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Bioorganic & Medicinal Chemistry Letters 17 (2007) 6305–6310
Bioorganic & Medicinal Chemistry Letters

Synthesis of (aryloxyacetylamino)-isonicotinic/nicotinic acid analogues as potent hypoxia-inducible factor (HIF)-1α inhibitors

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Received 18 May 2007; revised 14 August 2007; accepted 3 September 2007
Available online 7 September 2007

Abstract—We report a new series of HIF-1α inhibitors which were obtained through structural modifications of previously reported lead **1**. The in vitro inhibitory potencies of newly synthesized compounds were evaluated against hypoxia-induced HIF-1 activation using cell-based reporter assay in three human cancer cell lines including SK-Hep-1, Hep3B, and AGS cells. Several compounds displayed significant inhibitory activity in all the three tested cell lines. In particular, analogue **17** displayed potent inhibition of hypoxia-induced accumulation of HIF-1α protein in Hep3B cell line, in addition to the dose-dependent inhibition of HIF-1 target genes *VEGF* and *EPO*.
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Hypoxia-inducible factor (HIF)-1 is a key mediator for adaptation and survival process of cells to hypoxia (~1% O_2). The bioactivity of HIF-1, a heterodimer composed of HIF-1α and constitutively expressed HIF-1β (also known as ARNT), depends on the amount of HIF-1α protein.[1–4] The levels of HIF-1α are largely regulated on a post-translational level, by the rate of protein synthesis and degradation. Under normoxic conditions, HIF-1α protein is subjected to degradation via the von Hippel–Lindau tumor suppressor gene product (pVHL)-mediated ubiquitin–proteosomal pathway. The association of HIF-1α and pVHL under normoxia is triggered by the hydroxylation of prolines and the acetylation of lysine within oxygen-dependent degradation (ODD) domain.[5–7] Hypoxic conditions allow HIF-1α protein to escape proteolysis. Upon activation, the HIF complex with co-activator, such as p300/CBP, binds to HRE (Hypoxia Responsive Element 5′-RCGTG-3′) sequence within target genes, which leads to up-regulation of genes involved in angiogenesis, glucose metabolism, and pH regulation. Thus far, three isoforms of the α subunit have been cloned, with the well characterized being HIF-1α and HIF-2α. Although HIF-2α seems to be regulated in similar fashion with HIF-1α, it is suggested that there is little redundancy between two α subunits and that HIF-2α exhibits more tissue-specific expression.[8–11]

In human tumors, HIF-1α is over-expressed as a result of intratumoral hypoxia and genetic alterations affecting key oncogenes (HER2, FRAP, H-RAS, and c-SRC) and tumor suppressor genes (von Hippel–Lindau, PTEN, and p53).[6] Immunohistochemical analyses show that HIF-1α is present at higher levels in human tumors than in normal tissues.[12] The expression of HIF-1α in various solid tumors has been associated with tumor aggressiveness, vascularity, treatment failure, and mortality.[13] In addition, tumor growth and angiogenesis in xenograft tumors also depends on HIF-1 activity and on the expression level of HIF-1α.[14] All of these activities make the HIF-1 transcription factor an attractive target for the development of new anticancer therapeutics.[15–17]

Keywords: Hypoxia; Hypoxia-inducible factor; HIF-inhibitor; HIF-1; Isonicotinic/nicotinic acid analogues.
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doi:10.1016/j.bmcl.2007.09.005

