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DESIGN, SYNTHESIS, AND BIOLOGICAL ACTIVITY EVALUATION OF THIAZOLIDINONES CONTAINING ALKYNYL AND ALKENYL FURANS FOR DISRUPTING PROTEIN–PROTEIN INTERACTIONS BETWEEN HIF-1 α AND p300

Norihiko Takeda, Tomoyo Taguchi, Takeshi Nakajima,* and Mitsuyoshi Azuma

Senju Laboratory of Ocular Science, Senju Pharmaceutical Co., Ltd, 1-5-5, Minatojima-Minamimachi, Chuo-ku, Kobe, Hyogo 650-0047, Japan

E-mail: t-nakajima@senju.co.jp

Abstract – Based on the structure of the potent hypoxia-inducible factor (HIF) inhibitor **1**, a novel series of furanylidene-thiazolidinones **6** were designed and synthesized using Sonogashira or Suzuki–Miyaura cross-couplings, and subsequent Knoevenagel condensation. In particular, derivatives **6aA**, **6cA**, **6dA**, **6hA**, **6iA**, and **6cC**, bearing an alkynyl or alkenyl group on the furan ring, exhibited four- to five-fold higher activities than **1**. These potent compounds will serve as leads for the development of novel small molecules for targeting the HIF-1 α /p300 complex.

Hypoxia-inducible factor 1 (HIF-1) is a transcriptional activator that functions as a master regulator of oxygen homeostasis.¹ HIF-1 consists of an oxygen-sensitive α subunit and a constitutive β subunit.² Under normoxic conditions, the α subunits on proline residues are hydroxylated by prolylhydroxylases, ubiquitinated in a von Hippel–Lindau protein-dependent manner, and undergo proteasomal degradation.³ In contrast, under hypoxic conditions, HIF-1 α cannot be hydroxylated, because of the reduced molecular oxygen concentration, and translocates to the nucleus, where it dimerizes with the HIF-1 β subunit, and recruits coactivators p300/CBP, thereby inducing upregulating transcription of various hypoxia-responsive genes. These include various genes involved in the uptake and metabolism of glucose (GLUT1 and LDH), angiogenesis (VEGFA and PDGF), mitogenesis (TGF α), and erythropoiesis (EPO) in tumor biology.⁴ HIF-1 is therefore an attractive target for the suppression of hypoxia-mediated tumor biology, including angiogenesis, because of its central role in tumor biology. There are many reports on small-molecule inhibitors of HIF signaling. Early inhibitors for HIF signaling are indirect inhibitors such as heat shock protein 90 (HSP 90) and topoisomerase I inhibitors.^{5,6} Their lack of selectivities against HIF

makes it difficult to correlate molecular and clinical responses.⁷ Kung's group suggested that disruption of transcription factor HIF-1 α interactions with transcriptional cofactors p300/CBP could potentially downregulate the expression of genes encoding VEGFA and its receptor VEGFR2, which are involved in the induction of new blood vessels (angiogenesis) in solid tumors.⁸ Small-molecule inhibitors targeting HIF-1 α -p300 interaction have therefore attracted increasing levels of interest from medicinal and organic chemists over the past decade; for example, chetomin,⁹ KCN-1,¹⁰ KST012174,¹¹ novobiocin,¹² menadione,¹³ indole derivatives,¹⁴ and thiazolidinone compounds¹⁵ have been reported as inhibitors for blocking interactions between HIF-1 α and p300.^{16,17}

As a part of our program directed toward the development of new HIF inhibitors which have more potent activity, we describe the design, synthesis, and biological activity evaluation of novel alkynylfuran- and alkenylfuran-linked thiazolidinones as inhibitors for disrupting complex formation by HIF-1 α -p300 interactions. The target molecules **2**, containing alkynyl and alkenylfurans, were designed based on the structure of **1**¹⁵ (Figure 1). We considered the 5-furanylmethylene-2-thiazolidin-4-one motif to be a privileged structure of scaffold **1**, and therefore modified the *p*-cyanophenyl group (region I) of **1** with various alkynyl and alkenyl groups. Additionally, the introduction of a hydrophilic group on the thiazolidinone nitrogen (region II) was also investigated. We report the synthesis, structure-activity relationships, and in vitro evaluation of a new series of 5-[(5-substituted 2-furanyl)methylene]-2-thioxazolidin-4-ones **2**.

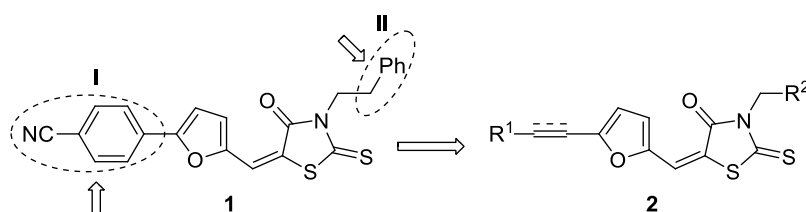
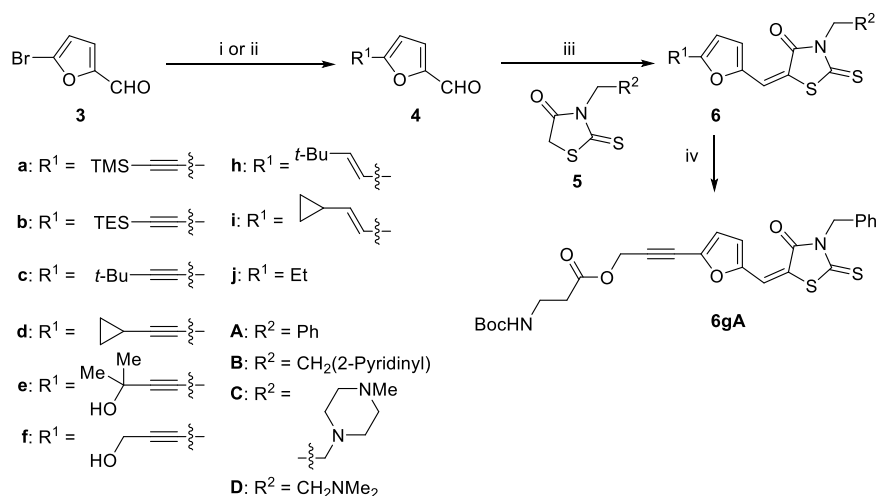


