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Design and synthesis of 4-*O*-methylhonokiol analogs as inhibitors of cyclooxygenase-2 (COX-2) and PGF_1 production

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This paper is dedicated to Professor Young-Ger Suh on the occasion of his 60th birthday

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ABSTRACT

A series of novel 4-*O*-methylhonokiol analogs were synthesized in light of revealing structure–activity relationship for inhibitory effect of COX-2 enzyme. The key strategy of the molecular design was oriented towards modification of the potential metabolic soft spots (e.g., phenol and olefin) or by altering the polar surface area via incorporating heterocycles such as isoxazole and triazole. Most of all exhibited the inhibitory effects on COX-2 and PGF_1 production but not macrophage NO production. Especially, aryl carbamates **10** and **11** exhibited more potent inhibitory activity against COX-2 and PGF_1 production.

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) exhibit their anti-inflammatory effect by inhibiting cyclooxygenases (COX) which catalyzes initially in arachidonic acid metabolic cascade.[1] There are two human enzymes, COX-1 and COX-2, which catalyze the same biochemical reaction but they are clearly different in terms of amino acids sequence, tissue distribution, and physiological function. The chronic use of NSAIDs to treat pain and inflammation is often accompanied by side effects such as gastrointestinal (GI) toxicities, bleeding, and suppressed renal function. These common side effects of NSAIDs are ascribed to their ability of inhibiting COX-1 and thus developing COX-2-selective inhibitors is the logical strategy for designing NSAIDS out the adverse events. Several COX-2 selective inhibitors such as celecoxib have shown excellent efficacy in humans with few side effects.[2] In spite of this initial success after the launch of selective COX-2 inhibitors, concerns were raised regarding the adverse thrombotic cardiovascular events. There has been a continuous progress made in the development of novel anti-inflammatory agents having reduced GI and cardiovascular risks.

The bark of the root and stem of Magnolia family has been used in traditional medicine to treat various diseases.[3,4] Among the bioactive compounds isolated from Magnolia family, biphenylneolignans such as magnolol (**1**), honokiol (**2**), 4-*O*-methylhonokiol (**3**) and obovatol (**4**) have been reported to have anti-inflammatory, anti-allergic, anti-bacterial and anti-depressant activities (Fig. 1).[5] 4-*O*-Methylhonokiol was isolated mainly from the bark of the root and stem of *Magnolia* species and exhibits anti-inflammatory, antioxidative and neurotropic activity. In a recent report, 4-*O*-methylhonokiol was capable of decreasing β-amyloid-induced memory impairment by reduction of oxidative damages in Aβ1-42-infused Alzheimer's disease mouse model.[5a,f,g] Moreover, 4-*O*-methylhonokiol was shown to have the higher anti-inflammatory activity

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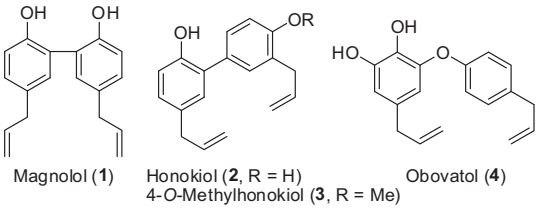


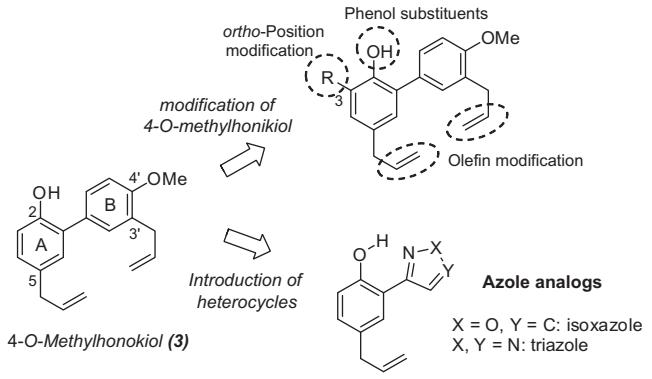
Figure 1. The structure of representative natural neolignans from *Magnolia* family.

(e.g., IC_{50} of 0.06 μM for COX-2) than honokiol (e.g., IC_{50} of 2.1 μM for COX-2) and a variety of honokiol analogs.[6] As 4-*O*-methylhonokiol is the attractive research candidate for the drug discovery against Alzheimer's disease and other inflammatory conditions such as rheumatoid arthritis (RA) and osteoarthritis (OA), we recently reported the efficient synthesis of 4-*O*-methylhonokiol.[7] Now this article deals with the structure–activity relationship (SAR) study aiming at identifying novel COX-2 selective 4-methylhonokiol analogs via modifying the potential metabolic soft spots[8] and increasing the polar surface area for the future assessment of structure–property relationship.

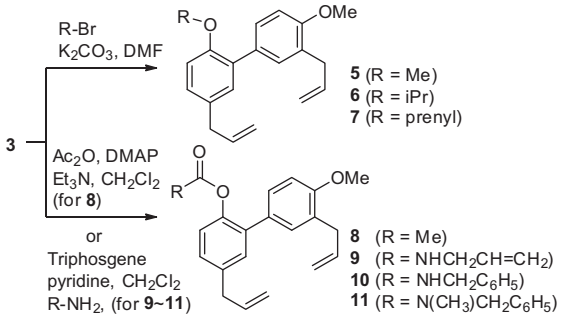
2. Result and discussion

4-*O*-Methylhonokiol consists of a 5,3′-diallyl-biphenyl skeleton bearing hydroxy group in C2 of A ring and methoxy group in C4 of B ring. The major route of metabolism in vivo for both **2** and **3** is via glucuronidation of the phenol group.[8] Thus we were interested in modification of the phenol group on A ring as well as the introduction of electron-withdrawing substituents at *ortho*-position of phenol group, which could reduce phase II metabolism (Scheme 1). Both of allyl groups would be replaced with appropriate surrogates in order to slow metabolic degradation while retaining potency at the same time. In addition, hydrogen bond of the phenol group on A ring with neighboring moiety was expected to lower a metabolic change. Consideration of other potential substituents that would fit the structural criteria required for biological activity led to the possibility of employing azole moieties, such as isoxazole and triazole.

Our synthesis of 4-*O*-methylhonokiol analogs commenced with the modification in three different ways; (1) the alkylation and acylation of phenol group in A ring, (2) *ortho*-halogenation of phenol group, and (3) olefin modification for the synthesis of more polar compounds. Thus alkyl phenyl ether (**5–7**) was obtained by treatment of the alkyl halide and potassium carbonate in DMF in good yield (Scheme 2).[9] Acetate **8** was prepared by acetic anhydride, Et_3N and DMAP.[9] For the synthesis of aryl carbamates **9–11**, **3** was treated with triphosgene and pyridine in CH_2Cl_2,



Scheme 1. Structure of 4-*O*-methylhonokiol (**3**) and schematic design of its analogs.

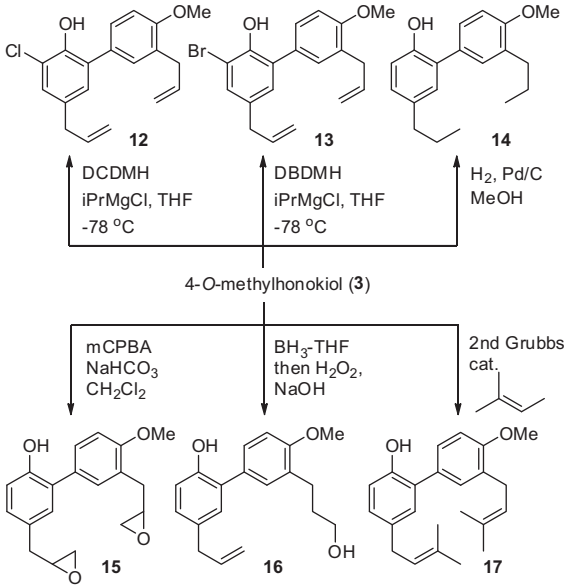


Scheme 2. Synthesis of alkyl ethers, acetate and aryl carbamates analogs.