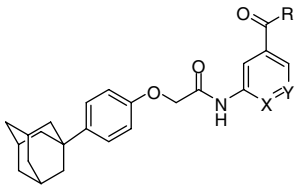
In this context, recently a molecular targeted HTS method was also reported, which coherently identifies small-molecule HIF-1 inhibitors.[18,19] In recent years several anticancer agents have been identified to inhibit HIF-1 activity in tumor xenografts including a soluble guanylyl-cyclase stimulator YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole), [20]the HSP90 inhibitor 17-AAG (17-allyl-aminogeldanamycin),[21–23] the microtubule destabilizer 2-methoxyoestradiol (2ME2),[24] the topoisomerase inhibitors camptothecin and topotecan,[18]the thioredoxin inhibitors pleurotin and 1-methylpropyl 2-imidazolyl disulfide,[25] the mammalian target of rapamycin (mTOR) kinase inhibitor CCI-779,[26] a 2,2-dimethylbenzopyran structural motif 103D5R,[27] and a small natural product chetomin.[28] In addition, a number of inhibitors targeting the HIF pathway with an emphasis on the inhibition of HIF hydroxylase activity to prevent HIF-1α degradation have also been reported.[29–34]

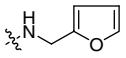
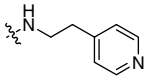
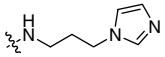
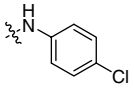
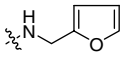
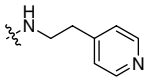
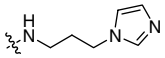
As part of our ongoing research in the identification of novel HIF-1 inhibitors, we have recently reported the identification of a novel series of potent HIF-1α inhibitors through closely related structural modifications of a screening hit.[35] Amongst them compounds **1** and **2** (Fig. 1) displayed potent HIF-1 inhibitory activity in human hepatocellular carcinoma Hep3B and human gastric adenocarcinoma AGS cell lines, in addition compound **1** potently suppressed the HIF-1α protein accumulation and its target gene expression under hypoxic conditions in Hep3B cells. The structure–activity relationship study of this series suggested that the presence of the adamantyl group on ring A is essential for effective inhibition of HIF-1 activation in hypoxia. We hypothesized that the replacement of phenol in ring B of **1** with pyridine while maintaining adamantyl moiety on ring A would be expected to modulate the inhibitory activity (Fig. 1) and on the basis of this strategy we prepared a series of pyridine-containing analogues and evaluated for their potential to inhibit HIF-1 in Hep3B, AGS, and SK-Hep-1 cell lines. In this communication, we report the design, synthesis, and HIF-1 inhibitory activity of a new series of isonicotinic and nicotinic acid derivatives.

The compounds described in this paper (Table 1) were prepared as outlined in Schemes 1–3.[36] As shown in Scheme 1, reaction of 4-adamantyl phenol **3** with ethyl chloroacetate followed by alkaline hydrolysis afforded the corresponding 4-adamantyl phenoxy acetic acid **4** in quantitative yield.

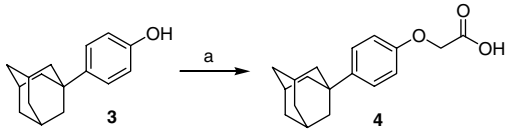
During our initial medicinal chemistry efforts we prepared intermediates **9** and **10** by following the chemistry described in Scheme 2.

Table 1. In vitro inhibition of HIF-1 transcriptional activity in cell-based reporter assay[a]



Compound	R	X	Y	IC_{50} (μM)		
				SK-Hep1	Hep3B	AGS
1	OCH_3	CH	CH	NA[b]	2.6	0.7
2	OH	CH	CH	NA	0.4	0.35
11	OCH_3	N	CH	1.03	1.2	2
12	OH	N	CH	2.0	1.0	0.8
13		N	CH	>30	>30	>30
14		N	CH	3.4	3.4	5.2
15		N	CH	0.9	11.2	11
16		N	CH	2.5	>30	18.6
17	NH_2	N	CH	0.6	3.1	1.8
20	OCH_3	CH	N	>30	5.9	5
21	OH	CH	N	>30	>30	>30
22		CH	N	4.1	>30	30
23		CH	N	>30	>30	1.3
24		CH	N	1.9	>10	>10
26	NH_2	CH	N	11.9	>10	0.9
YC-1				>30	13.8	2.0

[a] Values are means of three experiments.
[b] NA, data is not available.



