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Analogues of γ-Hydroxybutyric Acid. Synthesis and Binding Studies

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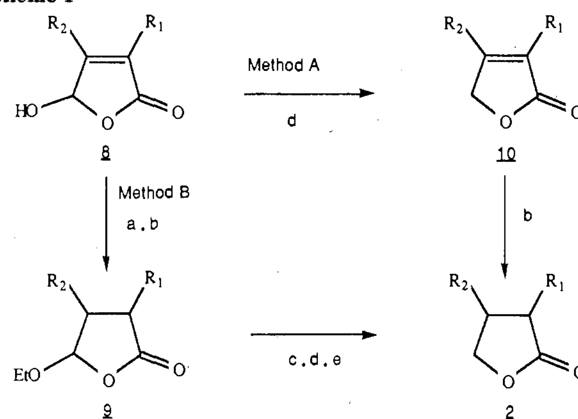
Substituted 4-hydroxybutyric (GHB) or *trans*-4-hydroxycrotonic acids (T-HCA) and structurally related compounds were synthesized and submitted to [^{3}H]GHB binding. Structure–activity relationship studies highlighted for [^{3}H]GHB binding (a) the necessity of a nonlactonic, relatively extended conformation of the γ-hydroxybutyric chain, (b) the existence of some bulk tolerance in the vicinity of the hydroxyl group, and (c) the high sensitivity toward isosteric replacements of the carboxyl or the hydroxyl groups. T-HCA has been recently identified as a naturally occurring substance in the central nervous system (CNS) and shows a better affinity than GHB. Our findings are in favor of the presence in the CNS of specific GHB binding sites, which are different from the GABA and the picrotoxin binding sites, and for which T-HCA may be an endogenous ligand.

γ-Hydroxybutyric acid (GHB) was first studied in 1960 by Laborit and his co-workers as an isostere of γ-aminobutyric acid (GABA) able to cross the blood–brain barrier and was proposed as an hypnotic and general anesthetic.[1] Later GHB was identified in the mammalian brain by Roth and Giarman as a reductive catabolite of succinic semialdehyde (SSA).[2] As GHB modulates dopaminergic activity[3] and plays a role in sleep regulation and in specific seizure states,[1] it is possible that GHB might be a neuromodulator or neurotransmitter in the central nervous system (CNS).[4,5] This hypothesis was then supported by the characterization for GHB of (a) a specific enzyme for the biosynthesis,[6] (b) a high affinity uptake system,[7,8] and (c) specific binding sites.[9,10] The present paper describes the synthesis of a series of GHB analogues and some structurally related compounds and examines structure–activity relationships with respect to [^{3}H]GHB binding.

Chemistry

The noncommerically available γ-butyrolactones **2** resulted from a sequence of $NaBH_4$[11] and catalytic reduction applied to 4-hydroxy-Δ^2-butenolides **8**[12–14] (method A) or from a $NaBH_4$ reduction of the 4-ethoxybutanolides **9**[12] (method B, Scheme I). Attempts to achieve reductions of 4-hydroxy-Δ^2-butenolides **8** to the corresponding butanolides **2** in a single step, using $NaBH_4$ in refluxing methanol,[15] were unsuccessful in our hands. As the 3-substituted 4-hydroxy-Δ^2-butenolides are now easily available,[12] method A constitutes a versatile and high-yield mode of preparation of 3-substituted butanolides **2** (R_1 = H) since other syntheses present some limitations.[16] On the other hand, the $NaBH_4$ reduction of 3-substituted 4-hydroxy-Δ^2-butenolides **8** in neutral medium represents an easy alternative to the Reformatsky-type or intramolecular Wittig syntheses of 3-substituted Δ^2-butenolides **10** (R_1 = H).[17–19] Attempts to prepare the sodium salt of the *cis*-4-hydroxycrotonic acid by ring opening of the crotonolactone gave usually rise to a complex mixture as a result of prototropic rearrangements.[11,20] Only the sodium salt of *cis*-3-methyl-4-hydroxycrotonic acid **11** (R_1 = H, R_2 = CH_3) was prepared in a satisfactory manner.

