*-pyran-3-carboxylates with 2-phenyl-6,7-dihydro-2*H*-indazol-4(5*H*)-one in good yields and their *in vitro* 2-DG uptake activity was evaluated. The methyl 8-(methylthio)-2-phenyl-6-p-tolyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate **5e** showed anti-hyperglycemic activity with 2-fold stimulation in 2-DG uptake at 10 μM and exhibited better glucose tolerance, fasting as well as postprandial blood glucose in SLM, STZ and *db/db* animal models. Further treatment with **5e** at the dose of 30 mg/kg caused a significant decrease in triglyceride and total cholesterol levels, and increased the HDL-C level in a significant manner. Mechanism of action of **5e** was found to be mediated via activation of IRS-1, Akt and GSK-3β pathways and possibly by the inhibition of PTP-1B, which is a major mediator of insulin signalling and insulin-resistance. For drug development perspectives, detailed pharmacokinetic and toxicity studies are in progress.

Experimental

Analysis and instruments

All the reactions were carried out under anhydrous conditions and were monitored by thin-layer chromatography (TLC), visualization was done with UV-light (254 nm). ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker Supercon Magnet DRX-300 spectrometer (operating at 400MHz for ^{1}H and 50, MHz for ^{13}C) using DMSO-d_6 or $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million. Signal pattern are indicated as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Coupling constant (*J*) are given in hertz. Infrared (IR) spectra were recorded in KBr disc and reported in wave number (cm^{-1}). ESIMS, EI spectrometer was used for mass spectra analysis. Infrared spectra were recorded on a Perkin-Elmer FT-IR RXI spectrophotometer. Electrospray ionization mass spectra (ESI-MS) were recorded on Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded on 6520 Agilent Q T of LC MS/MS (Accurate mass). Additionally, purity of compounds was measured by a RP- HPLC with the following conditions, and purity of the compounds was >98%. The Waters HPLC system, Milford USA consisted of a binary pump (model Waters 515 HPLC pump), auto sampler (model 2707 Auto sampler) and PDA detector (Waters 2998). Data collection and analysis were performed using Empower pro 2 software. The resolution and better peak shape were achieved on a X-Bridge C18 (5μm, 4.6 X 250 mm) column, protected with a C_{18} guard column. The system was analyzed in isocratic mode with a mobile phase consisting of acetonitrile-triple distilled water (60: 40, v/v) at a flow rate of 1.0 mL/min.

General procedure for the synthesis of functionalized methyl 8-(methylthio)-2-phenyl-6-aryl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylates (5a-h): A mixture of 6-aryl-2*H*-pyran-2-one **3a-h** (1 mmol), 2-phenyl-6,7-dihydro-2*H*-indazol-4(5*H*)-one (**4**) (1.2 mmol) and powdered KOH (1.2 mmol) in dry DMF (5 mL) was stirred at room temperature for 3-6 h. The progress of reaction was monitored by TLC, and on completion the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column using 10% chloroform in hexane as the eluent.

Methyl 8-(methylthio)-2,6-diphenyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5a): A mixture of **3a** (276 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 4h. Using the general procedure described above 234 mg (55%) of **5a** was obtained as an off

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white solid; mp = 173-175 °C; MS (ESI) 427 [M + H^+]; IR (KBr) ν = 3020, 1726, 1574 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.99 (s, 1H, ArH), 7.67 (d, J = 9.0 Hz, 2H, ArH), 7.38-7.50 (m, 5H, ArH), 7.24-7.35 (m, 3H, ArH), 7.19 (s, 1H, ArH), 4.01 (s, 3H, OCH_3), 2.81-2.93 (m, 4H, $2CH_2$), 2.48 (s, 3H, SCH_3), ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.66, 153.37, 143.93, 141.31, 140.38, 133.20, 132.49, 129.97, 129.48, 129.26, 128.77, 128.03, 127.97, 126.82, 123.45, 119.37, 117.24, 53.37, 27.75, 22.16, 18.79 ppm; HRMS (EI) calculated mass for $C_{26}H_{23}N_2O_2S$: 427.1480 found 427.1470.

