

Letter

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Magnolia extract, magnolol and metabolites: activation of cannabinoid CB₂ receptors and blockade of the related GPR55

Viktor Rempel,[§] Alexander Fuchs,[§] Sonja Hinz,[§] Tadeusz Karcz,^{§a} Matthias Lehr,⁺ Uwe Koetter,[#] and Christa E. Müller^{§*}

[§]PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany, [#]CH-8592 Uttwil, Oberdorfstrasse 14, Switzerland, ⁺Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Hittorfstrasse 58-62, D-48149 Münster, Germany. ^aT. Karcz was on leave from the Jagiellonian University, Cracow, Poland.

KEYWORDS bioactivation, biphenyls, CB₂ receptor agonists, Chinese traditional medicine, honokiol, magnolia extract, *Magnolia officinalis*, magnolol, magnolol metabolites

Supporting Information Placeholder

ABSTRACT: The bark of *Magnolia officinalis* is used in Asian traditional medicine, e.g., for the treatment of anxiety, sleeping disorders and allergic diseases. We found that the extract and its main bioactive constituents, magnolol and honokiol, can activate cannabinoid (CB) receptors. In cAMP accumulation studies magnolol behaved as a partial agonist (EC₅₀ 3.28 μM) with selectivity for the CB₂ subtype, while honokiol was less potent showing full agonistic activity at CB₁, and antagonistic properties at CB₂. We subsequently synthesized the major metabolites of magnolol and found that tetrahydromagnolol (**7**) was 19-fold more potent than magnolol (EC₅₀ CB₂: 0.170 μM) exhibiting high selectivity versus CB₁. Additionally, **7** behaved as an antagonist at GPR55, a CB-related orphan receptor (K_B 13.3 μM, β-arrestin translocation assay). Magnolol and its metabolites may contribute to the biological activities of *Magnolia* extract via the observed mechanisms of action. Furthermore, the biphenylic compound magnolol provides a simple novel lead structure for the development of agonists for CB receptors, and antagonists for the related GPR55.

Magnolia officinalis bark plays an important role in traditional Chinese and Japanese herbal medicine for the treatment of anxiety, sleep-related problems and allergic diseases.^{1,2} The pharmacological effects were proposed to be mainly mediated by the neolignans honokiol (**1**), 4-methoxyhonokiol (**2**), magnolol (**3**) and (R)-8,9-dihydroxydihydromagnolol (**4**).²⁻⁴ An intensive search for their molecular targets revealed an interaction of honokiol and magnolol with a variety of enzymes and receptors at micromolar concentrations.⁵⁻⁸

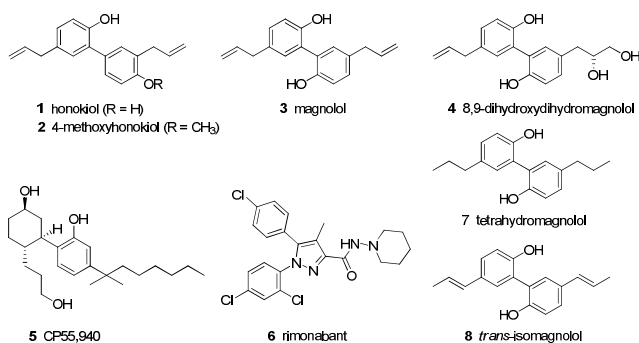


Figure 1. Structures of biologically active neolignans and synthetic CB receptor ligands.