Scheme I[a]



[a] (a) EtOH, HCl_g, reflux; (b) H_2, Pd/C; (c) $NaOH/H_2O$; (d) $NaBH_4$, MeOH; (e) H_3O^+, Δ; (f) NaOH/EtOH, H_2O, room temperature.

trans-4-Hydroxycrotonic acid **12** (T-HCA) was prepared in good yield by an optimized alkaline hydrolysis of 4-

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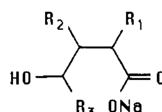
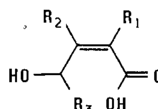
Table I. Physical Data of γ-Hydroxybutyrates 1



compd	R_1	R_2	mp, °C	^{1}H NMR[a]
1b	C_6H_5	H	230 dec	1.77–2.40 (m, 2 H), 3.50 (t, 2 H), 3.53 (t, 1 H), 7.28 (s, 5 H)
1c	H	CH_3	156	1.60–2.40 (m, 3 H), 0.90 (d, 3 H), 3.45 (d, 2 H)
1d	H	C_2H_5	160	0.73–1.10 (m, 3 H), 1.15–1.50 (m, 2 H), 1.66–2.40 (m, 3 H), 3.50 (d, 2 H)
1e	H	C_3H_7	146	0.63–1.06 (m, 3 H), 1.10–1.55 (m, 4 H), 1.73–2.27 (m, 3 H), 3.48 (d, 2 H)
1g	H	C_6H_5	260 dec	2.37–2.67 (m, 2 H), 2.97–3.57 (m, 1 H), 3.77 (m, 2 H), 7.33 (s, 5 H)
1h	H	$CH_2C_6H_5$	174	2.05–2.53 (m, 3 H), 2.60 (m, 2 H), 3.50 (d, 2 H), 7.25 (s, 5 H)
1i	CH_3	CH_3	132	0.93 (d, 3 H), 1.08 (d, 3 H), 1.43–2.47 (m, 2 H), 3.53 (dd, 2 H)

[a] NMR spectra were performed in D_2O for salts **1**.

Table II. Physical Data of γ-Hydroxycrotonic Acid Derivatives **11–13** and **16**



compd	R_1	R_2	R_3	yield, %	mp, °C	^{1}H NMR[a]
11[d]	H	CH_3	H	95[b]	220 dec	1.85 (d, 3 H), 4.32 (s, 2 H), 5.85 (m, 1 H)
12[22]	H	H	H	71	108 (AcOEt)	4.21 (dd, 2 H_4), 5.90 (dt, H_2), 6.90 (dt, H_3)
13a[39]	H	CH_3	H	78	110 ($CHCl_3$)	1.94 (s, 3 H), 3.90 (s, 2 H_4), 5.83 (m, H_2)
13b	H	C_6H_5	H	46	126 ($CHCl_3$/hexane)	4.23 (d, 2 H_4), 6.13 (m, H_2), 7.20 (s, 5 H)
16a	H	H	CH_3	*b, c*	>200	1.30 (d, 3 H), 4.39 (m, H_4), 5.85 (dd, H_2), 6.20 (m, H_3)
16b	H	H	*n*-C_6H_{11}	*c*	106 ($CHCl_3$)	1.1–2.0 (m, 1 OH), 2.6 (m, 1 H), 3.90 (m, H_4), 5.83 (dd, H_2), 6.77 (m, H_3)
16c	H	H	C_6H_5	*c*	94 ($CHCl_3$)	5.30 (dd, H_4), 6.05 (dd, H_2), 6.87 (dd, H_3), 7.38 (s, 5 H)

[a] NMR spectra were performed in $CDCl_3$ (**12**, **16b**), DMSO-d_6 (**13** and **16c**), and D_2O (**11**, **16a**). [b] Sodium salt. [c] See the Experimental Section. [d] All the compounds were in a trans configuration, except for the salt **11**.

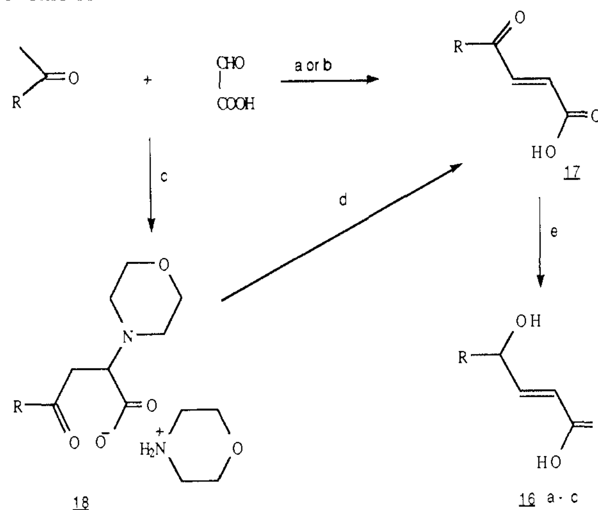
bromocrotonic acid. As observed by Allan in the 4-aminocrotonic series,[21] better yields are obtained when starting from the free 4-bromocrotonic acid rather than from ethyl 4-bromocrotonate as initially proposed.[22] The 3-substituted *trans*-4-hydroxycrotonic acids **13a**,**b** were prepared by performing the $NaBH_4$ reduction of **8** in alkaline aqueous medium.

