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Modulation of $GABA_A$-Receptors by Honokiol and Derivatives: Subtype Selectivity and Structure−Activity Relationship

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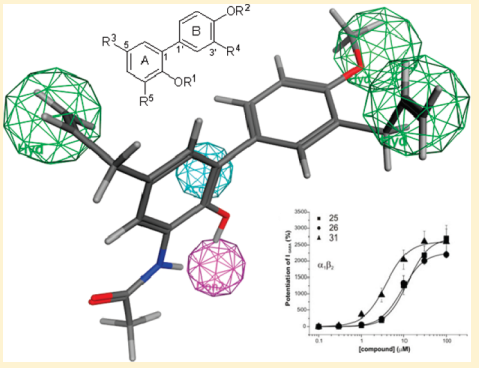
Supporting Information

ABSTRACT: A series of 31 analogues of the neolignan honokiol (a major constituent of *Magnolia officinalis*) was synthesized, and their effects on $GABA_A$ receptors expressed in *Xenopus* oocytes were investigated. Honokiol enhanced chloride currents (I_{GABA}) through $GABA_A$ receptors of seven different subunit compositions with EC_{50} values ranging from 23.4 μM ($\alpha_5\beta_2$) to 59.6 μM ($\alpha_1\beta_3$). Honokiol was most efficient on $\alpha_3\beta_2$ (maximal I_{GABA} enhancement 2386%) > $\alpha_2\beta_2$ (1130%) > $\alpha_1\beta_2$ (1034%) > $\alpha_1\beta_1$ (260%)). On $\alpha_1\beta_2$-receptors, *N*-substituted compounds were most active with 3-acetylamino-4′-*O*-methylhonokiol (**31**), enhancing I_{GABA} by 2601% (EC_{50} ($\alpha_1\beta_2$) = 3.8 μM). Pharmacophore modeling gave a model with an overall classification accuracy of 91% showing three hydrophobic regions, one acceptor and one donor region. Unlike honokiol, **31** was most efficient on $\alpha_2\beta_2$- (5204%) > $\alpha_3\beta_2$- (3671%) > $\alpha_1\beta_2$-receptors (2601%), suggesting a role of the acetamido group in subunit-dependent receptor modulation.



INTRODUCTION

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the mammalian central nervous system. GABA subtype A ($GABA_A$) receptors are ligand gated ion channels composed of five subunits, which coassemble by forming a chloride selective ion channel in the membrane. Several genes encoding for different subunits have been identified including α_{1-6}, β_{1-3}, γ_{1-3}, and δ with some of them existing in different splice variants.[1,2] The most widely distributed $GABA_A$ receptors in the human brain are composed of two α_1, two β_2, and one γ_2 subunit.[3] $GABA_A$ receptors are modulated by a variety of drugs such as benzodiazepines (BDZ), barbiturates, and anesthetics.[4,5] The pharmacological properties of $GABA_A$ receptors are largely determined by their subunit composition.[4−7] Benzodiazepine action requires the presence of a γ_2-subunit and an α_1-, α_2-, α_3-, or α_5-subunit in the receptor composition,[8,9] while barbiturates and anesthetics exert their modulating effects on $GABA_A$ receptors that do not comprise a γ-subunit.[4] The use of benzodiazepines is associated with undesirable effects including reduced coordination, cognitive impairment, increased accident proneness, physical dependence, and withdrawal symptoms.[10,11] $GABA_A$ receptors ligands with fewer side effects are therefore an unmet medical need.[11,12]

Natural products such as biphenyl neolignans may provide new scaffolds for pharmaceutical drug design.[13] The biphenyl substructure, which shows very good general binding affinity to proteins, is found in 4.3% of all known drugs and hence provides a suitable template for the discovery of new drugs.[14] Related structures such as polychlorinated biphenyls and bisphenol A have been previously shown to modulate $GABA_A$ receptors.[15,16] Various $GABA_A$ receptor modulators (flavonoids like methylapigenin or wogonin,[17,18] polyacetylene structures from *Cussonia zimmermannii*,[19] and valerenic acid in *Valeriana* spp.[20]) have been identified in plant extracts.

Plants from the Magnoliaceae were widely used by indigenous people both in America and Asia[21,22] and still play a major role in Asia, e.g. in the traditional Chinese medicine or in Japanese Kampo medicine. The bark from *M. officinalis* and *M. officinalis* var. *biloba* is used against inflammatory or neuronal diseases. The biphenyl neolignans magnolol and honokiol are considered to be the main active compounds. Besides various biological and pharmacological activities,[23−25] these isomers are reported to have antidepressant, anxiolytic, and muscle relaxant effects.[26−30] An involvement of the $GABA_A$ receptor system for the action of honokiol and magnolol has been reported[31] and most recently proposed for the pentobarbital-induced sleeping time in mice.[32] It was also demonstrated that the anxiolytic effect of honokiol in mice lacks diazepam-like side effects.[28] Additionally, honokiol was found to exhibit

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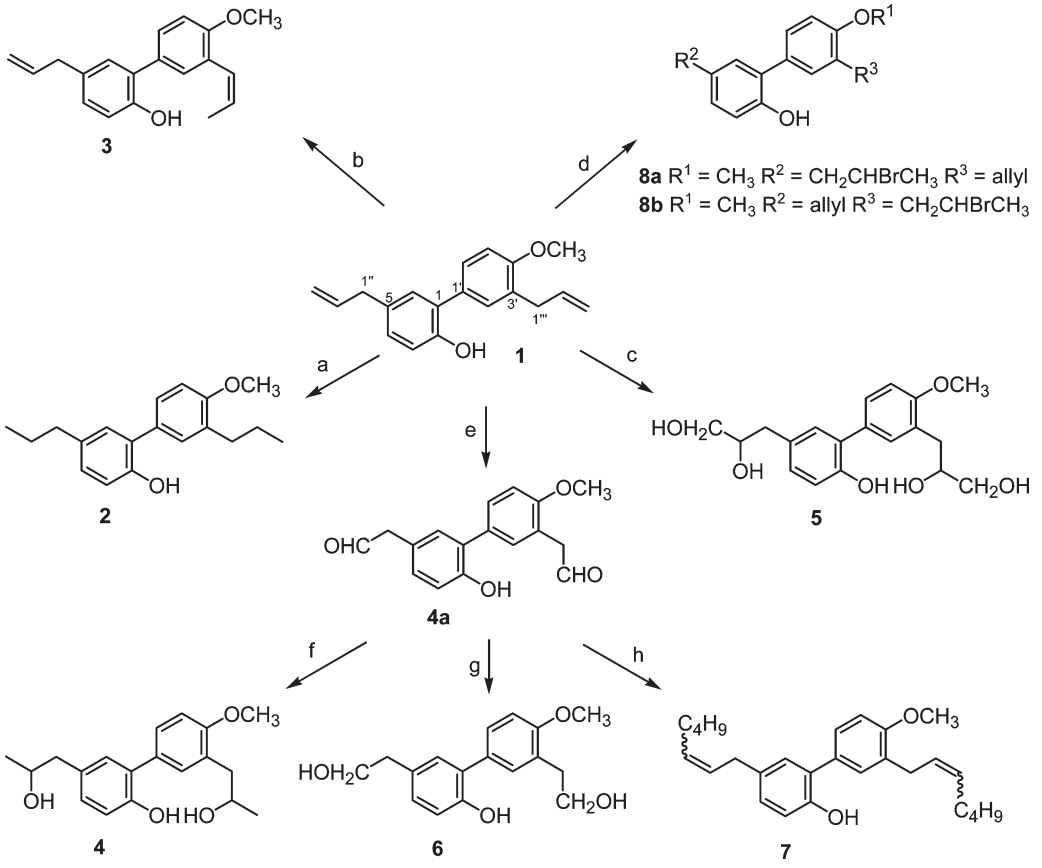
Scheme 1[a]



[a] Reagents and conditions: (a) 10% Pd/C, H_2, abs EtOH, RT; (b) KO*t*Bu, THF, reflux; (c) OsO_4, trimethylamine-*N*-oxide, pyridine/water, reflux; (d) (1) BBr_3 in CH_2Cl_2, −78 °C, (2) H_2O; (e) (1) O_3, MeOH/CH_2Cl_2, −78 °C, (2) SMe_2; (f) Grignard (CH_3I, Mg), abs Et_2O, RT; (g) $LiAlH_4$, abs Et_2O, RT; (h) (1) C_5H_{11}−PPh_3Br, (2) NaHMDS, (3) **4a**, −78 °C.

neurotrophic properties in fetal rat cortical neurons[33] and neuroprotective activity in a rat ischemia-reperfusion assay.[34]

First evidence for an interaction of honokiol and magnolol with $GABA_A$ receptors comes from behavioral studies by Kuribara et al.[26−28] and Maruyama et al.[35] Honokiol and its isomer magnolol have been reported to bind to and to modulate $GABA_A$ receptors in vitro.[31,36] The subunit dependence and the structure activity relationship of honokiol on $GABA_A$ receptors are yet unknown.

