Design, Synthesis, and Anti-Proliferative Evaluation of [1,1′-biphenyl]-4-ols as Inhibitor of HUVEC Migration and Tube Formation

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Abstract: Allylated biphenol neolignans contain a variety of chemopreventive entities that have been used as anti-tumor drug leads. Herein, 37 allylated biphenols were evaluated for anti-proliferative activity by the MTT assay and inhibitory effect on the migration and tube formation of HUVECs featuring anti-angiogenic properties. 3-(2-Methylbut-3-en-2-yl)-3′,5′-bis(trifluoromethyl)-[1,1′-biphenyl]-4-ol (5c) exerted an inhibitory effect on HUVECs compared to honokiol (IC50 = 47.0 vs. 52.6 μM) and showed significant blocking effects on the proliferation of C26, Hela, K562, A549, and HepG2 (IC50 = 15.0, 25.0, 21.2, 29.5, and 13.0 μM, respectively), superior to those of honokiol (IC50 = 65.1, 62.0, 42.0, 75.0, and 55.4 μM, respectively). Importantly, compound 5c inhibited the migration and capillary-like tube formation of HUVECs in vitro.

Keywords: biphenol; endothelial cell; anti-proliferative; migration; tube formation
1. Introduction

Angiogenesis is a complicated multistep process involving endothelial cell (EC) activation, invasion, migration, proliferation, tube formation, and finally capillary network formation in several solid tumors and haematological malignancies [1]. Tumor growth depends on the recruitment of new blood vessels from pre-existing vasculature. Without the development and progression of new blood vessels, tumors cannot deteriorate beyond a critical size or metastasize to other organs [2–4].

Endothelial cells play crucial roles in a series of physiological processes (e.g., wound healing, reproduction, and embryonic development) and pathophysiological events (e.g., solid tumor growth, psoriasis, and diabetic retinopathy). The well-established target of anti-angiogenesis is genetically stable, non-transformed ECs, which are less prone to acquire drug resistance [5,6]. When tumor cells secrete pro-angiogenic growth factors that bind to receptors on dormant ECs, leading to ECs activation, stimulation, vasodilatation and an up-regulation of vessel permeability, the activated ECs rapidly detach from the extracellular matrix and basement membrane by secreting proteases (matrix metalloproteinases). Subsequently, they migrate and proliferate to sprout and self-assemble into new branches from the pre-existing vasculatures [7–9]. Therefore, suppression of EC activation, invasion, migration, proliferation, tube formation, and finally capillary network formation seem to be an effective and relevant strategy for blocking tumor progression and cancer development. Up to now, more than 80 anti-angiogenic drugs are currently in clinical trials. Notably, many angiogenesis inhibitors have been discovered in natural resources, such as fungi, mushrooms, shark muscle and cartilage, sea corals, green tea, ginseng, and garlic by screening of EC cultures [10].

Biphenols are widely found in naturally products, including lignans, flavonoids, tannins, together with coumarins, peptides, glycopeptides, etc. [11]. Natural biphenol products contained a range of chemotherapeutic and chemo-preventive entities that have the capacity of preventing and inhibiting the development of malignancies [12–14]. However, only a few studies have focused on the structural modification and structure-activity relationship (SAR) of biphenols targeting angiogenesis or cancer [15,16]. Disconnections and chemical synthesis have been undertaken to develop potent allylated biphenols to improve biological activity or clarify the SAR in our previous study [17].

In this study, 37 allylated biphenols were designed, synthesized, and subsequently screened. Among our synthetic compounds, 3-(2-methylbut-3-en-2-yl)-3′,5′-bis(trifluoromethyl)-[1,1′-biphenyl]-4-ol (5c) exhibited a medium inhibitory effect on human umbilical vein endothelial cells (HUVECs; IC_{50} = 40.0 μM) in contrast to honokiol (IC_{50} = 57.0 μM) and blocked the proliferation of C26 (murine colon adenocarcinoma, IC_{50} = 15.0 μM), Hela (human cervical carcinoma, IC_{50} = 25.0 μM), K562 (human erythromyeloblastoid leukemia; IC_{50} = 21.2 μM), A549 (human lung carcinoma; IC_{50} = 29.5 μM), and HepG2 (human hepatocellular liver carcinoma; IC_{50} = 13.0 μM) showing superior activity to honokiol (IC_{50} = 65.1, 62.0, 42.0, 75.0, and 55.4 μM, respectively). Importantly, 5c showed much more potent inhibitory potency against the migration and tube formation of HUVECs than those of honokiol. The anti-proliferative activity of allylated biphenols led to the establishment of a structure-activity relationship. In contrast to our previous report, compound 5c exhibited comparable inhibitory activity to honokiol, but less potent in vitro inhibitory potency than 3,5′-diformalhonokiol (compound 8c in reference [17]) against HUVECs, A549, and, HepG2. Although compound 5c could significantly suppress HUVEC migration and tube formation, 5c was not found to prevent the
newly-grown segmental vessels from the dorsal aorta of zebrafish, and inappropriate vascularisation in
the transgenic zebrafish screening model.

