Novel High-Affinity and Selective Biaromatic 4-Substituted γ-Hydroxybutyric Acid (GHB) Analogues as GHB Ligands: Design, Synthesis, and Binding Studies

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γ-Hydroxybutyrate (GHB) is a metabolite of γ-aminobutyric acid (GABA) and has been proposed to function as a neurotransmitter or neuromodulator. GHB is used in the treatment of narcolepsy and is a drug of abuse. GHB binds to both $GABA_B$ receptors and specific high-affinity GHB sites in brain, of which the latter have not been linked unequivocally to function, but are speculated to be GHB receptors. In this study, a series of biaromatic 4-substituted GHB analogues, including 4′-phenethylphenyl, 4′-styrylphenyl, and 4′-benzyloxyphenyl GHB analogues, were synthesized and characterized pharmacologically in a [^{3}H](*E*,*RS*)-(6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid ([^{3}H]NCS-382) binding assay and in $GABA_A$ and $GABA_B$ receptor binding assays. The compounds were selective for the high-affinity GHB binding sites and several displayed K_i values below 100 nM. The affinity of the 4-[4′-(2-iodobenzyloxy)phenyl] GHB analogue **17b** was shown to reside predominantly with the *R*-enantiomer (K_i = 22 nM), which has higher affinity than previously reported GHB ligands.

Introduction

γ-Hydroxybutyric acid (GHB[a]) is a metabolite of γ-aminobutyric acid (GABA) and is present in micromolar concentrations in mammalian brain. GHB is also a registered drug for the treatment of excessive daytime sleepiness and cataplexy associated with narcolepsy,[1,2] a drug of abuse (Fantasy), and a so-called date rape drug.[3] Because of the biological and pharmacological importance of GHB, as well as the public health concerns regarding the illicit use of GHB, a more detailed knowledge on the mechanisms of action is desirable.

GHB binds to both specific high-affinity GHB sites and to $GABA_B$ receptors, making the understanding of its neuropharmacology quite complex. The presence of [^{3}H]GHB high-affinity binding sites with distinct ontogenesis[4] and distribution[5,6] argues in favor of a specific GHB molecular site of action (sometimes referred to as the GHB receptor). The existence of a distinct GHB binding site is further supported by the generation of selective GHB ligands such as (*E*,*RS*)-(6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid[7] (NCS-382) (Figure 1), an alleged GHB receptor antagonist[7,8] displaying nanomolar affinity for high-affinity GHB binding sites but no affinity for $GABA_B$ receptors.[9,10] GHB is a low affinity, partial agonist at $GABA_B$ receptors[11,12] and may also be converted into GABA *in vivo*, resulting in both $GABA_A$- and $GABA_B$-mediated receptor effects and thus complicating the understanding of pharmacology further.[13]

Many of the reported pharmacological and clinical effects of exogenously administered GHB appear to be mediated through $GABA_B$ receptors.[14] This has been confirmed by the finding that prominent pharmacological effects of GHB on behavior, temperature regulation, locomotion, and dopamine synthesis otherwise produced in wild type mice, are completely absent in mice lacking functional $GABA_B$ receptors,[15−17] underlining $GABA_B$ receptors as important pharmacological mediators of GHB effects. Intriguingly, however, is the finding that both [^{3}H]GHB and [^{3}H]NCS-382 binding sites are preserved in the brains of $GABA_{B(1)}$ receptor knockout mice,[15,17] substantiating that GHB and $GABA_B$ binding sites are separate entities. So far, the molecular identity of the overlapping [^{3}H]GHB/[^{3}H]NCS-382 binding sites, which from now on are referred to as high-affinity or specific GHB binding sites, is not coherently accounted for and has not been systematically linked to function, although reports pointing to the existence of specific GHB effects have emerged.[18−20]

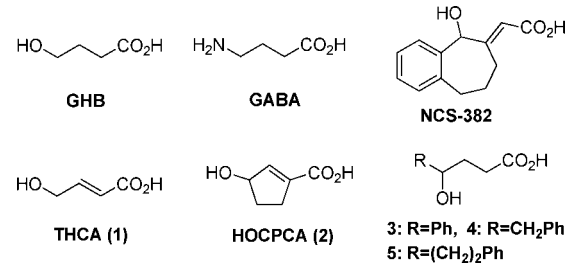

Figure 1. Structures of GHB and GABA, the reference compounds NCS-382 and **1**, the conformationally restricted GHB ligand **2**, and the 4-substituted GHB analogues **3**−**5**.

To distinguish the GHB specific effects from $GABA_B$ receptor-mediated ones, there is a need for developing pharmacological tools, i.e., high-affinity ligands with selectivity for the high-affinity GHB binding sites. Also, such high-affinity ligands could aid the cloning and hence molecular identification of the specific GHB binding sites.

With the aim of investigating the structural requirements of GHB binding sites, we have previously reported the pharmacological characterization of *trans*-4-hydroxycrotonic acid (THCA) (**1**) (Figure 1) and a series of conformationally restricted cyclic

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[a] Abbreviations: DCVC, dry column vacuum chromatography; DIAD, diisopropyl azodicarboxylate; FC, flash chromatography; GABA, γ-aminobutyric acid; GHB, γ-hydroxybutyric acid; HOCPCA, 3-hydroxycyclopent-1-enecarboxylic acid; NCS-382, (*E*,*RS*)-(6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid; SSA, succinic semialdehyde; THCA, *trans*-4-hydroxycrotonic acid.

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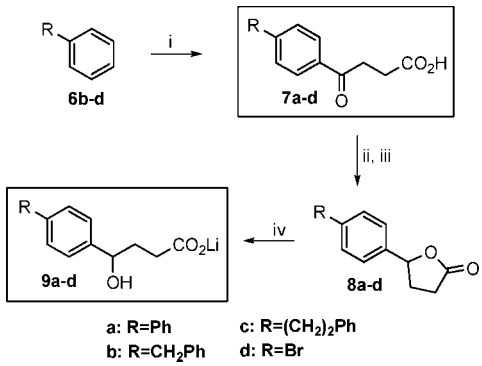
Scheme 1[a]



[a] Reagents: (i) succinic anhydride, $AlCl_3$; (ii) $NaBH_4$, EtOH; (iii) TFA, CH_2Cl_2; (iv) LiOH, H_2O, THF.

GHB/THCA analogues including the GHB analogue 3-hydroxycyclopent-1-enecarboxylic acid (HOCPCA) (**2**) (Figure 1) that binds to GHB binding sites with high affinity.[21] Several 4-substituted analogues of GHB and **2** have previously been reported to bind to specific GHB sites in mammalian brain,[22−27] including the 4-phenyl GHB analogue **3** (Figure 1) and the homologues **4** and **5** (Figure 1), all displaying similar affinity for GHB binding sites as GHB itself.[26] To expand our library of GHB ligands to include aromatic compounds and also synthetically more feasible structures, we decided to explore the effect of various substituents in the 4-position of GHB. In addition, we also investigated the importance of the 4-hydroxy substituent of these GHB analogues.

Results

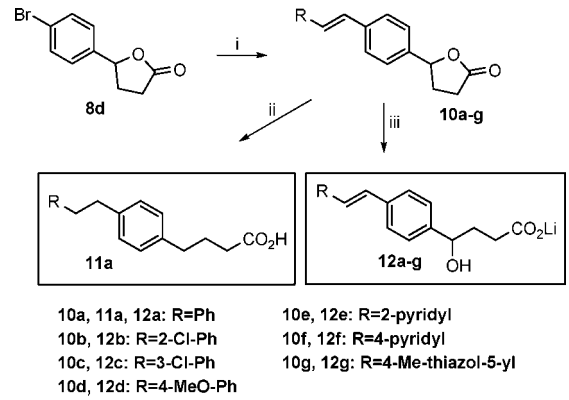
Synthesis. The synthesis of four GHB analogues substituted in the 4-position with 4′-biphenyl (**9a**), 4′-benzylphenyl (**9b**), 4′-phenethylphenyl (**9c**), or 4′-bromophenyl (**9d**) substituents, respectively, is outlined in Scheme 1. Friedel−Crafts acylation of the compounds **6b**−**d** led to the 4-oxobutanoic acids **7b**−**d**. Compounds **7a**−**d** were reduced to their corresponding 4-hydroxybutanoic acids. Acidic workup resulted in partial lactonization of the 4-hydroxybutanoic acids, and lactonization was completed by treatment with TFA. The resulting 4-butyrolactones **8a**−**d** were hydrolyzed with LiOH to give the target compounds **9a**−**d** as lithium salts.

