ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

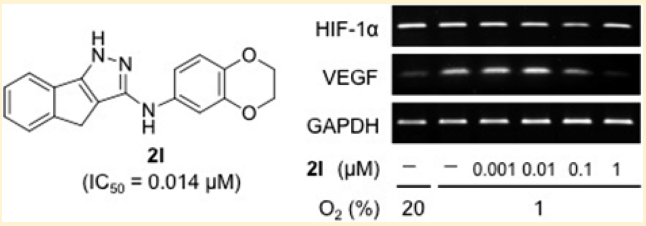
Discovery of Indenopyrazoles as a New Class of Hypoxia Inducible Factor (HIF)-1 Inhibitors

Hidemitsu Minegishi, Shinji Fukashiro, Hyun Seung Ban,[†] and Hiroyuki Nakamura*

Department of Chemistry, Faculty of Science, Gakushuin University, Mejiro, Toshima-ku, Tokyo 171-8588, Japan

Supporting Information

ABSTRACT: The indenopyrazole framework was investigated as a new class of HIF-1α inhibitors. Indenopyrazole **2l** was found to most strongly inhibit the hypoxia-induced HIF-1α transcriptional activity (IC_{50} = 0.014 μM) among all of the known compounds having relatively simple structures, unlike manassantins. Indenopyrazole **2l** suppressed HIF-1α transcriptional activity without affecting both HIF-1α protein accumulation and HIF-1α/HIF-1β heterodimerization in nuclei under the hypoxic conditions, suggesting that **2l** probably affected the transcriptional pathway induced by the HIF-1α/HIF-1β heterodimer.



KEYWORDS: *HIF-1, indenopyrazole, inhibitor, transcriptional activity*

Hypoxia inducible factor (HIF)-1 is a transcription factor that controls the expression of genes influencing angiogenesis, glucose metabolism, cell proliferation, survival, and invasion of solid tumors and is thereby considered to be a central regulator of major adaptive responses to hypoxia in cancer progression.[1] HIF-1 is a heterodimeric complex that consists of a hypoxia-inducible HIF-1α subunit and a constitutively expressed HIF-1β subunit.[2,3] Under the normoxic conditions, HIF-1α degradation is facilitated by the hydroxylation of proline residues at the oxygen-dependent degradation domain (ODDD) by prolyl hydroxylases (PHDs), and the hydroxylated HIF-1α is degraded by the von Hippel Lindau (VHL)-mediated ubiquitin proteasome system.[4,5] Under the hypoxic conditions, HIF-1α hydroxylation is impeded due to the reduced PHD activity. The stabilized HIF-1α is accumulated in cytosol and translocated to the nucleus where it dimerizes with HIF-1β. The complex binds to hypoxia response elements (HREs) together with coactivators to activate target genes, including vascular endothelial growth factor (VEGF), erythropoietin (EPO), glucose transporters, and insulin-like growth factors.[6−8] The fact that HIF-1α is detected at increased levels in many types of human tumors[9−14] makes it one of the potential targets of antitumor agents.[1,15−17]

Various compounds have the potential to inhibit HIF-1 transcriptional activity.[17] Such compounds as PX-478,[18] PX-866,[19] and EZN-2968[20] are currently undergoing phase I/II clinical trials. We recently found that *ortho*-carboranylphenoxyacetanilides, which were designed and synthesized based on the structure of CAY10585,[21,22] inhibited HIF-1 transcriptional activity through hypoxia-induced HIF-1α protein accumulation.[23] The identification of heat shock protein (Hsp) 60 as the primary target of *ortho*-carboranylphenoxyacetanilides revealed that Hsp 60 played an important role in the hypoxia-induced HIF-1α accumulation, both directly and indirectly.[24] We also found that VEGF receptor 2 (VEGFR2) tyrosine kinase inhibitors, such as AAL993, SU5416, and KRN633, suppressed HIF-1α transcriptional activity through the inhibition of Akt and/or ERK phosphorylation signaling pathways.[25] From those findings, it occurred to us that other VEGFR2 tyrosine kinase inhibitors might also have the potential to inhibit HIF-1α transcriptional activity. We also found that indenopyrazoles inhibited VEGFR2 tyrosine kinase activity.[26] In this study, we examined the inhibition of HIF-1α transcriptional activity by indenopyrazoles under the hypoxic conditions using the dual luciferase reporter gene assay[27] and found that compound **1** (GN2707) significantly inhibited the hypoxia-induced HIF-1α transcriptional activity, its IC_{50} being 0.214 μM (Figure 1). Although compound **1** displayed potent inhibition of HIF-1α

