Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *88* (6) 589–601 (2023)
JSCS–5648
Original scientific paper
Published 27 May 2023

In vitro study of redox properties of azolyl-lactones in human serum

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(Received 21 December 2022, revised 12 February, accepted 23 March 2023)

Abstract: Disruption of the redox balance in the body causes oxidative stress that can initiate many diseases. While antioxidants reduce the level of oxidizing compounds in the medium, prooxidants promote the opposite process and have been used in therapies in particular those of cancer diseases. In this study, a series of azolyl lactones, were tested in human serum as a biological matrix and the obtained values of their oxy scores (*OS*) were compared. The antioxidative properties of these compounds were also tested under conditions of induced oxidative stress using an external prooxidant, *t*-butylhydroperoxide. The results showed that the sulphur analogue 4-azolyl coumarin **5** has the best antioxidant properties (*OS* –2.2), while the halogenated derivatives of pyrazolyl-coumarin **7** and **8** act as prooxidants, but successfully resist oxidative stress (*OS* 2.7 and 2.0, respectively). Related phthalides and isocoumarins showed prooxidative properties, but azolyl isocoumarins **10** and **11** show the strongest resistance to oxidative stress, reflected in their negative oxy score value (*OS* –2.1 and –1.1, respectively). The results demonstrated that combining two pharmacophores with known redox properties can produce potent compounds in both directions, with the antioxidative and the prooxidative characteristics.

Keywords: oxidative stress; antioxidant; prooxidant; biological matrix; coumarins; azoles.

INTRODUCTION

Oxidative processes are common reactions that are the part of various metabolic transformations in the body. Small quantities of reactive oxidative species, such as free radicals, have their role in the body and participate in the regulation

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https://doi.org/10.2298/JSC221221017S

of certain biochemical processes, contributing in homeostasis maintenance.[1] Constitutive production of reactive oxygen species (ROS) is an inevitable phenomenon called physiological eustress.[2] However, if oxidative species are present in organism (tissues, cells) in a large amount and an imbalance occurs, oxidative stress can arise and cause organs' damage and thus many diseases such as cancer, diabetes, atherosclerosis and neurodegenerative processes.[3] Tumour cells proliferation takes place in high ROS environment, which is followed by antioxidants accumulation promoted by antioxidant transcription factors activation. Otherwise, critical ROS level will cause cancer cells' senescence and subsequently their death.[4] This tumour driven interplay between ROS and antioxidants, should be interrupted by cytostatic therapy, intended to increase cancer cells' ROS concentration. Ideal cytostatic should have high prooxidant activity with concomitant high selectivity for tumour cells in order to protect adjacent health tissue. This is a reason why the simultaneous use of antioxidants with cytostatic therapy should not be advised to patients, in order to preserve therapy potency. New cytostatic therapy design should rely on its prooxidant potency improvement and accompanied by new, sophisticated carriers to enable its cancer cells directed activity. Contrary to prooxidants, antioxidants promote the opposite process and can be used as prophylactic agents or as therapeutics that can reduce the side effects of anticancer drugs. Our previous study analysed several tyrosine kinase inhibitors with anticancer properties in order to reveal its redox activity, finding their clear prooxidant properties.[5]

Typical representatives of class of compounds with antioxidative properties are ascorbic acid and polyphenols. Compounds with the phenolic or stable enolic functionalities are capable of scavenging free radicals. A large number of various natural products are known as antioxidants, such as coumarins,[6–8] flavonoids,[9,10] phthalides,[11] stilbenes[12] and others. Antioxidative properties of vitamin E are also well known and have been extensively studied showing whole range of beneficial effects. Due to the importance of compounds with antioxidant properties, many synthetic compounds of these types have been produced and widely biologically profiled.[13,14] In this sense, coumarins are particularly interesting compounds as they can reduce the risk of diseases with high mortality rate such as cancer and cardiovascular diseases. This effect was attributed, at least in part, to their radical scavenging ability as the result of their antioxidative properties. Their appealing biological profile attracted much attention and this class of compounds was intensively studied in recent decades.[7,15–18]

Our involvement in this area encompassed the investigation of various azolyl derived coumarins and some structurally related lactones.[19] In our previous study, the coumarins possessing azole substituent at C-4 position showed anticancer properties against several tumour cell lines.[20] In continuation of that work we explored their antioxidative/prooxidative potential and the same properties of

related, structurally similar azolyl phthalides and azolyl isocoumarins in biological medium (serum pool of healthy subjects), and that work is outlined herein.