The high affinity of the 4′-phenethylphenyl analogue **9c** for specific GHB binding sites, and the feasibility in the synthetic route prompted the synthesis of a series of unsaturated analogues **12a**−**g**, which is outlined in Scheme 2. Compounds **12a**−**g** were prepared by microwave-assisted Heck reactions of the aryl bromide **8d** with corresponding alkenes, followed by hydrolysis of the resulting lactones **10a**−**g**. Olefin hydrogenation and hydrogenolysis of **10a** led to **11a**.

A feasible route to phenolic ether analogues of **9c** is shown in Scheme 3. The key step in the synthesis of the target compounds **17a**−**l** was Mitsunobu reactions of the phenol **15** with various benzylic alcohol equivalents, leading to lactones **16a**−**l**, which upon hydrolysis provided compounds **17a**−**l**. The phenol **15** required for these Mitsunobu reactions was synthesized in three steps from compound **13**: demethylation of compound **13** using pyridine hydrochloride provided the 4-substituted 4-oxobutanoic acid analogue **14**, which upon reduction followed by lactonization gave compound **15**.

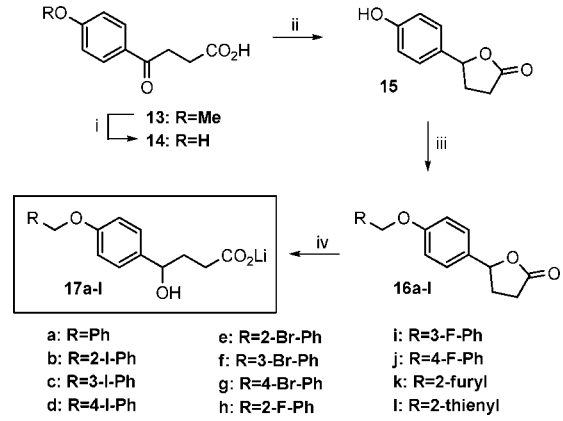
Chromatographic Resolution. Compound **16b** was resolved by chiral HPLC using a ChiralPak AS-H column into the enantiomers (+)**-16b** (99.7% ee) and (−)**-16b** (93.0% ee).

Scheme 2[a]



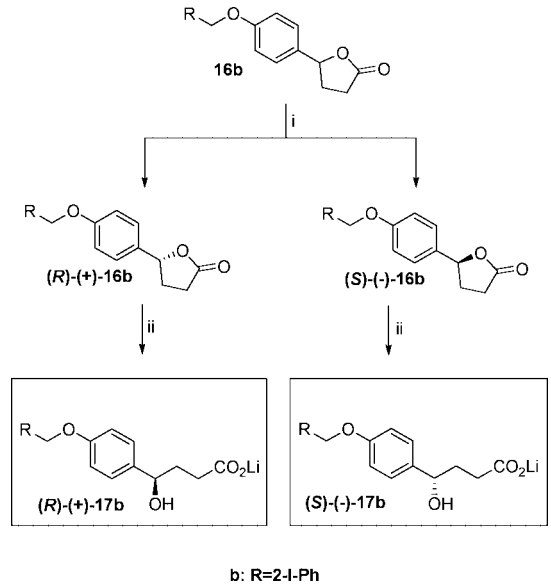
[a] Reagents: (i) appropriate alkenes, $Pd(OAc)_2$, K_3PO_4, THF; (ii) H_2, Pd−C, EtOH; (iii) LiOH, H_2O, THF.

Scheme 3[a]



[a] Reagents: (i) pyridine hydrochloride; (ii) $Ca(BH_4)_2$, MeOH; (iii) benzylic alcohol equivalents, Ph_3P, DIAD, THF; (iv) LiOH, H_2O, THF.

Scheme 4[a]



[a] Reagents: (i) chiral HPLC separation; (ii) LiOH, H_2O, THF.

Hydrolysis of (+)**-16b** and (−)**-16b** provided the enantiomers (+)**-17b** (99.6% ee) and (−)**-17b** (73.5% ee), respectively (Scheme 4).

X-ray Crystallographic Analysis. The absolute configuration of compound (+)**-16b** was established to be the (*R*)-configu-

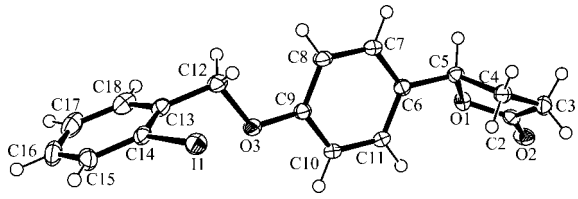
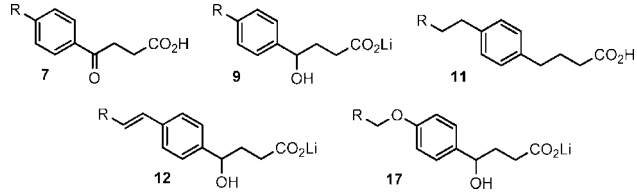


Figure 2. Perspective drawing (ORTEP-3[51]) of compound **(*R*)-(+)-16b**. Displacement ellipsoids of the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size.

Table 1. Inhibitory Affinities of GHB Ligands Measured as Inhibition of the Specific [^{3}H]NCS-382 Binding in Rat Brain Homogenate



compd	R	K_i (μM)[a]	(pK_i ± SEM)
GHB[b]		4.3	(5.4 ± 0.041)
NCS-382[b]		0.3	(6.5 ± 0.02)
THCA[b] (**1**)		1.1	(6.0 ± 0.02)
7a	Ph	20	(4.71 ± 0.05)
7b	CH_2Ph	15	(4.83 ± 0.04)
7c	$(CH_2)_2$Ph	8.8	(5.07 ± 0.08)
7d	Br	17	(4.61 ± 0.09)
9a	Ph	0.44	(6.36 ± 0.05)
9b	CH_2Ph	0.12	(6.94 ± 0.02)
9c	$(CH_2)_2$Ph	0.075	(7.13 ± 0.06)
9d	Br	1.1	(5.96 ± 0.06)
11a	Ph	1.2	(6.01 ± 0.1)
12a	Ph	0.16	(6.79 ± 0.04)
12b	2-Cl-Ph	0.054	(7.28 ± 0.08)
12c	3-Cl-Ph	0.079	(7.11 ± 0.05)
12d	4-MeO-Ph	0.18	(6.75 ± 0.02)
12e	2-pyridyl	0.23	(6.67 ± 0.09)
12f	4-pyridyl	0.34	(6.49 ± 0.09)
12g	4-Me-thiazol-5-yl	0.25	(6.62 ± 0.06)
17a	Ph	0.11	(6.96 ± 0.07)
17b	2-I-Ph	0.060	(7.22 ± 0.01)
(*R*)-(+)-17b	2-I-Ph	0.022	(7.68 ± 0.08)
(*S*)-(−)-17b	2-I-Ph	0.22	(6.66 ± 0.05)
17c	3-I-Ph	0.075	(7.12 ± 0.02)
17d	4-I-Ph	0.062	(7.21 ± 0.06)
17e	2-Br-Ph	0.053	(7.28 ± 0.06)
17f	3-Br-Ph	0.051	(7.32 ± 0.10)
17g	4-Br-Ph	0.034	(7.48 ± 0.07)
17h	2-F-Ph	0.041	(7.39 ± 0.04)
17i	3-F-Ph	0.063	(7.22 ± 0.10)
17j	4-F-Ph	0.093	(7.03 ± 0.01)
17k	2-furyl	0.20	(6.72 ± 0.10)
17l	2-thienyl	0.096	(7.03 ± 0.06)

[a] IC_{50} values were calculated from inhibition curves and converted to K_i values. Each value is mean (pK_i ± SEM) of at least three independent experiments each carried out in triplicate. [b] Data from ref 21.

ration at C5. Perspective drawing of the molecular structure of **(*R*)-(+)-16b** with atomic labeling is depicted in Figure 2.

Binding Affinity Studies. For measuring the affinity of the synthesized analogues for the GHB sites, we used the radioligand [^{3}H]NCS-382 to specifically label high-affinity GHB sites in rat brain membranes as previously described.[21] GHB and the known structural analogues NCS-382 and **1** were found to inhibit [^{3}H]NCS-382 binding in a concentration-dependent manner with order of K_i values, in agreement with previously reported studies[9,26] (Table 1). The majority of the synthesized GHB analogues inhibit [^{3}H]NCS-382 binding with affinities in the nanomolar range.