Scheme 1. Reagents: (a) i—Ethyl chloroacetate, K_2CO_3, DMF; ii—$LiOH{\cdot}H_2O$, dioxane, H_2O.

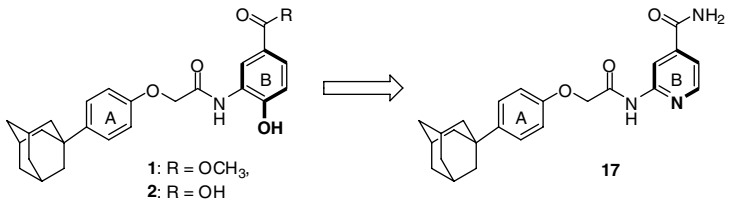
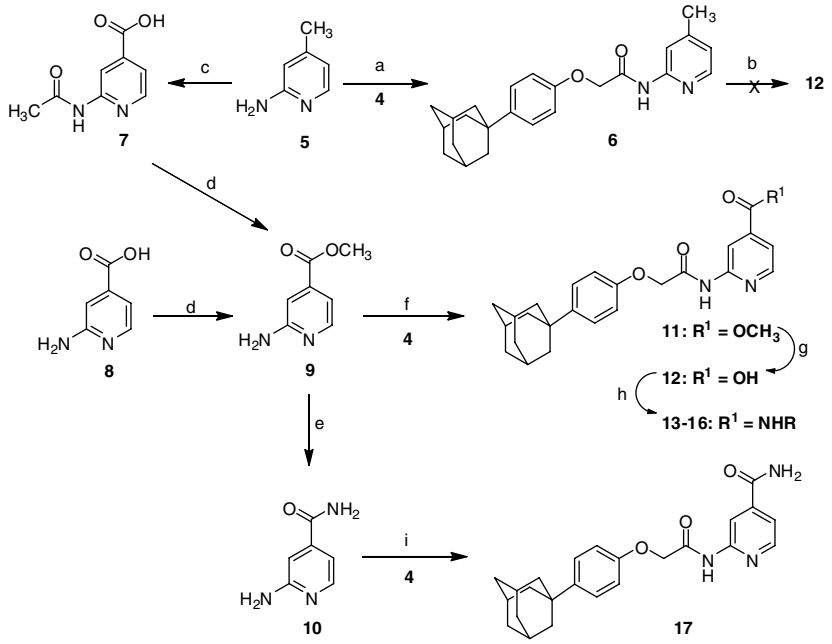
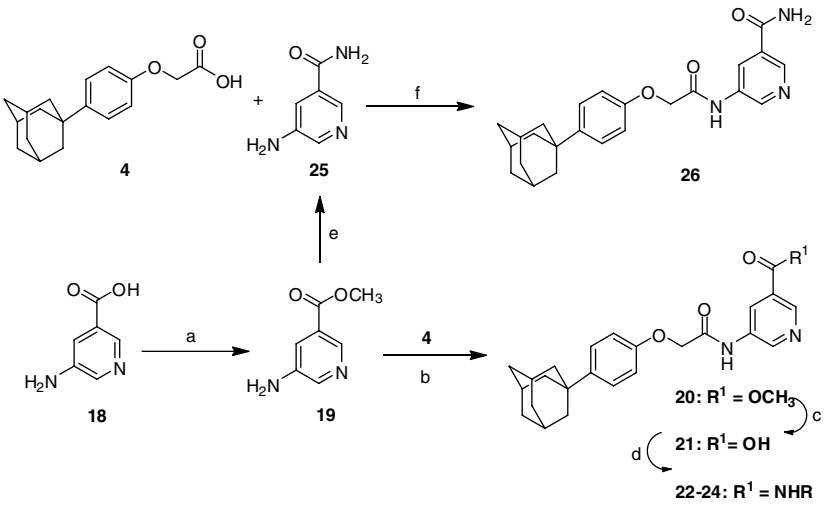


Figure 1. Structural modification of phenol to pyridine.