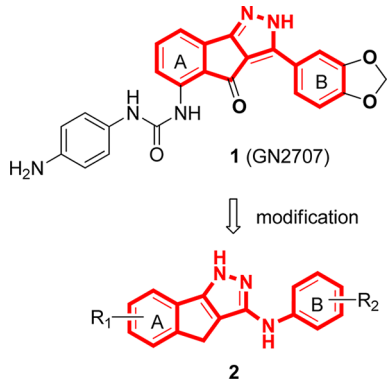


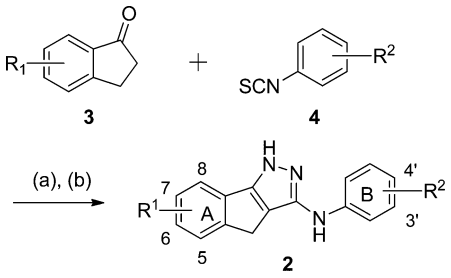
Figure 1. Modification of hit compound **1**.

Received: December 19, 2012
Accepted: January 27, 2013
Published: January 27, 2013

transcriptional activity, it was difficult to synthesize derivatives for structure−activity relationship studies due to the low chemical yields at the triketone formation step from corresponding phthalate esters and acetophenones.[28] Thus, in this study, we focused on the alternative indenopyrazole framework **2** and synthesized a series of indenopyrazole derivatives.[29] We evaluated the synthesized compounds for potential biological activity and found that they were potent inhibitors of the hypoxia-induced HIF-1 transcriptional activity with IC_{50} of nanomolar order. So far, manassantins isolated from the aquatic plant *Saururus cernuus* are known to most strongly inhibit the hypoxia-induced HIF-1 transcriptional activity in a T47D-cell-based dual luciferase reporter assay (IC_{50} = 3−10 nM).[30] Although manassantins are attractive inhibitors for HIF-1-related biological studies, they have complex structures with several chiral centers and thus require a considerable number of synthetic steps.[31,32] In this study, we present an alternative inhibitor that is readily available for HIF-1-related biological studies.

The synthesis of indenopyrazoles is shown in Scheme 1. Indenopyrazoles **2** were prepared from 2,3-dihydro-1*H*-inden-1-ones **3** and phenyl isothiocyanates **4** using the procedure in the literature with modification.[29] Compound **2a** was identified by comparison with the reported ^{1}H NMR spectra in the literature.[29] Indenopyrazoles **2b−g**, which have a substituent on aromatic ring A, and indenopyrazoles **2h−l**, which have a substituent on aniline ring B, were synthesized from corresponding substituted 1-indanones **3b−g** and **4a** and substituted phenyl isocyanates **4b−f** in a similar manner to the synthesis of **2a**, respectively. Furthermore, indenopyrazole **2m**, which has substituents on both aromatic rings A and B, was also synthesized by the reaction of **3b** and **4f** in 62% yield.

Scheme 1. Synthesis of Indenopyrazoles 2a−m[a]



[a]Reagents and conditions: (a) LiHMDS, THF, 12 h. (b) $H_2NNH_2{\cdot}H_2O$, AcOH, reflux, 24 h, 43−85%.

