A Novel Series of 2,6,7-Substituted 2,3-Dihydro-1,4-Benzodioxin and 2,6,7-Substituted 1,4-Benzodioxin Derivatives as Lipid Peroxidation Inhibitors. Structure−Activity Relationships for High Inhibition of Human Low-Density Lipoprotein Peroxidation

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A series of 6- or 7-substituted 2-carboxamido- or 2-(aminomethyl)-1,4-benzodioxin and -2,3-dihydro-1,4-benzodioxin derivatives were synthesized and evaluated to determine the necessary structural requirements for a high inhibition of human low-density lipoprotein copper-induced peroxidation. The most active compounds (**21**, **25**, **28**, **36**, and **37**) were found between 5 and >45 times more active than probucol itself. Due to both their potency and their structural features, compounds **25** and **36** were selected with others for complementary in vitro and in vivo investigations. Both of them exhibit calcium antagonist properties in the same range of potency as flunarizine itself. Compound **36** was also found to have significant hypolipaemic activity in mice at 100 and 300 mg/kg po, while compound **25** proved to be clearly active in a normobar hypoxia test.

Introduction

Too many people still suffer and die worldwide from coronary heart diseases such as atherosclerosis mainly due to hypercholesterolemia. In recent years, the pathogenesis of atherosclerosis lesions through oxidative modification of native low-density lipoproteins (LDLs) has been discussed.[1−5] In many cases heart disease and atherogenesis can be correlated with low levels of high-density lipoproteins (HDLs) and inversely elevated levels of LDLs. Oxidative modifications of the lipoproteins, and more particularly of LDLs (oxidized LDLs, ox-LDLs), appear to be one of the earliest phenomena in the development of atherosclerosis pathology. ox-LDLs are key components in endothelial injury.[6−10] Under pathological conditions (oxidative stress, free radicals) ox-LDLs are no longer recognized by the LDL receptor and are taken up in an unregulated manner by the subendothelial macrophages with a consequent accumulation of cholesterol esters and formation of foam cells. Not removed from the circulation, ox-LDL enhances the formation of damaged lesions on membrane lipids. Numerous available epidemiological, biochemical, and experimental data have led to the conclusion that antioxidant compounds able to protect lipids from peroxidation could be of value for the prevention and/or the treatment of atherosclerosis by attenuating foam-cell formation.[11−14] A number of natural or synthetic antioxidants (vitamin E, probucol; Figure 1) can lower coronary heart disease risk. Like vitamin E and probucol a lot of potent antioxidants have a structure based on hindered phenols or hydroxybenzopyrans.[15−17] This is the case, among others, for Trolox[18] and some of its analogues such as U-78517F[19,20] as well as some hydroxybenzylamine antioxidants[21] (Figure 2). Some of us have shown that 5-hydroxyindole-2-carboxamide derivatives[22] are potent inhibitors of human LDL peroxidation (Figure 3). 2,3-Dihydro-1,4-benzodioxin and 1,4-benzodioxin ring systems are present in a large number of structures of therapeutic agents possessing important biological activities.[23] Some of them are antagonists of α- or β-adrenergic receptors, giving them antihypertensive properties.[24−30] Others have affinities for serotonin receptors, which are involved in nervous breakdown and schizophrenia,[31−33] or represent an attractive thera-

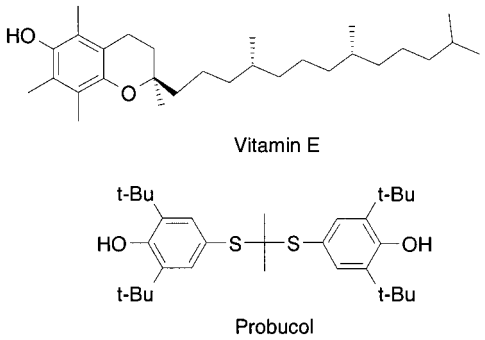

Figure 1. Natural and synthetic antioxidants.

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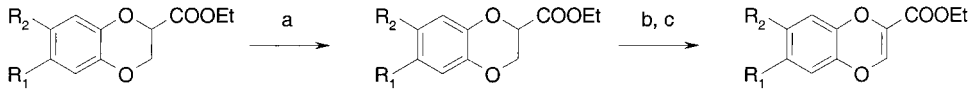
Scheme 1[a]



1-2 3-4 5-6

Compd	R_1	R_2	Compd	R_1	R_2	Yield, %	Compd	R_1	R_2	Yield, %
1	CH_3CO	H	**3**	CH_3COO	H	79	**5**	CH_3COO	H	97
2	H	CH_3CO	**4**	H	CH_3COO	78	**6**	H	CH_3COO	97

[a] Reagents and conditions: (a) *m*-CPBA, CH_2Cl_2, 40 °C; (b) NBS, AIBN, CCl_4 reflux; (c) NaI, acetone, 20 °C.

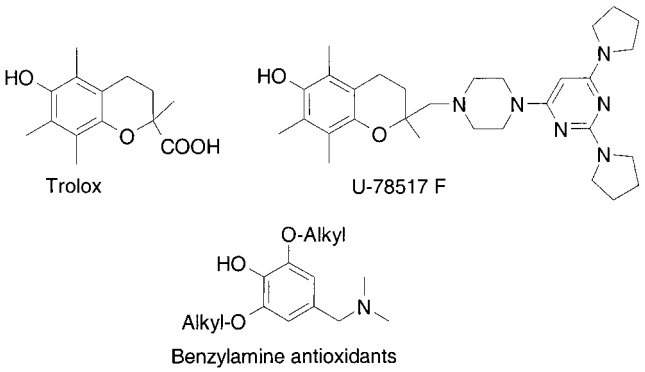


Figure 2. Antioxidants with a structure based on hydroxybenzopyran moieties or hindered phenol.

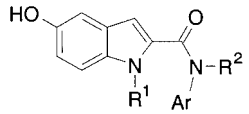


Figure 3. 5-Hydroxyindole-2-carboxamide derivatives.

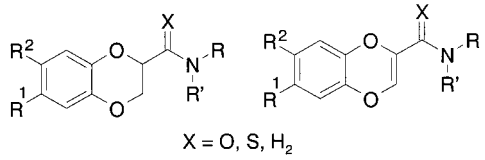
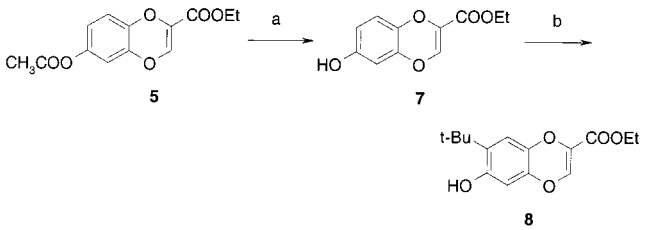


Figure 4. General formulas of new potent antioxidants.

peutic target for the treatment of glaucoma.[34] Recently, 2,3-dihydro-1,4-benzodioxins have been developed as inhibitors of 5-lipoxygenase, an enzyme involved in the oxygenation of arachidonic acid to the leukotrienes: they are also useful for the treatment of inflammatory diseases such as asthma and arthritis.[35] The occurrence of the 2,3-dihydro-1,4-benzodioxin structure in various naturally abundant compounds has also been reported.[36−41] As the hydroxybenzodioxin or hydroxydihydrobenzodioxin skeletons could be considered as bioisosters of hydroxychromane or hydroxyindole, we have described in this paper the synthesis and the pharmacological evaluation of new antioxidants having the general formulas given in Figure 4. A new series of 6- and 7-substituted 2-carboxamido- or 2-(aminomethyl)-1,4-benzodioxin and -2,3-dihydro-1,4-benzodioxin derivatives bearing at least a hydroxyl functionality have been developed.[42] The key synthetic precursors for the carboxamides are 2,3-dihydro-1,4-benzodioxin-2-carboxylic acid and 1,4-benzodioxin-2-carboxylic acid derivatives bearing a hydroxyl group on C6 or C7. We previously reported the regioselective functionalization on the aromatic ring at C6 and C7 by electrophilic

Scheme 2[a]



[a] Reagents and conditions: (a) C_2H_5ONa, C_2H_5OH, room temperature, 86%; (b) *t*-BuOH, TFA, room temperature, 84%.

substitution under typical Friedel−Crafts conditions.[43,44] On the other hand, experimental and clinical studies suggest that structurally disparate calcium channel blockers retard the progression of atherosclerosis. Calcium antagonists such as nifedipine, verapamil, and diltiazem exert antiperoxidative activity.[45−49] To counteract oxidative cardiovascular disease such as the development of atherosclerosis, our therapeutic approach suggests the use of mixed drugs.[50] The required amides were readily available via the condensation of 6- or 7-hydroxylated acids with amines possessing features that could potentially introduce calcium antagonist properties into the molecules.

