Synthesis and in Vitro Evaluation of New 8-Amino-1,4-benzoxazine Derivatives as Neuroprotective Antioxidants

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A series of new 8-amino-1,4-benzoxazine derivatives **5a**−**o** was synthesized and examined for their intrinsic cytotoxicity and their capacity to inhibit oxidative stress-mediated neuronal degeneration in neuronal cell cultures. In particular, substituent effects at the 3- and 8-positions of the 1,4-benzoxazine ring were investigated by in vitro evaluation. In this aim, 3-alkyl substituents seemed to be essential for efficient neuroprotective activity. Furthermore, within the subseries of substituted 3-alkyl benzoxazines, the most active derivatives were those bearing an 8-benzylamino substituent. From the combined results of both toxicity and neuroprotection expressed in terms of the safety index, 8-benzylamino-substituted-3-alkyl-1,4-benzoxazines were identified as the most promising compounds, owing to their potent neuroprotective activity without the manifestation of intrinsic cytotoxicity.

Reactive oxygen species are byproducts of normal metabolic processes in aerobic environments.[1] These species can exert cytotoxicity by damaging critical cellular components necessary for viability.[2] Examples of the reactive oxygen species are superoxide, hydrogen peroxide, and hydroxyl radicals produced by sources such as bioreductively activated xenobiotics or by low-molecular-weight complexes of transition metals such as iron and copper via the Fenton reaction.

Elimination of these oxidants is provided by enzymatic and nonenzymatic mechanisms. Enzymatic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase which catalytically detoxify oxidants, whereas nonenzymatic antioxidants are primarily reducing agents, such as vitamin C, vitamin E, and glutathione, which can scavenge oxidants by hydrogen atom donation in a stoichiometric manner.[3] Imbalances in the detoxification of reactive oxygen species relative to their production lead to what is a widely accepted phenomenon called "oxidative stress". In most cases, the defense provided by the enzymatic and nonenzymatic antioxidants is adequate. However, in acute situations, such as cerebral ischemia, an increased "oxidative load" results in "oxidative stress", culminating in accelerated neurodegenerative mechanisms. In addition, oxidative stress may play a role in the progression of many neurodegenerative pathologies of the central nervous system, including Parkinson's and Alzheimer's disease. Consequently, supplementation with exogenous antioxidants could represent an important therapeutic potential to minimize central nervous system damage and has prompted the search for such substances.

In this context, exifone (Adlone) was launched in France, in 1988, for the treatment of cognitive problems in the elderly[4,5] and was reported to have a number of pharmacological properties including activation of neuronal oxygen and glucose metabolism, as well as antagonism of aminergic neurotransmission impairment induced by temporary ischemia.[6,7] Furthermore, exifone demonstrated remarkable scavenger properties against free radicals.[8] Unfortunately, after reports of severe hepatotoxicity associated with the product, in 1990, the registration was revoked and exifone was withdrawn from the market.

On the basis of previous results concerning the possible role of highly electrophilic oxidation products (quinone or iminoquinone) in the toxicological effects of drugs,[9−11] we hypothesized that a potential toxic metabolite of exifone could be the transient reactive *o*-quinone species, which would bind irreversibly to the NH_2 and SH residues of cellular proteins to form conjugates.[12−14] Our objective was then to scavenge the transient reactive *o*-quinone by the formation of an adduct blocking the electrophilic sites generally attacked by NH_2 and SH membrane residues and consequently to prevent toxicity. A few years ago, we described the first electrochemical and chemical syntheses of novel 1,4-benzoxazine derivatives **I** and **II** structurally related to exifone (Chart 1) that have been identified as efficient antioxidants and significantly less hepatotoxic than exifone.[15,16]

To continue these investigations, we describe herein the electrochemical and chemical syntheses of a new set of 1,4-benzoxazine derivatives **5a**−**o** bearing a 8-amino substituent (Chart 1). Preliminary in vitro assays of these molecules have been performed in order to explore the structural requirements for efficient antioxidative activity, without the manifestation of intrinsic cytotoxicity.

Chemistry

General chemical methods currently available so far for the preparation of 1,4-benzoxazines[17−21] were not adapted to the synthesis of a series of 8-amino-1,4-benzoxazine derivatives for a rapid in vitro biological

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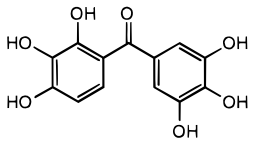
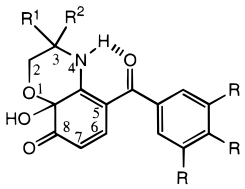
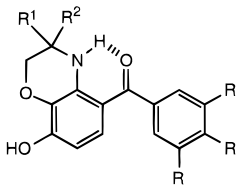
Chart 1



exifone (*Adlone*^{R})



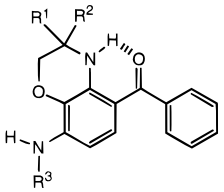
I



II

R^1 and R^2 = Me or CH_2OH

R = H or OH



5a-o

evaluation. These procedures would require, as the starting materials, polysubstituted *o*-aminophenols which are difficult to prepare. Consequently, it is for these reasons that we have recently developed a convenient general two-step one-pot electrochemical procedure for the synthesis of 8-amino-1,4-benzoxazine derivatives.[22] The pathway involved the initial oxidation of (3,4-dihydroxy-2-methoxyphenyl)(phenyl) methanone **1**, leading to the formation of the transient 3,4-quinone **2** (Scheme 1). The subsequent step consisted of a substitution reaction of the 2-methoxy group by an amino-alcohol [CH_2OH-C(R^1,R^2)-NH_2], affording, after intramolecular ring closure, the 1,4-benzoxazin-8-one derivatives **3a**−**c** which could be isolated in good yields ranging from 65 to 95% (Table 1, method A). However, the electrochemical procedure could be pursued without isolation of the latter that was allowed to react with an excess of amine R^3-NH_2, producing the Schiff base intermediate **4**. This intermediate was subsequently reduced through a two-electron transfer to 8-amino derivatives **5a**−**o** in moderate yields ranging from 15 to 60% (Table 2, method C).

To explore the influence of the amino chain at the C-8 position of the benzoxazine ring, the synthesis of 8-hydroxy derivatives **6a**−**c** was targeted from the corresponding 1,4-benzoxazin-8-ones **3a**−**c**. The electrochemical procedure involved a two-electron reduction of the carbonyl function at the C-8 position, producing, after elimination of one molecule of water, the phenolic derivatives **6a**−**c** in roughly 80% yields (Table 1, method F).

Last, in order to prepare the optimal candidate at the preparative scale for further in vivo evaluation, we looked for a chemical extrapolation of our electrochemical procedure. In agreement with the potential value used in the course of the controlled anodic potential electrolysis, Ag_2O was selected as a surrogate of the electrochemical oxidation. Nevertheless, this procedure gave lower yields of 1,4-benzoxazin-8-one derivatives **3a**−**c** ranging from 35 to 60% (Table 1, method B). Reduction of the carbonyl function at the C-8 position of 1,4-benzoxazin-8-one derivatives could be achieved using zinc in methanolic acetic acid solution, leading to the phenolic derivatives **6a**−**c** in roughly 60% yields (Table 1, method G).

Particular difficulty was noted with the chemical synthesis of 8-amino-1,4-benzoxazines **5a**−**o** which failed, in several cases, due to the instability of the Schiff base intermediate **4**. If the latter was sufficiently stable, it could be reduced using zinc in methanolic acetic acid solution at the end of the reaction with the excess of amine R^3-NH_2, giving the expected 8-amino derivatives (Table 2, method E). Conversely, when the Schiff base **4** was unstable, the electrochemical procedure (Table 2, method D) was preferred over the chemical one since the Schiff base **4** could be reduced as soon as it appeared in the electrolysis solution. All targeted compounds are detailed in Tables 1 and 2.