In the present study, we first expressed seven $GABA_A$ receptors composed of different subunits in *Xenopus laevis* oocytes and analyzed the modulation of chloride currents by honokiol with regard to subunit dependent effects on $GABA_A$ receptors using the two microelectrode voltage clamp technique. Subsequently, for the aim of understanding key features required for $GABA_A$ receptor modulatory activity with respect to particular subunit compositions as well as in search for compounds with higher potency than honokiol, the in vitro $GABA_A$ receptor modulatory activity of an array of 50 biphenyl derivatives was studied. Among the 31 compounds which were further pharmacologically evaluated and used in a modeling approach to elucidate the structural requirements of I_{GABA} modulation by biphenyl-type neolignans, 11 are here described for the first time.

CHEMISTRY

In an ongoing project investigating biological activities of derivatives and analogues of honokiol a broad set of substances was synthesized. Most of these compounds were screened for their $GABA_A$-receptor modulatory effect.

Synthetic modifications of the side chains of 4′-*O*-methylhonokiol (**1**) are shown in Scheme 1. Hydrogenation (a) and isomerization (b) were performed as given in the literature.[37] To obtain derivatives differing in chain length, **1** was subjected to ozonolysis and reductive workup with dimethylsulfide. This reaction was found to be very sensitive to the amount of ozone and reaction time. Wittig reaction of raw **4a** and an excess of the ylide derived from pentyltriphenylphosphonium bromide and NaHMDS[38] yielded the desired product with two 2-heptenyl side chains as an isomeric mixture in low yield. Reduction of **4a** with $LiAlH_4$ gave the corresponding diol **6**. Increase of the polarity of the side chains without shortening of the side chain of **1** was achieved by Grignard reaction of **4a** with MeMgI in diethylether/THF adopted from a literature procedure[39] to gain **4**.

A further increase in polarity was obtained by *cis*-dihydroxylation of the double bonds of both side chains by oxidation with trimethylamine-*N*-oxide and OsO_4 as catalyst according to a procedure described by Ray and Matteson,[40] yielding **5** as an isomeric mixture.

Synthesis of brominated derivatives **8a** and **8b** was achieved by successive addition of BBr_3 and water to **1**.[41] **10** and **11** were obtained by allylation and Claisen rearrangement of 2,2′-biphenol and 4,4′-biphenol, respectively, according to

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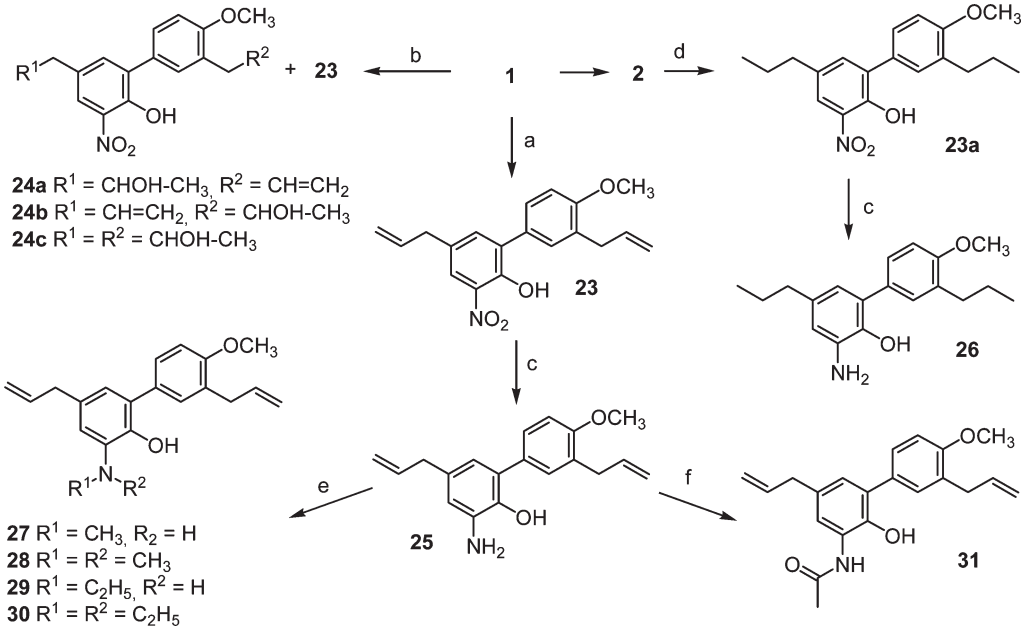
Scheme 2[a]



[a] Reagents and conditions: (a) nitric acid (65%), water, hexanes, RT; (b) (1) $Hg(NO_3)_2 \times H_2O$ in CH_3CN; (2) NaOH, $NaBH_4$ in EtOH, RT; (c) $SnCl_2$ in EtOH, RT; (d) $SOCl_2$/bismuth nitrate oxide in CH_2Cl_2, RT; (e) alkylation using $NaHCO_3$, MeI or EtI, acetonitrile, RT; (f) acetic anhydride/water, RT.

Chattopadhyay et al.[42] Mono- and di-*O*-alkylation of honokiol and magnolol leading to compounds **12**−**22** was performed using either Et_2SO_4 or alkylbromides as described elsewhere.[37,41]

As outlined in Scheme 2, the derivatives of **1** bearing nitrogen in position 3 were obtained from **1**. The amidomercuration−demercuration reaction of olefins with mercuric(II)nitrate in acetonitrile followed by reduction with $NaBH_4$ usually leads to the addition of acetamide to the double bond.[43] The aberrant use of the mercuric(II)nitrate monohydrate resulted in nitration of ring A in position 3 and partially in hydratation of the side chain to give **23**, **24a**, **24b**, and **24c**, each in very low yield.

The nitration of **2** according to Muathen et al.[44] with thionyl chloride and bismuth nitrate oxide yielded the crude nitrated product **23a**. Concentrated nitric acid in a two-phase system with hexanes adopted from Johnson and Corey[45] turned out to be the best nitration agent, yielding quantitatively pure **23** from **1**, within about one minute. **23** and **23a** were reduced with tin dichloride in ethanol to the corresponding amines **25** and **26**.[46] Methylation and ethylation of **25** were performed in acetonitrile[47] and resulted in a mixture of starting material **25**, dialkylated (**27** and **29**), and monoalkylated products (**28** and **30**). Even with an excess of amine **25**, the formation of the dialkylated products was favored over that of the monoalkylated product. The latter (**28**, **30**) turned out to be unstable. To prevent *O*-acetylation compound **31** was synthesized from **25** in analogy to a paracetamol synthesis with acetic anhydride in water according to literature.[48]

BIOLOGICAL ACTIVITY

Expression of $GABA_A$ Receptors in *Xenopus* Oocytes and Voltage Clamp Experiments. Preparation of stage V−VI oocytes from *Xenopus laevis*, synthesis of capped runoff poly(A) cRNA transcripts from linearized cDNA templates (pCMV vector), was performed as previously described.[49] Briefly, female *Xenopus laevis* frogs (NASCO, USA) were anesthetized by exposing them to a 0.2% solution of MS-222 (methane sulfonate salt of 3-aminobenzoic acid ethyl ester; Sigma Aldrich, Steinheim, Germany) for 15 min before surgically removing parts of the ovaries. Follicle membranes from isolated oocytes were enzymatically digested with 2 mg/mL collagenase (Type 1A, Sigma-Aldrich, Steinheim, Germany). One day after isolation, selected oocytes were injected with 10−50 nL of DEPC-treated water (diethyl pyrocarbonate, Sigma, Steinheim, Germany) containing the different cRNAs at a concentration of approximately 300−3000 pg/nL/subunit. The amount of cRNA was determined by means of a NanoDrop ND-1000 (Kisker-Biotech, Steinfurt, Germany).

To ensure expression of the γ_{2s} subunit in the case of $\alpha_1\beta_2\gamma_{2s}$ receptors, cRNAs were mixed in a ratio of 1:1:10. For most receptors comprising only α- and β-subunits ($\alpha_1\beta_2$, $\alpha_2\beta_2$, $\alpha_3\beta_2$, and $\alpha_5\beta_2$), the cRNAs were mixed in a ratio of 1:1. To ensure correct assembly in receptors composed of α_1 and β_1 subunits, cRNAs were mixed in a ration of 3:1.

Oocytes were stored at +18 °C in modified ND96 solution (90 mM NaCl, 1mM $CaCl_2$, 1 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, and 5 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); pH 7.4, all from Sigma-Aldrich, Steinheim, Germany).

Chloride currents through $GABA_A$ receptors (I_{GABA}) were measured at ambient temperature (+20−22 °C) by means of the two-microelectrode voltage clamp technique at a holding potential of −70 mV making use of a TURBO TEC-05X amplifier (npi electronic, Tamm, Germany). Data acquisition was carried out by means of an Axon Digidata 1322A interface using pCLAMP v.10 (Molecular Devices, Sunnyvale, CA, USA). The modified ND96 solution was used as bath solution. Microelectrodes were filled with 2 M KCl and had resistances between 1 and 3 MΩ.

