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Synthesis and Structure−Activity Relationship of (*E*)-Phenoxyacrylic Amide Derivatives as Hypoxia-Inducible Factor (HIF) 1α Inhibitors

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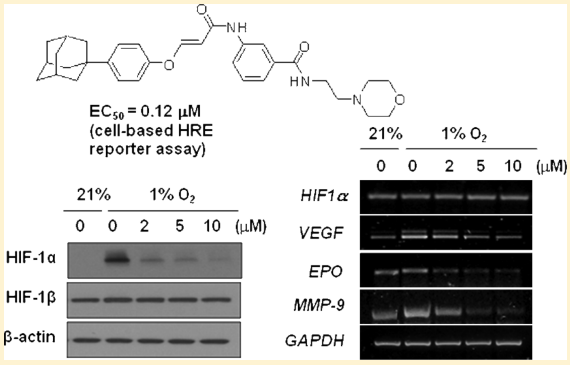
Supporting Information

ABSTRACT: A series of (*E*)-phenoxyacrylic amide derivatives were synthesized and evaluated as hypoxia inducible factor (HIF) 1α inhibitors. The present structure−activity relationship study on this series identified the morpholinoethyl containing ester **4p** as a potent inhibitor of HIF-1α under hypoxic conditions (IC_{50} = 0.12 μM in a cell-based HRE reporter assay) in HCT116 cells. The representative compound **4p** suppressed hypoxia-induced HIF-1α accumulation and targeted gene expression in a dose-dependent manner. The effect of HIF-1α inhibition by **4p** was further demonstrated by its inhibitory activity on in vitro tube formation and migration of cells, which may be valuable for development of novel therapeutics for cancer and tumor angiogenesis.



INTRODUCTION

Hypoxia inducible factor (HIF) 1 is a transcription factor that functions as a mast regulator in the response of growing tumor to hypoxia. HIF-1 activates genes that allow the cancer cells to survive and grow in hypoxic tumor environment. HIF-1 is a heterodimeric protein that consists of a constitutively expressed β-subunit, also called aryl hydrocarbon nuclear translocator (ARNT), and an α-subunit whose expression is regulated by O_2 levels.[1,2] Under normoxic condition, HIF-1α protein is subjected to degradation via the von Hippel−Lindau tumor suppressor gene product (pVHL) mediated ubiquitin−proteosomal pathway. Under hypoxic conditions, HIF-1α protein escapes proteolysis and rapidly accumulates in cells, which allows dimerization of HIF-1α and -1β subunits and subsequent binding to a cis-acting regulatory element referred to as hypoxia response element (HRE, 5′-RCGTG-3′) in the promoter region of a number of target genes, including vascular epidermal growth factor (VEGF) and erythropoietin (EPO).[3] Thus, HIF-1α plays a critical role during cancer development by regulating the expression of various genes associated with tumor angiogenesis, metastasis, invasion, proliferation, and apoptosis. Overexpression of HIF-1α has been observed in human cancers including brain, breast, colon, lung, ovary, and prostate cancers,[4] and HIF-1α has been implicated in resistance to treatment and poor prognosis in the hypoxic region around solid tumors. Accordingly, these findings have validated HIF-1α as an attractive target for treatment of cancer and tumor angiogenesis.[5,6]

Recently, considerable efforts have been directed toward the discovery of HIF-1 inhibitors using chemical libraries and natural products.[7−15] HIF-1 inhibitors reportedly regulate the HIF-1 signaling pathway through a variety of molecular mechanisms, including transcriptional regulation, mRNA translation, nuclear translocation, and HIF-1α degradation.

Our group has focused on the development of small molecule inhibitors targeting HIF-1α protein in solid tumors, which has resulted in several synthetic leads, **1**−**3**, as summarized in Figure 1.[10−12,16,17] To identify more potent and efficient HIF-1α inhibitors, we performed structural modifications on **1** and **2**, including a phenyl ring substituted with an adamantyl group (A-ring) and a N-aromatic ring (B-ring) linked with oxyacetylamide. In the current work, we focus on modifications that include replacement of the oxyacetylamide linker portion with a more conformationally constrained oxyacrylic amide linker, as represented by **4**. We present data from the synthesis and evaluation of (*E*)-phenoxyacrylic amide based compounds as potential HIF-1α inhibitors.

