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ARTICLE

Novel Potent HIF-1 Inhibitors for the Prevention of Tumor Metastasis: Discovery and Optimization of 3-Aryl-5-indazole-1,2,4-oxadiazole Derivatives

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Hypoxia inducible factor-1 (HIF-1) is the key transcription factor of cellular response to hypoxia and plays a critical role in tumor metastasis. We describe here the discovery and a structure-activity relationship study of a series of 3-aryl-5-indazole- 1,2,4-oxadiazole derivatives as novel HIF-1 inhibitors. The two most promising compounds **4g** and **4h** inhibit HIF-1 transcription with IC_{50} values of 0.62 and 0.55 μM *in vitro*, respectively, and they exhibit more efficient HIF-1 inhibition in xenograft tumors than YC-1, a potential anticancer drug targeting HIF-1. In addition, they also remarkably prevent the hypoxia-driven migration of SKOV3 cells *in vitro* and tumor metastasis *in vivo*. Further investigation of the mechanism revealed that the two inhibitors could decrease HIF-1α and VEGF expression. These results suggest that our newly synthesized HIF-1 inhibitors **4g** and **4h** are potential therapeutic agents with which to treat tumor metastasis.

1 Introduction

Hypoxia is a common characteristic of most solid tumors resulting from inefficient microvascular systems associated with rapid tumor growth. Tumor cells within these regions show resistance to both radiotherapy and chemotherapy, and are associated with poor prognosis in cancer patients.[1, 2] The hypoxia inducible factor-1 (HIF-1) is the key transcription factor activated in response to intratumoral hypoxia and plays a pivotal role in adaptation of tumor cells to hypoxia by orchestrating a number of cellular responses, such as angiogenesis, glycolysis, pH adaptation, cell proliferation and migration.[3]

HIF-1 is a heterodimeric basic helix-loop-helix transcription factor, with an oxygen sensitive HIF-1α subunit and a constitutively expressed HIF-1β subunit. Under normoxic conditions, HIF-1α continuously binds to pVHL, ARD1, SUMO and RACK1 and this leads to ubiquitination and proteasomal degradation. However, under hypoxic conditions, HIF-1α is stabilized and rapidly accumulated. After binding to transcriptional co-activators such as p300/CBP, HIF-1α is translocated to the nucleus and heterodimerized with HIF-1β to form an HIF-1 complex. HIF-1 specifically activates the transcription of more than 70 putative target genes, including glucose transporter 1 (*Glut1*), carboanhydrase IX (*CAIX*), vascular endothelial growth factor (*VEGF*) and erythropoietin (*EPO*) *via* binding to hypoxic response elements or (HRE)-containing promoter regions. In this way, anaerobic glycolysis, angiogenesis, cell proliferation and tumor invasion are mediated.[4, 5]

Increased expression of HIF-1 has been observed in many kinds of human cancers, including breast, brain, colon, lung and prostate cancers, and is strongly correlated with aggressive tumor growth, therapeutic resistance and poor patient prognosis.[5] Consequently, HIF-1 is regarded as an attractive target for the treatment of tumor hypoxia. A variety of compounds with different scaffolds have been identified as HIF-1 inhibitors by HIF-1-targeted cell based screens and empirical discoveries based on chemical libraries and natural products,[6-8] including the PI3K/mTOR inhibitor wortmannin,[9] the microtubule inhibitor 2-methoxyestradiol,[10] the topoisomerase I inhibitor topotecan,[11] the trypanocidal agent acriflavine,[12] and the arylsulfonamide derivative KCN1[13, 14]. In particular, the pyrazole-oxadiazole derivative BAY 87-2243 has been advanced into Phase I clinical trial as an antitumor agent[14] (Figure 1). These HIF-1 inhibitors regulate the HIF-1 signaling pathway through different molecular mechanisms, including transcriptional regulation, mRNA translation, nuclear translocation, HIF-1α degradation and HIF-1 dimerization. The majority of HIF-1 inhibitors identified so far are multi-target compounds and HIF-1 inhibition cannot be easily separated from other activities. Therefore, design of more specific HIF-1 targeting agents is of great significance and clinical importance in the investigation of potential therapeutic agents for solid tumors.

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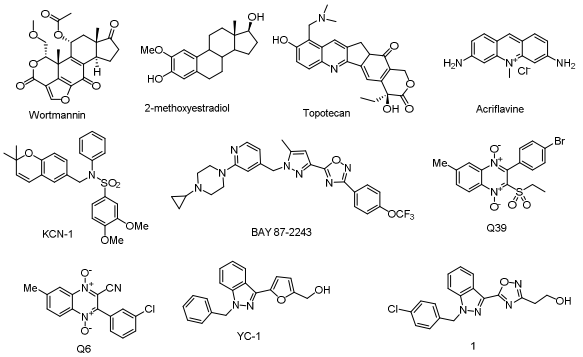


Figure 1. Structures of selected HIF-1 inhibitors

Tumor metastasis is a complex and multi-step process by which primary tumor cells invade other tissues or parts of the body, finally proliferating into macroscopic secondary tumors. It is estimated that metastasis is responsible for as much as 90% of cancer-associated mortality while only a few drugs exhibit metastasis-inhibitory activity in the clinic.[15-17] Although the pathogenesis of cancer metastasis remains unclear, abundant evidence proves that HIF-1 is the key regulator of tumor metastasis.[3, 18, 19] Recent research also has revealed that YC-1 and HSP90 inhibitor Ganetespib can efficiently inhibit tumor metastasis by blocking the HIF-1 pathway[20, 21] and consequently, development of novel HIF-1 inhibitors will provide specific therapeutic agents against tumor metastasis.

In the past few years, our group has focused on the development of small molecules targeting tumor hypoxia and has identified several hypoxia selective antitumor agents, including the quinoxaline-1,4-dioxide derivatives Q39 and Q6. In addition to their excellent hypoxia selective cytotoxic activities against a variety of tumor cell lines, these two compounds inhibit hypoxia-induced HIF-1α transcription or protein expression in vitro.[22-25] In our search for novel hypoxia-targeted antitumor agents, we recently designed the indazole-1,2,4-oxadiazole derivative **1** as a bioisostere analogue of the indazole-furan derivative YC-1, an attractive lead compound. This compound has dual reactions with HIF-1, decreasing HIF-1α protein accumulation and stimulating FIH-dependent p300 dissociation from HIF-1α.[26-28] Compound **1** exhibits comparable HIF-1 inhibitory activity (IC_{50} = 5.72 μM) to that of YC-1 (IC_{50} = 3.97 μM). Previous SAR study on YC-1 derivatives reveals that the substituted furan moiety on indazole skeleton as well as its substitution pattern is crucial for the high HIF-1 inhibition.[29] To identify more potent HIF-1 inhibitors for further pharmacological study as well as to verify the known structure-activity relationships, optimization of compound **1** was carried out through four modifications: 1) modification of the R_1 hydroxyethyl moiety of 1,2,4-oxadiazole, 2) modification or scaffold hopping of the indazole, 3) modification of R_2 or the chlorobenzyl moiety of the indazole and investigation of the tautomerism possibilities of the indazole (Figure 2).

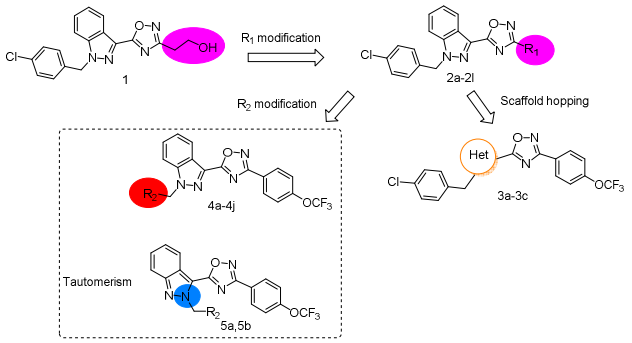
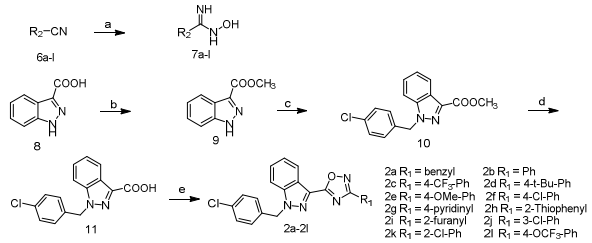


Figure 2. Diagram for the structural optimization strategy

2 Results and discussion

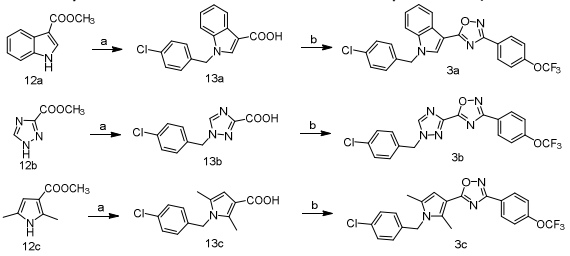
2.1 Chemistry

In the compounds **1** and **2a**-**2l,** the 5-(N-benzyl-indazole)-1,2,4-oxadiazole skeleton was retained and various alkyl and aryl groups were examined as an R_1 moiety to find an optimal substituent. Condensation of alkyl or aryl nitriles **6a-l** with hydroxylamine gave the intermediates **7a-l**.[30] Esterification of compound **8** with MeOH and thionyl chloride led to compound **9**, benzylation of which with 4-chlorobenzyl chloride gave compound **10.** Hydrolysis of **10** gave **11**, which was condensed with **7a-l** to afford compounds **2a-2l** (Scheme 1).



Scheme 1. Synthesis of compounds 2a-2l. *Reagents and conditions*: (a) NH_2OH-HCl, Na_2CO_3, EtOH, H_2O, reflux; (b) $SOCl_2$, CH_3OH, r.t., overnight; (c) p-chlorobenzyl chloride, NaH, DMF, r.t.; (d) 0.25 mol/L NaOH, THF, r.t., overnight; (e) **7a-l**, EDCI, HOBt, DMF, 140 °C.

While retaining the optimal 4-OCF_3-Ph moiety in the 3-position of the 1,2,4-oxadiazole, further modifications were made on the indazole moiety to probe their effect on activity (**3a-3c**). Compounds were prepared from the corresponding indole, 1,2,4-triazole or 2,5-dimethyl pyrrole 3-carboxylic acid ester **12a-12c** with the same synthetic route as was used for compound **2l** (Scheme 2).



Scheme 2. Synthesis of compounds 3a-3c. *Reagents and conditions*: (a) i) p-Chlorobenzyl chloride, NaH, DMF, r.t.; ii) 0.25 mol/L NaOH, THF, r.t., overnight; (b) **7l**, EDCI, HOBt, DMF, 140 °C.

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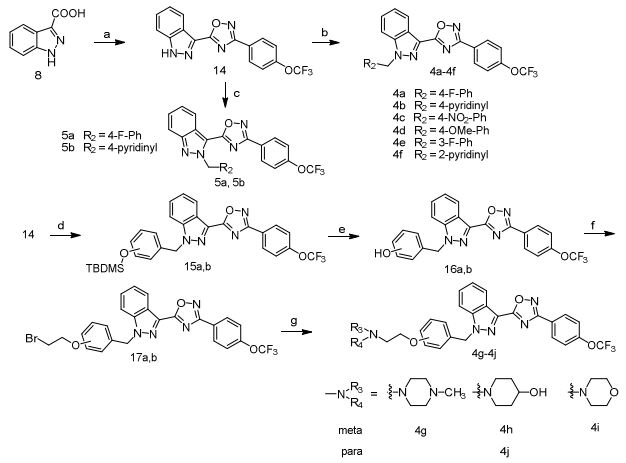
Variations of the R_2 indazole moiety were extensively explored with substituted aryl groups. First, compounds **4a-4f** were prepared through condensation of compound **14** with substituted benzyl chlorides and analogues in the presence of NaH in DMF**.** Meanwhile, compounds **5a** and **5b** were separated as minor products to probe the effect of tautomerisim on activity (Scheme 3). Benzylation of compound **14** with *p*- or *m*-TBDMS-O-benzyl bromide yielded compounds **15a** and **15b**, and deprotection with TBAF gave **16a** and **16b**, followed by alkylation with 1,2-dibromoethane gave compounds **17a** and **17b**. Subsequently, amination of **17a** and **17b** with various amines afforded compounds **4g-4j.**



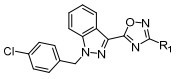
Scheme 3. Synthesis of compounds series 4 and 5. *Reagents and conditions*: (a) **7l**, EDCI, HOBt, DMF, 140 ^{o}C; (b) substituted benzyl chloride or analogues, NaH, DMF, r.t., major products; (c) 4-fluorobenzyl chloride or 4-chloromethyl pyridine, NaH, DMF, r.t, minor products were separated; (d) NaH, DMF, r.t. (e) TBAF, THF; (f) K_2CO_3, $BrCH_2CH_2Br$, acetone, reflux; (g) secondary amine, K_2CO_3, dry CH_3CN, reflux.

