

Theoretical and Experimental Studies on the Structure-Antioxidant Activity Relationship of Synthetic 4-Methylcoumarins

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The development of antioxidants as useful drugs for the treatment of neurodegenerative diseases such as Alzheimer's is extremely challenging in medicinal chemistry. Coumarins have attracted great attention as possible therapeutic tools against oxygen radicals in human degenerative diseases. In order to establish the possible structure-antioxidant activity relationship, a series of twenty four 4-methylcoumarin derivatives were examined by employing reducing power measurements, and comparison with bond dissociation enthalpy and ionization potential calculations. Based on the reducing potency of 4-methylcoumarin derivatives with respect to trolox, these compounds were classified into five groups as "most active", "more active", "moderately active", "less active" and "inactive" derivatives. The presence of hydroxyl groups is an essential requirement for the activity, and substitution of hydroxyl groups by methoxy groups leads to non-active derivatives. The results revealed that dihydroxyl groups in the *ortho* position show a better antioxidant activity with respect to dihydroxyl groups in the *meta* position. This is ascribed to the ability to construct more stable 4-methylcoumarin radical intermediates by rearrangement of intra-molecular hydrogen bonding. Our findings indicate that other important factors to enhance the antioxidant activity of coumarins are the number of hydroxyl groups, the presence of ester substitutions and a thiono functional group on the pyrone ring. However, bond dissociation enthalpy and ionization potential calculations alone are not sufficient to identify the best antioxidant structures. As a result, chemical and functional properties of molecules such as 4-methylcoumarins should be examined as a whole entity, considering all substitutions versus a single substitution to design functional compounds with good antioxidant activity.

Keywords: Coumarins, Reactive oxygen species, Antioxidant, Reducing power, Free radical, B3LYP method

INTRODUCTION

Coumarins (1,2-benzopyrone or *o*-hydroxycinnamic acid-

8-lactone) consist of fused benzene and α -pyrone rings, and are a group of natural phenolic compounds widely present in plants [1]. They have multiple biological activities including pharmacological effects [2] and they may be beneficial in different human diseases such as cancer, burns, brucellosis,

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cardiovascular and rheumatic diseases [3]. They also possess unique antioedema and anti-inflammatory activities [4], are capable of scavenging reactive oxygen species (ROS), and show protective effects in different cellular types, including models of amyloid β -peptide toxicities with respect to ROS damage [5,6], and they also protect against cytotoxicity induced by linoleic acid hydroperoxide [7]. Oxidative stress is an early and critical event in several neuropathologies [8] consisting in the chronic accumulation of ROS in different tissues leading to the onset and progression of Alzheimer's disease and other degenerative illnesses [9,10]. A decrease in ROS levels may be beneficial in the cure of neurodegenerative diseases [11].

Many different types of coumarins endowed with antioxidant activity have been reported; however, it has not yet been possible to elucidate the correlation between chemical structure and function [4,12,13]. Moreover, the tendency to form mutagenic and toxic 3,4-coumarin epoxide intermediates during metabolic degradation of coumarins has limited the pharmacological application of coumarins [14]. Designing different derivatives of coumarin as new drugs may be a good strategy to overcome this problem. There are many possible permutations through substitution and conjugation at any of the six available positions of coumarin molecules to produce different coumarin derivatives (Fig. 1).

Among different coumarin derivatives, 4-methylcoumarins have many advantages, for example, they are not substrates for the liver P-450 monooxygenase to metabolize mutagenic intermediates [15,16,17]. In addition, the series of 4-methylcoumarin derivatives that are reported in Table 1 are considered to have several beneficial pharmacological effects [18,19]. Chemical and biological effects of 4-methylcoumarin derivatives have been widely studied, and this is mainly due to the properties related to their highly free radicals/oxidant scavenging activity [20-22]. Recently, 24 derivatives of 4-methylcoumarins (C1-C24 in Table 1) were tested by our group using electron paramagnetic resonance (EPR) spectroscopy and measuring the kinetics of their reaction with two known standard radicals including galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [20]. The study of the antioxidant activity is an exciting challenge from both experimental and theoretical viewpoints. A comprehensive knowledge of the chemical and functional properties and antioxidant activities of methylcoumarin derivatives could

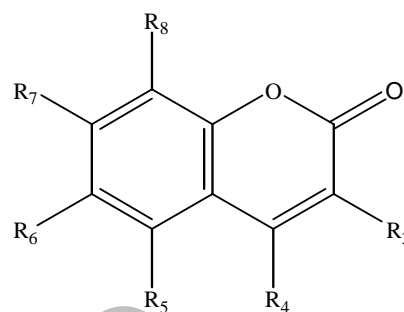


Fig. 1. The coumarin structure. Various potential permutations at any of the six possible positions (R_3 - R_8) lead to different coumarinic derivatives.

help the strategies for designing non-toxic coumarins with antioxidant activity. In this paper, we have studied structure-antioxidant activity relationship of new 4-methylcoumarin derivatives by a double approach: experimental through reducing power assessment, and theoretical using the computational density functional theory (DFT) for the calculation of quantum chemical features [23-25].

