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Structural analogues of the natural products magnolol and honokiol as potent allosteric potentiators of $GABA_A$ receptors

Alexander Fuchs [a], Roland Baur [b], Clara Schoeder [a], Erwin Sigel [b], Christa E. Müller [a,b,*]

[a] PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany
[b] Institute of Biochemistry and Molecular Medicine, University of Bern, Bühlstrasse 28, 3012 Bern, Switzerland

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ABSTRACT

Biphenylic compounds related to the natural products magnolol and 4′-*O*-methylhonokiol were synthesized, evaluated and optimized as positive allosteric modulators (PAMs) of $GABA_A$ receptors. The most efficacious compounds were the magnolol analog 5-ethyl-5′-hexylbiphenyl-2,2′-diol (**45**) and the honokiol analogs 4′-methoxy-5-propylbiphenyl-2-ol (**61**), 5-butyl-4′-methoxybiphenyl-2-ol (**62**) and 5-hexyl-4′-methoxybiphenyl-2-ol (**64**), which showed a most powerful potentiation of GABA-induced currents (up to 20-fold at a GABA concentration of 3 μM). They were found not to interfere with the allosteric sites occupied by known allosteric modulators, such as benzodiazepines and *N*-arachidonoylglycerol. These new PAMs will be useful as pharmacological tools and may have therapeutic potential for mono-therapy, or in combination, for example, with $GABA_A$ receptor agonists.

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1. Introduction

In the central nervous system of mammals, the neurotransmitter γ-aminobutyric acid (GABA, **1**, Fig. 1) is the main inhibitory agent.[1] It activates two different classes of membrane receptors: $GABA_A$ and $GABA_B$. The $GABA_A$ receptor is an ionotropic receptor which controls the influx of chloride ions. It has been shown to be modulated through different allosteric binding sites for benzodiazepines, barbiturates and ethanol.[2,3] In contrast, the $GABA_B$ receptor is a G protein-coupled receptor, which controls potassium and calcium channels via G protein activation.[2–4] Especially the $GABA_A$ receptor has been an important drug target since many years due to the anxiolytic, antidepressant, muscle relaxant and anti-convulsive effects that it conveys.[5] The $GABA_A$ receptors consist of five subunits of different composition depending on the receptor subtype.[6] The main subunits of $GABA_A$ α_1, β_2 and γ_2 are expressed in the entire central nervous system (CNS), while other isoforms have a more restricted expression pattern. The α_6 subunit, for example, is only expressed on granule cells of the cerebellum, and the ρ subunit is found on retinal cells.[7] Outside of the CNS $GABA_A$ receptors are also expressed, for example, on cells of the immune system, in liver, on smooth muscle and in the respiratory system.[8–10]

Recent findings showed that $GABA_A$ receptors contain binding sites for the endocannabinoid 2-arachidonoylglycerol (2-AG, **2**) and the endocannabinoid metabolite *N*-arachidonoylglycine (NAGLY, **3**).[11,12] Moreover, a new study reported that the natural products honokiol (**4**) and 4′-*O*-methylhonokiol (**5**) potentiate $GABA_A$ currents, **5** being superior to **4** (for structures see Fig. 1).[13] 4′-*O*-Methylhonokiol is also known to be a ligand of the cannabinoid receptor (CB) 2 where it acts as a partial agonist.[14]

The neolignans **4** and **5** as well as magnolol (**6**) are constituents of plants of the *Magnolia* family, and are found, for example, in *Magnolia officinalis* and *Magnolia grandiflora*.[15,16] Preparations of *Magnolia officinalis* are used in traditional Chinese medicine and Japanese Kampo medicine due to their antidepressant and anxiolytic effects.[15,17] Furthermore, magnolol, its metabolite tetrahydromagnolol and their synthetic analogs have been shown to interact with CB receptors, where they act as potent partial agonists, with a preference for CB_2.[14,18]

Abbreviations: iNOS, inducible NO-synthase; PAM, positive allosteric modulator; Ro15-1788, flumazenil, ethyl 8-fluoro-5-methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate.

* Corresponding author at present address: Universität Bonn, Pharmazeutisches Institut, An der Immenburg 4, D-53121 Bonn, Germany. Tel.: +49 228 732301; fax: +49 228 732567.
E-mail address: christa.mueller@uni-bonn.de (C.E. Müller).

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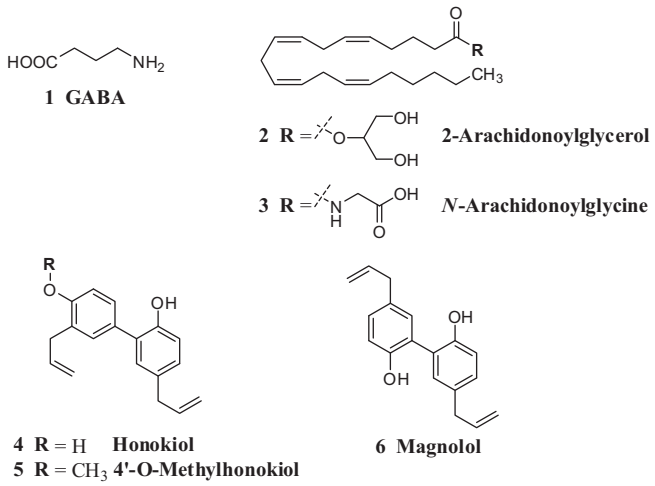


Figure 1. Structures of γ-aminobutyric acid (GABA, **1**) and (potential) positive allosteric modulators of $GABA_A$ receptors (**2**–**6**).

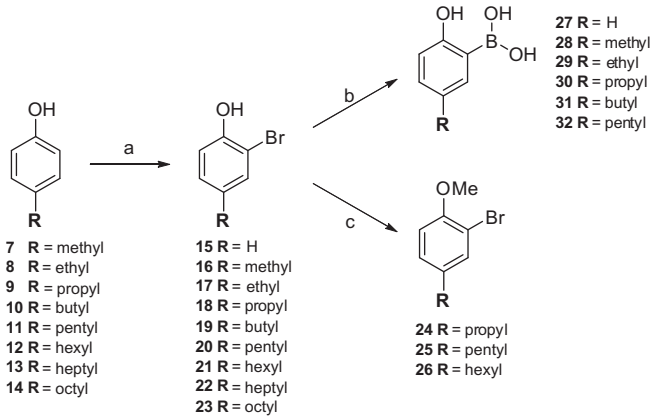
In the present study we synthesized analogs of magnolol and 4′-*O*-methylhonokiol with the goal to study their structure–activity relationships (SAR) as allosteric modulators of $GABA_A$ receptors and to optimize them with regard to potency and selectivity.

2. Results and discussion

2.1. Chemistry

In the present study 33 biphenylic compounds have been investigated, 15 of which are new compounds. The synthesis of **37**–**51**, **55**, **57** and **58** was described previously,[14] while the preparation and properties of **52**–**54**, **56**, **59**–**69** are described herein for the first time.

All magnolol and 4′-*O*-methylhonokiol analogs were synthesized by a previously published procedure,[14] starting from commercially available 4-alkylphenols (**7**–**14**) as shown in Scheme 1. The bromination of 4-alkylphenols via electrophilic aromatic substitution was carried out with elementary bromine in chloroform in the presence of sodium hydrogencarbonate to provide the 2-bromo-4-alkylphenols **16**–**23**.[19] Intermediates **15** and **27** are commercially available as well.



Scheme 1. Reagents and conditions: Syntheses of intermediates. Reagents and conditions: (a) Br_2, $NaHCO_3$, $CHCl_3$, 0 °C, 80%; (b) one pot three steps, (1) *n*-butyllithium, Et_2O, −78 °C; (2) $B(OCH_3)_3$, −78 °C to rt; (3) HCl, 60%; (c) CH_3I, NaOH, benzyl-tri-*n*-butylammonium bromide, CH_2Cl_2/H_2O (1:1), 12 h, rt, 90%.

