Hypocholesterolemic and Hypolipidemic Activity of Some Novel Morpholine Derivatives with Antioxidant Activity

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In this investigation, we study the synthesis and the evaluation of antioxidant and hypocholesterolemic activity of a number of 2-biphenylyl morpholine derivatives, which are structurally similar to some substituted morpholines possessing antioxidant activity, as well as to hypocholesterolemic 3-biaryl-quinuclidines. The novel derivatives are found to inhibit the ferrous/ascorbate induced lipid peroxidation of microsomal membrane lipids, the most potent derivative, 2-(4-biphenyl)-4-methyl-octahydro-1,4-benzoxazin-2-ol (compound **7**), having an IC_{50} value of 250 μM. In addition, these compounds demonstrate hypocholesterolemic and hypolipidemic action. The most active compound (**7**) decreases total cholesterol, low density lipoprotein, and triglycerides in plasma of Triton WR-1339 induced hyperlipidemic rats by 54%, 51%, and 49%, respectively, at 28 μmol/kg (ip). The above results indicate that the new molecules may be proven useful as leads for the design of novel compounds as potentially antiatherogenic factors.

Introduction

Hypercholesterolemia appears to be a serious risk factor for atheromatosis. High levels of low-density lipoprotein (LDL) are recognized as the initiating event in atherogenesis. LDL can undergo extensive lipid peroxidation, resulting in the generation of modified LDL and the formation of atheromatic lesions. Consequently, antioxidants such as α-tocopherol or carotenoids have been found to reduce LDL oxidation.[1] Furthermore, compounds that lower LDL and/or triglyceride levels could be useful in the treatment of atheromatosis.[2,3] Considering the above, we found it interesting to design molecules that would combine antioxidant and hypocholesterolemic activity. We have already studied the synthesis of several morpholine derivatives and their action as potential inhibitors of lipid peroxidation.[4,5] Moreover, the hypocholesterolemic potency of some substituted quinuclidines has been reported.[6] The latter compounds possess structural and physicochemical similarities with the antioxidant morpholines.

In this study, we report the synthesis of novel 2-biphenylmorpholine derivatives and their antioxidant and hypocholesterolemic activities. The examined parameters are the inhibition of lipid peroxidation in vitro and their effect on plasma total cholesterol, LDL, and triglyceride levels. To investigate the effect of lipophilicity on lipid peroxidation, we introduced in our study the previously synthesized[7,8] morpholines **1** and **2** (Chart 1). The objectives are to gain information about the structural features which are important for the specific actions and to draw further conclusions concerning the potential use of antioxidants against atheromatosis.

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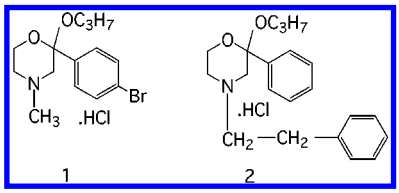
Chart 1



Results and Discussion

Chemistry. The synthesis of the compounds is shown in Scheme 1. The synthesis of the 2-hydroxy-2-(4-biphenyl)morpholines **6**−**8** involves the production of an intermediate hydroxyaminoketone, which undergoes ring closure to the cyclic hemiketal (average yield 80%). The derivatives **9**−**11** are formed from the hemiketals in an acid catalyzed ketal formation process (overall yield approximately 60%).

Structures are confirmed spectroscopically and by elemental analysis. There is no evidence (TLC, IR, UV) to suggest the presence of the keto alcohol tautomer of compounds **6**−**8**.

Lipid Peroxidation. The effect of different concentrations of the examined compounds on lipid peroxidation is given in Figure 1A−D. The observed inhibition of lipid peroxidation is time and concentration dependent. After 30 min of incubation, compounds **1** and **2** (1 mM) cause 70% and 40% inhibition, respectively, while compounds **6**−**11** (1 mM) can completely inhibit the peroxidation reaction. At 0.5 mM, compounds **6** and **10** inhibit lipid peroxidation by 96% and 78%. The most active antioxidant is compound **7**, having an IC_{50} of 250 μM after 30 min of incubation, while at the above concentration (250 μM) and under our experimental conditions, probucol inhibits lipid peroxidation about 16%.