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Table 1. Class Labels and Selected Physicochemical Descriptors

code	class	log *P* (o/w)	b_1rotN	apol	mr	H-acc	H_don	TPSA
honokiol	0	5.16	5	45.29	8.19	2	2	40.46
magnolol	0	5.16	5	45.29	8.19	2	2	40.46
1	0	5.43	6	48.38	8.69	2	1	29.46
2	0	6.01	6	51.05	8.79	2	1	29.46
3	0	5.45	5	48.38	8.70	2	1	29.46
4	0	3.64	6	52.65	9.05	4	3	69.92
5	0	1.57	8	54.26	9.38	6	5	110.38
6	0	2.72	6	46.46	8.19	4	3	69.92
7	1	8.36	12	73.13	12.42	2	1	29.46
8a	1	5.99	6	52.10	9.48	2	1	29.46
8b	0	5.99	6	52.10	9.48	2	1	29.46
10	0	5.09	5	45.29	8.19	2	2	40.46
11	0	5.16	5	45.29	8.19	2	2	40.46
12	0	5.43	6	48.38	8.69	2	1	29.46
13	0	5.69	7	51.47	9.19	2	0	18.46
14	0	5.77	7	51.47	9.15	2	1	29.46
15	0	5.77	7	51.47	9.15	2	1	29.46
16	0	6.37	9	57.66	10.11	2	0	18.46
17	0	6.38	8	54.57	9.61	2	1	29.46
18	0	6.38	8	54.57	9.61	2	1	29.46
19	0	7.60	11	63.85	11.03	2	0	18.46
20	0	7.27	10	60.75	10.54	2	1	29.46
21	0	7.27	10	60.75	10.54	2	1	29.46
22	0	9.37	15	76.22	12.88	2	0	18.46
23	0	5.40	7	50.42	9.26	2	1	75.28
24ab	1	4.50	7	52.55	9.44	3	2	95.51
25	0	4.79	6	50.15	9.00	2	2	55.48
26	1	5.38	6	52.81	9.10	2	2	55.48
27	1	5.11	7	53.24	9.49	2	2	41.49
28	0	5.38	7	56.33	9.93	2	1	32.70
29	1	5.45	8	56.33	9.95	2	2	41.49
30	0	6.06	9	62.52	10.87	2	1	32.70
31	1	4.75	7	55.80	10.04	3	2	58.56

Fast Perfusion System. GABA, honokiol, and the other compounds used were applied by means of the ScreeningTool (npi electronic, Tamm, Germany) fast perfusion system as described previously.[50] To elicit I_{GABA}, the chamber was perfused with 120 μL of GABA-containing solutions at a volume rate of 300 μL/s. The I_{GABA} rise time ranged between 100 and 250 ms.[49] Care was taken to account for possible slow recovery from increasing levels of desensitization in the presence of high GABA or drug concentrations. The duration of washout periods was therefore extended from 1.5 min (1−20 μM GABA, <10 μM compounds) to 30 min (≥100 μM GABA, ≥10 μM compounds), respectively. Oocytes with maximal current amplitudes >3 μA were discarded to exclude voltage clamp errors.

Analyzing Concentration−Response Curves. Stimulation of chloride currents by modulators of the $GABA_A$ receptor was measured at a GABA concentration eliciting between 3% and 7% of the maximal current amplitude (EC_{3-7}). The GABA EC_{3-7} was determined for each oocyte individually. Enhancement of the chloride current was defined as $(I_{(GABA+compound)}/I_{GABA}) - 1$, where $I_{(GABA+compound)}$ is the current response in the presence of a given compound and I_{GABA} is the control GABA current.

Concentration−response curves were generated and the data were fitted by nonlinear regression analysis using Origin Software (OriginLab Corporation, USA). Data were fitted to the equation $1/(1 + (EC_{50}/[compound])^{nH})$, where n_H is the Hill coefficient. Each data point represents the mean ± SE from at least 4 oocytes and ≥2 oocyte batches. Statistical significance was calculated using paired Student *t*-test with a confidence interval of <0.05.

Molecular Modeling and QSAR. Molecules were built using the builder module in MOE 2009.10 (Molecular Operating Environment; Chemical Computing Group, Montreal, Canada) and energy minimized using standard conditions (MMFF94x force field, adjust H and LP, gradient = 0.01, calculate forcefield partial charges). A database was built and a set of physicochemical parameters was calculated. These comprise log *P* (log *P*(o/w)), topological polar surface area (TPSA), polarizability (apol), molar refractivity (mr), number of rotable bonds (b_1rotN), as well as number of H-bond donors and acceptors. The data set was split into two classes, active/inactive, with an EC_{50} value of 10 μM as threshold. This resulted in six active and 27 inactive compounds. Class labels as well as selected physicochemical descriptors are given in Table 1.

Binary QSAR. Binary QSAR analysis (BQSAR) was performed as implemented in MOE 2009.10 using a set of simple physicochemical descriptors. The quality of the models was assessed by identifying the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN) obtained in leave-one-out cross validation runs. The overall prediction accuracy (A), the sensitivity (SE), which represents the accuracy on actives, and the specificity (SP), which illustrates the accuracy on inactives, were calculated as follows: A = (TP + TN)/(TP + TN + FP + FN), SE = TP/(TP + FN) and SP = TN/(TN + FP).

Pharmacophore Modeling. Pharmacophore models were established using MOE 2009.10. The number of conformers generated using the "best" feature of the program for each substrate was limited within the program to a maximum of 250 with an energy range of 15.00 kcal/mol beyond the calculated potential energy minimum. Automated pharmacophore elucidation was performed using standard settings. Annotation for active/inactive was achieved on basis of the same threshold used for BQSAR models. In addition, also EC_{50} values of 15 and 20 μM were applied. However, these did not result in models with acceptable overall accuracy values. The models obtained were ranked by accuracy, and thereupon two models were chosen for further consideration.

RESULTS AND DISCUSSION

Honokiol is a neolignan compound isolated from *Magnolia officinalis* and other *Magnolia* species that displays various pharmacological effects such as antitumor and antimicrobial effects.[51−54] Previous studies suggest an interaction of honokiol with $GABA_A$ receptors.[31,36] To investigate the subunit dependency of honokiol action, we analyzed the modulation of chloride currents though $GABA_A$ receptors (I_{GABA}) composed of seven different subunit compositions. To obtain insights into SAR of neolignan derivatives in general, a compound library featuring 50 biphenyls was screened, out of which 31 derivatives were further studied in more detail.

Modulation of $GABA_A$ Receptors by Honokiol Does not Require a γ_{2S}-Subunit. Incorporation of a γ_{2S} subunit was not

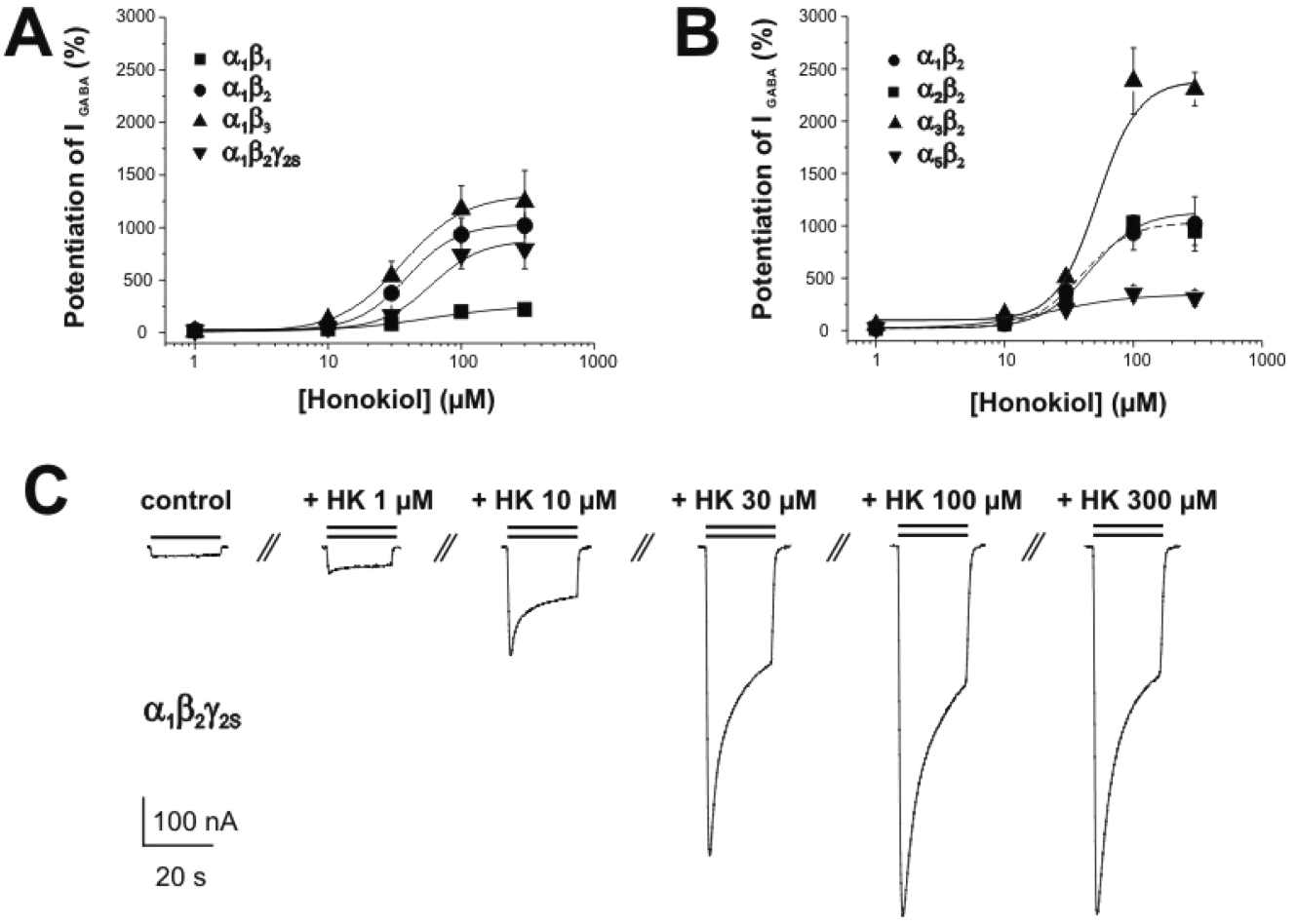

Figure 1. Concentration−response curves for I_{GABA} enhancement by honokiol on (A) $\alpha_1\beta_1$ (■), $\alpha_1\beta_2$ (●), $\alpha_1\beta_3$ (▲) and $\alpha_1\beta_2\gamma_{2S}$ (▼) and (B) $\alpha_1\beta_2$ (●, dashed line, data from (A)), $\alpha_2\beta_2$ (■), $\alpha_3\beta_2$ (▲), and $\alpha_5\beta_2$ (▼) receptors using a GABA EC_{3-7} (EC_{50} and n_H values are given in Table 2). Data points represent means ± SE from at least four oocytes from ≥2 batches. (C) Typical I_{GABA} recordings illustrating concentration-dependent modulation of GABA elicited chloride currents through $\alpha_1\beta_2$-receptors by honokiol.

