A Review on Quantitative Structure-Activity Relationships (QSARs) of Natural and Synthetic Antioxidants Compounds

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Abstract: During the last decade an increasing number of reports describe the roles of active oxygen species in the development or exacerbation of various kinds of diseases. Antioxidants are of great interest because of their involvement in important biological and industrial processes. They have been found to possess anticancer, anti-cardiovascular, anti-inflammatory and many other activities.

Many attempts have been made to elucidate the QSAR of antioxidants by using different physicochemical parameters. Unfortunately the limited number of antioxidants and the unavailable σ Hammett values of complex substituents did not lead to significant results in regression analysis. The redox potentials are well correlated to the antioxidant activities. In this report we will attempt to collect and discuss all the published results concerning the QSAR research on natural and synthetic antioxidants compounds.

Keywords: QSAR, natural and synthetic antioxidants, electronic effects.

INTRODUCTION

Radical reactions are generally chain reactions [1-3]. The radicals are generated in a step or steps called "initiation" and they participate in a sequence of "propagation" reactions in which their number is conserved; finally they are destroyed in a "termination" process or processes. Although for some molecules extreme conditions are required to form radicals, many others can be transformed into radicals under relatively mild conditions, including those that encountered in living organisms [4a].

DISEASES

Pathological and toxicological processes may disturb the equilibrium between production and use of free radicals and protection against their deleterious effects either by overproducing free radicals or by weakening the defense mechanisms. However, if free radicals are usually detrimental for the organism, they can also be useful because of their toxicity or as chemical intermediates in metabolic pathways. Any time that an organism is overexposed (either too much or for too long) to free radicals, it may become ill [4b].

Free radicals are linked with atherosclerosis. Cholesterol-rich low-density lipoproteins are clearly involved in the multistep process of coronary artery disease. Polyunsaturated fatty acids, which are susceptible to lipid peroxidation, can form free radicals that can injure the endothelium, damage heart muscle cells, and provoke proliferation of smooth muscle. These processes can be inhibited by antioxidants [5].

It is well known that inflammation is a complex process, which covers much different pathology. Numerous cell types and chemical mediators are involved, and the implication of free radicals has been clearly shown.

Free radicals are involved in many different ways in inflammation process. During the activation of phagocytes the superoxide anion radical, hydrogen peroxide and the hydroxyl radical are produced. The second and more complicated level of involvement is the synthesis of prostaglandins, which are important mediators of inflammation. Prostaglandins are produced following the initial transformation of arachidonic acid by cyclooxygenase. Other compounds are also derived from polyunsaturated fatty acids either as products of other enzymes (e.g. leukotrienes) or after chemical modification like oxidation by hydrogen peroxide or ultraviolet light. Prostaglandins are mediators of inflammation mostly because of their involvement in the regulation of vascular tone and tissue permeability. In fact, they can induce all the signs of inflammation, they are released during inflammatory reactions, and their synthesis is inhibited by known anti-inflammatory drugs.

ANTIOXIDANTS

Free radicals have been produced by living cells utilizing free oxygen, which have developed numerous mechanisms to control them. Life thus, requests a precarious equilibrium between production and use of free radicals and protection against their deleterious effects.

a. Protection by Enzymes

Superoxide dismutase Two types of enzymes exist to remove hydrogen peroxide within cells, the *catalases* and the *peroxidases.*

b. Protection by Small Molecules

Vitamins: α-tocopherol, ascorbic acid, quinones, carotenoids and others. Vitamin E [6a], vitamin A [4a], and vitamin C [4a] are the three major vitamins that serve as targets for radicals in biological systems.

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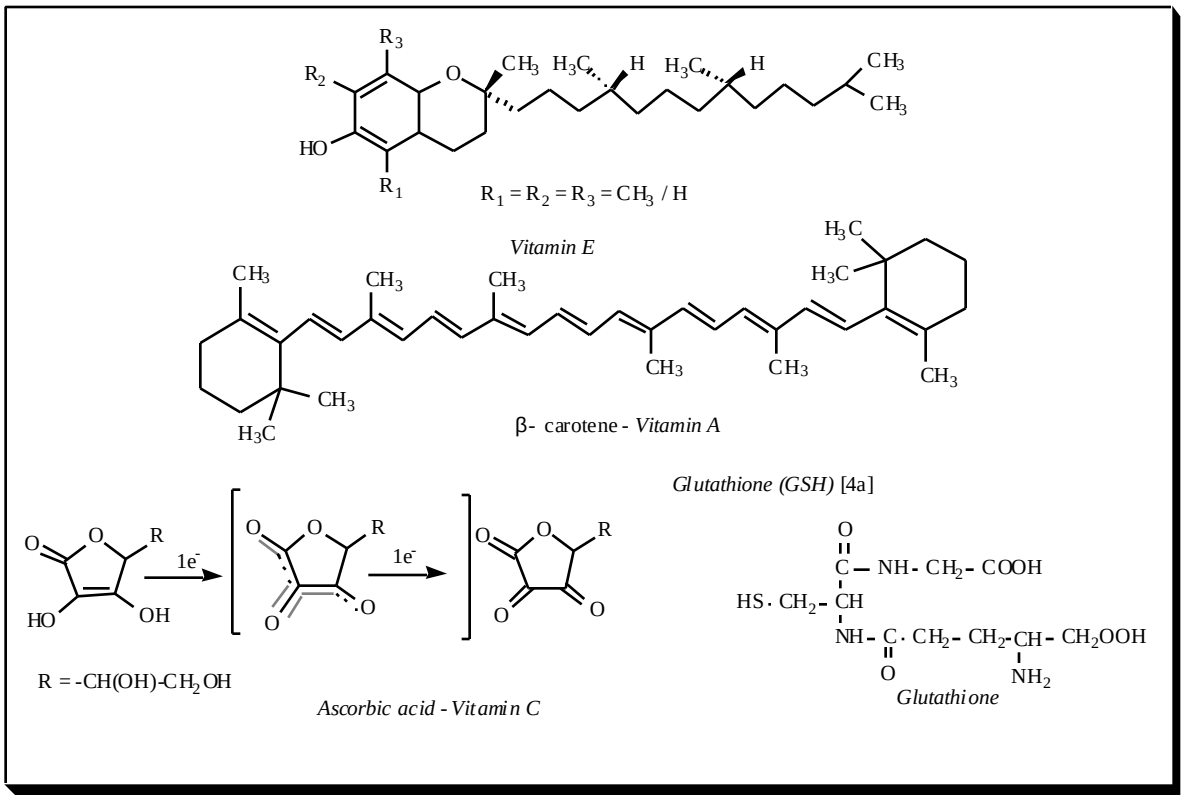


c. Protection by Sequestration of Metal Ions (Metal Binding Proteins) (6b)

Several metal-binding proteins as transferrin, lactoferrin, ceruloplamin and albumin reduce the effectiveconcentration of transition metals that are capable of reacting with hydroperoxides (LOOH) as follows:

$$LOOH + Fe^{2+} \rightarrow LO^{\cdot} + OH^{-} + Fe^{3+}$$

QSAR STUDIES

Quantitative structure-activity relationships (QSAR) derive models, which describe the structural dependence of biological activities can be determined either by physicochemical parameters (Hansch Analysis), or by indicator variables encoding different structural features (Free Wilson Analysis) or by three dimensional molecular property profiles of the compounds (Comparative Molecular Field Analysis – CoMFA).