We observed that the reported in vivo effects of *Magnolia officinalis* extracts and its constituents possess a striking analogy to the effects described for ligands acting at

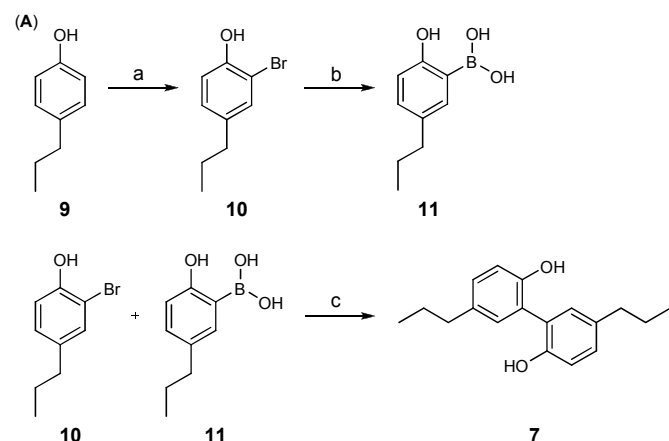
cannabinoid (CB) receptors (Figure 1).⁹ Furthermore the neolignans share structural similarity with some highly potent synthetic CB receptor ligands, such as CP55,940 (**5**) but also plant-derived biaryl cannabinoids.^{8,10,11}

Cannabinoid receptors belong to the G protein-coupled receptor (GPCR) superfamily and are divided into two distinct subtypes designated CB₁ and CB₂, both of which are linked to an inhibition of adenylate cyclase.¹² CB₁ activation mediates analgesia, stimulation of appetite, and euphoria, among other effects.¹³ CB₂ receptor activation results in analgesic and antiinflammatory effects.⁹ A related GPCR is the orphan GPR55, which has been described to be activated by lysophosphatidylinositol (LPI).¹⁴ Despite its low amino acid identity to CB₁ (13.5%) and CB₂ (14.4%) receptors, the GPR55 is reported to interact with certain cannabinoids.¹⁴ For instance, the CB receptor agonist CP55,940 (**5**) behaves as a GPR55 antagonist, while the CB₁-selective inverse agonist rimonabant (**6**) acts as an agonist at GPR55 (see SI).^{12,14} The receptor appears to be involved in cancer cell proliferation and migration, angiogenesis, regulation of bone mass and the onset of neuropathic pain.¹⁴⁻¹⁶

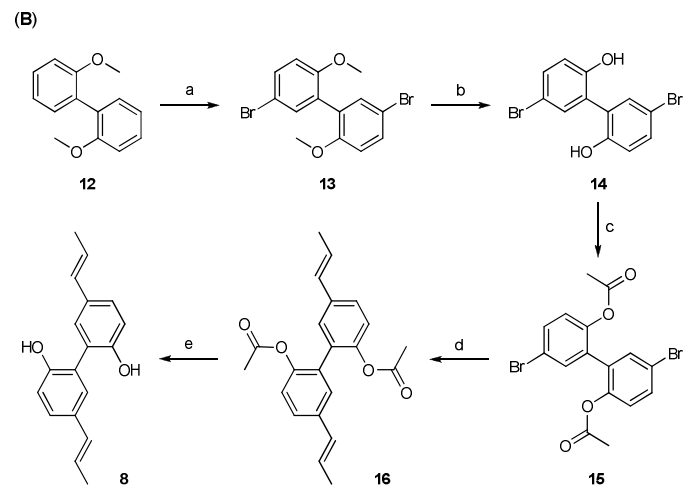
We investigated an ethanolic (90%) extract of *Magnolia officinalis* bark (magnolol content: 17.9%, honokiol: 22.8%, also see SI) for its affinity to CB receptors. In addition, magnolol and honokiol, the major neolignans found in *Magnolia* bark (0.78-7.65%, and 0.17-1.81%, respectively), were studied in radioligand binding and cAMP accumulation assays at CB₁ and CB₂ receptors, and in β-arrestin translocation assays at the GPR55.

Magnolol is known to be extensively metabolized by tissue and intestinal bacterial enzymes to hydrogenated and hydroxy derivatives, glucuronides and sulfates.² The main fecal metabolites of orally administered magnolol (**3**) in rats are tetrahydromagnolol (**7**) and *trans*-isomagnolol (**8**).¹⁷ Interestingly, the amount of tetrahydromagnolol (**7**) formed had been found to be increased in rats after repeated administration of magnolol (**3**), indicating the involvement of inducible enzymes in the metabolization of magnolol (**3**).^{17, 18} Since metabolites may contribute to the action of drugs we additionally synthesized and investigated **7** and **8**.