2. Results and Discussion

2.1. Chemistry

As depicted in Scheme 1, 1-allyloxy-4-bromobenzene (2) was prepared in a satisfactory yield (95%) by a convenient procedure starting from commercially available p-bromophenol and employing 1.1 equiv of allyl bromide in the presence of anhydrous K₂CO₃ (1.3 equiv) as base and acetone as solvent. The intermediates 3a–t were obtained through Suzuki-Miyaura reactions [17,18]. Two cross-coupling methods were developed for the condensation of appropriate arylboronic acids with 2 under palladium catalysis under a N₂ atmosphere. Method A involved the use of Pd(OAc)₂ as a cross-coupling catalyst, PPh₃ as a reducing agent and K₂CO₃ (2.0 M) as a base in isopropanol as solvent. The intermediates 3a–i was obtained by Method A in 55–80% yields. Another coupling method (used for 3j–t) was carried out by using Pd(PPh₃)₄ as a catalyst, 2.0 M K₃PO₄·3H₂O as a base and DMF as solvent. The intermediates 3j–t were obtained by Method B in much better yields (75–90%).

Scheme 1. Synthesis of compounds 4a–t and 5a–c.

Reagents and conditions: (a) Br-CH₂CH=CH₂, acetone, reflux, 5 h; (b) Method A: Pd(OAc)₂, PPh₃, K₂CO₃, isopropanol, H₂O, N₂ atmosphere, 90 °C; Method B: Pd(PPh₃)₄, DMF, H₂O, K₃PO₄·3H₂O, N₂ atmosphere, 100 °C; (c) N,N-diethylaniline, reflux, overnight.

The O-allylation of 3 with 3-bromoprop-1-ene or 1-bromo-3-methylbut-2-ene was performed in a good yield in the presence of anhydrous K₂CO₃. Next, the corresponding compounds 4a–t and 5a–c were synthesized through the Claisen rearrangement, which belongs to the [3,3]-sigmatropic concerted rearrangement category, in N,N-diethylaniline (boiling point = 216 °C) as solvent [19,20]. Moreover, the other allylated biphenols 6a–j and 7a–d were prepared following similar synthetic methods (Scheme 2).
Scheme 2. Synthesis of compounds 6a–j, and 7a–d.

Reagents and conditions: (a) CH$_3$I or Br-CH$_2$CH=CH$_2$, acetone, reflux, overnight; (b) N,N-diethylaniline, reflux, overnight.

2.2. Anti-Proliferative Activity and SAR Study

In order to find new potential angiogenic inhibitors, 37 allylated biphenols were primarily investigated for anti-proliferative activity on C26 and Hela tumor cells by the MTT assay and these results are shown in Table 1. In addition, predicted octanol/water log P (Clog P, miLog P, and Xlog P) were calculated using the ChemDraw software, Molinspiration online service and XlogP3 online service, respectively, to provide a measure of lipophilicity. Honokiol, a potent anti-angiogenic and anti-tumor drug lead, was selected as a positive control.

As for the twenty 3-allyl-[1,1′-biphenyl]-4-ols 4a–t, the preliminary IC$_{50}$ values indicated that only six substituted derivatives (i.e., 3′-SCH$_3$ in 4c, 2′-CF$_3$ in 4j, 2′,4′-diCF$_3$ in 4m, 3′,5′-diCF$_3$ in 4n, 2′-Cl,4′-CF$_3$ in 4o, and 3′-NO$_2$ in 4t) exhibited comparable or superior activity to honokiol (65.1 μM for C26, and 62.0 μM for Hela) on anti-proliferative activity, with 4n being the most efficient (34.5 μM for C26, and 36.0 μM for Hela). Importantly, 4n achieved an approximately 2.0-fold improvement in inhibitory activities against C26 and Hela compared to those of honokiol. With respect to all the O-alkylated biphenyls (compounds 6a–j), the introduction of a methyl or allyl group did not provide any advantage for the anti-proliferative effects. Although O-methylation of 4g (3′,4′,5′-triOCH$_3$) led to the corresponding derivative 6c, which showed weak inhibitory activity (76.0 μM for C26), it still was inactive in blocking the proliferation of Hela cells (>100.0 μM). Inspection of the structural features of 4a–t, and 6a–j and activity indicated that the phenolic hydroxyl group at the C-4 position of the biphenyl motif was very essential for anti-proliferation activity because the O-alkylated compounds were inactive (e.g., 4n vs. 6f; 4t vs. 6i) and the number of the trifluoromethyl substituents potentially affected the inhibitory potency (e.g., one CF$_3$ in 4j vs. two CF$_3$ in 4n).
Table 1. IC\textsubscript{50} values against C26 and Hela cells and calculated properties of allylated biphenols.

