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Molecular modeling parameters predict antioxidant efficacy of 3-indolyl compounds

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Abstract Many dietary constituents, such as indole-3-carbinol, are chemoprotective in toxicity and carcinogenicity bioassays. Indole-3-carbinol and related congeners appear to protect partly via radical and electrophile scavenging. To develop better chemoprotective indoles with lower intrinsic toxicity, we performed molecular graphic and quantum-mechanical analyses of model indolyl compounds to ascertain the determinant molecular features for antioxidant activity. We examined eight structurally related 3-indolyl compounds for relationships between antioxidation potential (using in vitro lipid peroxidation assays) and electronic, polar, and steric parameters, including bond dissociation energies, bond lengths, dipole moments, electronic charge densities, and molecular size parameters. Electronic features of the 3-methylene carbon and 1-nitrogen were not predictive of antioxidative potency due to extensive charge delocalization of the cation radical following electron abstraction from the nitrogen. Antioxidant efficacy of 3-indolyl compounds was most strongly predicted by molecular size parameters and by the energy of electron abstraction as calculated from the difference in heat of formation between the parent compound and its cation radical. A highly predictive multiple linear regression correlation model ($r^2 = 0.97$) was obtained using the parameters of heat of formation, molecular weight, log P, and diplole moment.

Key words Antioxidant · Indolyl compounds · Lipid peroxidation · Molecular modeling · Structure-activity relationships

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Introduction

The dietary indoles, indole-3-carbinol, indole-3-acetonitrile and 3,3′-diindolylmethane, occur naturally as glucosinolate conjugates in *Brassica* vegetables (Loub et al. 1975), and are released upon hydrolysis. They have been shown to protect against chemical carcinogenesis (Bailey et al. 1985; Wattenberg 1985, 1990) and chemically induced hepatotoxicity (Shertzer et al. 1987b, c, 1988, 1991). In the course of defining the mechanism by which indoles protect against chemically induced tissue damage, the compounds have been found to both inhibit (Shertzer 1980; Eisele et al. 1983; Shertzer et al. 1987c) and to induce (Shertzer 1982; Godlewski et al. 1985; Bradfield and Bjeldanes 1987; McDanell et al. 1987; Fong et al. 1990) the activities of several different enzymes. Although changes in the activities of enzymes, such as mixed-function oxidases and phase II enzymes, could alter the biological responses of tissues exposed to carcinogens and toxicants, indole-3-carbinol and/or its metabolites have also been shown to be capable of scavenging biologically reactive electrophiles (Shertzer et al. 1987a; Shertzer and Tabor 1988) and free radicals (Shertzer et al. 1988, 1991). Such scavenging provides an additional and broader-based protection mechanism for certain indole compounds.

To both elucidate the molecular and structural basis for radical quenching by 3-indolyl compounds, and identify and develop novel 3-indolyl compounds which exhibit enhanced antioxidant efficacy, we have examined a number of such compounds for their antioxidation potential . In this paper we report the evaluation of a set of 3-indolyl congeners for the relationships between antioxidation potential and electronic, polar, and steric parameters.

Materials and methods

Chemicals

All chemicals and solvents used in this study were obtained from Sigma Chemical Co., St. Louis, Mo., except as noted. Semipurified asolectin (soybean phospholipids) was supplied by Associated Concentrates, Woodside, Long Island, N.Y. Ascorbic acid and asolectin were purified as described previously (Shertzer et al. 1988). Malonaldehyde-bis-(dimethyl acetal), 3-methylindole (skatole), indole-3-carbinol, and 2,4,6-trimethylbenzyl alcohol were obtained from Aldrich Chemical Co., Milwaukee, Wis. 3-(4-N,N-Dimethylaminobenzyl)indole (Thesing and Mayer 1954), 3-(4-methoxybenzyl)indole (Pratt and Botimer 1957), and 3-benzylindole (Biswas and Jackson 1969) were synthesized according to published procedures. Structures of the synthesized indoles were verified using both high-resolution Fourier transformed nuclear magnetic resonance (NMR) and mass spectrometry (MS) techniques.

Additional indoles were synthesized as described below. In these syntheses, petrol refers to 60–80°C boiling range petroleum ether. All reaction and purification solvents were dried and distilled prior to use. Column chromatography for compound purification and isolation was achieved using Merck 7747 silica gel. For the compounds synthesized as described below, structural assignments and verifications were via NMR and MS techniques. ^{1}H NMR spectra were recorded at 270 MHz on a JEOL JNM Fourier transform instrument using trimethylsilylchloride as the internal standard. ^{13}C NMR spectra were obtained under identical conditions at 67.8 MHz. Mass spectra were measured using a VG 707OE mass spectrometer coupled to a VG 2000 data system.