Most often QSAR analyses and retrospective studies, whether they follow a rational design of investigated structure or not only offer performing syntheses and biological testing, a quantitative relationship is derived. Often the optimization of lead compound is step by step accompanied by QSAR analyses. QSAR helps to understand structure-activity relationships, in a quantitative manner and to find the borders of certain properties. The strategy and philosophy of QSAR enables medicinal chemists to look at their structures in terms of physicochemical properties instead of only considering certain pharmacophoric moieties in it.

With each QSAR the following statistics are given: the number of data points (n), the 95% confidence limits for each term in parentheses, the correlation coefficient (r), between observed values of the dependent and the values predicted from the equation, (r^2) the squared correlation coefficient, the (s) is the standard deviation, the (q^2) defines the cross-validated (r^2) (indication of the quality of the fit) and the F-values for the individual terms. F-value is a measure of the level of statistical significance of the regression model.

i) Phenols

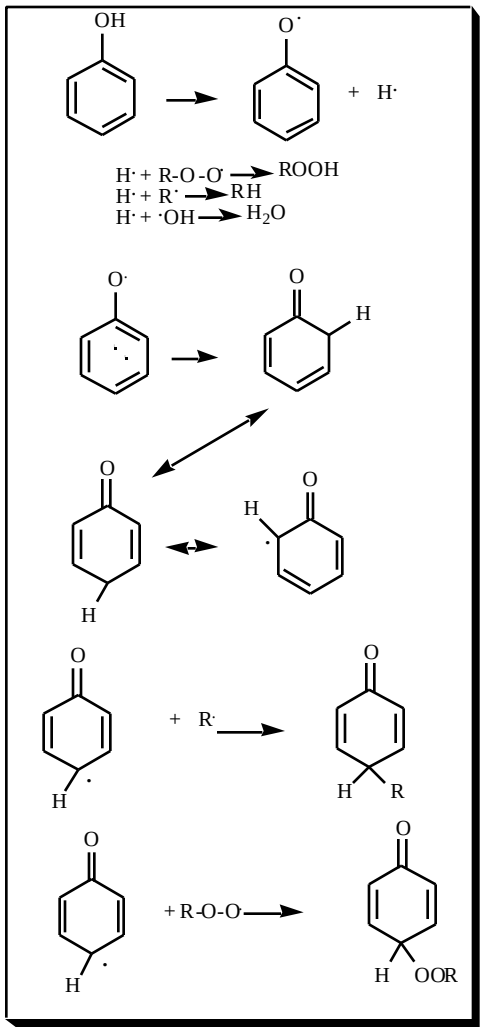
By Ruiz *et al*. [7] a QSAR study of phenols was performed in order to find out the existing relation between phenoxyl radical formation and the anti-inflammatory activity *via* an antioxidant mechanism. All molecules were minimized using the semiempirical AM1 method of MOPAC 6.0 ESP and the Discover programs with the Newton Raphson algorithm.

- The anti-inflammatory mechanism of phenols were studied by investigating the phenoxyl radical formation procedure which involves chain-breaking and radical neutralization or a conversion of them into non-radical products.

As inflammatory agents phenols present a double mechanism:

- The neutralization of harmful radicals *via* hydrogen donation
- The ability of the ketone radical derived from phenoxyl radical to scavenge intermediate radicals.

Significant correlations were calculated between the calculated thermodynamic reaction parameters and anti-inflammatory potency. All thermodynamic parameters were highly correlated with each other (r_s = 0.90 - 0.970)



$$pI_{50} = - 0.067\ (0.021)\ \Delta H\ (TS) + 0.915\ (0.900) \quad (1)$$

n = 15, r = 0.882, s = 0.3, F = 45.1

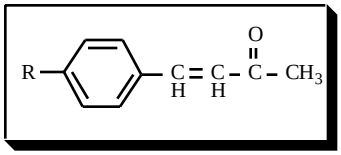
$$pI_{50} = -0.631\ (0.121)\ \Delta H_r + 14.050\ (3.073) \quad (2)$$

n = 15, r = 0.950, s = 0.242, F = 121.5

High correlation of ΔH_r with pI_{50} indicated that lower reaction energy increases anti-inflammatory activity. ΔH (TS) is enthalpy formation of transition state, ΔH_r is reaction energy in kcal/mol and r_s is the Spearman correlation.

ii) Phenyl-Butenones

Ring substituted phenyl-butenones (Table 1) possess good anti-inflammatory activity [8,9] and scavenge superoxide radicals to an appreciable extent. A good correlation was found to exist between superoxide scavenging (IC_{50}) and anti-inflammatory activity (A.A%).

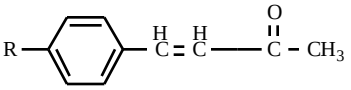


$$A.A\% = - 94.6\ IC_{50} + 80.9 \quad (3)$$

n= 8, r = 0.864, s= 14.19, $F_{1,6}$ = 17.8

According to the investigators this equation indicates that both the basic nucleus of phenyl-butenones and the substituents on the phenyl ring are contributing to the anti inflammatory activity significant. However, the equation fails to explain the high activity of halogenated compounds 4-Br and 2-Cl, which were deleted from the regression analysis.

Table 1. Phenyl-Butenones Derivatives



N	R		N	R	
1	4-OH	3-OCH_3	**6**	4-OCH_3	3-OH
2	4-$N(CH_3)_2$		**7**	4-OCH_3	
3	4-$OCOCH_3$	3-OCH_3	**8**	4-Br	
4	2-OH		**9**	2-OCH_3	4-OCH_3
5	4-OH		**10**	2-Cl	

For the superoxide radical scavenging activity of phenyl-butenones, the following correlation was derived:

$$\log 1/IC_{50} = - 0.354\ (0.267)\ \sum\pi + 0.295\ (0.164) \quad (4)$$

n = 8, r = 0.797, s = 0.162, $F_{1,6}$ = 10.509, π = 0.05

$\sum\pi$ represents the sum of π values of the substituents R, applies to substituents on all ring positions and explains 63.6% of the variance in the data approximately. The correlation coefficient is not good in view of (r), while the value of (s) is very small. This means that the observed log 1/IC50 from the data is in small variance. From eq. 4 we can see that hydrophilicity of R substituents (negative sign) is crucial.

iii) Polymethoxy-Flavones

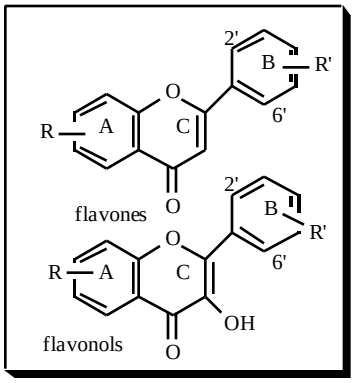
Recently [10] a qualitative structure-activity relationship study of polymethoxy-flavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation, induced by $FeSO_4$-cysteine in rat liver microsomes, was established. It was observed that the structural features for active polyhydroxylated compounds were different from those of polymethoxylated flavones, antiperoxydative flavonoids, possessing a high lipophilicity.

iv) Coumarins

Coumarins incorporate the styryl-carbonyl moiety into a rigid framework. From this point of view phenyl-butenones, as derivatives of styryl-carbonyls, are precursors of coumarins. Several styryl carbonyl derivatives, including cinnamic acids, possess anti-inflammatory/antioxidant activities [11,12].

