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Structure–activity studies of fluoroalkyl-substituted γ-butyrolactone and γ-thiobutyrolactone modulators of $GABA_A$ receptor function

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Abstract

Dihydro-2(3*H*)-furanones (γ-butyrolactones) and dihydro-2(3*H*)-thiophenones (γ-thiobutyrolactones) containing fluoroalkyl groups at positions C-3, C-4, and C-5 of the heterocyclic rings were prepared. The anticonvulsant/convulsant activities of the compounds were evaluated in mice. Brain concentrations of the compounds were determined and the effects of the compounds on [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to the picrotoxin site on $GABA_A$ receptors were investigated. The effects of the compounds on $GABA_A$ receptor function were studied using electrophysiological methods and cultured rat hippocampal neurons. Fluorination at C-3 results in either subtle or pronounced effects on the pharmacological activity of the compounds. When hydrogens are replaced with fluorines at the methylene carbon of an ethyl group, as in 3-(1,1-difluoroethyl)dihydro-3-methyl-2(3*H*)-furanone (**1**), the anticonvulsant actions of the compound are not much changed from those found for the corresponding alkyl-substituted analogue. In marked contrast, fluorination at the methyl carbon of the ethyl group, as in dihydro-3-methyl-3-(2,2,2-trifluoroethyl)-2(3*H*)-furanone (**3**), produces a compound having convulsant activity. This convulsant activity seems to be due to an increased affinity of the compound for the picrotoxin site on $GABA_A$ receptors caused by an interaction that involves the trifluoromethyl group. Results obtained with γ-butyrolactones containing either a 3-(1-trifluoromethyl)ethyl or a 3-(1-methyl-1-trifluoromethyl)ethyl substitutent indicate that the interactions of the trifluoromethyl group with the picrotoxin binding site are subject to both stereochemical and steric constraints. Sulfur for oxygen heteroatom substitution, as in the corresponding γ-thiobutyrolactones, affects the type (competitive, noncompetitive, etc.) of binding interactions that these compounds have with the picrotoxin site in a complex manner. Fluorination of alkyl groups at the C-4 and C-5 positions of γ-butyrolactones having convulsant activity increases convulsant potency.

Key words: γ-Butyrolactones, γ-thiobutyrolactones, picrotoxin, $GABA_A$ receptor, fluoroalkyl.

1. Introduction

Alkyl-substituted dihydro-2(3*H*)-furanones (γ-butyrolactones, GBLs) and dihydro-2(3*H*)-thiophenones (γ-thiobutyrolactones, TBLs) have been shown to be allosteric modulators of $GABA_A$ receptor (GR) function. Depending on the size and location of the alkyl substituents on the heterocyclic rings, these GBLs and TBLs either potentiate or block GABA-mediated chloride currents. In many cases, those compounds,

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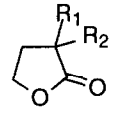
which potentiate these currents, have anticonvulsant properties and those that block these currents are convulsants [1–4].

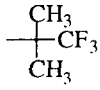
The structure-activity relationships governing the modulatory actions of these compounds at GRs are not understood completely. Whereas it was originally hypothesized that both the positive and negative modulatory actions of these compounds were explained by their binding to the picrotoxin binding site located on the GR [5,6], it is now clear from electrophysiological studies carried out on mutated forms of the GR that only their negative modulatory actions are correlated with binding to the picrotoxin site [7]. The positive modulatory effects of the compounds are a consequence of ligand binding to a separate site. This putative positive modulatory site is distinct from the benzodiazepine, barbiturate, and neurosteroid binding sites and is referred to by us as the 'lactone site' [8]. The development of new anticonvulsant drugs acting via the lactone site requires not only a detailed knowledge of those structural features required of a ligand for molecular recognition at the lactone site, but also an understanding of the structural features important for molecular recognition of ligands at the picrotoxin site. This information may make it possible to develop compounds which have high affinity for the lactone site and little or no affinity for the picrotoxin site. With this long term goal in mind, we have expanded on our earlier studies of fluoroalkyl-substituted GBLs and TBLs [9,10]. Reported herein is the synthesis of a group of fluoroalkyl-substituted GBLs and TBLs and their anticonvulsant/convulsant activities in mice. The effect of the compounds on [^{35}S]-*tert*-butylbicyclophosphorothionate ([^{35}S]TBPS) binding to the picrotoxin site on GRs and electrophysiological measurements of their actions on GR function in rat hippocampal neurons are also reported. In most cases, brain concentrations of the compounds were determined so that the relevance of their actions on GR function to their behavioral effects could be evaluated.

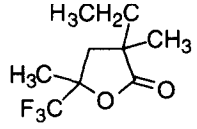
2. Chemistry

The structures of the fluoroalkyl-substitued GBLs and TBLs used in this study are shown in Chart 1. All compounds with ring stereocenters were used as racemates. Lactone **1** was prepared as described previously [9]. Lactones **2** and **3** were prepared by alkylation of dihydro-2(3*H*)-furanone and dihydro-3-methyl-2(3*H*)-furanone, respectively, using LDA as base and 2-iodo-1,1,1-trifluoroethane as the alkyl halide. Lactones **4a** and **4b** were prepared using ylid chemistry. The α-(O,O-diethylphosphonate) derivative of dihydro-2(3*H*)-furanone [11] was treated with NaH and reacted with 1,1,1-trifluoroacetone to give the intermediate dihydro-3-(1-trifluoromethylethylidene)-2(3*H*)-furanone. This intermediate was then hydrogenated in the presence of Pd-C catalyst to give the diastereomeric products **4a** and **4b** which were separated by preparative HPLC. The relative configuration of the hydrogens on the adjacent chiral centers in these diastereomers was not determined.

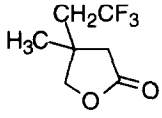
A general method that should be useful for the laboratory scale synthesis of compounds containing the



	R_1	R_2
1	CH_3	CF_2CH_3
2	H	CH_2CF_3
3	CH_3	$CH2CF_3$
4a	H	
4b	H	
5	H	



6a
6b



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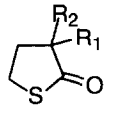


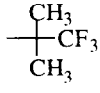
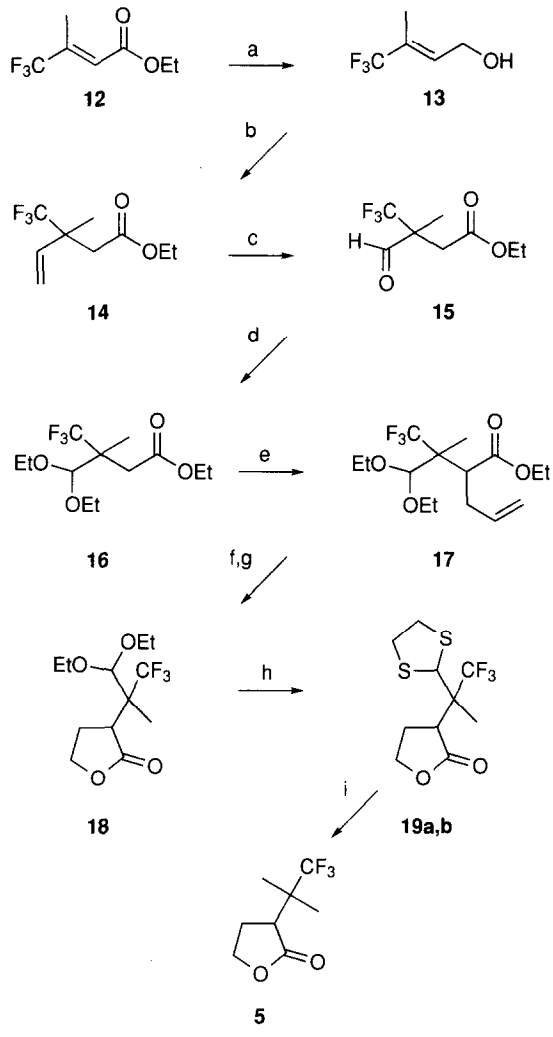
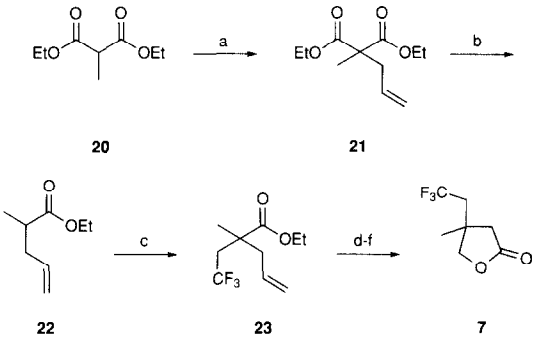
	R_1	R_2
8	CH_3	CF_2CH_3
9	CH_3	CH_2CF_3
10a	H	
10b	H	
11	H	

Chart 1.