Figure 1. Structures of target molecules **2** based on compound **1**

The synthesis of target compounds **6** is summarized in Scheme 1. A novel series of thiazolidinones **6aA–fA**, containing 5-alkynylfurans, was synthesized by Sonogashira cross-coupling, followed by Knoevenagel condensation. Thiazolidinones **6hA** and **6iA**, bearing 5-alkenylfurans, were synthesized using Suzuki–Miyaura cross-coupling and subsequent Knoevenagel condensation. Ethyl-substituted **6jA** was synthesized by condensation of commercially available **4j** with **5A** in the presence of a catalytic amount of piperidine. The Knoevenagel condensation of **5B–D** led to **6cB**, **6hB**, **6cC**, and **6cD**, bearing a hydrophilic group. Finally, the synthesis of Boc- β -alanine-tethered **6gA** was achieved through condensation of **6fA** with Boc- β -alanine-OH, using EDCI and HOBt in the presence of Et₃N.



Scheme 1. Synthesis of 5-[(5-substituted 2-furanyl)methylene]-2-thioxazolidin-4-ones **6aA–jA**, **6cB**, **6hB**, **6cC**, and **6cD**. Reagents and conditions: i, terminal alkyne, PdCl₂(PPh₃)₂, CuI, PPh₃, Et₃N, THF, rt, **4b** (82%), **4c** (84%), **4d** (84%), **4e** (92%), **4f** (99%); ii, boronic acid pinacol ester, Pd(PPh₃)₄, K₂CO₃, 1,4-dioxane/H₂O, reflux, **4h** (51%), **4i** (82%); iii, 2-thioxazolidin-4-one **5A–D**, piperidine, EtOH, rt, **6aA** (41%), **6bA** (97%), **6cA** (58%), **6dA** (87%), **6eA** (92%), **6fA** (44%), **6hA** (78%), **6iA** (81%), **6jA** (72%), **6cB** (71%), **6hB** (83%), **6cC** (25%), **6cD** (33%); iv, Boc-β-alanine-OH, EDCI, HOBT, Et₃N, THF, rt, **6gA** (92%).

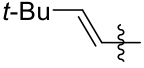
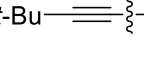
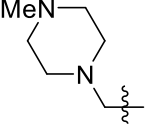
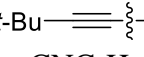
In this study, we designed and synthesized a series of 5-[(5-substituted 2-furanyl)methylene]-2-thioxazolidin-4-ones **6**. All these compounds were evaluated in a binding assay against the interactions between HIF-1α and p300 (Table 1). First, substitution of **6** in region I with various alkynyl, alkenyl, and alkyl groups was examined. Initial replacement of the aromatic ring of **1** with a trimethylsilylalkynyl group resulted in increased inhibitory activity of HIF-1α–p300, with an IC₅₀ value of 0.41 μM compared with 2.0 μM of compound **1** (entries 1 and 15). In our modified assay, the IC₅₀ value of **1** was similar to the previous report.¹⁵ Inhibition by the triethylsilylalkynyl analog **6bA** was slightly decreased by introducing a bulky group in region I, whereas the *tert*-butyl derivative **6cA** and cyclopropylalkynyl derivative **6dA** were similarly potent HIF-1α inhibitors, with IC₅₀ values of 0.48 and 0.59 μM, respectively (entries 2–4). In contrast, *gem*-dimethylhydroxypropynyl derivative **6eA**, hydroxypropynyl derivative **6fA**, and Boc-β-alanine-tethered derivative **6gA**, which introduced hydroxyl groups and Boc-β-alanine units at the propargylic position, were inefficient in improving the inhibitory activity (entries 5–7). Interestingly, the replacement of alkynyl groups in region I by *tert*-butyl and cyclopropylalkenyl groups led to an improvement in the HIF-1α inhibitory activity, compared with that of **1**. 5-[(5-Alkenyl-2-furanyl)methylene]-2-thioxazolidin-4-ones **6hA** and **6iA**, possessing *tert*-butyl and cyclopropylalkenyl groups, showed good inhibitory activities, with IC₅₀ values of 0.36 and 0.59 μM,