Results and Discussion

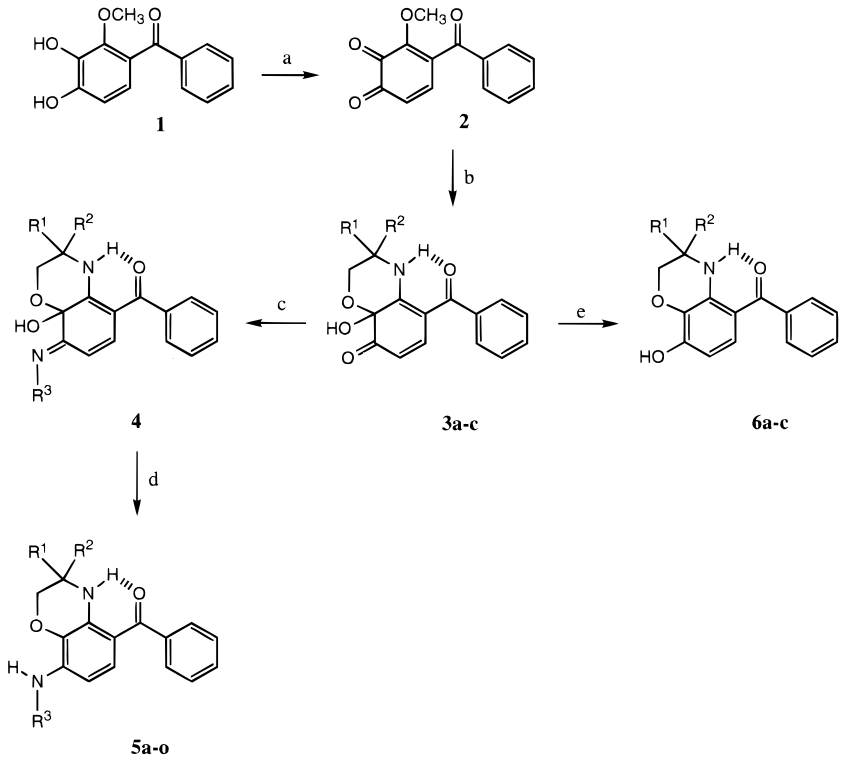
The intrinsic neurotoxicity as well as the neuroprotective activity of 1,4-benzoxazines were assessed in vitro on murine HT-22 hippocampal cell cultures and compared with those of parent exifone. The results are presented in Tables 3 and 4.

To estimate potential pro-oxidant effects[23] and to select a concentration range lacking intrinsic toxicity, suitable for studying the neuroprotective activity of the compound, the intrinsic neurotoxic effects of each compound was evaluated following two different methods. Neurotoxicity was monitored either by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reduction assay), which allows an evaluation of the "redox state" of the cells and emphazises oxidative stress, or by quantification of cellular lysis (death) after measurement of the lactate dehydrogenase (LDH) activity released from damaged cells into the culture supernatant. The maximum tolerated concentration (MTC) and the concentration producing 50% toxicity (TC_{50}) were estimated for each tested compound using both MTT and LDH determinations.

Neuroprotective properties of 1,4-benzoxazine derivatives were estimated through their protective effects against L-homocysteic acid (L-HCA) cytotoxicity. Previous cell culture toxicity studies have demonstrated that depletion of intracellular glutathione levels by the competitive inhibition of cystine uptake, via the cystine/glutamate antiporter Xc^-, and by exposure of immature primary neurons or certain neuronal cell lines to L-HCA results in oxidative stress mediated neuronal degeneration which was attenuated with antioxidants.[24,25] The concentration producing 50% protection (PC_{50}) was estimated for both MTT and LDH determinations. On the basis of these results, the MTC/PC_{50} ratio was calculated as an index of the pro- and antioxidant effects of each compound and was termed the "safety index".

As reported in Tables 3 and 4, the MTC determined from the MTT reduction assay appeared to be the most sensitive parameter for all evaluated compounds. With regard to this parameter, all targeted 1,4-benzoxazin-8-one derivatives **3a**−**c** as well as their 8-hydroxy

Scheme 1[a]



[a] Reagents and conditions: (a) MeOH, Ag_2O, or electrochemical oxidation at a platinum anode (E = +0.4 V vs sce), rt; (b) MeOH, CH_2OH-C (R^1, R^2)-NH_2, rt; (c) MeOH, R^3-NH_2, rt; (d) MeOH/AcOH, zinc, or electrochemical reduction at a mercury cathode (E = −1.0 V vs sce), rt; (e) MeOH/AcOH, zinc, or electrochemical reduction at a mercury cathode (E = −1.2 V vs sce), rt.

Table 1. Physical Properties of 2*H*-1,4-Benzoxazin-8-ones **3** and 2*H*-1,4-Benzoxazin-5-yl-(phenyl)-methanones **6**

Compd	R^1	R^2	method[a]	yield (%)	mp (°C)	crystn solvent
3a	H	Bu^t	A	95	185-187 dec	b
			B	60		
3b			A	60	208-210 dec	ether
			B	35		
3c	H	CH_2OH	A	65	140-142 dec	b
6a	H	Bu^t	F	85	160-162	ether-pentane
			G	60		
6b			F	80	171-173	ether
			G	57		
6c	H	CH_2OH	F	75	125-127	ether

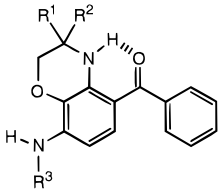
[a] Procedure is described in the Experimental Section. [b] Crystallized following concentration of the flash column chromatography solvent.

derivatives **6a**−**c** demonstrated MTC values of the same order of magnitude as the PC_{50} values, as indicated by the low safety index values. Similarly, with exifone, we were unable to determine, in our tests, a concentration range for which antioxidant effects could be achieved without the manifestation of intrinsic toxicity (compare PC_{50} with MTC).

Contrary to compounds **3** and **6**, the new series of 8-amino-1,4-benzoxazines **5a**−**o** showed promising results in vitro with, in most cases, very significant safety index values (up to 71 for compound **5d**).

First, apart from compounds **5c**, **5e**, and **5i** (which bear an 8-isopentylamino or an 8-isobutylamino substituent), introduction of an amino chain at the 8-position of the benzoxazine ring induced a significant decrease in in vitro toxicity when compared with both the 8-hydroxy analogues **6a**−**c** and exifone: compare compounds **5a** and **5b** (MTC = 100 μM) with their

Table 2. Physical Properties of 2*H*-1,4-Benzoxazin-5-yl-(phenyl)-methanones **5**



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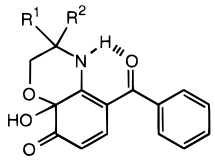
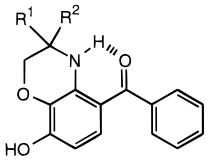
Compd	R^1	R^2	R^3	method[a]	yield (%)	mp (°C)	crystn solvent
5a	H	Bu^t	Bzl	C	30	87-89	petroleum ether[b]
5b	H	Bu^t	$(CH_2)_2$-Ph	C	50	107-109	ether-pentane
5c	H	Bu^t	Pe^i	C	45	95-97	petroleum ether[b]
5d	Me	Me	Bzl	C	15	131-133	ether-petroleum ether
5e	Me	Me	Pe^t	D	50	99-101	petroleum ether[b]
5f	Me	Me	CH_2-CH_2OH	E	60	134-136	pentane
5g	Me	Me	$CH(CH_2OH)_2$	D	45	177-179	ether
5h			Bzl	C	35	124-126	ether
5i			Bu^i	C	35	102-104	pentane[b]
5j	Ph	Ph	Bzl	C	50	149-151	methanol
5k	Ph	Ph	Pe^i	C	50	109-111	petroleum ether
5l	H	CH_2OH	$CH(CH_2OH)_2$	C	15	163-165	chloroform
5m	Me	CH_2OH	CH_2-CH_2OH	C	50	181-183	chloroform-ether
5n	Me	CH_2OH	$CH(CH_2OH)_2$	C	25	207-209	chloroform-ether
5o	CH_2OH	CH_2OH	CH_2-CH_2OH	E	60	177-179	ethyl acetate

[a] Procedure is described in the Experimental Section [b] Crystallized after cooling to −40 °C. Abbrevations: *tert*-butyl (Bu^t), isopentyl (Pe^i), isobutyl (Bu^i), benzyl (Bzl).