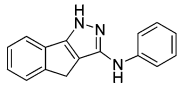
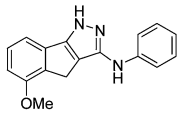
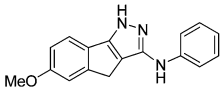
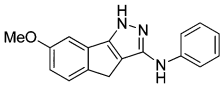
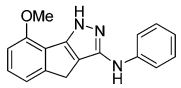
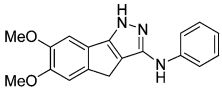
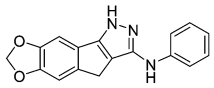
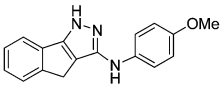
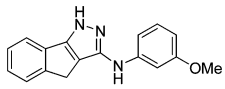
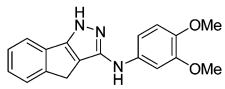
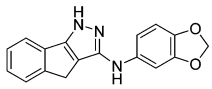
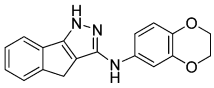
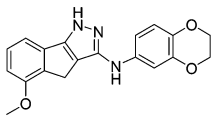
Synthesized indenopyrazoles **2a−m** were tested for their ability to inhibit HIF-1 transcriptional activity in HeLa cells under the hypoxic conditions (1% oxygen) using the dual luciferase reporter gene assay and their antiproliferative activities toward four human cancer cell lines, HCT116 (human colon cancer), HepG2 (human hepatoma), PC3 (human prostate cancer), and HeLa (human cervical carcinoma) using the MTT assay. 3-(5′-Hydroxymethyl-2′-furyl)-1-benzyl indazole (YC-1) and CAY10585[21] were used as positive controls for comparison. The results are summarized in Table 1. Indenopyrazole **2a** at 30 μM, which has no substituent on aromatic rings A and B, failed to inhibit HIF-1 transcriptional activity. Indenopyrazoles **2b−g**, which have a substituent on aromatic ring A, exhibited weak or no inhibition of HIF-1 transcriptional activity (0−41% inhibition at 30 μM). Compounds **2a−g** had high antiproliferative activities relative to their HIF-1 transcription inhibitory activities; the IC_{50} values for the former were lower than 30 μM. On the other hand, compounds **2h−l**, which have a substituent on aniline ring B, more strongly inhibited the hypoxia-induced HIF-1 transcriptional activity than compounds **2a−g**. Indenopyrazoles having the monomethoxy group (**2h** and **2i**), and the dimethoxy groups (**2j**) attached to aniline ring B inhibited the hypoxia-induced HIF-1 transcriptional activity with IC_{50} = 1.4, 6.1, and 3.2 μM, respectively. The antiproliferative activities of **2h−j** toward human cancer cell lines were similar to their HIF-1 transcriptional activities except for PC3 cells. Interestingly, a cyclic ether skeleton on aniline ring B dramatically enhanced the inhibition of the hypoxia-induced HIF-1 transcriptional activity (**2k−m**). Compound **2k** having a 3′,4′-methylenedioxy group attached to aniline ring B inhibited the HIF-1 transcriptional activity with IC_{50} of 0.27 μM. The best result was obtained for compound **2l**, which has a 3′,4′-ethylenedioxy group attached to aniline ring B: its IC_{50} was 0.014 μM, which was 20 times higher than that of compound **2k** and 100 times higher than that of YC-1. The methoxy group substitution at 5-position of aromatic ring A of **2l** decreased the inhibitory effect on HIF-1 transcriptional activity (**2m**), revealing that the 3′,4′-ethylenedioxy substituent on aniline ring B is essential for the strong inhibition of HIF-1 transcriptional activity. The antiproliferative activities of indenopyrazoles **2k−m** were weaker than their HIF-1α transcription inhibitory activities by 2 orders of magnitude, revealing that indenopyrazoles **2k−m** suppressed HIF-1 transcriptional activity without affecting cell viability.