Methylation by a phase transfer catalysis method with methyl iodide as an alkylating agent in a mixture of water and dichloromethane in the presence of sodium hydroxide and benzyl-tri-*n*-butylammonium bromide led to the 2-bromo-1-methoxy-4-alkylbenzenes **24**–**26**.[20] Boronic acid derivatives (**28**–**32**) were obtained from the 2-bromo-4-alkylphenols **15**–**23** by treatment with *n*-butyllithium and trimethyl borate yielding **28**–**32** after acid hydrolysis in moderate yields.[21] The final reaction step yielded products **37**–**69** via Suzuki cross-coupling (Scheme 2).[22]

The boronic acids **33**–**36** were commercially available. The final products were purified by flash chromatography, or preparative HPLC, respectively. The structures were confirmed by ^1H and ^{13}C NMR spectra and by HPLC coupled to electrospray ionization mass spectrometry (LC–ESI-MS). The purity of the compounds was determined by (LC–ESI-MS/UV) and was in all cases greater than >95% (for details see Section 4 and Supporting information).

2.2. Biological evaluation

2.2.1. Positive allosteric modulation of $GABA_A$ receptors

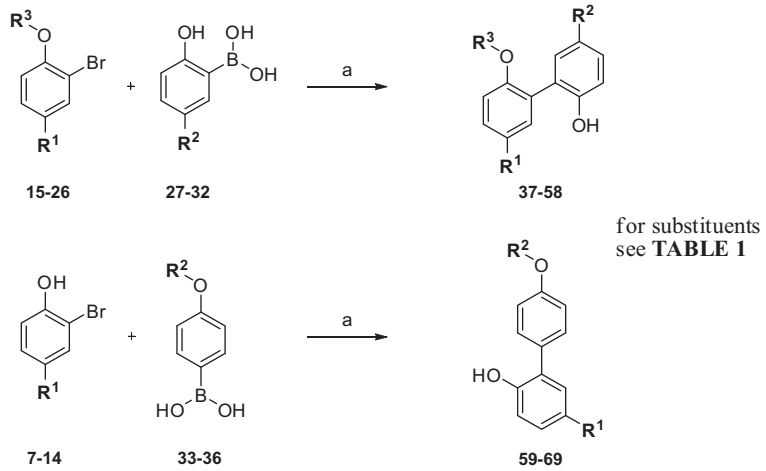
The compounds were initially screened at recombinant α1β2γ2 $GABA_A$ receptors expressed in Xenopus oocytes. We determined the potentiation of the GABA-induced current exerted by 3 μM of test compound. The agonist GABA (0.3 μM; EC_{50} about 30 μM; the applied concentration corresponds to the EC_1 at which 1% of the maximal effect of GABA is observed) was applied several times until a constant current response was observed. The same concentration of GABA was then co-applied with the test compound. Results are shown in Figure 2 and Table 1.

All of the tested biphenylic compounds showed a current potentiation in the Xenopus oocytes system. In Table 1 the compounds' structures and their percent increase in GABA-induced current are presented. In the first set of compounds — a series of magnolol analogs — compound **45**, with an ethyl and a hexyl residue, was the most potent one. The weak effects of compounds **37**, **38**, **39**, and **40** indicated that, for a high potency, an alkyl residue is required on both aromatic rings of the molecule, and that a methyl group on the one phenyl ring — in combination with a longer alkyl residue on the second ring — was too small. However, GABA-induced current potentiation also decreased when the molecule was too bulky. This was the case for compounds that bear a propyl or butyl residue on one phenyl ring combined with a pentyl or longer alkyl chain on the other phenyl residue (compare **46** to **51**). Further modification of the magnolol analogs by methylation of one of the phenolic groups resulted in a decreased effect (compare **52** to **58**).

In the second set of compounds — the 4′-*O*-methylhonokiol analogs — effects of structural modifications were even much more pronounced. Compound **64**, containing a hexyl residue and a methoxy group, was the most potent positive allosteric modulator (PAM) of GABA-induced currents in the present series. Again, the smallest compound (**59**), with a methyl residue and a methoxy group, as well as the most bulky compounds (**67**, **68**, **69**), with a hexyl residue and an ethoxy, propoxy, or isopropoxy group, respectively, were the least potent ones within this set of compounds.

As a next step, concentration-dependent effects were determined for the most efficacious potentiators of GABA-induced $GABA_A$ receptor currents, namely for the magnolol-derived 5-ethyl-5′-hexylbiphenyl-2,2′-diol (**45**), and the honokiol analogs 4′-methoxy-5-propylbiphenyl-2-ol (**61**), 5-butyl-4′-methoxybiphenyl-2-ol (**62**), and 5-hexyl-4′-methoxybiphenyl-2-ol (**64**) (see Fig. 3).

The compounds clearly showed concentration-dependent effects with extremely high potentiation by >1000% at the highest tested concentration of 10 μM. The most potent compound, **61**, increased the GABA-induced current by 5000% at 10 μM. Higher



Scheme 2. Reagents and conditions: Synthesis of $GABA_A$ modulators series I (magnolol analogs **37**–**58**) and II (honokiol analogs **59**–**69**). Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, H_2O, 100 °C, 18 h.

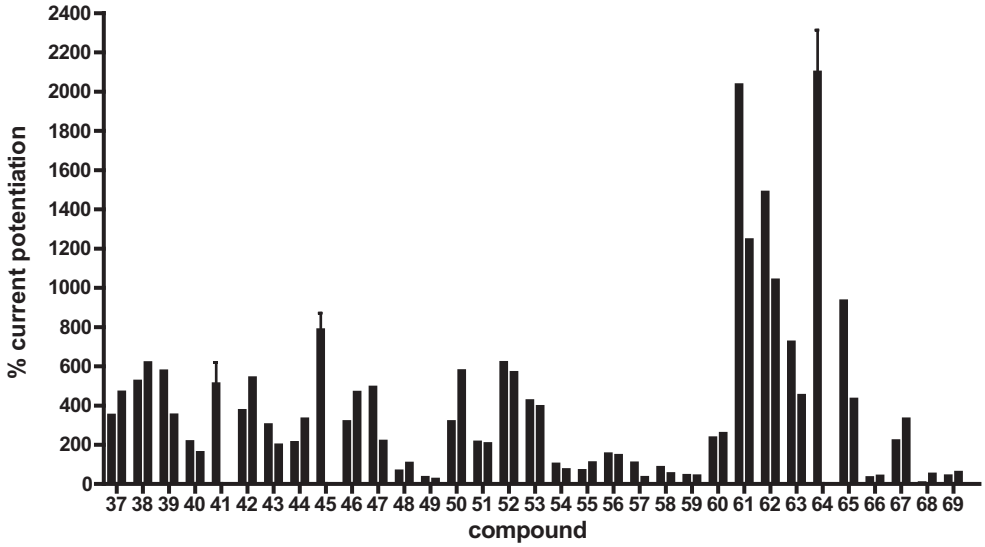


Figure 2. Screening of test compounds at recombinant α1β2γ2 $GABA_A$ receptors. The receptors were expressed in Xenopus oocytes and the current potentiation by 3 μM of test compound was determined. The agonist GABA (0.3 μM) was applied several times until a constant current response was obtained. The same concentration of GABA was then co-applied with the test compound. Each bar represents a single experiment. For compounds **41** (4 independent experiments), **45** and **64** (11 independent experiments each) mean values ± SD are shown).

concentrations could not be applied due to limited solubility of the rather lipophilic compounds. Only compound **64** appeared to reach the upper plateau of the curve with a maximal current potentiation in the range of nearly 3000% and an EC_{50} value of 1.8 μM was estimated. For compounds **45**, **61** and **62** a concentration of 10 μM was not sufficient to induce a maximal effect and to reach the upper plateau of the curve. At 10 μM the current potentiation for these compounds were in the range of 1000% (**45**), 5000% (**61**) and 4000% (**62**). A much smaller degree of potentiation may be biologically relevant already. A potentiation of 100% was reached below 0.7 μM in each case. *N*-Arachidonoylglycine was previously reported to exhibit concentration-dependent current potentiation showing a maximal current potentiation in the range of 400% at 10 μM (applying a GABA concentration of 1 μM), with an estimated EC_{50} value between 1 and 10 μM.[12] For 2-Arachidonoylglycerol a maximal current potentiation of 138% with an EC_{50} value of 2.1 μM was determined.[23] The natural product 4′-*O*-methylhonokiol was reported to strongly potentiate $GABA_A$ receptor currents. At a concentration of 10 μM the potentiation was in the range of 6000% using a GABA concentration of 0.5 μM.[13] Higher concentrations led to a reduction leading to a bell-shaped concentration–response curve.