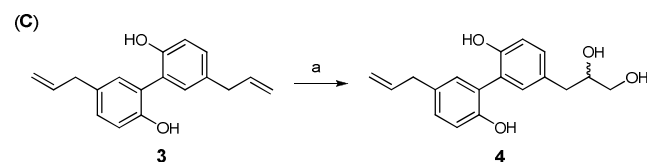
Scheme 1. Synthesis of tetrahydromagnolol^a, *trans*-isomagnolol^b and 8,9-dihydroxydihydromagnolol^c



^aReagents, Conditions: (a) Br₂, NaHCO₃, CHCl₃, 0°C, 80% yield. (b) three steps (1) butyl lithium, Et₂O, -78°C; (2) B(OCH₃)₃, Et₂O, -78°C to RT; (3) HCl, Et₂O, 50% yield. (c) Pd(PPh₃)₄, Na₂CO₃, toluene, EtOH, H₂O, 100°C, 25% yield.



^bReagents, Conditions: (a) N-bromosuccinimide, dimethylformamide, rt, 12h, 80% yield. (b) CH₂Cl₂, BBr₃, -78°C to rt, 75% yield. (c) acetic anhydride, 120 °C, 2h, 95% yield. (d) *trans*-1-propene-1-boronic acid, Pd(PPh₃)₄, CsF, dioxane, water, 85% yield. (e) NaHCO₃, water, methanol, 90% yield.



^cReagents, Conditions: (a) OsO₄, *t*-BuOH, acetone, water, 24h, 20%.

The synthesis of **7** by phenolic oxidative coupling had previously been described.¹⁹ In the present study we report a new synthetic route to obtain **7** in a higher yield of 25% (Scheme 1A). 2-Bromo-4-propylphenol (**10**) was prepared starting from 4-propylphenol (**9**) by electrophilic aromatic substitution.²⁰ Boronic acid (**11**) was obtained by treatment of **10** with butyllithium and subsequent reaction with trimethyl borate followed by acidic hydrolysis.²¹ The coupling of **10** with **11** was performed by a Suzuki-Miyaura cross-coupling reaction procedure, which had previously been used for the preparation of honokiol derivatives,²² affording the desired tetrahydromagnolol (**7**). *trans*-Isomagnolol (**8**) was obtained by a new procedure depicted in Scheme 1B. 2,2'-Dimethoxybiphenyl (**12**) was brominated with *N*-bromosuccinimide to give 5,5'-dibromo-2,2'-dimethoxybiphenyl (**13**).²³ After demethylation of **13** to 5,5'-dibromo-2,2'-diol (**14**)²⁴ and subsequent protection of **14** to the acetylated intermediate 5,5'-dibromo-2,2'-diacetate (**15**) a Suzuki-Miyaura cross-coupling reaction was performed with *trans*-propenylboronic acid to yield 5,5'-di-((*E*)-prop-1-enyl)biphenyl-2,2'-diacetate (**16**).²⁴ Product **8** was obtained by basic ester cleavage of **16**.²⁵ Another metabolite, primarily found in the human urine after oral administration of *Magnolia officinalis* bark preparations, is 8,9-dihydroxydihydromagnolol (**4**). Compound **4** was synthesized as depicted in Scheme 1C by treatment of **3** with osmium tetroxide to afford **4** in 20% yield after purification.²⁶

The ethanolic *Magnolia officinalis* bark extract ("M-extract") showed high affinity in radioligand binding assays for human CB₁ and CB₂ receptor subtypes recombinantly expressed in Chinese hamster ovary (CHO-K1) cells (for assay procedures see references).^{27, 28} As depicted in Figure 2 the extract preferentially displaced the radioligand at CB₂ receptors (K_i 0.165 µg/mL at CB₂, 1.21 µg/mL at CB₁; >7-fold difference).