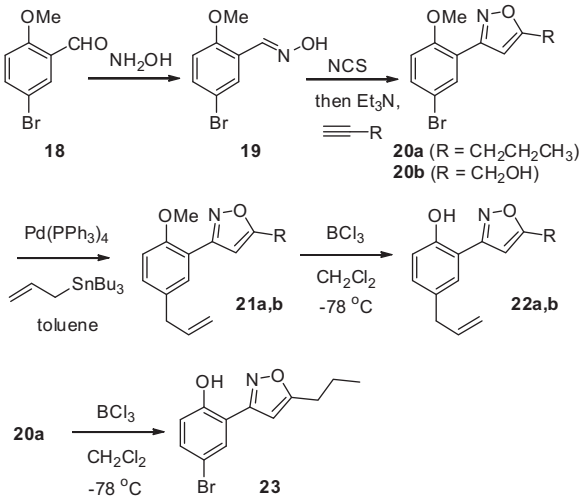
followed by the addition of allylamine, benzylamine, and *N*-benzymethylamine to give the corresponding aryl carbamates, respectively.[10]

Chloride **12** and bromide **13** were obtained by the *ortho*-halogenation of phenol in which DCDMH (1,3-dichloro-5,5-dimethylhydantoin) and DBDMH (1,3-dibromo-5,5-dimethyl-hydantoin) were used to serve as chloride and bromide source after the phenoxide of 4-*O*-methylhonokiol was generated with *i*PrMgCl (Scheme 3).[11] Two double bonds of **3** could be modified in terms of increasing the biological activity as well as the metabolic stability and/or the proper polarity (Scheme 3, compounds **14**–**17**). Thus, compounds **14** and **15** were obtained by hydrogenation and *m*-CPBA epoxidation, respectively. For the synthesis of alcohol **16** we performed hydroboration and subsequent oxidation to give two regioisomers. For diprenyl compound **17**, two allyl groups were smoothly converted to prenyl groups by cross metathesis using 2nd generation Grubbs catalyst and 2-methyl-2-butene in good yield.[12]

We chose five-member heterocyclic ring, especially isoxazole and triazole in replace of a phenyl ring of 4-*O*-methylhonokiol to be endowed with higher solubility and less reactivity of phenol in A ring through hydrogen bond with nitrogen of azole ring. In this connection, isoxazoles were synthesized from commercially available 5-bromo-2-methoxy-benzaldehyde **18** which was treated with hydroxylamine to give oxime **19** (Scheme 4). Isoxazoles **20a** and **20b** were prepared by cyclization of either 1-pentyne or



Scheme 3. *Ortho*-Halogenation of phenol group and olefin modification.

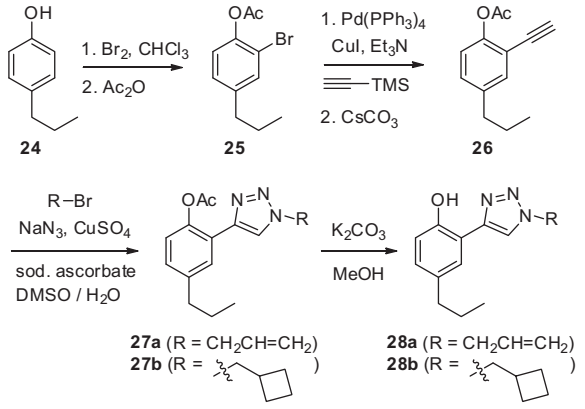


Scheme 4. Synthesis of isoxazole analogs of 4-*O*-methylhonokiol.

propargyl alcohol with oxime **19** in the presence of NCS and Et_3N, respectively.[13] Stille cross-coupling of compounds **20a** and **20b** with allyltributyltin and $Pd(PPh_3)_4$, followed by demethylation with BCl_3 provided isoxazole analogs **22a** and **22b**. In a similar manner, **20a** was treated with BCl_3 to give the resulting phenol **23**.

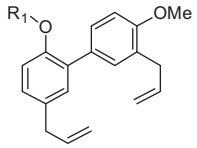
For the synthesis of 1,2,3-triazole analogs, one-pot method of Cu(I)-catalyzed azide–alkyne 1,3-dipolar cycloaddition was utilized[14] and thus 2-ethynylphenol (for A ring) and alkyl azide (for B ring) are required to access diverse 1,2,3-triazoles compounds (Scheme 5). 4-Propylphenol **24** could readily be converted into 2-ethynyl-4-propylphenyl acetate **26** by *ortho*-bromination and acetylation of phenol, followed by Sonogashira coupling reaction with ethynyltrimethylsilane, $Pd(PPh_3)_4$, and CuI. The formation of 1,2,3-triazole from compound **26** was accomplished by using $CuSO_4$, sodium ascorbate and an alkyl azide which was prepared in situ by the treatment of either allyl bromide or (bromomethyl)cyclobutane with NaN_3. Finally, the acetates **27a** and **27b** were hydrolyzed to afford the resulting 1,2,3-triazole compounds **28a** and **28b**, respectively.

The in vitro inhibitory activities of cyclooxygenase-2 (COX-2) enzyme and PGF_1 production were listed in Tables 1–5. Celecoxib and 4-*O*-methylhonokiol (**3**) were used as reference compounds. Inhibition of COX-2 enzyme by 100 nM of synthesized compounds was evaluated by using COX inhibitor screening kit. All of compounds showed the direct inhibition of COX-2 enzyme with the range of 22–65% inhibition at 100 nM treatment. Especially, mod-



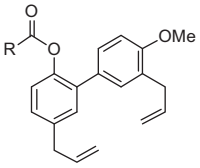
Scheme 5. Synthesis of 1,2,3-triazole analogs 4-*O*-methylhonokiol.

Table 1
Inhibitory activity of COX-2 enzyme and PGF_1 production in macrophages by 100 nM of the alkylated derivatives (**5–7**)



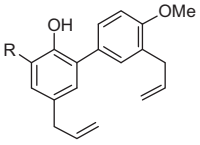
	R	COX-2 enzyme (%)	PGF_1 production (%)
Celecoxib	—	60	75
3	H	57	42
5	CH_3	46	48
6	$CH(CH_3)_2$	23	24
7	$CH_2CH{=}C(CH_3)_2$	44	19

Table 2
Inhibitory activity of COX-2 enzyme and PGF_1 production in macrophages by 100 nM of the acylated derivatives (**8–11**)



	R	COX-2 enzyme (%)	PGF_1 production (%)
8	CH_3	39	45
9	$NHCH_2CH{=}CH_2$	48	41
10	$NHCH_2C_6H_5$	65	63
11	$N(CH_3)CH_2C_6H_5$	63	41

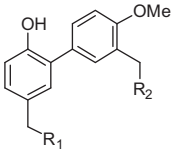
Table 3
Inhibitory activity of COX-2 enzyme and PGF_1 production in macrophages by 100 nM of the halogenated derivatives (**3–7**)



	R	COX-2 enzyme (%)	PGF_1 production (%)
12	Cl	ND[a]	52
13	Br	52	54

[a] ND, not determined

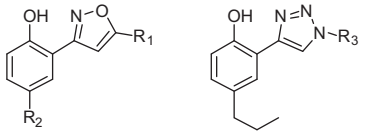
Table 4
Inhibitory activity of COX-2 enzyme and PGF_1 production in macrophages by 100 nM of the olefin-modified derivatives (**14–17**)



	R_1 and R_2	COX-2 enzyme (%)	PGF_1 production (%)
14	$R_1 = CH_2CH_3$ $R_2 = CH_2CH_3$	46	55
15	R_1 = oxiranyl R_2 = oxiranyl	58	51
16	$R_1 = CH{=}CH_2$ $R_2 = CH_2CH_2OH$	ND	61
17	$R_1 = CH{=}C(CH_3)_2$ $R_2 = CH{=}C(CH_3)_2$	41	42

a ND, not determined

Table 5
Inhibitory activity of COX-2 enzyme and PGF_1 production in macrophages by 100 nM of the isoxazole and triazole analogs