Chemistry

The synthetic pathway to the carboxylic acids **11**−**17** is described by three schemes. Pathway 1 (Scheme 1) was used to prepare the derivatives **3**−**6** bearing an acetoxy group at the 6 or 7 position. The benzodioxans **3** and **4**, obtained by Baeyer−Villiger oxidation[51] of suitably acylated starting materials[43] **1** and **2**, are converted to benzodioxins **5** and **6** in high yields via bromination−elimination.[52] Pathway 2 (Scheme 2) was used to convert unsaturated ester **5** into hydroxylated analogue **7** by treatment with sodium ethoxide in ethanol. The *tert*-butylation of compound **7** under acid-catalyzed conditions in trifluoroacetic acid (TFA) using *tert*-butyl alcohol[53] afforded the corresponding product **8** in good yield (84%). Pathway 3 (Scheme 3) leads to the carboxylic acids **11**−**17**. Alkaline hydrolysis of compounds **3**−**6** and **8**−**10** at room temperature, followed by acid workup, provided the corresponding acids in high yields. Protected aminated carboxylic acid **16** was obtained under the same conditions from the analogous ester previously described.[44]

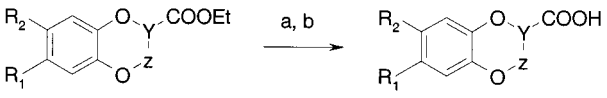
The carboxamides listed in Table 1 were synthesized by condensation of carboxylic acids **11**−**17** in *N*,*N*-dimethylformamide (DMF) with the appropriate amines **a**−**e** (Figure 5) in the presence of 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and

Table 1. 6- or 7-Substituted 2-Carboxamido- or 2-(Aminomethyl)-1,4-benzodioxins or -2,3-Dihydro-1,4-benzodioxins

compd	Y—Z	R^1	R^2	X	NRR′	yield, %	mp, °C	empirical formula	Anal.
18	CH—CH_2	HO	H	O	**a**	75	147	$C_{15}H_{13}NO_4$	C, H, N
19	CH—CH_2	H	HO	O	**a**	75	158	$C_{15}H_{13}NO_4$	C, H, N
20	CH—CH_2	HO	H	O	**b**	80	201	$C_{26}H_{24}N_2O_4F_2$	C, H, N
21	CH—CH_2	H	HO	O	**b**	73	136	$C_{26}H_{24}N_2O_4F_2$	C, H, N
22	CH—CH_2	H	HO	O	**c**	75	93	$C_{23}H_{28}N_2O_7$	C, H, N
23	C═CH	HO	H	O	**a**	72	210	$C_{15}H_{11}NO_4$	C, H, N
24	C═CH	H	HO	O	**a**	63	206	$C_{15}H_{11}NO_4$	C, H, N
25	C═CH	HO	H	O	**b**	85	163	$C_{26}H_{22}N_2O_4F_2$	C, H, N
26	C═CH	H	HO	O	**b**	85	221	$C_{26}H_{22}N_2O_4F_2$	C, H, N
27	C═CH	H	H	O	**b**	80	156	$C_{26}H_{22}N_2O_3F_2$	C, H, N
28	C═CH	HO	$(CH_3)_3C$	O	**b**	84	244	$C_{30}H_{30}N_2O_4F_2$	C, H, N
29	C═CH	NHBoc	H	O	**b**	78	220	$C_{31}H_{31}N_3O_5F_2$	C, H, N
30[a]	C═CH	NH_2	H	O	**b**	95	>240	$C_{26}H_{23}N_3O_3F_2$	C, H, N
31	C═CH	H	HO	O	**c**	73	88	$C_{23}H_{26}N_2O_7$	C, H, N
32	C═CH	HO	H	O	**d**	80	124	$C_{19}H_{19}NO_4$	C, H, N
33	C═CH	H	HO	O	**d**	83	156	$C_{19}H_{19}NO_4$	C, H, N
34	C═CH	HO	$(CH_3)_3C$	O	**d**	77	226	$C_{23}H_{27}NO_4$	C, H, N
35	C═CH	HO	H	O	**e**	80	166	$C_{27}H_{21}NO_4$	C, H, N
36	C═CH	HO	H	H_2	**b**	86	190	$C_{26}H_{24}N_2O_3F_2$	C, H, N
37	C═CH	HO	H	H_2	**e**	86	185	$C_{27}H_{23}NO_3F_2$	C, H, N

[a] Compound **30** was obtained by acidic hydrolysis of carbamate **29**.

Scheme 3[a]



Compd	Y-Z	R_1	R_2	Compd	Y-Z	R_1	R_2	Yield, %
3	CH-CH_2	CH_3COO	H	11	CH-CH_2	HO	H	89
4	CH-CH_2	H	CH_3COO	12	CH-CH_2	H	HO	88
5	C=CH	CH_3COO	H	13	C=CH	HO	H	87
6	C=CH	H	CH_3COO	14	C=CH	H	HO	88
8	C=CH	HO	$(CH_3)_3C$	15	C=CH	HO	$(CH_3)_3C$	86
9	C=CH	NHBoc	H	16	C=CH	NHBoc	H	95
10	C=CH	H	H	17	C=CH	H	H	88

[a] Reagents and conditions: (a) KOH, C_2H_5OH, 20 °C; (b) 2 N HCl.

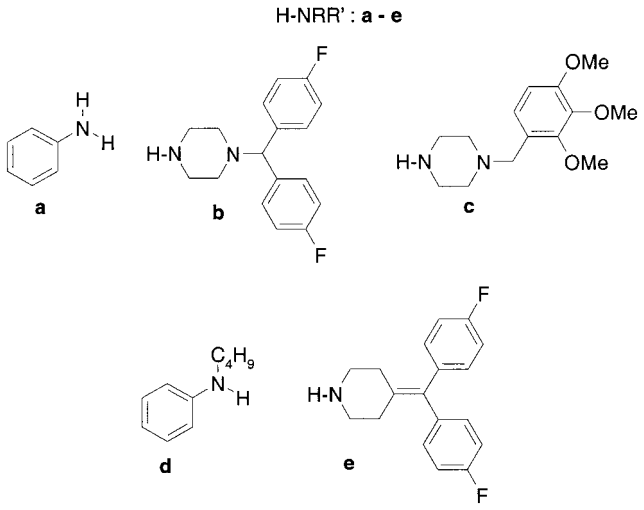
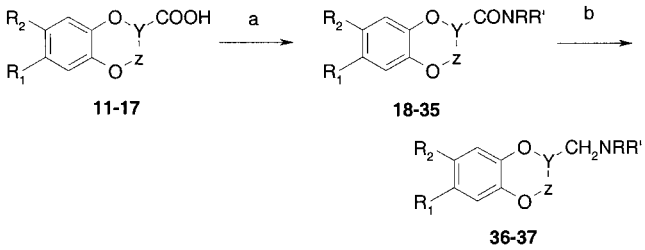


Figure 5. Amines to be condensed.

1-hydroxybenzotriazole[54] (HOBt) as shown in Scheme 4. Hydrolysis of the protected amino derivative **29** followed by a basic workup generated quantitatively the 6-aminocarboxamide **30**. Subsequent reduction of the amido group of compounds **25** and **35** with lithium aluminum hydride ($LiAlH_4$) provided high yields of the aminomethyl analogues **36** and **37**, respectively (Scheme 4). Compound **25** was structurally modified to enhance its antioxidant properties (Scheme 5). Compound **25** was exhaustively methylated to compound **38** in 83% yield by treatment with sodium hydride in DMF in the presence of methyl iodide. The thionation of carboxamide **25** using Lawesson's reagent[55] in boiling toluene gave a low yield (12%) of the thiocarboxamide **39**. Product **25** with nicotinoyl chloride hydrochloride in dichloroethane in the presence of pyridine gave the corresponding nicotine derivative **40** in high yield.

Scheme 4[a]



[a] Reagents and conditions: (a) HNRR′, EDCl, HOBt, DMF, 0 °C and then 20 °C; (b) $LiAlH_4$, THF reflux.

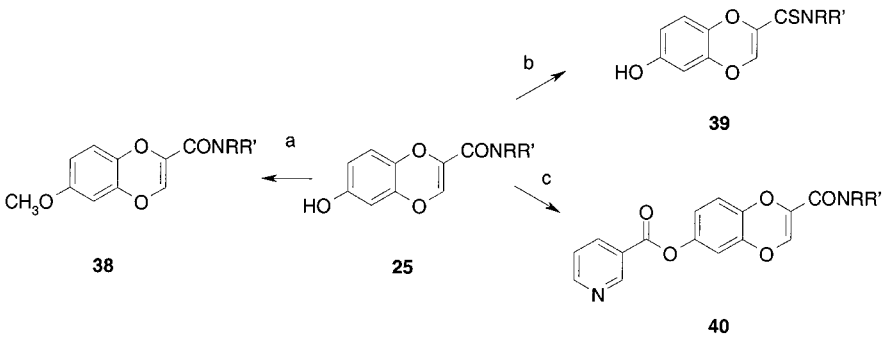
Results and Discussion

Twenty-two 2,3-dihydro-1,4-benzodioxin- and 1,4-benzodioxin-2-carboxamide derivatives were synthesized and evaluated. All were prescreened in vitro for their ability to protect human LDLs against copper-induced peroxidation.

Two tests were used for this preliminary evaluation.

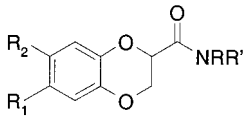
The first is based on an electrophoretic method (FPLC, fast protein liquid chromatography) that allows the separation of five different oxidized forms of LDLs.[56] Forms A and B correspond to the native forms of LDLs, and forms C, D, and E correspond to the different states of LDL oxidation (form E being the most oxidized). All the compounds were tested at a 10 μM concentration and evaluated for their ability to protect native human LDLs from copper (Cu^{2+})-induced oxidation. The results are expressed as the percentages of the LDLs corresponding to the different degrees of LDL oxidation, and

Scheme 5[a]



[a] NRR′ = **b**. Reagents and conditions: (a) NaH, CH_3I, DMF, room temperature, 83%; (b) Lawesson's reagent, toluene reflux, 12%; (c) pyridinylacyl chloride, DCE, pyridine, reflux, 93%.

