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Derivatives of schisandrin with increased inhibitory potential on prostaglandin E_2 and leukotriene B_4 formation in vitro

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ABSTRACT

Four derivatives of schisandrin, a major dibenzo[*a*,*c*]cyclooctadiene lignan of *Schisandra chinensis* (Turcz.) Baillon were synthesized and structurally characterized by means of NMR and mass spectroscopy. Furthermore, axial chirality of the biphenyl system was determined by comparison of calculated with measured circular dichroism (CD) spectra. Three of the obtained derivatives showed a ring contraction during chemical modification. While the original lignans were inactive on the performed bioassays, the compounds which showed the cycloheptadiene skeleton revealed remarkable activities. For the inhibition of LTB_4 production the IC_{50} values of a*R*-6,7-dihydro-6-(1′-hydroxyethyl)-3,9-dimethoxy-6-methyl-5*H*-dibenzo[*a*,*c*]cycloheptene-1,2,10,11-tetraol (**6**) and a*R*-6-(1′-iodoethyl)-1,2,3,9,10,11-hexamethoxy-6-methyl-5*H*-dibenzo[*a*,*c*]cycloheptene (**8**) were 4.2 ± 0.3 μM and 4.5 ± 0.2 μM, respectively. a*R*-6,7-Dihydro-6-(1′-hydroxyethyl)-6-methyl-5*H*-dibenzo[*a*,*c*]cycloheptene-1,2,3,9,10,11-hexaol (**5**) revealed dual inhibition on COX-2 (IC_{50} 32.1 ± 2.5 μM) and on LTB_4 production (37.3 ± 5.5% inhibition at 50 μM).

1. Introduction

Schisandra chinensis (Turcz.) Baillon (Wu Wei Zi, Magnolia Vine, Schisandraceae) is a plant commonly used in traditional Chinese medicine and also widely used in Russia, possessing various biological activities like antihepatotoxic, antibacterial and cardiovascular effects, as well as effects on CNS, uterus and immune regulatory actions.[1,2] The fruits of *Schisandra chinensis* are a rich source of lignans with concentrations ranging from 7.2% to 19.2% depending on the geographical origin and on the harvesting time.[3] Main constituents such as schisandrin (**1**), gomisin A (**2**), angeloylgomisin H (**3**), and tigloylgomisin H (**4**), which contain a dibenzo[*a*,*c*]cylooctadiene skeleton with a high degree of methoxylation/dioxomethylene bridges at the biphenyl system have been isolated and fully characterized by the group of Ikeya.[4–6] An overview on typical *Schisandra* constituents has been published by Tang et al.[3] Naturally occurring lignans show a*R* and a*S* configuration due to the presence of a chiral biphenyl axis leading to atropisomerism. Synthesis of various dibenzo[*a*,*c*]cyclooctadiene lignans has been accomplished by intramolecular or intermolecular coupling of the aryl units. The latter approach requires a subsequent formation of the eight-membered ring by C–C bond formation. Stereoselective synthesis of the axial conformation has been achieved only in few cases.[1] Therefore, derivatisation of enantiomerically pure biphenyl-type lignans isolated from natural sources allows to introduce higher diversity and variability of polarity into naturally occurring lignans without the necessity of stereoselective total synthesis.

In general, biphenyl-type lignans are of great interest due to their multiple pharmacological activities such as inhibition of LTB_4 production,[7] prevention of CCl_4-induced liver damage[8] and inhibition of P-glycoproteins.[9,10]

Cyclooxygenases are key enzymes of the arachidonic acid cascade, catalyzing the first two steps of arachidonic acid transformation into prostaglandins, thromboxanes and prostacyclins. Because of the negative gastrointestinal side effects caused by the inhibition of COX-1, selective COX-2 inhibitors have been the main focus in the development of anti-inflammatory drugs during the last decade.[11] However, recently also selective COX-2 inhibition has shown its deficiencies due to higher risks of myocardial infarction and cardiovascular thrombotic events.[12] Since inflammation is known to

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be a multifactorial process, also other pathways should be included in the search for new anti-inflammatory agents. Apart from the COX pathway, arachidonic acid can also be converted via the LOX pathway into leukotrienes, which are known to play a major role in numerous disorders such as rheumatoid arthritis, asthma, psoriasis, allergic rhinitis, colitis ulcerosa and other inflammatory processes.[13] Therefore, 5-LOX inhibitors are considered to possess therapeutic potential in some of these diseases. In search of new anti-inflammatory drugs, the simultaneous blockage of both the COX-2 and 5-LOX pathways by dual inhibitors has been suggested as a possible alternative approach to selective COX-2 inhibition.[14] Drugs possessing these characteristics are believed to be more effective with lower gastric toxicity due to a stronger anti-inflammatory effect on the one hand, and on the other hand because the enhanced conversion of arachidonic acid into leukotrienes, which is observed for drugs inhibiting only the COX pathway is avoided.[15] Recent reviews of COX and leukotriene biosynthesis inhibitory effects of natural products have been published by Jachak,[16] Adams,[17] and Werz.[18]

