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Synthesis of novel natural product-like diaryl acetylenes as hypoxia inducible factor-1 inhibitors and antiproliferative agents†

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The selaginellin derivatives are a type of novel natural pigments with an unusual alkynyl phenol skeleton from the genus *Selaginella*. Some of these natural compounds were previously reported to show important bioactivities, including anticancer activity, cardiovascular protection and phosphodiesterase-4 inhibition. We designed and synthesized fifteen biphenyl-containing diaryl acetylene derivatives mimicking the skeleton of natural alkynyl phenols. In MTT assay in cancer cells, compounds **1c**, **2d**, **2g**, **2h**, **2i** and **2j** exhibited potent antiproliferative activity. The evaluation of Hypoxia Inducible Factor-1 (HIF-1) pathway inhibitory activity in dual luciferase assay demonstrated that most tested compounds exhibited moderate to good activities. Compounds **1a**, **2f** and **2h** displayed high HIF-1 inhibitory activities and relatively low cytotoxicity, demonstrating great potential as HIF-1 inhibitors. These results afford a new strategy for the discovery of new HIF-1 inhibitors and anti-proliferative agents from natural or synthetic diaryl acetylene derivatives.

1. Introduction

Natural products and their synthetic derivatives have served as a consistent source of valuable new drug leads for centuries, and natural products with bioactive pharmacophores are biologically validated starting points for the development of new drugs.[1] In the last decade, a type of novel natural pigment with an unusual alkynyl phenol skeleton has been successively isolated from the genus *Selaginella*, mainly from *S. tamariscina* and *S. pulvinata* which are the two qualified species listed in Chinese Pharmacopoeia and used as traditional Chinese medicines for the treatment of dysmenorrhea, abdominal mass and traumatic injury. The pharmacological investigations of the genus *Selaginella* have revealed that it has anticancer, antivirus, antioxidant and anti-inflammatory activities.[2] The phytochemical investigations of the genus *Selaginella* have led to the identification of more than forty natural alkynyl phenols hitherto, including selaginellin,[3] selaginellins A–W,[4–19] selariscinins A–D,[14,17] and selaginpulvilins A–L.[20–25] All these natural products possess unique skeletons characterized by biphenyl-containing diaryl acetylene, as shown in Fig. 1.

The biological activities of these alkynyl phenol compounds have been demonstrated to involve hypoglycemic,[14] anti-inflammatory,[26] and anti-oxidant[27] activity, cardiovascular protection,[28] anticancer effects,[9] and neuroprotective effects.[18,29,30] Selaginellin and selaginellins A, C, M, N, O, Q have been reported to be cytotoxic against U251, HeLa, MCF-7 and BGC-823 tumor cells.[9–11] Selaginpulvilins A–L, with an unprecedented 9,9-diphenyl-1-(phenylethynyl)-9*H*-fluorene skeleton, exhibit remarkable phosphodiesterase-4 inhibitory activity with IC_{50} of 0.011–1.38 μM.[20,22,25] The previous work in our laboratory on the bioactive constituents from the genus *Selaginella* revealed selaginellins A and B were moderately cytotoxic against H322 cell, and selaginellin A displayed inhibitory activity on hypoxia inducible factor-1 (HIF-1) transcription in dual luciferase reporter assay (unpublished).

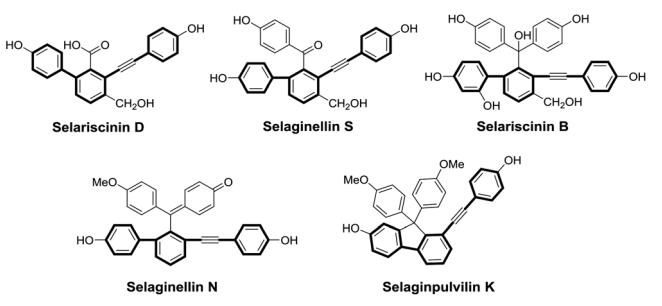

Fig. 1 Selected examples of natural diaryl acetylene compounds from the genus *Selaginella*.

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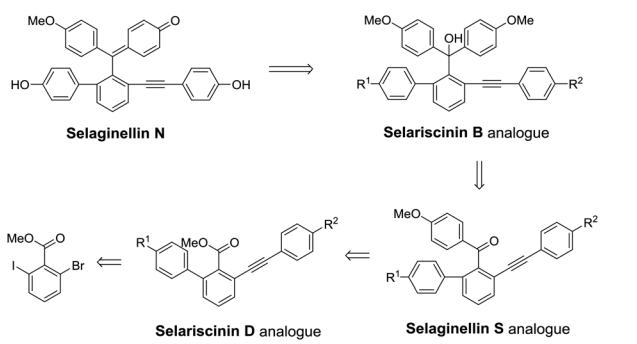
HIF-1 is a central regulator involved in detection and adaption to cellular oxygen stress through regulation of the hypoxic transcriptional program in angiogenesis, erythropoiesis, and metabolism.[31] The HIF-1 pathway is involved in many diseases, including anemia, ischemia, most tumors, and other hypoxic-ischemic diseases, making HIF-1 an attractive target for therapeutic application.[32] A considerable proportion of solid tumors are induced by hypoxia which results in overexpression of the HIF-1 pathway. Moreover, overexpression of the HIF-1 pathway has been correlated with poor prognosis, invasive tumor growth, and resistance to radiation for tumor patients.[33] Therefore, the inhibition of HIF-1 has been identified as an effective therapeutic strategy for various solid tumors.[34]

The unique skeletons associated with important bioactivities of this family of alkynyl phenols have attracted great attention of scientists from fields of organic synthesis and medicinal chemistry. Up to now, the reports on the total synthesis of natural alkynyl phenols are rare and all focus on the diarylfluorene derivatives selaginpulvilins,[21,23–25] whereas the total synthesis of selaginellins and selariscinins has not been reported yet. In our effort to develop synthetic method for selaginellins, we achieved the precursors of selaginellin N (**1a–1d**) with the skeleton of selariscinin D and selaginellin S. The difficulty in the synthesis of selaginellins with moiety of benzyl *para*-quinone methide is considered to result from the large steric hindrance of polycyclic skeleton. Considering the unique diaryl acetylene skeleton as a good privileged structure, we designed and synthesized a series of non-natural diaryl acetylene derivatives (**2a–2j**) analogous to selaginellins but with less steric hindrance. Herein we report the synthesis of this family of diaryl acetylene derivatives, their cytotoxic activity against tumor cells and HIF-1 inhibitory activity.