MATERIALS AND METHODS

Materials

All 4-methylcoumarins and 4-methylthionocoumarins were synthesized and characterized at the Department of Chemistry of the University of Delhi as described previously [14,20,26,27]. Trichloroacetic acid (TCA), potassium ferricyanide and ferric chloride were purchased from Merck.

Reducing Power

Reducing power assessment was based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} . The reducing power of 4-methylcoumarins was determined by analyzing their electron-donating potency according to the method of Oyaizu [28]. Potassium ferricyanide (1%) and 1:1 volume ratio of phosphate buffer saline (PBS) with different concentrations of 4-methylcoumarins (10, 25, 50, 75, 100 μ M) were used. Also trolox (TroH) was used as a strong reducing agent and a known "standard antioxidant" [29] in the same conditions to compare and arrange the antioxidant potency of these new 4-methylcoumarin derivatives into different groups. The samples were incubated for 30 min at 50 $^{\circ}$ C and then half volume

Table 1. Numbering of the 4-Methylcoumarins Studied (24 Derivatives, C1-C24)^a

Compound	R ₃	R ₅	R ₆	R ₇	R ₈
C1	CH ₂ COOCH ₂ CH ₃	OH	H	OH	H
C2	H	OH	H	OH	H
C3	CH ₂ CH ₂ COOCH ₂ CH ₃	H	H	OOCCH ₃	OOCCH ₃
C4	CH ₂ CH ₂ COOCH ₂ CH ₃	OOCCH ₃	H	OOCCH ₃	H
C5	H	H	H	OOCCH ₃	OOCCH ₃
C6	CH ₂ COOCH ₂ CH ₃	H	H	OOCCH ₃	OOCCH ₃
C7	CH ₂ COOCH ₂ CH ₃	OOCCH ₃	H	OOCCH ₃	H
C8	CH ₂ COOCH ₂ CH ₃	H	H	OH	OH
C9	H	H	OH	OH	H
C10	CH ₂ CH ₂ COOCH ₂ CH ₃	H	OH	OH	H
C11	CH ₂ CH ₂ COOCH ₂ CH ₃	OH	H	OH	H
C12	CH ₂ CH ₂ COOCH ₂ CH ₃	H	H	OCH ₃	OCH ₃
C13	H	OCH ₃	OOCCH ₃	OCH ₃	H
C14	CH ₂ CH ₂ COOCH ₂ CH ₃	H	OCH ₃	OCH ₃	H
C15	H	OCH ₃	H	OCH ₃	H
C16	CH ₂ COOCH ₂ CH ₃	H	H	OCH ₃	OCH ₃
C17	CH ₂ CH ₂ COOCH ₂ CH ₃	H	H	OH	OH
C18	CH ₂ COOCH ₂ CH ₃	OCH ₃	H	OCH ₃	H
C19	H	H	H	OCH ₃	OCH ₃
C20	H	H	H	OH	OH
C21 ^b	H	H	H	OOCCH ₃	OOCCH ₃
C22 ^b	H	H	H	OH	OH
C23 ^b	H	H	H	OOCCH ₃	H
C24 ^b	H	H	H	OH	H

^aPermutations at the six possible positions (R3-R8) of coumarin. All of them belong to the serie of methylated R₄ group and the other five available positions (R₃, R₅-R₈) are specified. ^b4-Methylthionocoumarins.

of TCA (10%) was added. After adding 200 μ l of ferric chloride (0.1%) to the final volume of 1.5 ml, the absorbance at 700 nm was recorded. The reduction of Fe³⁺ by antioxidants in the samples led to an increase in absorbance at 700 nm [30]. Hence, the absorbance of the mixture increased with increasing the reducing power of 4-methylcoumarins.

Quantum Chemical Calculations

In order to rationalize 4-methylcoumarin antioxidant behavior measured in the experimental stage of the study, we carried out quantum chemical calculations to determine both

the molecular geometry and stability (energetic properties or antioxidant related parameters) of these molecules. To this end, bond dissociation enthalpy (BDE) and ionization potential (IP) of the 24 compounds were calculated at two computational levels denoted as (RO)B3LYP/6-311+G(2d, 2p)//AM1/AM1 and B3LYP/6-31+G(d)//AM1/AM1 [31,32], respectively, which have the advantages of accuracy and economy. Previous studies [31,32] have demonstrated that these methods and basis sets are effective in calculating reliable geometries, energetic, and antioxidant parameters of such systems. Full details regarding the calculation procedures

are given in the following section.