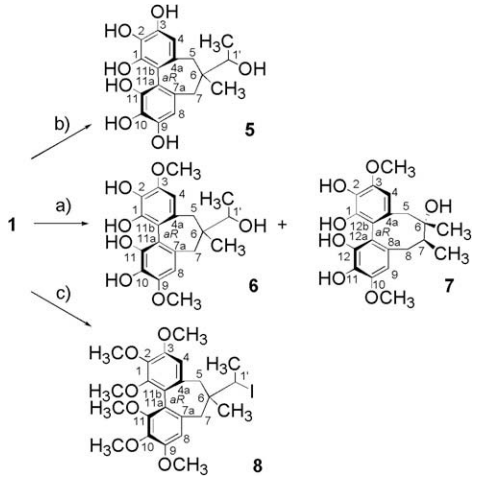
Biphenyl-neolignans such as magnolol and honokiol and derivatives thereof have been shown to act as potent inhibitors of COX-isoenzymes and 5-LOX.[19–22] In these compounds the biphenyl axis is more or less flexible, whereas in *S. chinensis* lignans this torsion angle is fixed by the presence of a cyclooctadiene ring. Apart from some reports indicating minor activities, no detailed studies revealing significant activity of *Schisandra* lignans and related structures on COX/LOX were carried out to date to the best of our knowledge.[23] Therefore, the aim of the present study was to evaluate the inhibitory activity of the main *S. chinensis* lignans and semi-synthetically prepared derivatives of schisandrin against COX-isoenzymes and 5-LOX mediated LTB_4 formation.

2. Results and discussion

Compounds **1**–**4** (Scheme 1) were isolated from *Schisandra chinensis* fruits in high yields and identified by comparison with literature NMR data.[24,25] Additionally, it was possible to confirm the structure of these compounds by means of electron impact mass spectrometry (EI-MS). Because of its relative abundance, schisandrin was selected for the intended derivatizations (Scheme 2).

In order to obtain more polar derivatives, schisandrin (**1**) was subjected to chemical modification. In view of modifications and synthesis of natural products mild reagents are absolutely indispensable. El-Feraly and Li proposed BBr_3 as a suitable demethylation reagent.[26] Selective demethylation with one equivalent of BBr_3 (reaction A) turned out to be unsuccessful, because a complex mixture of products was formed of which only some products could be isolated in pure form. Compound **6** was isolated as a medium polar derivative in about 30% HPLC yields. Compound **7** was formed in small amounts (5%).

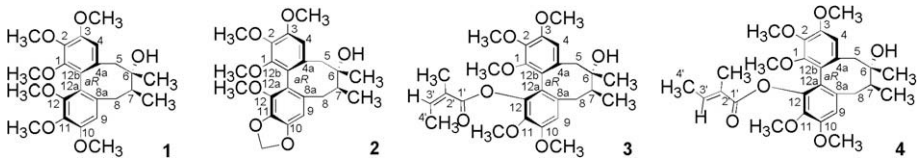
An excess of demethylation reagent (6.5 equiv BBr_3) led to a more uniform product distribution (reaction B). From the product mixture the major component **5** was isolated and its structure and constitution were determined by NMR spectroscopy. From 1D proton and 2D HSQC spectra it could be deduced that this compound showed a higher symmetry than the starting material. In



Scheme 2. Isolated and structurally confirmed products (**5**–**8**) of derivatisation of schisandrin (**1**). While absolute configuration at C-1′ in compounds **5**, **6**, **8** could not be determined, the C-6 atom is no longer a sterogenic center, but becomes prochiral. Axial stereochemistry was determined by comparison of calculated with measured CD spectra. Reaction conditions: (a) 1.1 equiv BBr_3; (b) 6.5 equiv BBr_3; (c) 5.3 equiv 9-iodo-9-BBN, $CH_2OHCH_2NH_2$.