respectively (entries 8 and 9). The ethyl-substituted **6jA** had significantly decreased activity (entry 10). We therefore concluded that the introduction of multiple bonds with π -orbitals in region I is favorable for blocking complex formation by interactions between HIF-1 α and p300, and led to a four- to five-fold increase in inhibitory activity compared with **1**. Given the improved potency of the alkynyl and alkenyl analogs, we decided to replace the benzyl group in region II with a hydrophilic group. We selected 2-pyridinylmethyl, (4-methylpiperazinyl)methyl, and *N,N*-dimethylaminomethyl as the hydrophilic group at R², and investigated the effect of *t*-butylalkynyl and *t*-butylalkenyl substituents at R¹. Derivatives **6cB**, **6hB**, and **6cC** displayed good IC₅₀ values of 0.68, 0.62, and 0.58 μ M, respectively (entries 11-13). When the nitrogen-containing heterocyclic group at R² was replaced by its acyclic group, the inhibitory activity of **6cD** was slightly reduced (entry 14). We therefore disclosed that the introduction of multiple bonds in region I improved the inhibitory activity against HIF-1 α -p300 interactions, and that substitution of a nitrogen-containing heterocyclic group in region II can be accomplished without loss of inhibitory activity.

Table 1. Inhibition of HIF-1-p300 binding interactions by 5-[(5-substituted 2-furanyl)methylene]-2-thioxazolidin-4-ones **6**

6

entry	compounds	R ¹	R ²	IC ₅₀ (μ M)
1	6aA	TMS—	Ph	0.41
2	6bA	TES—	Ph	1.0
3	6cA	<i>t</i> -Bu—	Ph	0.48
4	6dA	—	Ph	0.59
5	6eA		Ph	1.5
6	6fA		Ph	3.2
7	6gA		Ph	2.8
8	6hA	<i>t</i> -Bu—	Ph	0.36
9	6iA	—	Ph	0.59
10	6jA	Et	Ph	17
11	6cB	<i>t</i> -Bu—	(2-Pyridinyl)CH ₂	0.68

12	6hB		(2-Pyridinyl)CH ₂	0.62
13	6cC			0.58
14	6cD		Me ₂ NCH ₂	1.4
15	1	<i>p</i> -CNC ₆ H ₄	PhCH ₂	2.0

In conclusion, we designed and synthesized a new series of alkynylfuran- and alkenylfuran-containing thiazolidinones as novel HIF inhibitors for disrupting complex formation by HIF-1 α -p300 interactions. Several potent HIF inhibitors with alkynyl and alkenyl substituents at the 5-position of the furan ring were discovered. Most of these derivatives had good inhibitory activities for blocking interactions between HIF-1 α and p300. Further detailed studies on these compounds are in progress.

EXPERIMENTAL

Melting points were obtained using a Yanaco micro melting point apparatus MP-500V and are uncorrected. ¹H and ¹³C NMR spectra were recorded on a Bruker ADVANCE III (400 MHz and 100 MHz, respectively). IR spectra were recorded using an FTIR apparatus, Shimadzu IRPrestige-21. Mass spectra were obtained by the ESI method from Shimadzu LCMS-IT-TOF. Flash column chromatography (FCC) was performed using SiliaFlash® F60 (230–400 mesh). Medium-pressure column chromatography (MCC) was performed using UNIVERSAL™ column (silica gel 40 μ m 60Å, size M 20 I.D. \times 80 mm and size 2L 26 I.D. \times 170 mm packed length). 5-[2-(Trimethylsilyl)ethynyl]-2-furancarboxaldehyde **4a**,¹⁸ 3-(phenylmethyl)-2-thioxo-4-thiazolidinone **5A**,¹⁵ 3-[(2-dimethylamino)ethyl]-2-thioxo-4-thiazolidinone **5D**,¹⁹ and 4-(5-[[4-oxo-3-(2-phenylethyl)-2-thioxo-5-thiazolidinylidene]methyl]-2-furanyl)benzotrile **1**¹⁵ were prepared according to the literature procedures.

General procedure for preparation of 5-alkynyl-2-furaldehydes **4b-f**

To a mixture of 5-bromo-2-furaldehyde **3** (1.03 g, 5.9 mmol), PdCl₂(PPh₃)₂ (84.2 mg, 0.12 mmol), PPh₃ (62.9 mg, 0.24 mmol), and CuI (66.7 mg, 0.35 mmol) were added Et₃N (5.9 mL), THF (3.0 mL), and an appropriate terminal alkyne (7.13 mmol) under an argon atmosphere. After being stirred at room temperature for 2–4 h, the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated under reduced pressure. The residue was purified by FCC to give 5-alkynyl-2-furaldehydes **4b-f** as shown in Scheme.

5-[2-(Triethylsilyl)ethynyl]-2-furancarboxaldehyde (**4b**)

Yield 82%; pale yellow oil; IR (CHCl₃) 2155, 1685 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 9.61 (br s, 1H), 7.20 (d, *J* = 3.6 Hz, 1H), 6.71 (d, *J* = 3.6 Hz, 1H), 1.04 (br t, *J* = 7.6 Hz, 9H), 0.70 (br q, *J* = 7.6 Hz, 6H); ¹³C NMR (100 MHz, CDCl₃) δ: 177.3, 152.3, 141.7, 120.9, 117.4, 101.8, 94.0, 7.4, 4.1; HRMS (ESI, *m/z*) calcd for C₁₃H₁₈O₂SiNa 257.0968 [M+Na]⁺, found 257.0974.