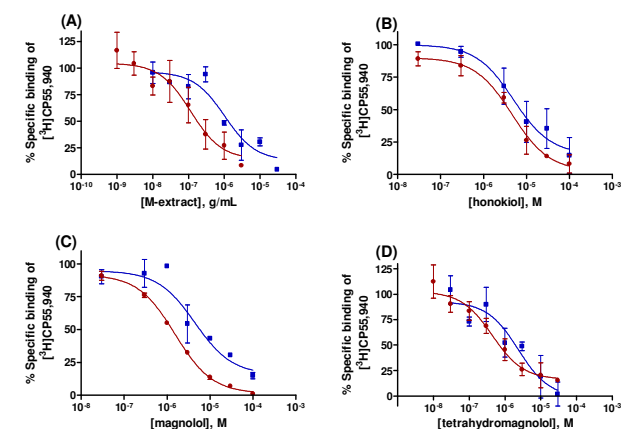


Figure 2. Concentration-dependent inhibition of specific [³H]CP55,940 binding at membrane preparations of CHO cells recombinantly expressing human CB₁ (■) or CB₂ (●) receptors by (A) magnolia extract, (B) honokiol (**1**), (C) magnolol (**3**), and (D) tetrahydromagnolol (**7**). Data points represent means ± SEM of three independent experiments, performed in duplicates.

Magnolol, but not honokiol, also appeared to show a certain preference for CB₂ in binding studies (K_i values of magnolol at CB₁: 3.19 µM; at CB₂: 1.44 µM; honokiol at CB₁: 6.46 µM, at CB₂: 5.61 µM, Figures 2B and 2C, and

Table 1). In cAMP assays the difference was even more pronounced, magnolol showing a 6-fold higher potency at CB₂ as compared to CB₁ receptors (Table 1 and Fig. 3A).

The functional studies indicated that magnolol (**3**) is a partial agonist at both receptor subtypes in comparison to the full agonist **5**. Although **3** possessed higher potency at CB₂ as compared to CB₁, it exhibited lower efficacy (CB₁: 62%, CB₂: 31%; Table 1 and Figure 3).

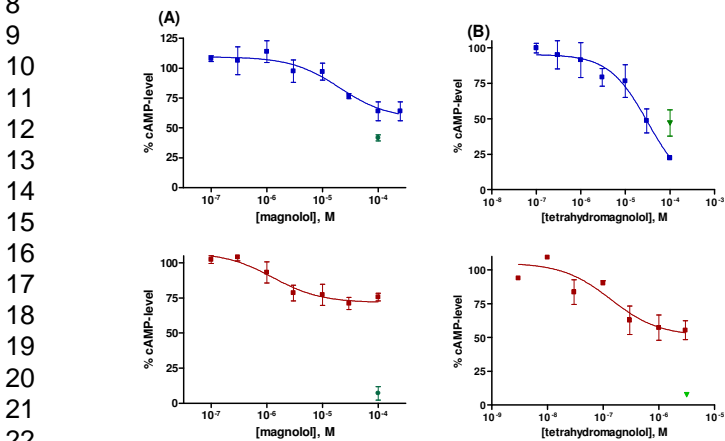


Figure 3. Concentration-dependent inhibition of forskolin (10 μM)-induced cAMP accumulation in CHO cells recombinantly expressing the human CB₁ (■) or CB₂ (■) receptor by (A) magnolol (**3**), and (B) tetrahydromagnolol (**7**). The effect of the full agonist CP55,940 (1 μM) is depicted by a green symbol (▼). Data points represent means ± SEM of at least three independent experiments, each performed in duplicates

In contrast, honokiol and the M-extract showed full agonistic properties at CB₁ receptors, while they acted as

antagonists with inverse agonistic activity at the CB₂ receptor subtype (Table 1 and SI).

