Anticonvulsant and Sodium Channel-Blocking Properties of Novel 10,11-Dihydro-5*H*-dibenz[*b,f*]azepine-5-carboxamide Derivatives

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A series of esters of the major metabolite of oxcarbazepine (**2**), 10,11-dihydro-10-hydroxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide, were synthesized and evaluated for their anticonvulsant and brain sodium channel-blocking properties. The compounds were assayed intraperitoneally and per os in rats against seizures induced by maximal electroshock (MES). Neurologic deficit was evaluated by the rotarod test. The enantiomeric acetates (*R*)-**11** and (*S*)-**12** were the most active of the series against MES-induced seizures with oral ED_{50} values at t_{max} of 10.9 ± 2.3 and 4.7 ± 0.9 mg/kg, respectively. After intraperitoneal administration, carbamazepine (**1**) behaved more potently than **2** and all other new dibenz[*b,f*]azepine-5-carboxamide derivatives in the MES test; compounds **2** and **12** were equally potent. In the rotarod test, low doses of **1** produced considerable motor impairment, which did not occur with **2**, enantiomeric alcohols (*S*)-**6**, (*R*)-**7**, and racemic alcohol **8**, or racemic acetate **10** or (*R*)-**11**. The potencies of the racemic and enantiomerically pure alcohols **8**, (*S*)-**6**, and (*R*)-**7** derived from **2** in the MES and rotarod test were found to be similar between them, and consequently they exhibit similar protective index values. All three forms of the alcohol and their corresponding acetates (pairs **8** & **10**, **6** & **12**, and **7** & **11**) were found to differ in the MES or rotarod tests; the ED_{50} value for (*S*)-**6** against MES-induced seizures was nearly 3-fold that for (*S*)-**12**. The protective index also differed markedly between all stereoisomers of the alcohol and their corresponding acetates, most pronouncedly for compound (*S*)-**12** which attained the highest value (12.5) among all compounds tested. Blockade of voltage-sensitive sodium channels was studied by investigating [³H]-batrachotoxinin A 20-α-benzoate ([³H]BTX) binding. Acetates (*R*)-**11** and (*S*)-**12** were more potent than the standards **1** and **2** at inhibiting the binding of [³H]BTX to sodium channels and the influx of ²²Na⁺ into rat brain synaptosomes. It is concluded that acetates (*R*)-**11** and (*S*)-**12** are not simple metabolic precursors of alcohols (*R*)-**7** and (*S*)-**6** in rodents but that they possess anticonvulsant and sodium channel-blocking properties in their own right.

Introduction

First synthesized by Schindler,[1] carbamazepine (**1**) (5*H*-dibenz[*b,f*]azepine-5-carboxamide) was added to the antiepileptic armamentarium in the early 1960s and since then has become the most frequently prescribed first-line drug for the treatment of partial and generalized tonic−clonic epileptic seizures.[2] Although **1** is very effective in the majority of cases, approximately 30−40% of patients with chronic epilepsies are not well-controlled or are largely unresponsive to treatment[3] and between 33% and 50% suffer from unwanted side effects of the medication.[4] Potent induction of hepatic microsomal enzymes[5] that cause self-induction of its own metabolism[6] together with slow and erratic absorption makes medication and especially polymedication with **1** more complicated.[7]

Chemical modifications of **1** were reported,[7] but it was not until the introduction of oxcarbazepine (**2**) in 1990 that another dibenz[*b,f*]azepine derivative with some improved antiepileptic properties appeared. This derivative seems to be equally effective as **1** in the treatment of seizures and is generally accepted as a better tolerated drug.[8,9] Differences in biotransformation

1   **2**
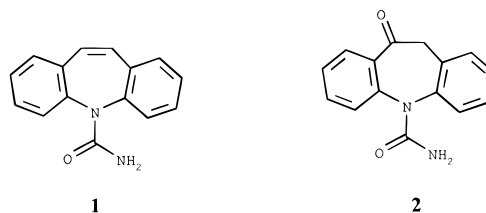
(Scheme 1) were suggested as an explanation for differences in toxicity and adverse effects seen between **1** and **2**.[10]

General and neuronal toxicity of **1** has been ascribed in part to the in vivo formation of the *meso*-epoxide **3**,[11] though in light of its chemical stability,[12] clinical results,[13] and DNA-binding studies,[14] this remains to be firmly established. This active main metabolite is further converted by a microsomal epoxide hydrolase into a pharmacologically inactive mixture of (10*S*,11*S*)-*trans*-diol **4** and its enantiomer **5** in the proportion of approximately 9/1 in humans.[15]

