Bioorganic & Medicinal Chemistry Letters 27 (2017) 3026–3029

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of kresoxim-methyl analogues as novel inhibitors of hypoxia-inducible factor (HIF)-1 accumulation in cancer cells

Sanghyuck Lee [a], Oh Seok Kwon [b], Chang-Soo Lee [b], Misun Won [c], Hyun Seung Ban [d,e,*], Choon Sup Ra [a,*]

[a] School of Chemistry and Biochemistry, Yeungnam University, 280 Daehak-Ro, Gyeongsan-si, Gyeongbuk 38541, Republic of Korea
[b] Hazards Monitoring Bionano Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea
[c] Personalized Genomic Medicine Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea
[d] Metabolic Regulation Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea
[e] Biomolecular Science, University of Science and Technology, Daejeon 34113, Republic of Korea

ARTICLE INFO

Article history:
Received 26 March 2017
Revised 6 May 2017
Accepted 8 May 2017
Available online 9 May 2017

Keywords:
Kresoxim analogues
Cancer
Hypoxia-inducible factor-1 inhibitor
Mitochondrial respiration

ABSTRACT

We designed and synthesized strobilurin analogues as hypoxia-inducible factor (HIF) inhibitors based on the molecular structure of kresoxim-methyl. Biological evaluation in human colorectal cancer HCT116 cells showed that most of the synthesized kresoxim-methyl analogues possessed moderate to potent inhibitory activity against hypoxia-induced HIF-1 transcriptional activation. Three candidates, compounds **11b**, **11c**, and **11d** were identified as potent inhibitors against HIF-1 activation with IC_{50} values of 0.60–0.94 μM. Under hypoxic condition, compounds **11b**, **11c**, and **11d** increased the intracellular oxygen contents, thereby attenuating the hypoxia-induced accumulation of HIF-1α protein.

Most solid tumors contain hypoxic regions within the tumor microenvironment and cancer cells adapted to hypoxia are highly resistant to chemotherapy and radiotherapy.[1] Hypoxia-inducible factor (HIF)-1α is a key regulator of the adaptation of cancer cells to hypoxia.[2] Under normoxic condition, hydroxylation of HIF-1α by prolyl hydroxylase (PHD) allows the recruitment of ubiquitin ligase von Hippel Lindau (VHL), and then HIF-1α undergoes ubiquitin and proteasome-dependent degradation.[3,4] Under hypoxic condition, stabilized HIF-1α translocates into the nucleus and dimerizes with HIF-1β. The HIF-1α/β heterodimer binds to hypoxia-response element (HRE) and induces transcription of a large number of genes implicated in tumor angiogenesis, glucose metabolism, cell survival and metastasis.[2] Therefore, HIF-1 is an attractive molecular target for cancer treatment, and various HIF-1 inhibitors have been developed.[5,6]

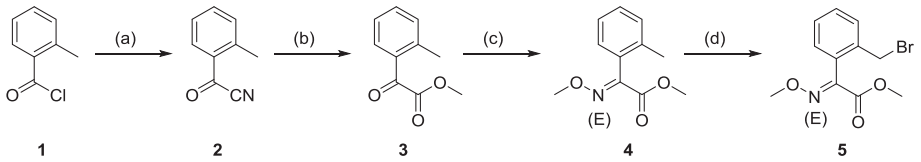
Strobilurins, natural products isolated from fungi, are commercial quinone outside inhibitor (Q_0I) fungicides.[7] The strobilurin mechanism of action is an inhibition of fungal respiration by binding to cytochrome b complex at the Q_0 site of complex III in the electron transport chain (ETC) of mitochondria.[8] Of note, in spite of a broad spectrum of fungicidal activity through the inhibition of respiration, strobilurins induce low toxicity towards mammalian cells.[8,9] Various biological activities of strobilurin derivatives have been reported including antimalarial,[10] antimicrobial,[11] and anticancer activity.[12] Until now, several synthetic strobilurins have been developed, and among them, kresoxim-methyl is one of the most widely used strobilurin fungicides.[13]

Our research has been focused on the development of a small molecule-based HIF-1 inhibitor having an inhibitory activity against mitochondrial respiration. From the results of our previous studies, the inhibition of mitochondrial respiration could lead to the degradation of the HIF-1α protein in cancer cells,[14–16] indicating that regulation of mitochondria respiration is a promising approach for treatment of hypoxic cancer. In this study, we designed and synthesized kresoxim-methyl analogues as inhibitors of HIF-1α accumulation, and evaluated their HIF inhibitory activity and anti-proliferative activity in cancer cells under hypoxic condition.

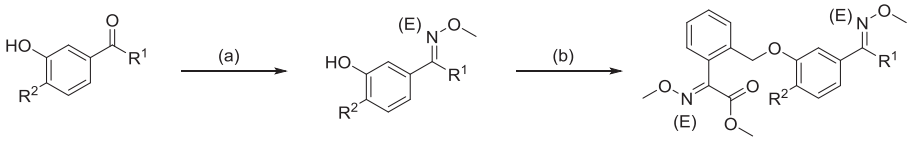
Chemical synthesis of a precursor **5**, (2-bromomethylphenyl) methoxyiminoacetic acid methyl ester, leading to kresoxim-methyl analogues was made using a well-known literature

* Corresponding authors at: Metabolic Regulation Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea (H.S. Ban) and School of Chemistry and Biochemistry, Yeungnam University, 280 Daehak-Ro, Gyeongsan-si, Gyeongbuk 38541, Republic of Korea (C.S. Ra).
E-mail addresses: banhs@kribb.re.kr (H.S. Ban), csra@yu.ac.kr (C.S. Ra).

http://dx.doi.org/10.1016/j.bmcl.2017.05.024
0960-894X/



Scheme 1. Reagents and conditions: (a) NaCN, Aliquat 336, DCM, H_2O, rt, 2 h; (b) 85% H_2SO_4, NaBr, rt, 2 h; MeOH, 65 °C, 2 h; (c) $MeONH_2$·HCl, MeOH, 65 °C, 12 h; (d) NBS, AIBN, CCl_4, 80 °C, 12 h, 70%.

