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Original Article

Synthesis, anticonvulsant properties and pharmacokinetic profile of novel 10,11-dihydro-10-oxo-5H-dibenz/b,f/azepine-5-carboxamide derivatives

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Abstract – A series of novel derivatives of oxcarbazepine (**5**), 10,11-dihydro-10-oxo-5H-dibenz/b,f/azepine-5-carboxamide was synthesised and evaluated for their anticonvulsant activity and sodium channel blocking properties. The oxime **8** was found to be the most active compound from this series, displaying greater potency than its geometric isomer **9** and exhibiting also the highest protective index value. Importantly, the metabolic profile of **8** differs from the already established dibenz/b,f/azepine-5-carboxamide drugs such as **1** and **5** which undergo rapid and complete conversion in vivo to several biologically active metabolites. In contrast **8** is metabolised to only a very minor extent leading to the conclusion that the observed anti-convulsant effect is solely attributable to **8**. It is concluded that **8** may be as effective as **1** and **5** at controlling seizures and that the low toxicity and consequently high protective index should provide the compound with an improved side-effect profile. © 2001 Éditions scientifiques et médicales Elsevier SAS

Anticonvulsant / dibenz/b,f/azepine-5-carboxamides / metabolism / sodium channel blockade / oxcarbazepine derivatives

1. Introduction

Carbamazepine (dibenz/b,f/azepine-5-carboxamide, **1**) has become established as an effective agent in the management of epilepsy, trigeminal neuralgia and affective disorders [1]. However, administration of **1** in humans is complicated by potent induction of hepatic oxidative enzymes, by adverse central nervous system effects and frequent and serious idiosyncratic reactions [2, 3]. Potent induction of hepatic microsomal enzymes [4] that cause self-induction of its own metabolism [5] together with slow and erratic absorption makes medication and especially polymedication with **1** more complicated [6]. General and neuronal toxicity of **1** has been ascribed in part to the in vivo formation of the *meso*-epoxide **2** (*figure 1*). This active main metabolite is further converted by a microsomal epoxide hydrolase into a pharmacologically inactive mixture of (10*S*,11*S*)-*trans*-diol **3** and its enantiomer **4** in the proportion of ca. 9/1 in humans [7].

Oxcarbazepine **5**, also structurally based upon the dibenz/b,f/azepine framework, was later found to possess comparable antiepileptic potency but importantly provokes generally fewer side-effects. This observation is attributable to the very different metabolic profile of **5** where oxidative attack leading to the epoxide **2** is avoided and the predominant pathway involves fast reduction of the ketone functionality. However, a crucial consequence of the non-stereoselective reduction of this apparently achiral prodrug is the undesirable formation of a mixture of two active enantiomeric alcohols (*S*)-**6** and (*R*)-**7** in

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the ratio of ca. 4/1, the anticonvulsant properties of which have been described previously [8].

We have recently described [9] the synthesis and anti-convulsant profile of enantiomerically pure ester derivatives of both (*S*)-**6** and (*R*)-**7**, which were designed to avoid such stereochemical complications during the metabolic pathway, with the objective to produce more effective alternative medications to **1** and **5** but endowed with the greater advantages of increased safety-profile and reduced side-effects. As part of an ongoing research programme concerning the discovery of improved anticonvulsive agents, the present study was conducted to evaluate potential differences in anticonvulsant activity and metabolic profile of novel derivatives of **5**. Structural modifications of **5** were carried out, primarily entailing replacement of the carbonyl moiety by selected substituted imino-groups (C=N–R) as a means to influence the metabolic profile from that described for **5** whilst maintaining high activity. These new compounds were tested in seizure models predictive of anticonvulsant efficacy in generalised tonic–clonic seizures, namely the maximal electroshock (MES) test. The most active compound identified from this series, the 10-hydroxyimino derivative **8** was thereafter further tested and compared with **1** and **5**. The putative mechanism of action of **8** was evaluated in experiments designed to study interference with voltage-gated sodium channels in rat brain membranes. Furthermore, the metabolic profile was studied in several species and compared to that for **1** and **5**.

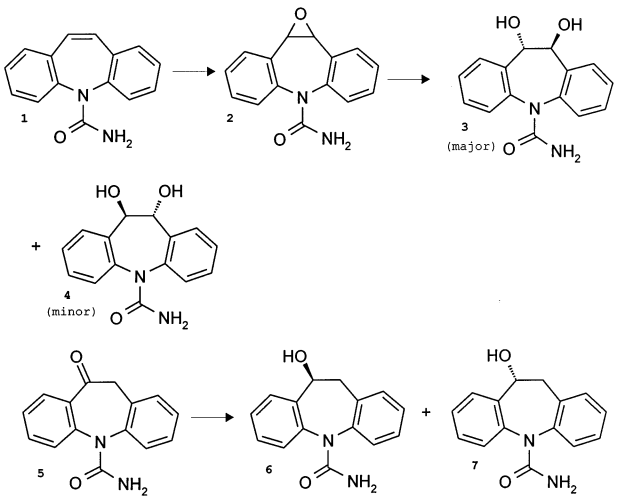


Figure 1. Metabolism of compounds **1** and **5**.

