STUDIA UBB. CHEMIA, LV, 4, 2010

EVALUATION OF THE ANTIOXIDANT CAPACITY OF A SERIES OF ACYL-HYDRAZONES BEARING 2-ARYL-THIAZOLE

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ABSTRACT. The antioxidant properties of a series of acyl-hydrazones bearing 2-aryl-thiazole are explored. Some specific parameters were measured: total oxidant status (TOS), total antioxidant response (TAR) and oxidative stress index (OSI). The study indicates antioxidant properties for compounds 4-6, 10, 12, while acyl-hydrazones 1 and 2 developed a prooxidant effect.

Keywords: *acyl-hydrazone, 2-aryl-thiazole, antioxidant activity*

INTRODUCTION

Reactive oxygen species (ROS) are produced in metabolic and physiological processes, and harmful oxidative reactions may occur in organisms, which are removed via enzymatic and non-enzymatic antioxidative mechanisms. Under some conditions, the increase in oxidants and decrease in antioxidants cannot be prevented, and the oxidative/ antioxidative balance shifts toward the oxidative status. Consequently, oxidative stress, involved in over 100 disorders, occurs [1-3].

ROS are implicated in the pathophysiology of ageing and oxidative stress associated pathologies such as diabetes, neurodegenerative diseases, atherosclerosis and cardiovascular complications [4-5].

ROS are normally produced throughout oxygen metabolism and play a major role in physiological and pathological cell redox signalling. Oxidative stress appears in the context of non-equilibrium of overproduction of ROS of various cellular sources (the mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate hydride oxidases (NADPHOXs or NOXs), xanthine oxidase, lipoxygenases, cytochromes P450, and other oxidases) and decreased cellular and plasma antioxidant defenses [6].

Hydroxyl group (OH) and its subsequent radicals are the most harmful ROS and they are mainly responsible for the oxidative injury of biomolecules. Alone hydrogen peroxide and superoxide molecules cannot directly oxidize

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lipids, nucleic acids and sugars. These species can lead to oxidative injury in biomolecules indirectly by producing ·OH via Fenton reaction and/or iron-catalyzed Haber–Weiss reaction [7]. Oxidized molecules generally form new radicals leading to radical chain reactions or they are neutralized by antioxidants.

Antioxidant molecules prevent and/or inhibit these harmful reactions remaining very efficient in preventing the early atherosclerotic lesions and inflammatory events implicated in the evolution of the lesions toward [8].

Hydrazones display diverse biological and pharmaceutical activities, such as antimicrobial, antitumoral, antiinflammatory, antioxidant properties [9, 10]. The antioxidant activity may be due to their capacity of metal chelating. Under certain abnormal conditions, activated oxygen species release iron from the transport and storage proteins, and the resulting “free” iron (Fe^{2+}) promotes the formation of the devastatingly reactive toxic ·OH. Thus, chelating the iron, hydrazones may inhibit free-radical formation and the consequent free radical tissue damage [11].

Chromone derivatives possess a wide spectrum of biological activities, such as anti-inflammatory, antifungal, antimicrobial, antiviral, antitumour, mainly due to their well-recognised antioxidant properties, which stem from their ability to neutralise active forms of oxygen and to cut off free radical processes. This potential health benefit is ruled by strict structure-activity/ structure-property relationships, which, apart from determining their biological action, modulate their systemic distribution and bioavailability in sites of oxidation within the cell [12, 13].

Prompted by these reports, we tested the antioxidant capacity of 14 acyl-hydrazones bearing 2-aryl-thiazole scaffold. Some of these hydrazones have a chromone moiety in their structures, too.