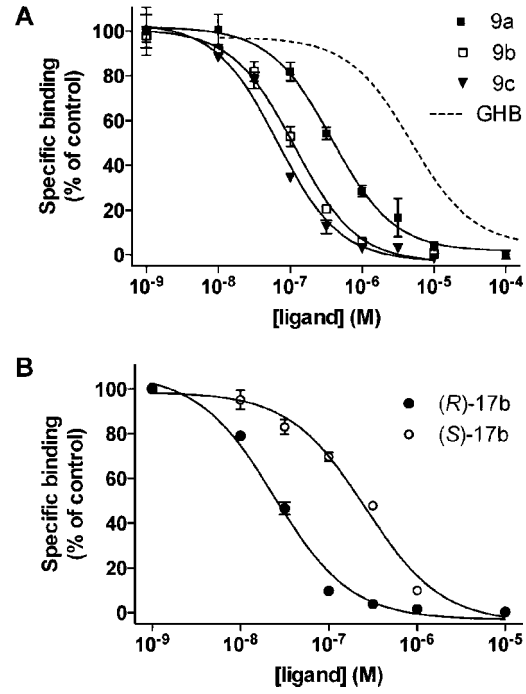


Figure 3. Concentration-dependent inhibition of [^{3}H]NCS-382 binding to rat cerebrocortical membranes by GHB and the novel GHB analogues **9a**−**c** (A) and the enantiomers **(*R*)-17b** and **(*S*)-17b** (B). The measurement of affinities was determined as described under Experimental Section. Results are expressed as mean ± SD of a single representative experiment performed in triplicate. Two additional experiments gave similar results (data summarized in Table 1).

Substitution of GHB at the 4-position with 4′-biphenyl to give compound **9a** resulted in a 10-fold increase in affinity compared to that of GHB itself. Separating the aromatic moieties of **9a** by replacing the 4′-biphenyl with 4′-benzylphenyl (**9b**) and 4′-phenethylphenyl (**9c**), respectively, led to a further 4- and 6-fold increase in affinity compared to that of **9a** as illustrated by the inhibition curves in Figure 3A. Compound **9c** with a K_i value of 75 nM provided a promising starting point for further modifications and structure−activity investigations. Compound **8d** was primarily synthesized for use in later Heck reactions but also led to the GHB analogue **9d**, displaying an affinity similar to GHB.

The 4-oxo analogues **7a**−**d** obtained in the synthesis of the 4-hydroxy compounds **9a**−**d** displayed much lower affinities than their corresponding 4-hydroxy analogues.

Introduction of a double bond in **9c** giving the styrylphenyl GHB analogue **12a** did not affect the affinity significantly. Likewise, the phenolic ether **17a** retained high affinity at specific GHB binding sites. Thus, these findings provided us with two novel high-affinity parent structures and feasible synthetic routes for introducing variation in the distal aromatic group of these compounds.

Introduction of halogens in the *ortho*-, *meta*-, or *para*-position of the distal phenyl groups of **12a** and **17a** led to minor increases in the affinities of these compounds, giving a series of high-affinity GHB ligands all displaying K_i values below 100 nM. Substitution of the distal aromatic group of **12a** and **17a** with various nonhalogen substituents did not improve affinity. This is illustrated by the introduction of a methoxy substituent in the *para*-position of the distal phenyl group of **12a** to give **12d**, replacement of the distal phenyl group of **12a** with a 2- or 4-pyridyl as in **12e** and **12f**, respectively, and with 4-methylthiazol-5-yl as in **12g**, as well as replacement of the distal phenyl

group of **17a** with a 2-furyl or a 2-thienyl as in **17k** and **17l**, respectively. Removing the hydroxyl group of **9c** gave **11a**, displaying much lower affinity than **9c** but higher affinity than the corresponding 4-oxo analogue **7c**.

Resolution of compound **16b** by chiral HPLC provided its enantiomers (ee ≥ 93%) and the absolute configuration of **(*R*)-(+)-16b** was established by an X-ray crystallographic analysis (Figure 2). Hydrolysis of the enantiomers of **16b** provided **(*R*)-17b** and **(*S*)-17b**. **(*R*)-17b** was found to display 10 times higher affinity than **(*S*)-17b** (Figure 3B, Table 1) and to have the highest affinity of any of the compounds tested. Because the enantiomeric excess of the tested (*S*)-form is 73%, the stereoselectivity in favor of the (*R*)-form may be underestimated.

Selectivity Studies. To examine the selectivity of the synthesized compounds, we investigated the affinities of four representative compounds from the series (**9c**, **12a**, **17a**, and **17b**) for $GABA_A$ and $GABA_B$ receptor sites.[21] None of these compounds displayed affinity for either $GABA_A$ or $GABA_B$ receptor sites (IC_{50} values >100 μM), whereas GABA in both cases concentration-dependently competed for binding as expected (data not shown).

Discussion

Although unique, high-affinity GHB binding sites distinct from $GABA_B$ receptors exist in mammalian brain,[4−6,9,15,17] the physiological role of these binding sites remains unclear. Indeed, most of the known physiological effects of GHB have been demonstrated to be a result of either direct or indirect $GABA_B$ receptor activation.[14−16] However, reports of specific GHB effects have emerged by the use of selective GHB ligands with no affinity for GABA receptors.[18−20] To further investigate the neuropharmacology of GHB and determine the molecular identity of the specific GHB binding sites, development of better tool compounds, i.e., selective ligands with improved affinity for high-affinity GHB binding sites are needed.[3,13]

In the present study, we have expanded the investigation of structure−affinity relationships for 4-substituted aromatic analogues of GHB at high-affinity GHB binding sites. For the three biaromatic analogues, **9a**−**c**, the affinity increased with increasing linker chain length, leaving compound **9c** as the most potent of the three (Figure 3A, Table 1) and among the GHB ligands with highest affinity known to date.

The unsaturated and the phenolic ether analogues of **9c**, **12a**, and **17a**, both retained high affinity at specific GHB binding sites. The comparable affinities of these three analogues indicates that some chemical diversity is allowed in the aromatic substituents in this class of GHB ligands upon binding to the specific GHB binding sites.

Introduction of halogens in the distal aromatic group of parent compounds **12a** and **17a**, providing the two compound series **12b**−**c** and **17b**−**j**, led to small increases in affinities. However, the affinities were not highly affected by the position nor the nature of the halogen. All halogen substituted compounds of the series **12b**−**c** and **17b**−**j** display K_i values below 100 nM, making these compounds among the highest affinity GHB ligands reported to date. Introduction of substituents other than halogens including heterocycles as in compounds **12d**−**g** and **17k**−**l** did not improve the affinities of these compounds. None of the changes we introduced in the distal aromatic group of the parent compounds **12a** and **17a** decreased the affinity dramatically, demonstrating that structural changes in this part of the lead structures **9c**, **12a**, and **17a** are fairly well tolerated with regard to binding to high-affinity GHB binding sites.

Although the highest-affinity GHB ligands reported so far all possess a hydroxyl group in a position equivalent to the hydroxyl group of GHB,[7,21,26] GHB ligands without a hydroxyl group have also been reported. Among these are 3-chloropropanoic acid[28] and ethers of 3-hydroxyphenylacetic acid.[29] Also, we recently found that derivatives of phenylacetic acid, including the nonsteroidal anti-inflammatory drug (NSAID) diclofenac, bind to specific GHB binding sites with affinities similar to GHB.[30] With the aim of investigating the importance of the hydroxyl group in the 4-position of GHB analogues, we found that the presence of an oxo group in the 4-position of compounds **7a**−**d** instead of a hydroxyl group as in compounds **9a**−**d** significantly decreased the affinity, similar to what we found for conformationally restricted analogues.[21] Intriguingly, although the absence of a functional group in the 4-position of **11a** dramatically decreased the affinity compared to the 4-hydroxy analogue **9c**, this compound displayed a significantly higher affinity than its 4-oxo analogue **7c**. In analogy with this, we recently found that butanoic acid and succinic semialdehyde (SSA), of which the latter is an intermediate of GHB/GABA metabolism,[3,31] are equipotent in terms of inhibiting [^{3}H]NCS-382 binding with K_i values of 24 and 22 μM, respectively.[30] Thus, whereas a 4-hydroxy substituent in GHB analogues may not be absolutely essential for obtaining affinity, it seems beneficial in obtaining high-affinity GHB ligands. With the aid of chiral HPLC and X-ray crystallographic analysis, we were able to resolve **16b** and determine the absolute configuration of the enantiomers; subsequent hydrolysis provided the enantiomers of **17b** (Figure 2, Scheme 4). Binding studies showed that **(*R*)-17b** displays at least an order of magnitude higher affinity than **(*S*)-17b** for the specific GHB binding sites (Figure 3B). This stereoselectivity corresponds well with earlier findings that the GHB binding affinity of **2**,[21] **4**,[26] and [^{3}H]NCS-382[10] predominantly resides with the (*R*)-enantiomers, demonstrating that the hydroxyl group of GHB analogues interact stereospecifically with high-affinity GHB binding sites.