2. Chemistry

New derivatives of 10,11-dihydro-10-oxo-5H-dibenz/b,f/azepine-5-carboxamide (**5**) were prepared by either direct oximation of oxcarbazepine or by condensation with free amino group-containing compounds, both reactions satisfactorily achieved in generally good yields via standard procedures. The parent oxime **8** subsequently underwent smooth acylation using various selected anhydrides although in certain cases the more readily available acid chlorides were used instead without detriment. Surprisingly, attempted *O*-alkylation of **8** (e.g. methylation, benzylation) proceeded in poor yields under standard reaction conditions used and condensation of **5** with the appropriate alkyloxyamine hydrochloride was found prudent in these cases. Interestingly, HPLC analysis of the oxime **8** indicated in each batch the presence of a minor product with similar retention time which could not be removed by recrystallisation and which was subsequently found to increase in concentration when a solution of the oxime was allowed to stand in daylight. This observation was confirmed when the solution was irradiated under conditions simulating exterior natural light and which facilitated isolation of the impurity by column chromatography in sufficient quantity for characterisation. The MS spectrum of the isolated product displayed the same molecular ion as for **8** leading to the suspicion that this substance was in fact the geometric isomer of the oxime. Unfortunately the NMR spectra of the isolated product and **8** were both conceivably compatible with either the *E*- or *Z*-isomer. However, a study of the results of NOE difference spectroscopy of the separate compounds implied that **8** exists as the *E*-isomer and the impurity **9** as the *Z*-isomer (*figure 2*).

Conclusive evidence was obtained by X-ray diffraction analysis of **8,** which unequivocally confirmed the *E*-configuration of this compound. The crystal structure contains two independent molecules of **8**, which are approximately related by a non-crystallographic inversion centre. However, the crystal structure cannot be centrosymmetric because the OH groups in the two associated molecules of ethanol are not suitably oriented as shown in *figure 3*. Both molecules of **8** have *E*- stereochemistry with respect to the oxime groups: the torsion angle C(11)–C(10)–N(10)–O(10) is −0.3(5) and −1.4(4)° in molecules **8a** and **8b**, respectively, while the torsion angle C(5A)–N(5)–C(5)–O(51) is −0.8(4) and −5.3(4)° in molecules **8a**

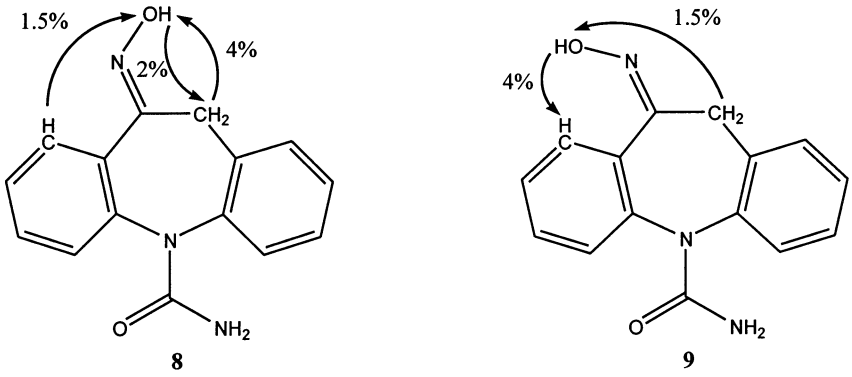


Figure 2. Results of NOE difference spectroscopy of compounds **8** and **9**.

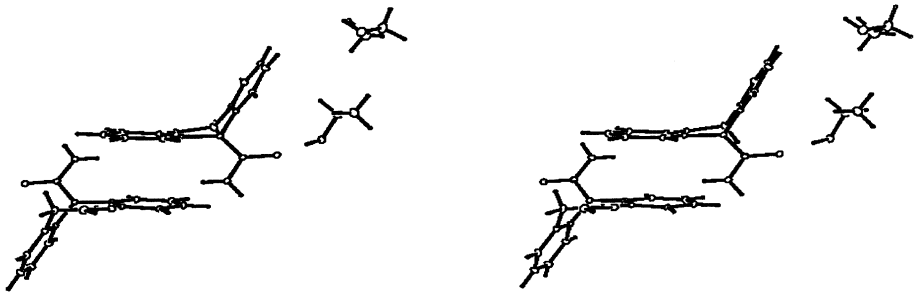


Figure 3. Figure 2. ORTEP [21] stereo diagram of the unit cell contents of the **8**·C_2H_6O crystal structure showing the non-crystallographic inversion centre between the two independent molecules of **8** (**8a** and **8b**) and the relative orientation of the OH groups of C_2H_6O which may be responsible for the lack of an crystallographic inversion centre in the crystal structure.

and **8b**, respectively. The asymmetric unit of this crystal structure further contains four solvent ethanol molecules (C_2H_6O). Molecular diagrams presenting the atomic numbering scheme and thermal ellipsoid plots for both independent molecules of **8** are shown in *figure 4*.

For the identification of metabolites, the synthesis of the unsaturated nitro-derivative **10** was achieved according to the literature procedure [10] which then underwent facile reduction with sodium borohydride to the saturated analogue **11**. Yields and physical properties of test compounds are given in *table I*.

