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Synthesis of symmetrical bis-alkynyl or alkyl pyridine and thiophene derivatives and their antiangiogenic activities

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Abstract—Fourteen symmetrical bis-alkynyl pyridine and thiophene derivatives were synthesized and their antiangiogenic activity was evaluated with the proliferation and tube formation inhibitory activity on the human umbilical vein endothelial cells (HUVEC). Compounds **6**, **8**, and **10**, rigid mimetic structure of curcumin, showed the potent growth inhibitory activity and the potent tube formation inhibitory activity.

Angiogenesis occurs during embryonic development, wound healing, and the menstruation cycle in physiologic conditions. Angiogenesis is an elaborately regulated phenomenon. Unregulated angiogenesis causes pathological conditions, such as diabetic retinopathy, psoriasis, arthritis, and cancer.[1] During the tumor growth, angiogenesis need for nourishment and removal of metabolic wastes from tumor sites. Tumor requires coordination of angiogenesis with continuous cancer cell proliferation. Tumor growth and metastasis strictly, which depend on angiogenesis reminds the idea that blocking tumor nourishment can be one of the ways to avoid its spread and growth.[2] Therefore, antiangiogenic treatment has received a lot of attention in cancer research.[3] A lot of antiangiogenic compounds were reported[4] and several antiangiogenic drugs are currently in clinical trials.[5]

Curcumin (**1**) is a natural product isolated from the spice turmeric. It inhibits several signal transduction pathways including protein kinase-C, transcription factor NF-κB, phospholipase A2, arachidonic acid metabolism, EGF receptor autophosphorylation, and Ca^{2+}-ATPase.[6] Thus curcumin has a potent antiangiogenic effect.

Arbiser et al. reported that curcumin (**1**) and its derivatives including demethoxycurcumin and tetrahydrocurcumin were discovered as potent antiangiogenic agents.[7] Kwon and co-workers reported that hydrazinocurcumin, which curcumin's diketone part is modified to imidazole ring has a potent antiangiogenic activity.[8] Recently, Bowen and co-workers reported that aromatic enone and dienone analogues of curcumin (**1**) have a potent antiangiogenic activity.[9] Therefore, although structure–antiangiogenic activity relationship of curcumin (**1**) is not completely understood, curcumin is a promising lead compound for structural modification. These studies suggest that phenolic group and aromatic enone or dienone group is essential to the antiangiogenic activity of curcumin (Fig. 1).

In addition, based on the previous results,[9] we hypothesized that the rigidity of symmetrical aromatic moieties plays an important role to enhance angiogenic activity. To certify our assumption, we synthesized symmetrical bis-aromatic alkynyl pyridine and thiophene derivatives (**2**) and bis-aromatic alkyl pyridine and thiophene

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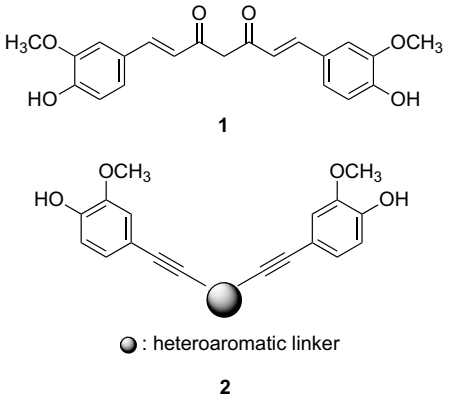


Figure 1.

derivatives. The bis-alkynyl compounds (**2**) are the rigid structure of curcumin.