Table 3. In Vitro Neuroprotective Activity of 2*H*-1,4-Benzoxazin-8-ones **3** and 2*H*-1,4-Benzoxazin-5-yl-(phenyl)-methanones **6**



3



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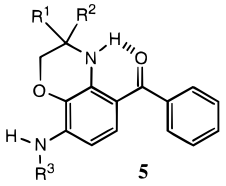
			Toxicity[a]				Protection *vs* 2mM L-HCA[b]		Safety index	
Compd	R^1	R^2	MTC (μM)		TC_{50} (μM)		PC_{50} (μM)		MTC / PC_{50}	
			MTT	LDH	MTT	LDH	MTT	LDH	MTT	LDH
exifone			10	50	34	91	> 10	> 10	< 1	< 1
3a	H	Bu^t	10	10	17.6	38.1	6.5	5.8	1.5	1.7
3b			50	50	109	> 250	47.4	40.9	1.1	1.2
3c	H	CH_2OH	10	25	39.1	134.3	15.1	17.0	< 1	1.5
6a	H	Bu^t	1	5	11.7	12.2	>1	>1	< 1	< 1
6b			25	25	37.5	36.4	25	18.4	1.0	1.4
6c	H	CH_2OH	5	10	55.5	111.3	>10	>10	< 1	< 1

[a] In vitro neurotoxicity monitored either by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reduction assay) or by quantification of cellular lysis after measurement of the lactate dehydrogenase (LDH) activity released from damaged cells into the culture supernatant. [b] In vitro neuroprotective activity estimated through their protective effects against L-homocysteic acid (L-HCA) cytotoxicity. Abbreviations: MTC, maximum tolerated concentration; TC_{50}, concentration producing 50% toxicity; PC_{50}, concentration producing 50% protection.

8-hydroxy counterpart **6a** (MTC = 1 μM); similarly, compare **5h** (MTC > 250 μM) with **6b** (MTC = 25 μM). From the data collected in Table 4, it could be concluded that substituted 8-benzylamino, 8-phenylethylamino,

Table 4. In Vitro Neuroprotective Activity of 2*H*-1,4-Benzoxazin-5-yl-(phenyl)-methanones **5**



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				Toxicity[a]				Protection *vs* 2mM L-HCA[b]		Safety index	
Compd	R^1	R^2	R^3	MTC (μM)		TC_{50} (μM)		PC_{50} (μM)		MTC / PC_{50}	
				MTT	LDH	MTT	LDH	MTT	LDH	MTT	LDH
exifone				10	50	34	91	>10	>10	< 1	< 1
5a	H	Bu^t	Bzl	100	>250	>250	>250	3.7	2.9	27	>86
5b	H	Bu^t	$(CH_2)_2$-Ph	100	250	>250	>250	8.8	8.9	11	28
5c	H	Bu^t	Pe^i	10	10	36	>250	8.9	6.1	1.1	1.6
5d	Me	Me	Bzl	>250	>250	>250	>250	3.5	3.1	>71	>81
5e	Me	Me	Pe^i	5	10	17	18	7.5	6.9	< 1	1.4
5f	Me	Me	CH_2-CH_2OH	25	100	88	234	35.5	30.5	< 1	3.3
5g	Me	Me	$CH(CH_2OH)_2$	100	>250	>250	>250	76.8	72.0	1.3	>3.5
5h			Bzl	>250	>250	>250	>250	6.9	7.1	>36	>36
5i			Bu^i	10	>250	>250	>250	>10	>10	< 1	< 1
5j	Ph	Ph	Bzl	50	>250	>250	>250	>100	>100	< 1	>2.5
5k	Ph	Ph	Pe^i	>250	>250	>250	>250	>100	>100	>2.5	>2.5
5l	H	CH_2OH	$CH(CH_2OH)_2$	100	>250	>250	>250	>100	>100	< 1	>2.5
5m	Me	CH_2OH	CH_2-CH_2OH	50	>250	210	>250	42.3	35.1	1.2	>7.0
5n	Me	CH_2OH	$CH(CH_2OH)_2$	100	>250	>250	>250	>100	>100	< 1	>2.5
5o	CH_2OH	CH_2OH	CH_2-CH_2OH	50	>250	250	>250	>100	85.1	< 1	>2.9

[a] In vitro neurotoxicity monitored either by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reduction assay), or by quantification of cellular lysis after measurement of the lactate dehydrogenase (LDH) activity released from damaged cells into the culture supernatant. [b] In vitro neuroprotective activity estimated through their protective effects against L-homocysteic acid (L-HCA) cytotoxicity. Abbreviations: MTC, maximum tolerated concentration; TC_{50}, concentration producing 50% toxicity; PC_{50}, concentration producing 50% protection.

and 8-hydroxyalkylamino benzoxazines exhibited a low toxicity with relatively high MTC values whereas, exclusive of compound **5k**, substituted 8-isobutylamino and 8-isopentylamino derivatives **5c**, **5e**, and **5i** were rather toxic. The nature of the substituents at the 3-position was apparently not critical for toxicity, since compounds **5g**, **5l**, and **5n**, all possessing an 8-[2-hydroxy-1-(hydroxymethyl)ethyl] amino substituent, showed the same MTC value (100 μM). Similar observations were made for compounds **5a**, **5d**, and **5h**, with an 8-benzylamino substituent.

Second, concerning the structure−activity relationships within the series of 8-amino-1,4-benzoxazine derivatives **5a**−**o**, the results reported in Table 4 showed that the requirements for the substitutions on both the 3- and 8-positions of the benzoxazine ring might be quite stringent. The 3-alkyl substituents seemed to be essential for efficient neuroprotective activity, since 3,3-diphenyl derivatives **5j** and **5k** or 3-hydroxyalkyl derivatives **5l**−**o** were clearly inactive. Furthermore, within the subseries of substituted 3-alkyl benzoxazines **5a**−**i**, the most active derivatives are those bearing an 8-benzylamino substituent (compounds **5a**, **5d**, and **5h**) and, to a lesser extent, those bearing an 8-phenylethylamino substituent (compound **5b**) or an 8-alkylamino group (compounds **5c**, **5e**, and **5i**). Interestingly, replacement of the 8-alkylamino by an 8-hydroxyalkylamino substituent resulted in a large decrease in activity (at least 5 times). So, it could be concluded that alcoholic substituents abolished neuroprotective activity, whatever their position (at C-3 or C-8) on the 1,4-benzoxazine ring.

As mentioned above, the results of both toxicity and activity were combined to determine a safety index defined as MTC/PC_{50}. The latter, reported in Table 4, was used to estimate the therapeutic potential of compounds **5a**−**o**. It rapidly appeared that compounds bearing 8-benzylamino as well as 3-alkyl substituents exhibited the highest safety index. Consequently, 8-amino-1,4-benzoxazine derivatives **5a**, **5d**, and **5h**, with safety index of 27, >71, and >36, respectively, were considered as the most attractive compounds from our in vitro evaluation.