Computational details. During the calculation of BDEs, the geometry optimization and the determination of vibrational frequencies were carried out using the semiempirical AM1 method [31]. Then, single-point electronic energies (SPEs) were obtained by DFT methods using (RO)B3LYP functional [23-25] at 6-311+G(2d,2p) level. Employing the molecular enthalpy in the gas-phase at 298.15 K, which consists of (RO)B3LYP/6-311+G(2d,2p)-calculated SPE, AM1-calculated zero point vibrational energy (ZPVE, scaled by a factor of 0.973) [33], vibrational contribution to energy (scaled by a factor of 0.973) [34], translational, rotational, and PV-work terms, hydroxyl BDE equals $H_r + H_h - H_p$, in which, H_r is the enthalpy of the radical generated after H-abstraction reaction, H_h is the enthalpy of the hydrogen-atom, -0.50216 hartree, and H_p is the enthalpy of the parent molecule. During the calculations of IPs, B3LYP functional at 6-31+G(d) level was used to calculate SPE on the basis of AM1-optimized structures. Thus, the molecular energy (E) in the gas-phase consists of B3LYP/6-31G+(d)-calculated SPE and AM1-calculated ZPVE (scaled by a factor of 0.973) [35]. The ionization potential was defined as $IP = E_c - E_p$, where E_c is the energy for the cation radical and E_p is the energy for the parent molecule. All of the quantum chemical calculations were carried out by the Gaussian 98 program [36].

RESULTS AND DISCUSSION

Reducing Power Potency of 4-Methylcoumarin Derivatives

The ability of a compound to effect the reduction of Fe(III) to Fe(II) is a significant indicator of its antioxidant activity [30,37,38]. Based on this reaction, we examined the potential antioxidant activity of 24 different derivatives of 4-methylcoumarins and 4-methylthionocoumarins. For all these compounds we measured the relationship between coumarin concentrations (10, 25, 50, 75 and 100 μ M) and the amount of Fe^{3+} reduced in the samples (Fig. 2). Since a linear correlation, with high R^2 values, was found between 4-methyl(thiono) coumarin concentrations and reducing power, we used the slope of lines to evaluate and compare their antioxidant potency (Fig. 2). We have divided these compounds into five groups based on their reducing potency with respect to TrOH.

According to this system, there are 5 coumarins classified as "most active" compounds (2 times more active than TrOH), 5 derivatives are "more active" (1.2-1.4 times more active than TrOH), 3 derivatives are "moderately active" (having activity similar to $TrOH \pm 0.15$), 3 derivatives are classified as "less active" (within the range of 0.3-0.7 of the activity of TrOH), while 9 compounds are "inactive" derivatives. To summarize, 15 derivatives of the newly designed coumarin derivatives were active antioxidants to some degree, while only 9 compounds out of 24 were inactive (Table 2).

These findings are consistent with our previous work on in vitro and intracellular ROS scavenging potency of some of the above mentioned compounds [20], confirming the validity of the proposed strategy to acquire information about antioxidant activity of 4-methylcoumarins.

A comparison of the chemical structures of the 24 compounds (C1-C24) in Fig. 3 shows that the compounds with two hydroxyl groups in *ortho* position are much more active than their *meta* substituted counterparts. In fact, all of the four most active compounds (C8, C10, C17 and C22) are *ortho* substituted dihydroxy compounds. It has been shown previously that dihydroxy-4-methylcoumarins produce dramatic inhibition of lipid peroxidation [17]. The excellent radical scavenging ability of the *ortho*-dihydroxy-derivatives of 4-methylcoumarins can be explained by the fact that the *ortho*-dihydroxy system is able to form a resonance-stable radical able to reduce oxy-compounds (see next section). A comparison of "most active" derivatives with "more active" compounds indicates that not only the presence of two hydroxyl groups in *ortho* position is an important feature to increase the antioxidant potency of these new drugs, but also ethylacetate or ethyl propionate groups in position 3 impose a higher activity. The experimental results also indicate that "more active" derivatives have *meta* substituted -OH groups (C1, C2, C11) and/or *ortho* substituted -OH groups without any additional substitution in position 3 (C20, C9). These results reveal two main factors important to enhance antioxidant behavior of 4-methylcoumarins; dihydroxy substitution in *ortho* position and an ester substitution in the pyrone ring. However, our data indicate that -OH substitution in *ortho* position is more important than the ester substitution. Our data also show that the dihydroxy-thiono-compound is more active than the oxo-compound analogue (compare the