| Compd | MW \textsuperscript{a} | cLogP \textsuperscript{b} | miLogP \textsuperscript{c} | xLogP \textsuperscript{d} | IC\textsubscript{50} (\textmu M) |
|--------|-----------------|-----------------|-----------------|-----------------|-----------------|
|        | C26 | Hela | K562 | C26 | Hela | K562 |
| Honokiol | 266.33 | 5.03 | 5.01 | 4.98 | 65.1 | 62.0 | 42.0 |
| 4a | 210.10 | 4.37 | 4.47 | 4.26 | 69.0 | 96.0 | NI |
| 4b | 240.12 | 4.25 | 4.51 | 4.23 | >100.0 | 82.0 | NI |
| 4c | 256.36 | 4.81 | 4.88 | 4.78 | 63.0 | 62.0 | 71.2 |
| 4d | 240.30 | 4.21 | 4.53 | 4.23 | 67.0 | 81.5 | NI |
| 4e | 270.03 | 4.12 | 4.51 | 4.21 | 96.0 | 58.0 | 90.0 |
| 4f | 270.13 | 4.12 | 4.12 | 4.21 | 90.0 | >100.0 | NI |
| 4g | 300.35 | 3.99 | 4.10 | 4.18 | 74.0 | 95.5 | 110.5 |
| 4h | 254.09 | 4.15 | 4.36 | 4.08 | 80.0 | 89.5 | NI |
| 4i | 268.31 | 3.88 | 3.98 | 3.98 | >100.0 | 76.5 | NI |
| 4j | 278.27 | 5.29 | 5.32 | 5.15 | 53.1 | 52.3 | 41.6 |
| 4k | 278.09 | 5.29 | 5.34 | 5.15 | 54.0 | 70.1 | 61.5 |
| 4l | 278.09 | 5.29 | 5.37 | 5.15 | 61.7 | 68.5 | 55.8 |
| 4m | 346.08 | 6.21 | 6.19 | 6.03 | 45.1 | 48.7 | 65.8 |
| 4n | 346.08 | 6.21 | 6.19 | 6.03 | 34.5 | 36.0 | 56.3 |
| 4o | 312.05 | 5.85 | 5.97 | 5.78 | 59.5 | 50.0 | 62.4 |
| 4p | 228.26 | 4.53 | 4.64 | 4.36 | 57.1 | 94.5 | 82.0 |
| 4q | 246.25 | 4.69 | 4.73 | 4.46 | 88.1 | 72.5 | 78.0 |
| 4r | 267.13 | 3.28 | 3.67 | 3.44 | 67.2 | >100.0 | NI |
| 4s | 303.09 | 2.59 | 3.68 | 3.23 | >100.0 | 80.0 | NI |
| 4t | 255.09 | 3.95 | 4.41 | 4.09 | 48.5 | 56.5 | 71.0 |
| 5a | 268.35 | 5.05 | 5.50 | 5.11 | 74.0 | 95.0 | 80.0 |
| 5b | 283.12 | 4.96 | 5.38 | 4.97 | 58.0 | 55.0 | 53.0 |
| 5c | 374.32 | 7.01 | 7.16 | 6.91 | 15.0 | 25.0 | 21.2 |
| 6a | 254.32 | 4.51 | 4.60 | 4.56 | >100.0 | >100.0 | NI |
| 6b | 280.15 | 5.20 | 5.24 | 5.20 | >100.0 | >100.0 | NI |
| 6c | 314.15 | 4.26 | 4.17 | 4.50 | 76.0 | >100.0 | NI |
| 6d | 268.11 | 4.41 | 4.43 | 4.40 | >100.0 | >100.0 | NI |
| 6e | 292.11 | 5.56 | 5.44 | 5.47 | >100.0 | >100.0 | NI |
| 6f | 386.11 | 7.17 | 7.17 | 7.00 | >100.0 | >100.0 | NI |
| 6g | 414.14 | 7.17 | 7.94 | 7.86 | >100.0 | >100.0 | NI |
| 6h | 281.35 | 3.54 | 3.74 | 3.77 | >100.0 | >100.0 | 58.0 |
| 6i | 269.11 | 4.47 | 4.48 | 4.42 | >100.0 | >100.0 | NI |
| 6j | 295.12 | 5.03 | 5.12 | 5.06 | >100.0 | >100.0 | NI |
| 7a | 295.12 | 5.04 | 4.95 | 5.16 | 44.7 | 51.3 | 55.2 |
| 7b | 280.15 | 5.30 | 5.07 | 5.31 | 65.0 | 72.0 | 73.4 |
| 7c | 386.33 | 7.27 | 6.74 | 7.10 | 25.5 | 36.0 | 37.3 |
| 7d | 414.38 | 8.07 | 7.71 | 7.98 | 29.0 | 40.0 | 42.1 |

\textsuperscript{a} Molecular weight; \textsuperscript{b} Calculated by ChemDraw Ultra, version 10.0; \textsuperscript{c} Calculated by Molinspiration online service; \textsuperscript{d} Calculated by XLOGP3 online service; \textsuperscript{e} no inhibition.

In order to explore the SAR information based on the aforementioned analysis, we further synthesized compounds 5a–c, in which the allyl group at the C-3 site was replaced by a 2-methylbut-3-en-2-yl
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substituent, and 7a–d with the introduction of one more allyl (compounds 7a–c) or a 2-methylbut-3-en-2-yl (7d) group at the C-5 position of the biphenol scaffold. Interestingly, 3-(2-methylbut-3-en-2-yl)-3′,5′-bis(trifluoromethyl)-[1,1′-biphenyl]-4-ol (5c) was found to exhibit more potent inhibitory activity against C26 and Hela tumor cells (IC50 = 15.0, and 25.0 μM, respectively) than those of honokiol and the chemical entity also contained the two trifluoromethyl (CF3) substituents. However, the further modification of 5c that led to 7c and 7d didn’t achieve an effective improvement in inhibitory potency compared to honokiol, indicating that the best number of allyl or 2-methylbut-3-en-2-yl moieties in the same phenyl ring seemed to be less than two, as well as highlighting that the introduction of allyl or 2-methylbut-3-en-2-yl groups at the C-5 position of the biphenol was forbidden.