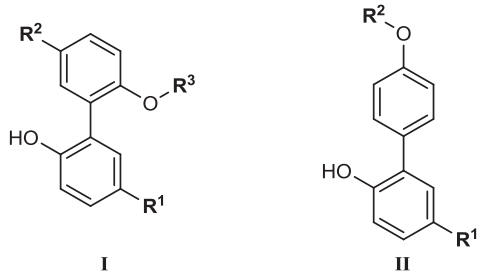
2.2.2. Direct activation of $GABA_A$ receptors

We subsequently investigated whether the most potent positive allosteric modulators additionally acted as agonists in their own right (see Fig. 4).

The investigated compounds **45**, **61**, **62** and **64** were found to be very weak direct agonists of $GABA_A$ receptors. All of them induced a concentration-dependent increase in current amplitude. A concentration of 10 μM was not sufficient to induce a maximal effect. At that concentration the compounds elicited currents amounting to about 2–6% of maximal effect achieved with GABA (=100%). Nevertheless their agonistic effect was higher than that of the parent compound 4′-*O*-methylhonokiol (0.21% at 3 μM).[13]

Interestingly, the rank order of efficacy for direct $GABA_A$ receptor activation was similar to that observed for potentiation of GABA effects: **61** ⩾ **62** > **64** > **45**. This indicates that the identified

Table 1
Activity of biphenylic compounds related to the natural products magnolol and honokiol as positive allosteric modulators of $GABA_A$ receptors



magnolol analogs honokiol analogs

Compound	R^1	R^2	R^3	$GABA_A$ activity[a]
Previously identified positive allosteric modulators				
2-arachidonolyglycerol[23]				+
N-arachidonoylglycine[12]				++
Magnolol[b,24]				++
Honokiol[13]				+
Methylhonokiol[13]				+++++
Series I: magnolol analogs				
37	H	Pentyl	H	++
38	H	Hexyl	H	+++
39	Methyl	Butyl	H	++
40	Methyl	Pentyl	H	+
41	Methyl	Hexyl	H	+++
42	Ethyl	Propyl	H	++
43	Ethyl	Butyl	H	+
44	Ethyl	Pentyl	H	+
45	Ethyl	Hexyl	H	++++
46	Propyl	Pentyl	H	++
47	Propyl	Hexyl	H	++
48	Propyl	Heptyl	H	(+)
49	Propyl	Octyl	H	(+)
50	Butyl	Pentyl	H	++
51	Butyl	Hexyl	H	+
52	Ethyl	Pentyl	CH_3	+++
53	Ethyl	Hexyl	CH_3	++
54	Propyl	Pentyl	CH_3	(+)
55	Propyl	Hexyl	CH_3	(+)
56	Pentyl	Ethyl	CH_3	+
57	Pentyl	Propyl	CH_3	+
58	Hexyl	Propyl	CH_3	(+)
Series II: 4′-O-Methylhonokiol analogs				
59	Methyl	Methyl	–	(+)
60	Ethyl	Methyl	–	+
61	Propyl	Methyl	–	+++++
62	Butyl	Methyl	–	+++++
63	Pentyl	Methyl	–	+++
64	Hexyl	Methyl	–	+++++
65	Heptyl	Methyl	–	+++
66	Octyl	Methyl	–	(+)
67	Hexyl	Ethyl	–	+
68	Hexyl	Propyl	–	(+)
69	Hexyl	Isopropyl	–	(+)

[a] allosteric potentiation at 3 μM concentration of test compound: 0–100% (+), 100–300% +, 300–500% ++, 500–700% +++, 700–900% ++++, >1000% +++++.
[b] Magnolol concentration 30–60 μM.

positive allosteric modulators are also weak allosteric agonists and may therefore be characterized as ago-allosteric modulators.[25,26]

2.2.3. Search for the allosteric binding site

Two of the most potent positive allosteric modulators at $GABA_A$ receptors of the present series, the magnolol analog **45** and the honokiol analog **64**, were investigated at $GABA_A$ receptors with different subunit composition, including $\alpha_1\beta_2\gamma_2$, $\alpha_2\beta_2\gamma_2$, $\alpha_3\beta_2\gamma_2$, $\alpha_4\beta_2\gamma_2$, $\alpha_5\beta_2\gamma_2$, $\alpha_6\beta_2\gamma_2$, $\alpha_1\beta_1\gamma_2$, $\alpha_1\beta_3\gamma_2$, $\alpha_1\beta_2$, $\alpha_1\beta_3\delta$ (see Fig. 5). Moreover, they were tested at receptors containing point mutations: $\alpha_1\beta_2$**N265S**γ_2, $\alpha_1\beta_2$**V436T**γ_2 (Fig. 5). The mutations were selected because other allosteric potentiators of $GABA_A$ receptors such as loreclezole and 2-arachidonoylglycerol were found to be no longer active at those mutated receptors, and we wanted to investigate whether the new magnolol and honokiol analogs shared the same binding site.[11,23,27,28]

To evaluate the subunit specificity of test compounds **45** and **64**, first the α-subunit of the $\alpha_1\beta_2\gamma_2$ isoform was replaced by several other α-subunits. Then, the β-subunit of the $\alpha_1\beta_2\gamma_2$ isoform was replaced by β_1- and β_3-subunits. Finally, the γ-subunit was omitted or replaced by a δ-subunit. For compound **64** a change in the α-subunit showed some alteration in its current potentiating properties. This indicates that the α-subunit has an effect on the binding of the compound. A change of the β_2-subunit to β_1 showed

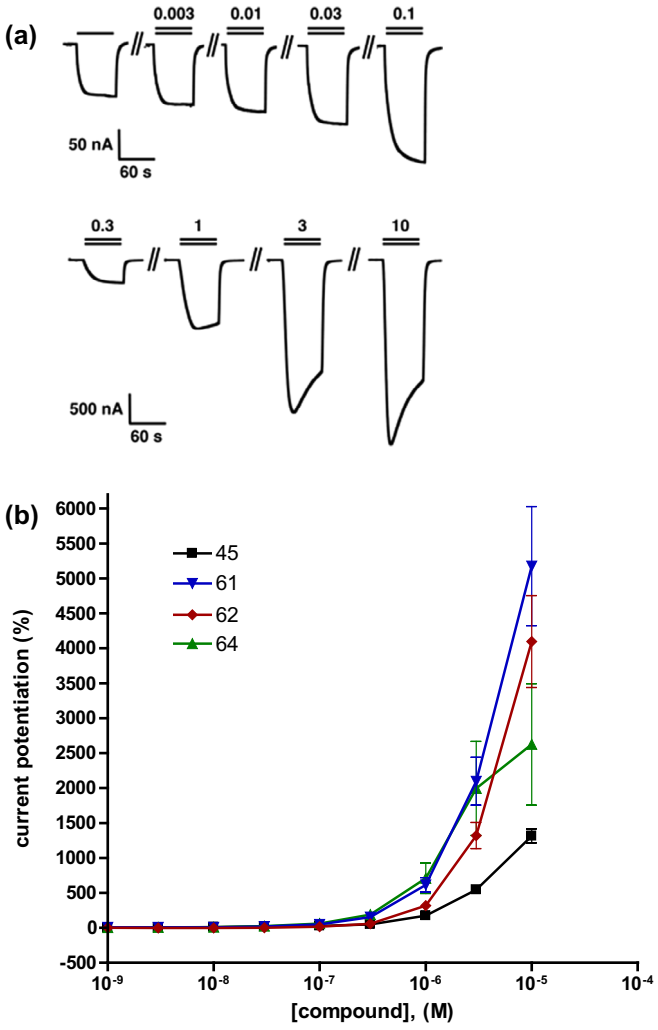


Figure 3. Concentration dependence of GABA-induced current potentiation at recombinant $\alpha1\beta2\gamma2$ $GABA_A$ receptors. The GABA concentration was 0.3 μM. (a) Original current traces of an experiment with **64**; numbers indicate applied concentrations of **64** (0.003–10 μM). (b) Averaged data ± SD for compounds **45**, **61**, **62** and **64** determined in four to five separate experiments.

a significant decrease in current potentiation, whereas a change to β_3 resulted in a small increase in current potentiation as compared to the $\alpha_1\beta_2\gamma_2$ isoform. This indicates that the β-subunit plays an important role in compound binding. When the γ-subunit was omitted or replaced by a δ-subunit this had almost no effect, indicating that the γ-subunit is not important for interaction with the allosteric modulator. Both investigated point mutations resulted in a decrease, but not a complete loss of current potentiation. These findings indicate that the honokiol analog **61** may not share its binding site with 2-arachidonoylglycerol and loreclezole, although its activity is influenced and modulated by these sites. For the magnolol analog **45** the subunit specificity was found to be similar to that of honokiol analog **64**. The current potentiation was influenced by a change of the α-subunit, but this effect was much smaller than that observed when changing the β-subunit. The receptor isoform in which β_2 was replaced by β_1 displayed the largest decrease in current potentiation by both compounds, **45** as well as **61**. The γ-subunit can be considered as less important for **45**, and likewise for **61**. A decrease in current potentiation observed with the isoforms containing point mutations indicates that the binding of **45** is influenced by these mutations but not abolished, indicating that **45** does not share this binding site with 2-arachidonoylglycerol and loreclezole.

