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Original Research Article

The antioxidant properties of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives under conditions of artificial oxidative stress *in vitro*

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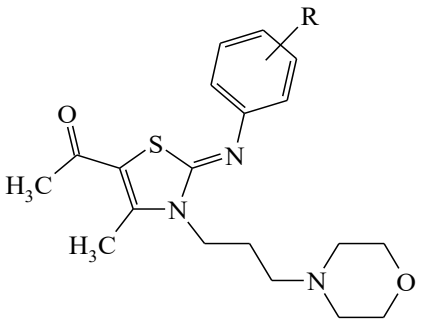
Abstract: Experimental pharmacological research of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives. Determination of the nature of the effect of electron-donating and electron-withdrawing substituents in the phenyl fragment of the molecule on expression of the antioxidant activity. Analysis of some aspects of the "structure–activity" relationship. Derivatives of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one were synthesized by Hantzsch method. Experimental pharmacological research was carried out on the model of artificial oxidative stress using the emulsion of egg-yolk lipoproteins *in vitro* as a substrate of oxidation. The substances of group 1 with methyl substituents and group 2 with chlorine substituent posseses pronounced antioxidant activity. The substances of group 2 with methoxy substituent posseses moderate antioxidant activity. Introduction of any substituents leads to a significant change in the level and direction of antioxidant activity of the substances; and it depends on the chemical nature and orientation of the substituents in the phenyl fragment of the molecule. Among the compounds under research four compounds can be recommended as potential antioxidants for further screening studies.
Keywords: 1,3-thiazole derivatives, in vitro, artificial oxidative stress, antioxidant activity

INTRODUCTION

Oxidation-reduction reactions are the basis for the leading metabolic processes of a living organism. Among them a special role is played by free radical processes, in which peroxide components causing the oxidative stress are formed. Peroxide compounds have the cytotoxic effect; they also contribute to development of the inflammatory response. The use of antioxidants helps to reduce the clinical picture of chronic inflammatory diseases. Currently, natural antioxidants are of great interest along with many synthetic drugs. In this regard, derivatives of thiazole are interesting; they are a part of numerous natural compounds, such as penicillins, the group of B1 vitamins (thiamine, phosphothiamine, cocarboxylase), althiomycine, sulfomycin, microcin B17, complex of peptide antibiotics, etc. In the recent years, many thiazole derivatives have been synthesized and revealed varied biological activities such as anticonvulsant [1], antioxidant [2-5], anti-inflammatory [6], antimicrobial [7], antihypertensive [8], antiviral [9], etc.

The aim of our research was to study the antioxidant activity of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives under conditions of artificial oxidative stress in vitro.



II

Where

R= a) H, b) 2-CH_3, c) 2,3-$(CH_3)_2$, d) 2,4-$(CH_3)_2$, e) 2,6-$(CH_3)_2$, f) 3,4-$(CH_3)_2$, g)3,5-$(CH_3)_2$, h) 2-OCH_3, i) 3-OCH_3, j) 4-OCH_3, k) 2-Cl, l) 3-Cl, m) 4-Cl.

Fig-1 Derivatives of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one IIa-m.

MATERIALS AND METHODS

Derivatives of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one IIa-m of the general formula (Figure 1) were synthesized by Hantzsch method based on the corresponding asymmetric thioureas and 3-chloropentane-2,4-dione. The general method of synthesis was described in article [10].

The assessment of the antioxidant activity (AOA) of pharmacological substances should include several stages, namely screening of biologically active substances *in vitro* on the models with generation of a certain radical and evaluation of the antioxidant activity of substances in different tissues in induction of free radical pathology [11].

Preclinical study of organic compounds is usually carried out *in vitro* using chemical test systems [11], which give information about the antiradical activity. However, there is interest to update this research by studying the ability of substances to inhibit free radical processes in animal tissues *in vitro*.

To study the antioxidant activity of potential drugs, particularly at the initial stages of their biological screening, the use of the initial assessment methods of the antioxidant and antiradical activity of compounds in experiments *in vitro* is substantiated [12-13].