Scheme 2. Reagents: (a) EDC, DIPEA, DMF; (b) SeO_2, dioxane or $KMnO_4$, NaOH, H_2O; (c) i—Ac_2O, DMAP; ii—$KMnO_4$, NaOH, H_2O; (d) $SOCl_2$, MeOH; (e) aqueous ammonia; (f) PyBOP, DMAP, DMF; (g) LiI, pyridine; (h) arylamine, PyBOP, DMAP, DMF; (i) PyBOP, DMAP, DMF.



Scheme 3. Reagents: (a) $SOCl_2$, MeOH; (b) PyBOP, DMAP, DMF; (c) LiI, pyridine; (d) arylamine, PyBOP, DMAP, DMF; (e) aqueous ammonia; (f) PyBOP, DMAP, DMF.

Commercially available picoline **5** was utilized to synthesize the requisite intermediates. Thus, acetylation of **5** followed by subsequent oxidation using $KMnO_4$ gave 2-acetylamino isonicotinic acid **7**,[37] which upon without further purification, subjected to esterification in the presence of thionyl chloride and methanol. This reaction affected both esterification along with deacetylation of acetylmino moiety to yield 2-amino isonicotinic acid methyl ester **9** in modest yield. Alternatively, this ester **9** was also prepared by the reaction of commercially available 2-amino isonicotinic acid **8** with thionyl chloride in refluxing methanol. Further reaction of this ester **9** with ammonium hydroxide provided the required 2-amino isonicotinamide **10** in good yield.[38]

Analogue **6** possessing picoline moiety was readily obtained by the reaction of 2-amino-4-picoline **5** with **4** in the presence of EDC and DIPEA. Attempting subsequent oxidation of the methyl group utilizing SeO_2 or $KMnO_4$ failed to give the desired acid **12**, consequently a two-step method was adopted as follows. 2-Amino isonicotinic acid methyl ester **9** was coupled with **4** to obtain the corresponding ester derivative **11**, which upon subsequent hydrolysis with LiI furnished the desired acid **12**. Subsequent coupling of acid **12** with the appropriate amines in the presence of PyBOP and DMAP afforded the corresponding amide analogues **13–16** in good yields.

A single-step coupling of **4** with amides **10** or **25** yielded the corresponding amide derivatives **17** and **26**,

respectively. Esterification of 3-amino-5-nicotinic acid **18** in the presence of thionyl chloride and methanol furnished the corresponding ester **19** in satisfactory yield, which upon subsequent aminolysis using aqueous ammonia afforded the desired amide analogue **25**. A similar sequence of reactions described for analogues **11**–**16** was followed to obtain nicotinic acid derivatives **20**–**24** in moderate to high yields (Scheme 3).

The in vitro inhibitory potencies of all the newly synthesized compounds were evaluated against hypoxia-induced HIF-1 activation in three cell lines including human hepatocellular carcinoma SK-Hep-1, human hepatocellular carcinoma Hep3B, and human gastric adenocarcinoma AGS cell lines. The inhibitory potencies were obtained using a HRE-mediated cell-based reporter assay under hypoxic conditions (1% O_2, 94% N_2, and 5% CO_2) and the results are tabulated as IC_{50} values in Table 1. All the assays were performed under standard assay conditions by employing hypoxic condition and following the previously described assay protocol.[39] Cell viability, as measured by the MTT assay, showed that all of the tested compounds had no significant cytotoxicity at their effective concentrations for the inhibition of HIF-1 activation. YC-1, a known small-molecule HIF-1 inhibitor,[20] was used as a reference compound for comparison, which showed IC_{50} 13.8 and 2.0 μM in Hep3B and AGS cells, respectively.