Conclusions

We have synthesized and evaluated a series of 1,4-benzoxazine derivatives as neuroprotective agents. Owing to their very low safety index, 1,4-benzoxazin-8-ones **3a**−**c** and 8-hydroxy-1,4-benzoxazines **6a**−**c** were not considered as promising compounds. It is possible that the 1,4-benzoxazin-8-one derivatives **3a**−**c** could undergo an opening reaction up to the 1,4-oxazine ring, to generate the putative toxic 3,4-quinone species. If such a species is the potential toxic metabolite of exifone as suggested in the Introduction, it was not surprising that 1,4-benzoxazin-8-one derivatives **3a**−**c** exhibited a po-

tent toxicity. Introduction of an amino chain at the 8-position of the 1,4-benzoxazine ring appeared to constitute a method of detoxification, except for alkyl amino chains such as isopentyl or isobutyl. However, the real role of the 8-substituent is still unclear so far. On the basis of structure−activity relationships within the 8-amino-1,4-benzoxazine series, the 3-alkyl functionality appeared to be essential for activity and we can conclude that the most suitable substituents to date are alkyl chains such as *tert*-butyl, methyl, or cyclopentyl. Since the 3-substituents play an important role on the intensity of the intramolecular hydrogen bonding that has been evidenced between the oxygen of the carbonyl group of the 5-benzoyl substituent and the N−H group of the 1,4-benzoxazine ring, it is also possible that some of these compounds may act in vivo via the formation of chelates with metals such as copper or iron. Additional structural biological studies (X-ray, for example) would be necessary to provide fresh insight into the structure−activity relationships within the 8-amino-1,4-benzoxazines series. These studies, as well as efforts to examine the ability of these molecules to afford chelates, are underway in our laboratories.

Finally, this study has culminated in the selection of 8-benzylamino-substituted-3-alkyl-1,4-benzoxazines with greater neuroprotective activity, without the toxicity exhibited by exifone.

Experimental Section

Chemistry. The syntheses of the compounds are described by way of typical examples: the methods are denoted in the same manner as in Tables 1 and 2. Melting points were determined on a Köfler block and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker AC 300 spectrometer operating at 300 MHz for ^{1}H observations. Chemical shifts (in ppm) are relative to internal tetramethylsilane. The measurements were carried out using the standard pulse sequences. The carbon type (methyl, methylene, methide, or quaternary) was determined by DEPT experiments. Mass spectra were recorded on a Nermag R 10-10C spectrometer equipped with desorption chemical ionization mode (DCI/NH_3). All compounds were analyzed for C, H, and N. Analytical results were within ±0.4% of the theoretical values unless otherwise indicated. Merck 60 (40−60 μm) and Merck 60 F_{254} silica gel were used for column chromatography and thin-layer chromatography, respectively. All reagents and solvents were of commercial quality or were purified before use.

Electrochemistry. The apparatus, cells, and electrodes were identical with those described previously.[26]

Method A. (*R*,*S*)-5-Benzoyl-3-*tert*-butyl-8a-hydroxy-3,4,8,8a-tetrahydro-2*H*-1,4-benzoxazin-8-one 3a. A solution of compound **1** (244 mg, 1 mmol), tetraethylammonium perchlorate (TEAP) (2.3 g, 10 mmol), and *S*-*tert*-leucinol (597 mg, 5 mmol) in methanol (500 mL) was oxidized, under nitrogen, at room temperature, at a platinum electrode [E = + 0.4 V vs saturated calomel electrode (sce)]. After exhaustive oxidation, i.e., when a steady-state minimum value of the current was recorded, the resulting solution was poured into a molar acetic acid buffered aqueous solution of pH ∼ 4.5 (100 mL). The resulting hydroalcoholic solution was concentrated to 100 mL, under reduced pressure, at 40 °C, and extracted with ethyl acetate (200 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica with toluene−acetone (98:2) as the eluent, leading to compound **3a** (310 mg, 95%) as an amorphous yellow solid: mp 185−187 (decomp). The latter contained a mixture (60:40) of two diastereoisomers A and B that could not be separated.

Diastereoisomer A: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.00 (s, 9H, Me, Bu^t), 3.65 (m, 1H, 3-H), 4.05 (m, 1H, 2-CH_2), 4.40 (m, 1H, 2-CH_2), 5.25 (d, 1H, J = 9 Hz, 7-H), 7.25 (d, 1H, J = 9 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.25 (s, 1H, OH, D_2O exchanged), 12.60 (s, 1H, NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.6 (Me, Bu^t), 35.1 (C_Q, Bu^t), 56.8 (C-3), 58.2 (C-2), 86.7 (C-8a), 99.5 (C-5), 109.3 (C-7), 128.8, 129.4 and 131.3 (CH, Ph), 140.3 (C_Q, Ph), 147.6 (C-6), 168.9 (C-4a), 190.9 (C-8), 194.0 (CO, 5-benzoyl).

Diastereoisomer B: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.00 (s, 9H, Me, Bu^t), 3.55 (m, 1H, 3-H), 3.95 (m, 1H, 2-CH_2), 4.30 (m, 1H, 2-CH_2), 5.15 (d, 1H, J = 9 Hz, 7-H), 7.15 (d, 1H, J = 9 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.20 (s, 1H, OH, D_2O exchanged), 12.70 (s, 1H, NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.2 (Me, Bu^t), 33.6 (C_Q, Bu^t), 58.1 (C-2), 58.5 (C-3), 87.2 (C-8a), 99.8 (C-5), 109.1 (C-7), 128.8, 129.4 and 131.3 (CH, Ph), 140.3 (C_Q, Ph), 147.1 (C-6), 169.6 (C-4a), 191.5 (C-8), 193.8 (CO, 5-benzoyl); MS (mixture of A and B) (DCI) m/z = 328 (MH^+). Anal. ($C_{19}H_{21}NO_4$) C, H, N.

Method B. To a solution of compound **1** (244 mg, 1 mmol) and S-*tert*-leucinol (597 mg, 5 mmol) in methanol (500 mL) was added Ag_2O (575 mg, 5 mmol). The mixture was stirred at room temperature for 2 h, under nitrogen. Then, the solid residue was removed by filtration, and the filtrate was poured into a molar acetic acid buffered aqueous solution of pH ∼ 4.5 (100 mL). The resulting aqueous alcoholic solution was treated according to method A and afforded compound **3a** (196 mg, 60%).

Compounds **3b** and **3c** were prepared following a procedure analogous to method A or method B (see Table 1).

5-Benzoyl-3-spiro-1′-cyclopentyl-8a-hydroxy-3,4,8,8a-tetrahydro-2*H*-1,4-benzoxazin-8-one 3b: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.70 to 2.10 (m, 8H, CH_2, cyclopentyl), 3.85 (d, 1H, J = 12 Hz, 2-CH_2), 4.30 (d, 1H, J = 12 Hz, 2-CH_2), 5.20 (d, 1H, J = 10 Hz, 7-H), 7.20 (d, 1H, J = 10 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.35 (s, 1H, OH, D_2O exchanged), 12.5 (s, 1H, NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 23.8, 24.2, 36.7 and 40.7 (CH_2, cyclopentyl), 61.8 (C-3), 65.0 (C-2), 87.9 (C-8a), 99.7 (C-5), 108.9 (C-7), 128.7, 129.5 and 131.3 (CH, Ph), 140.4 (C_Q, Ph), 147.1 (C-6), 167.8 (C-4a), 191.2 (C-8), 193.8 (CO, 5-benzoyl); MS (DCI): m/z = 326 (MH^+). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

(*R*,*S*)-5-Benzoyl-3-hydroxymethyl-8a-hydroxy-3,4,8,8a-tetrahydro-2*H*-1,4-benzoxazin-8-one 3c. Diastereoisomer A: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 3.40 to 3.90 (m, 3H, 3-CH_2 and 3-H), 4.10 (m, 1H, 2-CH_2), 4.40 (dd, 1H, J = 6 Hz, J = 12 Hz, 2-CH_2), 5.20 (d, 1H, J = 10 Hz, 7-H), 5.40 (s, 1H, 3-CH_2*OH*, D_2O exchanged), 7.15 (d, 1H, J = 10 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.30 (s, 1H, 8a-OH, D_2O exchanged), 12.30 (s, 1H, NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 50.9 (C-3), 58.2 (C-2), 62.8 (CH_2OH), 87.5 (C-8a), 99.8 (C-5), 108.8 (C-7), 128.7, 129.3 and 130.4 (CH, aromatic, Ph), 140.6 (C_Q, Ph), 147.7 (C-6), 191.9 (C-8), 193.8 (CO, 5-benzoyl).