5-(3,3-Dimethyl-1-butyn-1-yl)-2-furancarboxaldehyde (4c)

Yield 84%; white solid; mp 91–92 °C; IR (KBr) 2220, 1689 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 9.57 (s, 1H), 7.19 (d, *J* = 3.6 Hz, 1H), 6.59 (d, *J* = 3.6 Hz, 1H), 1.33 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ: 177.0, 151.9, 142.7, 121.5, 115.8, 106.3, 69.1, 30.4, 28.3; HRMS (ESI, *m/z*) calcd for C₁₁H₁₂O₂Na 199.0730 [M+Na]⁺, found 199.0724.

5-(2-Cyclopropylethynyl)-2-furancarboxaldehyde (4d)

Yield 84%; yellow solid; mp 56–57 °C; IR (KBr) 2220, 1674 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 9.56 (s, 1H), 7.18 (d, *J* = 3.6 Hz, 1H), 6.57 (br d, *J* = 3.6 Hz, 1H), 1.53–1.46 (m, 1H), 0.98–0.89 (m, 4H); ¹³C NMR (100 MHz, CDCl₃) δ: 177.1, 152.0, 142.7, 121.5, 116.1, 102.0, 65.5, 9.2, 0.3; HRMS (ESI, *m/z*) calcd for C₁₀H₈O₂Na 183.0417 [M+Na]⁺, found 183.0425.

5-(3-Hydroxy-3-methyl-1-butyn-1-yl)-2-furancarboxaldehyde (4e)

Yield 92%; brown solid; mp 76–77 °C; IR (KBr) 3396, 2219, 1674 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 9.61 (br s, 1H), 7.21 (d, *J* = 3.6 Hz, 1H), 6.68 (d, *J* = 3.6 Hz, 1H), 2.17 (br s, 1H), 1.63 (s, 6H); ¹³C NMR (100 MHz, CDCl₃) δ: 177.4, 152.3, 141.5, 121.4, 117.0, 101.2, 71.6, 65.5, 31.0; HRMS (ESI, *m/z*) calcd for C₁₀H₁₀O₃Na 201.0522 [M+Na]⁺, found 201.0520.

5-(3-Hydroxy-1-propyn-1-yl)-2-furancarboxaldehyde (4f)

Yield 99%; brown solid; mp 94–95 °C; IR (KBr) 3444, 2216, 1659 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 9.62 (s, 1H), 7.21 (d, *J* = 3.6 Hz, 1H), 6.72 (d, *J* = 3.6 Hz, 1H), 4.53 (d, *J* = 6.0 Hz, 2H), 1.79 (br t, *J* = 6.0 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ: 177.4, 152.5, 141.2, 121.0, 117.4, 94.9, 74.9, 51.3; HRMS (ESI, *m/z*) calcd for C₈H₆O₃Na 173.0209 [M+Na]⁺, found 173.0216.

General procedure for preparation of 5-alkenyl-2-furaldehydes 4h and 4i

To a mixture of 5-bromo-2-furaldehyde **3** (2.0 mmol), boronic acid pinacol ester (2.4 mmol), Pd(PPh₃)₄ (115.5 mg, 0.10 mmol), and K₂CO₃ (414.6 mg, 3.0 mmol) were added 1,4-dioxane (6 mL) and H₂O (1.2 mL) under an argon atmosphere. After being heated at reflux for 3.5–5 h, the reaction mixture was filtered through a pad of Celite and extracted with AcOEt. The organic phase was dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by MCC to give 5-alkenyl-2-furaldehydes **4h** and **4i** as shown in Scheme.

5-[(1E)-3,3-Dimethyl-1-buten-1-yl]-2-furancarboxaldehyde (4h)

Yield 51%; pale yellow oil; IR (KBr) 1662 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ: 9.54 (s, 1H), 7.20 (d, *J* =

3.6 Hz, 1H), 6.63 (br d, $J = 16.0$ Hz, 1H), 6.36 (d, $J = 3.6$ Hz, 1H), 6.20 (d, $J = 16.0$ Hz, 1H), 1.19 (br s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 175.2, 157.7, 149.8, 146.0, 122.4, 111.5, 107.3, 32.2, 27.5; HRMS (ESI, m/z) calcd for $\text{C}_{11}\text{H}_{14}\text{O}_2\text{Na}$ 201.0886 $[\text{M}+\text{Na}]^+$, found 201.0884.

5-[(1E)-2-Cyclopropylethenyl]-2-furancarboxaldehyde (4i)

Yield 82%; pale yellow oil; IR (KBr) 1669 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 9.50 (s, 1H), 7.19 (d, $J = 3.6$ Hz, 1H), 6.34 (d, $J = 16.0$ Hz, 1H), 6.28 (d, $J = 3.6$ Hz, 1H), 6.08 (dd, $J = 16.0, 9.6$ Hz, 1H), 1.62–1.54 (m, 1H), 0.94–0.89 (m, 2H), 0.63–0.59 (m, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ : 176.5, 158.9, 151.1, 141.7, 124.4, 114.9, 108.0, 15.0, 8.2; HRMS (ESI, m/z) calcd for $\text{C}_{10}\text{H}_{10}\text{O}_2\text{Na}$ 185.0573 $[\text{M}+\text{Na}]^+$, found 185.0577.

