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Synthesis of substituted 2*H*-benzo[e]indazole-9-carboxylate as a potent antihyperglycemic agent that may act through IRS-1, Akt and GSK-3 β 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 a 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 an antihyperglycemic effect in normal, sucrose challenged streptozotocin-induced diabetic rats and type 2 diabetic *db/db* mice. Treatment with **5e** at a dose of 30 mg kg⁻¹ 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 the 2*H*-benzo[e]indazole **5e** significantly stimulated insulin-induced signaling at the level of IRS-1, Akt and GSK-3 β in L6 skeletal muscle cells, 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.

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Introduction

Diabetes mellitus is a debilitating metabolic disorder which represents a huge social and economic burden owing to its long term complications and morbidity. The 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 has 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

The current regimen of drugs for the treatment of type 2 diabetes include the use of sulfonylureas,¹⁵ biguanides,^{16,17} alpha-glucosidase inhibitors,¹⁸ glitazones,¹⁹ glucagon-like peptide 1 (GLP-1) analogues,²⁰ gliptins²¹ 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 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

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new small-molecule insulin sensitizers, 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 L6 skeletal muscle cells (Fig. S1†). On the basis of a literature survey, we found that benzene derivatives fused with nitrogen containing five membered heterocyclic moieties in a rigid or flexible conformation constitute an interesting class of compounds, such as benzimidazole,²⁸ pyrazole,²⁹ and pyrazolopyrimidine,³⁰ that possess potential anti-hyperglycemic activity. Indazoles belong to the privileged class of molecules with diverse biological activity (Fig. 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 order to evaluate their anti-hyperglycemic activity and gain mechanistic insight. Herein, we report the discovery³⁵ of the 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*-Benzindazoles have attracted significant interest in the pharmaceutical industry owing to their potential biological activity. These molecules have been used for anti-cryptosporidial,³⁶ selective cyclooxygenase-2 inhibitor,³⁷ human dopamine D4 receptor,³⁸ antiproliferative,³⁹ and anti-fungal activities.⁴⁰ The reported strategies in the literature for the synthesis of 2*H*-benzo[*e*]indazoles generally require several steps which suffer from 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 esters⁴⁴ 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 the limitations of earlier procedures. Therefore, the development of mild and novel ap-

proaches 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 3a–h⁴⁷ from 2-phenyl-6,7-dihydro-2*H*-indazol-4(5*H*)-one (4)⁴⁸ 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 CO₂. Oxidation of the methylthio group to the methylsulfonyl group was achieved in 58–75% yield by stirring an equimolar mixture of 5e and 5g with *meta*-chloroperoxybenzoic acid (*m*-CPBA)⁴⁹ in dry dichloromethane for 30–60 minutes at ambient temperature to afford the methyl 8-(methylsulfonyl)-2-phenyl-6-aryl-4,5-dihydro-2*H*-benzo[*e*]indazole-9-carboxylates 6a and 6b (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 the newly synthesized 2*H*-benzo[*e*]indazole derivatives.

Biological evaluation

***In vitro* effect of the compound on insulin response in target tissues.** To evaluate the anti-hyperglycemic potential of the above synthesized compounds of the 4,5-dihydro-2*H*-benzo[*e*]indazole series, the effect on glucose uptake was monitored in L6 skeletal muscle cells (Fig. S2†). The activity profile of the screened compounds revealed that the 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 the methylthio group to the methylsulfonyl 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 and g and was selected for further study. In L6 myotubes, 5e increased the rate of glucose uptake in a concentration-dependent fashion with a 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 cells,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 (Fig. 2).

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, an effect comparable to the standard antidiabetic drug metformin (Fig. 3). Metformin has been established to exert its antidiabetic potential through regulating hepatic glucose

Fig. 1 Biologically active benzo[*e*]indazole derivatives.

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

Fig. 2 Dose-dependent effect of 5e on ^3H -2-deoxyglucose uptake by L6 myotubes. Significance: * $p < 0.05$, *** $p < 0.001$ relative to control conditions.