In conclusion, we have synthesized a series of selective GHB ligands with high affinity at the specific GHB binding sites. Of these compounds, **(*R*)-17b** exhibits higher affinity (K_i = 22 nM) for specific GHB binding sites than previously reported GHB ligands. Because the compounds are selective for high-affinity GHB binding sites over $GABA_A$ and $GABA_B$ receptors, they represent new tool compounds for studying the neuropharmacology of GHB both *in vitro* and *in vivo*. These compounds provide a promising basis for our further investigations into both medicinal chemistry and pharmacology of GHB ligands toward the goal of characterizing and understanding the physiological role of specific GHB binding sites.

Experimental Section

Chemistry. Materials and Methods. All materials were obtained from commercial suppliers and used without further purification unless otherwise stated. All reactions involving air-sensitive reagents were performed under N_2 using syringe-septum cap techniques or a manifold. Solvents were dried and glassware was flame-dried in vacuo prior to use. Microwave experiments were performed in sealed glass vials using a Biotage Initiator Sixty apparatus. Flash chromatography (FC) was performed on glass columns or on an Isco, Inc. CombiFlash Companion (Merck silica gel 60, 0.040−0.063 mm). Dry column vacuum chromatography (DCVC)[32] was performed on glass columns (Merck silica gel 60, 0.015−0.040 mm) using 0−100% EtOAc or CH_2Cl_2 in heptane or petroleum ether, with increments of 5%. The petroleum ether used had distillation range 80−100 °C. Compounds were visualized on TLC (Merck silica gel 60 F254 plates) using UV light and $KMnO_4$ spraying reagent. Melting points were determined in open capillary

tubes and are uncorrected or using a SRS MPA100 OptiMelt automated melting point system. NMR spectra were recorded on a 300 MHz Varian spectrometer in $CDCl_3$ solutions using TMS as an internal standard, in D_2O solutions using 1,4-dioxane as an internal standard, or in CD_3OD, acetone-d_6, or DMSO-d_6 solutions. Elemental analyses were performed at Analytical Research Department, H. Lundbeck A/S Denmark or by Mr. J. Theiner, Department of Physical Chemistry, University of Vienna, Austria, and are within ±0.4% of the calculated values, unless otherwise stated. Optical rotations were measured in thermostatted cuvettes on a Perkin-Elmer 241 polarimeter.

4-(4-Benzylphenyl)-4-oxobutanoic acid (7b).[33] Anhydrous $AlCl_3$ (5.34 g, 40 mmol) was added to a solution of succinic anhydride (2.24 g, 22 mmol) and diphenylmethane (3.36 g, 20 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was stirred at room temperature for 70 h and concentrated in vacuo. H_2O (30 mL) was added and the mixture cooled to 0 °C and acidified with HCl (37%, 15 mL). The mixture was allowed to warm to room temperature and stirred for 30 min. The pale-yellow solid was filtered off and dried. The crude product was subjected to FC (petroleum ether/EtOAc 5:1, AcOH 2%) giving 7b as a white solid (2.99 g, 56%); mp 124 °C (lit.[33] mp 124−125 °C). ^{1}H NMR ($CDCl_3$): δ 2.78 (t, 2H, J = 6.5 Hz), 3.26 (t, 2H, J = 6.5 Hz), 4.02 (s, 2H), 7.06−7.41 (m, 7H), 7.90 (d, 2H, J = 8.2 Hz). ^{13}C NMR ($CDCl_3$): δ 28.1, 33.1, 41.9, 126.3, 128.2, 128.5, 128.8, 129.0, 134.3, 139.8, 146.9, 178.9, 197.2. Anal. ($C_{17}H_{16}O_3$) C, H.

4-(4-Phenethylphenyl)-4-oxobutanoic acid (7c).[34] Anhydrous $AlCl_3$ (2.97 g, 22 mmol) was added to a solution of succinic anhydride (1.30 g, 13 mmol) and 1,2-diphenylethane (2.03 g, 11 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 6 days. H_2O (40 mL) was added to the reaction mixture at 0 °C, followed by HCl (37%, 18 mL). The mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was concentrated in vacuo to evaporate CH_2Cl_2, and the aqueous solution was filtered giving a brown solid. The crude product was subjected to FC (petroleum ether/EtOAc 3:1, AcOH 2%) giving **7c** as a white solid (1.24 g, 40%). An analytical sample for microanalysis was recrystallized (toluene); mp 129−130 °C (lit.[34] mp 125−127 °C). ^{1}H NMR ($CDCl_3$): δ 2.80 (t, 2H, J = 6.5 Hz), 2.87−3.04 (m, 4H), 3.29 (t, 2H, J = 6.5 Hz), 7.09−7.31 (m, 7H), 7.87 (d, 2H, J = 8.2 Hz). ^{13}C NMR ($CDCl_3$): δ 28.1, 33.1, 37.4, 37.9, 126.0, 128.1, 128.3, 128.3, 128.6, 134.1, 140.9, 147.5, 178.9, 197.2. Anal. ($C_{18}H_{18}O_3$) C, H.

4-(4-Bromophenyl)-4-oxobutanoic acid (7d).[35] Anhydrous $AlCl_3$ (25.0 g, 188 mmol) was added to a stirred mixture of succinic anhydride (10.1 g, 101 mmol) in bromobenzene (50 mL, 475 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 45 min. H_2O (150 mL) was added at 0 °C under stirring, followed by HCl (37%, 100 mL), and the mixture was stirred for 1 h at room temperature. The yellow solid was filtered off, washed with H_2O, and dried, giving a pale-yellow solid (25.8 g). The crude product was dissolved in hot toluene, filtered, and recrystallized from hot toluene to give **7d** as white crystals (18.3 g, 70%); mp 145−146 °C (lit.[35] mp 146−149.5 °C). ^{1}H NMR (acetone-d_6): δ 2.72 (t, 2H, J = 6.3 Hz), 3.31 (t, 2H, J = 6.3 Hz), 7.70 (d, 2H, J = 8.4 Hz), 7.95 (d, 2H, J = 8.4 Hz). ^{13}C NMR (acetone-d_6): δ 28.1, 33.9, 130.0, 130.4, 132.4, 136.5, 173.7, 197.5. Anal. ($C_{10}H_9BrO_3$) C, H.

General Procedure for Reduction and Lactonization of 4-Oxo Acids (8a−d). $NaBH_4$ (4 equiv) was added slowly to a solution of the 4-oxo acid in EtOH (99.9%, 5−10 mL/mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was concentrated in vacuo, H_2O was added, and pH adjusted to 2 using aqueous HCl (2N). The mixture was extracted with EtOAc (3×), and the combined organic phases were washed with brine, dried using $MgSO_4$, and concentrated in vacuo.

The resulting crude mixture of the 4-hydroxyacid and the corresponding lactone was dissolved in CH_2Cl_2 and cooled to 0 °C followed by the addition of a few drops of TFA. Stirring at room temperature overnight completed the lactonization, except for **8a**, which was stirred for 6 days to complete the lactonization. The reaction mixture was washed with saturated aqueous $NaHCO_3$ (3×) followed by H_2O (2×), and the aqueous layers were extracted with CH_2Cl_2 (1×). The combined organic layers were dried using $MgSO_4$ and concentrated in vacuo to give the crude lactonized 4-hydroxy acid.