A relevant strategy for anti-angiogenesis is effectively inhibition of the proliferation of ECs. Thus, the result of anti-proliferative activity against HUVECs was selected as the main index and the IC50 values against A549 and HepG2 cells were also outlined in Table 2. With comparable or superior inhibitory potency against C26 and Hela cells compared to honokiol, nine allylated biphenols were next selected for biological evaluation, with 5c being the most efficient. Compound 5c exhibited a moderate inhibitory activity against the proliferation of HUVECs (IC50 = 57.0 μM) in contrast to honokiol (IC50 = 40.0 μM) and exerted remarkable cytotoxic activities against A549 and HepG2 cells (IC50 = 29.5 and 13.0 μM, respectively). At the same time, 7c presented comparable inhibitory potencies. The anti-proliferative effects on HUVECs and the inhibitory activities against A549 and HepG2 of tested compounds showed consistent tendencies. In addition, there was a potential positive correlation between the lipophilicity (Log P and cLogP) and inhibitory potency.

Table 2. IC50 values against HUVECs and A549, HepG2 cells of the selected allylated biphenols.

| Compd. | HUVEC IC50 (μM) | A549 | HepG2 |
|--------|-----------------|------|-------|
| Honokiol | 40.0 | 75.0 | 55.4 |
| 4j     | 82.0 | 59.7 | 52.4 |
| 4k     | 76.0 | 73.0 | 58.3 |
| 4l     | 74.0 | 76.0 | 60.3 |
| 4n     | >100.0 | 50.0 | 37.5 |
| 4o     | >100.0 | 72.0 | 68.0 |
| 5b     | 77.0 | 64.0 | 56.0 |
| 5c     | 47.0 | 29.5 | 13.0 |
| 7c     | 48.0 | 30.0 | 26.5 |
| 7d     | 70.0 | 32.0 | 33.0 |

2.3. Effects on HUVEC Migration

EC migration is a relevant process in chemotaxis and an indispensable step to form new blood vessels. Inhibition on the process could block the formation of new blood vessels and further suppress the development of cancer. Therefore, to characterize the effects of allylated biphenols 5c and 7c on HUVEC migration, an in vitro migration assay was performed by the application of a slightly modified Boyden chamber. As depicted in Figure 1I, the HUVECs actively migrated to the serum-containing lower chamber within 6 h under the untreated conditions (control). Compared to the control, the mean
number of invaded HUVECs (% of control group) treated with 5c and 7c at the concentration of 40 μM were 7.1 and 14.2%, respectively, and the cell migration was not likely to occur [Figure 1(II)]. After treatment with 5c and 7c, even at 20 μM, the inhibitory effects on cell migration were also effective and the mean invasion rates remained below 50.0%. However, at 10 μM, the two biphenols hardly inhibited HUVEC migration. As a consequence, 5c and 7c exerted the potent inhibitory activity on the migration of HUVECs in a concentration-dependent manner and 5c was more potent than 7c.

**Figure 1.** Effects of 5c and 7c on the HUVECs migration. (I) HUVECs seeded in 24-well Chambers were incubated for 6 h with medium alone (control) or contained the indicated concentration (10, 20, and 40 μM) of 5c and 7c. The photographs exhibited the migrated cells on the lower surface of the filter stained with 1% crystal violet under a phase contrast microscopy (magnification: 200×). (II) Mean invaded HUVECs (% of control) were quantitatively analyzed by the inhibitory effects of 5c and 7c. Data represented the mean ± standard error (SE) from three independent experiments.

**P** < 0.01; ***P** < 0.005.
In the later stages of angiogenesis, ECs will assemble into an interconnected tubular network which is similar to in vivo capillary vascular beds. Inhibition on this formation of capillary-like tube networks will block the formation of new blood vessels. A tube formation assay was performed by plating HUVECs on Matrigel. In the blank control, the cells exhibited high mobility on Matrigel and constructed an intact tube network in 24 hours [Figure 2(I)]. Compared to the control, the mean number of tube formation (% of control) treated with 5c and 7c at the concentration of 40 μM were 3.7 and 4.7%, respectively, and the disrupted tubular structures were sparse and incomplete [Figure 2(II)]. After the treatment with 5c and 7c, even at 20 μM, the inhibitory effects were also effective and the mean tube formation remained at 23.4 and 40.6%, respectively. However, served at the low concentration of 10 μM, the two compounds were totally unable to inhibit tube formation of HUVECs. Our observation indicated that allylated biphenols (5c and 7c) could effectively terminate the formation of capillary-like tube networks and the effect of 5c was superior to that of 7c at the same concentration.