In radioligand binding experiments as well as in functional studies, the main fecal magnolol metabolite **7** showed a similar profile as the parent natural product **3**, but was considerably more potent (Table 1 and Figure 2). Like magnolol (**3**), metabolite **7** was CB₂-selective, being a partial agonist at CB₂ receptors, and a full agonist at CB₁. In cAMP assays at CB₂ receptors **7** (EC₅₀ 0.170 μM) was almost 20-fold more potent than **3** (Figure 3B). These findings indicate that the modestly potent magnolol (**3**) is bioactivated to the potent metabolite **7**, and can thus be envisaged as a pro- or co-drug. The second fecal magnolol metabolite, **8**, was also found to be active at CB receptors and displayed comparable functional properties as **3** and **7**, but possessed lower affinity and potency at both receptor subtypes (see Table 1 and SI). In contrast to tetrahydromagnolol (**7**) and *trans*-isomagnolol (**8**) the main urinary metabolite of magnolol in humans, 8,9-dihydroxydihydromagnolol (**4**) showed no affinity for CB receptors indicating that its described antidepressant effects are likely not mediated by CB receptors (Table 1).²

As a next step, the magnolia extract and the individual compounds were investigated for their potential to interact with the CB-like orphan receptor GPR55 by performing β-arrestin translocation assays. The M-extract, tested at a concentration of 50 μg/mL, showed complete inhibition of the LPI effect (Table 1), indicating that, in addition to CB receptors, interaction with the GPR55 may also be involved in mediating the biological effects of *Magnolia officinalis* extracts. As depicted in Figure 4A none of the pure biphenyls was able to significantly induce β-arrestin recruitment via GPR55 activation at a high concentration of 10 μM. This indicated that they did not act as GPR55 agonists.

Table 1. Interaction of magnolia extract and test compounds with cannabinoid CB₁ and CB₂ receptors and GPR55

Compound	radioligand binding vs. [³ H]CP55,940		cAMP accumulation assay		β-arrestin assay
	human CB ₁ receptor	human CB ₂ receptor	human CB ₁ receptor	human CB ₂ receptor	human GPR55
	K _i ± SEM (μM), n = 3		EC ₅₀ ± SEM (μM), n = 3 (efficacy at 100 μM compared to max. effect of full agonist 5 (1 μM) = 100%)		K _B ± SEM (μM), n = 3 (inhibition of LPI-induced β-arrestin translocation at 10 μM)
honokiol (1)	6.46 ± 3.54	5.61 ± 2.02	> 10.0 (168%)	n.d. ^a (0%)	n.d. ^a (42%) ^b
magnolol (3)	3.15 ± 1.65	1.44 ± 0.10	18.3 ± 8.6 (62%)	3.28 ± 2.01 (31%) ^c	n.d. ^a (0%) ^b
(<i>RS</i>)-8,9-dihydroxydihydromagnolol (4)	> 10.0	> 10.0	n.d. ^a	n.d. ^a	n.d. ^a (4%) ^b
tetrahydromagnolol (7)	2.26 ± 0.89	0.416 ± 0.089	9.01 ± 3.42 (124%)	0.170 ± 0.114 (49%) ^d	13.3 ± 2.0 (96%) ^b
<i>trans</i> -isomagnolol (8)	> 10.0	3.14 ± 0.12	> 10.0 (134%)	8.73 ± 3.39 (55%)	n.d. ^b (0%) ^b
CP55,940 (5)	0.00128 ± 0.00044	0.00142 ± 0.00075	0.00228 ± 0.00137 ^c	0.00100 ± 0.00019	1.89 ± 0.97 (92%) ^b
rimonabant (6)	0.0126 ± 0.0039	0.900 ± 0.320	n.d. ^a (0%) ^{c,d}	n.d. ^a (0%) ^d	n.d. ^a (agonist: EC ₅₀ = 2.01 ± 0.66)
magnolia extract	1.21 ± 0.48 μg/mL	0.165 ± 0.104 μg/mL	n.d. ^a (147%) ^e	n.d. ^a (0%) ^e	n.d. ^a (121%) ^b