3-(4-*Hydroxybenzyl*)*indole*

Trimethylsilyl indole (3.6 ml, 25 mmol) was added slowly to a solution of 3-(4-methoxybenzyl) indole (2 g, 8.4 mmol) in quinoline (20 ml) stirred in an ice-bath and protected by a nitrogen atmosphere. After the addition, the ice-bath was removed and the reaction mixture was heated to 180°C for 2 h. It was then cooled and poured onto ice-cold 2 M hydrochloric acid (50 ml) and extracted with diethyl ether (2 × 30 ml). The organic layers were combined and washed successively with 1 M hydrochloric acid (4 × 50 ml), water (2 × 50 ml), brine (1 × 50 ml), and dried over $MgSO_4$. Removal of the solvent gave a colorless residue, which was dissolved in methanol and treated with several drops of diethyl ether saturated with hydrogen chloride. The methanol was removed under reduced pressure and the resultant gum chromatographed. Elution with dichloromethane/petrol (1 : 1) gave 3-(4-hydroxybenzyl)indole (720 mg, 38%), which was crystallized from diethyl ether/petrol as colorless prisms, m.p. 142–143°C, λ_{max}nm (ε), 281 (9422), 224 (30, 640); ν_{max} ($CHCl_3$) cm^{-1}, 3495 (sh, N-H), 3300 (br, O-H). [Empirical formula – found: C, 80.7%; H, 5.9%; N, 6.3%, $C_{15}H_{13}NO$; requires: C, 80.3%; H, 5.95%; N, 6.5%].

3-(2,4,6-*Trimethylbenzyl*)*indole*

2,4,6-Trimethylbenzyl alcohol (2.5 g, 0.017 mol) in *p*-cymene (50 ml) was treated with powdered potassium hydroxide (0.8 g) and heated at reflux in a Dean and Stark apparatus. After 20 min the reaction mixture was cooled, and indole (1.95 g, 0.017 mol) and nickel metal (150 mg) were added. The mixture was then heated at reflux for a further 15 h. The solvent was removed and the residue distilled to afford the title compound as an oil (205 mg, 10%), b.p. 250–252°C/0.5–0.7 mmHg, which slowly crystallized to give colorless prisms, m.p. 134–135°C; λ_{max}nm (ε), 283 (5691), 224 (16, 885); ν_{max} ($CHCl_3$) cm^{-1}, 3500 (NH); m/z 249 (100% [M^+]), 130 (82%); δ_H ($CDCl_3$) ppm, 7.8–7.5 (2H,m), 7.3–7.1 (3H,m), 6.72 (2H,s, H-3′,H-5′), 6.35 (1H,s, 2-H), 3.90 (2H,s, CH_2), 2.22 (3H,s, *p*-CH_3), 2.18 (6H,s, 2 x *o*-CH_3). [Empirical formula – found: C, 86.9%; H, 7.5%; N, 5.5%, $C_{18}H_{19}N$; requires: C, 86.7%; H, 7.7%; N, 5.6%].

Lipid peroxidation assay

The cell-free lipid peroxidation assay utilized in this study has been described previously (Shertzer et al. 1988). Briefly, lipid peroxidation of purified soybean phospholipid vesicles in aqueous potassium phosphate buffer (pH 7.4), was initiated by the addition of 10 μM $FeNH_4(SO_4)_2$ in the presence of 100 μM ascorbic acid. The reaction was quenched with butylated hydroxytoluene, and the thiobarbituric acid-reacting products of lipid peroxidation were standardized using malonaldehyde-bis-(dimethylacetal) (MDA), and expressed initially as MDA equivalents. For each analysis of inhibition of lipid peroxidation by indole compounds, at least ten different concentrations of compound were tested, with at least four different concentrations above and below the concentration which gave 50% inhibition. After initial range-finder experiments, each titration was repeated at least three times. The results were calculated as the average percentage of control rates (no inhibitor) of lipid peroxidation versus concentration of inhibitor. SigmaPlot (Jandel Scientific) was used to fit the curve with a third order least-squares regression line and the concentration of compound required to inhibit lipid peroxidation by 50% (IC_{50}) was calculated.