Among the synthesized indenopyrazoles, **2l** most strongly inhibited the hypoxia-induced HIF-1 transcriptional activity. Then, we examined the effects of **2l** on the hypoxia-induced HIF-1α protein accumulation by Western blot analysis and the expression of HIF-1α and VEGFR mRNA by RT-PCR analysis in HeLa cells. The results are shown in Figure 2. Interestingly, **2l** did not suppress HIF-1α protein accumulation up to the concentration of 1 μM. Furthermore, RT-PCR analysis revealed that **2l** inhibited the hypoxia-induced VEGF mRNA expression in a concentration-dependent manner in the range of 0.001−1 μM. However, the HIF-1α mRNA expression levels were not affected by **2l**. These results clearly indicate that **2l** inhibits the hypoxia-induced VEGF expression without suppressing HIF-1α mRNA expression as well as HIF-1α protein accumulation.

As indenopyrazole **2l** was found to inhibit the hypoxia-induced HIF-1 transcriptional activity without suppressing HIF-1α protein accumulation, we next examined the effect of **2l** on the localization of HIF-1α protein in HeLa cells under the hypoxic conditions. Immunofluorescence analysis showed that the basal level of HIF-1α protein was low under the normoxic conditions, but the accumulated HIF-1α protein was translocated into nuclei under the hypoxic conditions (Figure 3a). Treatment with CAY10585 at 30 μM potentially suppressed HIF-1α protein accumulation and nuclear translocation under the hypoxic conditions. Interestingly, the treatment with **2l** at 1 μM did not affect the localization of HIF-1α protein, which was translocated into nuclei under the hypoxic conditions. To confirm whether indenopyrazole **2l** inhibited HIF-1α/HIF-1β heterodimerization, we performed immunoprecipitation (IP) analysis. As shown in Figure 3b, HIF-1α/HIF-1β heterodimerization was detected by immunoprecipitation using HIF-1α antibody. Whole cell lysates and immunoprecipitation products were immunoblotted with HIF-1α, HIF-1β, and tubulin antibodies. Although HIF-1α and HIF-1β proteins were

dx.doi.org/10.1021/ml3004632 | ACS Med. Chem. Lett. 2013, 4, 297−301

Table 1. Effects of Indenopyrazoles 2 on Proliferative Activities[a] of HCT116, HepG2, PC3, and HeLa Cell Lines and Hypoxia-Induced HIF-1 Transcriptional Activity in HeLa Cells[b]

Compound		Cytotoxicity IC_{50} (μM)[c]				HRE-Luc IC_{50} (μM)[c]
		HCT116	HepG2	PC3	HeLa	HeLa
	**2a**	17.5 ± 2.3	24.2 ± 6.5	>30	17.6 ± 1.8	>30 (0%)[d]
	**2b**	2.3 ± 0.01	5.2 ± 1.7	21.7 ± 4.9	1.6 ± 0.06	>30 (41%)[d]
	**2c**	<0.1	<0.1	<0.1	<0.1	>30 (28%)[d]
	**2d**	1.5 ± 1.9	15.4 ± 1.3	>30	13.4 ± 1.3	>30 (18%)[d]
	**2e**	0.57 ± 0.04	0.75 ± 0.05	3.2 ± 0.5	0.57 ± 0.02	>30 (3%)[d]
	**2f**	<0.1	0.24 ± 0.01	0.72 ± 0.09	<0.1	>30 (0%)[d]
	**2g**	5.2 ± 0.7	11.2 ± 0.2	22.3 ± 2.3	8.5 ± 0.4	>30 (28%)[d]
	**2h**	3.1 ± 0.2	9.1 ± 1.2	28.8 ± 2.2	2.6 ± 0.02	1.4 ± 0.6
	**2i**	2.2 ± 0.2	6.8 ± 1.3	>30	2.9 ± 0.1	6.1 ± 0.4
	**2j**	0.73 ± 0.03	3.8 ± 0.7	>30	1.8 ± 0.08	3.2 ± 0.6
	**2k**	6.9 ± 0.4	12.7 ± 0.9	23.7 ± 2.8	5.8 ± 0.4	0.27 ± 0.07
	**2l**	2.1 ± 0.1	3.7 ± 0.3	25.4 ± 2.1	1.8 ± 0.02	0.014 ± 0.006
	**2m**	5.9 ± 0.5	7.0 ± 0.16	6.9 ± 0.8	2.1 ± 0.03	0.034 ± 0.015
YC-1		2.2 ± 0.6	2.2 ± 0.2	>30	29.3 ± 0.7	1.5 ± 0.7
CAY10585		>30	>30	>30	>30	21 ± 0.9

