JPET Fast Forward. Published on July 12, 2005 as DOI: 10.1124/jpet.105.090472

JPET #90472

Novel cyclic γ-hydroxybutyrate (GHB) analogues with high affinity and stereoselectivity of binding to GHB sites in rat brain

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Running title: Novel and selective analogues of GHB with high affinity

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Abbreviations: GHB, γ-hydroxybutyrate; GABA, γ-aminobutyric acid; T-HCA, *trans*-4-hydroxycrotonic acid; NCS-382, (*E*,*RS*)-(6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid; HOCHCA, 3-hydroxycyclohex-1-enecarboxylic acid; HOCPCA, 3-hydroxycyclopent-1-enecarboxylic acid; HOCPrCA, *trans*-2-(hydroxymethyl)cyclopropane carboxylic acid.

Recommended section assignment: Neuropharmacology

Abstract

γ-Hydroxybutyrate (GHB) is a psychotropic compound endogenous to the brain. Despite its potentially great physiological significance, its exact molecular mechanism of action is unknown. GHB is a weak agonist at γ-aminobutyric acid subtype B ($GABA_B$) receptors, but there is also evidence of specific GHB receptor sites, the molecular cloning of which remains a challenge. Ligands with high affinity and specificity for the reported GHB binding site are needed for pharmacological dissection of the GHB and $GABA_B$ effects, and for mapping the structural requirements of the GHB receptor–ligand interactions. For this purpose, we have synthesized and assayed three conformationally restricted GHB analogues for binding against the GHB specific ligand (*E*,*RS*)-(6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid ([^{3}H]NCS-382) in rat brain homogenate. The cyclohexene and cyclopentene analogues, (*RS*)-HOCHCA and (*RS*)-HOCPCA, were found to be high-affinity GHB ligands, with IC_{50} values in the nanomolar range, and had respectively 9 and 27 times higher affinity than GHB. The stereoselectively synthesized *R*,*R*-isomer of the *trans*-cyclopropyl GHB analogue, HOCPrCA, proved to have 10-fold higher affinity than its enantiomer. Likewise, the *R*-enantiomers of HOCHCA and HOCPCA selectively inhibited [^{3}H]NCS-382 binding. The best inhibitor of these, (*R*)-HOCPCA, has an affinity 39 times higher than GHB and is thus among the best GHB ligands reported to date. Neither of the cycloalkenes showed any affinity (IC_{50} > 1 mM) for $GABA_A$ or $GABA_B$ receptors. These compounds show excellent potential as lead structures and novel tools for studying specific GHB receptor–mediated pharmacology.

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Introduction

γ-Hydroxybutyrate (GHB) is a naturally occurring substance in the mammalian brain, where it is currently believed to function as a neurotransmitter or neuromodulator (Bernasconi et al., 1999). GHB is a registered drug for the treatment of cataplexy associated with narcolepsy (Fuller and Hornfeldt, 2003) and has also displayed therapeutic potential for treating drug and alcohol dependence (Gallimberti et al., 2000). Furthermore, GHB is a drug of abuse (Wong et al., 2004), producing mild euphoria, muscle-relaxation, sedation and eventually coma with increasing dosage. Despite the obvious biological and pharmacological importance of GHB, its exact mechanism of action remains elusive.

Most of the reported GHB effects are mediated through the γ-aminobutyric acid (GABA) system, in particular $GABA_B$ receptors. GHB has been shown to be a low-affinity (Mathivet et al., 1997), weak partial agonist at $GABA_B$ receptors (Lingenhoehl et al., 1999), and may also be converted into GABA *in vivo*, and thus affect GABA receptors indirectly (Hechler et al., 1997). However, based on recent data, it would also seem that specific GHB receptor-mediated effects exist (Castelli et al., 2003; Kemmel et al., 2003; Brancucci et al., 2004). This, in combination with the presence of high-affinity [^{3}H]GHB binding sites in the brain (Benavides et al., 1982), suggests the existence of a distinct GHB receptor. In further support of this view, brains from $GABA_{B(1)}$ receptor knockout mice still exhibit [^{3}H]GHB binding (Kaupmann et al., 2003; Wu et al., 2004), demonstrating that GHB and GABA binding sites are separate entities. (*E*,*RS*)-(6,7,8,9-Tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid (NCS-382), a synthetic structural analogue of GHB, and a purported antagonist of the GHB receptor (Castelli et al., 2004), has been shown to compete with [^{3}H]GHB for the high-affinity sites. Radioligand binding studies have demonstrated [^{3}H]NCS-382 to be selective for GHB binding sites, making it a valuable tool for probing the putative GHB receptor (Mehta et al., 2001). One recent report claimed the cloning of a GHB-specific receptor, yet failed to show affinity of NCS-382 for the receptor (Andriamampandry et al., 2003). Furthermore, the *in situ* hybridization expression pattern of this

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claimed receptor in rat brain showed labeling in the cerebellum, whereas previous autoradiography studies performed with [^{3}H]GHB and [^{3}H]NCS-382 showed negligible binding in this region of the brain (Snead 1996; Gould et al., 2003). The dual action of GHB is a major obstacle when studying GHB receptor-mediated effects, and accentuates the need for potent and selective ligands. Also, higher-affinity compounds could aid the molecular cloning of GHB receptors, which remains the most prominent goal of this line of research.