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 ($p < 0.001$) (Fig. 4). Moreover, 5e exerted these cellular effects without any significant change in cell viability, as established by MTT

Fig. 3 Effect of 5e on glucose uptake in HepG2 liver cells. Significance: *** $p < 0.001$ relative to control conditions.

assay (Fig. S3†). The results validate the potential of 5e to enhance insulin response in sensitive tissues.

In vivo anti-diabetic effect of the compound in animal models. Before screening in *in vivo* models, compound 5e was checked for its purity by the HPLC method and was found to be >98% pure (Fig. S4 and S5†).

First, the effect of compound 5e on the improvement of oral glucose tolerance post sucrose load (SLM) on normal rats was evaluated. Fig. 5 shows 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 a dose of 100 mg kg⁻¹. Further the compound was evaluated for oral glucose tolerance in sucrose challenged streptozotocin-induced diabetic rats. Fig. 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 5 h post sucrose load in STZ-induced diabetic rat.

Fig. 4 Effect of 5e on glucose release from HepG2 liver cells. Significance: ### $p < 0.001$, *** $p < 0.001$ relative to control conditions.

Fig. 5 Effect of 5e at an oral dose of 100 mg kg^{-1} b.wt. on (A) blood glucose profile and (B) glucose AUC (0–2 h) of sucrose loaded (oral) normal rats. Values are the mean % change in 5 animals of a group; significance: $**p < 0.01$ compared with control.

Fig. 6 Effect of 5e at an oral dose of 100 mg kg^{-1} b.wt. on (A) blood glucose profile and (B) glucose AUC (0–24 h) of streptozotocin-induced diabetic rats with sucrose challenge. Values are the mean % change in 5 animals of a group; significance: $**p < 0.01$ when compared with control.

As an interesting chemical entity, compound 5e was further evaluated for its antihyperglycemic effect on *db/db* mice, a genetic model of type 2 diabetes. The animals were dosed orally with different doses ($3, 10, 30 \text{ mg kg}^{-1}$) of the compound or standard pioglitazone (10 mg kg^{-1}) 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. 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 Fig. 7. Treatment with compound 5e led to a continuous decrease in blood glucose level over time, in a dose-dependent manner. A significant decrease in blood glucose level was observed from day 7 upon treatment with the compound (30 mg kg^{-1}), whereas upon treatment with pioglitazone (10 mg kg^{-1}) significant lowering in blood glucose level was observed from day 4 (Fig. 7). There was no effect of the compound on the body weight and food intake during the course of the treatment (data not shown).

The 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 was found to be significantly lowered compared to that of the control group, in a dose-dependent manner. It showed a significant improvement in their glucose tolerance pattern in comparison to control animals. The overall improvement in the glucose toler-

Fig. 7 Antihyperglycemic effect of 5e in *db/db* mice. Blood glucose profile of *db/db* mice treated with vehicle, compound 5e at oral doses of 3 mg kg^{-1} , 10 mg kg^{-1} , 30 mg kg^{-1} b.wt. and the standard drug pioglitazone at an oral dose of 10 mg kg^{-1} b.wt. Values are the mean % change in 5 animals of a group; significance: $*p < 0.05$, $**p < 0.01$ when compared with control.

ance was calculated to be around 7.8%, 13.9%, and 25.7% at the doses of $3, 10$, and 30 mg kg^{-1} , at day 10 (Fig. 8A). The improvement was further enhanced to 13.2%, 20.1% and 31.6% at the doses of $3, 10$, and 30 mg kg^{-1} , respectively, at day 15 (Fig. 8B). These effects of 5e in *db/db* mice are associated with a decrease in fasting blood glucose and serum insulin levels, in a dose-dependent manner, and an improvement in the lipid profile of the animals. As shown in Fig. 9, treatment with 5e at a dose of 30 mg kg^{-1} 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 muscles. To investigate the signaling pathways involved in the glucose uptake by 5e in skeletal muscles, western blot analysis was carried out. From the literature, it is known that insulin stimulation in target tissues results in phosphorylation and activation of the signaling cascade IR/IRS1/PI3K/Akt/GSK3 β leading to enhanced glucose utilization in peripheral tissues and

Fig. 8 Dose dependent effect of compound 5e on oral glucose tolerance (OGTT) of *db/db* mice (A) Day 10, (B) Day 15. Values are the mean % change in 5 animals of a group; significance: $*p < 0.05$, $**p < 0.01$ when compared with control.