	R_1/R_2 or R_3	COX-2 enzyme (%)	PGF_1 production (%)
22a	$R_1 = CH_2CH_2CH_3$ $R_2 = CH_2CH{=}CH_2$	39	54
22b	$R_1 = CH_2OH$ $R_2 = CH_2CH{=}CH_2$	38	49
23	$R_1 = CH_2CH_2CH_3$ $R_2 = Br$	35	50
28a	$R_3 = CH_2CH{=}CH_2$	22	29
28b	R_3 = cyclobutylmethyl	33	43

ifications of 4-*O*-methylhonokiol by introducing methyl, isopropyl and prenyl group at phenol slightly decrease the inhibiting activity of COX-2 enzyme and PGF_1 production (Table 1). Both of **10** and **11** which contain a benzyl carbamate group in A ring showed slightly more potent inhibitory activity (65% and 63% inhibition, respectively) than celecoxib (60%) and 4-*O*-methylhonokiol (57%) (Table 2). When RAW 264.7 cells were treated with LPS in the presence of 100 nM analogs for 24 h and the concentrations of PGF_1 were measured, all of compounds showed the inhibitory activities with the range of 19–63% inhibition. There is no marked difference between COX-2 and PGF_1 production inhibition.

Replacing phenyl ring (B ring) with azole rings as a hydrogen bond acceptor to the potential metabolic soft spots phenol group (A ring) had the similar activity to 4-*O*-methyl-honokiol (Table 4). As the results, the identification of lead compounds for new anti-inflammatory agents from the current synthesized analogs would be achieved through in vitro/in vivo DMPK (drug metabolism and pharmacokinetic) studies.

The in vitro inhibitory activities of nitric oxide (NO) production and the cell viability were listed in Table 6. The synthesized analogs were evaluated for their ability to inhibit LPS-activated NO production by RAW264.7 macrophage cell line. Cell viability was examined by the propidium iodide (PI) nuclear staining method. Most of all show the inhibition of macrophage NO production at the IC_{50} value of 10–100 μM, while they have the cytotoxicity at the similar concentration (Table 6). Thus, it was noted that 4-*O*-methylhonokiol analogs were not able to inhibit NO production selectively without cytotoxicity in macrophage. In terms of structure–cytotoxicity relationship, 4-*O*-methylhonokiol analogs having the free phenol group showed more cytotoxicity than alkylated or acylated compounds.

Table 6
Inhibitory activity of compounds on macrophage NO production (IC_{50}, μM) and the cell viability (IC_{50}, μM)

Compounds	NO production	Viability
Celecoxib	12,090	11,130
3	18	27
5	40	>100
6	78	>100
7	>100	>100
8	24	29
9	>100	>100
10	>100	>100
11	33	44
12	40	81
13	28	33
14	11	13
15	24	64
16	41	53
17	13	15
22a	61	81
22b	36	>50
23	63	63
28a	>50	>50
28b	33	>50

3. Materials and methods

3.1. Synthetic procedures

All starting materials and reagents were obtained from commercial suppliers and were used without further purification. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using Silica Gel 60 (230–400 mesh, Merck) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). ^1H and ^{13}C NMR spectra were recorded on either a Bruker AVANCE III 400 MHz, Bruker AVANCE 500 MHz, or a JEOL JNM-EX400 (400 MHz) spectrometer as solutions in deuteriochloroform ($CDCl_3$). ^1H NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet and/or multiple resonances), number of protons, and coupling constant (*J*) in hertz (Hz).

3.1.1. 3′,5-Diallyl-2,4′-dimethoxy-1,1′-biphenyl (5)

To a DMF solution of 4-*O*-methylhonokiol (50 mg, 0.18 mmol) and potassium carbonate (73 mg, 0.53 mmol) was added at ambient temperature. After stirring for 30 min, methyl iodide (75 mg, 0.53 mmol) was added. After stirring for 3 h, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:8) to afford the methyl ether **5** (51 mg, 98%); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.37 (dd, 1H, J = 8.3, 2.2 Hz), 7.31 (ds, 1H, J = 2.2 Hz), 7.12 (ds, 1H, J = 2.1 Hz), 7.10 (dd, 1H, J = 8.2, 2.3 Hz), 6.90 (d, 2H, J = 8.3 Hz), 6.08–5.93 (m, 2H), 5.12–5.02 (m, 4H), 3.86 (s, 3H), 3.79 (s, 3H), 3.43 (d, 2H, J = 6.6 Hz), 3.37 (d, 2H, J = 6.7 Hz); the compound **5** was reported. See Ref. 7.

3.1.2. 3′,5-Diallyl-2-isopropoxy-4′-methoxy-1,1′-biphenyl (6)

To a DMF solution of 4-*O*-methylhonokiol (30 mg, 0.11 mmol) potassium carbonate (44 mg, 0.32 mmol) was added at ambient temperature. After stirring for 30 min, 2-bromopropane (220 mg, 0.16 mmol) was added. After stirring for 4 h, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:25) to afford the isopropyl ether product **6** (21 mg, 60%).; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, 1H, J = 2.3 Hz), 7.38 (dd, 1H, J = 8.3 and 2.3 Hz), 7.14 (ds, 1H, J = 2.3 Hz), 7.05 (dd, 1H, J = 8.3 and 2.3 Hz), 6.90 (d, 1H, J = 8.3 Hz), 6.88 (d, 1H, J = 8.3 Hz), 6.10–5.93 (m, 2H), 5.13–5.03 (m, 4H), 4.45–4.34 (m, 1H), 3.87 (s, 3H), 3.43 (d, 2H, J = 6.7 Hz), 3.37 (d, 2H, J = 6.7 Hz), 1.24 (d, 6H, J = 6.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.3, 153.3, 138.0, 137.2, 132.7, 131.9, 131.4, 131.3, 131.2, 128.3, 127.9, 127.8, 115.8, 115.6, 115.5, 110.0, 71.1, 55.6, 39.6, 34.4, 22.3; IR (neat): 2975, 1638, 1488, 1242, 811 cm^{-1}; LRMS (ESI) m/z 323.1 ($M+H^+$).

3.1.3. 3′,5-Diallyl-4′-methoxy-2-((3-methylbut-2-en-1-yl)oxy)-1,1′-biphenyl (7)

To a DMF solution of 4-*O*-methylhonokiol (56 mg, 0.20 mmol) potassium carbonate (55 mg, 0.40 mmol) was added at ambient

temperature. After stirring for 30 min, 3,3-dimethylallyl bromide (47 mg, 0.32 mmol) was added. After stirring for 5 h, the reaction mixture was diluted with EtOAc and washed with 1 N HCl and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:10) to afford the 3,3-dimethylallyl ether **7** (60 mg, 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (m, 2H), 7.15 (d, 1H, J = 2.4 Hz), 7.09 (dd, 1H, J = 8.3 and 2.4 Hz), 6.93 (m, 2H), 6.05 (m, 2H), 5.45 (t, 1H, J = 2.3 Hz), 5.08 (m, 4H), 4.50 (d, 2H, J = 2.3 Hz), 3.87 (s, 3H), 3.44 (d, 2H, J = 6.3), 3.39 (d, 2H, J = 6.8), 1.81 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 154.1, 137.8, 137.0, 136.6, 132.3, 131.1 130.88, 130.84, 130.7, 128.1, 127.77, 127.72, 120.3, 115.4, 115.2, 113.3, 109.8, 65.7, 55.4, 39.4, 34.2, 25.6, 18.1.