Oxcarbazepine (**2**) is rapidly and extensively metabolized to a mixture of the (10*S*)-alcohol **6** and its (10*R*)-enantiomer **7**, both appearing in plasma[16] and urine[17] in the proportion of approximately 4/1. Further oxidation to a *trans*-diol of undetermined absolute configuration and enantiomeric purity presents only a minor (3.6% of the dose) metabolic pathway in humans.[17] Glucuronides of (*S*)-**6** and (*R*)-**7** are the main end metabolites, and approximately 50% of the dose is excreted in this form. Oxcarbazepine thus presents an
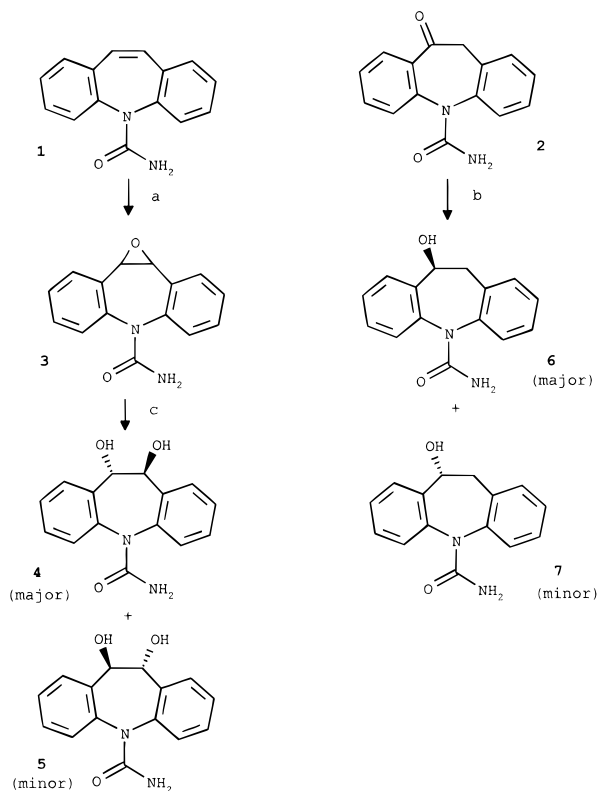
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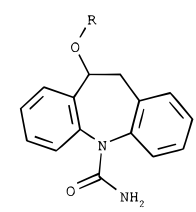|| Deceased.

Scheme 1. Metabolism of Compounds **1** and **2**[a]

[a] (a) Epoxidase; (b) cytosolic ketoreductase; (c) *meso*-epoxide hydrolase.

Table 1. Structure, Physical Properties, and Methods of Preparation of Compounds **6**–**32**[a]

compd	R	method	mp (°C)	yield (%)
6	H[c]	*d*	189–191	33[f]
7	H[b]	*d*	189–191	22[f]
8	H	*d*	185–188	91
9	CHO	A	203–205	72
10	COCH₃	A	158–159	85
11	COCH₃[b]	A	186–187	84
12	COCH₃[c]	A	186–187	82
13	COCH₂Cl	B	185–186	55
14	COCH₂Br	B	177–178	48
15	COEt	B	141–143	75
16	COnPr	B	167–169	80
17	COtBu	A	205–207	50
18	COCHClCH₃[g]	B	192–193	55
19	COCHnPr₂[g]	B	170–171	59
20	COCH(Et)nPr	B	148–149	50
21	CO(CH₂)₁₆CH₃	B	125–126	33
22	COPh	A	179–180	84
23	COCH₂Ph-4-OMe	B	162–163	60
24	COCH₂Ph-4-NO₂	B	200–203	52
25	CO(3-pyridyl)	B	196–198	76
26	COPh-2-OAc	A	*e*	56
27	CO(OAc-(*S*)-mandelate)[g]	A	199–200	52
28	CO(N-BOC-(*S*)-valinate)[g,h]	B	139–144	66
29	CO(l-menthoxyacetate)[g]	A	170–171	71
30	CO((1*S*)-camphanoate)[g]	A	197–198	90
31	COOEt	A	90–92	87
32	COO(l-menthyl)[g]	A	210–212	92

[a] Racemic compounds unless otherwise stated. [b] (*R*)-Enantiomer. [c] (*S*)-Enantiomer. [d] See Experimental Section. [e] Decomposition on heating without sharp melting point. [f] Yield for resolution of enantiomers as menthoxyacetates and following hydrolysis. [g] Mixture of diastereoisomers. [h] Anal. (C₂₅H₃₁N₃O₅) H, N; C: calcd, 66.21; found, 65.76.

example of an apparently achiral prodrug which is metabolically transformed into a mixture of the two active enantiomers (*S*)-**6** and (*R*)-**7**, which are probably responsible for a major part of the observed antiepileptic activity of **2**.[18] Anticonvulsant properties of the racemic alcohol were described and were reportedly comparable in efficacy to **2**.[18] Both (*S*)-**6** and (*R*)-**7** were reported to be active against electroshock-induced seizures in rodents and equally active on the blockade of penicillin-induced epileptiform discharges in hippocampal slices.[19]

The present study was conducted both to evaluate the differences in anticonvulsant activity of the racemic alcohol **8** and its enantiomers (*S*)-**6** and (*R*)-**7** and to assess their potential as starting compounds for the synthesis of corresponding esters that could be envisaged as prodrugs of the active metabolite of oxcarbazepine (**2**). To this end, a series of selected esters were prepared, covering a range of lipophilic, electronic, and steric factors that can influence absorption, distribution, and stability toward chemical and enzymatic hydrolysis and other transformations. These esters were tested in seizure models predictive of antiepileptic efficacy in generalized tonic–clonic seizures, namely, the maximal electroshock (MES) test. The optically pure enantiomers of the most active compound from this series, namely, the acetate **10**, were prepared and further tested and compared with (*S*)-**6**, (*R*)-**7**, **1**, and **2**. The putative mechanism of action of some of these compounds was evaluated in experiments designed to study their interference with voltage-gated sodium channels and sodium uptake in brain synaptosomes.