So far, the structural requirements of GHB binding sites are relatively unexplored (Bourguignon et al., 2000; Macias et al., 2004). Most attempts have been to generate GHB-selective compounds that do not undergo metabolism to GABAergic compounds. Albeit of high value for *in vivo* studies, these compounds have at best been equipotent with GHB (Wu et al., 2003; Carter et al., 2005). In addition, the conformational flexibility of previous ligands and limited stereospecificity data associated with them would hamper any attempt to map the GHB receptor pharmacophore. We therefore set out to synthesize a series of conformationally restricted GHB analogues, with close attention to stereochemistry. We report here the synthesis and pharmacological evaluation of the enantiomers of three highly selective GHB ligands. To our knowledge, one of these compounds, (*R*)-3-hydroxycyclopent-1-enecarboxylic acid [(*R*)-HOCPCA)] is one of the most high-affine GHB receptor ligand reported to date (structure is shown in Fig. 1).

Materials and Methods

Compounds and radioligands

GHB sodium salt, T-HCA, GABA and (*R*)-baclofen were purchased from Sigma-Aldrich (St. Louis, MO, USA); NCS-382 was from Tocris (Bristol, UK). [^{3}H]NCS-382 (20 Ci/mmol) was obtained from ARC (St. Louis, MO, USA). [^{3}H]GABA (27.6 Ci/mmol) and [^{3}H]muscimol (28.5 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA).

Synthesis

The racemic compounds (*RS*)-3-hydroxycyclopent-1-enecarboxylic acid [(*RS*)-HOCPCA] and (*RS*)-3-hydroxycyclohex-1-enecarboxylic acid [(*RS*)-HOCHCA] were synthesized in several steps (Supplemental data, Scheme 1). Ethyl 2-oxocyclopentanecarboxylate and ethyl 2-oxocyclohexanecarboxylate were converted into the respective unsaturated esters (Beagley et al., 1989; Palaty and Abbott, 1995). Allylic oxidation of the unsaturated ethyl esters with CrO_3 by a procedure similar to that described previously for the methyl esters (Lange and Otulakowski, 1982; Lange et al., 1989) gave the respective enone esters (Mori, 1978). These were converted by selective reductions (Gemal and Luche, 1981; Cossy et al., 1995) with the aid of $NaBH_4$-$CeCl_3$ to the respective allylic alcohols. Treatment with aqueous Na_2CO_3 gave the unsaturated hydroxy acids. Finally these were resolved by chiral HPLC using a ChiralPak AS-H column into the pure enantiomers (*S*)-HOCPCA (98.1% ee) and (*R*)-HOCPCA (97.6% ee), and (*S*)-HOCHCA (97.6% ee) and (*R*)-HOCHCA (96.4% ee), respectively. The absolute configurations of (+)-(*R*)-HOCPCA and (+)-(*R*)-HOCHCA were established by close agreement between the measured Electronic Circular Dichroism spectra and those predicted from first principles for the conformational ensembles in solution by Time Dependent Density Functional Theory, and supported by agreement between the signs of the measured and predicted optical rotations (Supplemental data, Figure 1). (1*S*,2*S*)-2-(Hydroxymethyl)cyclopropanecarboxylic acid [(*S,S*)-

HOCPrCA] and (1*R*,2*R*)-2-(hydroxymethyl)cyclopropanecarboxylic acid [(*R,R*)-HOCPrCA] were obtained by stereoselective synthesis of (–)-menthyl (1*S*,2*S*)-2-(hydroxymethyl)cyclopropanecarboxylate and (+)-menthyl (1*R*,2*R*)-2-(hydroxymethyl)cyclopropanecarboxylate, respectively (Pajouhesh et al., 2000), followed by ester hydrolysis.

Assigned structures were in agreement with the ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra. Elemental analyses were performed at the Analytical Research Department, H. Lundbeck A/S, Denmark, or by J. Theiner, Microanalytical Laboratory, Department of Physical Chemistry, University of Vienna, Austria. Further details concerning synthesis and purification are given in *Supplemental data*.

Binding Assays

Membrane preparations.

All binding assays were performed using rat brain synaptic membranes of the cortex and central hemispheres from adult male Sprague Dawley rats with tissue preparation as earlier described by Ransom and Stec (1988). 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 48,000*g* for 10 min at 4°C. This washing step was repeated four times. The final pellet was resuspended in incubation buffer for the relevant binding assay.

[^{3}H]NCS-382, [^{3}H]muscimol, and [^{3}H]GABA binding assays.