RESULTS AND DISCUSSION

Chemistry. The key intermediate (*E*)-phenoxyacrylic acid derivatives **9a**−**f** were synthesized from the phenol **7a**−**d**, according to the procedures in the literature and shown in

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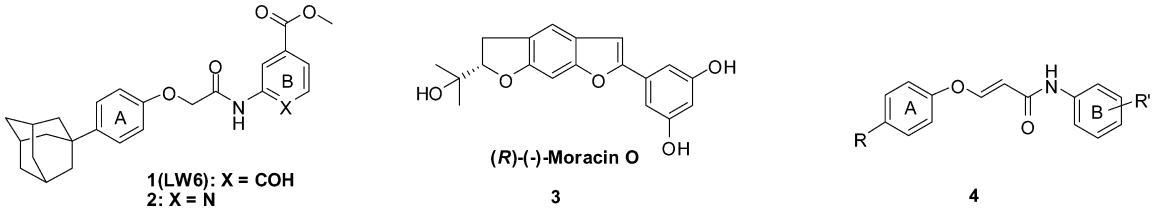
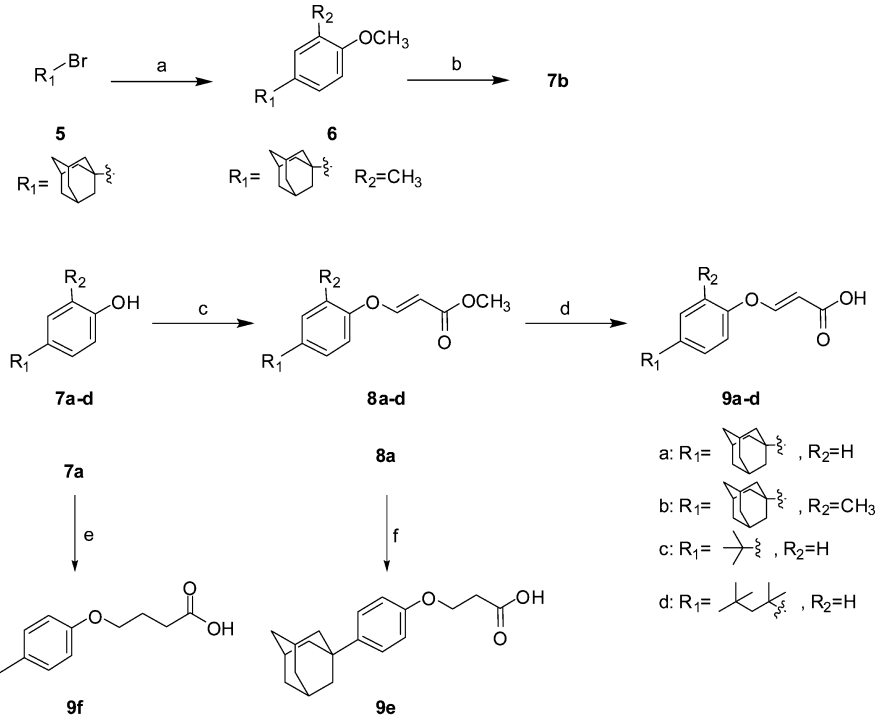


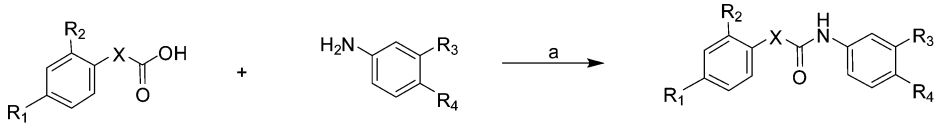
Figure 1. Structures of HIF-1α inhibitors.

Scheme 1. Synthesis of the Key Intermediates 9a–f[a]



[a]Reagents and conditions: (a) 1-methoxy-2-methylbenzene, Pd/C, K_2CO_3, reflux; (b) BBr_3, MC, −10 °C; (c) PPh_3, methyl propiolate, 115 °C; (d) LiOH, THF/H_2O; (e) (i) K_2CO_3, methyl 4-bromobutanoate, DMF; (ii) LiOH, THF/H_2O; (f) (i) Pd/C, H_2, MeOH; (ii) LiOH, THF/H_2O.

Scheme 2. Synthesis of (*E*)-Phenoxyacrylic Amide Derivatives 4a–g and 4j–k[a]



[a]Reagents and conditions: (a) HATU, DIPEA, DMF.

Scheme 1.[18] After the hydroxyl group of the phenol **7a–d** was subjected to alkylation with methyl propiolate in the presence of PPh_3 in toluene, the resulting (*E*)-phenoxyacrylic methyl ester **8a–d** was hydrolyzed with lithium hydroxide to form **9a–d**. The necessary **7b**, which is not commercially available, was prepared in two steps, including alkylation of 1-bromoadamantane **5** with 1-methoxy-2-methylbenzene in the presence of palladium followed by demethylation of **6** using boron tribromide with good yields.

