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Design and synthesis of ten biphenyl-neolignan derivatives and their in vitro inhibitory potency against cyclooxygenase-1/2 activity and 5-lipoxygenase-mediated LTB_4-formation

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ABSTRACT

A set of ten derivatives of methylhonokiol, an anti-inflammatory active biphenyl-type neolignan from *Magnolia grandiflora*, has been evaluated for their in vitro cyclooxygenase-1/2 (COX-1/2) inhibitory activity using assays with purified prostaglandin H synthase (PGHS)-1 and PGHS-2 enzymes as well as for their 5-lipoxygenase (5-LOX) mediated LTB_4 formation inhibitory activity using an assay with activated human polymorphonuclear leukocytes. The derivatization reactions included methylation, acetylation, hydrogenation, epoxydation and isomerization. Five of the derivatives are new to science. The most active compound against COX-1 and COX-2 was methylhonokiol with IC_{50} values of 0.1 μM, whereas the most active compound against LTB_4 formation was (*E*)-3′-propenyl-5-(2-propenyl)-biphenyl-2,4′-diol with an IC_{50} value of 1.0 μM. Structure–activity relationship studies showed that the polarity of the derivatives plays a crucial role in their activity towards COX-1/2 enzyme and 5-LOX mediated LTB_4 formation.

1. Introduction

Prostaglandins such as PGE_2 are produced in the cyclooxygenase pathway of the arachidonic acid cascade by the action of the isoenzymes COX-1 and COX-2. They are not only regulating physiological functions such as thromboxane production in platelets, wound healing, kidney function, blood vessel tone and others,[1] but are also involved in the development of inflammatory diseases such as acute and chronic arthritis.[2] While both isoforms COX-1 and COX-2 have a very similar protein structure and catalyze essentially the production of the same compounds, it can be stated in a very simplified way that COX-1 is constitutively expressed and physiologically active in many different tissues of the body whereas COX-2 is predominantly activated upon inflammatory stimuli.[3,4]

Among the drugs known since long time and used most widely in the treatment of inflammatory disorders are nonsteroidal anti-inflammatory drugs (NSAIDs, e.g., aspirin). Inhibition of COX-1 has been thought to be responsible for side effects of NSAIDs such as gastrointestinal bleeding, while inhibition of COX-2 has been associated with their anti-inflammatory properties. This was the rationale for the development of selective COX-2 inhibitors for the effective treatment of inflammatory disorders.[5] However, several selective COX-2 inhibitors have turned out to cause side effects such as cardiovascular problems,[6] and evidence has increased that not only COX-1, but also COX-2 has physiological functions and is constitutively expressed in certain tissues.[7]

Leukotrienes such as LTB_4 are important mediators in inflammatory pathways and in allergic responses. The formation of LTB_4 from arachidonic acid in human neutrophils by 5-LOX initiates a cascade of reactions which eventually causes inflammatory symptoms.[8] The biological properties of leukotrienes and their participation in a variety of diseases suggest that 5-LOX inhibitors should have a therapeutic potential in a range of allergic and inflammatory disorders. However, even though several of these compounds have turned out to be effective in the treatment of asthma, selective 5-LOX inhibitors seem to possess an insufficient therapeutic potential for the treatment of other inflammatory diseases.[9,10]

Therefore, dual inhibitors blocking both the COX and LOX pathway of the arachidonic acid cascade are recently regarded as a possible alternative in the treatment of inflammatory disorders.[9–11] Inhibition of both pathways might offer a broader range of anti-inflammatory effects, and several side effects known from NSAIDs and selective COX-2 inhibitors might be reduced: the shift of arachidonate metabolism towards the 5-LOX pathway observed for NSAIDs should be avoided, resulting in a decreased production of leukotrienes which damage the gastrointestinal mucosa and contribute to allergic reactions.[9,10]

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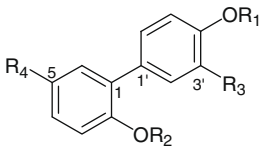
The impulse for the present investigation was derived from the fact that the active principles of *Magnolia* bark (e.g., from *Magnolia officinalis* Rehder et Wilson) honokiol and magnolol exhibit anti-inflammatory activities, for example, against pleurisy in mice in vivo.[12,13] Besides its strong antioxidative effect, honokiol is of major interest because of its various pharmacological activities.[14] *Magnolia* bark is widely known from traditional Asian medicinal systems such as Traditional Chinese Medicine (TCM) and Kampo medicine in Japan.[15] Based on literature reports about the anti-inflammatory activity (COX-1, COX-2 and 5-LOX) of honokiol[16–18] and founded on our own finding that the anti-inflammatory activity of methylhonokiol (**1b**) was even higher than that of honokiol,[19] we tried to fathom the effectiveness of derivatives of methylhonokiol, a major constituent of the seeds of the North American species *Magnolia grandiflora* L. Methylhonokiol (**1b**) turned out to be a promising lead structure suitable for derivatizations (Figs. 1 and 2). The design of derivatives was undertaken by utilization of the structural features of **1b**, which allowed the alteration of the double bond in the side chain as well as substitutions at the phenolic OH.

In a dual approach, the anti-inflammatory activity of honokiol-derived neolignans was evaluated against COX-1 and COX-2 enzyme activity as well as against 5-LOX-mediated LTB_4 formation in vitro. The presented data may help to better understand the structural requirements needed for anti-inflammatory activity of honokiol-type neolignans.