Figure 2. Effects of 5c and 7c on the HUVECs Tube Formation. (I) HUVECs (1 × 10⁴ cells) suspended in DMEM containing the tested compound (10, 20, and 40 μM) were added to the Matrigel. Control was treated with DMEM alone. After incubation for 24 hours at 37 °C, capillary networks were photographed and quantified (magnification: 200×). (II) The number of mean tube formation was counted in five randomly chosen regions and expressed as the percentage of the control. The results were expressed as mean ± SE.

* P < 0.05; ** P < 0.01; *** P < 0.005.
3. Experimental

3.1. Chemistry

Chemical reagents of analytical grade were purchased from Chengdu Changzheng Chemical Factory (Sichuan, China). $^1$H-NMR spectra were recorded at 400 MHz on a Varian Gemini 400 spectrometer (Varian, Palo Alto, CA, USA) and are reported in parts per million. Chemical shifts (δ) are quoted in ppm relative to the internal standard tetramethylsilane (TMS), where (δ) TMS = 0.00 ppm. The multiplicity of the signals is indicated as s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. Mass spectra were measured by a Premier quadrupole-time of flight (Q-TOF) mass spectrometer (Micromass, Manchester, UK) utilizing electrospray ionization (ESI). The purity of compounds was determined to be ≥97% by HPLC analysis using a photodiode array detector (Waters, Milford, MA, USA) and the chromatographic column was an Atlantis C18 (150 mm × 4.6 mm, i.d. 5 μm) (Waters, Milford, Ireland). All compounds were dissolved as 0.1 mg/mL solutions in HPLC quality methanol with 10 μL injected on a partial loop fill at a flow rate of 1 mL/min and the column chamber was kept at 20 °C for the analysis. The mobile phases were 70% methanol and 30% water (0.1% formic acid).

3.1.1. General Procedure Step I for the Preparation of 1-allyloxy-4-bromobenzene (2)

Allyl bromide (1.9 mL, 22 mmol) was slowly added into a solution of p-bromophenol (3.46 g, 20 mmol) and anhydrous K$_2$CO$_3$ (3.6 g, 26 mmol) in acetone (25 mL), and the mixture was refluxed for 5 hours (TLC monitoring). After completing and cooling, the mixture was filtered to remove the solid and the filtrate was evaporated to dryness. The residue was extracted with diethyl ether (20 mL × 3) and 10% NaOH (20 mL × 2) and then the organic layer was combined and washed by brine (20 mL × 2), dried over anhydrous MgSO$_4$, and concentrated under reduced pressure to afford a colorless oil (4.05 g, 19.01 mmol, 95.0%).

3.1.2. Step II for the Preparation of Intermediates 3 by Suzuki-Coupling Reaction

Method A (for 3a-i): Compound 2 (1.0 mmol) and arylboronic acids (1.2 mmol) were dissolved in isopropanol (4 mL) at room temperature and stirred for 10 min. After a clear solution was formed, Pd(OAc)$_2$ (0.01 mmol), PPh$_3$ (0.03 mmol) and anhydrous K$_2$CO$_3$ (2.0 mol/L, 1 mL) were quickly added under a N$_2$ atmosphere, and the resulting mixture was stirred for a further 18 hours at 90 °C (TLC monitoring). After completion of the reaction, the mixture was filtered, and extracted with ethyl acetate (10 mL × 3). The extracts were combined and washed with brine (10 mL × 2), dried over anhydrous MgSO$_4$, and concentrated under reduced pressure to afford a colorless oil (4.05 g, 19.01 mmol, 95.0%).

Method B (for 3j–t): Compound 2 (1.0 mmol) and arylboronic acids (1.2 mmol) were dissolved in DMF (4 mL) at room temperature and stirred for 5 min. After a clear solution was formed, Pd(PPh$_3$)$_4$ (0.01 mmol), and a solution of K$_3$PO$_4$.3H$_2$O (2.0 mmol) in water (1 mL) were quickly added under a N$_2$ atmosphere, and the mixture was heated for 18 h at 100 °C (TLC monitoring). After completion, the
reaction mixture was filtered, and extracted with ethyl acetate (10 mL × 3). The extract was combined and washed with brine (10 mL × 2), dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by gel chromatography (ethyl acetate/petroleum ether = 1:10) to give the intermediates 3j–t in satisfactory yields (75–90%).

3.1.3. Step III for the Preparation of 4a–t

Allyl bromide or 1-bromo-3-methylbut-2-ene (1.3 mmol) was added to the solution of 3 (1.0 mmol) and anhydrous K₂CO₃ (2.0 mmol) and refluxed for 5 hours. After completion (TLC monitoring) and cooling, the mixture was filtered, and the solution was evaporated to dryness. Next, the crude product was dissolved in N,N-diethylaniline and refluxed for 10 hours under a N₂ atmosphere (TLC monitoring). After completion and cooling, the solution was adjusted to pH = 4 with 2.5 N HCl and extracted with ethyl acetate (10 mL × 2) and the organic layer was combined and washed with water (10 mL × 2) and brine (10 mL × 1), dried over anhydrous MgSO₄, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/petroleum ether = 1:10) to give the targeted 4a–t. The yield represents the total yield of the above four steps. The chemical and structural elucidation of eighteen compounds (4a–g, 4j, 4t, 5a–b, 6a–b, 6d–e, 6i–j, and 7a–b) have been reported in our previous publication [17].