Scheme 1. [a](a) DIBALH, hexanes, −25°C; (b) $CH_3C(OEt)_3$, CH_3CH_2COOH, Δ; (c) O_3, CH_2Cl_2, −78°C; (d) $HC(OEt)_3$, *p*-TsOH; (e) LDA, $CH_2{=}CHCH_2Br$, THF; (f) O_3 CH_2Cl_2, −78°C; (g) $NaBH_4$, EtOH; (h) $HSCH_2CH_2SH$, BF_3 HOAc; (i) Raney Ni, EtOH, Δ.

(1-methyl-1-trifluoromethyl)ethyl (MTFME) group was developed for the synthesis of lactone **5** (Scheme 1). Ester **12** was prepared according to a literature procedure [12] and reduced with DIBALH to give the known alcohol **13** in 72% yield [13]. An ortho ester Claisen reaction [14], in which alcohol **13** was refluxed with triethylorthoacetate and propionic acid in DMF gave ester **14** (29%). The low yield for this reaction is chiefly explained by the difficulty encountered in the purification of the product. Ozonolysis of ester **14** gave the aldehyde-ester **15** in 93% yield. For our purposes, the aldehyde group of compound **15** was protected as the acetal derivative **16** (98%) so that the remaining carbon atoms needed to construct the lactone ring could be



Scheme 2. [a](a) NaH, $CH_2{=}CHCH_2Br$, THF; (b) $DMSO/H_2O$/NaCl, Δ; (c) LDA, CF_3CH_2I, THF; (d) O_3 1:1 $MeOH/CH_2Cl_2$, −78°C; (e) Jones oxidation; (f) DIBALH, toluene, −78°C.

introduced by an alkylation reaction. However, reaction of compound **15** with 1,2-ethanedithiol followed by desulfurization with Raney Ni can be utilized to prepare 3-methyl-3-trifluoromethylbutyric acid ethyl ester as a synthon for the preparation of compounds containing the MTFME group [15]. In the present case, the alkylation of compound **16** with allyl bromide/LDA was used to prepare pentenoic acid derivative **17** (65%), which was then converted into lactone **18** (76%) using a previously developed ozonolysis, reduction, and lactonization procedure [3]. Treatment of lactone **18** with 1,2-ethanedithiol in BF_3·HOAc gave a mixture of the readily separated lactone diastereomers **19a** and **19b** (75%), and desulfurization of this mixture with Raney Ni completed the synthesis of lactone **5** (66%).

Carbinols containing a trifluoromethyl group are readily prepared by reacting ketones with trimethyl(trifluoromethyl)silane [16]. Accordingly, lactone diastereomers **6a** and **6b** were prepared (94% yield) by acid catalyzed lactonization of the fluorinated carbinol that results from the reaction of 2-ethyl-2-methy-4-oxopentanoic acid methyl ester [17] with trimethyl(trifluoromethyl)silane. These lactone diastereomers were separated by semi-preparative HPLC. The relative configuration of the substituents at the chiral centers in the compounds was not determined.

Lactone **7** was prepared by the reaction sequence shown in Scheme 2. Diester **20** was alkylated using NaH/allyl bromide to afford diester **21** (70%) [18,19], and this diester was decarboethoxylated with DMSO/H_2O/NaCl to give the olefinic ester **22** (65%) [20]. Compound **22** was then alkylated with LDA/2-iodo-1,1,1-trifluoroethane to obtain olefinic ester **23** (56%), and this ester was directly converted into lactone **7** (55%) using a previously developed reaction sequence (ozonolysis, oxidation with Jones reagent, and DIBALH reduction) [3]. Thiolactones **8–11** were prepared by heating the corresponding lactone precursors with potassium thioacetate in DMA, as described previously [3].

3. Pharmacology

Results describing the convulsant or anticonvulsant/neurotoxicity profiles of fluorinated compounds **1–11**, their IC_{50} values for the displacement of [^{35}S]TBPS, and brain concentrations of the compounds when administered to mice at their respective convulsant CD_{50} or anticonvulsant ED_{50} values are summarized in Table 1. As reported previously, lactone **1**, which contains a 1,1-difluoroethyl group, is an anticonvulsant [9]. This compound is a weak displacer of [^{35}S]TBPS binding, and its brain concentration when administered at an ED_{50} dose is about one third of its IC_{50} value. Lactones **2** and **3**, which contain a 1,1,1-trifluoroethyl group, are convulsants. Only disubstitued lactone **3** was studied in greater detail. Lactone **3** is about 30 times more potent than lactone **1** as a displacer of [^{35}S]TBPS, and its brain concentration at a CD_{50} dose is ~60% of its IC_{50} value.

Lactone diastereomer **4a** is a convulsant, whereas lactone diastereomer **4b**, at neurotoxic concentrations, is an anticonvulsant. The convulsant diastereomer is more potent as a displacer of [^{35}S]TBPS having an IC_{50} value about 4 times smaller than the IC_{50} value of the anticonvulsant diastereomer. The brain concentrations of these compounds at their respective CD_{50} and ED_{50} doses are similar to their IC_{50} values. Lactone **5** is a convulsant. The small amount of this compound that was available for study limited its in vivo evaluation. In the [^{35}S]TBPS binding assay, the compound was found to be less potent than convulsant diastereomer **4a**, but more potent than anticonvulsant diastereomer **4b** in its ability to displace this radioligand.

Lactone diastereomers **6a** and **6b** are both convulsants. The CD_{50} of diastereomer **6a** is lower than that of diastereomer **6b**, but there is considerable overlap of the 95% fiducial limits associated with these values. The compounds are equally potent as displaceres of [^{35}S]TBPS and each compound at its CD_{50} dose is present in brain tissue at a concentration ~30% higher than the IC_{50} values. Lactone **7** is a potent convulsant. It is also the most potent displacer of [^{35}S]TBPS found in this study. At its CD_{50} dose, it is present in brain tissue at a concentration which is five times higher than its IC_{50} value.

The results shown in Table 1 for thiolactones **8–11** closely parallel those found for the lactone congeners. Notable differences are the enhanced neurotoxicity which thiolactone **8** has relative to that found for lactone **1** and the lower IC_{50} value which thiolactone **9** has relative to that found for lactone **3**. As was the case for lactone diastereomers **4a** and **4b**, one of the thiolactone

Table 1
Behavioral effects, [^{35}S]TBPS binding data, and brain concentrations of fluorinated compounds

Compound	Behavioral activity		Rotorod toxicity TD_{50} (mg/kg)[c]	[^{35}S]TBPS displacement IC_{50} (μM)[d]	Brain concentration (μM)[e]
	ED_{50} (mg/kg)[a]	CD_{50} (mg/kg)[b]			
1	243 (215–275)[f]	—	> 500	3160 ± 160	900 ± 90
2	—	90 (63–116)	—	NDg	ND
3	—	30 (20–40)	—	105 ± 4	62 ± 2
4a	—	30 (14–46)	—	142 ± 6	121 ± 15
4b	315 (261–379)	—	265 (195–347)	565 ± 61	857 ± 36
5	—	< 300 [4/5][h]	—	290 ± 20	ND
6a	—	47 (30–100)	—	224 ± 9	302 ± 5
6b	—	116 (90–138)	—	267 ± 23	370 ± 30
7	—	3 (2–3)	—	8 ± 9	41 ± 9
8	179 (131–250)	—	219 (131–350)	526 ± 32	355 ± 40
9	—	18 (14–22)	—	30 ± 1	44 ± 9
10a	211 (145–365)	—	> 330	139 ± 3	243 ± 41
10b	—	59 (31–82)	—	50 ± 6	141 ± 36
11	ND	ND	ND	600 ± 40	ND

[a]Dose at which 50% of the mice were protected from clonic seizures induced by pentylenetetrazole (85 mg/kg). At least four groups of six mice each were tested to obtain ED_{50}, CD_{50}, and TD_{50} values. Complete experimental details for these evaluations have been described previously [3].

[b]Dose at which 50% of the mice had clonic convulsions.

[c]Dose at which 50% of mice failed the rotorod toxicity test [3].

[d]Binding data are presented as the mean ± SEM of three experiments performed in triplicate. Other experimental details, including IC_{50} values for TBPS and picrotoxinin, were as described previously [4].

[e]Determined 30 min after an intraperitoneal injection of an ED_{50} dose (versus PTZ-induced seizures) or at the onset of clonic seizures (or within 5 min after injection) of a CD_{50} dose.

[f]Numbers in parentheses are the 95% fiducial limits.

[g]ND, not determined.