particular, the methylene groups C(5)H_2 and C(7)H_2 of **5** showed very similar proton and carbon resonance values. HMBC correlations of the methyl protons C(6)–CH_3 to both carbons C(5) and C(7) indicated a reduction of the ring size, resulting in a cycloheptadiene skeleton. The carbons C(1′) and C(2′) formed a 1-hydroxyethyl side chain. No resonances of *O*-methyl groups were observed. The demethylation led also to a series of mostly seven-membered side products, which could not be separated. The excess of reagents thus led to full demethylation and concomitant ring contraction. In reactions A and B there seems to be a concurrent reaction between demethylation and ring contraction, depending on reaction time and conditions (data not shown). In reaction A, the major component **6** was identified also as a cycloheptadiene containing structure, which could already be anticipated by the similar carbon resonances owing to the higher symmetry in this compound as compared to **1**. Compound **6** differed from compound **5** by the presence of *O*-methyl groups at positions C(3) and C(9). Compound **7**, which was isolated in very small amounts (5%) showed the same methoxylation pattern as **6** but had retained the cyclooctadiene skeleton. Comparison of the NMR shifts at C-5, 6, 7, 8 with those of compound **1** revealed that the relative configuration at C(6) and C(7) was retained in **7**. The seven-membered ring seems thermodynamically favoured over the eight-membered congener in the reactions A and B. We observed for this reaction that excess of reagent (BBr_3) led to complete demethylation, but already non stoichiometric amounts could induce ring contraction.

By reaction of 9-iodo-9-bora-bicyclononane (9-I-9-BBN) with **1**, a procedure proposed by Fürstner and Seidel[27] (reaction C), mainly three products were obtained of which only **8** could be structurally elucidated by NMR. Only the ring contraction occured but no demethylation. Additionally the OH group at C-1′ was replaced in **8** by an iodine. From 1D and 2D NMR spectra it was deduced that



Scheme 1. *S. chinensis* lignans: schisandrin (**1**), gomisin A (**2**), angeloylgomisin H (**3**), tigloylgomisin H (**4**), with numbering scheme (NMR table in Supplementary data) and axial configuration displayed in the formula.

the other products had retained the eight-membered ring skeleton, but their structure could not be fully elucidated. Due to the symmetric substitution pattern in the aromatic rings, atom C(6) is no longer a stereogenic center but becomes a pro-chiral center in compounds **5**, **6**, and **8**. Attempts to confirm the structure of the compounds **5**–**7** by means of EI-MS failed, since it was not possible to evaporate these compounds, which is in contrast to the behavior observed for compounds **1**–**4**. For compound **8**, the characterisation with EI-MS was again successful. Thus, EI-MS could be used for the characterisation of all methoxylated derivatives investigated within this work, while experiments with derivatives carrying phenolic groups generally failed. The problems with the evaporation of the derivatives **5**–**7** was attributed to the phenolic groups. The absolute configuration at C(1′) in all compounds with cycloheptadiene skeleton could not been determined yet. Compounds **5**–**8** neither have been isolated from natural sources nor have been synthesized so far.

Especially for the derivatives which show the ring contraction the question of axial chirality has to be clarified. In order to determine whether the axial configuration was retained or inverted, CD spectra for several derivatives were measured and compared with those calculated for model compounds (**M1**–**M3**) and **8** as well as with literature data for schisandrin (**1**).[5]

2.1. CD-Measurements and calculations

Simulated CD spectra are based on rotational strengths and excitation energies resulting from time-dependent density functional theory calculations. Exploratory calculations on unsubstituted dibenzocyclooctadiene model compounds **M1** and **M2** revealed that the overall appearance of the CD spectra is largely determined by their axial chirality (torsional angle C(8a)–C(12a)–C(12b)–C(4a) between the two phenyl rings) with little influence of the configurations at C(6) and C(7) (Fig. 1). Similarly, in seven-membered ring model compounds (**M3**), the chirality of the side chain—C(1′)—only marginally influenced the shape of the simulated CD curves. As already demonstrated by Chang et al.[1] and the group of Ikeya[5] the combination of a (+)-Cotton effect at around 210 nm and a (−)-Cotton effect at about 240 nm usually corresponds to *P* configuration (which is equal to a*S*) for the biphenyl axis. Actually, calculations on **8** indicated that besides a somewhat increased phenyl torsion angle, τ(C(7a)–C(11a)–C(11b)–C(4a)) = +60° compared with τ(C(7a)–C(11a)–C(11b)–C(4a)) = −45° in **M3**, and the presence of several additional electronic transitions, simulated CD curves should be similar to that one of the unsubstituted model compound **M3**. These results suggested that the methoxy groups do not affect the overall shape of the CD spectra. This was further corroborated by comparison of the CD spectra of schisandrin (**1**)[5] and model compound **M1**. Thus it must be concluded that the obtained compounds **5**, **6**, and **8** have retained their axial geometry during the ring contraction. A graphical representation of measured CD spectra is given in Figure 2.