2-Allyl-4-(benzo[d][1,3]dioxol-5-yl)phenol (4h). Yield 40.0%; HPLC: 99.3%. ¹H-NMR (CDCl₃) δ 7.29–7.26 (m, 2H, 2- and 6’-H), 7.02–6.98 (m, 2H, 2’- and 6-H), 6.86–6.84 (m, 2H, 5- and 5’-H), 6.09–6.02 (m, 1H, -CH=CH₂), 5.98 (s, 2H, -OCH₂O-), 5.23–5.17 (m, 2H, -CH=CH₂), 3.46 (d, 2H, J = 6.8 Hz, -CH₂CH); HRMS [M + Na]⁺ calcd. 255.1021; found 255.1028.

2-Allyl-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)phenol (4i). Yield 68.0%; HPLC: 97.8%. ¹H-NMR (CDCl₃) δ 7.31–7.26 (m, 2H, 2- and 6-H), 7.06–7.01 (m, 2H, 2’- and 6’-H), 6.90 (d, 1H, J = 8.4 Hz, 5-H), 6.85 (d, 1H, J = 8.0 Hz, 5’-H), 6.10–6.00 (m, 1H, -CH=CH₂), 5.23–5.17 (m, 2H, -CH=CH₂), 4.97 (s, 1H, -OH), 4.29 (s, 4H, -OCH₂CH₂O-), 3.46 (d, 2H, J = 6.4 Hz, -CH₂CH); HRMS [M + H]⁺ calcd. 269.1172; found 269.1178.

3-Allyl-2′-(trifluoromethyl)-[1,1′-biphenyl]-4-ol (4l). Yield 43.0%; HPLC: 97.8%. ¹H-NMR (CDCl₃): δ 7.72 (d, 1H, J = 7.2 Hz, 6′-H), 7.53 (t, 1H, J = 7.2 Hz, 3′-H), 7.43 (t, 1H, J = 7.2 Hz, 5′-H), 7.32 (d, 1H, J = 7.2 Hz, 4′-H), 7.10–7.08 (m, 2H, 2- and 6-H), 6.84 (d, 1H, J = 8.0 Hz, 5-H), 6.09–5.99 (m, 2H, -CH=CH₂), 5.19–5.15 (m, 2H, -CH=CH₂), 3.44 (d, 2H, J = 6.0 Hz, -CH₂CH); HRMS [M – H]⁻ calcd. 277.0840; found 277.0840.

3-Allyl-3′-(trifluoromethyl)-[1,1′-biphenyl]-4-ol (4k). Yield 59.8%; HPLC: 99.5%. ¹H-NMR (CDCl₃): δ 7.77 (s, 1H, 2′-H), 7.72–7.70 (m, 1H, 6′-H), 7.56–7.49 (m, 2H, 2- and 6-H), 7.39–7.35 (m, 2H, 3′- and 4′-H), 6.91 (d, 1H, J = 8.0 Hz, 5-H), 6.11–6.01 (m, 1H, -CH=CH₂), 5.24–5.19 (m, 2H, -CH=CH₂), 3.49 (d, 2H, J = 6.4 Hz, -CH₂CH); HRMS [M + H]⁺ calcd. 301.0816; found 301.0820.

3-Allyl-4′-(trifluoromethyl)-[1,1′-biphenyl]-4-ol (4l). Yield 51.8%; HPLC: 98.9%. ¹H-NMR (CDCl₃): δ 7.67–7.62 (m, 4H, Ar′-H), 7.40–7.36 (m, 2H, 2- and 6-H), 6.92 (d, 1H, J = 8.0 Hz, 5-H), 6.10–6.01
(m, 1H, -CH=CH₂), 5.24–5.19 (m, 2H, -CH=CH₂), 3.49 (d, 2H, J = 6.0 Hz, -CH₂CH); HRMS [M + H]⁺ calcd. 279.0997; found 279.0993.

3-Allyl-2',4'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-ol (4m). Yield 21.2%; HPLC: 98.7%. ¹H-NMR (CDCl₃): δ 7.90 (s, 1H, 3'-H), 7.38–7.35 (m, 2H, 2'- and 6'-H), 7.15–7.11 (m, 2H, 2- and 6-H), 6.95–6.81 (m, 1H, 5-H), 6.10–5.97 (m, 1H, -CH=CH₂), 5.20–5.10 (m, 2H, -CH₂CH), 3.48 (d, 2H, J = 6.4 Hz, -CH=CH₂); HRMS [M + H]⁺ calcd. 279.0997; found 279.0993.

3-Allyl-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-ol (4n). Yield 41.2%; HPLC: 98.0%. ¹H-NMR (CDCl₃): δ 7.95 (s, 1H, 4'-H), 7.40–7.37 (m, 2H, 2'- and 6'-H), 7.15–7.11 (m, 1H, 2-H), 6.95–6.81 (m, 2H, 5- and 6-H), 6.12–6.00 (m, 1H, -CH=CH₂), 5.23–5.14 (m, 2H, -CH₂CH), 3.50 (d, 2H, J = 6.0 Hz, -CH=CH₂); HRMS [M + H]⁺ calcd. 279.0997; found 279.0993.