Methyl 6-(4-chlorophenyl)-8-(methylthio)-2-phenyl-4, 5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5b): A mixture of **3b** (310 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 3h. Using the general procedure described above 266 mg (58%) of **5b** was obtained as an off white solid; mp = 160-162 °C; MS (ESI) 461 [M + H^+]; IR (KBr) ν = 1722, 1588 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.00 (s, 1H, ArH), 7.69 (d, J = 7.7 Hz, 2H, ArH), 7.41-7.51 (m, 4H, ArH), 7.14-7.35 (m, 4H, ArH), 4.05 (s, 3H, OCH_3), 2.85-2.89 (m, 4H, $2CH_2$), 2.52 (s, 3H, SCH_3) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.44, 153.11, 142.69, 140.33, 139.60, 134.10, 133.47, 132.42, 130.85, 129.91, 129.02, 128.29, 126.93, 123.52, 119.49, 117.13, 53.35, 27.75, 22.09, 18.79 ppm; HRMS (EI) calculated mass for $C_{26}H_{22}ClN_2O_2S$: 461.1091 found 461.1079.

Methyl 6-(4-bromophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5c): A mixture of **3c** (355 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 3.5h. Using the general procedure described above 297 mg (59%) of **5c** was obtained as an off white solid; mp = 164-166 °C; MS (ESI) 506 [M + H^+]; IR (KBr) ν = 3021, 1725, 1511 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.98 (s, 1H, ArH), 7.40-7.71 (m, 4H, ArH), 7.12-7.34 (m , 4H, ArH), 4.01 (s, 3H, OCH_3), 2.83-2.87 (m, 4H, $2CH_2$), 2.48 (s, 3H, CH_3), ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.42, 153.14, 142.68, 140.41, 140.12, 133.47, 132.38, 132.00, 131.18, 129.90, 129.07, 128.29, 126.88, 123.51, 122.23, 119.40, 117.11, 53.29, 27.75, 22.11, 18.83 ppm; HRMS (EI) calculated mass for $C_{26}H_{22}BrN_2O_2S$: 505.0585 found 505.0575.

Methyl 6-(4-methoxyphenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5d): A mixture of **3d** (306 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 3h. Using the general procedure described above 269 mg (59%) of **5d** was obtained as an off white solid; mp = 172-174 °C; MS (ESI) 457 [M + H^+]; IR (KBr) ν = 2923, 1722, 1503 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.98 (s, 1H, ArH), 7.63-7.71 (m, 2H, ArH), 7.41-7.52 (m, 2H, ArH), 7.16-7.34 (m, 4H, ArH), 6.95.-7.03 (m, 2H, ArH), 4.01 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 2.81-2.95 (m, 4H, $2CH_2$), 2.49 (s, 3H, CH_3) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 183.07, 170.23, 159.08, 152.90, 143.19, 133.14, 132.61, 132.26, 130.19, 129.46, 129.14, 126.34, 122.95, 118.96, 116.87, 113.75, 55.36, 52.81, 27.33, 21.73, 18.44, ppm; HRMS (EI) calculated mass for $C_{27}H_{25}N_2O_3S$: 457.1586 found 457.1557.

Methyl 8-(methylthio)-2-phenyl-6-p-tolyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5e): A mixture of **3e** (290 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 3.5h. Using the general procedure described above 237 mg (54%) of **5e** was obtained as an off white solid; mp = 178-180 °C; MS (ESI) 441 [M + H^+]; IR (KBr) ν = 2984, 2918, 1718, 1504 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.98 (s, 1H, ArH), 7.63-7.72 (m, 2H, ArH), 7.46 (t, J = 11.6 Hz, 2H, ArH), 7.21-7.33 (m, 5H, ArH), 7.18 (s, 1H, ArH), 4.01 (s, 3H, OCH_3), 2.80-2.94 (m, 4H, $2CH_2$), 2.48 (s, 3H, CH_3), 2.43 (s, 3H, CH_3) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.61, 153.32, 143.92, 140.43, 138.33, 137.74, 133.08, 132.56, 130.75, 129.88, 129.44, 129.36, 128.00, 126.76, 123.38, 119.38, 117.29, 53.24, 27.75, 22.14, 21.62, 18.79 ppm; HRMS (EI) calculated mass for $C_{27}H_{24}N_2O_2S$: 440.1559 found 440.1547.