The isonicotinic acid analogues **11** and **12**, which were prepared based on compound **1** as a starting point, displayed highly potent inhibition of hypoxia-induced HIF-1 activity in all the three tested cell lines with IC_{50} values ranging from 1 to 2 μM. Encouraged by this we further extended the analogue series characterized by an amide substitution at ring B. This derivatization produced amide derivatives **13**–**16**, comprising furfuryl, ethyl pyridine, propyl imidazole, and *p*-chloro phenyl moieties linked through an amide bond. It is worth noting that, while analogue **14** comprising ethyl pyridine moiety exhibited good inhibitory activities in all the three cell lines, derivatives **15** and **16** demonstrated high inhibitory potency against SK-Hep-1 cell line. In contrast, compound **13** in which it was linked to furfuryl ring was found to be a poor inhibitor.

We also investigated the effect of the free amide moiety at ring B on the HIF-inhibition. This modification resulted in the highly potent inhibitor represented by compound **17**, which demonstrated sub-micromolar inhibitory potencies in all the three tested cell lines. Additionally, **17** exhibited better potency (IC_{50} 3.1 μM) than the corresponding phenol compound[28] (IC_{50} > 30 μM) in Hep3B cell lines. From these results it is evident that pyridine moiety serves as a good bioisosteric replacement for phenol portion of **1** (Fig. 1). Further to confirm this hypothesis, we also prepared various pyridine analogues, wherein nitrogen atom was introduced at different position of ring B which resulted in nicotinic acid analogues **20**–**24** and **26**. Amongst these, compounds **20**, **23**, and **26** displayed significant inhibition in AGS cell line, while analogues **22** and **24** were found to be potent inhibitors in SK-Hep-1 cell line and rest of the compounds were found to be inactive or poor inhibitors. Of note, none of these nicotinic acid analogues exhibited inhibitory activity in all the three cell lines, suggesting that the position of nitrogen atom at pyridine ring is important for potency. Accordingly, a simple modification of phenol to pyridine resulted in a new series of potent HIF-1 inhibitors.

To confirm the HIF-1 inhibitory activity of these compounds, a representative analogue **17** which demonstrated significant inhibitory activity of HIF activation in all the three cell lines was chosen for further evaluation. Accordingly, this analogue was evaluated by Western blot analysis for its potential to inhibit hypoxia-induced accumulation of HIF-1α protein in Hep3B cell line.

As shown in Figure 2, analogue **17** blocked the accumulation of HIF-1α protein in a dose-dependent manner without affecting the expression level of HIF-1β. Thus, confirming the HIF-1α inhibitory property of this analogue.

HIF-1α responds to the hypoxia by binding to the HRE of target genes, including *VEGF* and *EPO*. Moreover, expression of these HIF-1 target genes, such as *VEGF*, stimulates new blood vessel formation from existing vasculature, increasing tumor oxygenation and, ultimately, tumor growth and metastasis.[40] Therefore, compounds **17** possessing potent HIF-1α accumulation inhibitory profile was further tested for its ability to inhibit the expression of HIF-1 target genes *VEGF* and *EPO* in Hep3B cells by RT-PCR analysis (Fig. 3). Interestingly, this inhibitor significantly suppressed the expression of *VEGF* and *EPO* in a dose-dependent manner without affecting mRNA expression level of HIF-1α and GAPDH, wherein potent inhibition was observed at the

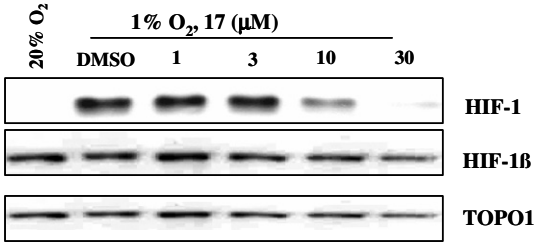


Figure 2. Western blot analysis of the effect of compound **17** on the hypoxia-induced HIF-1α protein level in Hep3B cells.