Table 2. Inhibition of Copper-Induced Lipid Peroxidation by 2,3-Dihydro-1,4-benzodioxin Derivatives. Evaluation by FPLC Methodology or MDA Dosage



				FPLC[a]					MDA	
compd	R_1	R_2	NRR′	A	B	C	D	E	IC_{50} (M)	r[b]
18	HO	H	**a**	–	–	–	76	24	NT[c]	–
19	H	HO	**a**	–	–	26	68	6	NT[c]	–
20	HO	H	**b**	–	–	–	88	12	3×10^{-6}	1.7
21	H	HO	**b**	–	–	29	59	12	2×10^{-7}	10
22	H	HO	**c**	–	–	6	73	21	1.7×10^{-6}	3

[a] Control without Cu^{2+}, 100% B; control with Cu^{2+}, 75% D, 25% E. Compounds were tested on 10 μM. [b] Activity ratio versus probucol. [c] NT = not tested.

a protective effect is demonstrated by a displacement of the E form toward the D form, or even toward the C and B forms for the most powerful compounds.

The second test is based on a malondialdehyde (MDA) dosage, and the results are expressed as IC_{50} (concentration inhibiting 50% of the copper-induced lipid peroxidation) determined using the thiobarbituric acid reactive substance (TBARS) and expressed as an MDA equivalent.[57] To correct slight variations in response between the same evaluation procedures not performed with the same batch of human LDLs, results were also expressed as an activity ratio versus probucol (*r*).

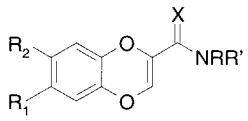
Results of these preliminary evaluations are reported in Tables 2 and 3.

In the first step we prepared anilide derivatives and investigated the influence of the hydroxyl group position with regard to the amide bond. Evaluation of compounds **18**, **19**, **23,** and **24** with the electrophoretic (FPLC) method showed that, in both benzodioxan and benzodioxin series, the best results were obtained for the 7-hydroxy-substituted compounds with a significant proportion of LDLs in the C form, especially for compounds **24** and **19** with around 20% of the LDLs in the C form.

In the second step we substituted the heterocycles with a "flunarizine-like" (fluorobenzhydryl)piperazine moiety to provide them with some calcium antagonist activity and consequently with additional cytoprotective potentialities.[21] These compounds were evaluated according to both FPLC and MDA methodologies.[56,57] Surprisingly, the structure−activity relationships concerning the 6- or 7-hydroxy-substituted compounds differ between the two series, 6-hydroxy-2-substituted benzodioxin **25** ($r > 45$) being more active in both tests than its 7-hydroxy-substituted counterpart **26** ($r = 2.9$), while in the benzodioxan series the best results are obtained in both tests with the 7-hydroxy-substituted **21** ($r = 10$). Compound **25** appeared to be a remarkably potent antioxidant compound with 95% B form observed in the FPLC test and a IC_{50} of 1×10^{-7} M in the MDA test (at least 45 times more potent than probucol itself). It is worth noting that carboxamide **25** is clearly more active than its benzodioxan analogue **20** ($r = 1.7$), which is only as active as probucol in the MDA test (the 7-hydroxy-substituted benzodioxane **21** being 10 times more active than probucol in the same test). The unsubstituted benzodioxane **27** appeared clearly less active in the FPLC test with 55% D form. As it appeared difficult to discriminate between the compounds with the FPLC method, all further compounds were exclusively evaluated with the MDA procedure. To determine structure−activity relationships, some chemical modulations were then performed with the very active compound **25** as the starting point by (1) introduction of a *tert*-butyl group on the aromatic ring, (2) modification of the hydroxyl group by substitution with functionalities exerting temporary interruption of cholesterol biosynthesis activities, (3) replacement of the hydroxyl group by an isostere amino group, (4) replacement of the N−C=O bond by a theoretically more metabolically labile $N-CH_2$, and (5) conversion of the N−C=O group to a N−C=S group.

Replacement of the hydroxyl function by a methoxy group, **38** ($r < 0.005$), or an amine group, **30** ($r = 0.1$), proved to be deleterious for the activity. Introduction of a *tert*-butyl group in the C-7 position, **28** ($r = 5$), or reduction of the amide to the aminomethylene group, **36** ($r = 5$), decreased, but did not suppress, the anti-

Table 3. Inhibition of Copper-Induced Lipid Peroxidation by 1,4-Benzodioxin Derivatives. Evaluation by FPLC Methodology or MDA Dosage



					FPLC[a]					MDA	
Compd	**R_1**	**R_2**	**NRR'**	**X**	**A**	**B**	**C**	**D**	**E**	**IC_{50} (M)**	**r[b]**
23	HO	H	**a**	O	-	-	-	74	26	NT[c]	-
24	H	HO	**a**	O	-	-	21	69	10	5×10^{-7}	4
25	HO	H	**b**	O	-	95	5	-	-	$<10^{-7}$	>45
26	H	HO	**b**	O	-	-	44	49	7	7×10^{-7}	2.9
27	H	H	**b**	O	-	-	-	55	45	NT[c]	-
28	HO	t-Bu	**b**	O	-	-	23	55	22	1×10^{-6}	5
30	NH_2	H	**b**	O	-	-	-	-	-	3.5×10^{-5}	0.1
36	HO	H	**b**	H_2	-	-	-	-	-	1×10^{-6}	5
38	MeO	H	**b**	O	-	-	-	60	40	$>10^{-4}$	<0.005
31	H	HO	**c**	O	-	-	-	-	-	3×10^{-6}	1.7
32	HO	H	**d**	O	-	-	-	-	-	1×10^{-6}	2
33	H	HO	**d**	O	-	-	-	-	-	4×10^{-7}	2
34	HO	t-Bu	**d**	O	-	-	-	-	-	2×10^{-7}	5
35	HO	H	**e**	O	-	-	-	-	-	5×10^{-7}	2
37	HO	H	**e**	H_2	-	-	-	-	-	$<10^{-7}$	>45
39	HO	H	**b**	S	-	-	-	-	-	9×10^{-6}	0.5
40		H	**b**	O	-	-	-	-	-	Prooxidant	-

[a] Control without Cu^{2+}, 100% B; control with Cu^{2+}, 75% D, 25% E. Compounds were tested on 10 μM. [b] Activity ratio versus probucol. [c] NT = not tested.

oxidant properties of these compounds, which were still 5 times more active than probucol. In contrast, replacement of the amide by a thioamide group resulted in a drastic (100-fold) decrease in activity (compound **39**, $r = 0.5$). Replacement of the benzhydrylpiperazine moiety by [bis(fluorophenyl)methylene]piperidine resulted in the moderately active compound **35** ($r = 2$), while additional reduction of the amide function surprisingly strongly enhanced the activity, the resulting compound **37** being at least 45 times more active than probucol ($r > 45$).

Amide formation with (2,3,4-trimethoxybenzyl)piperazine (compounds **22** ($r = 3$) and **31** ($r = 1.7$)) or *N*-butylaniline (**32** ($r = 2$), **33** ($r = 2$), and **34** ($r = 5$)) did not clearly modify the activity compared to that of the anilide derivative **24** (activity ratio between 1.7 and 5 compared to probucol). To provide our antioxidant compounds with additional hypolipaemic activity, we prepared analogue **40**, which can be considered as a prodrug of **25** and nicotinic acid. Unfortunately compound **40** was found prooxidant.

Table 4. Inhibition of KCl-Induced Rat Thoracic Aorta Contractions

compd	IC_{50} (μM)	compd	IC_{50} (μM)	compd	IC_{50} (μM)
20	3.0	**26**	5.0	**36**	0.57
21	2.2	**27**	1.8	flunarizine	0.27
25	0.56	**28**	3.1		

In conclusion to this preliminary evaluation, our chemical modifications led to the discovery of new compounds much more potent than probucol in their ability to prevent human LDLs from copper-induced peroxidation.

Complementary pharmacological evaluations were performed on some of them. Compounds **20**, **21**, **25**, **26**, **27**, **28**, and **36**, which are all substituted with a flunarizine-like (fluorobenzhydryl)piperazine moiety, were therefore evaluated for interactions with the calcium channel (Table 4).[58] All these compounds proved to inhibit the KCl-induced rat thoracic aorta contractions. Compounds **25** and **36** proved to be only 2 times less active than flunarizine (0.27 μM), with IC_{50} values

Table 5. Normobar Hypoxia Test[a]

compd	dose (mg/kg ip)	time (s) before the first gasps
untreated control		36 + 4
vincamine	2.5	inactive
vincamine	20	147 ± 14[b]
25	1	55 ± 12 (NS)
25	2.5	108 ± 29[b]
25	20	67 ± 19[a]
36	1	78 ± 11 (NS)
36	20	60 ± 10 (NS)
36	2.5	65 ± 11 (NS)

[a] NS = nonsignificant. [b] $p < 0.05$.

