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Anticonvulsant activity of some xanthone derivatives

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ABSTRACT

A series of appropriate alkanolamine and amide derivatives of xanthone were prepared and evaluated for anticonvulsant activity using maximal electroshock (MES) and subcutaneous pentylenetetrazole (scMet) induced seizures, and for neurotoxicity (TOX) using the rotorod test on mice and rats. The most promising compounds seem to be the appropriate aminoalkanolic derivatives of 6-chloroxanthone, among which the *R*-(−) and *S*-(+)-2amino-1-propanol derivatives of 6-chloro-2-methylxanthone (**2[a]** and **2[b]**) displayed anti-MES activity (in mice) with a protective index (TD_{50}/ED_{50}) of 6.23 < 6.85, corresponding to that of phenytoin, carbamazepine and valproate. The most active compound, **2[b]**, was determined to have an affinity to the benzodiazepine (BDZ) receptor and voltage-dependent Ca^{2+} channel (VDCC) by using radioligand binding assays. The enantiomeric purities of **2[a]** and **2[b]** were determined using an analytical liquid chromatography–mass spectrometry method.

1. Introduction

Knowing the mechanism of action of existing antiepileptic drugs provided a rational approach to the design of the new anticonvulsant agents. The most clinically used antiepileptic drugs act by more than one mechanism. Antiepileptic drugs can influence the inhibitory or excitatory neurotransmitter in the mammalian central nervous system (CNS) (γ-aminobutyric acid (GABA) or (*S*)-glutamic and aspartic acids, respectively[1–6] or ion transport across cell membranes.

While searching for compounds with potential antiepileptic action we directed our attention to the xanthone derivatives, which show several beneficial heterogenous and varied pharmacological properties, for example, analeptic,[7] antiallergic,[8] antilipidemic,[9] diuretic,[10] antitumor,[11] protein kinase C inhibitors,[12] antimycotics,[13,14] and antimicrobial.[15] In recent years, we have reported on the anticonvulsant activity of a series of aminoalkanolic derivatives of xanthone. Some of them provided excellent protection, mainly against MES-induced seizures, and had low neurotoxicity (TOX). The most promising compounds were (*R,S*)-2-amino-1-propanol derivatives of 6-chloro-2-methylxanthone and (*R,S*)-, (*R*)- or (*S*)-2-amino-, or 2-*N*-methylamino-1-butanol derivatives of 7-chloro-2-methylxanthone, with an anti-MES ED_{50} (mg/kg) value of 56.2 (PI (protection index) = 5.84), 104.6 (PI = 3.15), 76.5 (PI = 3.62), 27 (PI = 3.28), 33.4 (PI = 2.84) in mice dosed intraperitoneally (ip).[16,17] Positive anticonvulsant activity, with a lower neurotoxicity, was also observed for (*S*)-2-*N*-[(2,6-dimethyl)-phenoxyethyl]-amino-1-butanol, with an anti-MES ED_{50} (mg/kg) value of 7.57 (PI = 4.55) (mice, ip).[18] At this stage it seems that the anticonvulsant activity was mainly associated with the aminoalkanol type and its configuration.

In this paper, we report on the synthesis, structural characterization (Scheme 1), and preliminary evaluation of the anticonvulsant properties of a number of xanthone derivatives **1**–**19** including also the enantiomeric structures of the previously described racemic mixture.[16,19] Compound **2[b]**, which displayed the highest activity, was determined to have an affinity to the BDZ receptor and to the voltage-dependent Ca^{2+} channel (VDCC).

2. Chemistry

As we were interested in the relationship between structure and anticonvulsant activity we synthesized appropriate 2-substituted xanthones with the chiral or achiral moiety of the corresponding aminoalkanol (Table 1), in order to compare their anticonvulsant activity and to find out whether the activity is associated with steric structure and the type of compound (amine, amide). 6-Chloro-2-substituted xanthone, with the chiral moiety of the appropriate aminoalkanol, such as: (*R,S*)-1-amino-2-butanol, (*R*)-, (*S*)-2-amino-1-butanol, (*R*)-, (*S*)-1-amino-2-propanol, (*R*)-, (*S*)-2-amino-1-propanol (**2[a]** and **2[b]**), 3-amino-1-propanol, and other achiral aminoalkanols, for example, 2-aminoethanol, or 1-amino-pentanol and *trans*-2-amino-1-cyclohexanol, were synthesized using the

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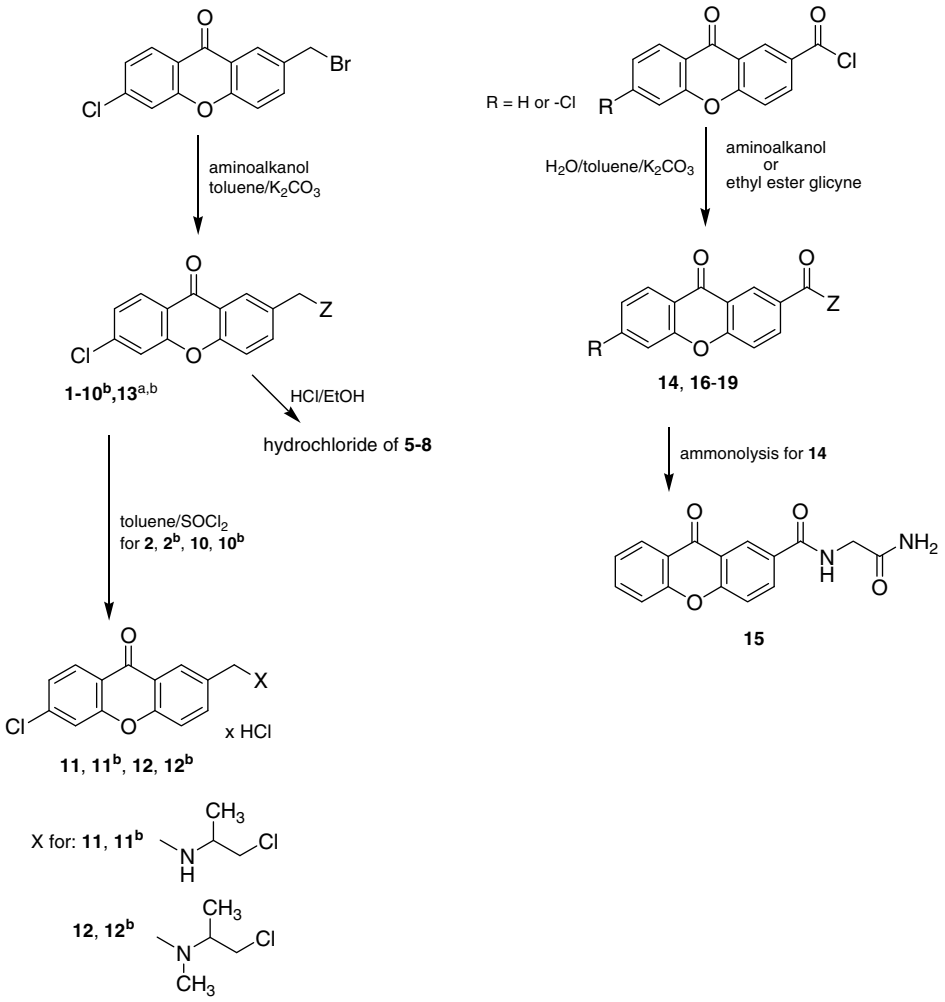


Scheme 1. Synthesis of xanthone derivatives **1**–**19**.

standard method from 2-bromomethyl-6-chloroxanthone,[16] with the corresponding aminoalkanol in toluene, in the presence of anhyd potassium carbonate. Some of these are enantiomers (**2**^{a,b}, **3**^{a,b}, and **13**^{a,b}). The anticonvulsant properties and physico-chemical data of suitable racemic mixtures were described previously.[16] The compounds with the highest anticonvulsant activity, that is, (*R*,*S*)[16] and (*S*)-(+)-2-amino-1-propanol derivatives of 6-chloro-2-methylxanthone (**3**), were subjected to N-methylation by the Leuckart reaction, in accordance with well-known procedures.[17] The replacement of the hydroxy group by a chloride in appropriate 2-amino-1-propanol xanthone derivatives with thionyl chloride gave the final product of hydrochlorides **11**–**12**^{b}. The enantiomers **2**^{a,b}, **3**^{a,b} **10**^{b}, **11**^{b}, and **12**^{b} were recrystallized at a constant rate of rotation. Additionally, the enantiomeric purity of **2**^{a} and **2**^{b} was determined by a high-performance liquid chromatography–mass spectrometry method with an electrospray ionization interface (ESI-LC/MS). Compounds of the series **14** and **16**–**19** were synthesized by N-acylation of glycine ethyl ester (**14**) and an appropriate aminoalkanol with 2-xanthone- or 6-chloro-2-xanthonecarboxy chloride (**16**–**19**), in a two-phase system (toluene/H_2O/K_2CO_3). The necessary chloride of the appropriate xanthone-2-carboxylic acid was prepared in advance by heating the corresponding acid under reflux with thionyl chloride in toluene solution. The raw product was used in the N-acylation process. Reaction yields were within the range 65–71%. The transformation of the ethyl ester group of **14** into the amide group of **15** was accomplished using ammonolysis (25% $NH_{3\ aq}$) in ethanol solution.

3. Results and discussion

3.1. Enantiomeric purity

An analytical liquid chromatography–mass spectrometry method was developed to determine the enantiomeric purity of the compounds under investigation. The separation of the two enantiomers (**2**^{a} and **2**^{b}) was carried out on the commercially available chiral stationary phase, cellulose tris (3,5-dimethylphenyl carbamate), chiralcel OD-RH.