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

decreased hepatic glucose production.⁵⁴ Given the sensitizer potential of 5e, we assessed its cellular activity in the insulin signal transduction pathway in skeletal muscles, 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 GSK-3 β (Ser-9) (Fig. 10). Results indicated the ability of 5e to activate insulin signaling pathway in skeletal muscle cells.

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

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 Compound 5e was found to inhibit the activity of the PTP-1B enzyme in a concentration-dependent manner and the IC₅₀ value was calculated to be 7.12 μ M (Table S1†). Thus, PTP-1B may be involved in 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 μ M 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^{−1} caused a significant decrease in triglyceride and total cholesterol levels and increased the HDL-C level in a significant manner. The mechanism of action of 5e was found to be mediated *via* activation of IRS-1, Akt and GSK-3 β 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 performed with UV light (254 nm). ¹H NMR and ¹³C NMR spectra were recorded using a Bruker Supercon Magnet DRX-300 spectrometer (operating at 400 MHz for ¹H and 50 MHz for ¹³C) using DMSO-*d*₆ or CDCl₃ as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million. Signal patterns are indicated as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Coupling constants (*J*) are given in hertz. Infrared (IR) spectra were recorded in KBr disc and reported in wave number (cm^{−1}). An 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 a Thermo Lcq Advantage Max-IT. High resolution mass spectra (HRMS) were recorded on a 6520 Agilent QT of LC MS/MS (accurate mass). Additionally, the purity of

the compounds was measured using a RP-HPLC with the following conditions, and the purity of the compounds was >98%. The Waters HPLC system (Milford, USA) consisted of a binary pump (model Waters 515 HPLC pump), an autosampler (model 2707 Autosampler) and a PDA detector (Waters 2998). Data collection and analysis were performed using Empower pro 2 software. The resolution and better peak shape were achieved using an X-Bridge C18 (5 μ m, 4.6 $\times$ 250 mm) column, protected with a C₁₈ 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⁻¹.

General procedure for the synthesis of functionalized methyl 8-(methylthio)-2-phenyl-6-aryl-4,5-dihydro-2H-benzo[e]indazole-9-carboxylates (5a–h). A mixture of 6-aryl-2H-pyran-2-one (3a–h, 1 mmol), 2-phenyl-6,7-dihydro-2H-indazole-4(5H)-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 the reaction was monitored by TLC, and upon 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-2H-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 4 h. Using the general procedure described above, 234 mg (55%) of 5a was obtained as an off-white solid; mp = 173–175 °C; MS (ESI) 427 [M + H⁺]; IR (KBr) ν = 3020, 1726, 1574 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 2.81–2.93 (m, 4H, 2CH₂), 2.48 (s, 3H, SCH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₆H₂₃N₂O₂S: 427.1480, found: 427.1470.

Methyl 6-(4-chlorophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2H-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 3 h. 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; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 2.85–2.89 (m, 4H, 2CH₂), 2.52 (s, 3H, SCH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₆H₂₂ClN₂O₂S: 461.1091, found: 461.1079.

Methyl 6-(4-bromophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2H-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 3.5 h. 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; ¹H NMR (300 MHz, CDCl₃) δ = 7.98 (s, 1H, ArH), 7.40–7.71 (m, 4H, ArH), 7.12–7.34 (m, 4H, ArH), 4.01 (s, 3H, OCH₃), 2.83–2.87 (m, 4H, 2CH₂), 2.48 (s, 3H, CH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₆H₂₂BrN₂O₂S: 505.0585, found: 505.0575.

