Synthesis and Evaluation of 2′-Hydroxyethyl *trans*-Apovincaminate Derivatives as Antioxidant and Cognitive Enhancer Agents

András Nemes,* László Czibula, Csaba Szántay, Jr., Anikó Gere, Béla Kiss, Judit Laszy, István Gyertyán, Zsolt Szombathelyi, and Csaba Szántay[†]

Gedeon Richter Ltd., Budapest 10, POB 27, H-1475, Hungary, and Budapest University of Technology and Economics, Gellért tér 4,H-1521, Budapest, Hungary

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A series of *trans*-2′-hydroxyethyl and 2′-acyloxyethyl apovincaminates **4b**−**f** and **7b**−**f** has been synthesized and evaluated for their antioxidant and antiamnesic effects. The new esters were prepared from **4a** and **7a** ethyl esters or from the corresponding carboxylic acid sodium salt. For starting materials **11a**,**b**, a new stereoselective *trans*-reduction was elaborated. From the combined results of the data obtained from in vitro and in vivo tests and examination of the metabolism, (3*R*,16*S*)-2′-hydroxyethyl apovincaminate (**7b**, **RGH-10885**) was identified as the most promising compound, owing to its potent neuroprotective and antiamnesic activities. The in vivo effectiveness of selected compounds on the cognitive functions was studied in a one-trial passive avoidance task and a water-labyrinth test.

Introduction

Vinpocetine (**1**, *cis*-(3*S*,16*S*)-ethyl apovincaminate), a derivative of vincamine (**2**), a main alkaloid of Vinca minor L., has successfully been used in the treatment of CNS disorders of cerebrovascular origin for decades.[1] Cerebrovascular, antihypoxic, antiamnesic, and antiischemic effects have been identified as the main components of the action of **1**.[2] Compound **1** is also known to have cytoprotective activity in vitro.[2,3] The experimentally proved components of the neuroprotective effect of vinpocetine are the inhibition of voltage-dependent sodium channels, the inhibition of PDE1 enzyme, and the stabilization of intracellular Ca^{2+} homeostasis.[4,5] However, the mechanism by which vinpocetine exerts its protective and cognitive effects is complex and it has not been fully understood.

Clinical and experimental data suggest that free radicals, reactive oxygen species (ROS[a]), and lipid peroxidation (LPO) are implicated in the pathogenesis of a wide variety of human diseases such as brain ischemia, trauma, stroke, subarachnoid hemorrhage, mild cognitive impairment, and chronic neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease.[6,7] The central nervous system (CNS) is particularly sensitive to oxygen free radicals induced damage because of the high levels of polyunsaturated lipids, high rate of oxygen consumption, and relatively low concentration of antioxidant enzymes and natural antioxidants. The free radicals induced oxidative stress leads to LPO, which is a pathological factor in mild cognitive impairment (MCI) and in the progression to Alzheimer's disease from MCI.[8,9]

Compounds possessing antioxidant activity exert significant protective action in events of cerebral ischemia [transient ischemia attack (TIA), stroke], where learning and memory defects may occur in addition to neurological symptoms of various severity. Compound **1** is successfully used in the therapy of memory disturbances following cerebral ischemic states, and several experimental data have confirmed that **1** prevents the impairment of cognitive functions caused by various impairing agents in animal models, too.[2,10] It has been shown that **1** exerts antioxidant/LPO inhibitory activity.[11] In our own experiments, **1** possessed a potent cognitive enhancing effect with moderate LPO inhibitory activity that may be beneficial in the treatment of cognitive decline.[12]

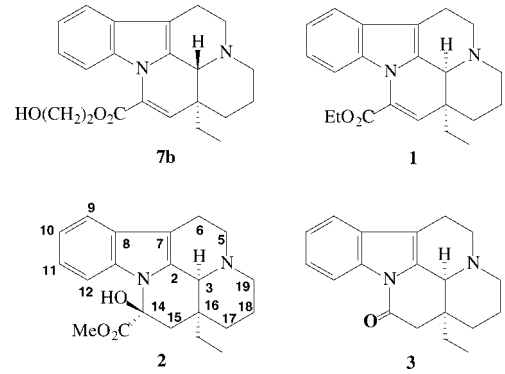

Figure 1. Structure of **7b**, Vinpocetine (**1**), Vincamine (**2**), and Vinburnine (**3**).

Earlier structure–activity relationship (SAR) studies showed the effects of different changes in the structure of the eburnane skeleton. The cerebrovascular activity is closely related to a specific configuration, namely, the natural *cis*-(3*S*,16*S*)-eburnane skeleton, which can be identified in **1** and **2**, as well as in (−)-eburnamonine (vinburnine, **3**),[13] the latter two compounds are also marketed as cerebrovasculars. (Figure 1). In contrast to *cis*-(3*S*,16*S*)-derivatives, *trans*-(3*S*,16*R*) ethyl ester (**4a**) and derivatives as (3*S*,16*R*)-dihydroeburnamenine-14-methanol, and its dinor-derivative (**5**) exhibit significant peripheral vasodilator effect.[14,15] Substitution of the carboxylic ester function with a dialkylamino-alkylaminomethyl chain on 14-C (**6**) increases the neuroprotective, LPO inhibiting activity, coupling with total loss of vascular and antihypoxic properties. The neuroprotective

* To whom correspondence should be addressed. Tel.: +36 1 431 4747. Fax: +36 1 432 6018. E-mail: a.nemes@richter.hu.

[†] Budapest University of Technology and Economics and Chemical Research Center, Hungarian Academy of Sciences, POB17, H-1525 Budapest.

[a]Abbreviations: AD, Alzheimer disease; BHT, 2,6-di-*tert*-butyl-4-methylphenol; LPO, lipid peroxidation; MCI, mild cognitive impairment; MDA, malondialdehyde; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SAR, structure−activity relationship; TBA, thiobarbituric acid; TBAR, thiobarbituric acid reactive substances; TIA, transient ischemia attack.

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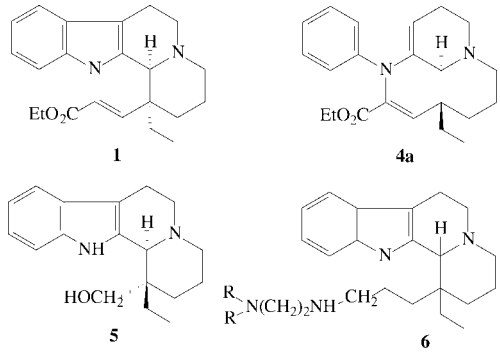


Figure 2. Structure–activity relationships.

effect is not configuration-dependent; *cis* and *trans*-compounds **6** showed comparable activities[16] (Figure 2).