Chemistry

The racemic alcohol **8** was prepared by sodium borohydride reduction of oxcarbazepine (**2**). Resolution into its enantiomers (*S*)-**6** and (*R*)-**7** was achieved by means of fractional crystallization of diastereoisomeric menthoxyacetate esters. Other esters of optically pure acids that were tried for resolution (camphanoate **30**, *O*-acetylmandelate **27**, N-BOC-valinate **28**, and menthyl carbonate **32**) did not provide significant resolution when crystallization was attempted from various solvent systems, although menthyl carbonate **32** and *O*-acetylmandelate **27** exhibited well-separated peaks of diastereoisomers on HPLC. None of the above-mentioned esters and corresponding Mosher esters provided sufficiently well-resolved ¹H NMR signals that could be used for assessment of optical purity. Proton spectra of all esters reveal two sets of multiplets for hydrogens at positions 10 and 11 that are ascribed to the presence of two conformers,[20] whereby the acyl group is pseudoaxial or pseudoequatorial with the ratio of the two conformers close to 1 at room temperature.

Esterifications of alcohols **6**–**8** were achieved by use of the appropriate acyl chloride or anhydride or by the acid according to the Steglich–Hassner procedure.[21] The physiochemical properties of new dibenz[*b,f*]azepine-5-carboxamide derivatives are reported in Table 1.

Table 2. Protection by **1**, **2**, and 10,11-Dihydro-10-hydroxy-5H-dibenz[b,f]azepine-5-carboxamide Ester Derivatives against Seizures MES-Induced[a]

compd	% protection
1	100.0 ± 0.0
2	68.3 ± 20.3
6	42.0 ± 17.9
7	22.5 ± 16.1
8	45.2 ± 19.5
9	32.1 ± 17.4
10	54.4 ± 20.2
11	5.9 ± 1.8
12	100.0 ± 0.0
13	0.5 ± 4.6
14	15.1 ± 4.0
15	40.6 ± 19.7
16	34.2 ± 20.9
17	6.5 ± 11.3
18	3.9 ± 26.4
19	0.2 ± 9.2
20	-12.8 ± 8.6
21	0.8 ± 5.8
22	17.2 ± 10.7
23	8.1 ± 4.9
24	13.8 ± 5.1
25	32.8 ± 19.0
26	10.2 ± 10.1
31	18.3 ± 18.7

[a] All compounds were given by gastric tube. Values are means $\pm$ SEM of 5–8 rats/group.

Results and Discussion

The compounds were tested for anticonvulsant activity using the procedures described previously.[22,23] In the first series of experiments, the compounds were administered by the oral route at a dose equimolar to 15 mg/kg oxcarbazepine (**2**). Two hours after their administration, the MES test was performed for each compound. Initial evaluation of the anticonvulsant activity of the synthesized racemic esters (Table 2) demonstrated that only the acetate **10** displayed activity comparable to or greater than that of the racemic alcohol **8**. The homologous aliphatic esters **15** and **16** exhibited reduced activity which was also observed with the less hydrolytically stable formyl derivative **9**. A marked sharp decrease in activity was associated with branched compounds **17** and higher homologue **19**; conversely, the 2-ethylhexanoate ester **20** showed surprising signs of proconvulsive activity. Attention was then turned to aromatic esters: the phenyl derivative **22** was endowed with low activity which was not improved by the incorporation of electron-donating (OCH_3, **23**) or electron-withdrawing (NO_2, **24**) groups or bulky electronegative substituents at the ortho position ($OCOCH_3$, **26**). The heteroaromatic pyridyl ester **25** showed marginal but not entirely encouraging improvement as was also the case for ethyl carbonate **31**. Consequently, the two enantiomers of the acetate, (R)-**11** and (S)-**12**, were thereafter evaluated and found to possess strikingly different properties: (S)-**12** was clearly the most potent anticonvulsant 2 h after administration with (R)-**11** almost devoid of activity and racemic **10** intermediate between both.