2.2 Biological evaluations

HIF-Luciferase activity *in vitro*. With a human colon carcinoma cell line HCT-116 stably expressing a hypoxia-inducible luciferase reporter gene (HRE element), the synthesized target compounds were evaluated for their potency in inhibiting HIF-1-mediated transcription. All of the assays were performed under standard hypoxic conditions (1% O_2). Cell viability, as measured by a sulforhodamine B assay, showed that all compounds effectively inhibited HIF-1 transcriptionbut had no significant cytotoxicity at the concentrations at which they were active (cell viability >10 μM). YC-1 was used as a positive control with IC_{50} value of 3.97 ± 0.13 μM, which was in substantial agreement with the reported value in reference (IC_{50} = 1.2 μM).[31]

The first series of compounds **2a-2l** were designed to assess the importance of the R_1 moiety to HIF-1 inhibition. In general, aryl substituents are favorable in this position. Except for compounds **2c** and **2j,** other aryl substituted compounds demonstrate moderate HIF-1 inhibitory activities with IC_{50} values ranging from 1.63 to 8.24 μM, while the alkyl substituted compounds **2a** showed a dramatic decrease in activity (Table 1). The results also reveal that the *p*-position substitution was more favorable than substitution at the *o*- or *m*-position (**2f** *vs* **2j**, **2k**). Three compounds (**2d, 2e, 2l**) exhibited more potent HIF-1 inhibition than YC-1 with IC_{50} values ranging from 1.63 μM to 3.16 μM. Compound **2l** was the most active compound in this series and the 4-CF_3O moiety was chosen as the optimal R_1 group for the further structural exploration.

Table 1. Structures and biological activities of compounds **2a-2l**.

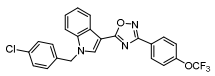
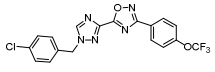
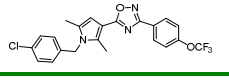


No.	R_1	HRE IC_{50} (μM) [a]	Cell Viability (μM)
YC-1	-	3.97 ± 0.13	> 10
1		5.72 ± 0.27	> 10
2a		> 10	> 10
2b		5.25 ± 0.24	> 10
2c		> 10	> 10
2d		2.67 ± 0.19	> 10
2e		3.16 ± 0.15	> 10
2f		5.48 ± 0.31	> 10
2g		4.83 ± 0.25	> 10
2h		6.80 ± 0.30	> 10
2i		5.08 ± 0.23	> 10
2j		> 10	> 10
2k		8.24 ± 0.52	> 10
2l		1.63 ± 0.09	> 10

[a] IC_{50} values are indicated as the mean ± SD (standard deviation) of three independent experiments.

Next, compounds **3a-3c** were designed to probe the effect of indazole moiety on activity. Replacement of the indazole with indole, 1,2,4-triazole or dimethylpyrrole moieties also clearly attenuated HIF-1 inhibition. In comparison with **2l**, compound **3a** (IC_{50} = 5.88 ± 0.41 μM) and **3c** (IC_{50} = 6.25 ± 0.38 μM) showed a 3- to 4-fold decrease in activity and compound **3b** (IC_{50} > 10.0 μM) was almost inactive. These results confirmed that the indazole moiety is essential to HIF-1 inhibitory activity and thus they were retained in future modifications.

Table 2. Structures and biological activities of compounds **3a-3c**.

No.	Structure	HRE IC_{50} (μM) [a]	Cell Viability (μM)
2l		1.63 ± 0.09	> 10
3a		5.88 ± 0.41	> 10
3b		> 10	> 10
3c		6.25 ± 0.38	> 10

[a] IC_{50} values are indicated as the mean ± SD (standard deviation) of three independent experiments.

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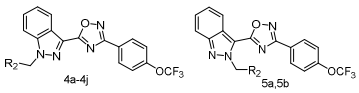
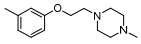
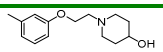
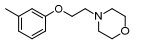
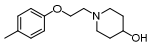
Compounds **5a** and **5b** were prepared to investigate the influence of indazole tautomerism on HIF-1 inhibitory activity. Compounds substituted at the N-2 position show an approximately 2-fold decrease in activity over those substituted at the N-1 position (**5a** *vs* **4a**, **5b** *vs* **4b**), indicating the N-1 substitution is more favorable for HIF-1 inhibition.

Compounds **4a-j** resulted from modification of the R_2 moiety in **2l** with various aryl substituents (Table 3). In general, **4a-j** all exhibited good to excellent HIF-1 inhibition, indicating that different variations of the phenyl group were well tolerated. In particular, compounds **4g-4j**, with IC_{50} values ranging from 0.55 to 1.94 μM *in vitro*, were more efficient HIF-1 inhibitors than YC-1. Such enhancement in HIF-1 inhibitory activity suggests that the introduction of hydrophilic aminoalkyl ether moiety that aims to improve physical chemistry property may be beneficial for the cell-based evaluation and in vivo biological evaluation. The weaker activity of compound **4j** compared to **4h** also revealed that substitution at the *m*-position is more favorable than at the *p*-position. Compounds **4g** and **4h** were the most potent HIF-1 inhibitors of all the designed compounds with IC_{50} values of 0.62 and 0.55 μM, respectively, and they were selected for further mechanism studies and *in vivo* evaluation.

Table 3. Structures and biological activities of compounds **4a-4j** and **5a, 5b**



No.	R_2	HRE IC_{50} (μM) [a]	Cell Viability (μM)
4a		3.84 ± 0.12	> 10
5a		7.65 ± 0.55	> 10
4b		4.65 ± 0.31	> 10
5b		10.3 ± 0.77	> 10
4c		1.74 ± 0.11	> 10
4d		5.67 ± 0.26	> 10
4e		6.01 ± 0.30	> 10
4f		5.00 ± 0.19	> 10
4g		0.62 ± 0.035	> 10
4h		0.55 ± 0.029	> 10
4i		1.00 ± 0.074	> 10
4j		1.94 ± 0.11	> 10

[a] IC_{50} values are indicated as the mean ± SD (standard deviation) of three independent experiments.

In order to determine the optimal adminstration manner for *in vivo* evaluation, the physicochemical properties of compounds **4g** and **4h** were calaulated by using ChemBioDraw and ACD/I-Lab prediction engine (https://ilab.acdlabs.com/iLab2/index.php). The results in Table 4 revealed that the two compounds possessed relatively large molecular weight, high LogP and TPSA values, which were unfavourable for oral admination. Therefore, the intravenous injection was chosen as the suitable method for them in *in-vivo* stdies.

Table 4. Predicted physicochemical properties of compounds **4g** and **4h.**

Compounds	4g	4h
Molecular Weight	578.6	579.6
Data predicted by ChemBioDraw	TPSA : 74.49 CLogP : 5.74	TPSA : 91.48 CLogP : 5.62
Data predicted by ACD/Labs	TPSA : 81.68 LogP : 5.56 ± 0.91	TPSA : 98.67 LogP : 5.65 ± 0.89

HIF-1 transcriptional activity *in vivo*. HCT116 colon carcinoma cells stably transfected with the hypoxia-inducible luciferase reporter gene (HRE-luciferase) were xenografted in nude mice. When the tumor volume reached 200 mm^3, the mice were divided into four groups, followed by *i.v.* administration of vehicle or test compounds for three consecutive days (50 mg/kg, once daily, Fig.3A). The luciferase activity was markedly decreased after treatment with **4g**, **4h** or YC-1 for 3 days compared to that of vehicle control group (Fig. 3B). These data demonstrate that **4g**, **4h** and YC-1 also inhibit the HIF-1 activity in tumor xenografts. More importantly, consistent with *in vitro* results, the inhibitory activities of **4g** and **4h** on HIF-1 transcriptional activity *in vivo* are much better than the positive control YC-1 (Fig. 3C).

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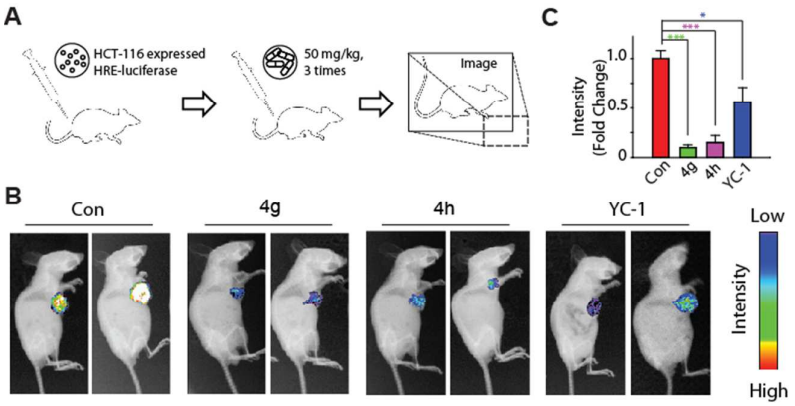

Figure 3. Compounds **4g** and **4h** inhibit HIF-1 transcriptional activity *in vivo*. (**A**) The scheme for evaluation of the inhibitory activity of the compound on the HIF-1 transcriptional activity *in vivo*. (**B-C**) Mice bearing HCT-116 xenografts expressing HRE-luciferase were treated with vehicle or compounds (50mg/kg) once daily for three days. (**B**) The HRE-driven luciferase activity was determined by Xenogen imaging after three days' treatment. The representative images in different groups are shown. (**C**) The intensity of luminescence was calculated in software, and the data are shown as the ratio vs. control group and expressed as the mean ± SD. *, vs. control (n≧3, *, p<0.05, ***, p<0.001)

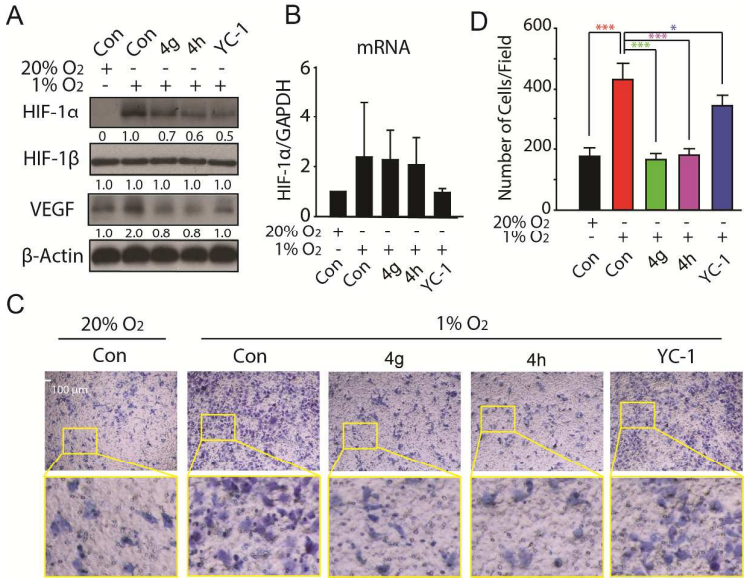

Figure 4. The effect of **4g** and **4h** on HIF-1 accumulation and hypoxia-driven cell migration. (**A**) The accumulation of HIF-1α, HIF-1β and VEGF was examined in HCT116 cells treated with **4g**, **4h** and YC-1 (5 μM) by Western blots analysis. β-actin was used as a loading control. The density of immunoreactive bands was calculated by Quantity One software, and the data are shown as the ratio versus β-Actin. (**B**) HCT116 cells were exposed to **4g**, **4h** and YC-1 (5 μM). Then, the total RNA was extracted and analyzed for HIF-1α mRNA expression by RT-PCR, using GAPDH as a control gene. (**C-D**) the effect of compounds on hypoxia-driven cell migration basing on transwell model was conducted. (**C**) The representative images in different groups are shown. (**D**) The number of cells was calculated, and the data are expressed as the mean ± SD. (n=3, *, p<0.05, ***, p<0.001)