Moreover, it is reasonable to carry out the pharmacological study of AOA of new substances on several models of initiation of free radical reactions in the experiments *in vitro* that present the various stages of a complex chain activation process of free radical oxidation.

When initiating free radical processes in the experiments *in vitro* antiradical activity and AOA of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives were studied by modeling artificial oxidative stress using the emulsion of egg-yolk lipoproteins as a substrate of oxidation [14]. This emulsion was placed in the culture medium with the optimal pH value of 7.5 for biosystems.

The yolk of eggs contains two types the lipid–protein complexes corresponding to very low-density and low density lipoproteins in the blood plasma by its lipid and protein composition. The model system selected has the following advantages: it is available, the release of lipoproteins is easy, it is stable in storage, and at the same time, highly oxidizable.

The experiment was conducted in the simulated conditions, variants of the experiment included control (dimethylsulfoxide solvent), solutions of the reference substances (ionol and quercetine) and the test substances with a titer of 0.3 mg/ml in the incubation medium.

As the reference substances the following substances were taken.

Ionol (2,5-di-tert-butyl-4-methylphenol) is used as an antioxidant in food production (food additive E321).

Quercetine (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-on-dihydrate) is a flavonol, which possesses the anti-edematous, spasmolytic, antihistamine, and anti-inflammatory action; it is an antioxidant, diuretic. It is included in the vitamin P group.

Dimethylsulfoxide of the analytical grade was used as a solvent of the substances under research.

To prepare the model system the yolk was isolated from a chicken egg, then it was mixed with the equal volume of potassium phosphate buffer solution (40 mM of KH_2PO_4 + 105 mM of KCl, pH 7.5). Before use the emulsion of egg-yolk lipoproteins obtained was diluted 25 times with the same buffer solution. The compounds studied, as well as the reference substances, were prepared as dimethylsulfoxide-solutions with the initial titer of 3 mg/ml.

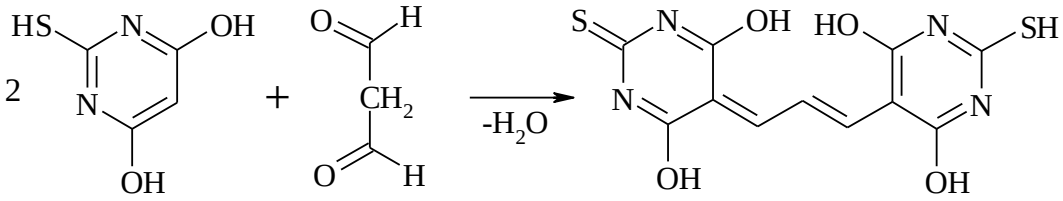
Modeling of oxidative stress was carried out as follows. To 1 ml of the emulsion of egg-yolk lipoproteins 0.5 ml of solutions of test substances, 0.5 ml of 0.5 mM of ferum (II) sulfate solution (the reactive oxygen intermediates generation system) and 3 ml of potassium phosphate buffer solution (40 mM of KH_2PO_4 + 105 mM of KCl, pH 7.5) were gradually added. The solution obtained was mixed and incubated for 60 min at 37ºC in a ITZh-0-03 water thermostat.

After incubation the solution was cooled and used to identify the products of lipid peroxidation, their intensity was estimated by accumulation of TBA-active products, in particular malondialdehyde by its reaction with thiobarbituric acid (TBA) [15]. For this purpose to 2 ml of the solution obtained after incubation 2 ml of cooled 20% trichloracetic acid was added. The solution obtained was refrigerated for 12 h, then the samples were centrifuged at 4000 rpm. After that 1 ml of freshly prepared 0.8% TBA solution was added to 2 ml of the trichloracetic acid-extract and placed in a boiling water bath for 10 min. It is known that at high temperature in the acidic medium malondialdehyde reacts with 2-thiobarbituric acid with formation of the azomethine complex with the absorption maximum at 532 nm (Scheme 1). The complex formed was extracted with alcohols, therefore, after cooling 4 ml of n-butanol were added to the sample, and optical density of butanol extracts was measured using a SF-46

spectrophotometer.