The administration by gastric tube of compounds listed in Table 3 conferred a dose-dependent protection of rats against MES-induced seizures. With the exception of compound (R)-**11**, t_{max} for anticonvulsant effect occurred at 2 h after the administration; this compound achieved its maximal activity 8 h after the administration. Compound (S)-**12** was found to be equally potent as **1** and more potent than **2**, **10,** and (R)-**11**. The oral

Table 3. ED$_{50}$, TD$_{50}$, and Protective Index Values for **1**, **2**, and Acetates **10**–**12**

compd	t_{max} (h)	MES ED$_{50}$ (mg/kg)	rotarod TD$_{50}$ (mg/kg)	protective index[b]
1	2	5.4 ± 0.8	251.0 ± 9.8	46.5
2	2	10.0 ± 1.9	>1000	>100.0
10	2	13.5 ± 2.2	427.9 ± 64.6	31.7
11	8	10.9 ± 2.3	>1000	>91.7
12	2	4.7 ± 0.9	358.7 ± 17.2	76.3

[a] All compounds were given by gastric tube. Data points are means $\pm$ SEM of 5–8 rats/group. For the calculation of the ED$_{50}$'s and TD$_{50}$'s, the parameters of the logistic equation were fitted to the experimental data. [b] TD$_{50}$/ED$_{50}$.

Table 4. ED$_{50}$, TD$_{50}$, and Protective Index Values for **1**, **2**, and Other Substituted 10,11-Dihydro-5H-dibenz[b,f]azepine-5-carboxamide Derivatives[a]

compd	t_{max} (min)	MES ED$_{50}$ (mg/kg)	rotarod TD$_{50}$ (mg/kg)	protective index[b]
1	15	3.4 ± 0.1	27.4 ± 0.1	8.1
2	5	6.1 ± 0.1	40.1 ± 1.2	6.6
6	30	17.1 ± 0.6	96.6 ± 4.1	5.6
7	30	17.9 ± 0.8	97.4 ± 5.2	5.4
8	15	14.6 ± 1.6	89.3 ± 0.5	6.1
10	5	13.1 ± 0.2	130.2 ± 1.2	9.9
11	60	18.0 ± 0.1	134.9 ± 3.5	7.5
12	15	6.3 ± 0.1	78.6 ± 4.2	12.5

[a] All compounds were given intraperitoneally. Data points are means $\pm$ SEM of 5–8 rats/group. For the calculation of the ED$_{50}$'s and TD$_{50}$'s, the parameters of the logistic equation were fitted to the experimental data. [b] TD$_{50}$/ED$_{50}$.

administration of (S)-**12** conferred a dose-dependent motor impairment of rats in the rotating rod apparatus. The TD$_{50}$ values for (S)-**12** at 2 h after the administration were greater than those for compound **1**, similar to that for compound **10**, but lower than those for **2** and (R)-**11**. Considering the protective index (TD$_{50}$ po/ED$_{50}$ po) as a measure of therapeutic tolerability, these data indicate that compound (S)-**12** should be better tolerated than **1** and **10**, but less so than **2** and (R)-**11**.

In another set of experiments, these most active compounds were given to rats by the ip route and tested at t_{max} (see Table 4). As shown in this table, **1** behaved more potently than **2** and all other new dibenz[b,f]-azepine-5-carboxamide derivatives on the MES test; compounds **2** and (S)-**12** were equally potent. In the rotarod test, low doses of carbamazepine (**1**) produced considerable motor impairment, which did not occur with oxcarbazepine (**2**), alcohols (S)-**6**, (R)-**7**, and **8**, and acetates **10** and (R)-**11**. The potencies of the enantiomerically pure alcohols (S)-**6**, (R)-**7**, and racemate **8** in the MES and rotarod test were found to be similar between them, and consequently they exhibit similar protective index values. Surprisingly, all three forms of the alcohol and their corresponding acetates (pairs **8** & **10**, **6** & **12**, and **7** & **11**) were found to differ markedly in the MES or rotarod tests (Table 4); the ED$_{50}$ value for (S)-**6** against MES-induced seizures was nearly 3-fold that for (S)-**12**. The protective index also differed markedly between all forms of the alcohol and their corresponding acetates, most pronouncedly for (S)-**12** which attained the highest value (12.5) among all compounds tested.

At therapeutic concentrations, **1** has a specific action to prevent seizures without diminishing normal electrical activity in the brain. Although the mechanism by which **1** exerts its antiepileptic effect is not clear, it has been proposed to inhibit voltage-dependent sodium

Table 5. Displacement of [3H]BTX (10 nM) Binding to Rat Cortical Synaptosomes by **1**, **2**, and Acetates **10**−**12**[a]

compd	IC$_{50}$ (μM)
1	210 ± 15
2	161 ± 16*
10	141 ± 9*
11	113 ± 39*
12	138 ± 32*

[a] Data points are means $\pm$ SEM of 4−5 determinations in triplicate. For the calculation of the IC$_{50}$'s, the parameters of the equation for one-site binding were fitted to the experimental data.[36] *Significantly different from values for carbamazepine (P < 0.05) using Student's t test.