3. Results and discussion

3.1. Pharmacology

The compounds were tested for anticonvulsant activity using the procedures described previously [11, 12]. In the first series of experiments, the compounds were administered by the oral route at a dose equimolar to 15 mg kg^{-1} of oxcarbazepine **5**. Two hours after their administration, the MES test was performed for each compound. Initial evaluation of the anticonvulsant activity of the new derivatives (*table II*) showed that the unsubstituted oxime **8** displayed activity comparable to or greater than the standards **1** and **5**. Interestingly, the acetate **12** derived from **8** displayed dramatically reduced activity which was inherently surprising since enzymatic hydrolysis in vivo was predicted to give origin to the parent oxime. In general the various acylated forms of the oxime, designed to span a broad range of hydrolytic and enzymatic stability exhibited reduced activity, with only glutaroyl derivative **19** showing the similar moderate activity of **12**. Of the carbonate series derived from **8** the t-butyl carbonate **22** was clearly superior to the n-butyl isomer **21** and lower homologue **20**, retaining activity comparable to **5**. Substitution of the free hydroxyl functionality of the oxime by a methyl group (**24**) resulted in a compound possessing mar-

ginally higher activity than most acylated derivatives but introduction of the bulky *O*-benzyl group (**25**) abolished activity completely. Further investigation revealed that changing the substituent on the nitrogen atom of the imino group from oxygen to nitrogen as in hydrazone **26** reduced activity substantially and introduction of a phenyl substituent (**27**) had the same effect as **25** in the carbonate series. Notably, of the metabolites of **8** identified from rats, the low activity associated with the unsaturated nitro-compound **11** was completely extinguished on saturation to the analogue **10**. The interesting observation was also noted of the low anti-convulsant activity of the pure (*Z*)-isomer **9**, ca. 50% that of (*E*)-isomer **8**. These results would indicate that the observed anti-convulsant effect is exclusively attributable to the oxime **8** itself and mainly to the (*E*)-isomer.

The administration by gastric tube of compounds listed in *table III* conferred a dose-dependent protection of rats against MES induced seizures. Compound **8** was found to be equally potent to **1** and more potent than **5** at 2 and 8 h after their administration. Four hours after administration compounds **8** and **5** had similar potency and both were less potent than **1**.

The oral administration of **1** and **5** conferred a dose and time dependent motor impairment of rats in the rotating rod apparatus. Compound **8** caused less motor impairment than **1** and **5** (*table III*). Due to the extremely low toxicity of **8**, with even 2000 mg kg^{-1} producing almost no motor impairment, the non-linear regressions for the determination of the TD_{50} could not be performed (*figure 5*). Considering the Protective Index (TD_{50} p.o./ED_{50} p.o.) as a measure of therapeutic tolerability, this data indicates that compound **8** should be better tolerated than **1** and **5**.

3.2. Metabolism

The metabolism and pharmacokinetic profile of compound **8** were evaluated in male Wistar rats, male CD-1 mice and female New Zealand rabbits (*figure 6*) and appear different in each of the three species. While the non-active nitro-derivative **11** was the major metabolite detected in the rat, by contrast the active 10-hydroxy compound **21** was found to be the most abundant metabolite in the rabbit (*table IV*). Mice appear incapable of metabolising compound **8** very effectively so that even 8 h post dose only traces of other products are detectable. The unsaturated nitro derivative **10** was only detected in the rat and its role as a possible intermediate between **11** and **5** has not yet been established.

3.3. Sodium channel blocking properties

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 channels

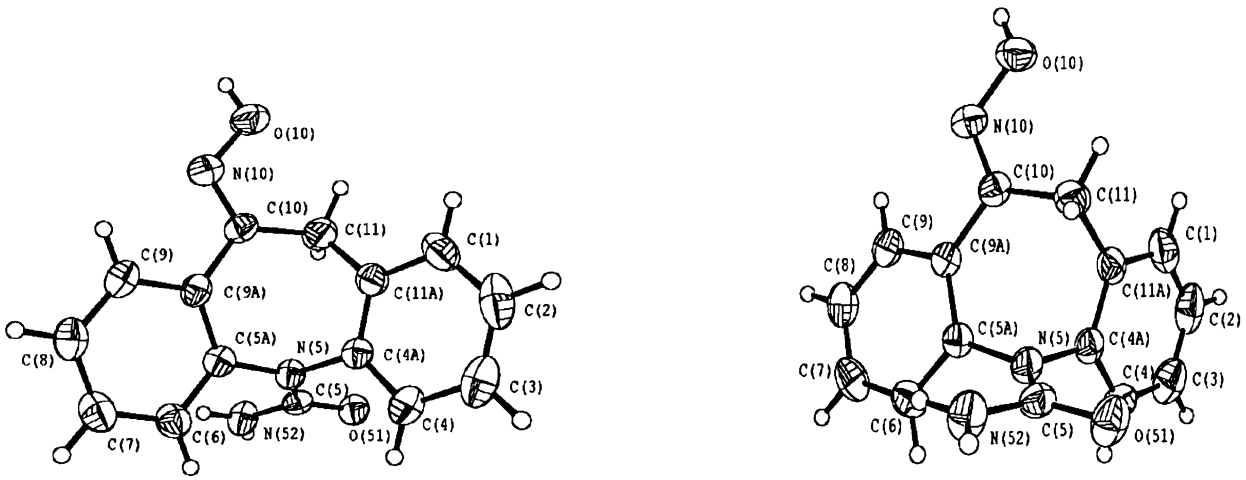
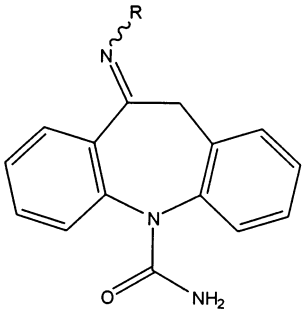