Western blotting and RT-PCR analysis. For further mechanistic studies, the structural based effects of compounds **4g** and **4h** were evaluated using biochemical techniques with YC-1 as positive control. Since most of the HIF-1 regulation usually occurs at the protein level, we investigated the effect of compounds on HIF-1α, HIF-1β and its downstream target protein VEGFR using Western blotting under normoxia or hypoxic conditions in HCT116 cells. As expected, the protein levels of HIF-1α are markedly increased under hypoxia compared with those under normoxia, while the protein levels of HIF-1β remained unchanged (Fig. 4A). Meanwhile, compounds **4g** and **4h** exhibit remarkable inhibitory activity on HIF-1α protein accumulation, similar to the effect of YC-1. To ascertain whether the reduction of HIF-1α by these compounds occurs at the transcriptional level, RT-PCR analysis was used to study the effect of compounds on the accumulation of HIF-1α mRNA. As shown in Fig. 4B, the HIF-1α mRNA levels are not significantly changed after **4g** and **4h** treatment in HCT116 cells. Of note, the inhibitory activity on HIF-1α downstream VEGF caused by **4g** and **4h** is better than that resulting from YC-1 (Fig. 4A), and this is consistent with the fact that **4g** and **4h** show more efficient inhibitory activities on HIF-1 transcription than does YC-1 both *in vitro* and *in vivo*. Therefore, these data further suggest that the decrease of HIF-1α protein maybe not the only reason for their strong activity. Additional work in this area is in progress and will be reported in due course.

Transwell assay. To confirm the activities of compounds **4g** and **4h** against tumor cell invasion and tumor metastasis, a cell

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migration assay based on transwell was carried out using human ovarian cancer cells SKOV3. As shown in Fig. 4C, compared to the control group, **4g**, **4h** and YC-1 significantly reduce the number of cells passing through the polycarbonate filter. In addition, **4g** and **4h** are more efficient than YC-1 in preventing cell migration with weak cytotoxicity (Fig. 4D and data not shown).

Pulmonary metastasis model of SKOV3 in nude mice. SKOV3 cells were *i.v.* injected into nude mice, and the mice started to receive *i.v.* injections of the compounds with dose of 50 mg/kg on the following two weeks (Fig. 5A). After two weeks' treatment, a transient decrease of the body weight of mice is only observed in the YC-1 treatment group (Fig. 5B). The mice were sacrificed on day 30 and the lungs were removed and fixed in paraformaldehyde. For analysis of pulmonary metastasis, lungs were cut into 5 μm sections and stained for HE. As shown in Fig. 5C and 5D, the numbers of pulmonary nodules in lungs are significantly decreased in treatment groups **4g** and **4h,** but not in the YC-1 group. These *in vivo* results are further confirmation that the newly synthesized HIF-1 inhibitors **4g** and **4h** are potential therapeutic agents for tumor metastasis.

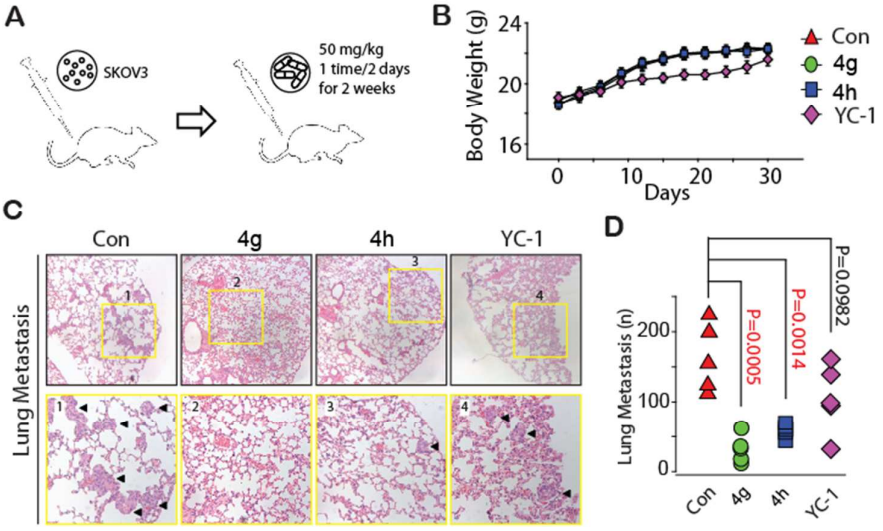

Figure 5. The effect of **4g** and **4h** on the development of pulmonary metastasis *in vivo*. (**A**) The scheme for evaluating the effect of **4g** and **4h** on the development of pulmonary metastasis *in vivo*. (**B**) The changes of body weight in the pulmonary metastasis model of SKOV3 in nude mice. (**C**) The representative images of lung in different groups are shown. (**D**) The numbers of pulmonary nodules in lung were calculated, and the data are expressed as the mean ± SD. (n=5)

3 Conclusion

In summary, a series of 3-aryl-5-indazole-1,2,4-oxadiazole derivatives has been designed, synthesized and identified as specific HIF-1 inhibitors. All the synthesized compounds show weak cytotoxic activities (IC_{50} > 10 μM) and a majority of them exhibit moderate to potent HIF-1 transcriptional inhibition. The two most potent HIF-1 inhibitors were **4g** and **4h** with IC_{50} values of 0.62 and 0.55 μM respectively, which is 6- to 7-fold more effective than YC-1. Consistent with the *in vitro* results, the two compounds **4g** and **4h** demonstrate much better HIF-1 inhibitory activities in xenografts tumor than YC-1. A cell migration assay based on transwell conducted in hypoxic conditions disclosed that compounds **4g** and **4h** can significantly reduce the number of SKOV3 cells passing through the polycarbonate filter, and are more efficient than YC-1. Furthermore, the *in vivo* cell metastasis experiment reveals that they can decrease the numbers of pulmonary nodules in lungs remarkably compared to vehicle control and YC-1. The mechanistic studies indicate that these two inhibitors influence the protein expression levels of HIF-1α and its downstream target gene VEGF. Our results indicate the newly synthesized HIF-1 inhibitors **4g** and **4h** are potential therapeutic agents against tumor metastasis.

4 Experimental Section

4.1 General Methods for Chemistry.

All reagents and solvents used were reagent grade and purchased from commercial resources. Flash chromatography was performed using silica gel (200-300 mesh). All reactions were monitored by TLC, using silica gel plates with fluorescence F254 and UV light visualization. Melting points were determined with a B-540 Buchi melting-point apparatus. ^{1}H NMR was recorded on a Brüker Advance DMX 500 MHz spectrometer. Coupling constants (*J*) are expressed in hertz (Hz) and chemical shifts (δ) of NMR are reported in parts per million (ppm) units relative to an internal control (TMS). Low resolution mass spectra (ESI-MS) were gathered on a Finnigan LCQ DecaXP ion trap mass spectrometer and HRMS spectra on an Agilent 6224 TOF LC/MS. The purity of compounds was determined by Agilent 1260 HPLC system and analysis to be over 95%. (Column: Eclipse XDB-C18, 5.0 μm, 4.6 × 250mm (Agilent); Flow rate: 1.0 mL/min; Mobile phase: A: MeOH, B: H_2O).

General Procedure for the Synthesis of Compounds 7a-7l. The substituted cyanide (**6a-6l**, 4.0 mmol) was added to a solution of hydroxylamine hydrochloride (0.69 g, 10 mmol), Na_2CO_3 (0.64 g, 6.0 mmol) in 8 mL H_2O and 4 mL EtOH. The mixture was refluxed for 6 h and extracted with EtOAc (3×20 mL). The combined organic layer was washed with saturated saline, dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give **7a-7l**.

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***N*-hydroxy-2-phenylacetimidamide (7a)**. White solid, 70% yield. Mp 65-66 °C. (lit.[32] 65-67 °C).

***N*-hydroxybenzimidamide (7b)**. White solid, 73% yield. Mp 73-75 °C. (lit.[30] 76-78 °C).

***N*-hydroxy-4-(trifluoromethyl)benzimidamide (7c)**. White solid, 92% yield. Mp 113-114 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.92 (s, 1H, OH), 7.90 (d, *J* = 8.5 Hz, 2H, ArH), 7.75 (d, *J* = 8.0 Hz, 2H, ArH), 5.98 (s, 2H, NH); MS (ESI), *m/z*: 205 $[M+H]^+$.

***N*-hydroxy-4-tert-butylbenzimidamide (7d)**. White solid, 73% yield. Mp 147-149 °C. ^{1}H NMR (500 MHz, $CDCl_3$)**:** δ 7.57 (d, *J* = 8.5 Hz, 2H, ArH), 7.43 (d, *J* = 8.5 Hz, 2H, ArH), 4.90 (brs, 2H, NH), 1.33 (s, 9H, 3×CH_3); MS (ESI), *m/z*: 193 $[M+H]^+$.

***N*-hydroxy-4-methoxybenzimidamide (7e)**. White solid, 65% yield. Mp 119-120 °C. (lit.[33] 116-117 °C).

4-Chloro-N-hydroxybenzimidamide (7f). White solid, 81% yield. Mp 133-134 °C. (lit.[33] 133-134 °C).

***N*-hydroxyisonicotinimidamide (7g)**. White solid, 90% yield. Mp 197-198 °C. (lit.[34] 197-199 °C).

***N*-hydroxythiophene-2-carboximidamide (7h)**. White solid, 73% yield. Mp 86-88 °C. (lit.[35] 90 °C).

***N*-hydroxyfuran-2-carboximidamide (7i)**. White solid, 44% yield. Mp 52-53 °C. (lit.[36] 57 °C).

3-Chloro-N-hydroxybenzimidamide (7j). White solid, 96% yield. Mp 111-112 °C. (lit.[30] 116-117 °C).

2-chloro-N-hydroxybenzimidamide (7k). White solid, 84% yield. Mp 116-118 °C. (lit.[37] 119-121 °C).

***N*-hydroxy-4-(trifluoromethoxy)benzimidamide (7l)**. White solid, 89% yield. Mp 107-109 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.76 (s, 1H, OH), 7.79 (d, *J* = 8.5 Hz, 2H, ArH), 7.38 (d, *J* = 8.5 Hz, 2H, ArH), 5.90 (s, 2H, NH); MS (ESI), *m/z*: 221 $[M+H]^+$.

Methyl 1*H*-indazole-3-carboxylate (9). To a stirred solution of 1*H*-indazole-3-carboxylic acid (**8**, 1.5 g, 9.3 mmol) in 33 mL MeOH was added $SOCl_2$ (2.2 g, 18.6 mmol) at 0 °C, the mixture was stirred overnight at room temperature. The solvent was removed *in vacuo* and the residue was partitioned between 20 mL EtOAc and 15 mL H_2O. The organic layer was separated, washed with saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give **9** as a white solid in 93% yield. Mp 163-165 °C. (lit.[38] 162-163 °C).