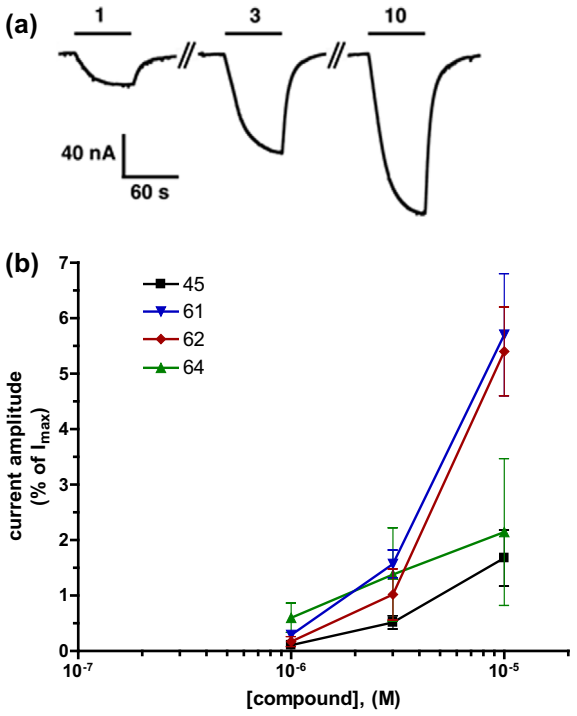


Figure 4. Direct activation of $GABA_A$ receptors by selected test compounds. Current amplitudes are expressed in % of the maximal current induced by GABA (≈100%) in the corresponding cell. (a) Original current traces of an experiment with **64**. (b) Individual experiments for **45**, **61**, **62** and **64**; data points are means ± SD from three to four separate experiments.

Finally, we tested whether the magnolol and honokiol analogs act via the allosteric benzodiazepine binding site. Thus, the current was potentiated by 3 μM of **45** or **64**, respectively, in the absence and in the presence of the benzodiazepine antagonist Ro15-1788 (1 μM). Relative potentiation amounted to 81 ± 5% (**45**) and 85 ± 12% (**64**), respectively ($n = 3$) in the presence of the benzodiazepine antagonist as compared to 100% in its absence. This minor effect indicates that both PAMs do not act interact with the benzodiazepine site (Fig. 6).

2.2.4. Interaction with other targets

Recently several other targets have been reported to be addressed by the *Magnolia* constituents magnolol, honokiol and 4′-*O*-methylhonokiol. A study investigated the effects of 4′-*O*-methylhonokiol (1 mg/kg) on lipopolysaccharide-induced neuroinflammation, in which it was found to reduce the expression of iNOS and COX2 and the formation of β-amyloid plaques in mouse CNS. In cultured astrocytes it was shown that these effects may be mediated via inhibitory effects on NF-κB.[29] In a further study methylhonokiol was shown to be an inhibitor of cyclooxygenases (COX) 1 and 2 with an IC_{50} of 2.4 μM for COX1 and 0.062 μM for COX2.[30] 4′-*O*-Methylhonokiol (1.5 mg/kg) as well as an ethanolic extract of *Magnolia officinalis* (5 mg/kg) were shown to reduce memory impairment effects of scopolamine via acetylcholine esterase inhibition.[31] Moreover, the biphenylic natural products were found to interact with cannabinoid (CB) receptors. Magnolol acts as partial agonist at both CB receptor subtypes (EC_{50}: CB_1 18.3 μM, CB_2 3.28 μM) whereas honokiol is a weak agonist at CB_1, and an antagonist at CB_2 receptors.[18] 4′-*O*-Methylhonokiol was found to show high affinity (0.043 μM) for CB_2 receptors where it behaved as a partial agonist.[14] Some synthetic analogs of magnolol were obtained by structural optimization of compounds which showed high affinity for CB receptors, for example, 5-hexyl-2′-methoxy-5′-propylbiphenyl-2-ol (K_i: CB_1 0.00957 μM, CB_2 0.0238 μM);[14] for affinity of

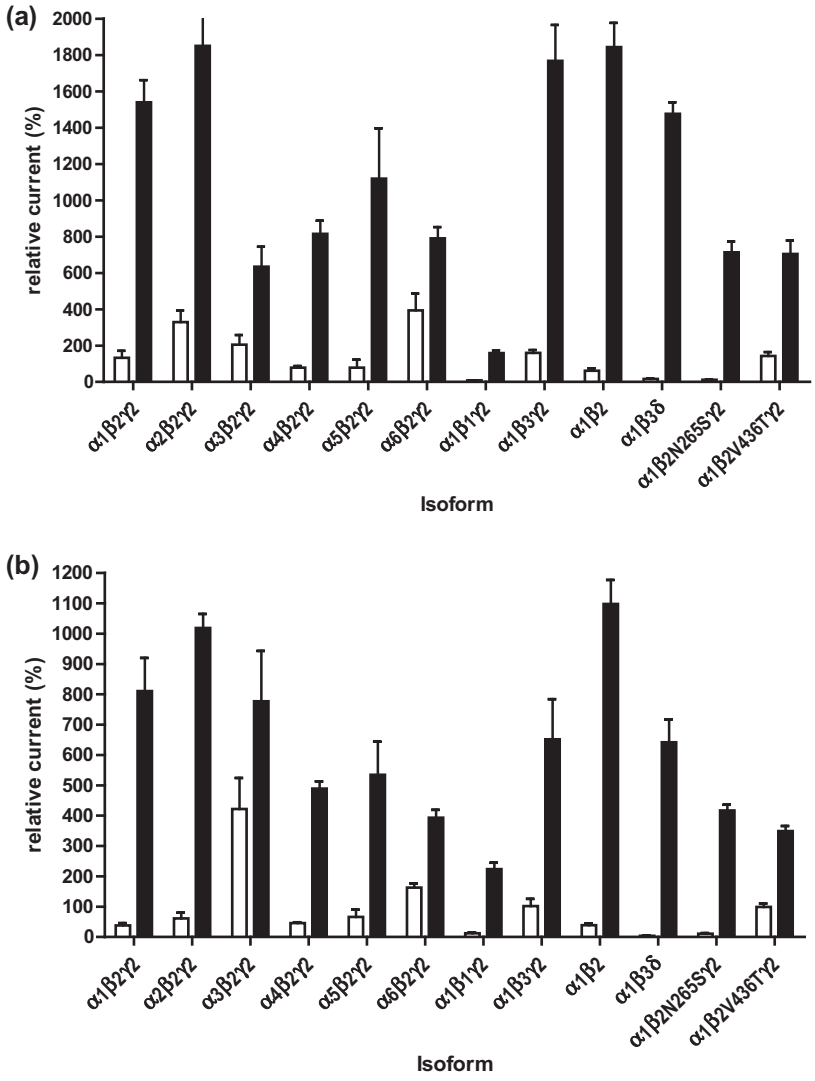


Figure 5. Subunit specificity of **64** (a) and **45** (b), and effects of specific point mutations on the positive modulatory activity of the compounds. For each subunit combination the agonistic effect (open bars; current elicited by 3 μM test compound divided by that elicited by $EC_{0.5}$ GABA = effect at which GABA shows 0.5% of its maximal effect) and the allosteric potentiation (closed bars; current elicited by 3 μM test compound in combination with $EC_{0.5}$ GABA divided by that elicited by the same concentration of GABA alone) were determined.