The [^{3}H]NCS-382 binding assay described by Mehta et al. (2001) was adapted to a 96 well-format filtration assay (Kaupmann et al., 2003). For saturation binding experiments,

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[^{3}H]NCS-382 concentrations ranged from 1 to 3000 nM, whereas for competition studies, 16 nM [^{3}H]NCS-382 was used. Aliquots of membrane preparation (50-70 μg protein/aliquot) in KH_2PO_4 buffer (50 mM, pH 6.0) were incubated with [^{3}H]NCS-382 in triplicate at 0°C for 1 h in a total volume of 200 μl. Non-specific binding was determined using unlabeled GHB (1 mM). The binding reaction was terminated by rapid filtration through GF/C unifilters (PerkinElmer Life Sciences), using a 96 well Packard FilterMate cell-harvester, followed by washing with 3 x 250 μl of ice-cold binding buffer. Microscint scintillation fluid (PerkinElmer Life Sciences) was added to the dried filters, and the amount of filterbound radioactivity was quantified in a Packard TopCount microplate scintillation counter.

The assay of [^{3}H]muscimol binding to the $GABA_A$ receptors followed a similar protocol. Membranes (100 μg protein/aliquot) in Tris-HCl buffer (50 mM, pH 7.4) were incubated with [^{3}H]muscimol (5 nM), and 1 mM of inhibitor at 0°C for 60 min in a total volume of 250 μl. GABA (1 mM) was used to determine non-specific binding. Filtration through GF/B filters, washing, and quantification was identical to that of the [^{3}H]NCS-382 assay.

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 1 mM of inhibitor at 25°C for 45 min in 1 ml total volumes. Isoguvacine serves to saturate $GABA_A$ receptors (Hill and Bowery, 1981). Non-specific 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 filter-bound radioactivity was counted in a Packard Tricarb 2100 liquid scintillation analyzer using 3 ml of Opti-fluor scintillation fluid (PerkinElmer LifeSciences).

The Bradford (1976) protein assay was used for protein determination using bovine serum albumin as a standard, according to the protocol of the supplier (Bio-Rad, Milan, Italy).

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Data analysis.

The binding data was analyzed by non-linear regression curve-fitting using Graphpad Prism 4.0b (GraphPad Software Inc., San Diego, CA, USA). Where indicated, K_i values were calculated from IC_{50} values by means of the Cheng-Prusoff equation (Cheng and Prusoff, 1973).

Results

Inhibition of [^{3}H]NCS-382 binding.

For measuring the specific GHB binding sites in brain, [^{3}H]NCS-382 was used as a radioligand, as this compound does not interfere with GABA receptors, neither directly nor indirectly and thus discriminates between GHB and GABA sites. Under our experimental conditions, [^{3}H]NCS-382 displayed a single high-affinity binding site (K_d of 430 $\pm$ 1 nM and a B_{max} of 57 $\pm$ 6 pmol/mg of protein; data not shown). We have previously demonstrated a single high-affinity binding site in mouse brain using the same radioligand with similar affinity (K_d of 360 $\pm$ 60 nM) (Kaupmann et al., 2003). All generated inhibition curves were therefore fitted using a one-site model. GHB and known structural analogues (Fig. 1) were found to inhibit [^{3}H]NCS-382 binding (16 nM) in a concentration-dependent manner, and the rank-order of affinity was NCS-382 > T-HCA > GHB (Fig. 2A and Table 1), which is in agreement with the literature (Bourguignon et al., 2000; Mehta et al., 2001). The specific amount of bound [^{3}H]NCS-382 inhibited by 1 mM GHB was at least 90% of total binding.

Having established our assay to be reliable and reproducible, the affinities of the three novel GHB analogues synthesized in our laboratory were measured (depicted in Fig. 1). HOCHCA and HOCPCA were initially tested as racemic mixtures, whereas for HOCPrCA, which was synthesized from asymmetric precursors, both the (*R*,*R*)- and (*S*,*S*)-forms were tested. Table 1 shows that the racemic mixtures of HOCHCA and HOCPCA inhibit [^{3}H]NCS-382 binding with K_i values of 0.48 and 0.16 μM, respectively, indicating that these cyclic GHB analogues

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have correspondingly 9 and 27 times higher affinity than GHB itself. Interestingly, for the cyclopropyl analogue HOCPrCA, the (*R*,*R*)-form (K_i of 1.1 μM) was approximately 10-fold better than the (*S*,*S*)-form (Fig. 2B; Table 1). This prompted us to resolve the racemic mixtures of HOCHCA and HOCPCA into (*R*)- and (*S*)-forms and measure their affinities. As illustrated in Figure 2C and 2D respectively, (*R*)-HOCHCA (K_i of 0.48 μM) has 70-fold higher affinity than (*S*)-HOCHCA for GHB binding sites, and similarly does (*R*)-HOCPCA (K_i of 0.11 μM) have 13-fold higher affinity than (*S*)-HOCPCA. We also analyzed the importance of the hydroxyl group at the 3-position of HOCPCA by testing the corresponding ketone, 3-oxocyclopent-1-enecarboxylic acid (OxCPCA) (Fig. 1). The presence of an oxo group instead of a hydroxyl group greatly attenuated the ability to compete for [^{3}H]NCS-382 binding (Table 1). This demonstrates that the GHB binding site interacts stereospecifically with its substrates, and that the hydroxyl group of HOCPCA is essential for the high affinity of this compound.