2. Results and discussion

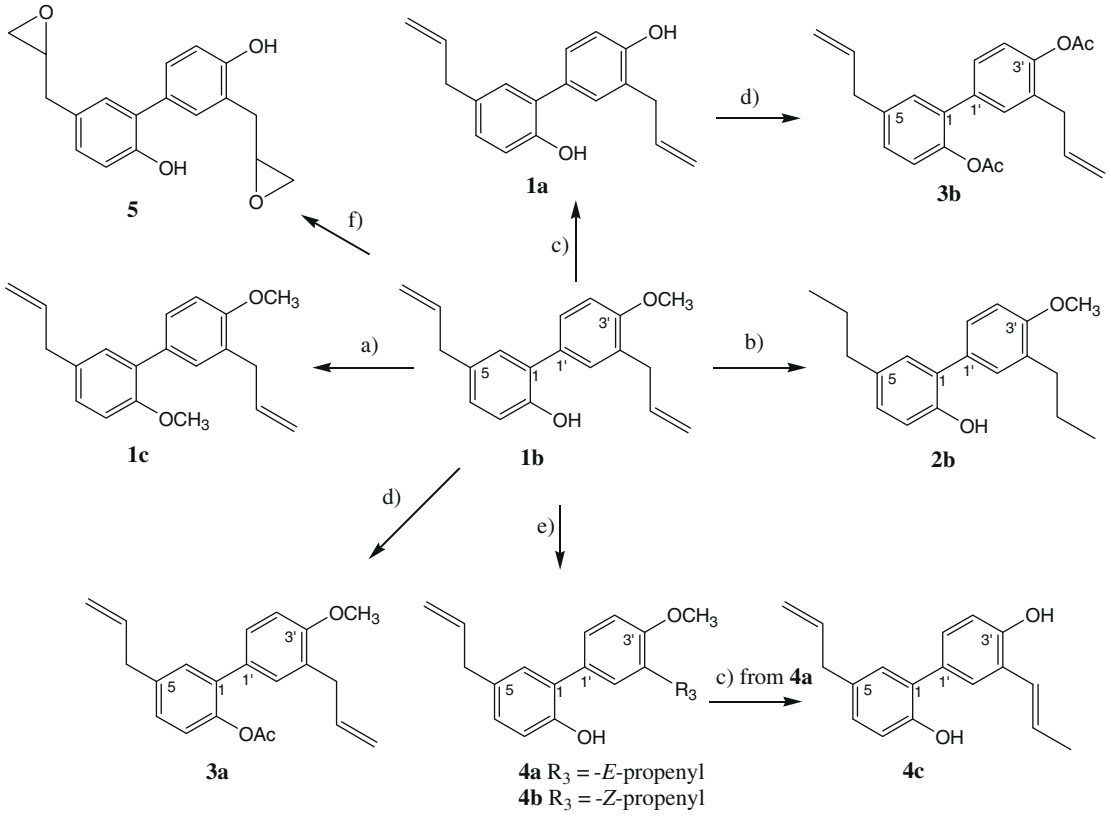
A set of 10 derivatives of methylhonokiol (**1b**) as lead compound has been synthesized and compared towards their inhibitory activity in COX-1/2 in vitro test systems and against 5-LOX mediated LTB_4 formation (Fig. 1). The derivatizations encompassed methylation and acetylation of the free phenolic OH-groups as well as hydrogenation, isomerization and epoxidation of the side chains (for a general scheme, see Fig. 2). The derivatizations led to yet undescribed or only partially described compounds. With the exception of **5** (epoxidation product) and **4c**, all derivatives were obtained in high purity and mostly high yield (67–97%). A separation of the diastereomeric mixture of **5** was not undertaken due to its loss of activity in both COX-1/2 and 5-LOX systems (see below). The pharmacological data for the derivatives are shown in Tables 1 and 2.

The overall best activity against COX-1 and COX-2 was exhibited by methylhonokiol (**1b**), which showed a slight selectivity towards COX-2. None of the derivatives showed a pronounced selectivity towards COX-1 or COX-2. The highest COX-2 selectivity was displayed by **4a** and **4b** with an IC_{50} for COX-2 about three times lower than for COX-1 (0.2 and 0.7 μM, respectively, for **4a** and 0.9 vs 2.8 μM for **4b**). A similar trend was observed for compounds **2c** and **3a**, however, the activity displayed by these compounds was much lower. Overall, for the inhibitory activity against COX-1/2, there are two distinct trends noticeable. First,



Compound	R_1	R_2	R_3	R_4
1a	-H	-H	-2-propenyl	-2-propenyl
1b	$-CH_3$	-H	-2-propenyl	-2-propenyl
1c	$-CH_3$	$-CH_3$	-2-propenyl	-2-propenyl
2a	-H	-H	-propyl	-propyl
2b	$-CH_3$	-H	-propyl	-propyl
2c	$-CH_3$	$-CH_3$	-propyl	-propyl
3a	$-CH_3$	$-COCH_3$	-2-propenyl	-2-propenyl
3b	$-COCH_3$	$-COCH_3$	-2-propenyl	-2-propenyl
4a	$-CH_3$	-H	-*E*-propenyl	-2-propenyl
4b	$-CH_3$	-H	-*Z*-propenyl	-2-propenyl
4c	-H	-H	-*E*-propenyl	-2-propenyl
5	$-CH_3$	-H	-oxiranylmethyl	-oxiranylmethyl

Figure 1. Biphenyl-type neolignan derivatives prepared from methylhonokiol.



a) $NaOEt / Me_2SO_4$; 70%

b) Pd on charcoal; 97%

c) Grignard in THF; 55% (**1a**), 16% (**4c** from **4a**)

d) Ac_2O / pyridine; 87% (**3a**), 67% (**3b**)

e) KO*t*Bu / THF, reflux; mixture of **4a** and **4b**; 94%

f) MCPBA / benzene; 87%

Figure 2. Scheme of syntheses of the biphenyl-neolignan derivatives **1**–**5**.

the very lipophilic compounds without OH group (**1c**, **2c**, **3a** and **3b**), did clearly show a reduced activity. At the low concentration used in the assay, a solubility problem could be ruled out. Second, the variation in the side chain (hydrogenation, isomerization) did not seem to considerably affect the overall activity against COX-1/-2. However, the introduction of the epoxy group in **5** led to a complete loss of activity in both COX-1/2 systems.