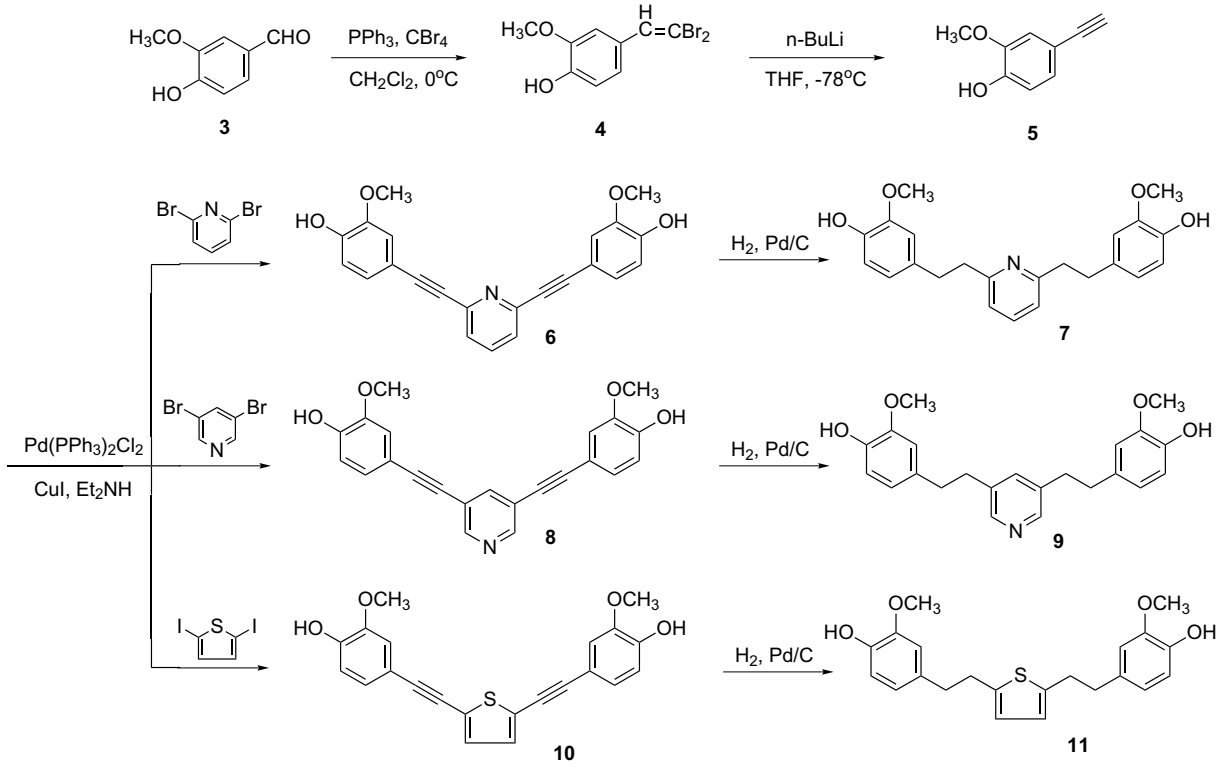
The antiangiogenic activities of those synthetic compounds were evaluated with the proliferation and tube formation inhibitory activities on the human umbilical vein endothelial cells (HUVEC).

Commercially available aldehyde (**3**) was reacted with carbon tetrabromide in the presence of triphenyl phosphine in CH_2Cl_2 at 0 °C for 2 h. The crude product was twice chromatographed ($CHCl_3$/MeOH = 95/5) on silica gel to afford dibromoalkene (**4**). This alkene (**4**) was dissolved in dry THF under *n*-BuLi and stirred at −78 °C for 5 h to give aromatic alkyne (**5**). Utilizing Sonogashira reaction,[10] bis-alkynyl pyridines (**6** and **8**), and bis-alkynyl thiophene (**10**) compounds were obtained by the reaction of **5** with two dibromo pyridines or diiodothiophene at room temperature for 15–17 h in the presence of dichloro bis-(triphenylphosphine) palladium, copper (I) iodide, and diethylamine, as shown in Scheme 1. ^1H, ^{13}C NMR spectrum, and GC/MS spectrum of bis-alkynyl compounds (**6**, **8**, and **10**) were investigated for structural identification.[11]