2.2. Inhibition of COX-1/2 and 5-LOX product formation

Testing of genuine *S. chinensis* lignans (compounds **1**–**4**) resulted in almost no or low activities at a screening concentration of 50 μM in the assay mixture. Compound **7**, also possessing an eight-membered ring, did not reveal activity in any of the performed assays either. However, some of the obtained derivatives possessing a cycloheptadiene ring exhibited remarkable activities (Fig. 3). While none of the compounds inhibited COX-1, compound **5** showed a good inhibition of COX-2 (IC_{50} 32.1 ± 2.5 μM) and a moderate inhibition of 5-LOX mediated LTB_4 formation.

Compounds **6** and **8** selectively inhibited LTB_4 production. For **8** an IC_{50} of 4.5 ± 0.2 μM was determined, and **6** showed an IC_{50} value of 4.2 ± 0.3 μM. This is in the range of Zileuton, a 5-LOX inhibitor which has been used as a positive control in this assay (IC_{50} 5.0 μM). Comparing the activity of **6** and **7**, possessing the same substitution pattern but differing in the ring size, shows the importance of the cycloheptadiene skeleton. This means that only the seven-membered ring derivatives (**5**, **6**, and **8**) showed strong but variable activities, while the eight-membered congeners (**1**–**4** and **7**) were completely inactive.

Interestingly, the absence or presence of the methyl groups at the positions C(1), (2), (10), (11) in the seven-membered rings hardly influenced the inhibitory activity, while the lack of the methoxy groups at positions C(3) and C(9) in compound **5** resulted in a reduced activity on LTB_4 production in comparison to **6** and **8**. However, compound **5** showing the seven-membered ring with the highest polarity, additionally inhibited COX-2.

Our study has revealed that not only biphenyl-lignans with flexible phenyl rings such as magnolol can act as inhibitors of COX and 5-LOX, but also more complex biphenyl-lignans with sterically fixed rings can be active. The cycloheptadiene skeleton seems to be necessary for LTB_4 formation inhibitory activity, while the different methoxylation/substitution pattern plays an ancillary role.

3. Conclusion

Schisandrin (**1**) was subjected to chemical modifications in order to obtain derivatives of different polarity and constitution. In vitro testing for COX-1 and COX-2 inhibition as well as for the inhibition of 5-LOX mediated LTB_4 production revealed that the original *Schisandra* lignans and the eight-membered ring (**7**) showed no activity, while only derivatives containing the cycloheptadiene

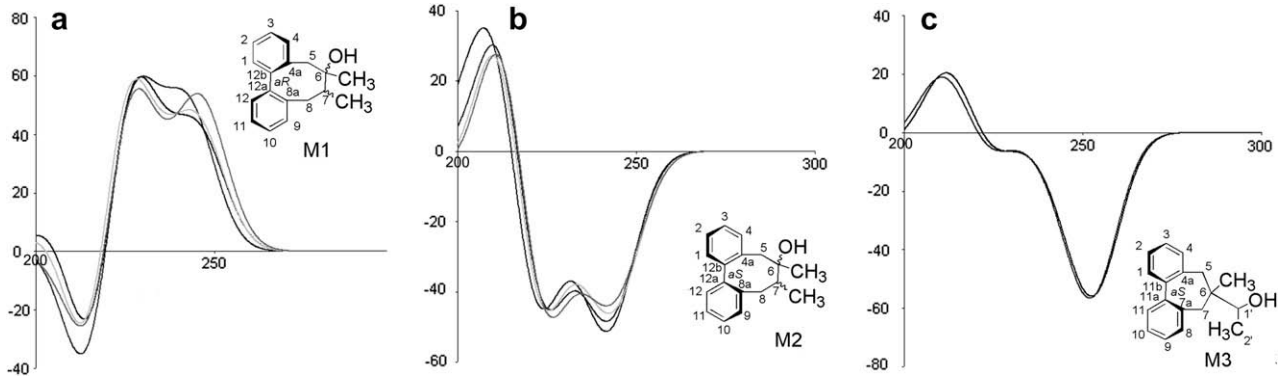


Figure 1. Simulated CD spectra of different stereoisomers of **M1**, **M2**, **M3** (chiral centers for model compounds **M1**, **M2**: C6, C7; and **M3**: C1′). The spectra a, b, and c are an overlay of the different stereoisomers of the respective model compound.