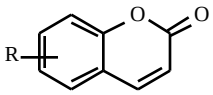
Coumarins affect the formation and scavenging of reactive substances derived from oxygen [13,14] and influence processes like lipid peroxidation, involving free radical-mediated injury (e.g. inflammation) as can some other phenolic compounds (flavonoids) [15,10]. Peroxidation and radical propagation lead to destabilization and disintegration of cell membranes. The propagation cycle is broken by

enzymic inactivation of oxygen species or by non - enzymic reactions due to the intervention of free radical scavengers and antioxidants [16,17,18]. The coumarins are recognized as inhibitors of the pro inflammatory lipoxygenase and cyclooxygenase pathways of arachidonate metabolism.



For the coumarins (Table **2**) of [9] the following equation was derived:

Table 2. Hydroxylated Coumarins



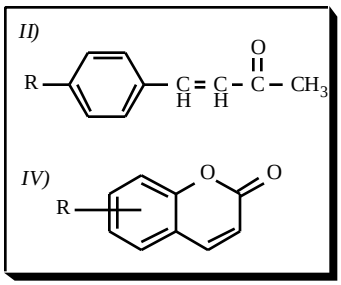
N	R		N	R	
1	4-OH		**7**	7,8-OH	6-OCH_3
2	7-OH		**8**	6,7-OH	
3	7-CH_3		**9**	6,7-OH	4-CH_3
4	7-OCH_3		**10**	7-OH	6-OCH_3
5	7-OH	4-CH_3	**11**	5,7-OH	4-CH_3
6	7-OCH3	4-CH_3	**12**	7,8-OH	
			13	7,8-OH	4-CH_3

$$\log LP\% = -2.969\ (0.725)\ \text{clog}\ P + 1.708\ (0.548)\ I_2 + 5.236\ (1.109) \quad (5)$$

$n = 13$, $r = 0.948$, $s = 0.364$, $F_{1,10} = 48.26$ ($\alpha = 0.01$), $F_{2,10} = 44.535$, $\alpha = 0.01$

Clog *P* accounts the overall lipophilicity of the molecule and is theoretically calculated according CLOG P program [19]. I_2 is an indicator variable, it takes the value of 1 for five compounds where R = 4 -CH_3.

The present results, show that within the set of phenyl-butenones (*ii*) and the natural-coumarins (*iv*), the most fundamental and common factor enhancing the superoxide scavenging and the lipid peroxidation are the hydrophilicity a) of substituents as $\sum n$ or b) the overall lipophilicity as Clog *P*. It seems that hydrophobicity of the substituents and /or of the molecules decreases the biological activities.



Generally for phenolic derivatives δ^+ values gives better correlations with radical reactions than δ or δ^-, if care is taken to include substituents whose δ^+ values differ significantly from δ as follows:

$$\log k = -0.71\delta^+ + 0.73 \quad (6)$$

$N = 7$, $r = 0.99$

$$\log k = -1.03\delta^+ + 2.82 \quad (7)$$

$n = 10$, $r = 0.885$

v) Anisidines

Komeshima *et al*. [20, 21] in order to search for anti inflammatory agents against autoimmune diseases, synthesized 4-alkylthio-o-anisidines possessing antioxidant activity. All of the listed compounds (Table 3) had nearly the same potency except methyl-thio derivative. This is a disadvantage in terms of QSAR.

$$\log 1/IC_{50} = -0.111\ (0.080)\ B_5 + 6.202\ (0.521) \quad (8)$$

$n = 8$, $r = 0.812$, $s = 0.232$, $F_{1,6} = 11.592$, $\alpha = 0.05$

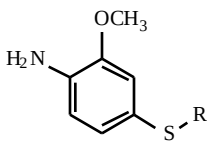
B_5 is the Verloop's [22] parameter for the width of the first atom of the substituent. In eq. 9 the inhibitory potency decreases as the width of the 4-alkylthio-group (B_5 variable) increases.

$$\log 1/IC_{50} = 2.739\ (1.452)\ E_s + 9.691\ (2.278) \quad (9)$$

$n = 8$, $r = 0.883$, $s = 0.218$, $F_{1,6} = 21.356$, $\alpha = 0.01$

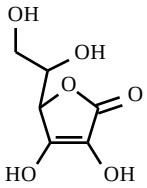
In eq. 9 [23] the E_s term for substituents para to -NH_2 does not formulate better correlation. However, it seems that the para-substituents depress the inhibition of the antioxidant activity as brought out by E_s. Since the values of E_s are all negative, the positive coefficient with E_s means steric hindrance (4-alkylthio- group).

Table 3. 4-Alkylthio-o-Anisidines



N	1	2	3	4	5	6	7	8	9	10	11	12
R	Me	Et	Pr	i-Pr	Allyl	Bu	i-Bu	Pent	Oct	Dec	Dodec	Benzyl

Table 4. 2-O-Alkyl-Ascorbic Acids



N	1	2	3	4	5	6	7
R	$(CH_2)_8Me$	$(CH_2)_9Me$	$(CH_2)_{10}Me$	$(CH_2)_{11}Me$	$(CH_2)_{12}Me$	$(CH_2)_{13}Me$	$(CH_2)_{14}Me$
N	8	9	10	11	12	13	
R	$(CH_2)_{15}Me$	$(CH_2)_{16}Me$	$(CH_2)_{17}Me$	$(CH_2)_{18}Me$	$(CH_2)_{19}Me$	$(CH_2)_{20}Me$	

$\log 1/IC_{50} = 0.945\ (0.543)\ \text{clog}\ P - 0.092\ (0.092)\ \text{clog}\ P^2 + 3.173\ (1.166)$ (10)

n = 9, r = 0.873, s = 0.156, Clog P_o = 5.146 (range from 4.744 to 5.843), $F_{2,6} = 9.61$, $\alpha = 0.05$

For the same data in eq. 10 the log *P* term accounts for 76% of the variance in the data but it is of special interest that the coefficient with the linear term log *P* is in the range (0.5 to 1.1) normally found for inhibitors in cell culture. For the calculation of the Clog *P* values the pKa values of the $-NH_2$ group of the o-anisidines were not considered, whereas π and B_5 are highly collinear.

vi) Ascorbic Acid Derivatives

For a series of ascorbic acid derivatives (Table **4**) [21,24], eq. 11, a parabolic relationship has been derived.