Methyl 1-(4-chlorobenzyl)-1*H*-indazole-3-carboxylate (10). NaH (0.08 g, 1.8 mmol) was added to a solution of 1*H*-indazole-3-carboxylate (**9**, 0.30 g, 1.7 mmol) in dry DMF (5 mL) at 0 °C. The mixture was stirred at room temperature for 30 min and 4-chlorobenzyl chloride (0.30 g, 1.8 mmol) was added. After stirring for 4 h at room temperature, the reaction was quenched with H_2O (20 mL) and the mixture was extracted with EtOAc (3×20 mL). The combined organic layer was washed with saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography to give **10** as white solid with 57% yield. Mp 156-157 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.28 (d, *J* = 8.0 Hz, 1H, ArH), 7.43 (t, *J* = 7.5 Hz, 1H, ArH), 7.36 (m, 2H, ArH), 7.30 (d, *J* = 8.0 Hz, 2H, ArH), 7.18 (d, *J* = 8.0 Hz, 2H, ArH), 5.69 (s, 2H, benzylic-CH_2), 4.07 (s, 3H, OCH_3); MS (ESI), *m/z*: 301 $[M+H]^+$.

1-(4-Chlorobenzyl)-1*H*-indazole-3-carboxylic acid (11). Aqueous NaOH (0.25 mol/L, 25.0 mL) was added to a solution of compound **10** (0.85 g, 2.9 mmol) in 25 mL THF. The mixture was stirred at room temperature overnight and concentrated. The residue was partitioned with EtOAc (30 mL) and H_2O (30 mL) and the organic layer was discarded. The aqueous layer was acidified with 2.0 mol/L HCl until the pH was adjusted to 2. The precipitate formed was collected by suction filtration, washed with H_2O and dried *in vacuo* to afford **11** as a white solid with 69% yield. Mp 196-197 °C (lit.[39] 196 °C).

General Procedure for the Synthesis of Compounds 2a-2l. Compound **7a-7l** (0.32 mmol) was added to a solution of **11** (72 mg 0.25 mmol), EDCI (62 mg, 0.32 mmol) and HOBt (44 mg, 0.32 mmol) in 8 mL dry DMF. The mixture was stirred at room temperature for 1h and then heated to 140 °C for 1 h. The completion of reaction was confirmed by TLC and the DMF was removed in vacumm. The residue was extracted with EtOAc (15 mL×3) and the combined organic layer was washed with H_2O and saturated saline, and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography to get **2a-2l**.

3-Benzyl-5-(1-(4-chlorobenzyl)-1*H*-indazol-3-yl)-1,2,4-oxadiazole (2a). White solid, 23% yield. Mp 120-121 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.17 (d, *J* = 8.5 Hz, 1H, ArH), 7.95 (d, *J* = 8.5 Hz, 1H, ArH), 7.58 (t, *J* = 7.5 Hz, 1H, ArH), 7.44 (d, *J* = 7.5 Hz, 1H, ArH), 7.37-7.41 (m, 6H, ArH), 7.32 (d, *J* = 8.5 Hz, 2H, ArH), 7.28 (t, *J* = 7.5 Hz, 1H, ArH), 5.86 (s, 2H, benzylic-CH_2), 4.23 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.92, 169.94, 140.40, 135.59, 134.20, 133.97, 130.47, 129.12, 128.70, 127.73, 127.11, 123.66, 123.09, 121.86, 109.96, 53.42, 32.45; MS (ESI), *m/z*: 401 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{17}ClN_4O$ $[M+H]^+$: 401.1164, found 401.1166. HPLC purity = 98.72%, Rt 8.57 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-phenyl-1,2,4-oxadiazole (2b). White solid, 77% yield. Mp 144-145 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 8.0 Hz, 1H, ArH), 8.19 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 2H, ArH), 7.99 (d, *J* = 8.5 Hz, 1H, ArH), 7.59-7.65 (m, 4H, ArH), 7.50 (t, *J* = 7.5 Hz, 1H, ArH), 7.42 (d, *J* = 9.0 Hz, 2H, ArH), 7.37 (d, *J* = 9.0 Hz, 2H, ArH), 5.91 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.96, 168.91, 140.57, 134.32, 134.09, 131.39, 130.64, 129.24, 128.94, 128.82, 127.89, 127.86, 126.90, 123.83, 123.33, 122.07, 110.13, 53.56; MS (ESI), *m/z*: 387 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{15}ClN_4O$ $[M+H]^+$: 387.1007, found 387.1005. HPLC purity = 100.00%, Rt 10.96 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (2c). White solid, 62% yield. Mp 185-186 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (d, *J* = 8.0 Hz, 2H, ArH), 8.37 (d, *J* = 8.5 Hz, 1H, ArH), 7.98-8.01 (m, 3H, ArH), 7.63 (t, *J* = 7.5 Hz, 1H, ArH), 7.51 (t, *J* = 7.5 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 2H, ArH), 7.38 (d, *J* = 8.5 Hz, 2H, ArH), 5.91 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.33, 167.81, 140.50, 134.30, 133.88, 130.25, 130.20, 129.17, 128.72, 128.09, 127.89, 125.86 (q, J_1 = 7.2 Hz, J_2 = 3.4 Hz), 125.18, 123.89, 123.23, 122.47, 121.83, 110.11, 53.53; MS (ESI), *m/z*: 455 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{14}ClF_3N_4O$ $[M+H]^+$: 455.0881, found 455.0871. HPLC purity = 99.03%, Rt 12.78 min.

3-(4-(Tert-butyl)phenyl)-5-(1-(4-chlorobenzyl)-1*H*-indazol-3-yl)-1,2,4-oxadiazole (2d). White solid, 61% yield. Mp 162-163 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 (d, *J* = 8.0 Hz, 1H, ArH), 8.10 (d, *J* = 8.5 Hz, 2H, ArH), 7.98 (d, *J* = 8.5 Hz, 1H, ArH), 7.65 (d, *J* = 8.5 Hz, 2H, ArH), 7.62 (t, *J* = 7.5 Hz, 1H, ArH), 7.50 (t, *J* = 7.5 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 2H, ArH), 7.36 (d, *J* = 8.5 Hz, 2H, ArH), 5.91 (s, 2H,

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benzylic-CH_2), 1.34 (s, 9H, 3×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.72, 168.75, 154.72, 140.47, 134.20, 134.02, 130.64, 129.14, 128.70, 127.76, 127.54, 125.80, 123.94, 123.68, 123.25, 122.01, 110.00, 53.45, 35.02, 31.23; MS (ESI), *m/z*: 443 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{26}H_{23}ClN_4O$ $[M+H]^+$: 443.1633, found 443.1639. HPLC purity = 96.78%, Rt 12.78 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(4-methoxyphenyl)-1,2,4-oxadiazole (2e). White solid, 57% yield. Mp 157-159 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.35 (d, *J* = 8.5 Hz, 1H, ArH), 8.12 (d, *J* = 8.5 Hz, 2H, ArH), 7.98 (d, *J* = 8.5 Hz, 1H, ArH), 7.62 (t, *J* = 7.5 Hz, 1H, ArH), 7.49 (t, *J* = 7.5 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 2H, ArH), 7.36 (d, *J* = 8.5 Hz, 2H, ArH), 7.17 (d, *J* = 9.0 Hz, 2H, ArH), 5.90 (s, 2H, benzylic-CH_2), 3.86 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.61, 168.50, 162.04, 140.47, 134.20, 134.02, 130.64, 129.37, 129.13, 128.70, 127.75, 123.66, 123.22, 122.00, 119.26, 114.24, 109.99, 55.40, 53.44; MS (ESI), *m/z*: 417 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{17}ClN_4O_2$ $[M+H]^+$: 417.1113, found 417.1107. HPLC purity = 97.72%, Rt 10.59 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(4-chlorophenyl)-1,2,4-oxadiazole (2f). White solid, 71% yield. Mp 175-176 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.34 (d, *J* = 8.0 Hz, 1H, ArH), 8.19 (d, *J* = 8.5 Hz, 2H, ArH), 7.99 (d, *J* = 8.5 Hz, 1H, ArH), 7.70 (d, *J* = 8.5 Hz, 2H, ArH), 7.62 (t, *J* = 7.5 Hz, 1H, ArH), 7.50 (t, *J* = 7.5 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 2H, ArH), 7.37 (d, *J* = 8.5 Hz, 2H, ArH), 5.91 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.05, 168.01, 140.48, 137.44, 134.26, 133.92, 130.36, 129.17, 129.05, 128.72, 127.85, 125.28, 123.81, 123.20, 121.89, 110.08, 53.50; MS (ESI), *m/z*: 421 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}Cl_2N_4O$ $[M+H]^+$: 421.0617, found 421.0619. HPLC purity = 100.00%, Rt 13.85 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(pyridin-4-yl)-1,2,4-oxadiazole (2g). White solid, 66% yield. Mp 182-184 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.86 (d, *J* = 6.0 Hz, 2H, ArH), 8.37 (d, *J* = 8.5 Hz, 2H, ArH), 8.11 (d, *J* = 6.0 Hz, 2H, ArH), 8.00 (d, *J* = 8.5 Hz, 1H, ArH), 7.63 (t, *J* = 7.5 Hz, 1H, ArH), 7.51 (t, *J* = 7.5 Hz, 1H, ArH), 7.42 (d, *J* = 8.5 Hz, 2H, ArH), 7.37 (d, *J* = 9.0 Hz, 2H, ArH), 5.92 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.61, 167.31, 150.70, 140.50, 134.32, 134.23, 133.82, 130.08, 129.19, 128.73, 127.94, 123.97, 123.21, 121.77, 121.51, 110.15, 53.56; MS (ESI), *m/z*: 388 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{21}H_{14}ClN_5O$ $[M+H]^+$: 388.0960, found 388.0958. HPLC purity = 96.11%, Rt 7.59 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(thiophen-2-yl)-1,2,4-oxadiazole (2h). White solid, 72% yield. Mp 173-174 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.43 (d, *J* = 8.5 Hz, 1H, ArH), 8.01 (dd, J_1 = 4.0 Hz, J_2 = 1.5 Hz, 1H, ArH), 7.58 (dd, J_1 = 5.0 Hz, J_2 = 1.0 Hz, 1H, ArH), 7.44-7.51 (m, 1H, ArH), 7.42-7.45 (m, 2H, ArH), 7.32 (d, *J* = 8.5 Hz, 2H, ArH), 7.21-7.24 (m, 3H, ArH), 5.77 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.78, 164.92, 140.46, 134.24, 133.94, 130.27, 130.05, 129.41, 129.16, 128.71, 128.33, 127.94, 127.83, 123.81, 123.22, 121.93, 110.05, 53.50; MS (ESI), *m/z*: 393 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{20}H_{13}ClN_4OS$ $[M+H]^+$: 393.0571, found 393.0573. HPLC purity = 100.00%, Rt 9.44 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(furan-2-yl)-1,2,4-oxadiazole (2i). White solid, 67% yield. Mp 191-192 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 9.0 Hz, 1H, ArH), 7.67 (d, *J* = 1.0 Hz, 1H, ArH), 7.46-7.50 (m, 1H, ArH), 7.40-7.43 (m, 2H, ArH), 7.34 (d, *J* = 3.0 Hz, 2H, ArH), 7.31 (d, *J* = 8.5 Hz, 2H, ArH), 7.23 (d, *J* = 8.5 Hz, 2H, ArH), 6.62 (dd, J_1 = 3.5 Hz, J_2 = 2.0 Hz, 1H, ArH), 5.74 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.86, 161.71, 145.29, 142.42, 140.46, 134.26, 133.89, 130.16, 129.16, 128.73, 127.84, 123.84, 123.17, 121.88, 114.35, 111.89, 110.06, 53.52; MS (ESI), *m/z*: 377 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{20}H_{13}ClN_4O_2$ $[M+H]^+$: 377.0800, found 377.0802. HPLC purity = 99.01%, Rt 6.88 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(3-chlorophenyl)-1,2,4-oxadiazole (2j). White solid, 67% yield. Mp 136-137 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (d, *J* = 8.0 Hz, 1H, ArH), 8.30 (t, *J* = 2.0 Hz, 1H, ArH), 8.16-8.18 (m, 1H, ArH), 7.50-7.53 (m, 1H, ArH), 7.46-7.49 (m, 2H, ArH), 7.41-7.45 (m, 2H, ArH), 7.31 (d, *J* = 8.5 Hz, 2H, ArH), 7.23 (d, *J* = 8.5 Hz, 2H, ArH), 5.76 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.22, 167.92, 140.59, 135.07, 134.37, 134.03, 131.43, 130.42, 130.28, 129.27, 128.83, 128.63, 127.95, 125.87, 123.94, 123.31, 122.00, 110.17, 53.61; MS (ESI), *m/z*: 421 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}Cl_2N_4O$ $[M+H]^+$: 421.0617, found 421.0620. HPLC purity = 100.00%, Rt 13.95 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(2-chlorophenyl)-1,2,4-oxadiazole (2k). White solid, 67% yield. Mp 132-133 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.44 (d, *J* = 8.0 Hz, 1H, ArH), 8.17 (dd, J_1 = 8.0 Hz, J_2 = 2.0 Hz, 1H, ArH), 7.61 (dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1H, ArH), 7.41-7.51 (m, 5H, ArH), 7.33 (d, *J* = 8.5 Hz, 2H, ArH), 7.25 (d, *J* = 9.0 Hz, 2H, ArH), 5.77 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.41, 167.60, 140.47, 134.24, 133.97, 133.65, 132.04, 131.78, 130.96, 130.33, 129.15, 128.75, 127.81, 126.88, 126.11, 123.80, 123.26, 121.91, 110.03, 53.48; MS (ESI), *m/z*: 421 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}Cl_2N_4O$ $[M+H]^+$: 421.0617, found 421.0619. HPLC purity = 99.14%, Rt 10.08 min.