Under these conditions, a mixture of the awaited *trans*-4-hydroxycrotonic acid **13** and **10** (lactone of the *cis*-4-hydroxycrotonic acid) was obtained, the acid **13** being easily separated from the neutral **10**. The 4-substituted *trans*-4-hydroxycrotonic acids **16a–c** were prepared by KBH_4 reduction of the corresponding *trans*-β-acylacrylic acids **17**. These latter compounds were prepared by condensing glyoxylic acid and methyl ketones by use of various reaction conditions (Scheme II). Refluxing a mixture of glyoxylic acid and morpholinium chloride in acetone afforded *trans*-β-acetylacrylic acid **17a** (R = CH_3). Heating glyoxylic acid and methyl cyclohexyl ketone with an excess of morpholine under experimental conditions described previously[23] gave the awaited β-hexahydrobenzoylacrylic acid **17b** via the Mannich base **18** (R = C_6H_{11}).

Other syntheses of substituted 4-hydroxycrotonates were recently reported.[24] However, they present the double disadvantage of being multistep syntheses leading to the ester or nitrile of the expected 4-hydroxycrotonic acid. A final hydrolysis step is then needed and can be a source of side reactions, as mentioned previously. The main physical data of the sodium salts **1** and 4-hydroxycrotonic acids **11**, **13**, and **16** are listed in Tables I and II respectively.

When 4-hydroxycrotonic acid derivatives **11–13** and **16** are compared with the butenolides **10** (doublet around 4.8

Scheme II[a]



[a] Access to 4-substituted THCA's: (a) R = CH_3, acetone, 1 equiv of morpholine hydrochloride, Δ, R = C_6H_5; (b) H_3PO_4, Δ; (c) R = C_6H_{11}, 2 equiv of morpholine base, EtOH, 60 °C; (d) AcOH, Δ; (e) KBH_4, $NaHCO_3$.

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Table III. GHB Receptor Affinities of GHB-Related Compounds

no.	compd	binding: % inhibition 10 μM	1 μM	IC_{50}, μM
	GHB	55	35	6.6 ± 0.2
1a	2-CH_3	24		
1b	2-C_6H_5	ns		
1c	3-CH_3	59		
1d	3-C_2H_5	43		
1e	3-C_3H_7	5		
1f	3-$CH(CH_3)_2$	ns		
1g	3-C_6H_5	ns		
1h	3-$CH_2C_6H_5$	ns		
1i	2,3-$(CH_3)_2$	ns		
1j	4-CH_3	63	12	
1k	4-C_6H_5	56	13	
3	$HO(CH_2)_2CO_2Na$	ns		
4	$HO(CH_2)_4CO_2Na$	53	22	
5	$HO(CH_2)_2SO_3Na$	ns		
6	$HO(CH_2)_3SO_3Na$	ns		
7	$HO(CH_2)_4SO_3Na$	ns		
11	*cis*-$HOCH_2C(CH_3)=CHCO_2Na$	ns		
12	*trans*-$HOCH_2CH=CHCO_2Na$ (T-HCA)	64	44	3.5 ± 0.4
13a	3-CH_3-T-HCA	55	11	
13b	3-C_6H_5-T-HCA	34	21	
16a	4-CH_3-T-HCA	60	36	11 ± 3
16b	4-C_6H_{11}-T-HCA	46	13	
16c	4-C_6H_5-T-HCA	70	39	3.3 ± 0.4
	GABA	ns		
	$HO(CH_2)_4OH$	ns		
	picrotoxin	ns		
	γ-BL	ns		
	γ-crotonolactone	ns		

ppm), they show an important downfield shift of 0.6–0.9 ppm for the allylic protons due to the absence of the electron-withdrawing effect of the lactone oxygen.[11] The same feature was observed in the saturated compounds series ($\Delta\delta \cong -0.9$ ppm for the γ-methylene protons between butanolides and their corresponding GHB sodium salts).