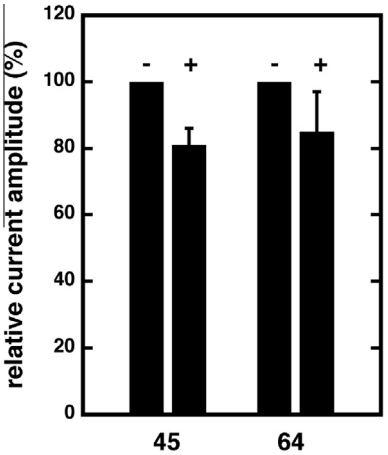


Figure 6. Effect of the benzodiazepine antagonist Ro15-1788. GABA at a concentration of 0.3 μM was applied together with 3 μM of compound **45** (left) or **64** (right) in the absence (−) and presence (+) of 1 μM Ro15-1788. Averaged data ± SD (n = 3).

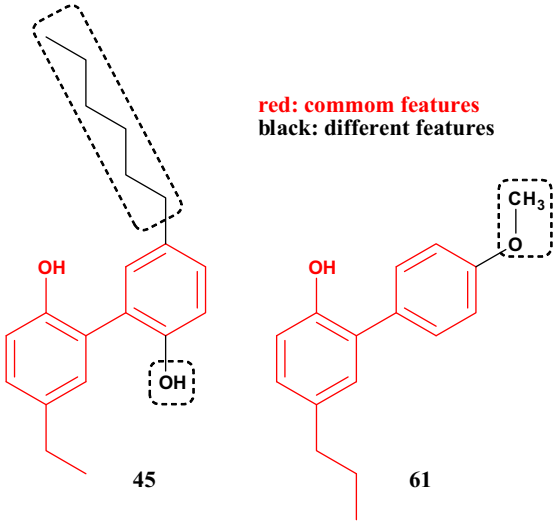


Figure 7. Comparison of structural features of magnolol and 4′-*O*-methylhonokiol analogs. Current potentiation at 10 μM: **45**, 1000%; **61**, 5000%.

compounds at human CB_1 and CB_2 see Table S1, Supplementary data. Another analog, 5′-hexyl-2′-methoxy-5-propylbiphenyl-2-ol, was developed to inhibit the lysophosphatidylinositol-activated receptor GPR55 (IC_{50} 3.25 μM).[14]

A dual activation of CB_1 and $GABA_A$ receptors could be therapeutically desirable, for example, for the treatment of convulsions, spasms, and associated pain. In this respect potent $GABA_A$ receptor PAMs that showed ancillary CB_1-agonistic activity, such as **45** (K_i CB_1: 0.386 μM) and **64** (K_i CB_1: 0.339 μM), may be suitable candidates for further evaluation, for example, in in vivo experiments.

It also appears warranted to evaluate the $GABA_A$-selective compound **61** (K_i CB_1: >10 μM), which is at the same time the most powerful allosteric modulator (up to 5000% current potentiation), in extended in vitro and in vivo studies. Its potency at CB receptors is significantly lower (>12-fold) as compared to the parent compound 4′-*O*-methylhonokiol (see Supplemenatary data).

3. Conclusions and outlook

The natural products magnolol and 4′-*O*-methylhonokiol were used as lead structures to study the SARs of this new class of extremely powerful $GABA_A$ receptor potentiators or PAMs. The target compounds were accessible by straightforward procedures. Minor modifications were found to have large effects on the compounds' biological activity. Except for magnolol analog **45** (Fig. 7), the most potent compounds, **61**, **62**, and **64**, were derived from the 4′-*O*-methylhonokiol scaffold. Whereas 4′-*O*-methylhonokiol has two allyl residues, one at each of the phenyl rings, we found that only one alkyl residue was sufficient for the compounds' $GABA_A$ potentiating effects. While methyl and ethyl residues were found to be too small, the optimal alkyl residue was found to be propyl, as in compound **61** (4′-methoxy-5-propylbiphenyl-2-ol). The two most powerful compounds from the two different scaffolds share several features, which appear to be a required for high $GABA_A$ potentiating activity. There are the directly connected phenyl rings and a free phenolic group *para* to a C2- or C3-alkyl residue (ethyl in **45**, propyl in **61**, see Table 1). The substitution pattern of the second ring, which differentiates magnolol from 4′-*O*-methylhonokiol analogs, was found to be responsible for high $GABA_A$ potentiating activity and selectivity over CB receptors. A single methoxy group in the *para*-position was found to be superior to an *ortho*-hydroxyl group in combination with an alkyl residue (see Fig. 7). Methylation of one of the hydroxyl groups in magnolol analogs resulted in a loss of activity, no matter whether the methoxy group was in the *para*-position to the short or the long alkyl residue (compare compounds **52**–**58**). Due to the different SARs it appears likely that both, magnolol and honokiol analogs exhibit—at least somewhat—different binding modes.

In case of the more efficacious 4′-*O*-methylhonokiol analogs (**61**, **62**, **64**) it could be interesting to evaluate the effects of further substituents on the methoxy-substituted phenyl ring in the future with the goal to potentially increase the PAM effect.

Alexeev et al., had reported on a current potentiation by honokiol of 351.2 ± 79.0% at 30–60 μM (hippocampal dentate granule neurons) which was in a similar range as in our study (192 ± 63% at 3 μM, *Xenopus* oocytes), considering that cell types and applied concentrations were different.[13,24]

The most potent compound of the present series, 4′-methoxy-5-propylbiphenyl-2-ol (**61**) potentiated the current amplitude of $GABA_A$ receptors ($\alpha_1\beta_2\gamma_2$ isoform) expressed in Xenopus oocytes by up to 5000% at 10 μM concentration. The compounds also acted as weak direct agonists at the $GABA_A$ receptor with a current potentiation at a concentration of 10 μM of up to 6% of the maximal effect of GABA; they can therefore be characterized as ago-allosteric modulators, although their direct effect was only moderate, in contrast to the extremely powerful indirect GABA-activating effect.

Investigation of the most potent compounds of the present series, **45**, **61**, **62**, **64**, at $GABA_A$ receptors of different subunit composition and with specific point mutations, indicated that they interacted with a different binding site than the benzodiazepines and the previously characterized allosteric PAM 2-arachidonoylglycerol. The interaction of the biphenylic compounds with CB receptors could be significantly reduced compared to the precursor 4′-*O*-methylhonokiol (K_i CB_1: 8.34 μM, CB_2: 0.0433 μM; see Supporting information).[14] This class of powerful PAMs of $GABA_A$ receptors has the potential for further development as anxiolytic, hypnotic, and/or muscle relaxant drugs.

4. Experimental section

4.1. Chemistry

4.1.1. Material and methods

All commercially available reagents and solvents were used without further purification. The reactions were monitored by thin layer chromatography (TLC) using aluminum sheets coated with silica gel 60 F_{254} (Merck). Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Preparative HPLC was performed on a C18 column (250 × 20 mm, particle size 10 μm, Eurospher 100) using a mixture of methanol and H_2O as eluent at a flow rate of 20 mL/min. Microwave reactions were performed in a CEM Discover microwave reactor. ^1H NMR and ^{13}C NMR data were recorded on a Bruker Avance spectrometer at 500 MHz for proton and 125 MHz for carbon. Shifts are given in ppm relative to the remaining protons of the deuterated solvents. Mass spectra were recorded on an API 2000 mass spectrometer (electron spray ion source, Applied Biosystems, Darmstadt, Germany) coupled with an Agilent 1100 HPLC system using a Phenomenex Luna HPLC C18 column (50 × 2.00 mm, particle size 3 μm). The purity of the tested compounds was determined by HPLC-UV obtained on an LC-MS instrument (Applied Biosystems API 2000 LC–MS/MS, HPLC Agilent 1100) using the procedure as follows: dissolving of the compounds at a concentration of 1.0 mg/mL in methanol and if necessary sonication to complete dissolution. Then, 10 μL of the solution was injected into a Phenomenex Luna C18 HPLC column (50 × 2.00 mm, particle size 3 μm) and elution was performed for 30 min at a flow rate of 250 μL/min with a gradient of water/methanol either containing 2 mM ammonium acetate from 90:10 up to 0:100, starting the gradient after 10 min (system A), or containing 2 mM ammonium acetate and 0.1% formic acid from 90:10 up to 0:100, starting the gradient after 10 min (system B), or containing 2 mM ammonium acetate from 60:40 up to 0:100 for 30 min, starting the gradient after 0 min and ending after 20 min (system C). UV absorption was detected from 220 to 400 nm using a diode array detector.