5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadiazole (2l). White solid, 74% yield. Mp 133-135 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, *J* = 8.0 Hz, 1H, ArH), 8.32 (d, *J* = 7.5 Hz, 2H, ArH), 8.00 (d, *J* = 8.5 Hz, 1H, ArH), 7.60-7.64 (m, 3H, ArH), 7.51 (t, *J* = 7.5 Hz, 1H, ArH), 7.43 (d, *J* = 8.5 Hz, 2H, ArH), 7.38 (d, *J* = 8.5 Hz, 2H, ArH), 5.92 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.26, 167.90, 151.51, 140.61, 134.40, 134.03, 130.47, 129.61, 129.29, 128.84, 127.98, 125.53, 123.95, 123.35, 121.99, 121.82 (q, *J* = 256.6 Hz), 121.19, 110.21, 53.63; MS (ESI), *m/z*: 471 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{14}ClF_3N_4O_2$ $[M+H]^+$: 471.0830, found 471.0826. HPLC purity = 98.63%, Rt 12.98 min.

2-(5-(1-(4-Chlorobenzyl)-1*H*-indazol-3-yl)-1,2,4-oxadiazol-3-yl)ethanol (1). Ethyl 2-(5-(1-(4-chlorobenzyl)-1*H*-indazol-3-yl)-1,2,4-oxadiazol-3-yl)acetate (120 mg, 3.0 mmol) was added to a solution of $LiAlH_4$ (152 mg, 3.27 mmol) in 10 mL of dry Et_2O at 0 °C. After stirring for 1 h, H_2O (2 mL) and saturated aqueous NH_4Cl (4 mL) were added to quench the reaction. The mixture was extracted with EtOAc (2×15 mL) and the organic layer was separated, washed with saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography to give **1** as pale yellow solid with 60% yield. Mp 118-119 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.33 (d, *J* = 8.0 Hz, 1H, ArH), 7.47-7.50 (m, 1H, ArH), 7.39-7.44 (m, 2H, ArH), 7.32 (d, *J* = 8.5 Hz, 2H, ArH), 7.23 (d, *J* = 8.5 Hz, 2H, ArH), 5.73 (s, 2H, benzylic-CH_2), 4.17 (t, *J* = 5.5 Hz, 2H, CH_2), 3.19 (t, *J* = 6.0 Hz, 2H, CH_2); MS (ESI), *m/z*: 355 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{18}H_{15}ClN_4O_2$ $[M+H]^+$: 355.0956, found 355.0955. HPLC purity = 100.00%, Rt 3.96 min.

1-(4-chlorobenzyl)-1H-indole-3-carboxylic acid (13a). With the same synthetic route to compound **11**, compound **13a** was prepared as white solid. Mp 204-206 °C. (lit.[40] 205-207 °C).

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1-(4-chlorobenzyl)-1H-1,2,4-triazole-3-carboxylic acid (13b). White solid, Mp 213-216 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 13.30 (s, 1H, COOH), 8.79 (s, 1H, ArH), 7.45 (d, *J* = 8.5 Hz, 2H, ArH), 7.35 (d, *J* = 8.5 Hz, 2H, ArH), 5.48 (s, 2H, CH_2). MS (ESI), *m/z*: 238 $[M+H]^+$.

1-(4-chlorobenzyl)-2,5-dimethyl-1H-pyrrole-3-carboxylic acid (13c). White solid, Mp 176-179 °C. ^{1}H NMR (500 MHz, DMSO): δ 11.55 (s, 1H, COOH), 7.41 (d, *J* = 8.5 Hz, 2H, ArH), 6.92 (d, *J* = 8.5 Hz, 2H, ArH), 6.16 (d, *J* = 1.0 Hz, 1H, pyrrole-H), 5.11 (s, 2H, CH_2), 2.34 (s, 3H, CH_3), 2.05 (s, 3H, CH_3). MS (ESI), *m/z*: 264 $[M+H]^+$.

General Procedure for the Synthesis of Compounds 3a-3c. Compound **7l** (0.50 mmol) was added to a solution of **13a**, **13b** or **13c** (0.60 mmol), EDCI (0.65mmol), HOBt (0.65 mmol) in dry DMF (5 mL). The mixture was stirred at room temperature for 1 h and then heated to 140 °C for 1 h. The mixture was partitioned between 25 mL EtOAc and 15 mL H_2O. The organic layer was separated, washed with H_2O and saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography.

5-(1-(4-Chlorobenzyl)-1*H*-indol-3-yl)-3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazole (3a). White solid, 44% yield. Mp 121-124 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.74 (s, 1H, ArH), 8.23-8.27 (m, 3H, ArH), 7.67 (d, *J* = 8.5 Hz, 1H, ArH), 7.62 (d, *J* = 8.5 Hz, 2H, ArH), 7.42 (d, *J* = 9.0 Hz, 2H, ArH), 7.38 (d, *J* = 8.5 Hz, 2H, ArH), 7.35 (t, *J* = 6.0 Hz, 2H, ArH), 5.61(s, 2H, benzylic-CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.38, 167.49, 151.25, 136.84, 134.44, 134.26, 131.98, 129.46, 129.39, 128.55, 126.20, 125.82, 124.00, 122.78, 121.80, 121.59 (q, *J* = 256.8 Hz), 121.17, 110.61, 101.86, 50.43; MS (ESI), *m/z*: 469 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{24}H_{15}ClF_3N_3O_2$ $[M+H]^+$: 470.0878, found 470.0879. HPLC purity = 99.40%, Rt 13.31 min.

5-(1-(4-chlorobenzyl)-1H-1,2,4-triazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadi-azole (3b). White solid, 65% yield. Mp 132-135 ℃. ^{1}H NMR (500 MHz, DMSO-d_6): 8.43 (s, 1H, ArH), 8.26 (d, *J* = 9.0 Hz, 2H, ArH), 7.64 (d, *J* = 8.5 Hz, 2H, ArH), 7.43-7.47 (m, 4H, ArH), 6.00 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.94, 165.76, 151.81, 145.00, 140.26, 134.82, 133.06, 129.56, 129.39, 129.19, 124.28, 121.64 (q, *J* = 257.4 Hz), 121.05, 53.65; MS (ESI), *m/z*: 422 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{18}H_{11}ClF_3N_5O_2$ $[M+H]^+$: 422.0626, found 422.0625. HPLC purity = 97.80%, Rt 9.18 min.

5-(1-(4-chlorobenzyl)-2,5-dimethyl-1H-pyrrol-3-yl)-3-(4-(trifluoro methoxy)phenyl)-1,2,4-oxadiazole (3c). White solid, 45% yield. Mp 127-129℃.^{1}H NMR (500 MHz, $CDCl_3$): 8.20 (d, *J* = 9.0 Hz, 2H, ArH), 7.29-7.33 (m, 4H, ArH), 6.87 (d, *J* = 8.5 Hz, 2H, ArH), 6.56 (s, 1H, ArH), 5.76 (s, 2H, benzylic-CH_2), 2.60 (s, 3H, CH_3), 2.18 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.33, 167.09, 150.97, 135.23, 133.50, 133.47, 129.63, 129.20, 129.15, 126.92, 126.35, 121.70 (q, *J* = 256.2 Hz), 120.96, 106.79, 105.41, 46.54, 12.20, 11.65; MS (ESI), *m/z*: 448 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{17}ClF_3N_3O_2$ $[M+H]^+$: 448.1034, found 448.1035. HPLC purity = 98.03%, Rt 10.52 min.

5-(1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazo le (14). N-hydroxy-4- (trifluoromethoxy)-benzimidamide (**7l**, 0.87 g, 3.9 mmol) was added to a solution of 1*H*-indazole-3-carboxylic acid (**8**, 0.7 g, 4.3 mmol), EDCI (0.90 g, 4.7 mmol) and HOBt (0.64g, 4.7 mmol) in dry DMF (10 mL). The mixture was stirred at room temperature for 1h and then heated to 140 °C for 1h. After removal of DMF under vacumm, the residue was partitioned with EtOAc (35 mL) and H_2O (25 mL). The organic layer was separated, washed with H_2O and saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography to get **14** as white solid with 52% yield. Mp 209-210 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.31 (s, 1H, NH), 8.36-8.32 (m, 2H, ArH), 7.79 (d, *J* = 8.5 Hz, 2H, ArH), 7.65 (d, *J* = 8.5 Hz, 2H, ArH), 7.59 (t, *J* = 8.0 Hz, 1H, ArH), 7.47 (t, *J* = 8.0 Hz, 1H, ArH). MS (ESI), m/z: 347 $[M+H]^+$.

General Procedure for the Synthesis of Compounds 4a-4f NaH (0.60 mmol) was added to a mixture of intermediate **14** (0.50 mmol) in dry DMF (5 mL) at 0 °C and the mixture was stirred at room temperature for 30 min. Substituted benzyl chloride (0.60 mmol) was added to the above solution and stirred for 4-6 h at room temperature. After quenching with 10 mL H_2O, the mixture was extracted with EtOAc (2×15 mL) and the organic layer was separated, washed with saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography.

5-(1-(4-Fluorobenzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadiazole (4a). White solid, 47% yield. Mp 128-130 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.36 (d, *J* = 8.5 Hz, 1H, ArH), 8.32 (d, *J* = 9.0 Hz, 2H, ArH), 8.02 (d, *J* = 8.5 Hz, 1H, ArH), 7.60-7.63 (m, 3H, ArH), 7.50 (t, *J* = 7.5 Hz, 1H, ArH), 7.40-7.43 (m, 2H, ArH), 7.20 (t, *J* = 9.0 Hz, 2H, ArH), 5.90 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.30, 167.90, 163.96 (d, *J* = 245.7 Hz), 151.50, 140.60, 131.35 (d, *J* = 3.0 Hz), 130.38, 129.61, 129.36 (d, *J* = 8.1 Hz), 127.92, 125.56, 123.91, 123.37, 121.98, 121.18, 116.17 (d, *J* = 22.0 Hz), 110.25, 53.64; MS (ESI), *m/z*: 455 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{14}F_4N_4O_2$ $[M+H]^+$: 455.1126, found 455.1128. HPLC purity = 99.42%, Rt 11.23 min.