^an.d.= not determined; ^binhibition of LPI (1 μM)-induced β-arrestin recruitment by test compounds (at 10 μM; 50 μg/mL for magnolia extract). LPI-effect is set at 100%; ^cn = 4; ^dconcentration of test compound (10 μM); ^econcentration of magnolia extract = 50 μg/mL.

However, when tested for potential inhibitory effects, honokiol (**1**) and - more strongly - tetrahydromagnolol (**7**) were able to inhibit LPI-induced GPR55 activation at a concentration of 10 μ M (Figure 4B), while magnolol (**3**), 8,9-dihydromagnolol (**4**) and *trans*-isomagnolol (**8**) were inactive. The more potent **7** was further investigated for its antagonistic properties at GPR55. Tetrahydromagnolol (**7**, 10 μ M) led to a significant parallel rightward shift of the curve for the agonist LPI at GPR55, indicating a competitive mechanism of inhibition. A K_B value of 13.3 μ M was determined (Figure 4C).

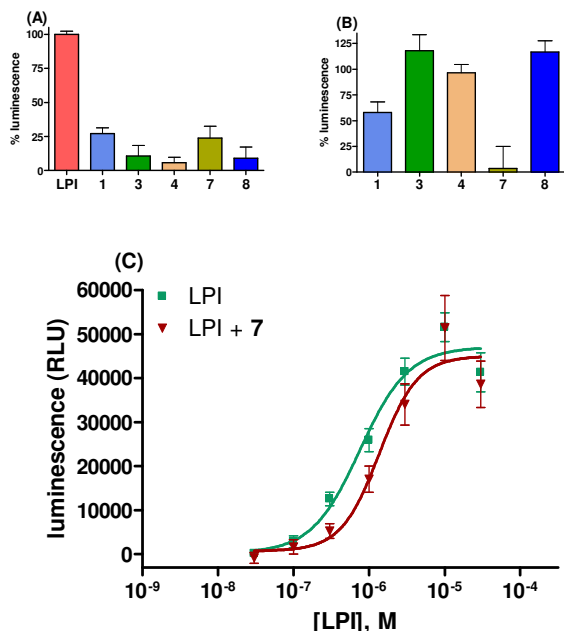


Figure 4. (A) Effects of LPI (GPR55 agonist, 1 μ M), honokiol (**1**), magnolol (**3**), 8,9-dihydroxydihydromagnolol (**4**), tetrahydromagnolol (**7**) and *trans*-isomagnolol (**8**) (10 μ M each) on β -arrestin recruitment to human GPR55 receptors recombinantly expressed in CHO cells. (B) Inhibition of LPI (1 μ M)-induced β -arrestin recruitment to human GPR55 by **1**, **3**, **4**, **7** and **8** (10 μ M). The results in (A) and (B) represent means \pm SEM of three independent experiments performed in duplicates. Data are expressed as percent luminescence related to the effect of LPI (1 μ M; ≥ 50 % of maximal effect; EC_{50} 0.769 μ M) set at 100 %. (C) Concentration-dependent effect of LPI on β -arrestin recruitment to human GPR55 receptors recombinantly expressed in CHO cells in the absence and in the presence of tetrahydromagnolol (**7**, 10 μ M). Data points are means \pm SEM of three independent experiments, performed in duplicates. A K_B value of 13.3 ± 2.0 μ M was determined for tetrahydromagnolol; RLU = relative luminescence units. Recruitment of β -arrestin to the receptor was detected by measuring luminescence emission, based on a β -galactosidase enzyme fragment complementation assay (DiscoverX[®]).