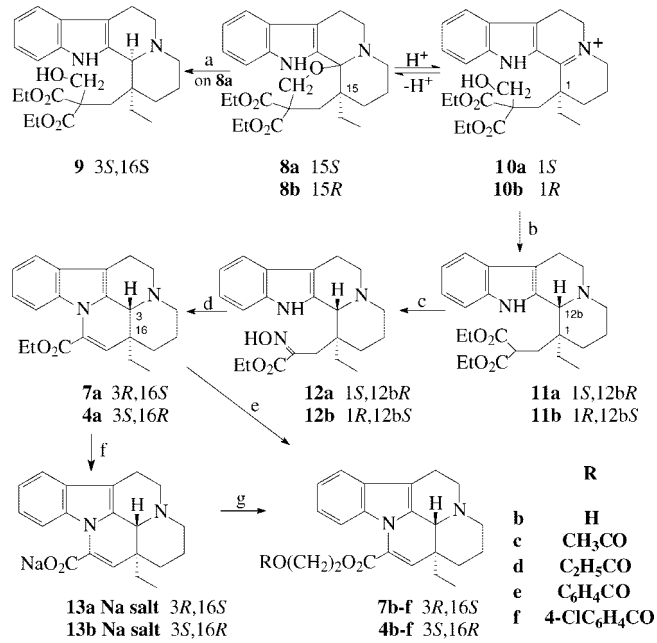
To continue these investigations, we describe herein the synthesis of a new series of substituted-alkyl esters of (3*S*,16*R*)- and (3*R*,16*S*)-*trans*-apovincaminic acids characterized with the general formulas **4** and **7**.[17] We hypothesized that the compounds with considerable LPO inhibitory efficacy may exhibit augmented cognition enhancing characteristics. The purpose of our work was to develop novel apovincaminate derivatives with antioxidant feature which can be used as drug candidates for the treatment and/or prevention of the neurodegenerative diseases. Preliminary in vitro assays of these molecules were performed in order to explore the structural requirements for efficient antioxidative activity. The antiamnesic effect of new compounds with trans configuration of eburnane skeleton was assessed using a one-trial passive avoidance test. Furthermore some selected *cis*- and *trans*-apovincaminates were tested in a complex, spatial learning paradigm: the water-labyrinth test. Some of the new compounds showed considerable antioxidant and antiamnesic activities. The most active compounds, **7b** and **7c**, were found among the (3*R*,16*S*)-*trans* series. The first tests concerning the metabolism demonstrated a quick metabolism of **7c** to **7b**. Hence, we selected compound **7b** (**RGH-10885**)[17] to investigate its in vivo efficacy in different cognitive tests.

Chemistry

The synthetic route followed to obtain the title compounds is summarized in Scheme 1. (15a*S*)-Diethyl ethyl-hexahydroindolo[2,3-*a*]pyrano[3,2-*i*]quinolizine-14,14-dicarboxylate (**8a**), previously reported as intermediate for the synthesis of **1** and **2**[18] was the starting material for compounds **7a**−**f**. Compound **8a** in the presence of a base as triethylamine exists as hexahydroindolopyranoquinolizine, catalytic reduction of which led stereoselectively to *cis*-octahydroindoloquinolizine **9**. In acidic medium, the pyrano-ring transforms to the open-chained form **10a** with addition of a proton.[18]

A modified stereoselectivity was achieved by the reduction of compound (15a*S*)-**8a** under mild acidic conditions with sodium tetrahydroborate. After treatment with ammonium hydroxide, *trans*-diethoxycarbonylethyl-octahydroindoloquinolizine **11a** was isolated. The transformation of indoloquinolizinyl diester (**11a**) into ethyl indoloquinolizinyl-pyruvate oxime (**12a**) was carried out without isolation of the intermediate monoester. Heating oxime **12a** in boiling toluene with *p*-toluenesulfonic acid furnished ethyl (3*R*,16*S*)-apovincaminate (**7a**).[14,18] Transesterification of compound **7a** to **7b**,[14] then acylation, or reaction of sodium *trans*-apovincaminate (**13a Na salt**) with a halo-alkyl reagent are the alternative methods to obtain compounds **7b**−**f**. Similarly, compounds **4b**−**f** were prepared from (15a*R*)-enantiomer **8b**. Finally, compounds **4b**−**f** and **7b**−**f** were isolated as mineral or organic acid salts (Table 1).

Scheme 1. Synthesis of Hydroxyethyl *trans*-Apovincaminate Derivatives[a]

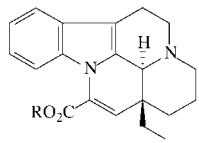


[a] Reagents and conditions: (a) Pd/C, Et_3N, DMF; (b) (i) $NaBH_4$, AcOH, EtOH; (ii) NH_4OH, 73%; (c) (i) KOH/H_2O, EtOH, 35 °C; (ii) $NaNO_2$/H_2O, AcOH, 15 °C; (iii) HCl/H_2O, 69%; (d) *p*-toluenesulfonic acid, toluene, reflux, 75%; (e) $HO(CH_2)_2OH$, KO*t*-Bu, 80 °C (R = H), 75%; (f) NaOH/EtOH, reflux, 96%; (g) $Cl(CH_2)_2OR$, DMF, 100 °C, 80–85%.

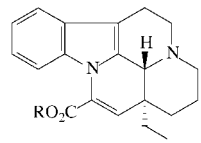
Results and Discussion

Biochemistry. Two in vitro methods, enzyme-catalyzed[19] lipid peroxidation (NADPH-induced LPO) and nonenzymic lipid peroxidation (Fe^{2+}-stimulated LPO)[20] in rat whole brain was used to study the antioxidant effect of the compounds. Different components of neuronal tissue were used as lipid substrates: plasma membrane (rat brain homogenate) and membrane from rat brain endoplasmic reticulum (microsomes). LPO, which is a chain reaction, was induced by a different mechanism, such as an enzymic (adding of NADPH in the presence of Fe^{3+}/ADP complex) and a nonenzymic way (by addition of Fe^{2+}). This method is based on the oxidation of polyunsaturated fatty acids (PUFAs) in biologic membranes, giving rise to a variety of lipid breakdown products such as malondialdehyde (MDA). By reacting MDA with thiobarbituric acid (TBA), a pink chromogene is formed, which can be detected by UV–vis spectrophotometry. The absorption of thiobarbituric acid reactive substances (TBARs) was measured spectrophotometrically at 532 nm.[6] On the basis of the concentration-effect correlations of the tested compounds, the IC_{50} values were determined. The results from both experiments are listed in Table 2. The cerebroprotective **1**, idebenone, DL-α-tocopherol, quercetin, and butylated hydroxytoluene or 2,6-di*tert*-butyl-4-methylphenol (BHT) were used as reference compounds. Idebenone, a benzoquinone derivative (coenzyme Q10 analog) with a potent free radical scavenger and antioxidant properties, has been used for the oral treatment of Alzheimer's type dementia.[21,22] DL-α-tocopherol is the best known isomer of natural antioxidant vitamin E.[23] Treatment with idebenone and α-tocopherol was also protective on Aβ-induced learning and memory deficits in rats.[24] The flavonol quercetin is an efficient LPO inhibitor and free radical scavenger.[24] Dialkyl phenol BHT has been used as

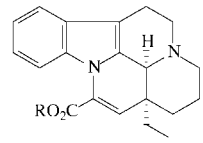
Table 1. Physical Properties of Apovincaminic Acids and Esters **4b**−**f**, **7b**−**f**, **13a**,**b**, and **14**



4b-f, 13b



7b-f, 13a



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cmpd	R	salt	procedure	yield (%)	mp (°C$^{solv.}$)	$[\alpha]_D$ (°solv., c)
4b	$HO(CH_2)_2$	HCl	A	85	235–237[b]	+123.0 (1)[a]
4c	$CH_3CO_2(CH_2)_2$	HCl	B	74	222–224[c]	+107.4 (1)[a]
4d	$C_2H_5CO_2(CH_2)_2$	HCl	C	75	226–228[e]	+107.3 (0.2)[a]
4e	$C_6H_5CO_2(CH_2)_2$	HCl	C	67	218–220[d]	+84.8 (0.2)[a]
4f	4-$ClC_6H_4CO_2(CH_2)_2$	CH_3SO_3H	C	77	200–202[e]	+73.3 (0.2)[a]
7b	$HO(CH_2)_2$	(+)-tartaric acid	A	82	157–159[b]	–72.1 (1)[f]
7c	$CH_3CO_2(CH_2)_2$	HCl	B	72	223–224[c]	–118.9 (0.2)[a]
7d	$C_2H_5CO_2(CH_2)_2$	HCl	C	69	219–220[e]	–111.9 (1)[a]
7e	$C_6H_5CO_2(CH_2)_2$	HCl	C	71	216–217[d]	–86.6 (1)[a]
7f	4-$ClC_6H_4CO_2(CH_2)_2$	CH_3SO_3H	C	74	197–199[e]	–73.3 (1)[a]
13a	H			94	201–202[g]	–133.0 (1)[f]
13b	H			92	200–201[g]	+130.8 (1)[f]
14	$HO(CH_2)_2$		A	67	170–171[b]	+172.5 (1)[f]

[a] Solvent: methanol. [b] Solvent: ethanol. [c] Solvent: 2-propanol. [d] Solvent: chlorobenzene. [e] Solvent: acetone. [f] Solvent: dimethylformamide. [g] Solvent: water.