Diastereoisomer B: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 3.40 to 3.90 (m, 3H, 3-CH_2 and 3-H), 3.80 (m, 1H, 2-CH_2), 4.10 (m, 1H, 2-CH_2), 5.15 (d, 1H, J = 10 Hz, 7-H), 5.40 (s, 1H, OH, 3-CH_2*OH*, D_2O exchanged), 7.25 (d, 1H, J = 10 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.20 (s, 1H, 8a-OH, D_2O exchanged), 12.20 (s, 1H, NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 52.3 (C-3), 58.4 (C-2), 61.7 (CH_2OH), 87.8 (C-8a), 99.8 (C-5), 108.9 (C-7), 128.7, 129.3 and 130.4 (CH, aromatic, Ph), 140.6 (C_Q, Ph), 147.3 (C-6), 191.9 (C-8), 193.9 (CO, 5-benzoyl); MS (mixture of A and B) (DCI) m/z = 302 (MH^+). Anal. ($C_{16}H_{15}NO_5$) C, H, N.

Method C. (*R*,*S*)-[8-Benzylamino-3-(*tert*-butyl)-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl] (phenyl)methanone 5a. A solution of compound **1** (244 mg, 1 mmol), TEAP (2.3 g, 10 mmol), and *S*-*tert*-leucinol (597 mg, 5 mmol) in methanol (500 mL) was oxidized, under nitrogen, at room temperature, at a platinum electrode (E = + 0.4 V vs sce). After exhaustive oxidation, i.e., when a steady-state minimum value of the current was recorded, benzylamine (5.3 g, 50 mmol) was added to the oxidized solution and allowed to react for 30 min. Then, the platinum anode was replaced by a mercury pool, and the

resulting solution was reduced at − 1.0 V vs sce. After exhaustive cathodic electrolysis, the solution was poured into a molar acetic acid buffered aqueous solution of pH ∼ 4.5 (100 mL). The resulting aqueous alcoholic solution was treated the same as above (method A) and afforded, after chromatography on silica with toluene as the eluent, compound **5a** (120 mg, 30%) as an amorphous yellow solid that was recrystallized from petroleum ether: mp 87−89 °C: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.00 (s, 9H, Me, Bu^t), 3.25 (m, 1H, 3-H), 3.85 (m, 1H, 2-CH_2), 4.30 (m, 1H, 2-CH_2), 4.40 (d, 2H, J = 6 Hz, CH_2, benzyl), 5.85 (d, 1H, J = 9 Hz, 6-H), 6.45 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 6.75 (d, 1H, J = 9 Hz, 7-H), 7.10 to 7.30 (m, 5H, aromatic, benzyl), 7.45 (m, 5H, aromatic, 5-benzoyl), 8.75 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.9 (Me, Bu^t), 34.0 (C_Q, Bu^t), 46.4 (CH_2, benzyl), 58.3 (C-3), 65.9 (C-2), 100.1 (C-7), 109.1 (C-5), 127.6 (C-8a), 127.8 to 130.9 (CH, aromatic, benzyl and 5-benzoyl), 139.8 (C_Q, 5-benzoyl), 141.2 (C_Q, benzyl), 142.0 and 142.3 (C-4a and C-8), 197.0 (CO, methanone); MS (DCI) m/z = 401 (MH^+). Anal. ($C_{26}H_{28}N_2O_2$) C, H, N.

Compounds **5b-d** were prepared following a procedure analogous to method C (see Table 2).

(*R*,*S*)-(3-*tert*-Butyl-8-phenylethylamino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5b: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.00 (s, 9H, Me, Bu^t), 2.85 (t, 2H, J = 8 Hz, CH_2Ph, phenylethyl), 3.25 (m, 1H, 3-H), 3.35 (q, 2H, J = 8 Hz, CH_2N, phenylethyl), 3.90 (m, 1H, 2-CH_2), 4.30 (dd, 1H, J = 3 Hz, J = 11 Hz, 2-CH_2), 5.75 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 6.05 (d, 1H, J = 9 Hz, 7-H), 6.90 (d, 1H, J = 9 Hz, 6-H), 7.25 (m, 5H, aromatic, phenylethyl), 7.50 (m, 5H, aromatic, 5-benzoyl), 8.80 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.90 (Me, Bu^t), 34.05 (C_Q, Bu^t), 36.40 (CH_2Ph, phenylethyl), 44.80 (CH_2N, phenylethyl), 58.20 (C-3), 65.80 (C-2), 99.70 (C-7), 109.5 (C-5), 127.2 to 130.9 (C-6 and CH, aromatic, phenylethyl and 5-benzoyl), 127.5 (C-8a), 139.8 (C_Q, 5-benzoyl), 140.2 (C_Q, phenylethyl), 142.0 and 142.2 (C-4a and C-8), 197.0 (CO, methanone); MS (DCI) m/z = 415 (MH^+). Anal. ($C_{27}H_{30}N_2O_2$) C, H, N.

(*R*,*S*)-(3-*tert*-Butyl-8-isopentylamino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5c: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 0.90 (d, 6H, J = 6 Hz, Me, isopentyl), 1.00 (s, 9H, Me, Bu^t), 1.40 (q, 2H, J = 8 Hz, CH_2, isopentyl), 1.60 (m, 1H, J = 6 Hz, CH, isopentyl), 3.10 (q, 2H, J = 8 Hz, CH_2N, isopentyl), 3.25 (m, 1H, 3-H), 3.90 (m, 1H, 2-CH_2), 4.25 (dd, 1H, J = 4 Hz, J = 11 Hz, 2-CH_2), 5.65 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 5.95 (d, 1H, J = 9 Hz, 7-H), 6.85 (d, 1H, J = 9 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.75 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 23.50 (Me, isopentyl), 26.40 (CH, isopentyl), 26.9 (Me, Bu^t), 34.0 (C_Q, Bu^t), 39.1 (CH_2, isopentyl), 41.2 (CH_2N, isopentyl), 58.3 (C-3), 65.8 (2-CH_2), 99.5 (C-7), 108.8 (C-5), 127.4 (C-8a), 129.0, 129.2 and 130.9 (C-6 and CH, aromatic, Ph), 139.7 (C_Q, Ph), 142.1 and 142.5 (C-4a and C-8), 196.7 (CO, methanone); MS (DCI) m/z = 381 (MH^+). Anal. ($C_{24}H_{32}N_2O_2$) C, H, N.

(8-Benzylamino-3,3-dimethyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5d: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.30 (s, 6H, 3-Me), 3.90 (s, 2H, 2-CH_2), 4.40 (d, 2H, J = 6 Hz, CH_2, benzyl), 5.85 (d, 1H, J = 9 Hz, 7-H), 6.55 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 6.75 (d, 1H, J = 9 Hz, 6-H), 7.10 to 7.30 (m, 5H, aromatic, benzyl), 7.35 to 7.50 (m, 5H, aromatic, 5-benzoyl), 8.40 (s, 1H, 4-NH, D_2O exchanged); MS (DCI) m/z = 373 (MH^+). Anal. ($C_{24}H_{24}N_2O_2$) C, H, N.