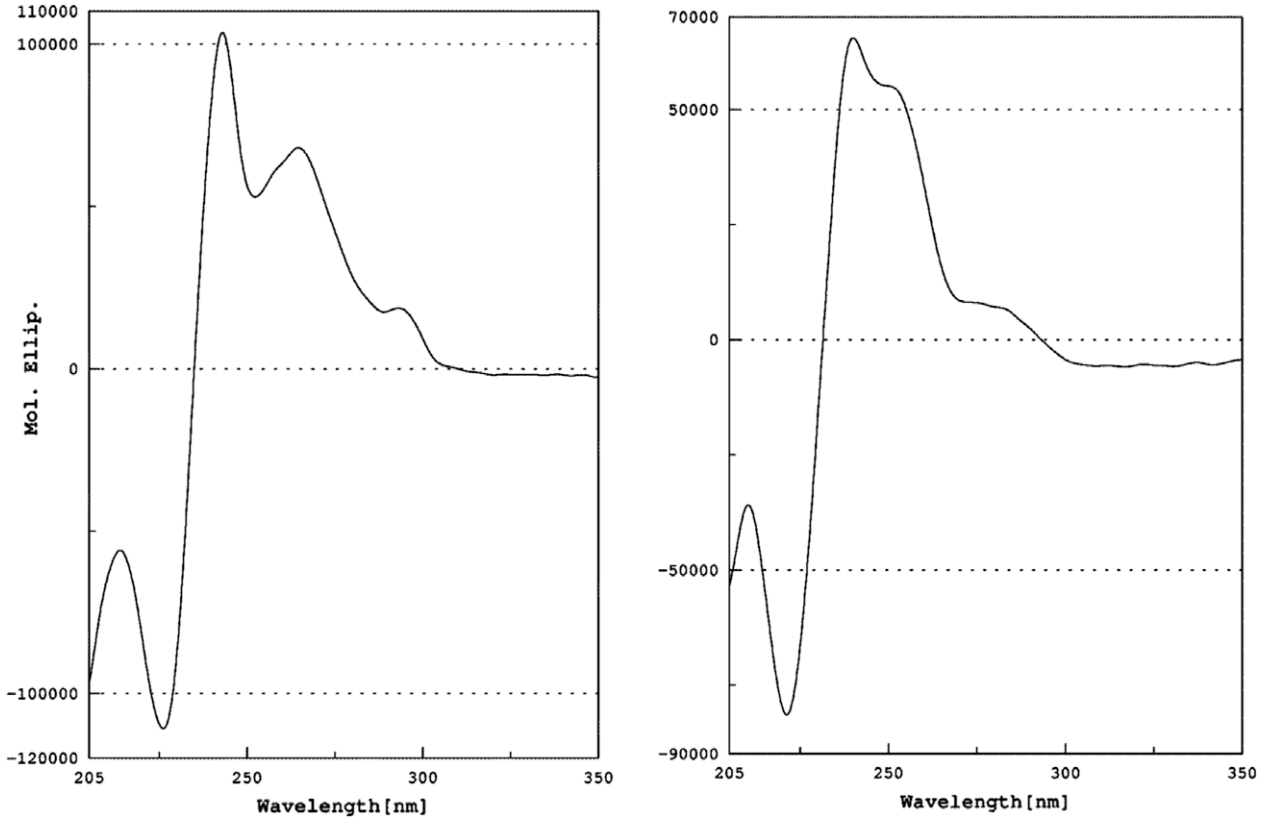


Figure 2. Measured CD spectra of compounds **6** (left) and **7** (right).

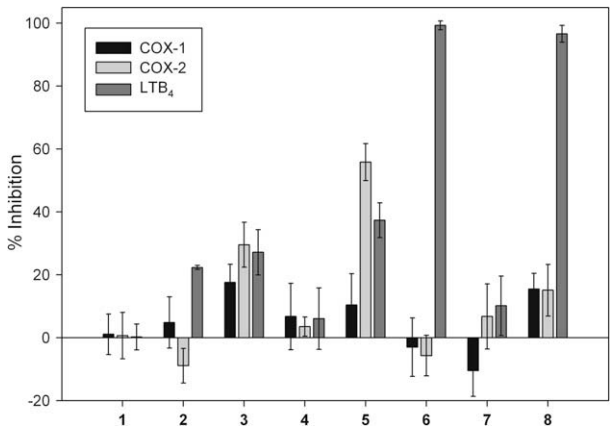


Figure 3. Comparison of the inhibition of compounds **1**–**8** to COX-1, -2 and LTB_4 formation assays. Samples were tested at a screening concentration of 50 μM in at least three independent experiments in duplicate. Data are expressed as mean values ± SD.

skeleton exhibited significant inhibitory potential on LTB_4 production (**5**, **6**, **8**). This work is another example that natural compounds act as leads which may be optimized by chemical modifications.[28]

Open to further investigation remains the question whether the configuration of the chiral axis is essential for biological activity. Our investigations were exclusively based on compounds with a*R* configuration. In comparison to studies conducted by Zhou and co-workers,[29] we assume a 5-lipoxygenase-activating-protein (FLAP) inhibitory activity for our active compounds. This hypothesis should be further corroborated by docking studies.