$\log 1/IC_{50} = 0.450\ (0.225)\ \text{Clog}\ P - 0.031\ (0.021)\ (\text{Clog}\ P)^2 + 3.721\ (0.503)$ (11)

r = 0.920, s =0.107, $F_{2,8} = 21.913$, $\alpha = 0.01$

Clog P_o = 6.458 (range from 5.907 to 7.899)

Log *P* and the size of substituents are perfectly collinear. Since the correlation with Clog *P* involves large changes in size (7C-20C carbon atoms as multiple methylenic groups in

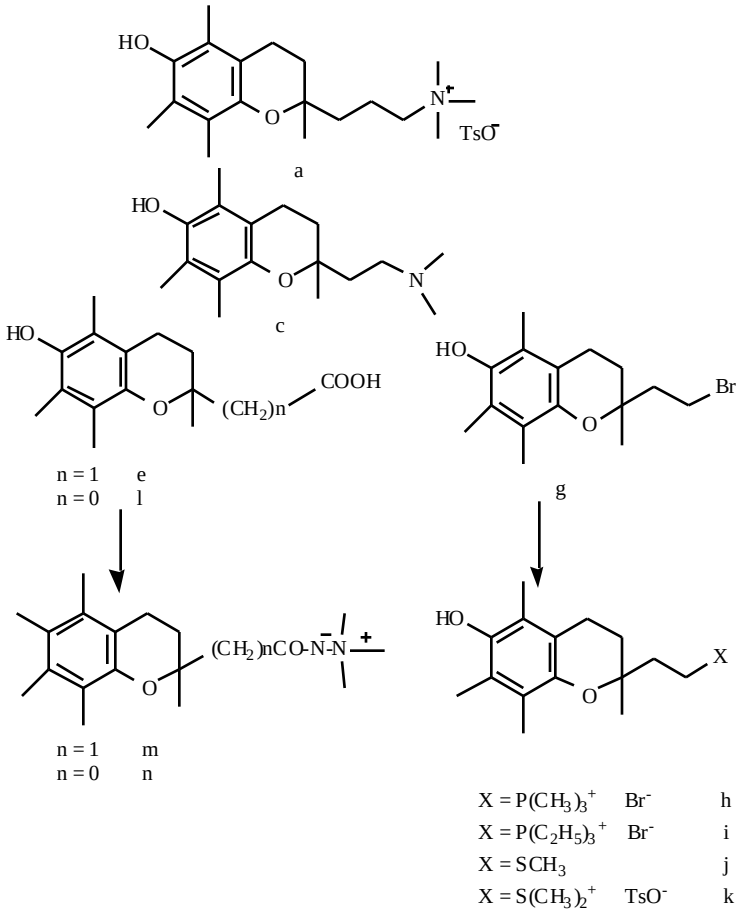


Fig. (1). α- Tocopherol Analogues.

a long chain), it is assumed that the size of R plays a major independent role. The activity decreases as the length of the alkyl chain at the 2-OH group increases. Equation 11 is the best fitted correlation. C log *P* - calculated log *P* values were used for the hydrophobicity of the whole molecule (neutral form), rather than π for substituents. Note that the calculated log *P* values for this set are unusually high 2.23 to 8.57. These calculated values could be somewhat high since they pertain to be in unprotonated form. At pH 7.4 log *P* would be different depending on the pKa of the enolic hydroxyl group at the 3-carbon of the ascorbic acid. An optimum range of Clog *P* was observed. Length (especially 17-CH_2-groups) and lipophilicity of the alkyl groups are essential for the inhibitory effect on lipid peroxidation in rat brain homogenates. A lipophilic moiety at the 2-OH carbon inhibits lipid peroxidation, by anchoring these molecules in the cellular and microsomal membranes and by reducing their ability to chelate with the metal ions (iron ions) existing in the extracellular site. However the inhibitory activity appears to decrease by an insufficient or excessive hydrophobicity. Lipid peroxidation is closely related to membrane fluidity [25]. Furthermore too high hydrophobicity of the compounds results in the decreased mobility of the radical scavenger in the lipid bilayer. Both superoxide anion radical and hydroxyl radical are highly hydrophilic and would localize in aqueous compartments. It may be that the extremely hydrophobic 2-O-alkylated ascorbic acids are localized in lipophilic sites so that the radicals being reduced must come by it.

In both data no role for the electronic effect of the substituents has been found as R were simple aliphatic alkyl