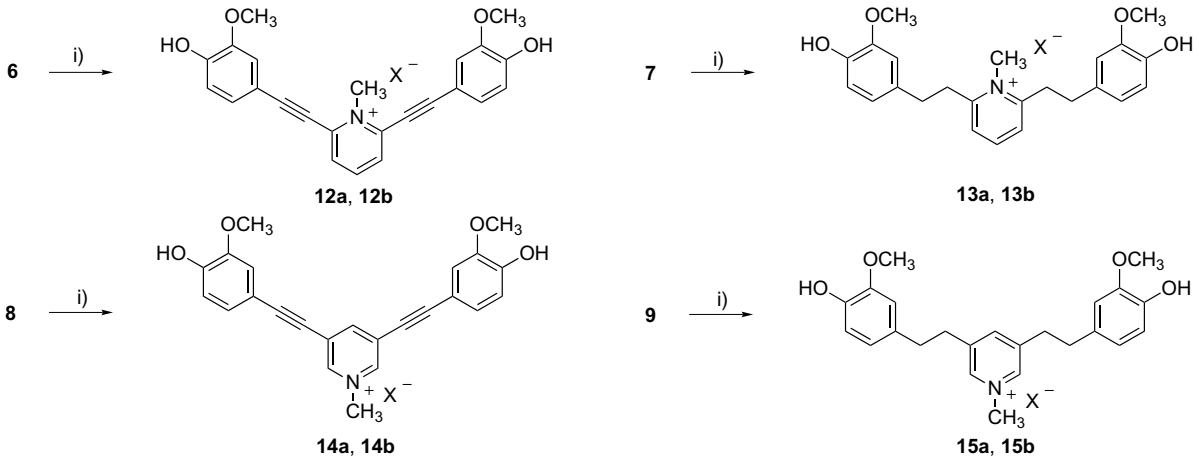
To study the relationship between structural rigidity and activity, we obtained the bis-alkyl compounds (**7**, **9**, and **11**), which are the hydrogenated compounds (**6**, **8**, and **10**) with Pd/C at room temperature in the H_2 atmosphere, respectively (Scheme 1). Also, to obtain water-soluble promising molecules, we reacted bis-alkynyl and bis-alkyl pyridines (**6**, **7**, **8**, and **9**) with trimethyloxonium tetrafluoroborate ($(Me)_3OBF_4$) in dichloroethane to transform into their salts (**12a**, **13a**, **14a**, and **15a**), and the treatment of methyl trifluoromethanesulfonate (MeOTf) on **6**, **7**, **8**, and **9** in diethyl ether afforded these salts (**12b**, **13b**, **14b**, and **15b**), as shown in Scheme 2.

Cell growth inhibitory effect of synthesized compounds was measured by MTT colorimetric method,[12] as shown in Table 1.

As we expected, the bis-alkynyl compounds (**6**, **8**, and **10**), rigid mimetic structure of curcumin (**1**), showed more potent growth inhibitory activity on HUVEC than curcumin (**1**). Especially, bis-alkynyl compounds (**6** and **8**) have a highly strong inhibition activity upon HUVEC. The bis-alkyl compounds (**7**, **9**, and **11**) having more flexible alkyl chain, hydrogenated compounds of



Scheme 1. Synthesis of symmetrical bis-alkynyl pyridine and thiophene derivatives, and their hydrogenated compounds.



Scheme 2. Synthesis of tetrafluoroborate and triflate salts of symmetrical bis-alkynyl pyridine and bis-alkyl pyridine derivatives. Reagents and conditions: (i) (a) $(Me)_3OBF_4$ (1.3 equiv), $ClCH_2CH_2Cl$, $X = BF_4^-$, (b) MeOTf (1.6 equiv), diethyl ether, $X = OTf^-$.

Table 1. Inhibitory activity of synthetic compounds[a] on HUVEC growth

Compounds	Growth inhibition IC_{50}, μg/mL
6	2.2
7	12.6
8	2.0
9	9.3
10	7.2
11	10.8
12a	>50
12b	>50
13a	>50
13b	>50
14a	9.2
14b	1.5
15a	22.8
15b	9.9
Curcumin	10.7

[a] IC_{50} was calculated from nonlinear regression by Graphpad Prism software.

6, **8**, and **10**, showed similar inhibition activity with curcumin.

When considered growth inhibition activity upon HUVEC, we concluded that the increase of the extent of unsaturation or rigidity of structure enhanced biological activity.

Finally, we examined the antiproliferative effect of salts (**12a**,**b**, **13a**,**b**, **14a**,**b**, and **15a**,**b**) with water solubility. Disappointingly, except of triflate salt (**14b**) of 3,5-bis-alkynyl pyridine, all salt-type molecules have a mild or weak inhibitory effect.