EXPERIMENTAL

Chemistry

Procedures for obtaining compounds **1**–**8**, **10**, **11**, **13** and **14** as well as their spectral characteristics, are described in our previous works.[19,20] Compounds **12** and **15** were synthesized according to literature procedures.[14,21] Commercially available 4-hydroxycoumarin (**9**) was purchased from Merck Schuchardt (Hohenbrunn, Germany).

Sample collection

Serum pool was formed by collecting samples from apparently healthy individuals, remaining after the every-day laboratory work. This study was created without using any patients' data.

The only samples included were of subjects whose basic biochemical parameters were within metabolite reference ranges, as a confirmation of subjects' good health. Serum pool's aliquots were frozen at −80 °C and used several months after the initial collection. Tested substances dissolved in dimethyl sulfoxide (DMSO, initial concentration 10 mmol/L), were mixed with serum pool aliquots (in 1:9 ratio in order to limit sample dilution at 10 %, because of bio-matrix preservation, thus final concentration for all tested substances were 1 mmol/L) and subjected to 2 h incubation at 37 °C. All analyses were performed in duplicate, alone or in combination with exogenously added prooxidant *tert*-butyl-hydroperoxide (TBH, conc. 0.25 mmol/L) in equi-volume ratio.

Evaluation of biochemical parameters

We performed four redox status parameters analyses, two of them were for prooxidants: total oxidative status (*TOS*) and prooxidant–antioxidant balance (*PAB*) and two of them were for antioxidants: total antioxidative status (*TAS*) and total sulfhydryl groups (SHG), by already published spectrophotometric methods.

Serum TOS presents a sum of lipid hydroperoxides and H_2O_2 concentrations and was determined using Erel's method and modified in our laboratory.[22,23] Oxidants from the sample oxidize the ferrous ion in *o*-dianisidine complex to ferric ion. The standard used for the assay calibration was water solution of hydrogen peroxide (2–200 μmol/L). Results are expressed as μmol H_2O_2 equivalent/L.

Serum PAB is a H_2O_2 concentration in an antioxidative environment and is measured according to a previously published method.[24] 3,3',5,5'-Tetramethylbenzidine (TMB) reacts with hydrogen peroxide and antioxidants like uric acid, simultaneously. Hydrogen peroxide and chromogen reaction is enzymatically catalysed with peroxidase, whereas the reaction of serum antioxidants and chromogen is non-enzymatic, *i.e.*, chemical reaction. Standard solutions were prepared by mixing varying proportions (0–100 %) of 1 mmol/L H_2O_2 with 6 mmol/L uric acid. The absorbance was measured at 450 nm. *PAB* values are expressed in arbitrary units, which correspond to the percentage of H_2O_2 in the standard solution. All measurement were performed using the micro-plate reader SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany).

TAS is a parameter which represents the total concentration of all reductive substances in blood and was measured using 10 mmol/L 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as a chromogen. ABTS molecule is oxidized to $ABTS^{\bullet+}$ radical cation using hydrogen peroxide in acidic medium (an acetate buffer 30 mmol/L, pH 3.6). Under defined

conditions, emerald green $ABTS^{\bullet+}$ molecules are stable for 6 months.[22-25] The antioxidants present in the sample cause the reagent discoloration to a degree proportional to their antioxidative potential. The reaction is calibrated with Trolox, a water-soluble vitamin E analog in the measurement range of 200–2000 μmol/L, the absorbance is measured at 660 nm, and the assay results are expressed in micromoles Trolox equivalent/L.

The levels of SHG were measured by Ellman's method modified by Kotur-Stevuljevic *et al.*, using 10 mM 5,5'-dithiobis(2-nitrodithiobenzoic acid) (DTNB) as a reagent.[23,26] DTNB reacts with aliphatic thiol compounds in a basic environment (pH 9.0) and this reaction generates equimolar quantities of mixed disulphide and 5-thio-2-nitrobenzoic acid (TNB) anion, which has absorbance maximum at 412 nm.[27,28] The method calibration was performed with the reduced glutathione as a standard, in the concentration range from 0.01–4.0 mM.