The propionic acid **9e** and the butanoic acid **9f** were synthesized from **7a** or **8a**, respectively (Scheme 1). Saturation of the phenoxy acrylic portion of **8a** was conducted by palladium carbon hydrogenation to provide **9e**. For the preparation of **9f** with three-carbon linker, the phenol **7a** was

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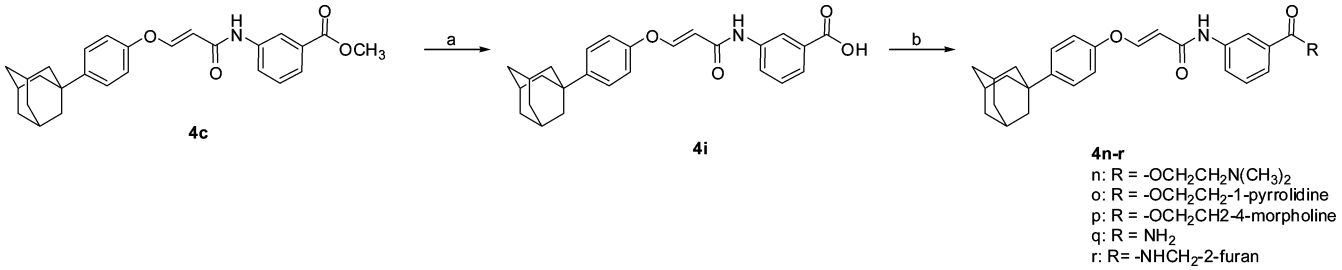
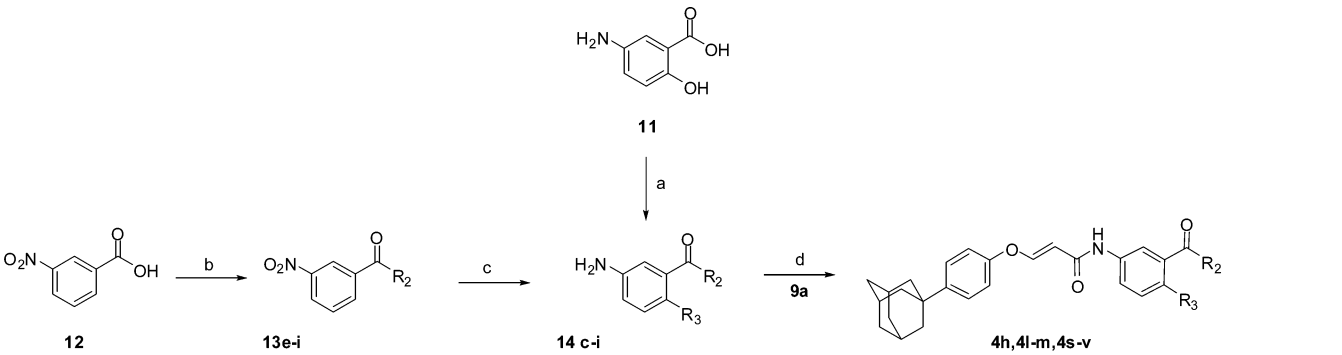
Scheme 3. Synthesis of (*E*)-Phenoxyacrylic Amide Derivatives 4i and 4n−r[a]



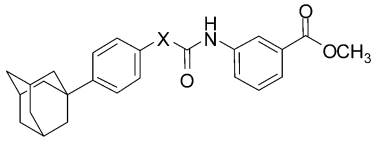
[a]Reagents and conditions: (a) LiOH, THF/H_2O for **4i**; (b) alkyl halides, K_2CO_3 for **4n−q**; amines, HATU, DIPEA, DMF for **4r**.

Scheme 4. Synthesis of (*E*)-Phenoxyacrylic Amide Derivatives 4h, 4l,m, and 4s−v[a]



[a]Reagents and conditions: (a) MeOH, $SOCl_2$ for **14c**; (b) 1-bromo-2-methoxyethane, K_2CO_3, Cs_2CO_3 for **13e**; amines, EDCI, HOBT, DIPEA for **13f−i**; (c) Pd/C, MeOH for **14e−i**; (d) **9a**, HATU, DIPEA, DMF.

Table 1. In Vitro HIF-1 Inhibitory Activity and Cytotoxicity of Linker Modified Derivatives



4a-c

No	Structure X	HRE IC_{50} (μM)[a]	Cell Viability (μM)[a]
4a		2.54±0.04	>20
4b		15.75±1.73	>20
4c		0.74±0.06	>20
15		5.03±0.21	>20
1 (LW6)		2.44±0.45	> 20

[a]Values are the mean of three experiments.

alkylated with methyl 4-bromobutanoate in the presence of potassium carbonate in DMF and then hydrolyzed to give **9f**.

With preparation of the key intermediates **9a−f**, the (*E*)-phenoxyacrylic amide derivatives were prepared by the methods shown in Schemes 2−4. Compounds with a variety of substitution patterns on the A- and B-rings or with different linkers (**4a−g** and **4j−k**) were synthesized by direct amide coupling of the requisite anilines **10a−d** with the newly synthesized acids **9a−f** using HATU as coupling agent (Scheme 2). Additionally, (*E*)-4-(1-adamantyl)phenoxyacrylic amide **4c** was converted to the corresponding carboxylic acid **4i** using lithium hydroxide and then further reacted with alkyl halides or anilines to provide the various ester and amide analogues **4n−r**, as shown in Scheme 3.

