Free Radical Scavenging and Antioxidant Activities of Substituted Hexahydropyridoindoles. Quantitative Structure−Activity Relationships

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New synthetic substituted hexahydropyridoindoles were studied for their radical scavenging ability in a system of an ethanolic solution of α,α′-diphenyl-β-picrylhydrazyl and for their lipid peroxidation inhibitory properties in a suspension of unilamellar dioleoylphosphatidylcholine liposomes. The activities in both in vitro systems were correlated with several structural parameters. In the homogeneous system of α,α′-diphenyl-β-picrylhydrazyl, the sum of aromatic substitution constants ($\sum\sigma^+$) and the hydration energy were shown to be effective predictors of the radical scavenging activity of the hexahydropyridoindole derivatives. Moreover, in the heterogeneous system comprising a model liposomal membrane, the overall antioxidant activity of the compounds was affected by their lipid-phase availability governed by the lipophilicity and basicity of the molecules.

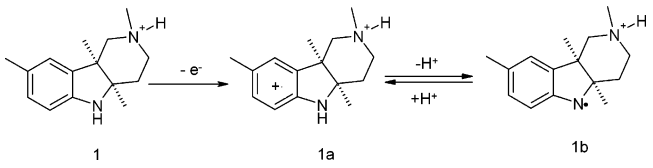
Introduction

Natural and synthetic indoles proved to be able to protect various tissues against oxidative stress, predominantly by scavenging deleterious reactive oxygen species.[1−11] A pulse-radiolysis study showed that the molecular mechanism of radical scavenging of the synthetic hexahydropyridoindole antioxidant stobadine (**1**) resided in the deprotonation of the indoline nitrogen of its oxidized form (**1a**), to give a resonance-stabilized, nitrogen-centered radical (**1b**) (Scheme 1).[12]

In several papers, including those on quantitative structure−activity relationship studies, researchers have reported on the close relation between the antioxidant activity of substituted indoles and their structure, predominantly due to the substituent effect on the stabilization of the nitrogen-centered primary radical.[11,13,14]

In the present work, we examined a set of 18 structural analogues of the hexahydropyridoindole stobadine (**1**; Table 1) for their antioxidant efficiency in two in vitro systems with different phase compositions. In a solution, we evaluated the ability of the hexahydropyridoindoles to scavenge stable free radicals of α,α′-diphenyl-β-picrylhydrazyl (DPPH) with the main interest to study the effect of aromatic substitution on the nitrogen-centered radical **1b** in Scheme 1. In a biphasic system comprising suspension of dioleoylphosphatidylcholine (DOPC) liposomes and peroxyl radicals continually generated from 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), we studied the antioxidant efficacy of **1** and its analogues against lipid peroxidation of the liposomal membranes. In this system the main focus was the overall antioxidant activity as affected by substituents at the synthetically accessible piperidinic nitrogen, which influence mainly the lipophilicity and basicity of the molecules. We report on the quantitative relationships between antioxidant activities of the compounds tested and their electronic properties, steric features, and lipophilicity parameters, using approaches of molecular modeling and multiple regression analysis.

Scheme 1. One-Electron Oxidation of Stobadine (**1**) Followed by Deprotonation of the Indole Nitrogen of Its Oxidized Form **1a** To Give a Resonance-Stabilized, Nitrogen-Centered Radical (**1b**)



Results

DPPH Assay. (Summary of all the calculated/measured parameters and their correlations is given in Tables 1−3 of the Supporting Information.) Radical scavenging potency of the compounds tested was assessed in vitro by the DPPH assay. This method is based on measuring the continual absorbance decrease of the ethanol solution of the stable free radical DPPH at 518 nm, in the presence of the derivatives tested. The values of the initial rates of absorbance decrease, which were used as the parameters of the antiradical reactivity, are summarized in Table 2. For typical kinetic curves corresponding to compounds **5**, **7**, and **9** see Figure 1 in the Supporting Information.

Inhibition of Lipid Peroxidation. (Summary of all the calculated/measured parameters and their correlations is given in Tables 1−3 of the Supporting Information.) The inhibitory efficiency of the compounds studied was evaluated in the system of unilamellar DOPC liposomes oxidatively stressed by peroxyl radical generated in the aqueous phase by thermal decomposition of the hydrophilic azo initiator AAPH. From the inhibition data represented by pIC_{50}, summarized in Table 2, it is apparent that the derivatives bearing the benzoyloxycarbonyl substituent at the piperidinic nitrogen (**15**−**19**) belong to the most potent antioxidants. A moderately lower activity was recorded for *N*-alkyloxycarbonyl analogues **8**−**14**, while derivatives substituted by a methyl group on the piperidinic nitrogen exerted the least antiperoxidative efficiency (**2**−**7**). For typical inhibition curves of compounds **3**, **7**, and **18** see Figure 2 in the Supporting Information.