5-(1-(Pyridin-4-ylmethyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadi-azole (4b). White solid, 50% yield. Mp 162-164 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.55 (d, *J* = 6.0 Hz, 2H, ArH), 8.40 (d, *J* = 8.5 Hz, 1H, ArH), 8.33 (d, *J* = 8.5 Hz, 2H, ArH), 7.97 (d, *J* = 8.5 Hz, 1H, ArH), 7.62-7.65 (m, 3H, ArH), 7.54 (t, *J* = 7.5 Hz, 1H, ArH), 7.23 (d, *J* = 5.5 Hz, 2H, ArH), 6.01 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.97, 167.81, 151.39, 150.46, 144.31, 140.73, 130.84, 129.48, 128.17, 125.32, 124.03, 123.12, 122.01, 121.74, 121.07, 109.77, 52.74; MS (ESI), *m/z*: 438 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}F_3N_5O_2$ $[M+H]^+$: 438.1172, found 438.1175. HPLC purity = 100.00%, Rt 7.63 min.

5-(1-(4-Nitrobenzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazole (4c). Yellowish solid, 46% yield. Mp 173-176 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (d, *J* = 8.0 Hz, 1H, ArH), 8.33 (d, *J* = 9.0 Hz, 2H, ArH), 8.24 (d, *J* = 8.5 Hz, 2H, ArH), 8.02 (d, *J* = 8.5 Hz, 1H, ArH), 7.63-7.65 (m, 3H, ArH), 7.57 (d, *J* = 8.5 Hz, 2H, ArH), 7.54 (t, *J* = 7.5 Hz, 1H, ArH), 6.11 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.93, 167.85, 151.44, 147.92, 142.50, 140.65, 130.98, 129.49, 128.26, 128.02, 125.30, 124.24, 124.09, 123.20, 122.10, 121.69 (q, *J* = 256.6 Hz), 121.08, 109.67, 53.15; MS (ESI), *m/z*: 482 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{14}F_3N_5O_4$ $[M+H]^+$: 482.1071, found 482.1072. HPLC purity = 97.11%, Rt 10.11min.

5-(1-(4-Methoxybenzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy)p henyl)-1,2,4-oxadi- azole (4d). White solid, 48% yield. Mp 143-145 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.34 (d, *J* = 8.0 Hz, 1H, ArH), 8.31 (d, *J* = 9.0 Hz, 2H, ArH), 8.00 (d, *J* = 8.5 Hz, 1H, ArH), 7.63 (d, *J* = 8.5 Hz, 2H, ArH), 7.59 (d, *J* = 8.0 Hz, 1H, ArH), 7.48 (t, *J* = 7.5 Hz, 1H, ArH), 7.34 (d, *J* = 8.5 Hz, 2H, ArH), 6.91 (d, *J* = 9.0 Hz, 2H, ArH), 5.81 (s, 2H, benzylic-CH_2), 3.70 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ

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171.30, 167.74, 159.98, 151.33, 140.43, 129.91, 129.48, 128.88, 127.56, 127.48, 125.49, 123.63, 123.28, 121.72, 121.04, 119.13, 114.30, 110.40, 55.27, 53.92; MS (ESI), *m/z*: 467 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{24}H_{17}F_3N_4O_3$ $[M+H]^+$: 467.1326, found 467.1324. HPLC purity = 97.21%, Rt 11.59 min.

5-(1-(3-Fluorobenzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazole (4e). White solid, 46% yield. Mp 126-129 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.39 (d, *J* = 8.0 Hz, 1H, ArH), 8.34 (d, *J* = 8.5 Hz, 2H, ArH), 8.02 (d, *J* = 8.5 Hz, 1H, ArH), 7.62-7.65 (m, 3H, ArH), 7.53 (t, *J* = 7.5 Hz, 1H, ArH), 7.39-7.43 (m, 1H, ArH), 7.22 (d, *J* = 9.5 Hz, 1H, ArH), 7.14-7.18 (m, 2H, ArH), 5.96 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.18, 167.79, 164.27 (d, *J* = 246.0 Hz), 151.37, 140.59, 137.93 (d, *J* = 7.0 Hz), 130.63 (d, *J* = 8.2 Hz), 130.42, 129.48, 127.89, 125.42, 123.84, 123.20, 122.89 (d, *J* = 3.0 Hz), 121.88, 121.70 (q, *J* = 256.8 Hz), 121.05, 115.42 (d, *J* = 20.8 Hz), 114.45 (d, *J* = 22.0 Hz), 110.06, 53.57; MS (ESI), *m/z*: 455 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{14}F_4N_4O_2$ $[M+H]^+$: 455.1126, found 455.1123. HPLC purity = 97.98%, Rt 11.48 min.

5-(1-(Pyridin-2-ylmethyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadi- azole (4f). White solid, 52% yield. Mp 167-169 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.51 (d, *J* = 4.0 Hz, 1H, ArH), 8.38 (d, *J* = 8.0 Hz, 1H, ArH), 8.33 (d, *J* = 8.5 Hz, 2H, ArH), 7.95 (d, *J* = 8.5 Hz, 1H, ArH), 7.82 (t, *J* = 7.5 Hz, 1H, ArH), 7.60-7.64 (m, 3H, ArH), 7.52 (t, *J* = 7.5 Hz, 1H, ArH), 7.30-7.34 (m, 2H , ArH), 6.02 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.20, 167.77, 155.41, 151.37, 149.58, 140.96, 137.18, 130.47, 129.48, 127.86, 125.42, 123.85, 123.11, 123.08, 121.76, 121.69 (q, *J* = 256.5 Hz), 121.65, 121.05, 110.58, 55.94; MS (ESI), *m/z*: 438 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}F_3N_5O_2$ $[M+H]^+$: 438.1172, found 438.1174. HPLC purity = 96.76%, Rt 8.58 min.

General Procedure for the Synthesis of Compounds 5a, 5b. When 4-fluorobenzyl chloride and 4-chloromethyl pyridine were used as starting materials, the minor products were collected through silica gel chromatography.

5-(2-(4-Fluorobenzyl)-2*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadiazole (5a). White solid, 27% yield. Mp 150-152 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.55 (d, *J* = 8.5 Hz, 1H, ArH), 8.46 (d, *J* = 9.0 Hz, 2H, ArH), 8.00-7.95 (m, 3H, ArH), 7.79 (d, *J* = 8.5 Hz, 1H, ArH), 7.58 (t, *J* = 7.5 Hz, 1H, ArH), 7.42-7.46 (m, 2H, ArH), 7.13 (t, *J* = 9.0 Hz, 2H, ArH), 6.03 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.31, 167.31, 163.86 (d, *J* = 246.0 Hz), 151.55, 148.35, 131.60 (d, *J* = 2.9 Hz), 129.87 (d, *J* = 8.2 Hz), 129.33, 127.27, 126.00, 125.07, 123.66, 121.68 (q, *J* = 257.2 Hz), 121.25, 120.64, 118.99, 118.63, 115.82 (d, *J* = 20.9 Hz), 55.98; MS (ESI), *m/z*: 455 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{23}H_{14}F_4N_4O_2$ $[M+H]^+$: 455.1126, found 455.1125. HPLC purity = 99.06%, Rt 14.10 min.

5-(2-(Pyridin-4-ylmethyl)-2*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadi- azole (5b). White solid, 25% yield. Mp 185-188 ^oC. ^1H NMR (500 MHz, $CDCl_3$)**:** δ 8.63 (d, *J* = 6.0 Hz, 2H, ArH), 8.55 (d, *J* = 8.5 Hz, 1H, ArH), 8.12 (d, *J* = 8.5 Hz, 2H, ArH), 7.96-8.00 (m, 3H, ArH), 7.71 (d, *J* = 8.5 Hz, 1H, ArH), 7.59 (t, *J* = 8.0 Hz, 1H, ArH), 7.21 (d, *J* = 5.5 Hz, 2H, ArH), 6.13 (s, 2H, benzylic-CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.04, 167.33, 151.60, 150.31, 148.53, 144.63, 129.30, 127.60, 126.31, 124.89, 123.58, 122.08, 121.27 (q, *J* = 216.5 Hz), 121.22, 120.63, 119.52, 118.67, 55.71; MS (ESI), *m/z*: 438 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{22}H_{14}F_3N_5O_2$ $[M+H]^+$: 438.1172, found 438.1173. HPLC purity = 100.00%, Rt 9.17 min.

General Procedure for the Synthesis of Compounds 15a, 15b. To a mixture of **14** (4.6 mmol) in dry DMF (15 mL) was added NaH (5.0 mmol) at 0 ^oC, and the mixture was stirred at room temperature for 30 min. (3 or 4-(bromomethyl)phenoxy)(tert-butyl)dimethylsilane[45] (5.0 mmol) was added to the above solution and stirred for 4-6 h at room temperature. After quenching with H_2O (5 mL), the mixture was extracted with EtOAc (2×15 mL) and the organic layer was separated, washed with saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography.

5-(1-(3-((Tert-butyldimethylsilyl)oxy)benzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy)- phenyl)-1,2,4-oxadiazole (15a). White solid, 31% yield. Mp 137-140 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.42 (d, *J* = 8.0 Hz, 1H, ArH), 8.35 (d, *J* = 9.0 Hz, 2H, ArH), 7.46-7.41 (m, 2H, ArH), 7.39 (d, *J* = 8.5 Hz, 2H, ArH), 7.19 (t, *J* = 8.0 Hz, 1H, ArH), 6.89 (d, *J* = 8.0 Hz, 1H, ArH), 6.76 (d, *J* = 8.0 Hz, 1H, ArH), 6.72 (s, 1H, ArH), 5.74 (s, 2H, benzylic-CH_2), 0.91 (s, 9H, 3×CH_3), 0.09 (s, 6H, 2×CH_3). MS (ESI), *m/z*: 567 $[M+H]^+$.

5-(1-(4-((Tert-butyldimethylsilyl)oxy)benzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy) phenyl)-1,2,4-oxadiazole (15b). White solid, 38% yield. Mp 141-143 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 8.0 Hz, 1H, ArH), 8.34 (d, *J* = 9.0 Hz, 2H, ArH), 7.46 (d, *J* = 8.0 Hz, 2H, ArH), 7.42-7.39 (m, 1H, ArH), 7.38 (d, *J* = 9.0 Hz, 2H, ArH), 7.19 (d, *J* = 8.5 Hz, 2H, ArH), 6.79 (d, *J* = 8.5 Hz, 2H, ArH), 5.72 (s, 2H, benzylic-CH_2), 0.95 (s, 9H, 3×CH_3), 0.16 (s, 6H, 2×CH_3). MS (ESI), *m/z*: 567 $[M+H]^+$.

General Procedure for the Synthesis of Compounds 16a, **16b.** A solution of tetrabutyl ammonium fluoride (1.2 mmol) in THF (5 mL) was added to a solution of intermediate **15a**, **15b** (1.0 mmol) in THF (10 mL) at 0 ^oC and the mixture was stirred at room temperature for 30 min. The completion of reaction was confirmed by TLC. The mixture was partitioned between 35 mL EtOAc and 15 mL H_2O. The organic layer was separated, washed with H_2O and saturated saline, dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography.

3-((3-(3-(4-(Trifluoromethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-1*H*-indazol-1-yl)methyl) phenol (16a). White solid, 95% yield. Mp 171-174 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.39 (d, *J* = 8.0 Hz, 1H, ArH), 8.28 (d, *J* = 9.0 Hz, 2H, ArH), 7.43 (d, *J* = 8.5 Hz, 2H, ArH), 7.40-7.37 (m, 1H, ArH), 7.36 (d, *J* = 8.5 Hz, 2H, ArH), 7.23 (t, *J* = 7.5 Hz, 1H, ArH), 6.89 (d, *J* = 8.0 Hz, 1H, ArH), 6.78-6.76 (m, 1H, ArH), 6.69 (s, 1H, ArH), 5.73 (s, 2H, benzylic-CH_2). MS (ESI), *m/z*: 453 $[M+H]^+$.