3-Allyl-4'-chloro-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-ol (4o). Yield 18.9%; HPLC: 97.5%. ¹H-NMR (CDCl₃): δ 7.93 (s, 1H, 3'-H), 7.41–7.36 (m, 2H, 2'- and 6'-H), 7.18–7.14 (m, 2H, 2- and 6-H), 7.05–7.01 (m, 1H, 5-H), 6.16–5.99 (m, 1H, -CH=CH₂), 5.25–5.16 (m, 2H, -CH₂CH), 3.54 (d, 2H, J = 6.4 Hz, -CH=CH₂); HRMS [M + Na]⁺ calcd. 335.0426; found 335.0422.

3-Allyl-4'-fluoro-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-ol (4p). Yield 23.9%; HPLC: 99.0%. ¹H-NMR (CDCl₃): δ 7.50–7.46 (m, 2H, 2- and 6-H), 7.32–7.30 (m, 2H, 2'- and 6'-H), 7.11–7.07 (m, 2H, 3'- and 5'-H), 6.88 (d, 1H, J = 8.4 Hz, 5-H), 6.11–6.00 (m, 1H, -CH=CH₂), 5.23–5.18 (m, 2H, -CH₂CH), 3.05 (s, 1H, -OH), 3.47 (d, 2H, J = 6.0 Hz, -CH₂CH); HRMS [M + H]⁺ calcd. 269.0754; found 269.0749.

N-(3'-Allyl-4'-hydroxy-[1,1'-biphenyl]-3-yl)acetamide (4r). Yield 56.9%; HPLC: 99.2%. ¹H-NMR (DMSO-d₆): δ 9.97 (s, 1H, -NH), 9.54 (s, 1H, -OH), 7.74 (s, 1H, 2'-H), 7.52 (d, 1H, J = 8.4 Hz, 4'-H), 7.32–7.28 (m, 2H, 2- and 6-H), 7.20 (d, 1H, J = 8.0 Hz, 5'-H), 6.89–6.87 (m, 1H, -OH), 3.36–3.34 (m, 2H, -CH₂CH), 2.05 (s, 3H, -CH₃); HRMS [M + Na]⁺ calcd. 269.1383; found 269.1347.

N-(3'-Allyl-4'-hydroxy-[1,1'-biphenyl]-3-yl)methanesulfonamide (4s). Yield 20.9%; HPLC: 99.7%. ¹H-NMR (CDCl₃): δ 7.91–7.33 (m, 5H, Ar-H), 7.18–7.16 (m, 1H, 2'-H), 6.89 (d, 1H, J = 8.0 Hz, 5-H), 6.74 (s, 1H, -NH), 6.05 (m, 1H, -CH=CH₂), 5.22–5.17 (m, 2H, -CH₂CH), 3.47 (d, 2H, J = 6.4 Hz, -CH=CH₂), 3.04 (s, 3H, -CH₃); HRMS [M + Na]⁺ calcd. 326.0827; found 326.0829.

3.1.4. Syntheses of 5a–c followed the general procedure

3-(2-Methylbut-3-en-2-yl)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-ol (5c). Yield 19.5%; HPLC: 98.9%. ¹H-NMR (CDCl₃): δ 7.95 (s, 1H, 4'-H), 7.41–7.37 (m, 1H, Ar-H), 6.95–6.91 (m, 3H, Ar-H), 6.73
(d, 1H, J = 8.0 Hz, 5-H), 6.11–5.97 (m, 1H, -CH=CH2), 5.07 (s, 1H, -CH=CH2), 4.83 (s, 1H, -CH=CH2), 1.74 (s, 3H, -CH3), 1.71 (s, 3H, -CH3). HRMS [M + H]+ calcd. 378.1184; found 378.1180.

3.1.5. Syntheses of 6a–j Following Step III of the General Procedure

3′-Allyl-3,4,4,5′-tetramethoxy-1,1′-biphenyl (6c). Yield 97.5%; HPLC: 98.4%. 1H-NMR (CDCl3): δ 7.39–7.37 (dd, 1H, J = 8.4 Hz, J = 2.4 Hz, 2-H), 7.32 (d, 1H, J = 2.4 Hz, 6-H), 6.91 (d, 1H, J = 8.4 Hz, 5-H), 6.72 (s, 2H, 2′- and 6′-H), 6.08–5.98 (m, 1H, -CH=CH2), 5.12–5.05 (m, 2H, -CH=CH2), 3.93 (s, 6H, -CH3), 3.88 (s, 3H, -CH3), 3.78 (s, 3H, -CH3), 3.74 (s, 3H, -CH3). HRMS [M + Na]+ calcd. 337.1416; found 337.1323.

3-Allyl-4-(allyloxy)-3′,5′-bis(trifluoromethyl)-1,1′-biphenyl (6f). Yield 95.8%; HPLC: 97.9%. 1H-NMR (CDCl3): δ 8.10 (s, 1H, 4′-H), 7.55–7.50 (m, 2H, 2′- and 6′-H), 7.35–7.31 (m, 1H, 2-H), 7.05–7.00 (m, 2H, 5- and 6-H), 6.15–6.00 (m, 2H, -CH=CH2 and -OCH2CH=CH2), 5.53–5.48 (m, 1H, -OCH2CH=CH2), 5.41–5.36 (m, 1H, -OCH2CH=CH2), 5.26–5.19 (m, 2H, -CH=CH2), 4.65 (d, 2H, J = 4.8 Hz, -OCH2CH=CH2), 3.54 (d, 2H, J = 6.4 Hz, -CH2CH); HRMS [M + H]+ calcd. 387.1184; found 387.1180.