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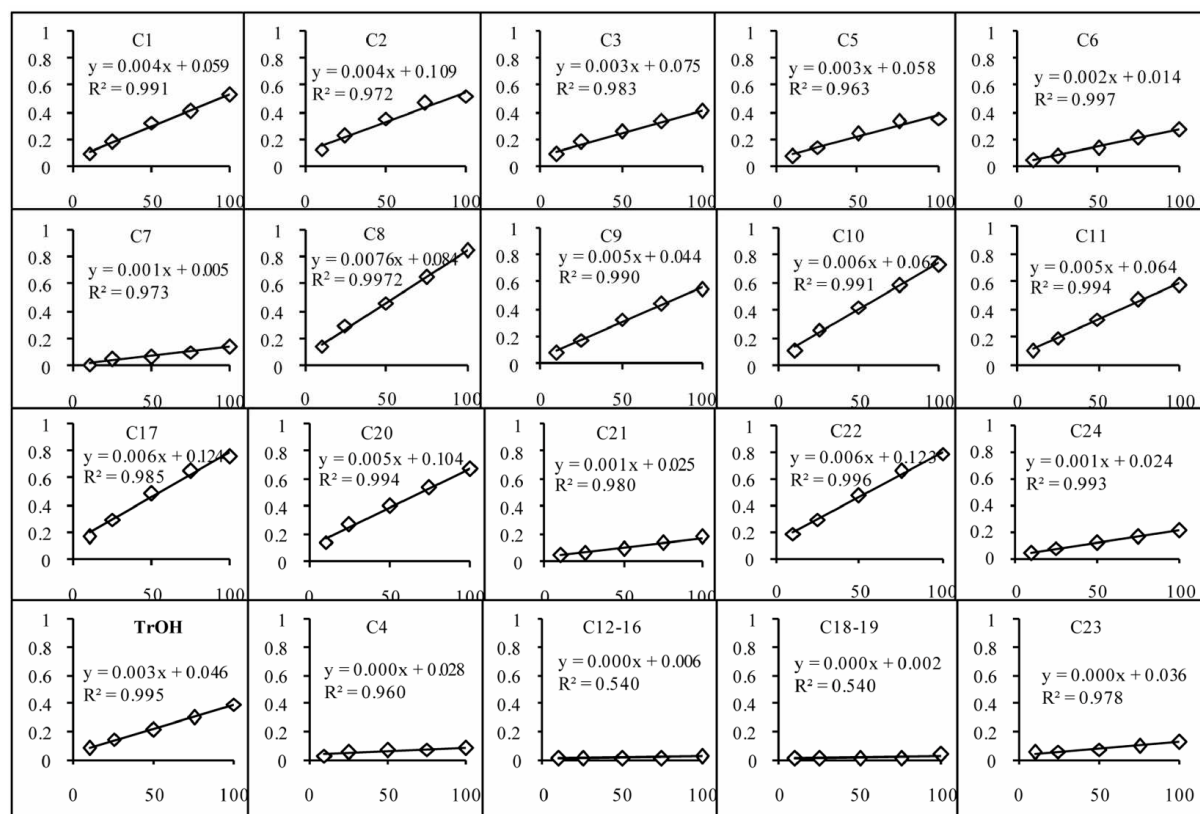


Fig. 2. Reducing power assay (antioxidant activity) of 24 different derivatives of 4-methylcoumarins (C1-C20) and 4-methylthionocoumarins (C21-C24) and trolox (TrOH). X-axis shows the concentration of the compounds (10, 25, 50, 75 and 100 μM) and Y-axis shows the absorption at 700 nm in the panels.

Table 2. Antioxidant Activity of 24 Derivatives of 4-Methylcoumarins (C1-C24)

Activity	4-Methylcoumarins
Most active	C8, C10, C17, C22
More active	C1, C2, C9, C11, C20
Moderately active	C3, C5, C6
Less active	C7, C21, C24
Inactive	C4, C12, C13, C14, C15, C16, C18, C19, C23

results of C20 and C22 in Table 2).

Altogether the presence of two -OH groups is an essential feature for this kind of antioxidants. The substitution of the active -OH group by methoxy (-OCH₃) causes suppression of the antioxidant activity leading to inactive molecules (see

Table 2 and Fig. 2). Interestingly, the substitution of -OH group in the *ortho* position or even *meta* substituted derivatives of 4-methylcoumarins by acetate esters (-OOCCH₃) does not eliminate the reducing potency of these compounds but results in moderately active (C3, C5 and C6