Methyl 8-(methylthio)-2-phenyl-6-(thiophen-2-yl)-4,5-dihydro-2*H* benzo[*e*]indazole-9-carboxylate (5f): A mixture of **3f** (282 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 5h. Using the general procedure described above 211 mg (49%) of **5f** was obtained as an off white solid; mp = 174-176°C; MS (ESI) 433 [M + H^+]; IR (KBr) ν = 3065, 2917, 1716, 1570 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.97 (s, 1H, ArH), 7.61-7.71 (m, 2H, ArH), 7.28-7.53 (m, 5H, ArH), 7.02-7.18 (m, 2H, ArH), 4.00 (s, 3H, OCH_3), 3.02-3.13 (m, 2H, CH_2), 2.82-2.92 (s, 2H, CH_2), 2.49 (s, 3H, SCH_3), ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.36, 153.16, 141.90, 140.39, 136.34, 133.55, 133.34, 131.48, 130.26, 129.90, 128.33, 127.87, 127.64, 126.83, 126.37, 123.50, 119.43, 117.11, 53.28, 27.75, 22.08, 18.82 ppm; HRMS (EI) calculated mass for $C_{24}H_{20}N_2O_2S_2$: 432.0966 found 432.0970.

Methyl 6-(4-fluorophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5g): A mixture of **3g** (294 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 4h. Using the general procedure described above 230 mg (52%) of **5g** was obtained as an off white solid; mp = 182-184°C; MS (ESI) 445 [M + H^+]; IR (KBr) ν = 2950, 1726, 1507 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.98 (s, 1H, ArH), 7.67 (d, J = 7.6 Hz, 2H, ArH), 7.46 (t, J = 7.8 Hz, 2H, ArH), 7.25-7.37 (m, 3H, ArH), 7.09-7.19 (m, 3H, ArH), 4.01 (s, 3H, OCH_3), 2.83-2.88 (m, 4H, $2CH_2$), 2.48 (s, 3H, SCH_3) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.10, 160.66, 152.78, 142.48, 139.96, 136.75, 136.71, 132.88, 132.13, 130.75, 130.65, 129.51, 128.90, 127.74, 126.45, 123.03, 119.00, 116.73, 115.47, 115.18, 52.90, 27.30, 21.68, 18.38 ppm; HRMS (EI) calculated mass for $C_{26}H_{22}FN_2O_2S$: 445.1386 found 445.1381.

Methyl 8-(methylthio)-2-phenyl-6-(pyren-1-yl)-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (5h): A mixture of **3h** (400 mg, 1 mmol, 1 equiv), compound **4** (212 mg, 1 mmol, 1 equiv) and KOH (84 mg, 1.5 mmol, 1.5 equiv) in dry DMF (5 mL) was stirred at room temperature for 5.5h. Using the general procedure described above 319 mg (58%) of **5h** was obtained as an off white solid; mp = 174-176°C; MS (ESI) 551 [M + H^+]; IR (KBr) ν = 2926, 1725, 1504 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ =

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8.16-8.29 (m, 3H, ArH), 8.14 (s, 2H, ArH), 7.99-8.08 (m, 3H, ArH), 7.90 (d, J = 7.7 Hz, 1H, ArH), 7.77 (d, J = 9.1 Hz, 1H, ArH), 7.68 (d, J = 7.5 Hz, 2H, ArH), 7.47 (t, J = 7.8 Hz, 2H, ArH), 7.23-7.35 (m, 2H, ArH), 4.08 (s, 3H, OCH_3), 2.61-2.78 (m, 4H, $2CH_2$), 2.49 (s, 3H, SCH_3) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 170.32, 152.92, 142.25, 140.00, 135.72, 133.45, 133.13, 131.42, 131.00, 130.92, 130.31, 129.53, 129.13, 128.99, 128.04, 127.75, 127.62, 127.36, 126.97, 126.46, 126.25, 125.47, 125.28, 124.92, 124.62, 123.11, 119.06, 116.82, 52.99, 27.32, 21.60, 18.11 ppm; HRMS (EI) calculated mass for $C_{36}H_{27}N_2O_2S$: 551.1793 found 551.1797.

General procedure for the synthesis of functionalized methyl 8-(methylsulfonyl)-2-phenyl-6-aryl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylates (6a-b): A mixture of methyl 8-(methylthio)-2-phenyl-6-aryl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylates **5** (1 mmol) and m-chloro perbenzoic acid (*m*-CPBA) (5 mmol) in dry DCM (5 mL) was stirred at room temperature for 30-60 min. The progress of reaction was monitored by TLC, and on completion the reaction mixture was neutralized with $NaHCO_3$ solution. The neutralized solution obtained was extracted with DCM solution; the organic layer was separated and purified on a silica gel column using 40% Chloroform in hexane as the eluent to afford **6a-c**.