More esters and amides with different substitution patterns on the B-ring (**4h**, **4l,m**, and **4s−v**) were prepared starting from 5-amino-2-hydroxybenzoic acid **11** or 3-nitrobenzoic acid **12** as described in Scheme 4. 2-Amino-5-hydroxybenzoic acid **11** was transformed to **14c** in the presence of thionyl chloride and methanol, whereas 3-nitrobenzoic acid **12** was converted to the substituted ester **13e** using 1-bromo-2-methoxyethane and to amides **13f−i** using various amines under standard coupling conditions, which were further reduced at the nitro group to produce anilines **14e−i**. With the addition of the commercially available 3-aminobenzoic acid ethyl ester **14d**, a series of anilines **14c−i** were coupled with the carboxylic acid **9a** in the presence of HATU and DIPEA to produce the final (*E*)-4-(1-

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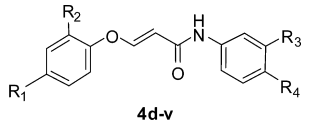
Table 2. In Vitro HIF-1 Inhibitory Activity and Cytotoxicity of (*E*)-Phenoxyacrylic Amide Derivatives



4d-v

No	Structure				HRE IC_{50}	Cell Viability
	R_1	R_2	R_3	R_4	(μM)[a]	(μM)[a]
4d		H	$COOCH_3$	H	6.03±0.2	> 20
4e		H	$COOCH_3$	H	1.04±0.24	> 20
4f		H	H	$COOCH_3$	2.17±0.45	> 20
4g		CH_3	$COOCH_3$	H	4.12±1.09	> 20
4h		H	$COOCH_3$	OH	> 20	> 20
4i		H	COOH	H	> 20	> 20
4j		H	SO_2CH_3	H	1.18±0.22	> 20
4k		H	SO_2NH_2	H	2.38±0.16	> 20

[a]Values are the mean of three experiments.

adamantyl)phenoxyacrylic amide derivatives **4h**, **4l**,**m**, and **4s**–**v**, respectively.

Cell-Based HRE Reporter Assay. The newly synthesized compounds were evaluated for their potential to inhibit hypoxia-induced accumulation of HIF-1α using a cell-based HRE reporter assay in the HCT116 cell line (Tables 1–3). All of the assays were performed under standard assay conditions by employing hypoxic conditions (1% O_2, 94% N_2, and 5% CO_2) and following the previously described assay protocol.[19] Cell viability, as measured by the sulforhodamine B assay, showed that most of the compounds had no significant cytotoxicity at the concentrations at which they effectively inhibited HIF-1 activation (>20 μM). Compound **1** (LW6, IC_{50} = 2.44 μM) was used as a positive control.[10,13]

We first examined the effect of increasing the length of the carbon linker between rings A and B, which may affect the overall conformation of the prototype compound, i.e., methyl 3-(2-(4-adamantylphenoxy)acetamido)benzoate **15** (Table 1).[10] Addition of one carbon resulted in a 2-fold increase in potency, as shown by the IC_{50} values (**15** vs **4a** giving 5.03 vs 2.54 μM, respectively). However, addition of two carbons resulted in a loss of potency, as shown in the case of **4b**. Additionally, introduction of a double bond linking the phenoxy group to the NH-phenyl portion (**4c**), which creates a more rigid conformation in the linker, resulted in an apparent increase in inhibitory activity (IC_{50} = 0.74 μM), as shown in Table 1. Encouraged by this result with **4c**, we further extended the synthesis of the series characterized by the (*E*)-4-(1-adamantyl)phenoxyacrylic amide moiety.

We previously defined the key structural requirements for HIF inhibition of aryloxyacetylbenzoic acid derivatives that involve the adamantyl group in the A-ring.[10] Because structural changes in the linker portion may affect those requirements, our next approach was to confirm the importance of the adamantyl group attached to the A-ring. The replacement of the adamantane ring with the smaller *tert*-butyl (**4d**) or octyl (2,4,4-trimethylpentan-2-yl) moiety (**4e**) on the 4-phenoxy group resulted in reduction or maintenance of HIF-1 activity (IC_{50} of 6.03 and 1.04 μM, respectively), indicating the importance of the lipophilic group such as the adamantyl on the A-ring.