Table 6. Evaluation of Hypolipaemic Activity in Mice

compd	dose (mg/kg po)	change in the total cholesterol (%)	change in the LDL−VLDL fraction (%)
25	300	−7	0
36	300	−48	−52
36	100	−33	−35
bezafibrate	100	−25	−20

of, respectively, 0.56 and 0.57 μM. This could be of great interest since calcium channel blockers such as nifedipine, diltiazem, and verapamil have already been shown to protect cultured lymphoid cells against the toxicity of oxidized LDLs.

Due to their in vitro activity profile, compounds **25** and **36** were evaluated in vivo for their antihypoxic activity in mice in a "normobar hypoxia test".[59] Briefly summarized, the mice received the compound to be tested 30 min before being placed in an atmosphere poor in oxygen. The results are expressed as the time until the first signs of suffocation (or "gasps") occurred, and vincamine is used as the reference drug (Table 5).

While compound **36** proved to be inactive at all the tested doses, compound **25** proved to be significantly active at 2.5 and 20 mg/kg ip (vincamine active at 20 mg/kg ip, inactive at 2.5 mg/kg ip).

These two compounds were also investigated in mice for their potential hypolipaemic activity at 300 and 100 mg/kg po (Table 6).[60] Compound **36** proved to be more active than bezabifrate at 100 mg/kg po with a decrease of 33% in total cholesterol and 35% in the LDL−VLDL fraction compared to 25% and 20% for bezafibrate. Compound **25** was found inactive.

Conclusion

The chemical modifications performed on the benzodioxan and benzodioxin series led us to new compounds **25** and **36** which were very efficient in preventing human LDLs from copper-induced peroxidation. Among the numerous compounds more active than probucol, compounds were selected for complementary in vitro and in vivo investigations that showed additional properties of great interest for the treatment and for the prevention of atherosclerosis and oxidative injuries.

Experimental Section

Chemical Synthesis. General Procedure. Melting points were determined on a Köfler hot-stage apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AM 300WB spectrometer. The coupling constants are recorded in hertz (Hz), and the chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Infrared spectra were obtained with a Perkin-Elmer 196 infrared spectrometer, and the data are reported in inverse centimeters. Mass spectra were recorded on an R 10-10 C Nermag (70 eV) apparatus. Organic solvents were purified when necessary by methods described by D. D. Perrin, W. L. F. Armarengo, and D. R. Perrin (*Purification of Laboratory Chemicals*; Pergamon: Oxford, 1986) or purchased from Aldrich Chimie. All solutions were dried over anhydrous magnesium sulfate and evaporated on a Büchi rotatory evaporator. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F_{254}), and the spots were visualized with UV light or an alcohol solution of ammonium cerium(IV) nitrate. Column chromatography was performed with Kieselgel 60 (70−230 mesh) silica gel for gravity columns. Where analyses in the tables are indicated by the symbols of the elements, analytical results obtained for those elements were ±0.3% of the theoretical values. All anhydrous reactions were performed in oven-dried glassware under an atmosphere of argon. The column chromatography solvents employed were distilled, and solvent mixtures were reported as volume-to-volume ratios. Compounds **1**, **2**, **9**, **10,** and **17** were prepared according to procedures described by V. Thiéry et al.[43, 44]

Typical Procedure for the Preparation of Acetoxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic Acid Ethyl Esters 3 and 4 (Scheme 1). To a solution of 7.5 mmol of acetyl-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid ethyl ester **1** or **2** (2 g) in anhydrous dichloromethane (30 mL) was added 16.5 mmol of *m*-chloroperbenzoic acid (3.05 g). The stirred mixture was heated at 40 °C for 18 h. The mixture was allowed to cool, and the chlorobenzoic acid was filtered off. The extract was washed with brine and saturated aqueous $NaHCO_3$. The organic phases were dried over $MgSO_4$. The solvent was removed under vacuum. The residue was purified by column chromatography with AcOEt/petroleum ether (PE) as eluent.

6-Acetoxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid ethyl ester (3) was obtained with AcOEt/petroleum ether (PE) (30/70) as eluent in 79% yield. Mp: 87−88 °C. IR (KBr): 1755 (C═O), 1745 (C═O). ^{1}H NMR ($CDCl_3$): δ 6.97 (1H, d, H_8, J = 8.3 Hz), 6.60−6.00 (2H, m, H_8, H_5), 4.79 (1H, t, H_2,J = 3.5 Hz) 4.37 (2H, d, H_3, J = 3.5 Hz), 4.26 (2H, d, CH_2CH_3, J = 7.1 Hz), 2.22 (3H, s, CH_3COO), 1.27 (3H, t, CH_3CH_2, J = 7.2 Hz). MS: m/z 267 (M + 1). Anal. ($C_{13}H_{14}O_6$) C, H.

7-Acetoxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid ethyl ester (4) was obtained with AcOEt/petroleum ether (PE) (30/70) as eluent in 78% yield (oil). IR (film): 1745 (C═O), 1715 (C═O). ^{1}H NMR ($CDCl_3$): δ 6.84 (1H, d, H_5, J = 8.2 Hz), 6.78 (1H, d, H_8, J = 3.1 Hz), 6.60 (1H, dd, H_6, J = 8.2 Hz, J = 3.1 Hz), 4.83 (1H, t, H_2, J = 3.5 Hz), 4.0.40−4.37 (2H, m, H_3), 4.27 (2H, q, CH_2CH_3, J = 7.1 Hz), 2.28 (3H, s, CH_3COO), 1.30 (3H, t, CH_2CH_3, J = 7.1 Hz). MS: m/z 267 (M + 1). Anal. ($C_{13}H_{14}O_6$) C, H.

Typical Procedure for the Preparation of Acetoxy-1,4-benzodioxin-2-carboxylic Acid Ethyl Esters 5 and 6 (Scheme 1). To a solution of 11.2 mmol of acetoxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid ethyl ester **3** or **4** (3 g) in anhydrous carbon tetrachloride (60 mL) were added 22.5 mmol of *N*-bromosuccinimide (4.4 g) and a catalytic amount of AIBN. The resulting mixture was stirred and heated with a bulb lamp (75 W) at reflux for 3 h. The mixture was allowed to cool and the succinimide filtered off. The filtrate was evaporated to yield a solid sufficiently pure to be used directly in the next step of the reaction. A solution of 11.8 mmol of dibromo compound (5 g) in 60 mL of dry acetone was stirred at room temperature for 4 h with 41.3 mmol of sodium iodide (7.2 g). The acetone was removed from the green slurry under reduced pressure, and then water (50 mL), diethyl ether (90 mL), and a 1 N solution of sodium hyposulfite (40 mL) were added to the resulting brown residue. After extraction the dried organic layers were removed. The residue was purified by column chromatography with AcOEt/petroleum ether (PE) as eluent.

6-Acetoxy-1,4-benzodioxin-2-carboxylic acid ethyl ester (5) was obtained with AcOEt/petroleum ether (PE) (30/70) as eluent in 97% yield. Mp: 97−98 °C. IR (KBr): 1765

(C═O), 1730 (C═O). ^{1}H NMR ($CDCl_3$): δ 6.93 (1H, s, H_3), 6.82 (1H, d, H_8, J = 7.7 Hz), 6.62 (1H, dd, H_7, J = 7.7 Hz, J = 2.5 Hz), 6.51 (1H, d, H_5, J = 2.5 Hz), 4.29 (2H, q, CH_2CH_3, J = 7.2 Hz), 2.26 (3H, s, CH_3COO), 1.34 (3H, t, CH_3CH_2, J = 7.2 Hz). MS: m/z 265 (M + 1). Anal. ($C_{13}H_{12}O_6$) C, H.

7-Acetoxy-1,4-benzodioxin-2-carboxylic acid ethyl ester (6) was obtained with AcOEt/petroleum ether (PE) (30/70) as eluent in 97% yield (oil). IR (film): 1760 (C═O), 1730 (C═O). ^{1}H NMR ($CDCl_3$): δ 6.94 (1H, s, H_3), 7.00 (1H, d, H_8, J = 3.1 Hz), 6.69 (1H, d, H_5, J = 8.2 Hz), 6.60−6.56 (2H, m, H_6, H_8), 4.27 (2H, m, CH_2CH_3), 2.25 (3H, s, CH_3COO), 1.31 (3H, t, CH_2CH_3, J = 6.9 Hz). MS: m/z 265 (M + 1). Anal. ($C_{13}H_{12}O_6$) C, H.

6-Hydroxy-1,4-benzodioxin-2-carboxylic Acid Ethyl Ester (7). To a solution of 1 g of ester **5** (3.78 mmol) in 15 mL of ethanol was added 0.5 mL of sodium ethoxide, prepared by addition of sodium (0.46 g, 20 mmol) to 200 mL of anhydrous EtOH. The resulting solution was stirred at room temperature for 6 h, then acidified at pH 6 with resin Dowex 50X8-400, and filtered off. The filtrate was evaporated to dryness. The resulting material was purified by column chromatography with AcOEt/petroleum ether (PE) (30/70) as eluent.