Selectivity studies.

The selectivity of HOCHCA and HOCPCA for GHB binding sites over $GABA_B$ and $GABA_A$ receptors was investigated in binding assays, using tritiated GABA and muscimol for respective labeling of the receptors. In each case GABA inhibited binding as expected. Figure 3 and Table 2 show that GHB binds to $GABA_B$ receptors with low affinity (IC_{50} of 230 μM) as reported by others (Mathivet et al., 1997). However, IC_{50} values of the racemic mixtures of HOCHCA and HOCPCA are not reached at concentrations of 1 mM (changes in specific binding of -17 ± 3% and -14 ± 1%, respectively) (Fig. 3 and Table 2). Nor do the two compounds possess any affinity for the GABA site of the $GABA_A$ receptor at 1 mM concentrations, as measured by [^{3}H]muscimol binding (no significant decrease in specific binding) (Table 2). These results demonstrate that HOCHCA and HOCPCA are selective for GHB binding sites/GHB receptors over GABA receptors.

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Discussion

Although the pharmacological effects of GHB have been the subject of many investigations in recent years, the role of specific GHB receptors remains unclear. It has been demonstrated in numerous studies that GHB causes stimulation of $GABA_B$ receptors, thus complicating, and possibly masking, the measurement of native GHB receptor-mediated effects (Carai et al., 2001; Jensen and Mody, 2001; Nava et al., 2001; Barbaccia et al., 2002; Kaupmann et al., 2003; Ren and Mody, 2003). Consequently, the biological significance and mere existence of a GHB receptor has been questioned. The fact that unique, overlapping [^{3}H]GHB and [^{3}H]NCS-382 high-affinity binding sites exist in mammalian brain (Mehta et al., 2001; Gould et al., 2003), and that these remain intact in mice lacking functional $GABA_B$ receptors (Kaupmann et al., 2003; Wu et al., 2004), however, stand in favor of a distinct GHB binding site or receptor. This has already been suggested based on the differential expression profile and ontogeny of $GABA_B$ and specific GHB binding sites (Snead 1996). In the GHB field there is therefore a clear need for the development of potent and selective compounds.

In this work, we have explored the affinity and stereoselectivity of three cyclic ligands for the putative GHB receptor with a view to obtaining new lead structures and pinning down the precise geometry required for activity. For the first time we have separately examined the *trans*-enantiomers of HOCPrCA (Bourguignon et al., 2000). The cyclopentene and cyclohexene compounds are entirely novel as GHB ligands, and were designed to lock in place as far as possible the hydroxyl and carboxylate moieties, as distinct from the flexibility of the carbon chain of GHB, greatly reducing the conformational degrees of freedom and providing more precise information about the topography of the binding site. Our findings confirm prior observations that the partially conformationally restricted analogues, T-HCA and NCS-382, possess higher affinity than GHB for the receptor sites labeled by either [^{3}H]NCS-382 or [^{3}H]GHB (Bourguignon et al., 2000; Mehta et al., 2001). Compared with GHB, T-HCA contains a double bond between the α- and β-carbons, eliminating folded conformations and increasing the affinity by a factor 4. NCS-

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382 is somewhat more constrained by a semi-rigid seven-membered ring system and has approximately 15 times higher affinity than GHB, part of which may be attributed to the incorporation of a benzene ring. Nonetheless, NCS-382 still leaves a number of possibilities open for the optimal geometric relationship between the main pharmacophore elements, the hydroxyl and carboxylate moieties. In keeping with this theme of conformational restriction, the newly characterized analogue offering the greatest spatial repertoire for these groups, HOCPrCA, is also the weakest inhibitor, but the introduction of two stereocentres and observation of 10-fold stereoselectivity further narrows down the pharmacophore. In the two novel GHB structures, the α and γ carbons of GHB are locked in a ring for the first time, and the *R*-isomer of the cyclopentene derivative HOCPCA has as much as 39 times higher affinity than GHB, indicating that this structure closely mimics the bioactive conformation of the endogenous ligand. Like HOCPrCA, and in accordance with the observations made by Castelli et al. (2002) for NCS-382, the new analogues bind to the GHB receptor in a stereoselective manner. Thus the alignment of GHB at its specific recognition sites is essentially solved, and the structural preferences of the receptor in this vicinity largely mapped out, with the corollary that GHB most likely takes on a different conformation when binding to $GABA_B$ receptors.