essential for allosteric modulation of $GABA_A$ receptors by honokiol as evident from the concentration−response curves shown in Figure 1A.

$\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_{2S}$ receptors were modulated with similar potency and efficiency (neither significant differences in the concentration eliciting half-maximal stimulation (EC_{50}) nor in maximum potentiation of I_{GABA} were observed, Figure 1A, Table 2).

Ethyl-12-fluoro-8-methyl-9-oxo-2,4,8-triazatricyclo[8.4.0.0^{2,6}]-tetradeca-1(10),3,5,11,13-pentaene-5-carboxylate (Flumazenil, 1 μM) did not significantly affect I_{GABA} modulation by honokiol and additive effects were observed when 7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3*H*)-onediazepam and honokiol were coapplied (data shown in Supporting Information Figure S1). Together these data indicate that the high-affinity benzodiazepine-binding site is not involved in the effect of honokiol on $GABA_A$ receptors.

Benzodiazepines[55] and compounds such as flavonoids, valerenic acid,[20] and many other natural products do not require a γ-subunit for modulation of $GABA_A$ receptors.[56] Subsequent studies were therefore performed on receptors composed of different α and β subunits.

Modulation of $GABA_A$ Receptors by Honokiol is More Efficient on Receptors Comprising β_2- and β_3-Subunits. The concentration−response curves for receptors comprising different β-subunits are illustrated in Figure 1A. I_{GABA} through $\alpha_1\beta_2$ and $\alpha_1\beta_3$ receptors was enhanced to comparable extents ($\alpha_1\beta_2$, 1034% vs $\alpha_1\beta_3$, 878%, $p > 0.05$). Honokiol was, however, significantly less efficient on receptors containing β_1 subunits (maximum I_{GABA} stimulation of 260%, $p < 0.05$). The respective EC_{50} values (57.0 μM for $\alpha_1\beta_1$, 39.3 μM for $\alpha_1\beta_2$, and 59.6 μM for $\alpha_1\beta_3$) were not significantly different (>0.05; see Tables 2 and 3).

α-Subunit Dependence of I_{GABA} Modulation by Honokiol. A potential α-subunit dependence of honokiol action was studied on $\alpha_X\beta_2$ receptors. Honokiol was found to be more efficient on receptors incorporating an α_3 subunit ($\alpha_3\beta_2$) with a maximum I_{GABA} enhancement of 2386% versus 1034% ($\alpha_1\beta_2$) and 1130% $\alpha_2\beta_2$ ($p < 0.05$). The lowest efficiency was observed for $\alpha_5\beta_2$ receptors (347%, $p < 0.05$). The EC_{50} values for the different receptor subtypes of 39.3 μM ($\alpha_1\beta_2$), 46.4 μM ($\alpha_2\beta_2$), 52.4 μM ($\alpha_3\beta_2$), and 23.4 μM ($\alpha_5\beta_2$) were not significantly different ($p > 0.05$; see Figure 1B and Tables 2 and 3).

Modulation of $\alpha_1\beta_2$ Receptors by Honokiol Derivatives. To obtain insights into the pharmacophores required for the activity of honokiol, 31 analogues and derivatives of honokiol out of a compound library were investigated (see Tables 4, 5, and 6 for structures). The basic scaffold of honokiol offers several possibilities for structural modifications: (i) the aliphatic side chains, (ii) the hydroxyl groups, and (iii) introduction of additional functional groups. As illustrated in Figure 1A, receptor

Table 2. Potency and Efficiency of Honokiol on $GABA_A$-Receptors of Different Subunit Compositions

subunit combination	EC_{50} (μM)	I_{max} (%)	Hill coefficient (n_H)	no. of experiments (n)
$\alpha_1\beta_2\gamma_{2s}$	36.2 ± 14.7	1315 ± 281	1.8 ± 0.6	7
$\alpha_1\beta_2$	39.3 ± 7.7	1034 ± 182	2.3 ± 0.4	8
$\alpha_1\beta_1$	57.0 ± 20.6	260 ± 52	1.3 ± 0.3	7
$\alpha_1\beta_3$	59.6 ± 21.5	878 ± 208	2.3 ± 0.9	8
$\alpha_2\beta_2$	46.4 ± 7.2	1130 ± 114	2.3 ± 0.4	8
$\alpha_3\beta_2$	52.4 ± 10.2	2386 ± 177	2.7 ± 0.9	8
$\alpha_5\beta_2$	23.4 ± 11.2	347 ± 81	1.4 ± 0.7	10

Table 3. Comparison of Efficiencies (Upper-Right) and Potencies (Lower-Left) of Honokiol for $GABA_A$ Receptors of Different Subunit Compositions

I_{max} / EC_{50}	$\alpha_1\beta_1$	$\alpha_1\beta_2$	$\alpha_1\beta_3$	$\alpha_1\beta_2\gamma_{2S}$	$\alpha_2\beta_2$	$\alpha_3\beta_2$	$\alpha_5\beta_2$
$\alpha_1\beta_1$		a	a	a	a	a	
$\alpha_1\beta_2$						a	a
$\alpha_1\beta_3$						a	a
$\alpha_1\beta_2\gamma_{2S}$						a	a
$\alpha_2\beta_2$						a	a
$\alpha_3\beta_2$							a
$\alpha_5\beta_2$							

[a] Indicates statistically significant ($p < 0.05$) differences.

modulation does not require a γ_{2s} subunit. We have therefore studied the structure−activity relationship of honokiol and its derivatives on $\alpha_1\beta_2$ receptors as these subunits are incorporated in the majority of $GABA_A$ receptors expressed in the central nervous system. In a first screening, we compared I_{GABA} modulation by honokiol, magnolol, and the 31 selected derivatives. All compounds were tested at a concentration of 30 μM for a first impression on respective activities (Figure 2 and Table 7). Twenty-three analogues stimulated I_{GABA} by >30% compared to honokiol (373%) and magnolol (357%). For these compounds, concentration−response relationships were established (see Figure 3 for selected concentration−response curves). The EC_{50} values and the maximum I_{GABA} potentiation by all 23 analogues are given in Table 7.

Structure−Activity Relationship of Honokiol and Derivatives Assessed by Determination of Potentiation of I_{GABA} on $GABA_A$ Receptors Containing $\alpha_1\beta_2$ Subunits. Structure−activity relationship was analyzed with respect to potency (EC_{50}) as this parameter reflects the apparent affinity. In a first step, we investigated whether changes in the aliphatic side chains (e.g., polarity, length) influence EC_{50} values. These derivatizations (Table 4, Scheme 1) started from 4′-*O*-methylhonokiol (**1**) and resulted in derivatives **2**−**8b**. The tetrahydromethylhonokiol **2** was twice as active as **1**. Isomerization of the double bond in the side chain attached to ring B (**3**) slightly decreased the EC_{50} value as compared to **1**. Introduction of hydroxy groups into both side chains (**4**, **5**, **6**), as well as elongation of the side chains (**7**) decreased activity (**4**) or rendered the compounds inactive (**5**−**7**). Interestingly, hydrobromination in one of the side chains (**8a**, **8b**), induces a remarkable potentiation of I_{GABA}, particularly if the double bond of the side chain at ring A was modified.

Compounds **10** and **11** (Table 5) were synthesized to analyze the role of different relative arrangements of phenolic groups and side chains at the phenyl rings. These neo-lignanoid compounds, which are not found in nature for biosynthetic reasons, are symmetric regioisomers of honokiol. Both compounds as well as magnolol, which also represents a regioisomer of honokiol, showed EC_{50} values comparable to the lead compound honokiol.