Table 6. Percent Inhibition of ^{22}Na$^+$ Uptake by Increasing Concentrations (30, 100, and 300 μM) of **1**, **2**, and Acetates **10**−**12** in Rat Cortical Synaptosomes[a]

compd	% inhibition		
	30 μM	100 μM	300 μM
1	22.6 ± 1.6	44.3 ± 2.8	64.8 ± 3.0
2	31.2 ± 8.2	50.2 ± 6.2	76.2 ± 5.0
10	42.2 ± 6.6	61.8 ± 7.2	82.1 ± 6.1*
11	17.9 ± 7.9	57.7 ± 3.5	73.2 ± 6.0
12	52.6 ± 4.5*	73.4 ± 6.3*	95.6 ± 2.6*

[a] Results are means $\pm$ SEM of 6 experiments in triplicate. *Significantly different from values for carbamazepine (P < 0.05) using Student's t test.

channels,[24] to inhibit calcium channels, and to interact with adenosine receptors,[25] among other mechanisms.[26]

In fact, sodium channel inhibition has been a natural candidate for the mechanism of action of **1** based on the reported interaction with receptor sites involved in the activation of sodium channels[27] and on the modulation of sodium entry[28] and sodium currents.[29,30] To confirm that sodium channels are a primary locus for the action of the new dibenz[b,f]azepine-5-carboxamide derivatives, it was considered necessary to compare the relative potency of these compounds as anticonvulsants with their relative potency to bind to sodium channels and to modulate sodium entry.

All dibenz[b,f]azepine-5-carboxamide derivatives listed in Table 5, within the concentration range tested (0.1−1000 μM), displaced [3H]BTX (10 nM) binding to rat cortical synaptosomes. As shown in this table, the relative potency (IC$_{50}$ in μM) of carbamazepine was significantly lower than that of compounds **2**, **10**, (*R*)-**11**, and (*S*)-**12**.

As shown in Table 6, all new dibenz[b,f]azepine-5-carboxamide derivatives inhibited in a concentration-dependent manner the uptake of ^{22}Na$^+$ by cortical synaptosomes. Within the concentration range tested, **2** was more potent than **1**. It was also observed that three new dibenz[b,f]azepine-5-carboxamide derivatives **10**, (*R*)-**11**, and (*S*)-**12** were more potent than **1**. Compound (*S*)-**12** was the most potent at inhibiting ^{22}Na$^+$ uptake by cortical synaptosomes, while **10** had a potency between that of (*S*)-**12** and (*R*)-**11**.

The rank order of potency of inhibitors of ^{22}Na$^+$ uptake obtained in the present study closely parallels their rank order of potency as displacers of [3H]BTX binding to rat cortical synaptosomes and their potency as anticonvulsants. This suggests that the interaction of these compounds with sodium channels produces inhibition of ^{22}Na$^+$ uptake into cortical synaptosomes and reduces seizure spread. Noteworthy, is the observation that compound (*R*)-**11**, despite its considerable

potency as an inhibitor of [3H]BTX binding and ^{22}Na$^+$ uptake, behaved less potently in producing immediate anticonvulsant effects.

Conclusion

A series of selected esters of the known metabolite (alcohol **8**) of oxcarbazepine **2** were synthesized. No member of this family of racemates was found to display greater activity than the parent itself except the acetate **10** through screening in vivo. The enantiomeric acetates (*R*)-**11** and (*S*)-**12**, however, conferred potent and pronouncedly different time-dependent protection. These data as well as enhanced protective index values suggest that acetates **10**, (*R*)-**11**, and (*S*)-**12** are not simple metabolic precursors of alcohols **8**, **7**, and **6** but that they may possess anticonvulsant activity in their own right or that their differing profiles could be attributed to complex absorption, distribution, metabolism, or excretion processes, which depend on the absolute stereochemistry at the C-10 chiral center. Subtle differences in the above processes might explain the profile of (*R*)-**11** as a slow-onset, long-acting anticonvulsant. A tentative mechanism of action for these compounds is proposed based on the observed interaction with sodium channels and inhibition of ^{22}Na$^+$ uptake into cortical synaptosomes.

Experimental Section

Chemistry. Melting points were measured in open capillary tubes on an Electrothermal model 9100 hot stage apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance DPX (400-MHz) spectrometer with solvent used as an internal standard, and data are reported in the order: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons, approximate coupling constant in hertz, and assignment of a signal. IR spectra were taken with a Bomem Hartmann & Braun MB series FTIR spectrometer using KBr tablets. Mass spectra were measured with a Hitachi-Perkin-Elmer RMU-6M low-resolution instrument (EI, 70 eV). Only molecular ions (M$^+$) and base peaks are given. Analytical HPLC was performed on a Gilson System equipped with a model 305 pump and 117 UV detector, using a LiChroCART 250-4 cartridge ChiraDex 5-μm column (Merck) and a 0.1 N aqueous Na$_2$HPO$_4$/methanol (8/2) mixture as mobile phase (optical purity assessment) and LiChrospher 100 RP-18 EcoCART 125-3 cartridges (Merck) in combination with acetonitrile/water mixtures. Analytical TLC was performed on precoated silica gel plates (Merck 60 Kieselgel F 254) and visualized with UV light. Preparative chromatography was done on Merck 60 Kieselgel (0.063−0.2 mm). Optical rotations were measured on a Jasco DIP 1000 polarimeter at the sodium d line (589 nm), at 20 °C using a 1-dm path length cell. Elemental analyses were performed on a Fisons EA 1110 CHNS instrument, and all analyses are consistent with theoretical values to within ±0.4% unless indicated. Solvents and reagents were purchased from Aldrich or E. Merck. Standard workup refers to washing of a reaction mixture in water-immiscible solvent consecutively with ice-cold 0.1 N aqueous hydrochloric acid, saturated aqueous sodium bicarbonate solution, and brine, followed by drying with sodium sulfate and evaporation at 45 °C at water aspirator vacuum.