7 was obtained in 86% yield. Mp: 187−188 °C. IR (KBr): 3560−3165 (OH), 1705 (C═O), 1670 (C═C). ^{1}H NMR ($CDCl_3$): δ 6.91 (1H, s, H_3), 6.69 (1H, d, H_8, J = 8.8 Hz), 6.33 (1H, dd, H_7, J = 8.8 Hz, J = 2.9 Hz), 6.26 (1H, d, H_5, J = 2.9 Hz), 4.50 (1H, s, OH), 4.27 (2H, q, CH_2CH_3, J = 7.3 Hz), 1.31 (3H, t, CH_2CH_3, J = 7.3 Hz). MS: m/z 223 (M + 1). Anal. ($C_{11}H_{10}O_5$) C, H.

6-Hydroxy-7-*tert*-butyl-1,4-benzodioxin-2-carboxylic Acid Ethyl Ester (8). To a solution of 2.2 mmol of ester **7** (1 g) in 5 mL of trifluoroacetic acid was slowly added 4.5 mmol of anhydrous *tert*-butyl alcohol (0.950 mL). The stirred solution was allowed to stand at room temperature for 24 h. The solvents were removed under vacuum, and ester **8** was isolated by column chromatography with AcOEt/petroleum ether (PE) (20/80) as eluent in 84% yield. Mp: 125−126 °C. IR (KBr): 3440−3215 (OH), 1705 (C═O). ^{1}H NMR (DMSO): δ 9.44 (1H, s, OH), 7.16 (1H, s, H_3), 6.55 (1H, s, H_8), 6.26 (1H, s, H_5), 4.18 (2H, q, CH_2CH_3, J = 7.3 Hz), 1.26 (9H, s, $(CH_3)_3C$), 1.22 (3H, t, CH_2CH_3, J = 7.3 Hz). MS: m/z 279 (M + 1). Anal. ($C_{15}H_{18}O_5$) C, H.

Typical Procedure for the Synthesis of Carboxylic Acids 11−16 (Scheme 3). A stirred solution of 4.5 mmol of esters **3**−**6** and **8**−**10** (2 g) in 20 mL of ethanol was treated with 9.0 mmol of 10% aqueous sodium hydroxyde. After 4 h at room temperature and evaporation under reduced pressure of ethanol, the aqueous phases were acidified with HCl (2 N). The combined extracts were dried over $MgSO_4$. Evaporation of the solvent yielded the crude acids, which were washed with dry dichloromethane.

6-Hydroxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid (11) was obtained in 89% yield. Mp: 165−166 °C. IR (KBr): 3650−2700 (OH), 1720 (C═O). ^{1}H NMR (DMSO): δ 13.00 (1H, br s, COOH), 9.50 (1H, s, OH), 6.69 (1H, d, H_8, J = 7.6 Hz), 6.27 (1H, dd, H_7, J = 7.6 Hz, J = 2.84 Hz), 6.23 (1H, d, H_5, J = 2.84 Hz), 4.88 (1H, t, H_2, J = 3.8 Hz), 4.27 (2H, d, H_3, J = 3.8 Hz). MS: m/z 197 (M + 1), 214 (M + 18). Anal. ($C_9H_8O_5$) C, H.

7-Hydroxy-2,3-dihydro-1,4-benzodioxin-2-carboxylic acid (12) was obtained in 88% yield. Mp: 205−206 °C. IR (KBr): 3650−2700 (OH), 1720 (C═O). ^{1}H NMR (DMSO): δ 13.20 (1H, br s, COOH), 9.00 (1H, s, OH), 6.61 (1H, d, H_5, J = 8.3 Hz), 6.30 (1H, d, H_8, J = 2.7 Hz), 6.21 (1H, dd, H_6, J = 8.3 Hz, J = 2.7 Hz), 4.94 (1H, t, H_2, J = 2.9 Hz), 4.3 (1H, dd, H_3, J = 11.4 Hz, J = 2.9 Hz), 4.13 (1H, dd, H_3, J = 2.9 Hz, J = 11.4 Hz). MS: m/z 197 (M + 1), 214 (M + 18). Anal. ($C_9H_8O_5$) C, H.

6-Hydroxy-1,4-benzodioxin-2-carboxylic acid (13) was obtained in 87% yield. Mp: 259−260 °C. IR (KBr): 3640−3010 (OH), 1670 (C═O). ^{1}H NMR (DMSO): δ 13.00 (1H, br s, COOH), 9.44 (1H, s, OH), 7.11 (1H, s, H_3), 6.66 (1H, d, H_8, J = 8.3 Hz), 6.33 (1H, dd, H_7, J = 8.3 Hz, J = 1.9 Hz), 6.24 (1H, d, H_5, J = 1.9 Hz). MS: m/z 195 (M + 1), 212 (M + 18). Anal. ($C_9H_6O_5$) C, H.

7-Hydroxy-1,4-benzodioxin-2-carboxylic acid (14) was obtained in 88% yield. Mp: 279−280 °C. IR (KBr): 3640−3010 (OH), 1670 (C═O). ^{1}H NMR (DMSO): δ 9.50 (1H, s, OH), 7.14 (1H, s, H_3), 6.65 (1H, d, H_5, J = 8.3 Hz), 6.27 (1H, dd, H_6, J = 2.9 Hz, J = 8.3 Hz), 6.21 (1H, d, H_8, J = 2,95 Hz). MS: m/z 195 (M + 1), 212 (M + 18). Anal. ($C_9H_6O_5$) C, H.

6-Hydroxy-7-*tert*-butyl-1,4-benzodioxin-2-carboxylic acid (15) was obtained in 86% yield. Mp: 129−130 °C. IR (KBr): 3600−2500 (OH), 1745(C═O). ^{1}H NMR (DMSO): δ 13.00 (1H, br s, COOH), 9.40 (1H, s, OH), 7.09 (1H, s, H_3), 6.55 (1H, s, H_8), 6.26 (1H, s, H_5), 1.28 (9H, s, $(CH_3)_3C$). MS: m/z 251 (M + 1). Anal. ($C_{13}H_{14}O_5$) C, H.

6-[*N*-(*tert*-Butoxycarbonyl)amino]-1,4-benzodioxin-2-carboxylic acid (16) was obtained in 95% yield. Mp: 228−230 °C. IR (KBr): 3345−2980, 2842−2095 (COOH, NH), 1676 (C═O). ^{1}H NMR (DMSO + D_2O): δ 7.17 (1H, s, H_3), 6.94 (1H, d, H_5, J = 2.3 Hz), 6.91 (1H, dd, H_7, J = 2.3 Hz, J = 8.7 Hz), 6.67 (1H, d, H_8, J = 8.7 Hz), 1.46 (9H, s, $(CH_3)_3C$). MS (IE, negative ions): m/z 292 (M − 1). Anal. ($C_{14}H_{15}NO_6$) C, H, N.

Typical Procedure for the Preparation of Amides 18−35 (Scheme 4). To a stirred solution of 5.0 mmol of carboxylic acids **11**−**17** (1 g) in anhydrous DMF (7 mL) were added at 0 °C 5.5 mmol (1.05 g) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 5.0 mmol (0.67 g) of hydroxybenzotriazole, and 5.5 mmol of amines **a**−**f**. The solution was stirred at room temperature for 18 h. After evaporation of the solvent, the residue was poured into water (50 mL) and extracted with ethyl acetate (2 × 50 mL). The extracts were dried over $MgSO_4$, and the solvent was removed under vacuum. The residue was purified by column chromatography with AcOEt/petroleum ether (PE) (30/70) as eluent.

6-Hydroxy-*N*-phenyl-2,3-dihydro-1,4-benzodioxin-2-carboxamide (18). With a similar procedure, **18** was prepared from acid **11** and amine **a** in 75% yield. Mp: 146−147 °C. IR (KBr): 3610−3100 (OH), 3370 (NH), 1665 (C═O). ^{1}H NMR (DMSO): δ 10.00 (1H, s, NH), 9.02 (1H, s, OH), 7.62 (2H, d, H_{arom}, H_{arom}, J = 7.6 Hz), 7.30 (2H, t, H_3, H_{arom}, J = 7.6 Hz), 7.07 (1H, t, H_{arom}, J = 7.1 Hz), 6.81 (1H, d, H_8, J = 8.3 Hz), 6.29−6.27 (2H, m, H_7, H_5) 4.81 (1H, dd, H_2, J = 2.7 Hz, J = 6.3 Hz), 4.78 (1H, dd, H_3, J = 2.7 Hz, J = 11.4 Hz), 4.50 (1H, dd, H_3, J = 11.4 Hz, J = 6.3 Hz). MS: m/z 272 (M + 1). Anal. ($C_{15}H_{13}NO_4$) C, H, N.

7-Hydroxy-*N*-phenyl-2,3-dihydro-1,4-benzodioxin-2-carboxamide (19). With a similar procedure, **19** was prepared from acid **12** and amine **a** in 75% yield. Mp: 157−158 °C. IR (KBr): 3500−3000 (OH, NH), 1635 (C═O). ^{1}H NMR ($CDCl_3$): δ 8.33 (1H, s, NH), 7.56 (2H, d, H_{arom}, J = 7.6 Hz), 7.35 (2H, t, H_{arom}, J = 7.6 Hz), 7.16 (1H, t, H_{arom}, J = 7.6 Hz), 6.79 (1H, d, H_5, J = 8.8 Hz), 6.57 (1H, d, H_8, J = 3.1 Hz), 6.41 (1H, dd, H_6, J = 8.8 Hz, J = 3.1 Hz), 5.10 (1H, s, OH), 4.79 (1H, d, H_2, J = 7.1 Hz, J = 3.1 Hz), 4.54 (1H, d, H_3, J = 11.9 Hz, J = 3.1 Hz), 4.24 (1H, dd, H_3, J = 11.9 Hz, J = 7.1 Hz). MS: m/z 272 (M + 1). Anal. ($C_{15}H_{13}NO_4$) C, H, N.