This method provides high specificity and selectivity for identifying isomers. Figures 1 and 3 show the extracted ion chromatograms for **2**^{a} and **2**^{b}, respectively, and Figures 2 and 4 show representative LC chromatograms obtained under the analytical conditions for the compounds studied. The protonated molecules $[M+H]^+$ of **2**^{a} and **2**^{b} were observed as base peak at 318.8 *m*/*z*. On the basis of the results obtained, the separation of the selected compounds gives enantiomers with a final purity of more than 99.9% (Table 1).

3.2. The anticonvulsant assays

The anticonvulsant activity and neurotoxicity of the synthesized compounds **1**–**19** were evaluated by the National Institute of Neurological Disorders and Stroke (NINDS) at the National Institute of Health (NIH) (Bethesda, MD, USA) using established procedures.[20] Phase I studies involved three tests: MES, ScMet (subcutaneous pentylenetetrazole-induced seizures) and TOX.

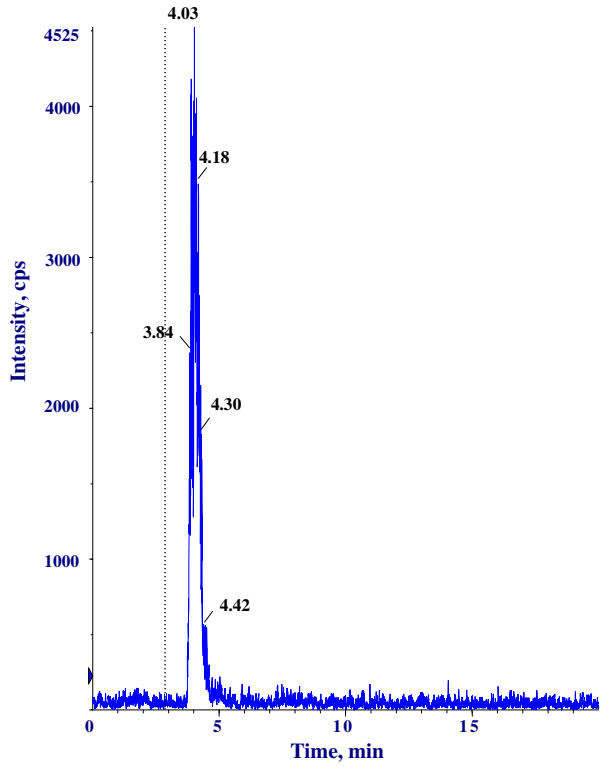


Figure 1. Selected ion chromatogram of **2**[a], $[M+H]^+$ at 318.8 *m/z*.

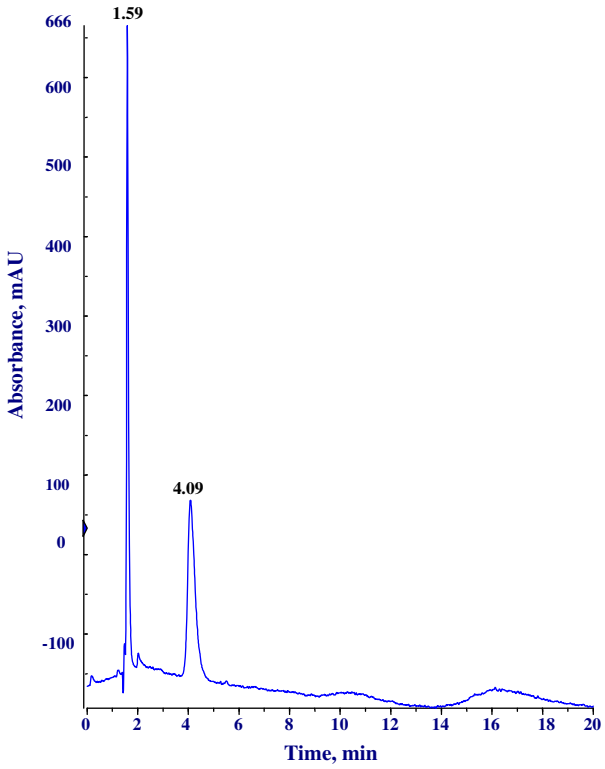


Figure 2. Selected chromatogram of **2**[a].

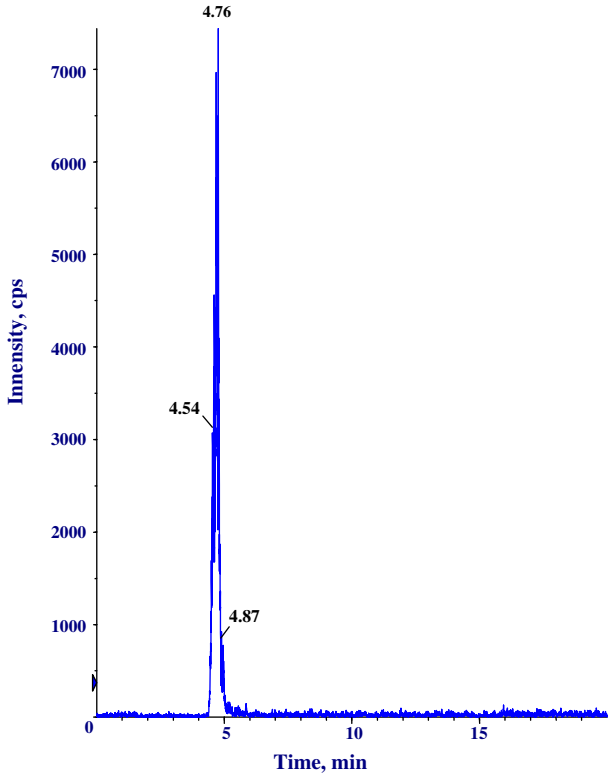


Figure 3. Selected ion chromatogram of **2**[b], $[M+H]^+$ at 318.8 *m/z*.

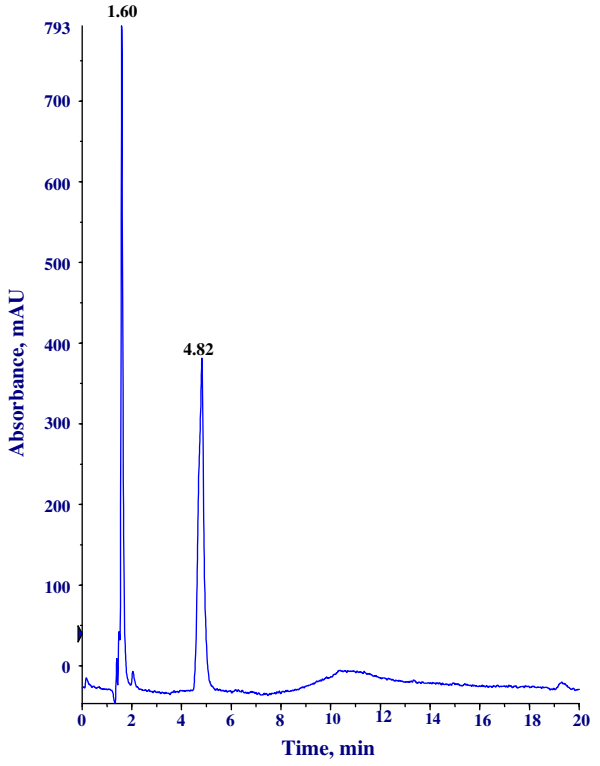
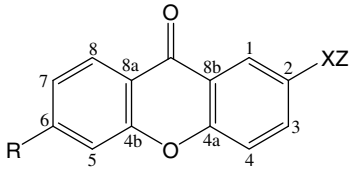
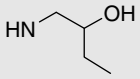
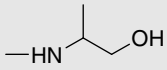
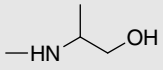
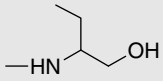
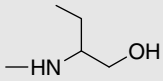
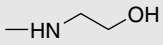
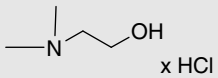
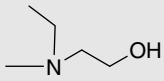
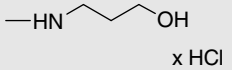
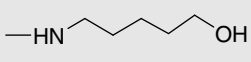
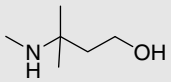
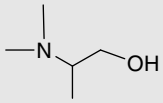
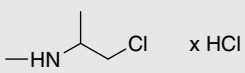
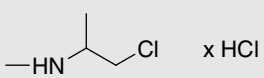


Figure 4. Selected chromatograms of **2**[b].