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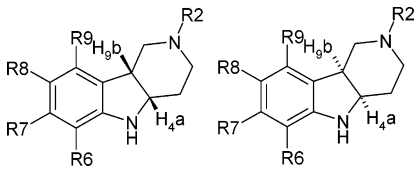
Table 1. General Formulas and List of Substituted Hexahydropyridoindoles **1**−**19**[a]



compd	R8	R2	R6	R7	R9	H4a, H9b
1	$-CH_3$	$-CH_3$	$-H$	$-H$	$-H$	4a(*R*),9b(*S*)-(−)-*cis*
2	$-CH_3$	$-CH_3$	$-NO_2$	$-H$	$-H$	(±)-*cis*
3	$-CH_3$	$-CH_3$	$-Br$	$-H$	$-H$	(±)-*cis*
4	$-CH_3$	$-CH_3$	$-NH_2$	$-H$	$-H$	(±)-*cis*
5	$-CH_3$	$-CH_3$	$-N(CH_3)_2$	$-H$	$-H$	(±)-*cis*
6	$-OH$	$-CH_3$	$-H$	$-H$	$-H$	(±)-*cis*
7	CH_3O-	$-CH_3$	$-H$	$-H$	$-H$	4a(*R*),9b(*S*)-(−)-*cis*
8	CH_3O-	*i*-BuO(CO)−	$-H$	$-H$	$-H$	(±)-*cis*
9	CH_3O-	$CH_3O(CO)-$	$-Br$	$-CH_3$	$-CH_3$	(±)-*cis*
10	CH_3O-	$CH_3O(CO)-$	$-H$	$-CH_3$	$-CH_3$	(±)-*cis*
11	CH_3O-	EtO(CO)−	$-H$	$-CH_3$	$-CH_3$	(±)-*cis*
12	CH_3O-	PrO(CO)−	$-H$	$-CH_3$	$-CH_3$	(±)-*cis*
13	CH_3O-	BuO(CO)−	$-H$	$-CH_3$	$-CH_3$	(±)-*cis*
14	CH_3O-	*i*-BuO(CO)−	$-H$	$-CH_3$	$-CH_3$	(±)-*cis*
15	CH_3O-	$PhCH_2O(CO)-$	$-H$	$-CH_3$	$-CH_3$	(±)-*cis*
16	CH_3O-	$PhCH_2O(CO)-$	$-H$	$-H$	$-H$	(±)-*cis*
17	CH_3O-	$PhCH_2O(CO)-$	$-CH_3$	$-H$	$-H$	(±)-*cis*
18	$-CH_3$	$PhCH_2O(CO)-$	$-CH_3$	$-H$	$-H$	(±)-*cis*
19	$-Br$	$PhCH_2O(CO)-$	$-CH_3$	$-CH_3$	$-H$	(±)-*cis*

[a] Notes: Et, ethyl; Pr, propyl; *i*-Pr, isopropyl; Bu, butyl; *i*-Bu, isobutyl (2-methylpropyl); Ph, phenyl; RO(CO)−, alkoxycarbonyl.

Table 2. Free Radical Scavenging and Antioxidant Activity of Hexahydropyridoindoles **1**−**19**[a]

compd	$\Delta A/\Delta t$ (s^{-1})	pIC_{50}	compd	$\Delta A/\Delta t$ (s^{-1})	pIC_{50}
1	0.012	4.60 ± 0.12	**11**	0.011	4.70 ± 0.01
2	$<1 \times 10^{-3}$	4.58 ± 0.02	**12**	0.020	5.16 ± 0.01
3	$<1 \times 10^{-3}$	4.80 ± 0.10	**13**	0.018	5.35 ± 0.02
4	0.040	4.90 ± 0.02	**14**	0.017	5.19 ± 0.02
5	0.045	4.66 ± 0.01	**15**	0.019	5.48 ± 0.05
6	0.040	4.68 ± 0.04	**16**	0.028	5.49 ± 0.05
7	0.025	4.48 ± 0.03	**17**	0.038	5.29 ± 0.04
8	0.029	5.12 ± 0.01	**18**	0.014	5.36 ± 0.05
9	0.002	4.71 ± 0.03	**19**	0.004	5.53 ± 0.04
10	0.019	4.71 ± 0.06			

[a] Notes: $-\Delta A/\Delta t$, initial rate of the absorbance decrease of the ethanolic DPPH solution in the presence of the hexahydropyridoindole tested at λ_{max} = 518 nm; pIC_{50}, −log value of the concentration of the antioxidant required to cause 50% inhibition of lipid peroxidation at 80 minute time interval.