(*RS*)-5-(Biphen-4-yl)dihydrofuran-2(3*H*)-one (8a).[34] **8a** was prepared from 4-(biphen-4-yl)-4-oxobutanoic acid (748 mg, 2.94 mmol) following the general procedure for reduction and lactonization of 4-oxo acids. The crude product was subjected to DCVC (petroleum ether → EtOAc), giving a white solid (691 mg), which was further purified by recrystallization (EtOAc/petroleum ether) providing **8a** as white crystals (465 mg, 66%); mp 105−106 °C (lit.[34] mp 105 °C). ^{1}H NMR ($CDCl_3$): δ 2.12−2.32 (m, 1H), 2.59−2.75 (m, 3H), 5.50−5.59 (m, 1H), 7.28−7.49 (m, 5H), 7.51−7.67 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 29.0, 31.0, 81.0, 125.6, 126.9, 127.3, 127.4, 128.7, 138.1, 140.2, 141.2, 176.7. Anal. ($C_{16}H_{14}O_2$) C, H.

General Procedure for Hydrolysis of Lactones (9a−d, 12a−g, and 17a−l). The lactone was dissolved in THF (2−10 mL/mmol). A solution of $LiOH \cdot H_2O$ (1 equiv) in H_2O (1−10 mL/mmol) was added, and the mixture stirred overnight at room temperature. If the reaction was not completed after 16 h, additional LiOH (0.1 equiv) was added and the mixture was stirred overnight to complete the reaction. The mixture was concentrated in vacuo to give the crude lithium salt of the 4-hydroxybutanoic acid, which was essentially pure by TLC. The crude lithium salt was recrystallized to obtain an analytically pure sample for use in binding studies.

Lithium (*RS*)-4-(Biphen-4-yl)-4-hydroxybutanoate (9a). 9a was prepared from **8a** (295 mg, 1.24 mmol) following the general procedure for hydrolysis of lactones. Recrystallization (EtOH/Et_2O) of the crude product gave **9a** as white crystals (256 mg, 79%); mp > 230 °C. ^{1}H NMR (D_2O): δ 1.93−2.12 (m, 2H), 2.13−2.27 (m, 2H), 4.68−4.74 (m, 1H), 7.34−7.57 (m, 5H), 7.58−7.75 (m, 4H). ^{13}C NMR (D_2O): δ 34.3, 34.9, 73.7, 127.08, 127.14, 127.3, 127.9, 129.4, 140.1, 140.4, 143.0, 182.9. Anal. ($C_{16}H_{15}LiO_3 \cdot 1H_2O$) C, H.

General Procedure for Heck Reactions (10a−g). The alkene (1.2 equiv) was added to a mixture of **8d**, $Pd(OAc)_2$ (0.01 equiv), and dry K_3PO_4 (1.4 equiv) in dry THF (3.0 mL). The vial was sealed, stirred for 1 min at room temperature, and subsequently irradiated in a microwave at 190 °C for 15 min.

(*E*,*RS*)-5-(4-Styrylphenyl)dihydrofuran-2(3*H*)-one (10a). 10a was prepared from **8d** (503 mg, 2.09 mmol) and styrene following the general procedure for Heck reactions. The reaction mixture was concentrated in vacuo and the crude product was subjected to FC (petroleum ether/EtOAc 2:1) providing **10a** as a white solid (430 mg, 78%). An analytical sample for microanalysis was recrystallized (EtOAc/Et_2O); mp 152−155 °C. ^{1}H NMR ($CDCl_3$): δ 2.08−2.30 (m, 1H), 2.56−2.80 (m, 3H), 5.43−5.57 (m, 1H), 7.09 (s, 2H), 7.20−7.42 (m, 4H), 7.43−7.64 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 29.0, 31.0, 81.0, 125.6, 126.4, 126.7, 127.6, 127.7, 128.6, 129.2, 136.9, 137.4, 138.3, 176.7. Anal. ($C_{18}H_{16}O_2 \cdot 0.1H_2O$) C, H.

4-(4-Phenethylphenyl)butanoic acid (11a). 10a (193 mg, 0.73 mmol) and Pd−C (0.023 g, 0.011 mmol) was added to a dry flask, which was charged with N_2. EtOH (99.9%, 30 mL) was added under N_2. H_2 was bubbled through the solution for a few minutes, and the reaction mixture was stirred under H_2 for 2 h. The reaction mixture was filtered through celite, and the solvent was evaporated giving **11a** as a white solid (182 mg, 93%). An analytical sample for microanalysis was recrystallized (EtOAc/petroleum ether); mp 85 °C. ^{1}H NMR ($CDCl_3$): δ 1.95 (pentet, 2H, J = 7.5 Hz), 2.37 (t, 2H, J = 7.5 Hz), 2.64 (t, 2H, J = 7.5 Hz), 2.89 (s, 4H), 7.05−7.13 (m, 4H), 7.14−7.21 (m, 2H), 7.23−7.30 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 26.3, 33.4, 34.6, 37.5, 38.0, 125.7, 128.2, 128.3, 128.3, 128.3, 138.5, 139.3, 141.6, 180.2. Anal. ($C_{18}H_{20}O_2$) C, H.

4-(4-Hydroxyphenyl)-4-oxobutanoic acid (14).[36] 4-(4-Methoxyphenyl)-4-oxobutanoic acid (**13**) (7.29 g, 35 mmol) and pyridine hydrochloride (21 g, 180 mmol) were heated in an oil bath at 200 °C for 4 h. The reaction mixture was allowed to cool to room temperature and H_2O (400 mL) was added. The mixture was filtered

and the pH adjusted to 2 with aqueous HCl (2N). The mixture was extracted with EtOAc (4 × 250 mL). The combined organic layers were dried using $MgSO_4$ and concentrated in vacuo, giving **14** as a pale-yellow solid (4.98 g, 73%). An analytical sample for microanalysis was recrystallized (acetonitrile), giving white crystals; mp 155−156 °C (lit.[36] mp 154−156 °C). ^{1}H NMR (acetone-d_6): δ 2.69 (t, 2H, J = 6.4 Hz), 3.25 (t, 2H, J = 6.4 Hz), 6.92 (d, 2H, J = 8.8 Hz), 7.91 (d, 2H, J = 8.8 Hz). ^{13}C NMR (acetone-d_6): δ 28.3, 33.3, 115.8, 129.7, 131.0, 162.3, 174.3, 196.6. Anal. ($C_{10}H_{10}O_4$) C, H.

(*RS*)-5-(4-Hydroxyphenyl)dihydrofuran-2(3*H*)-one (15).[37] A solution of $CaCl_2$ (2.2 g, 20 mmol) in MeOH (10 mL) was added to a solution of **14** (370 mg, 1.91 mmol) in MeOH (5 mL). $NaBH_4$ (0.80 g, 21 mmol) was added slowly and the reaction mixture was stirred overnight. H_2O (30 mL) was added, and the solution was acidified with aqueous HCl (2N). The solution was extracted with EtOAc (4 × 50 mL) and the combined organic layers were washed with saturated aqueous NaCl (1 × 80 mL) and dried using $MgSO_4$. The organic layers were concentrated in vacuo to give a white solid (410 mg), which was subjected to FC (petroleum ether/EtOAc), giving **15** as a white solid (240 mg, 71%). An analytical sample for microanalysis was recrystallized (EtOAc); mp 130−131 °C (lit.[37] mp 134−135 °C). ^{1}H NMR (acetone-d_6): δ 2.12−2.30 (m, 1H), 2.53−2.76 (m, 3H), 5.40−5.49 (m, 1H), 6.86 (d, 2H, J = 8.5 Hz), 7.26 (d, 2H, J = 8.5 Hz). ^{13}C NMR (acetone-d_6): δ 29.8, 31.5, 82.1, 116.1, 128.3, 131.6, 158.3, 177.2. Anal. ($C_{10}H_{10}O_3$) C, H.

Standard Procedure for Mitsunobu Reactions (Synthesis of 16a−l). Ph_3P (1.2 equiv) was added to a solution of **15** and the appropriate benzylic alcohol, furylmethanol, or thienylmethanol (1.2 equiv) in dry THF (5 mL/mmol) at 0 °C under N_2. Diisopropyl azodicarboxylate (DIAD) (1.2 equiv) was added dropwise and the reaction mixture was stirred for 1−2 h at 0 °C. The mixture was allowed to warm to room temperature and concentrated in vacuo.