[a]Cells were incubated with various concentrations (0.1−30 μM) of compounds for 72 h, and cell viability was determined by the MTT assay. [b]HeLa cells stably transfected with HRE-firefly luciferase and cytomegalovirus (CMV) promoter-renilla luciferase were incubated for 12 h with compounds under the hypoxic conditions. After the supernatant was removed, the luciferase assay was performed using the dual luciferase assay system. [c]Average values ± standard deviations of triplicate samples (n = 3). [d]Percentage (%) inhibition at 30 μM is indicated in parentheses.

detected in whole cell lysates, those proteins were also detected in immunoprecipitation products, and the inhibition of HIF-1α/HIF-1β heterodimerization by **2l** was not observed in HeLa cells under the hypoxic conditions. Together, the results indicate that indenopyrazole **2l** inhibits the hypoxia-induced HIF-1 transcriptional activity without affecting HIF-1α/HIF-1β heterodimerization in nuclei. Furthermore, we examined immunoprecipitation by anti-HIF-1α antibody to confirm

dx.doi.org/10.1021/ml3004632 | ACS Med. Chem. Lett. 2013, 4, 297−301

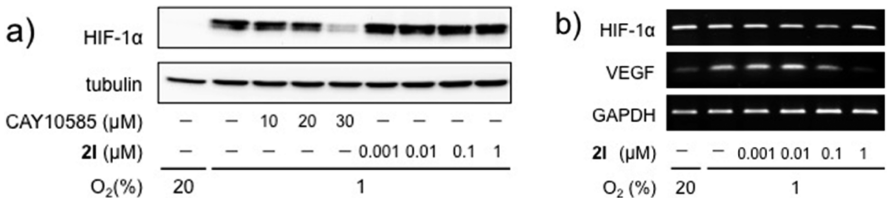


Figure 2. Effects of compound **2l** on HIF-1α protein and mRNA expression under the hypoxic conditions. HeLa cells were incubated for 4 h with compound **2l** at different concentrations under the hypoxic conditions. (a) HIF-1α protein expression was detected by immunoblot analysis with the specific antibody. CAY10585 was used as a positive control for the inhibition of HIF-1α protein expression. Tubulin was used as an internal control. (b) mRNA levels of HIF-1α, VEGF, and GAPDH were detected by RT-PCR. GAPDH was used as an internal control.