Considering the array of 5-LOX product formation inhibitory activity of the ten derivatives, the overall highest activity was found for **4b** and **4c** with IC_{50} values of 1.0 and 1.1 μM, respec-

Table 1
Inhibition of COX-1, COX-2 enzymes and 5-LOX mediated LTB_4 formation by derivatives of methylhonokiol at 8 μM

Compounds	COX-1 inhibition (% and SD)		COX-2 inhibition (% and SD)		LTB_4 formation inhibition (% and SD)	
Honokiol **1a**	91.0	1.5	88.3	3.8	94.0	0.9
Methylhonokiol **1b**	90.8	6.5	95.3	1.6	96.4	0.3
Dimethylhonokiol **1c**	45.1	5.8	47.8	6.0	27.8	8.5
Tetrahydrohonokiol **2a**	89.6	3.1	81.6	5.7	99.9	0.2
2b	56.5	5.6	11.6	3.8	97.1	4.0
2c	27.3	4.0	49.4	5.4	24.4	9.4
3a	18.0	7.0	56.3	6.3	76.3	6.1
3b	6.8	12.7	13.8	13.6	64.9	7.9
4a	80.5	2.4	81.5	3.1	99.3	0.5
4b	68.6	5.4	62.7	7.2	99.9	0.1
4c	84.8	3.0	75.1	6.0	100.0	0.0
5	0.5	6.5	4.2	4.8	4.0	4.6

SD: standard deviation.

Table 2
IC_{50} values of derivatives of methylhonokiol and positive controls in COX-1, COX-2 enzyme and LTB_4 formation assays in μM

Compounds	IC_{50} for COX-1 inhibition (μM)	IC_{50} for COX-2 inhibition (μM)	IC_{50} for LTB_4 formation inhibition (μM)
Honokiol **1a**	1.8	2.1	4.2
Methylhonokiol **1b**	0.1	0.06	1.5
Dimethylhonokiol **1c**	11.4	7.7	15.0
2a	0.8	2.1	1.7
2b	7.7	n.d.	1.4
2c	> 20	6.9	n.d.
3a	n.d.	4.9	2.2
3b	n.d.	n.d.	4.5
4a	0.7	0.2	2.1
4b	2.8	0.9	1.1
4c	0.6	2.7	1.0
5	n.d.	n.d.	n.d.
Indomethacin	0.9		
NS-398		2.6	
Zileuton			5.0

n.d.: not determined.

tively. For dimethylhonokiol (**1c**) and its hydrogenated sister compound (**2c**), a significantly reduced activity was observed in comparison to the less highly methylated compounds (**1a**, **1b**, **2a** and **2b**). However, this trend did not show its counterpart in the highly lipophilic acetylmethylhonokiol (**3a**) and diacetylhonokiol (**3b**), which showed a considerable IC_{50} of 2.2 and 4.5 μM, respectively. With the exception of the epoxide **5**, which lost all its LTB_4 formation inhibitory activity, the influence of the chemical character of the side chain on the activity seems to be relatively low. This could be seen from the comparable activities of, for example, **1a**, **1b**, **2a**, **2b** and **4a–c,** where the IC_{50} values range from 1.0–4.5 μM.

With the exception of **5**, which was inactive in all three test systems and **3b**, which selectively inhibited 5-LOX product formation, all tested derivatives were found to inhibit COX isoenzymes as well as 5-LOX mediated LTB_4 formation. From this finding it can be assumed that these compounds have a potential as dual inhibitors of the COX and the LOX pathway of the arachidonic acid cascade. Compounds **4a** and **4b** were found to be most promising so far due to their good inhibitory activities. However, for the interpretation of the present results it has to be considered that for assessing COX inhibitory activity, a cell-free test system with purified enzymes has been used, while the influence on LTB_4 formation has been investigated in a cellular system. Therefore, in order to be able to better evaluate the potential of these compounds as dual inhibitors, it will be necessary to perform further tests, for example, to investigate their activity in a cell-free assay on direct 5-LOX inhibition. This will also help to understand the mechanism of action underlying the observed LTB_4 formation inhibitory activity.

The formation of LTs can be reduced by direct inhibition of 5-LOX or by inhibition of a number of other mechanisms playing a role in LT formation. Indirect inhibition of LT formation can for instance be caused by an inhibition of PLA_2 leading to a decreased arachidonic acid release, by an inhibition of 5-LOX activating protein (FLAP), an enzyme facilitating the access of arachidonic acid to 5-LOX, by an inhibition of the translocation of 5-LOX from the cytosol to the nuclear membrane or by an inhibition of 5-LOX downstream enzymes such as LTA_4 hydrolase.[20,21] Direct 5-LOX inhibitors are classified according to their molecular mode of action as redox-active 5-LOX inhibitors, iron–ligand inhibitors, non-redox-type inhibitors and compounds acting by so far unrecognized mechanisms.[20,21]

As the assay used herein is cell-based and as LTB_4 concentration is measured for the determination of inhibitory activity, no statement can be made on the mechanism underlying the observed inhibition of LTB_4 formation inhibition so far. From the literature it is known that magnolol, an isomer of honokiol, inhibits LTB_4 formation in neutrophils by direct inhibition of 5-LOX.[21] The same mechanism was also suggested for the LT formation inhibitory activity of honokiol in RBL cells.[18] Further pharmacological studies will be necessary to investigate whether this is also true for the biphenyl derivatives presented herein.