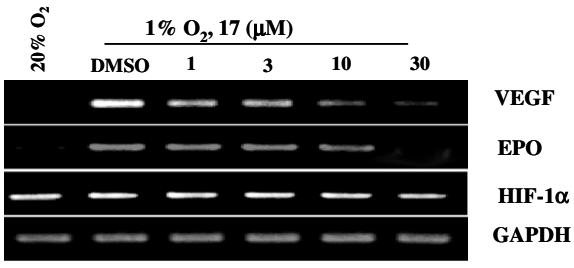


Figure 3. RT-PCR analysis of the effect of compound **17** on the hypoxia-induced expression of *VEGF* and *EPO* in Hep3B cells.

tested concentration of 30 μM. This result may suggest that **17** inhibits HIF-1α expression post-transcriptionally, not transcriptionally. However, more detailed studies are required for the mechanism of action of these inhibitors in the context of HIF-α isoforms as well as translational/post-translational level regulation.

In summary, herein we have disclosed a new class of potent HIF-1α inhibitors through a modification of a previously reported series, in which a pyridine moiety was introduced in place of a phenol in the lead structure **1**. Among these, analogues **11**, **12**, **14**, and **17** demonstrated potent HIF-1 inhibitory activity in all the three tested cell lines. HIF-1 inhibitory activity of these analogues was further established by potent inhibition of hypoxia-induced accumulation of HIF-1α protein in Hep3B cell line by a representative analogue **17**. In addition, this analogue **17** significantly suppressed the expression of *VEGF* and *EPO* dose-dependently without affecting the mRNA expression level of *HIF-1α*. Further investigations are warranted to establish more detailed mechanism of these analogues.

Acknowledgments

This study was supported by a grant from KRIBB Research Initiative Program, Korea, and the Molecular and Cellular BioDiscovery Research Program (M10601000155), Korea.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.005.