RESULTS AND DISCUSSION

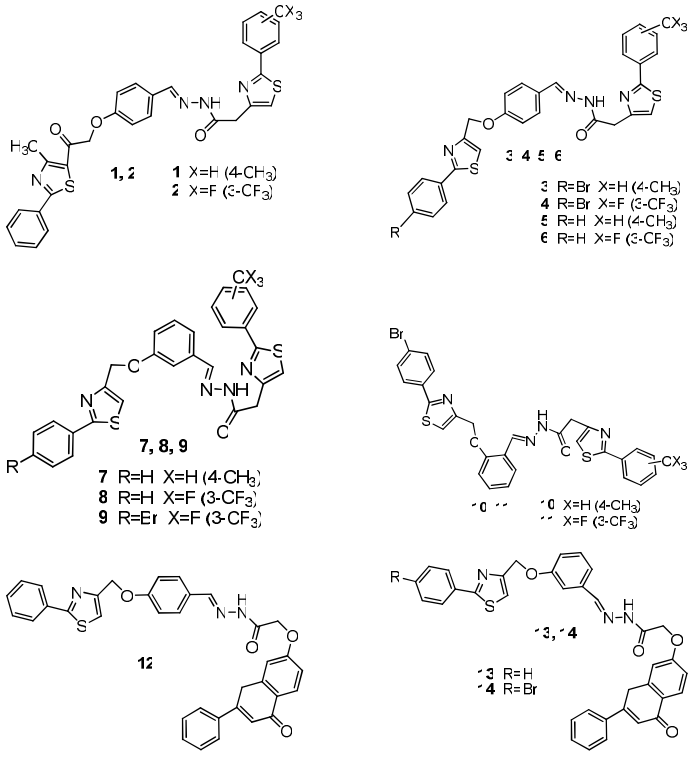
Our study investigated the effects of the acyl-hydrazones (Scheme 1) in an acute experimental inflammation, because of the close relationship between this process and ROS as endogenous mediators. The inflammation was induced by the i.m. injection of turpentine oil [14, 15]. The antioxidant effect of the tested compounds was assessed by evaluating some specific parameters: total antioxidant response (TAR), total oxidant status (TOS), and the index of oxidative stress (OSI) [OSI=(TOS/TAR)x100].

Determination of TAR in animal serum is done by a method that allows the simultaneous measurement of more molecules with an antioxidant potential, against the oxidants from serum. The method is based on the suppression of the obtention of dianisidil radicals from the oxidative process of orto-dianisidine, radicals colored in brown-yellow, by the antioxidant substances present in serum [16]. Therefore, a standardized solution of Fe^{+2}-o-dianisidine complex suffers a Fenton reaction with a standardized solution of H_2O_2, forming ·OH radicals. These radicals, in the presence of an acid, oxidize o-dianisidine to dianisidil radicals, which determine further oxidation reactions. The antioxidant

agents from the sample inhibit the oxidation reactions and determines the apparition of color. The method for evaluating the total antioxidant response is a colorimetric technique. The intensity of the color at the end of reactions is spectrophotometrically determined.

The method used to determine the total oxidant status is based on the oxidation of Fe^{+2}-o-dianisidine complex to Fe^{+3}, which forms a colored complex with xilenol-orange [17]. The intensity of this complex is colorimetrically determined and is in a direct relationship with the total quantity of oxidant molecules present in the sample.

The calculation of the index of oxidative stress is very useful for investigating and comparing the oxidant-antioxidant status of the tested compounds. Big values of OSI indicate oxidant properties, while small values suggest good antioxidant capacity.



Scheme 1

The study was performed on adult male Wistar-Bratislava albino rats, divided in groups, which received food and water *ad libitum*. The effects of the compounds were compared with those from the inflammation group (I), and with those from the group treated with Meloxicam (M), as a reference NSAID with an antioxidant activity. A negative control group (C) of healthy rats without any

treatment was also used. All the tests were performed in triplicate and the average was taken as final reading.

The total antioxidant response, as a measure of each organism to protect himself against the oxidant agents, by releasing different physiological antioxidant substances and/or using different exogenous ones, showed a higher value than that of the total oxidant status for the control group. This resulted in a small TOS/TAR ratio (Tabel 1). On the other hand, for the inflammation group, OSI had a bigger value, as expected, because of the important increase of TOS, doubled by the decrease of TAR.

Table 1. Effects of the compounds on the oxidative stress

Compound	TOS	TAR	OSI (TOS/TAR)x100
C	1.52±0.61	2.332±0.033	0.06518
I	30.25±2.2	1.0922±0.0029	2.769639
M	19.99±2.5	1.1017±0.0079	1.814469
1	33.54±2.97	1.0969±0.0026	3.057708
2	31.04±3.78	1.097±0.004 *	2.829535
3	27.27±3.52 *	1.1018±0.0029 **	2.475041
4	25.92±2.64 *	1.104±0.0065 **	2.347826
5	26.66±2.83 *	1.0989±0.0014 **	2.426062
6	22.57±3.44 **	1.0952±0.001 *	2.060811
7	28.04±3.3	1.0983±0.0007 **	2.553037
8	10.98±1.36 **, ***	1.0882±0.002	1.009006
9	35.77±3.3	1.0963±0.006	3.262793
10	27.62±2.03 *	1.0978±0.0022 **	2.515941
11	40.18±1.83	1.103±0.003	3.642792
12	20.35±0.69 **	1.0992±0.0032	1.851346
13	9.82±1.1 **, ***	1.0855±0.0047	0.904652
14	45.9±2.46	1.0979±0.0023	4.180709

* p<0.05, **p<0.001 (comparing with inflammation group)
*** p<0.001 (comparing with Meloxicam group)

The results registered for the tested acyl-hydrazones showed a significant decrease of TOS for the compounds **4**-**6**, **10**, **12** and also an increase of TAR, compared to the inflammation group (p<0.05) (Figures 1 and 2). These values resulted in an important reduction of OSI compared to inflammation (Figure 3). The results obtained for these compounds reflect their antioxidant properties.