Next we studied the role of the free phenolic group(s) and the effect of alkyl substituents attached. Whereas methylation of one of the two phenol hydroxyls does not yield a clear picture (slight increase of activity for honokiol/**1**, slight decrease for magnolol/**12**), long alkyl substituents (pentyl; compounds **20**, **21**) render the compounds almost inactive. In case of ring A, ethyl (**14**) seems to be the optimum, whereas for ring B with the exception of methyl derivative **1**, any type of alkylation leads to a decreased activity. With the exception of bis-methyl (**13**), bis-etherification generally leads to a remarkable decrease of activity (**16**, **19**, **22**). Thus, high overall lipophilicity of the compounds definitely is not linked to high biological activity (Tables 1 and 7). Furthermore, this clearly indicates that at least one phenolic OH group is necessary for high I_{GABA} potentiation, whereby it seems less important whether it is attached to ring A or to ring B. The most active compounds in this series were derivatives **8a**, **14**, and **2**.

The introduction of a nitro group as a strong electron-withdrawing moiety to ring A as in 4′-*O*-methyl-3-nitro-honokiol (**23**) (Scheme 2) slightly increased biological activity as compared to honokiol. Compounds **24a**/**b**, which are side products of a synthesis originally planned to introduce an acetylamino group into the side chain of **1**, were obtained in a 1:1 ratio, each molecule containing a hydroxy group in the side chain of subsystem A and B, respectively. This modification further increased potency, supporting the importance of hydrophilic interactions in this region of the molecule. However, the efficiency of the compounds was lower than that of honokiol.

Reduction of **23** led to 3-amino-4′-*O*-methylhonokiol (**25**), which turned out to potentiate I_{GABA} twice as strong (2179%) as observed so far in the data set. This promising activity encouraged us to further modify **25** into its hydrogenated derivative (**26**), alkyl derivatives (**26**−**30**), and the *N*-acetylated compound **31**. In analogy to compound **2**, **26** showed EC_{50} values in the same range as found for **25** (9.2 μM vs 12.4 μM for compound **25**). The monomethylated compound **27** demonstrated similar values but turned out to be insufficiently stable. The dimethylated compound **28** was stable but showed a remarkable decrease of activity. A very similar trend was observed for the mono- and diethyl derivatives **29** and **30**, where the monoethyl-derivative

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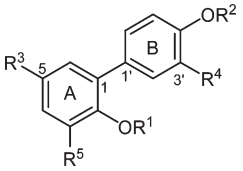
Table 4. Structures of Compounds Based on Honokiol for the Evaluation of $GABA_A$ Receptor Modulatory Activity



compound	R^1	R^2	R^3	R^4	R^5
Hon[a]	−H	−H	−2-propenyl	−2-propenyl	−H
1	−H	$-CH_3$	−2-propenyl	−2-propenyl	−H
2	−H	$-CH_3$	−propyl	−propyl	−H
3	−H	$-CH_3$	−2-propenyl	−*Z*-propenyl	−H
4	−H	$-CH_3$	−2-hydroxypropyl	−2-hydroxypropyl	−H
5	−H	$-CH_3$	−2,3-dihydroxypropyl	−2,3-dihydroxypropyl	−H
6	−H	$-CH_3$	−2-hydroxyethyl	−2-hydroxyethyl	−H
7	−H	$-CH_3$	−2-heptenyl (*Z* or *E*)	−2-heptenyl (*Z* or *E*)	−H
8a	−H	$-CH_3$	−2-bromopropyl	−2-propenyl	−H
8b	−H	$-CH_3$	−2-propenyl	−2-bromopropyl	−H
13	$-CH_3$	$-CH_3$	−2-propenyl	−2-propenyl	−H
14	$-C_2H_5$	−H	−2-propenyl	−2-propenyl	−H
15	−H	$-C_2H_5$	−2-propenyl	−2-propenyl	−H
16	$-C_2H_5$	$-C_2H_5$	−2-propenyl	−2-propenyl	−H
17	$-{}^nC_3H_7$	−H	−2-propenyl	−2-propenyl	−H
18	$-{}^nC_3H_5$	$-C_3H_7$	−2-propenyl	−2-propenyl	−H
19	$-{}^nC_3H_7$	$-{}^nC_3H_7$	−2-propenyl	−2-propenyl	−H
20	$-{}^nC_5H_{11}$	−H	−2-propenyl	−2-propenyl	−H
21	−H	$-{}^nC_5H_{11}$	−2-propenyl	−2-propenyl	−H
22	$-{}^nC_5H_{11}$	$-{}^nC_5H_{11}$	−2-propenyl	−2-propenyl	−H
23	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-NO_2$
24a	−H	$-CH_3$	−2-hydroxypropyl	−2-propenyl	$-NO_2$
24b	−H	$-CH_3$	−2-propenyl	−2-hydroxypropyl	$-NO_2$
25	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-NH_2$
26a	−H	$-CH_3$	−propyl	−propyl	$-NO_2$
26	−H	$-CH_3$	−propyl	−propyl	$-NH_2$
27	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-NHCH_3$
28	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-N(CH_3)_2$
29	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-NHC_2H_5$
30	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-N(C_2H_5)_2$
31	−H	$-CH_3$	−2-propenyl	−2-propenyl	$-NHCOCH_3$

[a] Hon = honokiol.

exhibited strong activity, whereas in the dialkylated compound **30**, activity was abolished. Finally, the acetylated amine 3-*N*-acetylamino-4′-*O*-methylhonokiol (**31**) turned out to be a very potent and stable derivative showing both high potency (EC_{50} = 3.8 μM) and high efficiency (2601%).

Although QSAR studies such as Hansch analysis, Free−Wilson analysis and hologram QSAR failed to retrieve statistically significant models, a binary QSAR model could be obtained which correctly annotates 32 out of 33 compounds as active/inactive based on a threshold of an EC_{50} of 10 μM. The model utilizes a set of simple physicochemical descriptors such as log *P*, molar refractivity, polar surface area, polarizability, number of rotatable bonds, and number of H-bond donors and acceptors (Table 1). The only compound misclassified is the bromo derivative **8a**, which is annotated as inactive, thus being considered as false negative prediction in the cross validation run. However, with respect to efficiency, **8a** shows only medium activity (1340%), which is also in line with its isomer **8b**. Furthermore, with an EC_{50} value of 8.4 ± 1.0, it is also on the borderline of being classified as active.

Finally, pharmacophore modeling as implemented in the software package MOE yielded two models which show excellent accuracies in classification of active/inactive according to the threshold used in the classification model (10 μM). Both consisted of five pharmacophores and featured an overall accuracy of 0.91 with accuracy on actives of 0.83 and accuracy on inactives of 0.93. Model 1 (see Figure 4) contains three hydrophobic regions, one acceptor and one donor region. The second model (see Figure 5) is defined by one aromatic region, two hydrophobic features, and two donor regions. Interestingly, although the compounds show some degree of pseudosymmetry, an overlay of molecules present in pharmacophore model 1 did

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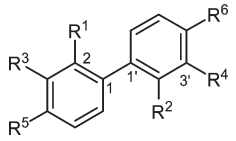
Table 5. Structures of Compounds Based on a General Biphenyl Structure for the Evaluation of $GABA_A$ Receptor Modulatory Activity



compound	R^1	R^2	R^3	R^4	R^5	R^6
10	−OH	−OH	−2-propenyl	−2-propenyl	−H	−H
11	−H	−H	−2-propenyl	−2-propenyl	−OH	−OH

Table 6. Structures of Compounds Based on Magnolol for the Evaluation of $GABA_A$ Receptor Modulatory Activity

compound	R^1	R^2	R^3	R^4
Magnolol	−H	−H	−2-propenyl	−2-propenyl
12	$-CH_3$	−H	−2-propenyl	−2-propenyl

not show any switch of rings A and B (Supporting Information Figure 2). Because all honokiol derivatives feature aromatic regions, we are in favor of model 1, which proposes that hydrophobic residues next to the hydroxy group on ring B increase activity. This is in line with the qualitative considerations on the structure−activity relationships discussed above. This model may thus serve as valuable tool for virtual screening of natural compound libraries.

Different α-Subunit Selectivity of Derivatives 26 and 31. Honokiol most efficiently modulated receptors containing α_3 subunits ($\alpha_3\beta_2 > \alpha_2\beta_2 > \alpha_1\beta_2 > \alpha_5\beta_2$; Figure 1A). Remarkably, the introduction of an amino group as in compounds **26** and **31** did not only enhance the potency but also affected the α-subunit selectivity by enhancing modulation of $\alpha_2\beta_2$ receptors (efficiency order: $\alpha_2\beta_2$ (**26**, 2975%; **31**, 5204%) > $\alpha_3\beta_2$ (**26**, 2378%; **31**, 3671%) > $\alpha_1\beta_2$ (**26**, 2260%; **31**, 2601%) > $\alpha_5\beta_2$; see Figure 6, Table 8). I_{GABA} modulation of receptors comprising α_5 and β_2 subunits by **26** and **31** was comparably weak as observed for honokiol effects, suggesting that interaction with α_5 subunits is not affected. Statistically significant differences compared to I_{GABA} enhancement through the respective receptor subtype by honokiol are given in Table 8.

CONCLUSIONS AND OUTLOOK

In conclusion, we have designed modulators of $GABA_A$ receptors with high subtype-selectivity by targeting key functionalities of honokiol for synthetic modification as, e.g., the phenolic group, the side chain, the polarity as well as ring positions for substitution. The amino group-containing compounds represent a novel type of potent and highly efficient $GABA_A$ receptor modulators among which some promising lead candidates may be found. Most of the synthetic derivatives have not been described so far. The potency of **26** and **31** was about 1 order of magnitude higher than that of honokiol. Both compounds display subtype-selectivity for α_2 and α_3 containing $GABA_A$ receptors, lower efficiency on $GABA_A$ receptors incorporating α_1-subunits, and may therefore provide ligands with anxiolytic and anticonvulsant properties but with reduced sedative, ataxic, and amnestic side effects.[57] Further studies, in vitro as well as in vivo will have to be performed in order to gain a more detailed insight into their mechanism of action and to confirm our encouraging data in vivo.