To summarize, within the set of investigated biphenyls, the structural prerequisites for anti-inflammatory activity against COX-1/2 seem to be a biphenylic system bearing two phenolic OH groups or one OH and one OCH_3 group together with apolar side chains of medium length. For 5-LOX LTB_4 formation inhibitory activity, the substitution of the phenolic ring could also include acetoxy groups. The denomination of one single clearly required pharmacophore in both systems could not be undertaken, however, the loss of all activity in the epoxy derivative **5** indicates that a dramatic change in the chemical character of the side chain abolishes the activity.

3. Experimental

3.1. General

IR spectra were taken as KBr pellets on a Perkin-Elmer 281 B spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer (400 and 100 MHz, respectively) using deuterated chloroform as solvent with TMS as internal standard. NMR signals marked with asterisk (*) may be interchanged. EI-MS were recorded on a Hewlett Packard HP 6890 instrument fitted with detector HP 7890. ESI-MS were measured in ESI positive and negative mode on a Thermo Finnigan LCQ™ Deca XP^{PLUS} instrument. For TLC analysis, precoated Si60 F_{254} plates (Merck, Darmstadt) were used. Detection was done by UV/254 nm and spraying with molybdato-phosphoric acid and subsequent heating. Compound mixtures were separated by column chromatography using cyclohexane/ethyl acetate mixtures and on a HPLC preparative column (Merck, Hibar Lichrospher RP-18, 10 μm, 250 × 25 mm) using an acetonitrile gradient in water.

Methylhonokiol (**1b**) as starting material was isolated from a dichloromethane extract of *M. grandiflora* seeds collected in northern Mississippi. A voucher (WS-4) is deposited at the Institute of Pharmaceutical Sciences, department of Pharmacognosy, in Graz. The crude methylhonokiol was obtained as an oil by chromatographing the extract on silica gel (40–63 μm) using *n*-hexane–ethyl acetate mixtures. Honokiol (**1a**) was obtained by demethylation via Grignard reaction of methylhonokiol,[22] its identity was demonstrated by comparison with literature data[22] and a reference sample. A general scheme (Fig. 2) gives an overview over the principal syntheses carried out in this investigation.

3.2. 2,4′-Dimethoxy-5,3′-di-(2-propenyl)-biphenyl (1c)

Na (25 mg, 1.1 mmol) was dissolved in abs. EtOH (16 mL) under argon and a solution of **1a** (213 mg, 0.75 mmol) in abs. EtOH (4 mL) was added. After stirring for 5 min, the solution was heated to reflux. $(CH_3)_2SO_4$ (195 μL, 230 mg, 1.0 mmol) was slowly added to the hot solution and refluxing continued for 5 h. After cooling to room temperature, the solvent was evaporated and water (20 mL) was added. The mixture was extracted with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4. Compound **1c** was obtained in 70% yield as a colorless oil. IR (KBr): 3446 (br), 2930, 1488, 1265, 1243, 1028, 913, 817 cm^{-1}; UV (15 μM, eth-

anol) λ_{max} (log ε) = 290.0 (3.87), 257.0 (4.13) 208.5 (4.60) nm; ^{1}H NMR ($CDCl_3$): δ 3.43 (d, J = 7.2 Hz, 2H, H-1‴), 3.47 (d, J = 7.2 Hz, 2H, H-1″), 3.78 (s, 3H, 2-OCH_3), 3.86 (s, 3H, 4′-OCH_3), 5.04 (d, J = 10.2 Hz, 1H, H-3‴), 5.05 (d, J = 10.2 Hz, 1H, H-3″), 5.09 (d, J = 18.0 Hz, 2H, H-3″ and H-3‴), 5.99 (m, 1H, H-2″), 6.03 (m, 1H, H-2‴), 6.90 (d, J = 8.4 Hz, 2H, H-3 and H-5′), 7.09 (dd, J = 8.4 and 1.8 Hz, 1H, H-4), 7.12 (d, J = 1.8 Hz, 1H, H-6), 7.31 (d, J = 2.0 Hz, 1H, H-2′), 7.37 (dd, J = 8.4 and 2.0 Hz, 1H, H-6′); ^{13}C NMR ($CDCl_3$): δ 34.6 (C-1‴), 39.7 (C-1″), 55.7 (4′-OCH_3), 55.9 (2-OCH_3), 110.1 (C-5′), 111.6 (C-3), 115.7 (C-3″), 115.7 (C-3‴), 128.1 (C-4), 128.5 (C-3′), 128.5 (C-6′), 130.7 (C-1′*), 131.0 (C-1*), 131.2 (C-2′), 131.2 (C-6), 132.5 (C-5), 137.3 (C-2‴), 137.9 (C-2″), 155.1 (C-2), 156.7 (C-4′); EI-MS 294 $[M]^+$ (100), 238 (37), 223 (19), 165 (18). The compound is yet incompletely described.[22]

3.3. 5,3′-Dipropyl-biphenyl-2,4′-diol (tetrahydrohonokiol, 2a)