The magnolia extract and biphenyls **1**, **3**, **4**, **7**, and **8** were further investigated for potential inhibition of the endocannabinoid-degrading enzymes fatty acid amide hydrolase (FAAH) and monoacylglyceride lipase (MAGL) in rat brain preparations.^{29, 30} No inhibition of the enzymes was observed by the compounds at a test concentration of 10 μ M. The extract, tested at a high concentration of 50 μ g/mL did not inhibit FAAH, and showed only weak inhibition (22%) of MAGL (for details see SI).

During the preparation of this manuscript a study appeared describing honokiol, 4-methoxyhonokiol and magnolol as CB₂ receptor ligands.⁸ 4-Methoxyhonokiol was found to exhibit high affinity (44 nM), while magnolol and honokiol were described to possess moderate (1-3 μ M) affinities for CB₂ receptors as determined in radioligand binding studies.⁸ These findings are in accordance with the results obtained in our laboratory. Furthermore, it was reported that magnolol and honokiol possess a significantly lower affinity for the CB₁ receptor.⁸ In our hands this observation was only true for magnolol, while honokiol possessed about equal affinity for both CB receptor subtypes.

No functional characterization of the main neolignan constituents of *Magnolia* bark, magnolol and honokiol, was described in the recently published study,⁸ neither at CB₁ nor at CB₂ receptors. In the present study we describe for the first time the functional properties of the natural products honokiol (CB₁ agonist, CB₂ antagonist) and magnolol (partial agonist at both receptors with preference for CB₂).

Moreover, we discovered that its main metabolites *trans*-isomagnolol and tetrahydromagnolol are selective partial CB₂ agonists. The main metabolite of magnolol, tetrahydromagnolol, is in fact a more potent partial CB₂ agonist than the parent drug magnolol, and also shows CB₂-selectivity. Partial CB₁ and CB₂ receptor agonists had been shown to reduce inflammation-associated hyperalgesia and to possess antiallodynic effects in animal models.^{31, 32} Thus, magnolol and its metabolites may have the same in vivo effects due to their action on CB receptors.

Furthermore, magnolia extract and its constituents magnolol and honokiol, as well as the main metabolites of magnolol **4**, **7** and **8** were investigated for the first time at the CB-like GPR55 using β -arrestin translocation assays, and at enzymes responsible for endocannabinoid degradation. Compound **7** was found to be a moderately potent GPR55 antagonist (K_B 13.3 μ M). It could serve as a lead structure for the development of potent and selective GPR55 antagonists.

Our results together with recently published reports provide a new potential mechanism of action for the known effects of *Magnolia* bark extracts. An involvement of further bioactive constituents, as well as synergistic effects have to be considered. As blood levels of **7** after magnolol intake have not been determined yet, pharmacokinetic studies are needed to corroborate our findings. Metabolite **7** exhibits an approximately 11-fold lower affinity for CB₂ receptors compared to the well-known plant-derived cannabinoid Δ^9 -THC. The lower affinity of **7** might be sufficient for biological activity, particularly since it may accumulate after repeated intake of magnolol-containing formulations.^{19, 20} Further investigations using in vivo models are warranted. The biphenyl magnolol provides a simple, novel lead structure for the development of agonists for CB receptors and antagonists for the related GPR55.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, analytical data, assay procedures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Address correspondence to: Dr. Christa Müller, Pharmazeutisches Institut, An der Immenburg 4, D-53121 Bonn, Germany, Phone: +49-228-73-2301, Fax: +49-228-73-2567, E-mail: christa.mueller@uni-bonn.de

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Table of Contents

Magnolia extract, magnolol and metabolites: activation of cannabinoid CB₂ receptors and blockade of the related GPR55

Viktor Rempel, Alexander Fuchs, Sonja Hinz, Tadeusz Karcz, Matthias Lehr, Uwe Koetter, and Christa E. Müller*

