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Design and synthesis of a vialinin A analog with a potent inhibitory activity of TNF-α production and its transformation into a couple of bioprobes

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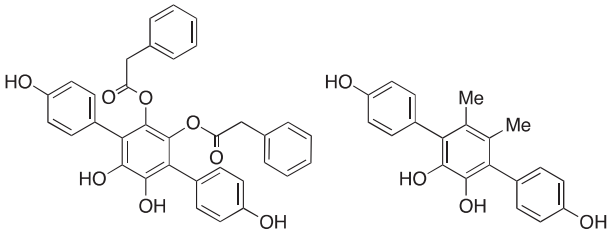
ABSTRACT

Vialinin A (**1**) is an extremely potent inhibitor against tumor necrosis factor (TNF)-α production in rat basophilic leukemia (RBL-2H3) cells. This Letter describes the design and synthesis of its advanced analog, 5′,6′-dimethyl-1,1′:4′1″-terphenyl-2′,3′,4,4″-tetraol (**2**) with a comparable inhibitory activity (IC_{50} = 0.02 nM) to that of **1**. The synthesis involved double Suzuki–Miyaura coupling as a key step, and required only five steps from commercially available 3,4-dimethylphenol. For identification of the target molecule, fluorescent and biotinylated derivatives of **2** were prepared through a 'click' coupling process.

Vialinin A (**1**) is an oxygenated *p*-terphenyl bearing phenylacetoxy groups which we isolated as a powerful DPPH free radical-scavenger (EC_{50} = 14 μM vs 10 μM for BHT) from the dry fruiting bodies of an edible Chinese mushroom, *Thelephora vialis* (Fig. 1).[1] This compound was also isolated from *Thelephora terrestris* and *Thelephora aurantiotincta* by Asakawa et al.[2] and by Norikura et al.,[3] respectively. The latter reported that this natural product had cytotoxicity against human hepatocellular carcinoma cells (HepG2) and human colonic carcinoma cells (Caco2), but not to noncancerous human hepatocytes, and suggested that **1** was an attractive compound for cancer prevention and/or treatment.[3]

Recently, we found that **1** strongly inhibited tumor necrosis factor (TNF)-α production in rat basophilic leukemia (RBL-2H3) cells: IC_{50} = 90 pM vs IC_{50} = 0.25 nM for FK-506.[4] TNF-α is a potent multifunctional cytokine that mediates a variety of biological actions with a central role in the pathogenesis of inflammatory diseases such as rheumatoid arthritis (RA).[5–8] In RA, TNF-α causes accumulation of inflammatory cells and self-perpetuation of inflammation, leading to cartilage and bone destruction.[9,10] Thus, inhibitors of TNF-α production in activated mast cells and basophils are promising candidates for a new type of anti-allergic agent, for example, RA treatment. The mode of action of **1** and its inhibition mechanism, however, remain unclear. In order to clarify the target molecule of **1** and its inhibition mechanism, development of a bioprobe is required. The core part in the probe is generally designed based on the structure of an active library member which would be gained through SAR studies of the original natural product, and has been modified by introduction of a tag such as biotin and a fluorescent label. Therefore, the first problem of this project was design of the core. In a previous paper, we reported that the positional isomers of **1** regarding the phenylacetyl group (ganbajunins D and E) showed no inhibitory activity against TNF-α production in RBL-2H3.[11] A similar result was also obtained from the case of *p*-terphenyl series carrying the benzoyl group,[12] suggesting the importance of the catechol moiety in the central benzene ring. The instability of the acyl groups toward several chemical modifications, in particular, basic conditions was observed in the course of total synthesis of **1** and its congeners.[12,13] These findings prompted us to design as a probe core a scalable *p*-terphenyl **2** in



Vialinin A (**1**) **2**

Figure 1. Structures of vialinin A (**1**) and its advanced analog **2**.