For binding assays performed at pH 6.0, GHB analogues were prepared as 1 M sodium salt solutions. ^1H NMR data discussed above were helpful for the evaluation of the lactone formation at a given pH and temperature in the study of GHB analogues. Between pH 8 (pH of the 1 M solution of sodium salt of GHB, **1c** or **1k**) and pH 5 (phosphate buffer in D_2O), no trace of lactone was detected by ^1H NMR analysis, even after an overnight standing at room temperature. However at 90 °C, an overnight heating at pH 5 of GHB, **1c**, or **1k** led to mixtures containing about 15% of lactonized compound. In a similar manner, no trace of **10b** was detected after an overnight standing of **11** at room temperature in the pH 9–5 range.

In other words, under the biological working conditions (pH 6 to pH 7.4), 4-hydroxybutyrate analogues can be assumed to be free from lactonic byproducts.

Results and Discussion

The compounds listed in Table III were first tested at a concentration of 10 μM for their ability to displace [^3H]GHB from its binding sites. If, at 10 μM, the compounds inhibited [^3H]GHB binding by more than 50%, compounds were studied at a concentration of 1 μM and then dose-inhibition curves were generated with eight concentrations of drug in triplicate incubations, and IC_{50} values were computerized by affinity spectra analysis[25] for the most active compounds. GHB binding studies[9,10] allowed the characterization of two subtypes of GHB binding sites. The high affinity binding site shows a K_D value of 95 nM with a low capacity (B_{max} = 0.56 pmol/mg of protein), whereas the lower affinity binding site (K_D = 16 μM) has a higher capacity (B_{max} = 46 pmol/mg of protein). In our binding assays, 20 nM concentrations of [^3H]GHB were used. Under these conditions, the high affinity, but also a part of the lower affinity binding sites, are labeled by [^3H]GHB. As a result of this binding-site heterogeneity, GHB shows a IC_{50} value of 6.6 μM. Nevertheless, our binding assays allow the identification of compounds that are more or less active than GHB. Moreover, they allow the identification of electronic and steric features leading to the loss of affinity of some GHB derivatives.

The fact that γ-butyrolactone and γ-crotonolactone were not recognized by [^3H]GHB-labeled binding sites suggests that an open form is necessary for recognition. Replacing the carboxylic acid of GHB by an isosteric sulfonic acid group (GHB vs **6**), or the hydroxyl function by an amino group (GHB vs GABA), and introducing a substituent in 2-position of GHB led to dramatic losses of activity. A methyl group is tolerated in the 3- or 4-position. A phenyl ring at the 4-position (GHB analogue **1k**) showed still some moderate affinity. As often seen in homologous series,[26] shortening the carbon chain of GHB (compound **3**) was detrimental for affinity, whereas lengthening it gave rise to a still reasonably potent compound (**4**).

T-HCA derivatives were systematically more active than their GHB homologoues. T-HCA showed a better affinity than GHB itself. As observed for GHB analogues, substitution at the 3-position led to less active compounds. Introduction of a methyl group in 4-position of T-HCA (compound **16a**) was also tolerated. Steric effects are illustrated by the decrease of affinity observed when the methyl group in **16a** was replaced by a cyclohexyl substituent (compounds **16b**). Interestingly, introduction of a phenyl ring in 4-position afforded a compound (**16c**) whose affinity was of the same order of magnitude than that of T-HCA. GHB binding sites seem to be specific, insofar that bioisosteric replacements of both the carboxylic acid or the alcoholic functions led to inactive compounds. The moderate affinity of 5-hydroxyvalerate (**4**) and the better affinity of T–HCA, when compared with GHB, are in favor of a semiextended or an extended conformation for GHB binding. As T-HCA and its 4-phenyl derivative **16c** are showing approximately the same activity in vitro, the latter compound can be considered as a more lipophilic T-HCA analogue and has been chosen for further in vivo pharmacological studies.

Taken together, our findings emphasize three points of importance for the GHB-ergic function. (a) GHB presents specific binding sites, particularly the fact that GABA does not compete with [^3H]GHB binding discards the old concept of GHB considered as a GABA isostere.[1,27] (b) It has been proposed that some alkyl-substituted butyrolactones presenting convulsant or anticonvulsant properties are ligands for the picrotoxin binding site.[28] As GHB is not acting on the [^3H]GHB binding sites under its lactonic form and as picrotoxin is not able to displace [^3H]GHB in our experiments, it cannot be further considered as a

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putative endogenous ligand of the picrotoxin binding sites chloride ionophore complex. However, the hypothesis that GHB may act on other biological systems under its lactonic form is not to be discarded. (c) T-HCA presents a higher affinity for the GHB binding sites than GHB itself; we have recently identified this compound as a naturally occurring substance in the CNS.[29] This point renders the system more complex, since T-HCA is not to be considered just as a synthetic semirigid analogue of GHB, but as a putative endogenous ligand competing with GHB for [^{3}H]GHB-labeled binding sites and possessing eventually specific functions.