(*RS*)-5-[4-(Benzyloxy)phenyl]dihydrofuran-2(3*H*)-one (16a).[38] Following the standard procedure for Mitsunobu reactions, **16a** was prepared from **15** (300 mg, 1.68 mmol) and benzyl alcohol. The crude product was purified by DCVC (heptane → CH_2Cl_2), giving **16a** as a white solid (303 mg, 67%); mp 96−97 °C. ^{1}H NMR ($CDCl_3$): δ 2.10−2.27 (m, 1H), 2.52−2.70 (m, 3H), 5.05 (s, 2H), 5.39−5.48 (m, 1H), 6.93−7.02 (m, 2H), 7.19−7.53 (m, 7H). ^{13}C NMR ($CDCl_3$): δ 29.3, 30.9, 70.0, 81.3, 114.9, 126.9, 127.3, 127.9, 128.5, 131.3, 136.5, 158.7, 176.7. Anal. ($C_{17}H_{16}O_3$) C, H.

Chiral Liquid Chromatography. Preparative chromatography was performed with an HPLC system consisting of a Jasco 880 pump (flow 1.0 mL/min), a Rheodyne 7125 injector, a 5 mL loop, and a Shimadzu SPD-6A UV detector (210 nm) attached to a Hitachi D-2000 chromato-integrator. For determination of the enantiomeric excess (ee), a TSP HPLC equipment consisting of an AS-3000 autoinjector, a P-2000 pump (flow 1.0 mL/min), and an SM-5000 photodiode array detector (229 nm) was used. Preparative resolution and analytical determination of the ee were performed on a ChiralPak AS-H column (250 mm × 4.6 mm) equipped with a ChiralPak AS-H guard column (10 mm × 4.0 mm) (Chiral Technologies, Europe). The columns were eluted with *n*-heptane/2-PrOH (80:20; v/v) for the preparative resolution to give **(*R*)-16b** (t_R = 38 min) and **(*S*)-16b** (t_R = 62 min), with *n*-heptane/2-PrOH (40:60; v/v) for the determination of the ee of **(*R*)-16b** (t_R = 14 min) and **(*S*)-16b** (t_R = 21 min), and with *n*-heptane/2-PrOH/TFA (90:10:0.1; v/v) for the ee determination of **(*R*)-17b** (t_R = 32 min) and **(*S*)-17b** (t_R = 37 min).

(*R*)-(+)-5-[4-(2-Iodobenzyloxy)phenyl]dihydrofuran-2(3*H*)-one [(*R*)-16b] and (*S*)-(−)-5-(4-(2-iodobenzyloxy)phenyl)dihydrofuran-2(3*H*)-one [(*S*)-16b]. The racemic compound **16b** (260 mg, 0.66 mmol) was dissolved in *n*-heptane/2-PrOH (80:20; v/v) (3 mg/mL), filtered through a Millex HV filter (0.45 μm, Millipore), and resolved using ChiralPak AS-H in up to 9.0 mg per injection. Fractions containing the first eluting enantiomer were pooled and evaporated. The crystals were dried to give **(*R*)-(+)-16b** (109 mg, 42%): 99.7% ee; $[\alpha]^{25}_D$ = +6.4° (c = 0.44; $CHCl_3$). ^{1}H NMR and ^{13}C NMR spectra were identical with those of the racemic compound **16b**. Anal. ($C_{17}H_{15}IO_3$) C, H, I. Fractions containing the second eluting enantiomer were pooled and evaporated. The crystals were dried in vacuo, giving **(*S*)-(−)-16b** (128 mg, 49%): 93.0% ee; $[\alpha]^{25}_D$ = −5.2° (c = 0.44; $CHCl_3$). ^{1}H NMR and ^{13}C NMR spectra were identical with those of the racemic compound **16b**. Anal. ($C_{17}H_{15}IO_3$) C, H, I.

Lithium (*R*)-(+)-4-hydroxy-4-[4-(2-iodobenzyloxy)phenyl]butanoate [(*R*)-17b]. **(*R*)-17b** was prepared from **(*R*)-16b** (79 mg, 0.20 mmol) following the general procedure for hydrolysis of lactones. Recrystallization (MeOH) of the crude product gave **(*R*)-17b** as white crystals: 99.6% ee; $[\alpha]^{25}_D$ = +2.2° (c = 0.226; H_2O). ^{1}H NMR and ^{13}C NMR spectra were identical with those of the racemic compound **17b.**

Lithium (*S*)-(−)-4-hydroxy-4-[4-(2-iodobenzyloxy)phenyl]butanoate [(*S*)-17b]. **(*S*)-17b** was prepared from **(*S*)-16b** (113 mg, 0.29 mmol) following the general procedure for hydrolysis of lactones. Recrystallization (MeOH) of the crude product gave **(*S*)-17b** as white crystals: 73.5% ee; $[\alpha]^{25}_D$ = −1.3° (c = 0.233; H_2O). ^{1}H NMR and ^{13}C NMR spectra were identical with those of the racemic compound **17b**.

X-ray Crystallographic Analysis of (*R*)-(+)-16b. Colorless single crystals were obtained from a solution in EtOAc. Crystal data: $C_{17}H_{15}IO_3$, M_r = 394.19, orthorhombic, space group $P2_12_12_1$ (no. 19), a = 5.8980(4) Å, b = 8.4160(10) Å, c = 30.842(3) Å, V = 1530.9(2) Å^3, Z = 4, D_c = 1.710 Mg m^{-3}, $F(000)$ = 776, μ(Mo $K\alpha$) = 2.099 mm^{-1}, T = 122.0 (5) K, crystal dimensions = 0.35 mm × 0.28 mm × 0.11 mm.

Data Collection and Processing. Diffraction data were collected on an Enraf-Nonius KappaCCD diffractometer using graphite monochromated Mo $K\alpha$ radiation (λ = 0.71073 Å).[39] The reflections were measured in the range $-10 \le h \le 10$, $-14 \le k \le 14$, $-53 \le l \le 53$, ($2.64° < \theta < 38.40°$). Data were reduced using the program EvalCCD.[40,41] Absorption correction was applied using the program NUMABS (T_{min} = 0.579; T_{max} =0.829).[42,43] A total of 56526 reflections were averaged according to the point group symmetry 222, resulting in 8553 unique reflections (R_{int} = 0.0378 on F_o[41]).

Structure Solution and Refinement. The structure was solved by the direct method using the program SHELXS97[44,45] and refined using the program SHELXL97.[46] Full matrix least-squares refinement on F^2 was performed, minimizing $\sum w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of all hydrogen atoms were located on intermediate difference electron density maps and refined with fixed isotropic displacement parameters, except for the hydrogen atoms connected to aromatic rings, which were included in calculated positions, riding on the parent atoms with fixed isotropic displacement parameters. The refinement (213 parameters, 8553 reflections) converged at R_F = 0.0232, wR_F^2 = 0. 0429 for 7530 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma^2(F_o^2) + (0.0224P)^2 + 0.0810P]$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.042. Absolute structure parameter is −0.016(8).[47] Correction for extinction has been performed (0.0015(3)).[46] In the final difference, Fourier map maximum and minimum electron densities were 0.596 and −0.827 e Å^{-3}, respectively (observed in the vicinity of iodine). Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.[46]

Fractional atomic coordinates, list of anisotropic displacement parameters, and a complete list of geometrical data have been deposited in the Cambridge Crystallographic Data Centre (no. CCDC 698436).

Pharmacology. Compounds and Radioligands. [^{3}H]NCS-382 (20 Ci/mmol) was purchased from Biotrend (Köln, Germany). [^{3}H]GABA (27.6 Ci/mmol) and [^{3}H]muscimol (28.5 Ci/mmol) were purchased from PerkinElmer (Boston, MA, USA). Baclofen, GABA, GHB, and isoguvacine were purchased from Sigma-Aldrich (St. Louis, MO, USA). NCS-382 was purchased from Tocris (Bristol, UK).

Membrane preparations. All binding assays were performed using rat brain synaptic membranes of cortex and the central hemispheres from adult male Sprague−Dawley rats with tissue preparation as earlier described.[48] On the day of the assay, the

membrane preparation was quickly thawed, suspended in 40 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) (for [^{3}H]muscimol and [^{3}H]GABA binding assays) or KH_2PO_4 buffer (50 mM, pH 6.0) (for [^{3}H]NCS-382 binding assay) using an UltraTurrax homogenizer, and centrifuged at 48000*g* for 10 min at 4 °C. This washing step was repeated four times. The final pellet was resuspended in incubation buffer and the binding assay carried out as detailed below. All experiments were performed in triplicate and repeated in at least two independent experiments.