The TOX was measured by the rotorod test. Twenty-three derivatives of xanthone (**1**–**19**) were subjected to the anticonvulsant screens. Phase I is a qualitative assay involving a small number of mice (1–8) at common dose levels of 3, 10, 30, 100. and 300 mg/kg (Table 2). Protective activity in the MES test in mice after intraperitoneal (ip) administration was found for compounds **1**–**19** (Table 2). The most active substances tested in the MES screen in mice were compounds **1** and **6**, which at a dose of 30

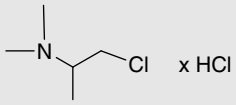
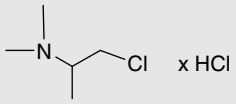
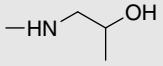
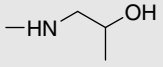
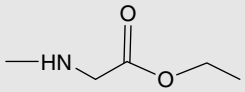
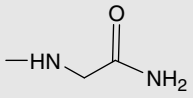
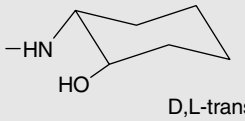
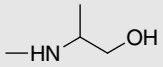
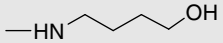
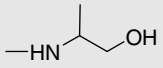
Table 1
Structure and chemical data of the tested compounds



Compounds	R	X	Z	Formula (MW) [mp (°C)]	Elemental analysis			$[\alpha]_{546}^{20}$
					% C Calcd Found	% H Calcd Found	% N Calcd Found	
1	Cl	CH_2		$C_{18}H_{18}ClNO_3$ (331.8) [126–128]	65.15 65.42	5.47 5.31	4.22 4.37	
2[a]	Cl	CH_2		$C_{17}H_{16}ClNO_3$ (317.8) [160–162]	64.25 64.50	5.07 5.02	4.41 4.23	−37.2° (c = 1%, $CHCl_3$) ee > 99.9%
2[b]	Cl	CH_2		$C_{17}H_{16}ClNO_3$ (317.8) [159–161]	64.25 64.37	5.07 5.29	4.41 4.28	+37.0° (c = 1%, $CHCl_3$) ee => 99.0%
3[a]	Cl	CH_2		$C_{18}H_{18}ClNO_3$ (331.8) [129–130]	65.16 64.50	5.47 5.24	4.22 4.40	−22.0° (c = 1%, $CHCl_3$)
3[b]	Cl	CH_2		$C_{18}H_{18}ClNO_3$ (331.8) [128–130]	65.16 64.17	5.47 5.14	4.22 4.40	+23.0° (c = 1%, $CHCl_3$)
4	Cl	CH_2		$C_{16}H_{14}ClNO_3$ (303.7) [155–157]	63.26 64.42	4.65 4.68	4.61 4.81	
5	Cl	CH_2		$C_{17}H_{17}Cl_2NO_3$ (354.2) [230–232] for base: [99–101]	57.64 57.29	4.83 5.11	3.95 3.77	
6	Cl	CH_2		$C_{18}H_{18}ClNO_3$ (331.8) [84–86] for hydrochloride: [230–232]	65.16 64.94	5.47 5.40	4.22 4.19	
7	Cl	CH_2		$C_{17}H_{17}Cl_2NO_3$ (354.2) [268–270] for base: [96–98]	57.54 57.70	4.84 4.84	3.95 3.90	
8	Cl	CH_2		$C_{19}H_{20}ClNO_3$ (345.8) [112–114] for hydrochloride: [271–273]	65.30 65.67	5.82 5.92	4.05 4.13	
9	Cl	CH_2		$C_{19}H_{20}ClNO_3$ (382.3) [107–109]	65.98 65.97	5.83 5.83	4.05 3.96	
10[b]	Cl	CH_2		$C_{18}H_{18}ClNO_3$ (331.8) [107–109]	65.15 64.88	5.47 5.18	4.22 3.98	+28.0° (c = 1%, $CHCl_3$)
11	Cl	CH_2		$C_{17}H_{16}Cl_3NO_2$ (372.7) [256–258]	54.79 54.81	4.33 4.52	3.76 3.83	
11[b]	Cl	CH_2		$C_{17}H_{16}Cl_3NO_2$ (372.7) [259–261]	54.79 54.96	4.33 4.57	3.76 3.93	−15.0° (c = 1%, MeOH)

(*continued on next page*)

Table 1 (*continued*)

Compounds	R	X	Z	Formula (MW) [mp (°C)]	Elemental analysis			$[\alpha]_{546}^{20}$
					% C Calcd Found	% H Calcd Found	% N Calcd Found	
12	Cl	CH_2		$C_{18}H_{18}Cl_3NO_2$ (386.7) [221–223] for base: [98–100]	55.90 55.94	4.69 4.80	3.62 3.65	
12[b]	Cl	CH_2		$C_{18}H_{18}Cl_3NO_2$ (386.7) [224–226] for base: [96–98]	55.90 55.78	4.69 4.73	3.62 3.67	−14.0° (*c* = 1%, MeOH)
13[a]	Cl	CH_2		$C_{17}H_{16}NO_3Cl$ (317.8) [122–124]	64.25 64.53	5.07 4.96	4.41 4.18	−18.2° (*c* = 1%, $CHCl_3$)
13[b]	Cl	CH_2		$C_{17}H_{16}NO_3Cl$ (317.8) [122–124]	64.25 64.06	5.07 4.85	4.41 4.20	+18.4° (*c* = 1%, $CHCl_3$)
14	H	CO		$C_{18}H_{15}NO_5$ (325.3) [172–174]	66.45 66.47	4.65 4.92	4.31 4.15	
15	H	CO		$C_{16}H_{12}N_2O_4$ (296.3) [287–289]	64.86 65.15	4.08 4.33	9.45 9.45	
16	H	CO	 D,L-trans	$C_{20}H_{19}NO_4$ (337.4) [222–224]	71.22 70.80	5.67 5.32	4.15 4.45	
17	H	CO		$C_{17}H_{15}NO_4$ (297.3) [204–206]	68.67 68.25	5.08 5.10	4.71 4.58	
18	H	CO		$C_{18}H_{17}NO_4$ (311.3) [164–166]	69.43 69.30	5.50 5.48	4.49 4.13	
19	Cl	CO		$C_{17}H_{14}NO_4Cl$ (331.7) [230-232]	61.54 61.60	4.25 4.39	4.22 3.98	

[a](*R*) isomer.
[b](*S*) isomer.

and 10 mg/kg, respectively, demonstrated anticonvulsant protection with neurotoxicity at 0.5 h for **1** and with no neurotoxic effects at 4 h for **1** and 0.5–4 h for **6**. Protective activity in the MES test on mice without, or with low, toxicity, was also shown for **2**[a,b] and **3**[b]. In the ScMet test on mice protective activity was seen only for **2**[b] at a dose of 100 mg/kg without toxicity at the dose studied. After the initial *i.p.* mouse screening, compounds **1, 2**[a], **2**[b], **3**[b], **6, 7**, 9, and **11** were selected for further oral evaluation in rats (phase VIa according to the Antiepileptic Drug Development (ADD) Program). The Anti-MES activity determined in rats treated with 30 mg/kg (po) of the compounds under investigation is summarized in Table 3. We found that only compound **1** showed anticonvulsant activity at a dose of 30 mg/kg for 0.25–4 h after administration (Table 3). No toxicity was observed at this dose for any of the tested compounds. A modification of the chemical structure of the most active compound, **2**[a,b] as well as (*R*,*S*)-2-*N*-(6-chloro-2-xanthonemethyl)-amino-1-propanol[16] by the introduction of a tertiary amine instead of secondary one, and the replacement of the hydroxy group in the relevant compounds mentioned above by a chloride did not increase or eliminate anti-MES activity (compounds **10**[b] and **11**[b] are active at 100 mg/kg; compound **11** is inactive), and greatly increased the neurotoxicity (neurotoxic at 100 mg/kg) (Table 2).

The most promising compounds, **2**[a] and **2**[b], which displayed anti-MES activity for up to 4 h after administration at a dose of 300 mg/kg, were selected for quantitative evaluation in mice dosed intraperitoneally (Table 4). Significantly, the PI for the enantiomers **2**[a] and **2**[b] was 6.23 and <6.85, respectively, which was similar to that for phenytoin (PI = 6.6) and higher than the PI values for the racemate of **2**[a,b] (PI = 5.84) and for carbamazepine and valproate (PI = 4.9 and 1.7, respectively) (Table 4).

3.3. In vitro studies

The radioligand [^{3}H]nitrendipine was used to estimate the voltage-dependent Ca^{2+} channel (VDCC), and [^{3}H]flunitrazepam was used as the specific ligand for estimating the benzodiazepine receptor (BDZ). The results obtained show that the compound under investigation, **2**[b], displaces, in micromolar concentrations, both [^{3}H]nitrendipine and [^{3}H]flunitrazepam from their binding sites. The affinity of compound **2**[b] to VDCC is in a concentration of low

Table 2
Anticonvulsant screening project (ASP), phase I: test results in mice after intraperitoneal injection