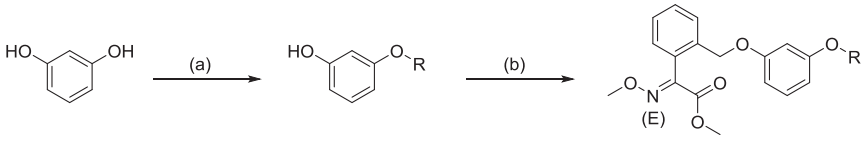


6a R^1 = H, R^2 = H
6b R^1 = CH_3, R^2 = H
6c R^1 = H, R^2 = OMe

7a (quant) R^1 = H, R^2 = H
7b (98%) R^1 = CH_3, R^2 = H
7c (92%) R^1 = H, R^2 = OMe

8a (80%) R^1 = H, R^2 = H
8b (73%) R^1 = CH_3, R^2 = H
8c (43%) R^1 = H, R^2 = OMe

Scheme 2. Reagents and conditions: (a) *o*-methoxyamine hydrochloride, MeOH, 65 °C, 12 h; (b) **5**, K_2CO_3, acetone, 60 °C, 12 h.



9

10a (81%); R = benzyl
10b (80%); R = 4-chlorobenzyl
10c (80%); R = 4-vinylbenzyl
10d (79%); R = 3,5-di(trifluoromethyl)benzyl
10e (83%); R = CH_2CN

11a (62%); R = benzyl
11b (55%); R = 4-chlorobenzyl
11c (51%); R = 4-vinylbenzyl
11d (39%); R = 3,5-di(trifluoromethyl)benzyl
11e (56%); R = CH_2CN
11f (72%); R = H

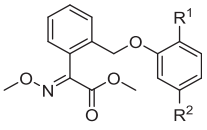
Scheme 3. Reagents and conditions: (a) R-Br, K_2CO_3, acetone, 60 °C, 12 h; (b) **5**, K_2CO_3, acetone, 60 °C, 12 h.

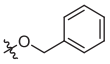
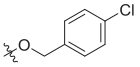
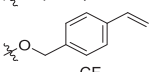
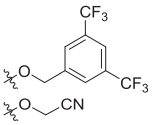
procedure.[17] In these sequences, commercially available *o*-toluoyl chloride was converted to **5** via a four-step route on a multigram scale without column chromatography (Scheme 1). Cyanation of *o*-toluoyl chloride **1** with sodium cyanide and aliquat 336 in dichloromethane and water (1:1 v/v) quantitatively yielded benzoyl cyanide **2**, which was converted to keto ester **3** by treatment with a methanolic solution of sodium bromide and sulfuric acid. Reaction of **3** with *o*-methylhydroxylamine hydrochloride gave the corresponding imine in a mixture of *E*/*Z* = 85:15, and following recrystallization from dichloromethane and hexane to afford (*E*)-methyl 2-methoxyimino-2-*o*-tolylacetate **4**. The benzylic radical bromination of **4** using the Wohl–Ziegler procedure with *N*-bromosuccinimide (NBS) yielded **5** in a good yield.

Reaction of phenolic carbonyl compounds **6** with *o*-methoxyamine hydrochloride in methanol gave their corresponding oximes **7** in good yields and resorcinol was converted into its benzylic derivatives **10** in modest to good yields. Finally oximes **7**, resorcinol (**9**) and its benzylic derivatives **10** were reacted with **5** in the presence of potassium carbonate in acetone to afford kresoxim-methyl analogues **8** and **11**, respectively, in modest yields (Schemes 2 and 3).

We next evaluated the biological activity of the synthesized kresoxim-methyl analogues. First, we examined the effects on hypoxia-induced transcriptional activation of HIF-1 using a cell-based HRE reporter gene assay in human colorectal cancer HCT116 cells; the results are shown in Table 1. Under hypoxic conditions, 2-hydroxy compound (**11f**) showed moderate inhibitory activity against HIF-1 activation. Replacement of the hydroxyl group to methyl oxime significantly increased the HIF-1 inhibitory activity (**8a** and **8b**). However, introduction of methoxy group into R^1 position (**8c**) resulted in reduction of the inhibitory activity. Furthermore, the addition of benzyloxy substituents on R^2 position (**11a**–**11d**) also showed significant inhibitory activity. Among

Table 1
Effects of kresoxim-methyl analogues on the hypoxia-induced HIF transcriptional activation and cancer cell growth.



Cmpd	R^1	R^2	HRE-Luc[a] IC_{50} (μM)[b]
11f	H	OH	9.16 ± 0.95
8a	H		3.40 ± 0.20
8b	H		4.52 ± 0.15
8c	OCH_3		14.88 ± 0.19
11a	H		1.12 ± 0.57
11b	H		0.60 ± 0.25
11c	H		0.87 ± 0.28
11d	H		0.94 ± 0.15
11e	H		>20

[a] HIF transcriptional activity was determined using HCT116 cells stably transfected with HRE-Luc.
[b] The drug concentrations required to inhibit HIF activation by 50% (IC_{50}) was determined from the semilogarithmic dose-response plots, and the results are means ± SD of triplicate samples.