3-[2-(2-Pyridinyl)ethyl]-2-thioxo-4-thiazolidinone (5B)

A suspension of bis(carboxymethyl)trithiocarbonate (5.0 g, 22 mmol), K_2CO_3 (1.4 g, 10 mmol), and 2-(2-aminoethyl)pyridine (2.4 mL, 20 mmol) in water was stirred at reflux for 4 h. After addition of AcOEt, the mixture was cooled to room temperature and extracted with AcOEt. The combined organic layers were dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by FCC (n -hexane /AcOEt = 3/1 \rightarrow 1/1) to give **5B** (2.4 g, 54%) as a pale yellow solid. mp 100–101 °C; IR (KBr) $1714, 1677\text{ cm}^{-1}$; ^1H NMR (400 MHz, CDCl_3) δ : 8.54 (br d, $J = 4.6$ Hz, 1H), 7.62 (td, $J = 7.8, 2.0$ Hz, 1H), 7.20 (d, $J = 7.8$ Hz, 1H), 7.16 (br dd, $J = 7.8, 4.6$ Hz, 1H), 4.39 (t, $J = 7.6$ Hz, 2H), 3.95 (s, 2H), 3.14 (t, $J = 7.6$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ : 200.9, 173.6, 157.6, 149.2, 136.9, 123.6, 122.0, 44.2, 35.3, 34.6; HRMS (ESI, m/z) calcd for $\text{C}_{10}\text{H}_{11}\text{N}_2\text{OS}_2$ 239.0307 $[\text{M}+\text{H}]^+$, found 239.0306.

3-[2-(4-Methyl-1-piperazinyl)ethyl]-2-thioxo-4-thiazolidinone (5C)

A solution of bis(carboxymethyl)trithiocarbonate (630 mg, 2.8 mmol), Et_3N (0.97 mL, 7 mmol) and 1-(2-aminoethyl)-4-methylpiperazine (463 mg, 2.6 mmol) in DME (25 mL) was stirred at reflux for 9 h. The mixture was diluted with water, and extracted with $\text{CH}_2\text{Cl}_2/\text{MeOH} = 5/1$. The combined organic layers were dried over Na_2SO_4 , and concentrated under reduced pressure. The residue was purified by FCC ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 10/1 \rightarrow 5/1$) to give **5C** (175.0 mg, 26%) as a purple solid. mp 85–86 °C; IR (KBr) 1740 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 4.13 (t, $J = 6.8$ Hz, 2H), 3.98 (s, 2H), 2.64 (t, $J = 6.8$ Hz, 2H), 2.59 (br m, 4H), 2.47 (br m, 4H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ : 201.2, 173.8, 55.0, 53.9, 52.9, 45.8, 41.7, 35.3; HRMS (ESI, m/z) calcd for $\text{C}_{10}\text{H}_{18}\text{N}_3\text{OS}_2$ 260.0886 $[\text{M}+\text{H}]^+$, found 260.0881.

General procedure for preparation of 5-[(5-substituted 2-furannyl)methylene]-2-thioxazolidin-4-ones 6

To a mixture of 5-substituted 2-furaldehyde **4** (1.0 mmol) and N -substituted 2-thioxazolidin-4-one **5** (1.0 mmol) in EtOH (8 mL) was added piperidine (0.030 mL, 0.30 mmol). After being stirred at room temperature for 24 h, the solvent was removed by an evaporator. The residue was purified by MCC to

afford 5-[(5-substituted 2-furanyl)methylene]-2-thioxazolidin-4-ones **6** as shown in Scheme.

3-Phenylmethyl-5-[[5-(2-trimethylsilyl)ethynyl-2-furanyl]methylene]-2-thioxo-4-thiazolidinone (6aA)

Yield 41%; orange solid; mp 133–134 °C; IR (KBr) 2147, 1699, 1606 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46–7.44 (m, 2H), 7.37 (s, 1H), 7.31–7.25 (m, 3H), 6.77 (d, $J = 3.6$ Hz, 1H), 6.71 (d, $J = 3.6$ Hz, 1H), 5.30 (s, 2H), 0.29 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.6, 167.9, 150.6, 141.4, 135.3, 129.3, 129.0, 128.5, 122.7, 119.7, 119.3, 117.6, 105.1, 93.7, 47.9, 0.4; HRMS (ESI, m/z) calcd for $\text{C}_{20}\text{H}_{19}\text{NO}_2\text{S}_2\text{SiNa}$ 420.0519 $[\text{M}+\text{Na}]^+$, found 420.0536.

3-Phenylmethyl-5-[[5-(2-triethylsilyl)ethynyl-2-furanyl]methylene]-2-thioxo-4-thiazolidinone (6bA)

Yield 97%; orange solid; mp 91–92 °C; IR (KBr) 2149, 1695, 1609 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46–7.44 (m, 2H), 7.38 (s, 1H), 7.33–7.27 (m, 3H), 6.77 (d, $J = 3.6$ Hz, 1H), 6.71 (d, $J = 3.6$ Hz, 1H), 5.31 (s, 2H), 1.06 (t, $J = 7.6$ Hz, 9H), 0.72 (q, $J = 7.6$ Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.2, 167.5, 150.1, 141.1, 134.9, 128.9, 128.6, 128.1, 122.2, 119.3, 118.8, 117.3, 102.7, 94.5, 47.5, 7.4, 4.1; HRMS (ESI, m/z) calcd for $\text{C}_{23}\text{H}_{25}\text{NO}_2\text{S}_2\text{SiNa}$ 462.0988 $[\text{M}+\text{Na}]^+$, found 462.1007.