By analyzing the structures of selaginellin and its reported derivatives, P. F. Tu *et al.* proposed a potential biosynthetic pathway of selaginellin and its derivatives.[35] We compared the different skeletons of natural alkynyl phenols as displayed in Fig. 1 and presumed that the formation of selaginellin N analogues might undergo the intermediates of selariscinin D analogues, selaginellin S analogues and selariscinin B analogues successively. Based on this presumption, a retrosynthetic analysis of selaginellin N was established as shown in Scheme 1, where selariscinin D analogues were synthesized starting with halogenated benzoate *via* the Suzuki coupling and Sonogashira reaction.



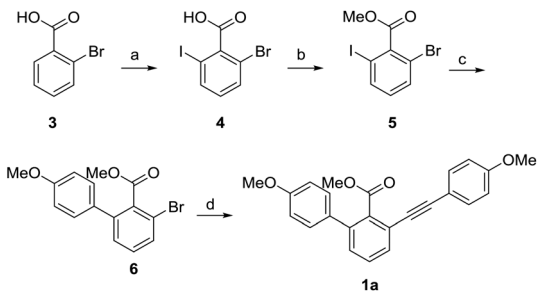
Scheme 1 Retrosynthetic analysis of diaryl acetylene compounds.

2. Results and discussion

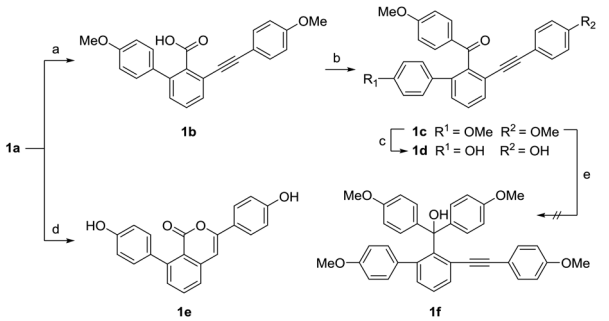
2.1. Chemistry

With the aim to synthesize selaginellin N, we first synthesized **1a** using *o*-bromobenzoic acid as the starting material *via* iodization, methyl esterification, regioselective Suzuki coupling reaction and Sonogashira reaction (Scheme 2). Using THF–H_2O as the solvent in Suzuki reaction, compound **6** was obtained under mild condition with a better yield than reported.[24] Compound **1a** has a skeleton similar to selariscinin D,[15] which exhibited stimulatory effect on glucose uptake in 3T3-L1 adipocyte cells and potent inhibitory effect against PTP1B.

We attempted to synthesize selaginellin N through the demethylation and dehydration of triphenyl methol intermediate **1f**. However, the nucleophilic addition of **1a** with 4-methoxyphenyl bromide under the presence of *n*-butyllithium produced the diphenyl ketone **1c** instead of triphenyl methol (Scheme 3). The steric hindrance of **1c** is presumably the dominated factor to prevent the further reaction to produce triphenyl methol. We tried using the more reactive acyl



Scheme 2 Synthesis of compound **1a**. Reagents and conditions: (a) $Pd(OAc)_2$, NIS, DMF, 100 °C, 12 h, 90%; (b) K_2CO_3, CH_3I, acetone, 40 °C, 5 h, 100%; (c) Na_2CO_3, 4-methoxyphenylboronic acid, $PdCl_2(PPh_3)_2$, THF/H_2O = 1 : 1, 60 °C, 8 h, 82%; (d) 4-methoxyphenylacetylene, CuI, $PdCl_2(PPh_3)_2$, PPh_3, Et_3N, DMF, 120 °C, 12 h, 48%.



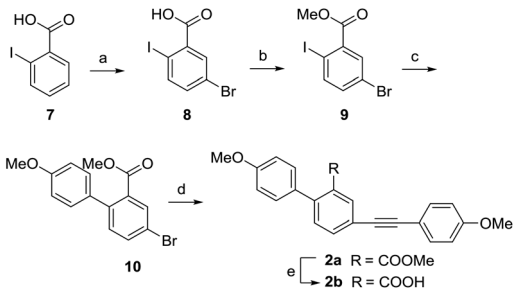
Scheme 3 Synthesis of compounds **1b–1e**. Reagents and conditions: (a) NaOH, CH_3OH/H_2O = 5 : 1, 80 °C, 8 h, 77%; (b) $SOCl_2$, CH_2Cl_2, r.t. 2 h, then *n*-BuLi, 1-bromo-4-methoxybenzene, THF, −83 °C, 2 h, 38%; (c) BBr_3, 0 °C, 30 min, 17%; (d) BBr_3, 0 °C, 2 h, 33%. (e) *n*-BuLi, 1-bromo-4-methoxybenzene, THF, −83–0 °C, 2 h, not detected.

chloride, which was prepared *via* the hydrolysis and chlorination of **1a**. As a result, **1c** was still yielded as the major product and no product of triphenyl methol was detected. Although the efforts to synthesize selaginellin N failed, the diphenyl ketones **1c** and **1d** are still important products since they bear the same skeleton with selaginellin S, which showed inhibitory effects against hepatitis B virus gene expression and replication.[16] During the demethylation of **1a** with boron tribromide, an unexpected isocoumarin **1e** was obtained as the main product.

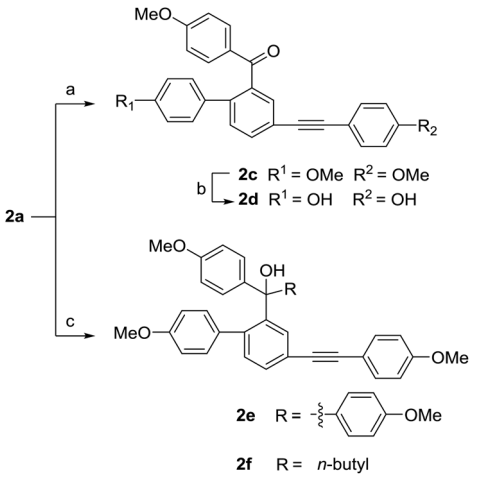
In view of the high steric hindrance in natural selaginellins, we designed and synthesized a series of selaginellin analogs (**2a–2j**) with a different attached position of 4-methoxyl phenylacetylene group (Scheme 4). Using the similar synthetic approach of **1a**, **2a** was obtained with a high yield starting with *o*-iodobenzoic acid *via* bromination, methyl esterification, regioselective Suzuki coupling and Sonogashira reaction. Compared with the yield of **1a** in Sonogashira reaction, the yield of **2a** was greatly increased due to less side reaction between *meta*-substituted carboxylic ester and alkynyl.[36]

In the next nucleophilic addition of **2a**, using 4-methoxyphenylmagnesium bromide as the nucleophilic regent yielded a diphenyl ketone product **2c**, whereas using 4-methoxyphenyl bromide under the presence of *n*-butyllithium produced the triphenyl methol **2e** along with a side product **2f** (Scheme 5). As we expected, the less steric hindrance of **2a** than that of **1a** allowed the production of triphenyl methol under the same conditions.