Prooxidative score, antioxidative score and oxy score

Oxy score (*OS*) is calculated as the difference between prooxidative score (average value of *Z* scores of all measured oxidants) and antioxidative score (average value of *Z* scores of all measured antioxidants). A larger oxy score means worse redox status (weaker antioxidative protection, higher prooxidants content). *Z* score is the difference between the original value and the control value divided by *SD* of control values (or population means and *SD*s).

Statistical analysis

Data are presented as median values (25^{th}–75^{th} percentile values). For the inter-groups comparison Kruskal–Wallis ANOVA and post-hoc Mann–Whitney U test were used. The *P* value below 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

A series of azolyl-coumarin derivatives including related isocoumarins and phthalides were previously synthesised an biologically profiled in various assays. As an extension of our interest in biological properties of these compounds, we further explored the antioxidative/prooxidative potential of these molecules as well. In fact, these compounds combine two pharmacophores, coumarin and diazole moieties, which are known to have redox properties individually, but their synergistic activity in this direction was not assessed.

As an addition, the redox properties of hydroxy analogues of coumarin, isocoumarin and phthalide were also tested due to their structure similarity to azolyl-coumarins.

The redox features of all these compounds in human serum as biological matrix were probed under conditions that mimic realistic physiological conditions. All structures are outlined in Fig. 1.

In order to determine the antioxidative and prooxidative properties of our compounds several parameters were measured as showed in Table I. The experiments were performed without (entries **a**–**o**, Table I) or with (entries **a′–o′**, Table I) the externally added *t*-butyl hydroperoxide in order to mimic conditions existing during pathological processes development.

The determined values for thePAB, TAS and SHG parameters were then used to calculate the prooxidative and antioxidative scores, as well as the oxy

scores, as the simple difference of the previous two factors (Table II). The calculation was performed for all compounds and experiments carried out without (entries **a**–**o**) and with the addition of TBH as exogenous prooxidant (entries **a′**––**o′**). Generally, a low value of the oxy score is associated with the pronounced antioxidant properties of tested compounds. The oxy scores with the addition of TBH indicate the ability of the system to resist the influence of exogenous prooxidant.

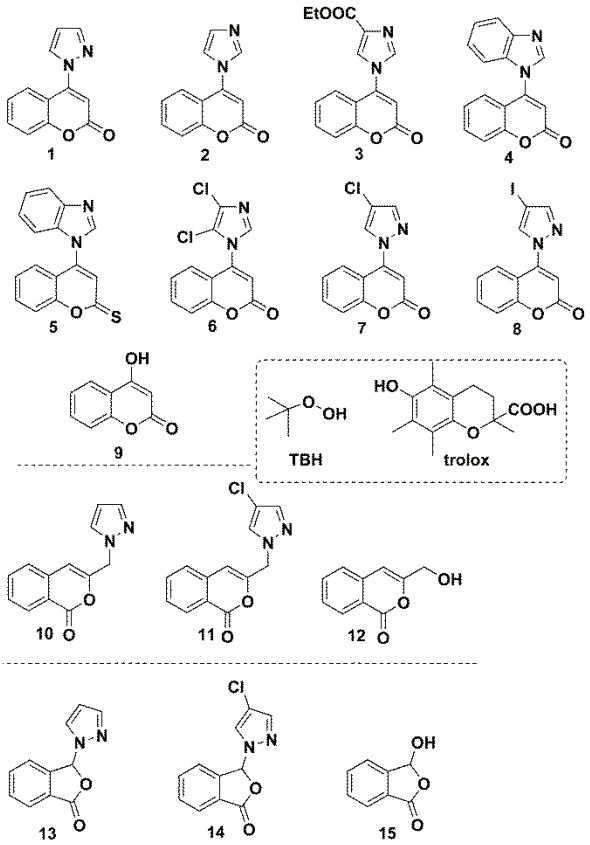

Fig 1. Structures of tested heterocycles, trolox and TBH.