Compound **2a** was obtained by demethylation of **2b**. To a stirred solution of **2a** (100 mg, 0.352 mmol) under argon, a 10% solution of BBr_3 in CH_2Cl_2 (1.0 mL, 0.6 mmol) was added dropwise at rt. The reaction mixture was poured into brine (6 mL) and stirred for 5 min. The organic layer was separated and the aqueous phase re-extracted with CH_2Cl_2. The combined phases were dried over Na_2SO_4 and purified by CC. **2a** was obtained as crystals in 74% yield. IR (KBr): 3357 (br, OH), 3019, 2927, 2870, 1609, 1498, 1429, 1375, 1198, 1126, 823 cm^{-1}; UV (25 μM, ethanol) λ_{max} (log ε) = 292.5 (4.12), 256.5 (4.30) 209.5 (4.76) nm; ^{1}H NMR ($CDCl_3$): δ 0.95 (t, J = 7.3 Hz, 3H, H-3″), 1.00 (t, J = 7.3 Hz, 3H, H-3‴), 1.65 (sext, J = 7.5 Hz, 2H, H-2″), 1.68 (sext, J = 7.5 Hz, 2H, H-2‴), 2.55 (t, J = 7.5 Hz, 2H, H-1″), 2.64 (t, J = 7.7 Hz, 2H, H-1‴), 4.86 (br s, 2H, OH), 6.84 (d, J = 8.0 Hz, 1H, H-5′), 6.90 (d, J = 7.7 Hz, 1H, H-3), 7.03 (s, 1H, H-6), 7.05 (dd, $J \sim 8$ and 2.2 Hz, 1H, H-4), 7.17 (dd, J = 8.1 and 2.2 Hz, 1H, H-6′), 7.23 (d, J = 2.2 Hz, 1H, H-2′); ^{13}C NMR ($CDCl_3$): δ 13.8 (C-3″), 14.0 (C-3‴), 22.8 (C-2‴), 24.8 (C-2″), 32.0 (C-1‴), 37.2 (C-1″), 115.3 (C-3), 115.9 (C-5′), 127.6 (C-6′), 127.7 (C-1), 128.5 (C-4), 129.5 (C-3′ and C-1′), 130.0 (C-6), 131.0 (C-2′), 134.9 (C-5), 150.3 (C-2), 153.2 (C-4′); EI-MS: 270 $[M]^+$ (57), 241 (100), 199 (34), 115 (12). ^{1}H NMR values without assignments are given in Kong et al.[23]

3.4. 4′-Methoxy-5,3′-dipropyl-biphenyl-2ol (2b)

To a solution of **1b** (200 mg, 0.714 mmol) in abs. EtOH (5 mL), 4 mg 10% Pd/activated charcoal was added and stirred for several minutes. The mixture was filtered and another 8 mg of 10% Pd on charcoal was added. The solution was stirred vigorously for 21 h at room temperature under H_2 at atmospheric pressure. The catalyst was filtered off through a Pasteur pipette filled with silica gel and Celite. The absorbents were washed with EtOH (5 mL) and the combined eluates were evaporated in vacuo to yield **2b** in 97% as colorless oil.[24] IR (KBr): 3443 (br, OH), 2958, 2930, 2870, 1607, 1492, 1245, 1029, 818 cm^{-1}; UV (66 μM, CH_2Cl_2) λ_{max} (log ε) = 289.0 (3.85), 253.5 (4.06), 231.5 (4.06) nm; ^{1}H NMR ($CDCl_3$): δ 0.96 (t, J = 7.7 Hz, 3H, H-3″*), 0.98 (t, J = 7.7 Hz, 3H, H-3‴*), 1.66 (sext, J = 7.7 Hz, 4H, H-2‴ and H-2″), 2.55 (t, J = 8.0 Hz, 2H, H-1″), 2.65 (t, J = 8.0 Hz, 2H, H-1‴), 3.86 (s, 3H, OCH_3), 5.19 (br s, OH), 6.90 (d, J = 8.4 Hz, 1H, H-3), 6.95 (d, J = 8.1 Hz, 1H, H-5′), 7.04 (s, 1H, H-6), 7.05 (dd, $J \sim 8$ and 2.1 Hz, 1H, H-4), 7.24 (d, J = 2.1 Hz, 1H, H-2′), 7.27 (dd, J = 8.2 and 2.1 Hz, 1H, H-6′); ^{13}C NMR ($CDCl_3$): δ 13.8 (C-3″*), 14.1 (C-3‴*), 22.9 (C-2‴), 24.8 (C-2″), 32.3 (C-1‴), 37.2 (C-1″), 55.4 (4′-OCH_3), 110.8 (C-5′), 115.3 (C-3), 127.3 (C-6′), 127.7 (C-1), 128.5 (C-4), 129.0 (C-1′), 130.0 (C-6), 130.6 (C-2′), 132.2 (C-3′), 134.8 (C-5), 150.5 (C-2), 157.2 (C-4′); EI-MS: 284 $[M]^+$ (84), 255 (100), 213 (42), 181 (20), 113 (32), 98 (27). ^{1}H NMR values without further assignments are given in Rao and Davis.[25]

3.5. 2,4′-Dimethoxy-5,3′-dipropyl-biphenyl (2c)