Racemic 10,11-Dihydro-10-hydroxy-5*H*-dibenz[b,f]azepine-5-carboxamide (8). Oxcarbazepine (172 g, 0.826 mol) was suspended in a mixture of water (0.4 L) and ethanol (0.7 L), sodium borohydride (20 g, 0.526 mol) was added in portions over 10 min without cooling, and stirring continued at 45 °C for 1 h. Acetone (150 mL) was added cautiously, and the reaction mixture was evaporated down to a small volume. Addition of water precipitated the product (157.8 g, 91%): mp

185−188 °C (lit.[31] mp 188−192 °C); ^{1}H NMR (CDCl$_3$) δ 7.7−7.1 (m, 8H, Ar−H), 5.3, 4.7 (2 × br s, 1H, C$_{10}$−H), 4.9 (br s, 2H, NH$_2$), 3.7, 2.7 (2 × br s, 1H, −OH), 3.5 (m, 1H, C$_{11}$−H), 3.0 (m, 1H, J = 15 Hz, C$_{11}$−H). Anal. (C$_{15}$H$_{14}$N$_2$O$_2$) C, H, N.

(R)-(−)-10,11-Dihydro-10-hydroxy-5H-dibenz[b,f]aze-pine-5-carboxamide (7). To a stirred solution of l-(−)-menthoxyacetic acid chloride (16.5 g, 77 mmol) in dichloromethane (160 mL) was added the racemic alcohol **8** (17.78 g, 70 mmol), followed by pyridine (8.13 mL, 100 mmol) and 4-(dimethylamino)pyridine (489 mg, 4 mmol), and the reaction mixture was stirred for 16 h. After standard workup and crystallization from dichloromethane/ethyl acetate to constant melting point and optical rotation, 7.57 g (24%) of l-(−)-menthoxyacetate was obtained as a white solid: mp 201−202 °C; ^{1}H NMR (CDCl$_3$) δ 7.6−7.15 (m, 8H, Ar−H), 6.5, 6.1 (2 × br s, 1H, C$_{10}$−H), 4.8 (br s, 2H, NH$_2$), 4.1 (m, 2H, −COCH$_2$O-), 3.6 (m, 1H, C$_{11}$−H), 3.2 (m, 2H, C$_{11}$−H, C$_{1'}$−H), 2.3 (m, 1H, C$_{5'}$-H), 2.1 (m, 1H, C$_{6'}$-H), 1.65 (m, 2H, C$_{3'}$-H, C$_{4'}$-H), 1.3 (m, 2H, C$_{2'}$-H, C$_{7'}$-H), 0.9 (m, 9H, -(CH$_3$)$_2$, C$_{3'}$-H, C$_{4'}$-H, C$_{6'}$-H), 0.7 (m, 3H, CH$_3$); $[\alpha]_D^{20}$ = 20.2° (c 0.51, pyridine); HPLC 98.5% of (R)-enantiomer. Anal. (C$_{15}$H$_{14}$N$_2$O$_2$) C, H, N.

The menthoxyacetate was hydrolyzed at room temperature by aqueous methanolic sodium hydroxide solution to the title compound (3.88 g, 91%): mp 189−191 °C; $[\alpha]_D^{20}$ = −196° (c 0.87, ethanol) (lit.[17] $[\alpha]_D^{20}$ = −197°, ethanol).

(S)-(+)-10,11-Dihydro-10-hydroxy-5H-dibenz[b,f]aze-pine-5-carboxamide (6). The mother liquors after preparation of the above diastereoisomer of menthoxyacetate were hydrolyzed as described above, and the (S)-enantiomer-enriched alcohol was resolved by D-(+)-menthoxyacetic acid chloride. The menthoxyacetate (11.0 g, 35%), mp 201−202 °C, $[\alpha]_D^{20}$ = −19.7° (c 0.54, pyridine), was hydrolyzed to the title compound (5.77 g, 93%): mp 189−191 °C; $[\alpha]_D^{20}$ = 199° (c 1.10, pyridine); HPLC 99.5% of (S)-enantiomer. Anal. (C$_{15}$H$_{14}$N$_2$O$_2$) C, H, N.