TABLE I. Redox status parameters concentration in serum samples with tested compounds; data presented as medians and 25th–75th percentile values in brackets; entries **a**–**o**: samples without TBH; entries **a′**–**o′**: samples with TBH

Entry	Compound	*PAB* / U L^{-1}	*TOS* / μmol L^{-1}	*TAS* / μmol L^{-1}	*SHG* / μmol L^{-1}
–	Blank (0)	78.6 (77.9–79.2)	<2	920 (878–963)	0.209 (0.207–0.210)
a	1	73.9 (73.7–74.1)	<2	930 (903–958)	0.212 (0.206–0.219)

TABLE I. Continued

Entry	Compound	*PAB* / U L^{-1}	*TOS* / μmol L^{-1}	*TAS* / μmol L^{-1}	*SHG* / μmol L^{-1}
b	2	73.0 (72.3–73.7)	<2	1058 (990–1125)	0.176 (0.157–0.196)
c	3	74.8 (73.4–76.3)	<2	858 (690–1025)	0.180 (0.175–0.184)
d	4	73.5 (72.9–74.0)	<2	903 (858–948)	0.157 (0.146–0.167)
e	5	67.3 (66.7–68.0)	<2	980 (950–1010)	0.244 (0.242–0.246)
f	6	73.9 (72.3–75.4)	2.2 (2.1–2.3)	878 (850–905)	0.222 (0.216–0.228)
g	7	166.4 (165.9–166.9)	29.1 (28.1–30.1)	1160 (1088–1233)	0.315 (0.310–0.320)
h	8	159.3 (158.0–160.7)	8.5 (6.8–10.2)	1069 (1048–1090)	0.271 (0.267–0.275)
i	9	72.9 (71.9–73.8)	3.6 (3.3–4.0)	789 (683–895)	0.151 (0.125–0.177)
j	10	146.9 (146.3–147.5)	8.7 (8.5–8.8)	1247 (1233–1260)	0.313 (0.299–0.328)
k	11	150.7 (150.5–150.8)	10.9 (9.9–11.9)	1248 (1120–1375)	0.295 (0.290–0.300)
l	12	161.3 (157.4–165.1)	7.0 (5.6–8.4)	1174 (1138–1210)	0.274 (0.244–0.304)
m	13	159.8 (158.3–161.4)	12.7 (11.2–14.3)	1147 (940–1353)	0.304 (0.296–0.312)
n	14	151.8 (151.7–152.0)	16.0 (15.7–16.3)	1145 (1120–1170)	0.307 (0.290–0.325)
o	15	160.3 (160.2–160.4)	12.4 (12.0–12.9)	1109 (1078–1140)	0.279 (0.270–0.289)
a′	101	79.5 (78.8–80.3)	65.8 (65.7–65.9)	987 (815–1158)	0.121 (0.116–0.126)
b′	102	80.0 (79.7–80.2)	69.5 (68.8–70.1)	1062 (963–1160)	0.117 (0.110–0.125)
c′	103	78.8 (77.5–80.1)	57.1 (56.4–57.9)	1087 (846–1328)	0.116 (0.099–0.133)
d′	104	77.9 (77.5–78.2)	62.9 (61.2–64.6)	868 (838–898)	0.116 (0.115–0.117)
e′	105	74.2 (74.0–74.5)	65.4 (57.0–73.8)	978 (833–1123)	0.125 (0.116–0.133)
f′	106	79.8 (78.9–80.7)	69.6 (66.0–73.3)	1179 (1165–1193)	0.146 (0.106–0.187)
g′	107	109.2 (108.9–109.4)	43.3 (42.4–44.1)	998 (985–1010)	0.188 (0.181–0.196)
h′	108	108.7 (104.2–113.3)	38.5 (36.2–40.9)	1015 (993–1038)	0.182 (0.162–0.202)
i′	109	78.9 (77.9–79.9)	74.5 (70.5–78.4)	1064 (960–1168)	0.054 (0.020–0.087)