Methyl 8-(methylsulfonyl)-2-phenyl-6-(p-tolyl)-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (6a): A mixture of methyl 8-(methylthio)-2-phenyl-6-p-tolyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (**5e**) (440 mg, 1 mmol) and *m*-CPBA (855 mg, 5 mmol) in dry DCM (5 mL) was stirred at room temperature for 60 min. Using the general procedure described above 354 mg (75%) of **6a** was obtained as an off white solid; mp = 228-230 ^oC; MS (ESI) 473 [M + H^+]; IR (KBr) ν = 2927, 1729, 1599 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (s, 1H, ArH), 7.81 (d, J = 1.7 Hz, 1H, ArH), 7.68 (d, J = 7.2 Hz, 2H, ArH), 7.48 (t, J = 6.7 Hz, 2H, ArH), 7.19-7.36 (m, 5H, ArH), 4.03 (s, 3H, OCH_3), 3.19 (s, 3H, SO_2CH_3), 3.01-3.05 (m, 2H, CH_2), 2.85-2.91 (m, 2H, CH_2), 2.43 (s, 3H, CH_3) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 169.27, 152.62, 143.76, 140.22, 139.79, 138.05, 136.44, 135.52, 129.59, 129.24, 128.91, 128.12, 127.19, 126.76, 123.43, 119.08, 116.11, 53.53, 45.10, 29.68, 28.14, 21.22 ppm; HRMS (EI) calculated mass for $C_{27}H_{25}N_2O_4S$: 473.1535 found 473.1530.

Methyl 6-(4-fluorophenyl)-8-(methylsulfonyl)-2-phenyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (6b): A mixture of methyl 6-(4-fluorophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylate (**5g**) (444 mg, 1 mmol) and *m*-CPBA (855 mg, 5 mmol) in dry DCM (5 mL) was stirred at room temperature for 60 min. Using the general procedure described above 280 mg (59%) of **6b** was obtained as an off white solid; mp = 245-247 ^oC; MS (ESI) 477 [M + H^+]; IR (KBr) ν = 2927, 1729, 1599 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (s, 1H, ArH), 7.78 (s, 1H, ArH), 7.67 (d, J = 7.4 Hz, 2H, ArH), 7.48 (m, J = 7.5 Hz, 2H, ArH), 7.12-7.24 (m, 5H, ArH), 4.03 (s, 3H, OCH_3), 3.20 (s, 3H, SO_2CH_3), 2.95-3.02 (m, 2H, CH_2), 2.86-2.92 (m, 2H, CH_2) ppm; ^{13}C NMR (50.3 MHz, $CDCl_3$) δ = 169.12, 152.47, 142.66, 140.23, 139.74, 135.67, 135.34, 130.84, 130.67, 129.61, 128.61, 128.02, 127.55, 126.83, 123.50, 119.10, 115.85, 115.42, 53.57, 45.09, 28.12, 21.15 ppm; HRMS (EI) calculated mass for $C_{26}H_{22}FN_2O_4S$: 477.1284 found 477.1277.

Ethical statement

The work with all these animals was cleared by Institutional Animal Ethics Committee (IAEC) and was conducted in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964.

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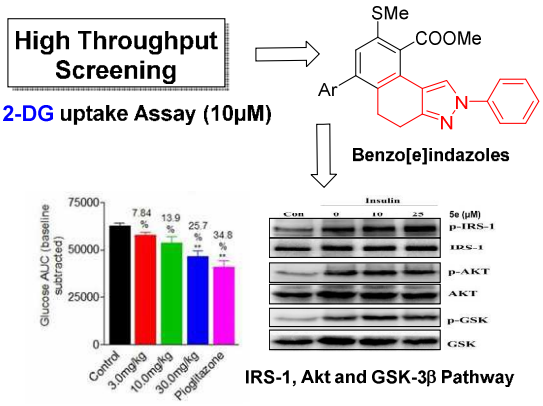

Synthesis, in vitro and in vivo antihyperglycemic activity of substituted 2*H*-benzo[*e*]indazole-9-carboxylate are described.