Compound	Dose (mg/kg)	Activity									
		MES[a]				ScMet[b]		Tox[c]			
		0.25 h	0.5 h	1 h	4 h	0.5 h	4 h	0.25 h	0.5 h	1 h	4 h
1	3		0/4						0/4		
	10		0/4						0/4		
	30		1/1		0/1	0/1	0/1		2/4		0/2
	100		3/3		3/3	0/1	0/1		6/8		1/4
	300		1/1		1/1	0/1	0/1		4/4		2/2
2[a]	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		2/3		0/3	0/1	0/1		1/8		0/4
	300		1/1		1/1	0/1	0/1		2/4		0/2
2[b]	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		2/3		2/3	2/5	0/1		0/8		0/4
	300		1/1		1/1	0/1	0/1		2/4		0/2
3[a]	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		2/3		0/3	0/1	0/1		5/8		¼
	300		1/1		1/1	0/1	0/1		4/4		½
3[b]	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		3/3		0/3	0/1	0/1		2/8		¼
	300		1/1		1/1	0/1	0/1		4/4		2/2
4	30		0/1		0/1	0/1	0/1		1/4		0/2
	100		1/3		0/3	0/1	0/1		1/8		0/4
	300		1/1		1/1	0/1	0/1		3/4		0/2
5	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		1/3		0/3	0/1	0/1		6/8		0/4
	300		0/4						4/4		1/1
6	3		0/4						0/4		
	10		1/1		0/1	0/1	0/1		0/4		0/2
	30		1/2		1/3	0/1	0/1		0/4		0/4
	100		1/1		1/1	0/1	0/1		1/8		2/2
	300								4/4		
7	30		0/1		0/1	0/1	0/1		0/4		0/2
	100	1/3	0/3	2/3	0/3	0/1	0/1	2/3	3/8	0/3	0/4
	300		1/1		0/1	0/1	0/1		4/4		2/2
8	30		0/1		0/1	0/1	0/1		1/4		0/2
	100		1/3		0/3	0/1	0/1		1/8		0/4
	300		1/1		1/1	0/1	0/1		4/4		½
9	30		0/1		0/1	0/1	0/1		1/4		0/2
	100		2/3		0/3	0/1	0/1		0/8		0/4
	300		1/1		0/1	0/1	0/1		3/4		0/2
10[b]	30		0/1		0/1	0/1	0/1		1/4		1/2
	100		3/3		1/3	0/1	0/1		5/8		0/4
	300		1/1		1/1	0/1	0/1		4/4		2/2
11	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		0/3		0/3	0/1	0/1		1/8		0/4
	300		0/1		0/1	0/1	0/1		1/4		1/2
11[b]	30		0/1		0/1	0/1	0/1		1/4	0/3	0/2
	100	2/3	0/3	0/3	1/3	0/1	0/1	0/3	2/8		0/4
	300		1/1		1/1	0/1	0/1		1/4		0/2
12	30		0/1		0/1	0/1	0/1		0/4		0/4
	100		1/3		0/3	0/1	0/1		4/8		4/8
	300		1/1		0/1	0/1	0/1		4/4		4/4
13[a]	30		0/1		3/3	0/1	0/1		1/4		1/2
	100		3/3		1/1	0/1	0/1		6/8		1/4
	300		1/1		0/1	0/1	0/1		4/4		2/2
13[b]	30		0/1		3/3	0/1	0/1		0/4		0/2
	100		3/3		1/1	0/1	0/1		7/8		0/4
	300		1/1		0/1	0/1	0/1		3/4		1/2
14	30		0/1		0/3	0/1	0/1		0/4		0/2
	100		0/3		0/1	0/1	0/1		0/8		0/4
	300		0/1		0/1	0/1	0/1		0/4		0/2
15	30		0/1		0/3	0/1	0/1		0/4		0/2
	100		0/3		0/1	0/1	0/1		0/8		0/4
	300		0/1		0/1	0/1	0/1		0/4		0/2
16	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		0/3		1/7	0/1	0/1		0/8		0/4
	300		0/1		0/5	0/1	0/1		1/4		0/2

(*continued on next page*)

Table 2 (*continued*)

Compound	Dose (mg/kg)	Activity									
		MES[a]				ScMet[b]		Tox[c]			
		0.25 h	0.5 h	1 h	4 h	0.5 h	4 h	0.25 h	0.5 h	1 h	4 h
17	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		0/3		0/3	0/1	0/1		0/8		0/4
	300		0/1		0/1	0/1	0/1		0/4		0/2
18	30		0/1		0/1	0/1	0/1		0/4		0/2
	100		0/3		0/3	0/1	0/1		0/8		0/4
	300		0/1		0/1	0/1	0/1		0/4		0/2
19	30		0/1		0/1	0/1	0/1		1/4		0/2
	100		0/3		0/3	0/1	0/1		0/8		0/4
	300		0/1		0/1	0/1	0/1		0/4		0/2

[a] Number of animals protected/number of animals tested in the MES test.
[b] Number of animals protected/number of animals tested in the ScMet test.
[c] Number of animals exhibiting toxicity/number of animals tested in the rotorod test.

Table 3
Anticonvulsant screening project; phase VIa: test results in rats (dose 30 mg/kg po)

Compound	Test	Time (h)				
		0.25	0.50	1.00	2.00	4.00
1	MES[a]	1/4	¼	1/4	2/4	2/4
	TOX[b]	0/4	0/4	0/4	0/4	0/4
2[a]	MES	0/4	0/4	0/4	1/4	1/4
	TOX	0/4	0/4	0/4	0/4	0/4
2[b]	MES	1/4	0/4	0/4	0/4	1/4
	TOX	0/4	0/4	0/4	0/4	0/4
3[b]	MES	0/4	0/4	1/4	0/4	2/4
	TOX	0/4	0/4	0/4	0/4	0/4
6	MES	1/4	0/4	0/4	0/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4
7	MES	0/4	0/4	0/4	0/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4
9	MES	0/4	0/4	0/4	0/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4
11	MES	1/4	0/4	0/4	0/4	3/4
	TOX	0/4	0/4	0/4	0/4	0/4

[a] Maximal electroshock test, number of animals protected/number of animals tested.
[b] Rotorod test for neurological toxicity, number of animals exhibiting toxicity, number of animals tested.

Table 4
Quantitative anticonvulsant activity and neurotoxicity in mice dosed intraperitoneally of **2[a]** and **2[b]** and some prototype AED_S

	TPE[a] (h)	TD_{50}*	ED_{50}[b]	ED_{50} ScMet[c]
2[a]	2, 2	482.62 (454.48–526.17) [27.82] PI 6.23	77.44 (62.29–98.53) [6.66]	>500.00 PI < 0.96
2[b]	1/4, 1	<500.00 (0.00) [0.00] PI < 6.851	72.97 (52.99–95.26) [5.28]	>350.00
Phenytoin**	1/2, 2	42.8 (36.4–47.5) [10.2]	6.48 (5.65–7.24) [12.4] PI 6.6	>50 PI < 0.9
Carbamazepine**	1/4, ¼	47.8 (39.2–59.2) [7.98]	9.85 (8.77–10.7) [20.8] PI 4.9	>50 PI < 1.0
Valproate**	1/4, ¼	483 (412–571) [12.3]	287 (237–359) [7.31] PI 1.7	290 (176–249) [8.51] PI 2.3

[a] Time to peak effect. The first value is for the rotorod test; the second is for the anticonvulsant tests. In the neurotoxicity assay, all doses of **2[a]** were tested at 1/4 h through 24 h; for **2[b]** were tested at 1/4 h through 8 h. For determination of TPE_{MES} four mice were used, for TPE_{TOX}—eight mice.
[b] Dose (mg/kg) eliciting the MES protection in 50% of animals (32 mice were used to determine of $ED_{50\ MES}$).
[c] Dose (mg/kg) eliciting the ScMet protection in 50% animals (16 mice were used to determine of $ED_{50\ scMet}$).
* Doses (mg/kg) eliciting evidence of minimal neurological toxicity in 50% of animals; 95% confidence interval is shown in parentheses; the slope regression line is shown in brackets. PI: neurotoxic dose/median effective dose (TD_{50}/ED_{50}) for anticonvulsant test; (32 mice were used to determine of toxicity (TOX)).
** Data from Ref. 21.

micromoles (EC_{50} = 7.6 μM) comparable to that of carbamazepine (EC_{50} = 1.2 μM). Compound **2[b]** also possesses an affinity to the BDZ receptor in a higher micromoles concentration (EC_{50} = 30 μM). The affinity to the BDZ receptor for compound **2[b]** was about five times less potent than that of diazepam (EC_{50} = 0.7 μM). The results are shown in Figures 5 and 6.

3.4. Lipophilicity

The role of the lipophilicity of the anticonvulsant agents was proven: the higher the lipophilicity (log*P*), the greater the anticonvulsant efficacy. The calculated partition coefficient (log*P*) of the most active anti-MES compounds, **1**, **2[a,b]**, **3[b]**, **6**, and **9**, was within the range 3.62–4.14 which corresponds to the calculated log*P* for carbamazepine (3.30).

4. Experimental

4.1. Chemistry

The melting points are uncorrected and were determined in open capillaries using Büchi SMP-20 apparatus. The IR spectra were recorded on a Perkin Elmer or FT Jasco IR 410 spectrometer using KBr discs and the ^{1}H NMR spectra—on a Bruker (500.13 MHz) spectrometer in DMSO-d_6 with TMS as an internal standard. MS were recorded using an AMD-604 mass spectrometer (70 eV). ^{13}C NMR spectra were recorded on a Brucker AMX spectrometer at 500.13 MHz and 125.17 MHz, respectively, using signals from DMSO in DMSO-d_6 and TMS in $CDCl_3$ as internal standards. TLC was performed on a silica gel 60 F_{254} (Merck), with an appropriate developing systems ([a]ethyl acetate/methanol (1:3); [b]ethyl acetate/methanol (1:1); [c]toluene/methanol (20:1), [d]methanol; [e]toluene/acetone (5:3)) and spots being visualized with UV light. Optical rotation measurements were carried out on a Polamat A polarimeter (Zeiss Jena). All of the new compounds were

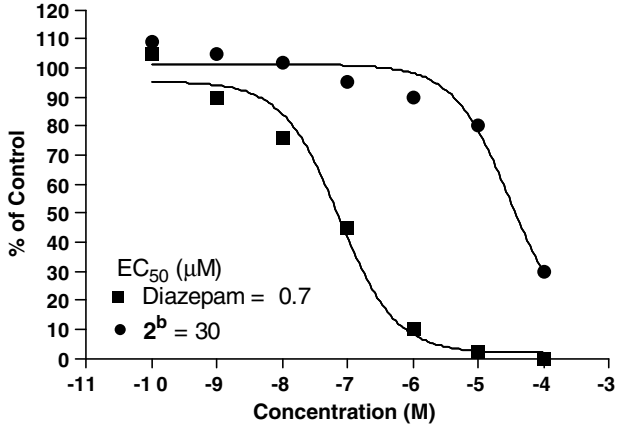


Figure 5. Displacement of [^{3}H]flunitrazepam (81 Ci/mmol) from its binding sites by diazepam and **2^b** in rat brain cortex.