Discussion

Free radicals have been suggested to be important mediating agents in aging and several human diseases, including cancer, pulmonary and cardiovascular diseases, cataracts, and neurological dysfunctions.[15−17] During the past decade, discovery of new antioxidants, both synthetic and natural in origin, has been a major focus in many laboratories. Many natural and synthetic indole derivatives have been reported to have protective effects in pathological processes caused by oxidative stress.[1−11]

Radical Scavenging Assay. In the first part of the present study we assessed the capability of a series of substituted hexahydropyridoindoles to reduce free radicals by using a model reaction with stable free DPPH radical.[18−20] The main focus was on the effect of aromatic substitution in the ortho and para positions to the indoline nitrogen reactivity center on the intrinsic antiradical activity of the molecules. As expected, the results indicated that the mesomeric stabilization of the N-centered radicals, corresponding to **1b** (Scheme 1), occurring via de-

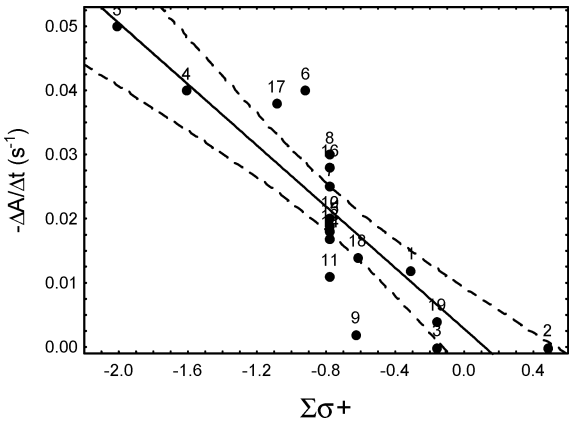


Figure 1. Free radical scavenging efficacy of the hexahydropyridoindole derivatives **1**−**19** tested as a function of the parameter $\sum\sigma^+$ ($R = -0.840$, $n = 19$). Dotted lines represent the borders of the area of the 95% confidence level. Antiradical efficacy is expressed as the initial rate of absorbance decrease of an ethanolic solution of DPPH (60 μM) in the presence of the compound tested (33.3 μM) at λ_{max} = 518 nm. The parameter $\sum\sigma^+$ was calculated as the sum of table values of substitution constants σ^+, defined by Brown,[36] pertaining to substituents at positions C6 and C8 (see the Experimental Section), with the assumption that $\sigma^+_{ortho} = \sigma^+_{para}$.

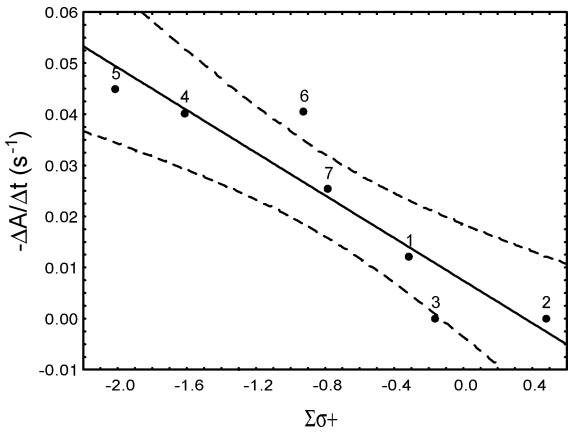


Figure 2. Free radical scavenging efficacy of the hexahydropyridoindole derivatives **1**−**7** tested as a function of the parameter $\sum\sigma^+$ ($R = -0.922$, $n = 7$). Dotted lines represent the borders of the area of the 95% confidence level. Antiradical efficacy is expressed as the initial rate of absorbance decrease of an ethanolic solution of DPPH (60 μM) in the presence of the compound tested (33.3 μM) at λ_{max} = 518 nm. The parameter $\sum\sigma^+$ was calculated as the sum of table values of substitution constants σ^+, defined by Brown,[36] pertaining to substituents at positions C6 and C8 (see the Experimental Section), with the assumption that $\sigma^+_{ortho} = \sigma^+_{para}$.

localization of their unpaired electron through the neighboring aromatic ring, was critical for the reactivity with DPPH. This was clearly documented by good simple linear correlation of the antiradical activity with the sum of aromatic substitution constants $\sum\sigma^+$ (R = −0.840), shown in Figure 1. (For the corresponding parametric equation of the regression see Table 3 in the Supporting Information).