The only major feature not pinpointed by these analogues is the hydroxyl proton. Since conversion of the hydroxyl group of HOCPCA to a carbonyl group greatly abolishes the affinity of this scaffold, it is tempting to conclude that the hydroxyl group donates an important hydrogen bond to the GHB receptor. However, it was recently shown that the less polar 3-chloropropanoic acid inhibits [^{3}H]NCS-382 binding with only slightly less efficiency than GHB, challenging the concept that hydrogen bond donation is essential for binding (Macias et al., 2004). Conversely, the oxo group in OxCPCA forces the distal hydrogen bond acceptor into the plane of the ring and the carboxylate group, a distinctly different arrangement from that of the active analogues, leaving the precise role of the hydroxyl proton yet to be determined.

With the demonstration that unlike GHB, the two best binding inhibitors HOCPCA and HOCHCA are devoid of affinity for the GABA receptors, these compounds represent exciting new tools for studying GHB pharmacology both *in vivo* and *in vitro*. An obvious advantage of HOCPCA and HOCHCA is their low-molecular weights, which makes them potential blood-brain-barrier penetrants, although this has not been tested as of yet. In this context, it will be necessary to determine the intrinsic activity of the compounds. Although isolated reports of functional assays have been described in the literature (Castelli et al., 2003; Kemmel et al., 2003; Brancucci et al., 2004), these remain to be validated by other laboratories. The compounds are also ideal lead structures, presenting ample opportunity for further substitution and structural elaboration, again with the advantage of low molecular weight and high binding efficiency of the scaffolds, bearing in mind that the synthesis of a radioligand with high specific activity could greatly aid in the molecular cloning of the GHB receptor, or lay the way for tomography studies. Also, combining the stereospecificity data obtained here with the structure-activity relationships from this and previous studies, paves the way for a more complete pharmacophore model of the extended GHB binding site. This work therefore offers a platform for further efforts in both pharmacology and medicinal chemistry, towards the final goal of characterizing and understanding the physiological role of the GHB receptor.

Acknowledgments

The authors would like to thank Shahrokh Padrah for technical assistance.

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Footnotes:

a)

This work was supported by the Danish Medical Research Council, Apotekerfonden of 1991, EU grant HPAW-CT-2002-80057 (PW), the Lundbeck Foundation, and the computer resources of the Danish Center for Supercomputing and the Australian Centre for Advanced Computing and Communications.

b)

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Legends for figures:

Fig. 1. Structures of γ-hydroxybutyrate (GHB), the reference compounds *trans*-4-hydroxycrotonic acid (T-HCA) and (*E*,*RS*)-(6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocyclohept-6-ylidene)acetic acid (NCS-382), and the novel GHB analogues 3-hydroxycyclohex-1-enecarboxylic acid (HOCHCA), 3-hydroxycyclopent-1-enecarboxylic acid (HOCPCA), 3-oxocyclopent-1-enecarboxylic acid (OxCPCA) and *trans*-2-(hydroxymethyl)cyclopropanecarboxylic acid (HOCPrCA). Chiral carbon atoms are marked by asterisk.

Fig. 2. Concentration-dependent inhibition of [^{3}H]NCS-382 binding to rat synaptic membranes by (A) the established compounds GHB, T-HCA and NCS-382, and by the novel cyclic analogues (B) (*S*,*S*)- and (*R*,*R*)-HOCPrCA, (C) (*S*)- and (*R*)-HOCHCA, and (D) (*S*)- and (*R*)-HOCPCA. Data shown are mean ± S. D. of a single representative experiment performed in triplicate. At least three individual experiments were carried out to calculate the average values summarized in Table 1.

Fig. 3. Comparison of the ability of GHB (—x—), (*RS*)-HOCHCA (■), and (*RS*)-HOCPCA (●) to inhibit [^{3}H]GABA binding from $GABA_B$ receptors in rat cerebrocortical membranes. Isoguvacine was added to saturate $GABA_A$ receptors. Data shown are from a single representative experiment performed in triplicate, which was repeated three times. Standard deviations were less than 5% and have been omitted for clarity. Average values are summarized in Table 2.

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TABLE 1.

Comparative inhibitory affinities of GHB and analogues on specific [^{3}H]NCS-382 (16 nM) binding in rat brain synaptic membranes.

IC_{50} values were calculated from inhibition curves and converted to K_i values. Each value is mean [pK_i ± S.E.M.] of at least three independent experiments carried out in triplicate.

Compound	K_i (μM) [pK_i ± S.E.M]	Affinity rel. to GHB
GHB	4.3 [5.4 ± 0.041]	1
T-HCA	1.1 [6.0 ± 0.02]	4.0
NCS-382	0.3 [6.5 ± 0.02]	15
(*R*,*R*)-HOCPrCA	1.1 [6.0 ± 0.03]	3.9
(*S*,*S*)-HOCPrCA	11 [5.0 ± 0.02]	0.41
(*RS*)-HOCHCA	0.48 [6.3 ± 0.08]	8.9
(*R*)-HOCHCA	0.48 [6.3 ± 0.0]	8.9
(*S*)-HOCHCA	34 [4.5 ± 0.05]	0.13
(*RS*)-HOCPCA	0.16 [6.8 ± 0.04]	27
(*R*)-HOCPCA	0.11 [6.9 ± 0.02]	39
(*S*)-HOCPCA	1.4 [5.8 ± 0.01]	3.0
OxCPCA	> 100	-

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TABLE 2.