As Scheme 6 described, the demethylation of **2e** with BBr_3 under different conditions gave three different products. By controlling the reaction temperature at −83 °C and using small equivalents of BBr_3, we obtained two demethylation products **2g** and **2h**. When the reaction temperature was set at 0 °C, the diarylfluorene derivative **2i**, the analog of selaginpulvilin K, was yielded, probably from the Friedel–Crafts reaction under the catalysis of boron tribromide. In order to hydrolyze more methyl groups, we used excessive equivalents of BBr_3 and slowly raised the reaction temperature from −83 °C to 0 °C. As we expected, the demethylation and dehydration occurred simultaneously to produce the benzyl *para*-quinone methide derivative **2j** which resembles selaginellin N in molecular skeleton.



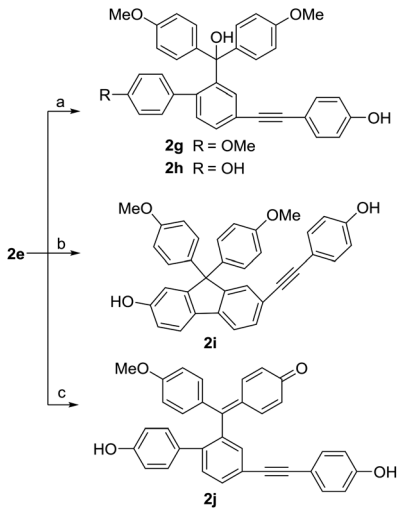
Scheme 4 Synthesis of compounds **2a** and **2b**. Reagents and conditions: (a) NBS, H_2SO_4, 60 °C, 2 h, 86%; (b) K_2CO_3, CH_3I, acetone, 40 °C, 5 h, 100%; (c) Na_2CO_3, 4-methoxyphenylboronic acid, $PdCl_2(PPh_3)_2$, $THF/H_2O = 1:1$, 60 °C, 12 h, 80%; (d) 4-methoxyphenylacetylene, CuI, $PdCl_2(PPh_3)_2$, PPh_3, Et_3N, DMF, 120 °C, 12 h, 78%; (e) NaOH, $CH_3OH/H_2O = 4:1$, 100 °C, 2 h, 99%.



Scheme 5 Synthesis of compounds **2c–2f**. Reagents and conditions: (a) 4-methoxyphenylmagnesium bromide, THF, −83 °C → r.t. 6 h, 36%; (b) BBr_3, 0 °C, 30 min, 61%; (c) *n*-BuLi, 1-bromo-4-methoxybenzene, THF, −83 °C → 0 °C, 4 h, **2e**: 82%, **2f**: 18%.

2.2. Biological results

2.2.1. MTT assay for tumor cell growth inhibition. The *in vitro* cytotoxic activity of the synthesized compounds against MCF-7, HepG2 and L-O2 cells was tested using MTT assay with cisplatin as a positive control. From the experiment data listed in Table 1, the tested compounds with different skeletons exhibited diverse cytotoxic effects. Compounds **1a–1e** showed weak cytotoxic effects with the exception of **1c** (IC_{50} = 7.18 μM against MCF-7). Among compounds **2a–2j**, **2a–2f** showed weak cytotoxic effects with the exception of **2d**, while **2g–2j** showed moderate cytotoxic effects. The cytotoxicity of **2d** was more potent than that of **1d**, suggesting that the attached position of phenylacetylene greatly influenced their cytotoxicity. Compounds **2g** and **2h** showed much more cytotoxic effects



Scheme 6 Synthetic route to compounds **2g–j**. Reagents and conditions: (a) BBr_3, −83 °C, 1 h, **2g**: 30%, **2h**: 50%; (b) BBr_3, 0 °C, 30 min, 27%; (c) BBr_3, −83 → 0 °C, 4 h, 48%.

Table 1 Anti-proliferative activity of synthesized compounds

Compounds	IC_{50}[a] (μM)		
	MCF-7	HepG2	L-O2
Cisplatin	7.24 ± 0.3	9.66 ± 0.1	3.64 ± 0.1
1a	>100	>100	>100
1b	>100	>100	>100
1c	7.18 ± 1.2	—[b]	—[b]
1d	51.16 ± 0.6	54.75 ± 1.9	64.00 ± 1.0
1e	95.57 ± 0.4	55.51 ± 1.9	92.51 ± 2.0
2a	>100	>100	>100
2b	>100	>100	>100
2c	>100	>100	>100
2d	6.49 ± 0.1	24.99 ± 0.1	7.83 ± 1.2
2e	>100	>100	>100
2f	>100	>100	>100
2g	12.98 ± 0.7	15.13 ± 0.6	16.36 ± 1.3
2h	11.90 ± 0.5	11.81 ± 0.8	10.58 ± 1.1
2i	9.23 ± 0.0	8.96 ± 0.4	10.91 ± 1.0
2j	12.10 ± 0.1	11.63 ± 0.6	11.07 ± 0.7

[a] Data are mean values of three independent experiments. Errors represent standard deviation. [b] The IC_{50} value was not determined owing to the nonlinear correlation between inhibition rates and concentrations.

than their methylated precursor **2e**, suggesting the presence of phenolic hydroxyl favors the antitumor activity of these compounds. Compounds **2h**, **2i** and **2j** showed similar cytotoxic effects although they possess different scaffolds. Interestingly, compound **2j** with a non-natural skeleton showed more cytotoxic effects than its natural analogs selaginellins, enabling **2j** to be a more promising anti-tumor agent due to its advantage of easier synthesis.