Figure 4. Figure 3. ORTEP [21] diagram of both independent molecules of **8** showing the atomic notation. The thermal ellipsoids of the non-hydrogen atoms have been drawn at the 40% probability level.

Table I. Structure, physical properties and methods of preparation of compounds.



Compound	R	Method	M.p. (°C)	Yield (%)
8	OH	A	238–240	86
9	OH	[a]		
12	OCOMe	B	176–177	85
13	OCO*n*-Pr	B	160–161	58
14	OCO*t*-Bu	B	192–193	61
15	OCOPh	B	170–171	53
16	OCO–3-OMePh	B [b]	185–186	55
17	OCO–2-Naphthyl	B	188–189	53
18	$OCO(CH_2)_2$-CO_2H	B	179–180	48
19	$OCO(CH_2)_3$-CO_2H	B	169–170	74
20	OCOOEt	B [b]	189–190	72
21	OCOOnBu	B [b]	167–168	72
22	OCOOtBu	B [c]	179–180	51
23	$OCOOCH_2Ph$	B [b]	189–190	34
24	OMe	A	158–160	87
25	OCH_2Ph	A	161–162	58
26	NH_2	C	209–211	46
27	NHPh	C	220–221	72
28	NH(2,4-di-NO_2Ph)	C	244–245	45
29	$NHCONH_2$	C	247–249	68
30	$NHCSNH_2$	C	238–240	61

[a] See Section 5.
[b] Corresponding chloride used instead of anhydride.
[c] Di-*t*-butyl dicarbonate used for acylation.

[13], to inhibit calcium channels and to interact with adenosine receptors [14] amongst other mechanisms [15].

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 [16], and on the modulation of sodium entry [17] and sodium currents [18, 19]. To confirm that sodium channels are a primary target 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 V*, within the concentration range tested (3–1000 μM), displaced [^{3}H]BTX (10 nM) binding to rat brain membranes. As shown in this table, the relative potency (IC_{50} in μM) of **1** was significantly lower than that of compound **8**. Compound **11**, the major metabolite of **8**, had no significant effect on the binding of [^{3}H]BTX to its binding site (*figure 7*).

Recently, Unverferth et al. have suggested a pharmacophore model for structurally different anticonvulsants containing aryl rings and electron donor and hydrogen bond donor/acceptor functions [20]. Carbamazepine **1** was found to satisfy the demands of this model and shares with **8** the dibenz/b,f/azepine-5-carboxamide nucleus with the amide bond serving as the hydrogen bond donor/acceptor. It could be envisaged that the nitrogen atom of the hydroxyimino group of the oxime **8** replaces the olefinic function of **1** as the electron donor so that

Table II. Protection by **1**, **5** and new 10,11-dihydro-10-oxo-5H-dibenz/b,f/azepine-5-carboxamide derivatives against MES-induced seizures [a].

Compound	Protection (%)
1	100.0 ± 0.0
5	68.3 ± 20.3
8	90.6 ± 9.4
12	19.9 ± 20.8
13	1.8 ± 9.9
14	3.5 ± 6.5
15	14.5 ± 4.8
16	-6.3 ± 9.0
17	13.4 ± 5.5
18	8.7 ± 9.1
19	20.6 ± 22.8
20	14.0 ± 11.6
21	13.3 ± 8.0
22	64.3 ± 22.1
23	8.2 ± 11.7
24	36.7 ± 21.4
25	-8.7 ± 2.7
26	23.6 ± 21.4
27	-2.4 ± 8.6
28	-13.1 ± 8.5
29	-1.3 ± 4.6
30	-8.6 ± 9.7
11	18.2 ± 17.7
10	36.0 ± 20.9
9	40.2 ± 24.5

[a] All compounds were given by gastric tube at 15 mg kg^{-1}. Values are mean ± S.E.M. for five to eight rats per group.

Table III. ED_{50}, TD_{50} and protective index values for **1**, **5** and **8** [a].

Compound	Time (h)	MES, ED_{50} (mg kg^{-1})	Rotarod, TD_{50} (mg kg^{-1})	Protective index [b]
1	2	5 ± 1	251 ± 10	50.2
	4	12 ± 1	390 ± 30	32.5
	8	18 ± 2	591 ± 91	32.8
5	2	10 ± 2	998 ± 96	99.8
	4	20 ± 3	1273 ± 85	63.7
	8	24 ± 5	1498 ± 80	62.4
8	2	12 ± 2	>2000	>166
	4	20 ± 2	>2000	>100
	8	17 ± 3	>2000	>117

[a] All compounds were given by gastric tube. Data points are mean $\pm$ S.E.M. for five to eight rats per group. For the calculation of the ED_{50} and TD_{50} values the parameters of the logistic equation were fitted to the experimental data.
[b] TD_{50}/ED_{50}.

this compound also meets the requisites of the proposed model thereby correlating with the high observed potency.