The decisive effect of the mesomeric (de)stabilization of the N-centered radicals on the reactivity with DPPH was demonstrated by improvement of the correlation $-\Delta A/\Delta t = f(\sum\sigma^+)$ from $R = -0.840$ to $R = -0.922$ for the group of derivatives **1**−**7**, with alterations exclusively in the aromatic substituents ($-NO_2$, $-Br$, $-NH_2$, $-N(CH_3)_2$, $-OH$, $-OCH_3$, $-CH_3$) at positions C6 and C8 (Figure 2). Exclusion of the hydroxy derivative **6** caused a slight increase of significance of the correlation to $R = -0.969$, suggesting that its phenolic structure

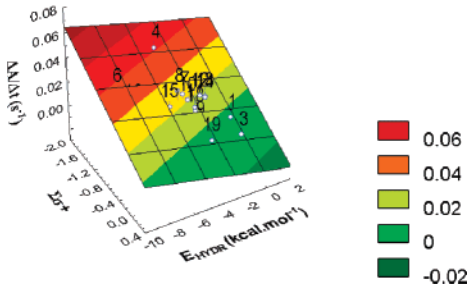


Figure 3. Regression plane and data points for the radical scavenging efficiency of the hexahydropyridoindole derivatives **1**−**19** ($R = 0.920$, $n = 19$) as a function of the parameters E_{HYDR} and $\sum\sigma^+$. Antiradical efficacy is expressed as the initial rate of absorbance decrease of an ethanolic solution of DPPH (60 μM) in the presence of the compound tested (33.3 μM) at $\lambda_{max} = 518$ nm. The color spectrum indicates the spread of values of antiradical activity assigned to individual points.

may dictate the mechanism of the reaction with free radicals, different from the typical one for hexahydropyridoindoles.

Consistent with the given mechanism of radical scavenging action of **1** (Scheme 1), we obtained good correlations of antiradical efficiency with other interdependent electronic parameters, ΔH (difference in the heat of formation between hexahydropyridoindole and its indolyl radical corresponding to **1b**, $R = -0.791$) and ϵ(HOMO) (energy of the highest occupied molecule orbital, $R = 0.840$). Both correlations were slightly improved by exclusion of the nontypical hexahydropyridoindole **6** ($R = -0.806$ and 0.862, respectively). Our results are in agreement with the data published by Westerlund et al.[11] for the redox potential of structurally related indeno[1,2-*b*]indole derivatives and with the data of Dorey et al.[21] for the antioxidant activity of substituted 1,2-dihydroquinolines.

The simple correlation given by the equation $-\Delta A/\Delta t = f(\sum\sigma^+)$ was improved by including the independent variable E_{HYDR} (hydration energy) in the double regression (Figure 3), yielding the value $R = 0.920$ with the corresponding parametric equation

$$-\Delta A/\Delta t = -0.0234\sum\sigma^+ - 0.0023E_{HYDR} - 0.0052 \quad (R = 0.920,\ s = 0.0058,\ p < 1 \times 10^{-6}) \quad (1)$$

This finding stresses the role of the solvation processes involved in the radical scavenging mechanism, which is in accordance with other reports on the kinetics of DPPH reaction with antioxidants.[22,23]

Paradoxically, the presence of additional electron-donating $-CH_3$ groups at positions C7 and C9 of the methoxy derivatives **10**−**15** resulted in a lower antiradical efficiency compared to that of their homologues lacking any substitution in these positions (**7**, **8**, **16**). This finding may be explained by distortion of the p_z orbital of the lone electron pairs of the methoxy group from the planarity of the aromatic ring, caused by steric hindrance with methyl groups at C7 and C9 (Figure 4), which may hinder conjugation of free electron pairs of CH_3O- with the aromatic ring. This conclusion is in good agreement with the finding of Van Acker et al.[24] for α-tocopherol derivatives, showing strong dependence of antioxidant activity on conjugation of the lone electron pair of a heteroatom of the fused heterocycle with the aromatic phenol ring.