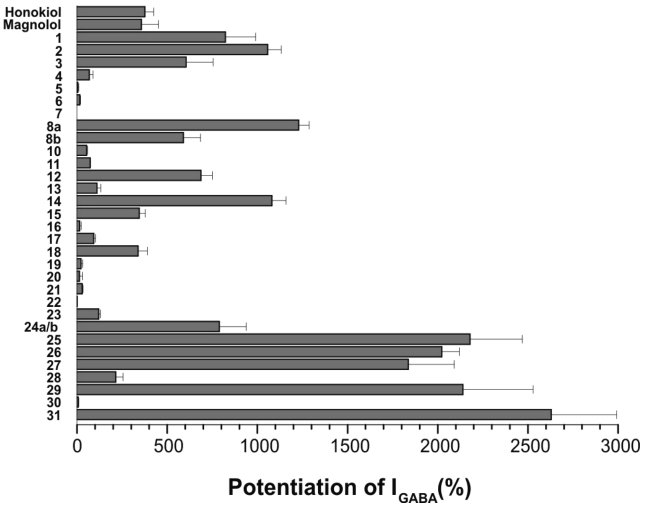


Figure 2. Modulation of chloride currents through $GABA_A$ receptors composed of α_1- and β_2-subunits by 30 μM of honokiol, magnolol, and the indicated derivatives. Each bar graph represents the mean ± SE from at least three oocytes and two oocyte batches.

EXPERIMENTAL SECTION

Chemicals. Honokiol for the investigation of subunit selectivity was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan. The structural formula is given in Tables 4−6. A stock solution (100 mM) was prepared in 100% DMSO (dimethyl sulfoxide, Sigma-Aldrich, Steinheim, Germany). Because of its poor solubility in the test medium, honokiol was only used in concentrations up to 300 μM. Derivatives of honokiol were synthesized as indicated in the Experimental Section. Equal amounts of DMSO were present in control and compound-containing solutions. The maximum DMSO concentration in the bath (0.3%) did not affect I_{GABA}. Diazepam and flumazenil were obtained from Sigma-Aldrich, Steinheim, Germany.

Syntheses. Compounds were synthesized as described below and under Supporting Information. Derivatives leading to chiral centers in the side chain were not further separated into diastereomers. Solvents were of pa quality, if not stated otherwise. Thin layer chromatography (TLC) was performed using aluminum foil coated with Silica 60 F_{254} (Merck, Darmstadt). Detection was done by UV/254 nm and spraying with molybdophosphoric acid and subsequent heating. For column chromatography, Silica Gel 60 (63−200 $\mu\mu$m, Merck, Darmstadt) was used. The purity of all synthesized compounds was verified using NMR, GC-MS, and analytical HPLC. Except for compounds **27** and **29**, which turned out to be rather instable, the purity exceeded 95%. Purity and LC retention times are given under Supporting Information.

^1H and ^{13}C NMR spectra were recorded in deuterated chloroform ($CDCl_3$ if not otherwise marked) with TMS as internal standard on a Varian 400 MHz UnityINOVA spectrometer (400 and 100 MHz, respectively). For convenience, ^{13}C NMR data are presented in Supporting Information Table S1. EI-MS were recorded on an Agilent Technologies HP 7890A instrument fitted with detector HP 5975C VL MSD (70 eV, ion source 250 °C, quadrupole temperature 150 °C). The column used was an Agilent HP-5MS 30 m, ID 0.25 mm, film 5% phenyl95%methylpolysiloxane 0.25 μm. The oven temperature was kept at 45 °C for 2 min and programmed to 300 °C at a rate of 3 °C/min, then kept constant at 300 °C for 20 min, with a total run time of 64.5 min.

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Table 7. Efficiency and Potency (EC_{50}) of I_{GABA} Modulation by 30 μM of the Indicated Compounds on $\alpha_1\beta_2$ Receptors[a]

compound	screening (% potentiation of I_{GABA} at 30 μM)	EC_{50} (μM)	I_{max} (%)	n_H	n
honokiol	376 ± 50	39.3 ± 7.7	1034 ± 182	2.3 ± 0.4	8
magnolol	357 ± 95	36.8 ± 14.0	963 ± 151	1.5 ± 0.4	9
1	822 ± 169	27.2 ± 11.5	1532 ± 218	1.0 ± 0.1	9
2	1057 ± 75	12.7 ± 2.5	1445 ± 103	1.3 ± 0.1	7
3	605 ± 150	19.7 ± 9.8	1001 ± 148	1.4 ± 0.5	4
4	68 ± 20	34.2 ± 9.9	161 ± 24	2.3 ± 0.1	3
5	4 ± 6	nd	nd	nd	3
6	15 ± 4	105.4 ± 22.6	177 ± 29	1.9 ± 0.4	4
7	−2 ± 2	nd	nd	nd	3
8a	1228 ± 59	8.4 ± 1.0	1340 ± 77	1.9 ± 0.2	4
8b	589 ± 95	16.9 ± 4.9	952 ± 120	1.1 ± 0.3	4
10	53 ± 6	35.2 ± 3.5	148 ± 5	2.1 ± 0.2	4
11	73 ± 3	23.7 ± 2.4	110 ± 8	2.8 ± 0.8	4
12	687 ± 64	54.3 ± 11.3	2800 ± 500	1.9 ± 0.3	6
13	110 ± 21	42.1 ± 2.7	368 ± 65	1.3 ± 0.1	4
14	1080 ± 79	14.3 ± 3.8	1396 ± 143	1.5 ± 0.2	4
15	345 ± 34	59.9 ± 23.0	865 ± 205	1.1 ± 0.2	4
16	14 ± 9	nd	nd	nd	4
17	93 ± 10	14.8 ± 6.4	145 ± 24	0.9 ± 0.1	4
18	339 ± 52	22.0 ± 7.7	608 ± 97	1.3 ± 0.2	4
19	22 ± 9	nd	nd	nd	4
20	13 ± 17	nd	nd	nd	3
21	28 ± 6	nd	nd	nd	3
22	1 ± 2	nd	nd	nd	3
23	120 ± 10	25.0 ± 5.7	227 ± 20	1.2 ± 0.3	4
24ab	789 ± 149	3.2 ± 1.0	814 ± 141	1.8 ± 0.6	6
25	2179 ± 290	12.4 ± 2.9	2707 ± 357	1.8 ± 0.2	5
26	2023 ± 97	9.2 ± 1.5	2260 ± 116	1.7 ± 0.2	5
27	1836 ± 256	9.3 ± 1.8	2031 ± 189	2.0 ± 0.2	5
28	215 ± 40	18.5 ± 4.4	326 ± 55	1.8 ± 0.2	4
29	2140 ± 389	7.7 ± 1.4	2288 ± 290	2.2 ± 0.2	4
30	7 ± 3	nd	nd	nd	3
31	2629 ± 363	3.8 ± 0.9	2601 ± 243	1.7 ± 0.2	6

[a] nd: not determined.

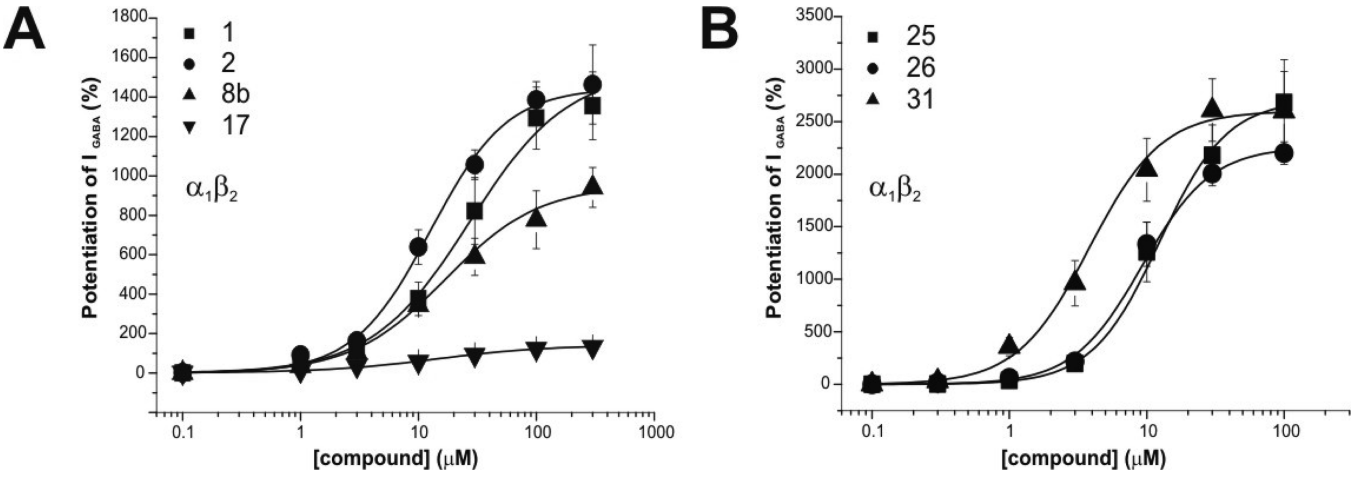

Figure 3. Concentration-dependent enhancement of I_{GABA} (EC_{3-7}) through $\alpha_1\beta_2$ channels for **1**, **2** **8b**, **17** (A); **25**, **26**, **31** (B). The EC_{50}, n_H, and maximal I_{GABA} enhancement are given in Table 7. Data points represent means ± SE from at least four oocytes from ≥2 batches.