[h]Four out of five animals had clonic seizures at this dose.

Table 2
Scatchard analysis for the effect of fluorinated isopropyl- and *t*-butyl-substituted compounds on [^{35}S]TBPS binding[a]

Compound	K_D (nM)		B_{max}	
	Control	Control + compound	Control	Control + compound
4a (140 μM)	25.0 ± 3.0	50.3 ± 8.8*	1.22 ± 0.16	1.23 ± 0.16
4b (560 μM)	20.8 ± 0.9	55.1 ± 5.9*	0.94 ± 0.05	0.96 ± 0.12
5 (290 μM)	21.6 ± 3.3	49.1 ± 6.0*	1.05 ± 0.26	0.78 ± 0.21*
10a (140 μM)	20.8 ± 0.9	62.3 ± 11.3*	0.94 ± 0.05	0.52 ± 0.12*
10b (50 μM)	20.8 ± 0.9	33.8 ± 2.9*	0.94 ± 0.05	0.79 ± 0.04
11 (600 μM)	21.6 ± 3.3	29.6 ± 2.6	1.05 ± 0.26	0.37 ± 0.32*

[a]Results are presented as the mean ± SEM of three experiments performed in triplicate. Scatchard analysis was carried out with IC_{50} concentrations of compounds. Statistical significance (*$p > 0.05$) was determined using a paired *t*-test. Other experimental details were as described previously [6].

10a, **10b** diastereomers is an anticonvulsant (**10a**) and the other is a convulsant (**10b**). Since a mixture of lactones (**4a** and **4b**) was used for the preparation of thiolactones **10a** and **10b**, and the diastereomers were assigned their compound numbers based on their order of elution during chromatographic separation, it is not possible to conclude that thiolactone diastereomer **10a** was derived from lactone precursor **4a**. Indeed, based on a comparison of the behavioral profiles found for these pairs of lactone and thiolactone diastereomers, it is most likely that anticonvulsant thiolactone **10a** was derived from anticonvulsant lactone **4b**. The behavioral effects of thiolactone **11** were not determined because of the small amount of compound available for evaluation. Thiolactone **11** was found to be a weaker displacer of [^{35}S]TBPS binding than its corresponding lactone analogue **5**.

Scatchard analyses were carried out to more completely investigate the binding interactions of lactones **4a**, **4b**, **5** and thiolactones **10a**, **10b**, **11** with the [^{35}S]TBPS binding site on GRs (Table 2). Each lactone was evaluated at its respective IC_{50} value. The K_D, but not the B_{max}, for [^{35}S]TBPS binding was affected by compounds **4a**, **4b**, and **10b**. These results are consistent with, but do not prove, that these compounds have a competitive interaction with the picrotoxin binding site. Compounds **5** and **10a** altered both the K_D and B_{max} for [^{35}S]TBPS binding, and are indicative of a mixed type of competitive/noncompetitive interaction of the compounds with the picrotoxin site. Thiolactone **11** altered only the B_{max} for [^{35}S]TBPS binding and this result is indicative of a noncompetitive interaction of the compound with the picrotoxin site.

The modulatory effects of the fluorinated compounds on GR function were examined using electrophysiological methods and cultured rat hippocampal neurons. The results of these experiments are summarized in Table 3. Except as noted, the concentrations of the compounds evaluated were close to the brain concentrations measured and reported in Table 1. In general, the GABA concentration used in the experiments was 30 μM, a concentration close to the ED_{50} for GABA (50 μM). Results from a more extensive electrophysiological evaluation of compounds **1**, **3**, and **8** on GR function in cultured rat hippocampal neurons have been reported previously [10]. Anticonvulsant lactone **1** was previously reported to potentiate the current mediated by 1 μM, but not 30 μM, GABA [10]. These earlier results were confirmed in this study. Also, as observed previously, the convulsant lactone **3**, which was evaluated at a concentration equal to its IC_{50} value for [^{35}S]TBPS displacement, was found to block the current mediated by 30 μM GABA [10]. Lactones **4a**, **4b**, and **5**, which like lactone **3** contain a trifluoromethyl group at the end of a two carbon side chain located at the C-3 position of the lactone ring, also blocked GABA-mediated currents. A comparison of the inhibition caused by a 1 mM concentration of lactone diastereomers **4a** and **4b** shows that diastereomer **4a** is the more potent blocker of GABA-mediated current. Results obtained with compound **4b** indicate that the extent of inhibition caused by the compound is only slightly increased at the lower GABA concentration. Lactone **5**, which was evaluated at its IC_{50} concentration for [^{35}S]TBPS displacement, blocked GABA-mediated currents. As was the case for lactone **4b**, the extent of inhibition caused by this compound was little affected by changes in GABA concentration.

The remaining lactones evaluated in the electrophysiology experiments contain fluoroalkyl groups at the C-5 (compounds **6a** and **6b**) or C-4 (compound **7**) positions of the lactone ring. All three compounds inhibited GABA-mediated currents. At a concentration of 300 μM, diastereomers **6a** and **6b** blocked these currents to an equal extent. Lactone **7** is the most potent blocker of these currents identified in this study.

As reported previously, thiolactone **8** potentiates currents mediated by 1 μM GABA [10]. Thiolactone **9**, like lactone **3**, blocks currents mediated by 30 μM GABA. Thiolactone diastereomers **10a** and **10b** have opposite

Table 3
Electrophysiological effects of fluorinated compounds on $GABA_A$ receptor function

Compound	Concentration	% response relative to current produced by GABA alone[a]	
		GABA (1 μM)	GABA (30 μM)
1	1 mM	146 ± 12(6)*[b]	95 ± 1 (3)
3	100 μM		76 ± 4 (7)**
4a	100 μM		94 ± 7 (4)*
	1 mM		35 ± 5 (4)*
4b	1 mM	57 ± 4 (4)*	69 ± 4 (4)*
5	300 μM	78 ± 5 (6)**	87 ± 5 (7)*
6a	300 μM		42 ± 5 (5)***
6b	300 μM		49 ± 3 (5)***
7	50 μM		64 ± 7 (7)*
8	300 μM	160 ± 17 (6)*	
9	50 μM		88 ± 5 (7)
	500 μM		48 ± 5 (6)**
10a	300 μM	190 ± 20 (8)*	88 ± 5 (7)
10b	300 μM		77 ± 5 (5)*
11	600 μM	91 ± 8 (4)	84 ± 4
	2 mM	48 ± 8 (4)*)	

[a]To calculate the % response, the magnitude of the peak current produced by GABA plus compound was normalized with respect to the peak current produced by GABA alone on the same cell. A % response of 100% reflects no change in the current compared to GABA alone. The effects of the compounds evaluated were reversible and all cells included in the analysis gave a GABA current after washout of the compound that was within 20% of the GABA current initially observed before the cell was exposed to the compound.
[b]Values reported are the mean ± SEM. The number of cells examined is given in the parentheses. Statistical significance (*$p > 0.05$; **$p > 0.01$; ***$p > 0.001$) was determined using a paired t-test.

effects on GABA-mediated currents. Thiolactone **10a**, like thiolactone **8** and lactone **1**, potentiates currents mediated by 1 μM ,but not 30 μM, GABA. By contrast, thiolactone **10b** has inhibitory effects on currents mediated by 30 μM GABA. Lastly, thiolactone **11**, which was evaluated at a concentration equal to its IC_{50} value for [^{35}S]TBPS displacement (600 μM) and at a higher concentration (2 mM), is observed to be a weak blocker of GABA-mediated currents. Statistical significance for the inhibitory effects of this compound was achieved only at the concentration that was more than three-fold higher than the IC_{50} for [^{35}S]TBPS displacement.

4. Discussion

The convulsant/anticonvulsant profiles, [^{35}S]TBPS binding properties, and some electrophysiological actions of the alkyl-substituted analogues of the fluoroalkyl compounds investigated in this study have appeared previously [2,3,6,21,22]. As was observed previously for lactone **1** and determined here for thiolactone **8**, fluorination at the methylene carbon in the C-3 ethyl group yields compounds which retain anticonvulsant activity against PTZ-induced seizures [9]. Also, as reported previously in greater detail, these compounds retain the ability to potentiate GABA-mediated currents [10]. In comparison to the corresponding alkyl-substituted analogues [3], the anticonvulsant potencies, neurotoxicities, and IC_{50} values for [^{35}S]TBPS displacement of the fluorinated compounds **1** and **8** are not markedly different. Thus, difluorination of the C-3 ethyl group in the methylene position has little effect on in vivo activity and pharmacological action at GRs. This conclusion is consistent with the hypothesis that there are no important binding interactions between a receptor binding site and this methylene group in the lactone ligands.