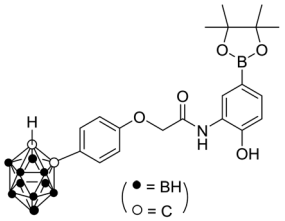
2.2.2. Cell-based reporter assays for hypoxia-inducible factor-1 (HIF-1) inhibition. Almost all solid tumors have a common feature, that is, hypoxia, resulting in overexpression of the HIF pathway in the tumor microenvironment.[37] Therefore, HIF inhibition has been suggested to be an attractive and promising therapeutic strategy for various solid tumors. To date, the mechanism underlying the anticancer effects of alkynyl phenol derivatives has not been investigated. We here explored the effect of synthesized alkynyl phenol derivatives on HIF pathway by dual luciferase reporter system. The carborane derivative **11**, an HIF-1 inhibitor reported by Nakamura,[38] was used as a reference substance. As the data listed in Table 2, all tested compounds exhibited moderate to strong HIF inhibitory activities except **2j**. Compounds **1a**, **2f** and **2h** displayed comparative HIF inhibitory activities and low cytotoxicity compared with compound **11**. Different from the rest synthetic derivatives, compound **2j** showed potent anti-proliferative effect, but little HIF inhibitory activity was determined, suggesting a distinct effect resulting from its *para*-quinone methide moiety.

3. Experimental section

3.1. Chemistry

All chemicals and solvents were commercially available, at least reagent grade. ^{1}H NMR and ^{13}C NMR spectra were recorded on

Table 2 HIF-1 inhibition in dual luciferase-reporter assay and cell growth inhibition of synthesized compounds



11

Compounds	HRE IC_{50}[a] (μM)	GI_{50}[a] (μM)
11	4.46 ± 0.2	10.80 ± 1.9
1a	5.41 ± 1.1	>100
1b	20.69 ± 2.5	>100
1c	2.36 ± 0.4	3.75 ± 0.2
1d	9.52 ± 0.4	44.81 ± 0.2
1e	13.66 ± 0.4	86.31 ± 0.5
2a	18.30 ± 1.6	>100
2b	15.57 ± 1.6	>100
2c	39.93 ± 3.1	>100
2d	4.47 ± 0.6	3.24 ± 0.1
2e	20.81 ± 0.8	>100
2f	1.89 ± 0.4	>100
2g	8.10 ± 1.4	15.48 ± 0.1
2h	2.52 ± 0.4	10.78 ± 0.3
2i	5.55 ± 1.3	7.58 ± 0.1
2j	—[b]	4.47 ± 0.5

[a] Data are mean values of three independent experiments. Errors represent standard deviation. [b] The IC_{50} value was not determined.

400 MHz NMR Spectrometer instrument (Bruker Avance II). HRMS data were detected with LTQ Orbitrap XL ETD Mass Spectrometer instrument (Thermo Scientific). The binary solvent system (A/B) of HPLC was as follows: water (A) and CH_3OH (B); gradient condition: from 60% B to 100% B in 60 min. The absorbance was detected at 254 nm. Reactions were monitored by the thin layer chromatography (TLC) from Qingdao Haiyang Chemical Co. Ltd (200 × 200 mm, 0.2–0.25 mm). Chromatograms were visualized by 254 nm UV light.

3.1.1. 2-Bromo-6-iodobenzoic acid (4). Compound **3** (6.03 g, 30.16 mmol), *N*-iodosuccinimide (8.10 g, 36 mmol), palladium acetate (673.5 mg, 3 mmol) and 50 mL DMF were added to a 100 mL sealed tube. Then the reaction mixture was stirred at 100 °C for 12 hours. The mixture was cooled down to room temperature and diluted with ethyl acetate. The solution was washed with 0.5 M HCl and water. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum and the residue was purified by silica gel column chromatography to afford compound **4** (8.84 g, 90%). White solid; mp 153–155 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.09 (dd, J = 7.8, 7.9 Hz, 1H), 7.68 (d, J = 7.9 Hz, 1H), 7.87 (d, J = 7.8 Hz, 1H). HRMS (ESI) (m/z): $[M - H]^-$ calcd for $C_7H_4BrIO_2$, 324.8367; found, 324.8369.

3.1.2. Methyl 2-bromo-6-iodobenzoate (5). Compound **4** (7.80 g, 23.93 mmol), potassium carbonate (6.66 g, 48.22 mmol), 50 mL acetone and excess methyl iodide were added to a 100 mL

round bottom flask. Then the reaction mixture was stirred at 40 °C for 5 h. Acetone was evaporated under vacuum. The residue was dissolved with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum to afford compound **5** (8.13 g, 100%). White solid; mp 87–89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.9 Hz, 1H), 7.56 (d, J = 8.1 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H), 3.99 (s, 3H). HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_8H_6BrIO_2$, 340.8669; found, 340.8675.

3.1.3. Methyl 3-bromo-4′-methoxy-[1,1′-biphenyl]-2-carboxylate (6). Compound **5** (8.13 g, 23.93 mmol), *p*-methoxyphenylboronic acid (4.36 g, 28.72 mmol), 50 mL THF and 50 mL water were added to a 250 mL three-necked flask and stirred at room temperature. Bis-triphenylphosphine palladium dichloride (842.3 mg, 1.2 mmol) was added under nitrogen atmosphere. When the temperature reached 60 °C, sodium carbonate (5.07 g, 47.86 mmol) was added. Then the reaction mixture was refluxed for 12 hours. The mixture was cooled to room temperature and diluted with ethyl acetate. The solution was washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel column chromatography to afford compound **6** (6.28 g, 82%). White solid; mp 89–91 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.64 (dd, J = 7.0, 1.9 Hz, 1H), 7.47–7.42 (t, 1H), 7.41 (d, J = 6.0 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 3.83 (s, 3H), 3.69 (s, 3H). HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{15}H_{13}BrO_3$, 321.0121; found, 321.0126.

3.1.4. Methyl 4′-methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-carboxylate (1a). Compound **6** (5.08 g, 15.88 mmol), triphenylphosphine (416.5 mg, 1.59 mmol), cuprous iodide (151.2 mg, 0.79 mmol) and 30 mL DMF were added to a 250 mL three-necked flask and stirred at room temperature. Bis-triphenylphosphine palladium dichloride (557.3 mg, 0.79 mmol) and 4-ethynylanisole (8.24 mL, 63.52 mmol) were added under nitrogen atmosphere. Then 30 mL triethylamine was added and refluxed at 120 °C for 12 hours. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The solution was washed with saturated ammonium chloride solution. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel column chromatography to afford compound **1a** (3.0 g, 48%). White solid; mp 98–99 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, J = 7.7, 1.1 Hz, 1H), 7.44 (d, J = 8.8 Hz, 2H), 7.40 (d, J = 7.7 Hz, 1H), 7.33 (d, J = 8.7 Hz, 2H), 7.32–7.30 (m, 1H), 6.94 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.57, 159.88, 159.11, 138.87, 135.18, 133.03, 131.29, 129.99, 129.58, 129.17, 120.65, 114.56, 114.16, 113.66, 92.89, 85.36, 55.34, 55.18, 52.21. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{24}H_{20}O_4$, 373.1434; found, 373.1439.