[^{3}H]NCS-382, [^{3}H]muscimol, and [^{3}H]GABA Binding Assays. For [^{3}H]NCS-382 binding to rat brain membranes, a 96-well filtration was used exactly as earlier described.[21] Briefly, aliquots of membrane preparation (50−70 μg protein/aliquot) in KH_2PO_4 buffer (50 mM, pH 6.0) in triplicate were incubated with [^{3}H]NCS-382 (16 nM) in the absence or presence of displacer at 0 °C for 1 h in a total volume of 200 μL. Nonspecific binding was determined using unlabeled GHB (1 mM). The binding reaction was terminated by rapid filtration through GF/C unifilters (Perkin-Elmer), using a 96-well Packard FilterMate cell-harvester, followed by washing with 3 × 250 μL of ice-cold binding buffer. The dried filters were added Microscint scintillation fluid (PerkinElmer), and the amount of filterbound radioactivity as counts per minute (CPM) was quantified in a Packard TopCount microplate scintillation counter.

$GABA_A$ receptor binding was carried out in a similar fashion using the selective radioligand [^{3}H]muscimol. Membranes (100 μg protein/aliquot) in Tris-HCl buffer (50 mM, pH 7.4) were incubated with [^{3}H]muscimol (5 nM), and 0.1 mM of displacer at 0 °C for 60 min in a total volume of 250 μL. GABA (1 mM) was used to define nonspecific binding. The reactions were terminated by filtration through GF/B filter plates (PerkinElmer), and the filters were washed × 3 with ice-cold incubation buffer, dried, and counted as described above.

For [^{3}H]GABA binding to the $GABA_B$ receptors, membranes (200 μg protein/aliquot) were suspended in Tris-HCl buffer (50 mM + 2.5 mM $CaCl_2$, pH 7.4) and incubated with [^{3}H]GABA (5 nM), isoguvacine (40 μM), and 0.1 mM of displacer at 25 °C for 45 min in 1 mL total volumes. Isoguvacine serves to saturate $GABA_A$ receptors.[49] Nonspecific binding was determined using 0.1 mM baclofen. Binding was terminated by filtration through Whatman GF/C filters, using a Brandell M-48R cell harvester, filters were washed with 3 × 3 mL of ice-cold buffer, and filterbound radioactivity was counted in a Packard Tricarb 2100 liquid scintillation analyzer using 3 mL of Opti-fluor scintillation fluid (PerkinElmer).

Data Analysis. The displacement data were analyzed using GraphPad Prism 4.0b, (GraphPad Software Inc., San Diego, CA). The curves were fitted by nonlinear regression using the formula for one-site competition. K_i values were calculated from IC_{50} values by means of the Cheng−Prusoff equation,[50] using the earlier determined K_d value of 430 nM for [^{3}H]NCS-382.[21] Data from each individual experiment were analyzed separately, and the means ± SEM were then calculated.

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Supporting Information Available: Experimental details for the synthesis of compounds **8b−d**, **9b−d**, **10b−g**, **12a−g**, **16b−l**, and **17a−l** and data from microanalysis. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Fuller, D. E.; Hornfeldt, C. S. From club drug to orphan drug: sodium oxybate (Xyrem) for the treatment of cataplexy. *Pharmacotherapy* **2003**, *23*, 1205–1209.

(2) Robinson, D. M.; Keating, G. M. Sodium oxybate: a review of its use in the management of narcolepsy. *CNS Drugs* **2007**, *21*, 337–354.

(3) Drasbek, K. R.; Christensen, J.; Jensen, K. Gamma-hydroxybutyrates − a drug of abuse. *Acta Neurol. Scand.* **2006**, *114*, 145–156.

(4) Snead, O. C., III. The ontogeny of [^{3}H]γ-hydroxybutyrate and [^{3}H]$GABA_B$ binding sites: relation to the development of experimental absence seizures. *Brain Res.* **1994**, *659*, 147–156.

(5) Castelli, M. P.; Mocci, I.; Langlois, X.; Gommeren, W.; Luyten, W. H.; Leysen, J. E.; Gessa, G. L. Quantitative autoradiographic distribution of γ-hydroxybutyric acid binding sites in human and monkey brain. *Brain Res. Mol. Brain Res.* **2000**, *78*, 91–99.

(6) Gould, G. G.; Mehta, A. K.; Frazer, A.; Ticku, M. K. Quantitative autoradiographic analysis of the new radioligand [^{3}H](2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[*a*][7]annulen-6-ylidene)ethanoic acid ([^{3}H]NCS-382) at γ-hydroxybutyric acid (GHB) binding sites in rat brain. *Brain Res.* **2003**, *979*, 51–56.

(7) Maitre, M.; Hechler, V.; Vayer, P.; Gobaille, S.; Cash, C. D.; Schmitt, M.; Bourguignon, J.-J. A specific γ-hydroxybutyrate receptor ligand possesses both antagonistic and anticonvulsant properties. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 657–663.

(8) Castelli, M. P.; Pibiri, F.; Carboni, G.; Piras, A. P. A review of pharmacology of NCS-382, a putative antagonist of γ-hydroxybutyric acid (GHB) receptor. *CNS Drug Rev.* **2004**, *10*, 243–260.

(9) Mehta, A. K.; Muschaweck, N. M.; Maeda, D. Y.; Coop, A.; Ticku, M. K. Binding characteristics of the γ-hydroxybutyric acid receptor antagonist[^{3}H](2*E*)-(5-hydroxy-5,7,8,9-tetrahydro-6*H*-benzo[*a*][7]annulen-6-ylidene) ethanoic acid in the rat brain. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 1148–1153.

(10) Castelli, M. P.; Mocci, I.; Pistis, M.; Peis, M.; Berta, D.; Gelain, A.; Gessa, G. L.; Cignarella, G. Stereoselectivity of NCS-382 binding to γ-hydroxybutyrate receptor in the rat brain. *Eur. J. Pharmacol.* **2002**, *446*, 1–5.

(11) Mathivet, P.; Bernasconi, R.; De Barry, J.; Marescaux, C.; Bittiger, H. Binding characteristics of γ-hydroxybutyric acid as a weak but selective $GABA_B$ receptor agonist. *Eur. J. Pharmacol.* **1997**, *321*, 67–75.

(12) Lingenhoehl, K.; Brom, R.; Heid, J.; Beck, P.; Froestl, W.; Kaupmann, K.; Bettler, B.; Mosbacher, J. γ-Hydroxybutyrate is a weak agonist at recombinant $GABA_B$ receptors. *Neuropharmacology* **1999**, *38*, 1667–1673.

(13) Crunelli, V.; Emri, Z.; Leresche, N. Unravelling the brain targets of γ-hydroxybutyric acid. *Curr. Opin. Pharmacol.* **2006**, *6*, 44–52.

(14) Carai, M. A.; Lobina, C.; Maccioni, P.; Cabras, C.; Colombo, G.; Gessa, G. L. γ-Aminobutyric $acid_B$ ($GABA_B$)-receptor mediation of different in vivo effects of γ-butyrolactone. *J. Pharmacol. Sci.* **2008**, *106*, 199–207.

(15) Kaupmann, K.; Cryan, J. F.; Wellendorph, P.; Mombereau, C.; Sansig, G.; Klebs, K.; Schmutz, M.; Froestl, W.; Putten, H. v. d.; Mosbacher, J.; Bräuner-Osborne, H.; Waldmeier, P.; Bettler, B. Specific γ-hydroxybutyrate-binding sites but loss of pharmacological effects of γ-hydroxybutyrate in $GABA_{B(1)}$-deficient mice. *Eur. J. Neurosci.* **2003**, *18*, 2722–2730.

(16) Queva, C.; Bremner-Danielsen, M.; Edlund, A.; Ekstrand, A. J.; Elg, S.; Erickson, S.; Johansson, T.; Lehmann, A.; Mattsson, J. P. Effects of GABA agonists on body temperature regulation in $GABA_{B(1)}^{-/-}$ mice. *Br. J. Pharmacol.* **2003**, *140*, 315–322.