References and notes

1. Jones, M. K.; Szabo, I. L.; Kawanaka, H.; Husain, S. S.; Tarnawski, A. S. *FASEB J.* **2002**, *16*, 264.
2. Wang, G. L.; Jiang, B.; Rue, E. A.; Semenza, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5510.
3. Maxwell, P. H.; Dachs, G. U.; Gleadle, J. M.; Nicholls, L. G.; Harris, A. L.; Stratford, I. J.; Hankinson, O.; Pugh, C. W.; Ratcliffe, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8104.
4. Semenza, G. L. *Annu. Rev. Cell Dev. Biol.* **1999**, *15*, 551.
5. Jaakkola, P.; Mole, D. R.; Tian, Y.-M.; Wilson, M. I.; Gielbert, J.; Gaskell, S. J.; von Kriegsheim, A.; Hebestreit, H. F.; Mukherji, M.; Schofield, C. J.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J. *Science* **2001**, *292*, 468.
6. Semenza, G. L. *Trends Mol. Med.* **2002**, *8*, S62.
7. Harris, A. L. *Nat. Rev. Cancer* **2002**, *2*, 38.
8. Hickey, M. M.; Simon, C. *Curr. Top. Dev. Biol.* **2006**, *76*, 217.
9. Carroll, V. A.; Ashcroft, M. *Cancer Res.* **2006**, *66*, 6264.
10. Tian, H.; McKnight, S. L. *Genes Dev.* **1997**, *11*, 72.
11. Makino, Y.; Kanopka, A.; Wilson, W. J.; Tanaka, H.; Poellinger, L. *J. Biol Chem.* **2002**, *277*, 39192.
12. Zhong, H.; De Marzo, A. M.; Laughner, E.; Lim, M.; Hilton, D. A.; Zagzag, D.; Buechler, P.; Isaacs, W. B.; Semenza, G. L.; Simons, J. W. *Cancer Res.* **1999**, *59*, 5830.
13. Birner, P.; Schindl, M.; Obermair, A.; Plank, C.; Breitenecker, G.; Oberhuber, G. *Cancer Res.* **2000**, *60*, 4693.
14. Maxwell, P. H.; Dachs, G. U.; Gleadle, J. M.; Nicholls, L. G.; Harris, A. L.; Stratford, I. J.; Hankinson, O.; Pugh, C. W.; Ratcliffe, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8104.
15. Giaccia, A.; Siim, B. G.; Johnson, R. S. *Nat. Rev. Drug Discov.* **2003**, *2*, 1.
16. Semenza, G. L. *Nat. Rev. Cancer* **2003**, *3*, 721.
17. Belozerov, A. E.; Van Meir, R. G. *Anti-Cancer Drugs* **2005**, *16*, 901.
18. Rapisarda, A.; Uranchimeg, B.; Scudiero, D. A.; Selby, M.; Sausville, E. A.; Shoemaker, R. H.; Melillo, G. *Cancer Res.* **2002**, *62*, 4316.
19. Shoemaker, R. H.; Scudiero, D. A.; Melillo, G.; Currens, M. J.; Monks, A. P.; Rabow, A. A.; Covell, D. G.; Sausville, E. A. *Curr. Top. Med. Chem.* **2002**, *2*, 229.
20. YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1 Yeo, E. J.; Chun, Y. S.; Cho, Y. S.; Kim, J.; Lee, J. C.; Kim, M. S.; Park, J. W. *J. Natl. Cancer Inst.* **2003**, *95*, 516.
21. Isaacs, J. S.; Jung, Y. J.; Mimnaugh, E. G.; Martinez, A.; Cuttitta, F.; Neckers, L. M. *J. Biol. Chem.* **2002**, *277*, 29936.
22. Mabjeesh, N. J.; Post, D. E.; Willard, M. T.; Kaur, B.; Van Meir, E. G.; Simons, J. W.; Zhong, H. *Cancer Res.* **2002**, *62*, 2478.
23. Zagzag, D.; Nomura, M.; Friedlander, D. R.; Blanco, C. Y.; Gagner, J. P.; Nomura, N.; Newcomb, E. W. *J. Cell. Physiol.* **2003**, *196*, 394.
24. Mabjeesh, N. J.; Escuin, D.; LaVallee, T. M.; Pribluda, V. S.; Swartz, G. M.; Johnson, M. S.; Willard, M. T.; Zhong, H.; Simons, J. W.; Giannakakou, P. *Cancer Cell* **2003**, *3*, 363.
25. Welsh, S. J.; Williams, R. R.; Birmingham, A.; Newman, D. J.; Kirkpatrick, D. L.; Powis, G. *Mol. Cancer Ther.* **2003**, *2*, 235.
26. Majumder, P. K.; Febbo, P. G.; Bikoff, R.; Berger, R.; Xue, Q.; McMahon, L. M.; Manola, J.; Brugarolas, J.; McDonnell, T. J.; Golub, T. R.; Loda, M.; Lane, H. A.; Sellers, W. R. *Nat. Med.* **2004**, *10*, 594.
27. Tan, C.; de Noronha, R. G.; Roecker, A. J.; Pyrzynska, B.; Khwaja, F.; Zhang, Z.; Zhang, H.; Teng, Q.; Nicholson, A. C.; Giannakakou, P.; Zhou, W.; Olson, J. J.; Pereira, M. M.; Nicolaou, K. C.; Van Meir, E. G. *Cancer Res.* **2005**, *65*(2), 605.
28. Kung, A. L.; Zabludoff, S. D.; France, D. S.; Freedman, S. J.; Tanner, E. A.; Vieira, A.; Cornell-Kennon, S.; Lee, J.; Wang, B.; Wang, J.; Memmert, K.; Naegeli, H. U.; Petersen, F.; Eck, M. J.; Bair, K. W.; Wood, A. W.; Livingston, D. M. *Cancer Cell* **2004**, *6*, 33.
29. Mole, D. R.; Schlemminger, I.; McNeill, L. A.; Hewitson, K. S.; Pugh, C. W.; Ratcliffe, P. J.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2677.
30. Epstein, A. C. R.; Gleadle, J. M.; McNeill, L. A.; Hewitson, K. S.; O'Rourke, J.; Mole, D. R.; Mukherji, M.; Metzen, E.; Wilson, M. I.; Dhanda, A.; Tian, Y. M.; Masson, N.; Hamilton, D. L.; Jaakkola, P.; Barstead, R.; Hodgkin, J.; Maxwell, P. H.; Pugh, C. W.; Schofield, C. J.; Ratcliffe, P. *J. Cell* **2001**, *107*, 43.
31. Elkins, J. M.; Hewitson, K. S.; McNeill, L. A.; Seibel, J. F.; Schlemminger, I.; Pugh, C. W.; Ratcliffe, P. J.; Schofield, C. J. *J. Biol. Chem.* **2003**, *278*, 1802.
32. Schlemminger, I.; Mole, D. R.; McNeill, L. A.; Dhanda, A.; Hewitson, K. S.; Tian, Y.-M.; Ratcliffe, P. J.; Pugh, C. W.; Schofield, C. J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1451.
33. Namal, C. W.; Shengde, W.; Angelique, B.; Richard, K.; Justin, Sheville.; Sean, R.; Kevin, Xu.; Matthew, Pokross.;