Modeling methods

The lowest energy molecular conformations of the 3-indolyl compounds were calculated using HyperChem Molecular Modeling software (Autodesk), using the Austin model 1, Polak-Ribiere conjugate gradient algorithm with UHF spin pairing and a 0.01 convergence limit in vacuo. The log P values were obtained using an additive-constitutive algorithm with ChemPlus software (Hypercube).

Regression analysis

For the purposes of these quantitative structure-activity relationships (QSAR), the in vitro antioxidant activities were converted to the form: $-\log IC_{50}$. First order regression analysis between indole antioxidant efficacy and physicochemical properties were computed using SigmaStat Software (Jandel Scientific). For those parameters showing relationships with <10% confidence that a linear correlation occurred by chance ($P < 0.1$), multiple linear regression analyses were performed using all possible combinations of descriptors.

Results and Discussion

Free radicals including reactive oxygen and lipid peroxidation products have been suggested to be important mediating agents in aging and several human diseases, including cancer, pulmonary and cardiovascular disease, cataracts, and neurological dysfunctions such as Parkinson's disease (Clark et al. 1985; Ames et al. 1993; Shigenaga et al. 1994; Halliwell and Cross 1994). While the major intracellular cytosolic antioxidant, reduced glutathione (GSH), is effective in preventing radical-induced damage to DNA, GSH is not effective in directly inhibiting lipid peroxidation

(Zhu et al. 1996). Although vitamin E normally serves to protect lipids from peroxidative damage, radical stress may promote lipid peroxidation during exposure to pro-oxidative pharmaceuticals, environmental agents, or in the course of inflammatory diseases. Therefore, the development and evaluation of novel or dietary antioxidants, for the purpose of prevention or treatment of such conditions is an important endeavor. This paper describes the essential features of 3-indolyl compounds that impart antioxidant potency against lipid peroxidation.

A typical lipid peroxidation inhibition curve is shown for 3-hydroxyethylindole (Fig. 1). Similar curves were obtained for each of the compounds studied. The molar concentrations required to inhibit by 50% the control levels of peroxidation (IC_{50}) were used to calculate the $-\log IC_{50}$ values, and these results are shown in Table 1. The $-\log IC_{50}$ was used as the parameter for the antioxidant potential of the 3-indolyl compounds on which to regress the parameters from the results obtained from molecular modeling.

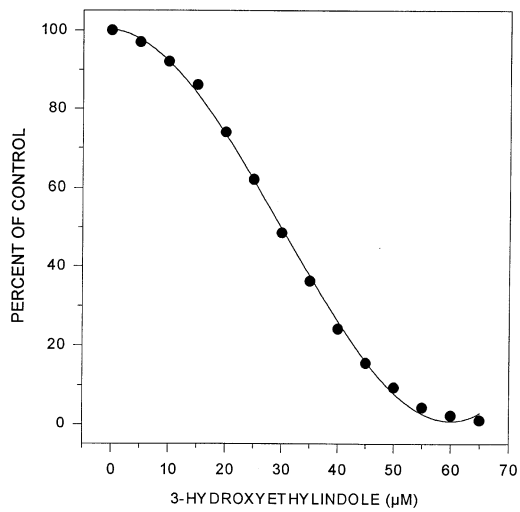

Fig. 1 Lipid peroxidation curve for 3-hydroxyethylindole. The curve represents the influence of 3-hydroxyethylindole on iron/ascorbate-initiated lipid peroxidation. Each value represents the average of three experiments (each performed in duplicate) expressed as a percentage of the control rates of lipid peroxidation (i.e., those that occurred in the complete assay systems, but in the absence of the test indolyl compound)

The calculated physicochemical parameters that best predict antioxidation efficacy are also reported in Table 1. Additional parameters that were calculated but not shown (since they were not predictive of antioxidation potency) are the atomic charges on each of the carbon atoms, and the bond lengths for carbon-hydrogen, carbon-nitrogen, and carbon-carbon bonds, and various other molecular dimensions and dipole moments. Several pairs of these most predictive descriptors are colinear (Table 2). This is to be expected when evaluating a series of congeners with rather hydrophobic constituents. Since several global molecular properties are colinear with molecular mass, they are hence colinear with each other. Linear least squares regression analyses of the data revealed two parameters to be the best individual predictors of antioxidant efficacy (Table 2). These were: (1) the energies of electron abstraction, as calculated from the differences in heats of formation between the parent compound and its cation radical (ΔHf); and (2) various parameters of size, for which the longest dimension (Z-dimension) of the smallest enclosing periodic box was the best predictor. The least linear regression equations for these data are:

$$-\log IC_{50} = 3.02\ \Delta Hf + 1.36\ (r^2 = 0.78,\ P = 0.004);$$

and

$$-\log IC_{50} = 0.21\ \text{Z-dimensional length} + 2.44\ (r^2 = 0.71,\ P = 0.009).$$