Inhibition of Lipid Peroxidation in Liposomes. Unilamellar DOPC liposomes were used to evaluate the antioxidant activity of the compounds tested, with the main interest in the effect of substituent variation at the synthetically accessible piperidinic nitrogen, which affected chiefly the lipophilicity and basicity of the molecules. Peroxidation of the liposomal membrane was triggered by peroxyl radicals generated by thermal decomposition of the hydrophilic azo compound AAPH. Our previous results[25] showed that, in a complete reaction system, DOPC liposomes/AAPH/phosphate buffer, pH 7.4, lipid peroxidation proceeded in the time interval 0−180 min at a constant rate, resulting in an approximately linear time-dependent increase of lipid hydroperoxides without any induction period. No accumulation of hydroperoxides was observed in the absence of AAPH or liposomes. Stobadine, as the hexahydropyridoindole representative, effectively suppressed the oxidation and produced a distinct inhibition period.[25] In the present study, the antioxidant activity of the hexahydropyridoindoles tested was expressed as $pIC_{50} = -[\log(IC_{50})]$, where IC_{50} is the concentration (μmol/L) which caused 50% inhibition of LOOH production in the 80 min incubation time.

The presence of a lipid−water interface was evidently responsible for the loss of significance of the correlation between the antioxidant efficacy and $\sum\sigma^+$ ($R = -0.010$), so crucial for antiradical reactivity. Obviously, an antioxidant molecule has to be lipophilic enough to be able to penetrate into the lipid phase. Indeed, the best predictors of antioxidant efficiency were found to be the lipophilicity parameters log *P* and R_M. The retention parameter R_M, obtained from reversed-phase thin-layer chromatography (RP TLC), showing collinearity with log *P* and log *D*, gave a highly significant correlation ($R = 0.927$, Figure 5).

The correlation of pIC_{50} vs R_M further improved for a group of closely related structural homologues (**10**−**15**) with substituent variability exclusively at the piperidinic nitrogen ($R = 0.992$, Figure 6). As shown in Figure 6, a gradual increase of lipophilicity resulted in a spread of antioxidant activities of the derivatives, with identical values of $\sum\sigma^+$, according to their availability in the lipid phase. The piperidinic nitrogen, isolated from the indoline reactivity center, offers a synthetically accessible site for variation of the lipophilicity of the hexahydropyridoindole molecules, without their intrinsic antiradical reactivity being significantly affected.

With regard to the two basic centers of hexahydropyridoindoles, represented by the indoline and piperidine nitrogens, the availability of the compounds in an oxidized liposomal membrane may be significantly affected by the presence of a positive charge on these nitrogens. For **1** and the *N*-methyl analogues **2**−**7**, an equilibrium with respect to the piperidine nitrogen is expected to be strongly shifted to its protonation at pH 7.4 (92.1% participation of the protonated form for **1** corresponding to stobadine $pK_{a2} = 8.5$[26]). The high degree of protonation of the *N*-methyl derivatives at physiological pH is expected to be reflected by low actual distribution ratios despite rather high partition coefficients (e.g., for **1**, calculated log $D = -0.05$ vs log $P = 1.95$; experimental log $D = 0.57 \pm 0.03$[27]). The indoline nitrogen, with $pK_{a1} = 3.2$[26] for **1**, remains unprotonated at pH 7.4.

In contrast, in compounds **8**−**19**, the alkoxycarbonyl substituent at N2 lowers the basicity of this site profoundly. According to calculations for the derivatives **8**−**19**, their pK_a values corresponding to piperidinic nitrogen may be expected to be around −3.7, while the pK_a of the indoline nitrogen remains around 5.4. Therefore, the protonation of these compounds is negligible at physiological pH, which is reflected by high distribution ratios at pH 7.4, reaching almost the values of the partition coefficients (log $D = 1.99$ vs log $P = 2$ as calculated for **10**).