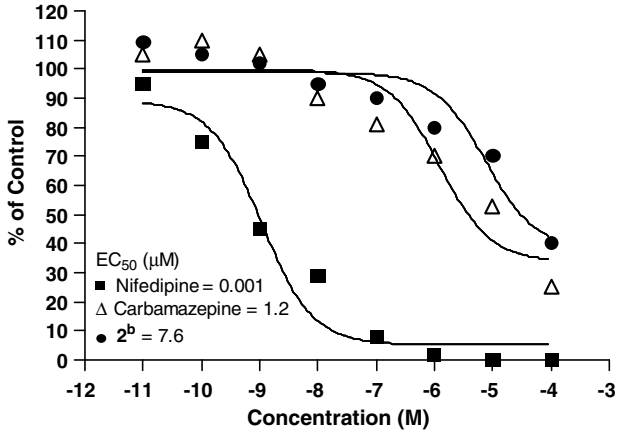


Figure 6. Displacement of [^{3}H]nitrendipine (87.6 Ci/mmol) from its binding sites by nifedipine, carbamazepine, and **2^b**.

analyzed for C, H and N and the results were within an acceptable range (±0.4% of theoretical values). Elemental analysis was performed on an Elemental Vario-El III apparatus. The reagents, enantiomeric 2-amino-1-butanols ($[\alpha]_{546}^{20}$: (*R*) = −11.25°; (*S*) = +11.15°), were obtained according to the literature.[22] Racemic 1-amino-2-butanol (bp 172–172 °C) was obtained from 1-nitro-2-butanol[23] by reduction with hydrogen under pressure (80 atm) on a nickel catalyst.[24] Racemic and enantiomeric 2-amino-1-propanol and 2-amino-1-propanol were commercial reagents (Aldrich, Lancaster). 2-Bromomethyl-6-chloroxanthone mp 219–221 °C (toluene)[25]; xanthone-2-carboxylic acid mp 303–305 °C and 6-chloroxanthone-2-carboxylic acid mp 360–362 °C were obtained by methods described in the literature.[8,26] Other reagents and solvents were commercially available materials at reagent grade. The theoretical values of log*P* combined (partition coefficient) were taken from the PALLAS program (version 1.2.).

4.2. General procedure for the synthesis of 1–19

4.2.1. Synthesis of aminoalkanol derivatives of xanthone (1–10^b and 13^{a,b})

A mixture of 24 mmol of an appropriate aminoalkanol and 20 mmol of bromomethyl-6-chloroxanthone in 60–80 ml of toluene was refluxed for 5–6 h in the presence of 16 mmol of anhydrous K_2CO_3. The inorganic salt precipitate was filtered from the hot mixture and washed with hot toluene (2× 10 ml). The precipitate was separated from the cooled filtrate, filtered off and recrystallized from a mixture of toluene and heptane (5:1) (v/v), yield 48–60%. Some bases were converted into salts (hydrochlorides) in *n*-propanol with an excess of EtOH saturated with HCl.

4.2.2. Synthesis of chloroalkyloamino derivatives of xanthone (11–12^b)

A mixture of 86 mmol of **2**[16], **2^b**, **10**[19], or **10^b** and 4 ml of thionyl chloride (d = 1.64 g ml^{-1}) in 20 ml of toluene was heated for 2 h. The solvent was then evaporated in vacuo. The reaction products (**11**, **11^b**, **12**, and **12^b** in the form of hydrochlorides) were recrystallized from acetone and ethanol (3:1) (v/v) with yield 43–48%. Products **12** and **12^b** were dissolved in 20 ml of water before adding NH_3 for neutralization. The solution was shaken out with toluene. The organic layer was separated and dried over Na_2SO_4. The residue after the evaporation of the toluene was recrystallized from *n*-hexane. In this way the free bases of **12** and **12^b** were obtained.

4.2.3. Synthesis of amide derivatives of xanthone (14 and 16–19)

A suspension of 20 mmol of glycine ethyl ester or an appropriate aminoalkanol and 40 mmol of anhydrous K_2CO_3 in 25 ml of water and 25 ml of toluene was cooled to 10–12 °C. After cooling a solution of 25 mmol of the appropriate raw xanthone-2-carboxy chloride in 30 ml of toluene was added with vigorous stirring at 10–12 °C. After the addition of 40 mmol of powdered anhydrous K_2CO_3, the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was then heated to 80 °C and left to cool down. The precipitated deposit was filtered off, stirred with a 10% solution of $NaHCO_3$ and, after drying, recrystallized from ethanol (**14**) and toluene (**16**–**19**).

4.2.3.1. (*R*,*S*)-6-Chloro-2-((2-hydroxybutylamino)methyl)-9*H*-xanthen-9-one (1). Yield: 56%; R_f = 0.46[a]; log*P* = 4.14; IR (*ν*, cm^{-1}): 3471, 3380, 3089, 2920, 1649, 1597, 1469, 1323, 1244, 1155; ^{1}H NMR (*δ*, ppm): 8.18 (d, *J* = 8.5 Hz, 1H, H-8), 8.12 (d, *J* = 2.3 Hz, 1H, H-1), 7.85 (dd, *J* = 8.6 Hz, *J* = 2.2 Hz, 1H, H-3), 7.82 (d, *J* = 2.0 Hz, 1H, H-5), 7.60 (d, *J* = 8.6 Hz, 1H, H-4), 7.51 (dd, *J* = 8.5 Hz, *J* = 2.0 Hz, 1H, H-7), 4.42 (d, *J* = 4.4 Hz, 1H, OH), 3.84 (s, 2H, Ar-CH_2), 3.50-3.44 (m, 1H, CH), 2.50 (dd, *J* = 11.7 Hz, *J* = 4.3 Hz, 1H, NC*H*H), 2.44 (dd, *J* = 11.7 Hz, *J* = 7.4 Hz, 1H, NHCH*H*), 2.21 (br s, 1H, NH), 1.49–1.39 (m, 1H, *H*CHCH$_3$), 1.36–1.26 (m, 1H, HC*H*CH$_3$), 0.85 (t, *J* = 7.4 Hz, 3H, CH_3).

4.2.3.2. (*S*)-Chloro-2((1-hydroxypropan-2-ylamino)methyl-9*H*-xanthen-9-one (2^b). Yield: 54%; R_f = 0.47[a]; log*P* = 3.62; IR (*ν*, cm^{-1}): 3460, 3360, 3029, 2928, 1642, 1582, 1470, 1320, 1224, 1155; ^{1}H NMR (*δ*, ppm): 8.18 (dd, *J* = 8.5 Hz, *J* = 0.3 1H, H-8), 8.13 (dd, *J* = 2.3, *J* = 0.4 Hz, 1H, H-1), 7.86 (dd, *J* = 8.6 Hz, *J* = 2.2 Hz, 1H, H-3), 7.84 (d, *J* = 2.0 Hz, 1H, H-5), 7.61 (d, *J* = 8.6 Hz, 1H, H-4), 7.48 (dd, *J* = 8.5 Hz, *J* = 2.0 Hz, 1H, H-7), 4.55 (t, *J* = 5.3 Hz, 1H, OH), 3.89 (d, *J* = 14 Hz, 1H, ArC*H*HN), 3.82 (d, *J* = 14 Hz, 1H, ArCH*H*N), 3.30 (dd, *J* = 5.5 Hz, *J* = 5.3 Hz, 2H, CH_2-OH), 2.63 (tq, *J* = 6.3 Hz, *J* = 5.5 Hz, 1H, C-H), 2.20 (s, 3H, NH), 0.97 (d, *J* = 6.3 Hz, 3H, CH_3-R); ^{13}C NMR (*δ*, ppm): 175.3 (C=O),155.8 (C4b), 154.3 (C4a), 139.6 (C6), 138.2 (C2), 135.6 (C3), 127.8 (C8), 124.7 (C7), 120.6 (C8a), 120.0 (C8b), 118.0 (C4), 117.8 (C5), 65.3 (CH_2OH), 53.7 (CH), 49.4 (Ar-CH_2-N), 17.2 (CH_3).

4.2.3.3. (*S*)-chloro-2((1-hydroxybutan-2-ylamino)methyl-9*H*-xanthen-9-one (3^b). Yield: 56%; R_f = 0.27[a]; log*P* = 4.14; IR (*ν*, cm^{-1}): 3058, 3360, 2937, 2820, 1650, 1620, 1480, 1472, 1320, 1224, 1058; ^{1}H NMR (*δ*, ppm): 8.19 (dd, *J* = 8.5 Hz, *J* = 0.4 1H, H-8), 8.14 (dd, *J* = 2.3, *J* = 0.4 Hz, 1H, H-1), 7.87 (dd, *J* = 8.6 Hz, *J* = 2.3 Hz, 1H, H-3), 7.84 (dd, *J* = 1.9 Hz, *J* = 0.4 Hz, 1H, H-5), 7.61 (d, *J* = 8.6 Hz, 1H, H-4), 7.52 (dd, *J* = 8.5 Hz, *J* = 1.9 Hz, 1H, H-7),

4.45 (dd, J = 5.2 Hz, J = 5.3 Hz, 1H, OH), 3.86 (s, 2H, CH_2N), 3.42 (ddd, J = 5.1 Hz, J = 5.2 Hz, J = 10.7 Hz,1H, C*H*HO), 3.34 (ddd, J = 5.3 Hz, J = 5.6 Hz, J = 10.7 Hz, 1H, CH*H*O), 2.40–245 (m, 1H, CH), 2.50 (br s, 1H, NH), 1.45–1.37 (m, 2H, CH_2 R), 0.86 (t, J = 7.5 Hz, 3H, CH_3).