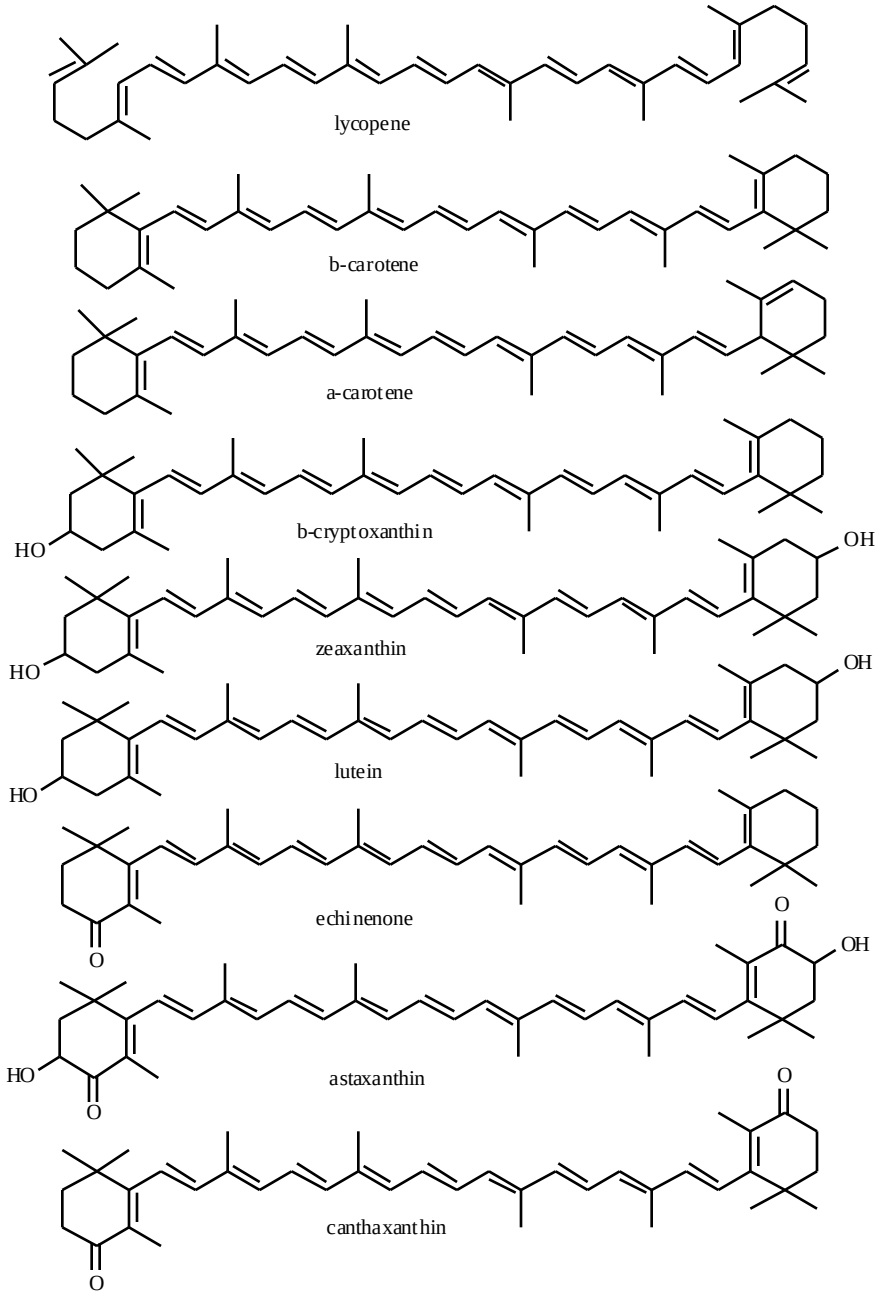


Fig. (2). All-trans carotenoids.

groups. Hydrophobicity as overall Clog *P* was the most significant physicochemical parameter. One could expect with whole cells to see a hydrophobic interaction of all parts of the molecule because of its importance in penetrating the membranes. The hydrophobic scavengers are well suited to neutralize hydrophilic radicals such as hydroxyl radical and vitamin E is well suited to scavenge hydrophobic radicals The more hydrophobic the better the scavenger other factors being equal. In opposition of this is the fact that the more hydrophobic the compound the more readily it is metabolized.

A parabolic relationship between k' values (k' hydrophobicity as obtained by HPLC using C_8- reversed phase column) and the anti-lipid peroxidative activity by 3-O alkylated ascorbic acid derivatives has also been reported [25].

vii) α-Tocopherol Analogues

The α- tocopherol analogues, in which the lipophilic phytyl ($C_{16}H_{33}$) side chain was replaced by a hydrophilic trimethylethanaminium moiety, was found to reduce myocardial infarct size in rats. Analogues of α- tocopherol and ascorbic acid with permanently cationic substituents eg. phosphonium, sulfonium, acylhydrazinium and ammonium were found to scavenge lipoperoxyl and superoxide radicals *in vitro* [26, 27].

For 17 tested α- tocopherol analogues (Fig. **1**), the following eq. was derived.

$$\log 1/IC_{50} = 12.401\ (7.125)\ L_x - 1.598\ (0.871)\ Lx^2 - 18.895\ (14.361) \quad (12)$$

$n = 16$, $r = 0.905$, $r^2 = 0.818$, $s = 0.200$, $F_{2,13} = 29.287$, $a = 0.01$, L_x optimum = 3.881(0.125) from 3.612 to 3.973

In Eq. 12 the parabolic dependence of L_x (the sterimol parameter of Verloop for the length of the first atom of substituent X) provides an optimum at 3.881. No role for an electronic factor was found.

QSARs are described for the antioxidant activity of a series of all-trans carotenoids: lycopene, β-carotene, α-carotene, β−cryptoxanthin, zeaxanthin, lutein, echinenone, astaxanthin, canthaxanthin (Fig. **2**) [28]. Their antioxidant activity is characterized by literature data: a) for their relative ability to scavenge the 2,2-azinobis (3-ethylbenzothiozoline-6-sulphonic acid)diazonium salt radical cation, reflected by the trolox equivalent antioxidant capacity (TEAC)value, b) their relative rate of oxidation by a range of free radicals, or c) their capacity to inhibit lipid peroxidation in multilamellar liposomes, leading to a decrease in formation of thiobarbituric acid reactive substances (TBARS). All the above antioxidant values for radical scavenging activity were correlated quantitatively with computer –calculated ionization potentials of the carotenoids. In all cases excellent correlations are obtained between: E_{HOMO} and TEAC ($r = 0.914$) TBARS: $r = 0.947$, the rate of carotenoid degradation by Fenton radicals: $r = 0.947$, peroxyl radicals: $r = 0.960$ and by phenoxyl radicals: $r = 0.992$. In line with theory, the plots show that an increased ionization potential decreases the rate of radical scavenging by the carotenoids.

viii) Redox Potentials of Substituted Phenols

Redox potentials of substituted phenols (Table 5) [29] at pH = 7 and calculated physicochemical parameters are used in the derivation of eq. 13.

$$E_7 = 0.249\ (0.087)\ H_{fr}/10 - 0.240\ (0.087)\ H_{fp}/10 + 0.357\ (0.200)E_{lumo\text{-}r} - 0.106\ (0.053)\ OH - 1.952\ (0.480) \quad (13)$$

$n = 31$, $r^2 = 0.914$, $s = 0.074$, $F_{4,26} = 69.49$, $p < 0.0005$

H_{fr} and H_{fp} are the heats of formation of phenoxyl radicals and the corresponding parent phenols, respectively. $E_{lumo\text{-}r}$ refers to the energy of lowest unoccupied molecular orbital of radicals. The number of hydroxyl group makes a negative contribution to redox potentials, suggesting that additional hydroxyl groups make phenols better antioxidants.

ix) Vitamin E Derivatives

AR and QSAR studies of vitamin E derivatives have been reported by several groups [30,31]. The following QSAR analysis on vitamin E derivatives was performed using calculated parameters.

$$\log (k_s \times 10^3) = -0.516\ (0.25)\ \Delta H_f/10 + 1.035\ (0.975)\ E_{homo} + 19.290\ (4.680) \quad (14)$$

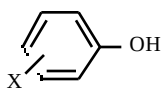
$n = 22$, $r^2 = 0.886$, $s = 0.164$, $F_{2,10} = 73.78$, $p = 0.0005$, $F_{1,19} = 4.96$, $p < 0.05$

Correlation parameters which are often used in QSAR studies with vitamin E are Hammett σ or Brown σ^+ of the substituents attached to the phenol ring.