6-Hydroxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-2,3-dihydro-1,4-benzodioxin (20). With a similar procedure, **20** was prepared from acid **11** and amine **b** in 80% yield. Mp: 200−201 °C. IR (KBr): 3680−3000 (OH), 1630 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.40−7.30 (4H, m, 2 × H_{arom}, H_{arom}), 7.00 (4H, t d, H_{arom}, J = 8.7 Hz), 6.71 (1H, d, H_8, J = 8.7 Hz), 6.40 (1H, d, H_5, J = 3.1 Hz), 6.32 (1H, dd, H_7, J = 3.7 Hz, J = 8.7 Hz), 4.92 (1H, s, OH), 4.72 (1H, dd, H_2, J = 2.3 Hz, J = 7.9 Hz), 4.43 (1H, dd, H_3, J = 2.3 Hz, J = 11.8 Hz), 4.29 (1H, dd, H_3, J = 5.9 Hz, J = 11.8 Hz), 4.27 (1H, s, N*CH*Ar), 3.82−3.50 (4H, m, $H_{piperaz}$), 2.50−2.32 (4H, m, $H_{piperaz}$). MS: m/z 467 (M + 1). Anal. ($C_{26}H_{24}N_2O_4F_2$) C, H, N.

7-Hydroxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-2,3-dihydro-1,4-benzodioxin (21). With a similar procedure, **21** was prepared from acid **12** and amine **b** in 73% yield. Mp: 135−136 °C. IR (KBr): 3480−3300 (OH), 1640 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.43−7.31 (4H, m, H_{arom}), 7.04−6.98 (4H, m, H_{arom}), 6.73 (1H, d, H_5, J = 8.2 Hz), 6.43 (1H, d, H_8, J = 2.7 Hz), 6.34 (1H, dd, H_6, J = 2.7 Hz, J = 8.2 Hz), 5.2 (1H, br s, OH), 4.81 (1H, dd, H_2, J_b = 2.5 Hz, J = 3.05 Hz), 4.37

(1H, d, H3, J = 3.05 Hz, J_b = 11.3 Hz), 4.30−4.12 (2H, m, *CH*Ar, H_3), 3.80−3.50 (4H, m, $H_{piperaz}$), 2.53−2.29 (4H, m, $H_{piperaz}$). MS: *m*/*z* 467 (M + 1). Anal. ($C_{26}H_{24}N_2O_4F_2$) C, H, N.

7-Hydroxy-2-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]oxo}-2,3-dihydro-1,4-benzodioxin (22). With a similar procedure, **22** was prepared from acid **12** and amine **c** in 75% yield. Mp: 92−93 °C. ^{1}H NMR ($CDCl_3$): δ 6.97 (1H, d, H_{arom}, J = 8.5 Hz), 6.74 (1H, d, H_{arom}, J = 8.5 Hz), 6.64 (1H, d, H_5, J = 8,1 Hz), 6.44 (1H, d, H_8, J = 2.5 Hz), 6.33 (1H, dd, J = 2.5 Hz, J = 8,2 Hz), 4.82 (1H, dd, H_2, J = 2.1 Hz, J = 8.3 Hz), 4.40 (1H, dd, H3, J = 2.1 Hz, J = 11.4 Hz), 4.21 (1H, dd, H_3, J = 8.3 Hz, J = 11.4 Hz), 3.69−3.89 (9H, 3s, 3 × CH_3O), 3.78−3.52 (4H, m, $H_{piperaz}$), 3.54 (s, 2H, NCH_2Ar), 2.52−2.49 (4H, m, $H_{piperaz}$). MS: *m*/*z* 445 (M + 1). Anal. ($C_{23}H_{28}N_2O_7$) C, H, N.

6-Hydroxy-*N*-phenyl-1,4-benzodioxin-2-carboxamide (23). With a similar procedure, **23** was prepared from acid **13** and amine **a** in 72% yield. Mp: 209−210 °C. IR (KBr): 3610−3000 (OH), 3380 (NH), 1675 (C═O). ^{1}H NMR (DMSO): δ 9.50 (1H, s, OH), 7.68 (d, 2H, H_{arom}, J = 7.9 Hz), 7.32 (t, 2H, H_{arom}, J = 7.9 Hz), 7.09 (t, 1H, H_{arom}, J = 7.9 Hz), 7.07 (1H, s, H_3), 6.78 (1H, d, H_8, J = 8.7 Hz), 6.37 (1H, dd, H_7, J = 8.7 Hz, J = 3.1 Hz), 6.27 (1H, d, H_5, J = 3.1 Hz), 4.64 (1H, s, NH). MS: *m*/*z* 270 (M + 1). Anal. ($C_{15}H_{11}NO_4$) C, H, N.

7-Hydroxy-*N*-phenyl-1,4-benzodioxin-2-carboxamide (24). With a similar procedure, **24** was prepared from acid **14** and amine **a** in 63% yield. Mp: 205−206 °C. IR (KBr): 3500−2980 (OH), 3310 (NH), 1640 (C═O), 1220. ^{1}H NMR ($CDCl_3$): δ 7.83 (1H, s, NH), 7.59 (2H, d, H_{arom}, J = 7.9 Hz), 7.36 (2H, t, H_{arom}, J = 7.9 Hz), 7.16 (1H, t, H_{arom}, J = 7.9 Hz), 7.08 (1H, s, H_3) 6.64 (1H, d, H_5, J = 8.3 Hz), 6.38 (1H, d, H_8, J = 2.8 Hz), 6.36 (1H, dd, H_6, J = 2.8 Hz, J = 2.3 Hz), 4.95 (1H, s, OH). MS: *m*/*z* 270 (M + 1). Anal. ($C_{15}H_{11}N\ O_4$) C, H, N.

6-Hydroxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (25). With a similar procedure, **25** was prepared from acid **13** and amine **b** in 85% yield. Mp: 162−163 °C. IR (KBr): 3610−2980 (OH), 1665 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.34 (4H, dd, H_{arom}, J = 8.7 Hz, J = 5.5 Hz), 6.98 (4H, t, H_{arom}, J = 8.7 Hz), 6.57 (1H, s, H_3), 6.48 (1H, d, H_8, J = 8.7 Hz), 6.29 (1H, dd, H_7, J = 8.7 Hz, J = 3.1 Hz), 6.23 (1H, d, H_5, J = 3.1 Hz), 5.60 (1H, s, OH), 4.25 (1H, s, N*CH*Ar), 3.65−3.40 (4H, m, $H_{piperaz}$), 2.39−2.29 (4H, m, $H_{piperaz}$). MS: *m*/*z* 465 (M + 1). Anal. ($C_{26}H_{22}N_2O_4F_2$) C, H, N.

7-Hydroxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (26). With a similar procedure, **26** was prepared from acid **14** and amine **b** in 85% yield. Mp: 220−221 °C. IR (KBr): 3300 (OH), 1650 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.36 (4H, dd, 2 × H_{arom}, H_{arom}, J = 8.7 Hz, J = 5.6 Hz), 7.00 (4H, t, H_{arom}, J = 8.7 Hz, J = 8.7 Hz), 6.58−6.52 (2H, m, H_3, H_5), 6.29 (1H, dd, H_6, J = 8.1 Hz, J = 2.5 Hz), 6.23 (1H, d, H_8, J = 2.5 Hz), 5.40 (1H, br s, OH), 4.27 (1H, s, *CH*Ar), 3.69−3.63 (4H, m, $H_{piperaz}$), 2.45−2.39 (4H, m, $H_{piperaz}$). MS: *m*/*z* 465 (M + 1). Anal. ($C_{26}H_{22}N_2O_4F_2$) C, H, N.

2-{{4-[Bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (27). With a similar procedure, **27** was prepared from acid **17** and amine **b** in 90% yield. Mp: 156−158 °C. IR (KBr): 1670 (C═O). ^{1}H NMR (DMSO): δ 7.43 (4H, dd, 2 × H_{arom}, H_{arom}, J = 8.8 Hz, J = 5.2 Hz), 7.12 (4H, t, H_{arom}, J = 8.8 Hz), 6.92−6.78 (4H, m, H_{arom}), 6.74 (1H, s, H_3), 4.43 (1H, s, N*CH*Ar), 3.70−3.40 (4H, m, $H_{piperaz}$), 2.30−2.15 (4H, m, $H_{piperaz}$). MS: *m*/*z* 449 (M + 1). Anal. ($C_{26}H_{22}N_2O_3F_2$) C, H, N.

6-Hydroxy-7-*tert*-butyl-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (28). With a similar procedure, **28** was prepared from acid **15** and amine **b** in 84% yield. Mp: 242−244 °C. IR (KBr): 3600−3000 (OH), 1640 (C═O). ^{1}H NMR (DMSO): δ 9.36 (1H, s, OH), 7.43 (4H, dd, H_{arom}, J = 8.3 Hz, J = 5.8 Hz), 6.98 (4H, t, H_{arom}, J = 8.3 Hz), 6.64 (1H, s, H_3), 6.52 (1H, s, H_8 or H_5), 6.24 (1H, s, H_5 or H_8), 4.44 (1H, s, N*CH*Ar), 3.60−3.46 (4H, m, $H_{piperaz}$), 2.50−2.10 (4H, m, $H_{piperaz}$), 1.25 (9H, s, $(CH_3)_3C$). MS: *m*/*z* 521 (M + 1). Anal. for ($C_{30}H_{30}N_2O_4F_2$) C, H, N.

