ORIGINAL ARTICLE

ANTIOXIDANT EVALUATION OF SOME COUMARIN DERIVATIVES

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Abstract

The present study evaluated the antioxidant potential of 28 coumarin derivatives, using three antioxidant assays: DPPH inhibition, total reducing power and NO inhibition. Out of the tested compounds, the most active DPPH free radicals scavengers were the coumarin hydrazide derivatives. These substances were also the only ones among the investigated derivatives that exhibited a moderate reducing power of ferricyanide to ferrocyanide, but their activity was inferior to that exhibited by the reference substance (ascorbic acid). Most of the tested substances were moderate NO inhibitors. The activity of 4-methyl-7-methoxy coumarin was remarkable, exceeding the inhibitory potential of ascorbic acid.

Rezumat

În această lucrare a fost investigat potenţialul antioxidant a 28 de derivaţi de cumarină, utilizând trei metode: determinarea capacităţii de *scavenger* faţă de radicalul DPPH, determinarea puterii reducătoare şi determinarea capacităţii de inhibiţie a oxidului nitric. Cei mai activi *scavengeri* faţă de radicalul liber DPPH au fost derivaţii ce au ca element comun radicalul hidrazidic. Aceste substanţe au fost de asemenea cele care au prezentat un potenţial reducător moderat, dar activitatea lor a fost inferioară celei prezentate de substanţa de referinţă (acidul ascorbic). Majoritatea derivaţilor testaţi au prezentat o capacitate moderată de inhibiţie a oxidului nitric, dar s-a remarcat 4-metil-7-metoxi-cumarina, potenţialul său inhibitor fiind superior celui al acidului ascorbic.

Keywords: antioxidant, coumarin derivatives, DPPH inhibition, total reducing power, NO inhibition

Introduction

Free radicals are molecular species capable of independent existence that contain an unpaired electron in an atomic orbital; they are usually unstable and very reactive. These species are normally produced in the human body from essential metabolic processes, but they may also occur from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals. The human organisms possess natural systems to annihilate these species, but when the body's ability to regulate them is overwhelmed, a condition known as oxidative stress appears, free radicals attacking important macromolecules leading to cell damage and homeostatic disruption [5].

Antioxidant compounds neutralize free radicals and are very important health-protecting agents, reducing the risk for chronic diseases. One mechanism through which this is achieved is by donating hydrogen to free radicals, removing the odd electron feature and reducing them to non-reactive species.

Many coumarin derivatives have special abilities to scavenge reactive oxygen species and to influence processes involving free radical-injury [9, 11]. The styryl carbonyl group in the structure of coumarin was found to be very important in scavenging reactive oxygen species, contributing to the prevention of oxidative damage caused by free radicals [6].

The objective of this paper was to evaluate the antioxidant potential of 28 coumarin derivatives. Three antioxidant assays were used: DPPH inhibition, total reducing power and NO inhibition.

Materials and Methods

All chemicals and solvents were purchased from commercial suppliers and used without purification. All spectrophotometric determinations were performed on a UV-VIS ABL & E Jasco V550 spectrophotometer. The synthesis of the investigated compounds and the elucidation of their structures are the subject of another scientific paper that is currently under review.

DPPH assay

The experimental procedure was adapted from literature [1, 2, 13], only slight modifications being made. Briefly, 2.5 mL solution of DPPH (2,2-di-

phenyl-1-picrylhydrazyl) radical 0.1 mM in methanol was added over 0.5 mL of methanolic solution of the tested compound (1mg/mL). The absorbance of the DPPH solution at 517 nm was determined spectrophotometrically before ($A_{control}$) and 15 minutes after adding the solutions of the compounds (A_{test}) and the percentage of activity was calculated. Ascorbic acid was used as a reference compound.

$$\% \text{ radical scavenging activity} = (A_{control} - A_{test}) \times 100 / A_{control}$$

where $A_{control}$ is the absorbance of the control sample (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution + test compound).

