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Short communication

Synthesis and evaluation of lipid peroxidase inhibition of 4-methyl substituted coumarins

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Introduction

Trough recent studies, aging, cancer, atherosclerosis, and some other serious diseases have been confirmed to correlate with low density lipoprotein (LDL), cell membranes, and DNA exposed to oxidative stress (Finkel et al., 2000). In conditions of oxidative stress, in interaction with generated free radicals, polyunsaturated fatty acids can undergo oxidative damage, which results in the initiation of lipid peroxidation (LP). Oxidative stress and the resulting LP are involved in the pathogenesis of numerous chronic and degenerative diseases which seriously impair the quality of life (Pinchuk et al., 2012). That is why numerous researches are aimed at finding substances with antioxidant properties.

Coumarins are scaffolds widely distributed in nature, possess numerous therapeutic applications including photochemotherapy, antitumor and anti-HIV therapy (Borges et al., 2005). They are proven antibacterial and anti-inflammatory agents, and anti-coagulants. In addition to a wide array of pharmacological activities, several evidences indicate that coumarins may act as antioxidants or regulator of antioxidant activity (Pérez-Cruz et al., 2012). Although reported papers can be related to an antioxidant activity of coumarins, the characterization of the antioxidant profile of coumarins is at present lacking. In this study the evaluation of antioxidative properties of four synthetic coumarin derivatives have been investigated by a LP assay.

Materials and methods

Chemicals

All of the starting materials, standards and solvents were of analytical reagent grade. Unless specified otherwise, all chemicals were purchased from Sigma-Aldrich Co. (Milan, Italy). Phospholipids (Phospholipon® 90 - PL90) were obtained by courtesy of Phospholipid GMBH, Cologne, Germany.

General synthetic procedure for the preparation of 4-methyl coumarin derivatives

Compounds **1-4** were synthesized by Pechmann condensation, according to the procedure previously reported in the literature (Bulut and Erk, 1996). The identity of **1-4** was confirmed by ^{1}H NMR and ^{13}C NMR analytical data. The purity of the synthesized derivatives was above 95%, which was confirmed by HPLC analysis.

Lipid peroxidation inhibition by thiobarbituric acid-malondialdehyde assay

Lipid peroxidation (LP) and the LP inhibition in the presence of coumarin derivatives as tested compounds, was measured by thiobarbituric acid-malondialdehyde (TBA-MDA) assay according to the procedure previously described in Lazarević et al. (2020).

Results and discussion

Four 4-methylcoumarin derivatives were synthesized according to previously published standard methodology.

Lipid peroxidation inhibition effect of coumarins **1**-**4**, was evaluated *in vitro* using the method based on TBA-MDA assay, selecting troxol and quercetin as positive

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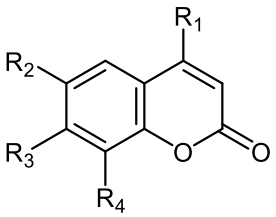
controls. The obtained results were plotted, IC_{50} values were calculated and reported in Table 1.

Table 1. Synthesized coumarin derivatives **1-4** along with the results of the MDA-TBA LP assay.



	R_1	R_2	R_3	R_4	IC_{50}(μM)
1	CH_3	H	OH	H	na*
2	CH_3	H	OCH_3	H	na
3	CH_3	OH	OH	H	39.1 ± 16.3
4	CH_3	H	OH	OH	51.0 ± 9.6

*na - no LP activity at concentration range 100 – 15 μM, however prooxidative effect was observed at concentrations 300 and 200 μM

The obtained results indicated that at tested concentrations two out of four compounds (**3** and **4**) had antioxidant properties. The most pronounced effect on the level of generated lipid peroxyl radicals and hydroperoxides was displayed by dihydroxy-4-coumarin derivatives **3** and **4** (IC_{50} = 39.1 ± 16.3 μM and 51.0 ± 9.6 μM, respectively). Those values were somewhat above the value obtained for standards trolox and quercetin (IC_{50} =22 ± 6 μM and 23 ± 6 μM, respectively). Interestingly, while compounds **3** and **4** had antioxidative effect, inhibiting LP, compounds **1** and **2** at concentrations 300 μM and 200 μM had prooxidative effect, while at concentrations below 100 μM had no activity on LP. The results we have obtained are in congruence with the results by Liu et al. (1999) who were investigating the peroxidation of human low-density lipoprotein (LDL) induced by different type of initiators. While 7-hydroxy-4-methylcoumarin (same as compound **1** in our study) exhibited prooxidative activity in AAPH-initiated peroxidation, 7,8-dihydroxy-4-methylcoumarin (compound **4** in our study)) was a good antioxidant for the AAPH-initiated peroxidation. The authors have concluded that the antioxidative and/or prooxidative activity of coumarin derivatives in LDL depends not only on their molecular structure but also on the initiation conditions.

Conclusion

Four synthetic 4-methylcoumarin derivatives were evaluated for *in vitro* scavenging activity of chemically generated, biologically relevant lipoperoxyl radicals (LP-OO•), with the greatest inhibitory activity detected for 6,7- and 7,8-dihydroxy-4-methylcoumarins (**3** and **4**). The antioxidative action of the coumarin derivatives in LP assay may include trapping initiating radicals or trapping the propagating lipid peroxyl radicals. However, only after integrating our results with the cellular assays, we can state the effect of coumarins as biological regulators of the oxidative stress.

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Author Contributions

KI, experimental work on synthesis and first-draft preparation; **JZ,** experimental work on LP; **JL** recording and analyzing spectral data, experimental work on LP, data curation, resources and final writing. All authors read and approved the final manuscript.

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