Method D. (3,3-Dimethyl-8-isopentylamino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5e. A solution of (*R*,*S*)-5-benzoyl-3,3-dimethyl-8a-hydroxy-3,4,8,8a-tetrahydro-2*H*-1,4-benzoxazin-8-one (235 mg, 0.8 mmol), TEAP (1.8 g, 8 mmol), and isopentylamine (4.5 mL, 40 mmol) in methanol (400 mL) was allowed to react for 30 min. Then, the resulting solution was reduced, under nitrogen, at room temperature, at a mercury pool (E = −1.0 V vs sce). After exhaustive reduction, i.e., when a steady-state minimum value of the current was recorded, the resulting solution was poured into a molar acetic acid buffered aqueous solution of pH ∼ 4.5 (100 mL). The resulting hydroalcoholic solution was treated according to method A and afforded, after chromatography on silica with toluene as the eluent, compound **5e** (141 mg, 50%) as an amorphous yellow solid that was recrystallized from petroleum ether: mp 99−101 °C: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 0.90 (d, 6H, J = 6 Hz, Me, isopentyl), 1.25 (s, 6H, 3-Me), 1.45 (q, 2H, J = 6 Hz, CH_2, isopentyl), 1.60 (m, 1H, J = 6 Hz, CH, isopentyl), 3.15 (q, 2H, J = 6 Hz, CH_2N, isopentyl), 3.85 (s, 2H, 2-CH_2), 5.75 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 5.95 (d, 1H, J = 9 Hz, 7-H), 6.85 (d, 1H, J = 9 Hz, 6-H), 7.45 (m, 5H, aromatic, Ph), 8.40 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 23.5 (Me, isopentyl), 26.4 (CH, isopentyl), 26.5 (3-Me), 39.1 (CH_2, isopentyl), 48.6 (C-3 and CH_2N, isopentyl), 73.5 (2-CH_2), 99.4 (C-7), 108.8 (C-5), 126.5 (C-8a), 129.0 (CH, *meta*, Ph), 129.1 (CH, *ortho*, Ph), 130.8 and 131.1 (C-6 and CH, *para*, Ph), 138.2 (C_Q, Ph), 142.2 and 142.6 (C-4a and C-8), 196.4 (CO, methanone); MS (DCI) m/z = 353 (MH^+). Anal. ($C_{22}H_{28}N_2O_2$) C, H, N.

Method E. (3,3-Dimethyl-8-hydroxyethylamino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5f. A solution of (*R*,*S*)-5-benzoyl-3,3-dimethyl-8a-hydroxy-3,4,8,8a-tetrahydro-2*H*-1,4-benzoxazin-8-one (225 mg, 0.75 mmol) and ethanolamine (2.5 mL, 37.5 mmol) in methanol (400 mL) was allowed to react for 2h. Then, 7 mL of concentrated acetic acid as well as zinc dust (250 mg, 3.7 mmol) were added, and the resulting mixture was stirred for 5 min at room temperature. The reaction mixture was filtered, and water (100 mL) was then added. The resulting aqueous alcoholic solution was treated the same as above (method A) and led to compound **5f** (147 mg, 60%) as an amorphous yellow solid that was crystallized from pentane: mp 134−136 °C: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.25 (s, 6H, Me), 3.15 (q, 2H, J = 6 Hz, CH_2N, hydroxyethyl), 3.55 (q, 2H, J = 6 Hz, CH_2N, hydroxyethyl), 3.85 (s, 2H, 2-CH_2), 4.80 (t, 1H, J = 6 Hz, OH, D_2O exchanged), 5.65 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 6.00 (d, 1H, J = 9 Hz, 7-H), 6.85 (d, 1H, J = 9 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.40 (s, 1H, 4-NH); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.5 (Me), 45.6 (CH_2N), 48.7 (C-3), 60.7 (CH_2O), 73.5 (C-2), 99.6 (C-7), 109.0 (C-5), 126.6 (C-8a), 129.0, 129.1, 130.9 and 131.0 (C-6 and CH, aromatic, Ph), 138.2 (C_Q, Ph), 142.1 and 142.6 (C-4a and C-8), 196.5 (CO, methanone); MS (DCI) m/z = 327 (MH^+). Anal. ($C_{19}H_{22}N_2O_3$) C, H, N.

{3,3-Dimethyl-8-[2-hydroxy-1-(hydroxymethyl)ethyl]-amino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl}(phenyl)methanone 5g. Compound **5g** was prepared following a procedure analogous to method D (see Table 2): ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.25 (s, 6H, Me), 3.35 (m, 5H, CH_2OH and CH, 8-amino chain), 3.85 (s, 2H, 2-CH_2), 4.80 (s, 2H, OH, D_2O exchanged), 5.30 (d, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 6.05 (d, 1H, J = 9 Hz, 7-H), 6.85 (d, 1H, J = 9 Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.40 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.5 (Me), 48.7 (C-3), 56.0 (CH, 8-amino chain), 61.0 (CH_2OH, 8-amino chain), 73.6 (C-2), 99.8 (C-7), 109.0 (C-5), 126.6 (C-8a), 129.0, 129.1 and 130.9 (C-6 and CH, aromatic Ph), 138.4 (C_Q, Ph), 141.9 and 142.1 (C-4a and C-8), 197.0 (CO, methanone); MS (DCI) m/z = 357 (MH^+). Anal. ($C_{20}H_{24}N_2O_4$) C, H, N.

Compounds **5h**−**n** were prepared following a procedure analogous to method C (see Table 2).

(8-Benzylamino-3-spiro-1′-cyclopentyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl(phenyl)methanone 5h. ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 1.60 to 1.80 (m, 8H, CH_2, cyclopentyl), 4.00 (s, 2H, 2-CH_2), 4.40 (d, 2H, CH_2, benzyl), 5.90 (d, 1H, J = 9 Hz, 7-H), 6.50 (t, 1H, J = 6 Hz, 8-NH, D_2O exchanged), 6.75 (d, 1H, J = 9 Hz, 6-H), 7.10 to 7.25 (m, 5H, aromatic, benzyl), 7.40 (m, 5H, aromatic, 5-benzoyl), 8.55 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 24.5 to 37.7 (CH_2, cyclopentyl), 46.4 (CH_2, benzyl), 59.8 (C-3), 71.6 (C-2), 100.1 (C-7), 109.2 (C-5), 127.3 (C-8a), 127.7, 128.0, 129.1, 129.4, 130.7 and 130.9 (C-6 and CH, aromatic, benzyl and 5-benzoyl), 138.6 (C_Q, 5-benzoyl), 141.2 (C_Q, 5-benzyl), 142.0 and 142.4 (C-4a and C-8), 196.0 (CO, methanone); MS (DCI) m/z = 399 (MH^+). Anal. ($C_{26}H_{26}N_2O_2$) C, H, N.

(8-Isobutylamino-3-spiro-1′-cyclopentyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5i: ^{1}H NMR

[300 MHz, $(CD_3)_2SO$] δ 0.90 (d, 6H, Me, isobutyl), 1.50 to 1.90 (m, 9H, CH, isobutyl and CH_2, cyclopentyl), 2.95 (t, 2H, $J = 6$ Hz, CH_2, isobutyl), 3.95 (s, 2H, 2-CH_2), 5.80 (t, 1H, $J = 6$ Hz, 8-NH, D_2O exchanged), 5.95 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.45 (m, 5H, aromatic, Ph), 8.55 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 21.2 (Me, isobutyl), 24.5 and 37.7 (CH_2, cyclopentyl), 28.9 (CH, isobutyl), 50.7 (CH_2, isobutyl), 59.7 (C-3), 71.6 (2-CH_2), 99.7 (C-7), 108.8 (C-5), 127.0 (C-8a), 129.0, 129.1 and 130.8 (C-6 and CH, aromatic Ph), 138.5 (C_Q, Ph), 142.1 and 142.8 (C-4a and C-8), 197.0 (CO, methanone); MS (DCI) $m/z = 365$ (MH^+). Anal. ($C_{23}H_{28}N_2O_2$) C, H. N.

(8-Benzylamino-3,3-diphenyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5j: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 4.35 (d, 2H, $J = 6$ Hz, CH_2, benzyl), 4.80 (s, 2H, 2-CH_2), 5.90 (d, 1H, $J = 9$ Hz, 7-H), 6.55 (t, 1H, $J = 6$ Hz, 8-NH, D_2O exchanged), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.10 to 7.30 (m, 5H, aromatic, benzyl), 7.35 to 7.55 (m, 15H, aromatic, 3-Ph and 5-benzoyl), 9.50 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 46.4 (CH_2, benzyl), 60.3 (C-3), 71.6 (C-2), 100.6 (C-7), 109.5 (C-5), 127.6 (C-8a), 127.8 to 131.2 (C-6 and CH, aromatic, 3-Ph, benzyl and 5-benzoyl), 138.0 (C_Q, 5-benzoyl), 141.0 (C_Q, benzyl), 141.7 and 142.6 (C-4a and C-8), 144.3 (C_Q, 3-Ph), 196.0 (CO, methanone); MS (DCI): $m/z = 497$ (MH^+). Anal. ($C_{34}H_{28}N_2O_2$) H, N; C: calcd, 82.85; found, 82.01.