4.2.3.4. 6-Chloro-2-((2-hydroxyethylamino)methyl)-9*H*-xanthen-9-one (4). Yield: 60%; R_f = 0.40[a]; log$P$ = 3.09; IR (ν, cm^{-1}): 3274, 3064, 2930, 2842, 1656, 1616, 1602, 1482, 1474, 1327, 1224, 1062; ^{1}H NMR (δ, ppm): 8.17 (d, J = 8.62 Hz, 1H, H-8), 8.11 (d, J = 1.9 Hz, 1H, H-1), 7.84 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H, H-3), 7.76 (d, J = 1.8 Hz, 1H, H-5), 7.57 (d, J = 8.6 Hz, 1H, H-4), 7.48 (dd, J = 8.5 Hz, J = 1.9 Hz, 1H, H-7), 4.95 (br s, 1H, OH), 3.86 (s, 2H, CH_2N), 3.52 (t, J = 5.8 Hz, 2H, CH_2O), 3.31 (s, 1H, NH+H_2O), 2.63 (t, J = 5.8 Hz, 2H, N-CH_2).

4.2.3.5. 6-Chloro-2-(((2-hydroxyethyl)(methyl)amino)methyl)-9*H*-xanthen-9-one (5). Yield: 54%; R_f = 0.57[a]; log$P$ = 3.24; IR (ν, cm^{-1}): 3238, 2846, 1654, 1603, 1490, 1447, 1416, 1372, 1352, 1329, 1295, 1256, 1209, 1121, 1074; ^{1}H NMR for base, (δ, ppm): 8.18 (d, J = 8.5 Hz, 1H, H-8), 8.07 (d, J = 2.0 Hz, 1H, H-1), 7.83 (dd, J = 9.1 Hz, J = 2.2 Hz, 1H, H-3), 7.82 (d, J = 1.9 Hz, 1H, H-5), 7.51 (dd, J = 2.0 Hz, J = 8.5 Hz, 1H, H-4), 7.49 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, H-7), 4.49 (br s, 1H, OH), 3.64 (s, 2H, $ArCH_2$), 3.55 (t, J = 6.4 Hz, 2H, CH_2O), 2.49 (t, J = 6.4 Hz, 6H, NCH_2), 2.19 (s, 3H, CH_3).

4.2.3.6. 6-Chloro-2-((ethyl(2-hydroxyethyl)amino)methyl)-9*H*-xanthen-9-one (6). Yield: 48%; R_f = 0.51[a]; log$P$ = 3.85; IR (ν, cm^{-1}): 3492, 3073, 2963, 2944, 2867, 2806, 1932, 1802, 1734, 1655, 1617, 1602, 1488, 1470, 1447, 1418, 1387, 1358, 1335, 1303, 1259, 1231, 1213; ^{1}H NMR for hydrochloride, (δ, ppm): 10.18 (br s, 1H, NH^+), 8.43 (d, J = 2.2 Hz, 1H, H-1), 8.19 (dd, J = 8.4 Hz, J = 0.4 Hz, 1H, H-8), 7.13 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H, H-3), 7.88 (dd, J = 2.0, Hz, J = 0.4 Hz, 1H, H-5), 7.75 (d, J = 8.6 Hz, 1H, H-4), 7.53 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H, H-7), 5.39 (br s, 1H, OH), 4.52 (d, J = 4.5 Hz, 2H, NCH_2Ar), 3.63–3.84 (m, 2H, CH_2O), 3.01–3.22 (m, 4H, CH_2N, CH_2(Et)), 1.28 (t, J = 7.2 Hz, 3H, CH_3).

4.2.3.7. 6-Chloro-2-((3-hydroxypropylamino)methyl)-9*H*-xanthen-9-one hydrochloride (7). Yield: 58%; R_f = 0.37[a]; log$P$ = 3.45; IR (ν, cm^{-1}): 3485, 2941, 2789, 2762, 2405, 1656, 1612, 1491, 1446, 1419, 1309, 1234, 1207, 1057; ^{1}H NMR for base, (δ, ppm): 8.14 (d, J = 8.5 Hz, 1H, H-8), 8.07 (d, J = 2.1 Hz, 1H, H-1), 7.81 (dd, J = 8.6 Hz, J = 2.1 Hz, 1H, H-3), 7.77 (d, J = 1.9 Hz, 1H, H-5), 7.56 (d, J = 8.6 Hz, 1H, H-4), 7.48 (dd, J = 8.5 Hz, J = 1.9 Hz, 1H, H-7), 5.50–2.50 (br s, 2H, NH, OH), 3.79 (s, 2H, CH_2Ar), 3.49 (t, J = 6.3 Hz, 2H, CH_2O), 2.57 (t, J = 7.0 Hz, 2H, CH_2N), 1.61 (tt, J = 7.0 Hz, J = 6.3 Hz, 2H, CCH_2C).

4.2.3.8. 6-Chloro-2-((5-hydroxypentylamino)methyl)-9*H*-xanthen-9-one (8). Yield: 52%; R_f = 0.08[a]; log$P$ = 4.47; IR (ν, cm^{-1}): 3237, 3061, 2924, 1664, 1604, 1448, 1420, 1329, 1309, 1202; ^{1}H NMR for base, (δ, ppm): 8.16 (d, J = 8.6 Hz, 1H, H-8), 8.09 (d, J = 2.3 Hz, 1H, H-1), 7.82 (dd, J = 8.6 Hz, J = 2.3 Hz, 1H, H-3), 7.81 (d, J = 2.0 Hz, 1H, H-5), 7.58 (d, J = 8.6 Hz, 1H, H-4), 7.50 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H, H-7), 4.35 (br s, 1H, OH), 3.79 (s, 2H, CH_2Ar), 3.38 (t, J = 6.0 Hz, 2H, CH_2O), 2.48 (t, J = 7.1 Hz, 2H, CH_2N), 2.18 (br s, 1H, NH), 1.48–1.37 (m, 4H, 2× CH_2), 1.35–1.27 (m, 2H, CH_2).

4.2.3.9. 6-Chloro-2-((4-hydroxy-2-methylbutan-2-ylamino)-methyl)-9*H*-xanthen-9-one (9). Yield: 56%; R_f = 0.12[a]; log$P$ = 3.62; IR (ν, cm^{-1}): 3294, 3081, 2964, 2925,1657, 1604, 1487, 1447, 1420, 1331, 1307, 1208; ^{1}H NMR (δ, ppm): 8.18 (d, J = 8.5 Hz, 1H, H-8), 8.12 (d, J = 2.3 Hz, 1H, H-1), 7.83 (dd, J = 8.5 Hz, J = 2.3 Hz, 1H, H-3), 7.82 (d, J = 2.0 Hz, 1H, H-5), 7.58 (d, J = 8.5 Hz, 1H, H-4), 7.51 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, H-7), 4.60 (br s, 1H, OH), 3.77 (s, 2H, $ArCH_2N$), 3.58 (t, J = 7.0 Hz, 2H, CH_2-O), 2.09 (br s, 1H, NH), 1.62 (t, J = 7.0 Hz, 2H, CH_2H_2O), 1.11 (s, 6H, 2× CH_3).

4.2.3.10. (*S*)-6-Chloro-2-(((1-hydroxypropan-2yl)(methyl)amino)methyl)-9*H*-xanthen-9-one (10[b]). Yield: 51%; R_f = 0.38[a]; log$P$ = 3.92; IR (ν, cm^{-1}): 3465, 3088, 2968, 2848, 2788,1658, 1604, 1487, 1448, 1331, 1263, 1208; ^{1}H NMR (δ, ppm): 8.15 (d, J = 8.6 Hz, 1H, H-8), 8.05 (d, J = 2.3 Hz, 1H, H-1), 7.83 (dd, J = 8.6 Hz, J = 2.3 Hz, 1H, H-3), 7.77 (d, J = 2.0 Hz, 1H, H-5), 7.57 (d, J = 8.6 Hz, 1H, H-4), 7.48 (dd, J = 8.6 Hz, J = 2.0 Hz, 1H, H-7), 3.72 (d, J = 14 Hz, 1H, ArC*H*HN), 3.65 (d, J = 14 Hz, 1H, ArCH*H*N), 3.52–3.58 (m, 1H, HOC*H*H), 3.32–3.38 (m, 1H, HO-CH*H*), 2.78 (tq, J = 6.7 Hz, J = 6.4 Hz, 1H, CH), 2.14 (s, 3H, CH_3-N), 1.00 (d, J = 6.7 Hz, 3H, CH_3-R); ^{13}C NMR (δ, ppm): 175.2 (C=O),155.7 (C4b), 154.4 (C4a), 139.6 (C6), 138.0 (C2), 1356.0 (C3), 127.8 (C8), 124.8 (C7), 124.7 (C1), 120.6 (C8a), 119.9 (C8b), 117.9 (C4), 117.8 (C5), 63.3 (CH_2OH), 59.0 (CH), 56.7 (CH), 36.7($ArCH_3$), 11.2 (CH_3).

4.2.3.11. (*R,S*)-6-Chloro-2-((1-chloropropan-2-yl(amino)methyl)-9*H*-xanthen-9-one hydrochloride (11). Yield: 48%; R_f = 0.83[b]; log$P$ = 5.30; IR (ν, cm^{-1}): 3433, 2940, 2697, 2660, 2561, 1654, 1617, 1605, 1590, 1466, 1419, 1300, 1233, 1207; ^{1}H NMR (δ, ppm): 9.79 (br s, 1H, NH^+), 9.71 (br s, 1H, NH), 8.43 (d, J = 2.1 Hz, 1H, H-8), 8.20 (d, J = 8.6 Hz, 1H, H-1), 8.18 (dd, J = 8.4 Hz, J = 2.1 Hz, 1H, H-3), 7.88 (d, J = 1.9 Hz, 1H, H-5), 7.74 (d, J = 8.7 Hz, 1H, H-4), 7.55 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, H-7), 4.43–4.36 (m, 2H, $ArCH_2$), 4.09–4.01 (m, 2H, CH_2Cl), 3.65–3.58 (m, 1H, CH), 1.44 (d, J = 6.6 Hz, 3H, CH_3); MS (m/z): 335 $(M-HCl)^+$, 286, 272, 245, 243 (100%), 215, 152, 56.