3-Allyl-4-((3-methylbut-2-en-1-yl)oxy)-3′,5′-bis(trifluoromethyl)-1,1′-biphenyl (6g). Yield 89.5%; HPLC: 98.5%. 1H-NMR (CDCl3): δ 8.01 (s, 1H, 4′-H), 7.45–7.40 (m, 2H, 2′- and 6′-H), 7.35–7.30 (m, 1H, 2-H), 7.08–7.02 (m, 2H, 5- and 6-H), 6.05–5.97 (m, 2H, -CH=CH2 and -OCH2CH=CH2), 5.50–5.45 (m, 1H, -OCH2CH=CH2), 5.40–5.35 (m, 1H, -OCH2CH=CH2), 5.27–5.21 (m, 2H, -CH=CH2), 4.55 (d, 2H, J = 4.8 Hz, -OCH2CH=CH2), 3.59 (d, 2H, J = 6.4 Hz, -CH2CH), 1.56 (s, 3H, -CH3), 1.53 (s, 3H, -CH3); HRMS [M + H]+ calcd. 415.1497; found 415.1495.

N-(3′-Allyl-4′-methoxy-[1,1′]-biphenyl)-3′-ylacetamide (6h). Yield 93.8%; HPLC: 99.5%. 1H-NMR (DMSO-d6): δ 9.97 (s, 1H, -NH), 9.54 (s, 1H, 2′-H), 7.74 (s, 1H, 2-H), 7.52 (d, 1H, J = 8.4 Hz, 5′-H), 7.33–7.28 (m, 2H, 6- and 6′-H), 7.20 (d, 1H, J = 8.0 Hz, 4′-H), 6.89–6.87 (m, 1H, 4-H), 6.05–5.95 (m, 1H, -CH=CH2), 5.11–5.02 (m, 2H, -CH=CH2), 3.86 (s, 3H, 4′-OCH3), 3.36–3.34 (m, 2H, -CH2CH), 2.05 (s, 3H, -COCH3); HRMS [M+Na]+ calcd. 304.1313; found 304.1298.

3.1.6. Syntheses of 7a–d Following Step III of the General Procedure

3,5-Diallyl-3′,5′-bis(trifluoromethyl)-[1,1′]-biphenyl]-4-ol (7e). Yield 65.4%; HPLC: 98.4%. 1H-NMR (CDCl3): δ 8.12 (s, 1H, 4′-H), 7.51-7.46 (m, 2H, 2′- and 6′-H), 7.30-7.24 (m, 1H, 2-H), 7.05–7.01 (m, 2H, 5- and 6-H), 6.16–6.03 (m, 2H, -CH=CH2), 5.29–5.15 (m, 4H, -CH=CH2), 4.65–4.43 (m, 4H, -CH=CH2); HRMS [M + H]+ calcd. 409.1003; found 409.0998.

3-Allyl-5-(2-methylbut-3-en-2-yl)-3′,5′-bis(trifluoromethyl)-[1,1′]-biphenyl]-4-ol (7d). Yield 65.4%; HPLC: 97.4%. 1H-NMR (CDCl3): δ 8.09 (s, 1H, 4′-H), 7.48–7.44 (m, 2H, 2′- and 6′-H), 7.21–7.18 (m, 1H, 2-H), 7.10–7.06 (m, 2H, 5- and 6-H), 6.13–6.04 (m, 2H, -CH=CH2), 5.25–5.13 (m, 4H, -CH=CH2), 4.55–4.36 (m, 4H, -CH=CH2), 1.66 (s, 3H, -CH3), 1.63 (s, 3H, -CH3); HRMS [M + H]+ calcd. 437.1316; found 437.1315.
4. Conclusions

To date, suppression of angiogenesis-dependent tumor growth has been a widely accepted strategy for cancer therapy. Although acquired drug resistance remains an insurmountable obstacle of tumor-targeting therapy, it is unlikely to occur or at least at a low rate if the genetically stable ECs are targeted. Thus, ECs have been proven to be an attractive and potent target for angiogenesis therapy [21]. In the present study, 37 allylated biphenols represented a novel biphenyl structural motif and possessed a unique mode of action in anti-angiogenic and anti-tumor activity. In detail, 3-(2-methylbut-3-en-2-yl)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-4-ol (5c) showed the strongest inhibitory effects on the proliferation, migration and tube formation of HUVECs featuring anti-angiogenic properties, and its average anti-proliferative activities against four tumor cell lines (C26, Hela, A549, and HepG2) demonstrated that the biologically active allylated biphenol molecule has dual functions and the inhibitory effect of 5c was specific on HUVEC migration and tube formation, rather than resulting from its cytotoxicity.

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*Sample Availability*: Samples of the compounds 4a–7d are available from the authors.

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