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Research paper

Protective effects of 4-methylcoumarins and related compounds as radical scavengers and chain-breaking antioxidants

Vessela D. Kancheva [a, *], Adriana K. Slavova-Kazakova [a], Silvia E. Angelova [a], Suraj K. Singh [b], Shashwat Malhotra [b], Brajendra K. Singh [b], Luciano Saso [c], Ashok K. Prasad [b], Virinder S. Parmar [b, d]

[a] Lipid Chemistry and Theoretical Chemistry Departments, Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[b] Bioorganic Laboratory, Department of Chemistry, University of Delhi, Delhi, 110 007, India
[c] Department of Human Physiology and Pharmacology "Vitorio Erspamer", University of Rome "La Sapienza", 00185 Rome, Italy
[d] Institute of Advanced Sciences, 86-410 Faunce Corner Mall Road, Dartmouth, MA 02747, USA

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ABSTRACT

The aim of this study is to determine, and to compare the protective effects of eight 4-methylcoumarins and four related compounds as radical scavengers and chain-breaking antioxidants. The main kinetic parameters of their radical scavenging activity (as % RSA, stoichiometry, n, and rate constants of reaction with DPPH radical, k_{RSA}) and of chain breaking antioxidant activity (as antioxidant efficiency, PF and reactivity, ID), have been determined and discussed. The RSA study has been conducted at physiological temperature (37 °C) towards DPPH radical and the tested compounds are separated into three main groups: with strong activity (% RSA > 40%); with moderate activity (20% < % RSA > 40%) and with weak activity (% RSA < 20%). Chain-breaking antioxidant activities of the studied compounds have been evaluated during bulk phase lipid (triacylglycerols of sunflower oil, TGSO) autoxidation at 80 °C. All results obtained are compared with those for standard and known inhibitors of oxidation processes, e.g. caffeic and *p*-coumaric acids, α-tocopherol and butylated hydroxytoluene (BHT). On the basis of a comparative analysis with standard antioxidants, the differences in the radical scavenging and antioxidant abilities of the studied compounds have been discussed and reaction mechanisms proposed. All structures are optimized at UB3LYP/6-31 + G(d,p) level in gas phase and in acetone solution to study the solvation effects.

1. Introduction

There is a growing interest during the last decade on the compounds with both - antioxidant and biological activities, i.e. bio-antioxidants [1–3]. The design of structural analogues of bioactive natural products with an improved pharmacological profile is a primary aim in medicinal chemistry [4,5]. Burlakova [6] suggested creation of the so called "hybrid compounds" by synthetic pathway. One possibility is to choose compounds with biological activity and by varying different substituents to get new bio-antioxidants. Another possibility is to choose compounds with antioxidant activity and by varying different substituents to get new set of bio-antioxidants [6,7].

Coumarins are secondary metabolites widely spread in nature, found in bacteria, fungi, green plants, in some animal species, in fruits (e.g. bilberry, cloudberry), green tea and other foods and spices [8,9]. Different derivatives of these compounds possess a wide range of biological activities [10] such as antioxidant [11–13], anti-inflammatory [14], antimicrobial [15], and *in vitro* [16] and *in vivo* platelet and anti-aggregation effects [17]. There are few studies evaluating the consumption of coumarins in foods and their clinical relevance, and these studies focus on parent coumarin molecule, which is found in many plants such as cinnamon, and is known to be a hepatotoxic and carcinogenic compound. The liver damage observed after injection of coumarin has been attributed to the formation of 3, 4-epoxide intermediates in rats, but

* Corresponding author. "Acad. G. Bonchev" str., bl. 9, 1113 Sofia, Bulgaria.
E-mail addresses: vessy.kancheva@abv.bg, vedeka@abv.bg (V.D. Kancheva).

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interestingly other modified coumarins such as 3, 4-dimethylcoumarin and 3, 4-dihydrocoumarin did not induce hepatotoxicity. Natural coumarins, such as osthole [18], esculetin [19] and umbelliferone [20] as well as several coumarin-containing plant extracts have been reported to inhibit the growth of cancer cells [21,22].

An interesting and successful idea of Parmar and co-workers [23] is to select the simple coumarin structure without any activity - neither antioxidant, nor biological, even more with some toxicity, and by varying the different substituents to get a series of new bio-antioxidants [24,25].

We have reported in our previous papers [26,27] that the position and number of OH groups in the coumarin ring is of great importance for their antioxidant activity. The catecholic structure in the structure of coumarins is the key factor for their high antioxidant potential. It was observed also that the substitution in ring A is of importance for their antioxidant potential, and the substitution in the ring B for their biological activity and does not affect their antioxidant properties. It was reported also [28] that esculetin having two OH groups at the *ortho*-position possesses a stronger radical scavenging activity compared with the methoxy-substituted coumarin analogue - scopoletin. Authors explained the highest effectiveness as radical scavengers of esculetin and 4-methylesculetin by the different resonance structures of their radicals, which are especially stable because of their *ortho*-quinonoid form.

27 Coumarin derivatives (mostly carrying the C-4 methyl moiety), including 7-hydroxy-4-methylcoumarins (7-HMCs), 7-acetoxy-4-methylcoumarins (7-AMCs) and different 7,8-dihydroxy-4-methylcoumarin (DHMC) and 7, 8-diacetoxy-4-methylcoumarin (DAMC) derivatives have been synthesized and examined by us previously for their cytotoxic effects on three human cancer cell lines [29]. An advantageous property of 4-methylcoumarins is that due to the presence of a methyl group at the C-4 position, they are less likely to be metabolized to the mutagenic derivative 3, 4-coumarin epoxide by the action of liver cytochrome P450 enzymes [30].