TABLE I. Continued

Entry	Compound	*PAB* / U L^{-1}	*TOS* / μmol L^{-1}	*TAS* / μmol L^{-1}	*SHG* / μmol L^{-1}
j′	110	104.6 (103.4–105.7)	28.4 (27.1–29.6)	1123 (1030–1215)	0.181 (0.172–0.188)
k′	111	105.2 (105.1–105.4)	35.7 (33.7–37.7)	1108 (1088–1128)	0.175 (0.167–0.183)
l′	112	107.5 (105.7–109.3)	35.6 (34.3–36.8)	1007 (963–1050)	0.168 (0.158–0.178)
m′	113	175.4 (172.5–178.3)	39.7 (39.0–40.4)	1102 (1050–1153)	0.170 (0.162–0.177)
n′	114	171.9 (170.8–173.1)	32.2 (31.7–32.7)	1053 (1038–1068)	0.177 (0.168–0.186)
o′	115	173.7 (172.3–175.0)	35.6 (32.3–38.8)	1019 (990–1048)	0.183 (0.175–0.190)
–	1000 (TBH)	81.4 (80.1–82.6)	126.4 (123.6–129.2)	873 (843–903)	0.046 (0.014–0.078)
–	Trolox	119.0 (118.0–120.0)	6.5 (5.9–7.1)	784 (750–818)	0.247 (0.203–0.291)

TABLE II. Calculated values of prooxy, antioxy and oxy score of azolyl lactones; data presented as medians and 25th–75th percentile values in brackets; entries **a**–**o**: samples without TBH; entries **a′**–**o′**: samples with TBH

Entry	Compound	Prooxy score	Antioxy score	Oxy score
–	Blank (0)	0.8 (0.40–1.3)	–0.7 (–1.0–(–)0.3)	1.5 (1.4–1.6)
a	1	–0.4 (–0.5–(–) 0.3)	–0.4 (–0.9–0.1)	0.0 (–0.4–0.4)
b	2	0.0 (–0.2–0.2)	–1.4 (–2.8–0.0)	1.4 (0.2–2.7)
c	3	0.1 (–0.3–0.6)	–2.5 (–3.8–(–)1.2)	2.6 (0.9–4.4)
d	4	0.1 (0.0–0.3)	–3.4 (–4.1–(–)2.6)	3.5 (2.9–4.1)
e	5	–0.8 (–0.9–(–)0.6)	1.5 (1.4–1.6)	–2.2 (–2.3–(–)2.2)
f	6	0.5 (0.4–0.5)	–0.3 (–0.8–0.2)	0.7 (0.3–1.1)
g	7	31.4 (31.1–31.8)	2.1 (1.2–2.9)	29.3 (28.1–30.6)
h	8	26.4 (25.6–27.2)	0.5 (0.2–0.8)	25.9 (25.3–26.4)
i	9	0.8 (0.6–1.0)	–4.4 (–6.4–(–)2.4)	5.2 (3.0–7.3)
j	10	19.7 (19.4–20.0)	3.1 (2.8–3.4)	16.6 (16.0–17.2)
k	11	21.9 (21.8–21.9)	3.0 (1.3–4.6)	18.9 (17.3–20.5)

TABLE II. Continued

Entry	Compound	Prooxy score	Antioxy score	Oxy score
l	12	27.3 (25.3–29.3)	1.9 (1.7–2.0)	25.5 (23.3–27.6)
m	13	26.9 (26.0–27.8)	1.8 (–0.8–4.4)	25.1 (23.4–26.8)
n	14	22.8 (22.7–22.9)	1.8 (1.7–2.0)	21.0 (20.8–21.2)
o	15	27.1 (27.1–27.2)	1.1 (0.6–1.6)	26.0 (25.5–26.6)
a′	101	12.8 (12.7–12.9)	–4.6 (–6.0–(–)3.3)	17.4 (15.9–18.8)
b′	102	13.5 (13.3–13.6)	–4.3 (–4.6–(–)4.1)	17.8 (17.4–18.2)
c′	103	11.1 (11.1–11.1)	–4.3(–6.6–(–)1.8)	15.4 (12.9–17.8)
d′	104	12.1 (17.8–12.4)	–5.6 (–5.8–(–)5.5)	17.7 (17.3–18.1)
e′	105	12.1 (10.6–13.6)	–4.5 (–5.0–(–)4.0)	16.6 (15.6–17.6)
f′	106	13.5 (12.7–14.2)	–2.1 (–4.1–(–)0.2	15.6 (14.4–16.8)
g′	107	1.6 (1.4–1.8)	–1.1 (–1.3–(–)0.9)	2.7 (2.6–2.8)
h′	108	1.1 (–1.2–3.4)	–0.9 (–1.4–(–)0.5)	2.0 (–0.7–4.8)
i′	109	14.3 (13.6–14.9)	–7.5 (–9.8–(–)5.2)	21.8 (18.8–24.7)
j′	110	–1.7 (–2.3–(–)1.2)	0.4 (–0.8–1.5)	–2.1 (–2.8–(–)1.5)
k′	111	–0.9 (–1.0–(–)0.9)	0.1 (0.0–0.3)	–1.1 (–1.3–(–)0.9)
l′	112	0.3 (–0.8–1.3)	–1.2 (–1.6–(–)0.7)	1.4 (–0.1–2.9)
m′	113	36.9 (35.3–38.5)	0.0 (–0.7–0.7)	36.9 (36.0–37.8)
n′	114	34.6 (34.0–35.2)	–0.5 (–0.8–(–)0.3)	35.1 (34.8–35.4)
o′	115	35.7 (35.2–36.2)	–0.9 (–1.2–(–)0.6)	36.6 (35.8–37.4)
–	1000 (TBH)	23.9 (23.6–24.3)	–9.1 (–10.5–(–)7.7)	33.0 (31.2–34.8)
–	Trolox	–11.7 (–12.0–(–) 11.5)	1.7 (0.8–2.6)	–12.7 (–13.2–(–)12.2)