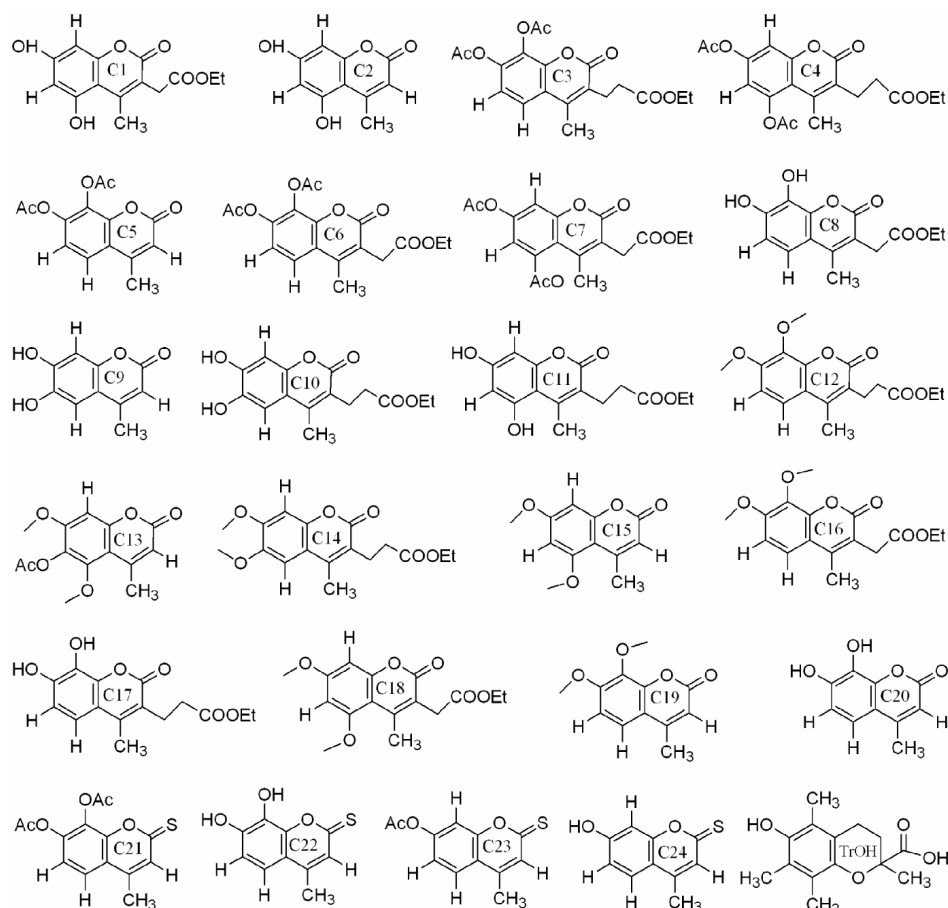


Fig. 3. Chemical structure of 24 different derivatives of 4-methylcoumarins (C1-C20), 4-methylthionocoumarins (C21-C24) and trolox (TrOH).

or less active (C7 and C21) compounds (Table 2). In order to better understand these findings and the possible antioxidant mechanism of 4-methylcoumarin derivatives, we have calculated their antioxidant-related parameters using theoretical methods as discussed in the following section.

Antioxidant-Related Parameters of 4-Methylcoumarins Based on B3LYP Calculations

The bond-dissociation enthalpy (BDE) of a hydroxyl group involves H-atom transfer whereas the ionization potential (IP) refers to an electron transfer process; these are two main accepted theoretical parameters for evaluating the possible antioxidant capacity of a molecule [39,40,41]. The weaker an O-H bond is and the lower the ionization potential, the more active will an antioxidant be in the reaction with radical

molecules. Hydroxycoumarins are believed to behave like classic phenol- or quinol-based antioxidants, in which the -OH group on an aromatic ring structure can take part in the H-atom transfer and/or electron transfer process for the reduction of a free radical. The results reported in Table 3 indicate that BDE values for all of the 4-methylcoumarin derivatives are in the range 77-87 kcal mol⁻¹. These antioxidants can easily react with hydroxyl radicals (HO[•]) due to the very high BDE of the HO-H bond in water, 119 kcal mol⁻¹ [39], which makes all possible reactions with HO[•] very exothermic. This leads to the high antioxidant activity of 4-methylcoumarin derivatives (MOH) according to:



Despite the higher reducing power of many synthetic 4-

Table 3. Bond Dissociation Enthalpy (BDE) and Ionization Potential (IP) for the 24 Derivatives of 4-Methylcoumarins (C1-C24) and TrOH^a