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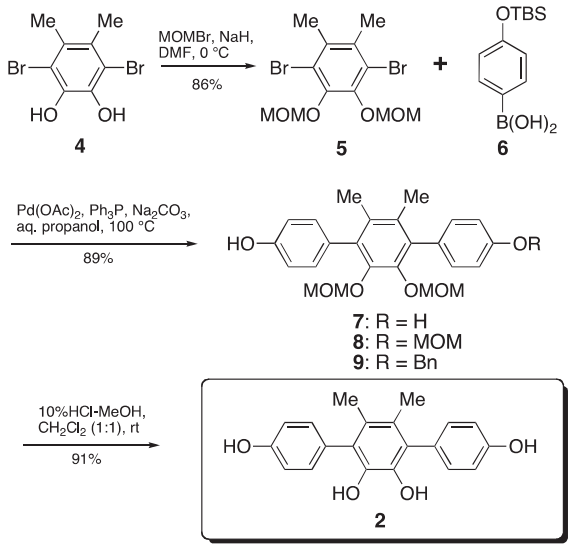


Scheme 1. Synthesis of advanced analog **2**.

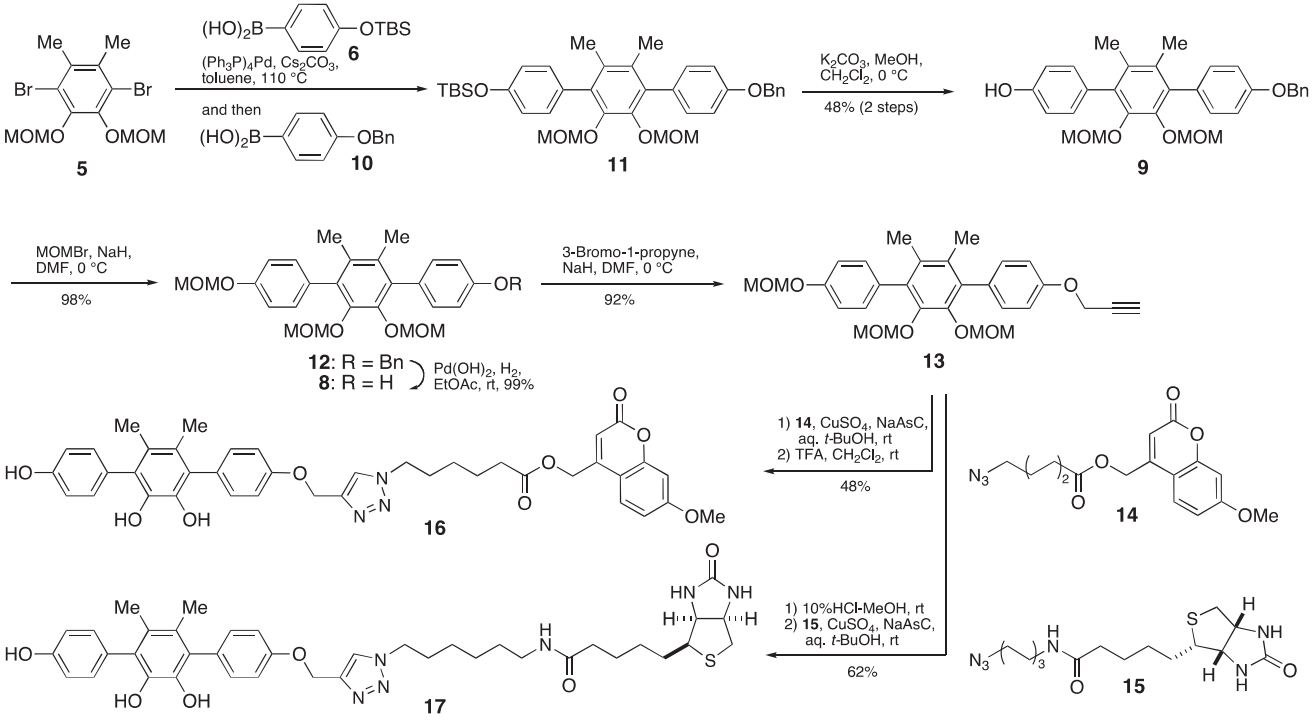
which the chemically reactive phenylacetoxyl groups in **1** were replaced by chemically stable dimethyl groups. Described herein are the synthesis of **2**, its inhibitory activity, and transformation into a couple of bioprobes **16** and **17**.