Experimental Section

Melting points (uncorrected) were determined on a Kofler WME type melting point apparatus. ^{1}H NMR spectra were recorded on a Bruker WP-80 instrument by using the δ scale with reference to Me_4Si for $CDCl_3$ solutions and 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionic acid sodium salt for D_2O solutions. IR spectra were taken on a Philips SP 300 spectrometer using KBr disks. α-Methyl- and γ-phenylbutyrolactones, γ- and δ-valerolactones, and propane and butane sulfone were purchased from Aldrich Chemical Co. and crotonolactone and GABA from Fluka and Sigma, Respectively. β-Benzoylacrylic acid is a commercially available product from Janssen Chimica. Glyoxylic acid hydrate was a generous gift from the Société Française Hoechst, Paris, France. Peroxide-free diisopropyl oxide was used. Lactonic compounds were purified by using a Büchi GKR-50 bulb-to-bulb distillation apparatus.

Substituted γ-Hydroxybutyric Acid Sodium Salts 1. The appropriate lactone or sultone (3 mmol) was dissolved in a minimum of ethanol and treated with 29.5 mL of 0.10 N NaOH solution. The solution was gently heated on a steam bath for 15 min. The medium was evaporated under reduced pressure. The crude residue was taken up in 2-propanol and evaporated to dryness. The resulting gummy solid was triturated with dry ethyl ether, rapidly filtered by suction, and dried under reduced pressure in a dessicator containing silica gel. When the salts were highly hygroscopic, they were stored as 1 M solutions.

Substituted Dihydro-2(3*H*)-furanones 2. Method A: From 2(5*H*)-Furanones 10. A solution of 5 mmol of 10 in 20 mL of MeOH with 50 mg of 10% Pd/C catalyst was vigorously stirred under atmospheric pressure of hydrogen over a 3–4-h period. The recovered crude lactone was further purified by bulb-to-bulb distillation under reduced pressure.

Method B: From 5-Ethoxydihydro-2(3*H*)-furanones 9.[12] Ten millimoles of 9 in 20 mL of MeOH were heated at 70 °C in a steam bath with 1.1 mL of 10 N NaOH solution for 20 min. Then, the reaction medium was cooled at 4 °C, and 1.13 g (0.03 mol) of $NaBH_4$ was added in small portions with stirring. After about 1 h, the reaction medium was acidified to pH 1 with concentrated HCl, heated at 80 °C for 15 min, and evaporated to dryness. The crude compound was extracted with ethyl ether. After the drying and evaporation of the organic layer, the awaited lactone was purified by distillation under reduced pressure with a bulb-to-bulb apparatus. The purity of the compound was essentially controlled by TLC and NMR analysis: IR (neat) 1775 ± 10 cm^{-1} (C=O). 2,3-Dimethyl-,[30] 3-methyl-,[31] 3-isopropyl-,[32] 3-benzyl-,[33] and 3-phenylbutyrolactones[30] are already described in the literature. **2** ($R_1 = C_6H_5$, R_2 = H): bp 210 °C (15 mmHg); NMR ($CDCl_3$) δ 2.50–3.25 (m, 2 H), 4.05–4.35 (m, 1 H), 4.5–4.95 (m, 2 H), 7.20 (s, 5 H).