3.1.4. 3′,5-Diallyl-4′-methoxy-[1,1′-biphenyl]-2-yl acetate (8)

To a CH_2Cl_2 solution of 4-*O*-methylhonokiol (47 mg, 0.17 mmol) 4-dimethylaminopyridine (5 mg, 34 μmol), triethylamine (86 mg, 0.85 mmol), and acetic anhydride (20 mg, 0.20 mmol) was added at ambient temperature. After stirring for 20 min, the reaction mixture was treated with sodium bicarbonate, diluted with ethyl acetate and dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:10) to afford the acetate (54 mg, 93%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.24 (dd, 1H, J = 8.3, 2.2 Hz), 7.21 (ds, 1H, J = 2.2 Hz), 7.20 (ds, 1H, J = 2.1 Hz), 7.14 (dd, 1H, J = 8.2, 2.2 Hz), 7.02 (d, 1H, J = 8.2 Hz), 6.89 (d, 1H, J = 8.3 Hz), 6.06–5.93 (m, 2H), 5.14–5.02 (m, 4H), 3.86 (s, 3H), 3.42–3.39 (m, 4H), 2.09 (s, 3H); The compound **8** was reported. See Ref. 7.

3.1.5. 3',5-Diallyl-4′-methoxy-[1,1′-biphenyl]-2-yl allylcarbamate (9)

To a CH_2Cl_2 solution (1 mL) of 4-*O*-methylhonokiol (42 mg, 0.15 mmol) and pyridine (80 mg, 1 mmol) was added triphosgene (89 mg, 0.3 mmol) at 0 °C. After stirring for 2 h at ambient temperature, allylamine (20 mg, 0.35 mmol) was added to the reaction mixture. After stirring for 12 h at ambient temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with aqueous NH_4Cl solution and water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:6) to afford the allylcarbamate **9** (35 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (m, 2H), 7.13 (S, 1H), 7.07 (m, 2H), 6.85 (d, 1H, J = 8.7 Hz), 6.01 (m, 2H), 5.77 (m, 1H), 5.09 (m, 6H), 4.87 (b, 1H), 3.81 (S, 3H), 3.74 (t, 2H, J = 5.8) 3.37 (d, 4H, J = 6.3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 154.5, 146.1, 137.6, 137.1, 137.0, 134.4, 134.0, 130.7, 130.5, 129.9, 128.2, 127.9, 127.8, 123.0, 116.1, 116.0, 115.3, 110.1, 55.4, 43.5, 39.6, 34.2; IR (neat) 2931, 1720, 1200 cm^{-1}; LRMS (ESI) m/z 386.1 (M+Na$^+$).

3.1.6. 3′,5-Diallyl-4′-methoxy-[1,1′-biphenyl]-2-yl benzylcarbamate (10)

To a CH_2Cl_2 solution (1 mL) of 4-*O*-methylhonokiol (42 mg, 0.15 mmol) and pyridine (80 mg, 1.0 mmol) was added triphosgene (89 mg, 0.30 mmol) at 0 °C. After stirring for 2 h at ambient temperature, benzylamine (32 mg, 0.30 mmol) was added to the reaction mixture. After stirring for 12 h at ambient temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with aqueous NH_4Cl solution and water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:4) to afford the benzylcarbamate **10** (50 mg, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 6H), 7.13 (S, 1H), 7.11 (m, 4H), 6.80 (d, 1H, J = 8.2), 5.98 (m, 2H), 5.12 (m, 4H), 4.29 (d, 2H, J = 6.3), 3.81 (S, 3H), 3.37(d, 2H, J = 6.8), 3.33 (d, 2H, J = 6.3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 154.7, 146.1, 138.1, 137.7, 137.1, 136.9, 134.6, 130.7, 130.4, 129.9, 128.5, 128.2, 127.9, 127.8, 127.5, 127.4, 123.0, 116.0, 115.3, 110.1, 55.3, 45.1, 39.6, 34.2. IR (KBr) 3629, 3346, 2931, 1710, 1529, 1490, 1261, 1219 cm^{-1}; LRMS (ESI) m/z 436.1 (M+Na$^+$).

3.1.7. 3′,5-Diallyl-4′-methoxy-[1,1′-biphenyl]-2-yl benzyl(methyl)carbamate (11)

To a CH_2Cl_2 solution (1 mL) of 4-*O*-methylhonokiol (42 mg, 0.15 mmol) and pyridine (80 mg, 1 mmol) was added triphosgene (89 mg, 0.3 mmol) at 0 °C. After stirring for 2 h at ambient temperature, *N*-benzylmethylamine (36 mg, 0.3 mmol) was added to the reaction mixture. After stirring for 12 h at ambient temperature, the reaction mixture was diluted with CH_2Cl_2 and washed with aqueous NH_4Cl solution and water, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:3) to afford the benzylmethylcarbamate **11** (38 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (m, 5H), 7.13 (m, 3H), 7.00 (m, 2H), 6.80 (m, 1H), 5.99 (m, 2H), 5.09 (m, 4H), 4.41 and 4.35 (S6, 2H), (S6, 2H), 3.82 (S, 3H), 3.38 (m, 4H), 2.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.5, 155.0, 146.7, 137.5, 137.3, 137.2, 137.0, 136.8, 136.7, 130.8, 130.7, 130.4, 130.1, 128.4, 128.0, 127.9, 127.6, 127.4, 127.3, 123.0, 115.9, 115.4, 109.9, 55.3, 52.6, 39.5, 34.2, 33.8; IR (neat) 3341, 3074, 2911, 2836, 1719, 1504, 1484, 1245, 1200 cm^{-1}; LRMS (ESI) m/z 450.2 (M+Na$^+$).

3.1.8. 3′,5-Diallyl-3-chloro-4′-methoxy-[1,1′-biphenyl]-2-ol (12)

To a THF solution of 4-*O*-methylhonokiol (48 mg, 0.17 mmol) isopropyl magnesium chloride (2.0 M solution in diethyl ether, 0.20 mL, 0.10 mmol) was added at −78 °C dropwise. After stirring for 30 min at −78 °C, the reaction mixture was heated to ambient temperature for 10 min. DCDMH (27 mg, 0.14 mmol) was added to the reaction mixture. After stirring for 2 h, the reaction mixture was treated with aqueous NH_4Cl, extracted with ethyl acetate and dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:10) to afford the chloride **12** (17 mg, 32%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, 1H, J = 8.4 and 2.3 Hz), 7.31 (d, 1H, J = 2.3 Hz), 7.13 (d, 1H, J = 2.2 Hz), 7.01 (d, 1H, J = 2.2 Hz), 6.94 (d, 1H, J = 8.4 Hz), 6.08–5.89 (m, 2H), 5.57 (s, OH), 5.14–5.04 (m, 4H), 3.88 (s, 3H), 3.44 (d, 2H, J = 6.7 Hz), 3.33 (d, 2H, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.1, 146.8, 137.1, 136.8, 132.9, 130.7, 129.5, 129.4, 129.3, 129.0, 128.2, 127.9, 120.5, 116.3, 115.8, 110.5, 55.7, 39.3, 34.4; IR (neat) 3511, 1470, 1244, 814, 675 cm^{-1}; LRMS (ESI) m/z 313.2 (M–H$^+$)

3.1.9. 3′,5-Diallyl-3-bromo-4′-methoxy-[1,1′-biphenyl]-2-ol (13)

To a THF solution of 4-*O*-methylhonokiol (50 mg, 0.18) isopropyl magnesium chloride (2.0 M solution in diethyl ether, 0.11 mL, 0.22 mmol) was added at −78 °C dropwise. After stirring for 30 min at −78 °C, the reaction mixture was heated to ambient temperature for 10 min. DBDMH (41 mg, 0.14 mmol) was added to the reaction mixture. After stirring for 2 h, the reaction mixture was treated with aqueous NH_4Cl, extracted with ethyl acetate and dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:15) to afford the bromide **13** (42 mg, 66%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, 1H, J = 8.4 and 2.3 Hz), 7.29 (d, 1H, J = 2.3 Hz), 7.26 (d, 1H, J = 2.1 Hz), 7.03 (d, 1H, J = 2.1 Hz), 6.93 (d, 1H, J = 8.4 Hz), 6.07–5.88 (m, 2H), 5.55 (s, OH), 5.13–5.02 (m, 4H), 3.87 (s, 3H), 3.42 (d, 2H, J = 6.7 Hz), 3.32 (d, 2H, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 147.7, 137.1, 136.8, 133.5, 131.0, 130.7, 130.3, 129.4, 129.3, 129.1, 128.2, 116.4, 115.8, 110.7, 110.5, 55.7, 39.2, 34.4; IR (neat) 3506, 2922, 1465, 1245, 815, 614 cm^{-1}; LRMS (ESI) m/z 357.1 (M–H$^+$)