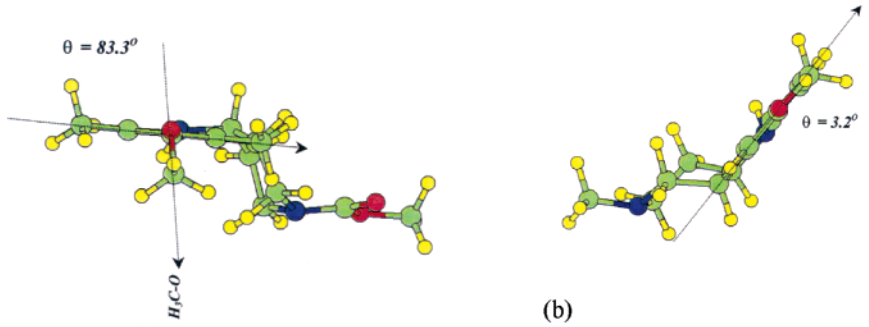


Figure 4. Low energy conformations of compounds **7** and **10** showing (a) steric hindrance of a methyl group of derivative **10** resulting in a decrease of conjugation of a lone electron pair of the CH_3O- group with an aromatic plane due to an increase of angle θ and (b) fully conjugated aromatic system of compound **7** lacking methyl substituents. Color key: oxygen, red; nitrogen, blue; hydrogen, yellow; carbon, green.

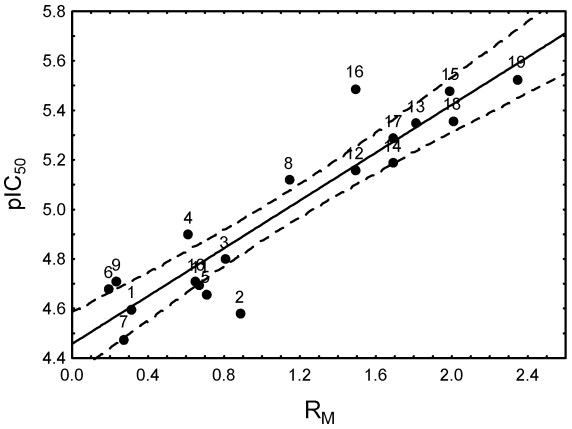


Figure 5. Regression line, 95% confidence interval, and data points for the antioxidant activity of the set of hexahydropyridoindole derivatives **1**−**19** ($R = 0.927$, $n = 19$) at lipid peroxidation of DOPC liposomes, as a function of the lipophilicity parameter R_M. Peroxidation of DOPC liposomes (0.8 mM in 20 mM buffer, pH 7.4) was induced by AAPH (10 mM) at 50 °C.

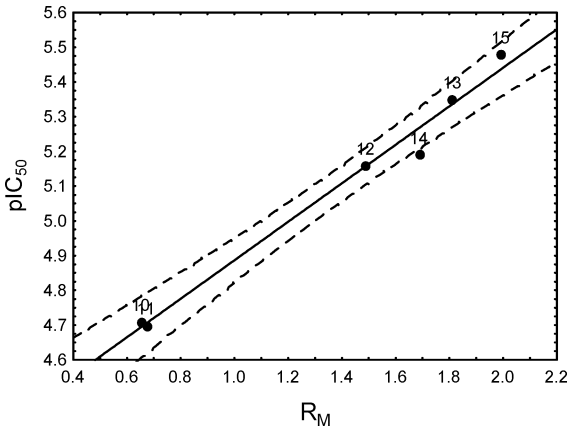


Figure 6. Regression line, 95% confidence interval, and data points for antioxidant activity in DOPC liposomes as a function of the lipophilicity parameter R_M concerning the homological series of hexahydropyridoindoles **10**−**15** ($n = 6$, $R = 0.992$), varying in the structure of the substituent at the piperidinic nitrogen. Peroxidation of DOPC liposomes (0.8 mM in 20 mM buffer, pH 7.4) was induced by AAPH (10 mM) at 50 °C.

The parameter of lipophilicity reflects only the availability of hexahydropyridoindoles in a peroxidatively damaged membrane regardless of their radical scavenging efficacy. We therefore investigated conjunction of the lipophilicity parameter R_M with the descriptor of intrinsic antiradical reactivity ($\sum\sigma^+$) in a double linear regression. However, no remarkable impact of electronic parameters on the correlations involving the