3.1.5. 4′-Methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-carboxylic acid (1b). Compound **1a** (722.0 mg, 1.94 mmol), 50 mL methanol and 10 mL 60% sodium hydroxide solution were added to a 100 mL round bottom flask. Then the reaction mixture was refluxed at 80 °C for 8 hours. The mixture was cooled to room temperature. The solvent was evaporated under vacuum. The residue was diluted with ethyl acetate and washed with dilute hydrochloric acid and saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **1b** (538.0 mg, 77%). White solid; mp 145–147 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.55 (dd, J = 7.7, 1.3 Hz, 1H), 7.52–7.45 (m, 3H), 7.45–7.41 (m, 2H), 7.38 (dd, J = 7.6, 1.3 Hz, 1H), 7.01–6.94 (m, 4H), 3.83 (s, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 169.79, 161.07, 160.45, 140.02, 137.66, 133.87, 133.11, 130.90, 130.45, 130.36, 129.97, 121.94, 115.67, 115.05, 114.66, 93.48, 86.65, 55.71, 55.56. HRMS (ESI) (m/z): $[M - H]^-$ calcd for $C_{23}H_{18}O_4$, 357.1132; found, 357.1138.

3.1.6. (4′-Methoxy-3-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)(4-methoxyphenyl)methanone (1c). Compound **1b** (365.4 mg, 1.02 mmol) was dissolved in 3 mL dichloromethane. Then the reaction mixture was stirred in an ice water bath and 0.2 mL thionyl chloride was added, followed by a drop of DMF. After being stirred for 2 hours, the solvent was evaporated under vacuum. The residue was dissolved in 2 mL THF under nitrogen atmosphere for use. Parabromoanisole (308 μL, 2.4 mmol) and 3 mL anhydrous THF were added to a 50 mL dry round bottom flask under nitrogen atmosphere. Then the reaction mixture was stirred at −83 °C and 1.7 mL *n*BuLi in *n*-hexane solution (1.6 M L^{-1}) was added. After being stirred for 30 min, the prepared acid chloride was added to the reaction mixture and stirred for 2 h. The reaction mixture was quenched by adding saturated ammonium chloride solution. The reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **1c** (170.3 mg, 38%). White solid; mp 48–49 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65 (d, J = 8.9 Hz, 2H), 7.61–7.56 (m, 2H), 7.46 (dd, J = 6.1, 2.9 Hz, 1H), 7.20 (d, J = 8.7 Hz, 2H), 7.03–6.97 (m, 4H), 6.89–6.83 (m, 4H), 3.80 (s, 3H), 3.74 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 195.25, 163.39, 159.65, 158.73, 141.09, 139.22, 132.50, 131.49, 131.41, 129.99, 129.92, 129.80, 129.33, 120.45, 114.30, 114.12, 113.78, 113.59, 93.82, 86.17, 55.55, 55.25, 55.02. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{30}H_{24}O_4$, 449.1747; found, 449.1744.

3.1.7. (4′-Hydroxy-3-((4-hydroxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)(4-methoxyphenyl)methanone (1d). Compound **1c** (150.8 mg, 0.34 mmol) was dissolved in 2 mL dry dichloromethane in a 10 mL chicken heart flask. Then the reaction mixture was stirred in an ice water bath and boron tribromide (320 μL, 3.38 mmol) was added. After being stirred for 30 min, 3 mL diethyl ether was added. The reaction mixture was stirred for 20 min and saturated sodium bicarbonate solution was slowly added. Then the reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **1d** (24.5 mg, 17%). White

solid; mp 91–93 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.73 (s, 1H), 8.39 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.57–7.51 (m, 2H), 7.42 (dd, J = 5.9, 3.0 Hz, 1H), 7.18 (d, J = 8.5 Hz, 2H), 7.01 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 6.75 (d, J = 8.5 Hz, 2H), 6.73 (d, J = 8.5 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 195.31, 163.70, 158.01, 157.02, 141.74, 140.12, 132.86, 131.63, 131.06, 130.77, 130.21, 129.81, 129.55, 128.90, 121.49, 115.47, 115.06, 113.75, 113.53, 94.09, 85.79, 55.02. HRMS (ESI) (m/z): $[M - H]^-$ calcd for $C_{28}H_{20}O_4$, 449.1289; found, 419.1293.

3.1.8. 3,8-Bis(4-hydroxyphenyl)-1*H*-isochromen-1-one (1e). Compound **1a** (186.5 mg, 0.50 mmol) was dissolved in 2 mL dry dichloromethane in a 10 mL chicken heart flask. Then the reaction mixture was stirred in an ice water bath and boron tribromide (470 μL, 4.97 mmol) was added. After being stirred for 2 hours, 3 mL diethyl ether was added. The reaction mixture was stirred for 20 min and saturated sodium bicarbonate solution was slowly added. The reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **1e** (54.9 mg, 33%). Light yellow solid; mp 297–299 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.87 (s, 1H), 8.36 (s, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.74 (t, J = 7.7 Hz, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.29 (d, J = 7.5 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.16 (s, 1H), 6.98 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H); ^{13}C NMR (101 MHz, acetone-d_6) δ 160.51, 160.13, 157.68, 154.57, 146.79, 140.77, 134.62, 133.82, 131.82, 130.85, 127.65, 126.18, 124.49, 117.92, 116.66, 115.25, 100.76. HRMS (ESI) (m/z): $[M - H]^-$ calcd for $C_{21}H_{14}O_4$, 329.0819; found, 329.0829.

3.1.9. 5-Bromo-2-iodobenzoic acid (8). Compound **7** (8.44 g, 34.03 mmol) and 60 mL H_2SO_4 were added to a 250 mL round bottom flask. NBS (4.82 g, 38.06 mmol) was then added at 60 °C and the mixture was stirred for 2 hours. Then the reaction mixture was cooled to room temperature and dropped into ice water. The mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **8** (9.54 g, 86%). White solid; mp 159–161 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, J = 2.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 8.4, 2.4 Hz, 1H). HRMS (ESI) (m/z): $[M - H]^-$ calcd for $C_7H_4BrIO_2$, 324.8367; found, 324.8369.