4-((3-(3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-1*H*-indazol-1-yl)methyl) phenol (16b). White solid, 92% yield. Mp 220-221 ^oC. ^1H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 8.5 Hz, 1H, ArH), 8.34 (d, *J* = 9.0 Hz, 2H, ArH), 7.47 (d, *J* = 2.5 Hz, 2H, ArH), 7.43-7.41 (m, 1H, ArH), 7.39 (d, *J* = 8.5 Hz, 2H, ArH), 7.28 (d, *J* = 8.5 Hz, 2H, ArH), 6.88 (d, *J* = 9.0 Hz, 2H, ArH), 5.73 (s, 2H, benzylic-CH_2). MS (ESI), *m/z*: 453 $[M+H]^+$.

General Procedure for the Synthesis of Compounds 17a and 17b. anhydrous K_2CO_3 (3.0 mmol) was added to a mixture of intermediate **16a**, **16b** (0.5 mmol), 1,2-dibromoethane (5.0 mmol) in dry acetone (20 mL) , and the mixture was refluxed for 24 h. The completion of the reaction was confirmed by TLC. The solvent was removed under vacuum and the residue was dispartitioned between 35 mL EtOAc and 15 mL H_2O. The organic layer was separated, washed with H_2O and saturated saline, dried over

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anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography.

5-(1-(3-(2-bromoethoxy)benzyl)-1*H*-indazol-3-yl)-3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazole (17a). White solid, 52% yield. Mp 162-165 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.43 (d, *J* = 8.5 Hz, 1H, ArH), 8.34 (d, *J* = 9.0 Hz, 2H, ArH), 7.48-7.45 (m, 2H, ArH), 7.43-7.40 (m, 1H, ArH), 7.39 (d, *J* = 8.0 Hz, 2H, ArH), 7.26-7.24 (m, 1H, ArH), 6.92 (d, *J* = 7.5 Hz, 1H, ArH), 6.85-6.83 (m, 2H, ArH), 5.76 (s, 2H, benzylic-CH_2), 4.24 (t, *J* = 7.5 Hz, 2H, CH_2), 3.60 (t, *J* = 7.5 Hz, 2H, CH_2). MS (ESI), *m/z*: 559 $[M+H]^+$.

5-(1-(4-(3-Bromoethoxy)benzyl)-1H-indazol-3-yl)-3-(4-(trifluoromethoxy)phenyl)-1,2,4-oxadiazole (17b). White solid, 46% yield. Mp 132-134 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.42 (d, *J* = 8.0 Hz, 1H, ArH), 8.34 (d, *J* = 8.5 Hz, 2H, ArH), 7.49-7.45 (m, 2H, ArH), 7.43-7.41 (m, 1H, ArH), 7.39 (d, *J* = 8.5 Hz, 2H, ArH), 7.28 (d, *J* = 8.5 Hz, 2H, ArH), 6.88 (d, *J* = 9.0 Hz, 2H, ArH), 5.73 (s, 2H, benzylic-CH_2), 4.27 (t, *J* = 6.0 Hz, 2H, CH_2), 3.62 (t, *J* = 7.5 Hz, 2H, CH_2). MS (ESI), *m/z*: 559 $[M+H]^+$.

General Procedure for the Synthesis of Compounds 4g-4j. Anhydrous K_2CO_3 (0.42 mmol) and secondary amine (0.42 mmol) was added to a solution of **17a** or **17b** (0.072 mmol) in dry CH_3CN (4 mL), then the mixture was refluxed for 24 h. The completion of reaction was confirmed by TLC. Then the mixture was partitioned between 15 mL EtOAc and 5 mL H_2O. The organic layer was separated, washed with H_2O and saturated saline, and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and the residue was purified by silica gel chromatography.

5-(1-(3-(2-(4-Methylpiperazin-1-yl)ethoxy)benzyl)-1H-indazol-3-yl)-3-(4-(trifluoro-methoxy)phenyl)-1,2,4-oxadiazole (4g). Yellowish solid, 52% yield. Mp 103-105 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 8.0 Hz, 1H, ArH), 8.33 (d, *J* = 8.5 Hz, 2H, ArH), 7.47-7.43 (m, 2H, ArH), 7.42-7.40 (m, 1H, ArH), 7.38 (d, *J* = 8.5 Hz, 2H, ArH), 7.24 (t, *J* = 7.5 Hz, 1H, ArH), 6.91-6.84 (m, 2H, ArH), 6.83 (d, *J* = 8.0 Hz, 1H, ArH), 5.74 (s, 2H, benzylic-CH_2), 4.03 (t, *J* = 6.0 Hz, 2H, CH_2), 2.76 (t, *J* = 6.0 Hz, 2H, CH_2), 2.57-2.46 (brd, 8H, piperazine), 2.70 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.25, 167.75, 159.21, 151.33, 140.56, 136.91, 130.04, 130.00, 129.49, 127.70, 125.43, 123.74, 123.20, 122.79 (q, *J* = 258.2Hz), 121.73, 121.06, 119.76, 114.20, 113.78, 110.39, 65.89, 57.03, 55.01, 54.97, 54.25, 53.53, 45.99; MS (ESI), *m/z*: 579 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{30}H_{29}F_3N_6O_3$ $[M+H]^+$: 579.2326, found 579.2328. HPLC purity = 97.90%, Rt 11.08 min.

1-(2-(3-((3-(3-(4-(Trifluoromethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-1H-indazol-1-yl)methyl) phenoxy)ethyl)piperidin-4-ol (4h). White solid, 86% yield. Mp 107-110 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.37 (d, *J* = 8.5 Hz, 1H, ArH), 8.33 (d, *J* = 8.5 Hz, 2H, ArH), 8.00 (d, *J* = 8.5 Hz, 1H, ArH), 7.65-7.60 (m, 3H, ArH), 7.50 (t, *J* = 7.5 Hz, 1H, ArH), 7.26 (t, *J* = 8.0 Hz, 1H, ArH), 6.94 (t, *J* = 1.5 Hz, 1H, ArH), 6.88-6.86 (m, 2H, ArH), 5.87 (s, 2H, benzylic-CH_2), 4.53 (d, *J* = 4.0 Hz, 1H, OH), 4.02 (t, *J* = 5.5 Hz, 2H, CH_2), 3.44-3.39 (m, 1H, piperidine), 2.74-2.72 (m, 2H, CH2), 2.61-2.59 (m, 2H, piperidine), 2.09-2.05 (m, 2H, piperidine), 1.68-1.65 (m, 2H, piperidine), 1.37-1.32 (m, 2H, piperidine); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.23, 167.75, 158.93, 151.33, 140.56, 136.98, 130.06, 129.48, 127.74, 125.38, 123.76, 123.18, 122.12 (q, *J* = 256.4Hz), 121.73, 121.07, 119.92, 114.13, 113.81, 110.38, 65.39, 56.58, 54.19, 50.94, 50.91, 33.50; MS (ESI), *m/z*: 580 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{30}H_{28}F_3N_5O_4$ $[M+H]^+$: 580.2166, found 580.2164. HPLC purity = 98.84%, Rt 9.64 min.

4-(2-(3-((3-(3-(4-(Trifluoromethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-1H-indazol-1-yl)methyl)phenoxy)ethyl)morpholine (4i). White solid, 61% yield. Mp 61-63 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 8.0 Hz, 1H, ArH), 8.33 (d, *J* = 9.0 Hz, 2H, ArH), 7.49-7.41 (m, 2H, ArH), 7.42-7.39 (m, 1H, ArH), 7.38 (d, *J* = 8.0 Hz, 2H, ArH), 6.89 (d, *J* = 7.5 Hz, 1H, ArH), 6.84-6.82 (m, 2H, ArH), 5.74 (s, 2H, benzylic-CH_2), 4.05 (t, *J* = 5.5 Hz, 2H, CH_2), 3.71 (t, *J* = 4.5 Hz, 4H, morpholine), 2.75 (t, *J* = 5.5 Hz, 2H, CH_2), 2.54 (t, *J* = 4.5 Hz, 4H, morpholine); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.24, 167.76, 159.14, 151.33, 140.56, 136.95, 130.06, 130.02, 129.48, 127.71, 125.40, 123.75, 123.20, 122.45 (q, *J* = 261.2Hz), 121.74, 121.07, 119.84, 114.20, 113.76, 110.37, 66.83, 65.68, 57.50, 54.23, 54.03; MS (ESI), *m/z*: 566 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{29}H_{26}F_3N_5O_4$ $[M+H]^+$: 566.2010, found 566.2014. HPLC purity = 96.35%, Rt 10.74 min.

1-(2-(4-((3-(3-(4-(Trifluoromethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-1H-indazol-1-yl)methyl) phenoxy)ethyl)piperidin-4-ol (4j). White solid, 62% yield. Mp 141-144 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.41 (d, *J* = 8.0 Hz, 1H, ArH), 8.34 (d, *J* = 9.0 Hz, 2H, ArH), 7.47 (d, *J* = 8.5 Hz, 2H, ArH), 7.42-7.39 (m, 1H, ArH), 7.39 (d, *J* = 8.5 Hz, 2H, ArH), 7.26 (d, *J* = 8.5 Hz, 2H, ArH), 6.86 (d, *J* = 9.0 Hz, 2H, ArH), 5.72 (s, 2H, benzylic-CH_2), 4.07 (s, 2H, CH_2), 2.87 (s, 2H, CH_2), 2.80 (s, 2H, piperidine), 2.30 (s, 2H, piperidine), 1.62 (s, 4H, piperidine), 1.39 (s, 1H, piperidine); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.30, 167,75, 158.76, 151.36, 140.44, 129.92, 129.48, 128.86, 127.62, 127.56, 125.49, 123.64, 123.28, 121.72, 121.04, 119.13, 114.97, 110.40, 67.66, 66.16, 56.96, 53.90, 51.49, 34.40; MS (ESI), *m/z*: 580 $[M+H]^+$; HRMS (TOF) *m/z* calcd for $C_{30}H_{28}F_3N_5O_4$ $[M+H]^+$: 580.2166, found 580.2169. HPLC purity = 98.70%, Rt 9.50 min.

4.2 Biological Materias and Methods

HIF-Luciferase Assay. Human colon carcinoma cell line HCT-116 provided by Zhejiang University was revived in DMEM containing 10% heat-inactivated fetal bovine serum, Penicillin (100 IU/mL) and Streptomycin (100 μg/mL). HCT-116 cells transfected with a luciferase report gene under the control of HIF-responsive element and a renilla luciferase (internal control for transfection efficiency) were seeded in 96-well plates with 6000 cells per well and incubated overnight under normoxic conditions (20% O_2). After 24 hours, test compounds with different concentrations were added into wells, and the cells were incubated under normoxic condition (20% O_2) for 1 hour followed by 24 hours in the hypoxic condition (1% O_2). Finally, the medium was aspirated, cells were lysed and reporter activities were measured using the Dual-Luciferase reporter assay system (Promega, Madison, Wis., USA). IC_{50} was calculated with Bliss method.

Western Blotting Assay. Human colon cancer cell line HCT116 was cultured under normoxia (20% O_2) or hypoxia (1% O_2) in the absence or presence of test compounds for 24 hours. Proteins were then extracted with lysis buffer and the lysates were centrifuged to get equivalent amounts of proteins which were analyzed by SDS-polyacrylamide gel electrophoresis. Proteins were visualized using enhanced chemiluminescence detection reagents (GE Healthcare, Buckinghamshire, UK).