(S)-(−)-10-Acetoxy-10,11-dihydro-5H-dibenz[b,f]azepine-5-carboxamide (12). General Method A: (S)-(+)-Alcohol **6** (25.4 g, 0.1 mol), pyridine (9.89 g, 0.125 mol), 4-(dimethyl-amino)-pyridine (0.3 g, 2.46 mmol), and acetyl chloride (9.82 g, 0.125 mol) in dichloromethane (200 mL) were stirred at room temperature for 1 h. Standard workup followed by crystallization furnished (S)-**12** (24.59 g, 83.5%): mp 186−187 °C; $[\alpha]_D^{20}$ = −20.5° (c 1.0, pyridine); ^{1}H NMR (CDCl$_3$) δ 7.6−7.1 (m, 8H, Ar−H), 6.4, 6.0 (2 × br s, 1H, C$_{10}$−H), 5.0 (br s, 2H, NH$_2$), 3.6, 3.2 (2 × m, 2H, C$_{11}$−H), 2.1 (s, 3H, −CH$_3$); ^{13}C NMR (CDCl$_3$) δ 171.1, 170.6 (CO), 157.4, 157.0 (NCON), 141.7, 141.0, 139.4, 134.8, 133.9 (quaternary C) 131.6, 131.4, 129.6, 129.2, 128.7, 128.6, 128.5, 128.2, (ArC), 72.7, 70.5 (C$_{10}$), 36.4, 36.1 (C$_{11}$), 21.6 (CH$_3$); MS m/z 296 (M$^+$), 193 (100); HPLC 99.8% of (S)-enantiomer. Anal. (C$_{17}$H$_{16}$N$_2$O$_3$) C, H, N.

Racemic 10,11-Dihydro-10-(nicotinoyloxy)-5H-dibenz-[b,f]azepine-5-carboxamide (25). General Method B: Racemic alcohol **8** (2.54 g, 10 mmol) was suspended in dichloromethane (50 mL), and dicyclohexylcarbodiimide (2.27 g, 11 mmol) was added followed by nicotinic acid (1.35 g, 11 mmol) and 4-(dimethylamino)pyridine (122 mg, 1 mmol). The reaction mixture was stirred at room temperature overnight and filtered, and after standard workup and chromatography using stepwise gradient from dichloromethane to 2% methanol in dichloromethane, the title compound **25** (2.74 g, 76%) was obtained as white crystals: mp 195−196 °C (ethyl acetate/ether); ^{1}H NMR (CDCl$_3$) δ 9.3, 9.1 (2 × br s, 1H, pyridine C$_2$−H), 8.8 (d, 1H, J = 5 Hz, pyridine C$_6$−H), 8.4, 8.2 (2 × br s, 1H, pyridine C$_4$−H), 7.65−7.1 (m, 9H, pyridine C$_5$−H and Ar−H), 6.7, 6.3 (2 × br s, 1H, C$_{10}$−H), 4.8 (br s, 2H, NH$_2$), 3.8, 3.3 (2 × br s, 2H, C$_{11}$−H). Anal. (C$_{21}$H$_{17}$N$_3$O$_3$) C, H, N.

Pharmacological Methods. All compounds were tested using male Wistar rats (Harlan-Interfauna Ibérica, Barcelona, Spain, or Gulbenkian Science Institute, Oeiras, Portugal) ranging from 6 to 8 weeks old.

1. MES Test. MES stimulation was applied for 0.2 s, using a Ugo Basile ECT unit 7801, with a frequency of 100 Hz, pulse width of 0.6 ms, and current of 150 mA through bipolar corneal electrodes. A drop of electrolyte/anesthetic, oxibuprocaine

chloride, was applied in the eyes of all animals immediately before placement of corneal electrodes. Abolition of the hindleg tonic extensor component was used as the endpoint. These experimental conditions produced tonic−clonic convulsions in 97% of animals tested, and only rats showing typical tonic−clonic convulsions were used.[22] All rats were submitted to a maximum of 3 MES sessions: the first MES session was performed to screen the animals and select those rats presenting a typical convulsive behavior. The day after, rats were given the compounds to be tested or the vehicle and submitted to a second MES session 2 or 4 h after the administration of test compounds. The third MES session was performed at 6, 8, or 12 h after the administration of test compounds. The time interval between each MES session was at least 4 h (rats tested at 2 h were retested at 6 h and rats tested at 4 h were retested at 8 h). The evaluation of the anticonvulsive profile of test compounds was based on the duration of the tonic phase (in seconds), each rat being its own control (internal control) as obtained in the first MES session. An external control group was also studied; in this particular case, rats were given the vehicle and submitted to the three MES sessions procedure as described above. All compounds used were suspended in 0.5% carboxymethylcellulose (4 mL/kg) and given by stomach tube. In some experiments, compounds used were dissolved in dimethyl sulfoxide (DMSO) (2 mL/kg) and given ip.