Table 1 Lipid peroxidation inhibition constants and physicochemical parameters for 3-indolyl compounds

Parameter	Compound number[a]							
	1	2	3	4	5	6	7	8
IC_{50} ($M \times 10^6$)	1.5	11	12	24	29	36	100	160
$-\log IC_{50}$	5.82	4.96	4.92	4.62	4.54	4.44	4.00	3.80
ΔHf (kcal/mol)	1.460	1.136	1.066	1.166	0.912	1.079	0.998	0.869
Z-dimension (Å)	13.96	12.32	11.58	13.20	8.68	11.31	7.27	7.57
MW (Da)	250.3	249.4	223.3	237.3	161.2	207.3	131.2	147.2
Log P	2.26	3.40	1.71	1.74	−0.37	1.99	0.38	−0.62
Z-dipole (Debyes)	1.86	0.78	−0.58	1.21	−1.80	0.07	−0.61	−1.11
Nitrogen charge	−0.225	−0.218	−0.218	−0.217	−0.216	−0.218	−0.218	−0.215

Determination of the values shown is as described in the Materials and methods. The Z-dimension is the longest dimension of the smallest periodic box that may surround a molecule, while Z-dipole is the dipole moment in that dimension. The parameter ΔHf is the difference in the heat of formation between the parent compound and the cation radical produced by electron abstraction. Log P is the $\log_{10}$ value of the partition coefficient. IC_{50} is the concn. of compound required to inhibit lipid peroxidation by 50%; MW is mol wt.

[a] Compound 1, 3-(4-N,N-dimethylaminobenzyl)indole; compound 2, 3-(2,4,6-trimethylbenzyl)indole; compound 3, 3-(4-hydroxybenzyl)indole; compound 4, 3-(4-methoxybenzyl)indole; compound 5, 3-hydroxyethylindole (tryptophol); compound 6, 3-benzylindole; compound 7, 3-methylindole (skatole); compound 8, 3-hydroxymethylindole (indole-3-carbinol)

Table 2 Colinearity analysis of the major physicochemical parameters used in this study (All parameters are as defined in Table 1)

	− Log IC_{50}	ΔHf	Z-dimension	MW	Log P	Z-dipole
− Log IC_{50}	1.00	0.78	0.71	0.68	0.43	0.41
ΔHf	–	1.00	0.73	0.62	0.51	0.81
Z-dimension	–	–	1.00	0.96	0.69	0.62
MW	–	–	–	1.00	0.78	0.54
Log P	–	–	–	–	1.00	0.61
Z-dipole	–	–	–	–	–	1.00

Linear least squares regression analyses among all pairs of major parameters used for the multiple linear regression analyses. The data are of r^2 values

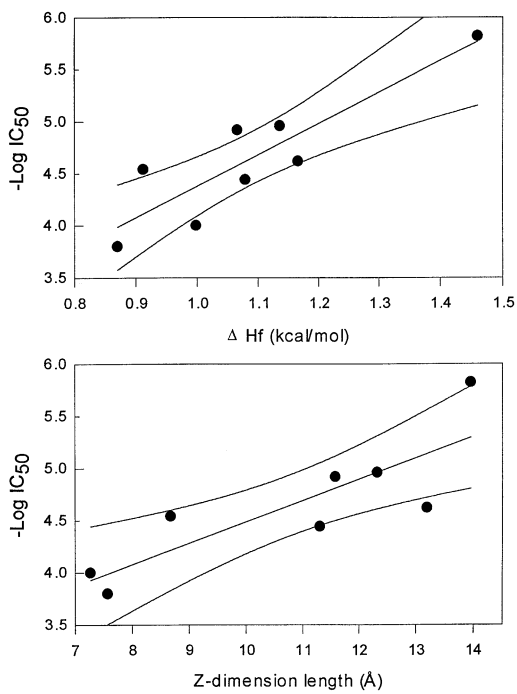

Fig. 2 The regression line and data points for ΔHf and Z-dimension length versus − log IC_{50}. These parameters represent the best individual predictors (simple linear regression) of antioxidant efficacy. The first order regression fit of the data and 95% confidence intervals are shown. (*ΔHf* Difference in heat of formation between parent compound and cation radical produced by electron abstraction)