5-[[5-(3,3-Dimethyl-1-butyn-1-yl)-2-furanyl]methylene]-3-phenylmethyl-2-thioxo-4-thiazolidinone (6cA)

Yield 58%; orange solid; mp 149–150 °C; IR (KBr) 2219, 1694, 1607 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46–7.44 (m, 2H), 7.37 (s, 1H), 7.33–7.27 (m, 3H), 6.77 (d, $J = 3.6$ Hz, 1H), 6.58 (d, $J = 3.6$ Hz, 1H), 5.31 (s, 2H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.3, 167.5, 149.5, 141.9, 135.0, 128.9, 128.5, 128.0, 121.1, 119.7, 117.5, 117.2, 107.1, 69.4, 47.4, 30.5, 28.4; HRMS (ESI, m/z) calcd for $\text{C}_{21}\text{H}_{19}\text{NO}_2\text{S}_2\text{Na}$ 404.0749 $[\text{M}+\text{Na}]^+$, found 404.0757.

5-[[5-(2-Cyclopropylethynyl)-2-furanyl]methylene]-3-phenylmethyl-2-thioxo-4-thiazolidinone (6dA)

Yield 87%; orange solid; mp 163–164 °C; IR (KBr) 2209, 1697, 1603 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46–7.44 (m, 2H), 7.35 (s, 1H), 7.33–7.27 (m, 3H), 6.76 (d, $J = 3.6$ Hz, 1H), 6.57 (d, $J = 3.6$ Hz, 1H), 5.30 (s, 2H), 1.56–1.50 (m, 1H), 1.00–0.90 (m, 4H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.5, 167.7, 149.7, 142.1, 135.1, 129.0, 128.7, 128.2, 121.5, 119.9, 117.6, 117.5, 102.9, 66.0, 47.6, 9.3, 0.6; HRMS (ESI, m/z) calcd for $\text{C}_{20}\text{H}_{15}\text{NO}_2\text{S}_2\text{Na}$ 388.0436 $[\text{M}+\text{Na}]^+$, found 388.0448.

5-[[5-(3-Hydroxy-3-methyl-1-butyn-1-yl)-2-furanyl]methylene]-3-phenylmethyl-2-thioxo-4-thiazolidinone (6eA)

Yield 92%; brown solid; mp 145–146 °C; IR (KBr) 3359, 2218, 1699, 1608 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46–7.44 (m, 2H), 7.37 (s, 1H), 7.35–7.27 (m, 3H), 6.77 (d, $J = 3.6$ Hz, 1H), 6.67 (d, $J = 3.6$ Hz, 1H), 5.31 (s, 2H), 2.04 (br s, 1H), 1.65 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.2, 167.5, 150.2, 140.7, 134.9, 128.9, 128.6, 128.1, 122.1, 119.3, 118.4, 117.3, 101.8, 72.1, 65.7, 47.5, 31.1; HRMS (ESI,

m/z) calcd for $C_{20}H_{17}NO_3S_2Na$ 406.0542 $[M+Na]^+$, found 406.0551.

5-([5-(3-Hydroxy-1-propyn-1-yl)-2-furanyl]methylene)-3-phenylmethyl-2-thioxo-4-thiazolidinone (6fA)

Yield 44%; brown solid; mp 174–175 °C (dec.); IR (KBr) 3312, 1698, 1603 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ : 7.46–7.44 (m, 2H), 7.38 (s, 1H), 7.34–7.27 (m, 3H), 6.78 (d, $J = 3.6$ Hz, 1H), 6.71 (d, $J = 3.6$ Hz, 1H), 5.30 (s, 2H), 4.56 (d, $J = 6.0$ Hz, 2H), 1.76 (br t, $J = 6.0$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 194.1, 167.5, 150.5, 140.3, 134.9, 128.9, 128.6, 128.1, 122.6, 119.1, 118.8, 117.1, 95.6, 75.5, 51.6, 47.5; HRMS (ESI, m/z) calcd for $C_{18}H_{13}NO_3S_2Na$ 378.0229 $[M+Na]^+$, found 378.0241.

5-([5-[(1E)-3,3-Dimethyl-1-buten-1-yl]-2-furanyl]methylene)-3-phenylmethyl-2-thioxo-4-thiazolidinone (6hA)

Yield 78%; orange solid; mp 160–161 °C; IR (KBr) 1696, 1601 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ : 7.47–7.44 (m, 2H), 7.42 (s, 1H), 7.33–7.27 (m, 3H), 6.84 (d, $J = 3.6$ Hz, 1H), 6.49 (d, $J = 16.0$ Hz, 1H), 6.36 (d, $J = 3.6$ Hz, 1H), 6.20 (d, $J = 16.0$ Hz, 1H), 5.33 (s, 2H), 1.16 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 194.4, 167.6, 158.8, 148.8, 146.6, 135.1, 128.8, 128.5, 128.0, 121.5, 119.0, 118.1, 113.1, 110.6, 47.5, 33.9, 29.3; HRMS (ESI, m/z) calcd for $C_{21}H_{21}NO_2S_2Na$ 406.0906 $[M+Na]^+$, found 406.0926.