4.2.3.12. (*S*)-6-Cloro-2-(((1-chloropropan-2-yl)(methyl)amino)-methyl-9*H*-xanthen-9-one (12[b]). Yield: 45%; R_f = 0.76[c]; log$P$ = 5.68; IR (ν, cm^{-1}): 3420, 2948, 2923, 2638, 1663, 1619, 1492, 1447, 1331, 1310,1206, 1106; ^{1}H NMR for base, (δ, ppm): 8.14 (d, J = 8.5, 1H, H-8), 8.07 (br s, 1H, H-1), 7.82 (dd, J = 8.6 Hz, J = 2.0, 1H, H-3), 7.76 (d, J = 2.0 Hz, 1H, H-5), 7.59 (d, J = 8.6 Hz 1H, H-4), 7.48 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, H-7), 4.27–4.36 (m, 1 H, CH), 3.67 (s, 2H, $ArCH_2N$), 2.55–2.71 (m, 2H, CH_2Cl), 2.22 (br s, 3H, NCH_3), 1.47 (d, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (δ, ppm): 175.1 (C=O),155.7 (C4b), 154.6 (C4a), 139.6 (C6), 136.1 (C2), 135.6 (C3), 127.7 (C8), 125.3 (C1), 124.7 (C7), 120.6 (C8a), 119.9 (C8b), 118.0 (C4), 117.9 (C5), 64.7 (CH_2Cl), 60.5 ($ArCH_2N$), 55.8 (CH), 56.7 (CH), 42.0(Ar-CH_3), 22.9 (CH_3).

4.2.3.13. (*R*)-6-Chloro-2-((2-hydroxypropylamino)methyl)-9*H*-xanthen-9one (13[a]). Yield: 64%; R_f = 0.24[d]; log$P$ = 3.63; IR (ν, cm^{-1}): 3444, 3302, 3066, 2973, 2909, 2821, 1651, 1609, 1493, 1451, 1331, 1264, 1206; ^{1}H NMR (δ, ppm): 8.14 (dd, J = 8.5, J = 0.4 Hz, 1H, H-8), 8.08 (dd, J = 2.2 Hz, J = 0.4 Hz, 1H H-1), 7.82 (dd, J = 8.6 Hz, J = 2.2, 1H, H-3), 7.76 (dd, J = 2.0 Hz, J = 0.4 Hz, 1H, H-5), 7.55 (dd, J = 8.6 Hz, J = 0.5 Hz, 1H, H-4), 7.49 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, H-7), 4.51 (br s, 1H, OH), 3.82 (s, 2H, $ArCH_2N$), 3.73 (dq, J = 6.3 Hz, J = 6.1 Hz, 1H, CH), 2.45 (d, J = 6.1 Hz, 2H, NCH_2C), 2.24 (br s, 1H, NH), 1.07 (d, J = 6.3 Hz, 3H, CH_3); ^{13}C NMR (δ, ppm): 175.2 (C=O),155.7 (C4b), 154.3 (C4a), 139.5 (C6), 137.8 (C2), 135.5 (C3), 127.8 (C8), 124.7 (C7), 124.3 (C1), 120.6 (C8a), 119.9 (C8b), 117.9 (C4), 117.8 (C5), 63.5 (CH), 56.6 (CH_2), 52.0 ($ArCH_2N$), 22.5 (CH_3).

4.2.3.14. Ethyl 2-(9-oxo-9*H*-xanthene-2-carboxamido)-acetate (14). Yield: 71%; R_f = 0.62[e]; log P = 3.11; IR (ν, cm^{-1}): 3394, 3068, 1739, 1666, 1612, 1540, 1488, 1402, 1376, 1355, 1340, 1301, 1267, 1106, 1068, 1010; ^{1}H NMR (δ, ppm): 9.26 (t, J = 5.7 Hz, 1H, NH), 8.76 (dd, J = 2.3 Hz, J = 0.4 Hz 1H, H-1), 8.33 (dd, J = 8.8 Hz, J = 2.3 Hz, 1H, H-3), 8.22 (ddd, J = 7.9 Hz, J = 1.7 Hz, J = 0.5 Hz, 1H,

H-8), 7.91 (ddd, J = 8.4 Hz, J = 7.0 Hz, J = 1.7 Hz, 1H, H-6), 7.76 (d, J = 8.8 Hz, J = 0.5, 1H, H-4), 7.68 (ddd, J = 8.4 Hz, J = 1.0 Hz, J = 0.5 Hz, 1H, H-5), 7.51 (ddd, J = 7.9 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H, H-7), 4.12 (q, J = 7.0 Hz, 2H, OCH_2), 4.02 (d, J = 5.7, 2H, NCH_2), 1.24 (t, J = 7.0 Hz, 3H, CH_3).

4.2.3.15. *N*-(2-Amino-2-oxoethyl)-9-oxo-9*H*-xanthene-2-carboxamide (15). Yield: 67%; R_f = 0.46[e]; log$P$ = 2.13; IR (ν, cm^{-1}): 3319, 1698, 1649, 1610, 1549, 1487, 1428, 1298, 1258, 1152, 1011; ^{1}H NMR (δ, ppm, DMSO-d_6): 9.01 (t, J = 5.9 Hz, 1H, NH), 8.75 (dd, J = 2.3 Hz, J = 0.5 Hz 1H, H-1), 8.32 (dd, J = 8.7 Hz, J = 2.3 Hz, 1H, H-3), 8.21 (ddd, J = 7.8 Hz, J = 1.7 Hz, J = 0.5 Hz, 1H, H-8), 7.89 (ddd, J = 8.4 Hz, J = 7.0 Hz, J = 1.7 Hz, 1H, H-6), 7.74 (d, J = 8.8 Hz, J = 0.5,1H, H-4), 7.68 (ddd, J = 8.4 Hz, J = 1.0 Hz, J = 0.5 Hz, 1H, H-5), 7.50 (ddd, J = 8.0 Hz, J = 7.1 Hz, J = 1.0 Hz, 1H, H-7), 4.42 (br s, 1H, N*H*H), 4.04 (br s, 1H, NH*H*), 3.87 (d, J = 5.9 Hz, 2H, CH_2).

4.2.3.16. (D,L)-*trans*-*N*-(2-Hydroxycyclohexyl)-9-oxo-9*H*-xanthene-2-carboxamide (16). Yield: 68%; R_f = 0.77[a]; log$P$ = 3.67; IR (ν, cm^{-1}): 3266, 2923, 2850, 1660, 1614, 1554, 1488, 1467, 1340, 1313, 1265, 1238, 1216, 1143, 1043; ^{1}H NMR (δ, ppm): 8.76 (dd, J = 2.3 Hz, J = 0.4 Hz, 1H, H-1), 8.46 (d, J = 8.1 Hz, 1H, NH), 8.34 (dd, J = 8.8 Hz, J = 2.3 Hz, 1 H, H-3), 8.23 (ddd, J = 8.0 Hz, J = 1.7 Hz, J = 0.5 1H, H-8), 7.90 (ddd, J = 8.4 Hz, J = 7.0 Hz, J = 1.7 Hz, 1H, H-6), 7.73 (dd, J = 8.8 Hz, J = 0.4 Hz, 1H, H-4), 7.69 (ddd, J = 8.4 Hz, J = 1.1 Hz, J = 0.4 1H, H-5), 7.52 (ddd, J = 8.0 Hz, J = 7.0 Hz, J = 1.1 Hz, 1H, H-7), 4.65 (t, J = 4.8 Hz, 1H, OH), 3.65–3.74 (m, 1H, CHO), 3.45–3.53 (m, 1H, CH-O), 1.91–1.97 (m, 1H, H-6a), 1.85–1.91 (m, 1H, H-3a), 1.63–1.71 (m, 2H, H-4a, H-5a), 1.21–1.34 (m, 4H, H-3e, H-4e, H-5e, H-6e).

4.2.3.17. *N*-(1-Hydroxypropan-2-yl)-9-oxo-9*H*-xanthene-2-carboxamide (17). Yield: 65%; R_f = 0.76[a]; log P = 2.50; IR (ν, cm^{-1}): 3319, 2937, 1612, 1541, 1487, 1314, 1237, 1134, 1106, 1053; ^{1}H NMR (δ, ppm,): 8.71 (d, J = 2.2 Hz, 1H, H-1), 8.00 (dd, J = 8.0 Hz, J = 0.4 Hz, 1H, NH), 8.32 (dd, J = 8.6 Hz, J = 2.2 Hz, 1H, H-3), 8.22 (dd, J = 8.0 Hz, J = 1.7 Hz, 1H, H-8), 7.90 (dd, J = 8.6 Hz, J = 7.0 Hz, J = 1.7 Hz, 1H, H-6), 7.73 (d, J = 8.6 Hz, 1H, H-4), 7.68 (dd, J = 8.6 Hz, J = 1.1 Hz, 1H, H-5), 7.51 (ddd, J = 7.9 Hz, J = 7.0, Hz, J = 1.1 Hz, 1H, H-7), 4.74 (t, J = 5.8 Hz, 1H, OH), 4.04–4.13 (m, 1H, CH), 3.50–3.55 (m, 1H, C*H*HO), 3.37–3.44 (m, 1H, CH*H*O), 1.19 (d, J = 6.8 Hz, 3H, CH_3).