3.1.10. 4′-Methoxy-3′,5-dipropyl-[1,1′-biphenyl]-2-ol (14)

A solution of 4-*O*-methylhonokiol (30 mg, 0.11 mmol) and 10% Pd/C (10 mg) in anhydrous MeOH was placed under an atmosphere of hydrogen. After stirring for 1 h, the reaction mixture was diluted with EtOAc, filtered through Celite pad and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:13) to afford the hydrogenated compound **14** (30 mg, 98%) as a colorless oil.; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (dd, 1H, J = 8.3 and 2.3 Hz), 7.23 (d, 1H, J = 2.2 Hz), 7.05–7.03 (m, 2H), 6.95 (d, 1H, J = 8.3 Hz), 6.89(d, 1H, J = 7.9 Hz), 5.16 (s, 1H), 3.87 (s, 3H), 2.64 (t, 2H, J = 7.5 Hz), 2.55 (t, 2H, J = 7.5 Hz), 1.68–1.61 (m, 4H), 0.99–0.94 (m, 6H); The compound **14** was reported. See Ref. 7.

3.1.11. 4′-Methoxy-3′,5-bis(oxiran-2-ylmethyl)-[1,1′-biphenyl]-2-ol (15)

To a CH_2Cl_2 solution of 4-*O*-methylhonokiol (51 mg, 0.18 mmol), *m*-chloroperoxybenzoic acid (94 mg, 0.54 mmol) and $NaHCO_3$ (76 mg, 0.90 mmol) were added at −78 °C dropwise. After stirring for 1 day at ambient temperature, the reaction mixture was diluted with water, extracted with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the epoxide **15** (30 mg, 53%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (dd, 1H, J = 8.3 and 2.2 Hz), 7.30 (d, 1H, J = 2.2 Hz), 7.12–7.09 (m, 2H), 6.97 (d, 1H, J = 8.4 Hz), 6.91 (d, 1H, J = 8.0 Hz); The compound **15** was reported. See Ref. 7.

3.1.12. 5-Allyl-3′-(3-hydroxypropyl)-4′-methoxy-[1,1′-biphenyl]-2-ol (16)

To an anhydrous THF solution of BH_3–THF (1.0 M solution in THF, 0.68 mL, 0.68 mmol) a THF solution of 4-*O*-methylhonokiol (190 mg, 0.68 mmol) was added during 5 min at 0 °C. After stirring for 2 h at ambient temperature, the reaction mixture was treated with 3 N NaOH and H_2O_2 at −10 °C. After heated to 70 °C for 2 h, the reaction mixture was extracted with diethyl ether three times and dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:1) to afford the alcohol **16** (77 mg, 36%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29–7.27 (m, 2H), 7.24 (d, 1H, J = 2.2 Hz), 7.22 (d, 1H, J = 2.3 Hz), 7.06–7.02 (m, 4H), 6.95 (d, 2H, J = 8.3 Hz), 6.91–6.88 (m, 2H), 6.04–5.93 (m, 2H), 5.18 (s, OH), 5.11–5.03 (m, 4H), 3.88 (s, 3H), 3.87 (s, 3H), 3.69 (t, 2H, J = 6.5 Hz), 3.64 (t, 2H, J = 6.2 Hz), 3.42 (d, 2H, J = 6.7 Hz), 3.35 (d, 2H, J = 6.8 Hz), 2.76 (t, 2H, J = 7.3 Hz), 2.66 (t, 2H, J = 7.5 Hz), 1.91–1.85 (m, 4H); LRMS (ESI) m/z 321.1 (M+Na$^+$)

3.1.13. 4′-Methoxy-3′,5-bis(3-methylbut-2-en-1-yl)-[1,1′-biphenyl]-2-ol (17)

To a CH_2Cl_2 solution (0.5 mL) of 4-*O*-methylhonokiol (28 mg, 0.1 mmol) and 2-methyl-2-butene (1.2 mL) in sealed tube was added 2nd generation Grubbs' catalyst (4 mg, 4.7 μmol). After stirring for 24 h, the reaction mixture was concentrated and the residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:9) to afford the prenylated compound **17** (32 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 2H), 7.19 (m, 2H), 7.10 (d, 1H, J = 8.2 Hz), 7.04 (d, 1H, J = 7.8 Hz), 5.49 (m, 2H), 4.02 (s, 3H), 3.51 (d, 2H, J = 7.3 Hz), 3.45 (d, 2H J = 7.3 Hz), 1.88 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 150.5, 133.8, 132.9, 132.2, 131.1, 129.9, 129.8, 128.9, 128.3, 127.8, 127.4, 123.5, 121.9, 115.3, 110.7, 55.4, 33.4, 28.4, 25.8, 25.7, 17.7; IR (neat) 2965, 1691, 1499, 1246 cm^{-1}; LRMS (ESI) m/z 337.1 (M+H$^+$).

3.1.14. 5-Bromo-2-methoxybenzaldehyde oxime (19)

To a CH_2Cl_2 (50 mL) solution of 5-bromo-2-anisaldehyde (2.15 g, 10 mmol) and pyridine (5 mL), hydroxylamine HCl (764 mg, 11 mmol) was added. After the reaction mixture was refluxed for 12 h, it was concentrated to a half of volume under reduced pressure. The mixture was poured to ice water and filtered using glass filter to afford the resulting oxime as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.76 (d, 1H, J = 2.4 Hz), 7.55 (br s, 1H), 7.37 (dd, 1H, J = 2.4 and 8.8 Hz), 6.72 (d, 1H, J = 8.8 Hz), 3.77 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 157.6, 144.5, 134.1, 129.1, 124.4, 114.1, 113.8, 56.3. IR (KBr) 3345, 1487, 1460, 1258, 968, 807 cm^{-1}; LRMS (ESI) m/z 229.9 (M+H$^+$)

3.1.15. 3-(5-Bromo-2-methoxyphenyl)-5-propylisoxazole (20a)

To a $CHCl_3$ (1 mL) solution of the crude aldoxime **19** (92 mg, 0.4 mmol) NCS (58 mg, 0.44 mmol) was added at 0 °C. After stirring for 3 h, pentyne (40 mg, 0.6 mmol) and triethylamine (0.11 mL, 0.8 mmol) was added to the solution and heated to 50 °C. After stirring for 12 h, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:10) to afford the isoxazole **20a** (75 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, 1H, J = 2.4 Hz), 7.44 (dd, 1H, J = 8.8 and 2.9 Hz), 8.83 (d, 1H, J = 8.8 Hz), 6.43 (s, 1H), 3.84 (s, 3H), 2.75 (t, 2H, J = 7.8 Hz), 1.79 (m, 2H), 1.00 (t, 3H, J = 7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 158.7, 156.2, 133.3, 131.8, 120.2, 113.1, 112.9, 102.0, 55.8, 28.6, 20.9, 13.6; IR (neat) 3742, 3680, 3621, 2371, 2317, 1741, 1691, 1514 cm^{-1}; LRMS (ESI) m/z 318.0 (M+Na$^+$).

3.1.16. (3-(5-Bromo-2-methoxyphenyl)isoxazol-5-yl)methanol (20b)

To a $CHCl_3$ (2 mL) solution of aldoxime **19** (164 mg, 0.76 mmol) NCS (120 mg, 0.9 mmol) was added at 0 °C. After stirring for 3 h, propargyl alcohol (84 mg, 1.5 mmol) and triethylamine (0.26 mL, 1.9 mmol) was added to the solution and heated to 50 °C. After stirring for 12 h, the reaction mixture was diluted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexanes = 1:2) to afford the isoxazole **20b** (90 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, 1H, J = 2.4 Hz), 7.49 (dd, 1H, J = 9.3, 2.9 Hz), 6.86 (d, 1H, J = 9.3 Hz), 6.71 (s, 1H), 4.80 (d, 2H, J = 5.4 Hz), 3.85 (s, 3H), 2.20 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 158.9, 156.2, 133.7, 131.9, 119.6, 113.1, 103.3, 56.6, 55.8, 29.6. IR (KBr) 3394, 2940, 2850, 1605, 1504, 1460, 1268, 1030, 813 cm^{-1}; LRMS (ESI) m/z 305.9 (M+Na$^+$).