For compounds **8** and **13**, the reduction of TOS/TAR ratio was ascribed to the more pronounced decrease of TOS. As for compound **7**, the reduction was assigned to the more pronounced increase of TAR. These three hydrazone derivatives bearing 2-aryl-thiazole may be considered for their antioxidant capacity, too.

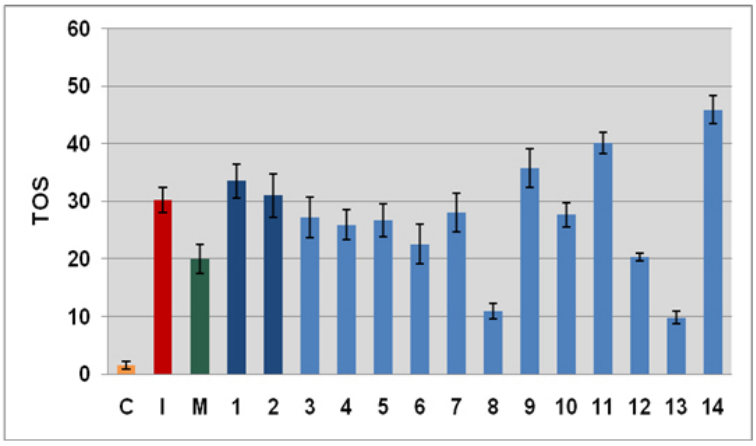


Figure 1. Influence of the compounds on the total oxidant status (TOS)

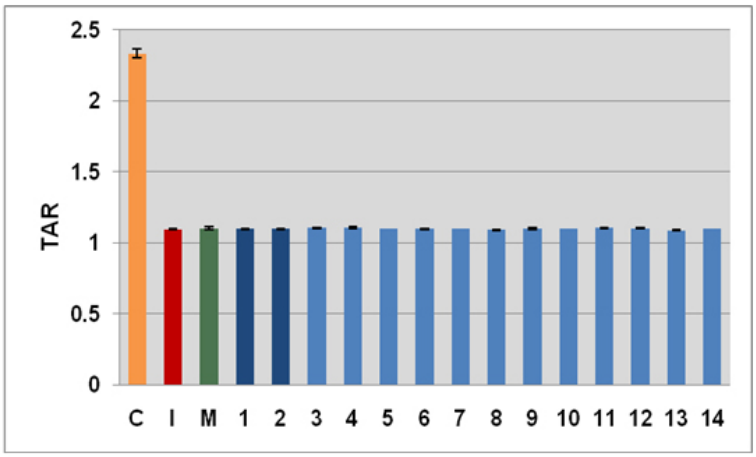


Figure 2. Influence of the compounds on the total antioxidant response (TAR)

Compounds **8** and **13** reduced TOS more powerful ($p<<0.001$) than meloxicam, the reference drug. This reduction of TOS determined the reduction of OSI, too. Values for TAR were superior to those from the group inflammation, for all the tested derivatives, excepting compounds **8** and **13**. For other compounds (**1**, **2**, **9, 11, 14**), both TOS and TAR increased, but in the case of TOS, the raise was more significant. This led to values of OSI bigger than for inflammation. Therefore, we could suspect a prooxidant effect of these compounds.

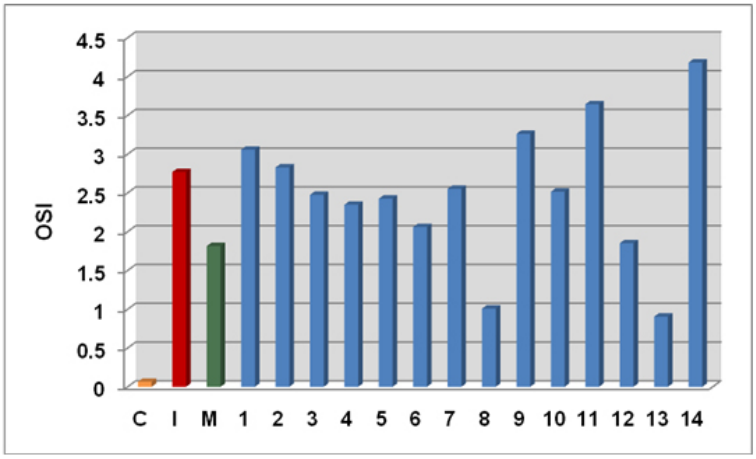


Figure 3. Variation of the index of oxidative stress (OSI) for the tested compounds