General Procedure for the Synthesis of Substituted 2-(5*H*)-Furanones 10. To a solution of 10 mmol of the corresponding 5-hydroxy-2(5*H*)-furanone **8**[12] in 20 mL of dry MeOH at 4 °C was added at 4 °C 1.13 g (30 mmol) of $NaBH_4$ in small portions. The mixture was stirred at room temperature for 1 h. After evaporation of MeOH, the crude residue was dissolved in water and acidified to pH 2 with HCl. Then, the product crystallized out **10a**[34] ($R_1 = C_6H_5$, R_2 = H) or **10**[35] (R_1 = H, R_2 = *i*-Pr) or was taken up with ethyl ether. Combined organic layers were washed with 10% $KHCO_3$ solution and then with brine and dried over $MgSO_4$. TLC of the crude (AcOEt/hexane, 1:2) may show by iodine visualization some traces (<5% by NMR) of the corresponding saturated lactone. Elimination of a head-distillation fraction, when bulb-to-bulb distillation was possible, or recrystallization in an appropriate solvent afforded the pure butenolide. After distillation, 3,4-dimethyl-2(5*H*)-furanone (**10e**)[17] ($R_1 = R_2 = CH_3$) gave rise to a solid: mp 35 °C; IR (film) 1745 ± 5 (C=O), 1635 ± 10 cm^{-1} (C=C). Physical data of the 3-methyl[36] [bp 190 °C (15 mmHg); NMR ($CDCl_3$) δ 2.11 (s, 3 H), 4.73 (s, 2 H), 5.78 (m, 1 H)] and 3-phenyl[37] [mp 92 °C from hexane; NMR ($CDCl_3$) δ 5.10 (d, 2 H), 6.25 (t, 1 H), 7.40 (s, 5 H)] compounds are consistent with those found in the literature.

Sodium Salt 11 of 4-Methyl-2(5*H*)-furanone (10b). The salt was prepared by a modified literature procedure.[20] To a solution of 97.1 mg (1 mmol) of **10b** in 10 mL of aqueous ethanol (1:1) was slowly added dropwise 10.5 mL of 0.1 N NaOH solution. The solution was left at room temperature for 10 min, carefully evaporated under vacuum, triturated with 2-propanol, and filtered by suction. TLC and ^{1}H NMR analyses did not show any trace of remaining butenolide **10b**. The NMR of **11** in the presence of TFA showed the quantitative recovery of **10b**.

***trans*-4-Hydroxycrotonic Acid (12, T-HCA).** To a cold solution of 12 g (72 mmol) of 4-bromocrotonic acid[38] in 120 mL of water was added dropwise 240 mL of a 2 M KOH solution in water. After the addition was completed, the solution was successively heated under reflux for 5 min (oil bath temperature 120 °C), cooled in an ice bath, and acidified with dilute H_2SO_4. The medium was evaporated under vacuum and extracted with ethyl ether. After the drying and evaporation of the solvent, the residue was chromatographied on a silica gel column eluted with a mixture of AcOEt/MeOH (97:3): yield 5.22 g of pure T-HCA (71%); mp (from AcOEt) 108 °C; ^{1}H NMR ($CDCl_3$) δ 7.05 (dt, 1 H, J_{12} = 15, J_{23} = 4 Hz), 6.05 (dt, 1 H, J_{13} = 2 Hz), 4.30 (dd, 2 H), 4.9–6.3 (br s, exchangeable D_2O, 2 H).

***trans*-3-Substituted-4-hydroxycrotonic Acids 13a and 13b.** In 260 mL of a $MeOH/H_2O$ (1:1) solution containing 3 g (75 mmol) of NaOH were dissolved 20 mmol of **10b** or **10d**. To the mixture was added 2.5 g (65.1 mmol) of $NaBH_4$ in five portions over a 20-min period. The reaction medium was left at room temperature with stirring for 2 h. After completion of the reaction, the mixture was acidified to pH 1 with cold HCl. The reaction medium was evaporated to dryness under vacuum and extracted with ethyl acetate. The combined organic layers were efficiently extracted with a 10% $KHCO_3$ solution. The aqueous phase was then collected, carefully acidified to pH 1 with HCl, and evaporated to dryness, and the residue was extracted with ethyl acetate. After the drying and evaporation of the solvent, the resulting solid was essentially pure as shown by TLC. A further appropriate recrystallization afforded pure 3-substituted T-HCA's **13a**[39] and **13b**.

***trans*-4-Substituted-4-hydroxycrotonic Acids 16a–c. Synthesis of *trans*-β-Acylacrylic Acids 17. (a) *trans*-β-Acetylacrylic Acid (17a).** A mixture of 9.2 g (0.1 mol) of glyoxylic acid hydrate and 12.3 g (0.1 mol) of ground morpholinium hydrochloride were stirred in 100 mL of acetone for 1 h. The medium was then refluxed in a balloon glass heater for 12 h. After the solution was cooled, the morpholinium hydrochloride crystallized out and was separated by filtration. After evaporation of the solvent, the gummy residue (13 g) was dissolved in water and extracted continuously with ethyl ether. Evaporation of ethyl

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ether afforded 7.1 g (61%) of β-acetylacrylic acid: mp 125 °C (lit.[40] mp 125–126 °C).