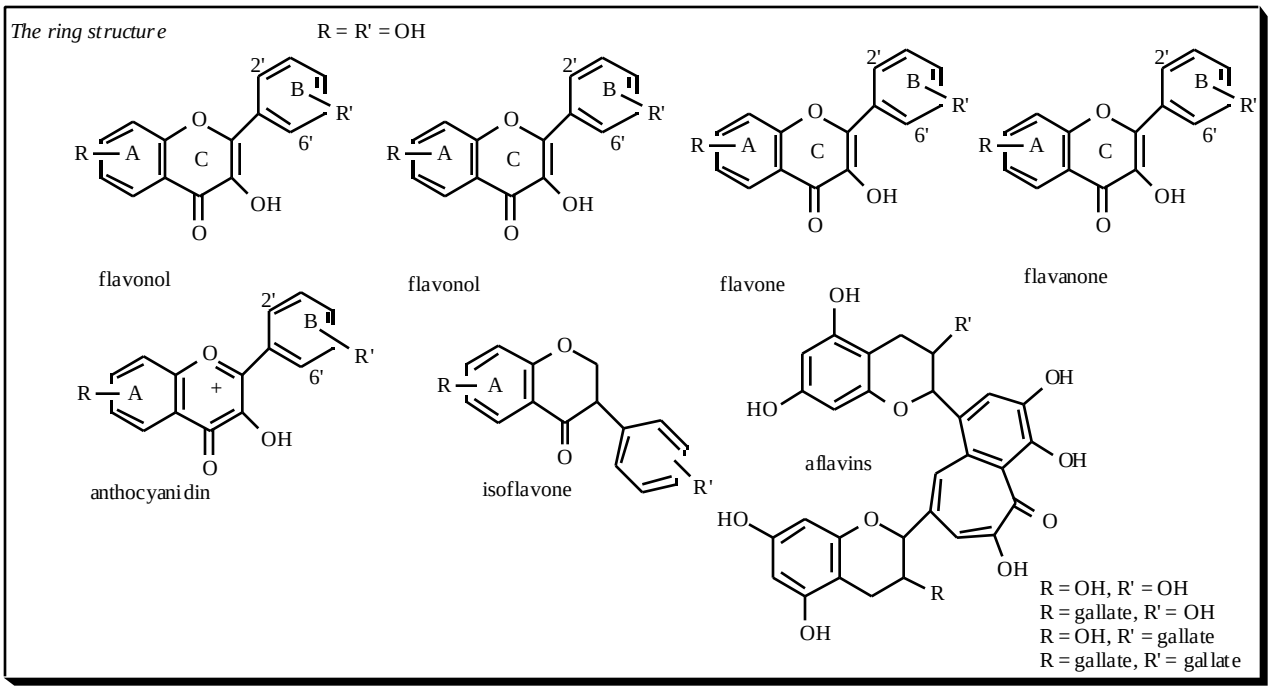
x) Flavonoids and Related Phenolic Compounds

Flavonoids and related phenolic compounds have a variety of physiological and pharmacological functions. Structure-activity relationship studies of flavonoids have shown that the dissociation of the hydroxyl functions occurs

Table 5.



1	2	3	4	5	6	7	8	9	10	11	12	13
3,5-Cl_2	4-CF_3	3-NO_2	4-PhCO	3-CN	3-CH_3CO	3-CH_3	3,5-$(CH_3)_2$	4-Ph	2-CH_3	4-t-Bu	2-O CH_3	2,6-$(CH_3)_2$



in the following sequence: 7- > 4'- > 5- positions. The presence of: o-dihydroxy structure in the B ring, the 2,3-double bond in conjugation with the 4-oxo function in the C ring and the 3- and 5- OH groups with the 4-oxo function in A and C rings are essential for effective free radical scavenging activity. Many authors have attempted to elucidate the QSAR of antioxidants by using different physicochemical parameters like the heat of formation (H_f), the number of hydroxyl groups (OH) and Hammett σ or Brown σ^+. Due to the limited number of antioxidants used in regression analysis and unavailable σ or σ^+ values of complex substituents the antioxidant activities and redox potentials can not be easily predicted from either the structures or other physicochemical properties Trolox equivalent antioxidant capacity (TEAC) has been used to determine the hierarchy of radical scavenging abilities of flavonoids as electron or H-donating agents through measuring their ability to scavenge radical cation 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid).

$$TEAC = 0.59\ (0.069) + 0.322\ (0.264)\ I - 0.17\ (0.505) \quad (15)$$

$n = 39,\ r^2 = 0.845,\ s = 0.574,\ F_{2,36} = 98.29,\ p < 0.0005$

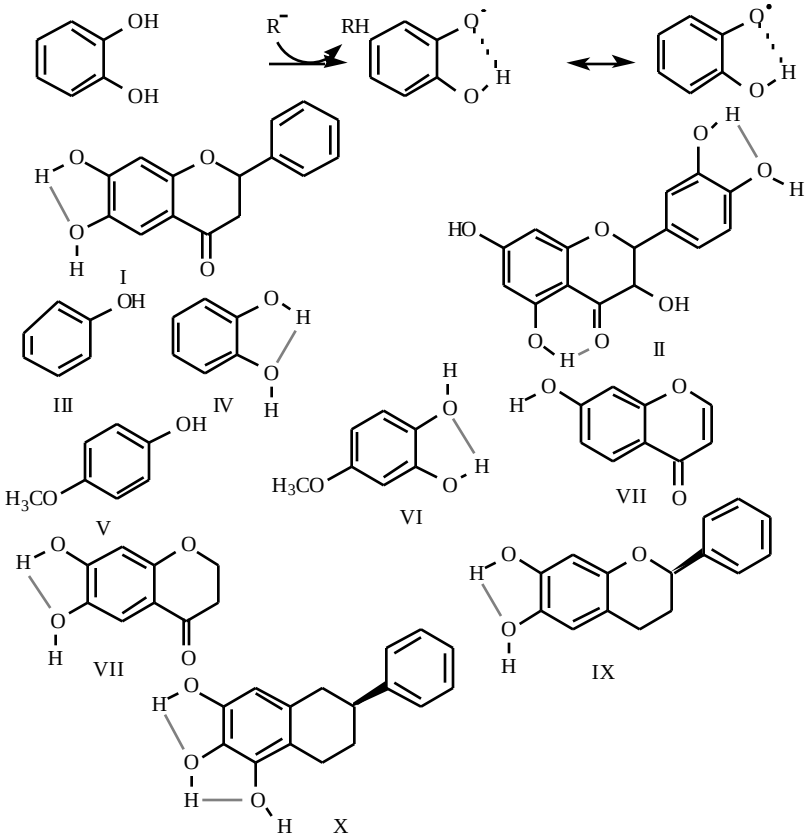
As demonstrated by eq. 15, the number of free phenolic OH groups makes a statistically significant contribution to TEAC. It has been reported that antioxidant activity of natural flavonoids is governed by the number and location of their aromatic hydroxyl groups [32]. Cao *et al*. [33] have reported that the relationship between peroxyl radical absorbing activity and the number of hydroxyl groups in flavonoids is linear for flavones and curvilinear for flavanones. Addition of an indicator variable I representing individually the presence (1) or absence (0) of the 2,3 double bond does not significantly improve the regression result. The derived equation include different members of flavonoids including flavanol, flavonol, flavanolol, flavanone, flavone, isoflavone, anthocyanidin and aflavins. The large standard deviation of 0.574 undoubtedly produces a large error when TEAC values are estimated using the above equation. However, this equation may be useful in explaining the physiological contribution factors to the antioxidant activity and in estimating the TEAC values and potential biological activities of flavonoids.

xi) Thiazolidinones

Thiazolidinones represent a novel class of calcium ion antagonists showing both Ca^{2+} overload inhibition and antioxidant activity [34]. In order to elucidate the structure-antioxidant relationships for thiazolidinones a set of phenolic analogues were contructed by adding various substituents to the phenol. The ILDL values used are the inhibition of soybean lipoxygenase-induced rabbit low-density lipoprotein LDL peroxidation % *in vitro* by a specified amount of thiazolidinones. The bond dissociation energy (kcal/mol) defined as BDE, was calculated employing ab initio molecular mechanics MMX method and full geometry optimization using the AM1 method. The lower the O-H bond dissociation energy, the higher was the antioxidant activity. The O-H BDEs of the phenols were determined mainly by the resonance effect and to a lesser extent by the field/inductive effect.