Yield 85%, colorless oil. Compound **2c** was obtained by hydrogenation of dimethylhonokiol (**1c**) with palladium on charcoal as catalyst in EtOH as described for **2b**. IR (KBr): 2957, 2929, 2870, 2834, 1608, 1508, 1492, 1463, 1267, 1242, 1138, 1031, 811 cm^{-1}; UV (10 μM, ethanol) λ_{max} (log ε) = 290.0 (3.92), 257.5 (4.13), 207.0 (4.71) nm; ^{1}H NMR ($CDCl_3$): δ 0.97 (t, J = 7.4 Hz, 3H, H-3″), 0.99 (t, J = 7.4 Hz, 3H, H-3‴), 1.66 (sext, J = 7.5 Hz, 4H, H-2″ and H-2‴), 2.58 (dd, J = 8.4 and 6.7 Hz, 2H, H-1″), 2.64 (dd, J = 8.4 and 6.7 Hz, 2H, H-1‴), 3.79 (s, 3H, 2-OCH_3), 3.85 (s, 3H, 4′-OCH_3), 6.89 (d, J = 8.4 Hz, 2H, H-3 and H-5′), 7.09 (dd, J = 8.1 and 2.2 Hz, 1H, H-4), 7.13 (d, J = 2.2 Hz, 1H, H-6), 7.32 (d, J = 2.1 Hz, 1H, H-2′), 7.36 (dd, J = 8.1 and 2.2 Hz, 1H, H-6′); ^{13}C NMR ($CDCl_3$): δ 13.9 (C-3″), 14.2 (C-3‴), 23.0 (C-2‴), 24.8 (C-2″), 32.4 (C-1‴), 37.2 (C-1″), 55.3 (4′-OCH_3), 55.7 (2-OCH_3), 109.8 (C-5′*), 111.1 (C-3*), 127.6 (C-4), 127.8 (C-6′), 130.4 (C-1**), 130.4 (C-3′**), 130.6 (C-1′**), 130.8 (C-6), 131.1 (C-2′), 134.9 (C-5), 154.6 (C-2), 156.6 (C-4′); EI-MS: 298 $[M]^+$ (100), 269 (79), 255 (5), 211 (22), 195 (19), 165 (21), 152 (21), 1120 (40), 105 (31).

3.6. 2-Acetoxy-4′-methoxy-5,3′-di-(2-propenyl)-biphenyl (3a)

Compound **3a** was obtained as colorless oil from **1b** in pyridine and acetic anhydride in a 87% yield.[26] IR (KBr): 2908, 1763 (carbonyl), 1507, 1487, 1246, 1215, 1190, 913 cm^{-1}; UV (9.9 μM, ethanol) λ_{max} (log ε) = 257.0 (4.40), 207.5 (4.88) nm. ^{1}H NMR ($CDCl_3$): δ 2.07 (s, 3H, acetyl-CH_3), 3.40 (m, 4H, H-1′ and H-1‴), 3.82 (s, 3H, 4′-OCH_3), 5.03 (d, J = 10.2 Hz, 1H, H-3‴), 5.06 (d, J = 18.0 Hz, 1H, H-3‴), 5.07 (d, J = 10.2 Hz, 1H, H-3″), 5.10 (d, J = 18.0 Hz, 2H, H-3″), 5.97 (m, 1H, H-2‴), 6.07 (m, 1H, H-2″), 6.86 (d, J = 8.4 Hz, 1H, H-5′), 7.01 (d, J = 8.4 Hz, 1H, H-3), 7.12 (d br, J = 8.1 Hz, 1H, H-4), 7.19 (s br, 1H, H-6), 7.21 (s br, 1H, H-2′), 7.23 (d, J = 8.4 Hz, 1H, H-6′); ^{13}C NMR ($CDCl_3$): δ 20.9 (CH_3-acetyl), 34.7 (C-1‴), 40.0 (C-1″), 55.5 (4′-OCH_3), 110.3 (C-5′), 115.5 (C-3‴), 116.2 (C-3″), 122.7 (C-3), 127.7 (C-6′), 128.0 (C-4), 128.4 (C-3′), 129.6 (C-1′), 130.6 (C-2′), 130.8 (C-6), 134.4 (C-1), 136.8 (C-2‴), 137.0 (C-2″), 138.2 (C-5), 146.1 (C-2), 156.7 (C-4′), 169.8 (CO acetyl); EI-MS: 322 $[M]^+$ (24), 280 (100), 251 (15), 238 (18), 223 (17), 198 (20), 165 (19), 43 (70); ESI$^+$ calcd for $C_{21}H_{22}O_3$: $[M+H]^+$ 323.16; found 323.14. Data incompletely given in El-Feraly and Li.[22]

3.7. 2,4′-Diacetoxy-5,3′-di-(2-propenyl)-biphenyl (3b)

Compound **3b** was obtained as colorless oil obtained from **1a** and acetic anhydride in pyridine in a 95% yield.[27] IR (KBr): 2978, 2923, 1763 (carbonyl), 1639, 1484, 1369, 1210, 1192, 1011, 914 cm^{-1}; UV (1.6 mM, EtOH) λ (log ε) = 243.0 (4.93), 207.5 (5.54) nm; ^{1}H NMR ($CDCl_3$): δ 2.08 (s, 3H, acetyl-CH_3), 2.32 (s, 3H, acetyl-CH_3), 3.33 (d, J = 6.6 Hz, 2H, H-1‴), 3.42 (d, J = 6.6 Hz, 2H, H-1″), 5.07 (m, 4H, H-3″ and H-3‴), 5.92 (m, 1H, H-2‴), 5.95 (m, 1H, H-2″), 7.04 (d, J = 8.1 Hz, 1H, H-3), 7.08 (d, J = 8.1 Hz, 1H, H-5′), 7.18 (dd, J = 8.2 and 2.2 Hz, 1H, H-4), 7.22 (d, J = 2.2 Hz, 1H, H-6), 7.27 (dd, $J \sim 8$ and 2.2 Hz, 1H, H-6′), 7.28 (s due to overlap, 1H, H-2′); ^{13}C NMR ($CDCl_3$): δ 20.9 (CH_3-acetyl), 20.9 (CH_3-acetyl), 34.7 (C-1‴), 39.6 (C-1″), 116.3 (C-3‴), 116.4 (C-3″), 122.3 (C-5′), 122.7 (C-3), 127.9 (C-6′), 128.7 (C-4), 130.9 (C-2′*), 130.9 (C-6*), 131.7 (C-3′), 133.8 (C-1), 135.6 (C-1′), 135.7 (C-2‴), 136.9 (C-2″), 138.2 (C-5), 146.0 (C-2), 148.3 (C-4′), 169.3 (CO acetyl), 169.6 (CO acetyl); EI-MS: 350 $[M]^+$ (8), 308 (32), 266 (100), 224 (12).