Compound	BDE		IP (eV)
	H1 (kcal mol ⁻¹)	H2 (kcal mol ⁻¹)	
C1	86.02	81.18	7.611
C2	84.91	81.54	7.878
C3	-	-	7.902
C4	-	-	7.803
C5	-	-	8.190
C6	-	-	8.339
C7	-	-	7.832
C8	83.56	78.97	7.684
C9	86.77	80.36	7.639
C10	80.93	76.69	7.462
C11	85.48	78.78	7.543
C12	-	-	7.457
C13	-	-	7.156
C14	-	-	7.156
C15	-	-	7.564
C16	-	-	7.413
C17	83.72	79.11	7.560
C18	-	-	7.299
C19	-	-	7.614
C20	84.31	79.57	7.959
C21	-	-	7.890
C22	83.06	79.63	7.909
C23	-	-	7.551
C24	82.45	-	7.402
TrOH	74.80	-	7.182

^aThe BDE and IP parameters have been computed using B3LYP calculations as described in the Materials and Methods Section. H1 and H2 stand for first and second phenolic hydrogen abstraction.

methylcoumarins compared to TrOH, all of them show higher BDE/IP values than TrOH (see Table 3). This is true not only of the “moderately active” and “more active” compounds but, interestingly, also the four “most active” compounds (two times more active than TrOH). Actually, almost all of 24 compounds have rather similar BDEs and IPs. Therefore, the important point that should be taken into account, is that the reason behind the higher activity of the 4-methylcoumarins is the number and position (best *ortho*) of hydroxyl groups on

the aromatic ring. At variance with this is that in the previous section we have shown and discussed the loss of activity due to substitution of hydroxy groups with methoxy groups (-OCH₃) on the aromatic ring of 4-methylcoumarins.

Highly active 4-methylcoumarins have two hydroxyl groups, whereas TrOH has only one hydroxyl group. Possibly, this is the main reason why these compounds have higher activity than TrOH in the experimental condition. For *ortho* derivatives there is an additional possible interaction due to

the formation of intramolecular hydrogen bonding, as indicated in Fig. 4. The presence of two -OH groups in the *ortho* position allows the formation of hydrogen bonding after the first H abstracting, leading to a more stable 4-methylcoumarin radical. A hydrogen bond can be formed if the hydrogen-donor distance is less than 3.2 Å. Considering the inter-atomic distances shown in Fig. 4, all "most active" compounds (C8, C10, C17, C22) and two "more active" compounds (C9, C20) can form an internal hydrogen bond, and actually C9 and C20 are very close to the arbitrary limit (twice the activity of trolox) that we use to distinguish "most active" from "more active" compounds. These hydrogen bonds are not seen in other derivatives and are also impossible in TrOH. Hence, in the highly active *ortho*-substituted antioxidants, after the first O-H bond is broken in the parent antioxidant, the radical is able to rearrange to the more stable conformation by intramolecular hydrogen bonding (Fig. 4).

Another mechanism by which an antioxidant can deactivate a free radical is electron transfer. A careful inspection of the calculated parameters in Table 3 suggests that IP does not play a significant role in the scavenging of free radicals by 4-methylcoumarins; in fact "non-active" compounds without any hydroxyl groups show IPs similar to "most active" compounds with two hydroxyl groups in the *ortho* position.

We agree with the commonly accepted idea that BDE and IP are useful parameters in determining chemical and functional antioxidant property, and we believe that one must first consider the BDE/IP, for designing good synthetic antioxidants. However, we propose that the following useful findings of this study be taken into account: a) Calculation of BDE is effectively limited to compounds with -OH dissociable groups. b) All of the 24 compounds studied here have sufficiently low BDEs and therefore small changes in their values are insignificant. The values were 77-87 kcal mol⁻¹ that are enough to cause exothermic reaction with HO[•] to produce HO-H (119 kcal mol⁻¹). Consequently, when compared with BDE of TrOH (74.8 kcal mol⁻¹), the small changes in BDE values are not remarkable for antioxidant activity, while other factors such as the presence of -OH, the number of -OH groups, substitution of -OH in *ortho/meta* positions and modification of the pyrone ring are important parameters. c) Although the IP value does not represent a limit for -OH

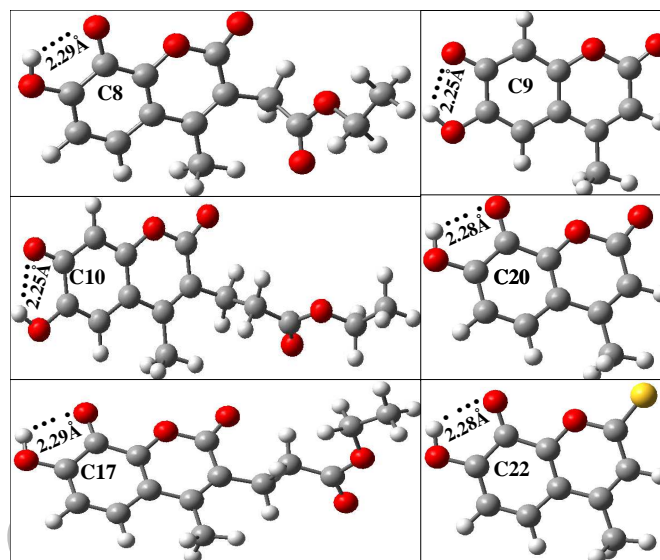


Fig. 4. Optimized three-dimensional structure of selected 4-methylcoumarins. Internal hydrogen bondings have been shown. Bond distances are in angstroms.