Table 2. Inhibition of Enzymic Lipid Peroxidation (400 μM NADHP-Induced LPO in Rat Brain Microsomes) and Nonenzymic Lipid Peroxidation (200 μM Fe^{2+}-Induced LPO in Rat Brain Homogenate) by (3*S*,16*R*)-*trans*-Apovincaminic Esters **4a**−**f** and (3*R*,16*S*)-*trans*-Apovincaminic Esters **7a**−**f** and the Corresponding Acids **13a** and **13b** and Reference Drugs, such as Vinpocetine, Idebenone, DL-α-Tocopherol, Quercetine, and BHT[a]

cmpd No.	R	configuration	NADH-induced LPO IC_{50} (μM)	Fe^{2+}−induced LPO IC_{50} (μM)
13b	H	3*S*,16*R*	>>300	258 (237–278)
4a	C_2H_5	3*S*,16*R*	3.0 (1.6–4.4)	13.4 (11.8–15.0)
4b	$HO(CH_2)_2$	3*S*,16*R*	9.1 (6.1–12.1)	13.6 (12.0–15.2)
4c	$CH_3CO_2(CH_2)_2$	3*S*,16*R*	3.8 (2.3–5.3)	15.2 (11.9–18.6)
4d	$C_2H_5CO_2(CH_2)_2$	3*S*,16*R*	4.4	15.8 (14.3–17.2)
4e	$C_6H_5CO_2(CH_2)_2$	3*S*,16*R*	3.4	14.8 (10.3–19.3)
4f	4-$ClC_6H_4CO_2(CH_2)_2$	3*S*,16*R*	2.7	15.7
13[a]	H	3*R*,16*S*	>>300	238 (237–239)
7a	C_2H_5	3*R*,16*S*	3.5 (2.8–4.1)	14.4 (14.0–14.8)
7b	$HO(CH_2)_2$	3*R*,16*S*	7.0 (5.0–9.1)	13.7 (11.6–15.8)
7c	$CH_3CO_2(CH_2)_2$	3*R*,16*S*	3.6 (2.3–4.9)	9.8 (7.8–11.9)
7d	$C_2H_5CO_2(CH_2)_2$	3*R*,16*S*	5.0	21 (7.5–34.5)
7e	$C_6H_5CO_2(CH_2)_2$	3*R*,16*S*	3.8	18.2 (15.3–21.0)
7f	4-$ClC_6H_4CO_2(CH_2)_2$	3*R*,16*S*	2.7	14.6 (9.2–19.9)
1	C_2H_5	3*S*,16*S*	137 (75.2–199)	209 (157–261)
14	$HO(CH_2)_2$	3*S*,16*S*	>>30	N.D.
idebenone			1.3 (0.9–1.6)	31.5 (19–44)
DL-α-tocopherol			273 (148–399)	35.7 (10.7–60.7)
quercetin			8.5 (6.9–10.1)	10.7 (8.9–12.6)
BHT			3.2 (2.1–4.4)	6.2 (5.3–7.1)

[a] Values are taken from 1−8 experiments performed in triplicate, expressed as means of IC_{50} in μM, with the confidence intervals in parenthesis. Values without confidence intervals are for a single determination. N.D. = not determined.

a radical scavenging antioxidant in food industry for many years and as an inhibitor of LPO in experimental pharmacology.[25,26] Figures 3 and 4 indicate the effect of (3*S*,16*R*)-*trans*-esters **4b**,**c** and (3*R*,16*S*)-*trans*-esters **7b**,**c** and reference drugs such as vinpocetine and idebenone on NADPH (400 μM) evoked LPO in rat brain microsomes and Fe^{2+} (200 μM) evoked LPO in rat brain homogenates, respectively.

It was found that all new compounds with *trans*-apovincaminate moiety (**4b**−**f**, **7b**−**f**) were active inhibitors of LPO both in rat cerebral microsomes and in rat brain homogenate induced by NADPH or Fe^{2+}, respectively. Compounds decreased the formation of TBARs in a concentration-dependent manner. IC_{50} values of the compounds were in micromolar range (Table 2). The inhibition of microsomal LPO could be explained by the withdrawal of electron from NADPH cytochrome *c* reductase, which is the enzyme involved in the generation of free radical and in the initiation of LPO in the presence of NADPH and Fe^{3+}/ADP complex. The compounds can react with the initiation, propagation, and termination steps of LPO evoked by Fe^{2+} in rat brain homogenate.

Trans-apovincaminic acid esters (**4a**−**f**, **7a**−**f**) were more active in inhibition of LPO than esters with *cis*-configuration (**1, 14**), as shown by their much lower IC_{50} values. It is therefore assumed that interaction of *trans*-ring-fused apovincaminates with membrane lipids is stronger than that of *cis*-ring-fused esters, thus, they more potently protect the brain membrane against the free radical-evoked oxidative damages.

There was no difference between the inhibitory action of alkyl-esters and substituted alkyl-esters of (3*R*,16*S*)-apovincaminate (**7a**−**f**) and (3*S*,16*R*)-apovincaminate (**4a**−**f**) on LPO. The configuration of the ethyl substituent at the D/E ring junction of eburnane skeleton did not change the inhibitory effects of these compounds on LPO.

On the one hand, esterification of the 14-carboxylic group has a marked effect on LPO. On the other hand, the acylation, for example, esterification of the 2-hydroxyethyl group of *trans*-

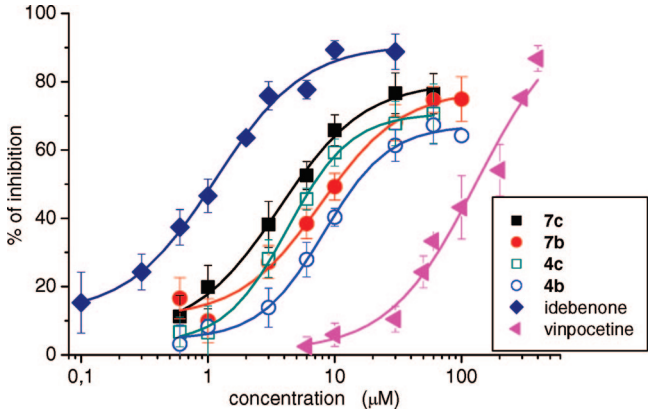

Figure 3. Effect of (3*S*,16*R*)-*trans*-apovincaminic esters **4b**,**c** and (3*R*,16*S*)-*trans*-apovincaminic esters **7b**,**c** and reference drugs vinpocetine and idebenone on NADPH (400 μM) evoked LPO in rat brain microsomes. Each point shown is the mean ± SEM of 3−8 separate experiments performed in triplicate, determined from 7−9 concentrations.

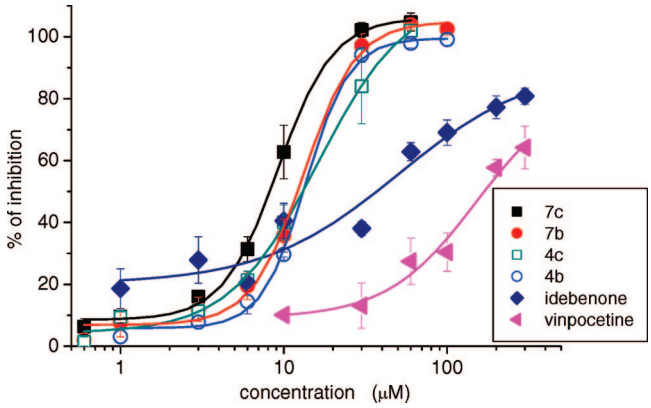

Figure 4. Effect of (3*S*,16*R*)-*trans*-apovincaminic esters **4b**,**c** and (3*R*,16*S*)-*trans*-apovincaminic esters **7b**,**c** and reference drugs vinpocetine and idebenone on Fe^{2+} (200 μM) evoked LPO in rat brain homogenates. Each point shown is the mean ± SEM of 3−8 independent experiments performed in triplicate, determined from 6−9 concentrations.

apovincaminate enantiomers with acetyl-, propionyl-, benzoyl-, and 4-chlorobenzoyl groups did not change the antioxidant efficacy of the compounds. Probably the esterification of the 14-carboxylic group enhances the lipophilicity of the compounds, which is involved in the increase of inhibitory potency of the compounds on LPO.