By contrast, trifluorination at the methyl carbon of the C-3 ethyl group profoundly affects pharmacological activity. Fluoroalkyl compounds **2**, **3**, and **9**, unlike their corresponding alkyl-substituted anticonvulsant analogues, are convulsants. In comparison to the anticonvulsant fluoroalkyl compounds **1** and **8**, which depending on the GABA concentration, either enhance or do not affect GABA-mediated currents, fluoroalkyl compounds **3** and **9** diminish GABA-mediated currents. Additionally, compounds **3** and **9** are 30-fold and 17-fold more potent than compounds **1** and **8**, respectively, as displacers of [^{35}S]TBPS. We interpret these results to indicate that trifluorination at the methyl carbon of the C-3 ethyl group enhances the binding of lactones to the picrotoxin site on GRs and that the behavioral and functional effects of these compounds on GR function are the result of their enhanced binding to this site. Since fluorine is an electronegative element, it is possible that the enhanced binding of the compounds to the picrotoxin site might be a consequence of a favorable electrostatic interaction between the trifluoromethyl group and this receptor site.

Compounds **4a**, **4b**, and **5** were prepared to investigate further the steric and stereochemical parameters of a potential electrostatic interaction between a trifluoromethyl group and the picrotoxin binding site. If one assumes that the trifluoromethyl groups in lactones **4a** and **4b** are similarly oriented when these compounds are bound to the picrotoxin site, then the methyl groups in these compounds must be oriented differently. For lactone **5**, a similar binding orientation for the trifluoromethyl group places the two methyl groups of this compound in the regions of space occupied by each of the methyl groups of diastereomers **4a** and **4b**.

Lactones **4a** and **4b** both appear to bind competitively at the picrotoxin binding site and the ability of both compounds to block GABA-mediated current at GRs is

reasonably explained by the binding of these compounds to this site. There is clearly diastereoselectivity in the actions of the compounds since they have different affinities for the picrotoxin site and different potencies in the electrophysiology experiments. Although it may be fortuitous, diastereomer **4a**, which has the strongest interactions with the picrotoxin site, is a convulsant. Diastereomer **4b**, which is not a convulsant, is also not a useful anticonvulsant because of its neurotoxicity. We suspect, but cannot prove, that this neurotoxicity is at least partly related to interactions of this compound with the picrotoxin site. The anticonvulsant activity is presumably the result of compound **4b** binding to the putative lactone site. These rationalizations are supported by the finding that the corresponding *i*-propyl-substituted compound, which is an anticonvulsant (ED_{50} = 150 mg/kg) at less than neurotoxic doses (TD_{50} > 400 mg/kg), is a much weaker displacer of [^{35}S]TBPS (IC_{50} = 2.0 mM) than either lactone **4a** or **4b** [3]. In summary, as was the case for lactone **3**, a trifluoromethyl group similarly located in compounds **4a** or **4b** increases the interactions of the compounds with the picrotoxin site. However, the strength of this interaction is diminished in a diastereostereoselective manner by the presence of the additional methyl group found in compounds **4a** and **4b**.

Convulsant lactone **5** blocks GABA-mediated current and it has an IC_{50} value for [^{35}S]TBPS displacement (290 μM) which is intermediate between the values found for lactones **4a** (142 μM) and **4b** (565 μM), and ~threefold lower than that found previously for the corresponding anticonvulsant *t*-butyl-substituted compound (IC_{50} = 990 μM, ED_{50} = 145 mg/kg) [3]. However, Scatchard analysis for the binding of compound **5** indicates a mixed competitive/noncompetitive binding interaction of the compound with the picrotoxin site. This last result may be a reflection of the binding of this lactone to both the picrotoxin and lactone sites, and we interpret the result to indicate that the hydrophobic interactions of the methyl groups increases the binding of the compound to the lactone site.

The effect that fluorination of alkyl groups located at ring positions other than C-3 has on biological activity was not investigated thoroughly in this study. Since lactones and thiolactones having alkyl substitutents at C-4 or C-5 are convulsants [3], and our major interest is in the development of new anticonvulsant agents, compounds **6a**, **6b**, and **7** were prepared and evaluated only to satisfy our expectation that compounds with fluoroalkyl groups in these positions would be convulsants. Our expectation was fullfilled since compounds **6a**, **6b**, and **7** are more potent convulsants that their corresponding alkyl-substituted analogues [3].

Finally, the results obtained with thiolactones **10a**, **10b**, and **11** yield information about the effect that replacement of the ring oxygen by sulfur has on the steric and stereochemical parameters of a potential electrostatic interaction between a trifluoromethyl group and the picrotoxin binding site. As was the case for lactones **4a** and **4b**, the trifluoromethyl group of thiolactones **10a** and **10b** increases, but to a lesser degree relative to the corresponding *i*-propyl-substituted compound (IC_{50} = 260 μM) [3], the potency of the compounds as displacers of [^{35}S]TBPS. However, whereas lactone diastereomers **4a** and **4b** both appear to bind competitively to the picrotoxin site and both inhibit GABA-mediated current, this is not the case for thiolactone diastereomers **10a** and **10b**. Scatchard analysis of the binding of the thiolactone diastereomers indicates that only the convulsant diastereomer **10b** has the binding characteristics expected for competitive binding to this site. The anticonvulsant and more weakly bound diastereomer **10a** shows mixed competitive/noncompetitive binding interactions with this site.

The difference in the binding interactions of thiolactones **10a** and **10b** with the picrotoxin site also manifests itself in the behavioral evaluations and the electrophysiology experiments. Thiolactone **10b**, which appears to competitively displace [^{35}S]TBPS, is a convulsant that diminishes GABA-mediated currents, and thiolactone **10a**, which does not competitively displace [^{35}S]TBPS, is an anticonvulsant that potentiates the current mediated by 1 μM GABA. Interestingly, thiolactone **10a** has a potency that is similar to, or perhaps somewhat weaker than (there is overlap in the 95% fiducial limits), that of the corresponding alkyl-substituted thiolactone analogue (ED_{50} = 145 mg/kg) [3]. Thus, for this diastereomer, the ring sulfur for oxygen subsitution largely counteracts the interactions of the compound's trifluoromethyl group with the picrotoxin site.

The effect that ring heteroatom substitution has on the interactions of the trifluoromethyl group of thiolactone **11** with the picrotoxin site is also prominent. Thiolactone **11** is the only fluorinated compound found in this study to be a weaker displacer of [^{35}S]TBPS than the corresponding alkyl-substituted thiolactone analogue (IC_{50} = 190 μM) [3]. It is also the only compound of the six examined by Scatchard analysis to be a noncompetitive displacer of [^{35}S]TBPS. Moreover, the effects of thiolactone **11** on GABA-mediated current were statistically significant only when the compound was evaluated at a concentration that was more than threefold higher than its IC_{50} value for [^{35}S]TBPS displacement. Unfortunately, due to the small amounts of material available, the behavioral effects of thiolactone **11** were not determined. Nevertheless, the electrophysiological results suggest that the compound would be either a convulsant or, perhaps like compound **4b**, an anticonvulsant at neurotoxic doses. Since the corresponding *t*-butyl-substituted analogue is a weak anticonvulsant (ED_{50} = 244 mg/kg, TD_{50} > 500 mg/kg) [3], it is also possible that this fluorinated compound has only

weak interactions with the GR lactone site. Additional data from future studies of the interactions of this compound with the putative lactone site in picrotoxin-resistant forms of GR would be useful for the evaluation of this possibility.

In conclusion, these fluorinated compounds readily enter the brain following peripheral administration and they attain brain concentrations that are similar to those used in our in vitro studies. Fluorination of C-3 substituted γ-butyrolactones and γ-thiobutyrolactones results in either subtle or pronounced effects on the pharmacological activity of the compounds. When hydrogens are replaced with fluorines at the methylene carbon of an ethyl group, as in compound **1**, the biological actions of the compound are not much changed from those found for the corresponding alkyl-substituted analogue. In marked contrast, fluorination at the methyl carbon of the ethyl group, as in compound **3**, produces a compound having convulsant activity. This convulsant activity seems to be due to an increased affinity of the compound for the picrotoxin site on GRs caused by an interaction that involves the trifluoromethyl group. Results obtained with compounds **4a**, **4b**, and **5** indicate that the interactions of a trifluoromethyl group at this position with the picrotoxin binding site are subject to both stereochemical and steric constraints. Sulfur for oxygen heteroatom substitution, as in the trifluoromethyl-substituted compounds **10a**, **10b**, and **11**, affects the type of binding interactions that these γ-thiobutyrolactones have with the picrotoxin site in a complex manner.