3.1.17. 3-(5-Allyl-2-methoxyphenyl)-5-propylisoxazole (21a)

To a DMF (1 mL) solution of the bromide **20a** (46 mg, 0.16 mmol) and allyltributyltin (103 mg, 0.31 mmol) tetrakis(triphenylphosphine)palladium(0) (10 mg, 7.8 μmol) was added. After stirring for 5 h at 90 °C, the reaction mixture was cooled to ambient temperature, diluted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:10) to afford the isoxzaole **21a** (30 mg, 71%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, 1H, J = 2.6 Hz), 7.22 (dd, 1H, J = 2.4 and 8.2 Hz), 6.93 (d, 1H, J = 8.8 Hz), 6.47 (s, 1H), 6.01 (m, 1H), 5.10 (m, 2H), 3.87 (s, 3H), 3.37 (d, 2H, J = 6.3 Hz), 2.78 (t, 2H, J = 7.3 Hz), 1.82(m, 2H), 1.04 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 159.9, 155.6, 137.4, 132.4, 130.8, 129.5, 118.2, 115.7, 111.5, 102.2, 55.6, 39.2, 28.7, 20.9, 13.7. IR (KBr) 3425, 2965, 2933, 1736, 1604, 1512, 1468, 1380, 1254 cm^{-1}; LRMS (ESI) m/z 296.0 (M+K$^+$).

3.1.18. (3-(5-Allyl-2-methoxyphenyl)isoxazol-5-yl)methanol (21b)

To a DMF (1 mL) solution of the isoxazole **20b** (80 mg, 0.3 mmol) and allyltributyltin (148 mg, 0.45 mmol) tetrakis-(triphenylphosphine)palladium(0) (17 mg, 15 μmol) was added. After stirring for 5 h at 90 °C, the reaction mixture was cooled to ambient temperature, diluted with ethyl acetate and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the isoxazole **21b** (60 mg, 82%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, 1H, J = 1.9 Hz), 7.24 (dd, 1H, J = 8.8 and 2.4 Hz), 6.94 (d, 1H, J = 8.8 Hz), 6.75 (s, 1H), 6.00 (m, 1H), 5.10 (m, 2H), 4.81 (br s, 2H), 3.81 (s, 3H), 3.37 (d, 2H, J = 6.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 160.1, 155.6, 137.3, 132.5, 131.2, 129.5, 117.6, 115.8, 111.5, 103.5, 56.6, 55.6, 39.1. IR (KBr) 3404, 2956, 2845, 1649, 1406, 1033 cm^{-1}; LRMS (ESI) m/z 268.0 (M+Na$^+$).

3.1.19. 4-Allyl-2-(5-propylisoxazol-3-yl)phenol (22a)

To a CH_2Cl_2 (2 mL) solution of the isoxazole **21a** (70 mg, 0.27 mmol) boron tribromide (1.0 M solution of CH_2Cl_2, 680 μL, 0.68 mmol) was added at −78 °C. After stirring for 1 h, the reaction mixture was heated to ambient temperature, quenched with methanol, and stirred for 30 min. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:15) to afford the phenol **22a** (40 mg, 61%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.47 (br s, 1H), 7.27 (d, 1H, J = 1.9 Hz), 7.16 (dd, 1H, J = 8.3 and 2.4 Hz), 7.01 (d, 1H, J = 8.3 Hz), 6.4 (s, 1H), 6.01 (m, 1H), 5.10 (m, 2H), 3.37 (d, 2H, J = 6.8 Hz) 2.81 (t, 2H, J = 7.3 Hz) 1.84 (m, 2H), 1.05 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 162.4, 154.9, 137.4, 131.7, 131.7, 130.9, 127.5, 117.3, 115.7, 113.2, 98.4, 39.1, 28.3, 20.8, 13.5; LRMS (ESI) m/z 244.1 (M+H$^+$) and 266.1 (M+Na$^+$).

3.1.20. 4-Allyl-2-(5-(hydroxymethyl)isoxazol-3-yl)phenol (22b)

To a CH_2Cl_2 (2 mL) solution of the isoxazole **21b** (55 mg, 0.22 mmol) boron trichloride (1.0 M solution of CH_2Cl_2, 880 μL, 0.88 mmol) was added at −78 °C. After stirring for 1 h, the reaction mixture was heated to ambient temperature, quenched with methanol, and stirred for 30 min. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the phenol **22b** (35 mg, 69%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (s, 1H), 7.27 (d, 1H, J = 1.9 Hz), 7.18 (dd, 1H, J = 8.8 and 2.4 Hz), 7.01 (d, 1H, J = 8.3 Hz), 6.66 (s, 1H), 6.00 (m, 1H), 5.10 (m, 2H), 4.84 (s, 2H), 3.36 (d, 2H, J = 6.8 Hz), 2.37 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 162.5, 154.8, 137.3, 132.1, 131.2, 127.7, 117.4, 115.9, 112.8, 99.6, 56.5, 39.1; LRMS (ESI) m/z 254.0 (M+Na$^+$).

3.1.21. 4-Bromo-2-(5-propylisoxazol-3-yl)phenol (23)

To a CH_2Cl_2 (2 mL) solution of the isoxazole **20a** (30 mg, 0.10 mmol) boron trichloride (1.0 M solution of CH_2Cl_2, 300 μL, 0.30 mmol) was added at −78 °C. After stirring for 1 h, the reaction mixture was heated to ambient temperature, quenched with methanol, and stirred for 30 min. The organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:15) to afford the phenol **23** (18 mg, 64%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (s, 1H), 7.58 (d, 1H, J = 2.4 Hz), 7.41 (dd, 1H, J = 2.4 and 8.7 Hz), 6.97 (d, 1H, J = 8.8 Hz), 6.37 (s, 3H), 2.82 (t, 2H, J = 7.8 Hz), 1.8 (m, 2H), 1.05 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 161.3, 155.7, 133.9, 130.1, 119.3, 115.3, 111.3, 98.4, 28.4, 20.8, 13.6; LRMS (ESI) m/z 281.0 (M+H$^+$).

3.1.22. 2-Bromo-4-propylphenyl acetate (25)

To a $CHCl_3$ (13 mL) solution of 4-propylphenol (1.36 g, 10 mmol) bromine (10 g, 7.8 mmol) was added at ambient temperature. After stirring for 12 h, the reaction mixture was quenched with aqueous $NaHSO_3$. The reaction mixture was extracted with ethyl ether, washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude bromide compound. To a CH_2Cl_2 (20 mL) solution of the crude 2-bromo-4-phenol (ca. 2.15 g, 10 mmol) triethylamine (2.8 mL, 20 mmol) and acetic anhydride (1.4 mL, 15 mmol) were added at 0 °C. After stirring for 2 h at ambient temperature, the reaction mixture was diluted with CH_2Cl_2 and the organic phase was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:12) to afford the acetate **25** (2.2 g, 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, 1H, J = 2.4 Hz), 7.11 (dd, 1H, J = 1.9 and 8.3 Hz), 7.00 (d, 1H, J = 7.8 Hz), 2.55 (t, 2H, J = 7.3 Hz), 2.32 (s, 3H), 1.65 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 145.9, 142.1, 132.9, 128.3, 123.1, 115.6, 36.9, 24.2, 20.6, 13.6. IR (KBr) 3424, 2965, 1773, 1491, 1372, 1191 cm^{-1}; LRMS (ESI) m/z 278.9 (M+Na$^+$).