Synthesis of **2** involved double Suzuki–Miyaura coupling[14] of **5** with boronic acid **6** as a key step (Scheme 1). The known bromide **4**[15] prepared from 3,4-dimethylphenol (**3**) in 48% overall yield (two steps) was protected as dimethoxymethyl ether to afford **5**. Suzuki–Miyaura coupling of **5** with **6** was performed by the action of 0.05 equiv of palladium acetate ($Pd(OAc)_2$)[16] in the presence of triphenylphosphine (0.15 equiv) and sodium carbonate (4.0 equiv) in aq. propanol at 100 °C to give terphenyl **7**[17] in high yield.[18] Hydrolysis of **7** provided **2**[19] in good yield. The overall yield of **2** from commercially available **3** attained 33%.

We next examined the effect of **2** on TNF-α production from RBL-2H3 Cells (Fig. 2) according to the method[4] previously described. As expected, analog **2** showed strong inhibitory activity with IC_{50} = 0.02 nM. Interestingly, its precursor **7** with protected catechol moiety also showed a medium inhibitory activity (IC_{50} = 0.32 nM) but the activity level was observed to reach saturation at 1 nM, although the reason for this is not clear. These results suggest that displacement of the phenylacetoxy function by the methyl group retained the activity to some extent, which is quite useful information for further rational design of artificial analogs of **1**. Synthesis and biological activity of similar methylated analogs of terprenin, an immunosuppressive *p*-terphenyl, were disclosed by Kawada et al.[20]

Encouraged by the above results, we planned to transform **2** into the corresponding probe molecules. Due to preservation of the catechol moiety and easy modification,[21] we thought that the tag including a functional group should be introduced to the hydroxyl group at the terminal benzene ring rather than the central one (e.g., **16**), and tried mono O-protection of **7** such as methoxymethylation and benzylation. Disappointingly, the corresponding phenol **8** or **9** was obtained in a low yield (~30%).

With this result, we turned our attention to preparing desymmetrical *p*-terphenyl by Suzuki–Miyaura coupling using different types of boronic acids. According to the method described before,[22] one-pot coupling was examined. Contrary to the previous case, the use of $Pd(OAc)_2$ was found to be ineffective for the coupling. The best result was obtained by using tetrakis(triphenylphosphine)palladium ($Pd(Ph_3P)_4$) as a catalyst. Thus, **5** was treated with 1.2 equiv of **6** in the presence of $Pd(Ph_3P)_4$ (0.05 equiv) and cesium carbonate (2.0 equiv) in toluene at 100 °C for 9 h, and after the addition of **10** (1.5 equiv), $Pd(Ph_3P)_4$ (0.05 equiv) and cesium carbonate (2.0 equiv), the reaction was further continued for 10 h to give the desired desymmetrical product **11** in ~50% yield. The yield was not improved even though the conditions (e.g., phosphine, additive and solvent) were changed. From a practical point of view, isolation of **9** after the following de-silylation was found to be more efficient. After methoxymethylation of **9**, the benzyl group



Scheme 2. Synthesis of a couple of bioprobes **16** and **17**.