5. Experimental

5.1 General chemistry methods

NMR spectra were recorded in $CDCl_3$ and were referenced to either $CHCl_3$ (δ 7.26, ^{1}H NMR) or $CFCl_3$ (δ 0.00, ^{19}F NMR). The Econosil HPLC column was purchased from Alltech Associates, Inc. (Deerfield, IL) and the Ultrasphere Si HPLC column was purchased from Beckman Instruments, Inc. (Fullerton, CA), respectively. Preparative HPLC was performed on a Waters Prep LC/System 500A liquid chromatograph, using a PrepPAK-500/Silica or C18 cartridge for normal and reverse-phase separations, respectively. Dry column grade silica gel obtained from Scientific Absorbents (Atlanta, GA) was used for column chromatography. Compound purity was determined with a Varian Model 3700 gas chromatograph equipped with a glass column (2 mm id, 6 ft length) packed with 1% SP2401 on 80/100 mesh Supelcoport (Supelco, Inc., Bellefonte, PA) and/or with a Hewlett Packard 5890A GC on an Ultra 1 capillary column (0.2 mm id, 0.11 micron film thickness, 25 m length). Microanalyses were carried out by either Galbraith Labs, Inc. (Knoxville, TN) or M-H-W Laboratories (Phoenix, AZ).

5.1.1 3-(1,1-Difluoroethyl)dihydro-3-methyl-2(3H)-furanone (1)

The compound was prepared as described previously [9].

5.1.2 Dihydro-3-(2,2,2-trifluoroethyl)-2(3H)-furanone(2)

This compound was prepared from dihyro-2(3*H*)-furanone and 2-iodo-1,1,1-trifluoroethane by the general alkylation method previously reported for the preparation of 2-ethyl-2,4-dimethyl-4-pentenoic acid methyl ester [3]. The oil recovered from the reaction was purified by column chromatography (silica gel, eluted with 40% EtOAc in hexanes). After solvent removal, the remaining liquid was vacuum distilled to yield pure product **2** (13%) as a colorless liquid: bp 68–70°C/2.8 mm Hg; IR 1773, 1200 cm^{-1}; ^{1}H NMR δ 4.45–4.36 (m, 1H), 4.25–4.15 (m, 1H), 2.92–2.73 (m, 2H), 2.63–2.48 (m, 1H), 2.21–1.98 (m, 2H); ^{19}F NMR δ–65.95 (t, ^3JHF = 11 Hz); Anal. calcd for $C_6H_7F_3O_2$: C, 42.86; H, 4.17; F, 33.89. Found: C, 43.10; H, 4.21; F, 33.76.

5.1.3 Dihydro-3-methyl-3-(2,2,2-trifluoroethyl)-2(3H)-furanone (3)

This compound was prepared from dihydro-3-methyl-2(3*H*)-furanone and 2-iodo-1,1,1-trifluoroethane by the above cited general alkylation method [3]. The oil recovered from the reaction was vacuum distilled to yield pure product **3** (53%) as a colorless liquid: bp 76–77°C/4.5 mm Hg; IR 1776, 1138, 1178 cm^{-1}; ^{1}H NMR δ 4.39–4.24 (m, 2H), 2.58–2.12 (m, 4H), 1.25 (s, 3H); ^{19}F NMR δ -61.24 (t, $^3J_{HF}$ = 11 Hz); Anal. calcd for $C_7H_9F_3O_2$: C, 46.16; H, 4.98; F, 31.29. Found: C, 46.50; H, 5.17; F, 30.96.

5.1.4 Dihydro-3-(1-trifluoromethyl)ethyl-2(3H)-furanone (4a and 4b)

The α-(O,O-diethylphosphonate) derivative of dihydro-2(3*H*)-furanone [11] (45.0 g, 203 mmol) was added to dry benzene (400 ml) in a 2 l, 3-neck round-bottom flask fitted with a reflux condenser, an overhead stirrer, and a dropping funnel. The temperature was raised to 50–60°C and NaH (4.9 g, 203 mmol) was added. After 1 h at 50–60°C, the reaction mixture was chilled to 0°C and 1.5 equiv of trifluoroacetone in dry benzene was added. A gummy yellow precipitate was produced. When addition was complete, the organic layer was decanted and the gummy residue was taken up in 6 N HCl (250 ml). The aqueous layer was extracted with benzene (3×200 ml) and the combined organic extract was washed with water (100 ml) and saturated NaCl (2×100 ml), and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a pale yellow oil which was dissolved in hexanes and hydrogenated (45 psi, room temperature) in the presence of 10% Pd-C

catalyst. Solvent removal followed by vacuum distillation gave a mixture of diastereomeric products **4a** and **4b** (10.2 g, 72%).

Compounds **4a** and **4b** were separated by preparative HPLC (silica gel, eluted at 0.3 l/min with 10% EtOAc in hexanes). Lactone **4a** eluted from the column before lactone **4b**. Boiling points and spectroscopic data for each diastereomer are given below. The relative configuration of the hydrogens on the adjacent chiral centers in these diastereomers was not determined.

Compound **4a** is a colorless liquid: bp 138–142°C/8 mm Hg; IR 1778, 1175 cm^{-1}; ^1H NMR δ 4.46–4.37 (m, 1H), 4.27–4.17 (m, 1H), 3.09–2.89 (m, 2H), 2.34–2.18 (m, 2H), 1.11 (d, $J=7$ Hz, 3H); ^{19}F NMR δ -72.54 (d, $^3J_{HF}=9$ Hz). Anal. calcd for $C_7H_9F_3O_2$: C, 46.16; H, 4.98; F, 31.29. Found: C, 45.95; H, 4.77; F, 31.12. Compound **4b** is a colorless liquid: bp 146–150°C/8 mm Hg; IR 1778, 1175 cm^{-1}; ^1H NMR δ 4.41–4.32 (m, 1H), 4.22–4.12 (m, 1H), 2.78–2.51 (m, 2H), 2.48–2.36 (m, 1H), 2.32–2.15 (m, 1H), 1.35 (d, $J=7$ Hz, 3H); ^{19}F NMR δ -69.68 (d, $^3J_{HF}=9$ Hz). Anal. calcd for $C_7H_9F_3O_2$: C, 46.16; H, 4.98; F, 31.29. Found: C, 46.17; H, 5.07; F, 31.07.

5.1.5 Dihydro-3-(1-methyl-1-trifluoromethyl)ethyl-2(3H)-furanone (5)

Unseparated diastereomers **19a** and **19b** (1.44 g, 5.03 mmol) were dissolved in warm absolute EtOH (15 ml), Raney nickel (25 g, thoroughly washed with distilled water and absolute EtOH) was added and the reaction mixture was refluxed for 3 h. After cooling, the mixture was filtered, and the Raney nickel was washed with EtOH. After solvent removal, the liquid residue was distilled bulb-to-bulb to give product **5** (650 mg, 66%) as a colorless liquid: 1775, 1191, 1142, 1118 cm^{-1}; ^1H NMR δ 4.39–4.32 (m, 1H), 4.18–4.08 (m, 1H), 2.74–2.67 (m, 1H), 2.37–2.27 (m, 2H), 1.52 (s, 3H), 1.19 (s, 3H); ^{19}F NMR δ -77.04 (s). Anal. Calcd for $C_8H_{11}F_3O_2$: C, 48.98; H, 5.65; F, 29.05. Found C, 49.03; H, 5.89; F, 28.78.

5.1.6 Dihydro-3-ethyl-3,5-dimethyl-5-trifluoromethyl-2(3H)-furanone (6a and 6b)

A stirred solution of 2-ethyl-2-methyl-4-oxopentanoic acid methyl ester [17] (4.02 g, 23.4 mmol) and 1.4 equiv of trimethyl(trifluoromethyl)silane (4.58 g, 32.2 mmol) in dry tetrahydrofuran (60 ml) was cooled to 0°C in an ice bath. Tetrabutylammonium fluoride was then added slowly until a yellow color persisted. Cooling was discontinued and the reaction mixture was stirred at room temperature (~2 h). Water (50 ml) was added, the liquid phases were separated, and the aqueous layer was extracted with EtOEt (2×50 ml). The combined organic layers were concentrated under reduced pressure and the residual oil was dissolved in 10% H_2SO_4 in acetone (120 ml) and stirred overnight. The following morning, water was added (100 ml), the acetone was removed on a rotary evaporator, and the aqueous solution was extracted with EtOEt (3×100 ml). The combined organic layers were washed with water (50 ml), 10% $NaHCO_3$, (50 ml), saturated NaCl (50 ml), and dried over $MgSO_4$. The solvent was removed under reduced pressure and the remaining liquid was vacuum distilled to yield a mixture of diastereomeric products **6a** and **6b** (4.9 g, 94%).