It has been found that 4-methylcoumarin derivatives have similar effects on three types of cancer cells. 7,8-DHMCs are the most active compounds. Thus, the presence of two OH groups at the C-7 and C-8 positions seems to improve the potency of 4-methylcoumarins as cytotoxic agents. In particular, the authors have previously reported that 7,8-dihydroxy-4-methylcoumarin induces apoptosis in cancer cells [31,32]. Umbelliferone (7-hydroxycoumarin), a natural coumarin compound, was also tested and did not show any cytotoxic effect on the tested cell lines. Previous studies showed that 7,8-DHMC is a potent anticancer agent that selectively targets leukemia cells [32,33]. Substitution of the hydroxyl groups with acetoxy groups reduced the cytotoxic activity. A similar phenomenon has been observed when the hydrogen is replaced with methyl moiety. 7,8-DHMCs bearing alkyl groups at the C-3 position are the most effective subgroup, out of which, the most potent compound is with an *n*-decyl chain at the C-3 position against K562, LS180 and MCF-7 cells. The second most active subgroup is 7,8-DAMCs containing ethoxycarbonylmethyl and ethoxycarbonylethyl moieties at the C-3 position.

Eight 4-methylcoumarins having one or two hydroxyl groups and four related compounds have been selected in the present study. Combination of different approaches: spectral (DPPH$^{\bullet}$ absorbance), kinetic (lipid autoxidation) and theoretical (quantum-chemical calculations) has been applied to study and to explain the structure-antioxidant activity relationship.

For the assessment of the antioxidant properties, it is customary to use both the spectral and the kinetic assays, the former technique refers, most prominently, to measuring the light absorption by the DPPH radical [34] in the presence of antioxidant analytes, while the latter methodologies pertain either to monitoring the chemiluminescence emission from the appropriate hydrocarbon substrates being oxidized [35] or to acquiring the time profiles of lipid peroxidation [36] upon the addition of antioxidants. The theoretical approaches to elucidation of the antioxidant's reactivity involve *ab initio* [37] or semiempirical [38] computations.

2. Materials and methods

2.1. Studied compounds

All compounds under study (Fig. 1 and Table 1) were synthesized and characterized at the Department of Chemistry, University of Delhi as described previously. Benzo-α-pyrones **a_1** and **a_3** were synthesized according to the previous reports [29] and **b_1**, **b_3**, **c_1**, **c_3** and **d_1** were prepared according to our previously reported work [39]. Benzo-α-thiopyrone **as_1** was prepared according to the procedure given by Parmar and coworkers [40], and the related compounds *i.e.* benzo-γ-pyrones **e** [41], **f** [42], and **g** [43] were synthesized according to the literature reports.

Standard antioxidants DL-α-tocopherol (**TOH**), butylated hydroxytoluene (**BHT**), caffeic acid (**CA**), *p*-coumaric acid (***p*-CoumA**), 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) were purchased from Sigma-Aldrich and used without further purification. Benzo-γ-pyrone **h** was purchased from Labnetwork, USA. All solvents were of HPLC grade purity.

2.2. Quantum chemical calculations

Among different combinations of density functional theory methods and basis sets the B3LYP/6-31 + G(d,p) method has proven reliable in reproducing the geometries, frequencies, and bond lengths [44]. Hence, the unrestricted open-shell approach UB3LYP and 6-31 + G(d,p) basis set [45–47] were used to optimize the geometry of compounds studied and their radicals without symmetry constraints with the default convergence criteria using the Gaussian 09 program [48]. Frequency calculations for each optimized structure are performed at the same level of theory. No imaginary frequency is found for the lowest energy configurations of any of the optimized structures. Unscaled thermal corrections to enthalpy are added to the total energy values. The BDEs for the generation of the respective radicals from the parent compounds are calculated by the formula,

$$BDE = H_{298}(AO^{\bullet}) + E_T(H^{\bullet}) - H_{298}(AOH) \quad (1)$$

where $H_{298}(AO^{\bullet})$ and $H_{298}(AOH)$ are enthalpies calculated at 298 K for radical species, $AO^{\bullet}$ and neutral molecule AOH, respectively, and $E_T(H^{\bullet})$ (calculated total energy of $H^{\bullet}$) is −313.93 kcal mol^{-1}.

Solvation effects have been accounted for by employing the polarizable continuum model (PCM) [49–51] as implemented in the Gaussian 09 suite of programs [48]: all structures are optimized in acetone surrounding environment. PyMOL molecular graphics system was used for generation of the molecular graphics images [52].

2.3. Radical scavenging activity (RSA)

One of the most popular rapid DPPH$^{\bullet}$ test has been applied. This approach gives information about the H atom abstraction from the phenolic O–H group of studied compounds (AOH): AOH + DPPH$^{\bullet}$ → DPPH-H + $AO^{\bullet}$; $AO^{\bullet}$ + DPPH$^{\bullet}$ → inactive product. The RSA determinations reported in literature are based on the DPPH$^{\bullet}$ absorbance decrease very often measured at different