5-([5-[(1E)-2-Cyclopropylethenyl]-2-furanyl]methylene)-3-phenylmethyl-2-thioxo-4-thiazolidinone (6iA)

Yield 81%; orange solid; mp 150–151 °C; IR (KBr) 1702, 1594 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ : 7.46–7.45 (m, 2H), 7.39 (s, 1H), 7.33–7.25 (m, 3H), 6.83 (d, $J = 3.6$ Hz, 1H), 6.32 (d, $J = 16$ Hz, 1H), 6.28 (d, $J = 3.6$ Hz, 1H), 6.02 (dd, $J = 16, 9.2$ Hz, 1H), 5.32 (s, 2H), 1.62–1.56 (m, 1H), 0.97–0.92 (m, 2H), 0.69–0.65 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 194.4, 167.6, 158.5, 148.7, 140.4, 135.1, 128.8, 128.5, 128.0, 121.8, 118.7, 118.1, 115.0, 110.0, 47.5, 15.2, 8.6; HRMS (ESI, m/z) calcd for $C_{20}H_{17}NO_2S_2Na$ 390.0593 $[M+Na]^+$, found 390.0609.

5-([5-(5-Ethyl-2-furanyl]methylene)-3-phenylmethyl-2-thioxo-4-thiazolidinone (6jA)

Yield 72%; brown powder; mp 140–141 °C; IR (KBr) 1700, 1610 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ : 7.46–7.44 (m, 2H), 7.41 (s, 1H), 7.33–7.27 (m, 3H), 6.77 (d, $J = 3.6$ Hz, 1H), 6.21 (d, $J = 3.6$ Hz, 1H), 5.31 (s, 2H), 2.75 (q, $J = 7.6$ Hz, 2H), 1.31 (t, $J = 7.6$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ : 194.7, 167.6, 164.1, 148.7, 135.1, 128.9, 128.5, 128.0, 120.6, 118.9, 118.7, 108.9, 47.4, 22.0, 11.5; HRMS (ESI, m/z) calcd for $C_{17}H_{15}NO_2S_2Na$ 352.0436 $[M+Na]^+$, found 352.0449.

5-([5-(3,3-Dimethyl-1-butyn-1-yl)-2-furanyl]methylene)-3-[2-(2-pyridinyl)ethyl]-2-thioxo-4-thiazolidinone (6cB)

Yield 71%; yellow solid; mp 163–164 °C; IR (KBr) 2213, 1701, 1608 cm^{-1} ; 1H NMR (400 MHz, $CDCl_3$) δ : 8.55 (br d, $J = 4.8$ Hz, 1H), 7.58 (br td, $J = 7.6, 1.6$ Hz, 1H), 7.33 (s, 1H), 7.17 (br d, $J = 7.6$ Hz, 1H),

7.12 (br dd, $J = 7.6, 4.8$ Hz, 1H), 6.76 (d, $J = 3.6$ Hz, 1H), 6.58 (d, $J = 3.6$ Hz, 1H), 4.52 (br t, $J = 7.6$ Hz, 2H), 3.20 (br t, $J = 7.6$ Hz, 2H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.4, 167.3, 157.9, 149.6, 141.9, 136.4, 123.4, 121.7, 121.2, 119.6, 117.3, 117.2, 107.1, 69.4, 44.1, 35.1, 30.5, 28.4; HRMS (ESI, m/z) calcd for $\text{C}_{21}\text{H}_{21}\text{N}_2\text{O}_2\text{S}_2$ 397.1039 $[\text{M}+\text{H}]^+$, found 397.1053.

5-([5-[(1E)-3,3-Dimethyl-1-buten-1-yl]-2-furanyl]methylene)-3-[2-(2-pyridinyl)ethyl]-2-thioxo-4-thiazolidinone (6hB)

Yield 83%; yellow solid; mp 157–158 °C; IR (KBr) 1700, 1597 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 8.56 (br d, $J = 4.8$ Hz, 1H), 7.59 (br td, $J = 7.6, 1.6$ Hz, 1H), 7.38 (s, 1H), 7.18 (br d, $J = 7.6$ Hz, 1H), 7.13 (br dd, $J = 7.6, 4.8$ Hz, 1H), 6.83 (d, $J = 3.6$ Hz, 1H), 6.50 (d, $J = 16$ Hz, 1H), 6.36 (d, $J = 3.6$ Hz, 1H), 6.20 (d, $J = 16$ Hz, 1H), 4.53 (br t, $J = 7.6$ Hz, 2H), 3.21 (br t, $J = 7.6$ Hz, 2H), 1.16 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.3, 167.3, 158.6, 157.9, 149.6, 148.8, 146.4, 136.4, 123.3, 121.7, 121.4, 119.0, 117.9, 113.1, 110.6, 44.0, 35.1, 33.9, 29.3; HRMS (ESI, m/z) calcd for $\text{C}_{21}\text{H}_{23}\text{N}_2\text{O}_2\text{S}_2$ 399.1195 $[\text{M}+\text{H}]^+$, found 399.1196.

5-([5-(3,3-Dimethyl-1-butyn-1-yl)-2-furanyl]methylene)-3-[2-(4-methyl-1-piperazinyl)ethyl]-2-thioxo-4-thiazolidinone (6cC)

Yield 25%; brown solid; mp 147–148 °C; IR (KBr) 2215, 1701, 1609 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.36 (s, 1H), 6.78 (d, $J = 3.6$ Hz, 1H), 6.59 (d, $J = 3.6$ Hz, 1H), 4.25 (br t, $J = 7.6$ Hz, 2H), 2.69 (br t, $J = 7.6$ Hz, 2H), 2.60 (br m, 4H), 2.45 (br m, 4H), 2.28 (s, 3H), 1.35 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.6, 167.5, 149.6, 141.9, 121.3, 119.6, 117.3, 117.2, 107.1, 69.4, 55.1, 54.1, 53.1, 45.9, 41.7, 30.5, 28.4; HRMS (ESI, m/z) calcd for $\text{C}_{21}\text{H}_{28}\text{N}_3\text{O}_2\text{S}_2$ 418.1617 $[\text{M}+\text{H}]^+$, found 418.1608.