All compounds were synthesized according to or in analogy to previously prescribed procedures.[14] Compounds **37**–**51**, **55**, **57** and **58** were prescribed previously.[14] Compounds **52**–**54**, **56**, **59**–**69** represent new compounds.

Analytical data for new products are described below. For detailed synthetic procedures, analytical data of compounds **15**–**32**, **37**–**51**, **55**, **57** and **58** as well as affinity of all compounds for human $CB_{1/2}$ receptors see Supplementary data.

4.1.2. Analytical data

4.1.2.1. 5-Ethyl-2′-methoxy-5′-pentylbiphenyl-2-ol, $C_{20}H_{26}O_2$, M_r: 298.42, (52). ^1H NMR (500 MHz, $CDCl_3$) δ 7.19 (dd, J = 8.3, 2.3 Hz, H_{ar}, 1H), 7.16 (d, J = 2.2 Hz, H_{ar}, 1H), 7.13 (dd, J = 8.2, 2.3 Hz, H_{ar}, 1H), 7.09 (d, J = 2.2 Hz, H_{ar}, 1H), 6.96 (d, J = 8.2 Hz, H_{ar}, 2H), 6.25 (s, OH, 1H), 3.88 (s, O-CH_3, 3H), 2.68–2.59 (m, ar-CH_2, 4H), 1.66–1.59 (m, CH_2, 2H), 1.38–1.31 (m, CH_2, 4H),

1.26 (t, J = 7.6 Hz, CH_3, 3H), 0.90 (t, J = 7.0 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.46 (C_{ar}-OCH_3), 151.68 (C_{ar}-O), 136.71 (C_{ar}), 136.60 (C_{ar}), 132.42 (C_{ar}), 130.49 (C_{ar}), 128.86 (C_{ar}), 128.52 (C_{ar}), 127.09 (C_{ar}), 126.24 (C_{ar}), 117.37 (C_{ar}), 111.45 (C_{ar}), 56.31 (O-CH_3), 35.05 (CH_2), 31.48 (CH_2), 31.34 (CH_2), 28.04 (CH_2), 22.52 (CH_2), 15.78 (CH_3), 14.03 (CH_3). LC/ESI-MS (positive mode) m/z 299 $(M+H)^+$ (negative mode) m/z 297 $(M-H)^-$ 96.3%, colorless liquid.

4.1.2.2. 5-Ethyl-5′-hexyl-2′-methoxybiphenyl-2-ol, $C_{21}H_{28}O_2$, M_r: 312.45, (53). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (dd, J = 8.3, 2.3 Hz, CH_{ar}, 1H), 7.16 (d, J = 2.2 Hz, CH_{ar}, 1H), 7.13 (dd, J = 8.2, 2.3 Hz, CH_{ar}, 1H), 7.09 (d, J = 2.2 Hz, CH_{ar}, 1H), 6.96 (d, J = 8.3 Hz, CH_{ar}, 2H), 6.25 (s, OH, 1H), 3.88 (s, CH_3, 3H), 2.65 (q, J = 7.5 Hz, ar-CH_2, 2H), 2.61 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.62 (dt, J = 15.4, 7.5 Hz, CH_2, 2H), 1.37–1.28 (m, CH_2 6H), 1.26 (t, J = 7.6 Hz, CH_3, 3H), 0.89 (t, J = 7.0 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.46 (C_{ar}-OCH_3), 151.68 (C_{ar}-O), 136.71 (C_{ar}), 136.60 (C_{ar}), 132.42 (C_{ar}), 130.49 (C_{ar}), 128.86 (C_{ar}), 128.52 (C_{ar}), 127.08 (C_{ar}), 126.24 (C_{ar}), 117.37 (C_{ar}), 111.45 (C_{ar}), 56.31 (O-CH_3), 35.08 (CH_2), 31.70 (CH_2), 31.63 (CH_2), 28.97 (CH_2), 28.04 (CH_2), 22.61 (CH_2), 15.78 (CH_3), 14.08 (CH_3). LC/ESI-MS (positive mode) m/z 313 $(M+H)^+$ (negative mode) m/z 311 $(M-H)^-$ 97.3%, colorless liquid.

4.1.2.3. 2′-Methoxy-5′-pentyl-5-propylbiphenyl-2-ol, $C_{21}H_{28}O_2$, M_r: 312.45, (54). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (dd, J = 8.3, 2.3 Hz, CH_{ar}, 1H), 7.16 (d, J = 2.2 Hz, CH_{ar}, 1H), 7.11 (dd, J = 8.2, 2.2 Hz, CH_{ar}, 1H), 7.08 (d, J = 2.2 Hz, CH_{ar}, 1H), 6.97 (d, J = 3.9 Hz, CH_{ar}, 1H), 6.95 (d, J = 3.7 Hz, CH_{ar}, 1H), 6.28 (s, OH, 1H), 3.88 (s, CH_3, 3H), 2.64–2.56 (m, 1H), 1.70–1.62 (m, 1H), 1.37–1.32 (m, 1H), 0.97 (t, J = 7.3 Hz, CH_3, 3H), 0.91 (t, J = 7.0 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.46 (C_{ar}-OCH_3), 151.69 (C_{ar}-O), 136.69 (C_{ar}), 135.07 (C_{ar}), 132.41 (C_{ar}), 131.09 (C_{ar}), 129.09 (C_{ar}), 128.83 (C_{ar}), 127.11 (C_{ar}), 126.16 (C_{ar}), 117.28 (C_{ar}), 111.48 (C_{ar}), 56.30 (O-CH_3), 37.28 (CH_2), 35.04 (CH_2), 31.48 (CH_2), 31.34 (CH_2), 24.75 (CH_2), 22.52 (CH_2), 14.02 (CH_3), 13.89 (CH_3). LC/ESI-MS (positive mode) m/z 313 $(M+H)^+$ (negative mode) m/z 311 $(M-H)^-$ 98.3%, colorless liquid.

4.1.2.4. 5′-Ethyl-2′-methoxy-5-pentylbiphenyl-2-ol, $C_{20}H_{26}O_2$, M_r: 298.42, (56). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24–7.20 (m, H_{ar}, 1H), 7.19 (d, J = 2.3 Hz, H_{ar}, 1H), 7.12 (dd, J = 8.2, 2.1 Hz, H_{ar}, 1H), 7.09 (d, J = 2.2 Hz, H_{ar}, 1H), 6.96 (dd, J = 14.1, 5.7 Hz, H_{ar}, 2H), 6.27 (s, OH, 1H), 3.89 (s, O-CH_3, 3H), 2.68 (q, J = 7.6 Hz, ar-CH_2, 2H), 2.60 (t, J = 7,5 Hz, ar-CH_2, 2H), 1.68–1.61 (m, CH_2, 2H), 1.39–1.34 (m, CH_2-CH_2, 4H), 1.27 (t, J = 7.6 Hz, CH_3, 3H), 0.92 (t, J = 6.9 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 153.49 (C_{ar}-OCH_3), 151.65 (C_{ar}-O), 137.98 (C_{ar}), 135.32 (C_{ar}), 131.92 (C_{ar}), 131.01 (C_{ar}), 129.05 (C_{ar}), 128.29 (C_{ar}), 127.19 (C_{ar}), 126.16 (C_{ar}), 117.29 (C_{ar}), 111.58 (C_{ar}), 56.32 (O-CH_3), 35.12 (CH_2), 31.56 (CH_2), 31.38 (CH_2), 28.01 (CH_2), 22.55 (CH_2), 15.79 (CH_3), 14.04 (CH_3). LC/ESI-MS (positive mode) m/z 299 $(M+H)^+$ (negative mode) m/z 297 $(M-H)^-$ 97.8%. mp 41 °C, off-white powder.