Scheme 1

Interaction of MDA with TBA



TBA MDA TBA-MDA adduct

Calculation of the antioxidant properties of the compounds studied was carried out taking into account formation of TBA-active products in the control samples containing dimethylsulfoxide, the samples of the compounds studied and inhibition of formation of TBA-products by the reference substances according to the formulas 1 and 2:

$$\%AOA_{\text{substance}} = \frac{D_{DMSO} \bullet D_{\text{substance}}}{D_{DMSO} \bullet D_{\text{ionol}}} \bullet 100\%$$

(assessment of AOA against ionol) (1)

and

$$\%AOA_{\text{substance}} = \frac{D_{DMSO} \bullet D_{\text{substance}}}{D_{DMSO} \bullet D_{\text{quercetine}}} \bullet 100\%$$

(assessment of AOA against quercetine) (2)

where:

D_{DMSO} – is the average value of the optical density of solutions containing the solvent DMSO;

$D_{substance}$ – is the average value of the optical density of solutions containing DMSO – solutions of the compounds studied;

D_{ionol} – is the average value of the optical density of solutions containing DMSO – solutions of ionol;

$D_{quercetine}$ – is the average value of the optical density of solutions containing DMSO – solutions of quercetine.

The mathematical processing of the data obtained was performed by calculating Student's t-test for independent samples [16]. In all cases there were 5 analytical replications (n=5). The probable effect of the compounds studied on inhibition of formation of TBA-active products was assessed for the significance level $p<0.05$.

RESULTS AND DISCUSSION

The studies concerning the presence of the antiradical and antioxidant activity were carried out not only for the target compounds – 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives II, and also for unsubstituted initial 3-[3-(morpholine-4-yl)propyl]-1-phenylthiourea I.

Since 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives differ by the presence of methyl-, methoxy- and chlorine-containing substituents in the phenylimino fragment of the molecule, the compounds were divided into three subgroups according to the structure and location of substituents. Thus, subgroup 1 contained compounds with methyl substituents in the phenyl fragment of the molecule in positions 2-, 2,3-, 2,4-, 2,6-, 3,4- and 3,5- (in relation to compound **II b-g**).

Subgroup 2 contained the methoxy substituent in the phenyl fragment of the molecule in positions 2-, 3- and 4- (compounds **II h-j**).

Subgroup 3 contained the chlorine substituent in the phenyl fragment of the molecule in positions 2-, 3- and 4- (compounds **II k-m**).

To assess the antioxidant properties of the compounds studied and the level of their activity in relation to ionol and quercetine the percent of inhibition of formation of TBA-active products (malondialdehyde) were calculated in relation to ionol and quercetine (Table 1).

Table-1: The antioxidant activity of 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one derivatives (%, inhibition of formation of TBA-active products) (M ±m, n=5)

No.	Contant of MDA (nmol/ml emulsion of egg-yolk lipoproteins)	AOA (% in relation to ionol)	AOA (% in relation to quercetine)
DMSO	7,54 ±0,03	-	-
Ionol	1,05 ± 0,02	-	-
Quercetine	2,44 ± 0,01	-	-
I	4,57 ± 0,023*	45.41	58.24
II a	4,77 ± 0,047*	42.68	54.31
II b	4,87 ± 0,061*	41.14	52.35
II c	5,14 ± 0,052*	36.98	47.06
II d	7,16 ± 0,059*	5.86	7.45
II e	6,12 ± 0,046*	21.88	27.84
II f	6,49 ± 0,032*	16.18	20.59
II g	6,22 ± 0,054*	20.34	25.88
II h	5,46 ± 0,057*	32.05	40.78
II i	5,48 ± 0,053*	31.74	40.39
II j	5,48 ± 0,068*	31.74	40.39
II k	4,63 ± 0,049*	44.84	57.06
II l	4,64 ± 0,032*	44.68	56.90
II m	6,01± 0,075*	23.57	30.00

*- $p<0.05$ – significance level in relation to the reference substance (ionol and quercetine).