5-([5-(3,3-Dimethyl-1-butyn-1-yl)-2-furanyl]methylene)-3-[(2-dimethylamino)ethyl]-2-thioxo-4-thiazolidinone (6cD)

Yield 33%; brown solid; mp 146–147 °C; IR (KBr) 2214, 1709, 1606 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.36 (s, 1H), 6.77 (d, $J = 3.6$ Hz, 1H), 6.58 (d, $J = 3.6$ Hz, 1H), 4.24 (br t, $J = 7.6$ Hz, 2H), 2.62 (br t, $J = 7.6$ Hz, 2H), 2.32 (s, 6H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.7, 167.5, 149.6, 141.9, 121.3, 119.6, 117.4, 117.2, 107.1, 69.4, 55.6, 45.7, 42.3, 30.5, 28.4; HRMS (ESI, m/z) calcd for $\text{C}_{18}\text{H}_{23}\text{N}_2\text{O}_2\text{S}_2$ 363.1195 $[\text{M}+\text{H}]^+$, found 363.1189.

N-[(1,1-Dimethylethoxy)carbonyl]- β -alanine 3-[5-(4-oxo-3-phenylmethyl-2-thioxo-5-thiazolidinylidene)methyl-2-furanyl]-2-propyn-1-yl ester (6gA)

To a solution of **6fA** (355.4 mg, 1.0 mmol) and Boc- β -alanine-OH (170.3 mg, 0.90 mmol) in THF (3.5 mL) were added Et_3N (0.38 mL, 2.75 mmol), HOBt (168.4 mg, 1.10 mmol), and EDCI (210.9 mg, 1.10 mmol) at room temperature. After being stirred at the same temperature for 19 h, the reaction mixture was diluted with AcOEt. The mixture was washed with 1N HCl, saturated NaHCO_3 , H_2O , and saturated NaCl.

The organic phase was dried over MgSO_4 , and concentrated in vacuo. The residue was purified by MCC (*n*-hexane/AcOEt = 1/1) to afford **6gA** (483.2 mg, 92%) as a yellow solid. mp 124–125 °C; IR (KBr) 3344, 2233, 1733, 1701, 1680, 1607 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ : 7.46–7.44 (m, 2H), 7.38 (s, 1H), 7.34–7.27 (m, 3H), 6.78 (d, $J = 3.6$ Hz, 1H), 6.76 (d, $J = 3.6$ Hz, 1H), 5.30 (s, 2H), 4.98 (s, 2H), 3.44 (br q, $J = 6.0$ Hz, 2H), 2.62 (br t, $J = 6.0$ Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 194.0, 171.6, 167.5, 155.8, 150.8, 139.8, 134.9, 128.9, 128.6, 128.1, 122.9, 119.5, 118.9, 117.0, 91.2, 79.5, 76.3, 52.5, 47.5, 36.0, 34.5, 28.4; HRMS (ESI, m/z) calcd for $\text{C}_{26}\text{H}_{26}\text{N}_2\text{O}_6\text{S}_2\text{Na}$ 549.1124 $[\text{M}+\text{Na}]^+$, found 549.1151.

In vitro assay for the inhibition of Biotin-HIF-1 α ⁷⁸⁶⁻⁸²⁶/p300³⁰²⁻⁵³¹

NUNC maxisorp 96-well plates (C96 Nunc Maxisorp plates) were incubated overnight with streptavidin (Sigma) to a final concentration of 1 $\mu\text{g}/\text{mL}$ in PBS buffer. Each well was subsequently washed three times with 300 μL of TBST buffer (10 \times Tris-buffered saline, 0.05% Tween-20). A 10 nM solution (100 μL) of biotinylated HIF-1 α ⁷⁸⁶⁻⁸²⁶ (Biologica) in TBSB (TBS with 5% BSA) was then added to each well and incubated for 1 h at 25 °C. In the last row of each plate, only the TBSB buffer was added. Each well was subsequently washed three times with 300 μL of TBST buffer. To a 2 nM solution (98 μL) of p300 in the incubation buffer (TBSB added with 0.1% Tween-20, 0.5 mM 1,4-dithioerythritol (DTT), and 10 mM ZnCl_2) were added 2 μL of the test compound dissolved in dimethyl sulfoxide (final concentrations: 0.1 to 50 μM), chetomin (final concentrations: 5 μM and 10 μM), and DMSO. The solutions were then mixed and added to each well. After incubation for 1 h at 25 °C, each well was washed three times with 300 μL of TBST buffer. Anti-6x Histidine (R&D) in 100 μL TBS with 5% BSA and 10 mM ZnCl_2 was then added to each well. After incubation for 1 h at 25 °C, each well was washed three times with 300 μL of TBST buffer and once with 300 μL of TBS buffer, and then 100 μL of detection solution (1-step Ultra TMB ELIZA) was added. The optical density (OD_{630 nm}) of the plate was measured using a Microplate reader (MTP-800) after 1 h. The inhibitory activity of the test compound was calculated from OD values. The data was shown as an average for three wells.

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