4. Materials and methods

4.1. Extraction and isolation

230 g raw dry plant material (fruits of *S. chinensis*) yielded 23.1 g raw extract by cold percolation with dichloromethane (DCM). The DCM extract was subjected to LC on silica gel with a hexane/ethylacetate/methanol gradient. 19 fractions were collected and monitored using analytical TLC and HPLC indicating four main constituents of interest (fractions F13–F16). After preliminary purification over RP-18 SPE, compounds **1**–**4** were isolated by semi-preparative HPLC on an RP-18 column (LiChrospher, 7 μm, 21 × 250 mm, Merck, Darmstadt, Germany) using an acetonitrile/water gradient (yields: 872 mg schisandrin (**1**), 142 mg gomisin A (**2**), 68 mg angeloylgomisin H (**3**), 32 mg tigloylgomisin H (**4**).

4.2. Derivatisation

Preparation of the derivatives was carried out in three different manners following typical literature procedures: A, B[26] and C[27] under inert conditions using absolute solvents.

4.2.1. Reaction A

To a cooled solution of **1** (45 mg, 0.10 mmol) in DCM (abs.) 1.1 equiv of BBr_3 (10 wt % solution in DCM, 0.10 ml, 0.11 mmol) were added over a period of 30 min and continuously stirred for another 30 min at −78 °C. The solution was allowed to warm to rt and stirred for 3 h and then poured onto 10 ml ice water. After stirring for 2 min the organic phase was separated and the water phase was extracted three times with DCM. The combined organic layers were successively washed with brine and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced

pressure yielding 34 mg of a brownish residue. **6** and **7** were isolated from the crude mixture in 30% and 5%, respectively.

4.2.2. Reaction B

To a cooled solution of **1** (52 mg, 0.12 mmol) in DCM (abs) 1 equiv of BBr_3 (10 wt % solution in DCM, 0.12 ml, 0.13 mmol) was added over a period of 30 min. The solution was stirred for 3 h with warming up to rt. The mixture was cooled down to −78 °C again and another 5.6 equiv of BBr_3 (10 wt % solution in DCM, 0.61 ml, 0.67 mmol) were added over a period of 30 min, stirred over night with warming up to rt and poured onto 10 ml ice water. After stirring for 2 min the organic phase was separated and the water phase was extracted three times with DCM. The combined organic phases were successively washed with brine and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure yielding 60 mg brownish residue. Compound **5** was obtained after chromatography in 75% yields.

4.2.3. Reaction C

To a solution of **1** (43 mg, 0.10 mmol) in 0.5 ml THF 9-iodo-9-BBN (9-iodo-9-bora-bicyclononane, 1 M in hexane) was added and stirred over night. The residue after evaporation was redissolved in diethylether and ethanolamine (25 μl, 25 mg, 0.41 mmol)—dissolved in 0.25 ml THF—was added. After stirring for 3 h the precipitate was filtered off and the filtrate dried in vacuum yielding 138 mg raw product. From the crude product mixture **8** was isolated in ca. 10% yield.

The reaction progress was monitored by TLC (ethyl acetate). All three reactions yielded complex mixtures, which were subjected to semiprep. HPLC workup from which the major products (**5**–**8**) could be isolated and identified by NMR and MS measurements (see NMR and MS part, Table 1 and Table 2).

4.3. NMR experiments

^1H, ^{13}C, and 2D NMR experiments (HSQC, HMBC, DQF-COSY) were performed on a Varian UnityInova spectrometer operating at a proton frequency of 600 MHz. Compounds were dissolved in $CDCl_3$ or methanol-d_4 and spectra were recorded at 25 °C. TMS was used as internal standard. Experimental parameters were as published in Seebacher et al.[30]

4.4. Mass spectrometry

Electron impact (EI, 70 eV) mass spectra were recorded on a Waters GCT Premier equipped with direct insertion (DI). Further MS data were obtained using a micrOTOF ESI-MS system (Bruker Daltonics) connected to an Agilent 1100 series HPLC in combination with HyStar 3.0 software (Bruker Daltonics). Table 2 gives a comparison of calculated and found HRMS data, as well as a listing of ions observed in the EI and ESI mass spectra.