3.8. General procedure for isomerization of side chain in 4a and 4b

To a solution of **1b** (45 mg, 0.17 mmol) in abs. THF (10 mL) under argon, KOtBu (57 mg, 0.51 mmol) was added and the solution

was refluxed vigorously for 21 h. After cooling to rt, a saturated solution of NH_4Cl (10 mL) was added. The reaction mixture was reduced to 10 mL and extracted with $CHCl_3$ (3 × 10 mL). The combined organic layers were washed with brine (20 mL), water (2 × 10 mL) and dried over $MgSO_4$.[27] A mixture of **4a** and **4b** was obtained in 94% yield. **4a** and **4b** were separated by preparative HPLC.

3.9. 4′-Methoxy-3′-(*E*)-propenyl)-5-(2-propenyl)-biphenyl-2-ol (4a)

Fine crystals, mp 81 °C; IR (KBr): 3448 (br, OH), 2934, 2910, 2836, 1638, 1604, 1490, 1246, 1180, 1121, 819 cm^{-1}; UV (10 μM, CH_2Cl_2) λ_{max} (log ε) = 298.0 (3.95), 244.5 (4.38), 222.5 (4.48), 205.5 (4.43) nm; ^1H NMR ($CDCl_3$): δ 1.91 (dd, *J* = 6.6 and 1.1 Hz, 3H, H-3‴), 3.35 (d, *J* = 6.6 Hz, 2H, H-1″), 3.88 (s, 3H, O*CH*$_3$), 5.05 (d, *J* = 10.5 Hz, 1H, H-3″), 5.08 (d, *J* = 16.8, 1H, H-3″), 5.17 (s br, 1H, 2-O*H*), 5.97 (ddt, *J* = 16.8, 10.3 and 6.6 Hz, 1H, H-2″), 6.27 (dq, *J* = 15.8 and 6.6 Hz, 1H, H-2‴), 6.74 (d, *J* = 15.8 Hz, 1H, H-1‴), 6.90 (d, *J* = 8.1 Hz, 1H, H-3), 6.94 (d, *J* = 8.4 Hz, 1H, H-5′), 7.05 (s, 1H, H-6), 7.06 (dd, *J* ~ 8 and 1.8 Hz, 1H, H-4), 7.25 (dd, *J* = 8.4 and 2.2 Hz, 1H, H-6′), 7.47 (s, 1H, H-2′); ^{13}C NMR ($CDCl_3$): δ 18.9 (C-3‴), 46.7 (C-1″), 55.6 (O*C*H$_3$), 111.4 (C-5′), 115.6 (C-3 and C-3″), 125.2 (C-1‴), 127.1 (C-2′), 127.5 (C-2‴), 127.8 (C-1*), 127.9 (C-1′*), 128.5 (C-6′), 128.8 (C-4), 129.2 (C-3′), 130.1 (C-6), 132.1 (C-5), 137.8 (C-2″), 150.8 (C-2), 155.9(C-4′); EI-MS: *m/z* 280 $[M]^+$ (100), 253 (19), 224 (17), 165 (15); ESI^+ calcd for $C_{19}H_{20}O_2$: $[M+H]^+$ 281.15; found 281.14.

3.10. 4′-Methoxy-3′-(*Z*)-propenyl)-5-(2-propenyl)-biphenyl-2-ol (4b)

Fine crystals, IR (KBr): 3529 (br, OH), 3449 (br), 3021, 2975, 2910, 2836, 1603, 1489, 1249, 1181, 1117, 819 cm^{-1}; UV (10 μM, EtOH) λ_{max} (log ε) = 297.0 (4.05), 221.5 (4.59) nm; ^1H NMR ($CDCl_3$): δ 1.84 (dd, *J* = 7.1 and 1.6 Hz, 3H, H-3‴), 3.35 (d, *J* = 6.7 Hz, 2H, H-1″), 3.88 (s, 3H, O*CH*$_3$), 5.06 (d, *J* = 10 Hz, 1H, H-3″), 5.09 (dq, *J* = 17 and 1.5 Hz, 1H, H-3″), 5.14 (s br, 2-O*H*), 5.89 (dq, *J* = 11.5 and 7.0 Hz, 1H, H-2‴), 5.96 (dq, *J* = 16.8, 10.1 and 6.6 Hz, 1H, H-2″), 6.56 (d, *J* = 11.6 Hz, 1H, H-1‴), 6.91 (d, *J* = 7.9 Hz, 1H, H-3), 6.98 (d *J* = 8.4 Hz, 1H, H-5′), 7.05 (s, 1H, H-6), 7.06 (dd, *J* ~ 8 and 2.0 Hz, 1H, H-4), 7.32 (dd, *J* = 8.3 and 2.2 Hz, 1H, H-6′), 7.35 (d, *J* = 2.0 Hz, 1H, H-2′); ^{13}C NMR ($CDCl_3$): δ 14.7 (C-3‴), 39.4 (C-1″), 55.6 (O*C*H$_3$), 111.0 (C-5′), 115.6 (C-3* and C-3″*), 124.7 (C-1‴), 127.1 (C-3′), 127.6 (C-2‴), 127.8 (C-1), 128.5 (C-6′), 128.5 (C-1′), 128.8 (C-4), 130.2 (C-6), 130.8 (C-2′), 132.2 (C-5), 137.7 (C-2″), 150.8 (C-2), 156.7 (C-4′); ESI^+ calcd for $C_{19}H_{20}O_2$: $[M+H]^+$ 281.15; found 281.38.