The established results were:

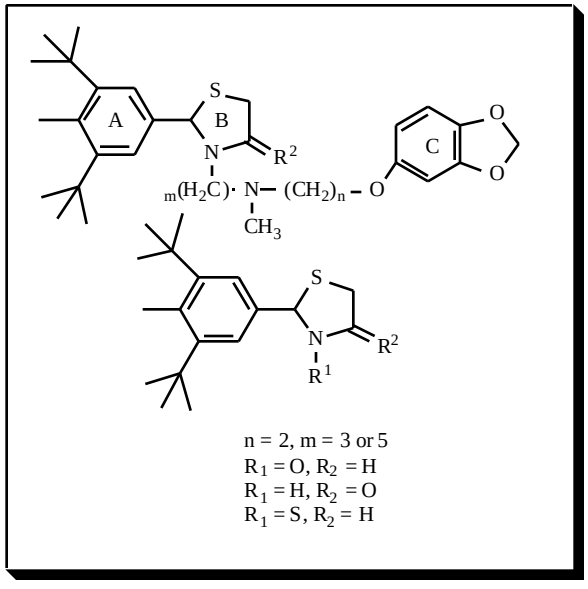
- t-Bu groups at the o-positions of OH were critical to lower the O-H BDE
- the existence of atoms N, S or O in the ring B increases the O-H BDE, which is non favorable for enhancing the antioxidant activity of thiazolidinones
- The length of the alkyl group adjacent to atom N of ring B has little effect on the O-H BDE of the compounds
- When O in ring B is replaced by S, the O-H BDE will increase.

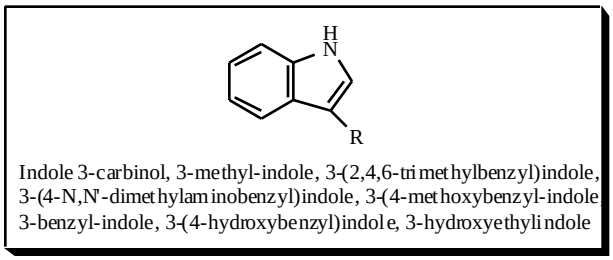


(Fig. 3) Stabilizing mechanisms of catecholic free radical. Molecular structures of 6,7-dihydroxyflavone (I), quercetin(II) and model structures.

xii) Indolyl Analogues Structure

Eight structurally related 3-indolyl compounds were subjected to a molecular modeling study [36] in order to develop novel chemoprotective indoles with improved activity. The IC_{50} values of the indolyl analogues required for 50% *in vitro* lipoxidation of purified soybean phospholipid vesicles were used. Indolyl 3-carbinol and related derivatives are known to be chemoprotective in toxicity and carcinogenicity assays.





The following linear equations were calculated.

$$pIC_{50} = 3.02\ \Delta Hf + 1.36 \quad (16)$$

$n = 8, r = 0.88, p = 0.004$

$$pIC_{50} = 0.21\ Z\text{-}dim + 2.44 \quad (17)$$

$n = 8, r = 0.84, P = 0.009$

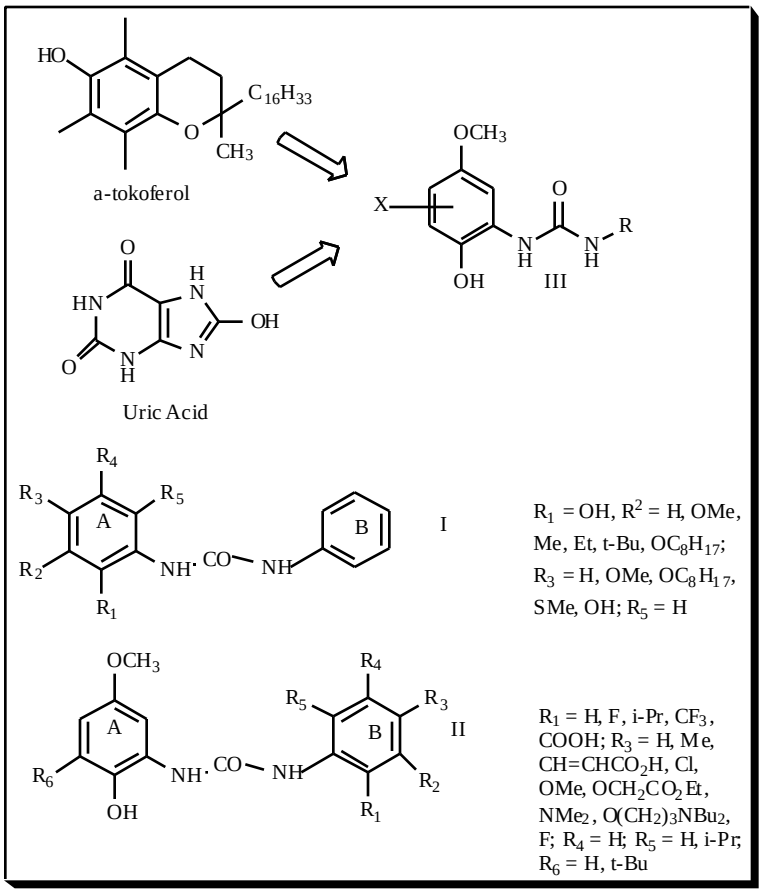
$$pIC_{50} = 2.09\ \Delta Hf + 0.8\ Z\text{-}dim + 1.54 \quad (18)$$

$n = 8, r = 0.90, P = 0.016$

$$pIC_{50} = 4.85\ \Delta Hf - 0.49\ Z\text{-}dip + 0.44 \log P + 0.004\ MW - 1.48 \quad (19)$$

$n = 8, r = 0.99, P = 0.013$

Log P was calculated using the ChemPlus software (hypercube), ΔH_f is the difference in the heat of the parent molecule and the cation radical produced by electron abstraccity and camol) expressing the energy of electron abstraction between the parent molecule and the cation radical, Z-dim is the longest dimension of the smallest periodic box that may surround a molecule (Å), Z-dip the



dipole moment in that dimension (D) and MW is the molecular weight.