Methyl 6-(4-methoxyphenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2H-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 3 h. 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; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 3.88 (s, 3H, OCH₃), 2.81–2.95 (m, 4H, 2CH₂), 2.49 (s, 3H, CH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₇H₂₅N₂O₃S: 457.1586, found: 457.1557.

Methyl 8-(methylthio)-2-phenyl-6-*p*-tolyl-4,5-dihydro-2H-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 3.5 h. 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; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 2.80–2.94 (m, 4H, 2CH₂), 2.48 (s, 3H, CH₃), 2.43 (s, 3H, CH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₇H₂₄N₂O₂S: 440.1559, found: 440.1547.

Methyl 8-(methylthio)-2-phenyl-6-(thiophen-2-yl)-4,5-dihydro-2H-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 5 h. 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; ¹H NMR (200 MHz, CDCl₃) 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, OCH₃), 3.02–3.13 (m, 2H, CH₂), 2.82–2.92 (s, 2H, CH₂), 2.49 (s, 3H, SCH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₄H₂₀N₂O₂S₂: 432.0966, found: 432.0970.

Methyl 6-(4-fluorophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2H-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 4 h. 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; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 2.83–2.88 (m, 4H, 2CH₂), 2.48 (s, 3H, SCH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₆H₂₂FN₂O₂S: 445.1386, found: 445.1381.

Methyl 8-(methylthio)-2-phenyl-6-(pyren-1-yl)-4,5-dihydro-2H-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 5.5 h. 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; ¹H NMR (300 MHz, CDCl₃) δ = 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, OCH₃), 2.61–2.78 (m, 4H, 2CH₂), 2.49 (s, 3H, SCH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₃₆H₂₇N₂O₂S: 551.1793, found: 551.1797.

General procedure for the synthesis of functionalized methyl 8-(methylsulfonyl)-2-phenyl-6-aryl-4,5-dihydro-2H-benzo[e]indazole-9-carboxylates (6a and b). A mixture of methyl 8-(methylthio)-2-phenyl-6-aryl-4,5-dihydro-2H-benzo[e]indazole-9-carboxylates 5 (1 mmol) and *m*-chloroperbenzoic acid (*m*-CPBA) (5 mmol) in dry DCM (5 mL) was stirred at room temperature for 30–60 min. The progress of the reaction was monitored by TLC, and upon completion, the reaction mixture was neutralized with NaHCO₃ solution. The neutral-

ized 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-2H-benzo[e]indazole-9-carboxylate (6a). A mixture of methyl 8-(methylthio)-2-phenyl-6-*p*-tolyl-4,5-dihydro-2H-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 °C; MS (ESI) 473 [M + H⁺]; IR (KBr) ν = 2927, 1729, 1599 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 3.19 (s, 3H, SO₂CH₃), 3.01–3.05 (m, 2H, CH₂), 2.85–2.91 (m, 2H, CH₂), 2.43 (s, 3H, CH₃) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₇H₂₅N₂O₄S: 473.1535, found: 473.1530.

Methyl 6-(4-fluorophenyl)-8-(methylsulfonyl)-2-phenyl-4,5-dihydro-2H-benzo[e]indazole-9-carboxylate (6b). A mixture of methyl 6-(4-fluorophenyl)-8-(methylthio)-2-phenyl-4,5-dihydro-2H-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 °C; MS (ESI) 477 [M + H⁺]; IR (KBr) ν = 2927, 1729, 1599 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 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, OCH₃), 3.20 (s, 3H, SO₂CH₃), 2.95–3.02 (m, 2H, CH₂), 2.86–2.92 (m, 2H, CH₂) ppm; ¹³C NMR (50.3 MHz, CDCl₃) 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 C₂₆H₂₂FN₂O₄S: 477.1284, found: 477.1277.

Ethical statement

The work with all these animals was cleared by the 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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