Compounds **6a** and **6b** were separated by HPLC (Ultrasphere Si, 5 μm, 10 mm×25 cm, eluted at 7 ml/min with 4% EtOAc in hexanes). Lactone **6a** eluted from the column before lactone **6b**. Boiling points and spectroscopic data for each diastereomer are given below. The relative configuration of the substituents at the chiral centers in these diastereomers was not determined.

Compound **6a** is a colorless liquid: bp 74–78°C/2.5 mm Hg; IR 1787, 1169 cm^{-1}; ^1H NMR δ 2.32 (d, $J=14.4$ Hz, 1H), 2.05–2.12 (m, 1H), 1.60–1.70 (m, 2H), 1.57 (broad s, 3H), 1.31 (s, 3H), 0.95 (t, $J=7.4$ Hz, 3H); ^{19}F NMR δ -83.27 (s). Anal. calcd for $C_9H_{13}F_3O_2$: C, 51.43; H, 6.23; F, 27.12. Found: C, 51.50; H, 6.21; F, 27.31.

Compound **6b** is a colorless liquid: bp 68–72°C/1.4 mm Hg; IR 1787, 1169 cm^{-1}; ^1H NMR δ 2.53 (d, $J=12$ Hz, 1H), 1.84–1.89 (m, 1H), 1.60–1.70 (m, 2H), 1.62 (broad s, 3H), 1.36 (s, 3H), 0.94 (t, $J=7.5$ Hz, 3H); ^{19}F NMR δ -83.19 (s). Anal. calcd for $C_9H_{13}F_3O_2$: C, 51.43; H, 6.23; F, 27.12. Found: C, 51.73; H, 6.31; F, 27.23.

5.1.7 Dihydro-4-methyl-4-(2,2,2-trifluoroethyl)-2(3H)-furanone (7)

Compound **7** was prepared from the ester **23** using previously described methods [3]. Briefly, ester **23** was treated with O_3 in 1:1 MeOH:CH_2Cl_2 at −78°C and then oxidized with Jones reagent. The diacid monoester thus formed was vacuum distilled and used without further purification in the next reaction. The diacid monoester (3.8 g; 16 mmol) dissolved in stirred, dry toluene (75 ml) was treated with DIBALH (50 mmol, 50 ml of a 1 M solution in toluene) at −78°C. After 2 h, the reaction was quenched with 6 N HCl (50 ml), and concentrated HCl was added until acidic (pH 1–2). The reaction mixture was stirred vigorously at room temperature for 1 h, the layers were separated, and the aqueous layer was extracted with EtOEt (2×100 ml). The combined organic extracts were washed with H_2O (50 ml), 10% $NaHCO_3$ (50 ml), saturated NaCl (50 ml), and dried over $MgSO_4$. After solvent removal and bulb-to-bulb vacuum distillation, compound **7** (2.1 g, 55%) was obtained as a colorless liquid: IR 1786, 1260, 1115 cm^{-1}; ^1H NMR δ 4.12–4.04 (m, 2H), 2.54–2.26 (m, 4H), 1.31 (s, 3H); ^{19}F NMR δ -61.15 (t, $^3J_{HF}=11$ Hz). Anal. Calcd for $C_7H_9F_3O_2$: C, 46.16; H, 4.98; F, 31.29. Found: C, 46.30; H, 5.21; F, 31.13.

5.2 General procedure for the preparation of dihydro-2(3H)-thiophenones

These compounds were synthesized by reaction of the corresponding dihydro-2(3*H*)-furanones with potassium thioactetate in DMA at 150–160°C for 4 h as described previously [3]. Purifications of individual compounds are reported below with yields and spectroscopic data.

5.2.1 Dihydro-3-(1,1-difluoroethyl)-3-methyl-2(3H)-thiophenone (8)

Column chromatography (silica gel, eluted with 12.5% EtOAc in hexanes) followed by bulb-to-bulb vacuum distillation yielded pure thiolactone **8** (59%) as a pale-yellow liquid: IR 1699, 1141 cm^{-1}; ^1H NMR δ 3.20–3.38 (m, 2H), 2.70–2.80 (m, 1H), 2.07–2.16 (m, 1H), 1.70 (t, $^3J_{HF}=20$ Hz, 3H), 1.28 (s, 3H); ^{19}F NMR δ -97.4 (d of q, $^2J_{FF}=248$ Hz, $^3J_{HF}=20$ Hz), -94.1 (d of q, $^2J_{FF}=248$ Hz, $^3J_{HF}=20$ Hz). Anal. calcd for $C_7H_{10}F_2OS$: C, 46.67; H, 5.56; F, 21.09; S, 17.80. Found: C, 47.02; H, 5.45; F, 20.83; S, 17.66.

5.2.2 Dihydro-3-methyl-3-(2,2,2-trifluoroethyl)-2(3H)-thiophenone (9)

Column chromatography (silica gel, eluted with 50% EtOAc in hexanes) followed by vacuum distillation gave pure thiolactone **9** (52%) as a colorless liquid: bp 65–66°C/3.2 mm Hg; IR 1699, 1261, 1148 cm^{-1}; ^1H NMR δ 3.33–3.16 (m, 2H), 2.52–2.10 (m, 4H), 1.19 (s, 3H); ^{19}F NMR δ -60.61 (t, $^3J_{HF}=12$ Hz). Anal. calcd for $C_7H_9F_3OS$: C, 42.42; H, 4.58; F, 28.76; S, 16.18. Found: C, 42.63; H, 4.53; F, 28.66; S, 16.37.

5.2.3 Dihydro-3-(1-trifluoromethyl)ethyl-2(3H)-thiophenone (10a and 10b)

Vacuum distillation of the crude reaction product gave a diastereomeric mixture (77%) of compounds **10a** and **10b**. The diastereomers were separated by column chromatography (silica-gel, eluted with 12.5% ethyl acetate in hexanes). Thiolactone **10a** eluted from the column before thiolactone **10b**. Boiling points and spectroscopic data for each diastereomer are given below. The relative configuration of the hydrogens on the adjacent chiral centers in these diastereomers was not determined.

Compound **10a** is a pale yellow liquid: bp 75–78°C/2.5 mm Hg; IR 1702, 1131 cm^{-1}; ^1H NMR δ 3.33–3.25 (m, 2H), 3.06–2.78 (m, 2H), 2.49–2.37 (m, 1H), 2.18–2.01 (m, 1H), 1.01 (d, $J=7$ Hz, 3H); ^{19}F NMR δ -71.62 (d, $^3J_{HF}=9$ Hz). Anal. calcd for $C_7H_9F_3OS$: C, 42.42; H, 4.58; F, 28.76; S, 16.18. Found: C, 42.45; H, 4.43; F, 28.61; S, 16.37.

Compound **10b** is a pale-yellow liquid: bp 85–88°C/2.5 mm Hg; IR 1703, 1134 cm^{-1}; ^1H NMR δ 3.37–3.20 (m, 2H), 2.98–2.80 (m, 1H), 2.58–2.42 (m, 2H), 2.28–2.08 (m, 1H), 1.30 (d, $J=7$ Hz, 3H); ^{19}F NMR δ -68.42 (d, $^3J_{HF}=9$ Hz). Anal. calcd for $C_7H_9F_3OS$: C, 42.42; H, 4.58; F, 28.76; S, 16.18. Found: C, 42.48; H, 4.54; F, 28.53; S, 16.09.

5.2.4 Dihydro-3-(1-methyl-1-trifluoromethyl)ethyl-2(3H)-thiophenone (11)

The crude product was purified by column chromatography (silica-gel, eluted with 10% EtOAc in hexanes) followed by HPLC (Ultrasphere Si, 5 μm, 10 mm×25 cm, eluted at 4 ml/min with 10% EtOAc in hexanes). Bulb-to-bulb vacuum distillation gave pure thiolactone **11** (53%) as a pale-yellow liquid: IR 1697, 1148, 1120 cm^{-1}; ^1H NMR δ 3.26–3.22 (m, 2H), 2.64–2.46 (m, 2H), 2.29–2.14 (m, 1H), 1.46 (s, 3H), 1.17 (s, 3H); ^{19}F NMR δ -76.35 (s). Anal. calcd for $C_8H_{11}F_3OS$: C, 45.28; H, 5.22; F, 26.86; S, 15.11. Found: C, 45.34; H, 5.24; F, 27.09; S, 15.27.

5.2.5 (E)-3-Trifluoromethyl-2-butenoic acid ethyl ester (12)

This compound was prepared as described previously [12]. bp 129.5–131°C, lit bp[13] 116–118°C (650 mm Hg). ^1H NMR data was consistent with reported data [13].

5.2.6 (E)-3-Trifluoromethyl-2-buten-1-ol (13) [13].