Reducing power assay

The solution of the test compound (0.5 mL) at different concentrations in methanol was mixed with phosphate buffer (1.25 mL, 0.2 mol/L, pH 6.6) and 1% potassium ferricyanide (1.25 mL) and the mixture was incubated at 50ºC for 20 min. At the end of the incubation period, 10% trichloroacetic acid (1.25 mL) was added to the mixture and centrifuged at 3000 rpm for 10 min. The upper layer solution was collected and 2.5 mL were mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). The absorbance was measured after 15 min at 700 nm against a blank (containing all the reagents, except the test compound) reagent. The EC_{50} values were calculated by linear interpolation between values above and below 50% activity. Ascorbic acid was used as reference [2, 4, 10, 13].

NO inhibition assay

0.5 mL of the tested coumarin derivative solution, as well as ascorbic acid (standard compound) were taken in separate test tubes and 2.0 mL of sodium nitroprusside (10 mM) and 0.5 mL phosphate buffer saline (pH = 7.4) were added to each tube. The solutions were incubated at 25ºC for 150 minutes. After the incubation, over 0.5 mL of the incubated solution 1 mL of 0.33% suphanilic acid was added and the mixture was left for 5 min. at room temperature; after this period of time, 1 mL 0.1% NED (naphthylethylene diamine HCl) Reagent was added and the solutions were incubated for another 30 min. The absorbance was measured at 546 nm [9].

Results and Discussion

Nine classes of coumarin derivatives were tested. The condensation products of 4-methyl and 4-propyl-7-hydroxy-coumarin (Ia-b) with bromethylacetate were obtained (IIa-b) and then their corresponding aceto-hydrazides (IIIa-b) and acids (VIIa-b). By treating acetohydrazides IIIa-b with carbonsulfide, compounds IVa-b – potassium dithiocarbazate derivatives - and their S-methyl analogues (Va-b) were obtained. The incorporation of another heterocyclic moiety into coumarin modifies the properties of the starting compound and, in general, the resulting substances have improved properties [8]. Therefore, two categories of coumarin derivatives with other heterocyclic rings - thiadiazole (VIa-b) and isatin (IXa-b) were obtained. The reaction scheme is presented in Figure 1and the structure of the tested coumarin derivatives is shown in Table I.