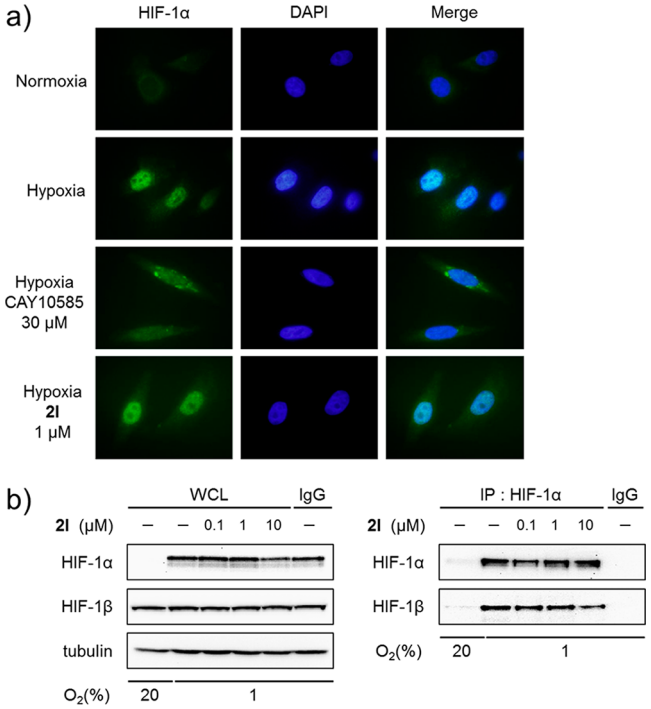


Figure 3. Effects of indenopyrazole **2l** on the localization of HIF-1α protein and HIF-1α/HIF-1β heterodimerization under the hypoxic conditions. HeLa cells were incubated for 4 h with compound **2l** under the hypoxic conditions. (a) HIF-1α protein localization was detected by immunofluorescence measurement with the specific antibody. Nuclei were visualized by staining with DAPI. (b) HIF-1α/HIF-1β heterodimerization was detected by IP using HIF-1α antibody. Whole cell lysate (WCL) and IP products were immunoblotted with HIF-1α, HIF-1β, and tubulin antibodies.

whether indenopyraziole **2l** inhibits the interaction between HIF-1α and p300, since indenopyrazole **2l** did not inhibit the formation of HIF-1α/β heterodimer complex. However, the interaction was not affected by **2l** (the data not shown).

In conclusion, we investigated the indenopyrazole framework as a new class of HIF-1α inhibitors. Indenopyrazole **2l** was found to most strongly inhibit the hypoxia-induced HIF-1α transcriptional activity (IC_{50} = 0.014 μM) among all of the known compounds having relatively simple structures, unlike manassantins. Indenopyrazole **2l** suppressed HIF-1α transcriptional activity without affecting both HIF-1α protein accumulation and HIF-1α/HIF-1β heterodimerization in nuclei under the hypoxic conditions, suggesting that **2l** probably affected the transcriptional pathway induced by the HIF-1α/HIF-1β heterodimer. The mechanism underlying the inhibition of the hypoxia-induced HIF-1α transcriptional activity by **2l** is under investigation in our laboratory.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and references. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +81-3-5992-1029. E-mail: hiroyuki.nakamura@gakushuin.ac.jp.

Present Address

†Biomedical Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Yuseong, Daejeon 305-806, Korea.

Funding

This work was partially supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Chemical Biology of Natural Products" from The Ministry of Education, Culture, Sports, Science and Technology, Japan.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Semenza, G. L. Targeting HIF-1 for Cancer Therapy. *Nat. Rev. Cancer* **2003**, *3*, 721−732.

(2) Wang, G. L.; Jiang, B. H.; Rue, E. A.; Semenza, G. L. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5510−5514.

(3) Wang, G. L.; Semenza, G. L. Purification and characterization of hypoxia-inducible Factor 1. *J. Biol. Chem.* **1995**, *270*, 1230−1237.

(4) Huang, L. E.; Gu, J.; Schau, M.; Bunn, H. F. Regulation of hypoxia-inducible factor 1α is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 7987−92.

(5) Ivan, M.; Kondo, K.; Yang, H.; Kim, W.; Valiando, J.; Ohh, M.; Salic, A.; Asara, J. M.; Lane, W. S.; Kaelin, W. G., Jr. HIFα Targeted for VHL-Mediated Destruction by Proline Hydroxylation: Implications for O_2 Sensing. *Science* **2001**, *292*, 464−468.

(6) Jiang, B.-H.; Rue, E.; Wang, G. L.; Roe, R.; Semenza, G. L. Dimerization, DNA Binding, and Transactivation Properties of Hypoxia-inducible Factor 1. *J. Biol. Chem.* **1996**, *271*, 17771−17778.

(7) 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. Overexpression of Hypoxia-inducible Factor 1α in Common Human Cancers and Their Metastases. *Cancer Res.* **1999**, *59*, 5830−5835.

(8) Wenger, R. H. Cellular adaptation to hypoxia: O_2-sensing protein hydroxylases, hypoxia-inducible transcription factors, and O_2-regulated gene expression. *FASEB J.* **2002**, *16*, 1151−1162.