(3,3-Diphenyl-8-isopentylamino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 5k: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 0.85 (d, 6H, $J = 6$ Hz, Me), 1.40 (q, 2H, $J = 8$ Hz, CH_2, isopentyl), 1.60 (m, 1H, $J = 8$ Hz, CH, isopentyl), 3.10 (q, 2H, $J = 8$ Hz, CH_2N, isopentyl), 4.70 (s, 2H, 2-CH_2), 5.80 (t, 1H, $J = 6$ Hz, 8-NH, D_2O exchanged), 6.00 (d, 1H, $J = 9$ Hz, 7-H), 6.95 (d, 1H, $J = 9$ Hz, 6-H), 7.20 to 7.40 (m, 10H, aromatic, 3-Ph), 7.50 to 7.60 (m, 5H, aromatic, 5-benzoyl), 9.50 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 23.5 (Me), 26.4 (CH, isopentyl), 38.9 (CH_2, isopentyl), 41.2 (CH_2N, isopentyl), 60.6 (C-3), 71.6 (C-2), 100.0 (C-7), 109.1 (C-5), 127.3 (C-8a), 127.8 to 131.3 (C-6 and CH, aromatic, 3-Ph and 5-benzoyl), 137.9 (C_Q, 5-benzoyl), 141.8 and 142.8 (C-4a and C-8), 144.4 (C_Q, 3-Ph), 197.0 (CO, methanone); MS (DCI) $m/z = 477$ (MH^+). Anal. ($C_{32}H_{32}N_2O_2$) C, H, N.

(*R*,*S*)-{8-[2-Hydroxy-1-(hydroxymethyl)ethyl]amino-3-hydroxymethyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl}-(phenyl)methanone 5l: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 3.40 to 3.60 (m, 8H, 3-CH_2OH, CH_2OH and CH, 8-amino chain), 4.10 (m, 2H, 2-CH_2), 4.80 (t, 2H, $J = 6$ Hz, OH, 8-amino chain, D_2O exchanged), 5.10 (t, 1H, $J = 6$ Hz, 3-CH_2*OH*, D_2O exchanged), 5.25 (d, 1H, $J = 8$ Hz, 8-NH, D_2O exchanged), 6.05 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.45 (m, 5H, aromatic, Ph), 8.60 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 51.6 (C-3), 56.0 (CH, 8-amino chain), 61.0 (CH_2OH, 8-amino chain), 62.2 (3-CH_2OH), 65.4 (C-2), 99.8 (C-7), 109.2 (C-5), 127.5 (C-8a), 129.0, 129.1 and 130.7 and 130.9 (C-6 and CH, aromatic, Ph), 138.4 (C_Q, Ph), 141.9 and 142.1 (C-4a and C-8), 196.0 (CO, methanone); MS (DCI) $m/z = 359$ (MH^+). Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

(*R*,*S*)-[3-Hydroxymethyl-3-methyl-8-(2-hydroxyethylamino)-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl](phenyl)methanone 5m: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 1.20 (s, 3H, Me), 3.15 (q, 2H, $J = 6$ Hz, CH_2N, hydroxyethyl), 3.25 (m, 1H, 3-CH_2OH), 3.40 (m, 1H, 3-CH_2OH), 3.50 (q, 2H, $J = 6$ Hz, CH_2, hydroxyethyl), 3.70 (d, 1H, $J = 9$ Hz, 2-CH_2), 4.10 (d, 1H, $J = 9$ Hz, 2-CH_2), 4.80 (t, 1H, $J = 6$ Hz, OH, hydroxyethyl, D_2O exchanged), 5.15 (t, 1H, $J = 6$ Hz, OH, 3-CH_2OH, D_2O exchanged), 5.65 (t, 1H, $J = 6$ Hz, 8-NH, D_2O exchanged), 5.95 (d, 1H, $J = 9$ Hz, 7-H), 6.80 (d, 1H, $J = 9$ Hz, 6-H), 7.40 (m, 5H, aromatic, Ph), 8.45 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 21.9 (Me), 45.6 (CH_2N, hydroxyethyl), 52.7 (C-3), 60.7 (CH_2, hydroxyethyl), 65.7 (C-2), 69.5 (3-CH_2OH), 99.6 (C-7), 109.1 (C-5), 126.9 (C-8a), 129.0, 129.1, 130.9 and 131.0 (C-6 and CH, aromatic, Ph), 138.3 (C_Q, Ph), 142.1 and 142.5 (C-4a and C-8), 198.0 (CO, methanone); MS (DCI) $m/z = 343$ (MH^+). Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

(*R*,*S*)-{3-Hydroxymethyl-3-methyl-8-[2-hydroxy-1-(hydroxymethyl)ethyl]amino-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl}(phenyl)methanone 5n: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 1.20 (s, 3H, Me), 3.25 to 3.50 (m, 7H, 3-CH_2OH, CH_2OH and CH, 8-amino chain), 3.70 (d, 1H, $J = 9$ Hz, 2-CH_2), 4.10 (d, 1H, $J = 9$ Hz, 2-CH_2), 4.80 (broad s, 2H, OH, 8-amino chain, D_2O exchanged), 5.10 (t, 1H, $J = 6$ Hz, OH, 3-CH_2OH, D_2O exchanged), 5.30 (d, 1H, $J = 6$ Hz, 8-NH, D_2O exchanged), 6.05 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.50 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 21.9 (Me), 52.7 (C-3), 56.0 (CH, 8-amino chain), 61.0 (CH_2OH, 8-amino chain), 65.7 (C-2), 69.5 (3-CH_2OH), 99.8 (C-7), 109.0 (C-5), 126.9 (C-8a), 129.0, 129.1 and 130.9 (C-6 and CH, aromatic, Ph), 138.5 (C_Q, Ph), 141.9 and 142.1 (C-4a and C-8), 196.0 (CO, methanone); MS (DCI) $m/z = 373$ (MH^+). Anal. ($C_{20}H_{24}N_2O_5$) C, H, N.

[8-(2-Hydroxyethylamino-3,3-dihydroxymethyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl](phenyl)methanone 5o: Compound **5o** was prepared following a procedure analogous to method E (see Table 2): ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 3.15 (q, 2H, $J = 6$ Hz, CH_2N, hydroxyethyl), 3.35 (d, 2H, $J = 6$ Hz, 3-CH_2OH), 3.50 (m, 4H, 3-CH_2OH and CH_2, hydroxyethyl), 3.90 (s, 2H, 2-CH_2), 4.80 (t, 1H, $J = 6$ Hz, OH, hydroxyethyl, D_2O exchanged), 5.05 (t, 2H, $J = 6$ Hz, OH, 3-CH_2OH, D_2O exchanged), 5.60 (t, 1H, $J = 6$ Hz, 8-NH, D_2O exchanged), 5.90 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.40 (m, 5H, aromatic, Ph), 8.45 (s, 1H, 4-NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 45.6 (CH_2N, hydroxyethyl), 52.7 (C-3), 60.7 (CH_2, hydroxyethyl), 65.7 (C-2), 69.5 (3-CH_2OH), 99.6 (C-7), 109.1 (C-5), 126.9 (C-8a), 129.0, 129.1, 130.9 and 131.0 (C-6 and *C*H, aromatic, Ph), 138.3 (C_Q, Ph), 142.1 and 142.5 (C-4a and C-8), 198.0 (CO, methanone); MS (DCI) $m/z = 359$ (MH^+). Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