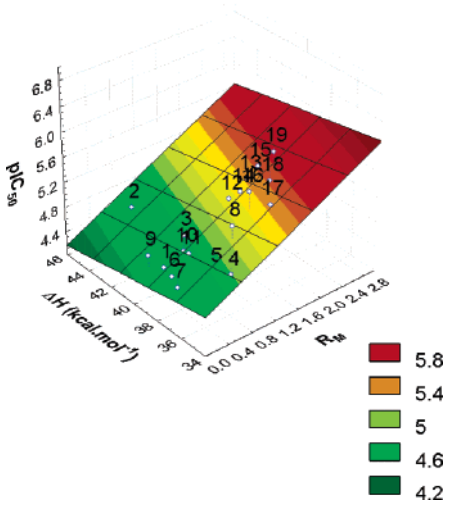


Figure 7. Regression plane and data points for the antioxidant efficiency of hexahydropyridoindole congeners **1**−**19** ($R = 0.950$, $n = 19$) in DOPC liposomes as a function of the parameters R_M and ΔH. Peroxidation of DOPC liposomes (0.8 mM in 20 mM buffer, pH 7.4) was induced by AAPH (10 mM) at 50 °C. The color spectrum indicates the spread of values of antioxidant activity assigned to individual points.

parameter of lipophilicity was shown, as documented by an increase of R from 0.927 for pIC_{50} vs R_M to 0.935 for double regression analysis of pIC_{50} vs R_M and $\sum\sigma^+$ (see also Table 1 in the Supporting Information). A slightly better significance ($R = 0.950$) was obtained after replacement of $\sum\sigma^+$ by a collinear ΔH (with a corresponding regression plane presented in Figure 7). This finding indicates that, for this type of compound, unlike for the group of structurally related 3-indolyl derivatives studied by Shertzer et al.,[14] the correlations in the heterogeneous liposomal system were predominantly dictated by lipophilicity parameters.

Triple correlation involving the parameters R_M, $\sum\sigma^+$, and E_{HYDR} gave a highly significant relationship, as shown in the following parametric equation:

$$pIC_{50} = 0.4889R_M - 0.0968\sum\sigma^+ - 0.0310E_{HYDR} + 4.272 \quad (R = 0.956,\ s = 0.1144,\ p < 1 \times 10^{-6}) \quad (2)$$

Conclusions

This work shows that simple and multiple linear regression analyses together with the molecular modeling approach may be useful tools for predicting the antioxidant efficiency in a series of substituted hexahydropyridoindoles. For the homogeneous conditions of the DPPH assay, the electronic parameters $\sum\sigma^+$, ϵ(HOMO), and ΔH showed the highest predictive power.

The sum of aromatic substituent constants $\sum\sigma^+$ and the hydration energy E_{HYDR} were shown to be effective predictors of the intrinsic radical scavenging efficiency of hexahydropyridoindole derivatives. Moreover, in a heterogeneous system of model membranes represented by DOPC unilamellar liposomes, the overall antioxidant activity was affected by the availability of the compounds in the lipid phase governed by the lipophilicity and basicity of the molecules.

Experimental Section

Chemicals. 1 and its structural analogues **2**−**19** (Table 1) were synthesized at the Institute of Experimental Pharmacology, Slovak Academy of Sciences,[28,29] and were available as appropriate salts (**2**, **4**, **5**, **7**, **8**, and **10**−**18** as hydrochlorides, **3**, **6**, **9**, and **19** as hydrobromides). Cumene hydroperoxide (∼80% in cumene) and AAPH were obtained from Fluka Chemie GmbH (Buchs, Switzerland). L-α-Phosphatidylcholine dioleoyl (C18:1, [*cis*]-9; DOPC; 99% grade), 2,6-di-*tert*-butyl-*p*-cresol (BHT), and DPPH radical were obtained from Sigma Chemical Co. (St. Louis, MO). Other chemicals were purchased from local commercial sources and were of analytical grade quality. All solvents used for lipid peroxidation studies were deaerated under nitrogen.

DPPH Test. To investigate the antiradical activity of the hexahydropyridoindole derivatives in homogeneous solution, 0.1 mL of a water solution of the antioxidant tested (1 mM) was added to 2.9 mL of an ethanolic solution of DPPH (62.1 μM) to give the final concentrations 33.3 and 60 μM for the antioxidant and DPPH, respectively. To release free bases from their salts, the stock solutions of the compounds tested were neutralized by an equimolar solution of NaOH before their addition to the reaction mixture. The absorbance decrease of the reaction mixture was continuously recorded at $\lambda_{max} = 518$ nm. The initial rate of the reaction was estimated from the approximately linear absorbance decrease during the initial 14 s reaction time.