Analyzing the structural profile of the tested acyl-hydrazones, it could be observed that N'-(4-(2-(2-phenyl-4-methyl-thiazole-5-yl)-2-oxoethoxi)-benzyliden-aryl-hydrazides **1** and **2** presented a prooxidant activity. The substitution in para of the benzylidene fragment from position 2 of thiazole with brome resulted in the reduction of the antioxidant potential of the N'-(2), (3), 4-((2-phenyl-thiazole-4-yl)-methoxi)-benzyliden-hidrazides **3**-**14**, compared with the unsubstituted compounds. Two compounds of this series, **8** and **13**, demonstrated a more potent antioxidant capacity than meloxicam, the reference drug. For these two derivatives, a significant decrease of OSI was registered.

CONCLUSIONS

The study on the effect of acyl-hydrazones on the oxidative stress indicated antioxidant properties for compounds **4**-**6**, **10**, **12**. These derivatives determined a significant reduction of the total oxidant status and an increase of the total antioxidant response. For compounds **8** and **13**, the decrease of the index of oxidative stress resulted from the more pronounced reduction of TOS and more pronounced increase of TAR, in the case of compound **7**. On the other hand, acyl-hydrazones **1** and **2** developed a prooxidant effect.

SAR study showed that the substitution of phenyl from position 2 of thiazole with brome, in position 4, led to a reduction of the antioxidant capacity.

EXPERIMENTAL SECTION

The experiments were performed on adult male Wistar-Bratislava albino rats, weighing 200–250g. The animals were obtained from the Biobase of University of Medicine and Pharmacy Cluj-Napoca and housed at 25 ± 2 C^{o},

50 ± 5% relative humidity and 12 h light/dark cycle. They were distributed in groups of ten and had free access to water and food. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of University of Medicine and Pharmacy Cluj-Napoca. Experiments were performed in triplicate and the average was taken as final reading.

For the group called Inflammation, each animal was injected i.m. with 0.6mL/100g (body weight) of turpentine oil, the pro-inflammatory substance. The same procedure and dose were used for the other groups, too. After that, a 3.2mg/kg dose, equivalent to 0.0091168mmol/kg of Meloxicam, the reference standard drug, was administered i.p. to the animals from the reference group. The test groups received the synthesized compounds in an equi-molar dose with Meloxicam, by the i.p. administration of its 1% carboxymethylcelullose suspension.

Determination of **TAR**: Serum (20 µl) is mixed with Fe^{+2}-o-dianisidine complex R_1 (obtained from the dissolution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and of 3.17 g o-dianisidine in a KCl solution) (800 µl) and solution of R_2 H_2O_2 7.5 mM (40 µl). The intensity of the color obtained after 3-4 minutes after the mixing is spectrofotometrically determined at λ=444 nm. The blank is represented by 860 µl of R_1. Calibration is done using serial dillutions of a 1mM/l Trolox solution (pH 7.4). The results are expressed in mmol equivalent Trolox/l.

Determination of **TOS**: Serum (140 µl) is mixed with xilenol-orange solution R_1 (obtained from the dissolution of 0.114 g xilenol-orange and of 8.18 g NaCl in 900 ml of H_2SO_4 25 mM) (900 µl) and a solution of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ R_2 (obtained from the dissolution of 1.96 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and of 3.17 g of o-dianisidine chlorhydrate in 1000 ml H_2SO_4 25 mM) (44 µl). The intensity of the color obtained after 3-4 minutes after the mixing is spectrofotometrically determined at λ=560 nm. The blank is represented by 900 µl R_1 and 184 µl distilled water. Calibration is done using serial dillutions of a 200 µmol/l H_2O_2 solution. The results are expressed in µmol H_2O_2 equivalent /l.

Determination of **OSI**: OSI = (TOS/TAR) x 100.

The values are expressed as mean±S.D. for Inflammation group, Meloxicam group and the healthy population, separately. The comparisons of parameters were performed with Student's *t*-test. A *p*-value < 0.05 was accepted as significant. Data were analyzed using the SPSS for Windows computing program (Version 11.0).

ACKNOWLEDGMENTS

The authors would like to thank The National University Research Council in Romania (scholarship BD109/2007) for the financial support.

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