The lowest value of oxy score (*OS*) in experiments performed without TBH was shown by derivative **5** (*OS* –2.2, entry **e**, Table II), which is the only one

with a thionoester group in the lacton ring. This is not surprising, bearing in mind the fact that thiocarbonyl compounds are known as good radical scavengers.[29–31] Additional unambiguous proof for the essential role of the thiono group is the antioxidative potential for compound **4**, the derivative with oxygen in place of sulphur, showing weaker antioxidative properties (*OS* 3.5, entry **d**, Table II). Further structure–activity relationship (SAR) analysis demonstrated some additional facts. The compound **2** possessing simple imidazole substituent showed better antioxidative properties (*OS* 1.4, entry **b**, Table II) than the benzimidazol derivative **4**. This might suggest that imidazole moiety directly contributes to the antioxidative properties and that this effect is hampered by the benzene ring in case of **4**. Going further along this line, the derivatives with chlorine **6** and carbethoxy group **3** compared with the parent **2** demonstrated different results. While the chloro derivative **6** showed slightly better antioxidative properties the ester derived compound **3** demonstrated worse profile, but in both cases the effect is relatively small (*OS* 2.6 and 0.7, entries **c** and **f**, respectively, Table II). It is known that amino acid histidine, which contains imidazole ring has antioxidative properties with the position C-2 prone to the oxidative transformation.[32] 2-Oxo histidine occurs in peptides as a product of its oxidation. Since this position in all our imidazole derived compounds is unsubstituted, it may contribute to the overall antioxidative feature of these derivatives. It was also interesting to compare the effect of pyrazole ring in place of imidazole. The compound **1** has simple pyrazole substituent and its oxy score is close to 0 (entry **a**, Table II), placing it in front of the complementary imidazole derivatives. It is also the most potent compound after the tiono derivative **5**. The preferred oxidative metabolic transformation of pyrazole leads to the formation of 4-hydroxy derivatives.[33] This could provide an explanation for the antioxidative properties of **1**, in particular when compared with the corresponding compounds **7** and **8** which demonstrated significantly lower antioxidative potential (entries **g** and **h**, Table II). Actually, these two compounds have prooxidative properties. It is noticeable that the influence of halogenated azole ring on antioxidative properties is more pronounced in the case of pyrazoles than in imidazoles. A possible explanation is that the halogen-occupied position C-4 in the compounds **7** and **8** cannot be oxidized as in the unsubstituted derivative **1** while in the case of compound **6**, as mentioned above, the unsubstituted position C-2 might be a key structural feature for the antioxidative character. In our previous study the compound **7** showed antiproliferative activity against tumor cell line K562 ($IC_{50} \approx 3.06$ μmol).[20]

In order to determinate the significance of the azole attached to the coumarin, the antioxidative properties of 4-hydroxycoumarin (**9**) in human serum were also examined.[34,35] Under our experimental conditions, it shows weaker antioxidant properties than most azolyl coumarins, but still doesn't have a high oxy score (*OS* 5.2, entry **i**, Table II).