Method F. (8-Hydroxy-3-*tert*-butyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 6a. A solution of compound **3a** (327 mg, 1 mmol) and TEAP (2.3 g, 10 mmol) in methanol (500 mL) was reduced, under nitrogen, at room temperature, at a mercury electrode ($E = -1.3$ V vs sce). After exhaustive electrolysis, i.e., when a steady-state minimum value of the current was recorded, the resulting solution was evaporated to dryness, under reduced pressure, at 35 °C. The residue was poured into water (50 mL) and extracted with ethyl acetate (100 mL). The organic phase was dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica with toluene as the eluent, leading to compound **6a** (265 mg, 85%) as an amorphous yellow solid that was recrystallized from ether−pentane: mp 160−162 °C: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 1.00 (s, 9H, Me, Bu^t), 3.25 (m, 1H, 3-H), 3.90 (m, 1H, 2-CH_2), 4.25 (m, 1H, 2-CH_2), 6.10 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.90 (s, 1H, NH, D_2O exchanged), 9.95 (s, 1H, OH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 26.9 (Me, Bu^t), 34.1 (C_Q, Bu^t), 58.2 (C-3), 65.4 (C-2), 105.4 (C-7), 110.9 (C-5), 129.1, 129.3 and 131.3 (CH, Ph), 129.6 (C-6), 130.4 (C-8a), 141.5 (C_Q, Ph), 142.3 (C-4a), 150.9 (C-8), 197.0 (CO, methanone); MS (DCI) $m/z = 312$ (MH^+). Anal. ($C_{19}H_{21}NO_3$) C, H, N.

Method G. To a solution of compound **3a** (327 mg, 1 mmol) in 250 mL of methanol was added, at room temperature, 10 mL of concentrated acetic acid. Then, zinc dust (327 mg, 5 mmol) was added, and the resulting mixture was stirred for 2 min at room temperature. The reaction mixture was filtered, and water (50 mL) was then added. The resulting solution was concentrated to 50 mL under reduced pressure at 35 °C, treated according to method F, and afforded compound **6a** (186 mg, 60%).

Compounds **6b** and **6c** were prepared following a procedure analogous to method F or method G (see Table 1).

(8-Hydroxy-3-spiro-1′-cyclopentyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl)(phenyl)methanone 6b: ^1H NMR [300 MHz, $(CD_3)_2SO$] δ 1.50 to 1.80 (m, 8H, CH_2, cyclopentyl), 3.90 (s, 2H, 2-CH_2), 6.10 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.70 (s, 1H, OH, D_2O exchanged), 9.95 (s, 1H, NH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 24.5 and 37.7 (CH_2, cyclopentyl), 59.8 (C-3), 71.3 (C-2), 105.4 (C-7), 111.1 (C-5), 129.1, 129.3 and 131.3

(CH, aromatic, Ph), 129.6 (C-6), 130.1 (C-8a), 141.0 and 141.6 (C_Q, Ph and C-4a), 150.9 (C-8), 197.0 (CO, methanone); MS (DCI) $m/z = 310$ (MH^+). Anal. ($C_{19}H_{19}NO_3$) C, H, N.

[(*R,S*)-8-Hydroxy-3-hydroxymethyl-3,4-dihydro-2*H*-1,4-benzoxazin-5-yl](phenyl)methanone 6c: ^{1}H NMR [300 MHz, $(CD_3)_2SO$] δ 3.00 to 3.70 (m, 3H and 3-CH_2OH), 4.10 (s, 2H, 2-CH_2), 5.10 (m, 1H, OH, CH_2OH, D_2O exchanged), 6.10 (d, 1H, $J = 9$ Hz, 7-H), 6.85 (d, 1H, $J = 9$ Hz, 6-H), 7.50 (m, 5H, aromatic, Ph), 8.75 (s, 1H, NH, D_2O exchanged), 9.90 (s, 1H, 8-OH, D_2O exchanged); ^{13}C NMR [75 MHz, $(CD_3)_2SO$] δ 51.6 (C-3), 62.1 (CH_2OH), 65.0 (C-2), 105.3 (C-7), 111.3 (C-5), 129.1, 129.2 and 131.3 (CH, aromatic, Ph), 129.6 (C-6), 130.4 (C-8a), 141.2 and 141.7 (C_Q, Ph and C-4a), 150.9 (C-8), 198.0 (CO, methanone); MS (DCI) $m/z = 286$ (MH^+). Anal. ($C_{16}H_{15}NO_4$) C, H, N.

Biological in Vitro Assays. Murine HT-22 Hippocampal Cell Cultures. HT-22 murine hippocampal cells,[27] a subclone of HT4, were obtained from Dr. Christian Behl, Max Plank-Institute of Psychiatry, Munich, Germany. Cells were grown to confluence in DMEM/F-12 supplemented with 10% FCS at 37 °C/5% CO_2 in a humidified atmosphere, as previously described.[28] Dissociated cells were plated at 1×10^4 cells/100 μL DMEM/F-12/well in the central 60 wells of 96-well poly-D-lysine-coated (20 μg/mL) culture plate and were maintained for 24 h in identical atmospheric condition.

Product Exposure Protocols. Toxicity. HT-22 cell cultures in 100 μL/well DMEM/F-12/2.5 %FCS were incubated with increasing concentrations of drugs for 48 h at 37 °C/5% CO_2. The maximum tolerated concentration (MTC) and the concentration producing 50% toxicity (TC_{50}) were estimated for each drug using MTT and LDH determinations by linear regression analyses. Each product was tested for toxicity in at least two separate experiments with $n = 6$ samples per condition, and fresh product samples were used for all protocols. Toxicity was estimated relative to mock-treated (0% toxicity) and 50 μM menadione-treated cultures (100% toxicity).[23]

Neuroprotection. HT-22 cell cultures in 100 μL/well DMEM/F-12/2.5% FCS were preincubated with different concentrations of antioxidant, over a 3-log range up to a maximum concentration corresponding to the MTC, for 1 h. Cells were then exposed to 2 mM L-HCA, in the presence of antioxidant, for 48 h, and neurotoxicity was estimated relative to 2 mM L-HCA plus 200 μM vitamin E treated cells (0% toxicity) and 2 mM L-HCA alone (100% toxicity). The concentration producing 50% protection (PC_{50}) was estimated for both MTT and LDH determinations by linear regression analyses. Each product was tested for protection in at least two separate experiments ($n = 6$ samples/condition).

Cell Viability Assay. Lactate Dehydrogenase (LDH) Activity Assay. Cellular lysis was quantified by measurement of LDH activity released from damaged cells into the culture supernatant 40−48 h after the initial exposure to L-HCA. An equivalent volume of assay buffer (125 mM NaH_2PO_4, pH 7.6 solution containing 5.675 mM pyruvate and 4 mg/mL βNADH) incubated at 37 °C was added to culture supernatant (100 μL) [samples were assayed within 2 h]. The microplate was then agitated for 15 s at 37 °C, and the decrease in absorbance at 340 nm, corresponding to the reduction in the absorbance of βNADH, was estimated in each well over a 2.5 min period at 15 s intervals in a Labinstruments SLC 340ATTC microplate reader. LDH activity values were calculated from the slope of the linear portion of absorbance curve (fit by linear regression) and scaled relative to the LDH release of control (0%) and L-HCA (100% toxicity) treated cultures.

MTT Reduction. Cytotoxicity of cells was assessed in parallel to LDH release with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reduction assay.[29] Cultures (100−150 μL) were treated with MTT (4 mg/mg) 10−20 μL/well and incubated at 37 °C/5% CO_2 for 3−4 h. The resultant formazan crystals were solubilized by the addition of 2-propanol [8% (v/v)] in 1 M HCl (100 μL/well) and agitated to dissolve remaining formazan crystals. Absorption readings were taken immediately at 540 nm with an automatic plate reader (SLC 340ATTC, Labinstruments).

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