The reaction was performed under an N_2 atmosphere in an oven-dried 1 l three-neck flask equipped with a magnetic stir bar, addition funnel and rubber septa. Compound **12** (30.0 g, 165 mmol) in hexane (100 ml) was cooled to −25°C and DIBALH (412 mmol, 412 ml of a 1 M solution in hexanes) was added from the addition funnel. Stirring was continued for 2 h at −25°C. After warming to 0°C, 1 N HCl (100 ml) was added slowly, then 12 N HCl (250 ml) was added, and the aqueous layer was saturated with NaCl. The organic layer was separated and the aqueous layer was extracted several times with EtOEt. The combined organic layers were washed with saturated $NaHCO_3$ (2×150 ml), saturated NaCl (2×150 ml) and dried over $MgSO_4$. The solvent was removed by flash distillation and the liquid remaining in the flask was distilled at atmospheric pressure through a Vigreux column to give the product **13** (72%) as a colorless liquid: bp 144–147°C.

5.2.7 3-Methyl-3-trifluoromethyl-4-butenoic acid ethyl ester (14)

Alcohol **13** (9.0 g, 64 mmol, 82% purity by GC), triethylorthoacetate (218 ml, 1.52 mol), and propionic acid (5 ml, 69 mmol) were dissolved in dry DMF (145 ml) and stirred at reflux overnight. After cooling, EtOEt (300 ml) was added and the solution was washed with 0.01 N HCl (3×150 ml), saturated NaCl (2×200 ml), and dried over $MgSO_4$. After solvent removal, the residual liquid was partially purified by chromatography (silica gel, eluted with a gradient of 1%–8% EtOAc in hexanes) to give product **14** (7.4 g, 64% purity by GC). Further

purification by distillation on a spinning band column gave product **14** of 92% purity by GC (3.92 g, 29%): bp 53–54.3°C/27 mm Hg.

An analytical sample of ester **14** was obtained by HPLC (Ultrasphere Si, 5 μm, 10 mm×25 cm, eluted at 2 ml/min with 5% EtOAc in hexanes): IR 1740, 1647, 1168, 1094, 1037, 997 cm^{-1}; ^1H NMR δ 5.92 (dd, J=10.9 Hz, J=17.4 Hz, 1H), 5.35 (d, J=10.6 Hz, 1H), 5.31 (d, J=17.5 Hz, 1H), 4.13 (q, J=7.2 Hz, 2H), 2.67 (dd, J=14.0 Hz, 1H), 2.54 (dd, J=14.0 Hz, 1H), 1.43 (s, 3H), 1.24 (t, J=7.1 Hz, 3H); ^{19}F NMR δ -77.82 (s). Anal. calcd for $C_9H_{13}F_3O_2$: C, 51.43; H, 6.23; F, 27.12. Found: C, 51.64; H, 6.39; F, 26.87.

5.2.8 3-Methyl-4-oxo-3-trifluoromethylbutanoic acid ethyl ester (15)

Compound **14** (3.73 g, 178 mmol, 92% purity) was dissolved in CH_2Cl_2 (80 ml) and treated with O_3 at −78°C until a blue color persisted. The excess O_3 was removed with a stream of O_2 and methyl sulfide (5.3 ml) was added. The solution was warmed to room temperature and the solvent was removed. EtOEt was added to the residue and the EtOEt solution was washed with water (2×), saturated NaCl (2×), and dried over $MgSO_4$. After solvent removal, the liquid residue was bulb-to-bulb distilled (room temperature to 120°C, 5 mm Hg) to give a colorless liquid (3.79 g, 93% yield, 90% purity by GC). Similarly prepared material of 92.5% purity had bp 85–87°C/32 mm Hg.

An analytical sample of ester **15** was obtained by HPLC (Ultrasphere Si, 5 μm, 10 mm×25 cm, eluted at 3 ml/min with 6% EtOAc in hexanes): IR 1746, 1181, 1101 cm^{-1}; ^1H NMR δ 9.71 (m, 1H), 4.16 (q, J=7.1 Hz, 2H), 3.11 (d, J=16.3 Hz, 1H), 2.69 (d, J=7.1 Hz, 1H), 1.46 (s, 3H), 1.26 (t, J=7.1 Hz, 3H); ^{19}F NMR δ -73.37. Anal. calcd for $C_8H_{11}F_3O_3$: C, 45.29; H, 5.22; F, 26.86. Found: C, 45.44; H, 5.33; F, 26.63.

5.2.9 4,4-Diethoxy-3-methyl-3-trifluoromethylbutanoic acid ethyl ester (16)

Compound **15** (3.69 g, 17 mmol, 93% purity), triethylorthoformate (10.31 g, 70 mmol), and *p*-TsOH (160 mg) were stirred at room temperature for 2.5 h. HCl (0.01 N, 40 ml) was added and after 15 min the reaction mixture was extracted with EtOEt (2×40 ml). The combined EtOEt extracts were washed with saturated $NaHCO_3$ (2×40 ml), saturated NaCl (2×40 ml), and dried over $MgSO_4$. After solvent removal, the liquid residue was bulb-to-bulb distilled to afford the product as a colorless liquid (4.76 g, 96%, 93% purity by GC).

An analytical sample of one diastereomer of ester **16** was obtained by column chromatography (silica gel, eluted with a gradient of 4%–8% EtOAc in hexanes): IR 1739, 1183, 1147, 1104, 1065 cm^{-1}; ^1H NMR δ 4.68 (s, 1H), 4.13 (q, J=7.2 Hz, 2H), 3.89–3.77 (m, 2H), 3.64–3.54 (m, 2H), 2.59 (dd, J=14.4 Hz, J=18.8 Hz, 2H), 1.30–1.20 (m, 12H); ^{19}F NMR δ -72.31. Anal. calcd for $C_{12}H_{21}F_3O_4$: C, 50.34; H, 7.39; F, 19.91. Found: C, 50.30; H, 7.42; F, 20.21.

5.2.10 2-(1-Diethoxymethyl-1-trifluoromethyl)ethyl-4-pentenoic acid ethyl ester (17)

The reaction was performed under an N_2 atmosphere in an oven-dried flask equipped with a stir bar and rubber septum. THF (10 ml) and diisopropylamine (0.85 ml, 6.1 mmol) were added to the flask and cooled to 0°C. *n*-Butyllithium (6.0 mmol, 2.40 ml of a 2.5 M solution in hexanes) was added and the solution was stirred for 15 min and then cooled to −78°C. A solution of compound **16** (1.62 g, 5.66 mmol) in THF (10 ml) was added and after 30 min, allyl bromide (0.56 ml, 6.5 mmol) in THF (5 ml) was added and stirring was continued for an additional 1 h. The reaction mixure was allowed to warm to room temperature and then stirred overnight. After cooling to 0°C, HCl (1.0 N, 25 ml) was added and the THF was removed under reduced pressure. The product was extracted from the remaining aqueous layer with EtOEt (2×80 ml), and the combined EtOEt layers were washed with saturated $NaHCO_3$ (2×), saturated NaCl (2×), and dried over $MgSO_4$. After solvent removal, the crude product was purified by column chromatography (silica gel, eluted with a 1–2.5% gradient of EtOAc in hexanes) to yield product **17** (1.2 g, 65%) as a colorless liquid: IR 1737, 1644, 1179, 1108, 1064 cm^{-1}; ^1H NMR δ 5.76–5.63 (m, 1H), 5.10–4.97 (m, 2H), 4.57 (s, 1H), 4.11 (q, J=7.2 Hz, 2H), 3.89–3.76 (m, 2H), 3.63–3.50 (m, 2H), 3.07 (dd, J=3.1 Hz, J=10.8 Hz, 1H), 2.74–2.65 (m, 1H), 2.49–2.31 (m, 13H); ^{19}F NMR δ -70.44. Anal. calcd for $C_{15}H_{25}F_3O_4$: C, 55.20; H, 7.72; F, 17.46. Found: C, 55.28, H, 7.64; F, 17.66.

5.2.11 Dihydro-3-(1-diethoxymethyl-1-trifluoromethyl)ethyl-2(3H)-furanone (18)

Compound **17** (1.2 g, 3.68 mmol) was dissolved in CH_2Cl_2 and treated with O_3 at −78°C until a blue color persisted. The excess O_3 was removed with a stream of O_2 and methyl sulfide (6.0 ml) was added. The solution was warmed to room temperature and the solvent was removed to give the crude ozonolysis product (1.15 g). Typically, this crude ozonolysis product is used without further purification or characterization.