Compounds (**4a**−**f**, **7a**−**f**) were 2–5-fold more potent in inhibition of LPO evoked by NADPH and Fe^{3+}−ADP complex in microsomes than in Fe^{2+}-induced LPO in brain homogenate. Fe^{2+} stimulates membrane LPO more than Fe^{3+} does. It can be explained by the greater solubility of Fe^{2+} salts in solution, higher reactivity of alkoxy radicals, and the faster rate of decomposition of lipid peroxides by Fe^{2+}.

Inhibitory effect of these compounds on LPO was comparable to antioxidant effect of well-known neuroprotective idebenone and flavonoid quercetine. *trans*-Apovincaminic acid esters (**4a**−**f**, **7a**−**f**) were more active in inhibition of LPO than synthetic DL-α-tocopherol.

These results indicate that a series of 2′-hydroxyethyl and 2′-acyloxyethyl *trans*-apovincaminates possess potent antioxidant activity shown by their strong inhibitory action on LPO induced by different agents. Structure–activity analysis within a series of analogues indicated a high tolerance toward structural modifications in the ester side-chain (R substituents), whereas the change the *cis*-ring fusion to *trans* in the apovincaminic skeleton increased the antioxidant activity of the compounds.

Table 3. Percent Value of Inhibition of Diazepam-Induced Amnesia by (3*S*,16*R*)-*trans*-Apovincaminic Esters (**4b**−**e**) and (3*R*,16*S*)-*trans*-Apovincaminic Esters (**7b**−**f**) in the One-Trial Passive Avoidance Test in Mice

	inhibition of diazepam-induced amnesia (%)	
cmpd No.	0.1 mg/kg p.o.	10.0 mg/kg p.o.
4b	50	56
4c	72	44
4d	64	24
4e	23	16
7b	74	65
7c	79	100
7d	16	4
7e	0	0
7f	3	15
1 vinpocetine	0	100

Behavioral Tests for Screening In Vivo Drug Effect. The in vivo effectiveness of the various chemical structures on the learning memory functions was studied in experimental amnesia models using a passive avoidance test and a water-labyrinth learning task in mice and rats, respectively.

In the passive avoidance paradigm, as the first step in testing of antiamnesic activity of the compounds, vinpocetine at an oral dose of 10 mg/kg exerted a marked protective effect against anterograde amnesia induced by diazepam. However, some of *trans*-derivatives also proved to be active (Table 3). Two compounds (**7b** and **7c**) with *trans*-(3*R*,16*S*)-apovincaminate moiety showed the most considerable antiamnesic activity against diazepam-induced cognitive impairment at both doses tested (Table 3).

Their (3*S*,16*R*)-enantiomers, **4b** and **4c** (Table 1), seemed to be also effective. The memory improving effect of **7b** and **7c** compounds appeared already at the lower 0.1 mg/kg oral dose and was comparable to that of vinpocetine (*cis*-configuration) exerted at 10 mg/kg dose. Whereas compound **7c** showed a quick metabolism to **7b**, this latter structure has been chosen for further in vivo examination.

The water-labyrinth test is a classical maze paradigm making use of the motivation of the rats to escape from the water.[27] The method is appropriate for measuring the potential improving effect of test compounds on memory disturbance induced by diazepam, as an impairing agent. Some selected compounds were investigated in this complex learning task. 3*S*,16*S*)-Apovincaminic acid ethylester, **1**, its 3-epimer 3*R*,16*S*)-apovincaminic acid ethylester **7a**, 3*R*,16*S*)-apovincaminic acid hydroxyethylester, **7b**, the 3-epimer (3*S*,16*S*)-apovincaminic acid hydroxyethylester **14** were compared with the primary in vivo metabolites of ethylesters,[28] the epimeric *cis*-apovincaminic[28] (**15**) and *trans*-apovincaminic (**13a**) acids.

The control animals produced a progressive improvement in the water-labyrinth acquisition from day to day, while diazepam at a dose of 5 mg/kg ip disrupted the normal learning process, resulting in a significant increase in the number of directional turning errors.

Figure 5 presents the percent reversal of diazepam-induced amnesia exerted by various apovincaminic acid derivatives given at an oral dose of 5 mg/kg. In the group of the compounds studied (3*R*,16*S*)-apovincaminic acid ethylester **7a** produced the most moderate protective effect (just missed the 5% level of statistical significance) against learning deficit induced by diazepam (Figure 5), while (3*R*,16*S*)-apovincaminic acid hy-

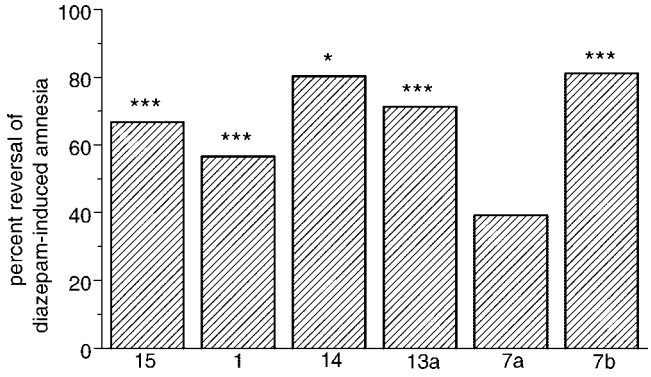

Figure 5. Percent restoring effect of vincaminic acid derivatives in diazepam-induced memory deficiency in the water-labyrinth test (calculated as described in Data Analysis). $*p < 0.05$, $***p < 0.001$, ANOVA followed by posthoc Duncan test was performed on group means pooled over days.

droxyethylester **7b** and (3*S*,16*S*)-apovincaminic acid hydroxyethylester **14** proved to be the most effective molecules with restoring effects of 81.1 and 80.3%, respectively. (3*S*,16*S*)-Apovincaminic acid ethylester **1** (vinpocetine) and the primary metabolites (**15** and **13a**) showed less but statistically significant memory improving activity against diazepam amnesia (their protective effect was measured to be 56.6, 66.7, and 71.3%, respectively).

It appears, that the alternation in the *cis–trans*-configuration of eburnane skeleton alone did not modify the memory improving property of the compounds, however, the hydroxyethylesters possess a higher biological activity in the water-labyrinth memory test. Putatively, the higher in vivo effectiveness of the hydroxyethylesters is due to their better metabolic stability.

Conclusions

In the present study, we showed that all new compounds with *trans*-apovincaminate moiety (**4b**−**f**, **7b**−**f**) were active inhibitors of LPO both in rat cerebral microsomes and in rat brain homogenate induced by enzymic (adding of NADPH in the presence of Fe^{3+}/ADP complex) and nonenzymic way (by addition of Fe^{2+}).

We have demonstrated by the present SAR study that probably both the change of the ring fusion to *trans* in the apovincaminate skeleton and the substitution of ester chain with 2′-hydroxyethyl and 2′-acyloxyethyl groups are responsible for the increasing inhibitory potency of the compounds on LPO.