dissociate-able groups and can be used for a broad range of compounds, our results indicate that IP is not a predictor for a good antioxidant activity. Taken together, experimental and theoretical findings show that the antioxidant properties of 4-methylcoumarins depend more on combinatorial parameters than on BDE and IP. This confirms results reported for other phenolic compounds [42], and is also in agreement with the different antioxidant rankings of 4-methylcoumarins obtained when their activities were determined against different types of radicals, and measured in different ways [43].

CONCLUSIONS

The results of this study demonstrate the abilities of some new synthetic 4-methylcoumarin derivatives to stimulate higher levels of antioxidant activity and to attenuate elevated levels of free radicals. Dihydroxy-4-methylcoumarins were found to possess remarkable ability in reducing power which is related to hydrogen atom transfer. The great antioxidant ability of the dihydroxy derivatives of 4-methylcoumarins can be explained by the fact that the *ortho* dihydroxy system is able to form a resonance-stable radical and intra-molecular H-

bonding, making a higher level of antioxidant activity feasible. The results lead to the conclusion that dihydroxy-4-methylcoumarin derivatives could be used as a model for the design of more active compounds with higher antioxidant potency.

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REFERENCES

- [1] R.D.H. Murray, in: W. Herz, G.W. Kirby, W. Steglich, C. Tamm, Vol. 58, Progress in the Chemistry of Organic Natural Products, Coumarins, New York, Springer-Verlag, 1991.
- [2] D. Egan, E. O'Kennedy, E. Moran, D. Cox, E. Prosser, R.D. Thornes, Drug Metab. Rev. 22 (1990) 503.
- [3] R.D.H. Murray, I. Mendez, S.A. Brawn, The Natural Coumarins, J. Wiley & Sons, New York, 1982.
- [4] K.C. Fylaktakidou, D.J. Hadjipavlou-Litina, K.E. Litinasc, D.N. Nicolaidis, Curr. Pharmaceut. Design. 10 (2004) 3813.
- [5] M.F. Molina-Jimenez, M.I. Sanchez-Reus, D. Andres, M. Cascales, J. Benedi, Brain Res. 1009 (2004) 9.
- [6] C.A. Kontogiorgis, Y. Xu, D. Hadjipavlou-Litina, Y. Luo, Free Radic. Res. 41 (2007) 1168.
- [7] T. Kaneko, N. Baba, M. Matsuo, Chem. Biol. Interact. 142 (2003) 239.
- [8] A. Nunomura, G. Perry, G. Aliev, K. Hirai, A. Takeda, E.K. Balraj, P.K. Jones, H. Ghanbari, T. Wataya, S. Shimohama, S. Chiba, C.S. Atwood, R.B. Petersen, M.A. Smith, J. Neuropathol. Exp. Neurol. 60 (2001) 759.
- [9] W.R. Markesbery, Free Radic. Biol. Med. 23 (1997) 134.
- [10] D.A. Butterfield, J. Drake, C. Pocernich, A. Castegna, Trends Mol. Med.7 (2001) 548.
- [11] J.V. Smith, Y. Luo, J. Alzheimer's Disease 5 (2003) 287.
- [12] I. Kostova, Mini Rev. Med. Chem. 6 (2006) 365.
- [13] F. Borges, F. Roleira, N. Milhazes, L. Santana, E. Uriarte, Curr. Med. Chem. 12 (2005) 887.
- [14] H.G. Raj, V.S. Parmar, S.C. Jain, S. Goel, P. Himanshu, S. Malhotra, A. Singh, C.E. Olsen, J. Wengel, Bioorg. Med. Chem. 6 (1998) 833.
- [15] S. Kumar, B.K. Singh, N. Kalra, V. Kumar, A. Kumar, A.K. Prasad, H.G. Raj, V.S. Parmar, B. Ghosh, Bioorg. Med. Chem. 13 (2005) 1605.
- [16] H.G. Raj, V.S. Parmar, S.C. Jain, S. Goel, A. Singh, K. Gupta, V. Rohil, Y.K. Tyagi, H.N. Jha, C.E. Olsen, J. Wengel, Bioorg. Med. Chem. 6 (1998) 1895.
- [17] H.G. Raj, R.K. Sharma, B.S. Garg, V.S. Parmar, S.C. Jain, S. Goel, Y.K. Tyagi, A. Singh, C.E. Olsen, J. Wengel, Bioorg. Med. Chem. 6 (1998) 2205.
- [18] S. Takeda, M. Aburada, J. Pharmacobiodyn. 4 (1981) 724.
- [19] A.A. Deana, G.E. Stokker, E.M. Schultz, R.L. Smith, E.J. Cragoe Jr., H.F. Russo, L.S. Watson, J. Med. Chem. 26 (1983) 580.
- [20] J.Z. Pedersen, C. Oliveira, S. Incerpi, V. Kumar, A.M. Fiore, P. De Vito, A.K. Prasad, S.V. Malhotra, V.S. Parmar, L. Saso, J. Pharm. Pharmacol. 59 (2007) 1721.
- [21] H.G. Raj, S. Gupta, A.K. Prasad, P.M. Boll, J. Wengel, G. Biswas, S.K. Singh, N.K. Sharma, K.S. Bisht, V.S. Parmar, Bioorg. Med. Chem. Lett. 5 (1995) 1567.
- [22] H.G. Raj, S. Gupta, G. Biswas, S. Singh, A. Singh, A. Jha, K.S. Bisht, S.K. Sharma, S.C. Jain, V.S. Parmar, Bioorg. Med. Chem. 4 (1996) 2225.
- [23] A.D. Becke, J. Chem. Phys. 98 (1993) 5648.
- [24] A.D. Becke, Phys. Rev. A 38 (1988) 3098.
- [25] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785.
- [26] V.S. Parmar, K.S. Bisht, R. Jain, S. Singh, S.K. Sharma, S. Gupta, S. Malhotra, O.D. Tyagi, A. Vardhan, H.N. Pati, D. Vanden Berghe, A.J. Vlietinck, Indian J. Chem. 35B (1996) 220.
- [27] V.S. Parmar, N.K. Sharma, M. Husain, A.C. Watterson,