4.1.2.5. 4′-Methoxy-5-methylbiphenyl-2-ol, $C_{14}H_{14}O_2$, M_r: 214.26, (59). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.41–7.37 (m, H_{ar}, 2H), 7.07–6.99 (m, H_{ar}, 4H), 6.87 (d, J = 8.0 Hz, H_{ar}, 1H), 5.02 (s, OH, 1H), 3.86 (s, O-CH_3, 3H), 2.31 (s, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.22 (Car-OCH_3), 150.23 (Car-OH), 130.65 (C_{ar}), 130.19 (C_{ar}, 2C), 129.87 (C_{ar}), 129.35 (C_{ar}), 129.20 (C_{ar}), 127.49 (C_{ar}), 115.43 (C_{ar}, 2C), 114.62 (C_{ar}), 55.33 (O-CH_3), 20.46 (CH_3). LC/ESI-MS (positive mode) m/z 299 $(M+H)^+$ 98.9%, colorless liquid.

4.1.2.6. 5-Ethyl-4′-methoxybiphenyl-2-ol, $C_{15}H_{16}O_2$, M_r: 228.29, (60). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.38 (m, CH_{ar}, 2H), 7.09–7.00 (m, CH_{ar}, 2H), 6.90 (d, J = 8.1 Hz, CH_{ar}, 2H), 5.04 (s, OH 1H), 3.86 (s, CH_3, 3H), 2.62 (q, J = 7.6 Hz, ar-CH_2, 2H), 1.24 (t, J = 7.6 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 159.23 (C_{ar}-OCH_3), 150.42 (C_{ar}-O), 136.45 (C_{ar}), 130.23 (C_{ar}, 2C), 129.50 (C_{ar}, 2C), 128.04 (C_{ar}), 127.52 (C_{ar}), 115.47 (C_{ar}), 114.63 (C_{ar}, 2C), 55.34 (O-CH_3), 27.99 (CH_2), 15.82 (CH_3). LC/ESI-MS (positive mode) m/z 229 $(M+H)^+$ (negative mode) m/z 227 $(M-H)^-$ 98.1%. mp 52 °C, off-white powder.

4.1.2.7. 4′-Methoxy-5-propylbiphenyl-2-ol, $C_{16}H_{18}O_2$, M_r: 242.31, (61). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.39 (m, CH_{ar}, 1H), 7.06–7.00 (m, CH_{ar}, 1H), 6.90–6.88 (m, CH_{ar}, 1H), 5.04 (s, OH, 1H), 3.86 (s, CH_3, 3H), 2.55 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.68–1.60 (m, CH_2, 2H), 0.96 (t, J = 7.3 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 159.22 (C_{ar}-OCH_3), 150.42 (C_{ar}-O), 134.92 (C_{ar}), 130.23 (C_{ar}, 2C), 130.10 (C_{ar}), 129.50 (C_{ar}), 128.64 (C_{ar}), 127.44 (C_{ar}), 115.39 (C_{ar}), 114.63 (C_{ar}, 2C), 55.34 (O-CH_3), 37.19 (CH_2), 24.76 (CH_2), 13.82 (CH_3). LC/ESI-MS (positive mode) m/z 243 $(M+H)^+$ (negative mode) m/z 241 $(M-H)^-$ 96.7%. mp 63 °C, off-white powder.

4.1.2.8. 5-Butyl-4′-methoxybiphenyl-2-ol, $C_{17}H_{20}O_2$, M_r: 256.34, (62). ^{1}H NMR (500 MHz, Chloroform-d) OH signal is missing δ 7.41–7.38 (m, H_{ar}, 2H), 7.06–7.00 (m, H_{ar}, 4H), 6.88 (d, J = 8.0 Hz, H_{ar}, 1H), 3.86 (s, O-CH_3, 3H), 2.57 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.63–1.55 (m, CH_2, 2H), 1.41–1.32 (m, CH_2, 2H), 0.93 (t, J = 7.3 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.19 (Car-OCH_3), 150.39 (Car-OH), 135.09 (C_{ar}), 130.22 (C_{ar}, 2C), 130.04 (C_{ar}), 129.53 (C_{ar}), 128.55 (C_{ar}), 127.44 (C_{ar}), 115.39 (C_{ar}, 2C), 114.59 (C_{ar}), 55.34 (O-CH_3), 34.76 (ar-CH_2), 33.88 (CH_2), 22.33 (CH_2), 13.95 (CH_3). LC/ESI-MS (positive mode) m/z 257 $(M+H)^+$ 97.9%, colorless liquid.

4.1.2.9. 4′-Methoxy-5-pentylbiphenyl-2-ol, $C_{18}H_{22}O_2$, M_r: 270.37, (63). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.42–7.38 (m, 2H), 7.06–7.00 (m, 4H), 6.88 (d, J = 8.1 Hz, 1H), 3.86 (s, 3H), 2.56 (t, J = 7.8 Hz, 2H), 1.63–1.58 (m, 2H), 1.36–1.31 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.19 (Car-OCH_3), 150.37 (Car-OH), 135.14 (C_{ar}), 130.22 (C_{ar}, 2C), 130.01 (C_{ar}), 129.51 (C_{ar}), 128.55 (C_{ar}), 127.43 (C_{ar}), 115.38 (C_{ar}, 2C), 114.60 (C_{ar}), 55.33 (O-CH_3), 35.05 (ar-CH_2), 31.49 (CH_2), 31.40 (CH_2), 22.52 (CH_2), 14.02 (CH_3). LC/ESI-MS (positive mode) m/z 271 $(M+H)^+$ 97.8%, colorless liquid.

4.1.2.10. 5-Hexyl-4′-methoxybiphenyl-2-ol, $C_{19}H_{24}O_2$, M_r: 284.39, (64). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42–7.38 (m, CH_{ar}, 2H), 7.06–7.00 (m, CH_{ar}, 2H), 6.88 (d, J = 8.1 Hz, CH_{ar}, 2H), 5.02 (s, OH 1H), 3.86 (s, CH_3, 3H), 2.56 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.65–1.57 (m, CH_2, 2H), 1.37–1.26 (m, CH_2-CH_2-CH_2, 6H), 0.89 (t, J = 6.9 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, CDCl3) δ 159.21 (C_{ar}-OCH_3), 150.36 (C_{ar}-O), 135.17 (C_{ar}), 130.22 (C_{ar}), 130.01 (C_{ar}), 129.48 (C_{ar}), 128.56 (C_{ar}), 127.43 (C_{ar}), 115.38 (C_{ar}), 114.62 (C_{ar}), 55.33 (O-CH_3), 35.09 (CH_2), 31.71 (CH_2), 31.68 (CH_2), 28.97 (CH_2), 22.58 (CH_2), 14.06 (CH_3). LC/ESI-MS (positive mode) m/z 285 $(M+H)^+$ (negative mode) m/z 283 $(M-H)^-$ 97.7%. mp 61 °C, off-white powder.

4.1.2.11. 5-Heptyl-4′-methoxybiphenyl-2-ol, $C_{20}H_{26}O_2$, M_r: 298.42, (65). ^{1}H NMR (600 MHz, Chloroform-d) δ 7.41–7.38 (m, 2H), 7.06–7.00 (m, 4H), 6.88 (d, J = 8.1 Hz, H_{ar}, 1H), 5.02 (s, OH), 3.86 (s, O-CH_3, 3H), 2.56 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.63–1.58 (m, 2H), 1.36–1.24 (m, 8H), 0.88 (t, J = 6.9 Hz, CH_3, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.21 (Car-OCH_3), 150.35 (Car-OH), 135.18 (C_{ar}), 130.23 (C_{ar}, 2C), 130.02 (C_{ar}), 129.47 (C_{ar}), 128.57 (C_{ar}), 127.42 (C_{ar}), 115.38 (C_{ar}), 114.62 (C_{ar}, 2C), 55.35 (O-CH_3), 35.11 (CH_2), 31.82 (CH_2), 31.76 (CH_2), 29.28 (CH_2), 29.18 (CH_2), 22.66

(CH_2), 14.10 (CH_3). LC/ESI-MS (positive mode) m/z 299 $(M+H)^+$ 97.7%, colorless liquid.