From the above correlations it is obvious that antioxidant potency of the indolyl analogs was most strongly determined by the molecular size parameters and by the ΔHf. The developed equations could be used to predict the antioxidant potencies of potential chemoprotective compounds.

xiii) Phenylureas

Novel 2-hydroxy-5-methoxyphenylureas of types I, II and III were designed and synthesized by combining the structural features of the natural antioxidant α-tocopherol and uric acid [37].

For structure III R_1 = all the above mentioned substituents (I + II) and R_2 = H, t-Bu, OMe

The novel analogues showed high inhibitory activity against lipid peroxidation. QSAR of the urea derivatives yielded significant linear regression equations using electronic and steric effects of substituents on the phenolic hydroxyl group.

$$pIC_{50} = -0.59\ (\pm 0.41)\ \Sigma\sigma^{+} - 0.24\ (\pm 0.23)\ \Sigma Es(AMD) + 4.98\ (\pm 0.68) \quad (20)$$

$n = 14,\ r = 0.80,\ s = 0.30,\ F_{2,11} = 9.9$

$$pIC_{50} = -1.20\ (\pm 0.48)\ \Sigma\sigma^{+} - 0.14\ (\pm 0.07)\ \Sigma Es(AMD) - 1.17\ (\pm 0.28)\ I_{COOR} + 5.25\ (\pm 0.35) \quad (21)$$

$n = 52,\ r = 0.88,\ s = 0.26,\ F_{3,48} = 56.8$

$$pIC_{50} = 1.01\ (\pm 0.57)\ R(O_{phenol}) - 0.20\ (\pm 0.07)\ \Sigma Es(AMD) - 1.16\ (\pm 0.31)\ I_{COOR} + 5.26\ (\pm 0.46) \quad (22)$$

$n = 54,\ r = 0.85,\ s = 0.30,\ F_{3,50} = 41.9$

I_{COOR} indicator variable 1 for the presence of carboxylic groups, $\Sigma\sigma^{+}$ combined electronic parameter defined as the sum of the σ^{+} for meta and para for the ortho substituents, E_s(AMD) constants representing the steric effect of ortho substituents on the phenolic hydroxy derived from the rate constants for the acidic hydrolysis of ortho substituted benzamides, $R(O_{phenol})$ electron-releasing reactivity index of phenolic oxygen.

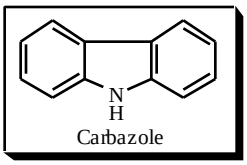
From the eqs 20-22 was indicated that an increase in the electron donating potency of substituents toward the phenolic hydroxyl group enhances the antioxidation activity by stabilization of an electron deficient radical type transition state. The derivatives containing a carboxyl group possess low activity as a result of an intermolecular ion-dipole interaction of the phenolic hydroxyl group with the carboxylate anion, which can retard the formation of the transition state.

xiv) Carbazoles

For a group of carbazoles eq. 23 was derived [38]

$$\log 1/C = 3.21\ (\pm 1.9)\ \varepsilon_{HOMO} + 3.05\ (\pm 0.16) \quad (23)$$

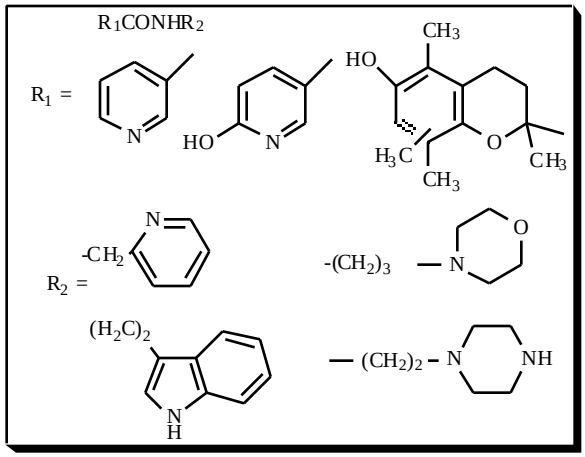
$n = 6,\ r = 0.916,\ s = 0.017,\ F_{1,4} = 21.2$



C is the concentration of carbazole given orally to mice required to inhibit lipid peroxidation by 50% compared to control, ε_{HOMO} the energy on the highest occupied molecular orbital.

xv) Radical Scavengers by 3-D Pharmacophore Generation

Three-dimensional QSAR studies were performed [39] for 13 radical scavengers, by 3-D pharmacophore generation (Apex –3-D) and CoMFA techniques. Two classical models with predictive cross-validated r^2 (q^2) over 0.96 indicated that the activity was attributed to the electronic C_{OH} and E_{LUMO}, steric molar refractivity (MR) and lipophilic log P. For Apex –3-D studies, two best models with high q^2 (0.94 and 0.97) were yielded. Structural properties contributing to the activity were not only lipophilic but also the optimum steric property and geometry of side-chain composition. For CoMFA studies the *sp*^{3} *C* (+1) probe provided the best q^2 of 0.79 with steric and electrostatic contributions of 42.3 and 57.7 % respectively.



$$\log (\% \text{ inhibition}) = 0.019 (\pm 0.049) \log P + 0.418 (\pm 0.202) E_{LUMO} - 12.878 (\pm 0.675) C_{OH} - 1.815 (\pm 0.154) \quad (24)$$

$n = 14$, $r^2 = 0.986$, $s = 0.266$, $F = 233.612$, $q^2 = 0.962$, Spress = 0.369

$$\log (\% \text{ inhibition}) = 0.005 (\pm 0.003) MR + 0.373 (\pm 0.179) E_{LUMO} - 12.624 (\pm 0.558) C_{OH} - 2.214 (\pm 0.282) \quad (25)$$

$n = 14$, $r^2 = 0.989$, $s = 0.202$, $F = 293.065$, $q^2 = 0.974$, Spress = 0.307

$$\log (\% \text{ inhibition}) = 0.007 (\pm 0.0) \log P + 0.022(\pm 0.001) E_{HOMO} - 2.779 (\pm 0.015) C_{OH} - 1.213 (\pm 0.003) \quad (26)$$

$n = 5$, $r^2 = 1.00$, $s = 9.44 \times 10^{-5}$, $F = 103560.591$, $q^2 = 0.963$, Spress = 0.180

CONCLUSIONS

We believe that this report and others like it [29, 33, 37, 39] are the beginning of a new focus in QSAR of antioxidants. Despite all of the investigations of antioxidants radical toxicity has been missed because the studies did not contain the proper substituents to bring it out. Our review illustrate the diagnostic value of Hammett constants and redox potentials, as well as various physicochemical parameters calculated with well-accepted programs [19,37]

From all models it was apparent that the descriptors that affected the electronic property of the molecule are most important for the design of radical scavengers. Steric descriptors that affected the electrostatic property or electron transfer of the molecule are found to be significant. However lipophilicity is also a significant factor that should be included in the molecular design to assure the availability of the drug at the target site.

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