RT-PCR. Total RNA from HCT116 cells was isolated using the Trizol reagent (Sangon Biotech Co., Ltd), and cDNA was synthesized using

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2 μg of total RNA with random hexamer primers and the Moloney murine leukemia virus reverse transcriptase (M-MuLV RT) (Fermentas International Inc., Burlington, Ontario, Canada). The conditions used for reverse transcription-PCR were as follows: 10 min at 25 °C, 60 min at 42°C and 15 min at 72 °C. The cDNA was subjected to PCR amplification using the following forward and reverse primer sets: HIF-1α, forward primer: 5′-TCACCACAGGACAGTACAGGATGC-3′ and reverse primer: 5′-CCAGCAAAGTTAAAGCATCAGGTTCC-3′; GAPDH, forward primer: 5′-GTCATCCATGACAACTTTGG-3′ and reverse primer: 5′-GAGCTTGACAAAGTGGTCGT-3′. The housekeeping gene GAPDH was used as the internal standard. Quantitative real-time RT-PCR analysis was performed by TAKARA SYBR Premix EXTaqTM. The reaction mixtures containing SYBR Green were composed following the manufacturer's protocol. The cycling program was 95 °C for 30 s, 58 °C or 70 °C (GADD153) for 20 s, and 72 °C for 30 s followed by 40 cycles using an Eppendorf epGradient Mastercycler (Eppendorf, Hamburg, Germany).

Cell Migration Assay. A human ovarian cancer cell SKOV3 migration assay was performed in a Transwell Boyden chamber containing a polycarbonate filter with a pore size of 8 μm. SKOV3 cells (10000 cells/ml) were maintained in the upper chamber with serum-free medium before treated with test compounds. The lower chamber contained medium plus 10% FBS as chemoattractant. The chamber was incubated under normoxia (20% O_2) for 1 hour before transferring to hypoxia (1% O_2), or continuously incubated under normoxia for another 24 hours. All nonmigrant cells were then removed by Phosphate Buffered Saline (PBS) washing. The migrated cells were fixed with 90% EtOH and stained with 0.1% crystal violet in 2% MeOH. The stained cells were subsequently photographed and the number of cells passed through the polycarbonate filter was calculated in five randomly selected, nonoverlapping fields under a light microscope.

HIF-1 transcriptional activity assay *in vivo*. HCT116 colon carcinoma cells stably transfected with the hypoxia-inducible luciferase reporter gene (HRE-luciferase) were implanted in twenty nude mice. When the tumor volume reached 200 mm^3, the mice were divided into five groups followed by *i.v.* administration of vehicle or test compounds for three consecutive days (50 mg/kg, once daily). HRE-driven FLuc activity was determined by Xenogen imaging and the intensity of luminescence was calculated by software. Animal study was approved by the Animal Research Committee at Zhejiang University (log number Zju2013101032), and animal care was provided in accordance with institutional guidelines.

Pulmonary metastasis model of SKOV3 in nude mice. Nude mice were *i.v.* injected with 1×10^7 SKOV3 cells, and received daily *i.v.* injection of the compounds (50 mg/kg) on the following day for two weeks. The body weight was recorded twice a week. The mice were sacrificed on day 30 and the lungs were removed and fixed in paraformaldehyde. For analysis of pulmonary metastasis, lungs were cut into 5μm sections, and stained for HE. One section every 100 μm throughout the whole lung was screened histologically, and the number of metastasis was counted (diameter > 0.1 mm). Animal study was approved by the Animal Research Committee at Zhejiang University (log number Zju2013101068), and animal care was provided in accordance with institutional guidelines.

Statistical analysis. For all parameters measured, the values for all samples in the different experiment conditions were averaged and the SD of the mean was calculated. ANOVA followed by Student's t test was used for the statistical analysis.

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Notes

The authors declare no competing financial interest.

Abbreviations

HIF-1, hypoxia inducible factor-1; pVHL, Von Hippel–Lindau tumor suppressor; ARD1, arrest defective 1; SUMO, small ubiquitin-related modifier; RACK1, receptor for activated C kinase 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; mTOR, mammalian target of rapamycin; HDAC, histone deacetylase; HRE, hypoxic response element; HSP90, heat shock protein 90; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; CAIX, carboanhydrase IX; EPO, erythropoietin; SD, standard deviation; SDS, Sodium Dodecyl Sulfonate; RT-PCR, reverse transcription-polymerase chain reaction; PBS, phosphate buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase ; DMEM, dulbecco's modified eagle medium; IC_{50}, the half maximal (50%) inhibitory concentration of a substance; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; rt, room temperature; DCM, dichloromethane; DMF, N,N-dimethylformamide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HOBT, hydroxybenzotriazole; TBDMS, tert-butyldimethylsilane; TBAF, tetrabutyl ammonium fluoride; DIPEA, N,N-Diisopropylethylamine.

References

1. A. Giaccia, B. G. Siim and R. S. Johnson, *Nat. Rev. Drug Discov.*, 2003, **2**, 803-811.
2. T. Hiraga, S. Kizaka-Kondoh, K. Hirota, M. Hiraoka and T. Yoneda, *Cancer Res.*, 2007, **67**, 4157-4163.
3. D. Liao, C. Corle, T. N. Seagroves and R. S. Johnson, *Cancer Res.*, 2007, **67**, 563-572.
4. K. J. Williams, R. L. Cowen, L. M. Brown, E. C. Chinje, M. Jaffar and I. J. Stratford, *Adv. Enzyme Regul.*, 2004, **44**, 93-108.
5. G. L. Semenza, *Nat. Rev. Cancer*, 2003, **3**, 721-732.
6. B. Onnis, A. Rapisarda and G. Melillo, *J. Cell. Mol. Med.*, 2009, **13**, 2780-2786.
7. Y. Xia, H. K. Choi and K. Lee, *Eur. J. Med. Chem.*, 2012, **49**, 24-40.
8. I. K. Nordgren and A. Tavassoli, *Chem. Soc. Rev.*, 2011, **40**, 4307-4317.
9. B. H. Jiang, G. Jiang, J. Z. Zheng, Z. Lu, T. Hunter and P. K. Vogt, *Cell Growth Differ.*, 2001, **12**, 363-369.
10. S. H. Kang, H. T. Cho, S. Devi, Z. Zhang, D. Escuin, Z. Liang, H. Mao, D. J. Brat, J. J. Olson, J. W. Simons, T. M. Lavallee, P. Giannakakou, E. G. Van Meir and H. Shim, *Cancer Res.*, 2006, **66**, 11991-11997.
11. A. Rapisarda, J. Zalek, M. Hollingshead, T. Braunschweig, B. Uranchimeg, C. A. Bonomi, S. D. Borgel, J. P. Carter, S. M. Hewitt, R. H. Shoemaker and G. Melillo, *Cancer Res.*, 2004, **64**, 6845-6848.
12. K. Lee, H. Zhang, D. Z. Qian, S. Rey, J. O. Liu and G. L. Semenza, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 17910-17915.
13. C. Tan, R. G. de Noronha, N. S. Devi, A. A. Jabbar, S. Kaluz, Y. Liu, S. R. Mooring, K. C. Nicolaou, B. Wang and E. G. Van Meir, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5528-5532.
14. J. Mun, A. A. Jabbar, N. S. Devi, S. Yin, Y. Wang, C. Tan, D. Culver, J. P.

View Article Online
DOI: 10.1039/C5RA15191K

Snyder, E. G. Van Meir and M. M. Goodman, *J. Med. Chem.*, 2012, **55**, 6738-6750.
15. C. A. Klein, *Science*, 2008, **321**, 1785-1787.
16. C. L. Chaffer and R. A. Weinberg, *Science*, 2011, **331**, 1559-1564.
17. S. Valastyan and R. A. Weinberg, *Cell*, 2011, **147**, 275-292.
18. C. C. Wong, D. M. Gilkes, H. Zhang, J. Chen, H. Wei, P. Chaturvedi, S. I. Fraley, C. M. Wong, U. S. Khoo, I. O. Ng, D. Wirtz and G. L. Semenza, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 16369-16374.
19. M. H. Yang, M. Z. Wu, S. H. Chiou, P. M. Chen, S. Y. Chang, C. J. Liu, S. C. Teng and K. J. Wu, *Nat. Cell Biol.*, 2008, **10**, 295-305.
20. L. Xiang, D. M. Gilkes, P. Chaturvedi, W. Luo, H. Hu, N. Takano, H. Liang and G. L. Semenza, *J. Mol. Med. (Berl.)*, 2014, **92**, 151-164.
21. D. H. Shin, J. H. Kim, Y. J. Jung, K. E. Kim, J. M. Jeong, Y. S. Chun and J. W. Park, *Cancer Lett.*, 2007, **255**, 107-116.
22. Q. Xia, L. Zhang, J. Zhang, R. Sheng, B. Yang, Q. He and Y. Hu, *Eur. J. Med. Chem.*, 2011, **46**, 919-926.
23. Y. Hu, Q. Xia, S. Shangguan, X. Liu, Y. Hu and R. Sheng, *Molecules*, 2012, **17**, 9683-9696.
24. Q. Weng, J. Zhang, J. Cao, Q. Xia, D. Wang, Y. Hu, R. Sheng, H. Wu, D. Zhu, H. Zhu, Q. He and B. Yang, *Invest. New Drugs*, 2011, **29**, 1177-1187.
25. X. W. Liu, T. Y. Cai, H. Zhu, J. Cao, Y. Su, Y. Z. Hu, Q. J. He and B. Yang, *Autophagy*, 2014, **10**, 111-122.
26. Y. S. Chun, E. J. Yeo and J. W. Park, *Cancer Lett.*, 2004, **207**, 1-7.
27. Y. S. Chun, E. J. Yeo, E. Choi, C. M. Teng, J. M. Bae, M. S. Kim and J. W. Park, *Biochem. Pharmacol.*, 2001, **61**, 947-954.
28. S. H. Li, D. H. Shin, Y. S. Chun, M. K. Lee, M. S. Kim and J. W. Park, *Mol. Cancer Ther.*, 2008, **7**, 3729-3738.
29. H. An, N. J. Kim, J. W. Jung, H. Jang, J. W. Park and Y. G. Suh, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 6297-6300.
30. Y. V. Kharchenko, O. S. Detistov and V. D. Orlov, *Chem. Heterocycl. Compd. (N. Y., NY, U. S.)*, 2008, **44**, 600-605.
31. H. An, N. J. Kim, J. W. Jung, H. Jang, J. W. Park and Y. G. Suh, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 6297-6300.
32. G. P. Moloney, J. A. Angus, A. D. Robertson, M. J. Stoermer, M. Robinson, C. E. Wright, K. McRae and A. Christopoulos, *Eur. J. Med. Chem.*, 2008, **43**, 513-539.
33. U. Girreser, M. Konig and B. Clement, *Magn. Reson. Chem.*, 2002, **40**, 202-206.
34. A. G. Koryakova, Y. A. Ivanenkov, E. A. Ryzhova, E. A. Bulanova, R. N. Karapetian, O. V. Mikitas, E. A. Katrukha, V. I. Kazey, I. Okun, D. V. Kravchenko, Y. V. Lavrovsky, O. M. Korzinov and A. V. Ivachtchenko, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3661-3666.
35. M. Flipo, M. Desroses, N. Lecat-Guillet, B. Dirie, X. Carette, F. Leroux, C. Piveteau, F. Demirkaya, Z. Lens, P. Rucktooa, V. Villeret, T. Christophe, H. K. Jeon, C. Locht, P. Brodin, B. Deprez, A. R. Baulard and N. Willand, *J. Med. Chem.*, 2011, **54**, 2994-3010.
36. J. Garfunkle, C. Ezzili, T. J. Rayl, D. G. Hochstatter, I. Hwang and D. L. Boger, *J. Med. Chem.*, 2008, **51**, 4392-4403.
37. D. M. Cottrell, J. Capers, M. M. Salem, K. DeLuca-Fradley, S. L. Croft and K. A. Werbovetz, *Bioorg. Med. Chem.*, 2004, **12**, 2815-2824.
38. Z. Liu, F. Shi, P. D. Martinez, C. Raminelli and R. C. Larock, *J. Org. Chem.*, 2008, **73**, 219-226.
39. A. Andreani, M. Rambaldi, D. Bonazzi, F. Andreani, R. Bossa and I. Galatulas, *Arch. Pharm. (Weinheim)*, 1984, **317**, 847-851.
40. V. V. Levin, A. A. Zemtsov, M. I. Struchkova and A. D. Dilman, *Org. Lett.*, 2013, **15**, 917-919.

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