6-[*N*-(*tert*-Butoxycarbonyl)amino]-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (29). With a similar procedure, **29** was prepared from acid **16** and amine **b** in 78% yield. Mp: 219−220 °C. IR (KBr): 3574−3302−2715 (NH), 1718 (C═O), 1642 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.36 (4H, dd, H_{arom}, J = 8.70 Hz, J = 5.3 Hz), 6.98 (4H, t, H_{arom}, J = 8.7 Hz), 6.86 (1H, d, H_5, J = 2.4 Hz), 6.76 (1H, dd, H_7, J = 8.7 Hz, J = 2.4 Hz), 6.56 (1H, s, H_3), 6.55 (1H, d, H_8, J = 8.7 Hz), 6.29 (1H, s, NH), 4.26 (1H, s, CHAr), 3.57−3.53 (4H, m, $H_{piperaz}$), 2.42−2.38 (4H, m, $H_{piperaz}$), 1.50 (9H, s, $(CH_3)_3C$). MS: *m*/*z* 564 (M + 1). Anal. for ($C_{31}H_{31}N_3O_5F_2$) C, H, N.

6-Amino-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (30). Compound **30** was prepared by acidic hydrolysis of 1 mmol of amide **29** in the presence of 2.5 mL of HCl (3 N). After 6 h at room temperature, the aqueous phase was basified with $NaHCO_3$. The product was extracted with ethyl acetate. The organic layers were dried over magnesium sulfate and evaporated in vacuo. Compound **30** was obtained in 95% yield. IR (KBr): 3600−3420−3310−3100 (NH_2), 1635 (C═O). ^{1}H NMR ($CDCl_3$ + D_2O): δ 7.33 (4H, dd, H_{arom}, J = 8.8 Hz, J = 5.1 Hz), 6.97 (4H, t, H_{arom}, J = 8.8 Hz), 6.54 (1H, s, H_3), 6.43 (1H, d, H_8, J = 8.4 Hz), 6.12 (1H, dd, H_7, J = 2.9 Hz, J = 8.4 Hz), 6.05 (1H, d, H_5, J = 2.9 Hz), 3.67−3.58 (4H, m, $H_{piperaz}$), 2.46−2.38 (4H, m, $H_{piperaz}$). MS: *m*/*z* 464 (M + 1), 481 (M + 18). Anal. ($C_{26}H_{23}N_3O_3F_2$) C, H, N.

7-Hydroxy-2-{[4-(2,3,4-trimethoxybenzyl)piperazin-1-yl]oxo}-1,4-benzodioxin (31). With a similar procedure, **31** was prepared from acid **14** and amine **c** in 73% yield. Mp: 87−88 °C. IR (KBr): 3610−3300 (OH), 1600 (C═O), 1590 (C═C). ^{1}H NMR ($CDCl_3$): δ 6.97 (1H, d, J = 8.3 Hz), 6.63 (1H, d, H_{arom}, J = 8.3 Hz), 6.54 (1H, s, H_3), 6.53 (1H, d, H_5, J = 8.3 Hz), 6.31 (1H, dd, H_6, J = 2.1 Hz, J = 8.3 Hz), 6.25 (1H, d, H_8, J = 2.1 Hz), 3.87−3.89 (9H, 3s, 3 · CH_3O), 3.61 (4H, m, $H_{piperaz}$), 3.51 (s, 2H, N*CH*$_2$Ar), 2.53 (4H, m, $H_{piperaz}$). MS: *m*/*z* 443 (M + 1). Anal. ($C_{23}H_{26}N_2O_7$) C, H, N.

***N*-Butyl-6-hydroxy-*N*-phenyl-1,4-benzodioxin-2-carboxamide (32).** With a similar procedure, **32** was prepared from acid **13** and amine **d** in 80% yield. Mp: 123−124 °C. IR (KBr): 3610−2980 (OH, NH), 1660 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.40−7.18 (5H, m, 5H_{arom}), 6.62 (1H, s, H_3), 6.14 (1H, d, H_5, J = 2.9 Hz), 6.1 (1H, dd, H_7, J = 8.3 Hz, J = 2.9 Hz), 5.71 (1H, d, H_8, J = 8.3 Hz), 5.14 (1H, s, OH), 3.90−3.70 (2H, m, N*CH*$_2$), 1.56−1.47 (2H, m, CH_2), 1.50−1.20 (2H, m, CH_2), 0.61 (3H, t, CH_3, J = 7.3 Hz). MS: *m*/*z* 326 (M + 1). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

***N*-Butyl-7-hydroxy-*N*-phenyl-1,4-benzodioxin-2-carboxamide (33).** With a similar procedure, **33** was prepared from acid **14** and amine **d** in 83% yield. Mp: 155−156 °C. IR (KBr): 3610−2980 (OH, NH), 1660 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.43−7.17 (m, 5H_{arom}), 6.60 (1H, d, H_8, J = 2.9 Hz), 6.44 (1H, d, H_5, J = 8.7 Hz), 6.42 (1H, s, H_3), 6.2 (1H, dd, H_6, J = 2.9 Hz, J = 8.7 Hz), 5.56 (1H, s, OH), 3.76 (2H, t, N*CH*$_2$, J = 7.8 Hz), 1.70−1.48 (2H, m, CH_2), 1.43−1.26 (2H, m, CH_2), 0.89 (3H, t, CH_3, J = 7.31 Hz). MS: *m*/*z* 326 (M + 1). Anal. for ($C_{19}H_{19}NO_4$) C, H, N.

***N*-Butyl-6-hydroxy-7-*tert*-butyl-*N*-phenyl-1,4-benzodioxin-2-carboxamide (34).** With a similar procedure, **34** was prepared from acid **15** and amine **d** in 77% yield. Mp: 225−226 °C. IR (KBr): 3610−3010 (OH), 1640 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.39−7.190 (5H, m, $H_{arom\ aniline}$), 6.63 (1H, s, H_3), 6.04 (1H, s, H_8), 5.73 (1H, s, H_5), 5.04 (1H, s, OH), 3.75 (t, 2H, NCH_2, J = 7.6 Hz), 1.60−1.25 (4H, m, CH_2), 1.22 (9H, s, $(CH_3)_3C$), 0.87 (3H, t, CH_3, J = 7.1 Hz). MS: *m*/*z* 382 (M + 1). Anal. ($C_{23}H_{27}NO_4$) C, H, N.

6-Hydroxy-2-{{4-[bis(4-fluorophenyl)methylene]piperidin-1-yl}oxo}-1,4-benzodioxin (35). With a similar procedure, **35** was prepared from acid **13** and amine **e** in 80% yield. Mp: 165−166 °C. IR (KBr): 3610−3010 (OH), 1645 (C═O). ^{1}H NMR ($CDCl_3$): δ 7.11−6.95 (m, 8H, H_{arom}), 6.57 (1H, s, H_3), 6.54 (1H, d, H_8, J = 8.6 Hz), 6.31 (1H, dd, H_7, J = 2.6 Hz, J = 8.6 Hz), 6.25 (1H, d, H_5, J = 2.6 Hz), 5.13 (1H, s, OH), 3.69−3.59 (4H, m, $H_{piperid}$), 2.48−2.38 (4H, m, $H_{piperid}$). MS: *m*/*z* 462 (M + 1). Anal. ($C_{27}H_{21}NO_4F_2$) C, H, N.

Typical Procedure for the Synthesis of Aminomethyl Derivatives 36 and 37 (Scheme 4). To a stirred suspension of 1.5 mmol (0.05 g) of lithium aluminum hydride in 30 mL

of anhydrous THF was added 2 mmol of compound **25** or **35**. The mixtures were stirred and refluxed for 1 h, cooled, and decomposed by adding dropwise ice−water. The mixtures were filtered through Celite and evaporated. Pure materials **36** and **37** were obtained by purification by column chromatography with AcOEt/petroleum ether (PE) as eluent.

6-Hydroxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}methyl}-1,4-benzodioxin (36) was prepared from amide **25** in 86% yield and obtained with AcOEt/petroleum ether (PE) (20/80) as eluent. Mp: 189−190 °C. IR (KBr): 3610−2985 (OH), 1695 (C═C). ^{1}H NMR ($CDCl_3$): δ 9.00 (1H, s, OH), 7.34 (4H, dd, H_{arom}, J = 8.6 Hz, J = 5.5 Hz), 6.96 (4H, t, H_{arom}, J = 8.6 Hz), 6.48 (1H, d, H_8, J = 8.5 Hz), 6.23 (1H, dd, H_7, J = 8.5 Hz, J = 2.8 Hz), 6.14 (1H, d, J = 2.8 Hz), 5.79 (1H, s, H_3), 4.23 (1H, s, NCHAr), 2.89 (s, 2H, CH_2N), 2.63−2.36 (8H, m, $8H_{piperaz}$). MS: m/z 451 (M + 1). Anal. ($C_{26}H_{24}N_2O_3F_2$) C, H, N.