Among the compounds based on the (*E*)-4-(1-adamantyl)-phenoxyacrylic amide structure, the methyl carboxylic ester containing analogue **4c** at the meta-position of ring B was more active (IC_{50} = 0.74 μM) than the corresponding para-position analogue **4f** (IC_{50} = 2.17 μM), which suggested that the ester at the meta-position positively affected HIF inhibition. Addition of a methyl substituent on phenyl ring A, as represented by **4g**,

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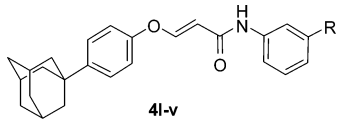
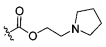
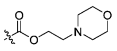
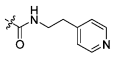
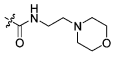
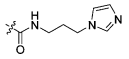
Table 3. In Vitro HIF-1 Inhibitory Activity and Cytotoxicity of (*E*)-Phenoxyacrylic Amide Derivatives



4l-v

No	Structure R	HRE IC_{50} (μM)[a]	Cell Viability (μM)[a]
4l		0.82±0.2	> 20
4m		1.84±0.18	> 20
4n		2.62±0.06	> 20
4o		1.35±0.14	> 20
4p		0.12±0.2	> 20
4q		> 20	> 20
4r		> 20	> 20
4s		> 20	> 20
4t		1.34±0.05	> 20
4u		1.07±0.02	> 20
4v		1.85±0.64	> 20

[a]Values are the mean of three experiments.

Table 4. In Vitro Water Solubility of the Representative Compounds at pH 3, 7.4, and 9

	solubility (μM)[a]		
compd	pH 2	pH 7.4	pH 9
4c	4.25 ± 2.12	6.56 ± 0.66	12.21 ± 0.77
4p	45.12 ± 2.64	113.09 ± 0.85	61.33 ± 1.68
1 (LW6)	68.15 ± 2.12	6.85 ± 1.00	44.91 ± 3.10

[a]Values are the mean of three experiments.

resulted in a 6-fold decrease in activity (IC_{50} = 4.12 μM) in comparison with **4c**. Similarly, an additional hydroxyl substituent on the B-ring also did not positively affect activity, as shown in **4h**. Of note, introducing a sulfonic ester (**4j**) to the aromatic ring B resulted in comparable potency (IC_{50} = 1.18 μM), whereas the sulfonamide **4k** showed less activity (IC_{50} = 2.38 μM) than **4c**. This result indicated that the sulfonic ester group is a good isosteric replacement for the carboxylic ester and yielded comparable potency.

Because **4c** displayed more inhibitory potency than LW6, we explored the effects of altering the methyl group of the ester in the B-ring with ethyl or substituted ethyl groups having a phenoxtacrylamide core **4l−p**. In general, the resulting ester compounds, including the ethyl (**4l**), methoxyethyl (**4m**), dimathylaminoethyl (**4n**), pyrrolininoethyl (**4o**), and morpholinoethyl ester (**4p**) compounds, possessed good to potent activity, with IC_{50} in the low micromolar range. The morpholine-substituted ethyl ester **4p** was the most potent (IC_{50} = 0.12 μM). It is noteworthy that **4p** has a secondary amine that helps improve its solubility in water. One of the shortcomings of the previous lead compounds (e.g., LW6) was their poor water solubility, which is a common issue with synthetic chemicals and often leads to difficulties in the further study of such compounds. Accordingly, the kinetic solubilities of LW6, **4c**, and **4p** were determined using an established nephlometry method,[20,21] and the results are provided in Table 4. As expected, the secondary amine-containing **4p** showed much better water solubility at the three different pH conditions than LW6 or **4a**, which merits further evaluation of this compound as a validated lead for the development of a novel anticancer agent.

In a similar fashion, a variety of benzamide derivatives with the (*E*)-phenoxyacrylic amide core structure were also prepared (**4q−v**) and evaluated for their HIF inhibitory activity. The free benzamide **4q** or secondary amide with a one or no carbon spacer (**4r,s**) displayed poor activity (Table 3). Of interest, the secondary amides on the B-ring, which feature insertion of two or three carbons between heterocycles/amines and NH in the amide portion (**4t−v**), restored the potency of the compound. Compound **4u** in which the secondary amide was substituted with an ethylene morpholine chain (IC_{50} = 1.07 μM) was of equal potency compared with the corresponding methyl ester **4c**. The most active analogues, including **4p** and **4u**, have a common substitution pattern with a morpholinoethyl group in the ester or amide in the B-ring, which seems to contribute to better biological activity in terms of the steric and electrostatic aspects of the pharmacophore, as well as to increased water solubility.