Besides that they may protect the brain against the free radical-induced oxidative membrane damage, results obtained in behavioral studies suggest that some compounds of the series of 2′-hydroxyethyl and 2′-acyloxyethyl *trans*-apovincaminates have considerable cognitive enhancer activity as well. However, comparing data of LPO assay with memory results, there is not a close correlation between antioxidant property and cognitive improving activity of the drugs tested. Although differences in pharmacokinetic parameters and metabolism of various molecules studied might at least partly explain the discrepancy between in vitro and in vivo data, it appears more probable that the antioxidant property does not underlie the cognitive enhancing profile of the compounds. The considerable influence of **7b** on lipid peroxidation may be an adventageous additional effect for the treatment of oxidative stress-related neurodegenerative disorders and mild cognitive impairments.

Experimental Section

Chemistry. Melting points were determined with a Büchi 510 apparatus and are uncorrected. The $[\alpha]_D$ observed using a Perkin-Elmer 243B polarimeter. IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT-IR spectrometer using KBr pellets. ^{1}H NMR spectra were recorded on a Varian INOVA 500 spectrometer (500 MHz for ^{1}H). MS spectrometric measurements were performed on a Finnigan MAT 95SQ mass spectrometer using EI (70 eV, 220 °C source temperature) ionization method. High-resolution MS measurements were carried out on a Finnigan MAT 95XP mass spectrometer; perfluorotributylamine was the reference compound using EI ionization technique. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were ±0.4% of the theoretical values. All reagents and solvents were of commercial quality. Starting (15a*S*) and (15a*R*) diethyl 15a-ethyl-hexahydroindolo[2,3-*a*]pyrano[3,2-*i*]quinolizine-14,14-dicarboxylates (**8a**,**b**) were prepared according to published method.[18]

(1*S*,12b*R*)-1-(2′,2′-Diethoxycarbonylethyl)-1-ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*] quinolizine 11a. To a mixture of compound **8a** (50 g, 0.11 mol), ethanol (500 mL), and acetic acid (15 mL) sodium tetrahydroborate (9g, 0.197 mol) in ethanol (300 mL) was added at 20–25 °C for 1 h under nitrogen, then 25% NH_4OH solution (15 mL) was added and the mixture was stirred for 2 h at 50 °C. After completion of the reaction, water (270 mL) was added at 20 °C and stirred for 1 h at 5 °C. The crystals obtained were filtered and washed with water (2 × 200) and ethanol (40 mL) to give 34.8 g (73%) of **11a**, mp 99 °C (ethanol), $[\alpha]_D = +25°$ (*c* 1, DMF). ^{1}H NMR (DMSO-d_6, $\delta_{TMS} = 0.00$ ppm; data are given according to the eburnane skeletal numbering): 0.52 (3H, t, H_3-21); 1.16 (1H, td, H_{ax}-17); 1.21 (6H, t, OCH_2CH_3 × 2); 1.43 (1H, m, H_{eq}-18); 1.46 (1H, m, H_{eq}-17); 1.51 (1H, m, H_x-20); 1.59 (1H, m, H_y-20); 1.67 (1H, m, H_{ax}-18); 2.35 (1H, m, H_{ax}-19); 2.39 (1H, dd, H_x-15); 2.49 (1H, m, H_{ax}-5); 2.53 (1H, dd, H_y-15); 2.54 (1H, m, H_{eq}-6); 2.75 (1H, m, H_{ax}-6); 2.95 (2H, m, H_{eq}-19, H_{eq}-5); 3.31 (1H, s, H-3); 3.67 (1H, t, H-14); ∼4.19 (4H, m, (4H, OCH_2CH_3 x 2); 6.94 (1H, t, H-10); 7.02 (1H, t, H-11); 7.34 (1H, d, H-9); 7.35 (1H, d, H-12); 9.98 (1H, brs, NH). MS (EI) *m*/*z* (%): 426 (M^+, 13.7), 425 (10.2), 411 (1.2), 381 (5.5), 353 (1.8), 267 (100), 253 (1.5), 237 (4.7), 197 (3.8), 170 (4.2), 169 (4.5). HRMS calcd for $C_{25}H_{34}N_2O_4$, 426.25131; found, 426.25221 (delta: 2.1 ppm). Anal. ($C_{25}H_{34}N_2O_4$) C, H, N.

(1*R*,12b*S*)-1-(2′,2′-Diethoxycarbonylethyl)-1-ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine 11b. From **8b**, mp 98 °C (ethanol), $[\alpha]_D = -21°$ (*c* 1, DMF). Anal. ($C_{25}H_{34}N_2O_4$) C, H, N.

Ethyl (1*S*,12b*R*)-1-Ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-(2′-hydroxy-imino)-propionate 12a. To (1*S*,12b*R*)-**11a** (30.0 g, 70 mmol) in EtOH (140 mL), a solution of potassium hydroxide (3.84 g, 70 mmol) in water (48 mL) was added and stirred for 2 h under nitrogen at 30–35 °C. The pH was adjusted to 7 with AcOH (2 mL) and the solution was evaporated in vacuo. The residue was dissolved in AcOH (230 mL), and a portion of aqueous acetic acid (80 mL) was distilled. A solution of sodium nitrite (9.2 g, 133 mmol) in water (25 mL) was added at 10 °C and stirred for 2 h at 10–15 °C, and then a mixture of concd HCl (25 mL) and water (48 mL) was added to give 25 g (69.6% yield) of **12a HCl**, mp 233–236 °C, $[\alpha]_D = +23°$ (*c* 1, DMF). Aqueous NH_4OH 25% solution (43 mL) was added to give a solution. After 30 min of stirring, water (85 mL) was added and the mixture was stirred for 1 h, then filtered, and washed with 20% EtOH/water (2 × 20 mL) to yield 22 g (65%) of **12a**, mp 168–170 °C, $[\alpha]_D = +53°$ (*c* 1, DMF). ^{1}H NMR (DMSO-d_6, $\delta_{TMS} = 0.00$ ppm; data are given according to the eburnane skeletal numbering): 0.58 (3H, t, H_3-21); 1.23 (3H, t, OCH_2CH_3); 1.37 (1H, td, H_{ax}-17); 1.40 (1H, m, H_{eq}-18); 1.50 (1H, m, H_x-20); 1.51 (1H, m, H_{eq}-17); 1.57 (1H, m, H_y-20); 1.64 (1H, m, H_{ax}-18); 2.33 (1H, td, H_{ax}-19); 2.48 (1H, td,

H_{ax}-5); 2.55 (1H, m, H_{eq}-6); 2.75 (1H, m, H_{ax}-6); 2.94 (1H, m, H_{eq}-5); 2.95 (1H, m, H_{eq}-19); 3.02 (1H, d, H_x-15); 3.23 (1H, d, H_y-15); 3.33 (1H, s, H-3); 4.22 (2H, q, OCH_2CH_3); 6.94 (1H, t, H-10); 7.02 (1H, t, H-11); 7.35 (1H, d, H-9); 7.40 (1H, d, H-12); 10.20 (1H, s, NH); 12.42 (1H, s, OH). MS (EI) *m/z* (%): 383 (M^+, 53.5), 382 (29.9), 366 (52.8), 354 (11.5), 321 (11.7), 310 (13.5), 307 (10.6), 292 (100), 278 (8.8), 267 (30.8), 253 (34.6), 237 (41.2), 223 (8.3), 197 (23.4), 184 (13.5), 170 (25.4), 169 (26.8). HRMS: calcd for $C_{22}H_{29}N_3O_3$, 383.22034; found, 383.21994 (delta: −1.1 ppm). Anal. ($C_{22}H_{29}N_3O_3$) C, H, N.

Ethyl (1*R*,12b*S*)-1-Ethyl-1,2,3,4,6,7,12,12b-octahydroindolo[2,3-*a*]quinolizine-1-(2′-hydroxy-imino)-propionate 12b. From **11b**, mp 168–169 °C, $[\alpha]_D$ = –53° (*c* 1, DMF). Anal. ($C_{22}H_{29}N_3O_3$) C, H, N.