4. Conclusion

A series of novel derivatives of 10,11-dihydro-10-oxo-5H-dibenz/b,f/azepine-5-carboxamide **5** were synthesised. Oxime **8** was found to exhibit greatest activity from this new family through screening in vivo. Most notable however is the fact that chemical modification of **5** has given rise to a compound exhibiting a unique metabolic profile when compared to the already established dibenz/b,f/azepine-5-carboxamide drugs such as **1** and **5** which undergo rapid and complete conversion in vivo to several biologically active metabolites. In contrast, **8** is metabolised to only a very minor extent in some species, leading to the conclusion that the high observed anti-convulsant effect is directly attributable to **8**. It is suggested that **8** may be as effective as **1** and **5** at controlling seizures and that the low toxicity and consequently high protective index should provide the compound with an improved side-effect profile. A tentative mechanism of action for this compound is proposed based on the observed interaction with sodium channels in rat brain membranes.

5. Experimental

5.1. 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 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 measured with a Bomem Hartmann & Braun MB Series FTIR spectrometer using KBr tablets. Analytical HPLC was performed on a Gilson System equipped with a Model 305 pump and 117 UV detector, 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 visualised with UV light. Preparative chromatography was done on Merck 60 Kieselgel (0.063–0.2 mm). Elemental analyses were performed on a Fisons EA 1110 CHNS instrument and all analyses are consistent with theoretical values to within $\pm 0.4\%$ unless indicated. Solvents and reagents were purchased from Aldrich or E. Merck. Standard work-up 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 sulphate and evaporation at 45°C at water aspirator vacuum. X-ray crystallography-data collection: reflection intensities for $\mathbf{8}\cdot C_2H_6O$ were measured with an Enraf-Nonius CAD-4 diffractometer and Ni-filtered Cu Kα radiation ($\lambda = 1.5418$ Å) using the ω–2θ scan technique. Data reduction included corrections for background, Lorentz and polarisation effects. No absorption corrections were deemed necessary ($\mu = 0.748$ mm^{-1}). The structure was determined by application of

direct methods (SHELXS-97) [21] and refined by full-matrix least-squares on F^2 for all observations (SHELXL-97) [22]. The non-hydrogen atoms were refined with anisotropic thermal motion parameters. The hydrogen atoms were placed in calculated positions and refined according to the riding or rotating group models. The geometric calculations were performed with the program

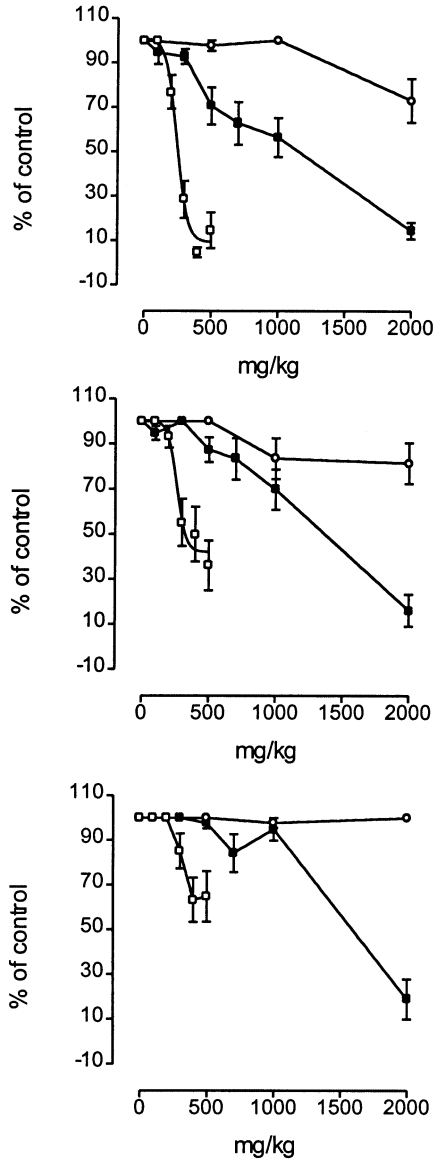


Figure 5. Dose response curves (p.o. administration) for **8** (■), **1** (□), **5** (○) in the rotating rod test at 2 h (upper panel), 4 h (middle panel) and 8 h (lower panel). Symbols are means and vertical lines show S.E.M.

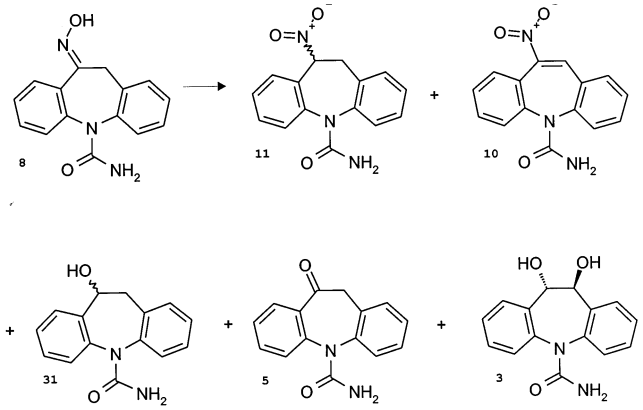


Figure 6. Metabolites of **8** formed in different species.