Effects of GABA, GHB and cyclic analogues (1 mM) on [^{3}H]GABA (5 nM) binding to $GABA_B$ receptors and [^{3}H]muscimol (5 nM) binding to $GABA_A$ receptors in rat brain synaptic membranes. Each value is mean IC_{50} (μM) [$pIC_{50} \pm$ S.E.M.] for $GABA_B$ binding or mean K_i (μM) [$pK_i \pm$ S.E.M.] for $GABA_A$ binding of at least three independent experiments in triplicate.

Compound	[^{3}H]GABA IC_{50} [$pIC_{50} \pm$ S.E.M] $GABA_B$ receptor	[^{3}H]muscimol K_i [$pK_i \pm$ S.E.M] $GABA_A$ receptor
GABA	0.013 [7.9 ± 0.05]	0.049 [7.3 ± 0.06]
GHB	230 [3.7 ± 0.11]	*NA*
(*RS*)-HOCHCA	*NA*	*NA*
(*RS*)-HOCPCA	*NA*	*NA*

NA: no affinity, meaning IC_{50} > 1 mM

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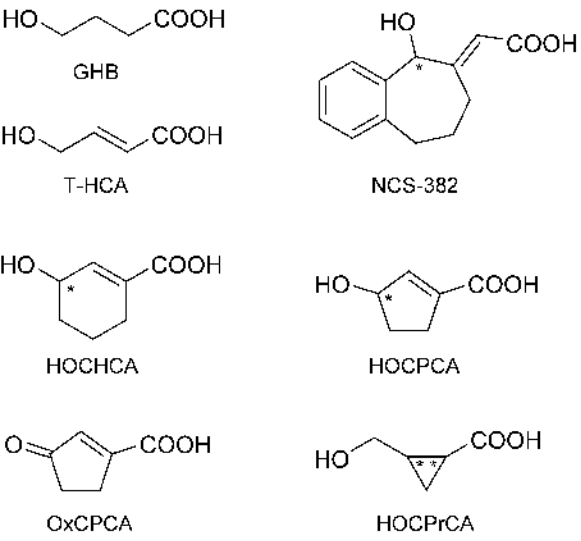

Figure 1

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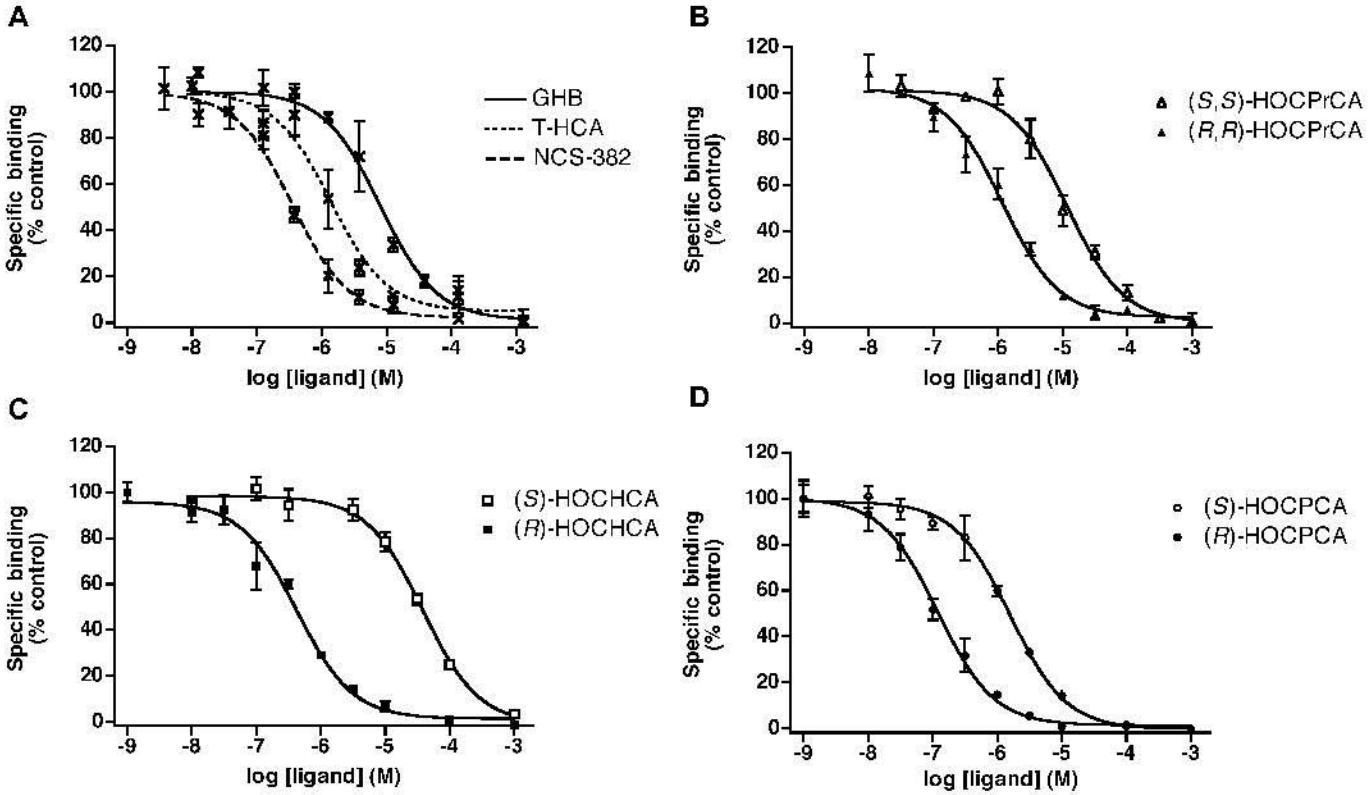