Inhibition of HIF-1α Accumulation by 4p. The representative compound **4p** was evaluated for its inhibitory effect on HIF-1α accumulation under hypoxic conditions in HCT116 colon cancer cells. As demonstrated by Western blot analysis, **4p** blocked HIF-1α accumulation in a dose-dependent manner (Figure 2A). Compared to LW6, **4p** inhibited hypoxia-induced accumulation of HIF-1α protein at a lower concentration of 50 nM. However, the expression of HIF-1β was not significantly affected by the presence of **4p**. The effects of **4p** on inhibition of HIF-1α accumulation in other cancer cells such as colorectal cancer LoVo cells, lung cancer A549 cells, cervical cancer HeLa cells, and prostate cancer PC-3 cells were investigated. As shown in Figure 2B, **4p** blocked HIF-1α accumulation in a dose-dependent manner in all of the cell lines tested. Of note, **4p** showed a greater effect on HIF-1α expression in LoVo and A549 cells, which warrants further mechanistic studies on the cell line specificity of this compound.

Previously, we presented evidence that LW6 promotes HIF-1α degradation via up-regulation of VHL, which interacts with prolyl-hydroxylated HIF-1α, leading to its ubiquitination and subsequent proteasomal degradation.[13] To examine whether **4p** has the same HIF-1α degradation signaling as LW6, a cell-based HRE reporter assay was carried out in the presence of dipyridol

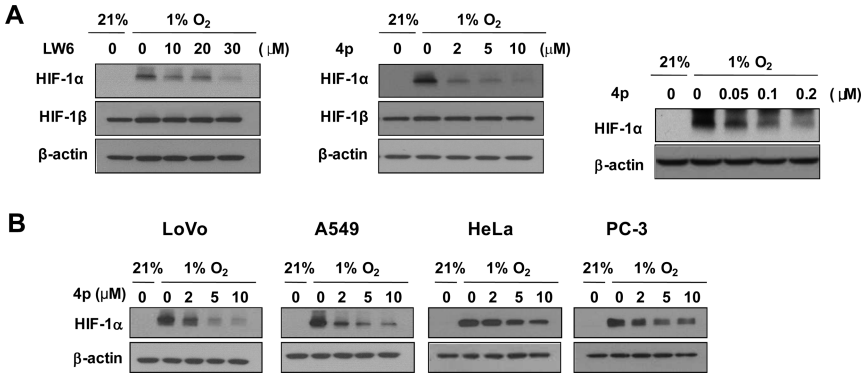


Figure 2. Effects of **4p** on hypoxia-induced HIF-1α accumulation. (A) The accumulation of HIF-1α was examined in the cells treated with **4p** at various concentrations under hypoxia. LW6 was used as a control. (B) The effect of **4p** on HIF-1α accumulation in various human cancer cell lines, LoVo, A549, HeLa, and PC-3, was determined by Western blot analysis.

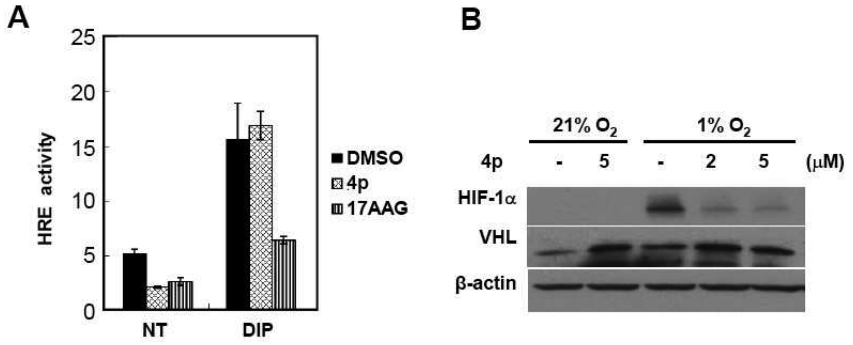


Figure 3. VHL-dependent HIF-1α degradation by **4p.** (A) Effect of **4p** on HRE-luciferase activity in the presence of DIP (dipyridol). The cells co-transfected with pGL3-HRE-luciferase and pRL-SV40 were incubated for 24 h. After incubation of cells under hypoxic conditions for 4 h, they were treated with 5 μM **4p** or 0.4 μM 17-AAG in the presence of 200 μM DIP for 12 h under hypoxia. Luciferase activity was measured using Renilla as a control. (B) Effect of **4p** on VHL expression. HCT116 cells treated with **4p** (0, 2, or 5 μM) under hypoxia were lysed with the RIPA buffer, and total lysates were prepared.

(DIP), a prolyl hydroxylase inhibitor. Compound **4p** did not reduce HRE activity when prolyl hydroxylation of HIF-1α was inhibited by 200 μM DIP, but **4p** did reduce HRE activity in the absence of DIP. However, 17-AAG, which leads to O_2/PHD/VHL-independent degradation of HIF-1α, reduced HRE activity regardless of whether DIP was present (Figure 3A). Furthermore, VHL expression was clearly increased in both normoxic and hypoxic conditions (Figure 3B). This result indicated that **4p** can abolish the accumulation of HIF-1α in a similar manner compared to LW6 by increasing VHL expression, which leads to the subsequent ubiquitination and proteasomal degradation of HIF-1α.