(9) Talks, K. L.; Turley, H.; Gatter, K. C.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J.; Harris, A. L. The Expression and Distribution of the Hypoxia-Inducible Factors HIF-1α and HIF-2α in Normal Human

Tissues, Cancers, and Tumor-Associated Macrophages. *Am. J. Pathol.* **2000**, *157*, 411−421.
(10) Birner, P.; Schindl, M.; Obermair, A.; Breitenecker, G.; Oberhuber, G. Expression of Hypoxia-inducible Factor 1α in Epithelial Ovarian Tumors: Its Impact on Prognosis and on Response to Chemotherapy. *Clin. Cancer Res.* **2001**, *7*, 1661−1668.
(11) Birner, P.; Gatterbauer, B.; Oberhuber, G.; Schindl, M.; Rössler, K.; Prodinger, A.; Budka, H.; Hainfellner, J. A. Expression of hypoxia-inducible factor-1α in oligodendrogliomas: Its impact on prognosis and neoangiogenesis. *Cancer* **2001**, *92*, 165−171.
(12) Giatromanolaki, A.; Koukourakis, M. I.; Sivridis, E.; Turley, H.; Talks, K.; Pezzella, F.; Gatter, K. C.; Harris, A. L. Relation of hypoxia inducible factor 1α and 2α in operable non-small cell lung cancer to angiogenic/molecular profile of tumours and survival. *Br. J. Cancer* **2001**, *85*, 881−890.
(13) Zagzag, D.; Zhong, H.; Scalzitti, J. M.; Laughner, E.; Simons, J. W.; Semenza, G. L. Expression of hypoxia-inducible factor 1α in brain tumors: Association with angiogenesis, invasion, and progression. *Cancer* **2000**, *88*, 2606−2618.
(14) Birner, P.; Schindl, M.; Obermair, A.; Plank, C.; Breitenecker, G.; Oberhuber, G. Overexpression of Hypoxia-inducible Factor 1α Is a Marker for an Unfavorable Prognosis in Early-Stage Invasive Cervical Cancer. *Cancer Res.* **2000**, *60*, 4693−4696.
(15) Folkman, J. Angiogenesis: An organizing principle for drug discovery? *Nat. Rev. Drug Discovery* **2007**, *6*, 273−286.
(16) Bertout, J. A.; Patel, S. A.; Simon, M. C. The impact of O_2 availability on human cancer. *Nat. Rev. Cancer* **2008**, *8*, 967−975.
(17) Ban, H. S.; Uto, Y.; Nakamura, H. Hypoxia-inducible factor inhibitors: A survey of recent patented compounds (2004−2010). *Expert Opin. Ther. Pat.* **2011**, *21*, 131−146.
(18) Koh, M. Y.; Spivak-Kroizman, T.; Venturini, S.; Welsh, S.; Williams, R. R.; Kirkpatrick, D. L.; Powis, G. Molecular mechanisms for the activity of PX-478, an antitumor inhibitor of the hypoxia-inducible factor-1α. *Mol. Cancer Ther.* **2008**, *7*, 90−100.
(19) Le Cras, T. D.; Korfhagen, T. R.; Davidson, C.; Schmidt, S.; Fenchel, M.; Ikegami, M.; Whitsett, J. A.; Hardie, W. D. Inhibition of PI3K by PX-866 Prevents Transforming Growth Factor-α induced Pulmonary Fibrosis. *Am. J. Pathol.* **2010**, *176*, 679−686.
(20) Greenberger, L. M.; Horak, I. D.; Filpula, D.; Sapra, P.; Westergaard, M.; Frydenlund, H. F.; Albæk, C.; Schrøder, H.; Ørum, H. A RNA antagonist of hypoxia-inducible factor-1α, EZN-2968, inhibits tumor cell growth. *Mol. Cancer Ther.* **2008**, *7*, 3598−3608.
(21) Lee, K.; Lee, J. H.; Boovanahalli, S. K.; Jin, Y.; Lee, M.; Jin, X.; Kim, J. H.; Hong, Y.-S.; Lee, J. J. Aryloxyacetylamino)benzoic Acid Analogues: A New Class of Hypoxia-Inducible Factor-1 Inhibitors. *J. Med. Chem.* **2007**, *50*, 1675−1684.