Ethyl (3*R*,16*S*)-Apovincaminate 7a. From a mixture of *p*-toluenesulfonic acid hydrate (25 g, 130 mmol) and toluene (360 mL), toluene (60 mL) was distilled. The solution was cooled to 80 °C and **12a** (20 g, 52 mmol) was added. The mixture was boiled under reflux for 4 h. After cooling to rt, water (165 mL) and 25% NH_4OH solution (16.5 mL) was added. The organic layer was separated, and the water was extracted with toluene (40 mL). The combined organic layers were washed with water (60 mL) and dried ($MgSO_4$), and the filtrate was clarified with Al_2O_3 (5 g) and Norit (1 g). The filtrate was evaporated and crystallized from ethanol–water 70:30 (120 mL). The separated crystals were filtered and washed with 70% aqueous ethanol (10 mL) to obtain 13.8 g (75%) of **7a**, mp 132–134 °C, $[\alpha]_D$ = –145° (*c* 1, $CHCl_3$), identical with an authentic sample.[14]

Ethyl (3*S*,16*R*)-Apovincaminate 4a. Compound **4a**[14] was obtained from **12b** under the same conditions.

(3*R*,16*S*)-Apovincaminic Acid 13a. A mixture of compound **7a** (5 g, 14 mmol), ethanol (30 mL), and sodium hydroxide (0.68 g, 17 mmol) was refluxed under nitrogen for 3 h. After completion of the hydrolysis, the solution was evaporated in vacuo. Acetone (20 mL) was added to the residue, and the mixture was refluxed for 10 min. The suspension was cooled to 10 °C, stirred for 1 h, and filtered to yield 4.6 g (95%) of **13a sodium salt**, mp 270 °C (dec.), $[\alpha]_D$ = −106° (*c* 1, CH_3OH). The crystals were dissolved in water (35 mL), and the solution was acidified with acetic acid 25% to pH 4 to afford **13a** (4.25 g, 94%), mp 201–202, $[\alpha]_D$ = –133° (*c* 1, DMF). **13a**, **13b**: ^{1}H NMR (DMSO-d_6, δ_{TMS} = 0.00 ppm): 0.58 (1H, m, H_x-20); 0.61 (3H, m, H_3-21); 1.44 (1H, td, H_{ax}-17); 1.57 (1H, d, H_{eq}-18); 1.79 (1H, m, H_{ax}-18); 1.81 (1H, m, H_y-20); 1.96 (1H, d, H_{eq}-17); 2.28 (1H, td, H_{ax}-19); 2.53 (1H, td, H_{ax}-5); 2.62 (1H, d, H_{eq}-6); 2.80 (1H, m, H_{ax}-6); 2.98 (1H, dd, H_{eq}-19); 3.01 (1H, s, H-3); 3.04 (1H, dd, H_{eq}-5); 6.28 (1H, s, H-15); 7.05 (1H, m, H-10); 7.07 (1H, m, H-11); 7.31 (1H, d, H-12); 7.41 (1H, d, H-9). MS (EI) *m/z* (%): 322 (M^+, 58.4), 321 (37.8), 307 (6.8), 294 (52.6), 293 (100), 292 (76.6), 277 (10.2), 262 (6.9), 252 (7.7), 249 (35.1), 248 (29.9), 219 (8.8), 123 (8.6). HRMS calcd for $C_{20}H_{22}N_2O_2$, 322.16758; found, 322.16783 (delta: 0.8 ppm).

(3*S*,16*R*)-Apovincaminic Acid 13b. From **4a**, mp 200–201, $[\alpha]_D$ = +130.8° (*c* 1, DMF).

2′-Hydroxyethyl (3*R*,16*S*)-Apovincaminate 7b. General Procedure for Compounds 4b, 7b, and 14 (R = Hydroxyethyl). Procedure A. Compound **7a** (15 g, 42 mmol) and KO*t*-Bu (1 g, 9 mmol) in ethylene glycol (300 mL) was heated for 4 h at 100 °C. The solution was cooled to rt, and water (300 mL) was added. The precipitated crystals were filtered and washed with water (3 × 200) to afford 14.1 g (91%) of **7b**: mp 87–88 °C, $[\alpha]_D$ = –95 ° (*c* 1, DMF).

7b Tartrate: The mixture of (+)-L-tartaric acid (9.65 g, 64 mmol) in water (10 mL) and ethanol (115 mL), and **7b** (23 g) in ethanol (190 mL) was stirred in 55 °C. The resulting crystals were filtered to obtain 30.7 g of **7b tartrate**: mp 157–159 °C, $[\alpha]_D$ = –72 ° (*c* = 1, DMF). ^{1}H NMR (DMSO-d_6, δ_{TMS}=0.00 ppm): 0.55–0.65 (4H, m, H_3-21+H_x-20); 1.45 (1H, td, H_{ax}-17); 1.58 (1H, d, H_{eq}-18); 1.80 (1H, m, H_{ax}-18); 1.84 (1H, m, H_y-20); 1.98 (1H, d, H_{eq}-17); 2.28 (1H, td, H_{ax}-19); 2.53 (1H, m, H_{ax}-5); 2.63 (1H, d, H_{eq}-6); 2.80 (1H, m, H_{ax}-6); 2.98 (1H, m, H_{eq}-19); 3.03 (1H, s, H-3); 3.05 (1H, m, H_{eq}-5); 3.70 (2H, m, OCH_2CH_2OH); 4.34 (2H, m, OCH_2CH_2OH); 6.39 (1H, s, H-15); 7.08 (1H, m, H-10); 7.09 (1H, m, H-11); 7.27 (1H, d, H-12); 7.42 (1H, d, H-9). MS (EI) *m/z* (%): 366 (M^+, 65.8), 365 (41.0), 351 (6.7), 337 (100), 293 (28.8), 292 (24.3), 277 (11.3), 262 (6.8), 249 (26.5), 248 (31.0). HRMS calcd for $C_{22}H_{26}N_2O_3$, 366.19379; found, 366.19351 (delta: −0.8 ppm).

2′-Hydroxyethyl (3*S*,16*R*)-Apovincaminate·HCl, 4b·HCl. See Table 1. ^{1}H NMR (DMSO-d_6, δ_{TMS} = 0.00 ppm): 0.71 (3H, t, H_3-21); 0.88 (1H, m, H_x-20); 1.73 (1H, td, H_{ax}-17); 1.91 (1H, d, H_{eq}-18); 2.02 (1H, m, H_{ax}-18); 2.10 (1H, d, H_{eq}-17); 2.13 (1H, m, H_y-20); 2.94 (1H, d, H_{eq}-6); 3.23 (1H, m, H_{ax}-19); 3.36 (1H, m, H_{ax}-6); 3.50 (2H, m, H_{ax}-5 + H_{eq}-19); 3.68 (1H, m, H_{eq}-5); 3.70 (2H, m, OCH_2CH_2OH); 4.36 (2H, m, OCH_2CH_2OH); 4.75 (1H, brs, H-3); 6.50 (1H, s, H-15); 7.16 (1H, m, H-10); 7.20 (1H, m, H-11); 7.36 (1H, d, H-12); 7.55 (1H, d, H-9); 10.65 (1H, brs, NH). MS (EI) *m/z* (%): 366 (M^+, 64.5), 365 (38.2), 351 (4.9), 337 (100), 293 (29.8), 292 (24.2), 277 (10.5), 262 (6.1), 249 (29.5), 248 (35.0). HRMS calcd for $C_{22}H_{26}N_2O_3$, 366.19379; found, 366.19337 (delta: −1.2 ppm). Anal. ($C_{22}H_{27}ClN_2O_3$) C, H, N.