3.11. (*E*)-3′-Propenyl)-5-(2-propenyl)-biphenyl-2,4′-diol (4c)

Compound **4c** was obtained as crystals from **4a** by demethylation using Grignard reagent[22] in 16% yield. IR (KBr): 3300 (OH), 2912, 1637, 1609, 1491, 1433, 1219, 825 cm^{-1}; UV (10 μM, EtOH) λ_{max} (log ε) = 299.0 (4.00), 244.5 (4.41), 222 (4.52), 204.0 (4.51) nm; ^1H NMR ($CDCl_3$): δ 1.92 (dd, *J* = 6.6 and 1.5 Hz, 3H, H-3‴), 3.34 (d, *J* = 6.6 Hz, 2H, H-1″), 5.05 (d, *J* = 10.3 Hz, 1H, H-3″), 5.08 (dq, *J* = 16.8 and 1.5 Hz, 1H, H-3″), 5.12 (s br, 1H, 2-O*H*), 5.19 (s br, 1H, 4′-O*H*), 5.96 (ddt, *J* = 16.9, 10.3 and 6.6 Hz, 1H, H-2″), 6.25 (dq, *J* = 15.8 and 6.6 Hz, 1H, H-2‴), 6.59 (dq, *J* = 15.8 and 1.5 Hz, 1H, H-1‴), 6.87 (d, *J* = 8.4 Hz, 1H, H-5′), 6.90 (d, *J* = 8.1 Hz, 1H, H-3), 7.02 (d, *J* = 1.8 Hz, 1H, H-6), 7.05 (dd, *J* = 8.1 and 2.2 Hz, 1H, H-4), 7.16 (dd, *J* = 8.0 and 2.2 Hz, 1H, H-6′), 7.37 (d, *J* = 2.2 Hz, 1H, H-2′); ^{13}C NMR ($CDCl_3$): δ 18.9 (C-3‴), 39.4 (C-1″), 115.6 (C-3‴* and C-3*), 116.5 (C-5′), 124.8 (C-1‴), 125.9 (C-3′), 127.7 (C-1), 128.0 (C-2′), 128.6 (C-6′), 128.9 (C-4), 129.2 (C-2‴), 129.5 (C-1′), 130.1 (C-6), 132.2 (C-5), 137.7 (C-2″), 150.7 (C-2), 152.1 (C-4′); ESI^+ calcd for $C_{18}H_{18}O_2$: $[M+H]^+$ 267.13; found 267.20.

3.12. 4′-Methoxy-5,3′-bis-oxiranylmethyl-biphenyl-2-ol (5)

Compound **5** was obtained as a dark yellow solid in a diastereomeric mixture from **1b** by oxidation with 3-chloroperoxybenzoic acid (MCPBA) in abs benzene in 87% yield (modified after O'Brian).[28] IR (KBr): 3423, 2920, 2850, 1733, 1608, 1492, 1245, 1027, 819 752 cm^{-1}; UV (14 μM, EtOH) λ_{max} (log ε) = 291.5 (3.79), 256.5 (3.98), 206.5 (4.58) nm; ESI-MS: 322 $[M]^+$ (24), 280 (100), 251 (15), 238 (18), 223 (17), 198 (20), 165 (19), 43 (70); ESI^- calcd for $C_{19}H_{20}O_4$: $[M-H]^-$ 311.12; found 311.23.

3.13. In vitro-assays

COX-1 and COX-2 inhibition assays were performed in a 96-well-plate format with purified prostaglandin H synthase (PGHS-1) from ram seminal vesicles for COX-1 and purified PGHS-2 from sheep placental cotyledons for COX-2 (both Cayman Chemical Company, Ann Arbor, USA) as previously described.[29,30] The concentration of PGE_2, the main arachidonic acid metabolite in the reaction, was determined by a competitive PGE_2 EIA kit (Assay Designs Inc., Ann Arbor, MI, USA). Indomethacin (ICN, Aurora, USA; IC_{50} COX-1 0.9 μM), a dual COX-1/2 inhibitor was used as positive control in COX-1 experiments and NS-398, a selective COX-2 inhibitor, (Cayman Chemical Company, IC_{50} COX-2 2.6 μM) was used as positive control.

The bioassay for inhibition of 5-LOX mediated LTB_4 formation was carried out in a 96-well-plate format with stimulated human polymorphonuclear leukocytes as described by Adams et al.[31] with slight modifications.[20] Zileuton (Sequoia, Oxford, UK; IC_{50} 5.0 μM) was used as positive control.

Test samples were dissolved in absolute ethanol. Pure compounds were tested at a final concentration of 8 μM. Samples were tested in at least 3 independent experiments run in duplicate. Results are given as means ± S.D. The samples possessing inhibition values higher than 50% at this 8 μM were then selected for the determination of IC_{50}. For IC_{50} determination, active samples were tested in at least 3 concentrations in at least 3 independent experiments, each time in duplicate. Calculation of IC_{50} values was performed by semilogarithmic presentation of dose vs. activity and logarithmic regression analysis.

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