It should be noted that the unsubstituted initial thiourea **I** has the highest values of AOA in relation to ionol and quercetine, and the target compound – 1-[2-(R-phenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one **IIa** obtained from it shows the lower values of AOA compared to it.

Introduction of both electron-donating and electron-withdrawing substituents leads to a significant change in the level and direction of AOA of the substances depending on the chemical nature and orientation of the substituents in the phenyl fragment of the molecule.

Therefore, for the substances of group 1 (with electron-donating methyl substituents) compound **II b** (2-methyl- substituent) has the highest values of AOA – 41.14% against ionol and 52.35% against quercetine, respectively, and compound **II d** (2,4-dimethyl-substituent) has the lowest values of AOA – 5.86 and 7.45%. Other compounds of this group have significantly higher values of AOA against both ionol and quercetine.

Thus, removal of the methyl substituent from the first carbon atom of the phenyl fragment weakens AOA of the substances, and its location closer to the thiazole ring significantly increases AOA of the substances under research.

It is worthy of note that for the substances of group 2 with the methoxy substituent also possessing the electron-withdrawing properties the location of this substituent almost has no effect on AOA, which is within 31.74–32.05% in all three compounds. These substances can be referred to antioxidants of the moderate strength.

The effect of electron-withdrawing substituent – chlorine on AOA should be noted. Moreover, the level of AOA in compounds **II k** (2-Cl- substituent) and **II l** (3-Cl-substituent) (44.84% and 44.68% against ionol) even exceeds the values of the substance with the unsubstituted phenyl fragment of the series – compound **II a** (42.68% against ionol) and probably approximates to that of the original substance **I** (45.41% against ionol). All substances exhibit similar dynamics in relation to quercetine.

Therefore, for chlorine-containing substances the proximity of the chlorine atom to the thiazole ring significantly increases AOA of compounds **II k** and **II l**.

CONCLUSIONS

On the model of artificial oxidative stress using the emulsion of egg-yolk lipoproteins *in vitro* as a substrate of oxidation it has been proven that:

- Introduction of any substituents leads to a significant change in the level and direction of AOA of the substances; and it depends on the chemical nature and orientation of the substituents in the phenyl fragment of the molecule.
- Among the substances of group 1 the highest values of AOA are characteristic for compounds **II b** and **II c** with the 2-methyl- and 2,3-dimethyl substituents in the phenyl fragment of the molecule. It is notable that removal of the methyl substituent from the first carbon atom of the phenyl fragment weakens AOA of the substances, and its location closer to the thiazole ring significantly increases AOA of the substances under research.

- For the substances of group 2 with the methoxy substituent having the electron-withdrawing properties the location of this substituent almost has no effect on AOA, which is within 31.74–32.05% in all three compounds. These substances can be referred to antioxidants of the moderate strength.
- The effect of electron-withdrawing substituent – chlorine on AOA is observed. Moreover, the level of AOA in compounds **II k** (2-Cl substituent) and **II l** (3-Cl-substituent) exceeds the values of the substance with the unsubstituted phenyl fragment of the series – compounds **II a** – and probably approximates to that of the original thiourea **I**. Therefore, for chlorine-containing substances the proximity of the chlorine atom to the thiazole ring significantly increases AOA of compounds **II k** and **II l**.
- Among the compounds under research the following compounds can be recommended as potential antioxidants for further screening studies: 1-[2-phenylimino-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one **II a**, 1-[2-(2- methylphenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one **II b**, 1-[2-(2-chlorophenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one **II k** and 1-[2-(3-chlorophenylimino)-4-methyl-3-(3-[morpholine-4-yl]propyl)-2,3-dihydro-1,3-thiazol-5-yl]ethane-1-one **II l**.

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