4.1.2.12. 4′-Methoxy-5-octylbiphenyl-2-ol, $C_{21}H_{28}O_2$, M_r: 312.45, (66). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.44–7.36 (m, H_{ar}, 2H), 7.08–6.99 (m, H_{ar}, 4H), 6.88 (d, J = 8.1 Hz, H_{ar}, 1H), 5.05 (s, OH, 1H), 3.86 (s, O-CH_3, 3H), 2.56 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.60 (m, CH_2, 2H), 1.33–1.25 (m, CH_2, 10H), 0.88 (t, J = 7.0 Hz, CH_3, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.20 (Car-OCH_3), 150.36 (Car-OH), 135.18 (C_{ar}), 130.23 (C_{ar}, 2C), 130.02 (C_{ar}), 129.49 (C_{ar}), 128.57 (C_{ar}), 127.43 (C_{ar}), 115.38 (C_{ar}), 114.61 (C_{ar}, 2C), 55.35 (O-CH_3), 35.11 (CH_2), 31.88 (CH_2), 31.75 (CH_2), 29.48 (CH_2), 29.32 (CH_2), 29.26 (CH_2), 22.66 (CH_2), 14.10 (CH_3). LC/ESI-MS (positive mode) m/z 313 $(M+H)^+$ 97.1%, colorless liquid.

4.1.2.13. 4′-Ethoxy-5-hexylbiphenyl-2-ol, $C_{20}H_{26}O_2$, M_r: 298.42, (67). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.40–7.36 (m, H_{ar}, 2H), 7.07–6.98 (m, H_{ar}, 4H), 6.88 (d, J = 8.1 Hz, H_{ar}, 1H), 5.04 (s, OH, 1H), 4.09 (q, J = 7.0 Hz, O-CH_2, 2H), 2.56 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.65–1.57 (m, CH_2, 2H), 1.45 (t, J = 7.0 Hz, CH_3, 3H), 1.37–1.28 (m, CH_2, 6H), 0.89 (t, J = 6.8 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.59 (Car-OCH_2), 150.36 (Car-OH), 135.14 (C_{ar}), 130.19 (C_{ar}, 2C), 129.99 (C_{ar}), 129.29 (C_{ar}), 128.53 (C_{ar}), 127.48 (C_{ar}), 115.35 (C_{ar}), 115.17 (C_{ar}, 2C), 63.53 (O-CH_2), 35.10 (ar-CH_2), 31.72 (CH_2), 31.70 (CH_2), 28.98 (CH_2), 22.59 (CH_2), 14.82 (CH_3), 14.08 (CH_3). LC/ESI-MS (positive mode) m/z 299 $(M+H)^+$ 97.7%, colorless liquid.

4.1.2.14. 5-Hexyl-4′-propoxybiphenyl-2-ol, $C_{21}H_{28}O_2$, M_r: 312.45, (68). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.40–7.36 (m, H_{ar}, 2H), 7.05–6.99 (m, H_{ar}, 4H), 6.88 (d, J = 8.1 Hz, H_{ar}, 1H), 5.03 (s, OH, 1H), 3.97 (t, J = 6.6 Hz, O-CH_2, 2H), 2.56 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.90–1.79 (m, CH_2, 2H), 1.63–1.57 (m, CH_2, 2H), 1.37–1.27 (m, CH_2, 6H), 1.06 (t, J = 7.4 Hz, CH_3, 3H), 0.88 (t, J = 7.0 Hz, CH_3, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.81 (Car-OCH_2), 150.37 (Car-OH), 135.14 (C_{ar}), 130.17 (C_{ar}, 2C), 129.99 (C_{ar}), 129.22 (C_{ar}), 128.52 (C_{ar}), 127.50 (C_{ar}), 115.34 (C_{ar}), 115.21 (C_{ar}, 2C), 69.61 (O-CH_2), 35.10 (ar-CH_2), 31.72 (CH_2), 31.70 (CH_2), 28.98 (CH_2), 22.60 (CH_2), 22.57 (CH_2), 14.08 (CH_2), 10.51 (CH_3). LC/ESI-MS (positive mode) m/z 313 $(M+H)^+$ 98.5%, colorless liquid.

4.1.2.15. 5-Hexyl-4′-isopropoxybiphenyl-2-ol, $C_{21}H_{28}O_2$, M_r: 312.45, (69). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.39–7.35 (m, H_{ar}, 2H), 7.05–6.98 (m, H_{ar}, 4H), 6.88 (d, J = 8.0 Hz, H_{ar}, 1H), 5.05 (s, OH, 1H), 4.60 (hept, J = 6.1 Hz, CH, 1H), 2.56 (t, J = 7.8 Hz, ar-CH_2, 2H), 1.63–1.57 (m, CH_2, 2H), 1.38 (d, J = 6.1 Hz, CH_3, 6H), 1.36–1.28 (m, 6H), 0.88 (t, J = 7.2 Hz, CH_3, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 157.57 (Car-OCH), 150.37 (Car-OH), 135.14 (C_{ar}), 130.22 (C_{ar}, 2C), 130.00 (C_{ar}), 129.14 (C_{ar}), 128.52 (C_{ar}), 127.49 (C_{ar}), 116.41 (C_{ar}, 2C), 115.34 (C_{ar}), 69.94 (O-CH), 35.12 (ar-CH_2), 31.73 (CH_2), 31.72 (CH_2), 29.00 (CH_2), 22.61 (CH), 22.06 (2CH_3), 14.10 (CH_3). LC/ESI-MS (positive mode) m/z 313 $(M+H)^+$ 99.2%, colorless liquid.

4.2. Biological experiments

4.2.1. Expression of $GABA_A$ receptors in Xenopus oocytes

Capped cRNAs were synthesized (Ambion, Austin, TX, USA) from the linearized plasmids with a cytomegalovirus promotor (pCMVvectors) containing the different subunits, respectively. A poly-A tail of about 400 residues was added to each transcript using yeast poly-A polymerase (United States Biologicals, Cleveland, OH, USA). The concentration of the cRNA was quantified on a formaldehyde gel using Radiant Red stain (Bio-Rad) for visualization of the RNA. Known concentrations of RNA ladder (Invitrogen) were loaded as standard on the same gel. cRNAs were precipitated in ethanol/isoamylalcohol 19:1, the dried pellet dissolved in water and stored at −80 °C. cRNA mixtures were prepared from these stock solutions and stored at −80 °C. *Xenopus laevis* oocytes were prepared, injected and defolliculated as described previously.[32,33] They were injected with 50 nL of the cRNA solution containing, in the case of αβ receptors α_1 and β_2 subunits at a concentration of 75 nM:75 nM, in the case of αβγ receptors α_1 or α_2, α_3, α_5, α_6 and β_2 and γ_2 subunits at a concentration of 10 nM:10 nM:50 nM and in the case of αβδ receptors α_1, β_3 and δ subunits at a concentration of 10 nM:10 nM:50 nM.[34] Subsequently, the oocytes were incubated in modified Barth's solution at +18 °C for at least 24 h before the measurements for αβ and αβγ receptors and 48 h for αβδ receptors.

4.2.2. Functional characterization of the $GABA_A$ receptors

Currents were measured using a modified two-electrode voltage clamp amplifier Oocyte clamp OC-725 (Warner Instruments) in combination with a XY-recorder (90% response time 0.1 s) or digitized at 100 Hz using a PowerLab 2/20 (AD Instruments) using the computer programs Chart (ADInstruments GmbH, Spechbach, Germany). Tests with a model oocyte were performed to ensure linearity in the larger current range. The response was linear up to 15 μA.

Electrophysiological experiments were performed by using the two-electrode voltage clamp method at a holding potential of −80 mV. The perfusion medium contained 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM Na-HEPES (pH 7.4) and was applied by gravity flow 6 ml/min. The perfusion medium was applied through a glass capillary with an inner diameter of 1.35 mm, the mouth of which was placed about 0.4 mm from the surface of the oocyte.

Allosteric modulation was measured at a GABA concentration eliciting 0.5–1.0% of the maximal GABA current amplitude in the corresponding receptor. GABA was applied for 20 s alone or in combination with allosteric compound. Potentiation of GABA currents was expressed as $(I_{(modulator+GABA)}/I_{GABA} - 1) * 100\%$. The perfusion system was cleaned between drug applications by washing with DMSO to avoid contamination. In each case DMSO was washed out before placing the next oocyte.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.10.027.

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