Table 1
Full names and NMR assignment of derivatives **5**–**8**

	5[b] a*R*-6,7-Dihydro-6-(1′-hydroxyethyl)-6-methyl-5*H*-dibenzo [*a*,*c*]cycloheptene-1,2,3,9,10,11-hexaol		**6**[b] a*R*-6,7-Dihydro-6-(1′-hydroxyethyl)-3,9-dimethoxy-6-methyl-5*H*-dibenzo[*a*,*c*]cycloheptene-1,2,10,11-tetraol		**8**[b] a*R*-6-(1′-iodoethyl)-1,2,3,9,10,11-hexamethoxy-6-methyl-5*H*-dibenzo[*a*,*c*] cycloheptene			**7**[a] (a*R*-6*S*,7*S*)-5,6,7,8-Tetrahydro-3,10-dimethoxy-6,7-dimethyldibenzo[*a*,*c*]cyclooctene-1,2,6,11,12-pentaol	
	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H		^{13}C	^1H
C(1)	143.1	—	n.d.	—	152.2	—	C(1)	146.9	—
C(2)	134.1	—	135.0	—	141.2	—	C(2)	138.5	—
C(3)	145.4	—	148.0	—	152.2	—	C(3)	148.8	—
C(4)	111.1	6.33 s	107.9	6.47 s	108.2	6.49 s	C(4)	111.8	6.70 s
C(4a)	130.4	—	130.3	—	133.8	—	C(4a)	n.d.	—
C(5)	42.7	1.91 d (12.4) 2.10 m	42.6	1.92 d (12.4) 2.23 d (12.4)	39.8	2.33 d (12.6) 2.04 d (12.6)	C(5)	42.4	2.36 m 2.58 d (13.6)
C(6)	50.4	—	50.6	—	49.5	—	C(6)	73.8	—
C(7)	43.5	1.85 d (12.4) 2.64 d (12.4)	43.7	1.88 d (13.2) 2.74 d (13.2)	45.3	2.69 d (13.2) 2.01 d (13.2)	C(7)	42.4	1.79 m
C(8)	110.3	6.51 s	106.6	6.61 s	107.4	6.81 s	C(8)	35.8	2.36 m 2.82 d (13.6)
	—	—	—	—	—	—	C(8a)	n.d.	—
C(9)	145.5	—	148.1	—	152.7	—	C(9)	111.7	6.64 s
C(10)	134.2	—	135.1	—	140.9	—	C(10)	148.8	—
C(11)	143.1	—	n.d.	—	151.6	—	C(11)	138.7	-
	—	—	—	—	—	—	C(12)	146.9	—
C(11a)	117.7	—	119.0	—	121.7	—	C(12a)	124.3	—
C(11b)	118.0	—	119.2	—	122.7	—	C(12b)	124.9	—
C(1′)	62.0	4.28 q (6.8)	61.8	4.26 q (6.8)	44.8	4.32 q (7.2)	C(1′)	—	—
C(2′)	21.1	1.64 d (7.2)	21.1	1.66 d (7.2)	23.5	1.92 d (7.2)	C(2′)	—	—
C(6)-CH_3	19.5	1.03 s	19.4	1.07 s	21.4	1.07 s	C(6)-CH_3	29.8	1.20 s
C(7a)	130.0	—	129.9	—	133.2	—	C(7a)	—	—
	—	—	—	—	—	—	C(7)-CH_3	16.5	0.81 d (7.2)
OMe(1)	—	—	—	—	60.8*	3.68* s	OMe(1)	—	—
OMe(2)	—	—	—	—	61.0	3.90 s	OMe(2)	—	—
OMe(3)	—	—	56.8	3.89 s	56.1	3.90 s	OMe(3)	56.5	3.90 s
OMe(9)	—	—	56.8	3.89 s	56.1	3.93 s	OMe(10)	—	—
OMe(10)	—	—	—	—	61.0	3.90 s	OMe(11)	56.5	3.88 s
OMe(11)	—	—	—	—	60.7*	3.66* s	OMe(12)	—	—

The resonance assignments of the two diastereotopic benzylic fragments in the seven-membered ring compounds (**5**, **6**, **8**) were arbitrary.
* Shifts exchangeable. Multiplicity: s singlet, d doublet, q quartet, m multiplet. Coupling constant *J* in parentheses (Hz).
[a] Chloroform-d_1.
[b] Methanol-d_4.