2′-Hydroxyethyl (3*S*,16*S*)-Apovincaminate 14. See Table 1. ^{1}H NMR (DMSO-d_6, δ_{TMS} = 0.00 ppm): 0.81 (1H, td, H_{ax}-17); 0.95 (3H, t, H_3-21); 1.33 (1H, m, H_{eq}-18); 1.49 (1H, d, H_{eq}-17); 1.58 (1H, m, H_{ax}-18); 1.84 (2H, q, H_2-20); 2.42 (1H, m, H_{eq}-6); 2.43 (1H, m, H_{ax}-19); 2.51 (1H, m, H_{eq}-19); 2.92 (1H, m, H_{ax}-6); 3.13 (1H, td, H_{ax}-5); 3.21 (1H, dd, H_{eq}-5); 3.70 (2H, m, OCH_2CH_2OH); 4.07 (1H, s, H-3); 4.32 (1H, m) and 4.38 (1H, m), [OCH_2CH_2OH]; 4.77 (1H, t, OH); 6.18 (1H, s, H-15); 7.06 (1H, m, H-10); 7.09 (1H, m, H-11); 7.21 (1H, d, H-12); 7.43 (1H, d, H-9). MS (EI) *m/z* (%): 366 (M^+, 41.8), 337 (100), 296 (62.0), 293 (7.7), 277 (2.7), 252 (18.7), 249 (7.5). HRMS calcd for $C_{22}H_{26}N_2O_3$, 366.19379; found, 366.19445 (delta: 1.8 ppm). Anal. ($C_{22}H_{26}N_2O_3$) C, H, N.

2′-Acetyloxyethyl (3*R*,16*S*)-Apovincaminate 7c. General Procedure for Compounds 4c and 7c. Procedure B. To **13a** sodium salt (4 g, 11.6 mmol) in DMF (15 mL), 2-chloroethyl acetate (3g, 24mmol) was added. The mixture was stirred under nitrogen at 100 °C for 4 h. After cooling to rt, water (25 mL) was added, and the solutions was stirred for 1 h, filtered, and washed with water (3 × 5 mL) to obtain 3.6g (76%) of amorphous **7c**.

7c·HCl: Crude **7c** was dissolved in 2-propanol (30 mL) and acidified with HCl 24% in 2-propanol to pH 4 to yield **7c·HCl** (3.4 g, 65%), mp 188–189 °C, $[\alpha]_D$ = –118.9° (*c* 0.2, MeOH). ^{1}H NMR (DMSO-d_6, δ_{TMS} = 0.00 ppm): 0.70 (3H, t, H_3-21); 0.88 (1H, m, H_x-20); 1.73 (1H, td, H_{ax}-17); 1.93 (1H, d, H_{eq}-18); 2.05 (1H, m, H_{ax}-18); 2.11 (1H, d, H_{eq}-17); 2.12 (1H, m, H_y-20); 2.95 (1H, d, H_{eq}-6); 3.23 (1H, m, H_{ax}-19); 3.32 (1H, m, H_{ax}-6); 3.50 (2H, m, H_{ax}-5 + H_{eq}-19); 3.67 (1H, m, H_{eq}-5); 4.35 (2H, m, OCH_2CH_2OCO); 4.55 (2H, m, OCH_2CH_2OCO); 4.74 (1H, brs, H-3); 6.45 (1H, s, H-15); 7.17 (1H, m, H-10); 7.20 (1H, m, H-11); 7.36 (1H, d, H-12); 7.55 (1H, d, H-9); 10.62 (1H, brs, NH). MS (EI) *m/z* (%): 408 (M^+, 69.6), 407 (40.5), 393 (2.6), 380 (55.0), 379 (44.5), 366 (5.5), 349 (5.1), 337 (8.5), 321 (11.0), 307 (7.0), 293 (100), 292 (59.7), 277 (12.1), 248 (28.3). HRMS calcd for $C_{24}H_{28}N_2O_4$, 408.20436; found, 408.20407 (delta: −0.7 ppm). Anal. ($C_{24}H_{29}ClN_2O_4$) C, H, N.

2′-Acetyloxyethyl (3*S*,16*R*)-Apovincaminate HCl, 4c·HCl. See Table 1. Anal. ($C_{24}H_{29}ClN_2O_4$) C, H, N.

2′-Benzoyloxyethyl (3*R*,16*S*)-Apovincaminate 7e. General Procedure for Compounds 4d−f and 7d−f. Procedure C. To a solution of **7b** (4.44 g, 12 mmol) in chlorobenzene (60 mL), triethylamine (1.83 g, 18 mmol) and benzoylchloride (2.5 g, 18 mmol) were added. The mixture was stirred for 30 min at 40 °C, then $NaHCO_3$ 15% solution (15 mL) was added. The organic layer was washed with water (2 × 20 mL), dried, handled with HCl in dioxane, then chilled, filtered, and washed with acetone to obtain **7e·HCl** (4.8 g, 79%), mp 216–217 °C, $[\alpha]_D$ = –111.9° (*c* 1, MeOH). ^{1}H NMR (DMSO-d_6, δ_{TMS} = 0.00 ppm): 0.65 (3H, t, H_3-21); 0.85 (1H, m, H_x-20); 1.66 (1H, td, H_{ax}-17); 1.91 (1H, d, H_{eq}-18); 2.01 (1H, m, H_{ax}-18); 2.03 (1H, d, H_{eq}-17); 2.09 (1H, m, H_y-20); 2.94 (1H, d, H_{eq}-6); 3.21 (1H, m, H_{ax}-19); 3.33 (1H, m, H_{ax}-6); 3.48 (1H, m, H_{eq}-19); 3.49 (2H, m, H_{ax}-5); 3.68 (1H, m, H_{eq}-

5); 4.62 (2H, m, OCH_2CH_2OCO); 4.70 (2H, m, OCH_2CH_2OCO); 4.70 (1H, brs, H-3); 6.41 (1H, s, H-15); 7.06 (1H, m, H-11); 7.13 (1H, m, H-10); 7.34 (1H, d, H-12); 7.53 (1H, d, H-9); 7.54 (2H, m), 7.68 (1H, m); 7.95 (2H, m) [ArH]. MS (EI) *m/z* (%): 470 (M^+, 69.2), 469 (43.5), 455 (4.5), 442 (64.3), 441 (100), 400 (3.1), 349 (5.1), 321 (3.2), 293 (6.0), 277 (10.0), 261 (9.3), 248 (29.7), 149 (48.4), 105 (29.7), 77 (11.9). HRMS calcd for $C_{29}H_{30}N_2O_4$, 470.22001; found, 470.21962 (delta: −0.8 ppm). MS (EI) *m/z* (%): 366 (M^+, 64.5), 365 (38.2), 351 (4.9), 337 (100), 293 (29.8), 292 (24.2), 277 (10.5), 262 (6.1), 249 (29.5), 248 (35.0). Anal. ($C_{29}H_{31}ClN_2O_4$) C, H, N.

2′-Benzoyloxyethyl (3*S*,16*R*)-Apovincaminate HCl 4e·HCl. See Table 1. Anal. ($C_{29}H_{31}ClN_2O_4$) C, H, N.

2′-Propionyloxyethyl (3*S*,16*R*)-Apovincaminate HCl, 4d·HCl. See Table 1. MS (EI) *m/z* (%, same as **7d·HCl**): 422 (M^+, 71.3), 421 (42.3), 407 (2.7), 394 (55.4), 393 (44.3), 349 (5.3), 321 (12.1), 307 (6.2), 293 (100), 292 (57.4), 277 (10.7), 248 (26.2). HRMS calcd for $C_{25}H_{30}N_2O_4$, 422.22001; found, 422.22004 (delta: 0.1 ppm).

2′-Propionyloxyethyl (3*R*,16*S*)-Apovincaminate HCl, 7d·HCl. See Table 1) Anal. ($C_{25}H_{31}ClN_2O_4$) C, H, N.

2′-(4-Chlorobenzoyl)-oxyethyl (3*S*,16*R*)-Apovincaminate Methanesulfonate, 4f·CH_3SO_3H. See Table 1. MS (EI) *m/z* (%, same as **7f·CH_3SO_3H**): 504 (M^+, 61.5), 503 (41.8), 476 (81.8), 475 (100), 434 (3.7), 349 (5.6), 321 (4.1), 293 (8.5), 277 (11.8), 261 (10.6), 249 (38.6), 183 (38.6), 139 (29.7), 111 (8.9). HRMS calcd for $C_{29}H_{29}N_2O_4Cl$, 504.18104; found, 504.18049 (delta: −1.1 ppm).