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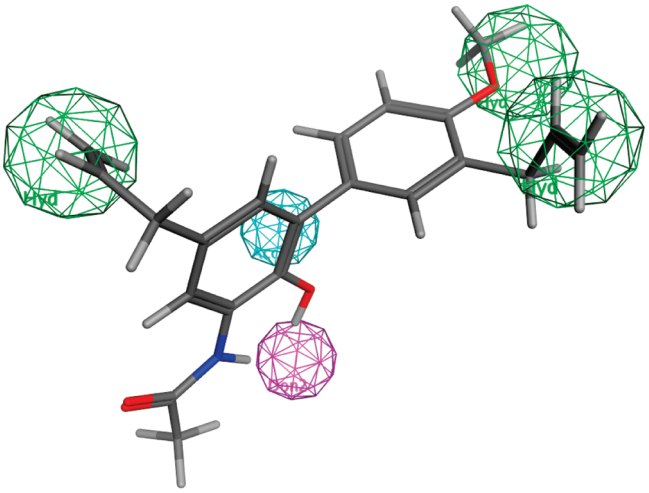

Figure 4. Pharmacophore model 1. Hydrophobic regions are represented by green areas (Hyd), the acceptor region is displayed in light-blue (Acc), and the donor region is represented by the pink area (Don).

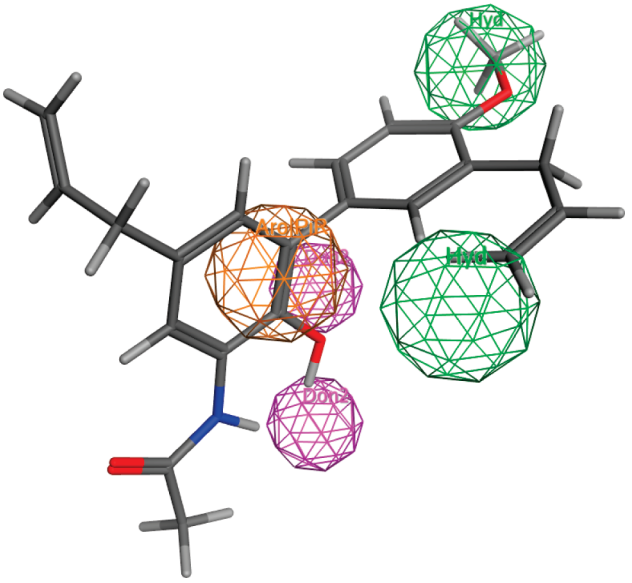

Figure 5. Pharmacophore model 2. Hydrophobic regions are represented by green areas (Hyd), donor regions are displayed in pink (Don), and the aromatic region is represented by the orange area (Aro).

Helium was used as a carrier gas. The injection volume was 1 μL (ca. 0.5% solution) and the split ratio 1:50.

Electrospray ionization (ESI) MS was carried out in positive mode on a Thermo Finnigan LQ Deca XP^{PLUS} mass spectrometer connected to a Surveyor LC system (Thermo-Finnigan) with autosampler. Analytical HPLC-DAD was performed on an Agilent 1100 series equipped with diode-array detector. For (ESI) MS and HPLC-DAD, an SB-C18 Zorbax column (3.5 μm; 150 mm × 2.1 mm; Agilent Technologies) equipped with guard column at a flow rate of 300 μL/min was used. The gradient elution program was as follows: CH_3CN in water (0→25 min/10→90%, 25→30 min/90→100%, 30→38 min/100%). Synthetic mixtures were prepurified on SPE (C18) cartridges, then separated using an HPLC preparative system (Dynamax/Varian solvent delivery system). The column used was a Merck VDSpher 100 C18, 10 μm, 250 mm × 25 mm and isocratic or gradient mixtures of acetonitrile in water at a flow rate of 20 mL/min with detection at 220 nm were used. Melting points (uncorrected) were determined on a Kofler apparatus.

The starting material for most of the syntheses was 4′-*O*-methylhonokiol (**1**), which was isolated from the seeds of *Magnolia grandiflora* L. as described previously.[25] From **1**, honokiol was obtained by Grignard reaction.[58]

4′-*O*-Methyl-3-nitro-honokiol (23). Aqueous nitric acid (65%, 0.7 mL, 0.97 g, 10 mmol) was added under intense stirring within ca. 15 s to a solution of **1** (112 mg, 0.4 mmol) in hexanes (15 mL) at RT. The reaction mixture was stirred for 60 s and quenched with $NaHCO_3$ (1 M, 15 mL). The organic phase was separated, and the aqueous phase was extracted with Et_2O (4 × 10 mL). The combined organic phases were washed with brine (5 mL), dried over Na_2SO_4, and concentrated under reduced pressure, resulting in 129 mg of **23**. **23**: orange oil, 99% yield. IR (KBr): 3424 (OH), 3080, 3004, 2926, 2904, 2851, 1541, 1609, 1541 (NO_2), 1504, 1320 (NO_2), 1251 (COC), 1143, 1133, 1031, 917 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.40 (d, 2H, *J* 6.6 Hz, 1″-H), 3.45 (d, 2H, *J* 6.6 Hz, 1‴-H), 3.88 (s, 3H, O*CH*$_3$), 5.05 (d, 1H, *J* ∼10 Hz, 3‴-H), 5.08 (d, 1H, *J* ∼17 Hz, 3‴-H), 5.12 (d, 1H, *J* ∼17 Hz, 3‴-H), 5.13 (dq, 1H, *J* ∼17, ∼1 Hz, 3‴-H), 5.95 (ddt, 1H, *J* 16.9, 10.3, 6.6 Hz, 2″-H), 6.00 (ddt, 1H, *J* 16.9, 9.9, 6.6 Hz, 2‴-H), 6.94 (d, 1H, *J* 8.4 Hz, 5′-H), 7.33 (d, 1H, *J* 2.2 Hz, 2′-H), 7.40 (dd, 1H, *J* = 8.4, 2.2 Hz, 6′-H), 7.45 (d, 1H, *J* 1.8 Hz, 6-H), 7.90 (d, 1H, *J* 1.8 Hz, 4-H), 11.02 (s br, 1H, O*H*). MS (EI) *m*/*z* (%): 325.2 ($M^{+\cdot}$, 100), 308.2 (4), 278.1 (10), 237.1 (10), 213.1 (68).

3-Amino-4′-*O*-methylhonokiol (25). $SnCl_2 \cdot 2H_2O$ (700 mg, 3.1 mmol) was added to a solution of crude **23** (96 mg, 0.3 mmol) in EtOH (20 mL). The reaction mixture was stirred for 66 h at RT and concentrated under reduced pressure and diluted with ethyl acetate (30 mL). The precipitate resulting from the addition of $NaHCO_3$ (1 M, 20 mL) was filtered off with Celite and rinsed with ethyl acetate (20 mL). The organic layer was separated from the combined filtrate and washings, dried over Na_2SO_4, and concentrated under reduced pressure. The crude product (71 mg) was purified by preparative HPLC. **25**: white crystals, 74% yield, mp 66−68 °C. IR (KBr): 3476 (NH), 3406 (NH), 3373 (NH), ∼3350 (br, OH), 3324 (NH), 3076, 3002, 2922, 2851, 1741 (br), 1639, 1614, 1503, 1487, 1245 (COC), 1225, 913 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 3.27 (d, 2H, *J* 6.9 Hz, 1″-H), 3.42 (d, 2H, *J* 6.6 Hz, 1‴-H), 3.87 (s, 3H, O*CH*$_3$), 5.02 (d, 1H, *J* ∼10 Hz, 3″-H), 5.05 (d, 1H, *J* ∼10 Hz, 3‴-H), 5.08 (2d, 2H, *J* ∼17 Hz, H-3″, 3‴-H), 5.95 (ddt, 1H, *J* ∼17, 9.9, 6.9 Hz, 2″-H), 6.00 (ddt, 1H, *J* 16.9, 10.3, 6.6 Hz, 2‴-H), 6.46 (d, 1H, *J* 1.8 Hz, 6-H), 6.55 (d, 1H, *J* 1.8 Hz, 4-H), 6.94 (d, 1H, *J* 8.1 Hz, 5′-H), 7.22 (d, 1H, *J* 1.8 Hz, 2′-H), 7.27 (dd, 1H, *J* 8.4, 1.8 Hz, 6′-H). MS (EI) *m*/*z* (%): 295.1 ($M^{+\cdot}$, 100), 266.1 (16), 254.1 (15), 237.1 (7), 213.1 (68). MS (ESI) *m*/*z* (%): 361.07 and 296.15 ($[M + H]^+$, 100).