In the tube-formation assay using HUVEC on the Matrigel,[13] as shown in Table 2, the bis-alkynyl compounds (**6**, **8**, and **10**), inhibited the tube formation on Matrigel as nearly 100% at the concentration of 10 μg/mL. Even at the concentration of 2.5 μg/mL, compound **8** inhibited about 60%. In expected water-soluble salts, only **14a** have a mild inhibitory activity of tube formation. The hydrogenated molecules (**7**, **9**, and **11**) and salt compounds failed to effectively inhibit the tube formation.

Table 2. The rate of tube-formation inhibition by synthesized compounds[a]

Compounds	Inhibition percentage (%)		
	At 10 μg/mL	At 5 μg/mL	At 2.5 μg/mL
6	97.4	79.4	31.7
8	98.2	79.2	59.9
10	89.3	71.7	37.8
14a	41.9	—	—

[a] Values expressed in percentage of HUVEC total tube length/field as compared to untreated control. Total tube length was measured using Image-pro plus version 3.0 (Media Cybernetics, MD, USA).

In conclusion, for the first time, we have shown that several symmetrical bis-alkynyl pyridine and thiophene derivatives (**6**, **8**, and **10**) readily synthesized by Sonogashira reaction, rigid mimetic structure of curcumin, have a strong antiangiogenic activity and rigidity of synthetic structures have improved the biological activity.

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11. Analytical data for **6**: mp >400 °C; TLC (chloroform/methanol = 95/5) $R_f = 0.31$; ^{1}H NMR ($CDCl_3$): δ 3.91 (6H, s, OCH_3), 5.82 (2H, s, OH), 6.89–6.91 (2H, d, $J = 8.2$ Hz, Ar–H), 7.14–7.18 (4H, m, Ar–H), 7.42–7.44 (2H, d, pyridine–(3,5)H), 7.66 (1H, t, $J = 7.8$ Hz, pyridine–(4)H) ppm; ^{13}C NMR (DMSO-d_6): δ 56.5, 87.5, 90.9, 112.3, 116.1, 116.7, 126.4, 126.7, 138.3, 144.0, 148.5, 149.4 ppm; GC/MSD (m/z) 371. For **8**: mp 175–177 °C; TLC (chloroform/methanol = 95/5) $R_f = 0.33$; ^{1}H NMR ($CDCl_3$): δ 3.94 (6H, s, OCH_3), 5.83 (2H, s, OH), 6.91–6.93 (2H, d, $J = 8.2$ Hz, Ar–H), 7.04 (2H, d, $J = 1.8$ Hz, Ar–H), 7.10–7.13 (2H, t, $J = 8.2$ Hz, Ar–H), 7.94–7.95 (1H, t, $J = 1.8$ Hz, pyridine–(4)H), 8.64 (2H, s, pyridine–(2,6)H) ppm; ^{13}C NMR ($CDCl_3$): δ 55.8, 86.8, 90.0, 111.3, 114.1, 118.6, 124.4, 125.8, 136.5, 143.8, 146.2, 149.0 ppm; GC/MSD (m/z) 371. For **10**: TLC (chloroform/methanol = 95/5) $R_f = 0.63$; ^{1}H NMR ($CDCl_3$): δ 3.91 (6H, s, OCH_3), 5.79 (2H, s, OH), 6.87–6.89 (2H, d, $J = 4.9$ Hz, Ar–H), 6.98–6.99 (2H, d, $J = 1.8$ Hz, Ar–H), 7.05–7.07 (2H, t, $J = 8.2$ Hz, Ar–H), 7.14 (2H, s, furan-H); ^{13}C NMR ($CDCl_3$): δ 56.0, 79.6, 95.1, 113.6, 114.0, 114.6, 125.6, 132.7, 137.0, 146.2, 146.7 ppm; GC/MSD (m/z) 376.
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13. Aoki, K.; Watanabe, K.; Sato, M.; Ikekita, M.; Hakamatsuka, T.; Oikawa, T. *Eur. J. Pharmacol.* **2003**, *459*, 131, Assay method: Tube formation of HUVEC on Matrigel (BD Biosciences, Bedford, MA) was assayed in a 96-well plate. HUVEC was seeded on 50 μL solidified Matrigel, and incubated for 16 h at 37 °C in a 5% CO_2 humidified atomosphere. After incubation, the cells were stained with toluidin blue in 4% paraformaldehyde, and photographed. A blinded observer quantified the number of tube branches. Each concentration of the control or test compound was assayed in triplicate.