Figure 2

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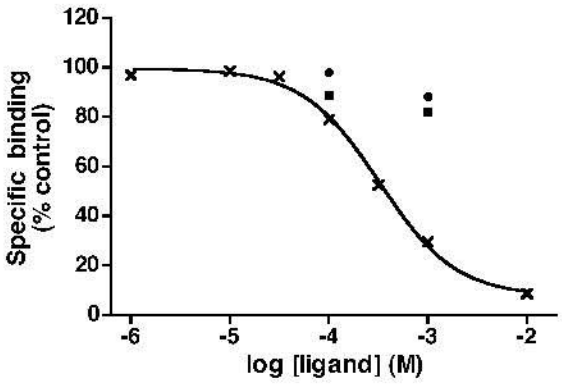
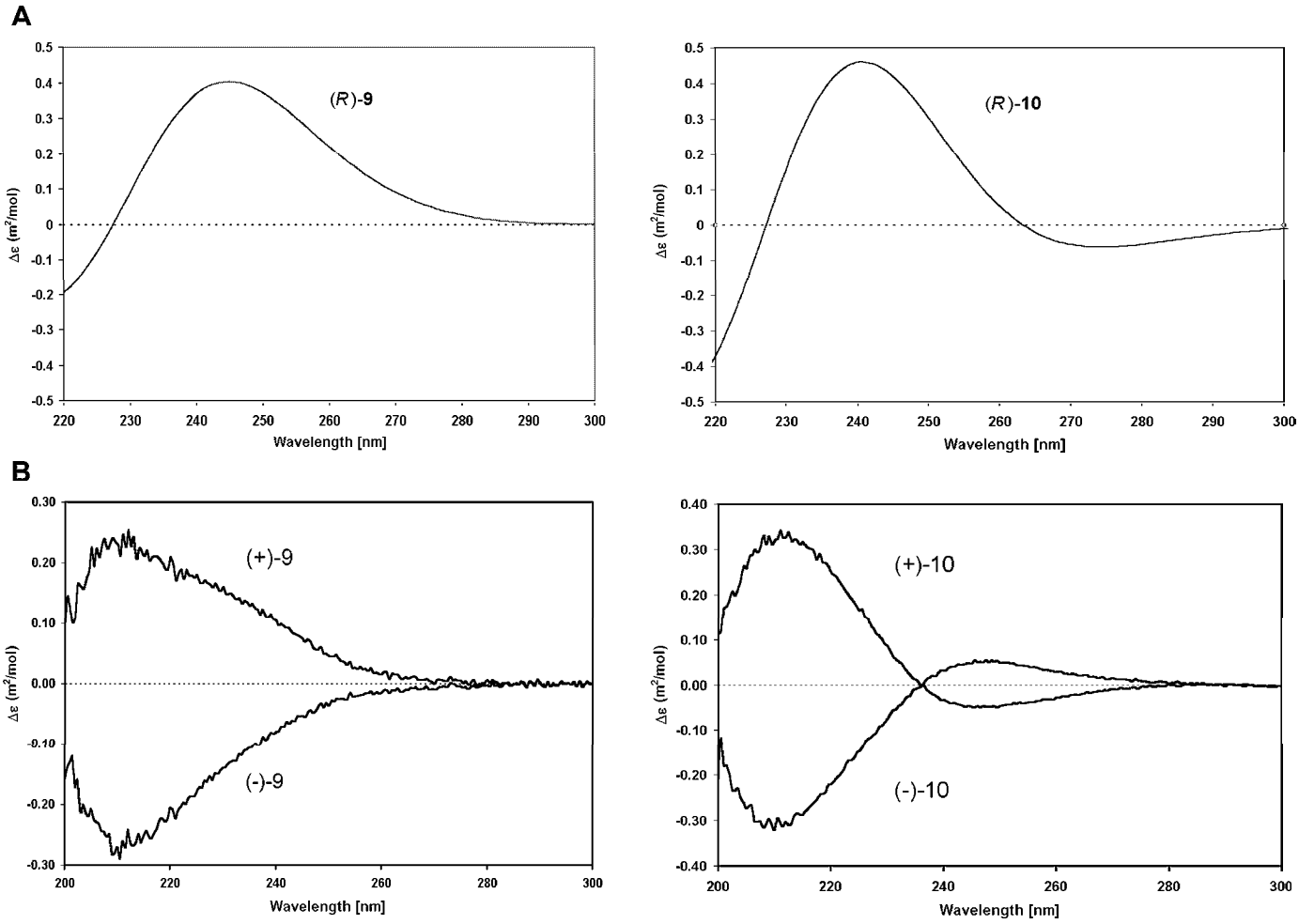