3.1.23. 4-Propyl-2-((trimethylsilyl)ethynyl)phenyl acetate

To a triethylamine (12 mL) solution of the bromide **25** (1.26 g, 4.9 mmol) and ethynyltrimethylsilane (727 mg, 7.4 mmol), bis(triphenylphosphine)palladium(II) dichloride (172 mg, 0.25 mmol) and CuI (48 mg, 0.25 mmol) were added. After stirring for 5 h at 80 °C, the reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:15) to afford the TMS-acetylene compound (1.09 g, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, 1H, J = 1.9 Hz), 7.14 (m, 1H), 7.02 (d, 1H, J = 8.3 Hz), 2.57 (dd, 2H, J = 15 and 7.8 Hz), 2.31 (s, 3H), 1.65 (m, 2H), 0.96 (dd, 3H, J = 15 and 6.8 Hz), 0.23 (s, 9H).

3.1.24. 2-Ethynyl-4-propylphenyl acetate (26)

To an acetonitrile (5 mL) solution of the TMS-acetylene compound (506 mg, 1.85 mmol) aqueous solution (1 mL) of Cs_2CO_3 (60 mg, 0.18 mmol) was added. After stirring for 2 h at ambient temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (diethyl ether/hexanes = 1:10) to afford the acetate **26** (220 mg, 59%) and the deacetylated compound (74 mg, 25%); For the acetate, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, 1H, J = 1.9 Hz), 7.12 (dd, 1H, J = 4.4 and 1.9 Hz), 6.97 (d, 1H, J = 8.2 Hz), 2.57 (dd, 2H, J = 15 and 7.8 Hz), 2.34 (s, 3H), 1.63 (m, 2H), 0.96 (dd, 3H, J = 15 and 6.8 Hz). For the deacetylated compound, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, 1H, J = 2.4 Hz), 7.07 (d, 1H, J = 8.3 Hz), 6.85 (d, 1H, J = 8.3 Hz), 5.63 (s, 1H), 3.43 (s, 1H), 2.50 (t, 3H, J = 7.3 Hz), 1.59 (m, 2H), 0.96 (t, 3H, J = 7.3 Hz).

3.1.25. 2-(1-Allyl-1*H*-1,2,3-triazol-4-yl)-4-propylphenyl acetate (27a)

A DMSO (2 mL) solution of allyl bromide (145 mg, 1.2 mmol) and sodium azide (78 mg, 1.2 mmol) was added to sealed tube. After stirring for 12 h at ambient temperature, aqueous solution (0.5 mL) of sodium ascorbate (25 mg, 0.1 mmol), $CuSO_4{\cdot}5H_2O$ (16 mg, 0.05 mmol) and the acetylene **26** (200 mg, 1 mmol) were added. After stirring for 1 h at ambient temperature and for 12 h at 70 °C, the reaction mixture was cooled to ambient temperature, quenched with cold 1 N NH_4OH solution (not to generate explosive CuN_3), and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (ethyl acetate/hexanes = 1:2) to afford the triazole **27a** (148 mg, 62%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, 1H, J = 1.9 Hz), 7.73 (s,

1H), 7.18 (dd, 1H, J = 8.2 and 2.4 Hz), 7.07 (d, 1H, J = 8.3 Hz), 6.09 (m, 1H), 5.39 (d, 1H, J = 10.2 Hz), 5.35 (d, 1H, J = 17.0 Hz), 5.03 (d, 2H, J = 6.3 Hz), 2.64 (t, 2H, J = 7.8 Hz), 2.3 (s, 3H), 1.72 (m, 2H), 0.97 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 145.1, 143.5, 140.9, 131.3, 129.0, 128.4, 122.8, 122.6, 121.3, 120.1, 52.6, 37.4, 24.4, 21.2, 13.8. IR (KBr) 3417, 2962, 2935, 2873, 1767, 1733, 1497, 1374, 1192 cm^{-1}; LRMS (ESI) m/z 308.1 (M+Na$^+$).

3.1.26. 2-(1-Allyl-1*H*-1,2,3-triazol-4-yl)-4-propylphenol (28a)

To a MeOH (1 mL) solution of the triazole **27a** (18 mg, 0.06 mmol) K_2CO_3 (20 mg, 0.15 mmol) was added. After stirring for 2 h at 50 °C, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (diethyl ether/hexanes = 1:1) to afford the triazole **28a** (10 mg, 68%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.6 (br s, 1H), 7.84 (s, 1H), 7.20 (d, 1H, J = 2.0 Hz), 7.06 (dd, 1H, J = 2.4 and 8.3 Hz), 6.98 (d, 1H, J = 8.3 Hz), 6.12 (m, 1H), 5.44–5.36 (m, 2H), 5.06–5.04 (m, 2H), 2.54 (t, 2H, J = 7.8 Hz), 1.66–1.57 (m, 2H), 0.95 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 137.1, 122.5, 119.7, 118.9, 114.4, 109.7, 107.5, 106.4, 102.4, 42.0, 26.1, 13.7, 27.3; LRMS (ESI) m/z 244.1 (M+H$^+$).

3.1.27. 2-(1-(Cyclobutylmethyl)-1*H*-1,2,3-triazol-4-yl)-4-propylphenol (28b)

A DMSO (1 mL) solution of (bromomethyl)cyclobutane (90 mg, 0.6 mmol) and sodium azide (40 mg, 0.6 mmol) was added to sealed tube. After stirring for 12 h at ambient temperature, aqueous solution (0.5 mL) of sodium ascorbate (13 mg, 0.05 mmol), $CuSO_4{\cdot}5H_2O$ (8 mg, 0.03 mmol) and the acetylene **26** (100 mg, 0.5 mmol) were added. After stirring for 1 h at ambient temperature and for 12 h at 70 °C, the reaction mixture was cooled to ambient temperature, quenched with cold 1 N NH_4OH solution(not to generate explosive CuN_3), and extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was used without further purification. To a MeOH (1 mL) solution of crude triazole compound K_2CO_3 (15 mg, 0.1 mmol) was added. After stirring for 2 h at 50 °C, the reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (diethyl ether/hexanes=1:1) to afford the phenol **28b** (90 mg, 67%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.6 (br s, 1H), 7.78 (s, 1H), 7.20 (d, 1H, J = 2.4 Hz), 7.05 (dd, 1H, J = 2.4 and 8.3 Hz), 6.97 (d, 1H, J = 8.3 Hz), 4.44 (d, 2H, J = 7.8 Hz), 2.93 (m, 1H), 2.55 (m, 2H), 2.17–2.10 (m, 2H), 2.04–1.81 (m, 4H), 1.66–1.56 (m, 2H), 0.95 (t, 3H, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.5, 147.6, 133.5, 129.7, 125.4, 118.5, 117.3 117.2, 55.5, 37.1, 35.4, 25.7, 24.7, 17.9, 13.7; LRMS (ESI) m/z 272.1 (M+Na$^+$).

3.2. Biologic assays

3.2.1. Inhibitory activity of COX-2 enzyme and PGF1 production in macrophages

Inhibition of cyclooxygenase-2 (COX-2) enzyme was performed by using COX inhibitor screening kit (Cat # 560131, Cayman Chemical Company, Ann Arbor, MI, USA). This kit directly measures prostaglandin $F_{1/2}$ produced by $SnCl_2$ reduction of COX-derived PGH_2 produced in the COX reaction. All procedures were performed as indicated in the assay procedures. Briefly, the reaction buffer (Tris–HCl buffer, pH 8.0, containing 5 mM EDTA and 2 mM phenol) and heme were placed in test tubes. The samples and recombinant human COX-2 were added to test tubes and pre-incubated for 10 min at 37 °C. After the substrate arachidonic acid was added, the test tubes were incubated for 2 min at 37 °C. The concentration of PGF_2 was measured using the EIA kit.[7] Inhibition of PGF_1 production in cells was determined by using RAW 264.7 cells. Cells were treated with LPS in the presence of compounds for 24 h. The concentration of PGF_1 in culture media was measured using EIA kit. COX-2 inhibitor celecoxib were used as positive control. Compounds were dissolved in DMSO and were tested in at least three independent experiments. Results were given as 50% inhibition concentration (IC_{50}) or means (GraphPad Software, San Diego, CA, USA).