3.1.10. Methyl 5-bromo-2-iodobenzoate (9). Compound **8** (9.54 g, 29.4 mmol), potassium carbonate (8.13 g, 58.8 mmol), 50 mL acetone and excess methyl iodide were successively added to a 100 mL round bottom flask. Then the reaction mixture was stirred at 40 °C for 5 h. Acetone was evaporated under vacuum. The residue was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum to afford compound **9** (9.99 g, 100%). White solid; mp 48–49 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.98 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.48 (dd, J = 8.4, 2.4 Hz, 1H), 3.92 (s, 3H). HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_8H_6BrIO_2$, 340.8669; found, 340.8675.

3.1.11. Methyl 4-bromo-4′-methoxy-[1,1′-biphenyl]-2-carboxylate (10). Compound **9** (9.99 g, 29.4 mmol), *p*-methoxyphenylboronic acid (5.36 g, 35.28 mmol), 50 mL THF and 50 mL water were successively added to a 250 mL three-necked flask and stirred at room temperature. Bis-triphenylphosphine palladium dichloride (1.03 g, 1.47 mmol) was then added under nitrogen atmosphere. When the temperature reached 60 °C, sodium carbonate (6.23 g, 58.8 mmol) was added. Then the reaction mixture was refluxed for 12 hours. The mixture was cooled to room temperature and diluted with ethyl acetate. The solution was washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **10** (7.53 g, 80%). White solid; mp 76–77 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 7.86 (d, J = 2.2 Hz, 1H), 7.75 (dd, J = 8.3, 2.2 Hz, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.28–7.21 (m, 2H), 7.02–6.95 (m, 2H), 3.84 (s, 3H), 3.66 (s, 3H). HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{15}H_{13}BrO_3$, 321.0121; found, 321.0126.

3.1.12. Methyl 4′-methoxy-4-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-carboxylate (2a). Compound **10** (7.53 g, 23.535 mmol), triphenylphosphine (617.3 mg, 2.35 mmol), cuprous iodide (224.1 mg, 1.18 mmol) and 40 mL DMF were added to a 250 mL three-necked flask and stirred at room temperature. Bis-triphenylphosphine palladium dichloride (826.0 mg, 1.18 mmol) and 4-ethynylanisole (9.5 mL, 73.25 mmol) were added under nitrogen atmosphere. Then 40 mL triethylamine was added and refluxed at 120 °C for 8 hours. The reaction mixture was cooled to room temperature and diluted with ethyl acetate. The solution was washed with saturated ammonium chloride solution. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2a** (6.84 g, 78%). White solid; mp 138–139 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.83 (d, J = 1.8 Hz, 1H), 7.69 (dd, J = 8.0, 1.8 Hz, 1H), 7.57–7.50 (m, 2H), 7.46 (d, J = 8.0 Hz, 1H), 7.32–7.26 (m, 2H), 7.03–6.96 (m, 4H), 3.86 (s, 3H), 3.85 (s, 3H), 3.67 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.05, 159.75, 158.97, 140.26, 133.48, 133.09, 131.73, 131.70, 131.26, 130.81, 129.31, 121.33, 114.45, 113.86, 90.81, 86.79, 55.30, 55.14, 52.14. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{24}H_{20}O_4$, 373.1434; found, 373.1442.

3.1.13. 4′-Methoxy-4-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-carboxylic acid (2b). Compound **2a** (372.1 mg, 1 mmol), sodium hydroxide (1.0 g), 20 mL methanol and 5 mL water were added to a 100 mL round bottom flask. Then the reaction mixture was refluxed at 100 °C for 2 hours. The mixture was cooled to room temperature. The solvent was evaporated under vacuum. The residue was diluted with ethyl acetate and washed with dilute hydrochloric acid and saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2b** (354.5 mg, 99%). White solid; mp 191–193 °C; the purity of the compound

was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (s, 1H), 7.79 (d, J = 1.7 Hz, 1H), 7.66 (dd, J = 8.0, 1.8 Hz, 1H), 7.56–7.51 (m, 2H), 7.41 (d, J = 8.0 Hz, 1H), 7.33–7.27 (m, 2H), 7.03–6.96 (m, 4H), 3.81 (s, 3H), 3.80 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 169.65, 160.20, 159.38, 140.51, 133.55, 133.38, 133.31, 132.63, 131.97, 131.24, 129.92, 121.66, 114.92, 114.44, 114.21, 91.06, 87.49, 55.77, 55.61. HRMS (ESI) (m/z): $[M - H]^-$ calcd for $C_{23}H_{18}O_4$, 357.1132; found, 357.1139.

3.1.14. (4′-Methoxy-4-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)(4-methoxyphenyl)methanone (2c). Compound **2a** (744.3 mg, 2 mmol) and 5 mL of anhydrous THF were added to a 50 mL dry round bottom flask under nitrogen atmosphere. Then the reaction mixture was stirred at −83 °C and 2.5 mL of *p*-methoxyphenylmagnesium bromide (1.0 M L^{-1} in THF) was added. The temperature was naturally raised to room temperature. After being stirred for 6 hours, the reaction mixture was quenched by adding saturated ammonium chloride solution. The reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2c** (319.2 mg, 36%). White solid; mp 153–155 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (dd, J = 8.0, 1.8 Hz, 1H), 7.64–7.59 (m, 2H), 7.52 (m, J = 4.8, 3.9 Hz, 4H), 7.23–7.16 (m, 2H), 7.01–6.97 (m, 2H), 6.97–6.93 (m, 2H), 6.88–6.82 (m, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 195.37, 163.36, 159.70, 158.83, 139.10, 139.06, 133.05, 132.40, 131.97, 131.31, 130.38, 130.28, 129.68, 129.32, 121.13, 114.43, 113.99, 113.95, 90.74, 87.15, 55.56, 55.29, 55.06. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{30}H_{24}O_4$, 449.1747; found, 449.1741.