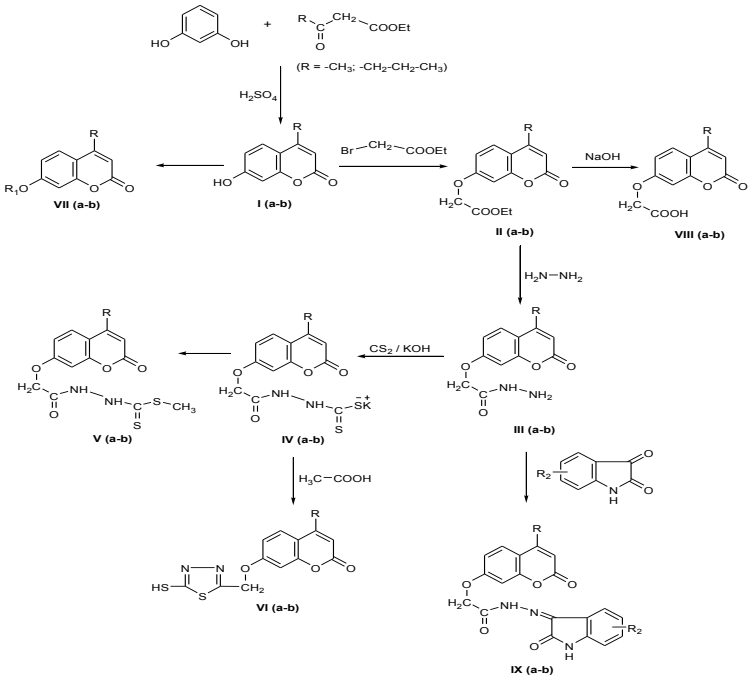

Figure 1.
Synthesis of the investigated compounds

Table I

The structure of the tested coumarin derivatives

Compound	R	R1	R2	Compound	R	R1	R2
Ia	H_3C-	-	-	VIIa2	H_3C-	CH_2=CH-CH_2-	-
Ib	H_3C-CH_2-CH_2-	-	-	VIIb	H_3C-CH_2-CH_2-	H_3C-	-
IIa	H_3C-	-	-	VIIb1	H_3C-CH_2-CH_2-	H_3C-CH_2-	-
IIb	H_3C-CH_2-CH_2-	-	-	VIIb2	H_3C-CH_2-CH_2-	CH_2=CH-CH_2-	-
IIIa	H_3C-	-	-	VIIIa	H_3C-	-	-
IIIb	H_3C-CH_2-CH_2-	-	-	VIIIb	H_3C-CH_2-CH_2-	-	-
IVa	H_3C-	-	-	IXa	H_3C-	-	H
IVb	H_3C-CH_2-CH_2-	-	-	IXa1	H_3C-	-	7 CH_3
Va	H_3C-	-	-	IXa2	H_3C-	-	5 Cl
Vb	H_3C-CH_2-CH_2-	-	-	IXa3	H_3C-	-	5 NO_2
VIa	H_3C-	-	-	IXb	H_3C-CH_2-CH_2-	-	H
VIb	H_3C-CH_2-CH_2-	-	-	IXb1	H_3C-CH_2-CH_2-	-	7 CH_3
VIIa	H_3C-	H_3C-	-	IXb2	H_3C-CH_2-CH_2-	-	5 Cl
VIIa1	H_3C-	CH_3-CH_2-	-	IXb3	H_3C-CH_2-CH_2-	-	5 NO_2

The DPPH assay is based on assessing the substances' ability to reduce the stable radical (diphenylpicryl-hydrazyl) to diphenylpicrylhydrazine. The DPPH free radical, bearing an odd electron, gives a strong absorption maximum at λ = 517 nm (purple colour). When the odd electron of the DPPH radical pairs with a hydrogen atom from an antioxidant, the reduced form DPPH-H is created, and the colour turns from purple to yellow [6, 10].

Out of the tested compounds, the most active DPPH free radicals scavengers were the coumarin hydrazide derivatives (IIIa-b, IVa-b, Va-b). The activities of IVa and Va were similar to that of the standard (reference substance, ascorbic acid, the inhibition percentage being over 90%, the introduction of sulphur atoms in the molecule having a positive influence on the scavenging potential. Substances IIIa, IVa, Va, containing a methyl group, were slightly more active than their analogues with propyl radicals (Figure 2).

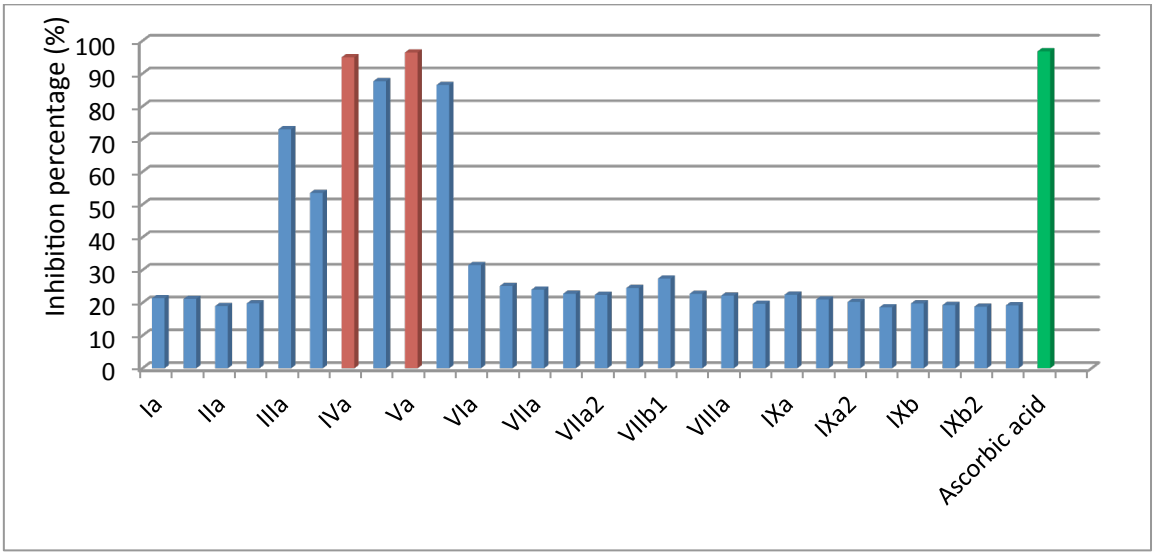

Figure 2.