SHELXL-97 [22]. The OSCAIL [23] interface was used as a front end to all calculations.

*5.1.1. 10-(E-Hydroxyimino)-10,11-dihydro-dibenz/b, f/azepine-5-carboxamide (**8**)*

General method A: oxcarbazepine (**5**) (1.40 g, 5.56 mM) and hydroxylamine hydrochloride (0.772 g, 11.1 mM) were suspended in ethanol (15 mL), and pyridine (0.757 g, 9.57 mM) was added. The reaction mixture was stirred and heated at reflux for 4 h. The solvent was evaporated under reduced pressure and water (20 mL) was added to the white precipitate that was then allowed to stand at room temperature overnight. The crystalline product was filtered off and washed by water (20 mL) and cold ethanol (10 mL) and then dried at 60°C under vacuum to afford the product as colourless crystals, 1.275 g, (86%), m.p. 238 to 240°C, ^{1}H-NMR (DMSO-d_6) δ 11.7 (br s, 1H, O–H), 7.7 (dd, 1H, $J = 8$Hz, $J = 1.5$ Hz, C_9–H), 7.45–7.2 (m, 7H, Ar–H), 5.9 (br s, 2H, $-NH_2$), 4.3 (d, 1H, $J = 10$ Hz, C_{10}–H), 3.8 (d, 1H, $J = 18$ Hz, C_{10}–H); ^{13}C-NMR (DMSO-d_6) δ 32.0 (C_{10}, 128.0, 128.1, 128.8, 128.9, 130.1, 130.4, 131.1, 132.5, 135.5 (C arom.), 142.2, 144.0 (C_{4a},C_{5a} arom.), 152.8 (C=O), 156.8 (C=N). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

*5.1.2. 10-(Z-Hydroxyimino)-10,11-dihydro-dibenz/b, f/azepine-5-carboxamide (**9**)*

Oxime (**8**) (0.5 g, 2.0 mM) was dissolved in acetonitrile/water (1/1, 500 mL) and the solution was irradiated in a Sunset CPS Apparatus (filter>290 nm, 765 W m^{-2}, three 1-h cycles). The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate. The *Z*-isomer was separated by column chromatography (silica gel, ethyl acetate) as a slower-moving

Table IV. Levels of **8** and metabolites in rat, mouse and rabbit plasma (in μg mL^{-1}) 1 h after an oral dose of 80 mg kg^{-1} (rat and rabbit) and 50 mg kg^{-1} (mouse), respectively [a].

	8	**11**	**10**	**31**	**5**	**3**
Rat	4.78 ± 0.60	3.35 ± 0.44	0.72 ± 0.14	0.24 ± 0.03	0.62 ± 0.09	0.00 ± 0.00
Rabbit	5.08 ± 0.63	0.41 ± 0.03	0.00 ± 0.00	0.98 ± 0.06	0.49 ± 0.03	0.06 ± 0.02
Mouse	3.04 ± 0.14	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

[a] Mean ± S.E.M., with $n = 4$ (rat, mouse) or 3 (rabbit).

band. The chromatographically homogeneous fractions were pooled, evaporated under reduced pressure and the residue was crystallised from ethanol, affording 84 mg (16.8%) of the pure *Z*-isomer as colourless crystals, m.p. 215–217°C, ^{1}H-NMR (DMSO-d_6) δ 11.0 (s, 1H, O–H), 7.8 (br d, 1H, $J = 7.9$ Hz, C_9–H), 7.45–7.2 (m, 7H, Ar–H), 5.75 (br s, 2H, $-NH_2$), 3.9 (br s, 2H, C_{10}–H); ^{13}C-NMR (DMSO-d_6) δ 38.5 (C_{10}, 127.9, 128.2, 128.4, 129.9, 130.7, 131.16, 131.22, 131.6, 135.4 (C arom.), 141.8, 142.1 ($C_{4a,}C_{5a}$ arom.), 150.7 (C=O), 157.1 (C=N). Anal. ($C_{15}H_{13}N_3O_2$) C, H, N.

5.1.3. 10-Acetoxyimino-10,11-dihydro-dibenz/b, f/azepine-5-carboxamide ***(12)***

General method B: a suspension of oxime (**8**) (0.50 g, 1.87 mmol) in dichloromethane (25 mL) was treated with pyridine (0.72 g, 9.16 mmol) and acetic anhydride (0.57 g, 5.61 mmol). The resulting mixture was stirred at room temperature overnight and then diluted with dichloromethane (10 mL). After standard workup and crystallisation from a mixture of dichloromethane and ethyl acetate the product was obtained as white crystals, 0.491 g, (85%), m.p.176–177°C, ^{1}H-NMR ($CDCl_3$) δ 8.0 (dd, 1H, $J = 7.9$ Hz, $J = 1.5$ Hz, C_9–H), 7.6–7.25 (m, 7H, Ar–H), 4.9 (br s, 2H, $-NH_2$), 4.4 (d, 1H, $J = 18$ Hz, C_{10}–H), 4.2 (d, 1H, $J = 18$ Hz, C_{10}–H), 2.4 (s, 3H, $COCH_3$); ^{13}C-NMR ($CDCl_3$) δ 20.3 (CH_3), 33.5 (C_{10}, 128.1, 128.7, 129.0, 129.2, 129.3, 129.8, 130.0, 131.0, 132.3, 135.5 (C arom.), 141.8, 142.5 ($C_{4a,}C_{5a}$ arom.), 156.4 (NCON), 160.5 (C=N), 169.1 (OCO). Anal. ($C_{17}H_{15}N_3O_3$) C, H, N.