(22) Won, M.-S.; Im, N.; Park, S.; Boovanahalli, S. K.; Jin, Y.; Jin, X.; Chung, K.-S.; Kang, M.; Lee, K.; Park, S.-K.; Kim, H. M.; Kwon, B. M.; Lee, J. J.; Lee, K. A novel benzimidazole analogue inhibits the hypoxia-inducible factor (HIF)-1 pathway. *Biochem. Biophys. Res. Commun.* **2009**, *385*, 16−21.
(23) Shimizu, K.; Maruyama, M.; Yasui, Y.; Minegishi, H.; Ban, H. S.; Nakamura, H. Boron-containing phenoxyacetanilide derivatives as hypoxia-inducible factor (HIF)-1α inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1453−1456.
(24) Ban, H. S.; Shimizu, K.; Minegishi, H.; Nakamura, H. Identification of HSP60 as a Primary Target of *o*-Carboranylphenoxyacetanilide, an HIF-1α Inhibitor. *J. Am. Chem. Soc.* **2010**, *132*, 11870−11871.
(25) Ban, H. S.; Uno, M.; Nakamura, H. Suppression of hypoxia-induced HIF-1α accumulation by VEGFR inhibitors: Different profiles of AAL993 versus SU5416 and KRN633. *Cancer Lett.* **2010**, *296*, 17−26.
(26) Uno, M.; Ban, H. S.; Nakamura, H. 1-[4-(*N*-Benzylamino)-phenyl]-3-phenylurea derivatives as a new class of hypoxia-inducible factor-1α inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3166−3169.
(27) Tang, J.; Luo, Z.; Zhou, G.; Song, C.; Yu, F.; Xiang, J.; Li, G. Cis-regulatory functions of overlapping HIF-1alpha/E-box/AP-1-like sequences of CD164. *BMC Mol. Biol.* **2011**, *12*, 44.
(28) Nugiel, D. A.; Vidwans, A.; Etzkorn, A.-M.; Rossi, K. A.; Benfield, P. A.; Burton, C. R.; Cox, S.; Doleniak, D.; Seitz, S. P. Synthesis and Evaluation of Indenopyrazoles as Cyclin-Dependent Kinase Inhibitors. 2. Probing the Indeno Ring Substituent Pattern. *J. Med. Chem.* **2002**, *45*, 5224−5232.
(29) Ho, C. Y.; Ludovici, D. W.; Maharoof, U. S. M.; Mei, J.; Sechler, J. L.; Tuman, R. W.; Strobel, E. D.; Andraka, L.; Yen, H.-K.; Leo, G.; Li, J.; Almond, H.; Lu, H.; DeVine, A.; Tominovich, R. M.; Baker, J.; Emanuel, S.; Gruninger, R. H.; Middleton, S. A.; Johnson, D. L.; Galemmo, R. A. (6,7-Dimethoxy-2,4-dihydroindeno[1,2-*c*]pyrazol-3-yl)phenylamines: Platelet-Derived Growth Factor Receptor Tyrosine Kinase Inhibitors with Broad Antiproliferative Activity against Tumor Cells. *J. Med. Chem.* **2005**, *48*, 8163−8173.
(30) Hossain, C. F.; Kim, Y.-P.; Baerson, S. R.; Zhang, L.; Bruick, R. K.; Mohammed, K. A.; Agarwal, A. K.; Nagle, D. G.; Zhou, Y.-D. Saururus cernuus lignans-potent small molecule inhibitors of hypoxia-inducible factor-1. *Biochem. Biophys. Res. Commun.* **2005**, *333*, 1026−1033.
(31) Hanessian, S.; Reddy, G. J.; Chahal, N. Total Synthesis and Stereochemical Confirmation of Manassantin A, B, and B1. *Org. Lett.* **2006**, *8*, 5477−5480.
(32) Kim, H.; Kasper, A. C.; Moon, E. J.; Park, Y.; Wooten, C. M.; Dewhirst, M. W.; Hong, J. Nucleophilic Addition of Organozinc Reagents to 2-Sulfonyl Cyclic Ethers: Stereoselective Synthesis of Manassantins A and B. *Org. Lett.* **2008**, *11*, 89−92.