The DPPH inhibition percentages for the investigated substances (1mg/mL)

A possible mechanism that can explain the antioxidant effect of the coumarin hydrazide derivatives is related to the keto-enol forms of the substances, the enol group being capable to easily donate the hydrogen (Figure 3) [3].

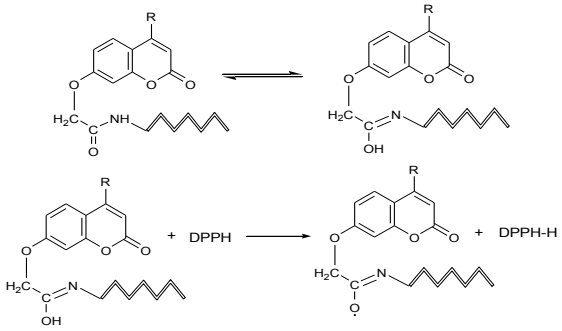

Figure 3.
Possible mechanism for the DPPH scavenger activity

Fe (III) reduction is often used as an indicator of electron donating activity. In the reducing power assay, antioxidants with electron-donating abilities reduce ferricyanide to ferrocyanide by donating an electron. The amount of ferrocyanide is monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing the absorbance at 700 nm indicates an increase in the reducing ability [4]. Within this assay, EC_{50} values are the effective concentrations at which the absorbance is 0.5 [1].

The reducing power of the tested compounds was modest and the results are presented in Table II. The only substances that were moderately active were the hydrazide derivatives IIIa, IVa, IVb and Va, but their activity was inferior to that exhibited by the reference substance (ascorbic acid). The calculated values for EC_{50} are shown in Figure 4. This method could not be applied to compound IIIb due to the formation of an abundant precipitate during the process.

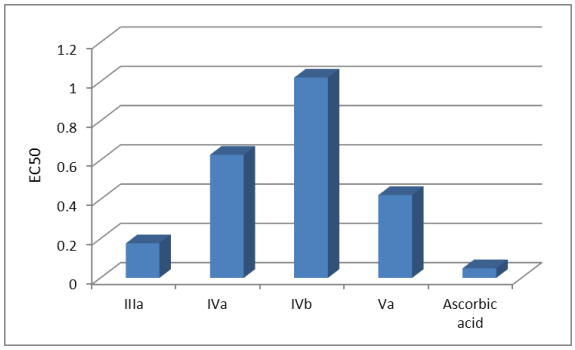

Figure 4.
EC_{50} values for the most active compounds in the reducing power assay

Table II
The reducing power of the investigated substances

Compound	EC_{50}	Compound	EC_{50}
Ia	>> 1mg/mL	VIIb	>> 1 mg/mL
Ib	>> 1mg/mL	VIIb1	> 1 mg/mL
IIa	>> 1mg/mL	VIIb2	>> 1 mg/mL
IIb	>> 1mg/mL	VIIIa	>> 1 mg/mL
IIIa	0.176 mg/mL	VIIIb	>> 1 mg/mL
IVa	0.627 mg/mL	IXa	>> 1 mg/mL
IVb	1.02 mg/mL	IXa1	>> 1 mg/mL
Va	0.422 mg/mL	IXa2	> 1 mg/mL
Vb	> 1 mg/mL	IXa3	>> 1 mg/mL
VIa	>> 1 mg/mL	IXb	>> 1 mg/mL
VIb	> 1 mg/mL	IXb1	>> 1 mg/mL
VIIa	>> 1 mg/mL	IXb2	>> 1 mg/mL
VIIa1	> 1 mg/mL	IXb3	>> 1 mg/mL
VIIa2	>> 1 mg/mL	Ascorbic acid 1 mg/mL	0.049 mg/mL

Nitric oxide is involved in a variety of biological functions (neurotransmission, vascular homeostasis, antimicrobial and antitumor activities). NO was primarily described as a regulator of vascular tone in the cardiovascular system. Beyond this function it can prevent platelet activation, limit leukocyte adhesion to the endothelium, regulate myocardial contractility and it is involved in immune system reactions.