3.1.15. (4′-Hydroxy-4-((4-hydroxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)(4-methoxyphenyl)methanone (2d). Compound **2c** (319.2 mg, 0.71 mmol) was dissolved in 5 mL dry dichloromethane added in a 50 mL round bottom flask. Then the reaction mixture was stirred in an ice water bath and boron tribromide (340 μL, 3.6 mmol) was added. After being stirred for 30 min, 10 mL diethyl ether was added. The reaction mixture was stirred for 20 min and saturated sodium bicarbonate solution was slowly added. Then the reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2d** (180.7 mg, 61%). White solid; mp 229–231 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 9.54 (s, 1H), 7.68 (dd, J = 8.0, 1.7 Hz, 1H), 7.58 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.1 Hz, 1H), 7.46 (d, J = 1.6 Hz, 1H), 7.39 (d, J = 8.6 Hz, 2H), 7.08 (d, J = 8.5 Hz, 2H), 6.93 (d, J = 8.9 Hz, 2H), 6.80 (d, J = 8.6 Hz, 2H), 6.65 (d, J = 8.5 Hz, 2H), 3.79 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 195.60, 163.25, 158.23, 157.08, 139.30, 138.94, 133.13, 132.31, 131.88, 130.34, 130.03, 129.75, 129.70, 129.35, 121.12, 115.77, 115.34, 113.89, 112.24, 91.16, 86.60, 55.51. HRMS (ESI) (m/z): $[M + H]^+$ calcd for $C_{28}H_{20}O_4$, 421.1434; found, 421.1432.

3.1.16. (4′-Methoxy-4-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)bis(4-methoxyphenyl)methanol (2e); 1-(4′-methoxy-4-((4-methoxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)-1-(4-methoxyphenyl)pentan-1-ol (2f). Parabromoanisole (2.03 g, 10.84 mmol) and 5 mL anhydrous THF were added to a 100 mL dry round bottom flask under nitrogen atmosphere. Then the reaction mixture was stirred at −83 °C and 7.45 mL *n*-BuLi in *n*-hexane solution (1.6 M L^{-1}) was added. After being stirred for 1 hour, 15 mL solution of compound **2a** (2.02 g, 5.42 mmol) in THF was added to the reaction mixture. The temperature was naturally raised to 0 °C. The reaction mixture was stirred for 4 hours and quenched by adding saturated ammonium chloride solution. The reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2e** (2.47 g, 82%) and compound **2f** (493.9 mg, 18%). The purity of the two compounds was detected by analytical HPLC to be over 95%. Compound **2e**: pink solid; mp 179–181 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (d, J = 1.5 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 7.34 (dd, J = 7.7, 1.4 Hz, 1H), 6.98 (d, J = 8.6 Hz, 2H), 6.85–6.89 (m, 3H), 6.68 (d, J = 8.7 Hz, 2H), 6.61 (d, J = 8.4 Hz, 2H), 6.44 (d, J = 8.2 Hz, 2H), 4.79 (s, 1H), 3.81 (s, 3H), 3.72 (s, 6H), 1.99–1.84 (m, 2H), 1.30–1.11 (m, 3H), 1.03–0.88 (m, 1H), 0.79 (t, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.51, 157.76, 157.67, 145.84, 140.71, 140.39, 134.13, 132.95, 132.33, 129.64, 128.64, 127.71, 120.74, 114.42, 114.37, 112.71, 112.17, 89.16, 88.54, 75.70, 55.28, 55.05, 54.98, 38.47, 25.73, 22.60, 14.11. ESI MS (m/z): $[M - OH]^+$ calcd for $C_{34}H_{34}O_4$, 489.24; found, 489.36. Compound **2f**: white solid; mp 113–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48–7.41 (m, 3H), 7.17 (d, J = 1.7 Hz, 1H), 7.03 (d, J = 7.8 Hz, 1H), 6.95–6.99 (m, 6H), 6.77 (d, J = 8.8 Hz, 4H), 6.64 (d, J = 8.7 Hz, 2H), 6.55 (d, J = 8.7 Hz, 2H), 5.76 (s, 1H), 3.78 (s, 3H), 3.72 (s, 6H), 3.67 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.54, 157.69, 157.31, 146.30, 142.35, 139.86, 134.99, 133.21, 132.94, 131.57, 130.02, 129.47, 128.87, 120.18, 114.36, 114.04, 112.66, 111.94, 89.35, 88.05, 80.72, 55.26, 54.99, 54.90. HRMS (ESI) (m/z): $[M - OH]^+$ calcd for $C_{37}H_{32}O_5$, 539.2222; found, 539.2209.

3.1.17. 4-((2-(Hydroxybis(4-methoxyphenyl)methyl)-4′-methoxy-[1,1′-biphenyl]-4-yl)ethynyl)phenol (2g); 2′-(hydroxybis(4-methoxyphenyl)methyl)-4′-((4-hydroxyphenyl)ethynyl)-[1,1′-biphenyl]-4-ol (2h). Compound **2e** (139.1 mg, 0.25 mmol) was dissolved in 2 mL dry dichloromethane in a 10 mL chicken heart flask. Then the reaction mixture was stirred at −83 °C and boron tribromide (140 μL, 1.4 8 mmol) was added. After being stirred for 1 hour, 5 mL diethyl ether was added. The reaction mixture was stirred for 20 min and saturated sodium bicarbonate solution was slowly added. Then the reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2g** (40.6 mg, 30%) and compound **2h** (66.0 mg, 50%). The purity of the two compounds was detected by analytical HPLC to be over 95%. Compound **2g**: pink solid; mp 199–201 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.79 (s, 1H), 7.44 (dd, J = 7.8, 1.5 Hz, 1H),

7.34 (d, J = 8.5 Hz, 2H), 7.09–7.11 (m, 2H), 7.06 (d, J = 8.8 Hz, 4H), 6.82–6.86 (m, 6H), 6.68–6.74 (m, 4H), 3.87 (s, 1H), 3.79 (s, 6H), 3.76 (s, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ 158.67, 158.65, 157.93, 146.40, 141.46, 139.97, 134.23, 133.13, 133.02, 132.11, 130.40, 129.59, 129.10, 121.43, 115.60, 113.74, 112.80, 89.68, 87.29, 82.03, 54.58. HRMS (ESI) (m/z): [M − H]$^-$ calcd for $C_{36}H_{30}O_5$, 541.2020; found, 541.2011. Compound **2h**: pink solid; mp 119–121 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.76 (s, 1H), 8.37 (s, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 8.5 Hz, 2H), 7.05–7.11 (m, 6H), 6.83–6.86 (m, 6H), 6.64 (q, J = 8.7 Hz, 4H), 3.78 (s, 6H), 3.75 (s, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 158.70, 157.90, 156.46, 146.40, 141.52, 140.03, 133.17, 133.01, 132.87, 132.13, 130.50, 129.59, 129.09, 121.35, 115.60, 114.43, 113.77, 112.82, 89.65, 87.32, 82.17, 54.59. HRMS (ESI) (m/z): [M − H]$^-$ calcd for $C_{35}H_{28}O_5$, 527.1864; found, 527.1866.