6-Hydroxy-2-{{4-[bis(4-fluorophenyl)methylene]piperidin-1-yl}methyl}-1,4-benzodioxin (37) was prepared from amide **35** in 86% yield and obtained with AcOEt/petroleum ether (PE) (20/80) as eluent in 80% yield. Mp: 185−186 °C. IR (KBr): 3605−3010 (OH), 1695 (C═C). ^{1}H NMR ($CDCl_3$): δ 7.09−6.92 (m, 8H, H_{arom}), 6.49 (1H, d, H_8, J = 8.8 Hz), 6.25 (1H, dd, H_7, J = 2.9 Hz, J = 8.8 Hz), 6.16 (1H, d, H_5, J = 2.9 Hz), 2.90 (s, 2H, NCH_2), 2.63−2.52 (4H, m, $H_{piperid}$), 2.48−2.36 (4H, m, $H_{piperid}$). MS: m/z 448 (M + 1). Anal. ($C_{27}H_{23}NO_3F_2$) C, H, N.

6-Methoxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}oxo}-1,4-benzodioxin (38). To a stirred suspension of 0.82 mmol of compound **25** (0.38 g) and 0.98 mmol of sodium hydride (60% dispersion in mineral oil, 0.04 g) in 10 mL of DMF was added dropwise 0.98 mmol of iodomethane (0.14 g). After 2 h at room temperature, the solvent was removed under reduced pressure, and the residue was hydrolyzed with water (15 mL) and extracted with ethyl acetate (2 × 15 mL). The organic layers dried over magnesium sulfate were evaporated in vacuo. The product **38** was obtained by purification by column chromatography with AcOEt/petroleum ether (PE) (50/50) as eluent in 83% yield. Mp: 144−145 °C. IR (KBr): 1660 (C═O), 1605 (C═C). ^{1}H NMR ($CDCl_3$): δ 7.34 (4H, dd, H_{arom}, J = 8.6 Hz, J = 5.4 Hz), 6.98 (4H, t, H_{arom}, J = 8.6 Hz), 6.56 (1H, d, H8, J = 8.8 Hz), 6.52 (1H, s, H_3), 6.36 (1H, dd, H_7, J = 8.8 Hz, J = 2.9 Hz), 6.28 (1H, d, H_5, J = 2.9 Hz), 4.25 (1H, s, N*CH*Ar), 3.70 (s, 3H, CH_3O), 3.72−3.66 (4H, m, $H_{piperaz}$), 2.50−2.30 (4H, m, $H_{piperaz}$). MS: m/z 479 (M + 1). Anal. ($C_{26}H_{22}N_2O_3F_2$) C, H, N.

6-Hydroxy-2-{{4-[bis(4-fluorophenyl)methyl]piperazin-1-yl}thioxo}-1,4-benzodioxin (39). A stirred solution of 1.1 mmol of product **25** (0.5 g) in 8 mL of anhydrous toluene was treated with 1.1 mmol of commercially avalaible Lawesson's reagent (0.4 g). The mixture was refluxed for 12 h. Then the solvent was evaporated to dryness and the resulting material purified by column chromatography with AcOEt/petroleum ether (PE) (30/70) as eluent to afford **39** in 12% yield. IR (KBr): 3600−2980−2600 (OH), 1280 (C═S). ^{1}H NMR ($CDCl_3$): δ 7.36 (4H, dd, H_{arom}, J = 8.6 Hz, J = 5.4 Hz), 7.00 (4H, t, H_{arom}, J = 8.6 Hz), 6.60 (1H, s, H_3), 6.48 (1H, d, H_8, J = 8.6 Hz), 6.30 (1H, dd, H_7, J = 2.9 Hz), 4.28 (1H, s, N*CH*Ar), 4.12−3.92 (4H, m, $H_{piperaz}$), 3.45 (1H, s, OH), 2.57−2.47 (4H, m, $H_{piperaz}$). MS: m/z 481 (M + 1). Anal. ($C_{26}H_{22}N_2O_3F_2S$) C, H, N.

6-(Nicotinoyloxy)-2-{{4-[bis(4-fluorophenyl)methyl]-piperazin-1-yl}oxo}-1,4-benzodioxin (40). To a solution of 1.37 mmol of compound **25** (0.635 g) in 25 mL of dry dichloroethane were added 4.5 mmol of nicotinoyl chloride hydrochloride (0.805 g) and 3.0 mmol of anhydrous pyridine (0.275 mL). The resulting mixture was refluxed for 8 h. After cooling, the organic layer was washed with a saturated solution of $NaHCO_3$, then dried over magnesium sulfate, and evaporated in vacuo. The residue was purified by column chromatography with AcOEt/petroleum ether (PE) (30/70) as eluent to give **40** in 93% yield. Mp: 141−142 °C. IR (KBr): 2950−2740 (CH_3, CH_2), 1740 (C═O), 1675 (C═O). ^{1}H NMR ($CDCl_3$): δ 9.32 (1H, d, H_{pyrid}, J = 1.9 Hz), 8.94 (1H, dd, H_{pyrid}, J = 2.0 Hz, J = 5.0 Hz), 8.39 (1H, ddd, H_{pyrid}, J = 8.0 Hz, J = 2.0 Hz), 7.45 (1H, dd, H_{pyrid}, J = 5.0 Hz, J = 8.0 Hz), 7.35 (4H, dd, H_{arom}, J = 8.5 Hz, J = 5.3 Hz), 6.99 (4H, t, H_{arom}, J = J = 8.5 Hz), 6.73 (1H, dd, H_7, J = 2.4 Hz, J = 8.9 Hz), 6.69 (1H, d, H_8, J = 8.9 Hz), 6.63 (1H, d, H_5, J = 2.4 Hz), 6.58 (1H, s, H_3), 4.26 (1H, s, N*CH*Ar), 3.70−3.50 (4H, m, $H_{piperaz}$); 2.60−2.30 (4H, m, $H_{piperaz}$). MS: m/z 570 (M + 1). Anal. ($C_{32}H_{25}N_3O_5F_2$) C, H, N.

Pharmacology. Antioxidant Properties: Electrophoretic Method (FPLC). The capacity of the compounds to decrease the proportions of oxidized LDLs was measured by incubating a preparation comprising native human LDLs, Cu^{2+} free radical generator, and the compounds to be tested for 24 h. The oxidation products were determined by FPLC in accordance with the method described by Vedie.[56] By this method it was possible to identify five peaks corresponding to different degrees of LDL oxidation. Peaks A and B correspond to the native forms of LDLs, and peaks C, D, and E correspond to the different states of oxidation of LDLs (peak E corresponding to the most oxidized form). The results are expressed as a percentage of the LDLs corresponding to those different states of oxidation. A protective effect of a compound with respect to oxidation induced by copper was demonstrated by a displacement of the E form toward the D form, or even toward the C form or the B form for the most powerful compounds.

Antioxidant Properties (MDA Dosage). LDLs were isolated by ultracentrifugation ($1.019 < d < 1.063$) from the pooled plasma of healthy normolipidemic human subjects with EDTA (0.04%). The LDL oxidation was promoted by copper (5 μmol^{-1}, 3 h, T = 37 °C). The compounds in DMSO were incubated at 10 concentrations just before the beginning of the oxidation. For each compound, protection against lipid peroxidation formation was estimated by dosing the inhibition of thiobarbituric acid reactive substance at each concentration according to the method of Yagi.[57] For each compound 10 concentrations between 10^{-7} and 10^{-3} M were evaluated in duplicate. The variability between the duplicates was less than 5% in all cases. IC_{50} values were calculated using linear regression analysis.

Calcium Channel Blocking Activity. Isolated rat aorta was contracted by hyperpotassic solution (KCl, 60 mM).[58] The inhibition of the contraction was evaluated in the presence of increasing concentrations of compound (eight concentrations between 10^{-8} and 3×10^{-5} M). Each concentration was performed in duplicate. Two different preparations were used. The concentration-related curve allowed us to calculate the IC_{50} values using linear regression analysis. The flunarizine was used as a reference compound and inhibits with an IC_{50} of 2.7×10^{-7} M.

Antihypoxic Activity in Vivo. Male mice (Swiss CD1) weighing from 25 to 30 g were, before experiment, housed for 1 week under conditions customary for animals (20−22 °C, 55% humidity, light/darkness cycle 12/12, commercial feed and water as desired).[59] The mice were placed in a box (7 × 5 × 5 cm) in which an atmosphere poor in oxygen was created by passing through a stream of air (96% N_2, 4% O_2, 12 L/min). The time taken until the first signs of suffocation (or gasps) occurred was measured. The mice received a dose of the compounds to be tested by the intraperitoneal route 30 min before the hypoxia was induced. Vincamine was used as the reference drug.

Hypolipaemic Activity. Groups each comprising six mice were rendered hypercholesterolaemic by a diet that was high in cholesterol and in cholic acid for 7 days.[60] The compounds were administered po (100 mg/kg) on the sixth and seventh days (half the dose being administered on day 6 and the other half on day 7). The animals were then left without food and drink for one night. The reduction in the concentration of cholesterol in the serum compared with that of hypercholesterolaemic control mice was then evaluated, as was the reduction in the concentration of lipoproteins (corresponding to the LDL−VLDL fractions), after precipitation by the addition of heparin to the serum, in the same hypercholesterolaemic mice compared with the control animals.

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