Despite the possible beneficial effects of NO, it also contributes to oxidative damage. In general, the overwhelming production of NO contributes to the pathogenesis of both acute and chronic inflammatory processes and NO has been recognized as one of the main signalling molecules involved in these processes [9, 12]. Therefore, compounds that act like nitric oxide inhibitors have beneficial effects.

The NO inhibition assay is based on the diazotization of sulphanilic acid at acid pH by nitric oxide. The reaction product is subsequently coupled stoichiometrically with N-(1-naphthyl) ethylene-diamine, forming a coloured azo compound which is measured spectrophotometrically at 548 nm [6].

Most of the investigated substances were moderate NO inhibitors (Figure 5).

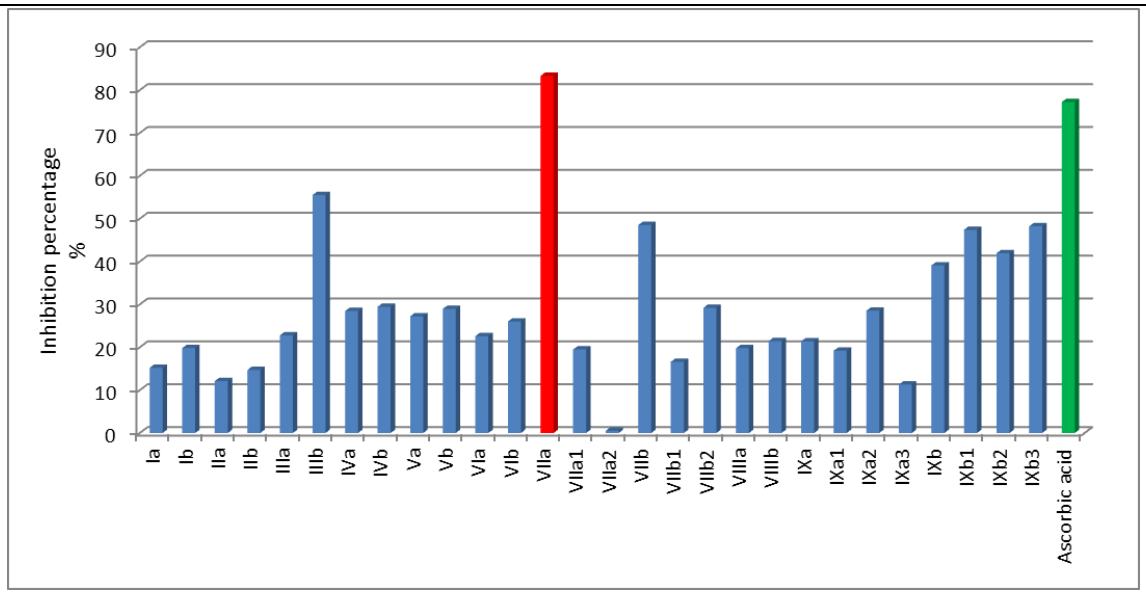

Figure 5.
The NO inhibition activity of the tested substances

A good NO inhibition activity was exhibited by compound IIIb (2-(2-oxo-4-propyl-2H-chromen-7-yloxy)acetohydrazide) and its condensation products with isatin and three substituted isatin derivatives (7-methyl, 5-chloro and 5-nitro isatin). The presence of a methoxy group in position 7 on the coumarin ring had a positive influence on the NO inhibition potential. The activity of 4-methyl-7-methoxy coumarin was remarkable, exceeding the inhibitory potential of ascorbic acid, used as a reference substance.

Conclusions

The antioxidant potential of 28 coumarin derivatives was assessed using three different methods: DPPH inhibition, total reducing power and NO inhibition. The results showed a significant antioxidant activity for some of the investigated derivatives. Compounds IVa-b (4-alkyl-7-(2-oxo-ethoxy-potassium-dithiocarbazate)-2H-chromen-2-one) and Va-b (4-alkyl-7-(2-oxo-ethoxy-methyl-dithiocarbazate)-2H-chromen-2-one) were very good DPPH free radicals' scavengers, while 4-methyl-7-methoxy coumarin was a more efficient NO inhibitor than vitamin C.

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