3.1.18. 7-((4-Hydroxyphenyl)ethynyl)-9,9-bis(4-methoxyphenyl)-9*H*-fluoren-2-ol (2i). Compound **2e** (155.7 mg, 0.28 mmol) was dissolved in 2 mL dry dichloromethane in a 10 mL chicken heart flask. Then the reaction mixture was stirred in an ice water bath and boron tribromide (110 μL, 1.16 mmol) was added. After being stirred for 30 min, 5 mL diethyl ether was added. The reaction mixture was stirred for 20 min and saturated sodium bicarbonate solution was slowly added. Then the reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2i** (38.8 mg, 27%). White solid; mp 125–127 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.75 (s, 1H), 8.53 (s, 1H), 7.71–7.75 (m, 2H), 7.46 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.8 Hz, 4H), 6.91–6.80 (m, 8H), 3.74 (s, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 158.61, 157.97, 157.77, 154.32, 151.69, 140.25, 137.69, 132.94, 130.90, 130.68, 129.01, 128.52, 121.54, 121.04, 119.21, 115.58, 115.05, 114.12, 113.57, 113.02, 89.44, 88.09, 63.93, 54.56. HRMS (ESI) (m/z): [M − H]$^-$ calcd for $C_{35}H_{26}O_4$, 509.1758; found, 509.1753.

3.1.19. 4-((4′-Hydroxy-4-((4-hydroxyphenyl)ethynyl)-[1,1′-biphenyl]-2-yl)(4-methoxyphenyl)methylene)cyclohexa-2,5-dien-1-one (2j). Compound **2e** (2.23 g, 4.0 mmol) was dissolved in 40 mL dry dichloromethane in a 250 mL round bottom flask. Then the reaction mixture was stirred at −83 °C and boron tribromide (7.6 mL, 80.39 mmol) was added. The temperature was slowly raised to 0 °C. After being stirred for 4 hours, 30 mL diethyl ether was added. The reaction mixture was stirred for 20 min and saturated sodium bicarbonate solution was slowly added. Then the reaction mixture was diluted with ethyl acetate and washed with saturated brine. The combined extract was dried by anhydrous sodium sulfate. Ethyl acetate was evaporated under vacuum. The residue was purified by silica gel chromatography to afford compound **2j** (943.4 mg, 48%). Red solid; mp 191–193 °C; the purity of the compound was detected by analytical HPLC to be over 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.01 (s, 1H), 9.49 (s, 1H), 7.66 (dd, J = 8.0, 1.6 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.41–7.33 (m, 4H), 7.22 (dd, J = 9.9, 2.6 Hz, 1H), 6.85–6.94 (m, 6H), 6.81 (d, J = 8.6 Hz, 2H), 6.59 (d, J = 8.5 Hz, 2H), 6.33 (dd, J = 10.0, 1.9 Hz, 1H), 6.29 (dd, J = 9.9, 2.0 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 185.55, 160.71, 159.69, 158.27, 156.77, 142.07, 139.27, 138.91, 138.67, 134.37, 133.29, 133.09, 132.20, 130.86, 130.66, 129.96, 129.59, 128.95, 128.06, 127.90, 121.07, 115.78, 115.01, 113.62, 112.16, 91.18, 86.62, 55.36. HRMS (ESI) (m/z): [M − H]$^-$ calcd for $C_{34}H_{24}O_4$, 495.1602; found, 495.1618.

3.2. Biological assays

3.2.1. MTT assay for cell growth inhibition. The inhibition of compounds **1a–1e** and **2a–2j** against MCF-7, HepG2, HeLa and L-O2 cells were evaluated using a standard MTT-based colorimetric assay. Three thousand corresponding cells per well were seeded into 96-well plates and incubated at 37 °C, 5% CO_2 for 24 h. Then 100 μL drug-containing medium with a series of concentration were dispensed into wells to maintain the final concentration as 100, 50, 25, 12.5, 6.25 and 3.125 μM, respectively. Each concentration was in triplicate. After 48 hours of incubation, cell survival was determined by the addition of 20 μL MTT (Sigma-Aldrich, St. Louis, USA) work solution (5 mg mL^{-1} MTT dissolved in phosphate buffer solution). After post-incubation at 37 °C for 4 hours, the medium was discarded followed by adding 150 μL DMSO (Sigma-Aldrich, St. Louis, USA). The plates were then vortexed for 10 min for complete dissolution. The optical absorbance was measured at 570 nm. The data represented the mean of three independent experiments in triplicate and were expressed as mean ± SD. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.

3.2.2. Cell-based reporter assays for hypoxia inducible factor-1 (HIF-1) inhibition. HeLa cells expressing HRE-dependent firefly luciferase reporter construct (HRE-Luc) and constitutively expressing CMV-driven Renilla luciferase reporter with SureFECT transfection reagent were established with Cignal™ Lenti Reporter (SABiosciences, Frederick, MD) according to the manufacturer's instructions. The consensus sequence of HRE was 5′-TACGTGCT-3′ from the erythropoietin gene. Cells stably expressing the HRE-reporter gene were selected with puromycin. The cells were incubated for 12 hours with or without drugs under the normoxic or hypoxic condition (1% O_2). After incubation, the luciferase assay was performed using a Luciferase Assay System (Promega, Madison, WI) according to the manufacturer's instructions. The drug concentration required to inhibit the relative light units by 50% (IC_{50}) was determined from semi-logarithmic dose–response plots, and the results represent means ± SD of triplicate samples.

4. Conclusion

In conclusion, we designed and synthesized a class of natural product-like biphenyl-containing diaryl acetylenes mimicking natural alkynyl phenols from the genus *Selaginella*. In MTT assay in cancer cells, compounds **1c**, **2d**, **2g**, **2h**, **2i** and **2j** exhibited potent cytotoxic activity. The evaluation of HIF-1 inhibitory activity demonstrated that all tested compounds exhibited moderate to good activities except **2j**. Compounds **1a**,

2f and **2h** displayed high HIF-1 inhibitory activities and relatively low cytotoxicity, demonstrating their great potential as HIF-1 inhibitors. These results afford us a new strategy for the discovery of new HIF-1 inhibitors and anti-proliferative agents from natural or synthetic diaryl acetylene derivatives.

Conflicts of interest

There are no conflicts to declare.

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