3.2.2. Inhibitory activity on macrophage NO production and the cell viability

The bioassay for inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-treated RAW264.7 macrophage cell lines was carried out by Griess assay.[15] RAW 264.7 macrophages were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's media supplemented with heat-inactivated 10% fetal bovine serum (FBS), benzylpenicillin potassium (143 U/ml) and streptomycin sulfate (100 μg/ml) under 37 °C and 5% CO_2 atmosphere. The cells were stimulated with LPS (1 g/ml) for 24 h in the presence or absence of compounds. Aliquots of the culture media were reacted with the same volume of 0.05% sulfanilamide and 0.05% *N*-(1-naphthyl)ethylenediamine, and then absorbance values at 540 nm were measured. Sodium nitrite used to make the standard curve. Cell viability was examined by the propidium iodide (PI) nuclear staining method. Cells were stained with 1 μg/ml of PI and analyzed with a FACSCanto flow cytometer (BD Biosciences, San Jose, CA, USA). Cells stained with PI were considered dead cells.[16]

4. Conclusion

18 compounds based on 4-*O*-methylhonokiol were designed and synthesized in order to improve metabolic stabilization and anti-inflammatory activity. They were synthesized through the direct modification of 4-*O*-methylhonokiol in three different ways that are phenol direct modification of A ring, *ortho*-halogenation of phenol group and olefin modification. Azole analogs such as isoxazole and triazole were replaced with B ring in order to give higher solubility and less reactivity of phenol in A ring through hydrogen bond with nitrogen of the azole rings. The inhibitory effects of the newly synthesized compounds on COX-2 and PGF_1 production were evaluated with the goal of identifying new anti-inflammatory agents. We found that aryl carbamates (**10** and **11**) exhibited a potent inhibitory activity against the production of COX-2 enzyme and the inflammatory modulator PGF_1 and could serve as new therapeutic agents for the treatment of diseases mediated by PGs. The structure–property relationship studies on these novel 4-*O*-methylhonokiol analogs are underway and the results of the DMPK study will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.03.028. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. (a) Griswold, D. E.; Adams, J. L. *Med. Res. Rev.* **1996**, *16*, 181; (b) DuBois, R. N.; Abramson, S. B.; Crofford, L.; Gupta, R. A.; Simon, L. S.; Van De Putte, L. B. A.; Lipsky, P. *FASEB J.* **1998**, *12*, 1063.

2. (a) FitzGerald, G. A. *Nat. Rev. Drug Disc.* **2003**, *2*, 879; (b) Flower, R. J. *Nat. Rev. Drug Disc.* **2003**, *2*, 179.
3. Song, W. Z.; Cui, J. F.; Zhang, G. D. *Yao Xue Xue Bao* **1989**, *24*, 295.
4. (a) El-Feraly, F. S.; Li, W.-S. *Lloydia* **1978**, *41*, 442; (b) Ito, K.; Iida, T.; Ichino, K.; Tsunezuka, M.; Hattori, M.; Namba, T. *Chem. Pharm. Bull.* **1982**, *30*, 3347; (c) Rao, K. V.; Davis, T. L. *Planta Med.* **1982**, *45*, 57; (d) Maruyama, Y.; Kuribara, H. *CNS Drug Rev.* **2000**, *6*, 35.
5. (a) Lee, Y. K.; Yuk, D. Y.; Kim, T. I.; Kim, Y. H.; Kim, K. T.; Kim, K. H.; Lee, B. J.; Nam, S.-Y.; Hong, J. T. *J. Natl. Med. Assoc.* **2009**, *63*, 274; (b) Schühly, W.; Khan, S. I.; Fischer, N. H. *Inflammopharmacology* **2009**, *17*, 106; (c) Oh, J. H.; Kang, L. L.; Ban, J. O.; Kim, Y. H.; Kim, K. H.; Han, S. B.; Hong, J. T. *Chem. Biol. Interact.* **2009**, *180*, 506; (e) Lee, Y. K.; Choi, I. S.; Kim, Y. H.; Kim, K. H.; Nam, S. Y.; Yun, Y. W.; Lee, M. S.; Oh, K. W.; Hong, J. T. *Neurochem. Res.* **2009**, *34*, 2251; (f) Lee, J. W.; Lee, Y. K.; Lee, B. J.; Nam, S. Y.; Lee, S. I.; Kim, Y. H.; Kim, K. H.; Oh, K. W.; Hong, J. T. *Pharmacol. Biochem. Behav.* **2010**, *95*, 31; (g) Lee, Y. K.; Choi, I. S.; Ban, J. O.; Lee, H. J.; Lee, U. S.; Han, S. B.; Jung, J.-K.; Kim, Y. H.; Kim, K. H.; Oh, K. W.; Hong, J. T. *J. Nutr. Biochem.* **2011**, *50*, 66; (h) Lee, Y.-J.; Lee, Y. M.; Lee, C. K.; Jung, J.-K.; Han, S. B.; Hong, J. T. *Pharmacol. Ther.* **2011**, *130*, 157. and references cited therein.
6. (a) Schühly, W.; Hüfner, A.; Pferschy-Wenzig, E. M.; Prettner, E.; Adams, M.; Bodensieck, A.; Kunert, O.; Oluwemimo, A.; Haslinger, E.; Bauer, R. *Bioorg. Med. Chem.* **2009**, *17*, 4459.
7. Kwak, J.-H.; Cho, Y. A.; Jang, J.-Y.; Seo, S.-Y.; Lee, H.; Hong, J. T.; Han, S.-B.; Lee, K.; Kwak, Y.-S.; Jung, J.-K. *Tetrahedron* **2011**, *67*, 9401.
8. The pharmacokinetics of 4-*O*-methylhonokiol was characterized by a high clearance, a high volume of distribution and a low oral bioavailability in rat. Also, it was shown to be metabolized rapidly by rat and human liver microsomes in the presence of NADPH and UDPGA, indicating cytochrome P450- and UDP-glucuronosyltransferase-mediated metabolism (Kwak, Y. S.; Han, S. B.; Hong, J. T.; Seo, S.-Y.; Jung, J.-K.; Lee, K. H. unpublished results).
9. **Compounds 5**, **8** and **14** were the known compounds; see Ref. 6.
10. Yoon, K. J. P.; Morton, C. L.; Potter, P. M.; Danks, M. K.; Lee, R. E. *Bioorg. Med. Chem.* **2003**, *11*, 3237.
11. Kwak, J. H.; In, J. K.; Lee, M. S.; Choi, E. H.; Lee, H.; Hong, J. T.; Yun, Y. P.; Lee, S. J.; Seo, S.-Y.; Suh, Y.-G.; Jung, J.-K. *Arch. Pharmacol. Res.* **2008**, *31*, 1559.
12. Tischer, S.; Metz, P. *Adv. Synth. Catal.* **2007**, *349*, 147.
13. Patrick, D. A.; Bakunov, S. A.; Bakunova, S. M.; Kumar, E. V. K. S.; Lombardy, R. J.; Jones, S. K.; Bridges, A. S.; Zhirnov, O.; Hall, J. E.; Wenzler, T.; Brun, R.; Tidwell, R. R. *J. Med. Chem.* **2007**, *50*, 2468.
14. (a) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897; (b) Kacprzak, K. *Synlett* **2005**, 943.
15. Chung, E.-Y.; Kim, B.-H.; Lee, I.-J.; Roh, E.; Oh, S.-J.; Kwak, J.-A.; Lee, Y.-R.; Ahn, B.; Nam, S.-Y.; Han, S.-B.; Kim, Y. *Br. J. Pharmacol.* **1971**, *2009*, 158.
16. Kim, J. Y.; Kang, J. S.; Kim, H. M.; Ryu, H. S.; Kim, H. S.; Lee, H. K.; Kim, Y. J.; Hong, J. T.; Kim, Y.; Han, S.-B. *Int. Immunopharmacol.* **2010**, *10*, 1185.