Artem, G. E.; Richard, W.; Marlene, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5598.
34. Namal, C. W.; Shengde, W.; Angelique, B.; Richard, K.; Justin, S.; Ritu, T. B.; Sean, R.; a Kevin, X.; Matthew, P.; Songtao, Z.; Richard, W.; Marlene, M.; Artem, G. E.; Stephen, E. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5616.
35. Lee, K.; Lee, J. H.; Boovanahalli, S. K.; Jin, Y.; Lee, M.; Jin, X.; Kim, J. H.; Hong, Y.-S.; Lee, J. H.; Lee, J. J. *J. Med. Chem.* **2007**, *50*, 1675–1684.
36. All of the newly synthesized compounds were characterized by ^{1}H NMR and ESIMS, and purified to a minimum purity of 96% as determined by RP-HPLC, either by flash column chromatography or by preparative thin layer chromatography: 2-[2-(4-Adamantan-1-yl-phenoxy) acetylamino]-isonicotinamide (**17**). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.62 (1H, s, CONH), 8.43 (2H, m, $CONH_2$), 8.19 (1H, s, pyridine-H), 7.67 (1H, s, pyridine-H), 7.49 (1H, d, J = 5.1 Hz, pyridine-H), 7.26 (2H, d, J = 9.3 Hz, aromatic-H), 6.89 (2H, d, J = 8.4 Hz, aromatic-H), 4.78 (2H, s, OCH_2), 2.03 (3H, br s, adamantyl-H), 1.82 (6H, s, adamantyl-H), 1.71 (6H, s, adamantyl-H); MS (ESI) *m*/*z* 406 (M+H)+, 428 $(M+Na)^+$, 404 $(M-H)^-$; Purity = >99.9% (as determined by RP-HPLC, Method C, t_R = 17.2 min).
37. Wagner, G. K.; Kotschenreuther, D.; Zimmermann, W.; Laufer, S. A. *J. Org. Chem.* **2003**, *68*, 4527.
38. Deardy, L. W.; Korytsky, O. L.; Rowe, J. E. *Aust. J. Chem.* **1982**, *35*, 2025.
39. All the assays were performed under standard assay conditions by employing hypoxic condition as described by Cai, X. F.; Jin, X.; Lee, D.; Yang, Y. T.; Lee, K.; Hong, Y.-S.; Lee, J. H.; Lee, J. J. *J. Nat. Prod.* **2006**, *69*, 1095.
40. Semenza, G. L. *Biochem. Pharmacol.* **2000**, *59*, 47–53.