Hemiketals **6**−**8**, although less lipophilic, are better inhibitors of lipid peroxidation than the corresponding

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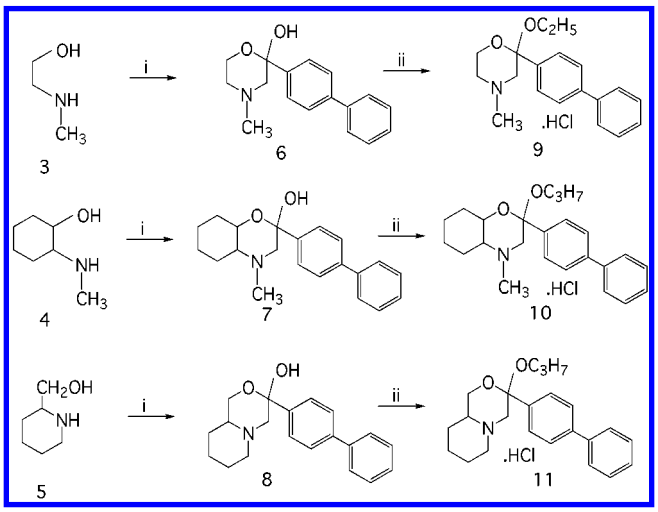
Scheme 1[a]



[a] Reagents and conditions: (i) 4-bromoacetylbiphenyl, acetone, room temperature, 15 h; (ii) gaseous HCl in Et_2O, EtOH, or *n*-PrOH, reflux, 12−15 h.

ketals **9**−**11**. However, it is known that the lipophilic character of antioxidant molecules is an important factor for protection against lipid peroxidation.[9]

Ketals **1**, **9**, and **2** have increasing clog*P* values[10] (3.98, 4.47, 5.06, respectively) and different substituents on the phenyl ring (Br, phenyl, H). It is found that the most lipophilic (**2**) is the weakest antioxidant. Furthermore, hemiketals **7** and **8**, with about the same clog*P* as ketal **9**, are significantly more potent inhibitors of lipid peroxidation than **9**. Therefore, it could be assumed that other structural characteristics of the studied compounds are more important than lipophilicity. We propose as a possible explanation of their antioxidant activity the formation of an intermediate radical generated during oxygen radical attack.

Plasma Levels of Cholesterol, LDL, and Triglycerides. The percent decrease of plasma total cholesterol, LDL, and triglyceride levels after the administration of the studied compounds and probucol to hyperlipidemic rats is demonstrated in Table 1. Compounds **6**−**11** significantly reduce cholesterol and triglyceride concentrations in plasma.

Compounds are administered by the same route to rats at a dose of 56 μmol/kg, twice in 24 h. All animals appeared normal macroscopically and by autopsy.

The most active of the examined compounds is **7**, which decreases cholesterol and triglyceride concentrations by ca. 50% at the lowest administered dose (28 μmol/kg, 10 mg/kg). Compounds **6**, **8**−**11** demonstrate no significant effect at the dose of 28 μmol/kg. Although the hemiketals **6**−**8** exert roughly the same effect on total cholesterol and triglyceride levels as the corresponding ketals **9**−**11**, derivatives **6**−**8** have a significantly higher effect on LDL levels than their ketal analogues **9**−**11**. This could be considered as an indication that the hydroxy derivatives may act selectively on LDL. It has been reported for a number of 3-biphenyl-3-hydroxyquinuclidines, compounds with structures similar to those of the present study, that the 3-hydroxyl is a critical structural element for their hypocholesterolemic action.[6]

Lipid peroxidation is particularly recognized to be a major causative factor in the pathogenesis of atherosclerosis.[11] Atheromatosis is connected with increased levels of cholesterol, especially LDL, as well as triglycerides. Since LDL constitutes the main cholesterol carrier of the plasma, it influences cholesterol plasma concentrations and is closely related to the aetiology of atherosclerosis. Oxygen derived radicals attack LDL, and this oxidatively modified LDL leads to the formation of foam cells and atheromatic plaques.