HIF-1α regulates transcription of target genes including *VEGF*, *EPO*, and *MMP-9*, which are associated with an aggressive tumor phenotype. To assess whether inhibition of HIF-1α by **4p** results in reduced expression of its target genes, mRNA levels of *VEGF*, *EPO*, and *MMP9* were examined (Figure 4A). Compound **4p** suppressed the hypoxia-induced mRNA expression of *VEGF*, *EPO*, and *MMP-9* in a dose-dependent manner without affecting expression of *HIF-1α* mRNA. Of particular interest, mRNA expressions of *VEGF*, *EPO*, and *MMP-9* were significantly suppressed by 2 μM **4p**.

Because **4p** inhibited the expression of *VEGF* and *EPO*, which are involved in angiogenesis, an in vitro tube formation assay was carried out using human umbilical vascular endothelial cells (HUVECs). When HUVECs were grown on a Matrigel surface, capillary-like structures such as those seen in angiogenesis were observed (Figure 4B). Cells treated with the positive control SU11248, a VEGFR inhibitor, did not form capillary-like tube structures, which was indicative of the inhibition of in vitro tube formation by HUVECs. Compound **4p** also inhibited in vitro tube formation, suggesting that **4p** has inhibitory activity against angiogenesis. It was clear that the growth of HUVECs was not affected by the presence of **4p** or SU11248 at the tested concentrations. Furthermore, a wound-healing assay was carried out to determine whether **4p** could inhibit the migration of HCT116 cells (Figure 4C). In the absence of **4p**, the scratch wound was almost completely covered by migrating cells after incubation for 24 h. However, the scratch wound was not filled with cells in the presence of **4p**, indicating that cell migration was inhibited by **4p** (Figure 4C). SU11248, a positive control, inhibited migration of cells and the scratch wound remained open, as expected.

■ CONCLUSIONS

We have synthesized a new series of (*E*)-phenoxyacrylic amide derivatives as potential HIF-1α inhibitors. Among the synthesized compounds, the morpholinoethyl ester analogue **4p** exhibited the highest activity in a cell-based HRE reporter assay in HCT116 cells and improved the solubility of the compound in water. The representative compound **4p** exhibited an inhibitory effect on hypoxia-induced HIF-1α accumulation via VHL-dependent HIF-1α degradation. Further, **4p** reduced target gene expression in a dose-dependent manner, which was consistent with its inhibitory effect on in vitro tube formation and migration of cells. The current work

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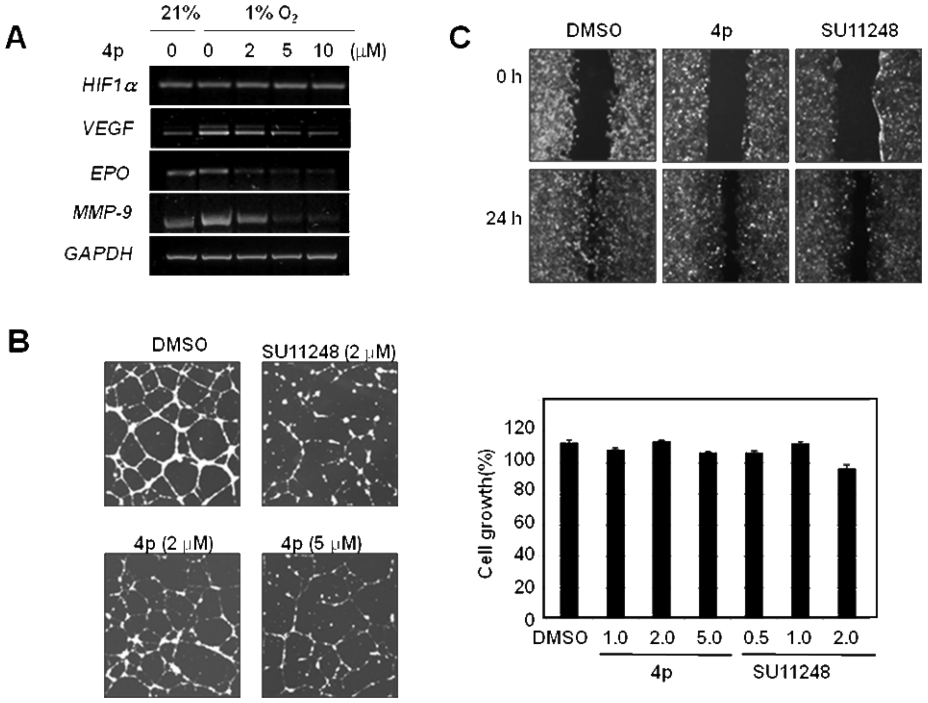