- J. Kumar, L.A. Samuelson, A.L. Cholli, Prasad, A.K. Kumar, A. Malhotra, S. Kumar, N. Jha, A. Singh, A. Singh, I. Himanshu, A. Vats, N.A. Shakil, S. Trikha, S. Mukherjee, S.K. Sharma, S.K. Singh, A. Kumar, H.N. Jha, C.E. Olsen, C.P. Stove, M.E. Bracke, M.M. Mareel, *Bioorg. Med. Chem.* 11 (2003) 913.
- [28] M. Oyaizu, *Jpn. J. Nutr.* 44 (1986) 307.
- [29] D. Villano, M.S. Fernández-Pachón, M.L. Moyá, A.M. Troncoso, M.C. García-Parrill, *Talanta* 71 (2007) 230.
- [30] Y. Zou, Y. Lu, D. Wei, *J. Agric. Food Chem.* 52 (2004) 5032.
- [31] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, *J. Am. Chem. Soc.* 107 (1985) 3902.
- [32] L.F. Wang, H.Y. Zhang, *Bioorg. Chem.* 33 (2005) 108.
- [33] G.A. DiLabio, D.A. Pratt, A.D. LoFaro, J.S. Wright, *J. Phys. Chem. A* 103 (1999) 1653.
- [34] G.A. DiLabio, D.A. Pratt, J.S. Wright, *J. Org. Chem.* 65 (2000) 2195.
- [35] G.A. DiLabio, D.A. Pratt, J.S. Wright, *Chem. Phys. Lett.* 311 (1999) 215.
- [36] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, J.L. Andres, C. Gonzalez, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian 98, Gaussian Inc: Pittsburgh, PA, 1998.
- [37] S. Meir, J. Kanner, B. Akiri, S. Philosoph-Hadas, *J. Agric. Food Chem.* 43 (1995) 1813.
- [38] M.T. Chua, Y.T. Tung, S.T. Chang, *Bioresour. Technol.* 99 (2007) 1918.
- [39] J.S. Wright, E.R. Johnson, G.A. Di Labio, *J. Am. Chem. Soc.* 123 (2001) 1173.
- [40] M. Leopoldini, T. Marino, N. Russo, M. Toscano, *J. Phys. Chem. B* 108 (2004) 4916.
- [41] M. Leopoldini, T. Marino, N. Russo, M. Toscano, *Theor. Chem. Acc.* 111 (2004) 210.
- [42] N. Nenadis, L. F. Wang, M. Z. Tsimidou, H.Y. Zhang, *J. Agric. Food Chem.* 53 (2005) 295.
- [43] G. Morabito, D. Trombetta, K.S. Brajendra, K.P. Ashok, S.P. Virinder, C. Naccari, F Mancari, A Saija, M. Cristani, O Firuzi, L. Saso, *Biochimie* 92 (2010) 1101.