The synthesized derivatives can considerably inhibit lipid peroxidation. They also possess a significant hypocholesterolemic and hypolipidemic effect. Probucol, a lipophilic antioxidant agent used in the treatment of atheromatosis,[12] demonstrates a minor effect on cholesterol, triglycerides, and LDL, in our experimental protocols. Therefore, it could be concluded that the design of these molecules, combining hypolipidemic and antioxidant activities, could be proven useful in the development of new, potentially antiatherogenic agents. In addition, further work is underway to assess the inhibitory activity after per os administration of the synthesized compounds.

Experimental Section

Materials. All chemicals are of the highest commercially available purity. 2-Thiobarbituric acid and diagnostic kits for total cholesterol, LDL, and triglyceride determination are purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents are purchased from Aldrich-Chemie (Steinheim, Germany). Commercial 2-piperidinemethanol (Aldrich) corresponds to the racemate.

Synthesis. Melting points (mp) are obtained on a MEL-TEMPII (Laboratory Devices USA) apparatus and are uncorrected. Infrared (IR) spectra are recorded on a Perkin-Elmer 597 infrared spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra are obtained with a Bruker AW-80 MHz and a Bruker 400 MHz spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS), and signals are given as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analyses are performed with a Perkin-Elmer 2400 CHN analyzer.

General Procedure for the Preparation of 2-Hydroxymorpholines 6, 7, and 8 and Their Alcoxy Derivatives 9, 10, and 11. A solution of 4-bromoacetylbiphenyl (10 mmol) and 2-methylaminoethanol **3**, or 2-methylamino-cyclohexanol **4**, or 2-piperidinemethanol **5** (22 mmol) in acetone (150 mL) was maintained at room temperature for 15 h.[7] Then, acetone was evaporated in vacuo, ether was added to the residue, the mixture was washed with saturated sodium chloride solution and dried (K_2CO_3), and the product was isolated as a salt.

Conversion into alcoxy derivatives **9**, **10**, and **11** was carried out by refluxing in acidified ethanol or *n*-propanol during 12−15 h.

2-(4-Biphenyl)-4-methyl-morpholin-2-ol, Hydrochloride, 6. It is isolated as a white solid and recrystallized from acetone and ether. Yield 67%, mp 138−140 °C. IR 2450, 1600 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 2.8 (3H, s), 3.05−3.65 (5H, m), 4.0 (1H, m), 4.1 (1H, m), 7.3−7.6 (3H, m), 7.65−7.9 (6H, m). Analysis ($C_{17}H_{20}ClNO_2$) C, H, N.

2-(4-Biphenyl)-4-methyl-octahydro-1,4-benzoxazin-2-ol, Hydrochloride, 7. The product is isolated as a white solid and recrystallized from acetone and ether. Yield 75%, mp 161−163 °C. IR 3150, 2450, 1600 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.2−1.6 (5H, m), 1.7−1.9 (3H, m), 2.23 (1H, d, J = 10.6 Hz), 2.8 (3H, s), 3.12 (1H, t, J = 18.0 Hz), 3.30 (1H, d, J = 12.5 Hz), 3.60, (1H, d, J = 12.5 Hz), 4.05−4.3 (1H, m), 7.3−7.6 (3H, m), 7.65−7.9 (6H, m). Analysis ($C_{21}H_{26}ClNO_2$) C, H, N.

3-(4-Biphenyl)-octahydro-1,4-pyrido[2,1-*c*]oxazin-3-ol, Hydrochloride, 8. The product is isolated as a white solid, recrystallized from acetone and ether. Yield 78%, mp 175−177 °C. IR 3300, 2550, 1600 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 1.35−1.8 (6H, m), 2.8−3.0 (2H, m), 3.10 (1H, t, J = 11.2 Hz), 3.2−