Table 2
Mass spectral data of compounds **1**–**6** and **8**

Compound	HRMS	*m/z* (%)
1[a]	$C_{24}H_{32}O_7$ calcd 432.2148 found 432.2165	432 (15) $[M]^+$, 414 (100) $[M-H_2O]^+$, 399 (17), 383 (8), 368 (1), 353 (11), 352 (10), 330 (13), 233 (7), 181 (5), 167 (6)
2[a]	$C_{23}H_{28}O_7$ calcd 416.1835 found 416.1844	416 (23) $[M]^+$, 398 (100) $[M-H_2O]^+$, 383 (18), 367 (9), 352 (8), 341 (11), 337 (8), 336 (11), 314 (20), 217 (6), 181 (22), 165 (10)
3[a]	$C_{28}H_{36}O_8$ calcd 500.2410 found 500.2427	500 (8) $[M]^+$, 482 (38) $[M-H_2O]^+$, 430 (6), 418 (4), 400 (40) $[M-C_5H_8O_2]^+$, 399 (14), 385 (6), 368 (14), 353 (9), 181 (4), 165 (3), 83 (100) $[C_5H_7O]^+$, 55 (29) $[C_4H_7]^+$
4[a]	$C_{28}H_{36}O_8$ calcd 500.2410 found 500.2441	500 (5) $[M]^+$, 482 (56) $[M-H_2O]^+$, 418 (3), 400 (55) $[M-C_5H_8O_2]^+$, 399 (20), 385 (7), 368 (19), 353 (14), 181 (5), 165 (3), 83 (100) $[C_5H_7O]^+$, 55 (28) $[C_4H_7]^+$
5-H_2O[a]	$C_{18}H_{20}O_7$ calcd 330.1103 found 330.1096	330 (100) $[M-H_2O]^+$, 298 (40), 287 (23), 271 (31), 243 (48), 229 (57), 215 (37), 193 (40)
5-H_2O+Na[b]	$C_{18}H_{18}NaO_6$ calcd 353.1001 found 353.0959	Traces
6-H_2O[a]	$C_{20}H_{22}O_6$ cald 358.1416 found 358.1429	358 (100) $[M-H_2O]^+$, 343 (22) $[M-H_2O-CH_3]^+$, 315 (28), 285 (20), 153 (28), 115 (30)
6-H_2O+Na[b]	$C_{20}H_{22}NaO_6$ calcd 381.1314 found 381.1298	Traces
8[a]	$C_{24}H_{31}IO_6$ calcd 542.1165 found 542.1193	542 (8) $[M]^+$, 414 (100) $[M-HI]^+$, 399 (18), 384 (15), 383 (16), 371 (12), 368 (13), 353 (11), 325 (7), 233 (5), 181 (11), 165 (5), 128 (7) $[HI]^+$, 127 (6) $[I]^+$

[a] DI-EI-MS (70 eV).
[b] TOF-ESI-MS.

4.5. CD-Measurements and calculations

CD measurements were performed on a Jasco-715 in a 0.1 cm cell at 25 °C with a resolution of 0.2 nm and a scan rate of 50 nm/min. Five spectra were accumulated and averaged for each sample. Calculation of the CD spectra was performed with the G03 program package.[31] Structures were optimized at the B3LYP/6-31G(d) and B3LYP/cc-pVTZ levels.[32–35] Electronic transitions and rotational strengths were derived by time-dependent density functional theory[36–40] at the B3PW91/TZVP//B3LYP/cc-pVTZ level of theory.[41] Solvent effects (MeOH) were simulated employing the IEF-PCM formalism.[42]

4.6. COX-1/-2 and 5-LOX product formation assays

The bioassays for inhibition of COX-1 and COX-2 were carried out in a 96-well-plate format with purified prostaglandin H synthase (PGHS-1) for COX-1 and purified PGHS-2 for COX-2 from ram seminal vesicles and sheep placental cotyledons, respectively (both Cayman Chemical Company, Ann Arbor, USA).[43,44] The concentration of the main arachidonic acid metabolite in the reaction, namely PGE_2 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) and NS-398 (Cayman Chemical Company, IC_{50} COX-2 2.6 μM) were used as positive controls.

The 5-LOX product formation assay was performed in a 96-well-plate format with stimulated human neutrophile granulocytes as described by Adams et al.[45] with slight modifications.[46] Briefly, polymorphonuclear leukocytes with 5-LOX activity were isolated from venous human blood based on sedimentation rates and lysis tolerance. Cell viability was checked with 0.4% trypan blue solution. The cell suspension (4500 cells/ml) was incubated with the sample, $CaCl_2$, calcimycin A23187 and arachidonic acid in a shaking water bath at 37 °C. After 10 min incubation was stopped by addition of 10% formic acid. After centrifugation, the samples were diluted and the concentration of LTB_4 formed during incubation was determined by means of a competitive LTB_4 EIA kit (Assay Designs Inc. Ann Arbor, USA). Positive control for this test system was Zileuton (Sequoia Oxford, UK) showing an IC_{50} value of 5.0 μM.

For the preliminary screening test samples were dissolved in absolute ethanol to a final concentration of 50 μM. Samples were tested in at least three independent experiments run in duplicate. Results are given as mean ± S.D. Samples showing moderate inhibitory activity were then subjected to further studies.

4.7. Statistics

IC_{50} determinations were performed in at least four concentrations, each in at least three independent experiments, every time in duplicate. The IC_{50} values were calculated with the SigmaPlot program package employing the 4-parameter logistic regression model.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.031.

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