2′-(4-Chlorobenzoyl)-oxyethyl (3*R*,16*S*)-Apovincaminate CH_3SO_3H, 7f·CH_3SO_3H. See Table 1. Anal. ($C_{30}H_{33}ClN_2O_7S$) C, H, N.

Pharmacology. In Vitro Assays. Experimental animals: male Hannover-Wistar rats (of our own breeding colony, Gedeon Richter) weighing 180–220 g were used.

1. Effect on the NADPH-Induced Lipid Peroxidation in Rat Brain Microsomes.[19] The whole rat brain was homogenized in a 10 vol (w/v) of ice-cold 0.25 M sucrose solution. The homogenate was centrifuged at 15000 g for 10 min at 4 °C. The supernatant was centrifuged at 78000 g for 60 min at 4 °C. The pellet, designated as microsomes, was suspended in 0.15 M KCI solution. The protein content was determined and then adjusted to 10 mg/mL concentration. The preparation was stored at −70 °C until use. The microsomes (0.2 mg protein) were incubated in 1.0 mL of medium (50 mM TRIS-HCI (pH 6.8)), 0.2 mM $FeCI_3$, 1 mM KH_2PO_4, and 0.5 mM ADP for 20 min at 37 °C with or without compounds. The LPO was induced by adding 0.4 mM NADPH. After 20 min, the reaction was stopped by adding 0.375 mL of a stopping solution containing trichloroacetic acid (TCA) of 40% and 5 M HCI in a 2:1 ratio. Acidified samples were mixed with 1 mL of 1% TBA solution and were placed in boiling water for 10 min. The samples were centrifuged at 2000 g for 10 min at 4 °C. Optical density was measured spectrophotometrically at 535 nm in a Hitachi 150-20 double beam spectrophotometer.

2. Effect on the Fe^{2+}-Induced Lipid Peroxidation in Rat Brain Homogenate.[20] The whole brain was homogenized in 9 vol of ice-cold Krebs−Ringer's buffer containing 15 mM HEPES, pH 7.4, 140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 0.7 mM $MgCl_2$, 1.4 mM KH_2PO_4, and 10 mM glucose. The rat brain homogenate (10 mg protein/ml) was used immediately. Cerebral homogenate (200 μL) was incubated at 37 °C for 20 min with or without compounds (added in a volume of 5 μL). The Fe^{2+}-induced LPO was induced by adding 5 μL of 8 mM $Fe_2(NH_4)_2(SO_4)_2$ solution. After 20 min, the reaction was stopped by addition of the stopping solution (12.5% TCA in 0.8 M HCl). Acified samples were centrifuged at 2000 g for 10 min at 4 °C. A total of 1 mL of 1% TBA solution was added to a 0.5 mL aliquot of the supernatant, and then the samples were placed in boiling water for 20 min. The optical density was determined at 535 nm with a Hitachi 150-20 spectrophotometer.

Data Analysis. The inhibitory effect of the compounds on LPO was determined using a minimum of six concentrations, and experiments were performed 1−8 times. Results are expressed as percent inhibition of TBARS formation (mean values of replicates were used for calculation) obtained in the presence of our compounds or well-known reference drugs. IC_{50} values (i.e., concentration of compound giving 50% inhibition of TBARS formation) were calculated from concentration−effect curves by sigmoid fitting using Origin 6.0 software (Microcal).

In Vivo Assays. Experimental animals: male Wistar rats (bred at Gedeon Richter Ltd.) and male NMRI mice (supplied by LATI Kft, Hungary) weighing 200–220 g and 25–28 g, respectively, were used in the behavioral studies. The animals, housed five per cage, were kept at 22 ± 1 °C, on a 12:12 h light/dark cycle (light on at 6:00 a.m.) in a laboratory animal care unit and commercial pellet rat-mouse feed and tap water were given ad lib.

All the procedures carried out on animals had been approved by the local ethical committee and conformed to the rules and principles of the 86/609/EEC directive.

1. Passive Avoidance Test in Mice. The passive avoidance apparatus consisted of an illuminated and a dark chamber (15 × 15 × 20 cm) separated by a guillotine door. During the habituation session, each mouse was placed in the lighted compartment and the time taken to enter the dark chamber was determined (step-through latency). The animals with higher latency than 30 s were excluded from the experiment. On the next day, the acquisition trial was performed. After entering the dark compartment, the door was closed and the mouse received a footshock (1 mA for 3 s) within 30 s. A total of 24 hours after the acquisition trial, a single retrieval trial was conducted and the step-through latency was measured (300 s was the upper cutoff time). For inducing anterograde amnesia, the animals were treated intraperitoneally with 3 mg/kg of diazepam 30 min before the acquisition trial. The test compounds were administered orally at 0.1 or 10 mg/kg doses, respectively, 1 h prior to the acquisition trial. All the experimental groups consisted of 10 animals. The percentage value of the protective effect (*P*%) was calculated by the following formula

$$P\% = \frac{T_{\text{treated+DIA}} - T_{\text{placebo+DIA}}}{T_{\text{placebo}} - T_{\text{placebo+DIA}}} \times 100 \qquad (1)$$

where *T* means the latency to enter the dark chamber in control (T_{placebo}), memory-impaired ($T_{\text{placebo+DIA}}$), and impaired, drug-treated ($T_{\text{treated+DIA}}$) groups.

2.Water-Labyrinth Test in Rats. In the water-labyrinth task, the rats had to maneuver through three choice points of a labyrinth system to reach a platform that allowed them to escape from the water. The water tank (1 m long, 60 cm wide, and 60 cm deep) was filled with water of 24 ± 2 °C to a depth of 30 cm and a removable labyrinth-system consisting of vertical metal plates was placed into the pool. The procedure for testing water-labyrinth acquisition process was carried out on 4 consecutive days. On day 1 (pretraining day), the rats were habituated to the test environment without plates and they were conditioned three times to swim in the tank from the start point to the platform. During the next days (days 2–4), the labyrinth system was in place and the animals were trained to swim through the labyrinth in three daily trials separated by approximately 25 min intertrial rest periods. The number of directional turning errors committed at all the three choice points was measured as a variable reflecting learning performance. If the rat did not find the platform within 5 min, it was assisted to the end of the labyrinth by the experimenter and the number of errors was recorded as 12.

The effect of compounds on the memory impairment elicited by diazepam was investigated at an oral dose of 5 mg/kg given 1 h prior to the first daily trial. Vinpocetine, *cis*-, and *trans*-apovincaminic acid were dissolved in 2% ascorbic acid solution, **7b** was dissolved in distilled water, while **7a** and **14** were dispersed in 5% (v/v) Tween 80. A dose of 5 mg/kg diazepam, suspended in 5% (v/v) Tween 80 solution, was injected intraperitoneally 30 min before the first swimming. Each study included a nonimpaired solvent control, a memory-impaired group, and an impaired group treated with the test compound. There were 10 animals in each experimental group. *trans*-Apovincaminic acid was examined in two separated experiments and data obtained were pooled.

Data Analysis. Results are given as percent reversal by the compound of the amnesia calculated from the group-means of pooled errors for all the trials in the training days using the following formula

$$\% = \frac{N_{\text{err}} \text{ of amnestic} - N_{\text{err}} \text{ of compound}}{N_{\text{err}} \text{ of amnestic} - N_{\text{err}} \text{ of control}} \times 100 \qquad (2)$$

where N_{err} means the number of errors.

Statistical comparisons between parameters of each group were made by ANOVA (two-way repeated measures analysis of variance) using "groups" as the independent between groups factor and "days" as the repeated measures factor. Posthoc comparisons (Duncan-test) were performed in case of a significant between-groups effect.

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Supporting Information Available: Table of elemental analyses, IR spectra, and NMR spectra for homologue compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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