5.1.4. 10-(Phenyl-hydrazono)-10,11-dihydro-dibenz/b, f/azepine-5-carboxamide(***26)***

General method C: a mixture of **5** (0.2 g, 0.8 mmol), phenylhydrazine (0.5 g, 4.6 mmol) and sodium acetate (0.5 g, 6 mmol) in a mixture of water (5 mL), ethanol (5 mL) and three drops of concentrated hydrochloric acid was heated at 60°C for 30 min and then allowed to cool to room temperature. The precipitate was then filtered and washed with cold water and ethanol to give the product (0.199 g, 72.5%) as yellow crystals of m.p. 220–221°C, ^{1}H-NMR (DMSO-d_6) δ 9.7 (br s, 1H, NHAr), 7.9 (m, 1H, C_9–H), 7. 5–7.2 (m, 11H, Ar–H), 6.8 (tt, 1H, $J = 6.5$, 1.8 Hz, ArH), 6.0 (br s, 2H, $-NH_2$), 4.1 (d, 1H, $J = 18$ Hz, C_{10}–H), 3.9 (d, 1H, $J = 18$ Hz, C_{10}–H); ^{13}C-NMR (DMSO-d_6) δ 34.1 (C_{10}, 128.1, 128.6, 128.7, 128.9, 129.2, 129.3, 129.8, 130.8 135.2, 135.5 (C arom.), 141.5, 144.2 ($C_{4a,}C_{5a}$ arom.), 140.0 (C=N), 156.9 (C=O). Anal. ($C_{21}H_{18}N_4O$) H, N; C: Calc., 73.66; Found, 72.83%.

Table V. Displacement of [^{3}H]BTX (10 nM) binding to rat brain membranes by **1**, **8** and **11**, the major metabolite of **8** [a].

Compounds	IC_{50} (μM)
1	390 (250, 608)
8	418 (314, 557)
11	2291 (1458, 3598)

[a] Data points are means with 95% confidence values for four to five determinations in triplicate. For the calculation of the IC_{50} values the parameters of the equation for one site binding were fitted to the experimental data [26].

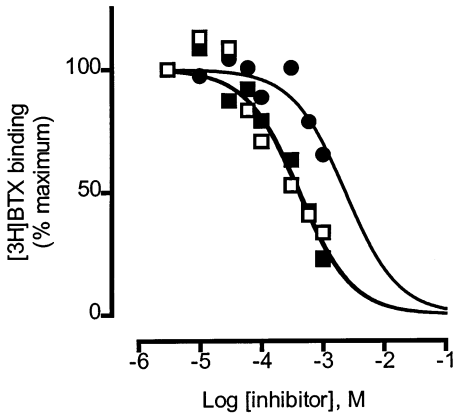


Figure 7. Displacement of [^{3}H]-BTX binding by **8** (■), **1** (□) and **11** (○) in % of maximum. Symbols are means of four to five experiments; S.E.M. were less than 10% of the correspondent mean.

*5.1.5. 10-Nitro-10,11-dihydro-dibenz[b, f]azepine-5-carboxamide (**11**)*

10-Nitro-dibenz[b,f]azepine-5-carboxamide (**10**) [10] (10.60 g, 37.7 mM) was dissolved in a warm mixture of THF and methanol (1/1, 380 mL) and then cooled to 5°C. Sodium borohydride (3.58 g, 94.3 mM) was added over 30 min at 5–7°C with stirring, and the reaction mixture was reacted for another 40 min at the same temperature. The resulting solution was concentrated on rotary evaporator under vacuum until crystallisation began and then kept overnight at 4°C. Filtration, washing with water and ethanol and drying gave 7.70 g (72%) of white crystals, m.p. 188–189°C, ^{1}H-NMR (DMSO-d_6) δ 9.7 (br s, 1H, NHAr), 7.9 (m, 1H, C_9–H), 7. 5–7.2 (m, 11H, Ar–H), 6.8 (tt, 1H, $J = 6.5$, 1.8 Hz, ArH), 6.0 (br s, 2H, $-NH_2$), 4.1 (d, 1H, $J = 18$ Hz, C_{10}–H), 3.9 (d, 1H, $J = 18$ Hz, C_{10}–H); ^{13}C-NMR (DMSO-d_6) δ 34.1 (C_{10}, 128.1, 128.6, 128.7, 128.9, 129.2, 129.3, 129.8, 130.8 135.2, 135.5 (C arom.), 141.5, 144.2 ($C_{4a,}C_{5a}$ arom.), 140.0 (C=N), 156.9 (C=O). Anal. ($C_{15}H_{13}N_3O_3$) C, H, N.