(17) Wu, Y.; Ali, S.; Ahmadian, G.; Liu, C. C.; Wang, Y. T.; Gibson, K. M.; Calver, A. R.; Francis, J.; Pangalos, M. N.; Carter Snead, O., III. γ-Hydroxybutyric acid (GHB) and γ-aminobutyric $acid_B$ receptor ($GABA_BR$) binding sites are distinctive from one another: molecular evidence. *Neuropharmacology* **2004**, *47*, 1146–1156.

(18) Castelli, M. P.; Ferraro, L.; Mocci, I.; Carta, F.; Carai, M. A. M.; Antonelli, T.; Tanganelli, S.; Cignarella, G.; Gessa, G. L. Selective γ-hydroxybutyric acid receptor ligands increase extracellular glutamate in the hippocampus, but fail to activate G protein and to produce the sedative/hypnotic effect of γ-hydroxybutyric acid. *J. Neurochem.* **2003**, *87*, 722–732.

(19) Brancucci, A.; Berretta, N.; Mercuri, N. B.; Francesconi, W. Presynaptic modulation of spontaneous inhibitory postsynaptic currents by gamma-hydroxybutyrate in the substantia nigra pars compacta. *Neuropsychopharmacology* **2004**, *29*, 537–543.

(20) Ferraro, L.; Tanganelli, S.; O'Connor, W. T.; Francesconi, W.; Loche, A.; Gessa, G. L.; Antonelli, T. γ-Hydroxybutyrate modulation of glutamate levels in the hippocampus: an *in vivo* and *in vitro* study. *J. Neurochem.* **2001**, *78*, 929–939.

(21) Wellendorph, P.; Høg, S.; Greenwood, J. R.; de Lichtenberg, A.; Nielsen, B.; Frølund, B.; Brehm, L.; Clausen, R. P.; Bräuner-Osborne, H. Novel cyclic γ-hydroxybutyrate (GHB) analogs with high affinity and stereoselectivity of binding to GHB sites in rat brain. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 346–351.

(22) Wu, H.; Zink, N.; Carter, L. P.; Mehta, A. K.; Hernandez, R. J.; Ticku, M. K.; Lamb, R.; France, C. P.; Coop, A. A tertiary alcohol analog of γ-hydroxybutyric acid as a specific γ-hydroxybutyric acid receptor ligand. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 675–679.
(23) Murphy, T. C.; Poppe, C.; Porter, J. E.; Montine, T. J.; Picklo Sr, M. J. 4-Hydroxy-*trans*-2-nonenoic acid is a γ-hydroxybutyrate receptor ligand in the cerebral cortex and hippocampus. *J. Neurochem.* **2004**, *89*, 1462–1470.
(24) Carter, L. P.; Wu, H.; Chen, W.; Matthews, M. M.; Mehta, A. K.; Hernandez, R. J.; Thomson, J. A.; Ticku, M. K.; Coop, A.; Koek, W.; France, C. P. Novel γ-hydroxybutyric acid (GHB) analogs share some, but not all, of the behavioral effects of GHB and $GABA_B$ receptor agonists. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1314–1323.
(25) Bourguignon, J. J.; Schoenfelder, A.; Schmitt, M.; Wermuth, C. G.; Hechler, V.; Charlier, B.; Maitre, M. Analogues of γ-hydroxybutyric acid. Synthesis and binding studies. *J. Med. Chem.* **1988**, *31*, 893–897.
(26) Bourguignon, J. J.; Schmitt, M.; Didier, B. Design and structure−activity relationship analysis of ligands of gamma-hydroxybutyric acid receptors. *Alcohol* **2000**, *20*, 227–236.
(27) Hechler, V.; Peter, P.; Gobaille, S.; Bourguignon, J.-J.; Schmitt, M.; Ehrhardt, J.-D.; Mark, J.; Maitre, M. γ-Hydroxybutyrate ligands possess antidopaminergic and neuroleptic-like activities. *J. Pharmacol. Exp. Ther.* **1993**, *264*, 1406–1414.
(28) Macias, A. T.; Hernandez, R. J.; Mehta, A. K.; MacKerell, A. D.; Ticku, M. K.; Coop, A. 3-Chloropropanoic acid (UMB66): a ligand for the gamma-hydroxybutyric acid receptor lacking a 4-hydroxyl group. *Bioorg. Med. Chem.* **2004**, *12*, 1643–1647.
(29) Chen, W.; Wu, H.; Hernandez, R. J.; Mehta, A. K.; Ticku, M. K.; France, C. P.; Coop, A. Ethers of 3-hydroxyphenylacetic acid as selective gamma-hydroxybutyric acid receptor ligands. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3201–3202.
(30) Wellendorph, P.; Høg, S.; Skonberg, C.; Bräuner-Osborne, H. Phenylacetic acids and the structurally related non-steroidal anti-inflammatory drug (NSAID) diclofenac bind to specific γ-hydroxybutyric acid (GHB) sites in rat brain. *Fundam. Clin. Pharmacol.* **2008**, in press.
(31) Bernasconi, R.; Mathivet, P.; Bischoff, S.; Marescaux, C. Gamma-hydroxybutyric acid: an endogenous neuromodulator with abuse potential. *Trends Pharmacol. Sci.* **1999**, *20*, 135–141.
(32) Pedersen, D. S.; Rosenbohm, C. Dry column vacuum chromatography. *Synthesis* **2001**, *16*, 2431–2434.
(33) Spencer, T. A.; Onofrey, T. J.; Cann, R. O.; Russel, J. S.; Lee, L. E.; Blanchard, D. E.; Castro, A.; Gu, P.; Jiang, G.; Shechter, I. Zwitterionic sulfobetaine inhibitors of squalene synthase. *J. Org. Chem.* **1999**, *64*, 807–818.
(34) Child, R. G.; Osterberg, A. C.; Sloboda, A. E.; Tomcufcik, A. S. Fenbufen, a new anti-inflammatory analgesic: synthesis and structure−activity relationships of analogs. *J. Pharm. Sci.* **1977**, *66*, 466–476.
(35) Kiryanov, A. A.; Sampson, P.; Seed, A. J. Synthesis and mesomorphic properties of 1,1-difluoroalkyl-substituted biphenylthienyl and terphenyl liquid crystals. A comparative study of mesomorphic behavior relative to alkyl, alkoxy and alkanoyl analogs. *J. Mater. Chem.* **2001**, *11*, 3068–3077.
(36) Fieser, L. F.; Gates, M. D.; Kilmer, G. W. Quinonyl derivatives of fatty acids. *J. Am. Chem. Soc.* **1940**, *62*, 2966–2969.
(37) Rosenmund, K. W.; Zymalkowski, F.; Engels, P. Über die synthese von γ-oxyphenyl- und γ-dioxyphenyl-butyrolactonen. *Chem. Ber.* **1951**, *84*, 711–718.
(38) Pelletier, J. C.; Rogers, J.; Wrobel, J.; Perez, M. C.; Shen, E. S. Preparation of highly substituted γ-lactam follicle stimulating hormone receptor agonists. *Bioorg. Med. Chem.* **2005**, *13*, 5986–5995.
(39) Collect, B. V. Nonius: Delft, The Netherlands, 1999.
(40) Duisenberg, A. J. M. EvalCCD. Ph.D. Thesis, University of Utrecht, The Netherlands, 1998.
(41) Duisenberg, A. J. M. Indexing in single-crystal diffractometry with an obstinate list of reflections. *J. Appl. Crystallogr.* **1992**, *25*, 92–96.
(42) Coppens, P. Evaluation of absorption and extinction in single-crystal structure analysis. In *Crystallographic Computing*; Hall, S. R., Ed.; Munksgaard: Copenhagen, 1970; pp 255−270.
(43) *Nonius 2003, maXus 1.2.1*; Nonius BV: Delft, The Netherlands; procedure NUMABS.
(44) Sheldrick, G. M. SHELXS97, Program for the Solution of Crystal Structures, 1997.
(45) Sheldrick, G. M. Phase annealing in SHELX-90: Direct methods for larger structures. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1990**, *46*, 467–473.
(46) Sheldrick, G. M. SHELXS97, Program for Crystal Structure Refinement, 1997.
(47) Flack, H. D. On enantiomorph-polarity estimation. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **1983**, *39*, 876–881.
(48) Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J. Neurochem.* **1988**, *51*, 830–836.
(49) Hill, D. R.; Bowery, N. G. ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature* **1981**, *290*, 149–152.
(50) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
(51) Farrugia, L. J. ORTEP-3 for Windows. *J. Appl. Crystallogr.* **1997**, *30*, 565.

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