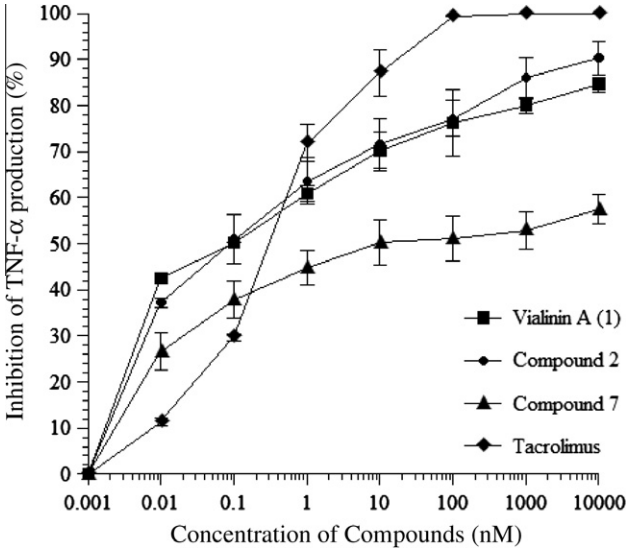


Figure 2. TNF-α Production Inhibition of **2** and **7**.

in **12** was removed to afford **8**. Upon treatment with 3-bromo-1-propyne and sodium hydride, **8** gave a key intermediate **13**.[19] 1,3-Dipolar Huisgen cycloaddition[23] of **13** with **14**[24] in the presence of copper sulfate and sodium ascorbate provided a coupling product, which was treated with TFA, giving a fluorescent probe **16**[19] in 48% yield from **13**. Similarly, reaction of **13** with **15**[25] provided a coupling product in a good yield. The final deprotection under acidic conditions, however, resulted in a complex mixture. On the other hand, the click reaction after deprotection of **13** proceeded without trouble to give the desired product **17**[19] in good yield. Both compounds showed an inhibitory activity against TNF-α production from RBL-2H3 cells at a promising level (IC_{50} = 0.55 nM for **16** and 8.15 nM for **17**). These bioprobes are expected to be a tool for identifying a vialinin A target. Now the research is under investigation.

We succeeded in synthesizing an advanced analog **2** with a comparable inhibitory activity to that of vialinin A (**1**), and transforming it to a couple of bioprobes **16** and **17**. The strategy developed here provides versatility for preparing a variety of functional molecules such as a photoactive probe as well as **16** and **17** and therefore is useful in medicinal chemistry.