5.2. Pharmacological methods

All compounds were tested using male Wistar rats (Harlan-Interfauna Ibérica, Barcelona, Spain) ranging from 6 to 8 weeks old.

5.2.1. 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 a current of 150 mA through bipolar corneal electrodes. A drop of electrolyte/anaesthetic, 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 [11]. 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 behaviour. The day after, rats were given the compounds to be tested or the vehicle and submitted to a second MES session 2, 4 or 8 h after the administration of test compounds. The evaluation of the anticonvulsive profile of test compounds was based on the duration of the tonic phase (in seconds) being each rat 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^{-1}) and given by stomach tube.

5.2.2. 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 rubberised cylinder until achieving to maintain the equilibrium for 3 min while rotating at 6 rpm. The day after, rats were given i.p. the test compound dissolved in DMSO 2 mL kg^{-1} and 15 min later were placed on the rotating rod at a speed of 6 r.p.m. 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 [24].

5.2.3. Blockade of voltage-sensitive sodium channels

Blockade of voltage-sensitive sodium channels was studied by investigating [^{3}H]batrachotoxinin A 20-α-benzoate ([^{3}H]BTX) displacement binding to rat brain membranes. Membrane preparation and binding assays were performed as described [25], with slight modifications. Binding studies were performed by incubation of [^{3}H]BTX for 30 min at 37°C with 200 μg of membrane protein in a final volume of 200 μL in a solution containing choline chloride (130 mM) KCl (5.5 mM), $MgSO_4$ (0.8 mM), glucose (5.5 mM), HEPES/TRIS (50 mM, pH 7.4), scorpion toxin (2 μM), tetrodotoxin (1 μM), [^{3}H]BTX 10 nM, bovine serum albumin (1 mg mL^{-1}), and varying concentrations of competing compounds (3–1000 μM), as previously described [16]. Nonspecific binding was determined in the presence of 300 μM veratridine. The binding reactions, performed in 96 wells EIA/RIA plates (Costar) with 300 μL capacity, were initiated by the addition of 50 μL of the membrane suspension to 150 μL of the reaction mixture. Plates were then incubated at 37°C for 1 h in a water bath with agitation. The binding reactions were stopped by vacuum filtration (Brandel 96 Harvester) through glassfibre filter mats A (1450–21 from Wallac) and washing of the filters and incubation tubes with 200 μL 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^{-1}) and 5 mM HEPES/TRIS (pH 7.4). The filter mats were dried, impregnated with MeltiLex™ a scintillation mixture (Wallac), inserted into plastic sample bags (Wallac), and the radioactivity was counted in a 1450 MicroBetaTM spectrophotometric detector for 2 min with an efficiency of 55–60%. [^{3}H]BTX (specific activity: 39.9 Ci mmol^{-1}) was from NEN Life Sciences. Tetrodotoxin, veratridine

and scorpion toxin from *Leiurus quinquestriatus hebraeus* were from Sigma. Scorpion toxin was resuspended in ice-cold distilled water (1 mg mL^{-1}), and incubated for 1 h at 0°C. The mixture was then centrifuged at $12\,000 \times g$ for 10 min and protein determined in the supernatant.

5.3. Metabolism studies

Blood taken from the vena cava (rat and mouse) or the ear vein (rabbit) was stabilised with one drop of heparin in the syringe and kept on ice until centrifuged at ca. $3000 \times g$ for 15 min (4°C). Of the supernatant plasma phase 1 mL was added to 1 mL of 0.1 M sodium phosphate buffer (pH 4.6), spiked with 2 ppm 10,11-dihydrocarbamazepine as internal standard.

These samples were then added to LiChrolut RP-18 SPE columns (3 mL, 200 mg, Merck) which were conditioned with 2 mL water–ACN (95:5) and 2×2 mL water (Milli-Q). After eluting the sample, the columns were washed with 2 mL water and 2 mL water–ACN (95:5) and dried under airflow for 5 min. The remainder was eluted with 2×250 μL acetone, the solvent evaporated (Speedvac, 35–40°C) and the residue reconstituted in 1 mL HPLC mobile phase and 20 μL injected into the HPLC.

Sample analysis was performed using LC-MS (HP 1100 Series, Agilent) with AP-ES ionisation (positive ion detection). At the same time, UV spectra were recorded with a diode array detector (monitoring wavelength 210 nm). The separation was performed on a RP-18 column (LiChroCART 125-4, Superspher 100 RP-18, 5 μm, Merck) with a 4×4 mm guard column containing the same stationary phase. Water–acetonitrile (73:27) as mobile phase (flowrate 1 mL min^{-1}) was freshly prepared, filtered (0.22 μm, NL 16, Schleicher & Schuell) and degassed (USB).

Selected ion monitoring (SIM) with one mass for each compound of interest was used for quantification with the fragmentor energy set to 100 V for maximal detection sensitivity for these compounds. Further settings were: capillary voltage 3500 eV, nebulizer gas temperature 350°C and nebulizer pressure 40 psi.

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