4.2.3.18. *N*-(4-Hydroxybutyl)-9-oxo-9*H*-xanthene-2-carboxamide (18). Yield: 65%; R_f = 0.79[a]; log$P$ = 2.83; IR (ν, cm^{-1}): 3299, 2948, 1659, 1632, 1541, 1473, 1298, 1220, 1139, 1108, 1059; ^{1}H NMR (δ, ppm): 8.77 (t, J = 5.6 Hz, 1H, NH), 8.72 (dd, J = 2.2 Hz, J = 0.4 Hz 1H, H-1), 8.31 (dd, J = 8.8 Hz, J = 2.3 Hz, 1H, H-3), 8.23 (ddd, J = 0.5 Hz, J = 1.8 Hz, J = 7.9 Hz, 1H, H-8), 7.91 (ddd, J = 7.4 Hz, J = 7.0 Hz, J = 1.8 Hz, 1H, H-6), 7.74 (d, J = 8.7 Hz, J = 0.4 Hz, 1H, H-4), 7.70 (ddd, J = 8.4 Hz, J = 1.0 Hz, J = 0.5 Hz, 1H, H-5), 7.52 (ddd, J = 7.9 Hz, J = 2.0 Hz, J = 1.0 Hz, 1H, H-7), 4.39 (t, J = 5.1 Hz, 1H, OH), 3.44 (dt, J = 6.5 Hz, J = 5.1 Hz, 2H, CH_2-OH), 3.32 (dt, J = 7.0 Hz, J = 5.6 Hz, 2H, CH_2 NH), 1.57–1.64 (m, 2H, N-CH_2CH_2), 1.47–1.53 (m, 2H, CH_2CH_2OH).

4.2.3.19. (*R*,*S*)-6-Chloro-*N*-(1-hydroxypropan-2-yl)-9-oxo-9*H*-xanthene-2-carboxamide (19). Yield: 67%; R_f = 0.85[a]; log$P$ = 3.32; IR (ν, cm^{-1}): 3276, 2969, 1666, 1604, 1562,1488, 1353, 1330, 1265, 1209, 1182, 1151; ^{1}H NMR (δ, ppm): 8.69 (t, J = 2.3 Hz, 1H, H-1), 8.45 (d, J = 7.9 Hz, 1H, NH), 8.32 (dd, J = 8.7 Hz, J = 2.3 Hz, 1H, H-3), 8.16 (d, J = 8.5 Hz, 1H, H-8), 7.79 (d, J = 7.4 Hz, J = 1.9 Hz, 1H, H-5), 7.67 (d, J = 8.7 Hz, 1H, H-4), 7.50 (dd, J = 8.5 Hz, J = 1.9 Hz, 1H, H-7), 4.75 (t, J = 5.8 Hz, 1H, OH), 4.14–4.05 (m, 1H, CH), 3.56–3.51 (m, 1H, CH*H*), 3.45–3.39 (m, 1H, CH*H*), 1.20 (d, J = 6.8 Hz, 3H, CH_3).

4.3. Liquid chromatographic conditions

Liquid chromatography was performed using an Agilent 1100 (Agilent Technologies, Waldbronn, Germany) LC system consisting of a degasser (G1322A), a gradient pump (G1312A), an autosampler (G1329A), and a DAD detector (G1315B). Chromatographic separation was carried out with a Chiralcel OD-RH analytical column (150 × 4.6 mm, 5 μm, Daicel Chemical Industries, Tokyo, Japan) at 40 °C. The autosampler temperature was set at 40 °C. The mobile phase consisted of acetonitrile and water (80:20, v/v) with the addition of 0.01% of formic acid and was set at a flow rate of 1 ml/min. A sample volume of 1 μl was injected into the LC/MS/ system.

4.4. Mass spectrometric conditions

Mass spectrometric detection was performed on an Applied Biosystems MDS Sciex (Concord, Ontario, Canada) API 2000 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) interface. ESI ionization was performed in the positive ion mode. The mass scanning mode was by multiple ions with a precursor ion of 317.8 m/z. The mass spectrometric conditions were optimized by continuously infusing the standard solution at 10 μl/min using a Harvard infusion pump. The ion source temperature was maintained at 550 °C. The ion spray voltage was set at 5500 V. The curtain gas (CUR) was set at 6, the collision gas (CAD) at 10 and the collision energy (CE) was set at 37 V. The following ion path parameters were set as optimal: declustering potential (DP) at 61 V, focusing potential (FP) at 360 V, entrance potential (EP) at 12 V, and the electron multiplier (CEM) at 2600 V. Data acquisition and processing were performed using the Applied Biosystems Analyst version 1.4 software.

5. Pharmacology

5.1. Anticonvulsant assays

Antiepileptic activity and neurological toxicity assays were carried out by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health in Bethesda, USA. Compounds were injected as suspensions in 0.5% methylcellulose at three dosage levels (30,100 and 300 mg kg^{-1}) intraperitoneally into mice, and orally at dosage rates of 30 mg kg^{-1}.

Phase I of the evaluation was a qualitative assay which used small groups of animals (1–8) and included three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (ScMet), and neurotoxicity, noted at 30 min and 4 h after administration.

The MES were elicited by 60 Hz alternating current at 50 mA (mice) or 150 mA (rats) delivered for 0.2 s via corneal electrodes. A drop of 0.9% NaCl solution was placed into each eye prior to applying the electrodes. Protection in the MES test was defined as the abolition of the hindlimb tonic extension component of the seizure. The ScMet was conducted by administering 85 mg/kg of pentylenetetrazole dissolved in 0.9% NaCl solution into the posterior midline of mice. A minimal time of 30 min subsequent to sc administration of pentylenetetrazole was used for seizure detection. A failure to observe even a threshold seizure (a single episode of clonic spasm of at least 5 s in duration) was regarded as protection. Neurological deficit was measured in mice by the rotorod test. The mouse was placed on a 1 in. diameter knurled plastic rod rotating at 6 rpm. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. In rats, neurological deficit was indicated by

ataxia and loss of placing response and muscle tone. Anticonvulsant quantification, that is, the doses of drug required to produce the biological responses in 50% of animals (ED_{50}), and the respective 95% confidence intervals, were determined for selected compounds displaying sufficient antiepileptic activity and low neurotoxicity in the above primary evaluations, by means of a computer program using probit analysis. The details of these procedures have been published.[20,27]

5.2. In vitro studies

5.2.1. [^{3}H]Flunitrazepam displacement by diazepam and 2^b

The receptor characteristics were investigated in crude synaptosomal fractions obtained from cerebral cortex tissue. The tissue was homogenized at 0 °C in 30 vol of 50 mmol/l Tris–HCl buffer, pH 7.6. The homogenate was centrifuged at 1000g for 10 min. The supernatant was decanted and recentrifuged at 25,000g for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The final pellet (fraction P2) was stored at −20 °C for 24 h. For binding studies the pellet was reconstituted in the Tris–HCl buffer, pH 7.6.

The radioligand [^{3}H]flunitrazepam (NEN, spec. act. 81 Ci/mmol) was used for estimating benzodiazepine binding sites. The incubation mixture (final volume 550 μl) consisted of 450 μl membrane suspension, 50 μl of radioligand solution at a concentration of 2 nmol/l and 50 μl of buffer containing seven concentrations of diazepam and the compound under investigation **2^b** (0.1 nM–100 μM). For measuring unspecific binding, diazepam at a concentration of 1 μmol/l was used. The incubation was carried out in duplicate, in a shaking water bath, at 0 °C for 45 min. The addition of the ligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 ml portions of ice-cold incubation buffer and placed in plastic scintillation minivials. Scintillation fluid (Akwascynt, BioCare) was added (3 ml) and the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter.

5.2.2. [^{3}H]Nitrendipine displacement by nifedipine, carbamazepine, and 2^b

The receptor characteristics were investigated in crude synaptosomal fractions obtained from cerebral cortex tissue. The tissue was homogenized at 0 °C in 30 vol of 50 mmol/l Tris–HCl buffer, pH 7.6. The homogenate was centrifuged at 1000g for 10 min. The supernatant was decanted and recentrifuged at 25,000g for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The final pellet (fraction P2) was stored at −20 °C for 24 h. For binding studies the pellet was reconstituted in the Tris–HCl buffer, pH 7.6.

The radioligand [^{3}H]nitrendipine (NEN, spec. act. 78.3 Ci/mmol) was used to estimate the voltage-dependent Ca^{2+} channel. The incubation mixture (final volume 550 μl) consisted of 450 μl of membrane suspension, 50 μl of radioligand solution [^{3}H]nitrendi pine at a concentration of 0.8 nmol/l prepared in the dark and 50 μl of buffer containing seven concentrations of nifedipine, carbamazepine, and the compound under investigation 2 (0.1 nM–100 μM). For measuring unspecific binding, nifedipine at a concentration of 10 μmol/l was used. The incubation was carried out in duplicate, in a shaking water bath, at 25 °C for 30 min. The addition of the ligand initiated the incubation, which was terminated by rapid filtration through GF/C Whatman fiberglass filters. The filters were then rinsed twice with 5 ml portions of ice-cold incubation buffer and placed in plastic scintillation minivials. Scintillation fluid (Akwascynt, BioCare) was added (3 ml) and the samples were counted for radioactivity in a Beckman LS 3801 scintillation counter.

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