Figure 4. Effect of **4p** on the mRNA levels of HIF-1 target genes. (A) The mRNA levels of HIF-1α and its target genes were determined by RT-PCR in the cells treated with **4p**. (B) Inhibition of in vitro tube formation assay. The 96-well plates were coated with ice-cold BD Matrigel matrix gel solution, and human umbilical vein endothelial cells (HUVECs) were seeded. HUVECs were incubated on the Matrigel in the presence of **4p** or SU11248 under 1% O_2 for 18 h, and tubule branches were photographed. At the same time, growth inhibition of HUVECs by **4p** or SU11248 was examined with the SRB assay. (C) A wound-healing assay was carried out using cell culture inserts. Migration of HCT116 cells treated with **4p** or SU11248 was observed for 24 h.

provides a valuable platform for development of small molecule inhibitors of HIF-1α-dependent tumor progression and angiogenesis.

EXPERIMENTAL SECTION

General Procedures. All of the commercial chemicals and solvents were of reagent grade and were used without further purification. All reactions were carried out under an atmosphere of dried argon in flame-dried glassware. Melting points were determined in open capillary tubes on an electrothermal apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian (300 MHz) spectrometer. Chemical shifts are provided in parts per million (ppm) downfield from tetramethylsilane (internal standard) with coupling constants in hertz (Hz). Multiplicity is indicated by the following abbreviations: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), pseudo triplet (ps-t), quartet (q), multiplet (m), broad (br). Mass spectra were recorded on a Finnigan ESI mass spectrometer, and HRMS (ESI-MS) was conducted with a Mariner instrument (Perseptive Biosystem). Products from all reactions were purified to a minimum purity of 96% as determined by high-pressure liquid chromatography (HPLC) either by flash column chromatography using silica gel 60 (230−400 mesh Kieselgel 60) or by preparative thin layer chromatography using glass-backed silica gel plates (1 mm thickness) unless otherwise indicated. Additionally, thin-layer chromatography on 0.25-mm silica plates (E. Merck, silica gel 60 F254) was used to monitor reactions. The chromatograms were visualized using ultraviolet illumination, exposure to iodine vapors, dipping in PMA, or Hanessian's solution. Purity was determined by HPLC analysis. See Supporting Information for details.

(*E*)-3-[3-(4-Adamantan-1-ylphenoxy)acryloylamino]benzoic Acid Methyl Ester (4c). **4c** was obtained as a white solid (236.1 mg, 88.5% yield) from (*E*)-3-(4-adamantan-1-ylphenoxy)acrylic acid **9a** and 3-aminobenzoic acid methyl ester **10a**. Mp 138.5−139.0 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.06 (1H, s, aromatic-H), 7.89−7.95 (2H, m, aromatic-H, CH), 7.77 (1H, d, J = 7.5 Hz, aromatic-H), 7.33−7.42 (3H, m, aromatic-H), 7.02 (2H, d, J = 8.7 Hz, aromatic-H), 5.68 (1H, d, J = 11.7 Hz, CH), 3.90 (3H, s, CH_3), 2.11 (3H, m, adamantly-H), 1.90 (6H, m, adamantly-H), 1.77 (6H, m, adamantly-H); MS (EI) m/z 431 (M^+); HRMS (EI) m/z calcd for $C_{27}H_{29}NO_4$ [M^+] 431.2097, found 431.2101.

(*E*)-3-[3-(4-Adamantan-1-ylphenoxy)acrylamido]benzoic Acid Morpholinoethyl Ester (4p). **4p** was obtained as a white solid (9.3 mg, 18.6% yield) from commercially available 4-(2-chloroethyl)-morpholine chlorohydride. Mp 166.1−166.5 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.13 (1H, s, NH), 8.27 (1H, m, aromatic-H), 7.86−7.89 (1H, m, aromatic-H), 7.76 (1H, d, J = 11.7 Hz, CH), 7.62 (1H, d, J = 7.8 Hz, aromatic-H), 7.41−7.48 (3H, m, aromatic-H), 7.14 (2H, d, J = 9.0 Hz, aromatic-H), 5.81 (1H, d, J = 12.0 Hz, CH), 4.38 (2H, t, J = 6.0 Hz, CH_2), 3.56 (4H, t, J = 5.1 Hz, CH_2), 2.68 (2H, t, J = 6.0 Hz, CH_2), 2.47 (4H, t, J = 4.8 Hz, CH_2), 2.06 (3H, m, adamantly-H), 1.86 (6H, m, adamantly-H), 1.74 (6H, m, adamantly-H); MS (EI) m/z 530 (M^+); HRMS (EI) m/z calcd for $C_{32}H_{38}N_2O_5$ [M^+] 530.2781, found 530.2783.

ASSOCIATED CONTENT

Supporting Information

Synthesis procedures and compound characterization, tables reporting the HPLC purity results of the final products, screening methods, and the biological experiments on the HIF inhibitory activity of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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