The oxy score was also determined in the presence of *t*-butyl hydroperoxide indicating potential of the compounds to resist to oxidative stress. It is interesting that the two coumarin derivatives with initial prooxidative properties (**7** and **8**) in the presence of TBH become antioxidants and their *OS* value becomes about ten times lower (2.7 and 2.0, entries **g'** and **h'**, Table II). This result could be attributed to the potential formation of an oxidized form of pyrazoles due to the action of strong exogenous prooxidants.[32] Namely, that would lead to the formation of hydroxypyrazoles, or some of their tautomeric forms, which are known to be strong radical scavengers.[36,37] Other coumarin derivatives in the presence of TBH lose their antioxidative properties and values of their oxy score increases.

The next small class of tested compounds have isocoumarin core linked to azole via a methylene group at C(3), as well as isocoumaryl alcohol itself. The isocoumarin derivatives with pyrazole (**10**) and chloropyrazole ring (**11)** don't show antioxidant properties, however they act as prooxidants (*OS* 19.7 and 21.9, entries **j** and **k**, Table II). The key difference compared to the above compounds is the presence of the benzylic C–H moiety which might be involved in the formation of radical intermediates. An interesting phenomenon which was observed with the coumarins **7** and **8**, also occurs here: in the presence of TBH, oxy score of azolyl-isocoumarins decreases even to a negative value (*OS* –2.1 and –1.1, entries **j'** and **k'**, Table II). This means that their role changes in the presence of exogenous prooxidants. Similar to them, isocoumaril alcohol shows better antioxidative properties with TBH, but its value of *OS* is positive. Based on these results, it can be concluded that azole ring has some influence on the antioxidative properties of that class of compounds.

As a part of overall SAR studies the phthalide derivatives, **13** and **14**, with an azolyl group as well as the hydroxyphthalide **15** were also investigated. All three phthalide derivatives are prooxidants, without and in the presence of TBH.

Oxy scores for all compounds are also outlined in Fig. S-7 of the Supplementary material to this paper which summaries the results from both experiments (without and with TBH) after 2 h incubation at 37 °C in comparison with trolox used as standard.

CONCLUSION

Our study of the substituted coumarines and the related isocoumarins and phthalides demonstrated the beneficial effect of azolyl substituents on antioxidative/prooxidative balance of these compounds. While in the case of azol-substituted coumarins majority of compounds, but not all, showed reasonable antioxidative properties, the effect of heterocyclic substituent was opposite in the case of isocoumarins and phthalides displaying prooxidative characteristics. Although some general trends can be recognised by analysing the current results,

further study would be necessary to understand the substituent effect on the antioxidative/prooxidative balance in detail, which is now underway.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12184, or from the corresponding author on request.

Acknowledgement. This research was funded by the Ministry of Education, Science and Technological Development, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-68/2022-14/200161.

ИЗВОД

IN VITRO СТУДИЈА РЕДОКС ОСОБИНА АЗОЛИЛ-ЛАКТОНА У ХУМАНОМ СЕРУМУ

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Поремећај редокс баланса у организму може узроковати оксидативни стрес, који је окидач за настанак многих болести. Антиоксиданси снижавају ниво оксидујућих једињења у медијуму у коме се налазе, док прооксиданси делују супротно и као такви могу наћи примену у терапији канцера. У овом истраживању, испитиване су антиоксидативне и прооксидативне особине серије азолил-лактона у хуманом серуму као биолошком матриксу. Антиоксидативне особине су представљене помоћу окси скорова (*OS*), а испитивано је и понашање ових једињења у условима индукованог оксидативног стреса насталог додатком *терц*-бутил-хидропероксида као спољног прооксиданса. Резултати су показали да сумпорни дериват, 4-бензимидазолил кумарин **5** има најизраженије антиоксидативне особине (*OS* –2,2), док халогеновани деривати пиразолил-кумарина **7** и **8** реагују као прооксиданси (*OS* 2,7 и 2,0). Утицају додатог прооксиданса се најбоље опиру једињења **7** и **8.** Испитивани деривати изокумарина и фталида такође показују прооксидативне особине, док се оксидативном стресу најбоље опиру азолил-изокумарини (*OS* < 0).

(Примљено 21. децембра 2022, ревидирано 12. фебруара, прихваћено 23. марта 2023)

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