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17. This coupling reaction in alkaline aqueous solutions was accompanied by hydrolysis of TBS group to afford **7** while the TBS group was retained under aprotic conditions (e.g., preparation of **11** in Scheme 2).
18. As initial attempts for Suzuki–Miyaura coupling of **5** and **6**, $Pd(Ph_3P)_4$ was employed as a catalyst. A considerable amount of the corresponding biphenyl, however, was always isolated along with the desired *p*-terphenyl **7**. These results made us to conceive the coupling using different types of boronic acids as shown in Scheme 2.
19. *Spectral data of representative compounds*
Compound **2**: mp >270 °C (*n*-hexane-ether-methanol); ^{1}H NMR (400 MHz, d_6-acetone): δ 8.35 (2H, s), 7.08 (4H, d, J = 8.8 Hz), 6.91 (4H, d, J = 8.8 Hz), 6.65 (2H, s), 1.93 (6H, s); ^{13}C NMR (100 MHz, d_6-acetone): δ 157.21, 141.05, 132.36, 129.64, 128.95, 126.50, 115.97, 17.41; HRMS (ESI) calcd for $C_{20}H_{18}O_4Na$ $[M+Na]^+$ 345.1103, found 345.1104.
Compound **13**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.24 (2H, J = 8.8 Hz), 7.22 (2H, d, J = 8.8 Hz), 7.10 (2H, d, J = 8.8 Hz), 7.04 (2H, J = 8.8 Hz), 5.22 (2H, s), 4.82 (2H, s), 4.81 (2H, s), 4.74 (2H, br s), 3.51 (3H, s), 2.92 (3H, s), 2.89 (3H, s), 2.54 (1H, t, J = 2.4 Hz), 2.04 (3H, s), 2.03 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.4, 156.1, 145.3, 136.0, 135.9, 131.9, 131.8, 131.67, 131.65, 131.6, 131.4, 115.8, 114.4, 98.88, 98.86, 94.6, 78.6, 75.4, 56.80, 56.77, 56.0, 55.9, 17.94, 17.92; HRMS (ESI^+) calcd for $C_{29}H_{32}O_7Na$ $[M+Na]^+$ 515.2046, found 515.2042.
Compound **16**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (1H, s), 7.40 (1H, d, J = 8.5 Hz), 7.23 (2H, d, J = 8.8 Hz), 7.17 (2H, d, J = 8.5 Hz), 7.10 (2H, d, J = 8.8 Hz), 6.97 (2H, d, J = 8.5 Hz), 6.87 (1H, dd, J = 8.5, 2.5 Hz), 6.85 (1H, d, J = 2.5 Hz), 6.30 (1H, t, J = 1.5 Hz), 5.62 (1H, br s), 5.27 (2H, s), 5.26 (2H, br d), 5.01 (1H, s), 4.97 (1H, s), 4.40 (2H, t, J = 7.1 Hz), 3.88 (3H, s), 2.47 (2H, t, J = 7.3 Hz), 2.01–1.96 (2H, m), 1.971 (3H, s), 1.967 (3H, s), 1.78–1.72 (2H, m), 1.46–1.39 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.4, 162.9, 161.0, 157.6, 155.5, 155.2, 149.3, 144.2, 138.62, 138.56, 131.55, 131.45, 129.2, 128.4, 127.41, 127.34, 126.75, 126.74, 124.4, 122.7, 115.9, 115.1, 112.7, 110.5, 109.8, 101.2, 62.1, 61.1, 55.8, 50.1, 33.6, 29.9, 25.9, 24.1, 17.17, 17.15; HRMS (ESI^+) calcd for $C_{40}H_{39}O_9N_3Na$ $[M+Na]^+$ 728.2584, found 728.2596.
Compound **17**: $[\alpha]_D^{23}$ +16.8 (c = 0.11, MeOH); ^{1}H NMR (500 MHz, d_4-methanol): δ 8.08 (1H, s), 7.15 (2H, d, J = 8.8 Hz), 7.08 (2H, d, J = 8.8 Hz), 7.05 (2H, d, J = 8.6 Hz), 6.87 (2H, d, J = 8.6 Hz), 5.22 (2H, s), 4.46 (1H, dd, J = 7.9, 4.9 Hz), 4.42 (2H, t, J = 7.1 Hz), 4.27 (1H, dd, J = 7.9, 4.4 Hz), 3.18–3.08 (3H, m), 2.89 (1H, dd, J = 12.7, 4.9 Hz), 2.68 (1H, d, J = 12.7 Hz), 2.18 (2H, t, J = 7.1 Hz), 1.95–1.87 (2H, m), 1.91 (3H, s), 1.90 (3H, s), 1.75–1.37 (12H, m); ^{13}C NMR (125 MHz, d_4-methanol): δ 175.9, 166.1, 158.7, 157.5, 145.2, 141.41, 141.37, 132.7, 132.6, 132.4, 130.23, 130.18, 129.6, 127.4, 127.3, 125.3, 116.2, 115.8, 63.4, 62.5, 61.6, 57.0, 51.3, 41.0, 40.2, 36.8, 31.2, 30.2, 29.8, 29.5, 27.3, 27.1, 26.9, 17.48, 17.46; HRMS (ESI^+) calcd for $C_{39}H_{48}O_6N_6SNa$ $[M+Na]^+$ 751.3254, found 751.3250.
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21. We had already gained the results of the inhibitory activity of vialinin A related compounds including its artificial regioisomers.[4,11–13] Furthermore, after several experiments, we found that the reactivity of two hydroxyl groups on the central benzene ring of **2** was low and that an efficient alkylation at such position was difficult. These results made us to choose the position of OH group for introduction of the tag without SAR studies on dimethyl analogs of **1** other than **2** and **7**.
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