4′-Methoxy-3-nitro-3′,5-dipropyl-biphenyl-2-ol (23a) and 3-Amino-4′-methoxy-3′,5-dipropyl-biphenyl-2-ol (26). $SOCl_2$ (73 μL, 1 mmol, 119 mg) and bismuth nitrate oxide (71−74% Bi basis, 220 mg, 0.125 mmol) were added successively to a stirred solution of **2** (142 mg, 0.5 mmol) in absolute CH_2Cl_2 (5 mL). After 20 min of stirring at RT, the precipitate was filtered off and extracted with CH_2Cl_2 (3 mL). The combined organic phases were washed with HCl (2 M, 2 × 2 mL) and brine (2 × 2 mL), dried over Na_2SO_4, and concentrated under reduced pressure, resulting in 168 mg crude **23a**, which was used without further purification.

Crude **23a** (156 mg) was reduced using $SnCl_2 \cdot 2H_2O$ (863 mg, 3.8 mmol) in analogy to **23**. The crude product (134 mg) was purified by preparative HPLC, yielding 19 mg of starting material **23a** and 35 mg **26**. **26**: waxy solid, 25% yield. IR (KBr): 3340 (OH), 3281 (NH), 2956 (NH), 2930, 2868, 2671, 1611, 1505, 1488, 1431, 1245 (COC), 1136, 1032, 806 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, *J* 7.3 Hz, 3‴-H), 0.96 (t, 3H, *J* 7.3 Hz, 3″-H), 1.61 (sext, 2H, *J* 7.7 Hz, 2″-H), 1.63 (sext, 2H, *J* 7.7 Hz, 2‴-H), 2.46 (t, 2H, *J* 7.7 Hz, 1″-H), 2.62 (t, 2H, *J* 7.7 Hz, 1‴-H), 3.85 (s, 3H, O*CH*$_3$), 6.47 (d, 1H, *J* 1.8 Hz, 6-H), 6.54 (d, 1H, *J* 1.8 Hz, 4-H), 6.92 (d, 1H, *J* 8.4 Hz, 5′-H), 7.22 (d, H, *J* 2.2 Hz, 2′-H), 7.26 (dd, 1H, *J* 8.1, 2.2 Hz, 6′-H). MS (ESI) *m*/*z* (%): 300.22 ($[M + H]^+$, 100).

Chemical data of **23a** are given under Supporting Information.

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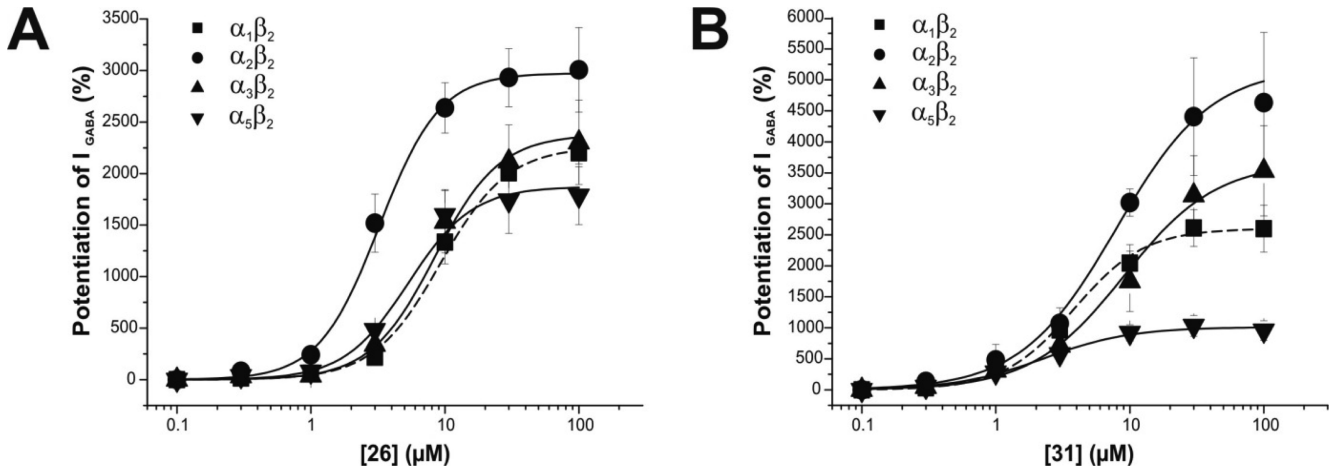

Figure 6. Concentration−response curves for the action of compounds **26** (A) and **31** (B) on $\alpha_1\beta_2$ (■), $\alpha_2\beta_2$ (●), $\alpha_3\beta_2$ (▲), and $\alpha_5\beta_2$ (▼) receptors using a GABA EC_{3-7} (EC_{50} and n_H values are given in Table 8). Data points represent means ± SE from at least four oocytes from ≥2 batches.

Table 8. Potency and Efficiency of 26 and 31 on $GABA_A$-Receptors of Different Subunit Compositions

compound	subunit combination	EC_{50} (μM)	I_{max} (%)	Hill coefficient (n_H)	no. of experiments (n)
26	$\alpha_1\beta_2$	9.3 ± 1.5 [a]	2260 ± 116 [a]	1.7 ± 0.2	5
26	$\alpha_2\beta_2$	3.2 ± 0.7 [a]	2975 ± 226 [a]	1.9 ± 0.3	5
26	$\alpha_3\beta_2$	8.4 ± 2.7 [a]	2378 ± 341	1.8 ± 0.3	6
26	$\alpha_5\beta_2$	5.3 ± 1.0	1874 ± 219 [a]	1.8 ± 0.2	6
31	$\alpha_1\beta_2$	3.8 ± 0.9 [a]	2601 ± 243 [a]	1.7 ± 0.2	6
31	$\alpha_2\beta_2$	7.9 ± 3.3 [a]	5204 ± 1166 [a]	1.2 ± 0.3	6
31	$\alpha_3\beta_2$	9.0 ± 3.0 [a]	3671 ± 679	1.3 ± 0.1	7
31	$\alpha_5\beta_2$	2.3 ± 0.6	1013 ± 116 [a]	1.3 ± 0.3	4

[a] Indicates statistically significant ($p < 0.05$) differences compared to I_{GABA} enhancement through indicated receptor subtypes by honokiol (see Table 2).

3-(*N*-Acetylamino)-4′-*O*-methylhonokiol (31). In a 10 mL round-bottom flask, **25** (120 mg, 0.41 mmol) was mixed with water (1.4 mL), and acetic anhydride (73 mg, 0.70 mmol, 66 μL) was added. The flask was allowed to rotate in 80 °C water bath for 10 min. After cooling to RT, the reaction mixture was extracted with Et_2O (3 × 1.5 mL). The combined extracts were washed with 1 M aqueous $NaHCO_3$ (1.5 mL) and water (1.5 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product (155 mg) was purified by HPLC, yielding 56 mg **31**. **31**: dark oil, 30% yield. IR (KBr): 3295 (NH), 3076, 3002, 2976, 2932, 2835, 1638 (C═O), 1608, 1540 (NH), 1503, 1477, 1247 (COC), 1032, 995, 914 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.16 (s, 3H, $COCH_3$), 3.26 (d, 2H, *J* 6.9 Hz, 1″-H), 3.41 (d, 2H, *J* 6.6 Hz, 1‴-H), 3.83 (s, 3H, OCH_3), 5.03 (m, 2H, 3″-H, 3‴-H), 5.06 (dq, 1H *J* ∼17, 2.2 Hz, 3″-H), 5.06 (dq, 1H, *J* ∼17, 1.8 Hz, 3‴-H), 5.91 (ddt, 1H, *J* 17.2, 10.3, 6.6 Hz, 2″-H), 6.00 (ddt, 1H, *J* 16.9, 10.4, 6.6 Hz, 2‴-H), 6.89 (d, 1H, *J* ∼8 Hz, 5′-H), 6.91 (s, 1H, 6-H), 7.20 (d, H, *J* 1.8 Hz, 4-H), 7.27 (d, 1H, *J* 2.2 Hz, 2′-H), 7.33 (dd, 1H, *J* 8.4, 2.2 Hz, 6′-H), 7.71 (s, br, 1H, O*H*), 8.21 (s, br, 1H, N*H*). MS (ESI) *m*/*z* (%): 338.08 ($[M + H]^+$, 100).

■ ASSOCIATED CONTENT

Supporting Information. Co-application of diazepam and honokiol in the presence of flumazenil (1 μM); HPLC/MS purity and retention times for target compounds; syntheses and spectroscopic data of compounds of non nitrogen-containing compounds and intermediary products (i.e., compounds **2**−**4**, **4a**, **5**−**7**, **23a**, and **24c**) together with carbon NMR shift values of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

BDZ, benzodiazepines; cDNA, complementary DNS; CNS, central nervous system; DMSO, dimethyl sulfoxide; DEPC, diethyl pyrocarbonate; EC_{50}, effective concentration 50%; I_{max}, maximal stimulation of I_{GABA}; GABA, γ-aminobutyric acid; GC-MS, gas chromatography−mass spectrometry; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HK, honokiol; HPLC, high performance liquid chromatography; I_{GABA}, chloride currents through $GABA_A$ receptors; MS-222, methane sulfonate salt

of 3-aminobenzoic acid ethyl ester; n_H, Hill coefficient; NMR, nuclear magnetic resonance; pCMV, cytomegalovirus promoter; SAR, structure−activity relationship; TLC, thin layer chromatography; TPSA, topological polar surface area

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