(b) *trans*-β-Hexahydrobenzoylacrylic Acid (17b). Morpholinium salt of α-morpholino-β-hexahydrobenzoylpropionic acid 18 was prepared via a procedure described previously.[24] An analytical sample was obtained by recrystallization from cyclohexane, mp 158 °C. The crude salt was generally used for the next step without any further purification. The crude **18** (50 mmol) was dissolved in 60 mL of AcOH containing 14 mL (3 equiv) of acetic anhydride and refluxed for 10 h. The disappearance of the starting material was monitored by TLC ($CHCl_3$/acetone, 9:1). The mixture was concentrated under vacuum, triturated with ethyl ether. The solid was separated by filtration, and the ethereal layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The crude solid **17b** was recrystallized from hexane: cummulative yield 50%; mp 122 °C, ^{1}H NMR (DMSO-d_6) δ 7.25 (d, 1 H), 6.69 (d, 1 H), 7.25 (d, 1 H, J_{AB} = 14.4 Hz), 2.1–1 (m, 11 H).

General Procedure for the Reduction of the *trans*-β-Acylacrylic Acids 17. To a well-stirred solution of 5 mmol of **17** in 10 mL of a 10% $KHCO_3$ solution at 4 °C was added 0.30 g (5.6 mmol) of KBH_4 in few portions. The medium was left at room temperature for 3 h. The mixture was cooled and acidified with a 6 N HCl solution. The aqueous layer was extracted with ethyl acetate portions. The combined extracts were washed with water, dried, and evaporated in vacuo, giving a crude oil, which generally crystallized over standing at room temperature. Recrystallization from $CHCl_3$ afforded the pure 4-substituted T-HCA derivative **16**. **16a** sodium salt: ^{1}H NMR (D_2O) δ 1.3 (d, 3 H), 6.58 (dd, 1 H), 5.93 (dd, 1 H, J_{23} = 16.8 Hz), 4.44 (dd, 1 H, J_{34} = 5.4, J_{24} = 2.4 Hz). **16b**: ^{1}H NMR ($CDCl_3$) δ 6.77 (dd, 1 H), 5.84 (dd, 1 H, J_{23} = 16.8 Hz), 3.90 (m, 1 H, J_{24} = 2.4, J_{34} = 5.6 Hz), 1.0–2.1 (m, 11 H), 6.7 (br s, OH). **16c**: ^{1}H NMR (DMSO-d_6) δ 7.38 (s, 5 H), 6.87 (dd, 1 H), 6.04 (dd, 1 H, J_{23} = 15 Hz), 5.30 (dd, 1 H, J_{24} = 2.4, J_{34} = 4.8 Hz), 8.1 (br s, exch/D_2O, OH).

Binding Studies. [2,3-^{3}H]GHB potassium salt (sp act. 40 Ci/mmol) was obtained from the CEA. Radiochemical purity was checked by thin-layer chromatography in three different solvent systems. Crude membranes from rat brain were prepared by homogenization with a polytron of a crude fraction (P_2 fraction) in ice-cold deionized water. Membranes were centrifuged at 50000*g* for 20 min and stored overnight at −20 °C. For binding assays, membrane suspensions were thawed, washed twice more with 20 volumes of ice-cold deionized water, and resuspended in the incubation medium. Buffer used for binding assays consisted of potassium phosphate (KH_2PO_4/K_2HPO_4), 50 mM, pH 6.0, at 0 °C. Different ions (Ca^{2+}, Mg^{2+}, Cl^-) were tested on the binding, but no significant variations were observed.

Binding experiments were carried out in small tubes, each containing 100 μL of membrane suspension (about 1 mg of protein), 300 μL of buffer containing [^{3}H]GHB at the desired concentration and 200 μL of buffer with or without the test substance. After incubation for 30 min at 0 °C, the tubes were centrifuged at 55000*g* for 20 min in a Beckman R 25 rotor. The supernatant was aspirated, and the pellet was rapidly washed twice with 800 μL of ice-cold incubation buffer. Pellets were then resuspended in 250 μL water by vortexing, and the tritium label was determined by liquid scintillation spectrometry in 10 mL of Rotiszint 22 (Roth, West Germany).