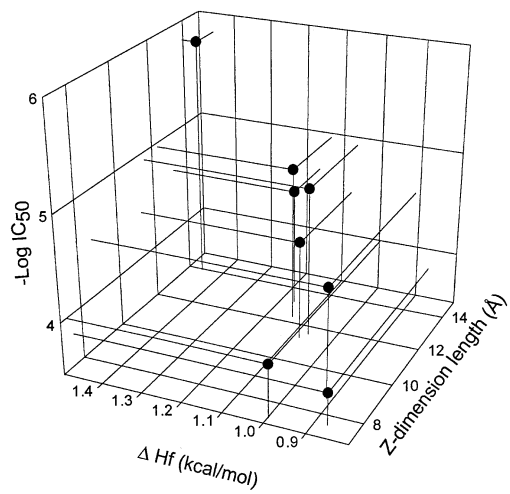

Fig. 3 The regression plane and data points for − log IC_{50} as a function of ΔHf and Z-dimension length. These two parameters represent the best pair of predictors (multiple linear regression) of antioxidant efficacy

These regression plots are shown in Fig. 2. Furthermore, the multiple linear regression of these two independent variables yielded an r^2 value that is better than either of the simple linear regressions whereas the *P*-value remained significant. The resultant regression plane is described by the following equation and presented graphically in Fig. 3

$$-\log IC_{50} = 2.09\ \Delta Hf + 0.08\ \text{Z-dimension length} + 1.54\ (r^2 = 0.81,\ P = 0.016).$$

The importance of the Z-dimension length may be related partly to its colinearity with the log of the partition coefficient (log P) ($r^2 = 0.69$, $P = 0.008$). A multiple linear regression using both of these parameters to predict − log IC_{50} did not significantly improve the predictive power ($r^2 = 0.71$, $P = 0.045$). This was not an expected result, since lipid peroxidation occurred in a hydrophobic liposomal membrane and it was expected that log P alone would have been a better predictor of antioxidation efficacy.

The charge on individual atoms and groups of atoms in the parent compounds and their cation radicals was investigated in depth. Of particular interest was the charge on the indolic nitrogen (Table 1), since electron abstraction from this site by lipid radicals would provide a reasonable mechanism for the chain-breaking antioxidant efficacy of these compounds. No significant correlation between any parameter of charge and antioxidant potential was found (data not shown). The lack of significant differences in the charge on any single atom or group of atoms between one indole and another after the loss of an electron from the nitrogen in the indole suggests an extensive delocalization of charge throughout the indole ring system. Although charge delocalization of the cation radical prevents atom-specific static charge from being used as a predictor of antioxidant efficacy, it serves two very important functions in enhancing radical chain-breaking

potency. Firstly, charge delocalization increases the ease of electron abstraction, and secondly, stabilization of the resulting radical reduces its ability to propagate the radical chain reaction. The stabilizing effect is predicted on a molecular level by the ΔHf, which increases with antioxidant efficacy. This relationship is best explained by the fact that as ΔHf increases, the relative stability of the cation radical increases in relation to the parent compound, making the loss of an electron by the parent compound more favored.

The best set of parameters for multiple linear regression to describe the antioxidant efficacy of the indolyl congeners is:

$$-\log IC_{50} = 4.85\ \Delta Hf - 0.49\ Z\text{-dipole} + 0.04 \log P + 0.004\ MW - 1.48\ (r^2 = 0.97,\ P = 0.013).$$

Although it is clear that ΔHf and to a lesser extent Z-dipole (the dipole moment along the Z-axis) are dominant (for these two parameters alone, $r^2 = 0.91$, $P = 0.002$), log P and mol.wt. also contribute to the best predictive equation. In summary, we have shown that molecular modeling in conjunction with multiple linear regression can be an important tool in predicting the antioxidant efficacy of 3-indolyl compounds. The antioxidant potential is highly predicted by ΔHf in conjunction with dipole moment, log P, and molecular size. Although extensive charge delocalization masks the single-atom effects of electron abstraction, the overall stability of the cation radical is predictive of the antioxidant potential. The multiple linear regression equations developed in this paper may be used to predict the antioxidant efficacies of potentially useful chemoprotective compounds.

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