A solution of $NaBH_4$ (0.29 g, 7.67 mmol) in absolute EtOH (15 ml) was slowly added at 0°C to a stirred solution of the ozonolysis product (1.09 g, 2.93 mmol) dissolved in absolute EtOH (10 ml). The reaction mixture was warmed to room temperature and stirred overnight. HCl (1.0 N, 15 ml) was added and the reaction mixture was stirred for 2 h. The EtOH was removed on a rotary evaporator under reduced pressure and the product was extracted from the aqueous layer with EtOEt (3×60 ml). The combined organic layers were

combined and washed with saturated $NaHCO_3$ (2×), saturated NaCl (2×), and dried over $MgSO_4$. After solvent removal on a rotary evaporator and bulb-to-bulb transfer under reduced pressure, product **18** was obtained as a colorless liquid consisting of two diastereomers. Spectroscopic data was recorded on the diastereomer mixture: IR 1772, 1189,1163, 1098, 1064 cm^{-1}; ^1H NMR δ 4.98 (broad s, 1H), 4.80 (broad s, 1H), 4.37–4.30 (m, 2H), 4.20–4.10 (m, 2H), 3.91–3.76 (m, 4H), 3.70–3.46 (m, 4H), 3.03 (dd, $J=9$ Hz, $J=11$ Hz, 1H), 2.82–2.72 (m, 2H), 2.62–2.48 (m, 1H), 2.34–2.25 (m, 2H), 1.47 (s, 3H), 1.36 (s, 3H), 1.25-1.19 (m, 12H); ^{19}F NMR δ -69.80 (s), -69.38 (s). Anal. calcd for $C_{12}H_{19}F_3O_4$: C, 50.70; H, 6.74; F, 20.05. Found: C, 50.58; H, 6.68; F, 20.20.

5.2.12 Dihydro-3-[1-(1,3-dithiolanyl)-1-trifluoromethyl)]ethyl-2(3H)-furanone (19a and 19b)

Compound **18** (1.60 g, 5.63 mmol) was placed in a 25 ml flask equipped with a magnetic stir bar under a N_2 atmosphere and cooled to 0°C. BF_3.HOAc complex (3.9 ml, 28 mmol) and 1,2-ethanedithiol (1.2 ml, 14 mmol) were added and the reaction was stirred for 1 h at 0°C and then at room temperature for 1.5 h. The reaction mixture was added to EtOEt (100 ml) and the EtOEt was extracted with 10% aqueous NaOH (3×30 ml), washed with water (2×30 ml), saturated NaCl (2×30 ml), and dried with $MgSO_4$. After solvent removal, the crude product (1.6 g) was purified by column chromatography (silica gel, eluted with a 6–24% gradient of EtOAc in hexanes) to afford the product as a white solid (1.2 g, 75% yield). The diastereomers were separated by HPLC (Ultrasphere Si, 5 μm, 10 mm ×25 cm, eluted at 3 ml/min with 20% EtOAc in hexanes).

Diastereomer **19a**, which eluted first from the HPLC column, had: mp 151–151.5°C; IR 2360, 1769, 1178 cm^{-1}; ^1H NMR δ 5.17 (s, 1H), 4.38–4.30 (m, 1H), 4.23–4.15 (m, 1H), 3.31–3.21 (m, 4H), 3.04–2.97 (m, 1H), 2.47–2.39 (m, 2H), 1.62 (s, 3H), ^{19}F NMR δ -66.79 (s). Anal. calcd for $C_{10}H_{13}F_3S$: C, 41.95; H, 4.58; F, 19.90; S, 22.39. Found: C, 41.76; H, 4.70; F, 19.77; S, 22.51.

Diastereomer **19b**, which eluted second from the HPLC column, had: mp 103–106°C; IR 1766, 1180, 1120 cm^{-1}; ^1H NMR δ 5.55 (s, 1H), 4.42–4.35 (m, 1H), 4.24–4.16 (m, 1H), 3.30–3.11 (m, 5H), 2.61–2.35 (m, 2H), 1.50 (s, 3H); ^{19}F NMR δ -68.67 (s). Anal. calcd for $C_{10}H_{13}F_3S$: C, 41.95; H, 4.58; F, 19.90; S, 22.39. Found: C, 41.78; H, 4.72; F, 19.78; S, 22.51.

5.2.13 Methyl-2-propenylpropanedioic acid diethyl ester (21)

In an oven dried 500 ml round-bottomed flask, methylpropanedioic acid diethyl ester (**20**, 20 g, 114 mmol), dissolved in dry THF (250 ml), was treated carefully with NaH (2.80 g, 114 mmol) under a N_2 atmosphere at 0–4°C. The mixture was warmed to room temperature and allyl bromide (16.6 g, 137 mmol) was added dropwise from a syringe. The reaction was stirred at room temperature for 4 h and quenched with H_2O (200 ml) and 6 N HCl (40 ml). The layers were separated and the aqueous layer extracted with hexane (2×100 ml). The organic layers were combined, washed with H_2O (50 ml), NaHCO3 (2×50 ml), saturated NaCl (2×50 ml), and dried over $MgSO_4$. After solvent removal and vacuum distillation product **21** (16.85 g, 70%) was obtained as a colorless liquid: bp 78–79°C/2 mm Hg. Spectroscopic data obtained for this compound was identical to previously reported data [18,19].

5.2.14 2-Methyl-4-pentenoic acid ethyl ester (22)

This compound was prepared by decarboethoxylation of diester **21** in heated DMSO-H_2O-NaCl, according to a previously published procedure [20]. Compound **22** (7.2 g, 65%) was obtained as a colorless liquid: bp 91–92°C/96 mm Hg. Spectroscopic data obtained for this compound was identical to previously reported data [18,19].

5.2.15 2-Ethyl-2-(2,2,2-trifluoroethyl)-4-pentenoic acid ethyl ester (23)

This compound was prepared from ester **22** and 2-iodo-1,1,1-trifluoroethane by the previously described general alkylation procedure [3]. The oil recovered from the reaction was column chromatographed (silica gel, eluted with 40% $CHCl_3$ in hexanes). After solvent removal and vacuum distillation ester **23** (6.12 g, 56%) was obtained as a colorless liquid: bp 91–94°C/45 mm Hg; IR 3078, 1737, 1642, 1263, 1154, 1111 cm^{-1}; ^1H NMR δ 5.74–5.60 (m, 1H), 5.14–5.05 (m, 2H), 4.15 (q, $J=7$ Hz, 2H), 2.71–2.54 (m, 1H), 2.42–2.17 (m, 3H), 1.27 (s, 3H), 1.24 (t, $J=7$ Hz, 3H); ^{19}F NMR δ -61.1 (t, $^3J_{HF}=11$ Hz). Anal. calcd for $C_{10}H_{15}F_3O_2$: C, 53.57; H, 6.74. Found: C, 53.86; H, 6.71.

5.3 Determination of brain concentrations of fluorinated compounds

The ED_{50} or CD_{50} doses of the test compounds were injected intraperitoneally into female CF-1 mice. The animals were sacrificed and decapitated 30 min after administration of anticonvulsant compounds or at the onset of clonic seizures (or within 5 min of the CD_{50} dose) for convulsant agents. Brain tissue was isolated and extracted with a 2:1 mixture of $CHCl_3$ and MeOH as described previously [3]. Internal standards were included in the extraction solvent to correct for solvent losses during the extractions. In the following list, the internal standards and their concentrations are given in parentheses after the number of the compound whose brain concentration was determined: **1** (dihydro-4-ethyl-

4-methyl-2(3*H*)-furanone, 50 μM), **3** (**23**, 10 μM), **4a** (**3**, 10 μM), **4b** (**3**, 50 μM), **6a** (**3**, 20 μM), **6b** (**3**, 20 μM), **7** (**3**, 10 μM), **8** (**9**, 50 μM), **9** (**10a**, 10 μM), **10a** (**9**, 10 μM), **10b** (**9**, 50 μM).

Analyses were performed with a Hewlett–Packard 5890A GC on an Ultra 1 capillary column (0.2 mm id, 0.11 micron film thickness, 25 m length). Helium was used as the carrier gas (1 ml/min) and splitless injection was employed. The operating temperatures were: injection port, 200°C; detector, 300°C. The oven temperature was programmed from 80–160°C at 8°C/min, for the 2(3*H*)-furanones and from 90–160°C at 8.5°C/min, for the 2(3*H*)-thiophenones. The detector response was obtained and its linearity validated over the appropriate concentration ranges. Brain concentration was determined from the ratio of the integration (HP 3393A integrator) of the flame ionization signal of the test compound and the internal standard with subsequent correction for detector response and tissue wet weight (specific gravity assumed to be 1.0).

5.4 Neurological evaluations, [^{35}S]TBPS binding, and electrophysiology

The methods used have been described previously [3,6,10].

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