2. Rotarod Test. Rats were examined for motor toxicity in the rotating rod apparatus (Accelerator Rota-Rod (Jones & Roberts) 7750; Ugo Basile). Naive rats were trained to hold onto the 5-cm diameter neoprene rubberized cylinder until able to maintain the equilibrium for 3 min while rotating at 6 rpm. The day after, rats were given ip the test compound (dissolved in DMSO, 2 mL/kg) and 15 min later were placed on the rotating rod at a speed of 6 rpm. In a compound-treated rat the neurological deficit is indicated by the inability of the rat to maintain equilibrium for 1 min in each of three trials.[32]

3. Blockade of Voltage-Sensitive Sodium Channels. Blockade of voltage-sensitive sodium channels was studied by investigating [3H]batrachotoxinin A 20-α-benzoate ([3H]BTX) displacement binding to rat cortical synaptosomes. Rat cerebral cortical synaptosomes were prepared by differential sucrose-Percoll density gradient centrifugations, as previously described.[33] Binding studies were performed by incubation of [3H]BTX for 30 min at 37 °C with 42−680 μg of membrane protein in a final volume of 200 μL in a solution containing KCl (130 mM), MgSO$_4$ (0.8 mM), glucose (5.5 mM), HEPES (50 mM, pH 7.4), scorpion toxin (0.5 μM), tetrodotoxin (1 μM), [3H]BTX (10 nM), bovine serum albumin (1 mg/mL), and varying concentrations of competing compounds (0.1−1000 μM), as previously described.[27] The binding reactions, performed in 96-well EIA/RIA plates (Costar) with 300-μL capacity, were initiated by the addition of 50 μL of the synaptosomal solution to 150 μL of the reaction mixture. The specific binding was calculated by subtraction of the nonspecific binding, which was determined in the presence of 300 μM veratridine. The binding reactions were stopped by vacuum filtration (Tomtec Harvester 96) through glass fiber filtermats A (1450-21 from Wallac) and washing of the filters and incubation tubes with 6 mL of an ice-cold wash solution consisting of choline chloride (130 mM), CaCl$_2$ (1.8 mM), MgSO$_4$ (0.8 mM), bovine serum albumin (1 mg/mL), and 5 mM HEPES/TRIS (pH 7.4). The filtermats were dried, impregnated with MeltiLex A scintillation mixture (Wallac), and inserted into plastic sample bags (Wallac), and the radioactivity was counted in a 1450 Micro-Beta spectrophotometric detector for 2 min with an efficiency of 55−60%. [3H]BTX (specific activity: 50.50 Ci/mmol) was from DuPont NEN. Tetrodotoxin, veratridine, and scorpion toxin from *Leiurus quinquestriatus hebraeus* were from Sigma. Scorpion toxin was resuspended in ice-cold distilled water (1 mg/mL) and incubated for 1 h at 0 °C. The mixture was then centrifuged at 12000g for 10 min and the supernatant added to the incubation medium. As previously reported,[34] this supernatant was composed by a major band of 7 kDa, spreading from 4 to 14 kDa, upon FPLC separation (data not shown).

4. Inhibition of $^{22}Na^+$ Uptake by Rat Cortical Synaptosomes. Sodium uptake through sodium channels was measured by the method described by Tamkun and Catterall[35] with slight modifications. Aliquots of 45 μL of synaptosomal suspension were preincubated at 37 °C with 5 μL of the test substances or with 5 μL of resuspension solution. After a preincubation of 9 min, sodium channels were opened by addition of veratridine (20 μM); nonspecific uptake of $^{22}Na^+$ (specific activity of 833 mCi/mmol; Amersham) was determined by simultaneous addition of veratridine (20 μM) and tetrodotoxin (1 μM). One minute after addition of 5 μL of veratridine or veratridine and tetrodotoxin solutions, 150 μL of incubation solution (resuspension solution containing 3.7 mM NaCl, 1.87 μCi/mL ^{22}NaCl, 6.7 mM ouabain, 26.7 μM veratridine, the appropriate concentration of the test compounds, and, only when measuring nonspecific $^{22}Na^+$ uptake, 1.33 μM tetrodotoxin). Incubation was performed for 5 s and terminated by addition of 3 mL of ice-cold wash solution (containing 163 mM choline chloride, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 50 mM HEPES/TRIS, pH 7.4, and 1 mg/mL BSA), immediately followed by filtration under reduced pressure through Whatman GF/C glass fiber (0.45-μm pore diameter) filters. The incubation tube and the filter were washed twice with 3 mL of washing solution. After drying the filters, $^{22}Na^+$ trapped on the filters was counted for 10 min by scintillation spectrophotometry (wide-open window) upon addition of 5 mL of scintillation cocktail (Optiphase HiSafe, Wallac). The effect of compounds was calculated as a percentage of the specific uptake of $^{22}Na^+$. The specific $^{22}Na^+$ uptake calculated upon subtraction of the nonspecific uptake determined in the presence of tetrodotoxin (1 μM) was 1.13 $\pm$ 0.12 pmol/mg of protein. All dibenz[b,f]azepine-5-carboxamide derivatives were made up as a 50 mM solution in ethanol, except compound **2** which was made up as a 50 mM solution in DMSO. Neither DMSO nor ethanol in concentrations up to 2% (v/v) had any effect on $^{22}Na^+$ uptake by rat cortical synaptosomes.

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