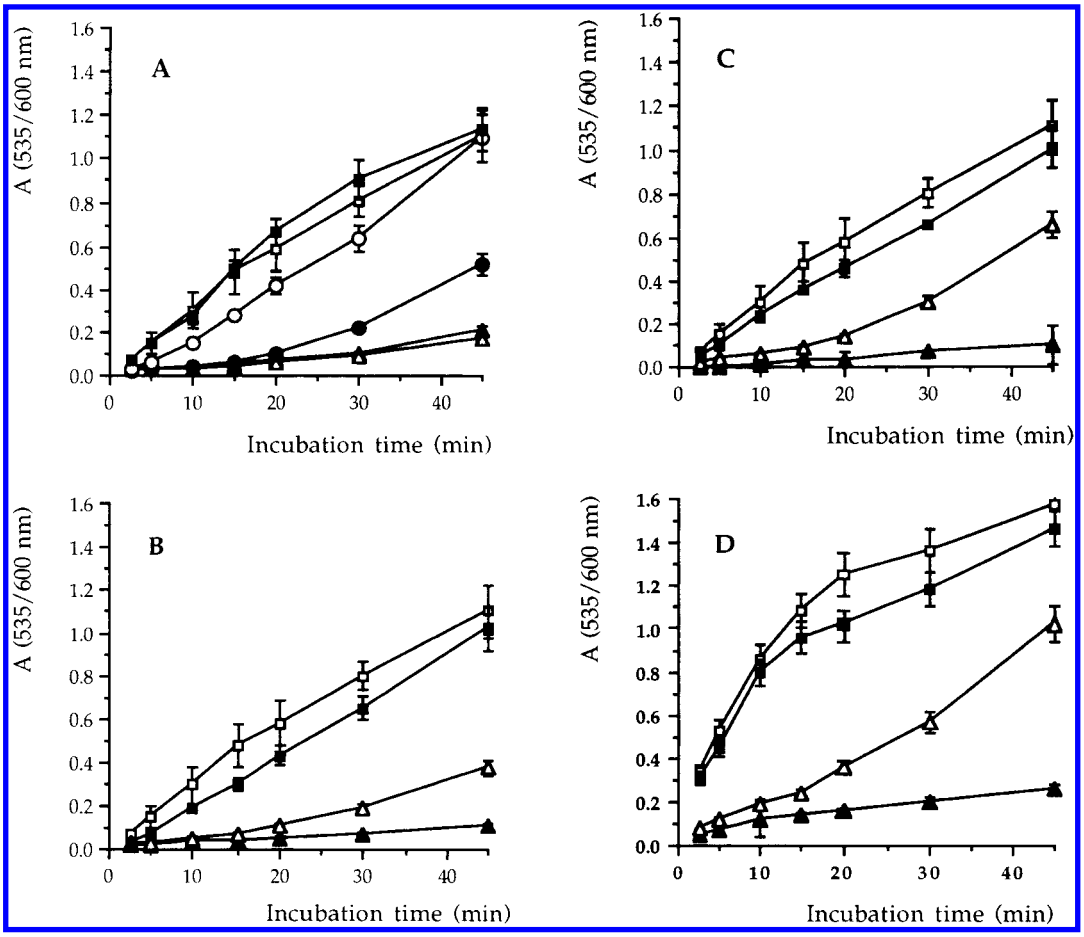


Figure 1. Time course of lipid peroxidation, as affected by the test compounds (panel A, **7**; B, **8**; C, **9**; D, **11**): control, open squares; 0.1 mM, filled squares; 0.2 mM, open circles; 0.4 mM, filled circles; 0.5 mM, open triangles; 1 mM, filled triangles.

Table 1. Effect of the Examined Compounds and Probucol on Plasma Total Cholesterol (TC), Triglyceride (TG), and Low Density Lipoprotein (LDL) Levels and clog *P* Values

compd	dose (μmol/kg, ip)	percent decrease compared to controls[a] TC	TG	LDL	clog P[10]
6	56	32**	42**	32*	3.28
7	28	54***	49**	51***	4.72
8	56	22**	20**	51**	4.20
9	56	30**	39**	22**	4.47
10	56	50**	41**	28**	6.43
11	56	36**	34**	17^{NS}	5.91
probucol	56	18**	11^{NS}	18^{NS}	10.75

[a] Each group is composed of 4−6 rats. Results are from two to three independent experiments. All determinations are performed at least in duplicate, and SD is always within ±10% of the absorbance values. Asterisks indicate statistical significance (Student's *t*-test) as follows: ***P < 0.005, **P < 0.05, *P < 0.1, ^{NS}not significant (P > 0.1).