Figure 3



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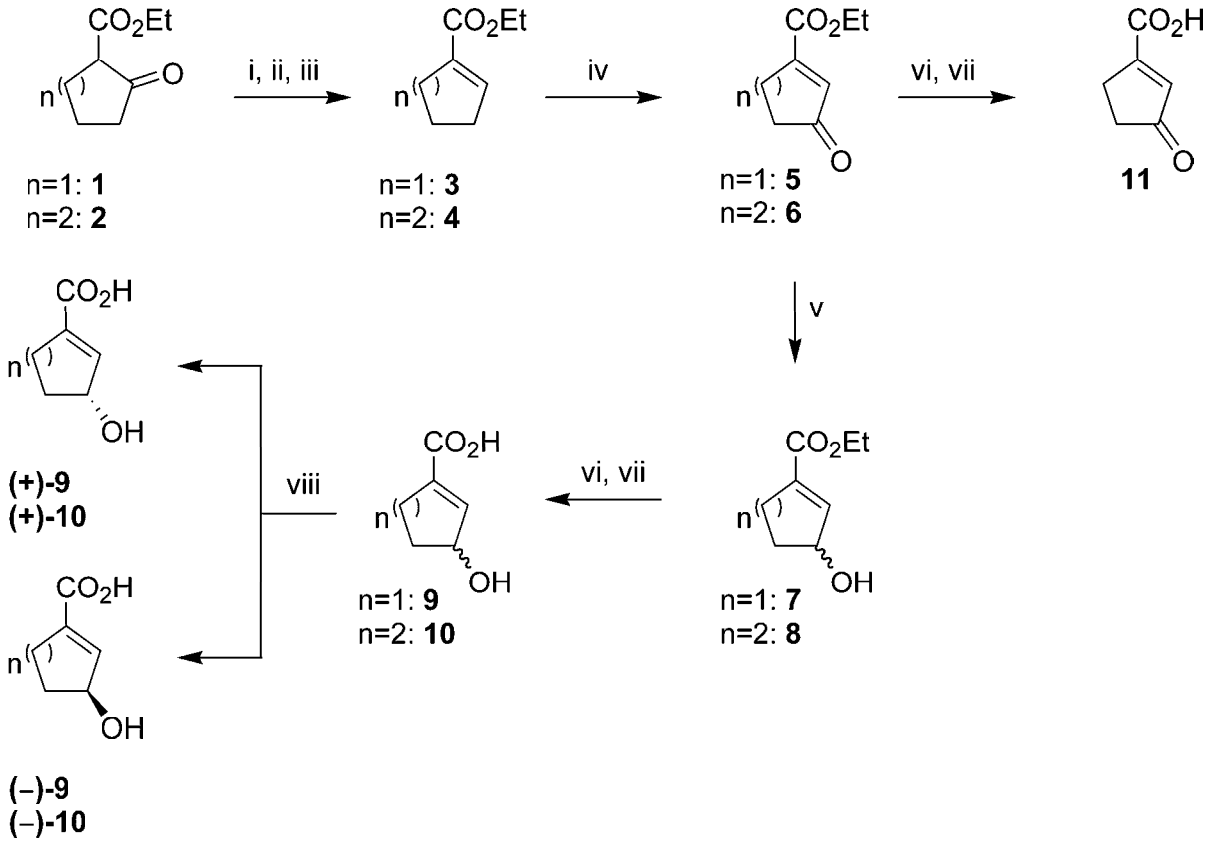

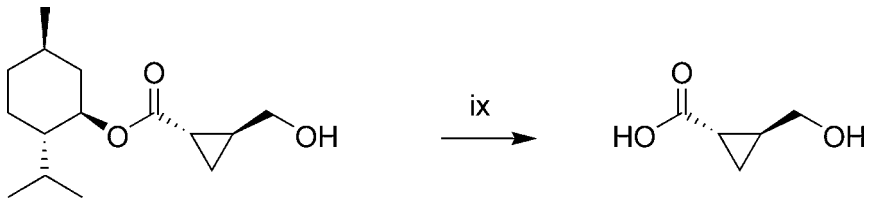

(–)-Menthyl (1*S*,2*S*)-2-(hydroxymethyl)-cyclopropanecarboxylate

Scheme1_supp_data