Liposome Preparation and Incubation. The methods were as described previously.[27,30] DOPC (15.7 mg) was placed in a round-bottom flask and dissolved in chloroform (5 mL). The solvent was subsequently removed under nitrogen, and the resulting thin film on the walls was dispersed in phosphate buffer (20 mL, 20 mM, pH 7.4) by vigorous stirring for 2 min followed by sonication for the same period of time. A suspension of unilamellar liposomes (1 mM DOPC) was thus obtained. The liposomes (final concentration 0.8 mM DOPC) were incubated in the presence of different concentrations of the antioxidants tested with the water-soluble initiator AAPH (final concentration 10 mM) at 50 °C for 80 min. The values of IC_{50} were obtained from the linear part of the semilogarithmic plot of I (%, percentage of inhibition) vs antioxidant concentration.

LOOH Determination. Aliquots (1 mL) of the incubation mixtures were extracted with 2 mL portions of the ice-cold mixture $CHCl_3$/MeOH (2:1, v/v) containing BHT (0.05%). The lipid hydroperoxide content was determined by the thiocyanate method according to Mihaljevic et al.[31] by sequentially adding the $CHCl_3$/MeOH (2:1, v/v) mixture (1.4 mL) and the thiocyanate reagent (0.1 mL). The reagent was prepared by mixing equivalent volumes of a methanolic solution of KSCN (3%) and a ferrous ammonium sulfate solution (45 mM in 0.2 mM HCl). After the mixture had been left at ambient temperature for at least 5 min, the absorbance at 500 nm was recorded with a Hewlett-Packard diode array spectrophotometer (8452A). The lipid peroxide value was determined using a calibration curve prepared with standard cumene hydroperoxide.

Determination of R_M Values. The lipophilicity parameters represented by R_M values were measured by the reversed-phase thin-layer chromatography technique.[32,33] The mobile phase consisted of a phosphate buffer solution (pH 7.4; 0.1 M) mixture with acetone (20:80, v/v). The stationary phase was obtained by impregnation of the layer of silica gel G F_{254} plates with a 5% solution of liquid paraffin in ether. The method of impregnating the plates was described elsewhere.[32,33] The compounds were dissolved in methanol, and about 1 μL of the solution was spotted onto the plates. The developed plates were dried, and the compounds were detected in UV light at 254 nm. The R_M values were calculated by the formula $R_M = \log(1/R_F - 1)$.

Computational Methods. The lowest energy molecular conformations of **1** and its derivatives **2**−**19** were calculated using HyperChem molecular modeling software,[34] applying the AM1 method and the Conformational Search module. The convergence limit for the Polak−Ribiere optimization method was 0.01. For optimal conformers of antioxidants the heats of radical formation H_A and the total energies E_A were calculated. As geometric optimization for radicals formed with the abstraction of hydrogen from the indole nitrogen was performed, the corresponding H_F and E_F were calculated, thus creating the theoretical measures of antioxidant activity (eqs 3 and 4).

$$\Delta H = H_F - H_A \quad (3)$$

$$\Delta E = E_F - E_A \quad (4)$$

Further calculated parameters for the compounds tested included values of the energy of the highest occupied molecule orbital ϵ(HOMO), energy of the lowest unoccupied molecule orbital ϵ(LUMO), hydration energy E_{HYDR}, volume V, surface area S, polarizability α, partial charges of the nitrogen q(N) and hydrogen q(H) pertinent to the >NH group, and spin density pertinent to the nitrogen radical derived from this group s(N). The parameter $\sum\sigma^+$ was calculated as the sum of table values of the substitution constants σ^+ defined by Brown.[35] The tabulated values of $\sum\sigma^+$ of the groups $-N(CH_3)_2$ (−1.7), $-NH_2$ (−1.3), and −OH (−0.92) obtained from bromination of aromatic derivatives in acetic acid and values for $-OCH_3$ (−0.778), $-CH_3$ (−0.311), −H (0), −Br (0.15), and $-NO_2$ (0.79) obtained from solvolysis of *tert*-cumyl chlorides were reported by Hansch and Leo.[36] Statistical evaluations of the correlation equations were made by the software package Statistica.[37] Values of the partition (log P) and distribution (log D) coefficients were calculated using the program Pallas.[38]

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Supporting Information Available: Typical DPPH kinetic curve and inhibition curve in an oxidatively stressed liposomal system for representative hexahydropyridoindoles, values of antioxidant activity in two in vitro systems and molecular parameters of all the compounds tested, and all the calculated simple and multiple linear regression equations and linear regression analyses of all pairs of variables included in the best simple and multiple linear regression equations. This material is available free of charge via the Internet at http://pubs.acs.org.

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