3.4 (3H, m), 3.7−3.9 (1H, m), 4.0 (1H, d, J = 10.4 Hz), 7.41 (1H, t, J = 7.32 Hz), 7.50 (2H, t, J = 15.2 Hz), 7.58, (2H, d, J = 8.2 Hz), 7.71 (2H, d, J = 7.6 Hz), 7.78, (2H, d, J = 8.2 Hz). Analysis ($C_{20}H_{24}ClNO_2 \cdot H_2O$) C, H, N.

2-(4-Biphenyl)-2-ethoxy-4-methylmorpholine, Hydrochloride, 9. It is isolated as a white solid and recrystallized from acetone and ether. Yield 68%, mp 150−151 °C. IR 2450, 1600 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 0.9 (3H, t, J = 8.9 Hz), 2.8 (3H, s), 3.0−3.4 (6H, m), 4.0 (1H, m), 4.1 (1H, m), 7.3−7.6 (3H, m), 7.65−7.9 (6H, m). Analysis ($C_{19}H_{24}ClNO_2$) C, H, N.

2-(4-Biphenyl)-2-propoxy-4-methyl-octahydro-1,4-benzoxazine, Hydrochloride, 10. The product is isolated as a white solid, recrystallized from acetone and ether. Yield 70%, mp 115−116 °C. IR 2350, 1600 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.9 (3H, t, J = 14.5 Hz), 1.2−1.8 (10H, m), 2.8 (3H, s), 2.9−3.4 (5H, m), 4.05−4.3 (1H, m), 7.3−7.6 (3H, m), 7.65−7.9 (6H, m). Analysis ($C_{24}H_{32}ClNO_2 \cdot H_2O$) C, H, N.

3-(4-Biphenyl)-3-propoxy-octahydro-1,4-pyrido[2,1-*c*]-oxazine, Hydrochloride, 11. The product is isolated as a white solid and recrystallized from *n*-propanol and ether. Yield 71%, mp 170 °C (dec). IR 2500, 1600 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, J = 14.8 Hz), 1.35−1.85 (7H, m), 1.95−2.15 (1H, m), 2.8−3.0 (2H, m), 3.11 (1H, t, J = 11.4 Hz), 3.2−3.4 (3H, m), 3.7−3.9 (2H, m), 4.03 (1H, d, J = 10.4 Hz), 7.41 (1H, t, J = 7.32 Hz), 7.50 (2H, t, J = 15.2 Hz), 7.58, (2H, d, J = 8.2), 7.71 (2H, d, J = 7.6 Hz), 7.78, (2H, d, J = 8.2 Hz). Analysis ($C_{23}H_{30}ClNO_2 \cdot 1/10H_2O$) C, H, N.

In Vitro Lipid Peroxidation. A heat-inactivated hepatic microsomal fraction from untreated male Fischer-344 rats (180−220 g) is prepared.[13] All compounds and the solvents are tested and found not to interfere with the assay. The incubation mixture contains the microsomal fraction corresponding to 2.5 mg of hepatic protein/mL (final concentration), or 4 mM fatty acid residues,[14] ascorbic acid (0.2 mM) in Tris-HCl/KCl buffer (50 mM, 150 mM, pH 7.4), and the studied compounds dissolved in dimethyl sulfoxide (DMSO) at various concentrations (0.1−1.0 mM). The peroxidation reaction is initiated by the addition of a freshly prepared FeSO4 solution (10 μM), and the mixture is incubated at 37 °C for 45 min. Aliquots are taken from the incubation mixture at various time intervals, and lipid peroxidation is assessed by spectrophotometric (535 against 600 nm) determination of the 2-thiobarbituric acid reactive material.[13,15]

In Vivo Evaluation of Hypocholesterolemic and Hypolipidemic Activity. An aqueous solution of Triton WR 1339 is administered ip (200 mg/kg) to male Fischer-344 rats (230−280 g),[16] and 1 h later the examined compounds (28 or 56 μmol/kg) dissolved in saline or saline only is given ip. After 24 h, blood is taken from the aorta and used for the determi-

nation of plasma total cholesterol (TC), LDL, and triglyceride (TG) concentrations.

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