The specific [^{3}H]GHB binding was linear in the range of protein concentrations used (between 0.5 and 2 mg/600 μL incubation medium). Specific binding reached equilibrium at 0 °C after 10 min of incubation. However, an incubation time of 30 min at 0 °C was usually adopted. Thin-layer chromatographic analysis of the bound [^{3}H]GHB displaceable by 5 mM nonradioactive GHB showed that at least 98% of the radioactivity was [^{3}H]GHB.

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Registry No. **1b**, 112895-80-4; **1c**, 112895-77-9; **1f**, 112895-78-0; **1g**, 40951-19-7; **1h**, 112895-79-1; **1i**, 112895-76-8; **1j**, 56279-37-9; **1k**, 83306-76-7; **2** (R_1 = R_2 = Me), 72693-07-3; **2** (R_1 = H, R_2 = Me), 1679-49-8; **2** (R_1 = H, R_2 = Pr-*i*), 10547-88-3; **2** (R_1 = H, R_2 = CH_2Ph), 22530-98-9; **2** (R_1 = H, R_2 = Ph), 1008-73-7; **2** (R_1 = Ph, R_2 = H), 6836-98-2; **3**, 6487-38-3; 4, 37435-69-1; **5**, 1562-00-1; **6**, 3542-44-7; **7**, 31465-25-5; **8** (R_1 = R_2 = Me), 1575-54-8; **8** (R_1 = H, R_2 = Me), 40834-42-2; **8** (R_1 = H, R_2 = Pr-*i*), 7755-27-3; **8** (R_1 = H, R_2 = CH_2Ph), 112895-74-6; **8** (R_1 = H, R_2 = Ph), 78920-11-3; **8** (R_1 = Ph, R_2 = H), 66661-71-0; **9** (R_1 = R_2 = Me), 112895-73-5; **9** (R_1 = H, R_2 = Me), 78920-23-7; **9** (R_1 = H, R_2 = Pr-*i*), 78920-26-0; **9** (R_1 = H, R_2 = CH_2Ph), 78939-70-5; **9** (R_1 = H, R_2 = Ph), 78920-27-1; **9** (R_1 = Ph, R_2 = H), 99558-18-6; **10** (R_1 = Ph, R_2 = H), 57200-23-4; **10** (R_1 = H, R_2 = Pr-*i*), 10547-89-4; **10** (R_1 = R_2 = Me), 1575-46-8; **10** (R_1 = H, R_2 = Me), 6124-79-4; **10** (R_1 = H, R_2 = Ph), 1575-47-9; (*Z*)-**11** (R_1 = R_3 = H, R_2 = Me)·Na, 112895-83-7; (*E*)-**12** (R_1 = R_2 = R_3 = H), 24587-49-3; (*E*)-**12** (R_1 = R_2 = R_3 = H)·Na, 112895-84-8; (*E*)-**13a** (R_1 = R_3 = H, R_2 = Me), 44647-19-0; (*E*)-**13a** (R_1 = R_3 = H, R_2 = Me)·Na, 112895-85-9; (*E*)-**13b** (R_1 = R_3 = H, R_2 = Ph), 99389-53-4; (*E*)-**13b** (R_1 = R_3 = H, R_2 = Ph)·Na, 112895-86-0; (*E*)-**16a** (R_1 = R_2 = H, R_3 = Me), 32748-41-7; (*E*)-**16a** (R_1 = R_2 = H, R_3 = Me)·Na, 112895-87-1; (*E*)-**16b** (R_1 = R_2 = H, R_3 = C_6H_{11}), 7465-09-0; (*E*)-**16b** (R_1 = R2 = H, R_3 = C_6H_{11})·Na, 112895-88-2; (*E*)-**16c** (R_1 = R_2 = H, R_3 = Ph), 99389-54-5; (*E*)-**16c** (R_1 = R_2 = H, R_3 = Ph)·Na, 112895-89-3; (*E*)-**17a**, 2833-28-5; (*E*)-**17b**, 112895-82-6; (*E*)-**17c**, 17812-07-6; 18 (R = C_6H_{11}), 112895-81-5; (*E*)-$BrCH_2CH{=}CHCO_2H$, 13991-36-1; 4-ethoxy-2,3-dimethyl-2-butenolide, 79379-71-8; 4-ethoxy-3-methyl-2-butenolide, 78920-13-5; 4-ethoxy-3-isopropyl-2-butenolide, 78920-16-8; 4-ethoxy-3-benzyl-2-butenolide, 78920-28-2; 4-ethoxy-3-phenyl-2-butenolide, 78920-17-9; 4-ethoxy-2-phenyl-2-butenolide, 112895-75-7.

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